GENETIC ANALYSIS OF THE GROUP IV *Rht* LOCI IN WHEAT

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A thesis submitted for the degree of Doctor of Philosophy

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June 2011

ABSTRACT

The introduction of the group IV semi-dwarf *Rht* alleles, *Rht-B1b* (formerly *Rht1*) and *Rht-D1b* (formerly *Rht2*) into bread wheat varieties from the donor line 'Norin 10' that began in the 1960s was a major contributor to the 'green revolution'. *Rht-B1b* and *Rht-D1b* were characterised and cloned over a decade ago (Gale and Youssefian 1985; Peng *et al*. 1999), however the *Rht-A1* locus has not been isolated and little is known regarding the genetic diversity of the group IV *Rht* loci or the genetic composition of the contiguous sequence surrounding Rht that was presumably introgressed into wheat varieties along with the dwarfing alleles.

To investigate the contiguous region around *Rht*, a hexaploid wheat ('Chinese Spring' (CS)) BAC library was screened using a PCR-based technique (Febrer *et al*. 2009). This identified several *Rht*-containing BAC clones, three of which (representing the A, B, and D genomes) were sequenced and found to contain one to two genes upstream of *Rht* in conserved order. Gene synteny was also highly conserved in rice, *Brachypodium distachyon*, sorghum, and maize. The previously unidentified *Rht-A1* homoeologue was physically mapped to the long arm of chromosome 4A using aneuploid lines and mapped relative to genetic markers.

To estimate genetic diversity, the entire coding regions of *Rht-A1, Rht-B1*, and *Rht-D1* and the flanking regions (approximately 1800 bp 5' and 450 bp 3') were sequenced in 40 diverse wheat accessions. Little polymorphism and few haplotypes were identified on the A and D genomes, but on the B genome a relative abundance of haplotypes and polymorphism were present, including insertions (relative to CS) of 160 bp and 197 bp within 600 nucleotides of the ORF. The *Rht-B1* insertions did not have a pronounced effect on RNA transcript level when assessed in seedlings. In an analysis of 368 lines from the INRA bread wheat core collection (BWCC) (Balfourier et al., 2007), lines with the *Rht-B1* insertions were associated with reduced heights and reduced GA sensitivity relative to lines without an insertion, but only the height reductions associated with the 197 bp insertion were significant ($p < 0.05$). GA sensitivity tests of the INRA BWCC did not reveal any novel GA insensitive mutants. An investigation of the origin of the 'Norin 10' alleles revealed potential discrepancies between the published pedigree and of 'Norin 10' and the genotypes of seed stocks.

Sequence, annotation, and comparative genomics of the *Rht*-containing BAC clones, the mapping of *Rht-A1*, and the discovery and investigation of novel genetic diversity provides greater insight into the *Rht* region and also provides tools for further analysis of this region and for the potential improvement of bread wheat.

ACKNOWLEDGEMENTS

I am indebted to my supervisor Margaret Boulton for her generous help and fantastic support throughout the thesis. Special thanks also to my co-supervisor Wayne Powell who made this thesis possible.

I gratefully acknowledge support from the NIAB trust and the BBSRC for a studentship for research funding.

Thank you to Andy Greenland and all my colleagues at NIAB for their technical help (especially Rhian Howells, bioinformatics) and for providing a wonderful working environment.

Thanks also to the rest of my PhD committee: Nadia Al-Kaff, JIC, for her encouragement and help; Ian Mackay, NIAB, for statistical advice; Simon Griffiths, JIC, and his lab (notably Michelle Leverington-Waite and Catherine Baker) for help with genetic mapping.

I'd also like to thank the members of Margaret Boulton's lab (in particular Andrey Korolev) for their help in performing experiments and Robert Saville for his help and fruitful discussions on *Rht*.

Thanks also to collaborators Francois Balfourier (Clermont-Ferrand, INRA) and Jizeng Jia (Chinese Academy of Agricultural Sciences, P.R. China)

Finally, I'm indebted to my wife for not divorcing me during this process, and to my wife and son for reminding me that life existed outside of the PhD.

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1. GENERAL INTRODUCTION

1.1. INTRODUCTION

Global food shortages prevalent in the 1960s were greatly abated by the development of high-yielding wheat (*Triticum aestivum*) varieties, one of the world's most important crops. The rapid and worldwide adoption of the new varieties overcame major food shortages and was termed the 'green revolution'. The utilisation of semi-dwarf wheat varieties was key to producing high-yielding varieties that were more resistant to lodging, allowing for greater agricultural inputs and more intense farming practices. The genetic basis of the semi-dwarf varieties are the *Reduced height* (*Rht*) alleles *Rht-B1b* and *Rht-D1b*, which are both derived from the Japanese variety 'Norin 10'. It is estimated that over 70% of the wheat acreage planted in the developing world contains at least one of these two alleles (Evans, 1998) and that 90% of the semi-dwarf varieties contain *Rht-B1b* and/or *Rht-D1b* (Worland *et al*., 1998b). The *Rht-B1b* and *Rht-D1b* alleles encode altered forms of the DELLA protein, resulting in plants with reduced gibberellin (GA) sensitivity and reduced stalk length while maintaining high yield. Although *Rht-B1b* and *Rht-D1b* have been characterised (Gale and Youssefian, 1985) and cloned (Peng *et al*., 1999), little is known regarding the allelic diversity at these loci. Less is known about *Rht-A1*, which has not been fully sequenced or genetically mapped and has not been associated with changes in plant height or GA sensitivity. In addition, the genetic makeup of the surrounding region that was presumably introgressed into modern wheat varieties along with the *Rht-B1b* and *Rht-D1b* alleles is not known.

1.2. DELLA PROTEINS IN PLANTS

1.2.1. DELLA loci in dicots and monocots

In *Arabidopsis*, there are five DELLA genes, *GAI* (*gibberellic acid insensitive*), *RGA* (*repressor of ga1-3*), *RGL1* (*RGA-like 1*), *RGL2*, and *RGL3*. These genes each encode proteins that have specific, but overlapping functions.

GAI and RGA reduce stem elongation (King *et al*., 2001), RGL2 inhibits seed germination (Lee *et al*., 2002; Tyler *et al*., 2004), and RGA, RGL1, and RGL2 modulate floral development (Tyler *et al*., 2004). The function of RGL3 is not specifically defined, although low temperature stress was found to increase expression of this gene (Achard *et al*., 2008a). While *Arabidopsis* has five DELLA genes, other plant species have only one or two DELLA genes (Table 1.1). In the dicots, a single DELLA-encoding gene is found in tomato (*Solanum lycopersicum*), grape (*Vitis vinifera*), and *Brassica rapa* while two genes are found in soybean (*Glycine max*) and pea (*Pisum sativum*).

Table 1.1 DELLA loci in dicot and monocot plant species

Type	Common Name	Latin Name	DELLA loci	Key Reference
Dicot	Arabidopsis	*Arabidopsis thaliana*	*GAI*	Pysh et al. (1999)
			RGA	Pysh et al. (1999)
			RGL1	Dill and Sun (2001)
			RGL2	Dill and Sun (2001)
			RGL3	Dill and Sun (2001)
	Grape	*Vitis vinifera*	*VvGAI*	Boss and Thomas (2002)
	Oilseed rape	*Brassica rapa*	*BrRGA1*	Muangprom *et al*., 2005
	Soybean	*Glycine max*	*GmGAI1*	Bassel *et al.* (2004)
			GmGAI2	Bassel *et al*. (2004)
	Tomato	*Solanum lycopersicum*	*LeGAI*	Bassel *et al*. (2004)
	Pea	*Pisum sativum*	*La*	Weston *et al.* (2008)
			Cry	Weston *et al*. (2008)
Monocot	Barley	*Hordeum vulgare*	*SLN1*	Chandler *et al.* (2002)
	Brachypodium	*Brachypodium distachyon*	*Bradi1g11090*	
	Maize	*Zea mays*	*d8*	Peng *et al.* (1999)
			d9	Lawit *et al.* (2010)
	Rice	*Oryza sativa*	*SLR1*	Ikeda *et al.* (2001)
	Sorghum	*Sorghum bicolor*	*SbD8*	Li *et al*. (2010)
	Pearl Millet	*Pennisetum glaucum*	*PgD8*	Li *et al.* (2010)
	Wheat	*Triticum aestivum*	*Rht-A1*	Febrer *et al.* (2009)
			Rht-B1	Peng *et al*. (1999)
			Rht-D1	Peng *et al*. (1999)

Most monocots have only a single gene encoding a DELLA protein, these are *SLN1* in barley, *SLR1* in rice (*Oryza sativa*), *SbD8* in *sorghum bicolor*, *PgD8* in Pearl Millet (*Pennisetum glaucum*), and *Bradi1g11090* in *Brachypodium distachyon.* Wheat is an allohexaploid (2n = 6x =42) containing A, B, and D genomes derived from three closely related ancestors (see section 1.6) and for this reason has three *Rht-1* homoeoloci, which are *Rht-A1*, *Rht-B1*, and

Rht-D1. Maize (*Zea mays*), due to a recent whole-genome duplication contains two DELLA genes, *D8* (*Dwarf8*) and *D9* (*Dwarf9*) (Lawit *et al*., 2010).

In a rice database search, two additional genes, *SLRL-1* (*SLR-like1*) and *SLRL-2* (*SLR-like2*) that closely resembled *SLR1* but lacked the DELLA motif were identified (Itoh *et al*., 2005). The authors reported that plants over-expressing SLRL-1 produced a dwarf phenotype and had other characteristics that suggested that SLRL-1 represses GA signalling in the over-expressed condition. However, SLRL-1 growth repression in this study was found to be much weaker than in transgenic plants expressing a truncated SLR1 protein lacking the DELLA domain. The role of the *SLRL* genes in rice is not clear, but Itoh *et al*. (2005) suggested that these genes might function as a safety mechanism preventing excessive GA-induced growth.

1.2.2. Conserved DELLA domains

DELLAs are members of the GRAS protein family, a diverse set of regulatory proteins affecting plant growth and regulation including hormone signalling, axillary meristem initiation, shoot meristem maintenance, root architecture, light signalling, and male gametogenesis (Tian *et al*., 2004). The GRAS family is named for the GAI, RGA, and SCR (SCARECROW) proteins, which were the first members of this family to be characterised. GRAS proteins contain several conserved domains including an LHR1 (leucine heptad repeat 1), NLS (nuclear localisation signal), VHIID, LHR2 (leucine heptad repeat 2), PFYRE, and SAW domain (Pysh *et al*., 1999) (Figure 1.1). The VHIID, PFYRE, and SAW domains (each named after conserved amino acids) act to repress plant growth (see section 1.2.3). In the DELLA proteins, the GRAS domain comprises the C-terminus, while the N-terminus contains the highly conserved DELLA, TVHYNP, and poly S/T/V (rich in serine, threonine, and valine) domains that distinguish these proteins from other members of the GRAS family (Tian *et al*., 2004). The DELLA and TVHYNP domains (named for highly conserved amino acid motifs) are important for GA signal perception (see section 1.2.3).

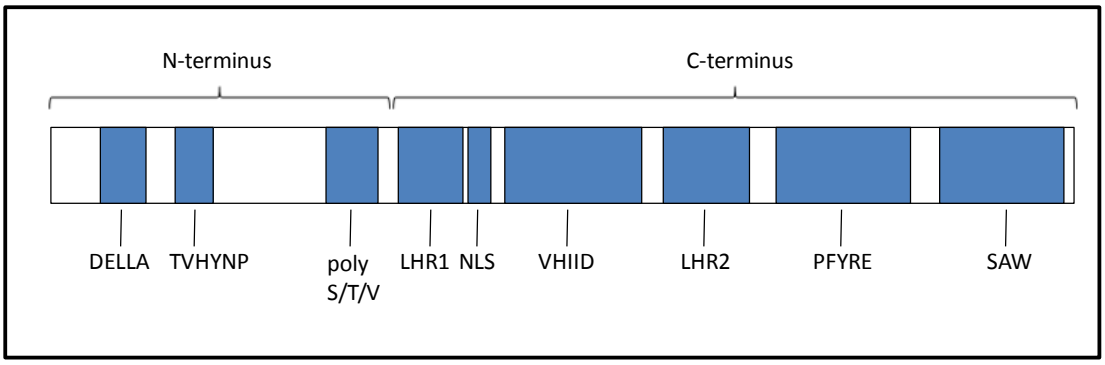


Figure 1.1. Conserved regions of the DELLA protein. Conserved domains are shown as blue rectangles. The N-terminus is unique to DELLA proteins and consists of the DELLA and TVHYNP domains (both named after conserved amino acid motifs) that participate in GA signalling and a poly S/T/V (Ser/Thr/Val rich) domain thought to regulate DELLA repression activity. The C-terminus, or GRAS domain, consists of LHR1 (leucine heptad repeat 1), NLS (nuclear localisation signal), LHR2, VHIID, PFYRE, and SAW domains (the latter three named after conserved amino acids), and functions as a growth repressor.

1.2.3. DELLA protein characterisation

Studies of DELLA mutants in *Arabidopsis* and in cereal species have shown how DELLA proteins affect plant phenotype and helped identify important regions in the DELLA protein. The *gai* mutant in *Arabidopsis* is a semi-dominant gain of function (GoF) mutant that encodes a DELLA protein lacking 17 amino acids in the DELLA domain (Peng *et al*., 1997). Plants with the *gai* allele are characterised by greater GA insensitivity (GAI), reduced plant height, and increased levels of endogenous GA (Koornneef *et al*., 1985). These are the same characteristics identified in *Rht* GoF mutants in other species, which also have N-terminal mutations. These mutant alleles include *Rht-B1b* and *Rht-D1b* in wheat, which each have a predicted stop codon in the DELLA domain (Gale and Youssefian, 1985); *D8-1* in maize, which contains a four amino acid deletion in the DELLA domain (Peng *et al*., 1999), and *Sln1d* in barley, which has a G to A amino acid substitution in the DELLA domain (Chandler *et al*., 2002). The role of the DELLA protein N-terminal domains in GA signalling was further investigated by overexpression of rice SLR1 cDNA-GFP fusion proteins that contained large deletions in specific N-terminal domains (Itoh *et al*., 2002). Overexpression of DELLA proteins with deletions in the DELLA or TVHYNP domain resulted in dwarfed plants with

decreased GA sensitivity. GFP fluorescence in proteins with DELLA or TVHYNP deletions was present in the nuclei even with the application of GA_3, indicating that these modified proteins are not degraded by GA whereas plants containing constructs of fully intact SLR1 fused to GFP were degraded in the presence of GA. A large deletion in the poly S/T/V also resulted in dwarf plants, however plants remained responsive to GA and the DELLA protein was degraded by GA. The Itoh *et al.* (2002) study indicates the importance of the DELLA and TVHYNP domains in GA signalling and suggests that the poly S/T/V domain serves a regulatory role in the repression activity of DELLA proteins.

Recessive loss of function (LoF) mutants have also been identified in *Arabidopsis*; however, due to the overlapping functions of the DELLA proteins, this required LoF mutations in four of the five DELLA proteins (RGL3 maintained a functional copy). The "DELLA LoF quadruple mutant" (*gai-t6+rga-t2+rgl1-1+rgl2-1*) has a phenotype closely resembling wild type plants treated with exogenous GA, having reduced seed dormancy and early flowering (Alvey and Harberd, 2008). A similar constitutive GA response was found in LoF DELLA mutants in rice *SLR1* and barley *SLN1*, which result in plants with tall, slender stems and slender leaves despite having reduced levels of GA. The rice *SLR1* LoF mutants include *slr1-1*, which has a frameshift in the NLS domain and *slr1-2*, *slr1-3*, and *slr1-4,* which each contain a premature stop codon in the C terminus (Ikeda *et al*., 2001). The barley mutant *sln1c* also has a premature stop codon in the C terminus of the protein (Chandler *et al*., 2002). While most LoF mutants are characterised by mutations in the C terminus of the DELLA protein, there are, as exceptions, C terminus mutants that lead to GoF mutants and a dwarf phentoype. These have been reported for the *Brassica rapa BrRGA1* (Muangprom *et al*., 2005) and maize *D9* loci (Lawit *et al*., 2010). Overall, the C-terminal mutants demonstrate the importance of this region in suppressing plant growth, although mutations in this region may also affect DELLA stability.

1.2.4. Regulation of DELLA protein levels

There is clear evidence of regulation of DELLA via mechanisms that control protein stability and regulation is also thought to occur at the transcriptional level and by post-translational modifications. In the absence of GA, DELLA proteins are stable and suppress GA responses and plant growth. In the presence of high levels of GA, the DELLA protein is degraded by ubiquitination (Figure 1.2). The process of ubiquitination begins with the binding of GA to GID1 (GA-Insensitive Dwarf 1), which was shown to occur in rice plants (Ueguchi-Tanaka *et al*., 2007). The GID1-GA complex then associates with DELLA, which is targeted by an $SCF^{SLY1/GID2}$ (Skp/Cullin/F-box; SLY1 in Arabidopsis; GID2 in rice) E3 ligase that results in DELLA ubiquitination and subsequent degradation by the 26S proteasome (Fu *et al*., 2004). The GID1-GA complex can also be inactivated by a proteolysis-independent pathway (Ueguchi-Tanaka *et al*., 2008).

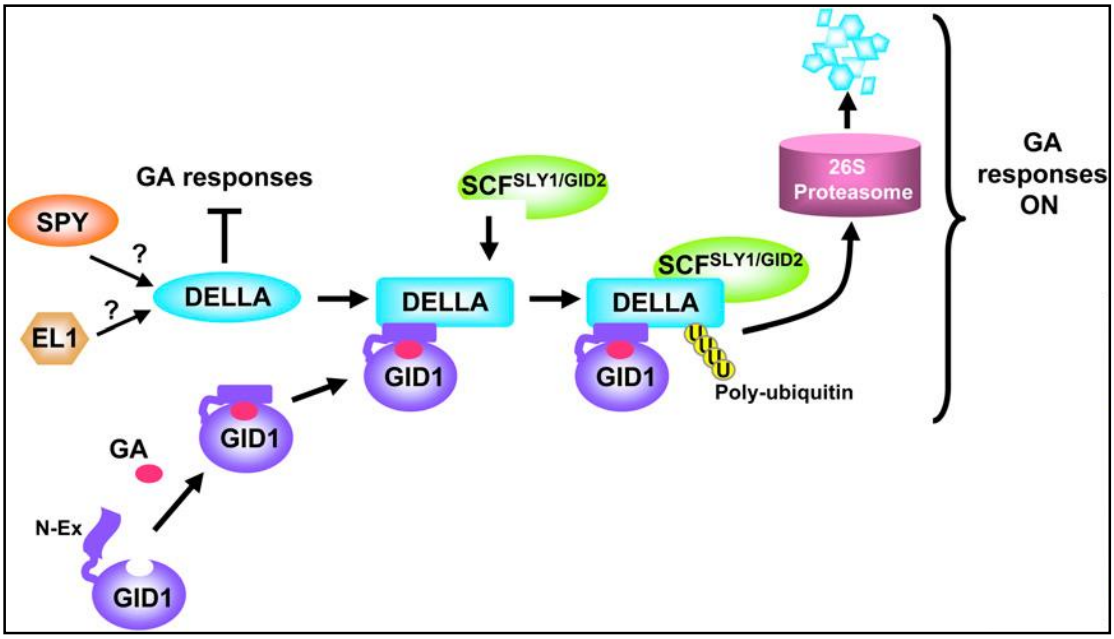


Figure 1.2. GA signalling and DELLA protein stability. DELLA inhibits GA responses in the absence of GA. When GA is present, it can interact with GID1 inducing a conformational change that allows for an interaction with the N-terminal domain of the DELLA protein, resulting in a conformation change in the GRAS domain of DELLA and allowing for interaction with the $SCF^{SLY1/GID2}$ complex. This leads to ubiquitination of DELLA and subsequent degradation by the 26S proteasome, thereby removing DELLA growth suppression. DELLA might be activated by SPY *O*-Glc-Nac modification or EL1 phosphorylation. Figure adapted from Sun *et al*. (2010).

Post-translational modifications of DELLA include phosphorylation and *O*-linked N-acetylglucosamine (*O*-Glc-Nac) modification. Phosphorylated DELLA proteins have been identified in Arabidopsis (Fu *et al*., 2004) and rice (Sasaki *et al*., 2003) and phosphorylation of DELLA by EL1 (Earlier Flowering 1) is thought to be needed for DELLA stability and activity (Sun *et al*., 2010) (Figure 1.2). SPY (Spindly), an *O*-Glc-Nac transferase was also proposed to increase the activity of RGA in Arabidopsis via *O*-Glc-Nac modification (Silverstone *et al*., 2007) (Figure 1.2)

Several studies also suggest that transcriptional control of DELLA is important. Abundant levels of RGL1, RGL2, and RGL3 in Arabidopsis were found only in germinating seeds and/or flowers and siliques, suggesting a potential transcriptional control (Tyler *et al*., 2004). In another study, GAI and RGA transcription in Arabidopsis was enhanced by direct binding of the transcription factor PIL5 (phytochrome-interacting factor3-like5) to the promoter regions of these genes (Oh *et al*., 2007). In rice, Ogawa *et al*. (2000) found that SLR1 expression levels differed based on development stage and tissue type. In wheat, transcriptional control of DELLA was found to be an important regulatory mechanism that affects seed germination (Saville, 2011), and DELLA expression levels were also found to vary between tissue types collected following ear emergence (Pearce *et al*., 2011).

1.2.5. DELLA protein function

While DELLA proteins are known to restrict plant growth in the absence of GA, the molecular mechanism is not well understood. As members of the GRAS family, DELLAs are putative transcription factors and DELLA proteins have been localised to the nucleus (Ogawa *et al*., 2000; Lawit *et al*., 2010). However, the presence of a DNA binding domain on the DELLA protein has not been shown (Achard and Genschik, 2009). *In vivo* association of DELLA with promoter regions of GA biosynthetic genes (including *GA20ox2* and *GA3ox1*), GA receptor genes (including *GID1*), and other early GA-responsive genes was detected using chromatin immunoprecipitation (ChIP) (Zentella *et al*., 2007). Based on the ChIP results, the authors proposed that DELLA

proteins repress GA signalling by activation of downstream genes thought to encode GA signalling repressors and that DELLA proteins modify GA homoeostasis by increasing expression levels of genes involved in GA biosynthesis and GA reception via a feedback mechanism (Zentella *et al*., 2007). In a more recent study, DELLA proteins were found to interact with PIF3 (phytochrome-interacting factor) (Feng *et al*., 2008) and PIF4 (De Lucas *et al*., 2008), members of the basic helix-loop-helix (bHLH) transcription factor family), reducing the ability of the PIFs to enhance expression of unidentified target genes involved in growth promotion. DELLA proteins have been shown to interact with three additional bHLH transcription factors (PIL2, PIL5, and SPT) (Gallego-Bartoleme *et al*., 2010), indicating DELLAs likely can interact with a broad class of bHLH proteins *in planta*.

1.2.6. Role of DELLAs in response to plant stress

Several studies in *Arabidopsis* indicate that DELLA proteins play a key role in plant survival under abiotic and biotic stress. The involvement of DELLA proteins in the regulation of salt-activated phytohormone signalling pathways was recently demonstrated (Achard *et al*., 2006). The GA insensitive/GoF *Arabidopsis* mutant, *gai* and the GA deficient mutant *ga1-3* were both found to be more tolerant of high salt than plants carrying the wild type allele at these loci. In a high salt environment, wild type *Arabidopsis* plants had a survival rate of 36% while the survival rate of the *gai* mutant was 82% and of *ga1-3* was 93% (Achard *et al*., 2006). In contrast, the Arabidopsis “DELLA LoF quadruple mutant” was less salt tolerant than the wild type with a survival rate of 7% (Achard *et al*., 2006). Similarly, barley GoF mutants at the *SLN* locus survive heat shock and salt stress better than wild type lines while LoF mutants have reduced survival rates relative to the wild type lines (T. Moore, JIC, pers. comm.). Under cold stress, increased DELLA protein accumulation is also associated with higher freezing tolerance in *Arabidopsis* plants (Achard *et al*., 2008a). Increased stress tolerance associated with DELLAs was hypothesised to be caused by an increase in the stability of the DELLA proteins, which restricts plant growth and thereby enhances plant survival (Achard and Genschik, 2008a). One mechanism by which DELLAs may

restrain growth and increase survival is by reducing levels of reactive oxygen species (ROS) (Achard *et al*., 2008b). ROS are small molecules created in response to stress that result in cell death as part of the plant defence response. DELLAs were found to reduce ROS accumulation and cell death after biotic or abiotic stress by increasing expression of ROS detoxification enzymes, thereby improving stress tolerance (Achard *et al*., 2008b).

In terms of biotic stress, DELLAs have been shown to promote increased resistance to necrotrophs, but increased susceptibility to biotrophs. The GoF (stabilised) DELLA protein found in the *Arabidopsis gai* mutant was found to play a role in growth inhibition as part of a defence response when plants perceived flg-22, a peptide signal associated with necrotrophs (Navarro *et al*., 2008). The authors also found that the *Arabidopsis* "DELLA LoF quadruple mutant" resulted in increased resistance to the biotroph *Pseudomonas syringae*. The mechanism is thought to be partially related to the balance of the jasmonic acid (JA)/salicylic acid (SA) defence pathways as high JA:SA generally results in enhanced resistance to necrotrophs and high SA:JA in enhanced resistance to biotrophs. Increased DELLA stability is thought to enhance JA signalling, possibly at the expense of SA signalling (Navarro *et al*., 2008). The barley (cv. 'Himalaya') *Sln1d* GoF DELLA mutant shows a significant increase in susceptibility to the biotroph *Blumeria graminis* (which causes powdery mildew) relative to the wild type while resistance of the LoF DELLA mutant *sln1c* was not significantly changed relative to the wild type (Saville, 2011). In wheat (cv. 'Mercia' and 'Maris Huntsman') infected with *B. graminis*, no change in resistance was evident between wild type plants and near isogenic lines (NILs) containing a single GoF allele (*Rht-B1c* or *Rht-D1c*, which each cause severe dwarfing), however, NILs containing two dwarfing alleles (*Rht-B1c*+*Rht-D1b*) were more susceptible than wild type plants (Saville, 2011). In addition, R. Saville also reported an increased resistance to necrotrophs *Oculimacula yallundae* and *O. acuformis* (which both cause eyespot disease) in wheat (cv. 'Mercia') GoF *Rht-B1c* and *Rht-D1c* NILs relative to the wild type and in the barley (cv. 'Himalaya') *Sln1d* GoF mutant relative to the wild type. These studies demonstrate not only the role of DELLA in abiotic and biotic stress, but also its role as an integrator of

hormonal and environmental signals. Plant responses to auxin and ethylene have also been attributed to DELLA function, suggesting that DELLA proteins may serve as important integrators of multiple phytohormone signalling inputs (Alvey and Harberd, 2005; Alvey and Boulton, 2008).

1.3. GROUP IV *RHT-1* LOCI AND DWARFING ALLELES IN WHEAT

1.3.1. *Rht-1* loci

The nomenclature of the Group IV *Rht* loci in wheat follows that of McIntosh (1988) with *Rht-1* serving as the generic locus name for the three homoeoloci. The loci on chromosomes 4A, 4B, and 4D are referred to as *Rht-A1*, *Rht-B1*, and *Rht-D1*, respectively. *Rht-B1* and *Rht-D1* have been mapped to the short arms of chromosomes 4B and 4D, respectively (Gale and Youssefian, 1985). *Rht-B1* has consistently been located near the centromere with estimates ranging from 10 to 22 cM distal (McVittie *et al.*, 1978; Borner *et al.*, 1997; Ellis *et al.*, 2002, Somers *et al.*, 2004). The location of *Rht-D1* is less clear with locations ranging from 15 cM distal of the centromere (McVittie *et al*., 1978) to having no linkage with the centromere (Izumi *et al*., 1983), and there is little consensus among mapping studies. No attempt has been made to map the *Rht-A1* locus owing to the absence of any detectable height variation associated with this locus and the lack of available sequence, which was only recently published (Febrer *et al*., 2009; Pearce *et al*., 2011). The location of the *Rht-A1* locus may not be colinear with either *Rht-B1* or *Rht-D1* because of multiple inversions and translocations on 4A that have resulted in the majority of the native short (S) arm of chromosome 4A being located on the “modern” long (L) arm of 4A (Miftahudin *et al.*, 2004).

Wild type alleles at each *Rht-1* locus, designated by an “*a”* (*Rht-A1a*; *Rht-B1a*; *Rht-D1a*), confer plants with a “tall” height that are GA sensitive (Table 1.2). The *Rht-B1a* and *Rht-D1a* genes (cv. Chinese Spring (CS)) consist of a single exon approximately 1.87 kb in length containing the highly conserved N- and C- terminal domains that are characteristic of DELLA proteins (Peng *et al*., 1999). The *Rht-D1a* sequence shares 62% amino acid identity with

Arabidopsis GAI and 58% with *RGA* (Peng *et al*., 1999). The CS *Rht-B1a* and CS *Rht-D1a* are the only *Rht-1*a alleles sequenced. The *Rht-B1a* and *Rht-D1a* allelic designations are not sequence-specific and other varieties having these designations may be polymorphic at these loci relative to CS. On the A genome, the likely presence of an *Rht-A1a* allele was demonstrated by Peng *et al*. (1999) using gel-blot hybridisation of the CS group 4 nullisomic-tetrasomic (NT) lines with wheat cDNA (C15-1) serving as the probe. More recently, an approximately 525 bp read from a putative *Rht-A1a* BAC clone from CS was published that was similar to, yet distinct from, the CS *Rht-B1a* and *Rht-D1a* sequences (Febrer *et al*., 2009 and Chapter 3).

Table 1.2. Summary of dwarfing alleles at the group IV *Rht-1* homoeoloci

Allele[a]	Prior Nomenclature	Source[b]	Phenotype	Chr. Loc[c]	GA sens.[d]	Key Reference
Rht-A1a		NA	tall	4A	sen.	Febrer *et al*. (2009)
Rht-B1a	*rht1*	NA	tall	4BS	sen.	Peng *et a*l. (1999)
Rht-B1b	*Rht1*	Norin 10	semi-dwarf	4BS	ins.	Gale and Youssefian (1985)
Rht-B1c	*Rht3*	Tom Thumb	severe dwarf	4BS	ins.	Gale and Youssefian (1985)
Rht-B1d	*Rht1S*	Saitama 27	semi-dwarf	4BS	ins.	Worland and Petrovic (1988)
Rht-B1e	*Rht Krasnodari 1*	Krasnodari 1	semi / severe dwarf	4BS	ins.	Worland (1986)
Rht-B1f	*Rht T.aethiopicum*	*T. aethiopicum*		4BS	ins.	Borner *et al*. (1995)
Rht-D1a	*rht2*	NA	tall	4DS	sen.	Peng *et al*. (1999)
Rht-D1b	*Rht2*	Norin 10	semi-dwarf	4DS	ins.	Gale and Youssefian (1985)
Rht-D1c	*Rht10*	Ai-bian 1	severe dwarf	4DS	ins.	Borner and Mettin (1988)
Rht-D1d	*Rht Ai-bian 1a*	Ai-bian 1a	semi-dwarf	4DS	ins.	Borner *et al.* (1991)

[a] The -*a* allele represents the wild type or tall alleles.

[b] Allelic source variety. NA = not applicable.

[c] Chromosomal location

[d] Gibberellic acid sensitivity, sensitive (sen.) or insensitive (ins.)

1.3.2. *Rht-B1b* and *Rht-D1b*

The *Rht-B1* and *Rht-D1* loci each contain a series of GAI alleles (Table 1.2) that confer varying degrees of height reduction relative to the wild type alleles (Figure 1.3). The five dwarfing alleles identified at the *Rht-B1* locus are designated by the lowercase letters *b*, *c*, *d*, *e*, and *f* following the locus name and the three dwarfing alleles at the *Rht-D1* locus are designated *b*, *c*, and *d*. By far the most economically important and most commonly used height reducing alleles in bread wheat are *Rht-B1b* and *Rht-D1b*, formerly known as

Rht1 and *Rht2*, respectively. These alleles are classified as semi-dwarf in that they confer moderate reductions in plant height (Figure 1.3). In a series of trials using four sets of NILs, *Rht-B1b* and *Rht-D1b* were 86% and 83% the height of wild type plants (Flintham *et al*., 1997). Sequences of the *Rht-B1b* and *Rht-D1b* semi-dwarf alleles reveal the presence of nucleotide substitutions that result in premature stop codons in the DELLA domain (Peng *et al*., 1999). In *Rht-B1b*, a C to T change converts amino acid 64 from glutamine to a stop codon and in *Rht-D1b*, a G to T change converts amino acid 61 from glutamic acid to a stop codon. The nucleotide sequences of the coding regions are otherwise identical to the respective *Rht-1a* alleles. It is thought that translation is reinitiated following the premature stop codons in *Rht-B1b* and *Rht-D1b*, leading to the synthesis of functional truncated DELLA proteins lacking the DELLA domain (Peng *et al*., 1999). The truncated protein is predicted to lack GA signal perception while maintaining the repressor function, which likely explains the reduced GA sensitivity of plants containing these proteins. The similarity of the *Rht-B1b* and *Rht-D1b* mutations may explain why both alleles confer a similar dwarfing phenotype.

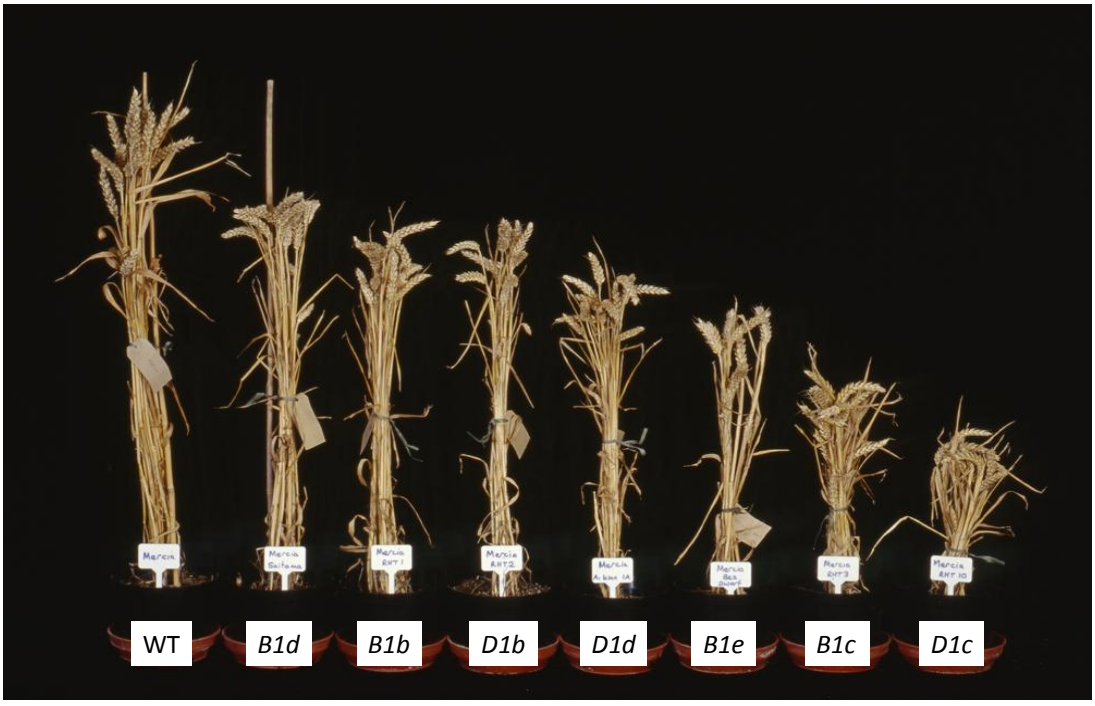


Figure 1.3. Mature plants of near isogenic lines containing a single *Rht-B1* or *Rht-D1* dwarfing alleles in the Mercia background.

Individual dwarfing alleles at the *Rht-B1* and *Rht-D1* loci were backcrossed into the Mercia wild type (WT). From left to right (with alternative nomenclature shown in parenthesis) are: Mercia (WT); *Rht-B1d* (Saitama); *Rht-B1b* (*Rht1*); *Rht-D1b* (*Rht2*); *Rht-D1d* (Ai-bian 1a); *Rht-B1e* (Bezostaya dwarf); *Rht-B1c* (*Rht3*); *Rht-D1c* (*Rht10*). Picture adapted from Pearce *et al.* (2011).

1.3.3. Alternative *Rht-B1* dwarfing alleles

Rht-B1c (previously *Rht3*) confers a greater degree of GA insensitivity than *Rht-B1b* or *Rht-D1b* and results in severe dwarfism (Figure 1.3; Table 1.2). The predominant source of the *Rht-B1c* allele is the American variety 'Tom Thumb'. Flintham *et al*. (1997) reported that *Rht-B1c* NILs were approximately 50% the height of wild type lines when averaged across four genetic backgrounds tested in multiple trials. Along with extreme dwarfism that is agronomically unfavourable, the *Rht-B1c* allele is also associated with reduced male fertility (J Flintham, JIC, pers. comm.). However, this allele was shown to reduce alpha-amylase activity in ripening grain (high alpha amylase activity is associated with pre-harvest sprouting and reduced grain quality) and may be of value to address this problem (Flintham and Gale, 1998). Recent work suggests that dwarfism caused by the *Rht-B1c* allele is likely due to a terminal repeat retro-transposons in miniature (TRIM) insertion of more than 2 kb in size that occurs in the DELLA domain (Wu *et al*., 2011).

The *Rht-B1d* allele (previously *Rht1S*) reduces height to a lesser extent than the other mutant alleles at the *Rht-1* locus (Figure 1.3; Table 1.2). Based on varietal comparisons, *Rht-B1d* was estimated to have an average plant height that is 89% of varieties not containing this allele (Worland, 1986). In addition, *Rht-B1d* is more sensitive to GA than *Rht-B1b* or *Rht-D1b* (GA-insensitivity of *Rht-B1d* was estimated to be approximately half that of the *Rht-B1b* or *Rht-D1b* alleles (Worland, 1986). *Rht-B1d* was first reported in the old Japanese variety 'Saitama 27', being introduced into European bread wheats in 1947, and currently is present primarily in Southern European wheat lines (Worland and Petrovic, 1988). The smaller decrease in height was suggested to have potential agronomic advantage under some conditions (Worland and Petrovic,

1988). *Rht-B1d* carries the *Rht-B1b* point mutation that leads to the premature stop codon in the DELLA domain (Pestsova *et al*., 2008). This result was confirmed by sequencing the entire open reading frame (ORF) of *Rht-B1d*, however no additional polymorphisms were found suggesting the presence of an undetected mutation that confers increased height relative to *Rht-B1b* (Pearce *et al*., 2011).

The semi-dwarf allele *Rht-B1e* (previously *Rht Krasnodari 1* or *Rht1*(*B.dw*)) results in plant heights intermediate between those of severe and semi-dwarfs (Figure 1.3; Table 1.2). Introduction of the *Rht-B1e* allele into four varietal backgrounds resulted in plant heights that were approximately 75% of the wild type, which is thought to be of potential commercial value (Worland and Sayers, 1995). The *Rht-B1e* allele resulted from a spontaneous mutation in the Russian line Bezostaya 1, with the mutant line named Krasnodari 1 (Worland, 1986). Sequencing of the ORF revealed the presence of a single nucleotide polymorphism (SNP) that introduces a premature stop codon in the DELLA domain (Pearce *et al*., 2011). The stop codon occurs at amino acid 61 (relative to *Rht-B1a* of CS), which is just three amino acids upstream of the *Rht-B1b* premature stop codon (Pearce *et al*., 2011).

The allele *Rht-B1f* was identified in two tetraploid Ethiopian accessions belonging to the subspecies *Triticum aethiopicum* JAKUBZ (Borner *et al*., 1995) (Table 1.2). The relative GA sensitivity of the allele and its effect on height has not yet been classified, nor has the genetic cause of dwarfism been established.

1.3.4. Alternative *Rht-D1* dwarfing alleles

The allele, *Rht-D1c* (previously *Rht10*) results in the greatest plant height reduction among the *Rht-1* GAI alleles (Figure 1.3; Table 1.2), limiting its agronomic value. The original source of dwarfism is the Chinese variety ‘Ai-bian 1’ (Izumi *et al*., 1981). *Rht-D1c* was found to carry the *Rht-D1b* point mutation leading to a predicted premature stop codon (Pestsova *et al*., 2008). More recently, quantitative reverse transcriptase PCR (qRT-PCR) transcript

analysis revealed an approximately four-fold increase in *Rht-D1c* copy number relative to lines with the *Rht-D1a* allele (Pearce *et al*., 2011). This was also confirmed by southern hybridisation using probes specific to *Rht-D1* and these results indicate that the severe dwarfism assocated with *Rht-D1c* is likely the result of increased accumulation of the GAI DELLA protein (Pearce *et al*., 2011).

The allele, *Rht-D1d* (previously *Rht Ai-bian 1a*) results in plants with a semi-dwarf stature with height intermediate between plants containing *Rht-D1b* or *Rht-B1e* (Figure 1.3; Table 1.2). This allele was derived from a spontaneous mutation in Ai-bain 1, which carries the *Rht-D1c* allele (Borner *et al*., 1991). Like *Rht-D1c*, *Rht-D1d* contains the *Rht-D1b* point mutation that leads to a predicted premature stop codon (Pestsova *et al*., 2008). However, unlike *Rht-D1c*, there does not appear to be an increase in *Rht-D1* copy number, which might explain the height relative *Rht-D1c* (Pearce *et al*., 2011). Taken together, these data suggest that the spontaneous mutation in *Rht-D1c* that produced *Rht-D1d* involved a reduction in gene copy number.

1.4. ADDITIONAL *RHT* LOCI AFFECTING PLANT HEIGHT IN WHEAT

According to the Catalogue of Gene Symbols (http://www.shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp), in addition to the *Rht-1* homoeoloci, there are a further 18 loci (*Rht4* to *Rht21*) that reduce height in wheat. These 18 additional loci are all classified as GA sensitive and none have been cloned. The semi-dwarf allele *Rht8* (the wild type allele at this locus will be referred to as *rht8*, according to Gasperini, 2010), located on chromosome 2D, is one of the few GA sensitive *Rht* alleles that reduce plant height without impacting grain yield (Worland and Law, 1986). *Rht8* is the predominant allele conferring reduced height in Italian wheat varieties and it is also commonly found in Southern and Eastern Europe, and in lower frequencies in other parts of the world (Borojevic and Borojevic, 2005). Relative to *Rht-B1b* and *Rht-D1b*, plants containing *Rht8* have a longer coleoptile and greater seedling vigour (Rebetzke *et al*., 2001). For these reasons, *Rht8* is being incorporated into wheat varieties in arid

regions as a replacement for *Rht-B1b* or *Rht-D1b* (Rebetzke *et al*., 2007). *Rht8* and the photoperiod insensitive *Ppd-D1a* allele were both introduced into Italian wheat via the Japanese variety 'Akakomugi' in the early 20th century by Nazareno Strampelli in an attempt to develop early maturing varieties with resistance to lodging (Giorgi *et al*., 1982; Worland, 1999). The genetic distance between *Ppd-D1* and *Rht8* is estimated at 21.7 cM (Gasperini, 2010). The *Ppd-D1a* allele contains a 2,089 bp deletion in its promoter region, which is the likely cause of the insensitivity (Beales *et al*., 2007). *Ppd-D1a* reduces the time to flowering by an average of six to eight days when October sown in the UK (Norwich) and is also associated with a reduction in plant height (Worland *et al*., 1998a).

To estimate the effects of *Rht8* and *Ppd-D1a* on plant height, a substitution line using Mara 2D was created in Cappelle Desprez (Cappelle Desprez/Mara 2D) by Korzun *et al*. (1998). Cappelle Desprez has the genotype *rht8*+*Ppd-D1b* (photoperiod sensitive) and owing to the substitution Cappelle Desprez/Mara 2D has the genotype *Rht8*+*Ppd-D1a*. Trial results showed that Cappelle Desprez/Mara 2D was on average 18 cm shorter than Cappelle Desprez with an estimated 10 cm of the height reduction attributable to *Ppd-D1a* and 8 cm to *Rht8* (Worland *et al*., 1998). Recently, Cappelle Desprez/Mara 2D recombinant inbred lines (RILs) were grown and scored at the *Ppd-D1* and *Rht8* loci (Gasperini, 2010). The data obtained by Gasperini showed that *Ppd-D1a*+*Rht8* RILs were 16 cm (15%) shorter than Cappelle Desprez, which was in agreement with those obtained by Worland *et al*. (1998b). However, in the Gasperini study, most of the height reduction was associated with *Rht8* as *Rht8* RILs were 14 cm (13%) shorter and *Ppd-D1a* RILs were 4 cm (3%) shorter than Cappelle Desprez. The reduced height in *Rht8* lines is due to a decrease in internode length throughout the stalk that is likely caused by an altered sensitivity to brassinosteriods (Gasperini, 2010).

The GA sensitive allele, *Rht9,* is also present in 'Akakomugi' and therefore is likely present in many of the same varieties that carry *Rht8* (Dalrymple, 1980; Gale and Youssefian, 1985). *Rht9* was originally thought to be located on

7BS (Worland *et al*., 1990), but more recently a quantitative trait locus (QTL) for height related to *Rht9* was found on 5AL (Ellis *et al*., 2005).

1.5. PHYSIOLOGICAL EFFECTS OF *RHT-B1B* AND *RHT-D1B*

1.5.1. *Rht-B1b* and *Rht-D1b* effects on wheat morphology and yield

In addition to reducing plant height, *Rht-B1b* and *Rht-D1b* have pleiotropic effects on plant morphology and grain yield. The reduced height in lines with the *Rht-B1b* or *Rht-D1b* alleles results from a reduced internode cell length, while lines with the more severe *Rht-B1c* allele also have reduced cell number (Flintham *et al*, 1997). Although stem length is reduced in *Rht-B1b* or *Rht-D1b* semi-dwarf varieties, there is no change in spikelet number (Gale, 1979; Brooking and Kirby, 1981; Youssefian *et al*., 1992). *Rht-B1b* and *Rht-D1b* alleles are associated with an increase in the number of fertile florets (in particular, distal florets) per ear, thereby increasing the total number of grains per ear relative to the wild type (Flintham and Gale, 1983; Miralles and Slafer, 1995). Although grain size is generally reduced in the lines with the *Rht-B1b* or *Rht-D1b* allele, the increase in grain number results in improved yield (Gale and Youssefian, 1985). Reduced grain size is attributed to intra-floral competition for assimilate rather than a direct effect of *Rht-1* gene expression in these tissues (Flintham *et al*., 1997). Hence, grain yield of *Rht-B1b* and *Rht-D1b* semi dwarf plants is largely dependent on the supply of assimilates and, under favourable conditions, semi-dwarf alleles are associated with increased harvestable yield beyond that which can be accounted for by reduced stalk lodging (Gale and Youssefian, 1985; Flintham *et al*., 1997; Chapman *et al*., 2007). There is no clear difference between the effects of *Rht-B1b* or *Rht-D1b* on yield (Gale and Youssefian, 1985), which may be due to the close similarity of these two mutations (described in section 1.3.2). Overall, for *Rht-B1b* or *Rht-D1b* semi-dwarf varieties, there is little or no change in total above ground biomass, but a larger proportion of this biomass is in the grain thereby increasing harvest index (Gale and Youssefian, 1985).

The effect of dwarfing alleles on root growth is not well understood. In some studies, wheat plants with *Rht-B1b* or *Rht-D1b* have reduced root mass (Subbiah *et al*., 1968; McKey, 1973; Siddique *et al*., 1990), while other studies suggest an association with increased root mass (Bush and Evans, 1988; Miralles *et al*., 1997). Several other studies showed no association between dwarfing alleles and root growth (Lupton *et al*., 1974; Cholick *et al*., 1977; Richards and Passioura, 1981; Wojciechowski *et al*., 2009; Saville, 2011). Root growth studies have differed in methodology and growth conditions, which have been shown to markedly affect the outcome (Wojciechowski *et al*., 2009).

1.5.2. *Rht-B1b* and *Rht-D1b* effects on wheat abiotic stress performance

Under low production environments, the yield advantages normally associated with *Rht-B1b* and *Rht-D1b* are often diminished or absent (Gale and Youssefian, 1985). This is particularly true of spring-type wheats containing semi-dwarfing alleles (Gale and Youssefian, 1985; Flintham *et al*., 1997). Chapman *et al*. (2007) grew 16 pairs of spring wheat NILs containing *Rht-B1b* or *Rht-D1b* and compared these to *Rht-B1a*+*Rht-D1a* NILs under differing irrigation regimes in Mexico. Their results showed that in the high yield (irrigated) environments, short-strawed varieties were higher yielding, but in the lowest yielding (drought) environments, the benefit of the short-strawed varieties disappeared. Loss of the yield benefit usually associated with *Rht-B1b* and *Rht-D1b* under drought conditions was also reported by Sojka *et al*. (1981), Richards (1992a), and Innes and Blackwell (1984). It was also reported that drought stress at various stages throughout the growing period reduces both grain number and grain weight to a greater extent in lines with semi-dwarfing alleles than in wild type lines, which is thought to result from decreased water use efficiency in the semi-dwarf lines (Nizam Uddin and Marshall, 1989; Richards 1992b). Heat stress during various stages can also be more detrimental to yield in lines with the *Rht-B1b* or *Rht-D1b* alleles than wild type lines. Heat stress during ear initiation reduces competent floret number, heat stress during meiosis reduces pollen viability, and post-anthesis

heat stress reduces grain size to a greater extent in semi-dwarfs than in wild type lines (Hoogendoorn and Gale, 1988).

Rht-B1b and *Rht-D1b* alleles are associated with a shortened coleoptile and reduced early seedling vigour (less leaf area) relative to GA sensitive alleles (Rebetzke *et al*., 2001). Poor stand establishment may occur when semi-dwarfs are planted at greater than 5 cm depth (Allan, 1989). This can be problematic when planting in dry soils that require a greater planting depth to reach moisture and can result in poor stand establishment if coleoptiles cannot bring the shoot to the soil surface (Schillinger *et al*., 1998). In addition, seedlings may emerge later and lack vigour needed for early leaf development (Kirby, 1993). In arid wheat regions, such as parts of Australia, wheat breeders have suggested that GA sensitive alleles such as *Rht8* may be more suitable than *Rht-B1b* and *Rht-D1b* (Ellis *et al*., 2004).

1.5.3. *Rht-B1b* and *Rht-D1b* associations with Fusarium Head Blight

Fusarium Head Blight (FHB) is an important fungal disease caused by *F. graminearum*, which results in substantial wheat yield losses and reduced grain quality due to increased concentrations of the mycotoxin deoxynivalenol (DON). Several studies have demonstrated a negative correlation between plant height and FHB severity (Mesterhazy, 1995; Miedaner 1997; Hilton *et al*., 1999; Buerstmayr *et al*., 2000; Somers *et al*., 2003; Gervais *et al*., 2003; Schmolke *et al*., 2005; McCartney *et al*., 2007; Miedaner & Voss, 2008; Haberle *et al*., 2009). The cause of this correlation has either been attributed directly to the height of the inflorescence above the soil surface (Yan *et al*., 2011) or to linkage associated with *Rht-D1b* or *Rht-B1b* (Srinivasachary *et al.*, 2009).

Lines with the *Rht-D1b* allele are reported to have decreased Type 1 resistance (resistance to initial infection) with no change in Type 2 resistance (resistance to spread in the ear) relative to lines containing *Rht-D1a* (Srinivasachary *et al.*, 2008; Srinivasachary *et al.*, 2009). During initial infection, the fungus is thought to be in a biotrophic stage (Brown *et al.,* 2010)

and during growth in the ear is in a necrotrophic stage (Boddu *et al.,* 2006). Srinivasachary *et al.* (2008) showed that the greatest effect (51% increased FHB susceptibility) was attributed to a QTL encompassing *Rht-D1b* in the Spark (*Rht-D1a*, moderately FHB resistant) × Rialto (*Rht-D1b*, susceptible) doubled haploid wheat population. Increased susceptibility to FHB associated with *Rht-D1b* was also reported by Hilton *et al.* (1999), Draeger *et al.* (2007), Holzapfel *et al*. (2008), Voss *et al*. (2008), and Meidaner and Voss (2008). This association is also suggested by the finding that UK winter wheat varieties, which predominantly carry the *Rht-D1b* allele, are generally susceptible to FHB (Gosman *et al*., 2007). Lines containing *Rht-B1b* were also associated with decreased Type 1 resistance, but were associated with increased Type 2 resistance relative to lines containing *Rht-B1a* (Srinivasachary *et al.*, 2009). The association of *Rht-B1b* with the level of plant FHB resistance in studies other than Srinivasachary (2009) is less clear. *Rht-B1b* was associated with a 19% increased severity of FHB disease, but this was not statistically significant (Medianer and Voss, 2008). Steiner *et al*. (2004) reported that a QTL thought to correspond to *Rht-B1b* accounted for just 7.4% of the phenotypic variance associated with FHB.

It was previously suggested that increased FHB susceptibility of semi-dwarf wheat plants was related to increased humidity surrounding the ear (Klahr *et al*., 2007; Somers *et al*., 2003a) or due to other microclimate effects directly related to plant height. However, height was not correlated to FHB resistance among plants when Draeger *et al*. (2007) compared *Rht-D1a* and *Rht-D1b* doubled haploid subpopulations separately (thereby removing the confounding effect of the *Rht-D1b* QTL). Hilton *et al*. (1999) also found no difference in relative humidity between tall and short NILs and suggested that microclimate did not explain the difference in FHB susceptibility between semi-dwarf and wild type NILs. In a recent study, however, Yan *et al*. (2011) attributed differences in FHB severity associated with *Rht* alleles to the direct or indirect effects of height *per se*. In this study, ten *Rht* NIL pairs, each consisting of an *Rht* line and a wild type line in an isogenic background, were evaluated for FHB severity in a controlled environment facility. A wide range of *Rht* alleles were evaluated that included alleles from *Rht-B1* and *Rht-D1*

loci (*Rht-B1b*, *Rht-D1b*, and *Rht-B1c*) and alleles from GA sensitive *Rht* loci (*Rht4*, *Rht5*, *Rht8*, *Rht8+9*, *Rht11*, and *Rht13*). In trial set 1, the wild type NIL resulted in significantly ($p < 0.05$) better FHB type 1 resistance than the *Rht* NIL in eight of the ten NIL pairs (*Rht5* and *Rht13* were not significantly better). Increased FHB susceptibility associated with *Rht* loci other than *Rht-B1* or *Rht-D1* indicates that susceptibility is not likely to be caused by genes linked to *Rht-D1b* or *Rht-B1b*. In trial set 2, the pots containing *Rht* NIL plants were physically raised so that ear height was the same as that of the wild type NIL. With the difference in ear height removed, there was no longer a significant ($p < 0.05$) difference in type 1 resistance between *Rht* and wild type plants in nine of the ten NIL pairs (*Rht8+9* was significantly different). However, in trail set 2, while FHB infection levels in the *Rht* NILs were slightly reduced, infection levels of the wild type NILs were nearly double that reported for trial set 1. The authors suggest that the possible cause of increased susceptibility in the *Rht* lines in sets 1 and 2 is related to microclimate differences (higher humidity or increased temperature) that may be present at lower ear heights or to morphological changes associated with ears of the *Rht* lines. The increased wild type NIL susceptibility appears to be the primary reason that differences in FHB susceptibility disappear in trial set 2. More work is required to resolve the effect of plant height on FHB susceptibility.

1.6. EVOLUTIONARY HISTORY AND GENETIC DIVERSITY OF WHEAT AND *POACEAE* SPECIES

The evolutionary history of wheat and related *Poaceae* (grass) species has implications on wheat genetic diversity and is the basis for comparative genomics. Determining genetic diversity among species at the genomic and individual gene level is critical in searches for useful genetic variation. Several genetic bottlenecks have occurred during wheat's evolutionary history that resulted in the loss of diversity (Reif *et al*., 2005; Haudry *et al*., 2007) and these were among the largest losses seen in crop species (Haudry *et al*., 2007). Hence, ancestral wheat lines represent key sources of allelic variation that could be used in future wheat breeding efforts. The radiation of the *Poaceae* from a common ancestor is revealed in the syntenic relationships

among grass species (Gale and Devos, 1998). The large amount of colinearity between genes of wheat and sequenced *Poaceae* species makes comparative genomics possible, which is an important tool for understanding the molecular genetics of wheat especially with the lack of a fully assembled wheat genomic sequence.

Wheat and the *Poaceae* have a complex evolutionary history that includes whole genome duplications and polyploidisation (Figure 1.4). The common ancestor of the *Poaceae* is thought to have contained five chromosomes (Salse *et al*., 2008). A whole genome duplication occurred in this ancestor approximately 70 million years ago (MYA), followed by several million years before divergence of the *Panicoideae* (sorghum and maize), *Pooideae* (wheat, barley, and *Brachypodium*), and *Oryzeae* (rice) lineages (Paterson *et al*., 2004). The divergence dates of the *Panicoideae* and *Oryzeae* lineages from the *Triticeae* lineage are estimated at 60 MYA and 50 MYA, respectively (Chalupska *et al*., 2008). Maize and sorghum did not diverge from each other until as recently as 12 MYA (Swigonova *et al*., 2004). Following the split in the maize and sorghum lineages, a whole genome duplication occurred in maize at least 4.8 MYA (Swigonova *et al*., 2004). Maize currently contains approximately 25% of its genes as duplicate homoeologues (Schnable *et al*., 2009). Divergence of *Brachypodium* from the wheat ancestor occurred approximately 32 to 40 MYA (Bossolini *et al*., 2007; Chalupska *et al*, 2008; The International *Brachypodium* Initiative, 2010). Within the *Triticeae*, most estimates of the divergence times of barley and wheat range from 10 to 15 MYA (Ramakrishna *et al*., 2002; Huang *et al*., 2002; Dvorak and Akhunov 2005; Chalupska *et al*., 2008).

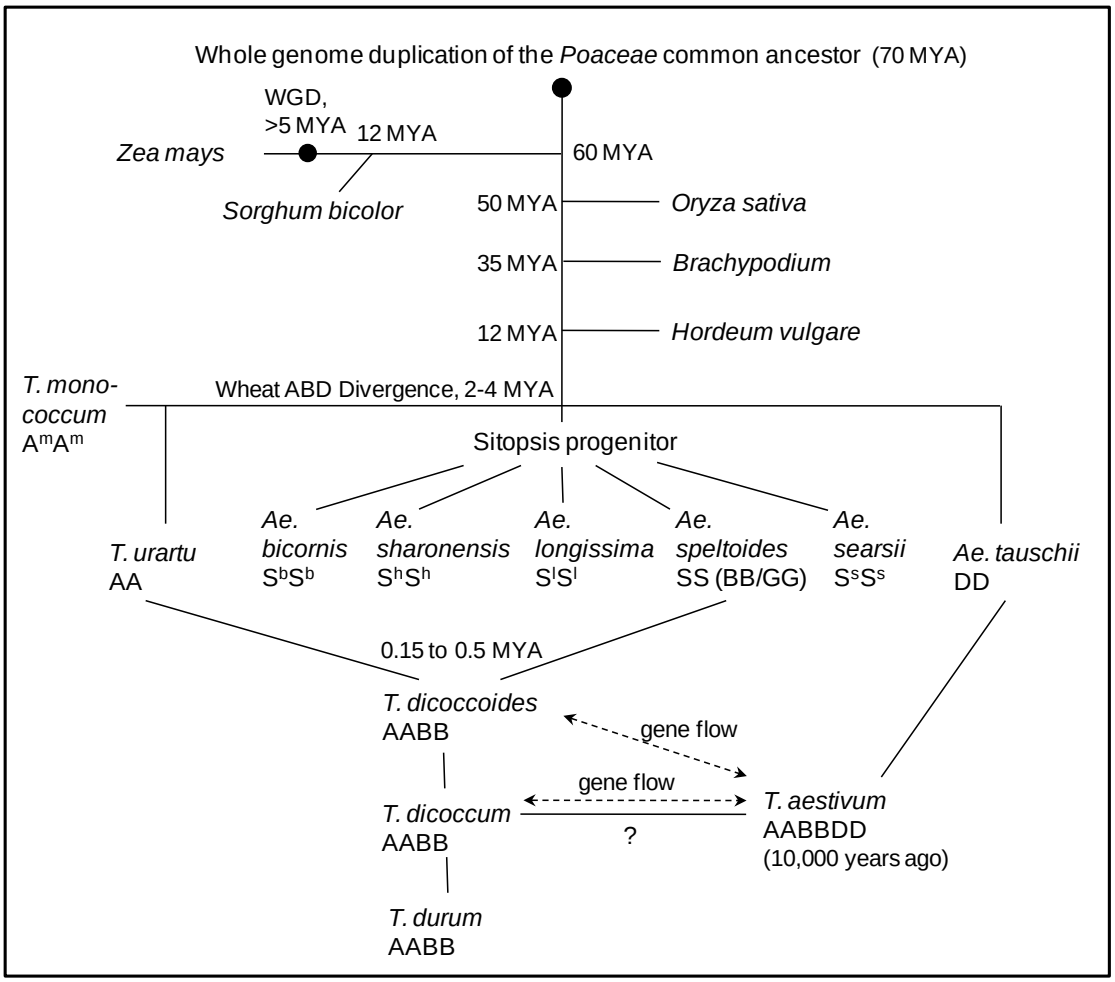


Figure 1.4. Evolutionary history of wheat and selected *Poaceae*

Estimated time of divergence is shown at branch points (MYA = millions of years ago). Black dots represent whole genome duplications (WGD). For wheat, genome designations are shown below species name. BB/GG indicates that *Aegilops speltoides* gave rise to species with BB or GG genomes. Dashed arrows indicate intermating and substantial gene flow between species. The question mark indicates that the tetraploid progenitor of wheat is not known, but is hypothesised to be *T. dicoccum*. Wheat divergences based on Charmet (2011).

Following separation from barley, the ancestral genomes that now compose *T. aestivum* diverged from one another approximately 2-4 MYA to form the A, B, and D genome lineages (Huang *et al*., 2002; Dvorak and Akhunov, 2005; Chalupska *et al*., 2008). The D genome lineage gave rise to *Aegilops (Ae.) tauschii* (DD genome), also known as *Ae. squarrosa*. The A genome lineages further subdivided to include *T. urartu* (AA), *T. boeoticum* (A^bA^b genome; common name: wild einkorn), and *T. monococcum* (A^mA^m, common name: einkorn). The B genome lineage of wheat is derived from the Sitopsis section of *Aegilops*, which have an SS genome designation. Several species make

up the Sitopsis including, *Ae. sharonensis* (S^hS^h), *Ae. longissima* (S^lS^l), *Ae. searsii* (S^sS^s), *Ae. bicornis* (S^bS^b), and *Ae. speltoides* (SS). The contributor of the *T. aestivum* B genome is not fully established, but a recent publication suggests that *Ae speltoides* is the likely progenitor (Kilian *et al*., 2007). *Ae. speltoides* is a natural outcrosser and has the highest level of haplotype diversity among the Sitopsis group (Kilian *et al*., 2007), which could have implications regarding the B genome diversity of *T. aestivum*. The polyploid *T. dicoccoides* (AABB genome, common name: wild emmer) is thought to have originated approximately 150,000 to 500,000 years ago (Levy and Feldman, 2002; Charmet, 2011) from the crossing and polyploidisation of *T. urartu* (AA) with *Ae. speltoides* (SS). The domesticated form of *T. dicoccoides* is known as *T. dicoccum* (AABB, common name: domesticated emmer). Domesticated emmer is the progenitor of modern durum wheat (*T. durum*, AABB). *T. aestivum* (AABBDD) arose from the interspecific cross and subsequent polyploidisation of *Ae. tauschii* with either *T. dicoccum* as suggested by Zohary and Hopf (2000) or *T. dicoccoides* approximately 8-10,000 years ago (Levy and Feldman, 2002).

Sequence analysis has revealed genetic bottlenecks during the domestication of wheat. A loss in genetic diversity was reported during the domestication of tetraploid wheat, with a 70% loss between *T. dicoccoides* and *T. dicoccum* and 69% loss between *T. dicoccoides* and *T. aestivum* when averaged across 21 loci (Haudry *et al*., 2007). An even greater loss (30-fold) in genetic diversity was reported between the D genome of *T. aestivum* varieties and *Ae. tauschii* at the *GSS* (*Granule-bound Starch Synthase*) locus (Caldwell *et al*., 2004). This likely is the result of the origin of bread wheat from just a small number of hexaploidisation events with the D genome donor *Ae. tauschii* (Talbert *et al*., 1998; Caldwell *et al*., 2004) and little inter-mating between bread wheat and *Ae. tauschii* (Dvorak *et al*., 1998), whereas a substantial amount of inter-mating appears to have occurred between *T. aestivum* and tetraploid wheat (Dvorak *et al*., 2006; Dubocovsky and Dvorak 2007; Luo *et al*., 2007). This may also explain why variation among D genomes of bread wheat varieties tends to be less than that of the A and B genomes (Caldwell *et al*., 2004; Dubcovsky and Dvorak, 2007; White *et al*.,

2008; Chao *et al*., 2009). In addition, further reductions in genetic diversity are likely to have occurred during selection of beneficial agronomic traits during domestication (Tanksley and McCouch, 1997).

1.7. ORIGIN AND DISTRIBUTION OF *RHT-B1B* AND *RHT-D1B*

1.7.1. Origin and spread of 'Norin 10'

The *Rht-B1b* and *Rht-D1b* alleles in modern wheat varieties are predominantly derived from the Japanese variety 'Norin 10'. By pedigree history, the oldest known ancestor of 'Norin 10' is the Japanese landrace 'Daruma', which is thought to be the source of both semi-dwarfing alleles (Dalrymple, 1980; Kihara, 1983; Dalrymple, 1986). 'Daruma' dates back to at least the turn of the 20th century with records showing that in 1894 it was used as a control in wheat trials in Japan (Kihara, 1983). According to Kihara (1983), 'Daruma' is short for 'Bodhi Dharma', the founder of Zen Buddhism in China, who is known for "sitting on the floor and facing to the wall in a cave for nine years, which took away his ability to walk". 'Daruma' has come to mean 'no legs' and 'do not tumble down' in Japan, which may be the reason lodging-resistant wheat and rice was given this name (Kihara, 1983). It has also been suggested that 'Daruma' may have originated in the Korean wheat population 'Anzunbaengimil', which means "crippled wheat", existing as early as the 3rd or 4th century AD (Cho *et al*., 1980). The authors speculated that it may have been introduced to Japan during the 16th century Korean-Japanese War or by Japanese agronomists dispatched to Korea from 1901 to 1905, although this latter date is subsequent to the date (1894) that 'Daruma' was used in Japanese wheat trials.

The *Rht-B1b* and *Rht-D1b* dwarfing alleles were introduced to the Western World when S.C. Salmon, a United States Department of Agriculture (USDA) advisor to the army in Japan, brought 'Norin 10' to the US in 1946 (Reitz and Salmon, 1968). 'Norin 10' was not suitable for cultivation in the USA, but it proved valuable in breeding. Orville Vogel, a USDA scientist at Washington State University, was the first person to utilise 'Norin 10' in the US,

incorporating it into his breeding programme beginning in 1949 (Reitz and Salmon, 1968). In Vogel's breeding programme, the cross 'Norin 10 x Brevor' was made and progeny of this cross became the predominant source of dwarfism in wheat in the US and worldwide. Vogel's work on short-strawed wheat coincided with the work of Norman Borlaug, then working at CIMMYT (International Maize and Wheat Improvement Center) in Mexico to improve wheat yields by the use of high levels of nitrogen fertilisation. Seed of a selection of 'Norin 10/Brevor' was sent to Borlaug in 1953 and the first successful crosses made in 1955 (Dalrymple, 1986). Beginning in 1962, a series of semi-dwarf bread wheat varieties was released from the CIMMYT programme and distributed to many regions around the world including Australia, the Middle East, Latin America, and Africa, representing the origin of the 'green revolution' in wheat. The end result was a rapid introgression of *Rht-B1b* and/or *Rht-D1b* into most of the world's hexaploid wheat varieties. For his contributions to the world food supply, Norman Borlaug was awarded the Nobel Peace Prize in 1970.

1.7.2. Alternative sources of *Rht-B1b* and *Rht-D1b*

There are several alternative, albeit relatively minor sources of the *Rht-B1b* and *Rht-D1b* alleles in addition to 'Norin 10'. Two alternative sources used in US wheat varieties include 'Suweon 92' (also referred to as 'Suwon 92') and 'Seu Seun 27', which were both derived from 'Daruma' (the putative source of *Rht-B1b* and *Rht-D1b*) and developed in Korea (Dalrymple, 1986). At least 132 additional 'Norin' lines exist in addition to 'Norin 10' and 102 of these were shown to contain *Rht-B1b* and/or *Rht-D1b* by test-crossing to *Rht-B1b* and *Rht-D1b* and measuring and GA response in F_2 seedlings (Yamada, 1990). The National Agricultural Experiment Stations in Japan used the 'Norin' numbering system in the early part of the 20th century (Nakamura *et al*., 1999), hence the term 'Norin' does not necessarily refer to plant height. 'Daruma' is shown in the parentage of only a small number of the 'Norin' lines examined by Yamada (1990), suggesting the possibility that non-'Daruma' sources of *Rht-B1b* and *Rht-D1b* may be present in the 'Norin' lines. The semi-dwarf lines 'Norin 16', 'Norin 26', and 'Norin 33' have had limited use in

the US (Dalrymple, 1980). 'Norin 16' contains 'Daruma' in its parentage while 'Norin 26' and 'Norin 33' have an unknown source of semi-dwarfism (Dalrymple 1980; Yamada 1990). Yamada (1989) also identified four Japanese landraces that contained the *Rht-D1b* allele that had an unidentified source. In China, 'Suwon 86', a Korean variety derived from 'Daruma', is a major souce of *Rht-B1b* and *Rht-D1b* (Zhang *et al*., 2006). The authors also reported that two other major sources of *Rht-D1b*, accessions 'Huixianhong', and 'Yaobaomai' have unknown sources of semi-dwarfism. 'Huixianhong' is a landrace that was a leading cultivar in the 1930s and 'Yaobaomai' is a variety released in 1964 (Zhang *et al*., 2006). Along with 'Norin 10', the Italian cultivar 'St 2422/464' may be a major source of *Rht-B1b* in China (Zhang *et al.,* 2006), however 'St2422/464' showed moderate response to GA and may in fact contain the *Rht-B1d* allele (Jia *et al*., 1992). These reports indicate that several sources of the *Rht-B1b* and *Rht-D1b* alleles not derived from 'Norin 10' are found in modern wheat varieties and in addition, some of these sources may not be derived from 'Daruma'.

1.7.3. *Rht-B1b* and *Rht-D1b* prevalence in the world

Several groups have estimated the prevalence of *Rht-B1b* and *Rht-D1b* in specific countries either by pedigree analysis, crossing to semi-dwarf tester lines and measuring GA response in F2 progeny, or more recently using the *Rht-B1b* and *Rht-D1b* perfect markers developed by Ellis *et al*., (2002). As discussed in section 1.7.1, most US semi-dwarf varieties are derived from 'Norin 10', but some are derived from other sources. The first semi-dwarf variety released in the US was 'Gaines' in 1961, which acquired its short stature from 'Norin 10/Brevor 14'. In 1964, 'Gaines' represented nearly all of the US semi-dwarf wheat acreage and 3% (1.6 million acres) of the total US wheat acreage (Dalrymple, 1980). By 1969, semi-dwarfs represented 7% of the US wheat acreage with 'Gaines' and its progeny 'Nugaines' representing over 70% of the semi-dwarf acreage (Dalrymple, 1980). By 1974 semi-dwarfs represented 22% of U.S acreage with 19 varieties planted to more than 100,000 acres each (Dalrymple 1980). Progeny of the Korean varieties 'Seu Seun 27' and 'Suweon 92', represented 3% (480,000 acres) and 12% (1.8

million acres), respectively, of the land planted to semi-dwarfs varieties in 1974 (Dalrymple, 1980). Most of the early US semi-dwarf lines are derived from breeding material created in the US and to a lesser extent from CIMMYT breeding lines (Dalrymple 1980).

Semi-dwarf wheat varieties currently used in the United Kingdom (UK) are thought to be predominantly derived from 'Vg9144' and 'Vg8058', which are two semi-dwarf varieties from Orville Vogel's breeding programme which have a 'Norin 10' ancestry (Gale and Youssefian, 1985). These lines were sent to Chile where they were crossed with tall French varieties before being sent to the Plant Breeding Institute (PBI), Cambridge, UK, in 1964 (Gale and Youssefian, 1985). This germplasm was not directly suitable for use in the UK, but was utilised as breeding material. In 1974, the first UK semi-dwarf varieties 'Fundin', 'Bilbo', and 'Durin' were released (Gale and Youssefian, 1985). Semi-dwarfs quickly dominated the market in the UK and by 1982 varieties carrying a 'Norin 10' dwarfing allele (later discovered to be *Rht-D1b*) represented over 75% of the winter wheat acreage (Gale and Youssefian, 1985). The PBI developed most of these varieties and eight of the nine semi-dwarf varieties in the 1981 NIAB Recommended List came from this group (Gale *et al*., 1982). The rapid adoption, limited sources of founding germplasm, and use of just the *Rht-D1b* allele represents a potential genetic bottleneck in UK wheat germplasm. A genome-wide screen of Diversity Array Technology (DArT) markers applied to US, UK, and Australian commercial bread wheat cultivars released between 1930-2005 indicates that the UK had the least genetic diversity among these countries and that UK wheat diversity reached a low-point during the period (1975-1985) that semi-dwarfs were rapidly replacing tall varieties in the UK (White *et al*., 2008).

In Australia, *Rht-B1b* or *Rht-D1b* are reported to be present in the majority of cultivars although a minority of other semi-dwarfing alleles exist (personal communication, Daryl Mares, School of Agriculture and Wine, University of Adelaide). The first semi-dwarf varieties in Australia were derived from CIMMYT lines of 'Norin 10' origin (Syme and Pugsley, 1974; Gale and King, 1988) and several hundred lines were imported from the CIMMYT breeding

programme in 1963 (Syme and Pugsley, 1974). In addition, Australian wheat breeders have repeatedly acquired additional semi-dwarf varieties from CIMMYT following the original introduction (Gale and King, 1988), which represents a greater donor base than seen in the UK. The hard red spring wheat 'WW15', which contains *Rht-B1b* derived from 'Norin 10' has been a major source of semi-dwarfism in Australia giving rise to 'Condor' and 'Egret', both released in 1973 (Gale and King, 1988). *Rht-D1b* was also present in the founding CIMMYT material used in Australia, but in 1985 was represented in only a minority of the varieties; however its prevalence is reported to be increasing over time (Gale and King, 1988).

Two other studies to determine *Rht-B1b* and *Rht-D1b* prevalence were carried out in Germany and China. In a survey of 94 German winter wheats, 6% carried *Rht-B1b*, 38% carried *Rht-D1b*, and none carried *Rht8* (Knopf *et al*., 2008). The authors found that varieties with *Rht-D1b* were shorter and higher yielding than varieties not carrying the allele, but were more susceptible to FHB. In a study of Chinese wheats, *Rht-B1b* and *Rht-D1d* were present in 24.5% and 45.5% of the varieties, respectively (Zhang *et al*., 2006).

1.8. LINKAGE DISEQUILIBRIUM AND SELECTIVE SWEEPS IN PLANTS

The worldwide introgression of the semi-dwarfing alleles, *Rht-B1b* and *Rht-D1b*, into most modern day wheat cultivars in less than 50 years represents an intense genetic selection on a global scale. The intense selection pressure over a short time frame likely means that extensive linkage disequilibrium (LD) is associated with *Rht-B1b* and *Rht-D1b*. The region of reduced variation associated with LD is often referred to as a "selective sweep" (Palaisa *et al*., 2003). Selective sweeps are associated with genes under selection, including crop domestication genes, and generally involve a loss in genetic diversity in these regions (Wang *et al*., 1999; Palaisa *et al*., 2003; Doebley *et al*., 2006).

The size of the region in LD varies widely depending on factors such as reproductive mode (outcrossing versus inbreeding), selection intensity, pre-existing levels of genetic diversity, and chromosomal location of the gene.

Several studies that have looked at average levels of LD in species indicate that self-pollinating or vegetatively propagated species tend to have a greater selective sweep than outcrossing species. Nordberg *et al*. (2002) analyzed 163 genome-wide SNPs in 76 *Arabidopsis* (an inbreeding species) accessions and found that average LD associated with the SNPs extended approximately 250 kb. In contrast, LD decay is much more rapid in the natural outcrosser maize with LD decaying within an estimated 1.5 kb when six genes were examined in a diverse set of 102 maize inbred lines (Remington *et al*., 2001). Genes under strong selection tend to have extended LD. The selective sweep of the rice *Waxy* gene, selected for its quality characteristics during domestication, was 250 kb and included six other genes (Olsen *et al*., 2006). Palaisa *et al*. (2004) examined *Y1*, a phytoene synthase gene that determines kernel colour in maize that was under strong selection in the 1930's in the US. The region of LD around *Y1* was large, especially considering the outcrossing nature of maize, extending 200 kb upstream and 700 kb downstream, and five linked genes in this region showed a pattern of reduced diversity. Recombination rates vary across the chromosome, thereby affecting LD across the genome (Flint-Garcia *et al*., 2003). Maize, for instance, contains recombination hot spots within genes but reduced levels of recombination in intergenic regions (Fu *et al*., 2002). Also, in wheat it was found that gene density and recombination rate increased with the relative distance from the centromere (Akhunov *et al*., 2003). In the case of *Rht-B1b*, and *Rht-D1b*, these genes have only been selected recently and with a high selection pressure suggesting that the region of LD surrounding these loci is large, especially considering the inbreeding nature of wheat.

1.9. PHD OBJECTIVES

The *Rht-B1b* and *Rht-D1b* alleles were important contributors to the green revolution in wheat and are the predominant height-reducing alleles used in modern day bread wheat varieties. Although these genes have been characterised and cloned, little is known regarding the genetic diversity of the *Rht-1* homeoloci or the genetic composition of the contiguous sequence that was presumably introgressed along with the dwarfing alleles. At the *Rht-A1*

locus, map location and full-length sequence is not currently available and genotypic and phenotypic variation at this locus has not been explored.

The first objective of this project is to determine the nucleotide sequences of the three *Rht-1* homoeologues along with that of the nearby genetic region in a common genetic background. This will be accomplished by screening the CS wheat BAC library to identify *Rht-1*-containing clones, which will be fully sequenced and annotated. The genetic information will be useful in identifying regions of high conservation, which will help identify key regulatory sequences.

A second objective of the project is to determine the genetic location of the *Rht-A1* homoeologue by deletion bin mapping and genetic mapping. Knowledge of the *Rht-A1* genetic location and associated markers opens up the possibility of exploring useful genetic variation in and around this locus.

A third objective is to explore the haplotype variation associated with the *Rht-1* homoeologues in ancestral wheat lines and in modern bread wheat varieties from the UK and from a world-wide collection. This will be accomplished by sequencing the ORF and flanking regions of all three *Rht-1* homoeologues. This information will be used to identify haplotypes and associated markers, which represent a potential resource for understanding phenotypic consequences of the genetic variation.

Lastly, this project will explore the origins of the ‘Norin 10’ alleles by determining the genotypes and phenotypes of ‘Norin 10’ ancestors. This will help identify the progenitor lines and should lead to a greater understanding of the genetic history of these alleles and potential sources for novel variation in and around the *Rht-B1b* and *Rht-D1b* alleles.

Determining variation at the *Rht-1* loci and developing genetic markers represents a key resource for identifying beneficial alleles at this locus and in surrounding genes. This knowledge can aid in our understanding of DELLA function and provides resources that be used to improve wheat.

2. IDENTIFICATION AND GENETIC ANALYSIS OF BAC CLONES CONTAINING *RHT-1*

Results from this chapter were part of a publication in the journal Genome (Febrer et al., 2009). Attached as a pdf and as a hard copy in thesis jacket)

2.1. INTRODUCTION

Due to their agronomic importance, *Rht-B1* and *Rht-D1* were among the first loci to be mapped and cloned in wheat (Peng *et al*., 1999). However, over a decade later, little is known regarding the immediate sequence surrounding the *Rht-1* loci and the presence of *Rht-1* on the A genome has not been established. Although wheat is the most important food crop worldwide after rice and many cereal crop species have a fully assembled sequence (e.g. rice, maize, sorghum), wheat still lacks an assembled sequence making molecular studies difficult. The absence of assembled wheat sequence is primarily due to the size and complexity of the genome. Bread wheat is an allohexaploid that consists of three genomes (A, B, and D) that together comprise >16 000 MB of sequence, 80% of which is repetitive (Gupta *et al.*, 2008). An effort is underway by the International Wheat Genome Sequencing Consortium (IWGSC; http://www.wheatgenome.org) to provide high quality reference sequence of Chinese Spring that is fully assembled into chromosomes and annotated. As of March 2011, reference sequence from the IWGSC is available for 25% of 3BS and 25% of 3BL. In addition, 5x 'shotgun' coverage of the CS genome is currently available from a separate collaboration (http://www.cerealsdb.uk.net). However, other than selected gene-rich regions this sequence is currently not assembled into contigs nor is the sequence physically mapped or annotated.

To obtain wheat sequence for assembly of specific genes and genetic regions, bacterial artificial chromosome (BAC) large-insert libraries have been used (Shizuya *et al*, 1992). BACs have become the favoured method to clone large genomic inserts (Xia *et al*., 2005) because of the high transformation efficiency of *Escherichia coli* and the stability of inserts, coupled with the ease

of BAC DNA purification and its compatibility with sequence determination protocols. Several BAC libraries useful to bread wheat studies have been created including *Aegilops tauschii* (the D genome ancestor) (Moullet *et al*., 1999; Xu *et al*., 2002, Akhunov *et al*., 2005), *Triticum urartu* (the A genome ancestor) (Akhunov *et al*., 2005), *Triticum monoccum* (diploid; A genome) (Lijavetzky *et al*., 1999), *Aegilops speltoides* (diploid; B genome) (Akhunov *et al*., 2005), and *Triticum turgidium* (AB) (Cenci *et al*., 2003). For *T. aestivum*, a bulked library was reported for genotype 'Hartog' (Ma *et al*., 2000) and gridded BAC libraries for genotypes 'Renan' (Chalhoub *et al*., 2002), 'Glenlea' (Nilmalgoda *et al*., 2003), 'CS' (Allouis *et al*, 2003), and 'Norstar' (Ratnayaka *et al*., 2005).

A high redundancy of genome coverage in BAC libraries is important for recovering a desired sequence, but high coverage along with the immense size of the wheat genome results in large libraries that are laborious to screen. Targeted BAC sequences can be identified by two methods: radioactive hybridisation and, more recently, PCR screening (Klein *et al*., 2000: Yim *et al*., 2007). Hybridisation relies on high-density filters that are screened with radioactive probes, however probes may lack specificity and the process can be cumbersome. PCR screening is relatively simple, fast, and sensitive, but first relies on the pooling of BAC clones in three or more dimensions. The 3-D pooling strategy was recently applied to a *Brachypodium distachyon* BAC library to facilitate PCR screening (Farrar and Donnison, 2007) and more recently to bread wheat (Febrer *et al*., 2009).

This chapter describes the identification and characterisation of BAC clones containing *Rht-1* from the CS wheat BAC library. The 'French' and 'UK' components of the library were screened using hybridisation and PCR, respectively, to identify *Rht-1*-containing clones from the A, B, and D genomes.

2.2. MATERIALS AND METHODS

2.2.1. The *Triticum aestivum* (cv Chinese Spring) BAC library

The CS bread wheat BAC library constructed by Allouis *et al*. (2003) as part of a collaboration between the BBSRC (UK) and INRA (France) was used in this study. The library coverage is 9.3 haploid genome equivalents and consists of 1,200,000 clones (3125 384-well microtitre plates) with an average insert size of 130 kb (ranging from 45 kb to 350 kb) (Allouis *et al*., 2003). After construction of the library, it was subdivided into 'French' and 'UK' components. The French component contains approximately 3 haploid equivalents of the hexaploid wheat genome and consists of 1332 384-well plates. To facilitate screening, BAC clones were gridded onto high-density colony filters in two dimensions as described by Allouis *et al*. (2003). A set of the colony filters from the French component is held at the John Innes Centre (JIC), Norwich, UK. The UK component is also held at the JIC Genome Centre and contains 715,776 clones (1864 384-well microtitre plates) and represents 5 haploid equivalents of the bread wheat genome. To facilitate rapid PCR screening of the UK component, clones were recently pooled to allow for a 3-D screening procedure (Febrer *et al*., 2009), although the pooled library had not been tested prior to the current study.

2.2.2. Screening of the French component of the CS BAC library by radioactive hybridisation

2.2.2.1. Screening strategy and probe construction

Filters containing the entire French component of the CS library held at the JIC were screened using a 195 bp radiolabelled [^{32}P] probe specific to *Rht-1* (probe 1). Probe 1 is from the N-terminal region of *Rht-1*, which is specific to this gene. Probe 1 was pooled with six additional probes from unrelated projects and the probe pool was used to screen the BAC library. The probes were pooled to reduce wear on the colony filters. BAC clones selected from the initial screen comprised a BAC sub-library. The sub-library was screened with probe 1 and also screened with a 202 bp (probe 2) and a 503 bp probe (probe 3). Probes 2 and 3 are also part of the N-terminal region of *Rht-1*.

All three probes are PCR amplification products from the CS D genome. Probe 1 (CS *Rht-D1* bp 49 to 243) was amplified using primers Rht-16-F and Rht-11-R and spans the highly conserved DELLA domain (Figure 2.1). Probe 2 (CS *Rht-D1* bp 264 to 465) was amplified using primers Rht-ABD-F1 and Rht-9-R and spans the highly conserved TVHYNP domain. The primer pair Rht-22-F and Rht-9-R was used to amplify probe 3 (CS *Rht-D1* bp -38 (upstream) to 465), which spans the DELLA and TVHYNP domains. Primer sequences are shown in Appendix I.

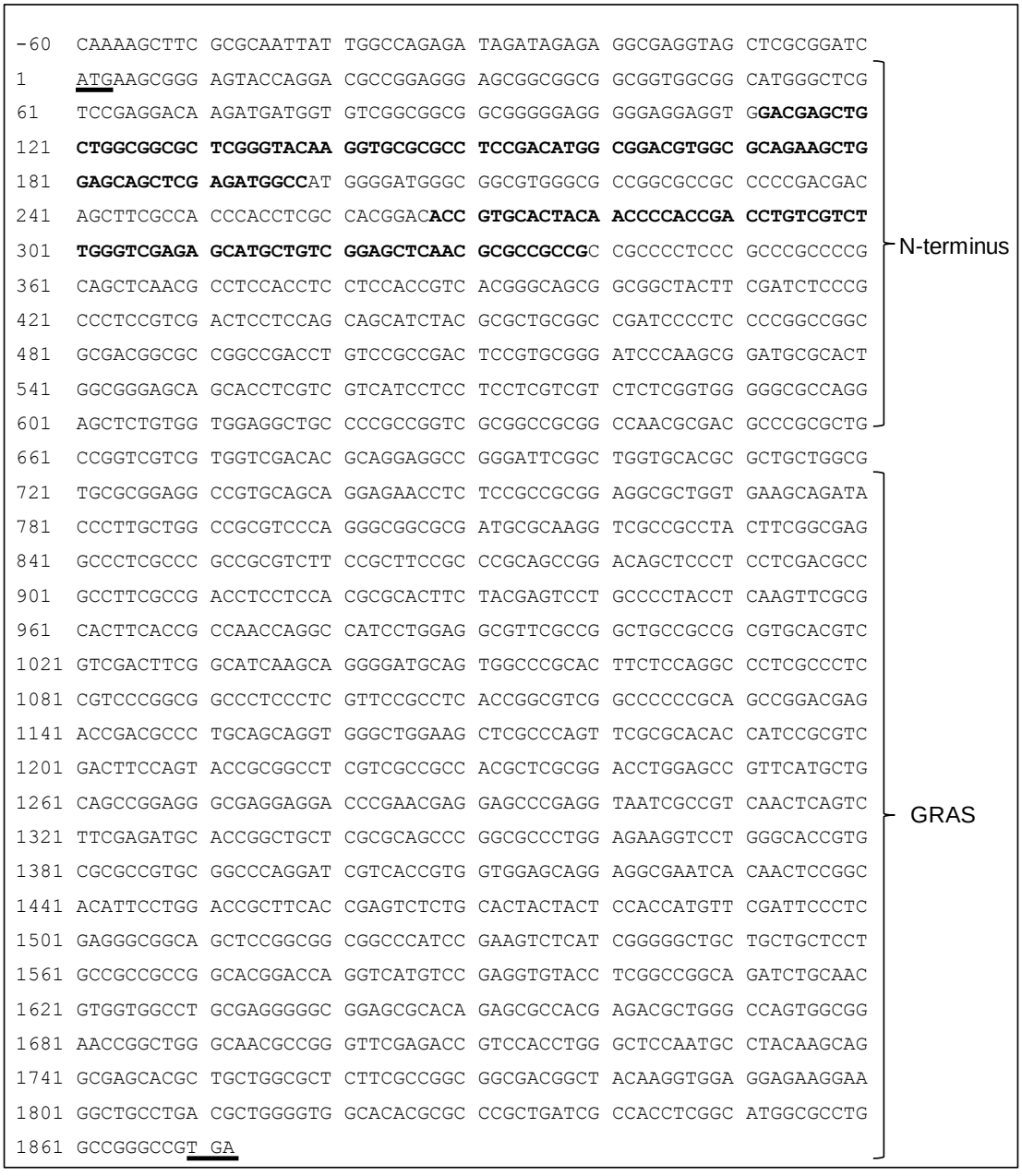


Figure 2.1. *Rht-D1a* coding and upstream sequence in the CS background. Coding sequence is from Genbank accession no. AJ242531 and upstream sequence is from N.

Harberd, Oxford University, unpublished data. Location (in basepairs (bp)) relative to the start of the *Rht-D1a* coding sequence is shown to the left of the first nucleotide in each row with negative numbers indicating 5' sequence. The *Rht-D1a* start and end codons are underlined. Sequence encoding the N-terminus and the GRAS functional protein domains are indicated. Conserved domains of the N-terminus shown in bold are: DELLA (bp 112 to 198) and TVHYNP (bp 268 to 339).

Probes were radiolabelled with the Rediprime II DNA labelling system (GE Healthcare Life Sciences). Purified PCR-amplified DNA (20 ng in 45 µl sterile distilled water) was denatured by heating to 95°C for 5 min. After cooling it was added to the Rediprime reaction tube, which contains dATP, dGTP, dTTP, Klenow enzyme and random primers. A 3 µl quantity of [^{32}P] dCTP was added to the tube and incubated at 37°C for 1 hr. The reaction was stopped and DNA denatured by adding 2.5 µl of 4N NaOH.

2.2.2.2. Screening of BAC library filters using radioactive probes

Southern hybridisation was carried out essentially as described by Sambrook *et al*., 1989). Colony filters were wetted in 2 × sodium chloride-sodium citrate (SSC) buffer in a 20 cm^2 plastic box after which they were equilibrated in Church & Gilbert pre-hybridisation buffer (Church and Gilbert, 1984) for two hours at 65°C with gentle shaking. Probe 1 was added and the hybridisation carried out for 16 hr at 65°C, with gentle agitation. The filters were placed in a clean box before immersion in Wash 1 (2 × SSC, 0.5% sodium dodecyl sulfate (SDS)). Next, filters were washed twice in 1 l of Wash 2 (0.5 × SSC, 0.5% SDS) with gentle shaking at 65°C. If filters still had high radioactivity, then Wash 2 was repeated. Wet filters were then wrapped in plastic wrap and exposed on Phosphor Image screens overnight. Exposed screens were visualised using a Typhoon phosphor imager (Amersham Biosciences).

BAC clones selected for repeated screening were grown overnight with shaking in 96-well plates containing Luria broth (LB) media with chloramphenicol (CAM) (12.5 µg/ml). A 5 µl quantity of culture was then spotted onto Hybond-N+ membrane (*Amersham* Biosciences), which was placed on LB agar and incubated overnight at 37°C. The membrane was

dried and DNA bound to the membrane by transferring the membrane (cell-side up) sequentially onto blotting paper soaked in 10% SDS (3 min); 0.5N NaOH, 1.5 mM NaCl (5 min), 0.5M Tris-Cl, 1.5M NaCl, pH 7.4 (5 min), and 2 × SSC (5 min). The membrane was air-dried for 30 min before washing in 0.5 × SSC, 0.1% SDS until no longer sticky (generally 4 to 5 washes). Replicate membranes were prepared for each probe. Radioactive [^{32}P] hybridisation was carried out with individual probes as described above except Wash 1 solution was 0.5 × SSC, 0.1% SDS and Wash 2 solution was 0.2 × SSC, 0.1% SDS.

2.2.2.3. Extraction of plasmid DNA

Selected BAC clones were streaked onto LB agar plates containing CAM (12.5 µg/ml) and incubated at 37°C for approximately 16 hr. Single colonies were then used to inoculate 15 ml of LB containing 12.5 µg/ml CAM and grown overnight at 37°C with shaking at 200 rpm. BAC plasmid DNA was extracted from bacterial pellets of BAC clones using the alkaline-lysis method (Sambrook *et al*., 1989) with buffers P1, P2, and P3 (QIAGEN Ltd) as follows. Cell cultures were centrifuged (3000 rpm, 20 min, 4°C) and the pellet resuspended in 200 µl P1 buffer. After adding 400 µl P2 buffer, samples were mixed by inversion and incubated for 5 min at room temperature. Buffer P3 (300 µl) was added and samples mixed before centrifuging (13 000 rpm, 15 min). A 600 µl volume of cold absolute ethanol was added to the supernatant and the DNA pelleted by centrifugation at 13 000 rpm for 10 min. After washing the pellet in 70% ethanol, it was air-dried, and the DNA resuspended in 50 µl 10mM Tris-HCl, pH 8.0.

2.2.2.4. Gel analysis of BAC clones

To determine if clones were unique and to estimate insert size and overlap between clones, DNA from each of the likely *Rht-1*-containing BAC clones was subjected to fingerprint analysis. BAC DNA (5 µl) was digested with 6 U *Hind III* and 2 µg RNase (DNase-free, Promega) to release the insert. A 16 µl volume of the digest was subjected to electrophoresis (100 V, 10 min then

75V, 27.5 hrs) on a 1% agarose gel at 4°C in 1 × TBE (Tris-Borate-EDTA) buffer. The DNA was visualised with CyberGold (Molecular Probes). Insert size was estimated by comparison with molecular weight markers HyperLadder IV (Bioline) and kb ladder (BRL).

The sizes of the likely *Rht-1*-containing clones were also estimated using Pulsed Field Gel Electrophoresis (PFGE). DNA (5 µl) was digested overnight with 6 U *NotI* (New England Biolabs) in the presence of RNase before 20 µl were loaded onto a 1% agarose gel. Samples were sealed into wells using 1% low melting point agarose and the gel immersed in 1 × TBE buffer. Electrophoresis was conducted at 165 V / 400mA / 40W with buffer kept at 13°C by circulation to a cooling tank. Pulsing consisted of alternating currents of North/South (5 s) and East/West (5 s) for 16 h followed by North/South (15 s) and East/West (15 s) for 1 min. DNA was stained with ethidium bromide and visualised under UV light. Insert size was estimated by comparison with molecular weight markers (Midrange II PFG, NEB Biolabs).

2.2.2.5. PCR amplification and sequencing of *Rht-1*

Selected BAC clones were screened using PCR amplification with primers Rht-ABD-F1 and Rht-ABD-R1, which amplifies a 489 bp product from the N-terminus of *Rht-1* (bp 264 to 752 in reference to CS *Rht-D1a*, Figure 2.1). Both primers completely matched the CS *Rht-D1a* sequence. Rht-ABD-F1 also completely matched the CS *Rht-B1a* sequence (N. Harberd, unpublished data) while Rht-ABD-R1 contained one mismatch (G/T at bp 5) with CS *Rht-B1a*. DNA of BAC clones was PCR-amplified using a 20 µl PCR mix containing 1 × Green GoTaq Reaction Buffer (Promega), 3% glycerol, 0.2 mM of each dNTP, 2 mM $MgCl_2$, 1 µM Rht-ABD-F1, 1 µM Rht-ABD-R1, 0.25 µl *Taq* polymerase, and approximately 100 ng BAC DNA. Glycerol was added to facilitate amplification of the G-C rich *Rht-1* sequence. The PCR profile consisted of initial denaturation of 5 min at 95°C, followed by 30 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 45 s. Amplification products were separated on a 1.5% agarose gel in 1 × Tris-acetic acid-EDTA (TAE) buffer and visualised under UV light with ethidium bromide.

To estimate the position of *Rht-1* on BAC clones, sequence of a pre-existing *Rht-D1*-containing BAC (BAC 1J9, J. Jia, CAAS, unpublished data) was utilised to create five primer pairs (D-BAC_F1/R1; D-BAC_F2/R2; D-BAC_F3/R3; D-BAC_F4/R4; D-BAC_F5/R5; Appendix I). Primer pairs were created at wide intervals along the length of the 1J9 BAC sequence. PCR reaction mixes and profiles were carried out as described above except substituting these primer pairs in the reaction mix.

BAC DNA was amplified with the Rht-ABD-F1 / Rht-ABD-R1 primer pair as described above and 1 µl used in a 10 µl BigDye (Applied Biosystems) sequencing mix, which also contained 1 × BigDye Sequence Buffer, 1 µl BigDye (ver. 3.1), and 1 µM Rht-ABD-F1 or 1 µM Rht-ABD-R1 primer. The PCR profile consisted of 96°C for 1 min, followed by 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. A forward and reverse read of each amplified product was performed to minimise potential PCR-induced errors. Sequencing was performed by the JIC Genome Sequencing Facility. Sequences were aligned using ContigExpress software of VectorNTI.

2.2.3. Screening of the UK component of the CS BAC library by PCR

To facilitate screening of the library, 384-well plate pools (PPs) were formed and organised in three dimensions (X, Y, Z) to form superpools (SPs) so that each PP is represented in three different SPs (Febrer *et al*., 2009) (Figure 2.2). A total of four cubes (one 9 × 9 × 9, two 8 × 8 × 8, and one 4 × 4 × 4) were created covering 1817 plates of the library and giving a total of 675 SPs. SP DNA was screened with the PCR primer pair Rht-ABD-F1/ Rht-ABD-R1a, which perfectly matched the sequences of *Rht-B1a* and *Rht-D1a* in the CS background. The primer pair was designed to the N-terminal region of *Rht-1* (bp 264 to 790 in reference to CS *Rht-D1a*, Figure 2.1). SPs were screened using the reaction mix and conditions described in section 2.2.2.5 except Rht-ABD-F1 and Rht-ABD-R1a were used as primers in a 10 µl volume mix with an annealing temperature of 58°C.

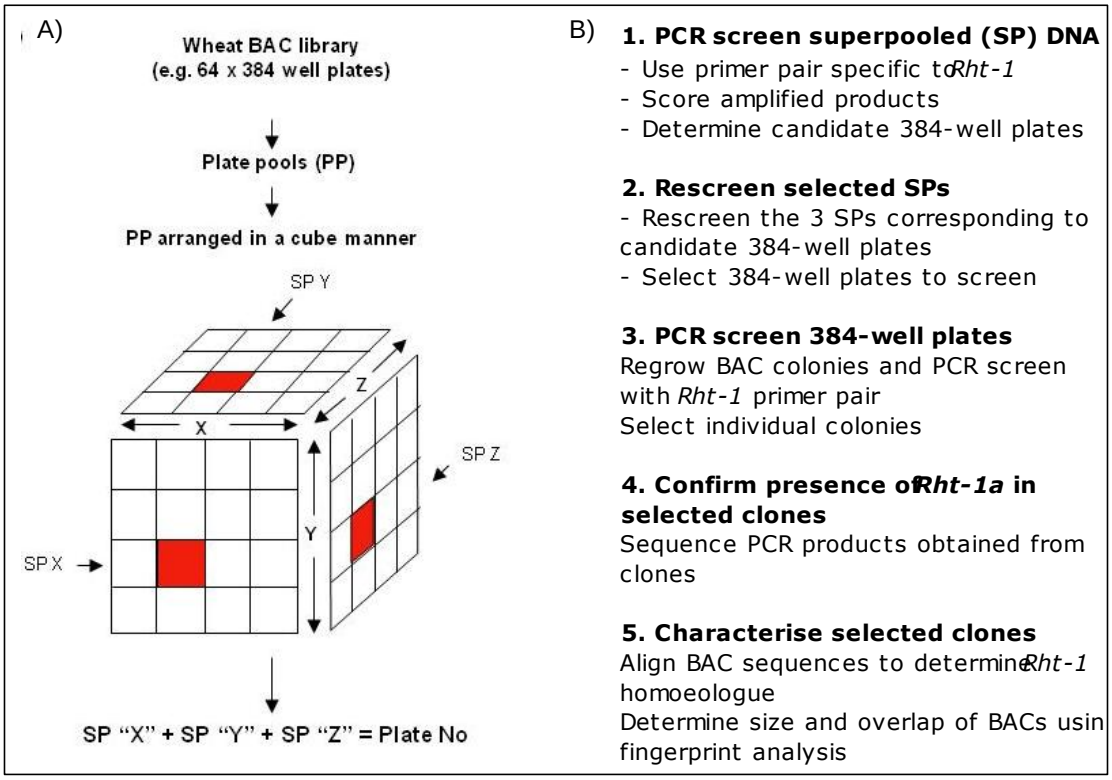


Figure 2.2. Overview of the screening strategy to identify and characterise *Rht-1*-containing BAC clones from the UK BAC library. (a) Representation of the 3-D pooling strategy for PCR screening of the UK BAC library (adapted from Febrer *et al*., 2009). A virtual 4 × 4 × 4 cube is depicted, which is sufficient for assessing the contents of 64 384-well plates in the library. Each plate was replicated and all 384 clones combined to form a plate pool (PP). Each PP is pooled in each of three dimensions (X, Y, Z) so that for a 4 × 4 × 4 cube, four PPs are present in each superpool (SP). For the 8 × 8 × 8 and 9 × 9 × 9 cubes, eight and nine PPs are present in each SP, respectively. The red squares depict a plate that is represented in SPs X, Y, and Z. (b) Summary of steps used to identify and characterise *Rht-1*-containing BACs.

To monitor the reliability of the PCR amplifications the following negative controls (NCs) and positive controls (PCs) were established in which BAC DNA was replaced with the following templates: NC1, water; NC2, DNA of BAC clones known not to contain *Rht-1*; PC1, DNA of BAC clones known to contain *Rht-1*; PC2, genomic DNA isolated from leaves of CS; PC3, a 1:3072 (v/v) dilution of PC1:NC2. The purpose of PC3 was to determine if a single BAC clone containing *Rht-1* could be identified in a SP from an 8 × 8 × 8 cube. A DNA concentration of 90 ngs per reaction was used for NC2 and PC3 and 20 ngs per reaction for PC1 and PC2.

The SP DNA samples were scored for the presence and intensity (strong (++) or weak (+)) of the approximately 525 bp amplification product. As DNA from each PP is represented in each of three SPs, identification of candidate plates likely to contain an *Rht-1*-containing clone was originally performed with a tabulated reference as described in Farrar and Donnison (2007). Later, a custom Perl script was written (Jon Wright, JIC) to facilitate 384-well plate identification. Once the candidate plates were selected, the corresponding SP DNAs were subjected to a second round of amplification to validate the original PCR screen. Individual BAC colonies in the candidate 384-well plates were screened by colony PCR using primers Rht-ABD-F1 and Rht-ABD-R1a as described above except that 1 µl of cells was collected directly from the plates to serve as template and initial denaturation time was 10 min.

For further characterisation, DNA was extracted from selected BAC clones using the method in section 2.2.2.3. Extracted DNA was used for fingerprint analysis (see section 2.2.2.4) except Hyperladder IV was used in place of Hyperladder I as a molecular marker. BAC DNA was also used in PCR reactions as specified above and sequenced as described in section 2.2.2.5 except that primers used in sequencing were Rht-ABD-F1 or Rht-ABD-R1a.

2.3. RESULTS

2.3.1. Identification and characterisation of *Rht-1*-containing BAC clones from the French component of the CS library

High-density colony filters containing the French component of the 'Chinese Spring' BAC library were screened by hybridisation using the *Rht-1*-specific probe 1 (described in section 2.2.2.1). A hybridisation signal was obtained for 267 clones (data not shown). The hybridisation contained six probes unrelated to *Rht-1* and therefore a BAC sub library, composed of the 267 clones was screened with *Rht-1*-specific probes 1 and 2 (Section 2.2.2.2). Screening of the BAC sub library with probe 1 resulted in a total of 42 clones that produced a positive signal, six of which were visually scored as strong

(++) and 36 as weak (+) (Table 2.1). The BAC sub library was then screened with probe 2, which hybridised with 46 clones (9 scored as ++ and 37 as +), 15 of which had also hybridised with probe 1.

DNA from 26 randomly selected positive BAC clones was extracted and digested with *Hind III* for fingerprinting. In addition, four randomly selected clones from the BAC sub library that did not hybridise to probe 1 or 2 were included as negative controls. In the fingerprint gel, none of the four negative control BAC clones produced a hybridisation signal. Of the 26 candidate *Rht-1* containing BAC clones, 17 hybridised with probe 1, including eight that gave a strong signal, but there was relatively little overlap of bands between the DNA of the different BAC clones (Figure 2.2). In several of the BAC clones, the probe also hybridised to more than one band. Hybridisation to multiple bands also occurred using probe 3 (figure not shown).

Table 2.1. *Rht-1* screening summary of the BAC sub library.

	Hybridisation signal[b]			
Clone[a]	Probe 1	Probe 2	Fingerprint	PCR[c]
0027_A23	+	+	NT	-
0035_J15	-	+	NT	NT
0043_F20	++	-	-	-
0153_M04	-	++	NT	-
0155_I24	++	++	++	+
0235_E17	+	-	-	-
0255_N19	++	-	+	-
0256_N08	-	+	NT	-
0273_G23	-	+	NT	NT
0273_N11 *	-	-	-	-
0299_K18	+	+	NT	-
0308_F10	-	+	NT	NT
0326_C07	+	-	-	-
0330_K20	-	++	NT	-
0398_C20	+	-	++	-
0402_E05 *	-	-	-	-
0434_O09	-	+	NT	NT
0440_L09	-	++	NT	-
0445_E11	+	+	+	-
0445_I22	+	+	-	-
0449_C19	+	-	NT	NT
0461_E17	+	++	NT	-
0467_L13	-	+	NT	NT
0476_I01	++	++	NT	+
0477_J17	-	+	NT	NT
0477_K17 *	-	-	-	-
0514_P03	-	+	-	-
0517_F21	-	+	NT	NT
0525_L01	+	-	+	-
0528_K10	+	-	+	-
0529_O05	+	-	-	-
0537_P07	-	++	NT	-
0563_P19	-	+	NT	NT
0583_D03	+	-	NT	NT
0583_F03	+	+	+	-
0609_F07	+	+	++	-
0616_M13	+	-	-	-
0625_L09	-	+	NT	-
0628_H12	+	-	NT	-
0631_D08	+	-	NT	NT
0642_O12	-	+	+	-
0648_M12	-	+	NT	NT
0651_A09	+	-	+	-
0651_J20	-	+	NT	NT
0661_F13	+	-	+	-
0661_P14	-	+	NT	NT
0667_N04	-	+	NT	NT
0668_J16	+	-	NT	-
0678_C21	-	+	NT	-
0682_L19	+	-	-	-
0706_G15	-	+	NT	-
0706_H06 *	-	-	-	-
0731_N07	+	+	-	-
0735_I16	+	+	NT	-
0833_B18	+	-	++	-
0852_O17	-	+	NT	NT
0855_G01	++	-	++	-
0900_P10	-	+	NT	NT
0933_I04	++	-	+	-
0946_F05	-	++	NT	-
0951_K01	+	++	NT	-
0959_D17	-	+	NT	NT
0968_J11	-	+	NT	NT
0990_A10	-	+	NT	NT
1023_E02	+	-	NT	NT
1028_C14	+	+	++	-
1083_O15	+	+	++	-
1153_H16	+	-	++	-
1202_I11	+	-	NT	NT
1221_H08	-	+	NT	-
1223_O22	+	+	NT	-
1229_N11	+	-	NT	-
1233_N01	+	-	NT	-
1249_I15	-	+	NT	NT
1250_G16	+	-	NT	NT
1253_G17	-	+	NT	NT
1253_P02	+	-	NT	-

[a] Plate number_address of BAC clones that hybridised to a *Rht-1* specific probe

[b] The presence and intensity of the hybridisation was scored visually: ++, strong; +, weak; -, no hybridisation; NT, not tested. 26 clones that hybridised to probe 1 or probe 2 were randomly selected for fingerprint analysis along with the 4 negative control clones (marked with an *).

[c] The 48 clones that were either ++ for probe 1, ++ for probe 2, or + for both probes were PCR-screened with a *Rht-1* specific primer pair and visually scored for the presence of the corresponding 490 bp band (+, present; -, absent; NT, not tested).

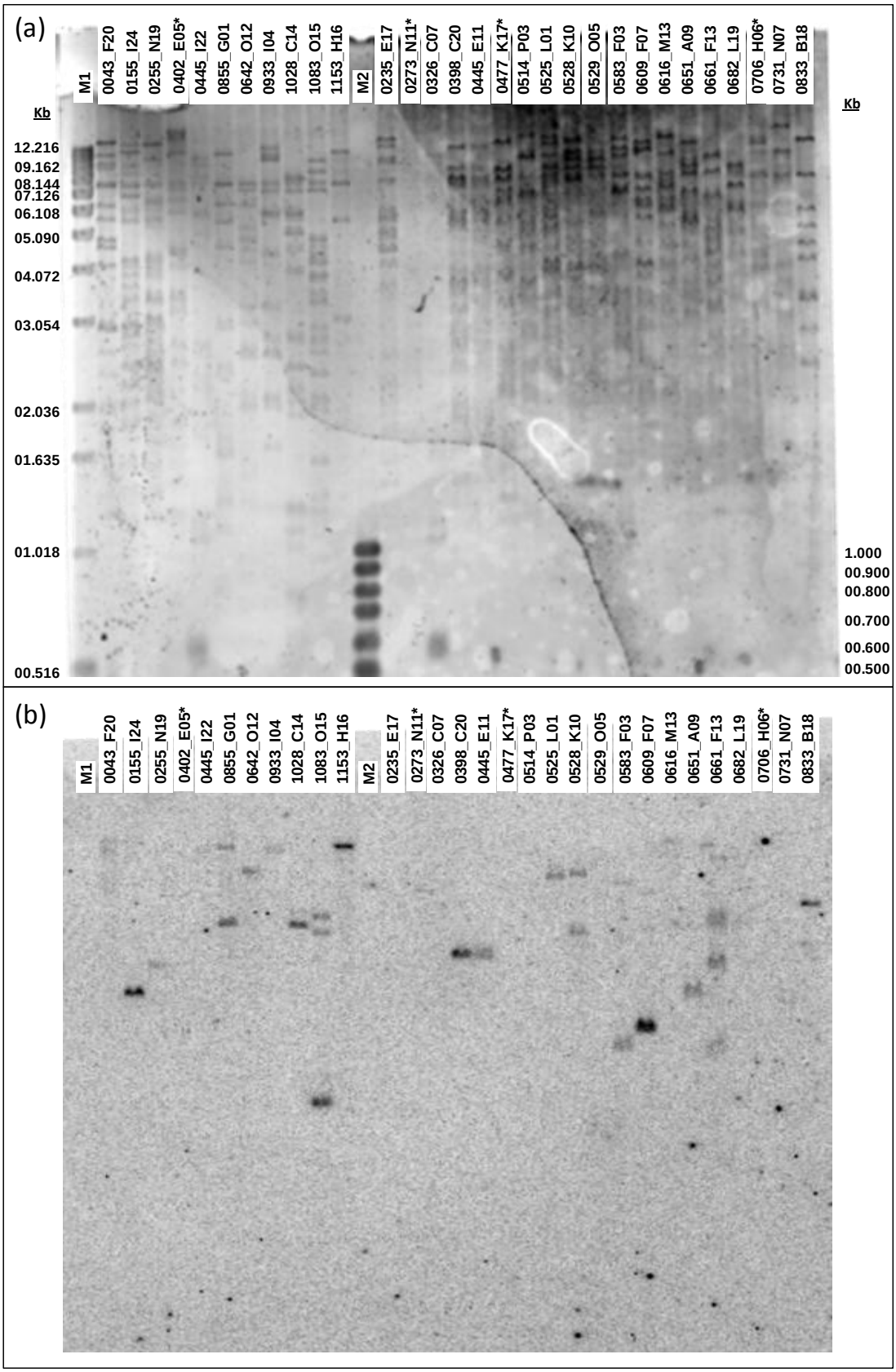


Figure 2.3. Fingerprint gel analysis and hybridisation pattern of candidate *Rht-1*-containing BAC clones and negative control clones. (a) DNA from clones was digested with *Hind III*, separated by electrophoresis, and visualised by staining. Molecular weight

marker M1 and M2 were Kb ladder (BRL) and Hyperladder IV (Bioline), respectively. M1 and M2 band sizes shown to the left and right of the gel, respectively. (b) DNA from (a) was transferred to a nylon membrane by Southern blotting and hybridised with the *Rht-1*-specific probe 1. Hybridisation signals were scored (absent, weak (+), or strong (++)) as shown in Table 2.1 (Fingerprint hybridisation). Negative control clones are marked with an (*).

Because the fingerprinting and hybridisation analysis did not clearly identify BAC clones that contained the *Rht-1* gene, PCR was used for clarification. The *Rht-1* specific primer pair Rht-ABD-F1 / Rht-ABD-R1 was used to assay the 30 BAC clones that had been fingerprinted. Only one BAC clone, 0155_I24, produced the approximately 490 bp product expected for *Rht-1* (Table 2.1). The primer pair was then used to screen 18 additional BAC clones from the BAC sub library. The total set of 48 included all 13 clones that produced a strong hybridisation signal with probe 1 or with probe 2 and the 11 clones that produced a signal (++ or +) with both probes. The approximately 490 bp product was amplified in a second BAC clone, 0476_I01 (Table 2.1).

The two *Rht-1*-containing BAC clones identified in the library were further characterised to determine the contributing genome, estimate the size of the insert, and locate *Rht-1* within the insert. To determine the contributing genome, the sequence of the PCR products amplified from each BAC clone using the Rht-ABD-F1 / Rht-ABD-R1 primer pair (bp 264 to 752 in reference to CS *Rht-D1a*) were compared with the sequences of *Rht-B1a* and *Rht-D1a*. The sequences of the amplified products were identical to that of *Rht-D1a* (accession AJ242531) and had several mismatches with *Rht-B1a*, indicating that both BAC clones were from the D genome (data not shown). The insert sizes of the *Rht-1* containing clones were estimated by PFGE and summed to be at least 175 kb for 0155_I24 and 105 kb for 0476_I01 (Figure 2.4). The location of *Rht-D1a* on the BAC clones was estimated using five PCR primer pairs designed at intervals based on sequence from BAC clone 1J9 (Table 2.2). Based on the absence or production of amplification products, the 5' end of insert of BAC 0155_I24 was predicted to be between 80 kb and 135 kb upstream of *Rht-D1a* and the 3' end was at least 8.5 kb downstream. The 5'

end of the insert in BAC 0476_I01 was estimated to be between 40 kb and 80 kb upstream and the 3' end was at least 8.5 kb downstream. Based on these data and the estimated length of the BAC inserts, *Rht-D1a* is likely to be near the middle of each insert.

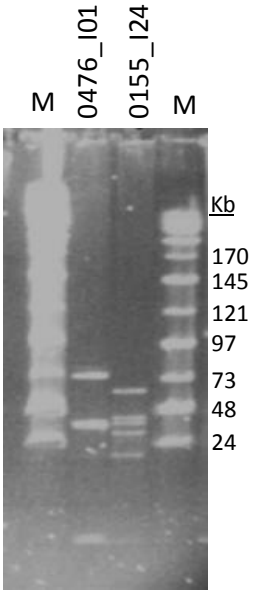


Figure 2.4. Sizing of the two *Rht-1*-containing BAC clones using pulsed field gel electrophoresis (PFGE). BAC DNA was digested overnight with *NotI*, separated with PFGE, and stained with ethidium bromide. BAC sizes were estimated by visual comparison with molecular marker Midrange II PFG (NEB Biolabs) denoted as 'M'.

Table 2.2. Estimated locations of *Rht-D1a* on BAC clones 0155_I24 and 0476_I01.

Primers			PCR amplification[b]	
Pair No.	Name	Location relative *Rht-D1* (kb)[a]	0155_I24	0476_I01
1	Rht_D-BAC_F1 Rht_D-BAC_R1	8.5	√	√
2	Rht_D-BAC_F2 Rht_D-BAC_R2	-10	√	√
3	Rht_D-BAC_F3 Rht_D-BAC_R3	-40	√	√
4	Rht_D-BAC_F4 Rht_D-BAC_R4	-80	√	x
5	Rht_D-BAC_F5 Rht_D-BAC_R5	-135	x	x

[a] Approximate location of primer pairs is shown relative to the start nucleotide of *Rht-D1a* of BAC 1J9. Negative numbers refer to sequence 5' or *Rht-D1a*.

[b] Presence of the expected PCR product is labelled as (√) and absence as (x) when using DNA of the indicated BAC clones as template.

2.3.2. Identification and characterisation of *Rht-1*-containing BAC clones from the UK component of the CS library

As only two *Rht-1*-containing clones were obtained by Southern hybridisation screening, an alternative approach, based on PCR amplification of *Rht-1,* was taken. Two primers, Rht-ABD-F1 and Rht-ABD-R1a, which both completely match *Rht-B1a* and *Rht-D1a* in the *Rht-1*-specific N-terminal region, were used to screen the library. Prior to screening the BAC library, the specificity of the primer pair for *Rht-1* was determined. The expected approximately 525 bp product was amplified from DNA extracted from leaf tissue of 'CS' (PC2, Figure 2.5). The primers were also shown to be capable of amplifying a single BAC clone containing *Rht-1* in a SP from an 8 × 8 × 8 cube as product was amplified in PC3, which is a 1:3072 (v/v) dilution of BAC DNA containing *Rht-1*:BAC DNA not containing *Rht-1* (PC3, Figure 2.5). The primers did not amplify a product from DNA of an *Rht-1* negative clone (NC2, Figure 2.5).

The initial PCR screen identified 49 SPs in which a single band of approximately 525 bp was amplified. A representative gel showing the screening results from SPs 385 to 675 is shown in Figure 2.5. Of the 49 SPs from which a PCR product was obtained, 26 were scored as strong amplifications and 23 as weak amplifications (Table 2.3). The SP data was de-convoluted to determine the corresponding 384-well plates that potentially held an *Rht-1*-containing BAC clone. Corresponding 384-well plates were selected for a second screening if two or three of the SPs resulted in a ~525 bp amplification product (weak or strong). A Perl script was used to identify the thirty-five 384-well plates in which at least two of the three corresponding SPs gave an amplification product (Table 2.4). To reduce the number of false positives, all of the SPs (except five that were inadvertently missed) associated with the 35 plates were re-screened with the same primer pair and the presence and intensity of the ~525 bp band scored (Table 2.4).

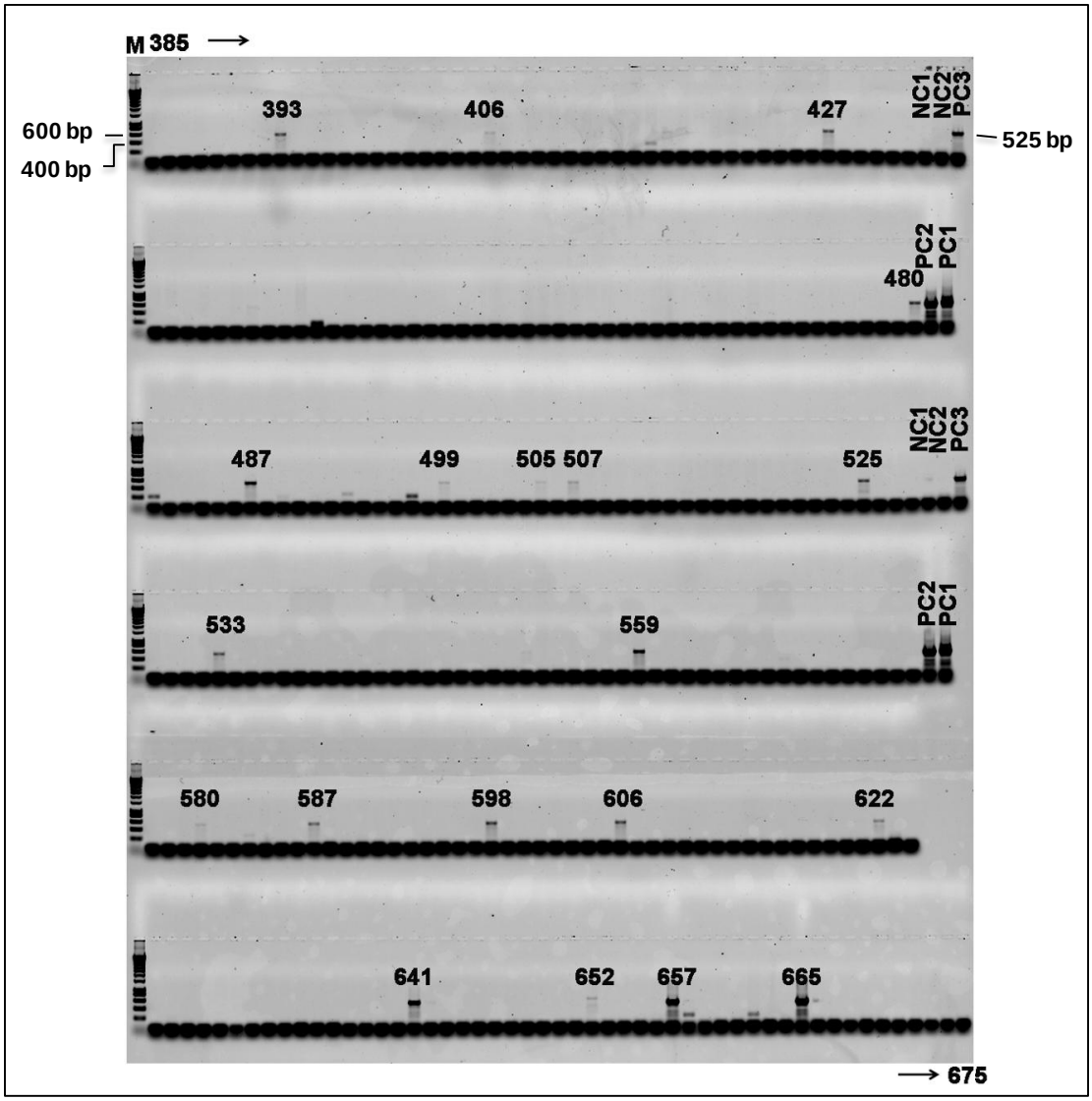


Figure 2.5. PCR screening results of BAC superpools (SPs) 385 to 675 using *Rht-1* specific primers. The SP number is indicated above any lanes in which the *Rht-1* (~525 bp) product was scored as present. Controls used to confirm the specificity of the PCR amplification are listed above the respective lane: NC1, water used in place of BAC DNA; NC2, DNA of BAC clones known not to contain *Rht-1*; PC1, DNA of BAC clones known to contain *Rht-1*; PC2, genomic DNA of 'Chinese Spring'; PC3, a 1:3072 (v/v) dilution of PC1:NC2 designed to mimic a SP that contained one *Rht-1*-containing clone. M, molecular weight marker (Bioline, Hyperladder I)

Table 2.3. Summary of superpools that amplified a *Rht-1* specific band

Rht-1 superpool screen[a]								
Strong					Weak			
18	141	332	533	657	96	245	317	505
21	237	393	559	665	155	258	329	507
28	242	427	587		177	269	353	580
39	305	480	598		184	272	357	622
87	308	487	606		187	301	406	652
97	325	525	641		192	310	499	

[a] The intensity of the approximately 525 bp product obtained from SPs was visually assessed as strong or weak.

Table 2.4. *Rht-1* screen summary

	Rht-1a superpool screen[a]									
	SP 1			SP 2			SP 3			
384-well plate no.[b]	No.	Screen 1	Screen 2	No.	Screen 1	Screen 2	No.	Screen 1	Screen 2	Result[c]
141	18	++	++	85	-	-	141	++	+	√
143	18	++	++	87	++	+	143	-	-	X
167	21	++	++	87	++	+	167	-	+	√
219	28	++	+	91	-	-	155	+	-	NT
224	28	++	+	96	+	+	160	-	-	√
248	31	-	-	96	+	+	184	+	+	NT
256	32	-	-	96	+	+	192	+	+	NT
305	39	++	++	97	++	++	177	+	-	√
312	39	++	++	104	-	NT	184	+	+	NT
679	201	ND	ND	269	+	+	325	++	+	√
711	205	ND	ND	269	+	+	357	+	+	NT
935	233	-	-	301	+	++	325	++	+	NT
963	237	++	++	297	-	-	353	+	+	NT
967	237	++	++	301	+	++	357	+	+	√
1003	242	++	++	305	++	+	329	+	-	X
1006	242	++	++	308	++	++	332	++	++	√
1008	242	++	++	310	+	-	334	-	-	NT
1027	245	+	+	305	++	+	353	+	+	NT
1030	245	+	+	308	++	++	356	-	NT	NT
1031	245	+	+	309	-	NT	357	+	+	NT
1032	245	+	+	310	+	-	358	-	-	NT
1063	249	-	-	317	+	-	325	++	+	NT
1095	253	-	NT	317	+	-	357	+	+	NT
1198	393	++	+	469	-	-	622	+	-	NT
1263	400	-	-	480	++	-	606	++	++	NT
1297	404	-	+	487	++	++	559	++	-	√
1315	406	+	++	487	++	++	577	-	-	X
1318	406	+	++	490	-	+	580	+	++	√
1360	411	-	NT	487	++	++	622	+	-	NT
1399	415	-	-	499	+	++	580	+	++	X
1417	417	-	++	499	+	++	598	++	++	√
1506	427	++	-	507	+	++	606	++	++	√
1668	445	-	-	525	++	+	606	++	++	X
1730	452	-	+	533	++	++	587	++	++	√
1905	641	++	++	657	++	++	665	++	++	√

[a] The presence and intensity of the approximately 525 bp product obtained from SPs was scored visually: ++, strong band; +, weak band; -, no band; ND, no DNA available; NT, not tested.

[b] 384-well plates were selected for further screening in which at least two of the three SPs scored + or ++ in the initial screen (screen 1) or re-screen (screen 2).

[c] The 384-well plates are marked as "positive" (√) if one or more clones amplified a *Rht-1*-specific PCR product. Plates in which no clones gave a product are designated (X) and plates that were not tested are marked NT.

To reduce the number of 384-well plates to be screened, 'best candidate' plates were chosen based on the following precepts: (1) At least two SPs were amplified in the original and repeat screens - with preference for those with strong amplifications, and (2) in cases where positive SPs were represented in more than one plate, the plate(s) most likely to have accounted for the positive SP result (based on amplification presence and intensity of the other two SPs) were chosen. The intention of the second precept was to eliminate plates most likely to have two positive SP results by chance. A total of 18 'best candidate' plates for containing an *Rht-1* BAC clone were selected.

PCR-screening of the BAC colonies from the 18 'best candidate' 384-well plates revealed that 13 BAC clones contained the *Rht-1* gene (Table 2.4). Figure 2.6 illustrates the identification of BAC clone 305_B11 (plate 305, address B11) as containing *Rht-1*. Sequence analysis of the amplified products of the 13 selected clones showed that seven were identical to *Rht-D1a* and four were identical to *Rht-B1a* (Figure 2.7). The remaining two sequences showed high homology to both *Rht-1* homoeologues, but contained several polymorphisms and were most likely the *Rht*-A1a homoeologue.

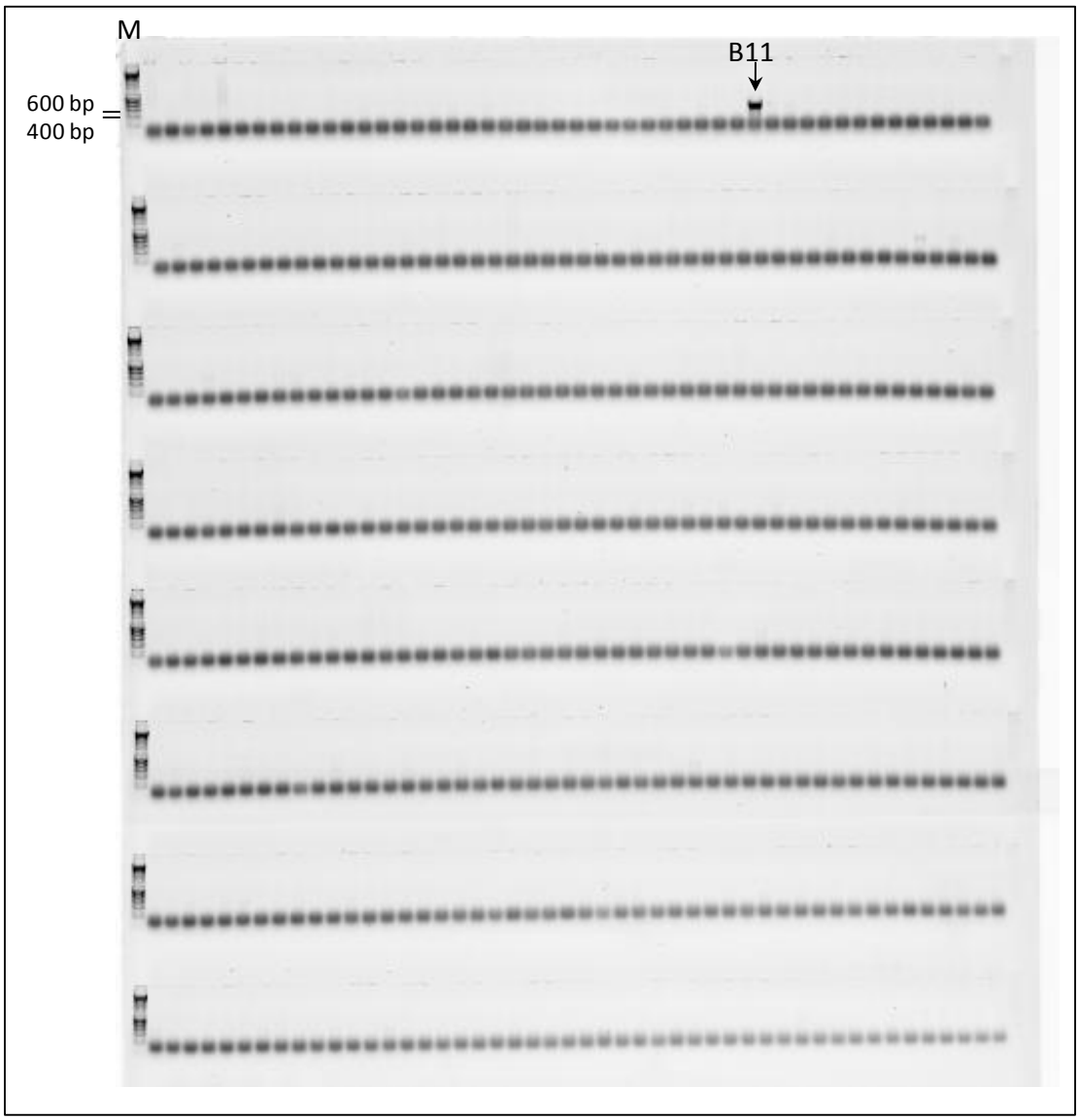


Figure 2.6. Colony PCR screening results of 384-well plate number 305 using *Rht-1* specific primers. The contents of each PCR were loaded into separate wells of an agarose gel and products identified by staining with ethidium bromide. A single clone (plate address B11) amplified the approximately 525 bp product specific to *Rht-1*. M, molecular weight marker (Bioline hyperladder I) with size in bp shown to the left.

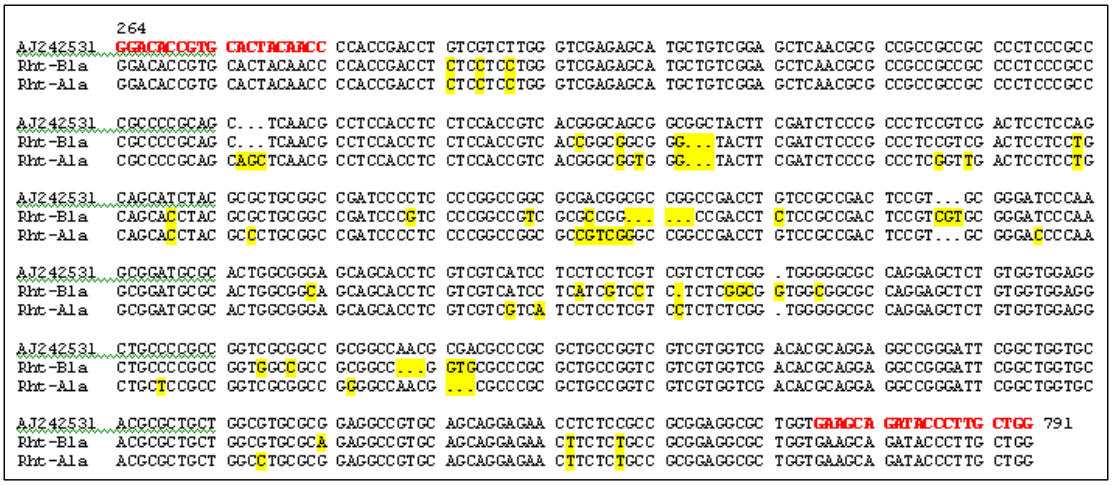


Figure 2.7. Nucleotide sequence alignment of the region amplified by the *Rht-1* specific primers in the 13 BAC clones. Homoeologue designation was determined from sequence of *Rht-D1a* (GenBank accession No. AJ242531) and *Rht-B1a* (N. Harberd, Oxford University, unpublished data). The designation of *Rht-A1a* is inferred. Primer sequences are shown in red font and polymorphisms are highlighted in yellow. Coordinates at the beginning and end of the sequence are relative to the start nucleotide of CS *Rht-D1a*.

The BAC clones were subjected to fingerprint analysis to determine the uniqueness of clones and which overlapping bands were shared by clones, and to estimate the size of the insert. Fingerprint analysis of DNA from the 13 BACs following *Hind III* digestion showed that although there was some overlap in the bands, the BACs were distinctive from each other (Figure 2.8). Estimates of the relative sizes of the BACs based on comparison of the fingerprint bands with the molecular marker ranged from 70 kb to 200 kb.

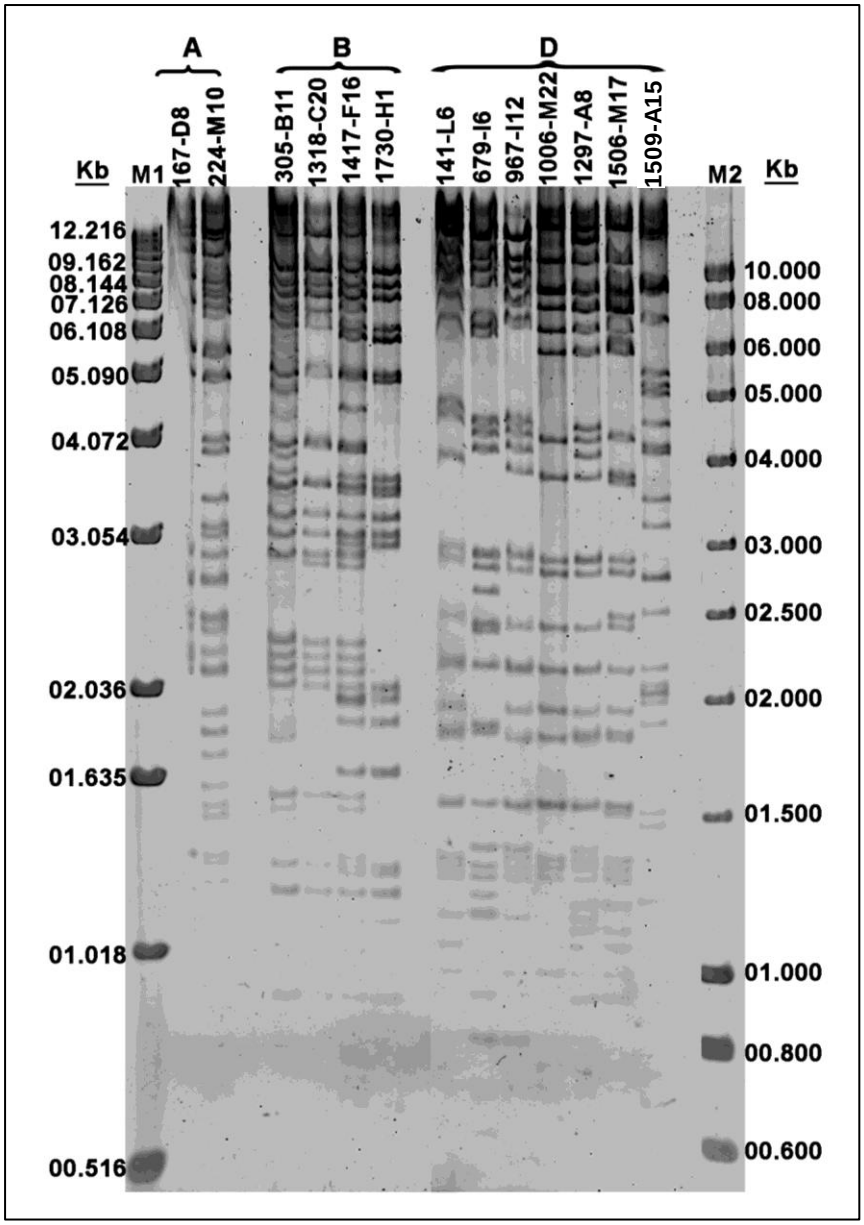


Figure 2.8. Fingerprint analysis of DNA extracted from BAC clones from which *Rht-1* sequence was obtained. DNA from clones was digested with *HindIII* and the gel stained with CyberGold. Molecular weight markers (M1 and M2) were Kb ladder (BRL) and Hyperladder I (Bioline), respectively. The genome derivation (A, B, or D) of the clones is also shown above their designations.

2.3.3. Summary of *Rht-1*-containing BAC clones identified in the French and UK components of the CS library

In total, 15 clones containing *Rht-1* were identified in the CS library with each of the three wheat genomes represented by at least two clones (Table 2.5).

Three clones were selected for full-length sequencing based on estimations of clone length and, where possible, *Rht-1* location within the insert.

Table 2.5. *Rht-1*-containing clones identified in the Chinese Spring ('CS') BAC library

Clone[a]	Library[b]	Genome	Sequencing[c]
0224_M10	'CS' (UK)	A	√
0167_D3	'CS' (UK)	A	
1318_C20	'CS' (UK)	B	
1417_F16	'CS' (UK)	B	√
1730_H1	'CS' (UK)	B	
0305_B11	'CS' (UK)	B	
0679_I12	'CS' (UK)	D	
1509_A15	'CS' (UK)	D	
0141_L6	'CS' (UK)	D	
0967_I6	'CS' (UK)	D	
1006_M22	'CS' (UK)	D	
1297_A8	'CS' (UK)	D	
1506_M17	'CS' (UK)	D	
0155_I24	'CS' (French)	D	√
0476_I01	'CS' (French)	D	

[a] Clones are named according to their position in the 384-well plate.

[b] BAC clones were identified in either the UK or French component of the CS library.

[c] BAC clones selected for full-length sequencing are marked with a (√)

2.4. DISCUSSION

A total of 13 clones were identified in the UK component before screening of the 384-well plates was discontinued. Based on the estimated 5 × haploid coverage of the UK component of the library (Febrer *et al*., 2009), 15 *Rht-1* clones would be predicted to be represented therein. Additional screening of the 384-well plates identified as candidates for containing *Rht-1* based on SP screening results (Table 2.4) is likely to uncover additional *Rht-1* containing clones. For example, plate 1027, has three corresponding SPs that score + or ++ that are not matched with an *Rht-1* containing clone. Our identification of 13 BAC clones containing *Rht-1* and the existence of additional 384-well plate candidates indicate that rigorous use of our screening procedure would identify all or most of the *Rht-1* clones in the UK library.

The French component of the CS BAC library consists of 3 haploid equivalents, yet only two *Rht-1* containing clones were identified compared

with the expected nine clones. Thus, it is likely that the Southern hybridisation screening method did not clearly identify all of the *Rht-1*-containing clones. The use of seven probes in the first screening could have decreased the efficiency of detection, especially if the hybridisation conditions were less favourable for the *Rht-1*-derived probe. Additionally, the *Rht-1* derived probes were not specific for *Rht-1* sequences even under the hybridisation conditions used for screening of the sub-library. This was made clear when *Rht-1* specific primers known to be suitable for amplification of all three *Rht-1* homoeologues identified only the two *Rht-1* containing clones out of 48 candidates identified by probes 1 and/or 2 (Table 2.1). Non-specific hybridisation was also evident when *Hind III* digested DNA of clones from the BAC sub-library were hybridised with probe 1 (Figure 2.3) or probe 3. In these cases, several BAC clones have multiple bands that hybridise to the probe even though there is no *Hind III* site within any of the *Rht-1* homoeologues and only a single band should be present. The apparent non-specificity of the hybridisation may be related to the high GC content (> 70%) of each the three probes used.

Both BAC clones identified by hybridisation were derived from the D genome and seven of the 13 clones identified by PCR screening were also derived from this genome. Since this bias was seen using two screening methods this may be a true representation of the composition of *Rht-1*-containing clones in the library. However, additional clones may also have been present in the two parts of the library, and especially in the French component, making it difficult to accurately define the relative proportions of the three homoeologues in this library.

This study shows that PCR-based screening was more efficient in discovering *Rht-1*-containing clones than Southern hybridisation but it depends upon the availability of appropriate primers, which ideally should be capable of amplifying all three homoeologues of the target gene. Screening using PCR also has the advantage that it does not require the use of radioactivity. However, although the PCR screening technique was rapid, this is only true if the SPs are already available.

3. GENETIC COMPOSITION AND COMPARATIVE ANALYSIS OF THE *RHT-1* LOCI AND ADJOINING SEQUENCE

3.1. INTRODUCTION

The *Rht-1* semi-dwarf alleles, *Rht-B1b* and *Rht-D1b*, were introduced into wheat varieties in the Western world as part of the green revolution beginning in the 1960s. These alleles were rapidly incorporated into wheat varieties worldwide and within 40 years, an estimated 70% of planted wheat acreage thought to contain *Rht-B1b* or *Rht-D1b* (Evans, 1998). While these two alleles along with the wild type alleles *Rht-B1a* and *Rht-D1a* in the CS background have been cloned and sequenced (Peng *et al.*, 1999), little is known regarding the composition of the adjoining genetic region at these loci. The genetic regions tightly linked to *Rht-B1b* and *Rht-D1b* presumably were integrated into most wheat varieties. On the third homoeolocus, *Rht-A1*, the sequence of the open reading frame (ORF) and adjoining region remain unknown.

Understanding the genetic composition of the *Rht-1* homoeoloci and the surrounding region is critical because alleles at these loci are associated with changes in the level of resistance to biotic and abiotic stress. *Rht-B1b* and *Rht-D1b* have both been associated with changes in the level of resistance to Fusarium Head Blight (FHB), a major disease in wheat. A quantitative trait locus (QTL) containing *Rht-D1b* was associated with increased susceptibility to initial infection (Type I resistance) of FHB (Srinivasachary *et al.*, 2008; Srinivasachary *et al.*, 2009). Increased susceptibility to FHB associated with *Rht-D1b* was also reported by Hilton *et al.* (1999) and Draeger *et al.* (2007). The association of *Rht-D1b* with increased FHB susceptibility is thought to result from a linked gene (Paul Nicholson, Project Leader, Disease and Stress Biology, JIC, personal communication). Similarly, a QTL encompassing *Rht-B1b* was associated with increased Type I susceptibility to FHB and, conversely, increased Type II resistance (resistance to fungal growth within the spike) to FHB was also associated with a QTL that contained *Rht-B1b* (Srinivasachary *et al.*, 2009). In *Arabidopsis thaliana*, the GA insensitive *gai* mutant in Arabidopsis and the GA deficient mutant *ga1-3* were both found to

be more tolerant of salinity than plants carrying the wild type alleles (Achard *et al*., 2006). When Arabidopsis is exposed to cold stress, increased DELLA protein accumulation is also associated with greater tolerance (Achard *et al*., 2008a). In relation to biotic stress in Arabidopsis, stabilised DELLA proteins have been shown to promote increased resistance to necrotrophs and increased susceptibility to biotrophs (Navarro *et al*., 2008). A similar finding was also reported in wheat where the stabilised DELLA proteins encoded by *Rht-B1b* and *Rht-D1b* were more resistant to necrotrophs and more susceptible to biotrophs than plants with wild type DELLA proteins (R. Saville, 2011). Functionally, the DELLA proteins are thought to integrate hormonal and environmental signals and play a key role in plant growth and survival (Alvey and Boulton, 2008), hence knowledge of the *Rht-1* loci and the surrounding region is important.

It is well established that gene synteny of the three wheat genomes is well conserved and that this colinearity extends to other members of the grass (*Poaceae*) family (Moore *et al.*, 1995; Gale and Devos, 1998; Kumar *et al.*, 2009), while transposable elements (TEs) are generally not conserved among *Poaceae* species (Devos *et al.*, 2008). Fully assembled and annotated sequences of the *Poaceae* family currently include rice (*Oryza sativa*), *Brachypodium distachyon*, maize (*Zea mays*), and sorghum (*Sorghum bicolor*). For barley (*Hordeum vulgare*), one of wheat's closest relatives, assembled sequence is not yet available; however a large collection of barley expressed sequence tags (ESTs) exists. These genomic resources enable comparative analysis between genomes, which facilitates identification of important functional and regulatory sequences associated with genes and aids in determining the genetic relatedness of species. The majority (approximately 80%) of the wheat sequence, however, is made up of TEs (Gupta *et al.*, 2008), which are rapidly evolving relative to genes (Devos *et al.*, 2008). TEs can play an important role in plant genomes by reshaping overall genome structure and by altering gene function and regulation (Bennetzen 2000).

The aim of this chapter is to determine the genetic composition of the *Rht-1* ORF and flanking regions of the wheat homoeologues within a common genetic background. To accomplish this, three *Rht-1*-containing BAC clones (one from each homoeologue) identified in the CS BAC library (Chapter 2) were sequenced, assembled, and annotated. Comparative analysis of the genic and TE composition among the three BAC sequences is performed. In addition, the CS wheat BAC sequences are compared to *Rht-1* containing BAC sequences derived from *T. urartu* (the A genome ancestor of wheat) and from the D genome of 'Aibai/10*CS'. The level of sequence conservation and phylogenetic relationships of the genes found on the CS wheat BACs are determined in relation to orthologues in rice, *Brachypodium*, maize, sorghum, and barley.

3.2. MATERIALS AND METHODS

3.2.1. Sequencing of *Rht-1*-containing BAC clones

Rht-1a containing clones 0224_M10 (A genome), 1417_F16 (B genome), and 0155_I24 (D genome) identified in the CS BAC library (Chapter 3) were sequenced to 8 × coverage by shotgun sequencing at the Genome Center, Washington University, St. Louis, USA (Appendix II). The CS-A, CS-B, and CS-D genome BACs were assembled into 3, 3, and 4 contigs, respectively, with estimated gaps between contigs of no more than 500 bp (personal communication, William Courtney, Washington University Genome Center). The *Rht-1*-containing BAC sequences derived from *Triticum urartu* and from the D genome of 'Aibai/10*CS' (BAC 1J9; Wu *et al.*, 2004) were provided by Jizeng Jia (Chinese Academy of Agricultural Sciences, P.R. China).

3.2.2. Annotation of *Rht-1* containing wheat BAC sequences

Location and identity of repeat elements was determined by blastn and tblastx searches of the TREP cereal repeat database (http://wheat.pw.usda.gov/ITMI/Repeats). Retrotransposon LTRs (long terminal repeats) and TSDs (target site duplications) were identified using the

default settings of LTR Finder (http://tlife.fudan.edu.cn/ltr_finder; Zhao and Wang, 2007). Gene predictions were made across entire BAC inserts using Softberry FgeneSH software (http://linux1.softberry.com/berry.phtml). Nucleotide sequences of predicted genes were queried against the TREP cereals repeated database, the NCBI nucleotide collection (nr/nt) (http://blast.ncbi.nlm.nih.gov/Blast.cgi), the TIGR plant transcript assemblies (TAs) monocot collection (http://plantta.jcvi.org), and the KOME rice cDNA collection (http://cdna01.dna.affrc.go.jp/cDNA) to identify regions of homology. Predicted genes were considered true 'genes' if a known orthologue was identified, not considered a gene if sequence overlapped a repeat element, or remained 'predicted genes' if no significant similarity was found in the database searches. Intron-exon boundaries of genes were determined by alignment with TIGR wheat and barley TAs, and annotated orthologues in rice, *Brachypodium*, sorghum, and maize (B73 inbred) where sequence was available. The remaining BAC sequence that did not correspond to a TE, gene, or predicted gene was blast searched against the NCBI database nucleotide collection (nr/nt) to determine if any similarity existed. Annotation of BAC sequences was facilitated with Invitrogen Vector NTI v10.1.1 software.

3.2.3. Comparative analysis of BAC sequences

Poaceae orthologues of genes identified on the wheat BAC clones were identified by querying the wheat ORF sequences in BLAST searches of the fully assembled genomes of rice (http://rice.plantbiology.msu.edu), *Brachypodium* (http://www.modelcrop.org), sorghum (http://www.phytozome.net/sorghum), and maize B73 inbred (http://www.maizegdb.org). To identify orthologues in the barley genome, wheat gene ORFs were used in a BLAST search of the NCBI nucleotide (nr/nt) collection, the NCBI *Hordeum vulgare* EST collections, and the TIGR *Hordeum vulgare* TA collection. For the *Rht-1* orthologue in barley, the *SLN1* full-length coding sequence was utilised (GenBank accession no. AF460219; Chandler *et al.*, 2002). To construct the barley zinc finger (*ZnF*) and *DUF6* orthologous sequences, matching ESTs and TAs were identified and aligned with the ORFs of the wheat, rice, *Brachypodium*, sorghum, and maize

orthologues. The barley *ZnF* orthologue ORF sequence was assembled by combining TA38367_4513 (bp 34-640), TA38365_4513 (bp 487-504), and TA38366_4513 (bp 11-807), which together cover the entire length of the wheat ORF. The barley *DUF6* orthologue was assembled using EST GH226632 (bp 71-428) (Close *et al.*, 2008) and TA54310_4513 (1-785). Alignment with the *Poaceae* consensus *DUF6* revealed that the barley TA and EST sequences did not cover an estimated 165 bp region in the middle of the gene presumably due to the unavailability of barley sequence. To maximise alignment with the *Poaceae* consensus *DUF6* sequence, the code 'N' (IUPAC [International Union of Pure and Applied Chemistry; http://www.chem.qmul.ac.uk/iupac/] convention for 'unknown nucleotide'), is used to designate each of the estimated 165 missing nucleotides. In the translated barley *DUF6* amino acid sequence, the code 'X' (IUPAC convention for 'any amino acid') is used whenever a codon contained an 'N' nucleotide designation.

Nucleotide and amino acid identities were calculated with the AlignX application of VectorNTI (v10.1.1) following alignment of sequence pairs. Alignments of multiple orthologues were made using ClustalX v2.0.12 (Larkin *et al.*, 2007) and manually adjusted where necessary to improve alignments using GeneDoc v2.6.002 software (Nicholas and Nicholas, 1997). Locations of protein motifs in the *Rht-1* genes were based on Tian *et al*. (2004). Locations of protein motifs on the *DUF6* and *ZnF* amino acid sequences were determined using the European Bioinformatics Institute (EBI) InterPro scan (http://www.ebi.ac.uk/Tools/InterProScan/) with amino acid sequences serving as query. Phylogenetic relationships among orthologous genes was determined using the following applications of PHYLIP v3.6 software (Felsenstein, 2004): 'Seqboot' was used to calculated bootstrap values (1000 replicates); genetic distance between species was calculated using 'Dnadist' (1000 multiple data set values); 'Neighbor', which employs the neighbor-joining method, was used to construct trees; 'Consense' was used to determine the consensus tree.

Alignment of the 10 kb of sequence 5' and 3' of *Rht-1* was performed using an on-line version of ACT: the Artemis Comparison Tool (Carver *et al.*, 2005). Comparison files were created using Double ACT v2 (http://www.hpa-bioinfotools.org.uk/pise/double_act.html). Identification of homologous regions among wheat BACs, rice, and *Brachypodium* was performed with the NCBI multiple sequence alignment tool.

3.3. RESULTS

3.3.1. Genetic composition and comparative analysis of the *Rht-1* containing wheat BACs

Sequence analysis revealed that the inserts in the three 'CS' BAC clones ranged from 164 kb to 213 kb in length with *Rht-1* located near the middle of each clone (Table 3.1; Appendix III). The *T. urartu* BAC clone contains a 100 kb insert in which *Rht-1* is positioned near the middle. The D genome-derived BAC from 'Aibai/10*CS' contains a 207 kb insert in which *Rht-1* is located near the 3' end. Transposable elements (TEs) constitute 47% to 66% of each BAC insert while gene content accounts for 1.7% to 4.6% of any BAC sequence. Between 32% and 48% of each BAC sequence did not have homology to annotated genes or to TEs.

Table 3.1. Summary of *Rht-1*-containing wheat BAC sequences.

Genetic background[a]	Address[b]	Length (bp)	Gene coordinates[c]			Insert compostion[d]		
			ZnF	*DUF6*	*Rht-1*	TE	ORF	Other
Ta 'CS' (A)	0224-M10	164257	24659	48779	79748	56%	2.8%	41%
Ta 'CS' (B)	1417-F16	187310	NA	53952	77662	63%	1.7%	35%
Ta 'CS' (D)	0155-I24	213794	6381	40731	96445	66%	2.2%	32%
T. urartu (A)	NA	100141	19834	42927	59411	47%	4.6%	48%
Ta 'Aibai/10*CS' (D)	1J9	207533	108486	142474	198722	59%	2.2%	39%

[a] *Ta* 'CS' (A), *Ta* 'CS' (B), and *Ta* 'CS' (D) are the *Triticum aestivum* BAC sequences from the A, B, and D genomes, respectively, of Chinese Spring. *T.urartu* (A genome) and *Ta* 'Aibai/10*CS' (D genome) sequences were received from Jizeng Jia, CAAS.

[b] NA = not available.

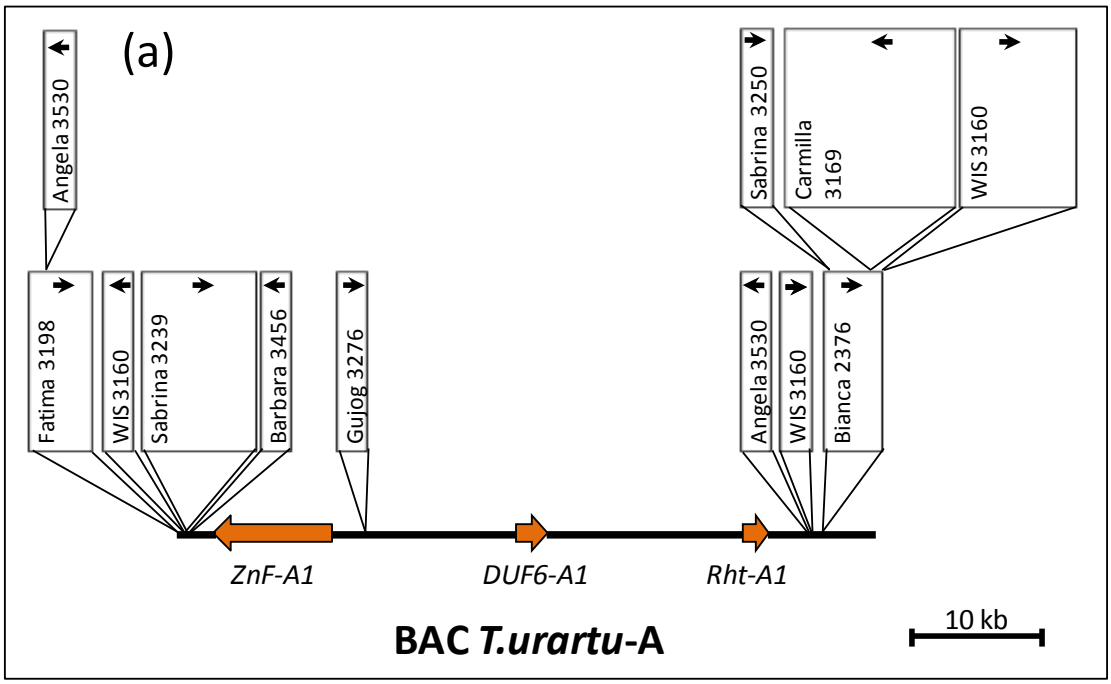
[c] Location of the most 5' nucleotide of the Zinc finger family (*ZnF*), Domain of Unknown function family (*DUF6*), and *Reduced height* (*Rht-1*) genes relative to the first nucleotide of

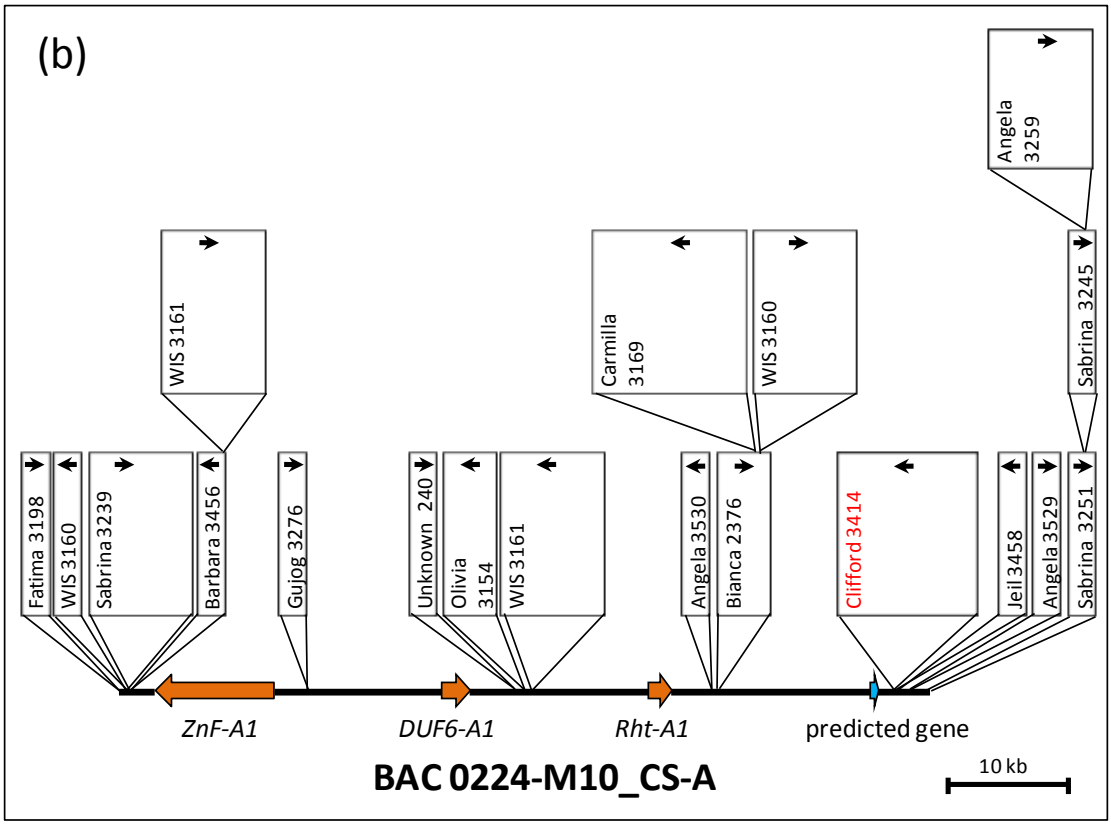
the insert (coordinate 1). *DUF6* and *Rht-1* are on the plus stand of each BAC and coordinates match the start nucleotide. *ZnF* is not present of the B genome BAC and is on the minus strand of the remaining BACs with coordinates matching the end nucleotide.
[d] TE = transposable element; ORF = open reading frame of genes. Other = sequence without homology to TEs or known genes.

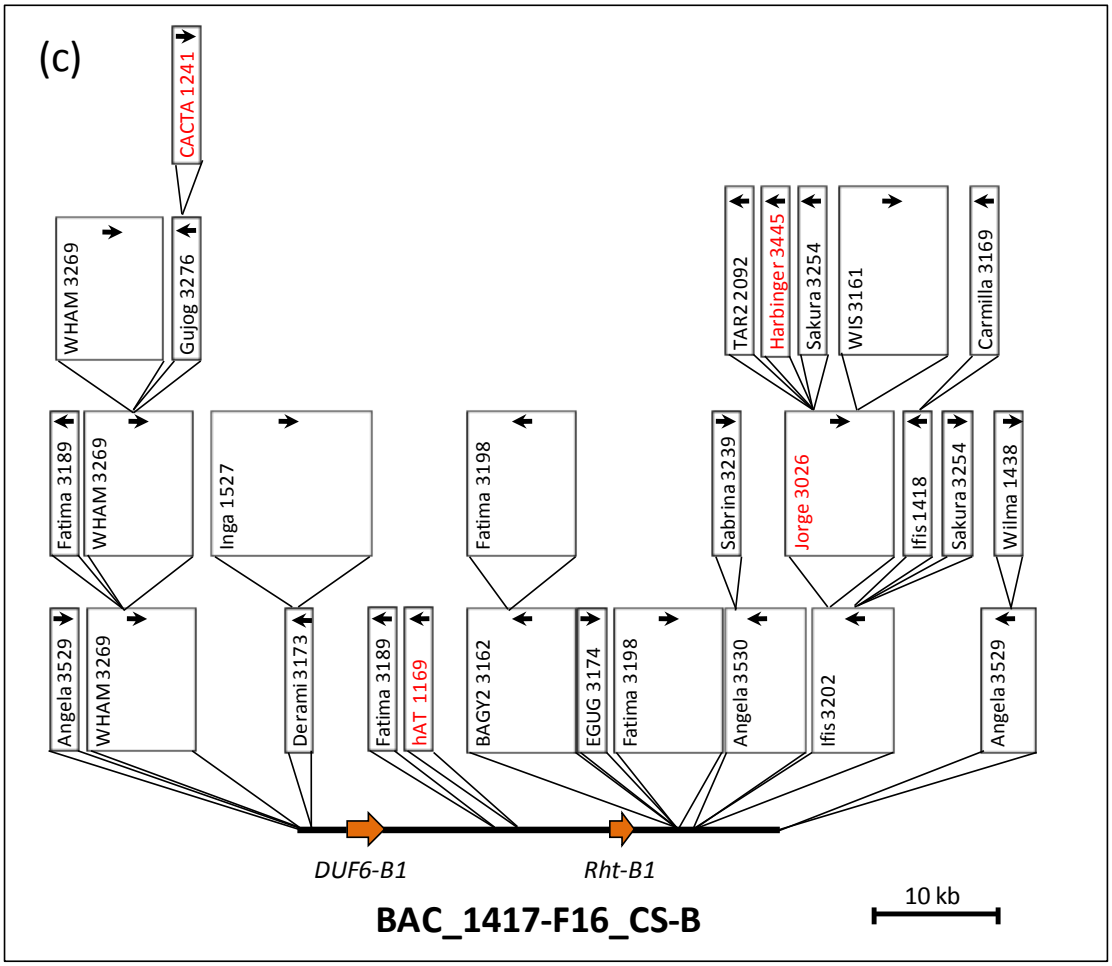
Along with *Rht-1*, each 'CS' BAC sequence also contained one to two additional genes, which were in synteny among BAC clones (Table 3.1). CS-A and CS-D contain the same three genes in conserved order and orientation, which from 5' to 3' are: a zinc finger family protein (TIGR rice locus Loc_*Os03g49900;* herein referred to as *ZnF*), a domain of unknown function family protein (TIGR rice locus Loc_*Os03g49940*; herein referred to as *DUF6*), and *Rht-1* (TIGR rice locus Loc_*Os03g49990*). In this text, references to the A, B, and D genome homoeologues of the three genes will be indicated by attaching the suffixes -*A1*, -*B1*, and -*D1*, respectively, to the end of the gene names. The *ZnF* gene is named for a C3HC4 type zinc-finger (RING finger) domain, which is a cysteine-rich domain of 40 to 60 residues that coordinates two zinc ions (http://www.ebi.ac.uk/interpro/IEntry?ac=IPR018957). The *DUF6* family, also known as the *EamA*-like transporter family are predicted integral membrane proteins that are part of the drug/metabolite transporter super-family, but have no known function (http://www.ebi.ac.uk/interpro/IEntry?ac=IPR000620). Each *DUF6* gene contains two regions that encode for the *DUF6*-type domains. The CS-B BAC contains *DUF6* and *Rht-1*, but sufficient sequence is not available to determine if *ZnF* is present even though approximately 54 kb of sequence exists upstream of *DUF6*. In addition to these three genes, a predicted gene also exists 3' of *Rht-A1* on the CS-A BAC and is described in section 3.3.3. Although gene synteny is conserved among the CS BAC clones, the physical distance encompassing the three genes varies widely from 42 kb in CS-A to 92 kb in CS-D.

Comparison of the three CS BACs containing *Rht-1* revealed that although gene synteny is highly conserved, there is no apparent conservation of repeat elements or nesting structure in the intergenic regions (Figure 3.1b, c, d). The

region between the *ZnF* and *DUF6* genes contains a single LTR retrotransposon on CS-A (Figure 3.1b), whereas five LTR retrotransposons and a DNA transposon were identified on CS-D (Figure 3.1d). In this region of the CS-B BAC (upstream of *DUF6*; Figure 3.1c), there are three insertions of the WHAM LTR retrotransposon (TREP no. 3269) comprising 23 kb of sequence that is distinct from the equivalent regions on the A and D genomes. Analysis of the region between the *DUF6* and *Rht-1* genes shows no conservation of TE sequence among the CS homoeologues. In this region, CS-D contains sequence of five TEs, including a 9.6 kb Romani LTR retrotransposon and an 8.6 kb WIS LTR retrotransposon while CS-A contains only three TE sequences and CS-B contains just two short TE sequences. 3' of *Rht-1*, each of the CS BAC sequences contain large numbers of transposons, with no similarity among BACs. Similar to the other regions, the region upstream of *ZnF* (only available on the A and D genomes) has no conservation of TE sequence.




(b)
Angela 3259
Sabrina 3245
WIS 3161
Carmilla 3169
WIS 3160
Fatima 3198
WIS 3160
Sabrina 3239
Barbara 3456
Gujog 3276
Unknown 240
Olivia 3154
WIS 3161
Angela 3530
Bianca 2376
Clifford 3414
Jeil 3458
Angela 3529
Sabrina 3251
ZnF-A1
DUF6-A1
Rht-A1
predicted gene
BAC 0224-M10_CS-A
10 kb


(c)
CACTA 1241
WHAM 3269
Gujog 3276
TAR2 2092
Harbinger 3445
Sakura 3254
WIS 3161
Carmilla 3169
Fatima 3189
WHAM 3269
Inga 1527
Fatima 3198
Sabrina 3239
Jorge 3026
Ifis 1418
Sakura 3254
Wilma 1438
Angela 3529
WHAM 3269
Derami 3173
Fatima 3189
hAT 1169
BAGY2 3162
EGUG 3174
Fatima 3198
Angela 3530
Ifis 3202
Angela 3529
DUF6-B1
Rht-B1
BAC_1417-F16_CS-B
10 kb

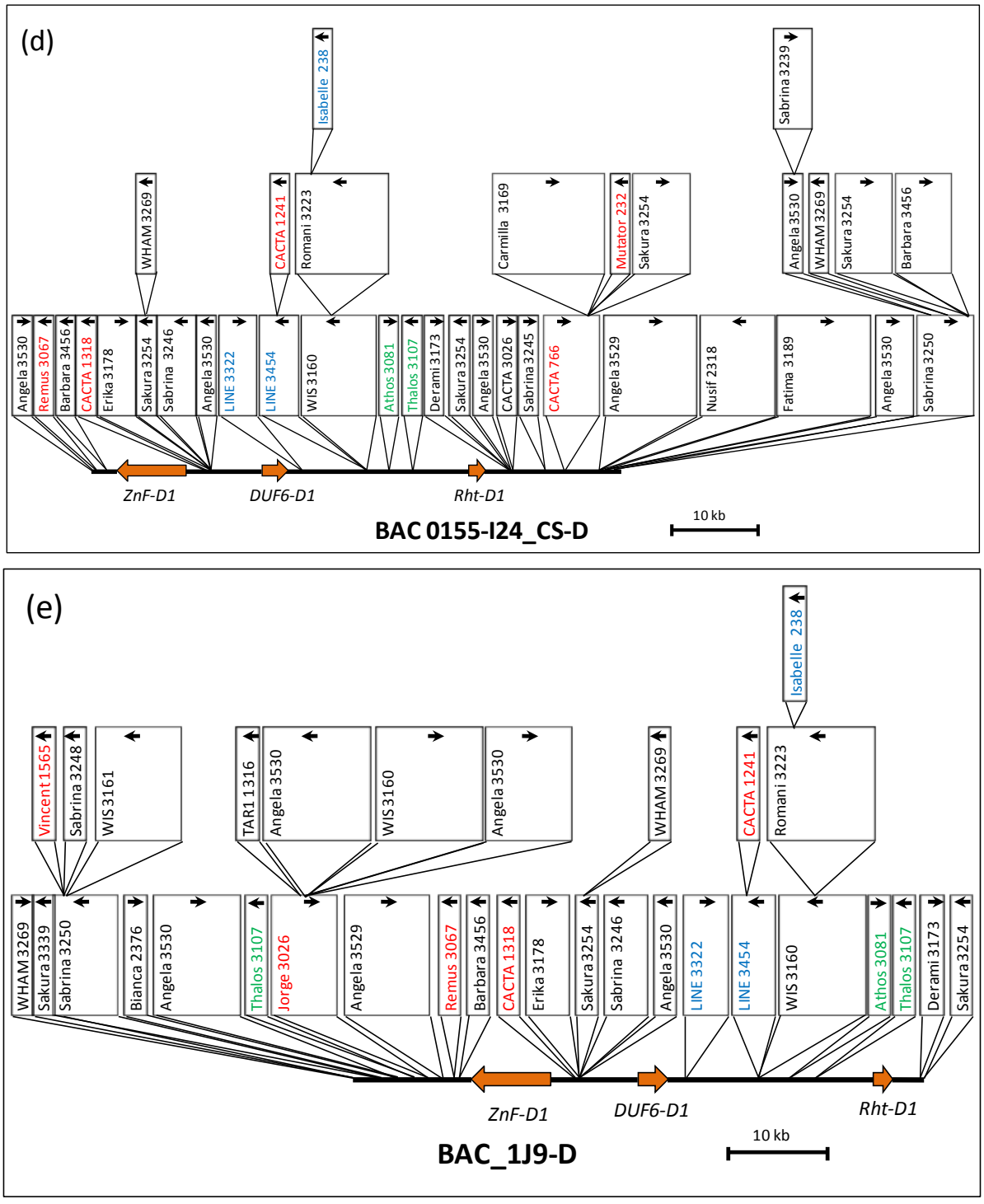


Figure 3.1. Gene and transposable element (TE) nesting structure of the five *Rht-1* wheat BAC sequences. (A) *T. urartu*, A genome BAC; (B) Chinese Spring (CS) A genome BAC 0224-M10; (C) CS B genome BAC 1417-F16; (D) CS D genome BAC 0155-I24, (E) BAC 1J9, a D genome-derived BAC from Aibai/10*CS, and. In each figure, the thick black line denotes intergenic sequence that does not correspond to TEs or genes. On this line, orange arrows indicate genes and blue arrows indicated predicted genes. TEs are shown in boxes and identified by common name and Triticeae Repeat (TREP) number. The lengths of the BACs, genes, repeat elements, and the position of the insertions, are approximately to scale.

Arrows indicate orientation of transcription. LTR retrotransposons are shown in black font, DNA transposons are shown in red font, non-LTR retrotransposons are shown in blue font, and intergenic MITES (Miniature Inverted-repeat TEs) in green font. Intronic MITEs are shown in Figures 3.7 and 3.10.

Approximately 35.6% (201 kb) of the sequences from the CS BACs have low homology (Expectation (E) < 1×10^{-10}) to known genes or to TEs (Table 3.1). There are several blocks of low homology sequence greater than 5 kb in size. On all three CS BACs, the region between *DUF6* and *Rht-1* contains large stretches of low homology sequence and each homoeologue has at least 8 kb of low homology sequence that is upstream of and adjoining *Rht-1* (Figure 3.1b, c, d). This low homology region is also present on the BACs derived from *T. urartu* and 'Aibai/10*CS' (Figure 3.1a, e). Other blocks of low homology include 14 kb of sequence located between 37.2 and 51.6 kb downstream of *Rht-A1*, which extends to and includes the predicted gene (Figure 3.1b) (Appendix IIIA, bp 117038 to 131305). In addition, on the CS-A genome BAC (and on the *T. urartu* BAC) is a low homology region of nearly 11 kb that is upstream of and adjoining *DUF6-A1* (Figure 3.1b) (Appendix IIIA, bp 37820 to 48778).

Gene order and orientation is very highly conserved between the CS-A and *T. urartu* BAC inserts whereas TE composition is only generally conserved and there are several large differences (Figure 3.1a, b). In the region between *ZnF-A1* and *DUF6-A1*, TE composition is nearly identical as both BACs each contain just 400 bp of the TE Gujog (TREP no. 3276), which has 98% nucleotide identity between the two sequences. In the region between *DUF6-A1* and *Rht-A1*, the most striking difference in TE composition between the two A genome BACs exists as *T. urartu* is devoid of TEs in this region, but three TEs totaling 13 kb are present on CS-A. Upstream of *ZnF-A1*, the overlapping regions of the two BACs have similar TE content with the exception of an 8.7 kb WIS LTR retrotransposon (TREP no. 3161) that is only present on CS-A. Downstream of *Rht-A1*, the TE content is again similar in the overlapping regions of the BAC sequences, except for the presence of

sequences of WIS (TREP no. 3160) and Sabrina (TREP no. 3250) in *T. urartu*, but not CS-A.

The CS-D and 'Aibai/10*CS' BACs have 105 kb of overlapping sequence in common that is nearly identical, having 99.83% of the nucleotides conserved. The *ZnF-D1*, *DUF6-D1*, and *Rht-D1* genes are contained in the overlapping regions and are conserved in synteny and orientation of transcription. The *ZnF-D1* and *DUF-D1* ORFs are identical on the two BACs. The *Rht-D1* ORFs differ by a single base substitution as Aibai/10*CS contains the SNP associated with the *Rht-D1b* allele whereas CS contains the SNP associated with the *Rht-D1a* allele. The intergenic sequences of the overlapping regions have the same TE composition and nesting structure is completely conserved.

3.3.2. Comparative analyses of *Rht-1* and the surrounding region in wheat and *Poaceae*

Comparisons of the wheat BAC sequences to fully-assembled and annotated members of the *Poaceae* family (rice, *Brachypodium*, sorghum, and maize) resulted in identification of orthologous regions in each species. A summary of the orthologous regions in the *Poaceae* is contained in Appendix IV and shown in Figure 3.2. In maize, the region was found to be present in duplicate, on chromosomes 1 (*Zm*-1) and 5 (*Zm*-5), while only a single copy was present in the other *Poaceae*. In the text, orthologues will be referenced by the wheat locus name preceded by the first letters of the genus and species they are derived from (e.g. *OsRht-1* refers to the rice orthologue of *Rht-1* and *Zm*-1*ZnF* refers to the maize orthologue of *ZnF* found on chromosome 1). The *ZnF-DUF6-Rht-1* synteny was retained in all orthologues, with the exception of *Zm*-1, in which *DUF6* is absent. In rice, an expressed hypothetical gene (TIGR rice locus LOC_*Os03g49960*) is located between *OsDUF6* and *OsRht-1* on the reverse strand. The distance encompassing the *ZnF-DUF6-Rht-1* linkage group varies widely among these species. In *Brachypodium*, the genes are contained within 38 kb of sequence, while on *Zm*-1 the genes exist in a space of 191 kb, with most of this

difference due to a vast increase in the amount of intergenic sequence between *Zm*-1*ZnF* and *Zm*-1*DUF6* relative to the other species.

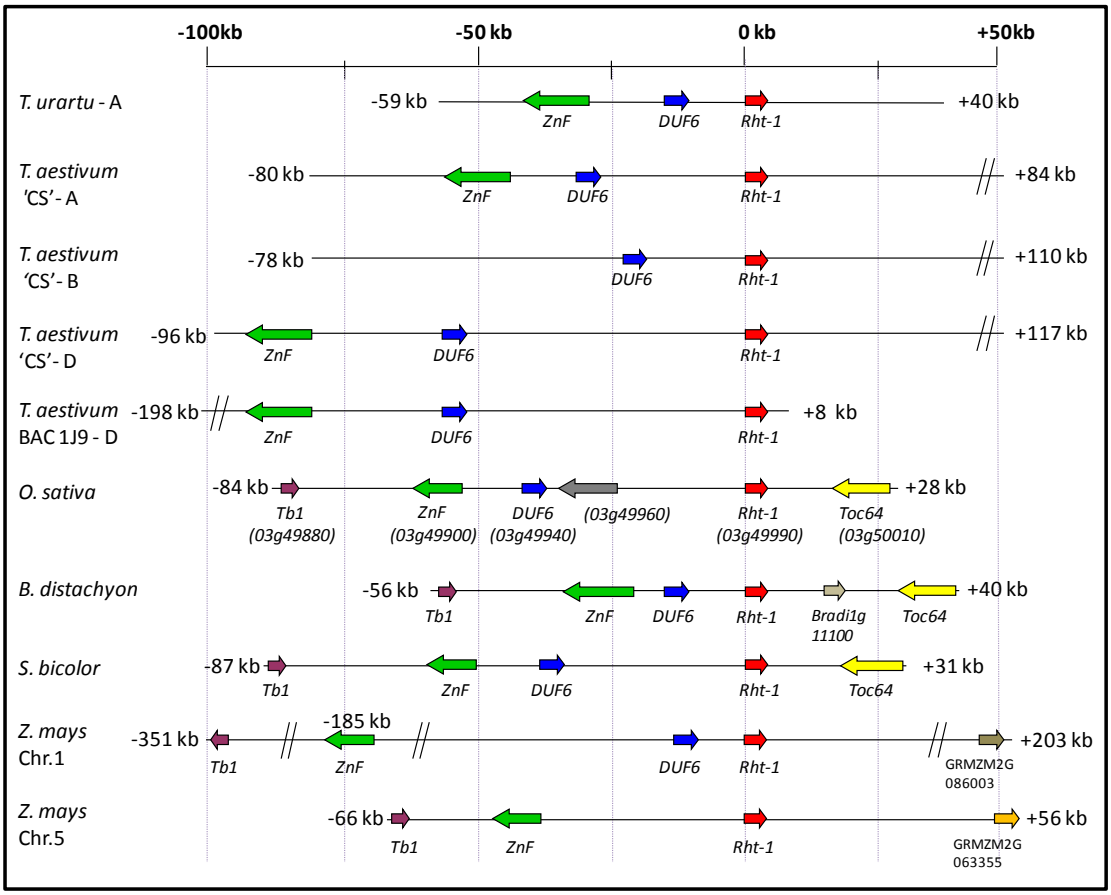


Figure 3.2. Summary of microsynteny in the *Rht-1* region. The five *Rht-1*-containing wheat BACs are shown in alignment with orthologous regions of four additional species of the *Poaceae* family. The species and genome derivation are indicated to the left of the panel. For wheat, the entire BAC insert sequences are depicted. For the remaining *Poaceae* species, the orthologous region is shown and extended to include the genes adjacent to the *ZnF* and *Rht-1* orthologues. The solid lines denote the sequence lengths, the presence of // within these lines denotes a foreshortened depiction of the sequence length relative to the scale in kb. The length preceding (-kb) and following (+kb) *Rht-1* is indicated at the beginning and end of each sequence. Genes are depicted as solid arrows that are oriented according to DNA strand (forward or reverse). Orthologous genes are colour-coded across species with the abbreviated wheat locus name beneath. For rice orthologues, the locus names (LOC_*Os*) are shown in parentheses. Loci without an orthologue in this region are named according to species convention. Nucleotide coordinates (NCs) and additional details of the genes are provided in Appendix IV.

To determine which loci in wheat are most likely 5' of *ZnF* and 3' of *Rht-1*, co-linear regions of the sequenced genomes of the *Poaceae* family were examined. The next gene upstream of *ZnF* in all species is a *Tb1* orthologue. The *Tb1* orthologues are all a single exon consisting of 1080 to 1167 bp and are oriented in the same direction as *Rht-1*, except the *Zm*-1 orthologue, which is on the opposite strand. The distance between *Tb1* and *Rht-1* is less than 90 kb in all orthologous regions, except for *Zm*-1, where the intergenic space in substantially expanded to 350 kb. A *Tb1* orthologue is not present on any of the wheat BAC inserts even though one of the sequences (BAC 1J9) extends 198 kb upstream of *Rht-1* and 108 kb upstream of *ZnF*.

In rice and sorghum the nearest gene downstream from *Rht-1* is a *Toc64*-like gene. In *Brachypodium*, *Toc64* also exists downstream of *Rht-1*, but the two genes are separated by a predicted gene, *Bradi1g11100*. In maize, no similarity was found to *Toc64* or to *Bradi1g11000* in the region near *Rht-1*. In rice, *Brachypodium*, and sorghum, *Toc64* is composed of 13 exons with ORFs containing between 1761 and 1767 nucleotides. In these three species, *Toc64* is within 40 kb of *Rht-1*, but is located on the opposite strand. The *Toc64* and *Bradi1g11000* genes are not present on the CS wheat BAC clones even though the BAC inserts each have at least 84 and up to 117 kb of sequence downstream of *Rht-1*.

3.3.2.1. Comparative analysis of the *Rht-1* ORF in wheat and *Poaceae*

Rht-1 in CS is composed of a single exon with lengths of 1863 (620 residues) in *Rht-A1*, 1866 (621 residues) in *Rht-B1*, and 1872 bp (623 residues) in *Rht-D1* (Table 3.2). The coding sequences of *Rht-A1*, *Rht-B1*, and *Rht-D1* in the CS background are highly conserved with 94.1% of the nucleotide identities and 96.8% of the amino acid identities shared among the three homoeologues. Of the three CS *Rht-1* homoeologous nucleotide sequences, the *Rht-A1a* and *Rht-D1a* sequences are the most similar, having 96.4% identity (differing by 40 SNPs and the need to insert 27 gaps for alignment over 1875 sites), followed by *Rht-A1a* and *Rht-B1a* (96.0% identity; differing by 61 SNPs and 15 gaps over 1878 sites), and *Rht-B1a* and *Rht-D1a* are the

most dissimilar (95.6% identity; differing by 56 SNPs and 26 gaps over 1882 sites). Among the homoeologous peptide sequences, *Rht-B1a* and *Rht-D1a* have the most residue differences at 16 (8 amino acid changes and 8 gaps; 97.4% identity) while *Rht-B1a* and *Rht-A1a* differ by 12 residues (5 amino acid changes and 7 gaps; 98.1% identity) and *Rht-A1a* and *Rht-D1a* differ by 12 residues (7 amino acid changes and 5 gaps; 98.1% identity). Only one amino acid substitution among the homoeologues (Figure 3.3, coord. 273) was found in the conserved domains described by Tian *et al.* (2004), with the substitution occurring in the Leucine Heptad Repeat I (LHR I) domain of the DELLA-specific N-terminal region. Of the remaining amino acid polymorphisms, 17 occur outside of the defined domains of the N-terminal region and two occur outside of the defined domains of the GRAS C-terminal region. Alignment of the *Rht-A1* ORF nucleotide and amino acid sequences from CS and *T. urartu* revealed a total of five SNPs (99.7% similarity) and no predicted amino acid changes (Table 3.2).

Table 3.2. Relatedness of *Rht-1* orthologues in wheat and *Poaceae* species.

		Nucleotide \ Amino acid identitities (%)[a]									
Locus Name[b]	Species [c]	*Ta*-A	*Ta*-B	*Ta*-D	*Tu*	*Hv*	*Bd*	*Os*	*Sb*	*Zm*-1	*Zm*-5
Rht-A1 (CS)	*Ta*-A		98.1	98.1	100.0	94.9	89.1	85.1	83.5	84.9	83.7
Rht-B1 (CS)	*Ta*-B	96.0		97.4	98.1	95.5	88.5	85.3	82.9	84.0	82.4
Rht-D1 (CS)	*Ta*-D	96.4	95.6		98.1	93.9	88.5	84.7	82.7	84.2	82.9
Rht-A1	*Tu*	99.7	95.8	96.7		94.9	89.1	85.1	83.5	84.9	83.7
SLN1	*Hv*	93.2	93.4	92.6	93.1		88.1	85.6	82.7	84.5	83.6
Bradi1g11090	*Bd*	88.0	87.7	87.5	88.0	87.0		86.5	85.9	87.5	83.6
Os03g49990	*Os*	86.2	85.8	85.5	86.1	85.4	85.7		84.3	85.2	83.5
Sb01g010660	*Sb*	84.7	84.5	84.6	84.8	84.2	85.1	85.1		93.8	90.4
GRMZM2G144744	*Zm*-1	84.9	84.5	84.6	85.0	84.1	85.2	85.2	93.6		92.2
GRMZM2G024973	*Zm*-5	85.2	84.7	84.9	85.2	84.5	84.2	84.7	90.6	92.0	
Nucleotides (no.)		1863	1866	1872	1863	1857	1872	1878	1884	1893	1878
Amino Acids (no.)		620	621	623	620	618	623	625	627	630	625

[a] Percentage of nucleotide and amino acid identities shared between orthologues is shown below and above the slash in the grid, respectively. Background colour of cells in the grid is based on % identity: red (≥95%); blue (90-94.9%); yellow (85-89.9%); white (≤85%).

[b] CS = Chinese Spring; Locus pseudonyms: *Os03g49990 = SLR1; GRMZM2G144744 = D8; GRMZM2G024973 = D9*.

[c] Species names are shown below and to the right of the header. *Ta*-A, *Ta*-B, and *Ta*-D = the A, B, and D genomes of *Triticum aestivum*, respectively; *Tu* = *Triticum urartu*; *Hv* = *Hordeum vulgare*; *Bd* = *Brachypodium distachyon*; *Os* = *Oryza sativa*; *Sb* = *Sorghum bicolor*; *Zm*-1 = *Zea mays* chromosome 1 (B73 inbred); *Zm*-5 = *Zea mays* chromosome 5 (B73 inbred).

Nucleotide and amino acid sequences of the *Rht-1* orthologues from barley (*HvRht-1*), *Brachypodium (BdRht-1)*, rice (*OsRht-1*), sorghum (*SbRht-1*), maize chromosome 1 (*Zm*-1*Rht-1*), and maize chromosome 5 (*Zm*-5*Rht-1*) were compared to the wheat *Rht-1* loci from Chinese Spring and *T. urartu* (Table 3.2; Figure 3.3). The ORFs of all *Rht-1* orthologues are composed of a single exon with lengths ranging from 1857 to 1893 nucleotides (618 to 630 amino acids). Among the *Rht-1* ORFs, 68.5% of the nucleotides and 70.9% of the amino acid residues are shared among all of the orthologues with greater conservation occurring in the GRAS carboxyl termini than in the DELLA-specific N-terminal regions. There are no frameshifts or large indels in any of the *Rht-1* proteins and the amino acids encoding the defined protein domains (Tian *et al.*, 2004) are highly conserved. The majority of the *Rht-1* amino acid sequence divergences occur in three locations: (1) a 32 bp region upstream of the DELLA domain (Figure 3.3, coords. 14-45), (2) a 112 bp region (coords. 129-240), in-between the TVHYNP and LHR I domains, and (3) a 25 bp region between the FY and RE domains (coords. 526-550). Relative to the three CS *Rht-1* homoeologues, *HvRht-1* was the most similar of the non-wheat sequences with 93.1% nucleotide and 94.8% amino acid identity when averaged over homoeologue comparisons (Table 3.2). The rank of nucleotide similarity among *Rht-1* orthologues when averaged over the three CS homoeologues is *Hv* > *Bd* > *Os* > *Zm*-5 > *Zm*-1 > *Sb* and amino acid similarity rank is *Hv* > *Bd* > *Os* > *Zm*-1 > *Sb* = *Zm*-5. A phylogenetic comparison of nucleotide sequences depicts this ranking and also show that the wheat *Rht-1* sequences are more closely related to *HvRht-1* than any other orthologue (Figure 3.4).

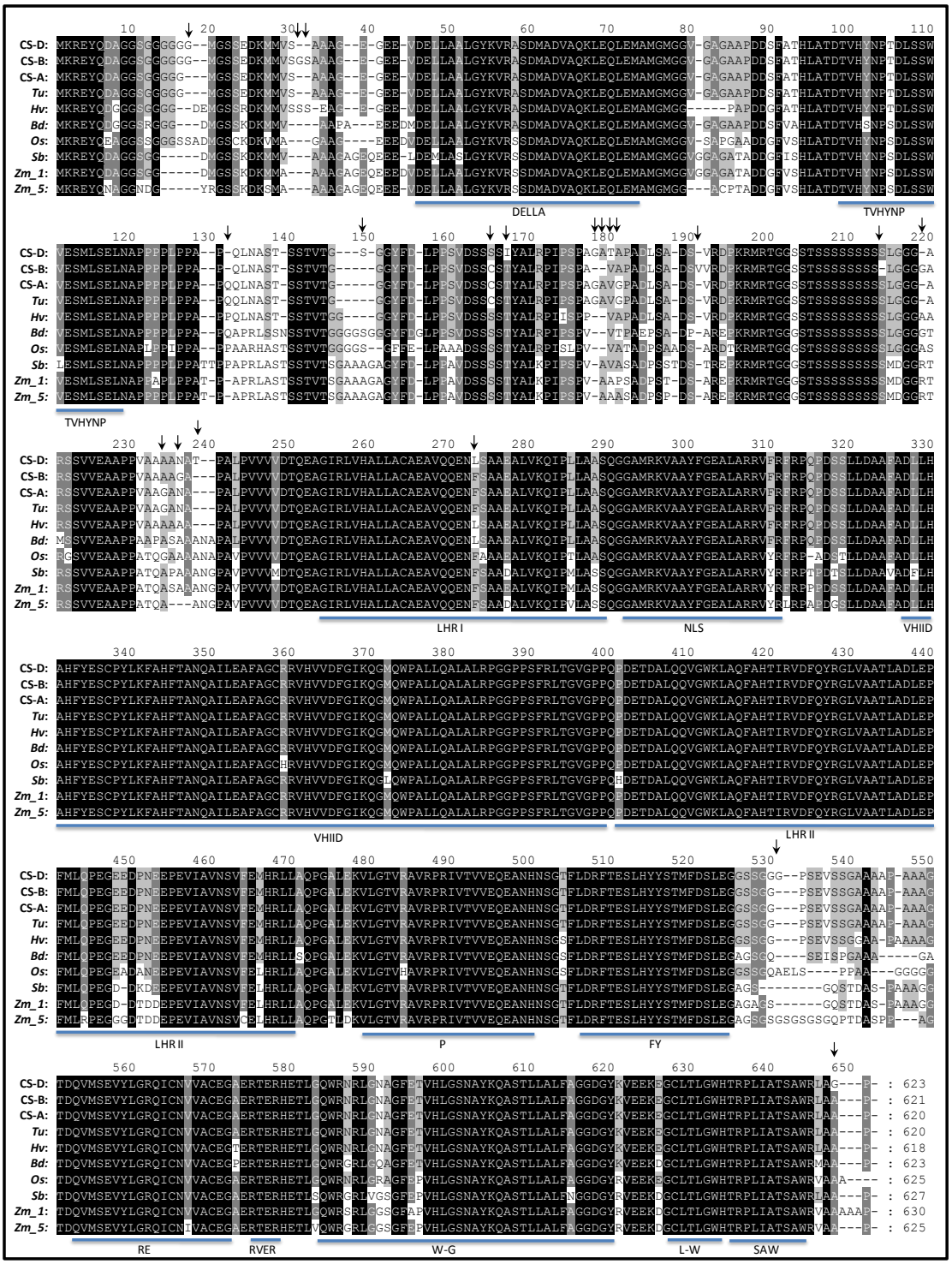


Figure 3.3. Peptide sequences of *Rht-1* from wheat and *Poaceae* orthologues. Amino acid sequences of three Chinese Spring wheat homoelogues of *Rht-1* (CS-D, CS-B, and CS-A), and orthologues in *T. urartu* (*Tu*), *Hordeum vulgare* (*Hv*), *Brachypodium distachyon* (*Bd*), *Oryza sativa* (*Os*), *Sorghum bicolor* (*Sb*), chromosome 1 of *Zea mays* (*Zm*_1), and chromosome 5 of *Zea mays* (*Zm*_5) were aligned using ClustalX and adjusted manually to minimise mismatches. Gaps inserted to allow for alignment are indicated by a (-). Residue conservation levels among the 10 sequences is shown by the following shading and font colours: Absolute sequence identity = white font with black background; Identity shared by 8

to 9 orthologues = white font with dark grey background; Identity shared by 6 to 7 orthologues = black font with light grey background; Identity shared by 5 or less orthologues = black font with white background. Coordinates are shown above the sequence for referral in the text, but because of the introduction of gaps to facilitate alignment they do not correspond to any one sequence. Total peptide length is shown following the last residue in each sequence. Arrows indicate differences among the three CS homoeologues. Previously established protein motifs (Tian *et al.*, 2004) are underlined in blue and with names below. The DELLA-specific N-terminal region includes coords. 1 to 320 and the GRAS carboxyl terminus includes coords. 321 to 652. Accessions used for each *Rht-1* orthologue are shown in Appendix IV.

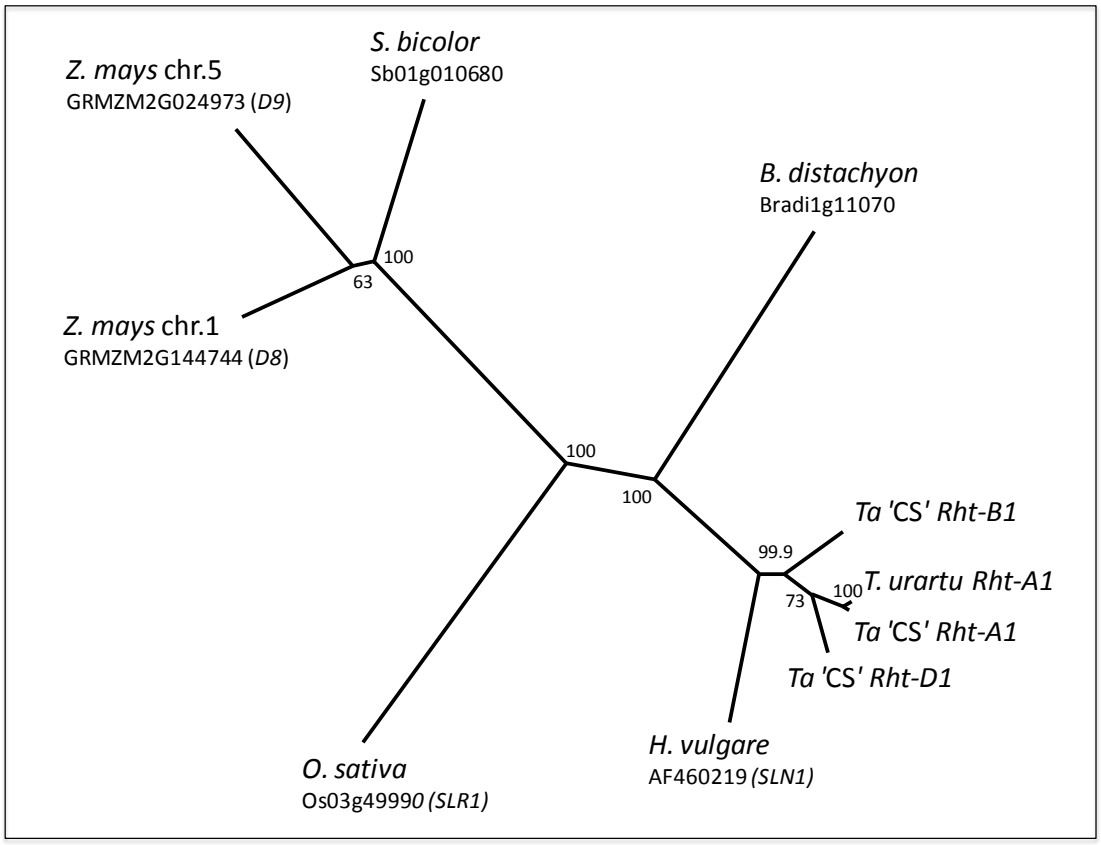


Figure 3.4. Unrooted neighbor-joining tree of wheat *Rht-1* and *Poaceae* orthologues. Nucleotide sequences of the *Rht-1* ORFs were aligned with ClustalX and bootstrap values and genetic distances determined using the seqboot application and neighbour joining method of PHYLIP (v3.6). *Ta* 'CS' *Rht-A1*, *Ta* 'CS' *Rht-B1*, and *Ta* 'CS' *Rht-D1* are, respectively, the A, B, and D homoeologues of the *Rht-1* gene identified in the Chinese Spring wheat BAC library. *T. urartu Rht-A1* sequence is also from a BAC clone. The remaining sequences are shown by species name with locus or genbank accession number below. If a species-specific *Rht-1* pseudonym exists, it is shown in parenthesis. Chromosome number containing the *Rht-1* orthologue is also given for maize. Branch lengths indicate relative genetic distance. Bootstrap values per 100 trees are given at each node if available.

3.3.2.2. Comparative analysis of the *Rht-1* flanking region (10 kb 5' and 10kb 3') in wheat and *Poaceae*

Sequence 5' and 3' of *Rht-1* was examined to determine the degree of similarity among the three CS wheat genomes. It was hypothesised that regions of high conservation close to the ORF could reflect regulatory regions for expression of *Rht-1*. Comparison of the *Rht-1* regions of the three CS genomes showed that the 5' region beyond approximately 10 kb upstream of the ORF (9.6 kb 5' of *Rht-A1*, 8 kb 5' of *Rht-B1*, and 11.1 kb 5' of *Rht-D1*) had high similarity to TEs. TE sequences are unlikely to be regulatory regions for *Rht-1* function, and therefore comparative analysis was restricted to the region from 0 to 10 kb upstream of the ORF. CS BAC sequences from 0 to 10 kb upstream of *Rht-A1, Rht-B1, and Rht-D1* were compared using the NCBI BLAST alignment tool and regions of similarity were defined as those with hits ≥ 100 and nucleotide identity greater than 80% (Figure 3.5). The CS-A and CS-D regions 5' of *Rht-1* were the most alike, with 61% of the sequences defined as similar in the 10 kb interval. In contrast, the percentage of the 5' region defined as similar between CS-B and CS-D is 25% and between CS-B and CS-A is 22%. Sequence similarity shared among all three CS genomes was 21% from ten hits ranging from 69 to 651 bp in length (Table 3.3). Two-thirds of the sequence conserved among the three homoeologues is located between 793 and 2251 bp upstream of *Rht-B1*.

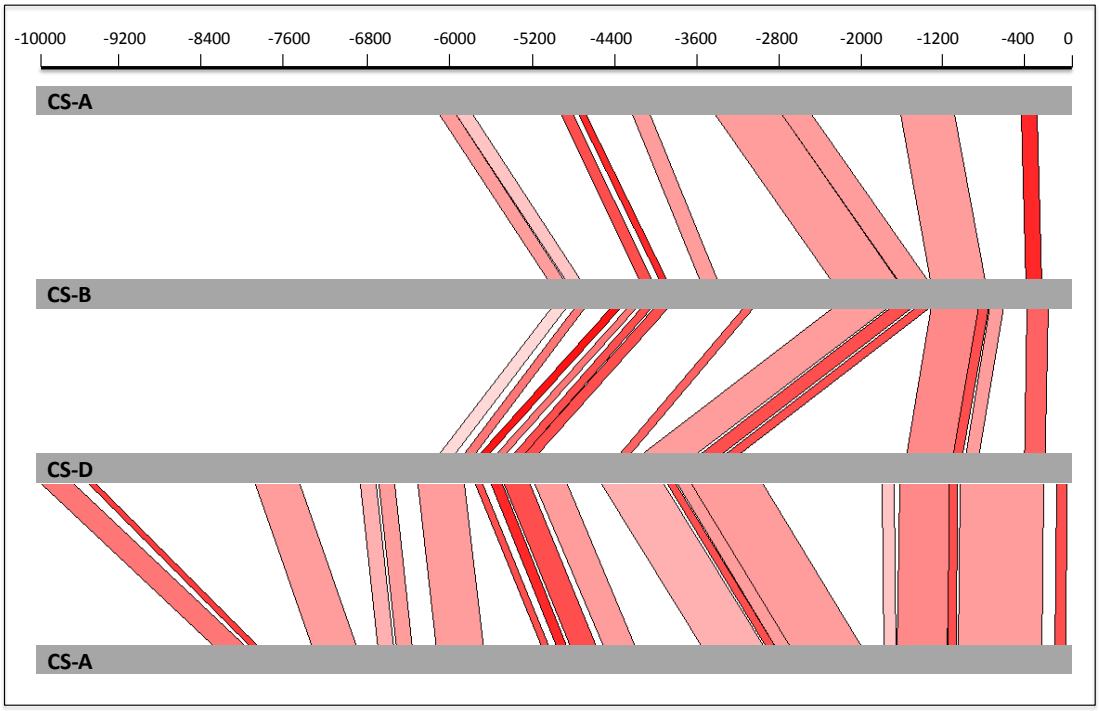


Figure 3.5. Sequence similarity 5' of *Rht-1* on the Chinese Spring (CS) A, B, and D genomes. Sequences from 0 kb to 10 kb upstream of *Rht-1* from each genome were compared using ACT (Artemis Comparison Tool) software (Carver *et al.*, 2005) and comparison files created with Double ACT v2 (http://www.hpa-bioinfotools.org.uk/pise/double_act.html). Genomic regions from 10,000 bp upstream (-10,000) up to the first nucleotide of the translational start codon (0) of *Rht-1a* are shown as grey bars. Regions with BLAST scores ≥100 and similarity > 80% are connected by red rectangles; the width of the rectangle represents the length of the hit and colour intensity is correlated to percent sequence similarity, with darker red corresponding to higher percentage similarity.

Table 3.3. Summary of 5' regions within 10 kb of *Rht-1* that are conserved among the Chinese Spring (CS) A, B and D genomes.

		CS-A		CS-B		CS-D	
BLAST hit[a]	Length (bp)	Start	Finish	Start	Finish	Start	Finish
1	156	-6083	-5931	-4991	-4844	-6280	-6124
2	101	-5854	-5767	-4785	-4687	-6058	-5957
3	100	-4907	-4807	-4123	-4015	-5581	-5481
4	69	-4736	-4667	-3931	-3862	-5423	-5354
5	651	-3412	-2770	-2251	-1619	-4442	-3791
6	283	-2772	-2482	-1608	-1320	-3780	-3497
7	509	-1618	-1092	-1318	-793	-1592	-1083
8	70	-694	-623	-593	-524	-676	-607
9	152	-450	-298	-400	-249	-431	-279

[a] Sequences from 0 kb to 10 kb upstream of *Rht-1* from each genome were compared using the NCBI BLAST alignment tool to identify regions of similarity shared among the three homoeologues. BLAST hits and lengths of conserved regions are listed from 5' to 3'.

Conserved regions were designated as having BLAST scores ≥100 and sequence similarity > 80%. The coordinates of the hits are shown for each of the genomes relative to the start nucleotide of *Rht-1*. Negative numbers refer to the distance upstream of *Rht-1*.

Nucleotide sequence comparison of the region from 0 to 10 kb 3' of *Rht-1* among the three CS BACs (using the same BLAST score criteria as for the 5' region) revealed high sequence similarity up to 4.5 kb downstream of the stop codon of *Rht-1* (Figure 3.6). Beyond this, similarity breaks down between any two genomes, with the exception of approximately 500 bp of similarity between the A and D genomes. Beyond roughly 6 kb 3' of *Rht-1* (7.4 kb, 6.1 kb, and 5.9 kb in the A, B, and D genomes, respectively), the majority of sequence has high similarity to known TEs. Among the three genomes, 27% of the 0 to 10 kb downstream sequence is defined as similar and all this occurs with 4 kb of the end of *Rht-1* (Table 3.4).

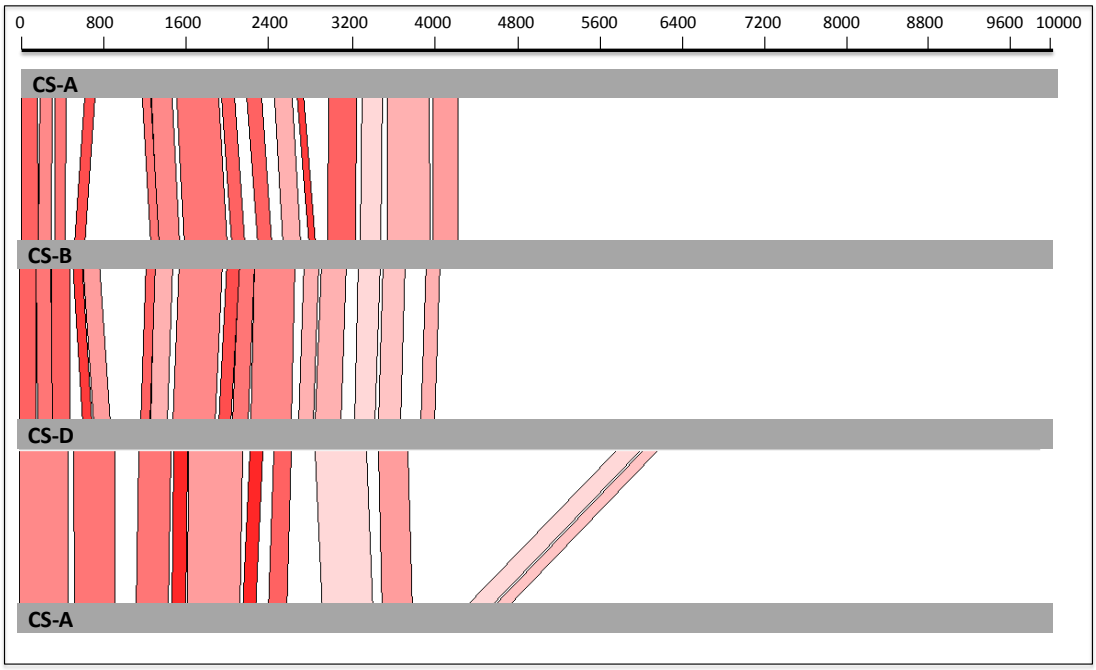


Figure 3.6. Sequence similarity 3' of *Rht-1* on the Chinese Spring (CS) A, B, and D genomes. Sequences from 0 kb to 10 kb downstream of *Rht-1* from each genome were compared using ACT (Artemis Comparison Tool) software (Carver *et al.*, 2005) and comparison files created with Double ACT v2 (http://www.hpa-bioinfotools.org.uk/pise/double_act.html). Genomic regions from the end nucleotide of the *Rht-1* ORF (0) to 10,000 bp downstream (10,000) are shown as grey bars. Regions with BLAST scores ≥100 and similarity > 80% are connected by red rectangles; the width of the

rectangle represents the length of the hit and colour intensity is correlated to percent sequence similarity, with darker red corresponding to higher percentage similarity.

Table 3.4. Summary of 3' regions within 10 kb of *Rht-1* that are conserved among the Chinese Spring (CS) A, B and D genomes.

		CS-A		CS-B		CS-D	
BLAST hit[a]	Length (bp)	Start	Finish	Start	Finish	Start	Finish
1	481	1	483	2	483	3	484
2	289	623	895	515	789	616	905
3	298	1161	1455	1247	1538	1192	1490
4	135	1495	1631	1581	1717	1513	1648
5	416	1649	2057	1739	2165	1664	2080
6	120	2194	2314	2307	2424	2267	2387
7	174	2440	2620	2537	2712	2496	2670
8	492	2978	3468	2965	3451	2905	3397
9	293	3563	3856	3567	3861	3521	3814

[a] Sequences from within 10 kb downstream of *Rht-1* from each genome were compared using the NCBI BLAST tool to identify regions of similarity shared among all three genomes. BLAST hits and lengths of conserved regions are listed from 5' to 3'. Conserved regions were designated as having BLAST scores ≥100 and sequence similarity >80%. Coordinates of the hits are shown for each of the genomes relative to the last nucleotide of *Rht-1*.

To determine whether regions upstream or downstream of *Rht-1* are conserved between the three CS wheat BAC sequences and the orthologous regions in rice and *Brachypodium*, comparisons were made using the NCBI BLAST alignment tool with conservation defined as BLAST hits ≥ 100 and nucleotide similarity ≥ 70. For each sequence the regions up to 10 kb upstream and up to 10 kb downstream of *Rht-1* (not including the ORF) were compared. Upstream of *Rht-1*, only one conserved region (Region 1, table 3.5) was identified among the five genomes. This region is approximately 120 bp in length and in each genome is located in the interval between 300 and 500 bp upstream of *Rht-1*. Using CS-D BAC sequence as a reference (arbitrarily chosen), nucleotide similarity of this upstream region with rice and *Brachypodium* was 84% and 92%, respectively. Downstream, two conserved regions (Regions 2 and 3, table 3.5) were identified among the five genomes. Region 2, the nearest downstream region, was approximately 100 bp in length and located between 300 and 450 bp 3' of the *Rht-1* stop codon in each sequence. Nucleotide similarities of region 2 relative to the CS-D reference sequence were 81% with rice and 84% with *Brachypodium*. Region 3 was

approximately 250 bp in size and located between 1.5 and 2 kb downstream of *Rht-1* in each genome. Nucleotide similarities for this region relative to the CS-D reference sequence were 70% for rice and 75% for *Brachypodium*.

Table 3.5. Sequence similarities among the CS wheat genomes, rice, and *Brachypodium* for the 5' and 3' regions within 10 kb of *Rht-1*.

		Coordinates[b]		BLAST Comparison[c]		
Region[a]	Genome	Start	End	Score	E-value	Similarity
1	CS-D	-435	-314	244	3×10^{-63}	100%
	CS-A	-452	-333	230	2×10^{-57}	98%
	CS-B	-400	-283	206	5×10^{-51}	94%
	Brachypodium	-430	-310	194	3×10^{-47}	92%
	rice	-474	-349	156	2×10^{-36}	84%
2	CS-D	328	428	202	5×10^{-52}	100%
	CS-A	327	432	172	7×10^{-42}	92%
	CS-B	327	427	176	6×10^{-43}	95%
	Brachypodium	238	305	108	5×10^{-24}	84%
	rice	256	353	100	3×10^{-21}	81%
3	CS-D	1627	1901	550	1×10^{-145}	100%
	CS-A	1610	1879	432	6×10^{-112}	91%
	CS-B	1696	1973	416	1×10^{-107}	89%
	Brachypodium	1612	1885	202	5×10^{-49}	75%
	rice	1456	1759	126	8×10^{-28}	70%

[a] Sequences from 0 kb to 10 kb upstream of *Rht-1* and from 0 to 10 kb downstream of *Rht-1* from each genome were compared using the NCBI BLAST tool to identify regions of similarity. Lengths of conserved regions are listed from 5' to 3'. Conserved regions were designated as having BLAST scores ≥100 and sequence similarity ≥70%.

[b] Coordinates of 5' hits are shown for each of the genomes relative to the start nucleotide of *Rht-1* with negative numbers referring to sequence upstream of *Rht-1*. Coordinates of 3' hits are shown relative to the end nucleotide of *Rht-1*.

[c] BLAST scores, expectation (E) values, and percentage of shared nucleotides (similarity) relative to CS-D are given. BLASTs of CS-D to itself are included for comparative purposes.

3.3.2.3. Comparative analysis of *ZnF* in wheat and *Poaceae*

A comparison of the *ZnF* genes present on the CS-A, CS-D, and *T. urartu* (A genome) BAC inserts reveals that the intron-exon structure is highly conserved among all four BACs with each *ZnF* gene occurring on the minus strand and consisting of 14 exons and 13 introns (Figure 3.7). The CS and 'Aibai/10*CS' *ZnF-D1* exon sequences are identical and the intron sequences differ by only 4 polymorphisms (3 SNPs and 1 bp deletion), so only CS *ZnF-D1* will be used in comparisons. The gene lengths (introns + exons) of the wheat *ZnF* genes vary widely with a 9032 bp size in *T. urartu,* a 9894 bp size in CS-A, and a 8298 bp size in CS-D,. All of the *ZnF* indels occur in the introns and the four largest indels, which range from 85 to 800 bp, account for almost all of the size differences found among the *ZnF* loci. The first indel (relative to the *ZnF* start codon) occurs in intron 4 and involves an 800 bp insertion on the two *ZnF-A1* sequences relative to *ZnF-D1*. A BLAST search of the inserted sequence against the NCBI nucleotide collection (nr/nt) and the TREP cereals repeat database revealed no matches with significant similarity. Also in intron 4 is a partial sequence (91 bp) of a Tantalos MITE (Miniature Inverted-*repeat* Transposable Element) on *ZnF-D1*. The second indel is an 87 bp insertion on CS *ZnF-A1* intron 5 of a Hades MITE (87 bp), which is degenerate. The third *ZnF* indel occurs in intron 6 where a full-length copy of the Thalos MITE (165bp) is present on the two *ZnF-A1* genes but absent on *ZnF-D1*. In the same region of intron 6, the *ZnF-D1* contains a full-length copy of the Athos MITE (85 bp). The fourth *ZnF* indel is a 770 bp insertion on *ZnF-A1* of CS that occurs between the Thalos MITE and exon 7. No matches were found to the 770bp sequence in the NCBI nucleotide collection (nr/nt) or TREP cereals repeat database. In contrast to the intronic Tantalos, Hades, and Thalos MITEs which differ between *ZnF-A1* and *ZnF-D1*, the Icarus MITE is conserved between these two homoeologues. The conservation of the intronic Icarus MITE also contrasts with the transposon composition among the intergenic regions of the CS *Rht*-containing BACs, in which no transposons were discovered that were conserved between any two homoeologues.

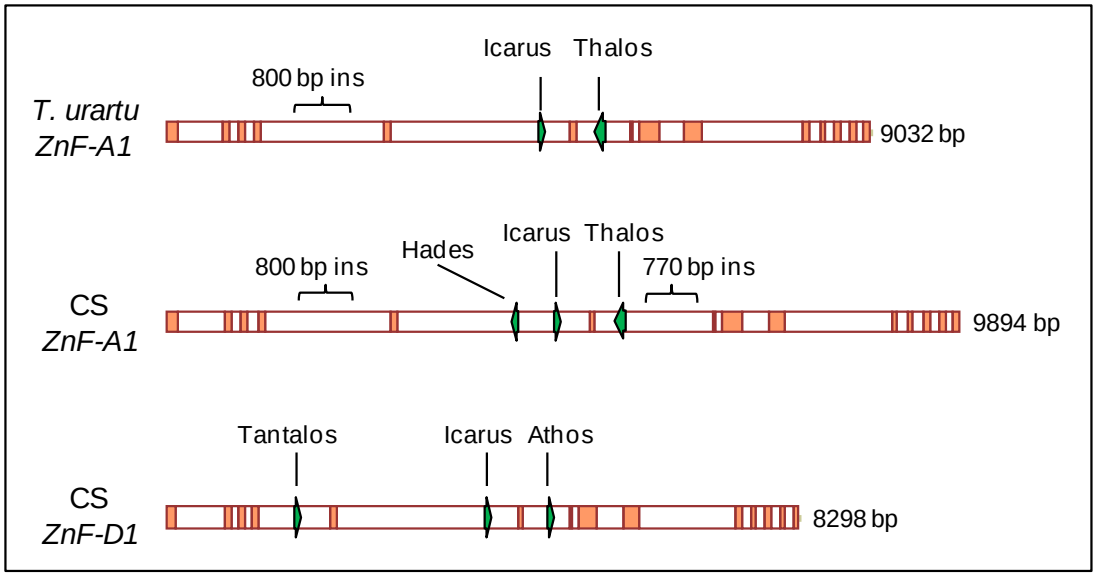


Figure 3.7. Diagrammatic representation of the wheat zinc-finger (*ZnF*) gene structure and intronic MITES. The genes (outlined in orange) from *T. urartu* and the A and D genomes of Chinese Spring (CS) are each located on the minus strand and are shown oriented from 5' to 3' in scale to one another with total length shown in basepairs (bp) at the end of each gene. Filled orange rectangles correspond to the 14 exons and unshaded regions represent the 13 introns present in each copy. MITES (Miniature Inverted-repeat Transposable Elements) are indicated by green arrows pointing in the direction of transcription relative to *ZnF* with names shown above. The locations of 770 bp and 800 bp insertions (ins) are also indicated.

The nucleotide and amino acid sequences of the ORFs of the *ZnF* wheat genes found on the four BAC sequences are also highly conserved and all ORFs are 1422 nucleotides (473 amino acids) in length (Table 3.6). The *ZnF* ORFs of the CS-A and CS-D homoeologues have 98.5% nucleotide identity (differing by only 21 SNPs) and 99.8% amino acid similarity (1 residue difference). The only predicted amino acid change between the CS-A and CS-D *ZnF* ORFs is a valine (*ZnF-A1*) to leucine (*ZnF-D1*) change (Figure 3.8, coord. 315) that occurs outside of the C3HC4 (RING) zinc finger type domain. The CS-A and *T. urartu ZnF-A1* sequences differ by 5 SNPs (99.6% identity) and the amino acid sequences are identical.

Table 3.6. Nucleotide and amino acid identities shared among *Poaceae ZnF* orthologues

		Nucleotide \ Amino acid identitities (%)[a]								
Locus Name[b]	Species[c]	*Ta*-A	*Ta*-D	*Tu*	*Hv*	*Bd*	*Os*	*Sb*	*Zm*-1	*Zm*-5
ZnF-A1 (CS)	*Ta*-A		99.8	100.0	99.4	95.1	90.9	92.4	92.4	92.0
ZnF-D1 (CS)	*Ta*-D	98.5		99.8	99.2	94.9	90.7	92.2	92.2	91.8
ZnF-A1	*Tu*	99.6	98.6		99.4	95.1	90.9	92.4	92.4	92.0
HvZnF	*Hv*	97.3	97.5	97.4		94.9	90.5	92.0	92.0	91.3
Bradi1g11070	*Bd*	92.5	92.3	92.3	92.5		90.5	92.6	93.0	91.8
Os03g49900	*Os*	88.5	88.4	88.5	88.5	88.8		94.9	94.1	93.2
Sb01g010680	*Sb*	88.5	88.4	88.5	88.1	89.5	89.1		98.3	96.8
GRMZM2G704032	*Zm*-1	88.2	87.8	88.3	88.0	88.8	89.0	97.0		97.3
GRMZM2G024690	*Zm*-5	88.0	88.0	88.0	87.9	88.3	88.6	96.0	96.1	
ORF nucleotides (no.)		1422	1422	1422	1422	1425	1422	1422	1422	1419
Amino Acids (no.)		473	473	473	473	474	473	473	473	472

[a] Percentage of nucleotide and amino acid identities shared between orthologues is shown below and above the slash in the grid, respectively. Background colour of cells in the grid is based on % identity: red (≥95%); blue (90-94.9%); yellow (85-89.9%).

[b] Locus names corresponding to each species. CS = Chinese Spring; *HvZnF* is the consensus sequence of expressed sequence tags (ESTs) TA38367_1353, TA38365_1353, and TA38366_1353.

[c] Species abbreviations are shown below and to the right of the header. *Ta*-A and *Ta*-D = the A and D genomes of *Triticum aestivum*, respectively; *Tu* = *Triticum urartu*; *Hv* = *Hordeum vulgare*; *Bd* = *Brachypodium distachyon*; *Os* = *Oryza sativa*; *Sb* = *Sorghum bicolor*; *Zm*-1 = *Zea mays* chromosome 1; *Zm*-5 = *Zea mays* chromosome 5.

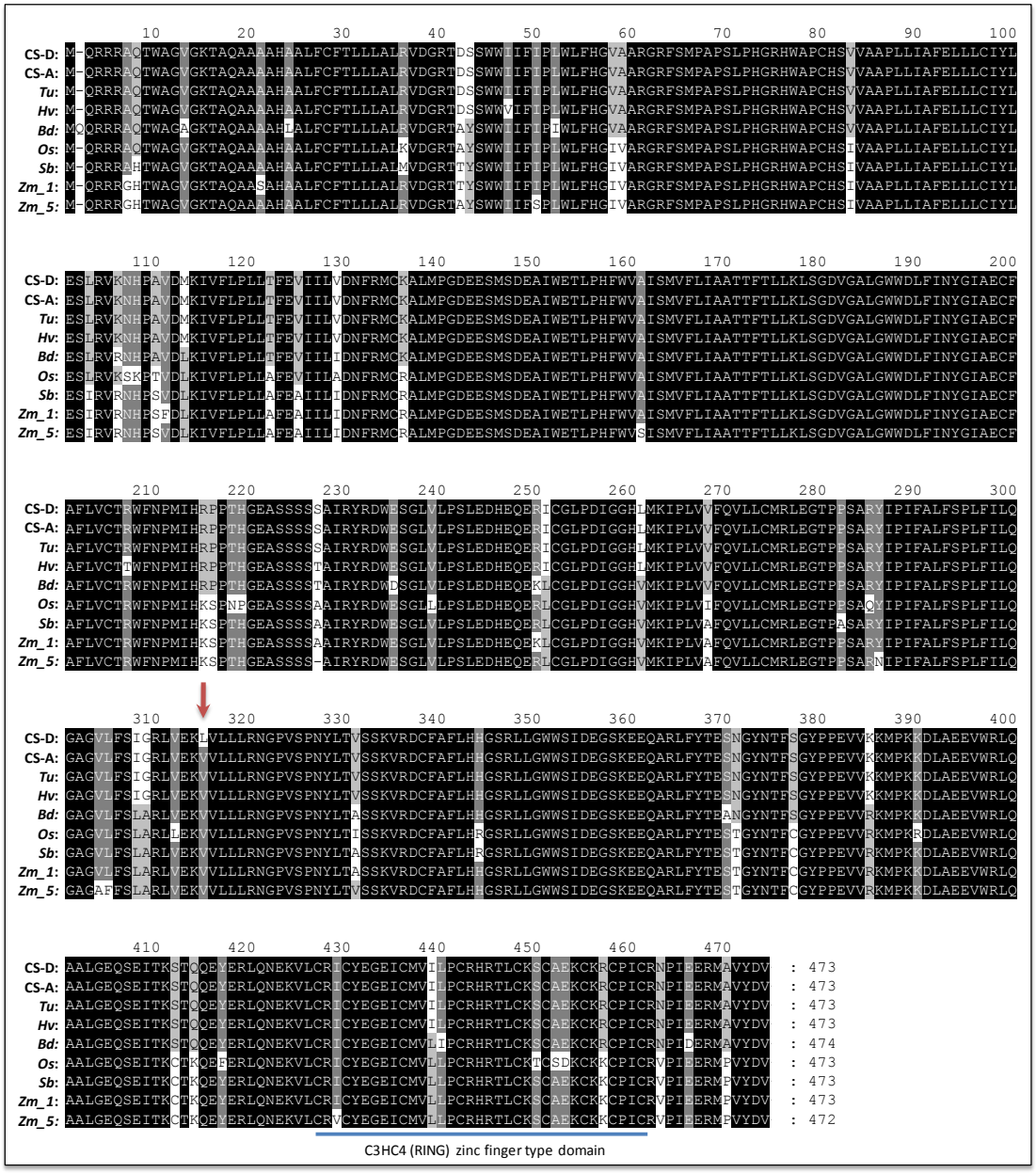


Figure 3.8. Peptide sequences of *ZnF* from wheat and *Poaceae* orthologues. Amino acid sequences of two Chinese Spring (CS) wheat homoelogues of *ZnF* (CS-D and CS-A) and orthologues in *T. urartu* (*Tu*), *Hordeum vulgare* (*Hv*), *Brachypodium distachyon* (*Bd*), *Oryza sativa* (*Os*), *Sorghum bicolor* (*Sb*), chromosome 1 of *Zea mays* (*Zm*_1), and chromosome 5 of *Zea mays* (*Zm*_5) were aligned using ClustalX and adjusted manually to minimise mismatches. Gaps inserted to allow for alignment are indicated by a (-). Residue conservation of the nine sequences is shown by the following shading and font colours: Absolute sequence identity = white font with black background; Identity shared by 7 to 8 orthologues = white font with dark grey background; Identity shared by 5 to 6 orthologues = black font with light grey background; Identity shared by 4 or less orthologues = black font with white background. Coordinates are shown above the sequence. Total peptide length is shown after each sequence.

The blue line indicates amino acids that encode the C3HC4 (RING) zinc finger type domain based on an InterProScan (http://www.ebi.ac.uk/Tools/InterProScan/) of the amino acid sequences. The red arrow at coordinate 315 indicates the lone amino acid substitution identified among the CS-A, CS-D, and *T.urartu* sequences. Accessions used for each orthologue are shown in Appendix IV.

Nucleotide and amino acid sequences of the *ZnF* orthologues from barley (*HvZnF*), *Brachypodium* (*BdZnF*), rice (*OsZnF*), sorghum (*SbZnF*), maize chromosome 1 (*Zm*-1*ZnF*), and maize chromosome 5 (*Zm*-1*ZnF*) were compared to the CS *ZnF-A1,* CS *ZnF-D1 and T. urartu ZnF-A1* sequences to determine the level of conservation (Table 3.6). All nine of the *ZnF* genes have 14 exons and 13 introns and the nucleotide lengths are in a narrow range of 1419 to 1425 bp (472 to 474 amino acids). The nine sequences share 78.0% nucleotide identity and 85.1% amino acid identity. Seven of the amino acids contained in the C3HC4 (RING) zinc finger domain are not wholly conserved across the species including three amino acid changes that only occur in rice (Figure 3.8, coords. 450, 452, and 453). Only the *HvZnF* amino acid sequence completely matches the three wheat sequences in the RING domain. Over the entire ORF, the *HvZnF* sequence is the most similar to the *T. aestivum* sequences, having 97.4% nucleotide identity and 99.3% amino acid identity when percent identities are averaged across the CS *ZnF* sequences. In relation to the wheat *ZnF* ORF nucleotide sequence, the order of similarity among the non-wheat species is *Hv* > *Bd* > *Os* = *Sb* > *Zm*-1 = *Zm*-5 and for the amino acid sequence the similarity to wheat ranking is *Hv* > *Bd* > *Sb* = *Zm*-1 > *Zm*-5 > *Os*. Phylogenetic analyses of the *ZnF* sequences showed a similar ranking of similarity with the *HvZnF* sequence being closest to the wheat sequences (Figure 3.9).

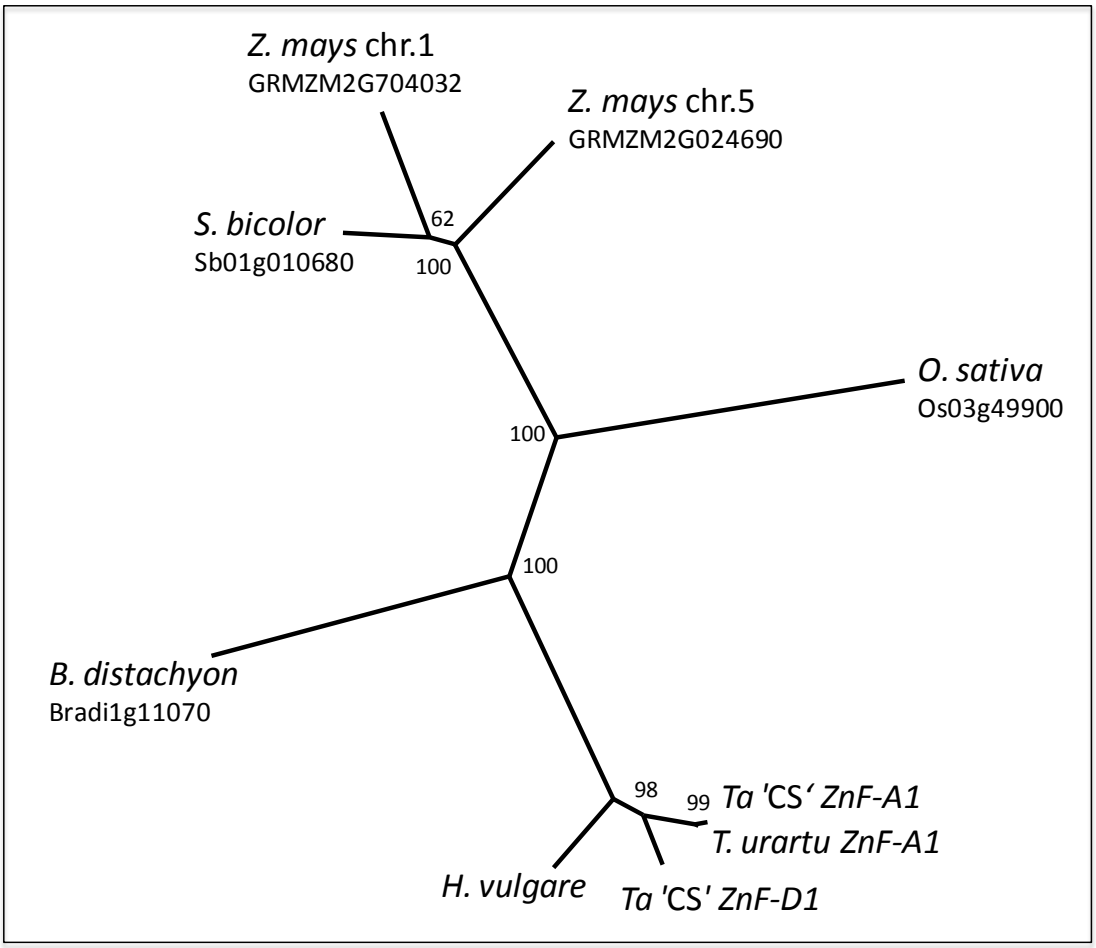


Figure 3.9. Unrooted neighbor-joining tree of wheat *ZnF* and *Poaceae* orthologues. Nucleotide sequences of the *ZnF* ORFs were aligned with ClustalX and bootstrap values and genetic distances determined using the seqboot application and neighbour joining method of PHYLIP (v3.6). *Ta* 'CS' *ZnF-A1* and *Ta* 'CS' *ZnF-D1* are, respectively, the A and D homoeologues of the *ZnF* gene identified from the wheat BAC library derived from Chinese Spring. *T. urartu ZnF-A1* is *ZnF* sequence from a BAC clone. *H. vulgare* sequence was assembled from TA38367_4513, TA38365_4513, and TA38366_4513. The remaining sequences are shown by species name with locus number below. The chromosome carrying the *ZnF* orthologue is indicated for maize. Branch lengths indicate relative genetic distance. Bootstrap values of 100 trees are given at each node if available.

3.3.2.4. Comparative analysis of *DUF6* among wheat BACs and *Poaceae*

The *DUF6* genes found upstream of *Rht-1* are present on the CS-A, CS-B, CS-D, and *T. urartu* (A genome derived) BAC sequences. On all the wheat BAC inserts, *DUF6* is on the plus strand and has 8 exons and 7 introns (Figure 3.10). The CS-D and 'Aibai/10*CS' *DUF6* intron and exon sequences are completely identical, so only CS-D is included in the comparative analysis.

The gene length (exons + introns) of CS *DUF6-B1* is 2382 bp, which is 192 bp shorter than both *DUF6-A1* genes and 181 bp shorter than CS *DUF6-D1*. The difference is primarily due to the presence of a 162 bp Thalos MITE in the first intron of *DUF6-A1* and *DUF6-D1* that is not present in *DUF6-B1*. Of the three CS homoeologues, *DUF6-A1* and *DUF6-D1* are the most similar having 96.9% nucleotide identity (41 SNPs) and 96.8% amino acid identity (14 substitutions) (Table 3.7). *DUF6-B1* and *DUF6-A1* have 95.9% nucleotide identity (42 SNPs and a 12 bp indel) and 95.2% amino acid identity (17 substitutions and a 4 amino acid indel). *DUF6-B1* and *DUF6-D1* have 96.5% nucleotide identity (34 SNPs and a 12 bp indel) and 94.8% amino acid identity (19 substitutions and a 4 amino acid indel). Among the three CS *DUF6* homoeologues, 94.7% of the nucleotide identities and 93.6% of the amino acid identities are conserved (Table 3.7). The most striking polymorphism between the CS *DUF6* ORFs is the 12 bp (4 amino acid) deletion that occurs in exon 1 of *DUF6-B1* (Figure 3.11, coords. 19 to 22), which results in *DUF6-B1* having a reduced gene length (1311 bp; 436 residues) relative to *DUF6-A1* or *DUF6-D1* (1323bp; 440 residues). The *DUF6-A1* genes from CS-A and *T. urartu* differ by 6 SNPs (99.5% nucleotide identity), which result in 3 amino acid substitutions (99.3% amino acid identity).

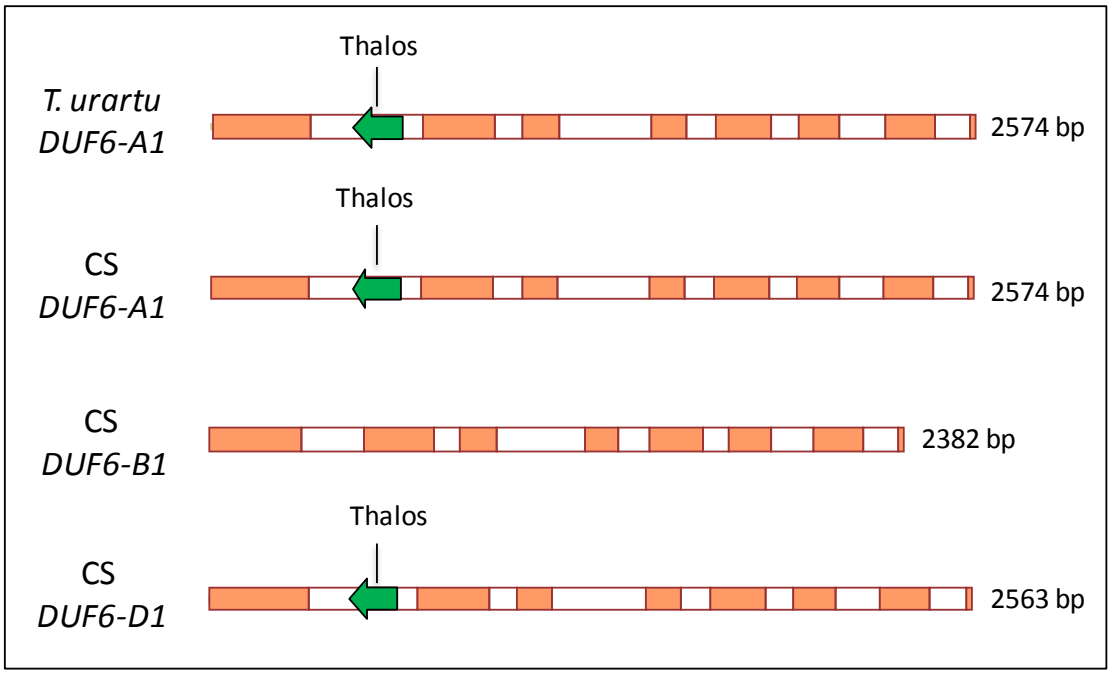


Figure 3.10. Diagrammatic representation of the wheat *DUF6* gene structure and intronic MITES. The genes (outlined in orange) from *T. urartu* and the A, B, and D genomes of Chinese Spring (CS) are in scale to one another with total length shown in basepairs (bp) at the end of each gene. Filled orange rectangles correspond to the 8 exons and unshaded regions represent the 7 introns present in each copy. MITES are shown as green arrows depicting their orientation with names above.

Table 3.7. Nucleotide and amino acid identities shared among *Poaceae DUF6* orthologues.

		Nucleotide \ Amino acid identitities (%)[a]								
Locus Name[b]	Species[c]	*Ta*-A	*Ta*-B	*Ta*-D	*Tu*	*Hv*	*Bd*	*Os*	*Sb*	*Zm*-1
DUF6-A1 (CS)	*Ta*-A		95.2	96.8	99.3	91.1	85.2	75.4	73.4	73.3
DUF6-B1 (CS)	*Ta*-B	95.9		94.8	94.5	92.3	85.2	75.8	74.4	74.0
DUF6-D1 (CS)	*Ta*-D	96.9	96.5		96.4	92.4	85.5	76.0	74.0	73.9
DUF6-A1	*Tu*	99.5	95.8	96.7		90.6	84.8	74.7	73.1	73.1
HvDUF6	*Hv*	92.5	94.0	93.6	92.4		83.8	74.2	73.6	71.9
Bradi1g11080	*Bd*	87.5	88.0	88.0	87.5	86.3		76.0	73.8	74.6
Os03g49940	*Os*	77.7	77.9	78.1	77.5	77.2	76.8		74.5	73.4
Sb01g010670	*Sb*	78.4	78.9	78.8	78.3	78.8	79.0	79.3		85.3
GRMZM2G093849	*Zm*-1	77.1	77.8	77.6	77.0	77.6	77.5	78.3	90.2	
ORF nucleotides (no.)		1323	1311	1323	1323	1308	1320	1365	1326	1344
Amino Acids (no.)		440	436	440	440	435	439	454	441	447

[a] Percentage of nucleotide and amino acid identities shared between orthologues is shown below and above the slash, respectively. Background colour of cells in the grid is based on % identity: red (≥95%); blue (90-94.9%); yellow (85-89.9%); white (≤85%).

[b] Locus names corresponding to each species. CS = Chinese Spring. The barley sequence is composed of two non-overlapping ESTs with an estimated gap of 165 bp. The predicted length of the barley coding sequence and the peptide residue number include the envisaged 165 bp, but these were excluded from identity calculations. *Z. mays* sequence is from chromosome 1 of inbred B73.

[c] Species abbreviations are shown below and to the right of the header. *Ta*-A, *Ta*-B and *Ta*-D = the A, B, and D genomes of *Triticum aestivum*, respectively; *Tu* = *Triticum urartu*; *Hv* = *Hordeum vulgare*; *Bd* = *Brachypodium distachyon*; *Os* = *Oryza sativa*; *Sb* = *Sorghum bicolor*; *Zm*-1 = *Zea mays* chromosome 1

The wheat BAC *DUF6* exon and intron sequences were also compared with orthologues in barley (*HvDUF6*), *Brachypodium (BdDUF6)*, rice (Os*DUF6*), sorghum (*SbDUF6*), and maize chromosome 1 (*Zm*-1*DUF6*) (Table 3.7). Unlike *Rht-1* and *ZnF*, no *DUF6* orthologue is present on maize chromosome 5. In barley, a consensus of EST sequences was made, but relative to the other alignments, there was a gap of an estimated 165 bp (approximately 13% of the *HvDUF6* sequence) that likely results from the absence of EST

sequence in the databases searched. The missing 165 nucleotides, along with three other missing nucleotides, (one in exon 2 and two in exon 4) were designated 'N' for 'unknown nucleotide' (IUPAC nomenclature) and the corresponding 59 ambiguous amino acids were designated 'X', for 'any amino acid' according to IUPAC nomenclature (Figure 3.11, coords. 144-199, 205, 256-257). Regions containing nucleotides designated 'N' or amino acids designated 'X' were not used in identity comparisons that included *HvDUF6*. Identity analysis that excluded *HvDUF6* showed that nucleotide and amino acid identities in this region are 4.6 and 7.8 percentage points higher, respectively, than in the overall sequence. Each of the *Poaceae DUF6* orthologues has 8 exons and 7 introns. The coding sequences range from 1308 nucleotides (the estimated number in *HvDUF6*) to 1365 nucleotides in Os*DUF6* (Table 3.7). The nine *Poaceae* sequences have 58.4% of the nucleotides and 56.5% of the amino acid identities in common. Among the non-wheat orthologues, *HvDUF6* is the most similar to the CS wheat *DUF6* sequences having 93.4% nucleotide and 91.9% amino acid similarity when averaged across the barley-CS *DUF6* homoeologue comparisons. The nucleotide similarity ranking of the *Poaceae DUF6* orthologues relative to the CS *DUF6* homoeoloci is *Hv* > *Bd* > *Sb* > *Os* > *Zm*-1 and the amino acid ranking is *Hv* > *Bd* > *Os* > *Sb* > *Zm*-1. The majority of the residue changes occur near the 5' end of the gene (Figure 3.11, coords. 10-100). In this divergent region, among all the DUF6 sequences, nucleotide identity is only 30% and residue identity is only 12.1% (11 of 91 amino acids conserved) relative to the remainder of the sequence in which nucleotide identity is 65.7% and amino acid identity is 67.4%. A phylogenetic tree of the *DUF6* orthologues shows wheat to be most closely related to barley (Figure 3.12). The phylogenetic tree also indicates that *BdDUF6* is the next closest sequence to wheat and barley.

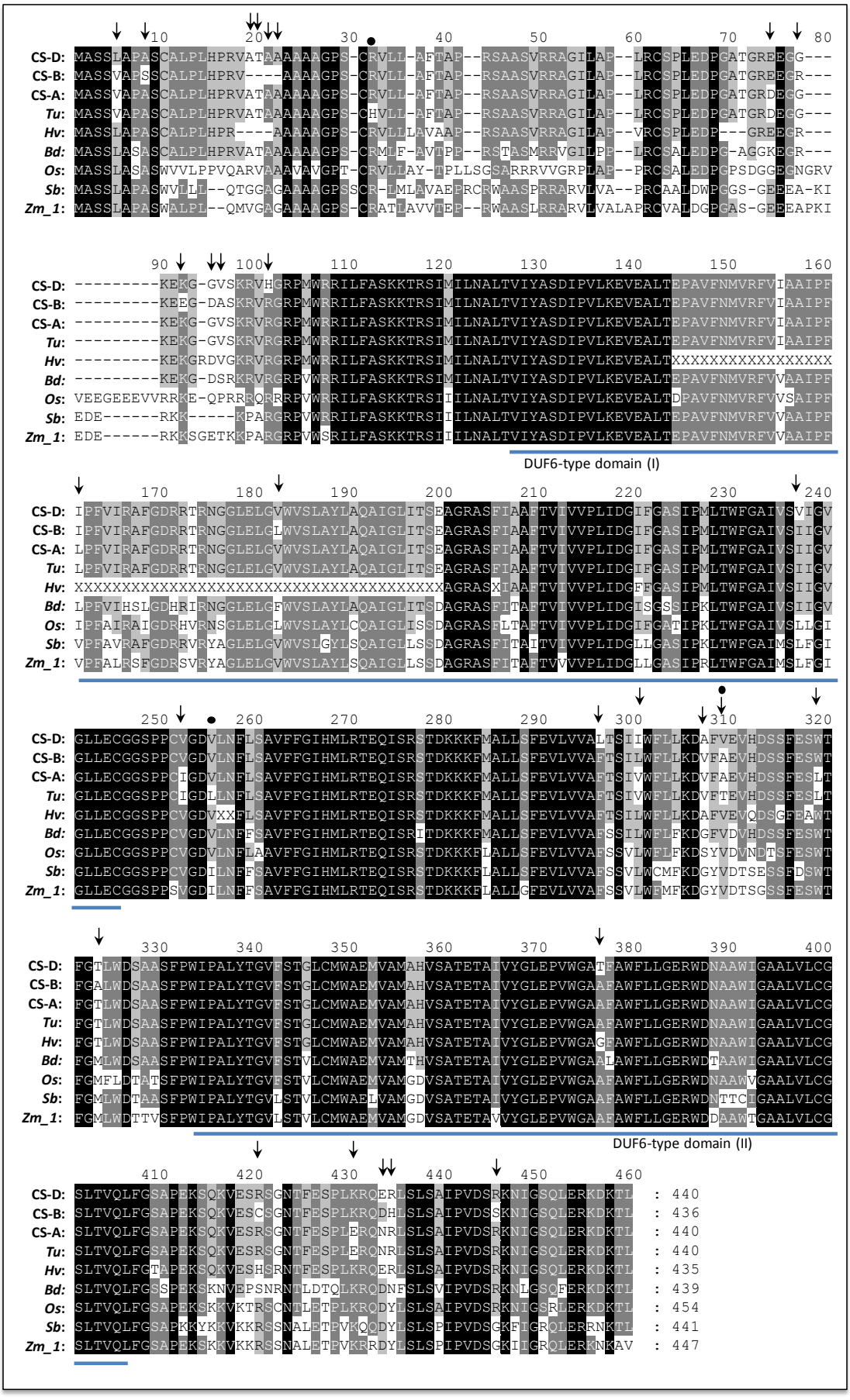

DUF6-type domain (I)
DUF6-type domain (II)

Figure 3.11. Alignment of predicted amino acid sequences of the CS *DUF6* homoeologues and *Poaceae* orthologues. Amino acid sequences of the three Chinese Spring wheat homoelogues of *DUF6* (CS-A, CS-B, and CS-D), and orthologues in *T. urartu* (*Tu*), *Hordeum vulgare* (*Hv*), *Brachypodium distachyon* (*Bd*), *Oryza sativa* (*Os*), *Sorghum bicolor* (*Sb*) and *Zea mays* from chromosome 1 (*Zm*_1) were aligned using ClustalX and adjusted manually to minimise mismatches. Gaps inserted to allow for alignment are indicated by a (-), or in the case of undetermined sequence in *Hv*, by (X). Residue conservation of the nine sequences is shown by the following shading and font colours: Absolute sequence identity = white font with black background; Identity shared by 7 to 8 orthologues = white font with dark grey background; Identity shared by 5 to 6 orthologues = black font with light grey background; Identity shared by 4 or less orthologues = black font with white background. However, this cannot be accurately assessed for coordinates 144-199, 205, 256, and 257 because of a lack of sequence information. Coordinates relative to the start of the ORF are shown above the sequence. The total number of residues per sequence is shown following the last residue. For *Hv*, residue number is estimated. The blue lines represents the amino acids that encode for the two *DUF6*-type domains (I and II) found in the gene as identified in an InterProScan (http://www.ebi.ac.uk/Tools/InterProScan/) of the amino acid sequences. Arrows above the sequences indicate residue differences between CS-A, CS-B, and CS-D homoeologues, Black dots show residue differences between CS-A and *Tu*. Accessions used for each *DUF6* orthologue are shown in Appendix IV.

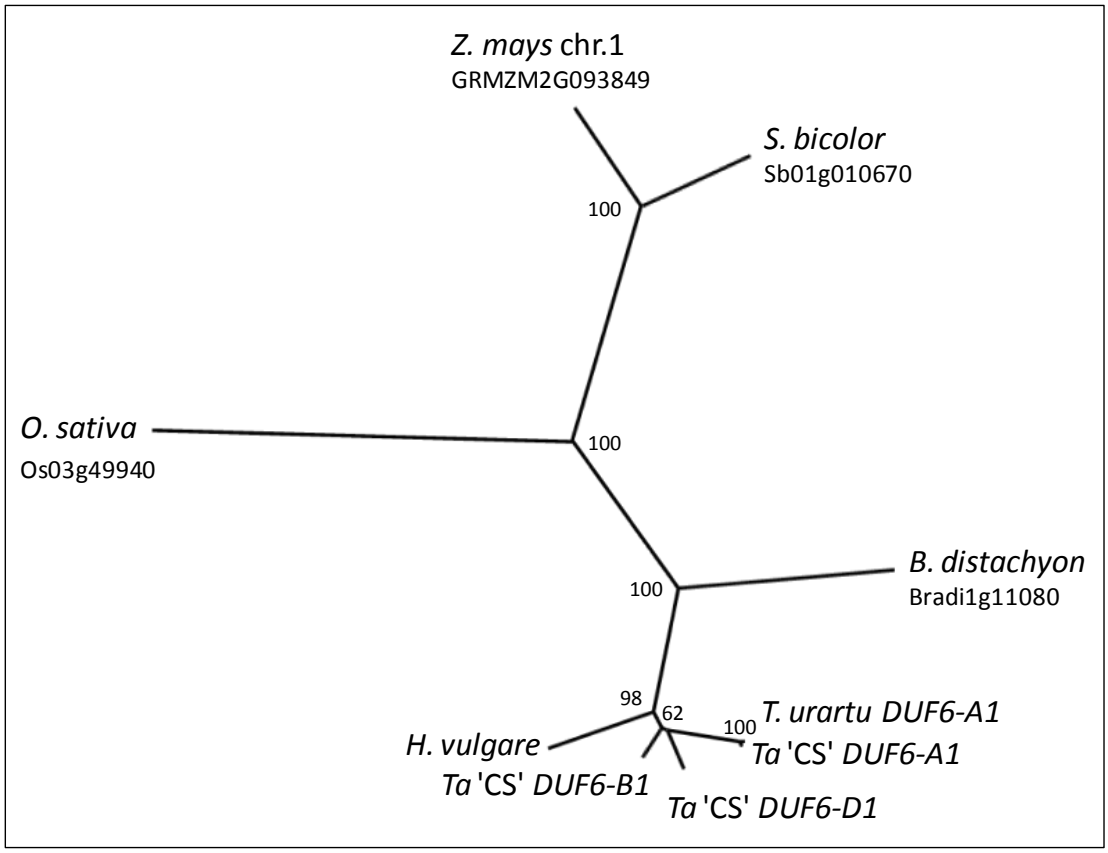


Figure 3.12. Unrooted neighbor-joining tree of wheat *DUF6* and *Poaceae* orthologues. Nucleotide sequences of the *DUF6* ORFs were aligned with ClustalX and bootstrap values and genetic distances determined using the seqboot application and neighbour joining method of PHYLIP (v3.6). *Ta* 'CS' *DUF6-A1*, *Ta* 'CS' *DUF6-B1* and *Ta* 'CS' *DUF6-D1* are, respectively, the A, B and D homoeologues of the *DUF6* gene identified from the wheat BAC library derived from Chinese Spring. *T. urartu DUF6-A1* sequence is from a BAC clone. The *H. vulgare* sequence is composed of two non-overlapping ESTs with an estimated gap of 165 bp, which were excluded when determining the genetic distance of *H. vulgare*. The remaining sequences are shown by species name with locus number below. The chromosome carrying the *DUF6* orthologue is indicated for maize. Branch lengths indicate relative genetic distance. Bootstrap values of 100 trees are given at each node if available.

3.3.3. Predicted genes on the CS wheat BAC sequences

In additon to the *ZnF*, *DUF6*, and *Rht-1* genes, one predicted gene was identified that could not be discounted due to homology with TEs. The predicted gene (a single exon) is located only on the CS-A BAC on the plus strand near the 3' end of a 14 kb stretch of low homology sequence (Figure 3.1b; Appendix IIIA, bp 129602 to 130183). Two nucleotide matches with E values more significant than 1×10^{-10} were found when the predicted ORF was queried to the sequence databases. The highest match ($E = 5 \times 10^{-26}$) is with wheat EST (wEST) CK209908 and the match extends over the full length of the predicted gene. CK209908 is annotated as a homeobox protein DLX-2 related cluster. Another full-length match ($E = 9 \times 10^{-23}$) is with wEST CK209889, which is a predicted signal transduction protein containing EAL and modified HD-GYP domains. No sequence with significant similarity to this predicted gene was found on any of the other BAC sequences.

3.4. DISCUSSION

Genetic analysis of *Rht-1*-containing wheat BAC sequences from the A, B, and D homoeologues of CS revealed a well-conserved gene synteny among the BAC inserts and little conservation of TEs (Figure 3.1b, c, d). A similar pattern of conservation was reported for the bread wheat acetyl-CoA carboxylase (*Acc*) homoeoloci (Chalupska *et al.*, 2008), the bread wheat

Hardness (*Ha*) homoeoloci (Ragupathy and Cloutier, 2008), and the high molecular weight glutenin (*Glu-1*) loci from *Triticum durum* (A and B genomes) and *Ae tauschii* (D genome) (Gu *et al.*, 2004). Combined, the *Rht-1*-containing CS BAC sequences were composed of approximately 62.2% TE, 2.2% ORF, and 35.6% unknown sequence (Table 3.1). The percentage of sequence that encodes genes is similar to previous reports for bread wheat chromosome 3B (1.2%; Paux *et al.*, 2006) and for *Ae. tauschii* (2.5%; Li *et al.*, 2004). Over the three CS BAC sequences, there is an average of 1 gene per 71 kb of sequence, which closely resembles the density of 1 gene per 75 kb estimated by Devos *et al.* (2005) in an analysis of four randomly chosen CS wheat BACs. The percentage of TE sequence on the CS-B BAC (63%) is lower than was calculated for known TEs on chromosome 3B (76%) by Paux *et al.* (2006) while the TE percentage of the CS-D BAC (66%) is similar to that calculated for *Ae. tauschii* (68%) by Li *et al.* (2004).

The CS-A and CS-D genome BAC inserts each contain (from 5' to 3') a zinc finger (*ZnF*) family gene, a domain of unknown function (*DUF6*) family gene, and *Rht-1* (Figure 3.1b, d). An additional predicted gene also exists on the CS-A genome, but no known *Poaceae* orthologues were found in the databases searched. The CS-B BAC insert contains the *DUF6-B1* and *Rht-B1* genes, but the *ZnF-B1* homoeologue was not present in the 54 kb of sequence upstream of *DUF6-B1* (Figure 3.1c). In contrast, the *ZnF-A1* gene is only 14 kb upstream of *DUF6-A1* and *ZnF-D1* gene is only 26 kb upstream of *DUF6-D1* in CS. The proliferation of TEs (most notably the WHAM LTR retrotransposon) in this region of the B genome relative to intergenic collinear regions on the A and D genomes appears to have expanded the region upstream of *DUF6-B1*, which provides the most likely explanation for the absence of *ZnF-B1* on the BAC insert. As an example of this expansion between genes, *DUF6-D1* and *Rht-D1* are separated by 53 kb while the A and B genome homoeologues of these two genes are only separated by 28 kb and 21 kb, respectively. Alternatively, it is also possible that synteny breaks down among the wheat homoeologues and *ZnF-B1* is not the next gene upstream on CS-B. Synteny of the genes in the *Rht-1* region is also

completely conserved in *T. urartu* and on the D genome of Aibai/10*CS (Figure 3.1a, e).

The *Rht-1* region is more conserved between the CS-A and *T. urartu* genomes than between CS-A and the CS-B or CS-D bread wheat homoeologues. The CS-A and *T. urartu* amino acid sequences of *Rht-A1* (Figure 3.3) and *ZnF-A1* (Figure 3.8) are identical while the *DUF6-A1* sequences (Figure 3.11) differ by only 3 residues. Most of the TEs are conserved between the two wheat A genome BACs (Figure 3.1a, b), which is in contrast to the lack of conservation amongst A, B, and D genome homoeologues. Similarly, *Glu-1* was more highly conserved between bread wheat genomes and the corresponding ancestral lines than amongst the three bread wheat genomes (Gu *et al.*, 2006). These results reflect the closer relationship of the A genomes with divergence dated to less than 0.5 million years ago while the A, B, and D genomes of wheat diverged 2-4 million years ago (Huang *et al*., 2002; Dvorak and Akhunov, 2005; Chalupska *et al*., 2008).

To determine if the micro-synteny of genes around *Rht-1* is conserved in other *Poaceae* species, orthologous regions were identified in *Brachypodium*, rice, sorghum, and maize (Figure 3.2). In maize, this region is in duplicate on chromosomes 1 and 5, and is in single copy in the other species. Gene order and orientation is conserved in all orthologous regions except on maize chr. 5 where *DUF6* is absent, and in rice where an additional gene is present between *DUF6* and *Rht-1*. The next gene 5' of the *ZnF* gene in all five orthologous regions is *Teosinte Branched 1* (*Tb1*), which in maize is an important domestication gene that controls branch number (Doebley *et al.*, 2004). Downstream of *Rht-1*, synteny among the *Poaceae* is not as highly conserved. *Toc64* is the next gene 3' of *Rht-1* in rice and sorghum while in *Brachypodium*, *Toc64* is downstream of *Rht-1*, but proceeded by the predicted gene *Bradi1g11100*. The Toc64 protein is a subunit of the pre-protein translocon of the outer envelope of chloroplasts (Toc complex; Sohrt and Soll, 2000). In maize, neither *Toc64* nor *Bradi1g11100* are in the vicinity of *Rht-1* in either orthologous region from chromosome 1 or 5. While synteny in general is well-conserved near *Rht-1* in the *Poaceae*, gene densities vary

widely. The region that includes the *ZnF-DUF6-Rht-1* linkage group and the flanking intergenic sequences leading up to the nearest adjacent genes has a density of 1 gene / 28 kb in *Brachypodium*, 1 gene / 34 kb in rice and sorghum, 1 gene / 59 kb on maize chr.5, and 1 gene / 183 kb on maize chr. 1. In bread wheat, based on the BAC sequences, the density of this region is estimated at 1 gene / 55 kb on the A genome, 1 gene / 94 kb on the B genome, and 1 gene / 105 kb on the D genome; however, the BAC sequences almost certainly do not include all of the intergenic sequence adjacent to the *ZnF-DUF6-Rht-1* block meaning that the wheat gene densities are likely to be lower than these estimates. The low gene densities in this region of wheat and maize relative to *Brachypodium*, rice, and sorghum are the result of a higher TE content. TE content of the *Rht-1* region is reflective of the overall genome contents of these species as high TE content in wheat relative to *Brachypodium*, rice, and sorghum was previously reported when analyzing whole genomes (The International *Brachypodium* Initiative, 2010).

A comparison of the *ZnF*, *DUF6*, and *Rht-1* genes among the CS wheat and *Poaceae* species reveals no large indels, stop codons, or frame-shift mutations in any of the ORFs and no changes in the number of introns or exons. For *Rht-1*, the CS wheat homoeologues share 94.1% of the nucleotide identities and 96.8% of the amino acid identities. Most nucleotide differences between any two homoeologues are SNPs, but 30% of the polymorphisms are indels that translate into 1 to 2 amino residue indels causing overall peptide lengths among wheat homoeologues to vary from 620 to 623 residues (most clearly depicted in Figure 5.3). The amino acid sequences of *Rht-1* were particularly well conserved in the protein motifs previously identified by Tian *et al.* (2004). Of the three *Rht-1* homoeologues, *Rht-A1* and *Rht-D1* have ORFs slightly more similar to each other than to *Rht-B1*, and in the adjoining 10 kb upstream of *Rht-1*, the differences are very marked with the A and D genomes having 61% of the sequence defined as similar whereas less than 26% of the B genome sequence is defined as being similar to the A or D genomes. Analysis of the flanking 5' and 3' regions within 10 kb of *Rht-1* also revealed several regions conserved among the *Rht-1* homeologues (Figures 3.5 and 3.6; Tables 3.3 and 3.4) that may represent

important regulatory regions in wheat. Three regions within 10 kb of *Rht-1* were conserved in the three CS homoeologues, *Brachypodium,* and rice (Table 3.5). Among all the *Poaceae Rht-1* ORF sequences (wheat included), amino acid lengths vary from 618 to 630 residues and 68.5% of nucleotide identities and 70.9% of amino acid residues are shared.

The *ZnF* nucleotide and amino acid sequences are the most conserved of the three genes among the *Poaceae* species. The two available CS orthologues (*ZnF-A1* and *ZnF-D1*) do not vary in sequence length and share 98.5% nucleotide identity and 99.8% residue identity (caused by a single amino acid substitution). Among all *ZnF Poaceae* sequences, the amino acids lengths vary by no more than 2 residues (472 to 474 residues) and 78.0% of nucleotide and 85.1% of amino acid identities are in common. Between any two orthologues, at least 90.5% amino acid identity is maintained (Table 3.6) whereas in *Rht-1* identities are frequently lower than 90% and as low as 82.4% (Table 3.2). The high level of conservation and the presence of a C3HC4 zinc finger domain normally associated with modulating protein levels via the ubiquitination pathway (Lorick *et al.*, 1999) indicate this gene likely performs a critical function in the *Poaceae* family.

Of the three genes, *DUF6* has the least conserved ORFs among the CS wheat homoeologues (nucleotides shared = 94.7%; amino acids shared = 93.6%; residue number ranging from 436 to 440) and among the *Poaceae* (nucleotides shared = 58.4%; amino acids shared = 54.2%; residue number ranging from 436 to 454). Similar to *Rht-1*, the *DUF6-A1* and *DUF6-D1* ORF nucleotide and amino acid sequences are the most similar of the three homoeologues. The most notable differences in *DUF6-B1* relative to the A and D homoeologues are: (1) a 12 bp deletion in the first exon, which reduces overall protein length from 440 amino acids to 436 amino acids, and (2) the absence of a 162 bp MITE in intron 1. Conservation of amino acids between orthologues was in most cases less than 80% and as low as 71.9%. The least conserved region of *DUF6* is in the 5’ end of the gene (Figure 3.11, coords. 10 to 100, which are in the first exon).

The genetic relatedness of the *Poaceae* species is similar in the *ZnF*, *DUF6* and *Rht-1* ORFs for each of the measures used: nucleotide identity and amino acid identity (Tables 3.2, 3.6, and 3.7) and phylogeny based on nucleotide sequence (Figures 3.4, 3.9, and 3.11). In each gene comparison, the ORFs of the *T. urartu* and CS A genome sequences are the most similar (99.5% to 99.7% nucleotide identity) and the CS A, B, and D ORF sequences also have high similarity, but are clearly distinct from each other having nucleotide identities ranging from 95.9% to 98.5% between any two homoeologues. For each of the three genes, the barley orthologue is the most closely related to wheat among the *Poaceae* followed by *Brachypodium*. For each gene, the rankings of relatedness based on the average number of shared nucleotides averaged across the three CS homoeologues (shown in parenthesis) are: *Rht-1* = *Hv* (93.5%) > *Bd* (87.7%) > *Os* (85.8%) > *Zm*-5 (84.9%) > *Zm*-1 (84.7%) > *Sb* (84.6%); *ZnF* = *Hv* (97.4%) > *Bd* (92.4%) > *Sb* (88.5%) = *Os* (88.5%) > *Zm*-1 (88.0%) = *Zm*-5 (88.0%); *DUF6* = *Hv* (93.4%) > *Bd* (87.8%) > *Sb* (78.7%) > *Os* (77.9%) > *Zm*-1 (77.5%). In all three locus comparisons, the wheat nucleotide identities shared with rice, sorghum, or maize never differ by more than 1.2 percentage points indicating these three species are approximately equally genetically distant to wheat. The phylogenetic relationships among the *Poaceae Rht-1* (Figure 3.4), *ZnF* (Figure 3.9), and *DUF6* (Figure 3.12) closely resemble the phylogenetic relationships among *Poaceae* depicted by Paterson *et al* (2009) (Figure 3.13), which is based on morphological and DNA sequence data from the Angiosperm Phylogeny Website (v9) (http://www.mobot.org/MOBOT/Research/APweb) (Stevens 2008). Among the *Poaceae* species with a fully-assembled genome sequence, the three genes described here are most genetically similar to *Brachypodium*. In addition, *Brachypodium* genes are syntenic with the *Poaceae* species examined here and *Brachypodium* has the highest gene density. These features indicate that in this genetic region, *Brachypodium* is the best model species for wheat among the *Poaceae* that are fully sequenced and assembled.

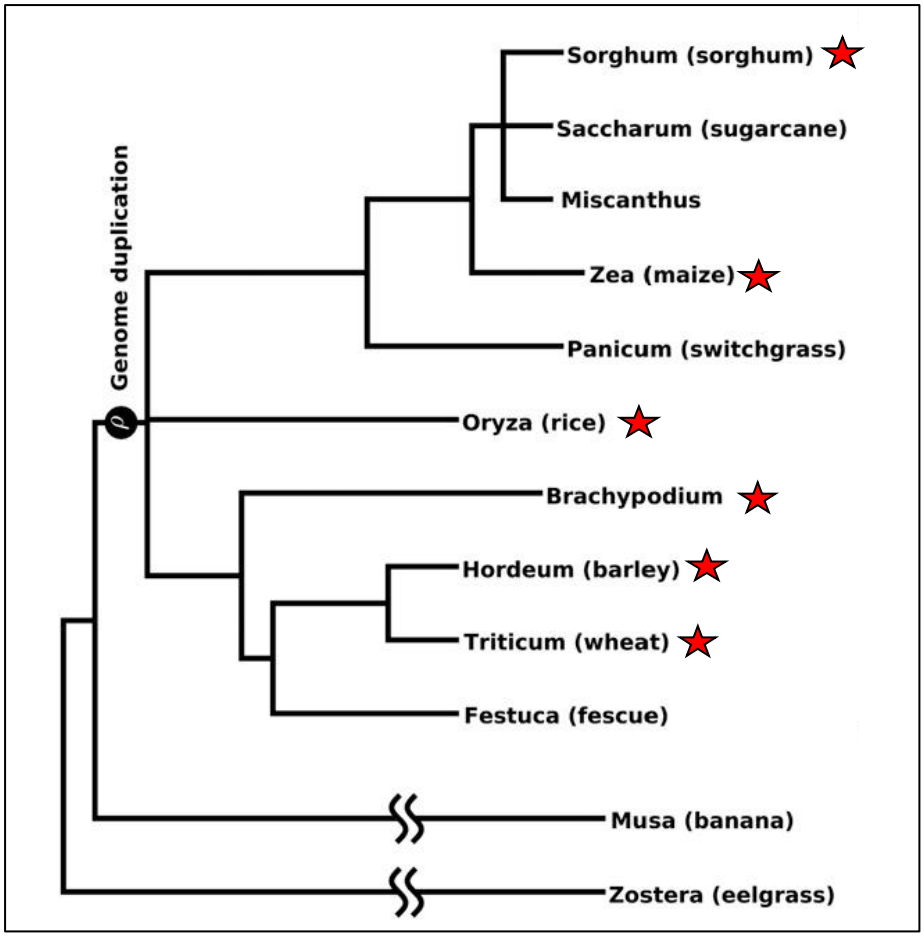


Figure 3.13. Phylogenetic relationship between members of the *Poaceae* family and two outgroup species. Species used in the text for orthologous comparisons of *Rht-1* (Fig. 3.4), *ZnF* (Fig. 3.9), and *DUF6* (Fig. 3.12) are indicated with a red star. Branch lengths of outgroups have been shortened. Figure adapted from Paterson *et al.* (2009) using data originally obtained from (http://www.mobot.org/MOBOT/Research/APweb).

4. PHYSICAL AND GENETIC MAPPING OF *RHT-A1*

4.1. INTRODUCTION

The *Rht-B1b* and *Rht-D1b* alleles located on wheat chromosomes 4B and 4D, respectively, are gibberellin (GA) insensitive. These alleles were utilised in breeding programmes beginning in the 1960s to produce semi-dwarf wheat varieties that helped alleviate a major worldwide food shortage (Hedden, 2003). The *Rht-B1* and *Rht-D1* loci have been mapped (Gale *et al.*, 1975; Gale and Marshall, 1976; McVittie *et al*, 1978) and cloned (Peng *et al.*, 1999). Due to the hexaploid nature of wheat, a third *Rht-1* homoeologue on 4A was long ago postulated to exist (McVittie *et al.*, 1978). Several years later, the presence of an *Rht-1* locus on chromosome 4A in bread wheat was demonstrated by hybridisation of an *Rht-B1*a-containing clone (C15-1) in nullisomic lines (Peng *et al.*, 1999). Recently, an *Rht-1* containing clone was isolated from a *T. urartu* (the A genome ancestor of bread and pasta wheat) BAC library (Jizeng Jia, unpublished data). As described in chapter 2, an *Rht*-containing clone (0224_M10) was identified in a CS hexaploid wheat BAC library with sequence similar to, but distinct from *Rht-B1* and *Rht-D1*, indicating this is likely the *Rht-A1* homoeologue. The *Rht* ORF sequence of clone 0224_M10 was nearly identical to the *T. urartu Rht-1* sequence (Chapter 3) further indicating that this clone contains *Rht-A1*.

Loci in wheat can be physically mapped using CS aneuploid lines and CS deletion lines or genetically mapped relative to genetic markers. Genetic markers are also useful for physical mapping if these have a known physical location or are linked to physically mapped markers. A large collection of wheat genetic maps can be found on the Graingenes website (http://wheat.pw.usda.gov) and several marker types are available. Of these, SSR (Simple Sequence Repeat) markers are widely used in wheat because they are publicly accessible, co-dominant, and often contain a large numbers of marker variants. For physical mapping, publicly available aneuploid stocks (described in Sears, 1954) consist of nullisomics (2n - 2; entire chromosome pair is absent), monosomics (2n -1; a single chromosome is absent), and

ditelosomics (either both short or both long arms missing from a chromosome pair). Deletion lines have terminal deletions of a particular chromosome arm from one or both chromosomes of a pair (described in Endo and Gill, 1996).

The physical locations of *Rht-B1* and *Rht-D1* on 4BS and 4DS were established several decades ago using monosomic analyses (Morris *et al.*, 1972; Gale *et al.*, 1975; Gale and Marshall, 1976). In addition, several groups have genetically mapped *Rht-B1* and/or *Rht-D1* (Borner *et al.*, 1997; Peng *et al.*, 1999; Ellis *et al.*, 2002; Somers *et al.*, 2004; Quarrie *et al.*, 2005; Draeger *et al.*, 2007; Cuthbert *et al.*, 2008; Srinivasachary *et al.*, 2008; 2009; Cao *et al.*, 2009; Raquin *et al.*, 2009). The *Rht-B1* locus has been consistently mapped to a region near the centromere on 4BS. Telocentric F_2 mapping analysis by McVittie *et al.* (1978) placed *Rht-B1b* and *Rht-B1c* (both *Rht-B1* alleles) 13 cM from the centromere. Using genetic markers, Borner *et al.* (1997) estimated the location of *Rht-B1c* to be 17 to 22 cM from the centromere. This estimation was based on linkage to an RFLP previously mapped by Gale *et al.* (1995). Ellis *et al.* (2002), developed diagnostic markers for *Rht-B1b* and *Rht-D1b* and placed *Rht-B1b* approximately 10 cM from the centromere based on linked SSR markers. In a consensus map of SSR markers, Somers *et al.* (2004) placed *Rht-B1* an estimated 11 cM from the centromere.

Unlike *Rht-B1*, the position of *Rht-D1* relative to the 4D centromere is less clear. The *Rht-D1b* (semi-dwarf phenotype) and *Rht-D1c* (severe dwarf phenotype) alleles at the *Rht-D1* locus have been used in mapping studies. *Rht-D1b* was estimated to be 15 cM from the centromere by McVittie *et al.* (1978) using telocentric F_2 mapping. In contrast, Izumi *et al.* (1983) found no linkage between *Rht-D1c* and the centromere using telocentric mapping. Borner *et al.* (1997) found *Rht-D1c* to be linked by less than 1 cM to an RFLP (*Xpsr 921*) that was mapped 45 cM from the centromere by Gale *et al.* (1995). Borner *et al.* (1997) also reported that *Rht-D1c* and *Rht-D1b* were 28.0 cM and 41.1 cM, respectively, distal to the SSR *Xgwm165*, which maps near the centromere. Ellis *et al.* (2002) placed *Rht-D1b* approximately 30 cM distal of the centromere. Somers *et al* (2004) mapped *Rht-D1b* approximately 18 cM

from the centromere and in a subsequent publication (Cao *et al.*, 2009) found *Rht-D1c* to map near the *Ms2* male sterility gene at approximately 31 cM from the centromere. These studies indicate that the location of the *Rht-D1* locus relative to the centromere is not precisely known.

Chromosome 4A has a complex rearrangement history, having undergone at least two reciprocal translocations and three inversions (Figure 4.1). A 4AS-4AL pericentric inversion that likely occurred in tetraploid wheat (Devos *et al.,* 1995) resulted in the re-location of the majority of the native short arm of 4A to the long arm of 4A (Mickelson-Young *et al.*, 1995; Devos *et al.*, 1995; Nelson *et al.*, 1995). Only a small segment near the telomere of the native 4AS still appears to be present in the modern 4AS arm (Miftahudin *et al.*, 2004). Hence, in hexaploid wheats, the majority of loci on 4AS are homologous to 4BL and 4DL. Following this inversion on 4A, a smaller pericentric inversion near the centromere is thought to have occurred within the initial pericentric inversion in tetraploid wheat (Miftahudin *et al.*, 2004). Reciprocal translocations have occurred between 4AL/5AL and 4AL/7BS and a paracentric inversion has occurred on 4AL (Naranjo *et al*., 1987; Mickelson-Young *et al.*, 1995; Devos *et al.*, 1995; Nelson *et al.*, 1995; Miftahudin *et al.*, 2004). The 4AL/5AL translocation is thought to have occurred in diploid wheat while the paracentric inversion and the 4AL/7BS translocation is thought to have occurred in tetraploid wheat (Miftahudin *et al*., 2004). Major chromosomal re-arrangements following the delineation of the A, B, and D genomes of wheat have not been detected on 4D while a pericentric inversion has been detected on 4B. The 4B pericentric inversion likely involves a short region near the centromere as evidenced by the map locations of the genome-generic marker *Xwg212* in the proximal 37% of 4BS and in the proximal 9% of 4DL, whereas markers outside of these boundaries had co-linear positions on 4B and 4D (Mickelson-Young *et al*., 1995).

For *Rht-A1*, the absence of sequence information or of any alleles that alter phenotype have precluded its identification. However, with the identification (Chapter 2) and characterisation (Chapter 3) of a third homoeologue of *Rht-1*, mapping of this locus is now possible. In this chapter, *Rht-A1* is genetically

mapped alongside SSR markers and physically mapped with CS aneuploid and deletion stocks. In addition, the bin map location of *Rht-D1* is estimated using the CS aneuploid and deletion stocks.

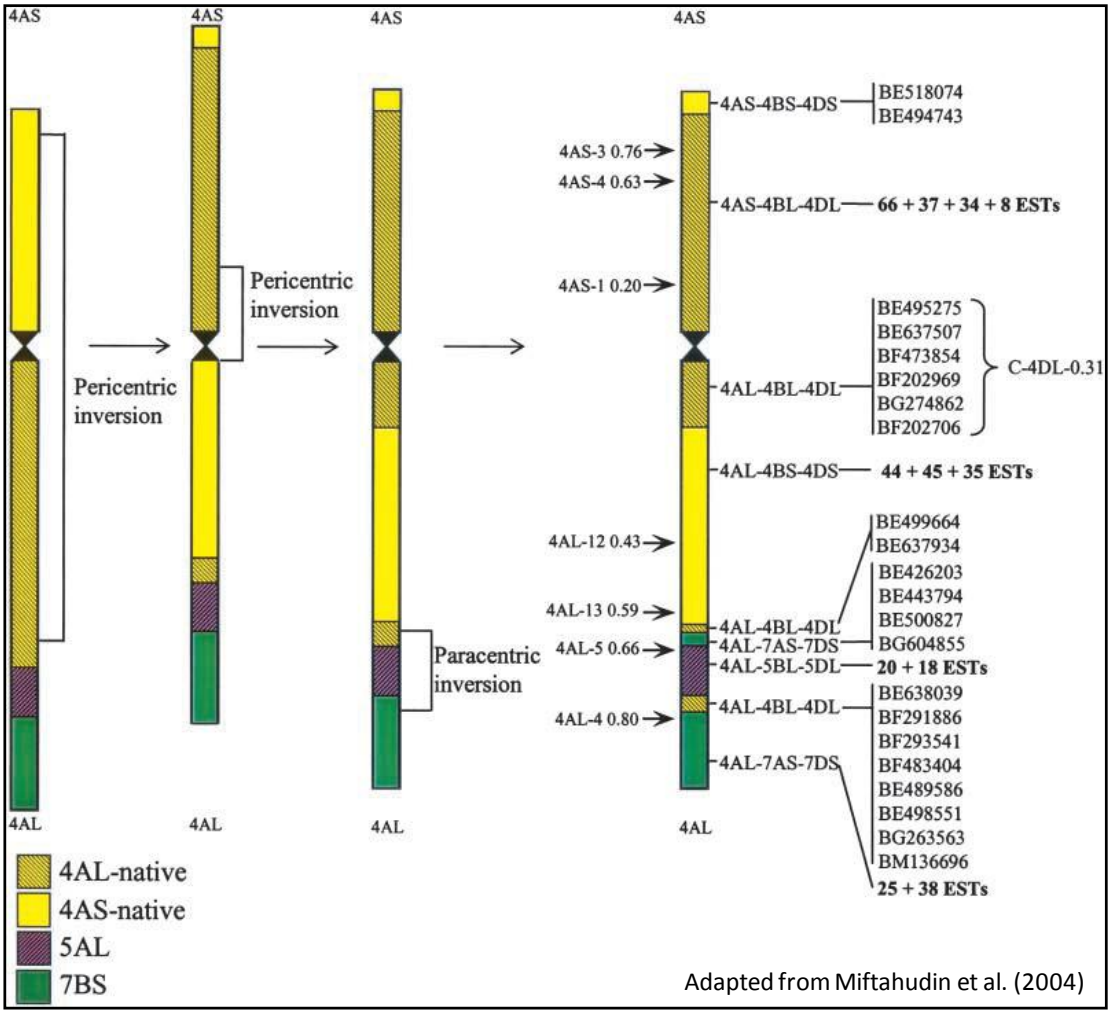


Figure 4.1. Rearrangement history of wheat chromosome 4A. Chromosomes segments native to chromosomes 4AL, 4AS, 5AL, and 7BS are colour-coded based on EST bin-mapping as shown by Miftahudin *et al*. (2004). On the left side of the rightmost chromosome, deletion line breakpoints are indicated by arrows labeled with the name of the breakpoint followed by the proportion of chromosome arm remaining. On the right side of the rightmost chromosome, bin-map locations are shown for wheat ESTs. Where greater than ten ESTs are mapped to a particular segment, the total number is shown with "+" referring to ESTs mapped to each bin in a segment when more than one deletion breakpoint is present.

4.2. MATERIALS AND METHODS

4.2.1. Plant Materials

Aneuploid and deletion line stocks were all in the CS background. Aneuploid stocks consisted of the nullisomic-tetrasomic (NT) lines N4AT4D and N4DT4B and the ditelosomic (Dt) lines Dt4AS (containing two 4AS arms and no 4AL arms), Dt4AL, Dt4BL, and Dt4DL. These lines were supplied by S.Reader, JIC. All aneuploid lines received were homozygous except Dt4BL, which due to the presence of a male sterility gene on 4BS is maintained in the heteromorphic condition with one complete 4B chromosome and one chromosome missing 4BS. Deletion stocks used were obtained from the Wheat Genetics Resource Center (WGRC), Kansas State University, USA and shown in Figure 4.2. Homozygous deletion stocks on 4BS were not available due to male sterility.

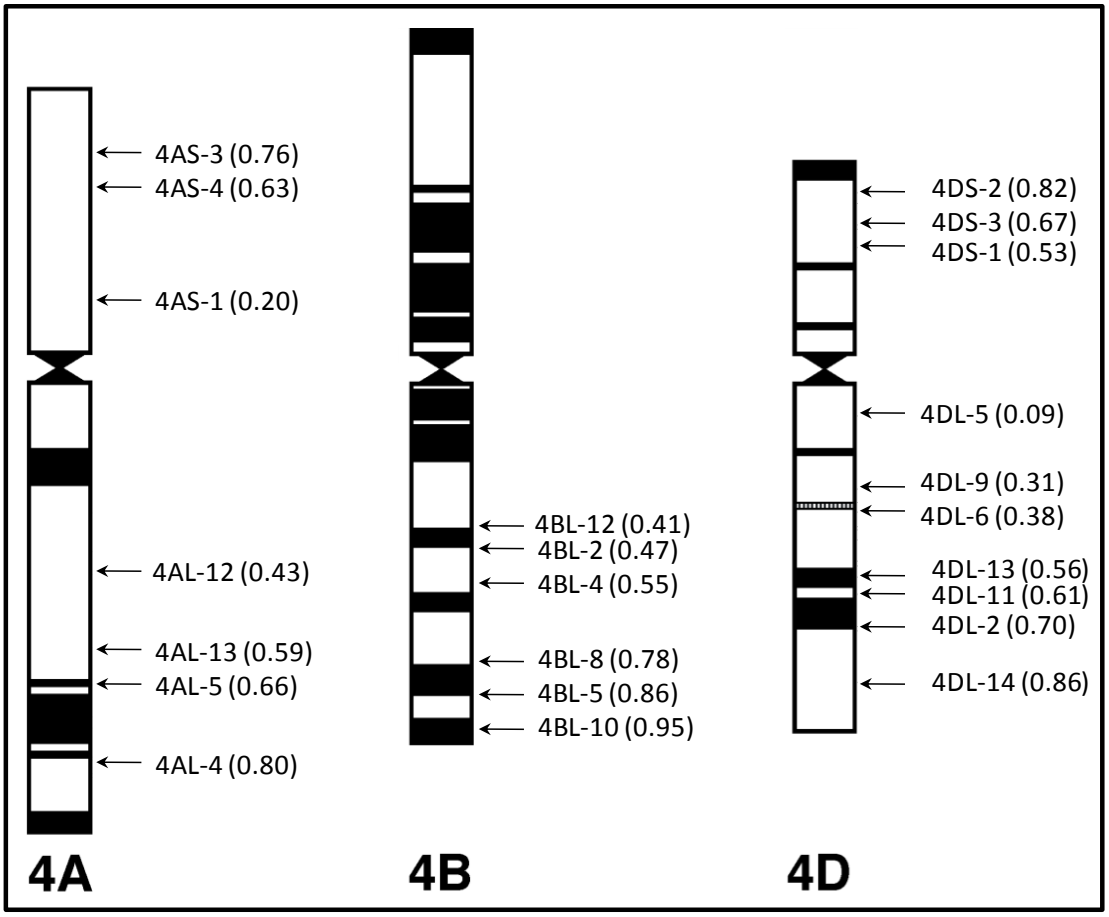


Figure 4.2. Wheat group IV deletion lines. Chromosomes are oriented with the short arm at the top and long arm at the bottom. The centromere is designated by a constriction. Deletion line breakpoints are indicated by arrows with the proportion of arm remaining shown in parentheses. Black and white rectangles show the C-banding pattern. Figure adapted from http://www.k-state.edu/wgrc.

The biparental F_5 population 'Sears Synthetic 7010073 × Paragon' ('SS7010073 × Paragon') was utilised for the genetic mapping of *Rht-A1*. This population was developed by the Department for Environment Food and Rural Affairs (DEFRA) and funded by the Wheat Genetic Improvement Network (WGIN). 'SS7010073' is a synthetic hexaploid of *T. dicoccum* × *Ae. tauschii* created by Ernie Sears, University of Missouri, USA (S. Reader, JIC pers. comm.). 'Paragon' is an elite UK spring wheat. Seed of 'SS7010073' was received from the JIC and seed of 'Paragon' was received from NIAB.

4.2.2. DNA extraction

Genomic DNA (gDNA) of aneuploid stocks, deletion lines, 'SS7010073', and 'Paragon' was extracted using a modification of the method described by Fulton *et al*. (1995) and described as follows. gDNA was extracted from leaf tissue collected from 2-4 week old seedlings. Leaf tissue was placed in 96-well deep-well plates along with a steel ball bearing and frozen at -80°C. Frozen tissue was disrupted by shaking on a SPEX 2000 Geno Grinder ® mill until tissue was powdery (20 s at 500 strokes per min). A 500 µl quantity of extraction buffer was added to each sample and tissue disrupted again (20 s, 500 strokes per min). The extraction buffer was made fresh and for a 96-well plate required 25 ml sorbitol extraction buffer (0.35 M sorbitol; 0.1 M Trizma base; 5 mM ethylenediaminetetraacetic acid (EDTA)); 25 ml nuclei lysis buffer (0.2 M Trizma base; 50 mM EDTA; 2 M NaCl; 2% Cetyl trimethylammonium bromide (CTAB); 10 ml 5% N-lauroylsarcosine sodium salt; 0.2 g sodium bisulfite; and 840 U RNAse A (Qiagen). Following tissue disruption, samples were incubated 30-60 min at 65°C. Samples were cooled to room temperature and 300 µl of 24:1 chloroform:isoamylalcohol added to each sample. Samples were mixed and centrifuged at 1500 × g for 5 min. The aqueous phase (approximately 400 µl) was recovered and an equal volume of ice cold isopropanol was mixed with the sample before centrifuging at 6000 × g for 5 min. After tipping off the isopropanol, pellets were washed by adding 500 µl of 70% ethanol, vortexing briefly, and centrifuging at 6000 × g for 5 mins. The ethanol was tipped off and pellets air dried. DNA was eluted in a

final volume of 100 µl TE elution buffer (10 mM Tris-Cl, pH 7.5; 1 mM EDTA). gDNA of 'SS7010073 × Paragon' was extracted using the DNeasy kit (Qiagen) and was supplied by M. Leverington-Waite (Crop Genetics, JIC). DNA from BAC clones was extracted as described in section 2.2.2.3.

4.2.3. PCR amplification

For the physical mapping of *Rht-1*, gDNA extracts from aneuploid and deletion lines were used as templates in PCR reactions containing two primers in which one primer was designed to be genome specific (Table 4.1). Primers were designed with primer3 software (http://frodo.wi.mit.edu/primer3). PCR reactions were performed in 10 µl volumes containing 1 × Green GoTaq Reaction Buffer (Promega), 3% glycerol, 0.2 mM of each dNTP, 2 mM $MgCl_2$, 1 µM forward primer, 1 µM reverse primer, 0.25 µl *Taq* polymerase, and 20 ng DNA. The PCR profile consisted of 5 min at 95°C, followed by 40 cycles of [95°C for 30 s, a primer pair-dependent annealing temperature (shown in Table 4.1) for 30 s, and 72°C for 1:30 min], and concluded with 72°C for 5 min. Amplified products were separated in a 1.5% agarose gel in 1 × TBE buffer and visualised under UV light with ethidium bromide.

Table 4.1. Summary of primers used for physical mapping of *Rht-1*.

Primer Pair	Genome Specificity	Forward Primer[a]	Reverse Primer	Anneal Temp	Product (bp)
1	A	Rht-A-F2*	Rht-ABD-R5	60°C	674
2	A	Rht-A-F3*	Rht-ABD-R6	64°C	1304
3	A	Rht-ABD-F3	Rht-A-R1*	64°C	1236
4	A	Rht-ABD-F9	Rht-A-R2*	66°C	1041
5	B	Rht-B-F2*	Rht-ABD-R5	60°C	694
6	B	Rht-B-F1*	Rht-ABD-R6	64°C	1105
7	B	Rht-ABD-F3	Rht-B-R1*	62°C	1472
8	D	Rht-D-F1*	Rht-ABD-R6	64°C	1118
9	D	Rht-ABD-F3	Rht-D-R1*	60°C	1495

[a] the (*) indicates genome specific primers. Primer sequences are provided in Appendix I.

For mapping *Rht-A1*, a genetic marker was created based on a 3 bp nucleotide deletion (TTC) in SS7010073 relative to the intact Paragon allele. The indel occurs 156 bp upstream of the *Rht-1* start codon in CS (Table 5.6, coord -156; Paragon is haplotype A2 and SS7010073 is haplotype A10) and

reverse primers were designed based on this indel. The reverse primer PS-Rht-R2 (primer sequences shown in Appendix I) in conjunction with the genome-specific Rht-A-F3 primer was designed to amplify a 120 bp product in the Paragon sequence. The reverse primer PS-Rht-R4 in conjunction with the genome-specific Rht-A-F3 primer was designed to amplify a 120 bp product in the SS7010073 sequence. Both reverse primers match their target sequences except for the introduction of a 'strong' (T/C) penultimate mismatch that was introduced into each primer according to Ye *et al.* (2001). Reaction mixtures and conditions were as described above with an annealing temperature of 60°C and a 30 s extension time for both primer pair mixes.

4.2.4. SSR linkage mapping

SSR primer sequences were acquired from the Graingenes database (http://wheat.pw.usda.gov). Forward primers were labeled with the dyes FAM, VIC, NED, or PET (Applied Biosystems [ABI]) according to Schuelke (2000). PCR mixes were in 6.25 µl volumes that consisted of 3.125 µl HotstarTaq Master Mix (Qiagen), 0.75 µM of each primer, and 12.5 ng gDNA. The PCR profile consisted of 15 min at 95°C, followed by 35 cycles of [95°C for 1 min, a primer pair-dependent annealing temperature according to Graingenes for 1min, and 72°C for 1 min], and concluded with 72°C for 10 min. Products were measured on an ABI 3730 DNA Analyzer with a POP-7™ polymer column. Linkage maps were created using JoinMap® with an LOD score of 5. SSR marker amplifications and linkage mapping was funded by WGIN and carried out by C. Baker and M. Leverington-Waite (JIC).

4.3. RESULTS

4.3.1 Physical mapping of *Rht-1* loci using aneuploid lines

Prior to determining the physical location of the *Rht-1* loci, locus specificity of each of the nine primer pairs listed in Table 4.1 was tested using DNA extracted from CS BAC clones 0224-M10, 1417-F16, and 0155-I24, which contain *Rht-A1*, *Rht-B1*, and *Rht-D1*, respectively (Chapter 2). Reactions

were carried out for all primer pair/BAC DNA combinations and each primer pair amplified a product only from the BAC DNA it was designed to amplify, thereby validating the specificity of the primers.

To determine *Rht-A1* chromosomal location, gDNA of CS and N4AT4D were used in separate PCR reaction mixes along with an *Rht-A1* specific primer pair. All four *Rht-A1* specific primer pairs shown in Table 4.1 amplified the expected product in gCS, but not in N4AT4D or a water control, implying the location of *Rht-A1* on chromosome 4A. To determine which 4A chromosome arm contains *Rht-A1*, gDNA of Dt4AS and Dt4AL were used in PCR reactions containing primer pair 1. The amplification of the *Rht-A1* specific product in the reaction containing gDNA of Dt4AL and not in the reaction containing gDNA of Dt4AS indicated that *Rht-A1* is located on chromosome 4AL. Amplification products obtained with the chromosome 4A aneuploid lines using primer pair 1 are shown in Figure 4.3.

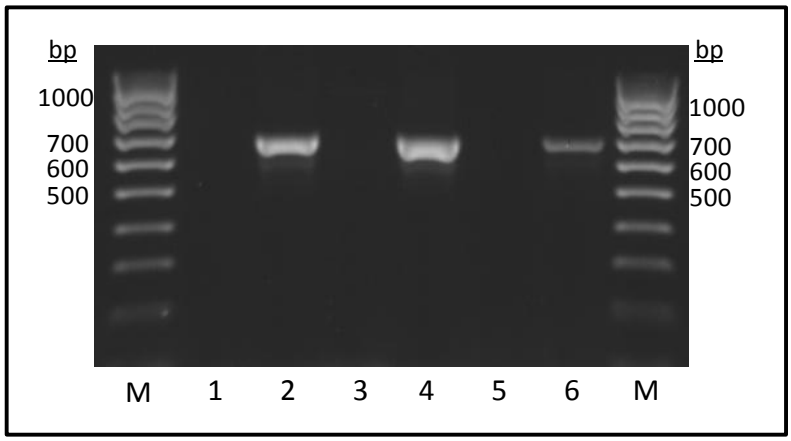


Figure 4.3. Products obtained following PCR amplification of aneuploid and control lines using Rht-A-F2/Rht-ABD-R5 (primer pair 1). Rht-A-F2/Rht-ABD-R5 was used in PCR reactions containing either water (reaction 1) or genomic DNA extracted from CS (2), N4AT4D (3), N4DT4B (4), Dt4AS (5), or Dt4AL (6). Products were separated on an agarose gel and visualised under UV light with ethidium bromide. M = molecular weight marker Hyperladder IV (Bioline) with size in basepairs (bp) as indicated.

To further delineate the location of *Rht-A1* on 4AL, gDNA extracts of the chromosome 4A deletion lines shown in Figure 4.2 were used in PCR mixes containing A-genome specific primer pairs 2, 3, or 4. gDNA of three to five plants per deletion line was initially assayed with each primer pair. The short

arm deletion lines 4AS-3 (0.76), 4AS-4 (0.63), and 4AS-1 (0.20) produced the *Rht-A1* specific product with all primer pairs (Table 4.2, chromosome arm 4AS). This indicates that *Rht-A1* is not between the telomere and breakpoint 0.20 on 4AS, which partially supports the earlier finding that *Rht-A1* is not on 4AS. When the long arm deletion lines 4AL-4 (0.80), 4AL-5 (0.66), 4AL-13 (0.59), and 4AL-12 (0.43) were tested for the presence of *Rht-A1* with the same primer pairs, a product was amplified from the 4AL-12, 4AL-5 and 4AL-4 lines (Table 4.2, 4AL), but not from 4AL-13. These results are seemingly contradictory because 4AL-12 has a larger deletion than line 4AL-13. To help determine whether the 4AL results were due to a mislabeling, the four 4AL deletion lines were ordered a second time from WGRC and gDNA of 2-3 plants per line was assayed with primer pair 1. All plants gave results consistent with those previously obtained (Table 4.2, 4AL).

Table 4.2. Summary of deletion line PCR amplifications.

Chromosome Arm	Accession _Line	Deletion	Deletion Breakpoint[a]	Product[b]
4AS	TA4528_3	4AS-3	0.76	√
	TA4528_4	4AS-4	0.63	√
	TA4528_1	4AS-1	0.20	√
4AL	TA4529_12	4AL-12	0.43	√
	TA4529_13	4AL-13	0.59	x
	TA4529_5	4AL-5	0.66	√
	TA4529_4	4AL-4	0.80	√
4BL	TA4531_12	4BL-12	0.41	√
	TA4531_2	4BL-2	0.47	√
	TA4531_4	4BL-4	0.55	√
	TA4531_8	4BL-8	0.78	√
	TA4531_5	4BL-5	0.86	√
	TA4531_10	4BL-10	0.95	√
4DS	TA4532_2	4DS-2	0.82	x
	TA4532_3	4DS-3	0.67	x
	TA4532_1	4DS-1	0.53	x
4DL	TA4533_5	4DL-5	0.09	√
	TA4533_9	4DL-9	0.31	√
	TA4533_6	4DL-6	0.38	√
	TA4533_13	4DL-13	0.56	√
	TA4533_11	4DL-11	0.61	√
	TA4533_2	4DL-2	0.70	√
	TA4533_14	4DL-14	0.86	√

[a] The values represent the proportion of the original chromosome arm remaining.

[b] Amplification of the *Rht-1* product pertaining to a particular chromosome is indicated by a (√) and absence of a product is marked (x).

The physical locations of *Rht-B1* and *Rht-D1* were estimated using gDNA of CS aneuploid and deletion lines in PCR mixes with locus specific primer pairs. For *Rht-B1*, the lack of a homozygous nulli-4B line necessitated the use of the heteromorphic 4B/Dt4BL line. gDNA extracts from ten selfed F_2 progeny of the 4B/Dt4BL line were first tested with an *Rht-1* genome-generic pair, which amplified a product in all 10 extracts, confirming the presence of sufficient gDNA for PCR amplifications. The gDNA from the F_2 progeny was then screened using primer pair 5 (Table 1), which is specific to *Rht-B1*. A product was amplified in nine of the ten plants indicating that the remaining plant was homozygous for Dt4BL and the absence of product indicates that *Rht-B1* is on 4BS. The reduced frequency of Dt4BL/Dt4BL plants (0.10 instead of 0.25) is not unexpected due to reduced viability of male gametes that carry the Dt4BL chromosome fragment (S. Reader, pers. comm.). To partially confirm this finding, gDNA extracts of the six homozygous 4BL deletion lines (one plant per line) were used in separate PCR reactions containing an *Rht-B1* specific primer pair. Primer pairs 5, 6, and 7 were used for each 4BL deletion line (Table 4.1) and the expected product was amplified in each case, confirming that *Rht-B1* is not located beyond breakpoint 0.41 on 4BL (Table 4.2).

The chromosomal location of *Rht-D1* was determined using *Rht-D1* specific primer pairs 8 and 9 (Table 4.1) in PCR reactions with gDNA of CS, N4AT4D, and N4DT4B. The predicted product was amplified from gCS and N4AT4D, but not from N4DT4B indicating *Rht-D1* is located on chromosome 4D. To determine chromosomal arm location, primer pair 9 was then used in a PCR reaction with Dt4DL and no amplification product resulted, indicating *Rht-D1* is on 4DS. In addition, gDNA of seven 4DL deletion lines (breakpoints 0.09 to 0.86; Figure 4.2) were used in PCR mixes with the same primers and the *Rht-D1* specific product was amplified from all 4DL deletion lines, verifying that *Rht-D1* is not on 4DL beyond breakpoint 0.09 (Table 4.2). To further delineate the physical location of *Rht-D1* on 4DS, gDNA of three 4DS deletion lines (breakpoints 0.53, 0.67, 0.82; Figure 4.2) were used in PCR reactions with primer pairs 8 and 9. No product was amplified in any of the 4DS deletion lines using either primer pair, which indicates *Rht-D1* is distal to breakpoint 0.82 on 4DS.

4.3.2 *Rht-A1* mapping in the 'SS7010073 × Paragon' F_5 population

To clarify the physical location of *Rht-A1* on chromosome 4A and to determine linked markers, *Rht-A1* was mapped in the 'SS7010073 × Paragon' F_5 population. Markers based on DNA polymorphisms present between the parental lines were designed (see section 4.2.3.) and tested to determine if the SS7010073 and Paragon *Rht-A1* sequences could be distinguished. The Paragon *Rht-A1* specific primer pair Rht-A-F3/PS-Rht-R2 amplified the expected ~120 bp product using gDNA from Paragon, CS-Dt4BL, and CS-N4DT4A and did not amplify a product using water or gDNA from SS7010073 or N4AT4B (Figure 4.4), demonstrating specificity for the Paragon *Rht-A1* sequence, which is also shared by CS. The SS7010073 *Rht-A1* specific Rht-A-F3/PS-Rht-R4 primers amplified the expected ~120 bp product in reactions using SS7010073 gDNA as a template and did not amplify a product when water or gDNA of Paragon or CS-N4AT4D served as templates. These results confirm that the primer pairs distinguish the Paragon and SS7010073 *Rht-A1* sequences and can be used for screening the bi-parental population.

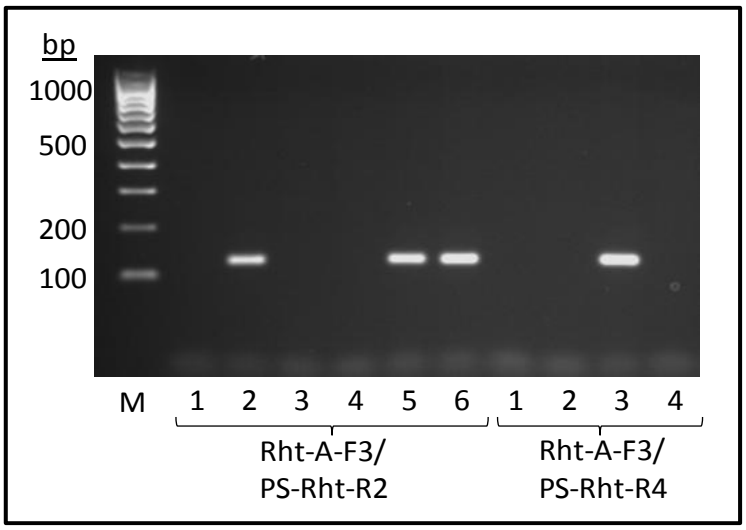


Figure 4.4. Validation of *Rht-A1* haplotype-specific primers used to screen the 'SS7010073 × Paragon' F_5 population. The primer pairs Rht-A-F3/PS-Rht-R2 and Rht-A-F3/PS-Rht-R4, designed to specifically amplify the *Rht-A1* sequences of Paragon and SS7010073, respectively, were used in PCR reactions containing the following templates: water (1) or gDNA extracted from Paragon (2), SS7010073 (3), CS-N4AT4D (4) CS-Dt4BL (5), and CS-N4DT4B (6). Amplification products were run out on a 1% gel stained with ethidium bromide and visualised under UV light. M = Molecular marker Hyperladder I (Bioline) with weights shown in basepairs (bp).

A total of 288 individuals from the 'SS7010073 × Paragon' population were screened by PCR with the Paragon *Rht-A1* specific primers and then separately with SS7010073 *Rht-A1* specific primers. Of the 278 individuals that amplified a product, 139 were homozygous for the Paragon allele, 126 were homozygous for the SS7010073 allele, and 13 were heterozygous (Table 4.3). The observed chi square value of the segregation classes fits the expected chi distribution for an F_5 population (p =.40) (Table 4.3).

Table 4.3. Summary of the results for *Rht-A1* genotyping of the 'SS7010073 × Paragon' F_5 population

	Individual Plants		
Rht-A1 haplootype	Observed	Expected[a]	Chi square
Homozygous Paragon	139	130.3	0.58
Homozygous Synthetic	126	130.3	0.14
Heterozygous	13	17.4	1.10
No Product	10		
Sum	288	278.0	1.82[b]

[a] Expected values based for 278 F_5 individuals in Hardy-Weinberg equilibrium.

[b] Chi distribution p (1.82, 2 df) = 0.40

Due to the absence of pre-existing markers on the 'SS7010073 × Paragon' population, SSR markers mapped in other bi-parental populations were selected for screening. A set of 28 SSR markers that spanned chromosome 4A were genotyped in both parents (Table 4.4). For ten of the markers, no polymorphism existed between the parental lines. Of the 18 polymorphic markers, 12 markers that were the most easily scored and/or widely spaced across the chromosome were selected for screening a set of 94 individuals from the 'SS7010073 × Paragon' population.

Table 4.4. Summary of SSR markers considered for screening the 'Paragon × SS7010073' population and genetic locations taken from other maps.

			Map Location (cM)[c]	
SSR marker	Parental screen[a]	Scored on population[b]	Consensus SSR	Synthetic × Opata
Xgpw4545-4A	polymorphic	√	-	10.5
Xgwm4-4A	monomorphic		0	17.1
Xgpw2302-4A	polymorphic		-	18.3
Xgpw2140-4A	monomorphic		-	18.7
Xgpw2166-4A	polymorphic		-	18.7
Xgpw2283-4A	monomorphic		-	18.7
Xbarc206-4A	polymorphic	√	4	-
Xbarc138-4A	monomorphic		6	-
Xwmc89-4A	polymorphic	√	7	-
Xwmc420-4A	polymorphic	√	7	-
Xwmc48-4A	polymorphic	√	8	-
Xwmc491-4A	monomorphic		8	-
Xcfa2256-4A	polymorphic		10	-
Xgwm44-4A	polymorphic	√	10	-
Xgwm610-4A	polymorphic	√	12	-
Xwmc617-4A	polymorphic	√	13	-
Xgwm397-4A	polymorphic	√	18	39.6
Xbarc170-4A	monomorphic		27	-
Xwmc258-4A	polymorphic		39	67.5
Xwmc161-4A	polymorphic	√	46	-
Xcfd88-4A	monomorphic		60	96.8
Xwmc232-4A	monomorphic		68	110.3
Xbarc70-4A	polymorphic	√	71	-
Xgwm160-4A	polymorphic		79	120
Xwmc313-4A	monomorphic		83	-
Xwmc497-4A	polymorphic	√	85	-
Xwmc219-4A	polymorphic		88	-
Xgwm350-4A	monomorphic		-	-

[a] DNA of the parental lines SS7010073 and Paragon were used in PCR reactions with each SSR marker pair and products for each marker scored as either monomorphic (no detectable difference) or as polymorphic (difference detected).

[b] Markers used to screen the 'SS7010073 × Paragon' population are marked with an (√).

[c] Previously mapped SSR marker locations are indicated for the wheat SSR consensus map (Somers *et al.*, 2004) and the 'Synthetic × Opata' population using GPW (Wheat Genoplante) markers (Sourdille *et al.*, 2005). Unmapped markers are indicated by a (-).

The SSR and *Rht-A1* marker scores of the 94 individuals are shown in Appendix V and a summary is shown in Table 4.5. The Chi square test based on two degrees of freedom for an F_5 population under Hardy-Weinberg equilibrium indicates segregation distortion ($p < 0.05$) is present for eight of

the 12 markers. The segregation distortion in *Xwmc161-4A* and *Xwmc89-4A* is the result of a high number of heterozygotes, 22 and 16, respectively, when only six each are expected. Five markers have a significantly ($p < .05$) disproportionate representation of the homozygous alleles, with three markers (*Xbarc206-4A*; *Xwmc89-4A*; *Xbarc70-4A*) favouring the Paragon allele and two markers (*Xgwm397-4A*; *Xwmc161-4A*) favouring the SS70100073 allele. For these five markers the number of unscored amplifications averaged 18, while the rest of the markers averaged five unscored amplifications.

Table 4.5. Summary of marker scores for 94 individuals from the 'SS7010073 × Paragon' population.

	Marker score[a]				Chi square[b]	
Marker	P/P	S/S	P/S	U	X^2	*p*
Xbarc206-4A	58	21	3	12	18.7	8E-05*
Xgwm397-4A	23	46	1	24	10.8	0.004*
Xwmc617-4A	41	53	0	0	7.9	0.019*
Xwmc48-4A	47	42	0	5	6.2	0.044*
Xgwm610-4A	47	43	4	0	0.8	0.664
Rht-A1	43	43	6	2	0.0	0.994
Xgpw4545-4A	39	40	6	9	0.1	0.948
Xwmc161-4A	20	43	22	9	62.6	3E-14*
Xwmc89-4A	45	14	16	19	42.8	5E-10*
Xbarc70-4A	52	13	5	24	23.3	9E-06*
Xwmc420-4A	38	38	0	18	5.1	0.079
Xgwm44-4A	37	55	0	2	9.9	0.007*
Xwmc497-4A	50	40	2	2	3.8	0.152

[a] Number of individuals classified as Paragon homozygotes (P/P), SS7010073 homozygotes (S/S), heterozygotes (P/S), or unscored (U) are indicated.

[b] Chi square test based on two degrees of freedom with probability (*p*) shown that the markers are in Hardy-Weinberg equilibrium.

The SSR and *Rht-A1* marker data was used to construct a linkage map that contained eight SSR markers in linkage with *Rht-A1* and spanned 49 cM (Figure 4.5). One marker, *Xgwm610-4A* was very tightly linked to *Rht-A1*, having the same marker score in 90 instances and two instances where a crossover was detected (Appendix V, Individuals 28 and 49). The markers *Xwmc48-4A* and *Xgpw4545-4A* were also tightly linked, being separated from *Rht-A1* by an estimated 3 and 2 cM, respectively. Markers *Xgwm610-4A* and

Xgpw4545-4A did not show statistically significant ($p < .05$) segregation distortion while segregation distortion of *Xwmc48-4A* was significant ($p = .04$).

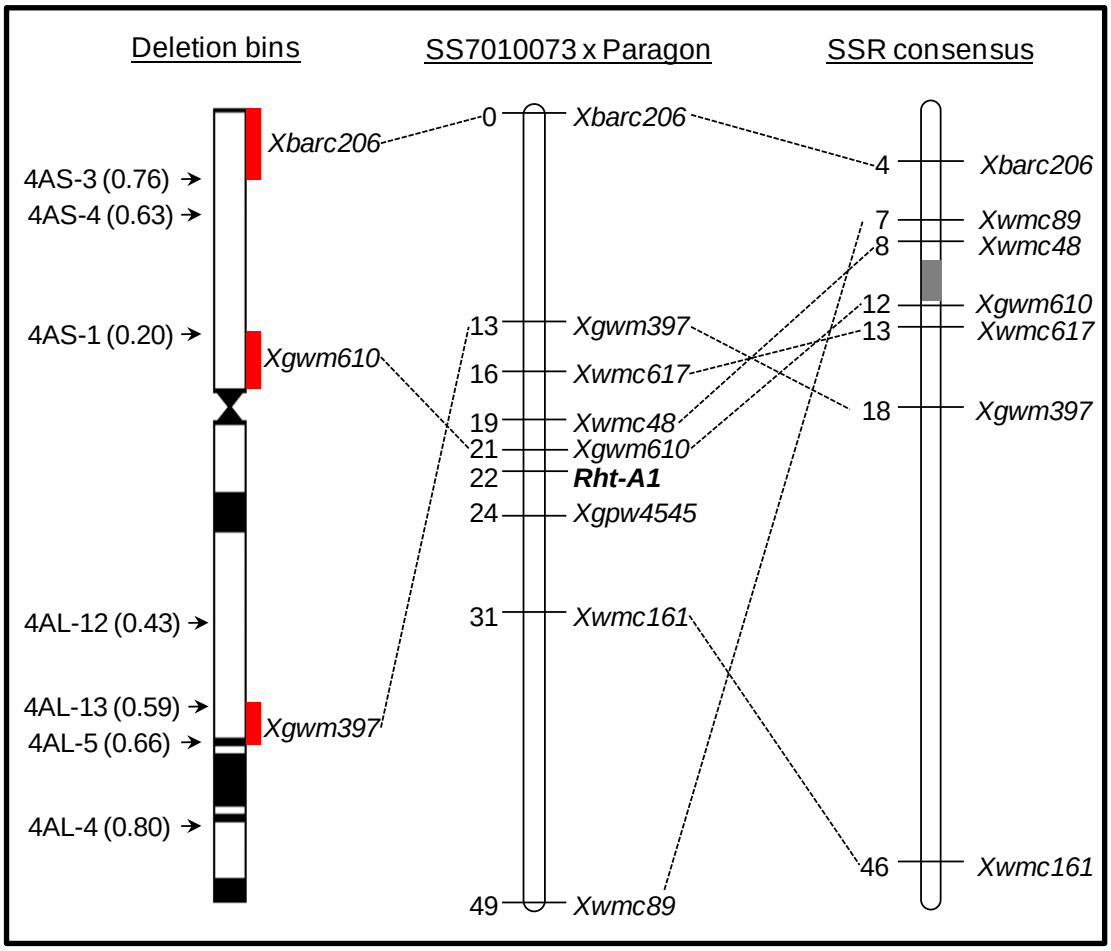


Figure 4.5. Linkage map of chromosome 4A in the 'SS7010073 × Paragon' population aligned to previously mapped SSRs. The 'SS7010073 × Paragon' linkage map was constructed using JoinMap® with an LOD score of 5 and is based on 94 individual plants. Marker names are shown to the right of the linkage group with distance (in centimorgans) indicated to the left. Previously bin-mapped SSR marker intervals (Sourdille *et al*., 2004) are shown by the red rectangles in the ideogram to the left with the centromere designated by a constriction and deletion line breakpoints indicated by arrows with the proportion of arm remaining shown in parentheses. Black and white rectangles show the C-banding pattern. Dotted lines connect identical markers between maps. The linkage group to the right is from the SSR consensus map (Somers *et al.*, 2004) with the approximate location of the centromere indicated with a grey rectangle.

The 'SS7010073 × Paragon' SSR markers have some shared colinearity with the SSR consensus map (Somers *et al.,* 2004) with the notable exception of *Xwmc89-4A*, which is located at nearly opposite ends of the two linkage groups. The location of *Xgwm397-4A* relative to the other SSR markers also

differs slightly between the two maps. Three of the SSR markers were previously mapped to bins using the CS deletion stocks (Sourdille *et al.*, 2004). In the bin map, these markers are widely separated with *Xbarc206-4A* mapped distal to breakpoint 0.76 on 4AS, *Xgwm610-4A* mapped proximal to breakpoint 0.20 on 4AS, and *Xgwm397-4A* mapped between breakpoints 0.59 and 0.66 on 4AL. The order of *Xgwm610-4A* and *Xgwm397-4A* is reversed relative to the 'SS7010073 × Paragon' linkage group.

4.4. DISCUSSION

The previously unmapped *Rht-A1* locus in wheat has been identified and physically and genetically mapped. *Rht-A1* is located on chromosome 4AL between the centromere and breakpoint 0.66 based on mapping using CS aneuploid and deletion lines. The location of *Rht-A1* on 4AL differs relative to the homoeologous *Rht-B1* and *Rht-D1* loci, which are located on the short arms of 4BS and 4DS. However, this is not surprising due to a pericentric inversion that resulted in the majority of the native 4AS being transferred to 4AL (Figure 4.1). The region of 4AL containing *Rht-A1* could not be further narrowed using homozygous deletion lines because of the apparently contradictory result where 4AL-13 (breakpoint 0.59) did not amplify the *Rht-A1*-specific product that the shorter 4AL-12 (breakpoint 0.43) did amplify. This result introduces the possibility that a previously unidentified interstitial deletion may exist in 4AL-13 between the centromere and 4AL-12, although a misidentification of seed stock cannot be fully ruled out despite having tested two separate sources ordered from the WGRC. To determine if stock misclassification has occurred, genetic markers previously assigned to bins delineated by the 4AL-13 and 4AL-12 breakpoints could be screened or these deletion lines could be cytologically screened.

Genetic mapping of *Rht-A1* in the 'SS7010073 × Paragon' population identified eight linked SSR markers (Figure 4.5), including three (*Xwmc48-4A, Xgwm610-4A,* and *Xgpw454-4A*) within 3 cM or less of *Rht-A1*. The order between the seven markers in common between the 'SS7010073 × Paragon' map and SSR consensus map (Somers *et al.*, 2004) were in general

agreement, with the exceptions of *Xwmc89-4A*, which is considerably more distal in the 'SS7010073 × Paragon' map and *Xgwm397-4A*, which differs slightly in order. While the 'SS7010073 × Paragon' SSR markers were in general agreement with the SSR consensus map, the three markers (*Xbarc206-4A*, *Xgwm397-4A*, and *Xgwm610-4A)* that were previously bin-mapped (Sourdille *et al.,* 2004) are assigned to bins that are physically separated by large distances even though these markers were contained within 21 cM in the 'SS7010073 × Paragon' population (Figure 4.5). *Xgwm610-4A,* which is separated from *Rht-A1* by 1 cM in 'SS7010073 × Paragon', was previously mapped to the 4AS bin located between the centromere and breakpoint 0.20 (Sourdille *et al.*, 2004). This result seems to conflict with the 4AL map location of *Rht-A1* in 'SS7010073 × Paragon', however if *Rht-A1* and *Xgwm610-4A* are close to the centromere, actual physical distance may be greater due to suppressed recombination associated with the centromere (Devos *et al.*, 1995; Akhunov *et al.*, 2003) making it possible that *Rht-A1* and *Xgwm610-4A* are on different chromosome arms. A close proximity of *Rht-A1* and *Xgwm610-4A* to the centromere and a 4AL location of *Xgwm610-4A* is also indicated by the SSR consensus map (Figure 4.5). The previously mapped bin location of *Xgwm397-4A* (between breakpoints 0.59 and 0.66 on 4AL) clearly differs from its location in 'SS7010073 × Paragon' and the SSR consensus map. If an interstitial deletion in 4AL-13 exists, this could provide one possible explanation for the difference in location.

The differences described above between the 'SS7010073 × Paragon' linkage group, the SSR consensus map (Somers *et al*., 2004), and the bin-mapped SSRs (Sourdille *et al*., 2004) could also be related to actual genetic differences between the populations. 'SS7010073' is a synthetic line with the A genome derived from the tetraploid *T. dicoccum* (see section 5.3.4.4). Paragon is a bread wheat cultivar. The bin-mapping of SSRs by Sourdille *et al.* (2004) was performed using deletion lines from the bread wheat cultivar CS and the SSR consensus map (Somers *et al*., 2004) is composed of data from four bi-parental populations, three of which are composed solely of bread wheat cultivars and the fourth is a cross between a bread wheat ('Opata 85')

and a synthetic wheat containing the *T. durum* (4x) line 'Altar84'. The timing of the 4A inversions shown in Figure 4.1 are not known, but are thought to have occurred during the tetraploid stage of wheat (Devos *et al*., 1995; Miftahudin *et al*., 2004). It is not known whether SS7010073 contains the 4A inversion and therefore the locations of *Rht-A1* and the SSR markers may differ greatly relative to Paragon. This could provide a possible explanation for the difference in the mapping positions of *Xwmc89-4A* and *Xgwm610-4A* between 'SS7010073 × Paragon' and the SSR consensus map or deletion line bin map location shown in Figure 4.5.

Several SSR markers exhibited significant segregation distortion in the 'SS7010073 × Paragon' population, although the two markers most closely flanking *Rht-A1* (*Xgwm610-4A* and *Xgpw454-4A*) did not show segregation distortion (Table 4.5). The pattern of distortion did not consistently favour one parent over the other and there was no clear distortion pattern associated with marker location. The large number of unscored PCR amplifications associated with several of the distorted markers provides a likely explanation for much of the distortion seen, although segregation distortion of markers on 4AL and 4AS was also previously reported by Quarrie *et al.* (2005). The high level of segregation distortion for markers such as *Xwmc89-4A* also provides a possible explanation for the loss of colinearity of this or other markers as described above.

While *Rht-B1* and *Rht-D1* are both located on the short arms of the respective chromosomes, the locations within the arms appear to differ. For *Rht-B1*, the results from the Dt4BL aneuploid PCR amplification support earlier studies that showed that this locus is located on 4BS (Morris *et al.*, 1972; Gale *et al.*, 1975; Gale and Marshall, 1976; McVittie *et al.*, 1978; Izumi *et al.*, 1983). Mapping to a specific 4BS bin was not attempted because of the lack of homozygous deletion lines on 4BS. However, previous physical mapping using telocentric F_2 mapping of either *Rht-B1b* or *Rht-B1c* has placed this locus within 13 cM of the centromere (McVittie *et al.*, 1978) and genetic mapping of this locus indicates this locus is near the centromere (Borner *et al.*, 1997; Ellis *et al.*, 2002; Somers *et al.*, 2004).

For *Rht-D1*, the physical mapping reported in this chapter places the locus on 4DS between breakpoint 0.82 and the telomere. The distal location agrees with the telocentric F_2 mapping of *Rht-D1c* by Izumi *et al*. (1983), who found no linkage between *Rht-D1* and the centromere, but differs from the telocentric F_2 mapping of *Rht-D1b* by McVittie *et al.* (1978) who estimated a 15 cM distance between *Rht-D1* and the centromere. The differences in telocentric mapping could result from the mapping of different alleles. *Rht-D1c* has stronger GA response than *Rht-D1b* and this may have aided in mapping precision as seedling length based on GA sensitivity was used to score for this locus. In addition, ditelosomic lines may not have breakpoints that end precisely at the centromere (S. Reader, pers. comm.), which may affect telocentric mapping and could be misleading when assigning chromosomal arm location as performed herein. The cause of the apparent difference in *Rht-B1* and *Rht-D1* map location is not clear. A pericentric inversion was reported on 4B near the centromere (Mickelson-Young *et al*., 1995). However, the affected region appears to be confined to the proximal 37% of 4BS, which does not provide an explanation for the distal location of *Rht-D1* relative to *Rht-B1*. Deletion-bin mapping of the *Rht-B1* locus would be a useful step in more precisely determining if the physical locations of *Rht-B1* and *Rht-D1* actually differ. Genes located near the telomere have higher recombination rates and increased rates of duplicaton relative to proximal regions (Akhunov *et al.*, 2003). The distal location of *Rht-D1* relative to the proximal *Rht-A1* and *Rht-B1* locations would be expected to increase recombination near this locus.

In summary, the *Rht-A1* homoeologue identified in Chapter 2 has been mapped to chromosome 4AL near the centromere, and SSR markers tightly linked to *Rht-A1* have been identified. The flanking markers represent a valuable tool for studying *Rht-A1* and the nearby genetic regions, and could be useful in marker assisted breeding. *Rht-D1* has been mapped to the distal arm of 4DS, which suggests the possibility of an additional translocation in the group 4 chromosomes as *Rht-B1* has previously been physically mapped near the centromere.

5. GENETIC DIVERSITY AT THE *RHT-1* LOCI IN WHEAT

5.1. INTRODUCTION

In plants, DELLA proteins serve a key biological function by integrating hormonal and environmental signals that affect overall plant growth (Alvey and Harberd, 2005; Achard *et al.*, 2006; Alvey and Boulton, 2008) and are associated with abiotic and biotic stress tolerance (Achard and Genschik, 2009). In wheat, DELLA proteins are encoded by the group IV homoeologous *Rht-1* loci. Two alleles at these loci, *Rht-B1b* and *Rht-D1b* are major adaptative alleles that reduce plant height and were key to the 'green revolution' in wheat. Both alleles confer a degree of gibberellic acid insensitivity (GAI) and were derived from a single cultivar, 'Norin 10', which is the primary source of semi-dwarfism in wheat varieties in the Western world (Gale *et al*., 1981; Dalrymple, 1986). An estimated 70% of wheat acreage in developing nations is planted to varieties that carry one of these two alleles (Evans, 1998). Genetic diversity of the *Rht-1* locus and the surrounding region is of particular concern not only due to the important biological and adaptative functions of this gene, but also because the *Rht-B1b* and *Rht-D1b* alleles have been associated with susceptibility to a major disease in wheat, Fusarium Head Blight (FHB) (Hilton *et al.*, 1999; Draeger *et al.*, 2007; Srinivasachary *et al.*, 2008; Srinivasachary *et al.*, 2009). The recent introgression of both alleles from a single source would be expected to substantially decrease genetic diversity at the *Rht-B1* and *Rht-D1* loci as a reduction in diversity is characteristic of crop domestication genes and genes under selection relative to ancestral populations (Doebley *et al.*, 2006; Palaisa *et al.*, 2003; Wang *et al.*, 1999).

Wheat is an allohexaploid that arose from 8,000 to 10,000 years ago from a cross between tetraploid wheat (AB genomes) and *Ae. tauschii* (D genome) (Levy and Feldman, 2002). *T. dicoccum* (domesticated emmer) has been suggested as the possible tetraploid donor (Zohary and Hopf, 2000) and *T. dicoccoides* (wild emmer) has been identified as the wild progenitor of domesticated wheat (Haudry *et al.*, 2007). The A genome ancestral line, *T.*

urartu, is estimated to have diverged from bread wheat approximately 0.5 million years ago (Huang *et al.*, 2002; Dvorak and Akhunov, 2005; Chalupska *et al.*, 2008). Previous studies have shown a great loss in genetic diversity between hexaploid wheat and tetraploid wheat (Haudry *et al.*, 2007) and between hexaploid wheat and *Ae. tauschii* (Caldwell *et al.*, 2004).

At the wheat group IV *Rht-1* loci, the sequences of *Rht-B1a* (CS background) and *Rht-B1b* (N. Harberd, University of Oxford, unpublished data) and *Rht-D1a* (CS background) and *Rht-D1b* (Peng *et al.*, 1999) are available. The prevalence of *Rht-B1b* and *Rht-D1b* alleles in regional collections of bread wheat accessions has been estimated using the GA sensitivity tests (Worland 1986; Yamada 1990; Chrpova *et al.*, 2003) developed by Gale and Gregory (1977) and more recently using genetic markers (Zhang *et al.*, 2006; Pestsova *et al.*, 2008) that were developed by Ellis *et al.* (2002). Additional GAI alleles that reduce height at the *Rht-B1* and *Rht-D1* loci have been identified, but the sequences are unknown. Outside of the *Rht-B1a, Rht-B1b, Rht-D1a,* and *Rht-D1b* ORF sequences and genotyping of varieties for the presence of these alleles, little is known regarding the genetic variation in the *Rht-1* ORF and the flanking region in wheat. The absence of this information largely results from the lack of an available *Rht-A1* sequence, which has prevented the creation of primers needed for locus-specific amplification. The lack of a complete set of homoeologous sequences is the case for most genes in wheat, hence comparisons of genetic diversity across the three genomes is rare. Determining existing genetic variation is an important first step toward finding useful variation that may affect *Rht-1* expression, protein function, and plant phenotype, which may be key to tackling issues such as FHB susceptibility.

The isolation and characterisation of wheat BAC clones containing *Rht-A1*, *Rht-B1*, and *Rht-D1* along with flanking sequences (Chapters 2 and 3) enables the development of locus-specific primers to assess natural variation within the *Rht-1* region. The objective for this chapter is to measure nucleotide diversity and to identify unique polymorphisms and haplotypes associated with the *Rht-1* homoeoloci using three sets of wheat germplasm:

'Bread Wheat 1' (BW1), composed primarily of bread wheats that are either of agronomic significance in the UK or are closely related to 'Norin 10'; 'Bread Wheat 2' (BW2), a subset of diverse accessions from the INRA worldwide bread wheat collection (Balfourier *et al*., 2007); 'Tetraploid/ Diploid Wheat' (TDW), a small set of tetraploid and diploid wheat accessions. For each homoeologue, sequence was obtained for the entire *Rht-1* ORF along with the adjoining 1760 bp upstream and 439 bp downstream. These flanking regions encompass the two most highly conserved non-coding regions shared among the wheat homoeologues, *Brachypodium*, and rice that were identified in Chapter 3. The effect on *Rht-1* expression of indels of 160 bp and 197 bp discovered upstream of *Rht-B1* are investigated using quantitative RT-PCR (qRT-PCR).

5.2. MATERIALS AND METHODS

5.2.1. Sources of accessions used for sequencing the *Rht-1* region

Wheat germplasm used for sequencing *Rht-1* and the flanking region (*Rht-1*+flank) was received from the John Innes Centre (JIC) BBSRC cereals collection (http://www.jic.ac.uk/GERMPLAS/bbsrc_ce), the USDA-ARS National Plant Germplasm System (http://www.ars-grin.gov/npgs), the National Institute for Agricultural Research (INRA, France) worldwide core collection of bread wheats (INRA BWCC; Balfourier *et al.*, 2007), or the National Institute of Agricultural Botany (NIAB) Distinct, Uniform, and Stable (DUS) collection (http://www.niab.com). *Rht-1*+flank sequences from the A, B, and D genomes of CS are from BAC clones 0224-M10, 1417-F16, and 0155-I24, respectively (Chapter 2). The sequence of *T. urartu* was received from Jizeng Jia (Chinese Academy of Agricultural Sciences (CAAS), P.R. China), and was derived from a BAC clone (described in Chapter 3). Details of germplasm sources are shown in Table 5.1.

Table 5.1. Sequenced accessions and haplotypes

	Accessions[b]						
Set[a]	Source and Reference No.	Name	Geog. Origin	Growth Habit	Reg. Year	Type	Haplotype[c]
BW1	CS BAC library	Chinese Spring	CHN	S	na	L	A1 B1 D1
BW1	JIC_W748	Fultz	USA	W	1871	F	A2 B2 D2
BW1	JIC_W814	Gaines	USA	W	1961	F	A2 B3 D3
BW1	JIC_W741	Kanred	USA	W	1917	F	A2 B4 D1
BW1	JIC_W7208	Krasnodari 1	RUS	W	na	F	A2 B5 D1
BW1	USDA_PI156641	Norin 10	JPN	W	1935	F	A2 B6 D3
BW1	USDA_CItr13253	Norin 10/Brevor-14	USA	W	1949	F	A2 B6 D3
BW1	JIC_W614	Siete Cerros	MEX	S	1966	F	A4 B6 D2
BW1	JIC_W613	Sonora 64	MEX	S	1964	F	A3 B7 D3
BW1(UK)	NIAB DUS	Alchemy	GBR	W	2005	F	A2 B8 D3
BW1(UK)	JIC_W2	April Bearded	GBR	S	na	L	A2 B9 D4
BW1(UK)	NIAB DUS	Avalon	GBR	W	1979	F	A2 B9 D3
BW1(UK)	NIAB DUS	Cadenza	GBR	S	1992	F	A2 B3 D1
BW1(UK)	NIAB DUS	Cappelle Desprez	FRA	W	1946	F	A2 B10 D4
BW1(UK)	NIAB DUS	Hobbit 'Sib'	GBR	W	1975	F	A2 B10 D3
BW1(UK)	JIC	Mercia	GBR	W	1984	F	A2 B9 D4
BW1(UK)	NIAB DUS	Paragon	GBR	S	1998	F	A2 B11 D4
BW1(UK)	NIAB DUS	Robigus	GBR	W	2003	F	A2 B6 D2
BW1(UK)	NIAB DUS	Soissons	FRA	W	1987	F	A2 B6 D5
BW1(UK)	JIC_W8551	Squarehead's Master	GBR	W	1911	F	A2 B3 D1
BW1(UK)	NIAB DUS	Xi19	GBR	W	2002	F	A2 B3 D3
BW2	INRA_00537	CH62022	CHE	W	na	F	A2
BW2	INRA_00748	A.4	AFG	W	na	F	A5
BW2	INRA_00822	Aifeng-4	CHN	W	1971	F	A2
BW2	INRA_00957	Arawa	NZL	W	1955	F	B10
BW2	INRA_01192	Balkan	YUG	W	1979	F	D1
BW2	INRA_01697	Bung Epi Blanc	NPL	W	na	L	D1
BW2	INRA_01974	CF4563-1-5-3-2-5	FRA	W	na	F	A2
BW2	INRA_02411	Daeraad	ZAF	S	1958	F	A6
BW2	INRA_03170	Fronthatch	USA	S	1963	F	B1
BW2	INRA_03220	G72300	GRC	S	na	F	D2
BW2	INRA_03485	H93-70	ESP	W	na	F	A2
BW2	INRA_03942	JO3045	FIN	S	na	F	D1
BW2	INRA_03970	Jufy II	BEL	S	1954	F	B9
BW2	INRA_04645	Mars de Suede Rouge Barbu	FRA	S	1922	F	D1
BW2	INRA_04796	Miche	FRA	W	1954	F	A2
BW2	INRA_04901	Mocho de Espiga Bianca	PRT	S	1928	F	A2
BW2	INRA_05096	N67M2	ISR	S	na	F	D3
BW2	INRA_05260	Norin 60	JPN	S	1965	F	A2
BW2	INRA_05816	Precoce a Barbe Blanche	PRT	S	1955	F	A2
BW2	INRA_06047	Redman	CAN	S	1946	F	D1
BW2	INRA_06318	Rouge de Marchissy	FRA	W	1929	F	B1
BW2	INRA_06396	S975-A4-A1	ZWE	S	na	F	D1
BW2	INRA_06740	Strubes Dickkopf	DEU	W	1880	F	A2
BW2	INRA_07040	Tremesino Meira	ESP	W	na	L	B13
BW2	INRA_08194	Neelkant	SYR	W	1980	F	D2
BW2	INRA_08287	DC147U	FRA	W	na	F	D3
BW2	INRA_09077	Non Plus Extra	AUT	W	1919	F	B9
BW2	INRA_13310	Fruh Weizen	DEU	W	na	F	B11
BW2	INRA_13436	Fondard Crespin	FRA	W	1948	F	D1
BW2	INRA_13445	Volt	FRA	W	1994	F	D1
BW2	INRA_13471	Ornicar	FRA	W	1998	F	A7
BW2	INRA_13812	W7984 (Synthetic)	MEX	S	na	F	A6 B6 D6
BW2	INRA_13861	Auguste	FRA	W	1998	F	B10

Table 5.1 (continued). Sequenced accessions and haplotypes

	Accessions[b]						
Set[a]	Source and Reference No.	Name	Geog. Origin	Growth Habit	Reg. Year	Type	Haplotype[c]
BW2	INRA_15950	AS68VM4-3-2/TJB636 13	FRA	W	na	F	D3
BW2	INRA_23891	landrace	ARM	S	na	L	B1
BW2	INRA_23896	landrace	TUR	S	na	L	D7
BW2	INRA_23909	landrace	MAR	S	na	L	A8
BW2	INRA_23964	Thori 212-Var.8/1	PAK	S	1934	F	A9
BW2	INRA_23989	landrace	GEO	S	1931	L	D1
BW2	INRA_23995	landrace	RUS	S	1950	L	B12
BW2	INRA_23996	Guisuiskaya Syao-Bai-Mai	CHN	S	1953	F	A2 B1
BW2	INRA_24056	landrace	TUR	I	na	L	B1
BW2	INRA_24180	Palestinskaya	PAL	S	1927	F	B1
BW2	INRA_24184	landrace	PAL	S	1927	L	B1
BW2	INRA_24185	landrace	TKM	S	na	L	B1
TDW	INRA_13812	W7984 (Synthetic)	MEX	S	na	F	D6
TDW	JIC_7010073	SS7010073 (Synthetic)	na	W	na	F	A10 B14 D8
TDW	USDA_PI428054	*T. dicoccoides* 57	TUR	W	na	L	A11 B15
TDW	USDA_PI428097	*T. dicoccoides* 65	ISR	W/S	na	L	A12 B16
TDW	BAC clone	*T. urartu*	unk	unk	na	L	A13

[a] Set: These are as described in section 5.2.2. BW1 = Bread Wheat 1; BW1 (UK) denotes BW1 varieties with agronomic significance in the United Kingdom. BW2 = Bread Wheat 2; TDW = Tetraploid/ Diploid Wheats. The *Rht-D1* sequence of INRA_13812 was included as part of the BW2 and TDW sets. All BW1 and BW2 accessions are natural hexaploids except: INRA_13812 (synthetic of *T. durum* × *Ae. tauschii*) and INRA_03485 (*T. turgidum/Ae. ventricosa//T. aestivum* altarnense).

[b] *T. urartu* sequence was received from J. Jia (CAAS). JIC = John Innes Centre; INRA = French National Institute for Agricultural Research; NIAB DUS = NIAB Distinct, Uniform, and Stable germplasm collection; USDA = US Dept. of Agriculture; Geographical (Geog.) origin is shown using three-letter nation abbreviations; na = not available; unk = unknown; Reg. Year = Year of Registration; S = spring; W = winter; I = indeterminate; L= landrace; F = fixed

[c] Haplotypes were determined for the A (A1-A13), B (B1-B16), and/or D (D1-D8) genome of each accession based on *Rht-1*+flank sequence. Polymorphisms associated with each haplotype are shown in Tables 5.6 (*Rht-A1*+flank), 5.7 (*Rht-B1*+flank), and 5.8 (*Rht-D1*+flank).

CS and CS wheat group IV nullisomic-tetrasomic (NT) lines N4AT4B, N4AT4D, and N4DT4B and the CS group IV ditelosomic line Dt4BL, were supplied by S. Reader, JIC, Norwich, UK (these lines were described in section 4.2.1). DNA of CS and CS aneuploid lines were used to validate locus specific primers. CS was also used as a phenotypic and genotypic control and to verify BAC sequence.

5.2.2. Diversity sets

5.2.2.1. Bread Wheat 1 (BW1) set: Accessions and experimental design

BW1 is composed of 21 natural hexaploid wheat varieties from a mixture of backgrounds in which all three *Rht-1*+flank homoeologous regions were sequenced in each accession (Table 5.1, BW1 set). The BW1 accessions include a 'UK' subset of 12 varieties that either originated, or have been widely grown, in the UK in the last 100 years (Table 5.1, BW1(UK) set); a 'Norin 10'-related subset of seven varieties associated with the origin or early spread of the 'Norin 10'-derived *Rht-B1b* and *Rht-D1b* alleles; one variety containing the *Rht-B1e* semi-dwarf allele (Krasnodari 1); and CS. Seeds of each BW1 variety were stratified for three days at 4°C on wetted filter paper and then transferred to room temperature for one week. Following vernalisation (6 weeks at 4°C) seedlings were transferred to 1-litre pots and placed in the JIC glasshouse (supplementary lighting, 16 h daylength, 22°C day / 15°C night) beginning Nov. 10, 2008. Plants were visually assessed throughout the growth phase and off-types discarded. At maturity, plant height was measured as the distance from the soil surface to the tip of the longest tiller, excluding awns. DNA was extracted from individual seedlings as described in section 4.2.2 and individual plants were genotyped for the *Rht-B1a*/*b* and *Rht-D1a*/*b* alleles (section 5.2.3). DNA of one to four plants from each accession was bulked and used to amplify PCR products used in sequencing.

5.2.2.2. Bread Wheat 2 (BW2) set: Accessions and experimental design

Accessions that composed the BW2 set were selected by examining chromosome 4 DArT and SSR marker scores received from F. Balfourier, combined with PCR analysis to detect the presence/absence of *Rht-B1b* and *Rht-D1b* and the presence/absence of the *Rht-B1* indels identified in this Chapter. In an effort to maximise the number of *Rht-A1*+flank haplotypes selected, the sixteen accessions chosen for sequencing of this region contained the greatest combined number of chromosome 4A alleles, which

was determined using the line selection feature, simulated annealing method (N = 1000) of Powermarker v2.5 (Liu and Muse, 2005). This procedure was also used to choose accessions for sequencing of the *Rht-B1*+flank and *Rht-D1*+flank regions using markers from 4B and 4D, respectively. Two accessions that are part of the INRA BWCC, Cadenza and CS, were already sequenced in the BW1 set, so to maximise the likelihood of finding new haplotypes, alleles associated with these two lines were excluded from the selection criterion. For 4B and 4D, markers appearing to be linked to *Rht-1* were given priority over apparently unlinked markers. Unlike the BW1 set in which *Rht-1*+flank sequences of the A, B, and D genomes of each accession were sequenced, in the BW2 set only a single *Rht-1*+flank region from each accession was sequenced with the exceptions of INRA_23996 (*Rht-A1*+flank and *Rht-B1*+flank) and INRA_13812 (*Rht-A1*+flank, *Rht-B1*+flank, and *Rht-D1*+flank) (Table 5.1, BW2 set). The BW2 accessions are natural hexaploids with the following exceptions: (1) INRA_13812, which is the synthetic line 'W7984' composed of *T. durum* (AABB genomes) 'Altar 84' and *Ae. tauschii* (DD) and (2) INRA_03485 (A genome sequenced), which is a cross between *T. turgidum* (AABB) / *Ae. ventricosa* (DDNN) and *T. aestivum*. The BW2 set was grown in pots in an outdoor experiment at NIAB along with the remainder of the INRA BWCC set to collect plant height (distance from the soil surface to the tip of the longest tiller, excluding awns) data (described in section 6.2.3). For GA sensitivity data, the BW2 set was grown in the JIC glasshouse along with the rest of the INRA BWCC set (described in section 6.2.4). DNA was extracted as described in section 4.2.2 from both replicates of the INRA BWCC outdoor experiment and both replicates were genotyped for *Rht-B1a/b* and *Rht-D1a/b* (section 5.2.3) and for the *Rht-B1* promoter insertions (section 6.2.2). One DNA replicate was used for sequencing with forward primers and the other for reverse primers to provide 2× coverage.

5.2.2.3. Tetraploid/Diploid Wheat (TDW) set: Accessions and experimental design

To gauge *Rht-1* diversity in a wider pool of germplasm, *Rht-1*+flank regions that originated in tetraploid or diploid wheat accessions were sequenced. The

TDW set consisted of nine accessions used for *Rht-1*+flank region sequencing (in total, four A genome, three B genome, and two D genome *Rht-1*+flank sequences) (Table 5.1, TDW set). The *Rht-1*+flank region of all genomes was sequenced in the following lines: *T. urartu* (A genome), *T. dicoccoides* 57 (A and B genomes), *T. dicoccoides* 65 (A and B genomes), and SS7010073 (A, B, and D genomes) (Table 5.1, TDW). Ernie Sears, University of Missouri (Columbia, Missouri, USA) created SS7010073 from a cross between a *T. dicoccum* and an *Ae. tauschii* accession. It is thought that the *T. dicoccum* accession used in SS7010073 corresponds to 'JIC 1070026' and that the *Ae. tauschii* accession corresponds to 'JIC 2220053' (S. Reader, JIC, pers. comm.). The second *Rht-D1*+flank sequence used as part of the TDW set is from the synthetic INRA_13812, which was also part of the BW2 set. The D genome of this line is from *Ae. tauschii*. SS7010073 and the two *T. dicoccoides* lines were grown alongside the BW1 set. Plant phenotyping, DNA extraction, and genotyping was performed as described for the BW1 set (section 5.2.2.1).

5.2.3. *Rht-B1a/Rht-B1b* and *Rht-D1a/Rht-D1b* PCR assays

Accessions were genotyped for *Rht-B1a*/*Rht-B1b* and *Rht-D1a*/*Rht-D1b* alleles using four separate assays (Table 5.2). Each assay utilised a forward primer that was locus-specific and a reverse primer designed to be specific to the semi-dwarf causative SNP. The location of the 3' end of the reverse primers was the same as described by Ellis *et al.* (2002). For each reverse primer, a mismatch was introduced at the 3rd nucleotide from the 3' end according to Ye *et al.* (2001). PCR reactions were performed in 10 µl volumes containing 1 × Green GoTaq Reaction Buffer (Promega), 3% glycerol, 0.2 mM of each dNTP, 2 mM $MgCl_2$, 1 µM forward primer, 1 µM reverse primer, 0.25 µl *Taq* polymerase, and 20 ng DNA. The PCR profiles consisted of an initial denaturation of 5 min at 95°C, followed by 40 cycles of [95°C for 30 s, 60°C for 30 s, and 72°C for 30 s], and concluded with 72°C for 5 min. Amplification products were separated on a 1.5% agarose gel in 1 × TBE buffer and visualised under UV light with ethidium bromide.

Table 5.2. PCR primers used to identify *Rht-B1a*/*B1b* and *Rht-D1a*/*D1b*

	Primer name[a]		Expected product size (bp)[b]			
Assay	Forward	Reverse	*Rht-B1a*	*Rht-B1b*	*Rht-D1a*	*Rht-D1b*
Rht-B1a	Rht-B-F1	Rht-B1a-R2	265	NP	NP	NP
Rht-B1b	Rht-B-F1	Rht-B1b-R2	NP	265	NP	NP
Rht-D1a	Rht-D-F5	Rht-D1a-R2	NP	NP	385	NP
Rht-D1b	Rht-D-F5	Rht-D1b-R2	NP	NP	NP	385

[a] Primer sequences are shown in Appendix 1.

[b] The product sizes expected for plants containing the specified *Rht-1* allele. NP = no product

5.2.4. PCR amplification and sequencing of *Rht-1* and the flanking region

Locus-specific primers were designed to amplify products of sufficient length for sequencing of the *Rht-1* ORF and at least 1760 bp upstream and 439 bp downstream sequence in each genome. Primers were designed using primer3 software (http://frodo.wi.mit.edu/primer3). Primer pairs for amplifying PCR products are shown in Table 5.3 along with expected product sizes and coordinates. PCR products were designed to overlap each other by a minimum of 85 bp. Reaction mixtures, PCR profiles, and gel electrophoreses were as described in section 5.2.3 except annealing temperature and extension times are as shown in Table 5.3.

PCR products were purified (primers and dNTPs removed) in 10 µl reaction mixes that contained 1 × Exonuclease I buffer, 0.75 U Exonuclease I (NEB Biolabs), 0.25 U shrimp alkaline phosphatase (Promega), and 7.5 µl of PCR product by incubating at 37°C for 30 min before heating to 80°C for 15 min to deactivate enzymes. Sequencing reactions were performed in 10 µl Big Dye (Applied Biosystems (ABI) sequencing mixes that contained 1 × BigDye Sequence Buffer, 1 µl BigDye (ver. 3.1), 5% dimethyl sulfoxide (DMSO), 1 µM primer, and 2 µl purified PCR product. Sequencing primers and PCR products used in the reactions are shown in Table 5.3. PCR profiles consisted of 98°C for 1 min, followed by 25 cycles of 98°C for 10 s, 50°C for 5 s, and 60°C for 4 min. The DMSO and high denaturation temperature (98°C instead of 96°C) were used to facilitate sequencing of the G-C rich *Rht-1* PCR products.

Table 5.3 Primers used for *Rht-1* locus specific amplification and sequencing

Genome	DNA amplification					Sequencing primers	
	F/R primers[a]	Ann. temp.[b]	Ext. time[c]	Product coordinates[d]	Exp. prod. size (bp)[e]	Forward	Reverse
A	Rht-A-F4 / Rht-ABD-R10	63	01:00	-1823 to -943	880	Rht-A-F4	Rht-ABD-R10
						Rht-F04	Rht-R04
	Rht-A-F5 / Rht-R01	63	00:45	-1088 to -386	702	Rht-A-F5	Rht-R01
	Rht-A-F2 / Rht-ABD-R5	60	00:45	-593 to 81	674	Rht-A-F2	Rht-ABD-R5
	Rht-A-F3 / Rht-ABD-R6	64	01:30	-261 to 1043	1304	Rht-ABD-F4	Rht-ABD-R9
						Rht-ABD-F8	Rht-ABD-R1b
						Rht-ABD-F6	Rht-ABD-R6
						Rht-ABD-F2	
	Rht-ABD-F3 / Rht-A-R1	64	01:15	921 to 2157	1236	Rht-ABD-F3	Rht-ABD-R7
						Rht-ABD-F7	Rht-ABD-R8
	Rht-ABD-F9 / Rht-A-R2	66	01:15	1783 to 2824	1041	Rht-ABD-F9	Rht-A-R3
B	Rht-B-F4 / Rht-ABD-R10	60	01:30	-1888 to -648	1240	Rht-B-F4	Rht-R02
						Rht-F01	Rht-R03
							Rht-ABD-R10
	Rht-B-F5 / Rht-B-R3	60	01:15	-986 to -177	809	Rht-B-F5	Rht-R05
						Rht-F06	Rht-B-R3
	Rht-F03 / Rht-ABD-R5	60	00:45	-449 to 84	533	Rht-F03	Rht-ABD-R5
	Rht-B-F1 / Rht-ABD-R6	64	01:15	-58 to 1047	1105	Rht-ABD-F4	Rht-ABD-R9
						Rht-ABD-F8	Rht-ABD-R1b
						Rht-ABD-F6	Rht-ABD-R6
						Rht-ABD-F2	
	Rht-ABD-F3 / Rht-B-R1	62	01:30	925 to 2397	1472	Rht-ABD-F3	Rht-ABD-R7
						Rht-ABD-F7	Rht-ABD-R8
						Rht-ABD-F9	Rht-B-R1
D	Rht-D-F3 / Rht-R08	63	01:00	-1919 to -1049	870	Rht-D-F3	Rht-R04
						Rht-F04	Rht-R08
	Rht-D-F6 / Rht-ABD-R5	64	01:30	-1162 to 84	1246	Rht-F07	Rht-R05
						Rht-D-F2	Rht-R01
							Rht-ABD-R5
	Rht-D-F1 / Rht-ABD-R6	64	01:15	-70 to 1048	1118	Rht-ABD-F4	Rht-ABD-R9
						Rht-ABD-F1	Rht-ABD-R1b
						Rht-ABD-F2	Rht-ABD-R6
	Rht-ABD-F3 / Rht-D-R1	60	01:30	927 to 2422	1495	Rht-ABD-F3	Rht-ABD-R7
						Rht-ABD-F7	Rht-ABD-R8
						Rht-ABD-F9	Rht-D-R1

[a] Primer sequences are shown in Appendix 1.

[b] Annealing temperature in °C.

[c] Extension time in minutes:seconds.

[d] Product coordinates refer to nucleotide number relative to the start codon of *Rht-1* of 'Chinese Spring' (CS) in the respective genome with negative numbers indicating 5' sequence.

[e] Expected product size in basepairs (bp) relative to CS.

Sequenced products were precipitated in microtitre plates using the BigDye v3.1 Cycle Sequencing Kit ethanol/EDTA/sodium acetate method. Using this method, 1 µl of 125 mM EDTA, 1 µl of 3 M sodium acetate, and 50 µl of 100% ethanol were added to 10 µl of sequenced product before mixing and centrifuging at 3000 × g (4°C) for 30 min. To remove supernatant, microtitre plates were inverted and centrifuged up to a rate of 40 × g and then slowly stopped. Precipitates were washed with 70 µl of 70% ethanol and re-pelleted by centrifugation at 3000 × g (4°C) for 15 min. Ethanol was removed by inverting plates and centrifuging at 40 × g for 1 min. Pellets were air dried in the dark and resuspended in 10 µl Hi-Di formamide (ABI). Sequencing was performed using an ABI 3730 DNA Analyzer and bases called with ABI Sequencing Analysis Software, v5.1.

5.2.5. Assembly of *Rht-1* contigs and bioinformatic analyses

Nucleotide contigs were assembled using the ContigExpress package of Vector NTI (Invitrogen) and assembled sequences aligned using ClustalX (Larkin *et al.*, 2007). Amino acid alignments were performed using GeneDoc v2.6.002 software (Nicholas and Nicholas, 1997). Nucleotide diversity per site (π) (Tajima 1983) and Watterson's theta per site (θ) (Nei, 1987) were calculated using DnaSP v5 software (Librado and Rozas, 2009) on aligned sequences. Haplotype diversity (*h*) was calculated using the following formula: $h = n(1-\Sigma f^2)/(n-1)$, where *n* is the sample size and *f* is the frequency of each haplotype (Nei, 1987). Fisher's Exact Test was used to determine the probability value (two-tailed distribution) of the synonymous to non-synonymous SNP ratios between diploid/tetraploid and natural hexaploid lines and calculated using http://www.graphpad.com/quickcalcs/contingency1.cfm.

The 160 bp and 197 bp insertion sequences were queried against the TREP cereal repeat database (http://wheat.pw.usda.gov/ITMI/Repeats) and the NCBI nucleotide collection (nr/nt) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The NCBI BLAST tool was used to determine homology between the insertion

sequences and orthologous regions on the *Rht-1* BAC, *Brachypodium*, and rice sequences.

5.2.6. *Rht-1* Transcript analysis

Seeds of CS, Cadenza, SS7010073, Mercia, Paragon, Kanred, and Cappelle Desprez (sources shown in Table 5.1) were surface sterilised and stratified (4°C, two days) on wetted filter paper. Germinated seeds were transferred to petri dishes lined with filter paper and placed in a controlled environment room (22°C 16/8 hr light/dark) for five days. Shoot tissues were collected into eppendorf tubes containing a stainless steel bead and immediately snap frozen in liquid nitrogen and stored at -80°C. Three replicates, each consisting of ten seedlings, were collected for each accession; however one replicate of Cadenza and one replicate of SS7010073 were lost during the RNA extraction process.

For RNA extraction, frozen tissue was ground to a fine powder with a TissueLyser LT (Qiagen) for 30 s and RNA extracted using the RNEasy plant mini kit method (Qiagen). To remove contaminating DNA, the 'rigorous DNAse treatment' protocol was performed as described in the TURBO DNA-free procedure (Ambion). Following DNAse treatment, the concentration and purity of RNA of each sample was determined using a Picodrop spectrophotometer (Picodrop Limited, Saffron Walden, UK). Only samples with a 260/280 absorbance ratio (a measure of purity) between 1.6 and 2.0 were used for cDNA synthesis. A total of 1 µg of RNA was used to synthesise cDNA using the SuperScript III first strand synthesis reverse transcription kit (Invitrogen) following the manufacturer's instructions with a random nonamers (Invitrogen) concentration of 50 µM. RNA was removed from the RNA-cDNA duplex using RNase-H (Invitrogen). The resulting single-stranded cDNA was diluted 1:20 with nuclease-free water prior to quantitative real time PCR (qRT-PCR).

The qRT-PCR reactions were performed on a DNA engine Opticon2 Contiguous Fluorescence Detector (MJ Research Inc., Alameda, CA, USA).

cDNA was amplified using SYBR Green JumpStart Taq ReadyMix (Sigma-Aldrich) using an initial activation step of 95°C for 3 min, followed by 40 cycles of [94°C for 20 s, a primer-dependent annealing temperature for 20 s, and 72°C for 30 s], and a final polymerisation of 72°C for 10 min. Melt-curve analysis was performed from 65°C to 95°C and read every 0.5°C with a 1 s hold time. For *Rht-B1* specific amplification, a forward primer (5' – CAC TAC TAC TCC ACC ATG TTC GAT TCT CTG – 3') and reverse primer (5' – GCG GCA GGA GCA GCA GCC – 3') were used in reaction mixes with a 68.5°C annealing temperature. For *Rht-D1* specific amplification, a forward primer (5' – CCA CGA GAC GCT GGG C – 3') and reverse primer (5' – CCT TCC TTC TCC TCC ACC TTG TAG – 3') were used in reaction mixes with a 64°C annealing temperature. *Rht-B1b* and *Rht-D1b* primers were designed and qRT-PCR reaction conditions developed by Robert Saville (Saville, 2011). The internal controls for normalisation of expression were *GAPDH (Glyceraldehyde 3-phosphate dehydrogenase*) and *EF1a* (*Elongation factor 1α*) using the following primers: [*GAPDH;* forward: 5' – CCT TCC GTG TTC CCA CTG TTG – 3'; reverse: 5' – ATG CCC TTG AGG TTT CCC TC – 3'; 60°C anneal temp (McGrann *et al.*, 2009)] and [*EF1a;* forward: 5' – TGG TGT CAT CAA GCC TGG TAT GGT – 3'; reverse: 5 –ACT CAT GGT GCA TCT CAA CGG ACT – 3'; 60°C anneal temp (Coram *et al.*, 2008)]. The normalisation factor was calculated as the geometric mean of the two normalisation genes. Two technical replicates were analysed for each sample.

The average threshold cycle (C_t) was calculated from the technical replications of each sample. Data were analysed using Opticon Monitor analysis software (v 2.02; MJ Research Inc.). *Rht-B1* and *Rht-D1* expression levels were calculated relative to the normalisation factor using the ΔC_t method (Pfaffl, 2001) and corrected for primer efficiencies ($\Delta\Delta C_t$). Normalised data is presented as the mean of the three biological replicates. Statistical significance among *Rht-B1* transcript levels and among *Rht-D1* transcript levels was determined using analysis of variance (Genstat, 12th edition) by inputting normalised replicate values. RNA extraction, qRT-PCR

amplification, and data calculations were performed with the guidance of R. Saville, JIC.

5.3. RESULTS

5.3.1. PCR amplification and sequencing

Locus specificity of each primer pair used to amplify DNA of the *Rht-1*+flank region listed in Table 5.3 was confirmed using water and the following DNA templates: CS, CS-N4AT4B (or CS-N4AT4D), CS-Dt4BL, CS-N4DT4B, BAC clone 0224-M10 (CS-A), BAC clone 1417-F16 (CS-B), and BAC clone 0155-I24 (CS-D). For each primer pair, the expected product was amplified only from the specified genome. An example of locus specific primer amplification for *Rht-B1* is shown in Figure 5.1.

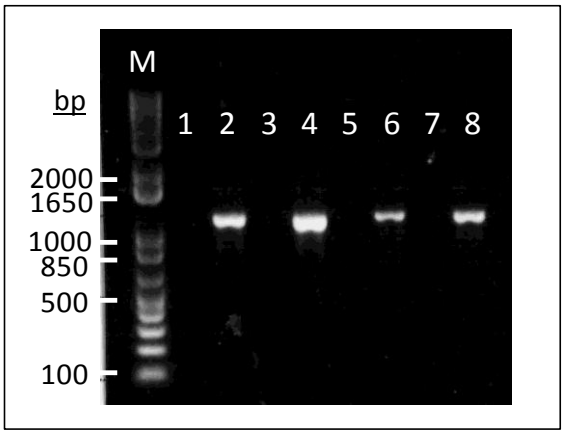


Figure 5.1. Example of *Rht-1* locus-specific amplification.
The primer pair Rht-B-F1/Rht-ABD-R6 was tested for locus-specificity using PCR. The following templates were used in PCR reactions and visualised following electrophoresis on a 1.5% agarose gel stained with ethidium bromide: [1] water; [2] genomic DNA (gDNA) of Chinese Spring (CS); [3] BAC DNA from *Rht-A1*-containing clone 0224-M10; [4] BAC DNA from *Rht-B1*-containing clone 1417-F16; [5] BAC DNA from *Rht-D1*-containing clone 0155-I24; [6] gDNA of CS nulli-tetra (NT) N4AT4D; [7] gDNA of CS ditelosomic 4BL; [8] gDNA of CS N4DT4B. The expected product size was 1105 bp. M = Invitrogen '1 Kb Plus' DNA marker with fragment sizes indicated to the left in basepairs (bp).

Locus-specific products were used for sequencing of the ORF and the flanking 5' and 3' regions of *Rht-A1*, *Rht-B1*, and *Rht-D1* in three sets of

wheat accessions. All *Rht-A1*+flank and *Rht-B1*+flank sequences are contiguous with 2 × coverage from one forward and one reverse read. To specify sequence locations in the text, nucleotide coordinates (NCs) are relative to the *Rht-1* start codon of the respective CS genome with negative numbers referring to sequence 5' of the *Rht-1* ORF. The *Rht-A1*+flank sequences of each accession cover 4122 bases from NC -1760 to NC 2362 (Figure 5.2). The *Rht-B1*+flank sequences are 4137 bp in length, extending from NC -1815 to NC 2322. The *Rht-D1*+flank sequence of each accession extends from NC -1809 to NC 2311, but there is a gap that corresponds to a 29 bp length in CS (NCs -1062 to -1034) in all D genome sequences derived from genomic DNA (all sequences except CS-D). This region contains a 19 bp long poly-C chain in CS. It was possible to amplify a PCR product that spanned this gap in each accession, but the sequencing reactions all terminated in the poly-C chain. The D-genome PCR products spanning this region from each accession were subjected to electrophoresis alongside CS in an agarose gel and stained with ethidium bromide, which did not reveal any visual evidence of a size polymorphism among the accessions (data not shown). Flanking the gap are regions (NC -1128 to -1063 and -1033 to -968) in each accession in which sequence was obtained in only one direction. For the D-genome sequence of SS7010073, single coverage upstream of the gap extended from NC -1416 to NC -1063.

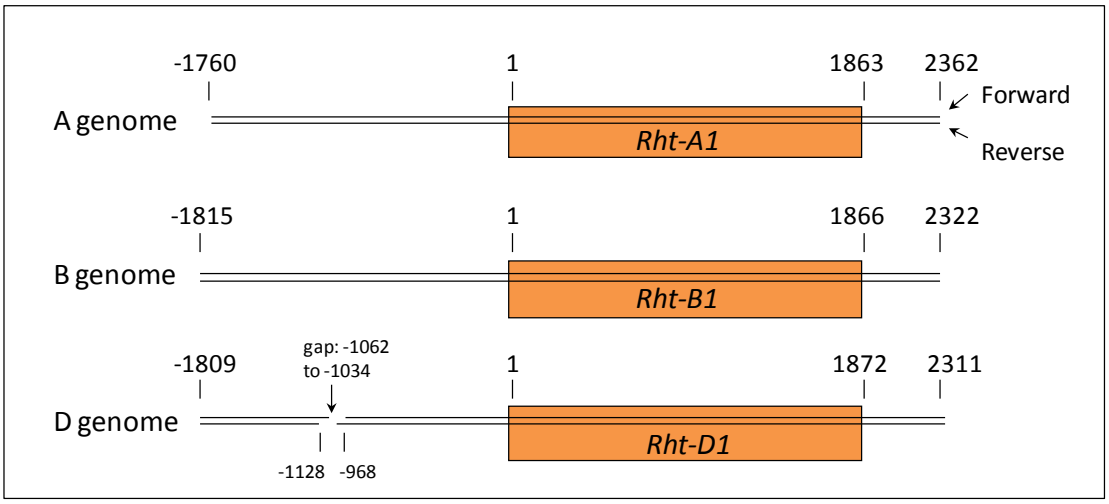


Figure 5.2. *Rht-1* and flanking regions sequenced on the A, B, and D genomes in wheat accessions. Regions of 2× coverage (one forward read and one reverse read) obtained in the accessions are indicated by the double line. *Rht-1* open reading frames (ORFs) are

indicated by orange rectangles. Nucleotide coordinates (NCs) of each genome relative to the *Rht-1* translational start codon of Chinese Spring (CS) are shown with negative numbers indicating sequence 5' of the ORF. On the D genome, an arrow indicates the location of a sequencing gap of 29 bp relative to CS that is also surrounded by a region of single coverage with boundaries indicated. The gap is present in all D genome sequences except the D genome BAC sequence from CS. On the D genome of SS7010073, the single coverage 5' of the gap extends from NC -1416 to NC -1063.

5.3.2. Genetic diversity of the *Rht-1*+flank region of the BW1 set

5.3.2.1. BW1 set: Genotypes and phenotypes

Prior to sequencing, all accessions were genotyped for *Rht-B1a/b* and *Rht-D1a/b* (Table 5.4) and only homozygous plants with the expected genotype were used for sequencing. Although Kransnodari 1 has an *Rht-B1a* genotype using this assay, it is known to contain the *Rht-B1e* allele (Worland, 1986). A previous study that used the primers specific to the *Rht-B1b* SNP developed by Ellis *et al*., 2002 also showed that the *Rht-B1a* product is amplified in accessions carrying the Kransodari 1 *Rht-B1e* allele (Pestsova *et al.*, 2008). Mean plant height varied widely among the BW1 accessions, ranging from 46 cm to 115 cm (Table 5.4). *Rht-B1* insertion allele presence/absence was determined by sequencing as described later in the chapter.

Table 5.4. Phenotype and genotype scores of the BW1 and TDW accessions.

		Genotype[b]			Height[c]			
Set[a]	Accession	*Rht-B1* ins.	*Rht-B1a/B1b*	*Rht-D1a/D1b*	Plants (no.)	Mean (cm)	Range (cm)	CV
BW1	Chinese Spring	NP	*B1a*	*D1a*	11	82	76-92	5%
BW1	Fultz	NP	*B1a*	*D1a*	6	115	101-129	8%
BW1	Gaines	NP	*B1a*	***D1b***	6	68	64-73	5%
BW1	Kanred	197	*B1a*	*D1a*	5	95	85-109	10%
BW1	Krasnodari 1	NP	*B1a*	*D1a*	6	47	40-52	10%
BW1	Norin 10	NP	***B1b***	***D1b***	7	46	43-47	3%
BW1	Norin 10/Brevor-14	NP	***B1b***	***D1b***	7	51	48-57	6%
BW1	Siete Cerros	NP	***B1b***	*D1a*	6	55	48-61	9%
BW1	Sonora 64	NP	*B1a*	***D1b***	6	60	54-64	6%
BW1(UK)	Alchemy	160	*B1a*	***D1b***	na	na	na	na
BW1(UK)	April Bearded	160	*B1a*	*D1a*	6	107	95-118	8%
BW1(UK)	Avalon	160	*B1a*	***D1b***	6	70	68-73	2%
BW1(UK)	Cadenza	NP	*B1a*	*D1a*	12	69	61-86	9%
BW1(UK)	Cappelle Desprez	197	*B1a*	*D1a*	5	90	81-93	5%
BW1(UK)	Hobbit 'Sib'	197	*B1a*	***D1b***	7	74	67-77	5%
BW1(UK)	Mercia	160	*B1a*	*D1a*	6	77	72-80	4%
BW1(UK)	Paragon	160	*B1a*	*D1a*	3	80	72-87	9%
BW1(UK)	Robigus	NP	***B1b***	*D1a*	5	64	63-67	2%
BW1(UK)	Soissons	NP	***B1b***	*D1a*	6	71	67-78	6%
BW1(UK)	Squarehead's Master	NP	*B1a*	*D1a*	5	86	76-93	8%
BW1(UK)	Xi19	NP	*B1a*	***D1b***	5	57	54-58	3%
TDW	SS7010073	197	*B1a*	*D1a*	6	82	71-93	10%
TDW	*T. dicoccoides* 57	197	*B1a*	*D1a*	4	69	61-78	10%
TDW	*T. dicoccoides* 65	197	*B1a*	*D1a*	2	87	82-91	7%

[a] Set: These are as described in section 5.2.2. BW1 = Bread Wheat 1; BW1(UK) denotes BW1 varieties with agronomic significance in the United Kingdom. TDW = Tetraploid/ Diploid Wheats.

Accession numbers are shown in Table 5.1.

[b] 160, 197 indicate the presence of 160 bp and 197 bp insertions 5' of *Rht-B1*; NP = 160 bp or 197 bp insertions are not present. *Rht-B1a/B1b and Rht-D1a/D1b* genotypes were determined using primers (shown in Table 5.2) specific to the *Rht-B1b* and *Rht-D1b* causative SNPs. Semi-dwarf (*Rht-B1b* and *Rht-D1b*) alleles are shown in bold font. Krasnodari 1 contains the *Rht-B1e* allele, but the *Rht-B1a/B1b* primer pair does not distinguish *Rht-B1a* from *Rht-B1e* (Pestsova *et al.*, 2008)

[c] Plant height was measured from soil surface to the tip of the tallest tiller for the indicated number of plants. CV = coefficient of variation. na = not available.

5.3.2.2. BW1 set: *Rht-A1*+flank diversity

Over the 4122 bp of *Rht-A1*+flank sequence there were four haplotypes (A1-A4) and 18 polymorphic sites (PS; 15 SNPs and three indels) among the 21 BW1 varieties with a nucleotide diversity (π) of 0.35×10^{-3} and haplotype diversity of 0.27 (Table 5.5, BW1 set). All SNP changes discussed in the text

will show the CS nucleotide preceding the slash "/" and the new nucleotide following the "/". CS is the only member of haplotype A1 in the BW1 set, which differs from all other BW1 haplotypes by the absence of a T nucleotide at NC -1046 (Table 5.6). To rule out the possibility that the NC -1046 T indel was due to a sequencing error in the CS-A genome BAC 0224-M10, DNA from leaf tissue of CS seedlings was amplified from this region and sequenced. A forward and reverse sequencing read of the genomic DNA confirmed the absence of the T base in CS (data not shown). Haplotype A2 is the predominant BW1 haplotype, being present in 18 of the 21 accessions (Table 5.1), and all 12 accessions in the UK subset have this haplotype. The only difference between haplotype A2 and CS is the T indel at NC -1046. Haplotype A3 is present in only one accession, Sonora 64, and differs from haplotype A2 by a single polymorphism (two polymorphisms relative to CS), a C/A SNP at NC 1430 in the ORF. The SNP results in a predicted amino acid change from serine in residue 477 of *Rht-A1* in CS to tyrosine in Sonora 64 (S477Y; as shown here, nomenclature for amino acid changes in the text will show CS residue followed by CS amino acid number followed by new amino acid). This residue is located between the conserved P and FY domains identified by Tian *et al*. (2004) (Figure 5.3, residue 477 of *Rht-A1*, coord. 483 in the figure due to insertion of gaps during alignment). The A4 haplotype is present only in Siete Cerros and contains 16 polymorphisms not found in any other BW1 haplotype and 17 polymorphisms relative to CS. All polymorphisms in Siete Cerros occur upstream of the *Rht-A1* ORF and consist of 14 SNPs and three single bp indels.

Table 5.5. Summary of diversity measurements of the sequence sets

Region (seq. length)[a]	Set[b]	Seqs. (no.)	Diversity[c]		Polymorphic sites[d]					Haplotypes		
			$\pi \times 10^{-3}$	$\theta \times 10^{-3}$	Indels (no.)	Indels (total bp)	SNPs (no.)	Total (no.)	AA changes	no.	Designations	Diversity
Rht-A1 +flank (4122 bp)	BW1	21	0.35	1.01	3	3	15	18	1	4	A1-A4	0.27
	BW1 (UK)	12	0.00	0.00	0	0	0	0	0	1	A2	0.00
	BW2	16	0.68	1.46	3	3	20	23	2	6	A2;A5-A9	0.62
	BW1+BW2	37	0.49	1.22	4	4	21	25	3	9	A1-A9	0.43
	TDW	4	5.94	6.35	16	39	48	64	0	4	A10-A13	1.00
	Overall	41	1.24	3.17	18	41	56	74	3	13	A1-A13	0.54
Rht-B1 +flank (4137 bp)	BW1	21	0.96	0.99	6	368	16	22	5	11	B1-B11	0.90
	BW1 (UK)	12	1.13	1.11	4	364	15	19	4	6	B3;B6;B8-B11	0.88
	BW2	16	0.76	0.94	5	365	14	19	5	7	B1;B6;B9-B13	0.75
	BW1+BW2	37	0.86	0.85	7	369	16	23	6	13	B1-B13	0.89
	TDW	3	4.15	4.15	4	28	27	31	1	3	B14-B16	1.00
	Overall	40	1.43	2.17	10	394	39	49	6	16	B1-B16	0.91
Rht-D1 +flank (4092 bp)	BW1	21	0.26	0.20	1	3	3	4	1	5	D1-D5	0.78
	BW1 (UK)	12	0.29	0.24	1	3	3	4	1	5	D1-D5	0.80
	BW2	16	0.20	0.29	1	1	4	5	2	5	D1-D3;D6;D7	0.67
	BW1+BW2	37	0.24	0.29	2	4	5	7	2	7	D1-D7	0.76
	TDW	2	5.59	5.59	7	11	23	30	3	2	D6-D8	1.00
	Overall	38	0.52	1.56	9	17	27	36	4	8	D1-D8	0.77

[a] Diversity measurements are for sequence lengths that cover the entire open reading frame and 5' and 3' flanking regions of *Rht-A1* (*Rht-A1*+flank), *Rht-B1* (*Rht-B1*+flank), and *Rht-D1* (*Rht-D1*+flank). Total length in basepairs (bp) is shown in parentheses.

[b] BW1 = Bread Wheat 1; BW1(UK) = subset of of BW1 lines relevant to UK wheat production; BW2 = Bread Wheat 2; TDW = Tetraploid/diploid wheats. The D genome of the synthetic wheat INRA_13812 was analyzed as part of the BW2 set and the TDW set.

[c] π = nucleotide diversity per site; θ = Watterson's theta per site.

[d] Indels = Insertion-deletions; SNPs = single nucleotide polymorphisms; AA changes = predicted amino acid changes.

Table 5.6. Summary of polymorphisms and haplotypes in the *Rht-A1*+flank region

		Haplotypes[c]											
Coord.[a]	CS (A1)[b]	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13
-1741	A			G					G		G		
-1729	C			T					T	T	T	T	T
-1714	A												T
-1671	C												T
-1654	A												-
-1635	G			A					A		A	A	A
-1620	C												T
-1610	A												-
-1527	C												G
-1413	G			C					C		C		
-1366	G			A					A		A		
-1329	T									G			
-1318	A												G
-1303	G												A
-1289	A												C
-1286	G												A
-1202	A												G
-1193	A			G					G		G		
-1155	T			C					C		C		
-1139	C			A					A		A		A
-1088	A			G					G	G	G	G	G
-1087	T			C					C	C	C	C	C
-1065	T			-					-				
-1064	-												T
-1062	C											G	
-1057	C			-					-		-		
-1056	-												CCCCCC
-1056	T												C
-1050	T			G					G		G	G	G
-1046	-	T	T	T	T	T	T	T	T	T	T	T	T
-1046	C					T							
-1037	T											C	
-1027	T												A
-1013	G			A					A		A	A	A
-998	T								G		G	G	G
-907	C												G
-894	G								A		A		
-877	A												G
-835	-									A			
-820	-								A		A	A	
-817	T												C
-808	A												T
-749	C												T
-741	T												A
-738	G												A
-732	C												G
-639	GAAAAA												-
-633	G									A			
-599	A					T		T					
-497	A												G

Table 5.6 (continued). Summary of polymorphisms and haplotypes in the *Rht-A1*+flank region

		Haplotypes[c]											
Coord.[a]	CS (A1)[b]	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13
-490	A												-
-485	T												C
-462	-												CAGTA
-455	T												-
-377	T			C					C		C		
-298	C												A
-297	C												T
-234	C												T
-183	TCGC											-	-
-156	TTC									-			
-130	C												-
-33	A			G					G		G	G	G
291	C												G
294	C												G
297	C												T
565 **aa189**	T **S**				A **T**								
994 **aa332**	G **G**						A **S**						
1245	A											G	G
1311	G												A
1430 **aa477**	C **S**		A **Y**										
2024	C											T	T
2219	ACTGT											-	-
2281	A												T
2348	G												-
Sets[d]:	bw1	bw1 UK bw2	bw1	bw1	bw2	bw2	bw2	bw2	bw2	tdw	tdw	tdw	tdw

[a] Nucleotide coordinates that mark the locations of polymorphisms present amongst the accessions are shown relative to the start nucleotide of the Chinese Spring (CS) *Rht-A1* sequence with negative numbers referring to sequence 5' of the open reading frame (ORF). Nucleotide changes occurring in the ORF are indicated in orange background with predicted amino acid (aa) changes shown in bold font directly below with affected residue.

[b] Nucleotides present in the reference sequence of CS are as shown with missing sequence relative to other haplotypes denoted by a "-". Where predicted amino acid changes occur, the amino acid present in CS is shown as a 1-letter code in bold font directly below the causative nucleotide change. The (A1) denotes that CS is representative of haplotype A1.

[c] For haplotypes A2-A13, nucleotides and amino acids are indicated in red background where haplotypes differ from CS.

[d] The sets of accessions containing the haplotypes are indicated. bw1 = bread wheat 1; UK denotes bw1 varieties with agronomic significance in the United Kingdom. bw2 = bread wheat 2; tdw = tetraploid/ diploid wheats. Accessions and haplotypes of each set are shown in Table 5.1.

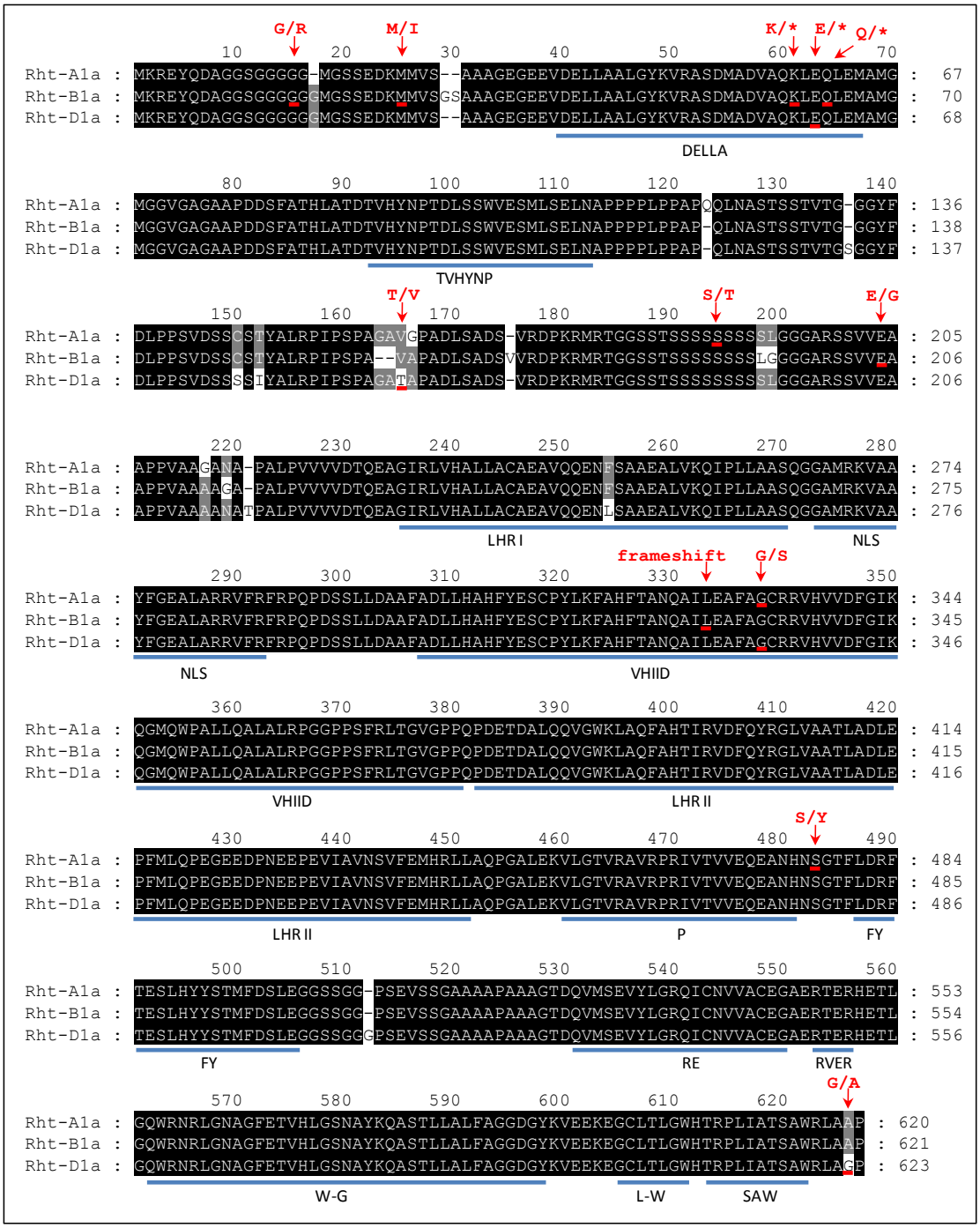


Figure 5.3. Predicted amino acid changes in the *Rht-1* ORF identified among the wheat diversity sets. The open reading frame (ORF) sequences of *Rht-A1a*, *Rht-B1a*, and *Rht-D1a* from the Chinese Spring (CS) background were aligned using ClustalX. Amino acids in black, grey, and white background are present in 3, 2, and 1 homoeologue, respectively. Coordinates to the right of each row show the amino acid number of the preceding residue in each homoeologue. Coordinates are also shown above the sequence for referral in the text, but because of the introduction of gaps (-) to facilitate alignment they do not correspond to any one sequence. Conserved protein domain motifs (Tian *et al*., 2004) are underlined in blue with name indicated below. Red arrows indicate the location of amino acid changes that occur in the diversity sets relative to CS. The affected amino acids are underlined in red

below the arrows and the amino acid change is indicated above the arrow. 'Frameshift' refers to a 1 bp nucleotide insertion that occurs at coordinate 333 in an *Rht-B1* accession.

5.3.2.3. BW1 set: *Rht-B1*+flank diversity

The 4137 bp of *Rht-B1*+flank sequence from the 21 BW1 accessions revealed 11 haplotypes (B1-B11) and haplotype diversity was 0.90 (Table 5.5, BW1 set). Nucleotide diversity was 0.96×10^{-3} and there were 22 PS (16 SNPs and 6 indels) among the accessions, which included a 160 bp and a 197 bp indel within the upstream region and five predicted amino acid changes in the *Rht-B1* ORF. Haplotype B1 consisted only of CS, which differed from the other BW1 haplotypes by one to 15 polymorphisms (Table 5.7). There were four *Rht-B1*+flank haplotypes (B2, B3, B6, and B7) that each differed from CS by only one polymorphism. Haplotype B2 differs from CS by a 3 bp deletion downstream of the ORF (NC 1982) and is only present in the line 'Fultz'. The B3 haplotype is found in four BW1 varieties (Table 5.1) and has a G/T SNP 5 bp downstream of the ORF (NC 1871). The B6 haplotype contains *Rht-B1b* and all five lines with this allele have identical sequences in the *Rht-B1*+flank region. As first reported by Peng *et al.* (1999), the semi-dwarf nature of *Rht-B1b* results from a C/T SNP at NC 190 that converts *Rht-B1* AA64 from a glutamine (Q) to a stop codon (*) (Figure 5.3, coord. 64). The B7 haplotype is only present in Sonora 64 and differs from CS by a 3 bp deletion at NC 2191, which is part of a CTA repeat-rich region 3' of the ORF.

The semi-dwarf line, Krasnodari 1 contains the *Rht-B1*e allele and is the only member of haplotype B5 in the BW1 set. Relative to CS, Krasnodari 1 has three polymorphisms including an A/T SNP in the ORF at NC 181. The ORF SNP is a nonsense mutation that converts residue 61 of *Rht-B1* from lysine (K) to a stop codon (*) (Figure 5.3, coord. 61). The stop codon occurs in the DELLA domain at a position that is only three amino acids upstream of the residue that is converted to a stop codon in *Rht-B1b* and only two amino acids upstream of the equivalent residue on the D genome that is converted to a stop codon in *Rht-D1b*.

Table 5.7. Summary of polymorphisms and haplotypes in the *Rht-B1*+flank region

		Haplotypes[c]														
Coord.[a]	CS (B1)[b]	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16
-1487	A							T		T				T		
-1210	T															C
-1198	G			T				T		T				T	T	T
-1180	G															A
-1177	T													C		
-1140	-				C											
-1053	A													G		
-1003	T															C
-911	T			C						C				C		C
-907	G														A	
-867	TTTCT														-	
-738	G			A						A				A	A	A
-694	intact													-16		
-638	C													T		
-634	T			C						C						
-629	C													T		
-591	-			+197						+197				+197	+197	+197
-591	-			G						G				A	G	G
-591	-			C						C				C	G	C
-591	-			A						A				A	G	A
-591	-			T						T				C	T	T
-460	A															G
-391	G													C		
-356	-							+160	+160		+160					
-322	G													T		
-243	C													-		
-231	T														G	
-131	C			T						T			T	T	T	T
-68	C													A		
-39	T															C
43 **aa15**	G **G**			C **R**						C **R**			C **R**			
75 **aa25**	G **M**			A **I**						A **I**				A **I**		A **I**
181 **aa61**	A **K**				T *****											
190 **aa64**	C **Q**					T *****										
614 **aa205**	A **E**							G **G**	G **G**		G **G**					
618	T															C
723	A			G						G				G	G	G
984 **aa328**	- **L**											T **FS**				
1584	C													G		
1734	G														A	
1761	A			C						C				C	C	
1871	G		T													
1877	T			-						-						
1931	G													A		
1982	AAG	-														

Table 5.7 (continued). Summary of polymorphisms and haplotypes in the *Rht-B1*+flank region

		Haplotypes[c]														
Coord.[a]	CS (B1)[b]	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16
2086	C														G	
2167	C			T						T				T	T	T
2191	CTA CTA			CTA	CTA		CTA			CTA	CTA CTA CTA		CTA CTA CTA	-		
2221	A			T						T				T	T	T
Sets[d]:	bw1 bw2	bw1	bw1 UK	bw1	bw1	bw1 UK bw2	bw1	bw1 UK	bw1 UK bw2	bw1 UK bw2	bw1 UK bw2	bw2	bw2	tdw	tdw	tdw
Alleles[e]:					*B1e*	*B1b*										

[a] Nucleotide coordinates that mark the locations of polymorphisms present amongst the accessions are shown relative to the start nucleotide of the Chinese Spring (CS) *Rht-B1* sequence with negative numbers referring to sequence 5' of the open reading frame (ORF). Nucleotide changes occurring in the ORF are indicated in orange background with predicted amino acid (aa) changes shown in bold font directly below with affected residue. Coordinate -591 marks a 197 bp insertion along with 4 SNPs that occur within the insertion.

[b] Nucleotides present in the reference sequence of CS are as shown with missing sequence relative to other haplotypes denoted by a "-". Intact refers to the presence of sequence in CS that is deleted in another haplotype. Where predicted amino acid changes occur, the amino acid present in CS is shown as a 1-letter code in bold font directly below the causative nucleotide change. The (B1) denotes that CS is representative of haplotype B1.

[c] For haplotypes B2-B16, nucleotide and amino acids are indicated in red background where haplotypes differ from CS. "+197" indicates the presence of a 197 bp insertion; "+160" indicates the presence of a 160 bp insertion; "-16" indicates a 16 bp deletion; "*" = predicted stop codon; "FS" = amino acid frameshift.

[d] Accession sets containing each haplotype are indicated. bw1 = bread wheat 1; UK denotes bw1 varieties with agronomic significance in the United Kingdom. bw2 = bread wheat 2; tdw = tetraploid/ diploid wheats. Accessions and haplotypes of each set are shown in Table 5.1.

[e] Previously identified GA insensitive alleles are indicated.

Three of the remaining haplotypes (B8, B9, and B11) in the BW1 set contain a 160 bp insertion relative to CS at NC -356. No sequences with significant similarity were found when the 160 bp was queried against the TREP cereal repeat database, the NCBI nucleotide collection (nr/nt), or the CS *Rht-1*-containing BAC sequences. Each of the three haplotypes also contains an A/G SNP in the ORF (NC 614) that gives rise to a predicted E205G change (Figure 5.3, coord. 209). The change occurs in the N-terminal region of *Rht-B1* and is outside of the conserved domains. These two polymorphisms

constitute the only difference between CS and haplotype B9, which is present in three BW1 accessions (April Bearded, Avalon, and Mercia). The accession Alchemy is the only member of haplotype B8, which contains two SNPs (NC -1487 and -1198) in addition to those found on haplotype B9. Haplotype B11 is only represented in Paragon among the BW1 set, differing from haplotype B9 by a CTA insertion in the repeat rich region 3' of the ORF (NC 2191).

The remaining two haplotypes found in the BW1 set (B4 and B10) each contain a 197 bp insertion upstream of the *Rht-B1* ORF relative to CS (NC -591). No sequences of significant similarity were found when the 197 bp insertion sequence (from Cappelle Desprez) was queried against the TREP cereal repeat database, the NCBI nucleotide collection (nr/nt). However, homologous sequence is present in nearly equivalent regions on the *Rht-1*-containing BAC sequences from the A and D genomes. On the A genome, the region (NC -883 to -702) has significant similarity (2×10^{-55}) and 86% identity to the 197 bp insertion. On the D genome, significant homology (1×10^{-57}) and 86% identity to the insertion was found between NC -872 to -685. No homology was found when the 197 bp insertion was queried with the upstream regions (1 bp to 10 000 bp upstream) of rice or *Brachypodium*. The B4 haplotype, in addition to the 197 bp insertion, contains 13 polymorphisms relative to CS (11 SNPs, indels of 1 and 3 bp) and is present only in the accession Kanred. Four of the polymorphisms (all SNPs) occur in the *Rht-B1* ORF and two result in predicted amino acid changes (G15R (Figure 5.3, coord. 15) and M25I (Figure 5.3, coord. 25)) that occur upstream of the DELLA domain. The B10 haplotype is composed of two accessions (Cappelle Desprez and Hobbit 'Sib') and differs from the B4 haplotype by an A/T SNP at NC -1487. The UK subset of *Rht-B1*+flank sequences contained six haplotypes (B3, B6, B8-B11) of the eleven present in the BW1 set and 19 of the 22 PS (Table 5.5). The PS in the UK subset included both of the large indels and four of the five predicted amino acid changes present in the full BW1 set (the haplotype B5 stop codon is absent). Among the UK lines, haplotype diversity was 0.88 and $\pi = 1.13 \times 10^{-3}$.

5.3.2.4. BW1 set: *Rht-D1*+flank diversity

For the 4092 bp of *Rht-D1*+flank sequence, a total of four PS (3 SNPs; 1 indel) and five haplotypes (D1-D5) exist among the 21 BW1 accessions with $\pi = 0.26 \times 10^{-3}$ and haplotype diversity = 0.78 (Table 5.5, BW1 set). CS and four other bread wheat accessions comprise the D1 haplotype, which differs from the other haplotypes by one to two polymorphisms (Table 5.8).

Table 5.8. Summary of polymorphisms and haplotypes in the *Rht-D1*+flank region

		Haplotypes[c]						
Coord.[a]	CS (D1)[b]	D2	D3	D4	D5	D6	D7	D8
-1809	G							A
-1795	-						A	AA
-1720	G							A
-1567	G							A
-1508	C							G
-1501	-							CTA
-1368	G							A
-1367	T				C			
-1360	G							-
-1326	A							G
-1055	C							A
-1053	A							C
-970	A							T
-740	-							A
-680	A							-
-630	G							A
-479	A					G		G
-383	G	T		T				
-322	G							A
-304	C							A
-137	T							C
-106	C							T
-32	GA							-
181 **aa61**	G **E**		T *					
483 **aa162**	GACG **T**							CGTC **V**
1000 **aa334**	G **G**					A **S**		
1266	G							A
1865 **aa622**	G **G**							C **A**
1987	AAG							-
2066	T							C
2137	TTC			-				
2306	G							T
Sets[d]:	bw1 UK bw2	bw1 UK bw2	bw1 UK bw2	bw1 UK	bw1 UK	bw2 tdw	bw2	tdw
Alleles[e]:			*D1b*					

[a] Nucleotide coordinates that mark the locations of polymorphisms present amongst the accessions are shown relative to the start nucleotide of the Chinese Spring (CS) *Rht-D1* sequence with negative numbers referring to sequence 5' of the open reading frame (ORF). Nucleotide changes occurring in the ORF are indicated in orange background with predicted amino acid (aa) changes shown in bold font directly below with affected residue indicated.
[b] Nucleotides present in the reference sequence of CS are as shown with missing sequence relative to other haplotypes denoted by a "-". Where predicted amino acid changes occur, the amino acid present in CS is shown as a 1-letter code in bold font directly below the causative nucleotide change. The (D1) denotes that CS is representative of haplotype D1.
[c] For haplotypes D2-D8, nucleotide and amino acids are indicated in red background where haplotypes differ from CS.
[d] The sets of accessions containing the haplotypes are indicated. bw1 = bread wheat 1; UK denotes bw1 varieties with agronomic significance in the United Kingdom. bw2 = bread wheat 2; tdw = tetraploid/diploid wheats. Haplotype D6, which contains only INRA_13812 was used as part of the bw2 and tdw sets. Accessions and haplotypes of each set shown in Table 5.1.
[e] Previously identified GA insensitive alleles are indicated.

Haplotype D2 of the BW2 set differs from CS by a single G/T SNP at NC -383. Haplotype D3 consists of the eight accessions that contain the *Rht-D1b* semi-dwarf allele (Table 5.1). The D3 sequence is identical to CS with the exception of the *Rht-D1b* causative SNP (G/T) at residue 61 of *Rht-D1* (Peng *et al.*, 1999), which converts a glutamic acid (E) residue in CS to a stop codon (*) (Figure 5.3, coord. 63). Haplotype D4 is present in four BW1 accessions and contains the upstream SNP (NC -383) found in haplotype D2 and a 3 bp deletion downstream of the *Rht-1* ORF (NC 2137). Haplotype D5 is present in a single accession (Soissons) and differs from CS by only a T/C SNP (NC -1367). The UK subset of accessions contain all of the *Rht-D1*+flank BW1 haplotypes and PS with $\pi = 0.29 \times 10^{-3}$ and haplotype diversity = 0.80.

5.3.3. Genetic diversity of the *Rht-1*+flank region of the BW2 set

5.3.3.1. BW2 set: Genotype and phenotype results

Chromosome IV marker data was used in an attempt to select accessions with the most diverse 4A, 4B, and 4D chromosomes amongst the INRA BWCC for sequence analysis. The lines selected for sequencing of the *Rht-*

A1+flank region contained 116 of the 147 4A alleles found amongst the INRA BWCC, the lines selected for sequencing of *Rht-B1*+flank contained 72 of the 93 4B alleles, and the lines selected for sequencing of *Rht-D1*+flank contained 39 of the 52 4D alleles (data not shown). A wide range of plant heights (54-189 cm when grown in the UK) and GA treatment differences (difference in mean seed-to-first-ligule (STFL) lengths of seedlings in the GA+ treatment minus STFL lengths of seedlings in the GA- treatment, which is a measure of GA sensitivity; described in Chapter 6) of 1.5 to 50.3 mm occur among the sequenced accessions (Table 5.9). The GA sensitivity and plant heights of the sequenced accessions cover the ranges found in the entire INRA BWCC as presented in Chapter 6. Among the accessions selected for *Rht-B1*+flank sequencing, the alleles *Rht-B1a*, *Rht-B1b*, the 160 bp insertion, and the 197 bp insertion are each represented. Among the *Rht-D1*+flank sequences, the *Rht-D1a* and *Rht-D1b* alleles are both represented.

Table 5.9. Genotype and phenotype summary of the Bread Wheat 2 (BW2) set

Accessions[a]			Genotype[b]			Phenotype[c]	
Code	Name	Genomes seq.[d]	*Rht-B1* ins.	*Rht-B1a/B1b*	*Rht-D1a/D1b*	Plant ht. (cm)	GA trt. diff. (mm)
INRA_00537	CH62022	A	160	*B1a*	*D1a*	114	19.6
INRA_00748	A.4	A	NP	*B1a*	*D1a*	160	25.7
INRA_00822	Aifeng-4	A	197	*B1a*	***D1b***	86	13.9
INRA_00957	Arawa	B	197	*B1a*	*D1a*	137	27.3
INRA_01192	Balkan	D	NP	*B1a*	*D1a*	107	36.2
INRA_01697	Bung Epi Blanc	D	NP	*B1a*	*D1a*	157	50.3
INRA_01974	CF4563-1-5-3-2-5	A	NP	***B1b***	*D1a*	94	2.2
INRA_02135	Chinese Spring	-	NP	*B1a*	*D1a*	136	38.5
INRA_02411	Daeraad	A	197	*B1a*	*D1a*	130	33.5
INRA_03170	Fronthatch	B	NP	*B1a*	*D1a*	140	41.0
INRA_03220	G72300	D	NP	*B1a*	*D1a*	146	21.3
INRA_03485	H93-70	A	NP	*B1a*	*D1a*	172	24.3
INRA_03942	JO3045	D	NP	*B1a*	*D1a*	146	23.2
INRA_03970	Jufy II	B	160	*B1a*	*D1a*	127	29.7
INRA_04645	Mars De Suede Rouge Barbu	D	NP	*B1a*	*D1a*	183	33.3
INRA_04796	Miche	A	160	*B1a*	*D1a*	121	22.8
INRA_04901	Mocho De Espiga Bianca	A	197	*B1a*	*D1a*	135	38.3
INRA_05096	N67M2	D	NP	***B1b***	***D1b***	54	1.5
INRA_05260	Norin 60	A	NP	*B1a*	***D1b***	100	1.7
INRA_05816	Precoce A Barbe Blanche	A	197	*B1a*	*D1a*	153	15.8
INRA_06047	Redman	D	NP	*B1a*	*D1a*	137	17.7
INRA_06318	Rouge De Marchissy	B	NP	*B1a*	*D1a*	189	23.8
INRA_06396	S975-A4-A1	D	NP	*B1a*	*D1a*	89	17.4
INRA_06740	Strubes Dickkopf	A	160	*B1a*	*D1a*	146	20.0

Table 5.9 (continued). Genotype and phenotype summary of the Bread Wheat 2 (BW2) set

Accessions[a]			Genotype[b]			Phenotype[c]	
Code	Name	Genomes seq.[d]	*Rht-B1* ins.	*Rht-B1a/B1b*	*Rht-D1a/D1b*	Plant ht. (cm)	GA trt. diff. (mm)
INRA_07040	Tremesino Meira	B	NP	*B1a*	*D1a*	155	36.3
INRA_08194	Neelkant	D	NP	*B1b*	*D1a*	121	19.0
INRA_08254	Cadenza	-	NP	*B1a*	*D1a*	105	31.0
INRA_08287	DC147U	D	197	*B1a*	***D1b***	118	4.7
INRA_09077	Non Plus Extra	B	160	*B1a*	*D1a*	149	28.7
INRA_13310	Fruh Weizen	B	160	*B1a*	*D1a*	167	20.0
INRA_13436	Fondard Crespin	D	NP	*B1a*	*D1a*	165	27.0
INRA_13445	Volt	D	NP	***B1b***	*D1a*	91	11.3
INRA_13471	Ornicar	A	NP	***B1b***	*D1a*	90	7.7
INRA_13812	W7984	A B D	NP	***B1b***	*D1a*	112	14.2
INRA_13861	Auguste	B	197	*B1a*	***D1b***	80	8.4
INRA_15950	AS68VM4-3-2/TJB636 13	D	197	*B1a*	***D1b***	106	5.7
INRA_23891	Landrace	B	NP	*B1a*	*D1a*	142	15.0
INRA_23896	Landrace	D	NP	*B1a*	*D1a*	161	24.8
INRA_23909	Landrace	A	NP	*B1a*	*D1a*	144	40.7
INRA_23964	Thori 212-Var.8/1	A	NP	*B1a*	*D1a*	129	32.5
INRA_23989	Landrace	D	NP	*B1a*	*D1a*	171	21.3
INRA_23995	Landrace	B	NP	*B1a*	*D1a*	150	21.8
INRA_23996	Guisuiskaya Syao-Bai-Mai	A B	NP	*B1a*	*D1a*	126	34.2
INRA_24056	Landrace	B	NP	*B1a*	*D1a*	176	36.3
INRA_24180	Palestinskaya	B	NP	*B1a*	*D1a*	139	33.7
INRA_24184	Landrace	B	NP	*B1a*	*D1a*	184	27.0
INRA_24185	Landrace	B	NP	*B1a*	*D1a*	152	30.0

[a] The BW2 accessions are a subset of the INRA BWCC and the genotype and phenotype data for the whole collection are presented in Chapter 6.

[b] 160, 197 indicate the presence of a 160 or a 197 bp insertion 5' of *Rht-B1*; NP = 160 or 197 bp insertion not present. Semi-dwarf (*Rht-B1b* and *Rht-D1b*) alleles are shown in bold font.

[c] Plant heights were measured using plants grown outdoors in pots at NIAB (Cambridge, UK) as described in section 6.2.3. GA treatment difference (GA trt. diff) refers to difference in mean seed-to-first-ligule (STFL) lengths of seedlings in the GA+ treatment minus STFL lengths of seedlings in the GA- treatment (described in section 6.2.4).

[d] Cadenza and Chinese Spring (both listed as "-") were sequenced as part of the BW1 set using different seed sources and are included for genotypic and phenotypic comparisons.

5.3.3.2. BW2 set: *Rht-A1*+flank diversity

The 16 *Rht-A1*+flank sequences from the BW2 set contained 23 PS (20 SNPs and 3 indels) and six haplotypes (A2 and A5-A9) with $\pi = 0.68 \times 10^{-3}$ and haplotype diversity = 0.62 (Table 5.5, BW2 set). Five of the haplotypes (A5-

A9) and seven of the polymorphisms (Table 5.6), including two predicted amino acid changes were not present in the BW1 set. Similar to the BW1 set, all accessions contained the NC -1046 T insertion, which was not present in CS. Also similar to the BW1 set, the most common BW2 haplotype is A2, which is present in 10 of the 16 accessions. (Table 5.1). The five novel haplotypes in BW2 differed from CS by 2 to 20 polymorphisms. Haplotype A5 is present in one BW2 line (INRA_00748) and differed from A2 by a single SNP in the ORF (NC 565, T/A) that leads to a S189T predicted amino acid change. The change occurs outside of a conserved domain and in the middle of a 9-residue poly-serine string (Figure 5.3, coord. 194). Haplotype A6 is contained in two BW2 lines and has two SNPs (NC -1046 and -599) not present in haplotype A2. Haplotype A7 is represented by a single line (INRA_13471) and differs from haplotype A2 by a single SNP in the ORF (G/A at NC 994, G332S). The predicted change occurs in the VHIID protein domain (Figure 5.3, coord. 338). Haplotype A8 differs from haplotype A2 by a single SNP at NC -599 (also found in the A6 haplotype) and is present in only INRA_23909. The last haplotype of the BW2 set, A9, is represented by a single accession, INRA_23964, and has 19 polymorphisms not found in any other BW2 line and differs from CS by a total of 20 polymorphisms, all of which are 5' of *Rht-A1*. This haplotype is most closely related to the A4 haplotype (from BW1), differing by only three PS (NC -998, -894, and -820).

5.3.3.3. BW2 set: *Rht-B1*+flank diversity

The 16 accessions of the BW2 set that were sequenced for *Rht-B1*+flank contained seven haplotypes (B1, B6, and B9-B13) and 19 PS (14 SNPs and 5 indels) with $\pi = 0.76 \times 10^{-3}$ and haplotype diversity = 0.75 (Table 5.5, BW2 set. Only two haplotypes (B12 and B13) and one PS not present in the BW1 set were discovered in this set (Table 5.7). The novel PS is a 1 bp T insertion in the *Rht-B1* ORF that leads to a predicted frameshift mutation in haplotype B12, which is present in only INRA_23995. The *Rht-B1*+flank nucleotide sequence of haplotype B12 is otherwise identical to CS. The T insertion precedes NC 984 and occurs near the beginning of the C terminus of the *Rht-*

B1 protein (residue 328 of *Rht-B1*) in the VHIID protein motif (Figure 5.3, coord. 333). The resulting protein is predicted to contain 633 amino acids with a stop codon encoded by NCs 1899 to 1901. The other new haplotype in the BW2 set, haplotype B13, differs from CS by three polymorphisms previously identified in the BW1 set: a C/T SNP (NC -131), a G/C SNP (NC 43) leading to a predicted G15R change, and a CTA insertion (NC 2191), which did not occur together in other haplotypes. This haplotype is present only in the line INRA_07040. The remaining 14 accessions have previously identified haplotypes (Table 5.1). Eight of the accessions contained the B1 haplotype, which was only found in CS among the BW1 set. A single accession (INRA_13812) contained the *Rht-B1b* semi-dwarf allele and it has sequence identical to the BW1 lines carrying this allele (haplotype B6). The remaining accessions belonged to haplotypes B9 (two accessions), B10 (two accessions), and B11 (one accession).

5.3.3.4. BW2 set: *Rht-D1*+flank diversity

Among the 16 BW2 *Rht-D1*+flank sequences there are 5 haplotypes (D1-D3, D6-D7) and 5 PS (4 SNPs and 1 indel) with $\pi = 0.20 \times 10^{-3}$ and haplotype diversity = 0.67 (Table 5.5, BW2 set). Two of the haplotypes (D6 and D7) and three of the PS were not found in the BW1 set (Table 5.8). Two of the novel PS are found in haplotype D6, which otherwise is identical to the CS haplotype. One of these is a G/A SNP that occurs in the ORF at NC 1000 leading to a predicted G334S change (Figure 5.3, coord. 338). The residue change occurs in the VHIID conserved domain and at the equivalent residue where a glycine/serine substitution occurs on the A genome in haplotype A7. The second SNP (A/G) in this haplotype occurs upstream of the *Rht-D1* ORF (NC -479). The sole member of this haplotype is the synthetic hexaploid INRA_13812. The second novel haplotype, D7, contains the third polymorphism not present in the BW1 set, a 1 bp insertion relative to CS at NC -1795. This is the only polymorphism that distinguishes this haplotype (present only in INRA_23896) from CS. Nine of the remaining 14 accessions have sequence identical to CS, belonging to haplotype D1 (Table 5.1). Two

accessions belong to haplotype D2. Lastly, three BW2 accessions contain the *Rht-D1b* allele and have sequence identical to the BW1 accessions carrying the *Rht-D1b* allele and belong to haplotype D3.

5.3.4. Genetic diversity of the *Rht-1*+flank region of the TDW set

5.3.4.1. TDW set: Genotype and phenotype results

Three of the TDW lines (*T. dicoccoides* 57, *T. dicoccoides* 65, and SS7010073) were phenotyped and genotyped alongside the BW1 lines (Table 5.4). The D genome of INRA_13812 is derived from *Ae. tauschii* so the *Rht-D1*+flank sequence was analysed as part of the TDW set in addition to being analysed as part of the BW2 set. Phenotype and genotype data of INRA_13812 is shown in Table 5.9. *T. urartu* was not grown. *Rht-B1b* and *Rht-D1b* were not present among the TDW sequences.

5.3.4.2. TDW set: *Rht-A1*+flank diversity

There are 64 PS (16 indels and 48 SNPs) among the four A genome sequences of the TDW set and 49 of the PS are not present in the two BW (BW = combined BW1 and BW2) sets and $\pi = 5.94 \times 10^{-3}$ (Table 5.5). The four *Rht-A1*+flank sequences from the TDW set each comprise unique haplotypes (A10-A13) not present among the BW sets (Table 5.6). There are no *Rht-A1*+flank PS that distinguish all of the TDW sequences from all of the BW sequences. Only five of the 64 PS found among the TDW sequences are located in the *Rht-A1* ORF and none result in a predicted amino acid change. Of the 16 indels present in the TDW *Rht-A1*+flank sequences, the largest is six bp in length. The TDW *Rht-A1*+flank sequence most similar to CS is SS7010073 (haplotype A10), which has an A genome derived from domesticated emmer (*T. dicoccum*). Haplotype A10 differs from CS by 8 PS (7 SNPs and a 3 bp indel), four of which were not present in any of the BW sequences. The wild emmers *T. dicoccoides* 57 (haplotype A11) and *T. dicoccoides* 65 (haplotype A12) differ from CS by 19 polymorphisms (18

SNPs and 1 bp indel) and 16 polymorphisms (14 SNPs, a 4 bp indel, and a 5 bp indel), respectively. Haplotype A11 most closely resembles the BW haplotypes A9 (differing by only a single bp insertion at NC -1065) and A4 (four polymorphisms). Haplotype A12 contains six polymorphisms not found in the BW sets. Of all the A genome sequences, *T. urartu* (haplotype A13) contained the largest number of PS (53) relative to CS and 39 of these, which is more than half of all the *Rht-A1*+flank PS found among all the accessions, are unique to *T. urartu*. The TDW lines share several of the polymorphisms that separate them from CS and the other BW sequences, but also differ from one another by at least 15 polymorphisms.

5.3.4.3. TDW set: *Rht-B1*+flank diversity

The three TDW *Rht-B1*+flank sequences are each unique haplotypes (B14-B16) that are not present in the BW sets (Table 5.7). Among these sequences $\pi = 4.15 \times 10^{-3}$ and there are 31 PS (27 SNPs and 4 indels), 27 of which are not are not present among any of the BW sequences (Table 5.5). All three of the TDW haplotypes contain the 197 bp insertion and none contain the 160 bp insertion. A 16 bp deletion also occurs in SS7010073 (haplotype B14) relative to CS and this deletion is not found in any other accession. Similar to the A genome, there are no B genome PS that distinguished all of the TDW accessions from all of the BW lines. Among the 31 PS in the *Rht-B1*+flank region of the TDW lines, there are five in the ORF and only one of these leads to a predicted amino acid change. The PS leading to a predicted amino acid change (M25I) was previously identified in the BW accessions (Figure 5.3, coord. 25) and is present in haplotype B14 and *T. dicoccoides* 65 (haplotype B16). Haplotype B14 is the most dissimilar of the three TDW haplotypes relative to the CS haplotype, differing by 23 polymorphisms while *T. dicoccoides* 57 (haplotype B15) and haplotype B16 differed from CS by 13 and 15 polymorphisms, respectively. The fewest PS found between a BW haplotype and a TDW haplotype is 11, which occurs between haplotypes B4 and B16.

5.3.4.4. TDW set: *Rht-D1*+flank diversity

The two *Ae. tauschii* D genome *Rht-D1*+flank sequences analysed in the TDW set are derived from the synthetic hexaploids SS7010073 and INRA_13812 (W7984). Each *Ae. tauschii* sequence represents a unique haplotype (SS7010073 = haplotype D8 and INRA_13812 = haplotype D6; Table 5.8). There are 30 PS (23 SNPs and 7 indels) between the two *Rht-D1*+flank haplotypes and $\pi = 5.59 \times 10^{-3}$ (Table 5.5, TDW set). None of the PS found in haplotypes D6 and D8 were present among the BW accessions (haplotype D6 is considered only as part of the TDW set in this and the following comparison). Unlike the A and B genome *Rht-1*+flank sequences, a polymorphism was identified (an A/G SNP at NC -479) that distinguishes the D genome haplotypes of the TDW set from the D genome haplotypes of the BW set.

As described previously in the BW2 set, haplotype D6 differs from CS by only two PS (Table 5.8). In contrast, haplotype D8 differs by 30 PS, which consist of 23 SNPs and seven indels that range in size from 1 to 3 bp. Six of the D8 haplotype SNPs occur in the *Rht-D1* ORF and result in two predicted amino acid changes. Four of the ORF SNPs are consecutive (NC 483 to 486) resulting in a GACG/CGTC change. The SNPs at NC 484 to 486 result in a T162V change on *Rht-D1* (Figure 5.3, coord. 165). The second predicted amino acid change that occurs between CS and haplotype D8 involves a G/C SNP at NC 1865 that results in a G622A predicted amino acid change (Figure 5.3, coord. 626). Both of the amino acid changes occur outside of conserved protein domains. The SS7010073 nucleotides and amino acids associated with the T162V and G622A changes on *Rht-D1* match the *Rht-A1* and *Rht-B1* sequences of CS (Figure 5.3), indicating that the SS7010073 *Rht-D1* sequence is more closely related to the ancestral *Rht-1* sequence of wheat than are CS or the other BW *Rht-D1* sequences.

To determine the sources of the *T. dicoccum* and *Ae. tauschii* lines used to create the SS7010073 synthetic, the *Rht-1* genes of *T. dicoccum* accession

'JIC 1070026' and *Ae. tauschii* accession 'JIC 2220053', which are thought to be the SS7010073 parental lines (S. Reader, JIC), were sequenced. For 'JIC 1070026', the sequence of the *Rht-A1* and *Rht-B1* ORFs and the flanking 500 bp upstream and 439 bp downstream perfectly matched the colinear sequences in SS7010073. Similarly, for 'JIC 2220053', the *Rht-D1* ORF and flanking 500 bp 5' and 439 bp 3' perfectly matched the colinear D genome sequence of SS7010073. These results suggest that 'JIC 1070026' and 'JIC 2220053' are likely the parents of SS7010073.

5.3.5. Comparisons of the *Rht-1*+flank regions among the A, B, and D genomes of the bread wheat sets

Among the three genomes of the two BW sets, the B genome contains the greatest level of nucleotide diversity ($\pi = 0.86 \times 10^{-3}$), followed by the A ($\pi = 0.49 \times 10^{-3}$) and D ($\pi = 0.24 \times 10^{-3}$) genomes (Table 5.5, BW1+BW2). However, in terms of Watterson's theta, the genomes rank A > B > D. The B genome contains the greatest number of haplotypes (13) relative to the A (9 haplotypes) and D (7 haplotypes) genomes in BW1+BW2 and has the highest haplotype diversity (0.89) followed by the D (0.76) and A genomes (0.43). The low haplotype diversity among the A genome sequences results from the predominance of a single haplotype (A2) that accounts for 28 of the 37 (76%) of the *Rht-A1*+ flank sequences among the BW1+BW2 sets (Figure 5.4). Similarly, the D genome sequences belong mainly to two haplotypes (D1 and D3), which contain 25 of the 37 (68%) of the *Rht-D1*+ flank sequences. However, on the B genome, no haplotype accounts for more than nine (25%) of the *Rht-B1*+ flank sequences.

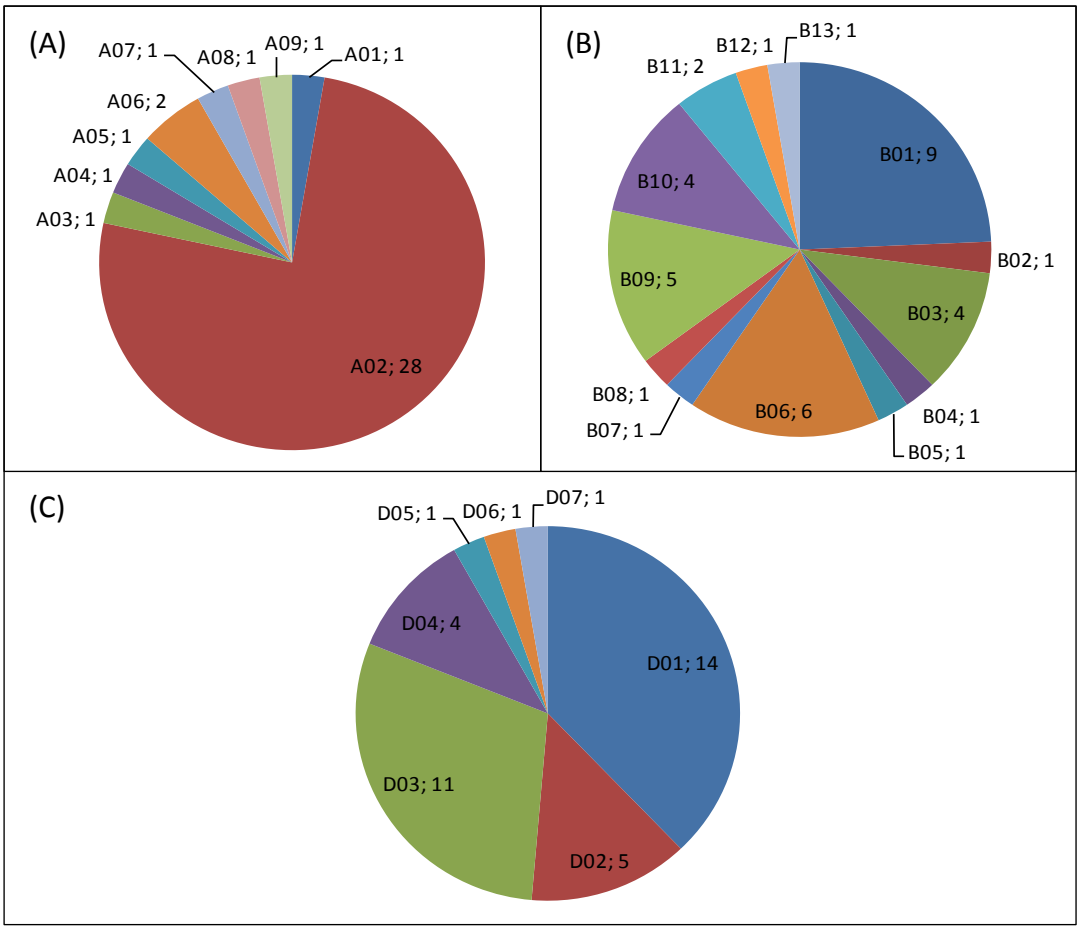


Figure 5.4. Distribution of haplotypes among the (A) A genome, (B) B genome, and (C) D genome sequences of the bread wheat (BW1 and BW2) sets. Haplotype designations are shown followed by the number of accessions (37 accessions per genome) containing that haplotype.

In the bread wheat sequences, the largest number of *Rht-1* PS occur on the A genome (25 PS; 0.61% of the sites polymorphic) followed by the B genome (23 PS; 0.56% of the sites polymorphic), and the D genome (7 PS; 0.17% of the sites polymorphic) (Figure 5.5). The distribution of PS among the 5', ORF, and 3' regions varied greatly among the genomes of the bread wheats. For the A genome, the frequency of PS in the 5' region was seven fold larger than in the ORF and no PS were found in the 3' region. In the B genome, the frequency of PS was greatest in the 3' region. For each genome, the frequency of PS in the ORF was less than that of the non-coding regions; however, this difference was less pronounced in the B genome. The B genome contains eight of the 13 ORF PS and six of the 11 predicted amino acid changes among the bread wheats. Averaged across the three genomes

of the BW accessions (Figure 5.5, Bread Wheat ABD), the frequency of PS in the *Rht-1* ORF was less than half of that found in the 5' or 3' regions. This trend was even more pronounced in the TDW lines, in which the frequency of PS in the 5' and 3' regions was 11 times and 3 times higher, respectively, than in the ORF (Figure 5.5; Tetraploid/Diploid wheat ABD).

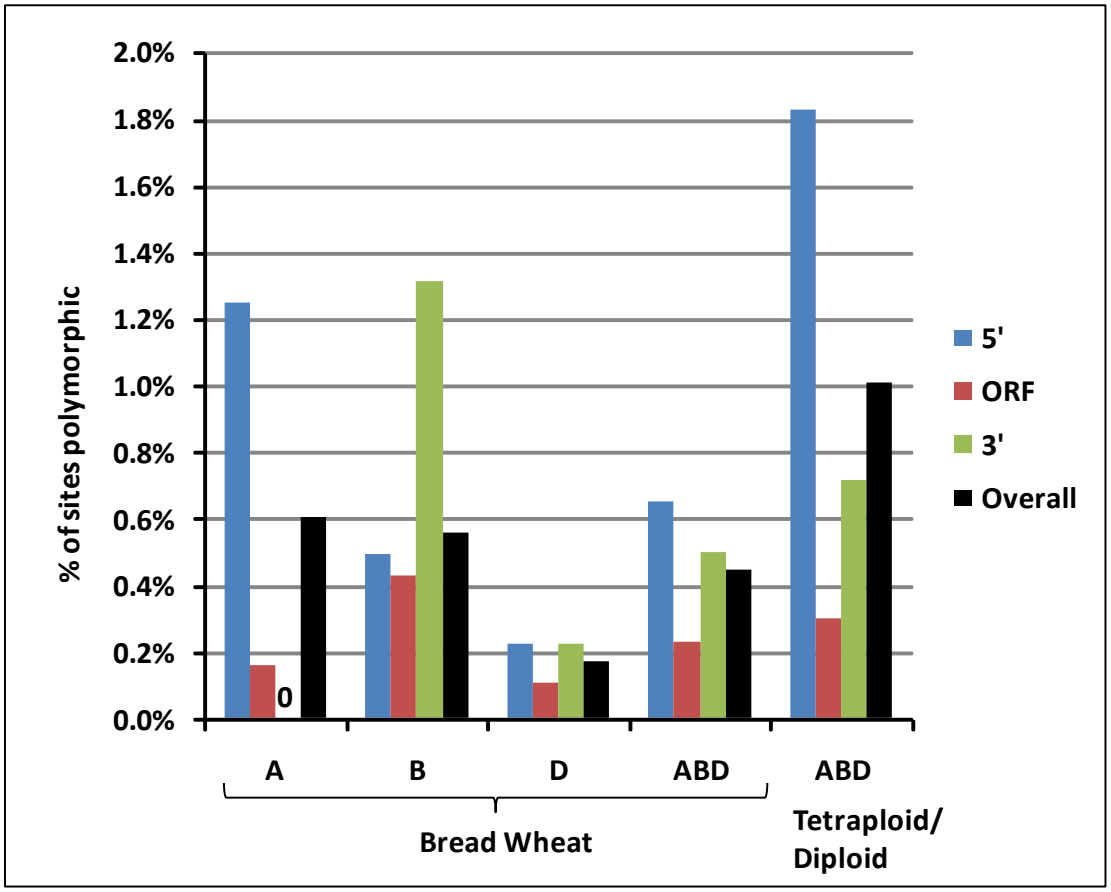


Figure 5.5. Percentage of sites that are polymorphic in the *Rht-1* open reading frame (ORF) and flanking 5' and 3' regions in the bread wheat and tetraploid/diploid accessions. For the bread wheat accessions, the A, B, and D genomes are shown separately and then combined (ABD).

5.3.6. Predicted amino acid changes in *Rht-1*

Among the *Rht-1*+flank sequences of the natural hexaploids (all sequences from the BW sets excluding the D genome of INRA_13812), there are twelve ORF polymorphisms that result in ten predicted amino acid changes (Tables 5.5 to 5.8). Among the nine TDW sequences, there are 15 ORF polymorphisms and four predicted amino acid changes (Tables 5.5 to 5.8).

Excluding the frameshift mutation and the three PS from previously known semi-dwarfing alleles the non-synonymous: synonymous SNP ratios for the natural hexaploid wheats is 6:2 and for TDWs is 4:11 (Figure 5.6). Using Fisher's exact test, the association between SNP type (non-synoymous versus synonymous) and wheat type (TDW versus BW) was found to be statistically significant at $p = 0.04$.

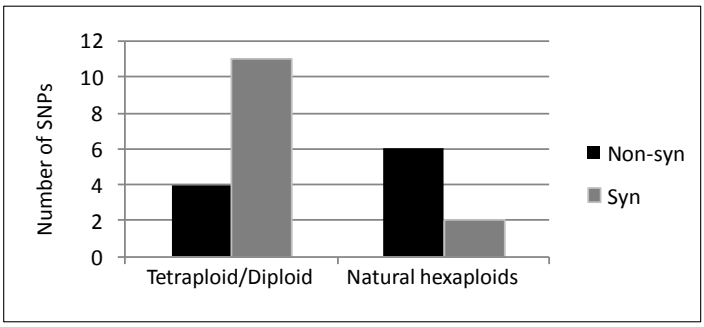


Figure 5.6. Number of *Rht-1* ORF missense SNPs leading to predicted amino acid changes in the tetraploid/diploid (TDW) and natural hexaploids.

5.3.7. Effect of the 160 bp and 197 bp Insertions on expression of *Rht-B1*

To determine if the 160 bp and 197 bp *Rht-B1* insertions affected *Rht-B1* expression, *Rht-B1* and *Rht-D1* transcript abundance was measured using qRT-PCR on three sets of lines: (1) lines with no insertion (CS and Cadenza); (2) lines with the 160 bp insertion (Mercia and Paragon); (3) lines with the 197 bp insertion (Kanred, Cappelle Desprez, and SS7010073). The SS7010073 line also carries a 16 bp deletion relative to the other lines. Transcript abundance was normalised relative to *GAPDH* and *EF1a*. Of the five lines with the *Rht-B1* insertions, only Kanred shows a statistically significant difference ($p < .01$) in normalised *Rht-B1* transcript amount relative to CS or Cadenza (Figure 5.7). Whilst normalised Kanred *Rht-B1* transcript levels were less than 25% of those found in CS or Cadenza, the remaining four lines with *Rht-B1* insertions have only a slight reduction in *Rht-B1* transcript level relative to CS and Cadenza, which is not significant at $p = .05$. To determine if the *Rht-B1* insertions differentially affected the expression levels of *Rht-B1* relative to other *Rht-1* homoeologues, *Rht-D1* transcript levels were measured. Similar to the *Rht-B1* results, Kanred showed the lowest transcript

level after normalisation (less than 25% CS or Cadenza), which was significant at $p < .01$. The Cappelle-Desprez *Rht-D1* transcript level was also significantly reduced relative to CS and Cadenza ($p < 0.05$), but was still 64% and 75% of Cadenza and CS, respectively. In Kanred, the expression levels of *GAPDH* and *EF1a* normalisation genes were unusually high relative to the other accessions, which accounts for most of the reduction in *Rht-B1* and *Rht-D1* expression levels relative to the other accessions.

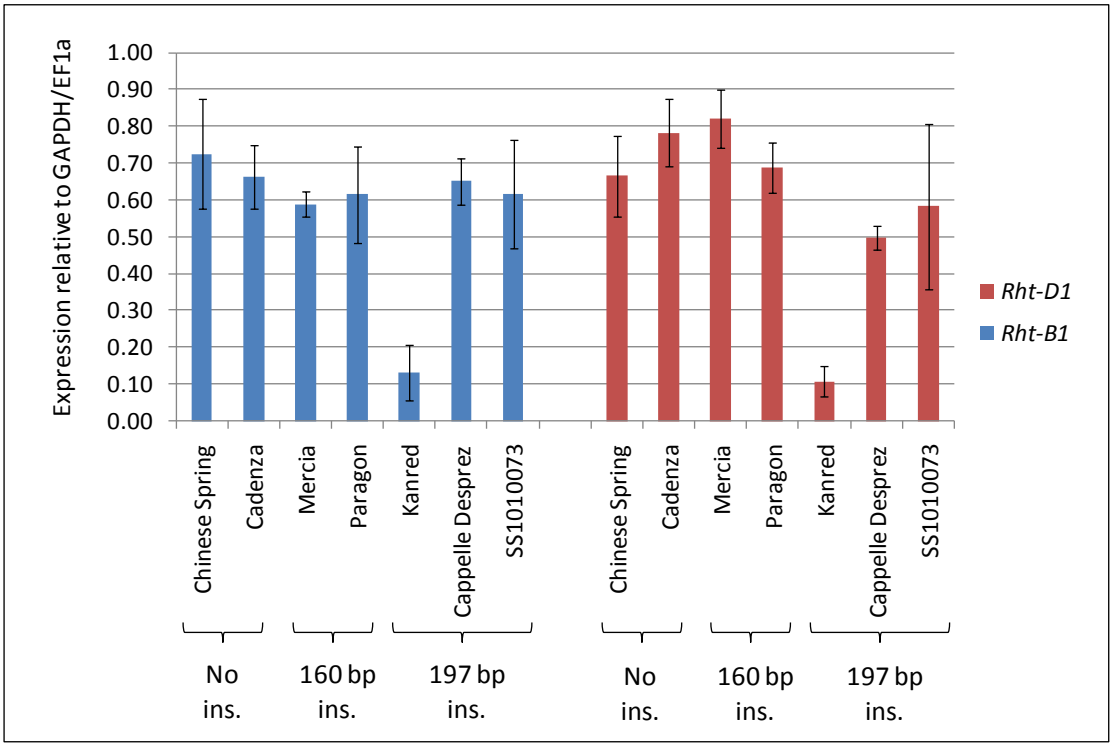


Figure 5.7. Expression of *Rht-B1* and *Rht-D1* in accessions with the *Rht-B1* 160 bp insertion (160 bp ins.), *Rht-B1* 197 bp insertion (197 bp ins.), or neither insertion (No ins.). Normalised data is presented as the mean of the three biological replicates and error bars denote the 95% confidence interval (2 × the standard error of the mean) of each sample. GAPDH = Glyceraldehyde 3-phosphate dehydrogenase, EF1a = Elongation factor 1α.

5.4. DISCUSSION

Over the combined distances of the A, B, and D genome *Rht-1*+flank regions (12,351 bp) from the Bread Wheat sets (BW1+ BW2; 37 sequences from each genome), the total number of polymorphic sites (PS) is 55 (42 SNP sites; 13 indel sites) and nucleotide diversity (π) is 0.54×10^{-3}. The frequency of *Rht-1*

SNP sites (1 SNP/294 bp) and PS (1 PS/225 bp) falls within the range reported in comparable bread wheat diversity studies while π values are relatively low. The following values were reported in studies in which genes (coding and non-coding sequences) from the A, B, and D genomes were represented: 1 SNP/91 bp, $\pi = 1.83 \times 10^{-3}$ for the *Spa1* homoeologues among 42 accessions (Ravel *et al.*, 2009); 1 SNP/212 bp, $\pi = 0.9 \times 10^{-3}$ for 21 genes among 27 accessions (Ravel *et al.*, 2006); 1 SNP/441 bp for the *GAMYB* homoeologues from 42 accessions (Haseneyer *et al.*, 2008); 1 SNP/540 bp of wheat EST sequence from 12 accessions (Somers *et al.*, 2003). In a study that examined only the A and B genomes of bread wheat, 1 PS/362 bp and $\pi = 0.83 \times 10^{-3}$ existed among 41 accessions (Haudry *et al.*, 2007). The combined distance of the BW *Rht-1* ORF homoeologous sequences (5601 bp) contained a low frequency of polymorphic sites relative to non-coding regions, a pattern also seen in other bread wheat genes (Ravel *et al.*, 2006; Haseneyer *et al.*, 2008; Ravel *et al.*, 2009).

The nucleotide diversity ($\pi = 0.41 \times 10^{-3}$) of the BW *Rht-1* ORF accessions is only a fraction of the values reported for the ORF of *Rht-1* orthologues in maize, sorghum, or pearl millet (*Pennisetum glaucum*). For maize, the nearly full-length *D8* (an *Rht-1* orthologue) sequences of 92 inbreds from three diverse gene pools had a π value of 1.8×10^{-3} (4 times that in wheat *Rht-1*) (Thornsberry *et al.*, 2001). For sorghum, the partial-length sequences of the *Rht-1* orthologue analysed in 26 inbreds from West and Central Africa averaged had $\pi = 1.63 \times 10^{-3}$ (4 times that in wheat *Rht-1*) (Li *et al.*, 2010). In pearl millet, the partial-length sequences of the *Rht-1* orthologue measured in 20 inbreds from West and Central Africa had $\pi = 7.04 \times 10^{-3}$ (17 times more than wheat *Rht-1*) (Li *et al.*, 2010). The relatively low diversity observed in the bread wheat *Rht-1* coding region relative to the *Panicoideae* species may be the result of an overall reduction in the diversity of bread wheat relative to these species and is in agreement with the findings of Wicker *et al.* (2009) that relative to maize, sequence diversity within wheat genes is minimal. Reduced *Rht-1* diversity in wheat relative to maize, sorghum, and pearl millet may also have resulted from extremely strong selection for the semi-dwarfing

alleles *Rht-B1b* and *Rht-D1b* over the last 50 years that would be expected to increase the frequency of these two haplotypes at the expense of other haplotypes, although evidence of some selection at the *D8* locus has also been reported in maize (Thornsberry *et al.*, 2001). Differences in experimental sets (*e.g.* unequal representations of the total diversity in each of the crops) between studies represents another factor that contributes to the interspecific differences reported here.

The A genome of the BW sets contained an intermediate number of haplotypes (9), the lowest haplotype diversity (0.43), an intermediate nucleotide diversity value ($\pi = 0.49 \times 10^{-3}$), and the highest number (25) of polymorphic sites (Table 5.5, *Rht-A1*:BW1+BW2). Although the bread wheat A genome sequences contain the largest number of PS among the genomes, 80% result from only two haplotypes (A4 and A9) and 76% of all A genome sequences belong to a single haplotype (A2) (Figure 5.4). For the UK subset, all 12 *Rht-A1*+flank UK sequences were identical and belonged to single haplotype, A2 (Table 5.5). The lack of *Rht-A1*+flank diversity in the UK subset relative to the other BW sequences is surprising because the UK bread wheats captured most of the *Rht-B1*+flank and *Rht-D1*+flank diversity and haplotypes. In contrast, genetic diversity of the A genome of UK bread wheat varieties was previously found to be significantly higher than on the B and D genomes (White *et al.,* 2008). Also, while selection at *Rht-B1* and *Rht-D1* is known to have occurred, no known selection has occurred at the *Rht-A1* locus. The lack of *Rht-A1* diversity in UK wheats suggests the possibility that this locus may be linked to an additional trait that has been selected for in UK bread wheat varieties. The A4 and A9 haplotypes, which contain 80% of the *Rht-A1*+flank PS in the BW sets differ by only three polymorphisms and are each present in only one accession. Both haplotypes are closely related to the A11 haplotype from *T. dicoccoides 57* with A9 differing by one polymorphism and A4 differing by four polymorphisms. A4 is present in ‘Siete Cerros’, a Mexican line released from the CIMMYT programme in the 1960s that also contains the *Rht-B1b* allele. Of the Mexican varieties exported to developing nations, offspring of ‘Siete Cerros’ (also known as ‘cross 8156’

and by several other designations) was the most widely planted as of 1974 (Dalrymple, 1976) suggesting this haplotype may be fairly widespread. Haplotype A9 is present in INRA_23964 (Thori 212-Var.8/1), a fixed line that was released in Pakistan in 1934 (F. Balfourier, pers. comm.), which does not contain *Rht-B1b* or *Rht-D1b* (see Appendix VII). A4 and A9 represent potentially useful sources of diversity in the *Rht-A1* region, which could prove useful considering the overall lack of haplotype diversity around *Rht-A1*.

Among the three genomes sequenced from the BW accessions, the *Rht-B1*+flank sequences contained the greatest number of haplotypes (13), the highest haplotype diversity (0.89), the highest nucleotide diversity ($\pi = 0.86 \times 10^{-3}$), and an intermediate number of polymorphic sites (23) (Table 5.5, *Rht-B1*+flank:BW1+BW2). Indels of 16 bp, 160 bp, and 197 bp exist among the BW *Rht-B1*+flank sequences whereas no indel greater than 3 bp exists among the equivalent *Rht-A1*+flank or *Rht-D1*+flank BW genome sequences. In addition, six of the eleven polymorphisms predicted to alter the *Rht-1* amino acid sequence in the BW sets occur on the B genome. In previous studies, higher genetic diversity on the B genome relative to the A and D wheat genomes was found based on nucleotide sequence (Ravel *et al.*, 2006; Haseneyer *et al.*, 2008; Li *et al.*, 2010) and genetic marker (Huang *et al.*, 2002; Wang *et al.*, 2007) comparisons. The UK subset of 12 bread wheats represented much of the diversity found among the 37 bread wheats as six of the 13 B genome haplotypes and 19 of the 23 PS were found in the UK subset, including both large indels and five of the six predicted amino acid changes.

The three large *Rht-B1*+flank indels (16 bp, 160bp, and 197 bp) are all located within 1 kb of the start codon (Table 5.7). The furthest upstream indel (NCs -694 to -679 relative CS) is a 16 bp deletion that occurs in only the synthetic line SS7010073, which has a B genome derived from *T. dicoccum*. The largest indel is 197 bp (inserted between NC -592 and -591 of CS) and the insertion is present in five of the bread wheat lines (Kanred; Cappelle Desprez; Hobbit 'Sib'; INRA_00957 (‘Arawa’); INRA_13861 (‘Auguste’)) and

all three TDW accessions ('SS7010073'; *T. diccocoides* 57; *T. dicoccoides* 65). Presence of the 197 bp insertion in all of the sequenced ancestral lines and homologous sequence in colinear regions upstream of *Rht-A1* and *Rht-D1* indicate that the intact sequence is the ancestral condition and that CS and the majority of the bread wheat accessions examined here have the 197 bp sequence deleted. Colinear regions in rice and *Brachypodium* do not have significant homology to the 197 bp sequence and no similarity to the sequence was found in BLAST searches of the TREP cereal repeat database or the NCBI nucleotide collection (nr/nt). Of the three indels, the 160 bp indel occurs nearest to the *Rht-B1* start codon (inserted between NCs -357 and -356 of CS). Eight bread wheat lines (Alchemy; April Bearded; Avalon; Mercia; Paragon; INRA_03970 ('Jufy II'); INRA_09077 ('Non Plus Extra'); INRA_13310 ('Fruh Weizen')) and no TDW lines contain the insertion. There is no significant homology to the 160 bp insertion on the *Rht-A1*- or *Rht-D1*-containing BAC clones indicating that the 160 bp indel is an insertion relative to the ancestral condition. No similarity to the 160 bp insertion was found in BLAST searches of the TREP cereal repeat database or the NCBI nucleotide collection (nr/nt). Interestingly, the 160 bp insertion falls in the middle of the only sequence 5' of *Rht-1* that is highly conserved among the three CS *Rht-1* homoeologues, rice and *Brachypodium* (Table 4.4, BLAST hit 1), suggesting the possibility that a *cis*-regulatory region may be disrupted by the insertion.

The *Rht-B1* indels did not clearly affect *Rht-B1* transcript levels using stem and leaf tissue collected from 5-day old seedlings (Figure 5.7). A decrease in normalised expression levels was seen in accessions with the 16 bp, 160 bp, or 197 bp insertions relative to lines with the insertion; however, except for Kanred (197 bp insertion), these reductions were slight and not below the significance threshold of $p = 0.05$. In Kanred, normalised *Rht-B1* transcript levels were reduced 75%, however Kanred had unusually high transcript levels of the two normalisation genes (*GAPDH* and *EF1a*) while absolute *Rht-B1* and *Rht-D1* expression levels were only slightly reduced relative to the other lines. Hence, further experiments are required to confirm this reduction. While no clear effect of the *Rht-B1* insertion alleles on *Rht-B1* transcript level was evident in this experiment, this could relate to the age and tissue type

assayed. *Rht-1* expression patterns in wheat have previously been shown to differ based on tissue type and developmental stage (R. Saville, 2011) and expression of *SLN1* and *SLR1* (the *Rht-1* orthologues in barley and rice, respectively) were also found to differ based on tissue type (Chandler *et al*., 2002; Kaneko *et al*., 2003). To more fully determine whether the *Rht-B1* upstream indels affect expression of this gene it will be necessary to analyze more tissues and to sample at multiple developmental stages. The effect of the *Rht-B1* indels on plant height is not easily determined with the limited set of data available and this will be investigated further in a larger accession set in Chapter 6.

For the *Rht-1*+flank region of the bread wheat varieties, the D genome contained the least genetic variation, having the fewest haplotypes (7), an intermediate haplotype diversity value (0.76), the lowest nucleotide diversity ($\pi = 0.24 \times 10^{-3}$), and the lowest number of polymorphic sites (7) (Table 5.5, *Rht-D1*:BW1+BW2). There were no large indels in the bread wheat sequences and no amino acid changes outside of the previously described *Rht-D1b* causative SNP. No haplotypes on the D genome differed by more than four polymorphisms and two haplotypes represent 68% of the sequences. Other studies of wheat have also found the D genome to be the least diverse based on nucleotide sequence (Chao *et al.*, 2009) or marker variation (Bryan *et al.*, 1997; Huang *et al.*, 2002; Wang *et al.*, 2007; White *et al.*, 2008). In the current study, reduced diversity in the D genome of the BW sets may be artificially enhanced by the inclusion of 11 lines containing *Rht-D1b* whereas only six lines with *Rht-B1b* were included in the B genome set. The 12 UK bread wheats represented most of the *Rht-D1*+flank diversity found among the bread wheats, containing five of the six haplotypes and four of the five PS (excluding the INRA_13812 synthetic accession, which has a D genome derived from *Ae. tauschii*). The paucity of polymorphisms in the *Rht-D1*+flank area and the lack of predicted amino acid changes in the bread wheat D genomes relative to the tetraploid/diploid wheats suggest that ancestral lines will be important sources of diversity in this region.

The nine *Rht-1*+flank sequences derived from the TDW accessions represented haplotypes not present in the BW sets (Tables 5.6, 5.7, and 5.8). Although the TDW set consisted of only nine sequences (D genome sequence of INRA_13812 synthetic included), it contained a combined total of 126 PS (2.4 times more than found among the 110 bread wheat sequences) and an average of 1 PS/98 bp. Among the four A genome TDW sequences, there were 64 PS in the *Rht*+1 flank region (2.5 times more than among the 37 bread wheat A genomes) and 49 were found only in the TDW accessions. Relative to the CS *Rht-A1*+flank sequence, *T. urartu* differed by 52 polymorphisms while the *T. dicoccoides* lines differed by 16 to 19 polymorphisms, which agrees with prior reports that the A genome of bread wheat is more closely related to *T. dicoccoides* than *T. urartu* (Dvorak and Akhunov, 2005). Excluding the *T. urartu* sequence, the *Rht-A1*+flank nucleotide diversity among the remaining emmer wheats ($\pi = 3.08 \times 10^{-3}$) was still over six times greater than among the A genome sequences of the BW sets. The *Rht-A1*+flank haplotypes of the TDW lines deviated from the bread wheat haplotypes (excluding A4 and A9, described above) by seven or more polymorphisms. The increased diversity of the three emmer sequences compared to the BW set was seen also in the *Rht-B1*+flank sequences, with nucleotide diversity ($\pi = 4.17 \times 10^{-3}$) of the B genomes of the *T. dicoccoides* and *T. dicoccum* accessions being over four times greater than among BW B genome sequences. The most similar *Rht-B1*+flank sequences between the TDW and BW sets differed by 11 polymorphisms. At the *Rht-1*+flank region over both the A and the B genomes, 78% of the nucleotide diversity of the *T. dicoccoides* lines ($\pi = 3.20 \times 10^{-3}$) is absent in the bread wheat lines ($\pi = 0.7 \times 10^{-3}$), which is similar to the 69% loss found between *T. dicoccoides* and the A and B genomes of bread wheat (Haudry *et al.*, 2007). The two D genome TDW sequences are from SS7010073 and INRA_13812 (W7984), which are synthetics that both have D genomes derived from *Ae. tauschii*. Between these two sequences there are 30 PS (over seven times more than found among the BW D genome sequences) and none of the PS are present among any other D genome sequences. This reduced diversity in the D genome of bread wheat relative to *Ae. tauschii* is in agreement with the work of Caldwell

et al. (2004) who reported a 30-fold reduction in genetic diversity of bread wheat relative to *Ae. tauschii* based upon analysis of the diversity of *granule-bound starch synthase* (*GBSS*). The *Rht-D1*+flank sequences of INRA_13812 and CS differ by only two polymorphisms whereas the *Rht-D1*+flank regions of SS7010073 and CS differ by 30 polymorphisms. A high degree of similarity between the D genomes of INRA_13812 and bread wheat was also reported for *GAMYB* where two polymorphisms were present (Haseneyer *et al.*, 2008) and for nine of ten D-genome derived genes from bread wheat that differed from the INRA_13812 by one or no polymorphisms (Ravel *et al.*, 2006).

A total of 13 polymorphisms (12 SNPs and a 1 bp indel) that occur in the *Rht-1* coding region resulted in amino acid changes. Three of the SNPs occur in the DELLA motif and result in a stop codon (nonsense mutation) and these are associated with the *Rht-B1b*, *Rht-D1b*, and *Rht-B1e* semi-dwarfing alleles. No sequence variation occurred among the *Rht-B1b* haplotypes or among the *Rht-D1b* haplotypes, most likely due to the recent introduction (beginning in 1960) of both alleles into Western wheat varieties from a single source, Norin 10 (Gale and Youssefian, 1985; Dalrymple, 1986). The *Rht-B1b* and *Rht-D1b* haplotypes each differ from CS by only the causative SNP, indicating that the semi-dwarf mutations may have arisen in a variety closely related to CS. Similar geographic origins of CS (a Chinese landrace) and Norin 10 (a Japanese line that may have been derived from Korea (Cho *et al*., 1980)) support this hypothesis. The third nonsense mutation occurs on the B genome of the accession Krasnodari 1, which contains the *Rht-B1*e semi-dwarfing allele (Worland, 1986). The presence of the stop codon at *Rht-1* amino acid coordinate 61 (Figure 5.3) in the DELLA domain just three residues upstream of the *Rht-B1b* stop codon and two residues upstream of the respective position of the *Rht-D1b* stop codon on the D genome indicates that this polymorphism likely causes or contributes to the semi-dwarf stature of *Rht-B1e*. The 1 bp insertion leading to a frameshift occurs on the B genome of INRA_23995 at *Rht-1* amino acid coordinate 333 (Figure 5.3), which is in the conserved VHIID protein domain. INRA_23995 is a Russian landrace that has a height near the median of the BW2 accessions that do not

contain the *Rht-B1b* or *Rht-D1b* semi-dwarf alleles (Table 5.9). The nearest stop codon following the frameshift occurs at coordinates 1899-1901, resulting in a predicted protein length of 633 amino acids. The VHIID domain is part of the C-terminus, which acts as the repression domain in SLR1 in barley (Itoh *et al.*, 2002). A frameshift mutation in this location would be expected to lead to a LoF, GA-constitutive growth response phenotype of elongated and slender stems and leaves (Ikeda *et al*., 2001; Gubler *et al*., 2002), although mutations in the C-terminus that reduce GA sensitivity and produce a dwarf phenotype have been reported in *Brassica* rapa (Muangprom *et al*., 2005) and maize (Lawit *et al*., 2010). However, due to the buffering effects of *Rht-A1* and *Rht-D1*, only a dominant or semi-dominant mutation is likely to produce an observable phenotype. However, the *Rht-B1* LoF mutation in combination with the *Rht-D1b* GAI allele could reduce the buffering capacity at this locus (1 GAI DELLA protein: 1 GA sensitive DELLA protein instead of 1 GAI: 2 GA sensitive), which may result in a stronger dwarfing phenotype.

The remaining nine PS in the *Rht-1* ORFs are missense changes, with only one of these (Figure 5.3 coord. 338 (G to S) occurring in a conserved domain. G338S occurs in the VHIID domain and occurs in the *Rht-A1* ORF of INRA_13471 and in the *Rht-D1* ORF of INRA_13812. This amino acid is otherwise conserved in the CS wheat homoeologues, and in the *Brachypodium*, barley, rice, maize, and sorghum *Rht-1* orthologues (Figure 3.3, coord. 357) and the *Arabidopsis GAI* orthologue (Tian *et al.*, 2004), suggesting this may be an important amino acid. The remaining missense polymorphisms occur outside of the conserved domains, although three of these (Figure 5.3 coords. 194 (S to T), 209 (E to G), and 483 (S to Y)) are conserved in the *Rht-1* orthologues found in *Brachypodium*, barley, rice, maize, and sorghum indicating these changes could be significant. Overall, six of the missense PS occur in the natural hexaploids and four in the TDW lines, which is surprising considering that among the sequenced natural hexaploid ORFs, eight SNPs were identified whereas 15 SNPs were found in the TDW ORFs. This is the result of a significantly higher (p = .04) proportion of non-synonymous to synonymous SNPs in the natural hexaploid ORFs relative to the TDW ORFs (Figure 5.6). In rice, a nearly two-fold increase in

the ratio of non-synonymous to synonymous substitutions was found among domesticated rice cultivars than among wild relatives with 25% of these found to be deleterious and the result of genetic 'hitchhiking' (Lu *et al.*, 2006).

In summary, diversity and haplotypes in the *Rht-1* region was higher on the B genome than on the A and D genomes. Knowledge of alternative *Rht-1* haplotypes is important when searching for novel sources of variation in this region. This is directly applicable at the *Rht-D1* locus where *Rht-D1b* is thought to be linked to increased FHB susceptibility (Srinivasachary *et al.,* 2009). Also, on the A genome, the presence of only a single haplotype in UK bread wheats could be problematic if this haplotype is associated with detrimental agronomic issues. For future wheat breeding programmes, a pre-existing knowledge of genetic variation around the *Rht-1* loci will aid in screening for useful alleles. The novel haplotypes and polymorphisms discovered here require further characterisation to determine their usefulness in wheat breeding. Characterisation of the two largest indels discovered here, the *Rht-B1* 160 bp and 197 bp insertions was carried out here by measuring *Rht-B1* transcript levels and these alleles will be further characterised based on plant height, flowering date, and GA sensitivity in Chapter 6.

6. *RHT-1* AND *PPD-D1* ASSOCIATIONS WITH HEIGHT, GA SENSITIVITY, AND HEADING DATE IN A WORLDWIDE BREAD WHEAT COLLECTION.

6.1. INTRODUCTION

Alleles at the *Rht-B1*, *Rht-D1,* and *Ppd-D1* loci have a major influence on the adaptability of wheat to climate and modern agricultural practices. It is well established that the homoeologous group IV *Rht-B1b* and *Rht-D1b* semi-dwarf alleles reduce plant height (Gale and Youssefian, 1985; Flintham *et al.*, 1997). Plants carrying *Rht-B1b*, *Rht-D1b*, or additional alleles at these loci that reduce plant height (summarised in Table 1.2) have reduced GA sensitivity, which is associated with suppressed plant growth. The GA insensitive (GAI) nature of plants containing a dwarf allele relative to the GA sensitive (GAS) wild type alleles was first determined by Allan *et al.* (1959). This finding led to the development of GA sensitivity tests to identify GAI alleles based on stem elongation response to GA (Gale and Gregory, 1977). GA insensitivity and semi-dwarfism in plants containing *Rht-B1b* and *Rht-D1b* are caused by SNPs that lead to premature stop codons in the DELLA domain, which is involved in GA signalling (Peng *et al.*, 1999). Based on these SNPs, perfect PCR primers were created to discriminate between accessions carrying *Rht-B1a* or *Rht-B1b* and between accessions carrying *Rht-D1a* or *Rht-D1b* (Ellis *et al*., 2002).

Ppd-D1 is mapped to the short arm of chromosome 2D, where the photoperiod insensitive *Ppd-D1a* allele and the photoperiod sensitive allele *Ppd-D1b* reside (Worland *et al.*, 1988). The *Ppd-D1a* allele, which contains a 2,089 bp deletion in the promoter region, is associated with early flowering under both short and long days, with the strongest effects occurring under short days (Beales *et al.*, 2007). Worland *et al.* (1988) reported that *Ppd-D1a* varieties had ear emergence accelerated by six to eight days when October-sown in the UK (Norwich). Perfect PCR markers have been developed to detect the presence/absence of the 2,089 bp deletion (Beales *et al.*, 2007). The *Ppd-D1a* allele is also associated with a reduction in plant height, but the effect has been difficult to estimate because in most varieties the allele is

present with *Rht8*, a GA sensitive semi-dwarf allele. The *Ppd-D1* and *Rht8* loci are linked at estimated genetic distances of 20.9 cM by Worland *et al.*, (1998) and 21.7 cM by Gasperini (2010). *Ppd-D1a* and *Rht8* were introduced together into European wheats through the Japanese variety Akakomugi in the early 20th century (Giorgi *et al.*, 1982; Worland, 1999). Worland *et al.* (1998) reported an 18 cm reduction in substitution lines containing *Rht8*+*Ppd-D1a* with 10 cm of the height reduction contributed by *Ppd-D1a* and 8 cm by *Rht8*. More recently, Gasperini (2010) genotyped the same substitution line population used by Worland *et al.* (1998) using the *Ppd-D1a* perfect markers to differentiate the effects of *Rht8* and *Ppd-D1a* on height. The author reported that the presence of *Rht8* resulted in a 14 cm (13%) height reduction while *Ppd-D1a* only accounted for a 4 cm (3%) height reduction.

In Chapter 5 of this thesis, sequencing of the *Rht-B1* region in 37 diverse wheat accessions led to the discovery of varieties that contained insertions within 600 bp of the start codon. One of the insertions was 160 bp in size, occurring 356 bp upstream of the *Rht-B1* ORF (CS reference sequence) and was present in eight varieties. The second insertion was 197 bp in size, occurred 591 bp upstream of the ORF and was found in six accessions including all three of the tetraploid wheats. The locations of the insertions just upstream of *Rht-B1* suggest that they could be within the promoter region. The 160 bp insertion occurs in the middle of a 120 bp stretch of sequence that is highly conserved in all three wheat *Rht-1* homoeologues and in *Rht-1* orthologues in rice and *Brachypodium* (section 3.3.2.2., Table 3.5) and the insertion could thus affect regulation of *Rht-B1* expression, although this could not be confirmed by analysis of one-week old seedlings (section 5.3.7). The effect of these alleles on plant phenotype, however, is yet to be investigated.

The INRA core collection of 372 bread wheat accessions (INRA BWCC) is a subset that contains an estimated 98% of the genetic diversity of a larger set of 3,942 accessions that originated from 45 geographical areas (Balfourier *et al.*, 2007). The INRA BWCC has been genotyped with genome-wide sets of DArT (578 polymorphic) and SSR markers (approximately 100 polymorphic) allowing population structure to be estimated (F. Balfourier, INRA, pers.

comm.). In addition, plant heights and ear emergence of the full set was recorded in Clermont-Ferrand, France (Bordes *et al.*, 2008). The collection represents a valuable resource for evaluating agronomic characteristics and allelic frequencies and for performing association analyses.

The objectives of this Chapter are to (1) characterise the INRA BWCC for plant height, GA sensitivity, and heading date, (2) determine the prevalences of *Rht-B1b*, *Rht-D1b*, *Ppd-D1a,* and the *Rht-B1* 160 bp and 197 bp insertions in the collection, (3) determine associations that exist between phenotype and genotype, and (4) search for novel GAI alleles in the collection.

6.2. MATERIALS AND METHODS

6.2.1. Germplasm

Seed of 368 bread wheat varieties from the INRA BWCC were provided by F. Balfourier, INRA, Clermont-Ferrand, France. This represents the entire set of bread wheat lines in the INRA 372CC collection (four of the original accessions were later found to not be hexaploid (F. Balfourier, pers. comm.)). Several control lines were utilised in the experiments including *Rht-1* NILs in the backgrounds of Cappelle Desprez (CD), Mercia, April Bearded (AB), and Bersee, which were provided by M. Ambrose (JIC). The NILs with introgressed semi-dwarf alleles will be given the nomenclature 'Variety name_Introgressed allele' in the text. The controls also include the *Ppd-D1a* substitution lines (SLs) in the backgrounds of Mercia and CS, which were provided by D. Laurie (JIC). The SLs were developed by C. Law and T. Worland at the JIC and contain a whole 2D chromosome substitution from Ciano 67 (D. Laurie, pers. comm.) and are referred to as 'Mercia_C67 2D' and 'CS_C67 2D' in the text. Additional control lines included Norin 10 (*Rht-B1b*+*Rht-D1b*; USDA-ARS PI156641) and Xi19 (*Rht-B1a*+*Rht-D1b*) and Robigus (*Rht-B1b*+*Rht-D1a*), obtained from the NIAB DUS collection.

6.2.2. DNA extraction and genotyping

All DNA extractions were performed as described in section 4.2.2 using the CTAB method. For the INRA BWCC lines, DNA of fixed lines was extracted from bulks of four plants and DNA of landraces was extracted from individual plants. Both replicates of the INRA BWCC fixed lines and four plants of each landrace were genotyped for *Rht-B1a/b*, *Rht-D1a/b*, *Ppd-D1a/b*, and the *Rht-B1* 160 bp and 197 bp insertions. Each control replicate (four plants) was bulked and genotyped for *Rht-B1a/b*, *Rht-D1a/b*, and *Ppd-D1a/b*.

The *Rht-B1a/b* and *Rht-D1a/b* PCR assays were performed as described in section 5.2.3. The *Ppd-D1a/b* assay is slightly modified from Beales *et al.* (2007). The multiplex PCR reaction was performed in a 10 µl volume containing 1 × Green GoTaq Reaction Buffer (Promega), 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.5 µM each of primer 414F-F10 and primers 414F-R4 and DgR3 (see Appendix I for sequences), 0.125 µl Taq Polymerase, and 2 ng of DNA template. The PCR profile consisted of 95°C for 2 min, followed by 40 cycles of [95°C for 20 s; 55°C for 20 s, and 72°C for 40 s], and 5 min at 72°C. Amplified products were separated in a 1.5% agarose gel in 1 × TBE buffer and visualised under UV light with ethidium bromide. A 297 bp product is amplified in accessions with *Ppd-D1a* and a 414 bp product is amplified in accessions with *Ppd-D1b*.

A multiplex PCR assay was developed to detect the *Rht-B1* 160 bp and 197 bp insertions. PCR reactions utilised a 10 µl reaction mix that contained 1 × Green GoTaq Reaction Buffer (Promega), 3% glycerol, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 1 µM primer Rht-F11, 0.33 µM primers 160-R1, 197-R1 and Rht-ABD-R9 (sequences shown in Appendix I), 0.125 µl Taq Polymerase, and 2 ng of DNA template. The PCR profile consisted of 95°C for 5 min, followed by 40 cycles of [95°C for 30 s; 60°C for 30 s, and 72°C for 1 min], and 5 min at 72°C. Amplified products were separated in a 1.5% agarose gel in 1 × TBE buffer and visualised under UV light with ethidium bromide. Primers Rht-F11 and Rht-ABD-R9 flank the two insertions. In lines without an insertion these primers amplify a 1050 bp product. Primer 160-R1 lies within the 160 bp

insertion and is designed to anneal only in lines with this insertion, thereby amplifying a 449 bp product. Primer 197-R1 lies within the 197 bp insertion and is designed to amplify a 361 bp product only in lines with this insertion. In lines with an insertion, the flanking Rht-F11/Rht-ABD-R9 primer pair could theoretically amplify products of 1210 bp (160 bp assay) or 1247 bp (197 bp assay), but under the stated amplification conditions this does not occur.

6.2.3. INRA BWCC outdoor experiment

The INRA BWCC was grown outdoors in 4l pots at NIAB, Cambridge, UK, and plants were sown 25 November 2008 or 18 February 2009. The November sowing consisted primarily of winter wheat types and the February sowing consisted primarily of spring wheat types. In each sowing, the experimental unit was a 4l pot containing four plants of a variety grown in coarse compost mixed with 1 tsp of 11-11-18 (N%-P%-K%) controlled-release fertiliser.

The November sowing contained 249 INRA BWCC accessions representing 235 winter, 8 facultative, and 6 spring growth habit types at two replicates each (inclusion of accessions with the spring growth habits was accidental). As controls, 22 winter/facultative lines were grown in the experiment, although only 15 were analyzed (see Table 6.1, section 6.3.1) because seven lines were discovered to be segregating at the *Rht-B1* or *Rht-D1* locus following sowing. Seeds were planted in compost in 96-well trays in the glasshouse under natural lighting (21°C, 16 hr period that included all daylight; 17°C, 8 hr) to promote uniform germination. Following emergence (5 December) seedlings were exposed to ambient air temperature and natural lighting in the glasshouse before healthy seedlings were transplanted to 4l pots (6 Jan. 2009). On 21 January, the pots were moved outside to “plunge beds” and positioned according to the experimental design (Figures 6.1 and 6.2). The experimental design of the November sowing was an incomplete block design that consisted of two main blocks with 3 row blocks (plunge beds) per main block and 105 pots per row block (630 experimental units in total). The 22 controls were represented once in each plunge bed and the 249 INRA BWCC varieties were represented once in each main block. Experimental units were

randomised to achieve maximum efficiency using:
http://biometrics.hri.ac.uk/DesignOfExperiments/UnstructuredTreatmentDesigns.html

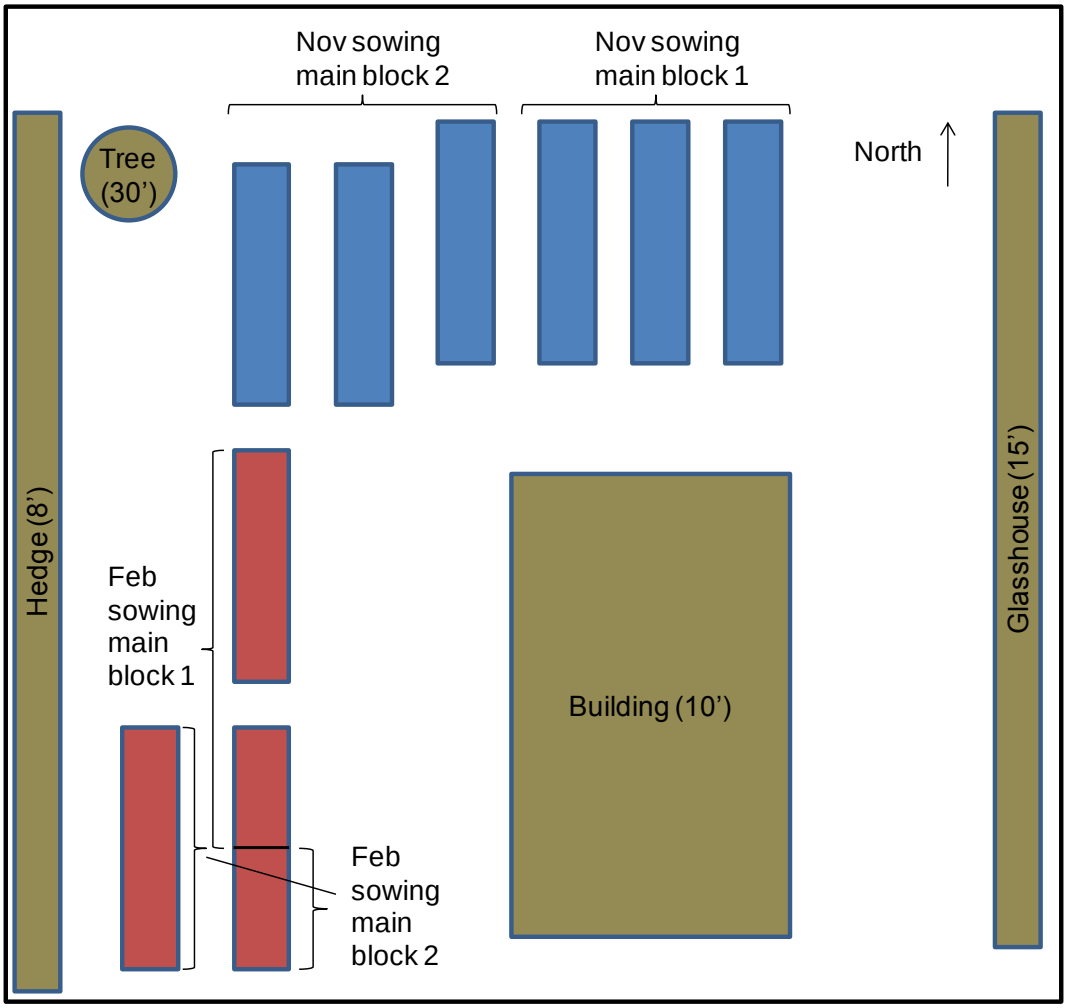


Figure 6.1. Experimental layout of the November 2008 and February 2009 sowings of the INRA BWCC. Plants were grown outside in 4l pots placed in plunge beds. Blue rectangles indicate plunge beds from the November sowing and red rectangles represent plunge beds from the February sowing. In the February sowing, one plunge bed was split between the two main blocks. All plunge beds were capable of holding 161 4l pots (7 pots wide by 23 pots long). In brown, are features that shaded a portion of the plunge beds for periods of the day with approximate height in feet (') shown in parentheses. Spatial location and dimensions are show in approximate scale except glasshouse width and length, which are not to scale.

Figure 6.2. The INRA BWCC plants sown November 2008. The image was taken at midday, 16 April 2009. The six plunge beds of the November 2008 sowing are shown in the middle of the picture. Pots in the bottom right corner are part of the February 2009 sowing.

The February sowing consisted of 119 accessions (117 spring and two facultative types) at two replicates each and 15 spring/facultative wheat control lines at three replicates each, although only 13 controls were analyzed (Table 6.1) because two lines were discovered to be segregating at *Rht-B1* or *Rht-D1* following sowing. Seeds were planted directly into pots and immediately positioned in the plunge beds according to the experimental design (Figures 6.1 and 6.3). The experimental design for the February sowing consisted of two main blocks, each consisting of 1.5 plunge beds (one plunge bed was split between two main blocks). The 15 control lines were represented once in each plunge bed and the 119 INRA BWCC varieties were represented once in each main block (238 experimental units total). Controls and INRA BWCC varieties were assigned a random location within plunge beds and main plots using the Microsoft Excel random number function.

Figure 6.3. The INRA BWCC plants sown February 2009. The image was taken late afternoon 1 April 2009. The three plunge beds that composed the February 2009 sowing are in the foreground. Plunge beds from the November 2008 sowing are in the background.

For both sowings, 4l pots of Xi19 wheat (4 plants per pot) were placed along the perimeters of the plunge beds to reduce edge effects. For all accessions, plant roots were allowed to grow through holes in the pot bottoms to obtain moisture and additional nutrients from the plunge beds, which were filled with peat and irrigated as needed. To prevent lodging of plants, bamboo poles were erected and baling twine strung between poles to create a grid that kept plants erect. To control powdery mildew, fungicides were applied as follows: 3 April 2009 (Fortress at 1 ml l^{-1}, November sowing); 15 April 2009 (Flexity at 1 ml l^{-1}, November sowing), 22 May 2009 (Fortress at 1 ml l^{-1}, both sowings); 6 June 2009 (Flexity at 1 ml l^{-1}; both sowings). Fungicides were selected that did not contain known growth regulators. Both sowings were sprayed to control aphids as follows: 29 May (Decis at 0.7 ml l^{-1}) and 3 July (Gazelle at 0.5 g l^{-1}). Both sowings were covered with netting in July to prevent bird damage. Plants were harvested and threshed in August 2009. Heading date was recorded as the number of days from the 1st of January until the inflorescence of the primary tiller from each pot was 50% emerged from the

flag leaf. Plant height was recorded as the distance from the soil surface to the tip of the tallest tiller in each pot (excluding awns) at harvest. Obvious off-types based on plant appearance or flowering date were excluded from height and flowering date measurements.

6.2.4. GA sensitivity experiments

Three experiments were conducted in the glasshouses at the JIC, Norwich, UK to examine the GA responsiveness of the INRA BWCC lines. The first experiment contained all 368 INRA BWCC varieties, the second experiment contained 161 INRA BWCC varieties and the third contained 19 INRA BWCC varieties. Seed used in the GA sensitivity experiments was collected from bagged ears of plants grown in the INRA BWCC outdoor experiment. Seed sourced from plants homozygous at the *Rht-B1* and *Rht-D1* loci (based on the *Rht-B1a/b* and *Rht-D1a/b* PCR assays) was available for all but three accessions. Each GA sensitivity experiment also contained the following control lines: AB, AB_*Rht-D1b*, AB_*Rht-B1c*, Bersee, Bersee_*Rht-B1b*, Mercia, and Mercia_*Rht-D1b*. The control lines were all sourced from plants homozygous at the *Rht-B1* and *Rht-D1* loci, seeds were collected from bagged heads to ensure foreign pollen was excluded.

The GA responsiveness tests were based on those described by Gale and Gregory (1977), but along with a GA+ treatment also consisted of a GA- treatment. The GA+ treatment contained 10 ppm GA_3 (Sigma product number G-7645) dissolved in 0.1% ethanol. The GA- treatment consisted only of 0.1% ethanol. GA treatments were assigned to whole trays and trays were watered with a GA+ or GA- treatment as plants needed moisture. Each tray contained 60 wells filled with a peat/sand mixture that was wetted with the GA+ or GA- solution prior to transplanting of seedlings (one seedling per well) to begin the experiments. The experimental unit consisted of an individual seedling. Trays were placed in the glasshouse under artificial lighting (300 to 400 µmol m^{-2} s^{-1}) with day/night temperatures of 20°C/15°C, 16 h daylength.

In GA sensitivity experiment 1 (GA expt. 1), seeds were stratified at 4°C on wetted filter paper for two days beginning 3 October 2009 before transplanting to 60-well trays to begin the experiment. GA expt. 1 consisted of three main blocks with seven trays designated as GA+ treatment and seven trays designated as GA- treatment per main block (42 trays total). The locations of GA+ and GA- trays were randomly assigned within main blocks. Each INRA BWCC accession was represented in each main block treatment (in total, three GA+ plants and three GA- plants per INRA BWCC accession). Each control was represented once per tray (42 GA+ and 42 GA- plants per control). On 21 October, plants were uprooted and the length from the seed-to-first-ligule (STFL) was recorded as shown in Figure 6.4.

Figure 6.4. Seed-to-first-ligule (STFL) length measured in the GA sensitivity experiments. The measured length (shown by arrows) extended from the seed (bottom white line) to the first ligule (top white line).

In GA sensitivity experiment 2 (GA expt. 2), seeds were stratified for three days at 4°C on wetted filter paper beginning 3 December 2009 and transferred to room temperature for three days before transplanting to 60-well trays. GA expt. 2 consisted of three main blocks with three GA+ trays and

three GA- trays per main block (18 trays total). GA+ and GA- trays were randomly assigned a location within main blocks. Each INRA BWCC accession was represented once per main block (three GA+ and three GA- plants per INRA BWCC accession) and each control was represented once per tray (nine GA+ and nine GA- plants per control). STFL lengths were recorded on 21 December.

In GA sensitivity experiment 3 (GA expt. 3), seeds were stratified at 4°C on wetted filter paper beginning 29 April 2010 and moved to room temperature on 2 May where they remained for four days but for a single day (4 May) when they were returned to 4°C to limit growth prior to transplanting. GA expt. 3 consisted of six main blocks for the GA+ and GA- treatments with a main block consisting of 1/3 of a tray (four trays total were used). Each INRA BWCC accession was represented once in each main block (six GA+ and six GA- plants per INRA BWCC accession) and each control was represented once on each tray (two GA+ and two GA- plants per control). However, due to tray dimensions or poor seed germination, the following INRA BWCC accessions were not represented in all main blocks: INRA_03752 (two GA+ and two GA- plants), INRA_00347 and INRA_01065 (four GA+ and four GA- plants each). STFL lengths were recorded on 19 May.

6.2.5. Statistical analyses

In GA expt. 1 and 2, the GA sensitivity classification of plants was determined using a mixed model (MM) and a least significant difference (LSD). In GA expt. 3, only the LSD was used. In the MM, a mixture model consisting of two independent normal distributions with different means and variances (George *et al*., 2000) was fitted with a probability (α) of an observation belonging to one distribution and a probability (1- α) of belonging to a second distribution. An observation consisted of the mean STFL length of an accession in the GA+ treatment in an experiment. All five parameters (μ_1, σ_1^2, μ_2, σ_2^2, and α) were fitted using maximum log likelihood (MLL) in Microsoft Excel using the Solver function. Posterior probabilities of any individual observation belonging to either of the two distributions were calculated from the probability density

functions of each using the estimated means and variances as: *p*(dist 1) / (*p*(dist 1) + *p*(dist 2)) and *p*(dist 2) / (*p*(dist 1) + *p*(dist 2)). A threshold posterior probability greater than or equal to 0.95 was used to assign individual observations to one or the other of the two GA classes (GAI or GAS). Individuals with posterior probabilities < 0.95 for either of the two distributions were classified as having an intermediate (INT) GA response.

In the LSD test, a t-value was calculated for each accession by rearranging the LSD formula to solve for t: t = LSD/SED (Snedecor and Cochran, 1967), where SED (standard error of the difference between two means) = $((s^2/n_a) + (s^2/n_b))^{1/2}$ where s^2 = the estimated variance in a GA experiment; n_a = the number of GA+ plants of an accession; n_b = the number of GA- plants of an accession. GA experimental variances and SEDs were determined using a REML (Restricted Maximum Likelihood) mixed model in Genstat, 12th edition (VSN International) with GA treatment, variety, and the interaction as fixed effects and replicate as a random effect. The GA treatment difference of an accession (STFL length in the GA+ treatment minus STFL length in the GA- treatment) was substituted for LSD in the formula and t values calculated. The probability that a t-value fit a normal two-tailed t distribution with null hypothesis of treatment difference = 0 was calculated using the TDIST function in Excel. For GA expts. 1 and 2, 100 degrees of freedom (d.f.) were used for the t-test and for GA expt. 3, 26 d.f. were used. When the probability of the null hypothesis being true was ≤ 0.05, accessions were classified as GA sensitive and when the probability was > 0.05, accessions were classified as having no significant treatment difference (NSTD).

For plant height, heading date, and GA treatment difference (GA expts. 1, 2, and 3), the effect of alleles at the *Rht-B1*, *Rht-D1*, and *Ppd-D1* loci were estimated using these loci and all possible interactions as fixed effects and variety as a random effect in a REML mixed model (referred hereafter as *Rht-B1*Rht-D1*Ppd-D1* REML). Only accessions homozygous at these three loci (352 accessions) were included in the analysis. To separate mean differences among *Rht-B1* alleles, *Rht-B1* REML analyses were performed for each phenotype using the following fixed terms in each model: (1) *Rht-B1*, (2)

the locus (*Rht-D1* or *Ppd-D1*) that accounted for most of the remaining variation in the *Rht-B1*Rht-D1*Ppd-D1* REML, and (3) the interaction of (1) and (2). To separate mean differences among *Ppd-D1* alleles and among *Rht-B1*+Rht-D1* allelic combinations, *Ppd-D1*RHT* REML analyses were performed for each phenotype. Three fixed terms were used in each model: (1) *Ppd-D1*, (2) *RHT*, which was composed of the *Rht-B1a*+Rht-D1a*, *Rht-B1a*+Rht-D1b*, *Rht-B1b*+Rht-D1a*, and *Rht-B1b*+Rht-D1b* genotypes, and (3) the interaction of (1) and (2). For the *Rht-B1* REML and *Ppd-D1*RHT* REML analyses, the locus with the greatest effect in the *Rht-B1*Rht-D1*Ppd-D1* REML was placed first in the model and variety served as the random term. The LSD threshold for main effect means was 0.05 and no attempt was made to separate means of interactions. All of the above statistical analyses were carried out with the guidance of Ian Mackay, Statistical Geneticist, NIAB.

Genotype/phenotype association analysis was also performed using mean plant height, mean heading date, and mean GA treatment differences (data from expts. 1 and 2 only) along with presence/absence scores for the 160 bp insertion, the 197 bp insertion, *Rht-B1b*, *Rht-D1b*, and *Ppd-D1a*. Associations were considered significant at a threshold of $p < 0.001$. To control for population structure and kinship among varieties, a mixed model was fitted in which relationships among varieties were accounted for by the inclusion of a kinship matrix estimated from a genome-wide set of DArT and SSR markers (Yu *et al*., 2006). Kinship was estimated using a simple matching coefficient. As this is proprietary information that was not made available to this project, these calculations were carried out by F. Balfourier, INRA. This association analysis, which controls for population structure is termed AA-PS in the text.

6.3. RESULTS

6.3.1. *Rht-1* and *Ppd-D1* genotyping of control lines

All controls used in the INRA BWCC outdoor experiment were genotyped for *Rht-B1a/b*, *Rht-D1a/b*, and *Ppd-D1a/b* to confirm the presence of these alleles (Table 6.1). Nine accessions that were segregating at one of these loci or

that did not contain the expected allele were excluded from the phenotypic analysis. Controls containing *Rht-B1* and *Rht-D1* dwarfing alleles other than *Rht-B1b* or *Rht-D1b* could not be clearly identified using the *Rht-B1a/b* and *Rht-D1a/b* PCR assays. At the *Rht-B1* locus, no product was obtained using the *Rht-B1a/b* primer pairs when gDNA from any of the four control lines with the *Rht-B1c* allele was used in PCR reactions. *Rht-B1a/b* PCR reaction mixes containing gDNA of CD_*Rht-B1d* as template amplified the *Rht-B1b* product; hence the *Rht-B1d* control was indistinguishable from *Rht-B1b* controls using these assays. *Rht-B1a/b* PCR reactions containing gDNA of CD_*Rht-B1e* as template amplified only the *Rht-B1a* product, and therefore the *Rht-B1e* control was indistinguishable from the *Rht-B1a* controls. At the *Rht-D1* locus, *Rht-D1a/b* reactions with gDNA of Mercia_*Rht-D1c* as template resulted in amplification of only the *Rht-D1b* product and therefore the *Rht-D1c* control was indistinguishable from *Rht-D1b* controls using this assay. At the *Ppd-D1* locus, PCR assay results demonstrated the presence of *Ppd-D1a* in 'Norin 10', CS_C67 2D, and Mercia_C67 2D, and the presence of *Ppd-D1b* in the remaining controls.

Table 6.1. Summary of genotypic and phenotypic data of control lines used in the INRA 368 BWCC outdoor experiment.

			Genotyping[b]			Plant ht.[c]			Heading date (days)[d]		
Variety[a]	Sow-ing	Reps	*Rht-B1*	*Rht-D1*	*Ppd-D1*	Mean (cm)	% of WT	Range (cm)	Mean	Rel WT	Range
CD	Nov08	1	*B1a*	*D1a*	*D1b*	114		114	148		148
CD_*Rht-B1b*	Nov08	6	***B1b***	*D1a*	*D1b*	108	95%	104-122	148	-1	146-150
CD_*Rht-D1b*	Nov08	5	*B1a*	***D1b***	*D1b*	97	85%	92-102	148	0	144-151
CD_*Rht-B1c*	Nov08	4	NP	*D1a*	*D1b*	53	46%	38-88	153	+5	146-157
CD_*Rht-B1d*	Nov08	4	***B1b***	*D1a*	*D1b*	106	93%	101-111	148	-1	146-149
CD_*Rht-B1e*	Nov08	5	*B1a*	*D1a*	*D1b*	80	71%	70-89	149	1	144-150
Mercia	Nov08	6	*B1a*	*D1a*	*D1b*	100		94-104	144		142-146
Mercia_*Rht-B1b*	Nov08	2	***B1b***	*D1a*	*D1b*	96	96%	91-100	143	-1	142-144
Mercia_*Rht-D1b*	Nov08	6	*B1a*	***D1b***	*D1b*	74	74%	65-81	146	+2	143-148
Mercia_*Rht-B1c*	Nov08	6	NP	*D1a*	*D1b*	40	40%	35-45	150	+6	144-153
Mercia_*Rht-D1c*	Nov08	6	*B1a*	***D1b***	*D1b*	76	76%	71-81	146	+1	143-148
Mercia_C67 2D	Nov08	6	*B1a*	*D1a*	***D1a***	97	97%	86-102	138	-7	135-140
Norin 10	Nov08	4	***B1b***	***D1b***	***D1a***	87		75-112	134		131-135
Robigus	Nov08	4	***B1b***	*D1a*	*D1b*	90		86-100	142		126-149
Xi19	Nov08	5	*B1a*	***D1b***	*D1b*	91		86-99	146		145-148

Table 6.1 (continued). Summary of genotypic and phenotypic data of control lines used in the INRA 368 BWCC outdoor experiment.

			Genotyping[b]			Plant ht.[c]			Heading date (days)[d]		
Variety[a]	Sow-ing	Reps	*Rht-B1*	*Rht-D1*	*Ppd-D1*	Mean (cm)	% of WT	Range (cm)	Mean	Rel WT	Range
AB	Feb09	3	*B1a*	*D1a*	*D1b*	175		170-181	155		153-157
AB_*Rht-B1b*	Feb09	2	***B1b***	*D1a*	*D1b*	145	83%	145-145	153	-2	152-154
AB_*Rht-D1b*	Feb09	3	*B1a*	***D1b***	*D1b*	142	81%	137-145	157	+2	155-158
AB_*Rht-B1c*	Feb09	3	NP	*D1a*	*D1b*	53	30%	50-56	161	+6	161-162
Bersee	Feb09	3	*B1a*	*D1a*	*D1b*	125		123-129	158		153-163
Bersee_*Rht-B1b*	Feb09	3	***B1b***	*D1a*	*D1b*	91	73%	87-95	156	-3	153-159
Bersee_*Rht-D1b*	Feb09	3	*B1a*	***D1b***	*D1b*	91	73%	85-96	160	+2	157-162
Bersee_*Rht-B1c*	Feb09	2	NP	*D1a*	*D1b*	47	38%	42-52	168	+9	167-168
CD	Feb09	2	*B1a*	*D1a*	*D1b*	108		106-110	164		162-166
CS	Feb09	3	*B1a*	*D1a*	*D1b*	131		121-138	145		143-148
CS_C67 2D	Feb09	3	*B1a*	*D1a*	***D1a***	133	102%	122-146	142	-4	141-143
Norin 10	Feb09	1	***B1b***	***D1b***	***D1a***	66		66	147		147
Xi19	Feb09	3	*B1a*	***D1b***	*D1b*	83		76-89	155		153-157

[a] Introgressed *Rht-1* alleles are shown following the underscore. Mercia_C67 2D and CS_C67 2D refer to Mercia and Chinese Spring varieties with a 2D substitution from Ciano 67. CD = Cappelle Desprez; AB= April Bearded

[b] *Rht* genotypes based on PCR with *Rht-B1a/b* and *Rht-D1a/b* primers; NP = no product; semi-dwarf ("*-b"*) alleles are shown in bold font. *Ppd-D1* genotype based on PCR with *Ppd-D1a/b* primers with photoperiod insensitive ("*-a"*) alleles shown in bold font.

[c] Plant heights of lines with a dwarf or photperiod insensitive allele are shown as a percentage of the wild type (% of WT) from the same genetic background.

[d] Heading date is the number of calendar days after January 1. 'Rel WT' = Difference in days to heading relative to the WT line in the same genetic background (where appropriate).

6.3.2. Multiplex PCR identifies *Rht-B1* insertion types

The multiplex PCR assayed designed to detect the upstream *Rht-B1* insertions was tested using varieties known (see Table 5.7) to have no insertion (CS), a 160 bp insertion (Mercia, AB, Paragon), or a 197 bp insertion (CD, Hobbit Sib, SS7010073). A 1050 bp product was specifically amplified using gDNA of the line with no insertion (Figure 6.5, CS; lane 2), a 449 bp product was specifically amplified using gDNA of lines with the 160 bp insertion (Figure 6.5, Mercia, AB, or Paragon; lanes 3, 4, 5), and a 361 bp product was specifically amplified using gDNA of lines with the 197 bp insertion (Figure 6.5, CD or Hobbit Sib; lanes 6, 7). SS7010073 contains the 197 bp insertion and a 16 bp deletion in this region, and a 345 bp product is amplified when gDNA of this line serves as template (Figure 6.5, lane 8).

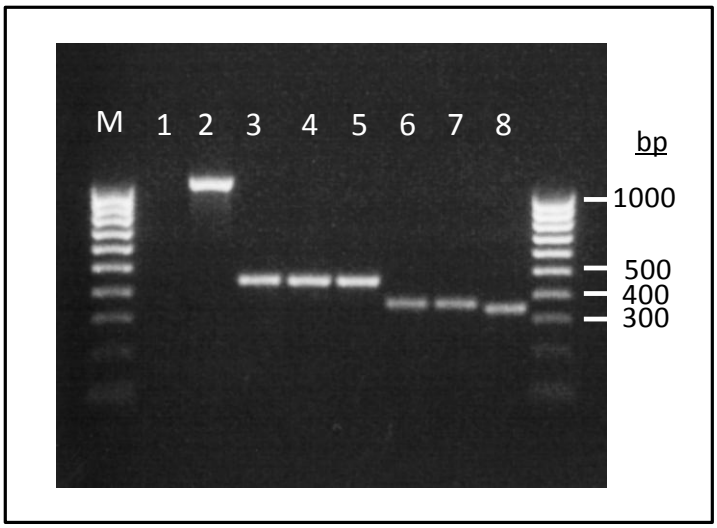


Figure 6.5. Validation of the *Rht-B1* 160 bp and 197 bp insertion PCR assay. The PCR mix included the forward primer, Rht-F11, in combination with reverse primers 160-R1, 197-R1, and Rht-ABD-R9. Templates were: water (lane 1); gDNA of CS (no *Rht-B1* insertion; 2); gDNA of Mercia, April Bearded, or Paragon (all have the 160 bp insertion; 3, 4, 5); gDNA of Cappelle Desprez or Hobbit Sib (both have the 197 bp insertion; 6, 7); gDNA of SS7010073 (197bp insertion and a 16 bp deletion; 8). Reaction mixtures were subject to electrophoresis on a 1.5% agarose gel stained with ethidium bromide and visualised under UV light. M = molecular marker Hyperladder IV (Bioline) with size in bp indicated.

6.3.3. Frequency of *Rht-1* and *Ppd-D1* alleles in the INRA BWCC

Among the 368 INRA BWCC accessions, the 160 bp insertion was present in 59 accessions (16%), and the 197 bp insertion was present in 44 accessions (12%) (Table 6.2A). The two insertions never occurred together in the same accession. The *Rht-B1* insertion data was then correlated with the *Rht-B1a/b*, *Rht-D1a/b*, and *Ppd-D1a/b* genotype data. With a single exception, the *Rht-B1* insertions always occurred in accessions containing the *Rht-B1a* allele (Table 6.2). *Rht-B1a_160* and *Rht-B1a_197* will be used to refer to the 160 bp and 197 bp insertions, respectively, in the *Rht-B1a* background. The single exception was INRA_06986 ('Tom Thumb'), which amplified the product associated with the 197 bp insertion, but did not amplify a product with the *Rht-B1a/b* primer pairs. 'Tom Thumb' is known by pedigree to contain the *Rht-B1c* allele and this result is similar to that reported for the *Rht-B1c* control lines (section 6.3.1). *Rht-B1a_0* (*Rht-B1a* with no insertion) was the most frequent *Rht-B1* allele among the INRA BWCC lines, occurring in

214 (58.2%) of the accessions while *Rht-B1b* was present in 45 (12.2%) of the accessions. *Rht-D1a* occurred in 318 (86.4%) accessions and *Rht-D1b* occurred in 47 (12.8%) accessions (Table 6.2B). *Rht-B1b* and *Rht-D1b* were present together in nine of the accessions. Six of the lines were not homozygous at the *Rht-B1* locus and three were not homozygous at the *Rht-D1* locus, including two lines that were not homozygous at either locus.

Table 6.2. Summary of INRA BWCC *Rht-B1* (A), *Rht-D1* (B), and *Ppd-D1* (C) genotypes.

		Accessions[a]	
A)	*Rht-B1* locus[b]	No.	% of total
	Rht-B1a_0	214	58.2%
	Rht-B1a_160	59	16.0%
	Rht-B1a_197	43	11.7%
	Rht-B1_197	1	0.3%
	Rht-B1b	45	12.2%
	Not homozygous	6	1.6%
		Accessions	
B)	*Rht-D1* locus[c]	No.	% of total
	Rht-D1a	318	86.4%
	Rht-D1b	47	12.8%
	Not homozygous	3	0.8%
		Accessions	
C)	*Ppd-D1* locus[d]	No.	% of total
	Ppd-D1a	95	25.8%
	Ppd-D1b	265	72.0%
	450bp product	1	0.3%
	No product	6	1.6%
	Not homozygous	1	0.3%

[a] For all 368 accessions, genotypes were determined using DNA extracted from plants grown during the INRA BWCC outdoor experiment. For fixed lines, two bulks of four plants were separately genotyped. For landraces, four separate plants were genotyped.

[b] *Rht-B1a* and *Rht-B1b* designations based on the *Rht-B1a/b* PCR assays. *Rht-B1a_0, Rht-B1a_160,* and *Rht-B1a_197* refer to accessions containing *Rht-B1a* with no insertion, a 160 bp insertion, or a 197 bp insertion, respectively. *Rht-B1*_197 refers to an accession ('Tom Thumb') that contains the 197 bp insertion but did not amplify a product associated with *Rht-B1a* or *Rht-B1b*. 'Not homozygous' refers to accessions in which the majority of samples were heterozygous or genotype scores for samples did not match.

[c] *Rht-D1* locus designations based on the *Rht-D1a/b* PCR assays.

[d] *Ppd-D1* locus designations based on the *Ppd-D1a/b* PCR assay (Beales *et al.*, 2007). The ~450 bp product does not correspond to the *Ppd-D1b* (414 bp product) or *Ppd-D1a* (297 bp product) allele. 'No product' indicates that a product was not obtained in either replicate.

At the *Ppd-D1* locus, the photoperiod sensitive *Ppd-D1b* allele was present in 265 varieties (72.0%) while the insensitive *Ppd-D1a* allele was present in 95 varieties (25.8%) (Table 6.2C). An approximately 450 bp product was amplified when gDNA of the accession INRA_13812 served as template in the *Ppd-D1a*/b PCR assay. The ~450 bp product is larger than the expected *Ppd-D1b* (414 bp) or *Ppd-D1a* (297 bp) products. Using the *Ppd-D1a*/b primer pair, no product was amplified from gDNA of three fixed lines (two replicates of four bulked plants were tested separately) and from three landraces (four plants were tested separately).

To determine the geographical prevalence of alleles, the 368 INRA BWCC accessions were classified according to geographical area as originally designated by Balfourier *et al.* (2007). The accessions come from 44 geographical regions with the largest proportion (28%) originating in France (Table 6.3). Among the *Rht-B1* alleles, *Rht-B1a_160* is most prevalent in accessions from Europe, central Russia, and North America, absent in accessions from Africa, South America, and Australia-New Zealand, and in low frequency or absent in the remaining regions. *Rht-B1a_197* is most prevalent in accessions from France and the USA, which combined have 29 of the 42 (69%) accessions containing this allele. In the remaining geographical areas, *Rht-B1a_197* is generally in low prevalence or not represented. *Rht-B1b* is in highest frequency in accessions from Mexico-Guatemala, Syria, and Israel-Lebanon-Palestine, in moderate frequency in several European countries, and absent or infrequent in the remaining geographical regions. *Rht-D1b* is most prevalent in Mexico-Guatemala and Israel-Lebanon-Palestine, and occurs in moderate frequency in many regions, but is absent in the majority of the regions. *Ppd-D1a* is most common in accessions from Japan, Mexico-Guatemala, China-Korea-Mongolia, Australia-New Zealand, and countries bordering the Mediterranean. For the accessions that did not amplify a product with the *Ppd-D1a*/b primers, the landraces were collected from the Black Sea region (one each from Azerbaijan, Georgia, and Turkey) and the three fixed lines were from Russia, Ethiopia, and France.

Table 6.3. Distribution of *Rht and Ppd* alleles by geographical area in the INRA BWCC

		Rht-B1[a]					*Rht-D1*			*Ppd-D1*[b]		
Geographical area[c]	Total	*B1a_160*	*B1a_197*	*B1a_0*	*B1b*	Other	*D1a*	*D1b*	Other	*D1a*	*D1b*	Other
FRA	103	22	25	42	13	SEG(1)	86	17		25	77	NP(1)
NLD	4	1		3			3	1			4	
DEU	6	3	1	2			6				6	
GBR–IRL	5	2	2			SEG(1)	2	3			5	
BEL	3	3					3				3	
SWE	2	1		1			2				2	
NOR–DNK	1			1			1				1	
FIN	6	1		5			6				6	
CHE	6	1	1	3	1		6				6	
POL	6	4	1	1			6			1	5	
CSK	6	3	1		2		6			3	3	
AUT	6	1		3	2		6				6	
ROM	3			2	1		3			2	1	
BGR	5			3	2		5			5		
UKR–BLR	4			4			2	2		2	2	
YUG–HRV	2	1		1			2			2		
HUN	6	3		1	2		6			1	5	
ESP	11	1	1	7	1	SEG(1)	10	1		3	8	
PRT	4		2	2			4				4	
GRC–ALB–MAD	2			2			2			2		
ITA	5			3	2		5			2	3	
USA	12	2	4	3	2	*B1c* (1)	11	1		1	11	
CAN	9	1		8			9			1	8	
AUS–NZL	13		1	9	2	SEG(1)	11	2		4	9	
RUS–Central	15	5		10			14	1		1	13	NP(1)
Caucasus	7			7			7				5	NP(2)
TUR	7			7			7			1	5	NP(1)
NPL	24	1		23			24			2	21	SEG(1)
CHN–KOR–MNG	16		1	14	1		14	2		5	11	
JPN	12	1		8	2	SEG(1)	7	3	SEG(1)	9	3	
PAK–KSM	5			5			4	1		1	4	
SYR	4			1	3		4			3	1	
ISR–LBN–PAL	7			4	3		4	3		3	4	
AFG–IRN–IRQ	1			1			1				1	
IND	5	1		4			5			1	4	
DZA–MAR	2			2			2				2	
EGY–TUN	4			3		SEG(1)	1	2	SEG(1)	3	1	
ETH–NER	4		1	3			4				3	NP(1)
KEN	2			2			2				2	
ZAF–ZWE	3		1	1	1		2	1		2	1	
BRA	3		1	2			3			1	2	
COL–PER	2			2			2			1	1	
MEX–GTM	10			5	5		4	6		7	2	450(1)
ARG–URY	5	1		4			4	1		1	4	
SUM	**368**	**59**	**43**	**214**	**45**	**7**	**318**	**47**	**3**	**95**	**265**	**8**

[a] *Rht-B1a_0, Rht-B1a_160,* and *Rht-B1a_197* refer to accessions containing *Rht-B1a* with no insertion, a 160 bp insertion, or a 197 bp insertion, respectively. Segregants (SEG) at each locus are shown with the number of accessions in parentheses. The *Rht-B1c* (*B1c*) designation is based on pedigree. *Rht-B1c* contains the 197 bp insertion.

[b] NP = No product; 450 = 450 bp product amplified using the *Ppd-D1a*/*b* primers with number of accessions in parenthesis.

[c] Geographic areas of accessions as shown by Balfourier *et al.* (2007). RUS–Central (Russia central) consists of TJK–TKM–KAZ–KIR–UZB and the Caucasus consist of ARM-GEO-AZE. AFG Afghanistan; ALB Albania; ARG Argentina; ARM Armenia; AUS Australia; AUT Austria; AZE Azerbaijan; BEL Belgium; BGR Bulgaria; BLR Belarus; BRA Brazil; CAN Canada; CHE Switzerland; CHN China; COL Colombia; CSK Czech and Slovak Republics; DEU Germany; DNK Denmark; DZA Algeria; EGY Egypt; ESP Spain; ETH Ethiopia; FIN Finland; FRA France; GEO Georgia; GBR Great Britain; GRC Greece; GTM Guatemala; HUN Hungary; HRV Croatia; IND India; IRL Ireland; IRN Iran; IRQ Iraq; ISR Israel; ITA Italy; JPN Japan; KAZ Kazakhstan; KEN Kenya; KIR Kyrgyzstan; KOR Korea; KSM Kashmir; LBN Lebanon; MAD Macedonia; MAR Morocco; MEX Mexico; MNG Mongolia; NER Niger; NLD Netherlands; NOR Norway; NPL Nepal; NZL New Zealand; PAL Palestine; PAK Pakistan; POL Poland; POR Portugal; PER Peru; ROM Romania; SYR Syria; SWE Sweden; TJK Tajikistan; TKM Turkmenistan; TUN Tunisia; TUR Turkey; URY Uruguay; UKR Ukraine; USA United States; UZB Uzbekistan; YUG Yugoslavia; ZAF South Africa; ZWE Zimbabwe.

6.3.4. Plant heights and heading dates of INRA BWCC accessions and controls in relation to genotype

The majority of control lines used in the INRA BWCC performed as expected with lines containing GAI alleles having reduced height and lines with the *Ppd-D1a* allele having earlier heading dates than wild type lines in the same genetic background (Table 6.1). Averaged over the CD, Mercia, AB, and Bersee genetic backgrounds, the *Rht-B1b*+*Rht-D1a* lines were 13% shorter and ear emergence 1.6 days earlier than wild type lines. Averaged over the same four backgrounds, the *Rht-B1a*+*Rht-D1b* lines were 22% shorter and ears emerged 1.3 days later than wild type lines. The *Rht-B1c* NILs were on average 62% shorter and had heading dates 6.4 days later than the wild type lines. For the *Ppd-D1a* substitution lines, CS_C67 2D flowered four days earlier and was 102% the height of CS. Mercia_C67 2D flowered seven days earlier and was 97% the height of Mercia. Xi19 and Norin 10 were the only two controls present in both sowings. Norin 10 from the February 2009 sowing was 76% the height and flowered 13 days later than Norin 10 from the November 2008 sowing. Similarly, the February 2009 sowing of Xi19 was 91% the height and flowered 9 days later than the November 2008 sowing.

Among the 368 INRA BWCC accessions, plant heights ranged from 54 to

200.5 cm with a mean of 133 cm (Appendix VI). A histogram of plant heights appeared to have a bi-modal distribution (Figure. 6.6A), but accessions could not be easily split into two groups using the MM approach (see section 6.2.5), so this was not performed. Nine accessions with the *Rht-B1a+Rht-D1a* genotype have plant heights less than the mean of accessions containing *Rht-B1b* or *Rht-D1b* (100 cm) and INRA_13481 was the shortest *Rht-B1a+Rht-D1a* accession with a plant height of 84.5 cm. Conversely, there were no accessions containing a semi-dwarfing allele that were taller than the *Rht-B1a+Rht-D1a* mean plant height (144 cm). INRA_07092, which contains the *Rht-B1b* allele, was the tallest semi-dwarf accession at 135.5 cm.

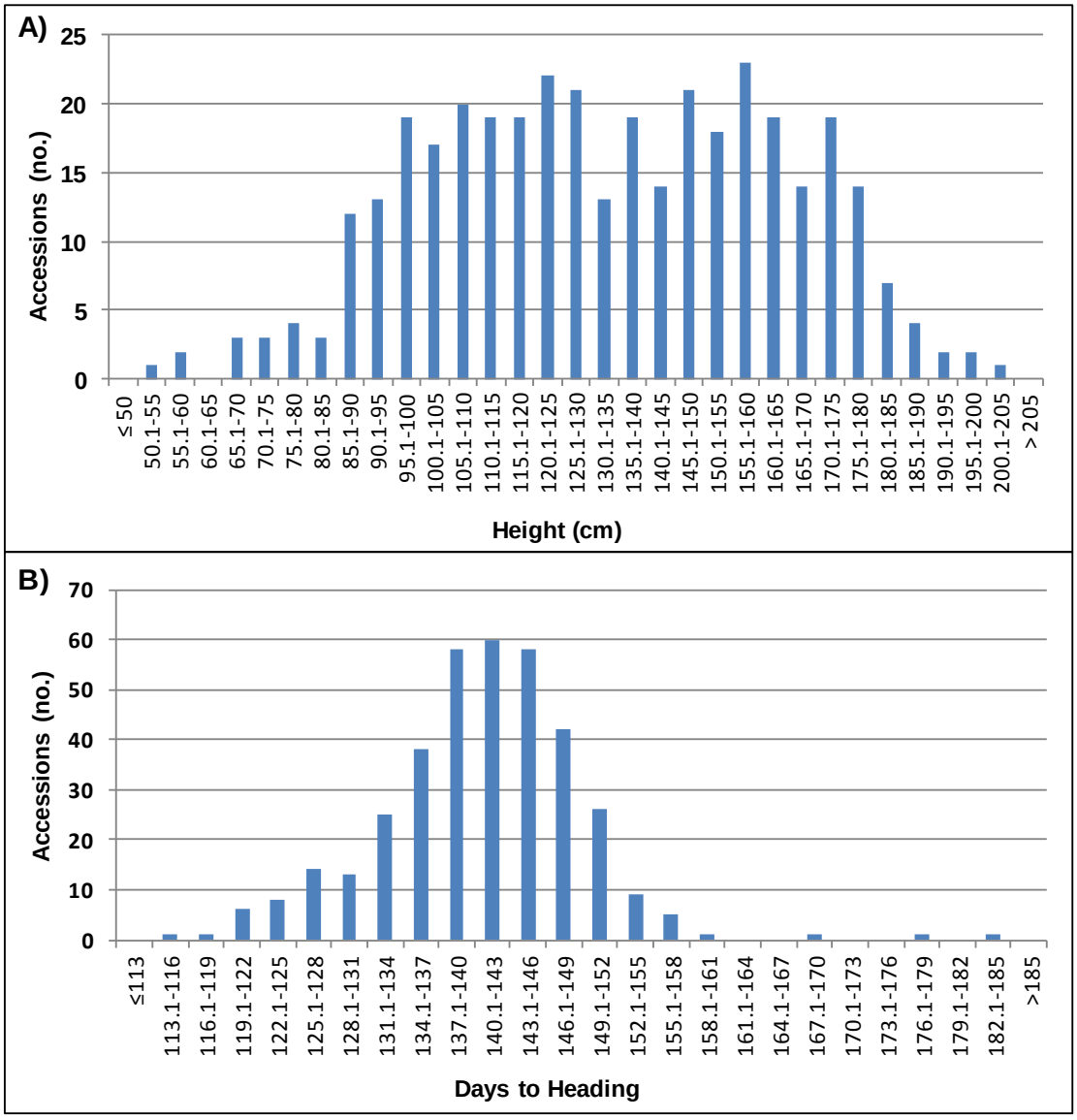


Figure 6.6. Distributions of heights (A) and heading dates (B) of the INRA BWCC lines.

Heading dates of the INRA BWCC lines ranged from 116 to 185 days with a mean of 141 days (Appendix VI). Heading dates were normally distributed; however there was a skew toward early ear emergence and three accessions had very late heading dates (nine to 25 days later than the next latest line; Figure 6.6B). The three late accessions were from the February 2009 sowing, indicating these lines may be winter types that flowered late due to a lack of vernalisation. Among all INRA BWCC lines, INRA_08227 (*Ppd-D1a* genotype) had the earliest ear emergence, three days earlier than any other line. The *Ppd-D1a* accession with the latest heading date was INRA_03306 with a heading date of 153 days. The earliest flowering accession with the *Ppd-D1b* allele was INRA_08233 with a heading date of 121 days.

6.3.5. GA sensitivities among INRA BWCC and control accessions

6.3.5.1 GA sensitivities of accessions in GA experiment 1

In GA expt. 1, the STFL lengths of the INRA BWCC and control lines from the GA+ treatment revealed an apparent bi-modal distribution (Appendix VII; Figure 6.7A). Because the distribution was bi-modal, the MM approach was used to calculate the probability that controls and INRA BWCC lines were GAI (*p*GAI) or GAS (*p*GAS = 1 - *p*GAI) based on GA+ treatment STFL lengths as described in section 6.2.5. For the controls, the MM procedure identified the three GAS *Rht-B1a+Rht-D1a* lines as GAS with *p*GAS = 1 in all three lines (Table 6.4). Using the MM, three of the four accessions with dwarfing alleles had a response classified as GAI (AB_*Rht-B1c, p*GAI=1; Mercia_*Rht-D1b*, *p*GAI = 0.98; AB_*Rht-D1b*, *p*GAI = 0.93); however unexpectedly, Bersee_*Rht-B1b* GA+ treatment values more closely resembled a GAS response (*p*GAS = 0.91). In a similar analysis of GA sensitivity, an LSD test was used to calculate the probability that an accession's GA treatment difference was equal to zero. A low probability of no GA treatment difference is indicative of a GAS response. The conclusions drawn from the LSD analysis were similar to those obtained using MM for all control lines, including Bersee_*Rht-B1b,* which had a 0.09 probability of no GA treatment difference. The *Rht-B1a+Rht-D1a* control lines all had large GA treatment differences, ranging

from 24.2 to 30.8 mm. GA treatment differences also existed for the *Rht-B1b*, *Rht-D1b*, and *Rht-B1c* NILs, but were smaller, ranging from 2.3 to 9.0 mm.

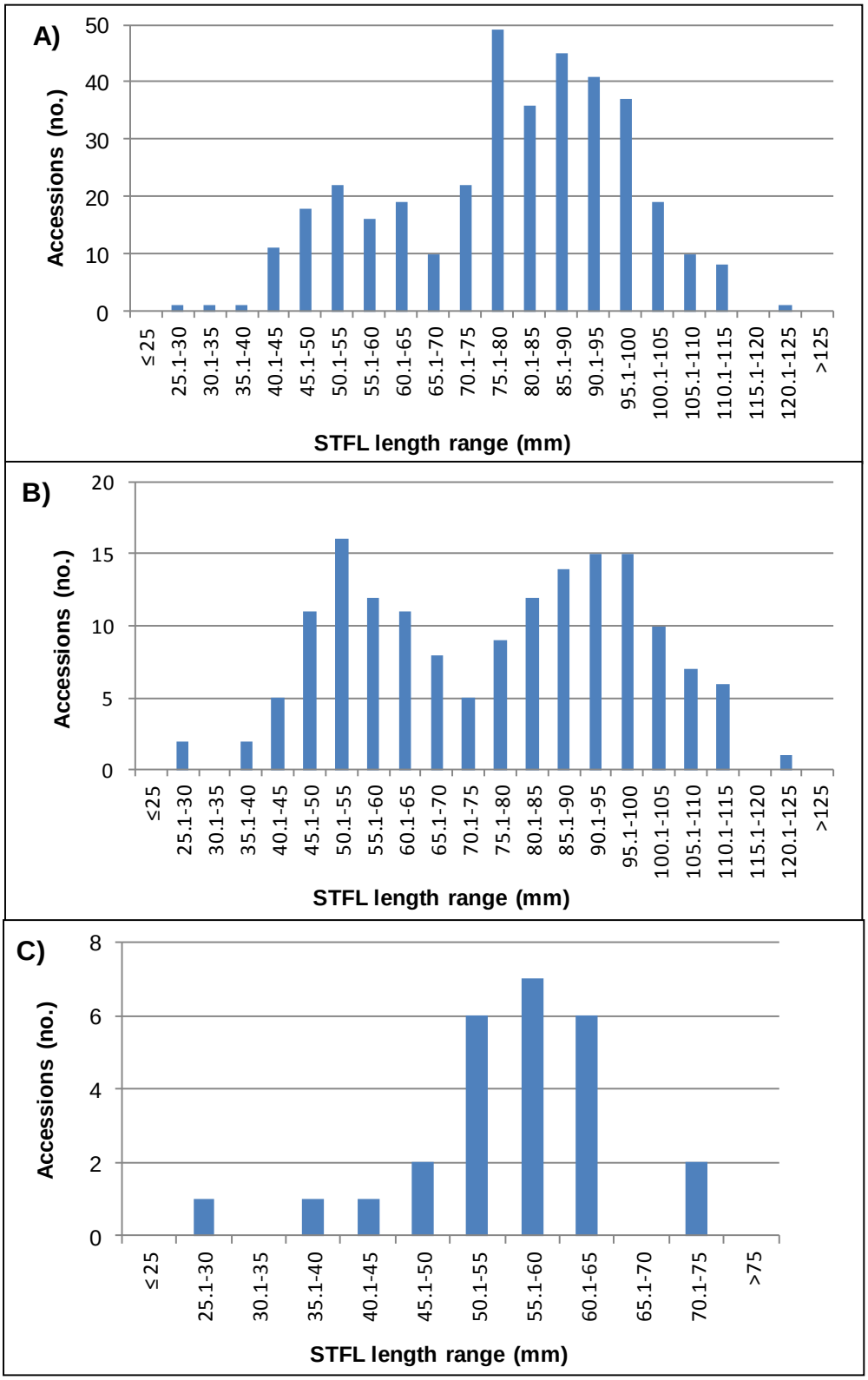


Figure 6.7. Distributions of seed to first ligule (STFL) lengths of the GA+ treatment of INRA BWCC and control lines in GA sensitivity experiment 1 (A), 2 (B), and 3 (C).

Table 6.4. Summary of GA treatment effects on control lines

GA Expt.	Control Line[d]	Rht genotype[e]	Mean STFL length (mm)[a]			MM[b]		LSD[c]
			GA+ trt	GA- trt	GA trt diff.	*p*GAI	*p*GAS	*p*GA trt diff =0
1	AB	*B1aD1a*	88.1	57.3	30.8	0.00	1.00	0.00
	AB_*Rht-D1b*	*B1aD1b*	55.5	51.8	3.7	0.93	0.07	0.48
	AB_*Rht-B1c*	*D1a*	29.3	27.0	2.3	1.00	0.00	0.66
	Bersee	*B1aD1a*	94.1	64.6	29.4	0.00	1.00	0.00
	Bersee_*Rht-B1b*	*B1bD1a*	70.8	61.8	9.0	0.09	0.91	0.09
	Mercia	*B1aD1a*	81.6	57.4	24.2	0.00	1.00	0.00
	Mercia_*Rht-D1b*	*B1aD1b*	49.2	46.7	2.6	0.98	0.02	0.62
	Mean		**66.9**	**52.4**	**14.6**			
2	AB	*B1aD1a*	93.9	63.7	30.2	0.00	1.00	0.00
	AB_*Rht-D1b*	*B1aD1b*	54.9	52.2	2.7	1.00	0.00	0.49
	AB_*Rht-B1c*	*D1a*	28.6	26.2	2.3	1.00	0.00	0.55
	Bersee	*B1aD1a*	95.6	69.8	25.8	0.00	1.00	0.00
	Bersee_*Rht-B1b*	*B1bD1a*	68.1	58.3	9.8	0.78	0.22	0.02
	Mercia	*B1aD1a*	90.4	60.7	29.8	0.00	1.00	0.00
	Mercia_*Rht-D1b*	*B1aD1b*	46.4	45.0	1.4	1.00	0.00	0.71
	Mean		**68.3**	**53.7**	**14.6**			
3	AB	*B1aD1a*	61.0	36.5	24.5	N/A	N/A	0.00
	AB_*Rht-D1b*	*B1aD1b*	45.5	37.5	8.0	N/A	N/A	0.12
	AB_*Rht-B1c*	*D1a*	28.0	26.0	2.0	N/A	N/A	0.69
	Bersee	*B1aD1a*	72.5	36.0	36.5	N/A	N/A	0.00
	Bersee_*Rht-B1b*	*B1bD1a*	59.0	36.5	22.5	N/A	N/A	0.00
	Mercia	*B1aD1a*	60.0	29.5	30.5	N/A	N/A	0.00
	Mercia_*Rht-D1b*	*B1aD1b*	35.5	29.0	6.5	N/A	N/A	0.20
	Mean		**51.6**	**33.0**	**18.6**			

[a] Mean seed to first ligule (STFL) lengths for the GA+ treatment, GA- treatment, and the GA treatment difference (trt diff; GA+ STFL length minus GA- STFL length).

[b] *p*GAI is the probability that a line is classified as GA insensitive based on a mixed model (MM) approach using the GA+ treatment values. *p*GAS = 1-*p*GAI. N/A = not applicable.

[c] An LSD test with a critical t value of $p = .05$ was used to determine the probability that the GA treatment difference is significantly greater than zero.

[d] Introgressed alleles are shown following the underscore.

[e] *Rht* genotype based on the *Rht-B1a/b* and *Rht-D1a/b* PCR assays. *Rht-B1c* did not amplify a product in the *Rht-B1a/b* assays.

For the INRA BWCC accessions in GA expt. 1, STFL lengths in the GA+ treatment ranged from 34 to 123 mm (mean of 79.8 mm) and GA treatment differences ranged from -15 mm to 69 mm (mean of 23.3 mm) (Table 6.5, GA expt. 1; Appendix VII). Mean STFL lengths in the GA+ treatment and GA- treatment, and GA treatment differences all had the same ranking among *Rht-*

1 genotypes with *Rht-D1a+Rht-B1a* > *Rht-B1b+Rht-D1a* > *Rht-B1a+Rht-D1b* > *Rht-B1b+Rht-D1b*. GA- treatment differences were the least pronounced. Using the MM approach, 254 accessions were classified as GAS, 45 as GAI, and 60 as INT. With the LSD approach, 253 accessions were classified as GAS (i.e. *p* no treatment difference ≤ 0.05) and 102 accessions were classified as NSTD (*p* of no treatment difference > 0.05), which is indicative of an insensitive or an intermediate GA response.

Table 6.5. Summary GA responses of INRA BWCC lines in GA experiments 1, 2, and 3

			Mean STFL length (mm)[a]			MM class.[b]			LSD class.[c]	
GA Expt	*Rht* Genotype[d]	Statistic[e]	GA+ trt	GA- trt	Trt Diff (mm)	GAS	GAI	INT	GAS	NSTD
1	*B1aD1a*	count	272	276	268	249	0	23	239	29
		min;max	58;123	31;84	0;69					
		mean	87.8	59.4	28.2					
	B1bD1a	count	36	36	36	2[f]	10	24	9[g]	27
		min;max	45;75	27;60	-3;26					
		mean	59.3	47.5	11.8					
	B1aD1b	count	38	38	38	1[h]	25	12	2[i]	36
		min;max	40;76	36;60	-12;30					
		mean	51.9	46.6	5.3					
	B1bD1b	count	9	9	9	0	9	0	0	9
		min;max	42;54	37;59	-15;10					
		mean	45.6	43.6	2.0					
	All genotypes	count	359	363	355	254	45	60	253	102
		min;max	34;123	27;84	-15;69					
		mean	79.8	56.3	23.3					
2	*B1aD1a*	count	91	91	91	76	1[j]	14	88	2
		min;max	53;123	48;89	5;51					
		mean	92.0	63.4	28.6					
	B1bD1a	count	33	33	33	0	26	7	4[k]	29
		min;max	39;77	38;72	-3;20					
		mean	58.6	51.3	7.3					
	B1aD1b	count	23	23	23	0	22	1	2[l]	21
		min;max	44;65	40;59	-7;16					
		mean	53.2	49.0	4.1					
	B1bD1b	count	3	3	3	0	3	0	0	3
		min;max	38;50	36;47	-5;3					
		mean	42.8	42.6	0.2					
	All genotypes	count	154	154	154	77	54	23	95	59
		min;max	27;123	48;89	-7;51					
		mean	77.1	57.9	19.3					
3	*B1aD1a*	count	19	19	19	NA	NA	NA	19	0
		min;max	44;73	24;45	12;31					
		mean	56.9	31.7	19.7					

[a] STFL = Length from seed to first ligule. Treatment difference (Trt Diff) is GA+ treatment STFL length minus GA- treatment STFL length averaged across all accessions within an *Rht*-genotype. Trt diffs in GA expt. 3 were multiplied by 0.78 to allow for comparisons to be made with GA expts. 1 and 2 as described in section 6.3.5.3.

[b] A mixed model (MM) approach was used to classify accessions based on GA+ STFL lengths in expts 1 and 2. Accessions were classified as GA insensitive (GAI) if *p*GAI ≥ 0.95; GA sensitive (GAS) if *p*GAS ≥ 0.95, or intermediate (INT) if *p*GAI < 0.95 and *p*GAS < 0.95.

[c] An LSD was used to determine the probability that an accession's treatment difference was equal to zero. Accessions were classified as GAS if the probability of no treatment difference was ≤ 0.05 or as 'no significant treatment difference' (NSTD) if p > 0.05. NSTD is indicative of a GAI or INT response.

[d] *Rht* genotype determined using *Rht-B1a/b,* and *Rht-D1a/b* primers. Accessions heterozygous at these loci or that contained alternative alleles are only included under "All genotypes".

[e] Count refers to the number of accessions belonging a specified genotype or classification group. Min = minimum value; Max = maximum value.

[f] INRA BWCC lines 13811 (Opata 85); 13812 (W7984)

[g] INRA BWCC lines 01747 (114/62); 02424 (Danubia); 03050 (Flamura-85); 03176 (Fukuho-komugi); 07092 (Tyler); 08194 (Neelkant); 13210 (Solaris); 13476 (Taldor); 13811 (Opata 85)

[h] INRA BWCC line 20224 (Fantaziya-Odesskaya)

[i] INRA BWCC lines 00822 (Aifeng-4); 20224 (Fantaziya-Odesskaya)

[j] INRA BWCC line 03752 (IAR W83-2)

[k] INRA BWCC lines 02345 (Corsodor); 06027 (Recital); 08194 (Neelkant); 13811 (Opata 85)

[l] INRA BWCC lines 01647 (BT2281); 20276 (Equinox)

MM and LSD GA response classifications were evaluated against the *Rht-B1+Rht-D1* genotype to determine if any unexpected GA responses occurred (Table 6.5, GA expt. 1). Differences between *Rht-1* genotype and GA response could be indicative of the presence of a novel allele affecting GA sensitivity at these or other loci. Using the MM approach, 249 *Rht-B1a+Rht-D1a* accessions were classified as GAS, 23 as INT, and none as GAI. Similarly, the LSD classified 239 of the *Rht-B1a+Rht-D1a* accessions as GAS and 29 as NSTD. These results suggest the absence of any novel GAI alleles. For the 36 accessions with the *Rht-B1b+Rht-D1a* genotype, only ten were classified as GAI using MM, 24 as INT, and two as GAS (INRA_13811; INRA_13812). The LSD approach classified nine *Rht-B1b+Rht-D1a* accessions (listed in Table 6.6, footnote g) as GAS including INRA_13811 and 27 as NSTD. For the 38 accessions with the *Rht-B1a+Rht-D1b*

genotype, the MM approach classified 25 as GAI, 12 as INT, and one (INRA_20224) as GAS. Using the LSD approach, two lines (INRA_20224; INRA_00822) with the *Rht-B1a*+*Rht-D1b* genotype were classified as GAS and 36 as NSTD. All nine of the accessions with the *Rht-B1b*+*Rht-D1b* genotype were categorised as GAI in the MM approach and as NSTD in the LSD approach.

6.3.5.2 GA sensitivities of accessions in GA experiment 2

Based primarily on results from GA expt. 1, 154 INRA BWCC lines along with the same seven control lines used in GA expt. 1 were selected for a second GA sensitivity test. The objective of GA expt. 2 was to increase the number of replicates of: (1) any *Rht-B1a*+*Rht-D1a* variety not classified as GAS in the MM or LSD analysis, (2) any variety with a semi-dwarf allele classified as INT in the MM procedure or with a probability of no treatment difference of 0.50 or less based on LSD, or (3) any variety in which zero or one replicate of either GA treatment was obtained (72 accessions).

The STFL lengths from the GA+ treatment of the INRA BWCC and control lines in GA expt. 2, revealed an apparent bi-modal distribution similar to GA expt. 1 (Figure 6.7B). As for GA expt. 1, the *Rht-B1a*+*Rht-D1a* controls were all identified as belonging to the GAS group using the MM and LSD tests (Table 6.4, GA expt. 2). Three of the four lines with a dwarfing allele (AB_*Rht-D1b*, AB_*Rht-D1*c, and Mercia *Rht-D1b*) were classified as GAI (p = 1) using MM, and GA treatment difference was not significantly greater than zero using LSD. For Bersee_*Rht-B1b*, the MM placed the probability of this line being GAI as 0.78, whereas the LSD approach classified this control as GA sensitive (p = 0.02 of no GA treatment difference). Similar to GA expt. 1, GA treatment differences of plants containing *Rht-B1a*+*Rht-D1a* ranged from 25.8 to 30.2 mm and GA treatment differences for the *Rht-B1b*, *Rht-D1b*, and *Rht-B1c* NILs ranged from 2.3 to 9.8 mm.

In GA expt. 2, when all INRA BWCC genotypes were assessed, the STFL lengths in the GA+ treatment ranged from 27 to 123 mm (mean of 77.1) and

GA treatment differences ranged from -7 to 51 mm (mean of 19.3) (Table 6.5, GA expt. 2; Appendix VII). The mean STFL lengths of the GA+ treatment, GA- treatment and GA treatment difference all had the same *Rht-1* genotype rankings as for GA expt. 1, with *Rht-D1a*+*Rht-B1a* > *Rht-B1b*+*Rht-D1a* > *Rht-B1a*+*Rht-D1b* > *Rht-B1b*+*Rht-D1b*. Using the MM approach, 77 accessions were classified as GAS, 54 as GAI, and 23 as INT. With the LSD approach, 95 accessions were classified as GAS and 59 were classified as NSTD.

Analysis of GA classifications in light of *Rht-1* genotypes in GA expt. 2 revealed that of 91 accessions with the *Rht-B1a*+*Rht-D1a* alleles, 76 were categorised as GAS, 14 as INT, and one (INRA_03752) as GAI using the MM approach (Table 6.5, GA expt. 2). LSD classifications of *Rht-B1a*+*Rht-D1a* accessions placed 88 in the GAS class and three into the NSTD class. Of the 33 accessions with the *Rht-B1b*+*Rht-D1a* genotype, 26 were classified as GAI, seven as INT, and none as GAS using the MM approach. With the LSD test, four *Rht-B1b*+*Rht-D1a* accessions were classified as GAS (INRA lines 02345, 06027, 08194, and 13811) and 29 as NSTD. For the 23 accessions with the *Rht-B1a*+*Rht-D1b* genotype, the MM method classified 22 as GAI, one as INT, and none as GAS. Using the LSD test on this same group, two of the 23 INRA BWCC lines (INRA_01647; INRA_20276) were classified as GAS and 21 as NSTD. The three accessions with the *Rht-B1b*+*Rht-D1b* genotype were all categorised as GAI in the MM procedure and as NSTD by the LSD.

6.3.5.3 GA sensitivities of accessions in GA experiment 3

To more fully determine if any of the *Rht-B1a*+*Rht-D1a* INRA BWCC lines tested in GA expt. 1 or 2 potentially contained a novel GAI allele, a third GA sensitivity experiment was performed. GA expt. 3 consisted of the same seven controls used in GA expts. 1 and 2, and 19 *Rht-B1a*+*Rht-D1a* INRA BWCC lines with a short height in the INRA BWCC outdoor experiment and a small GA treatment difference when averaged across GA expts. 1 and 2.

A bi-modal distribution of STFL lengths of the INRA BWCC accessions and controls was not apparent in GA expt. 3 (Figure 6.7C); hence the MM

approach was not used to classify the GA response. As in GA expts. 1 and 2, controls with the *Rht-B1a+Rht-D1a* genotype had probabilities of no GA treatment difference equal to zero (Table 6.4, GA expt. 3). For the controls lines containing a dwarfing allele, the probability values of no GA treatment difference were reduced relative to GA expts. 1 and 2 (*i.e.* the dwarf control NILs appeared more GA sensitive in expt. 3). The mean treatment difference among GA expt. 3 controls was 18.6 mm, which is four mm greater than in GA expts. 1 or 2. Overall, the control results indicate that it will be more difficult to distinguish GAI accessions in GA expt. 3 than in GA expts. 1 or 2. As mean treatment differences among controls in GA expts. 1 and 2 were 78% that of GA expt. 3, GA expt. 3 treatment differences were "adjusted" by multiplying by 0.78 to facilitate comparisons with GA expts. 1. and 2.

All INRA BWCC lines in GA expt. 3 had the *Rht-B1a+Rht-D1a* genotype and all 19 were classified as GAS based on the LSD (Table 6.5, GA expt. 3). The STFL lengths in the GA+ treatment ranged from 44 to 73 mm (mean of 56.9) and "adjusted" GA treatment differences ranged from 12 to 31 mm (mean of 19.7; Appendix VII). In GA expt. 3, INRA_03752 (the only accession classified as GAI in a previous GA experiment) had the longest STFL length of any accession in the GA+ treatment (72.5 mm), which was over double that of its GA- length (33.5 mm). Mean GA treatment differences of each accession averaged across the three experiments are shown in Appendix VI.

6.3.6. Phenotype-genotype associations of INRA BWCC accessions

6.3.6.1. *Rht-B1*Rht-D1*Ppd-D1* REML analysis

A REML analysis was conducted to estimate the effects of the *Rht-B1*, *Rht-D1*, and *Ppd-D1* genotypes on heading date, plant height, and mean GA treatment difference. For heading date, *Ppd-D1* had a statistically significant effect (F probability (F pr) < 0.001 of no effect) and accounted for the majority of the genotype variance (Table 6.6A). *Rht-B1* was also significantly (F pr = 0.023) associated with heading date while *Rht-D1* and the genotype interactions were not significant at F pr ≤ 0.05. For height, significant effects

(all with F pr < 0.001) were associated with *Rht-B1*, *Rht-D1*, *Ppd-D1*, and *Rht-B1*.*Ppd-D1* (Table 6.6B) with most of the genotype variation due to the main effects of *Rht-B1* and *Rht-D1*. The remaining interaction terms were not significant at F pr ≤ 0.05, although *Rht-B1*.*Rht-D1* was nearly significant (F pr = 0.084). For mean GA difference, *Rht-B1*, *Rht-D1,* and *Rht-B1*.*Rht-D1* all had F pr values < 0.001 (Table 6.6C) while the remaining genotypic effects were not significant at F pr ≤ 0.05. *Rht-B1* and *Rht-D1* main effects constituted nearly the entire variance component among genotype classes.

Table 6.6. Summary of *Rht-B1*Rht-D1*Ppd-D1* REML mixed model analyses for heading date (A), plant height (B), and mean GA difference (C) in the INRA 368 BWCC.

A) HEADING DATE

Effect	Term[a]	Wald statistic[b]	ndf[c]	F statistic	ddf[d]	F pr[e]
Fixed	Ppd-D1	62.2	1	62.2	337	<0.001
Fixed	Rht-B1	9.64	3	3.21	337	0.023
Fixed	Rht-D1	0.47	1	0.47	337	0.496
Fixed	Ppd-D1.Rht-B1	0.17	3	0.06	337	0.983
Fixed	Ppd-D1.Rht-D1	0.5	1	0.5	337	0.478
Fixed	Rht-B1.Rht-D1	0.52	3	0.17	337	0.914
Fixed	Ppd-D1.Rht-B1.Rht-D1	4	2	2	337	0.137

Residual variance (variety)[f] = 59.16 with SE = 4.56

B) PLANT HEIGHT

Effect	Term	Wald statistic	ndf	F statistic	ddf	F pr
Fixed	Rht-B1	238.03	3	79.34	337	<0.001
Fixed	Rht-D1	145.03	1	145.03	337	<0.001
Fixed	Ppd-D1	57.6	1	57.6	337	<0.001
Fixed	Rht-B1.Rht-D1	6.69	3	2.23	337	0.084
Fixed	Rht-B1.Ppd-D1	19.07	3	6.36	337	<0.001
Fixed	Rht-D1.Ppd-D1	1.37	1	1.37	337	0.243
Fixed	Rht-B1.Rht-D1.Ppd-D1	1.31	2	0.65	337	0.52

Residual variance (variety) = 399.7 with SE = 30.8

C) MEAN GA TREATMENT DIFFERENCE

Effect	Term	Wald statistic	ndf	F statistic	ddf	F pr
Fixed	Rht-B1	266.22	3	88.74	337	<0.001
Fixed	Rht-D1	318.93	1	318.93	337	<0.001
Fixed	Ppd-D1	0.48	1	0.48	337	0.488
Fixed	Rht-B1.Rht-D1	27.45	3	9.15	337	<0.001
Fixed	Rht-B1.Ppd-D1	1.06	3	0.35	337	0.788
Fixed	Rht-D1.Ppd-D1	1.93	1	1.93	337	0.166
Fixed	Rht-B1.Rht-D1.Ppd-D1	0.54	2	0.27	337	0.762

Residual variance (variety) = 53.58 with SE = 4.13

[a] *Rht-B1* consists of *Rht-B1a_0* (no insertion), *Rht-B1a_160* (160 bp insertion), *Rht-B1a_197* (197 bp insertion), and *Rht-B1b* alleles. *Rht-D1* consists of *Rht-D1a* and *Rht-D1b* alleles. *Ppd-D1* consists of *Ppd-D1a* and *Ppd-D1b* alleles. The (.) indicates an interaction term.

[b] the Wald statistic is a measure of variation

[c] ndf = numerator degrees of freedom

[d] ddf = denominator degrees of freedom

[e] F pr = F probability of no treatment difference.

[f] Residual variance between varieties within genotypic terms was estimated using variance as the random term in the model. SE = Estimated standard error of the residual variance.

6.3.6.2. *Rht-B1* genotype REML and means analysis

Mean effects of the *Rht-B1* alleles on heading date, plant height, and GA treatment difference were compared using the INRA BWCC accession data. As other loci were found to significantly influence these phenotypes (Table 6.6), mean *Rht-B1* allelic effects were estimated in relation to either *Rht-D1* or *Ppd-D1*, depending on which locus accounted for the largest portion of the remaining genotype variation in the *Rht-B1*Rht-D1*Ppd-D1* REML analysis. For each phenotype, a fitted mean was calculated for each *Rht-B1* allele as the average value across both allelic classes of the second locus. Fitted means were declared to be significantly different (SD) or not significantly different (NSD) using an LSD with a probability threshold of ≤ 0.05.

For mean heading date, *Rht-B1* alleles were compared across the *Ppd-D1* backgrounds as *Ppd-D1* was previously shown (Table 6.6A) to account for most of the heading date variation. The mean heading dates of *Rht-B1a_160* accessions and *Rht-B1a_197* accessions were later than that of *Rht-B1_0* or *Rht-B1b* accessions regardless of *Ppd-D1* allele (Table 6.7A). Comparing *Rht-B1* fitted means, heading dates of *Rht-B1a_160* accessions were 1.1 days later than *Rht-B1a_197* accessions (NSD), 2.9 days later than *Rht-B1a_0* accessions (SD), and 2.8 days later than the *Rht-B1b* accessions (NSD). *Rht-B1a_197* accessions also flowered later than *Rht-B1a_0* accessions (1.8 days, NSD) or *Rht-B1b* accessions (1.7 days, NSD) when comparing fitted means.

Table 6.7. Mean effects of *Rht-B1* alleles on heading date (A), plant height (B), and GA treatment difference (C) among INRA BWCC accessions.

A) MEAN DAYS TO HEADING[a]

	Ppd-D1a	*Ppd-D1b*	Fitted mean[b]	Fitted means significantly different at $p \leq 0.05$[c]
Rht-B1a_160	137 (11)	145.4 (47)	141.2	*Rht-B1a_160* > *Rht-B1a_0*
Rht-B1a_197	136.5 (11)	143.7 (31)	140.1	
Rht-B1a_0	134.8 (43)	141.7 (165)	138.3	
Rht-B1b	134.6 (25)	142.2 (19)	138.4	

B) MEAN PLANT HEIGHT (cm)

	Rht-D1a	*Rht-D1b*	Fitted mean	Fitted means significantly different at $p \leq 0.05$
Rht-B1a_160	142.1 (54)	89.2 (4)	115.7	*Rht-B1a_160* > *Rht-B1b*
Rht-B1a_197	126.4 (31)	94.9 (11)	110.7	*Rht-B1a_197* > *Rht-B1b*
Rht-B1a_0	147.3 (187)	103.6 (21)	125.4	*Rht-B1a_0* > *Rht-B1a_197*,*Rht-B1b*
Rht-B1b	100.7 (35)	72.2 (9)	86.4	

C) MEAN GA TRT DIFF (mm)[d]

	Rht-D1a	*Rht-D1b*	Fitted mean	Fitted means significantly different at $p \leq 0.05$
Rht-B1a_160	28.3 (54)	0.6 (4)	14.4	*Rht-B1a_160* > *Rht-B1b*
Rht-B1a_197	27.4 (31)	5.3 (11)	16.3	*Rht-B1a_197* > *Rht-B1b*
Rht-B1a_0	29.4 (187)	5.1 (21)	17.3	*Rht-B1a_0* > *Rht-B1b*
Rht-B1b	9.4 (35)	0.7 (9)	5.0	

[a] Days from January 1. *Rht-B1a_160*, *Rht-B1a_197*, and *Rht-B1a_0* refer to *Rht-B1a* alleles with a 160 bp, a 197 bp, or no insertion, respectively. The numbers of accessions possessing each genotypic combination are indicated in parentheses.

[b] Fitted mean refers to the average effect associated with an *Rht-B1* allele across the *Ppd-D1* alleles (box A) or across the *Rht-D1* alleles (boxes B and C).

[c] Significant differences are based on an LSD with a probability threshold of 0.05. Direction of significance is shown with by >.

[d] Mean GA treatment difference (trt diff) refers to the increase in seed-to-first-ligule (STFL) length of the GA+ treatment minus the GA- treatment.

For mean plant height, the effects of the *Rht-B1* alleles were compared across *Rht-D1* backgrounds (Table 6.7B). Relative to the three *Rht-B1a* alleles, the *Rht-B1b* allele was associated with shorter plant heights in both *Rht-D1* backgrounds. The reductions in fitted mean plant height associated with *Rht-B1b* ranged from 24.3 to 39 cm relative to the *Rht-B1a* alleles, and all differences were significant at $p \leq 0.05$. *Rht-B1a* alleles with an insertion were associated with height reductions relative to *Rht-B1a_0*. Comparing fitted means, *Rht-B1a_197* accessions were 14.7 cm (12%) shorter (SD) than *Rht-B1a_0* accessions and *Rht-B1*_160 accessions were 9.7 cm (8%) shorter

(NSD) than *Rht-B1a_0* accessions. Between the two insertion alleles, *Rht-B1*_160 was associated with a 15.7 cm height increase over *Rht-B1*_197 in the *Rht-D1a* background, but also a 5.7 cm height decrease in the *Rht-D1b* background, indicative of an interaction. However, *Rht-B1a_160*+*Rht-D1b* is present in only four lines and may be an unreliable estimate of the true mean. For the fitted means of the *Rht-B1a* insertion alleles, *Rht-B1a_160* was 5 cm taller than *Rht-B1a_197* (NSD).

For GA sensitivity, the effects of *Rht-B1* alleles were estimated across the *Rht-D1* loci (Table 6.7C). Similar to plant height, the GA treatment difference associated with *Rht-B1b* was significantly lower ($p < 0.05$) than each of the *Rht-B1a* alleles, with reductions ranging from 9.4 to 12.3 cm. Comparing fitted means, a reduced GA treatment difference is associated with *Rht-B1a_160* (-2.9 mm; -17%) and *Rht-B1a_197* (-1.0 mm; -6%) relative to *Rht-B1a_0*; however these differences were NSD. When comparing among the *Rht-B1a* alleles, *Rht-B1a_197* has the smallest mean GA treatment difference in the *Rht-D1a* background and the largest mean GA treatment difference in the *Rht-D1b* background, which indicates an interaction.

6.3.6.3. *RHT*Ppd-D1* REML and means analysis

A means analysis was performed to estimate the changes in heading date, plant height, and GA treatment difference associated with *Rht-B1*+*Rht-D1* semi-dwarf allele combinations in differing *Ppd-D1* backgrounds (Table 6.8). In this analysis, *Rht-B1a* allelic classes were combined due to the small effects of the insertions relative to *Rht-B1b*. Accessions with the *Ppd-D1a* allele had a fitted mean heading date that was 6.6 days earlier than accessions with the *Ppd-D1b* allele (SD, using an LSD threshold of $p < 0.05$). In contrast, there was only a 1.6 day range in heading dates among the *Rht-1* genotype classes when comparing fitted means. The six accessions that did not amplify a PCR product using the *Ppd-D1* primers had a mean heading date of 142.2 days and the accession (INRA_13812) that amplified a 450 bp product had a mean heading date of 142 days. These heading dates are similar to the fitted mean heading date of *Ppd-D1b* (142.3 days).

Table 6.8. Gentoypic effects of *Rht-1* and *Ppd-D1* alleles on heading date (A), plant height (B), and GA treatment difference (C) among INRA BWCC accessions.

A) MEAN DAYS TO HEADING[a]

	Ppd-D1a	*Ppd-D1b*	Fitted mean[b]	Fitted means significantly different at $p \leq 0.05$[c]
Rht-B1a+Rht-D1a	135.6 (46)	142.6 (226)	139.1	
Rht-B1a+Rht-D1b	135.2 (19)	143.8 (17)	139.5	
Rht-B1b+Rht-D1a	133.4 (19)	142.5 (16)	137.9	
Rht-B1b+Rht-D1b	138.7 (6)	140.3 (3)	139.5	
Fitted mean	135.7	142.3		*Ppd-D1b* > *Ppd-D1a*

B) MEAN PLANT HEIGHT (cm)

	Ppd-D1a	*Ppd-D1b*	Fitted mean	Fitted means significantly different at $p \leq 0.05$
Rht-B1a+Rht-D1a	119.6 (46)	148.8 (226)	134.2	*Rht-B1a+Rht-D1a* >all *Rht-1* genotypes
Rht-B1a+Rht-D1b	100.7 (19)	97.8 (17)	99.3	*Rht-B1a+Rht-D1b* > *Rht-B1b+Rht-D1b*
Rht-B1b+Rht-D1a	98.8 (19)	103 (16)	100.9	*Rht-B1b+Rht-D1a* > *Rht-B1b+Rht-D1b*
Rht-B1b+Rht-D1b	70.3 (6)	75.8 (3)	73.1	
Fitted mean	97.4	106.4		*Ppd-D1b* >*Ppd-D1a*

C) MEAN GA TRT DIFF (mm)[d]

	Ppd-D1a	*Ppd-D1b*	Fitted mean	Fitted means significantly different at $p \leq 0.05$
Rht-B1a+Rht-D1a	27.8 (46)	29.2 (226)	28.5	*Rht-B1a+Rht-D1a* >all *Rht-1* genotypes
Rht-B1a+Rht-D1b	5.7 (19)	3.4 (17)	4.6	*Rht-B1a+Rht-D1b* < *Rht-B1b+Rht-D1a*
Rht-B1b+Rht-D1a	9.6 (19)	9.1 (16)	9.3	*Rht-B1b+Rht-D1a* > *Rht-B1b+Rht-D1b*
Rht-B1b+Rht-D1b	2.4 (6)	-2.6 (3)	-0.1	
Fitted mean	11.4	9.8		

[a] Days from January 1. The numbers of accessions possessing each genotypic combination are indicated in parentheses.

[b] Fitted mean refers to the average effect associated with an allele across genotypes.

[c] Significant differences are based on an LSD with a probability threshold of 0.05. Direction of significance is shown with by > or <.

[d] Mean GA treatment difference (trt diff) refers to the increase in seed-to-first-ligule (STFL) length of the GA+ treatment minus the GA- treatment.

For plant height, the *Rht-B1a+Rht-D1a* combination was taller than all other *Rht-1* genotype classes in both *Ppd-D1* backgrounds (Table 6.8B). The fitted means of *Rht-B1a+Rht-D1a* accessions were 34.9 (SD), 33.3 (SD), and 61.1 (SD) cm taller than the fitted means of *Rht-B1a+Rht-D1b*, *Rht-B1b+Rht-D1a*, and *Rht-B1b+Rht-D1b*, respectively. Similarly, accessions with the *Rht-B1b+Rht-D1b* alleles were shorter than all other *Rht-1* genotype classes regardless of *Ppd-D1* background. The fitted means of *Rht-B1b+Rht-D1b* accessions were 27.8 cm (SD) and 26.2 cm (SD) shorter than *Rht-B1a+Rht-*

D1b and *Rht-B1b+Rht-D1a* accessions, respectively. Comparing accessions with a single *Rht-1b* allele, the fitted mean plant height of *Rht-B1b+Rht-D1a* accessions was 1.6 cm (NSD) greater than that of *Rht-B1a+Rht-D1b* accessions. Accessions with the *Ppd-D1a* allele showed a 9 cm reduction in plant height relative to *Ppd-D1b* accessions when comparing fitted means (SD). However, a clear interaction with *Rht-1* is evident as *Ppd-D1a* accessions are 29.2 cm shorter than *Ppd-D1b* accessions in the *Rht-B1a+Rht-D1a* background, but in genetic backgrounds containing *Rht-B1b* and/or *Rht-D1b, Ppd-D1a* mean plant heights are from 5.5 cm shorter to 2.9 cm taller than *Ppd-D1b* mean plant heights.

For mean GA treatment difference, significant differences were found among *Rht-1* genotypes but not between *Ppd-D1* genotypes (Table 6.8C). *Rht-B1a+Rht-D1a* accessions were associated with an increased mean GA treatment difference in both *Ppd-D1* backgrounds. Comparing fitted means, *Rht-B1a+Rht-D1a* lines had increases in mean GA treatment difference of 23.9 (SD), 19.2 (SD), and 28.6 (SD) mm relative to *Rht-B1a+Rht-D1b*, *Rht-B1b+Rht-D1a*, and *Rht-B1b+Rht-D1b*, respectively. The *Rht-B1b+Rht-D1b* accessions had lower mean GA treatment differences than all other *Rht-1* genotype classes in both *Ppd-D1* backgrounds. Comparing fitted means, GA treatment differences of *Rht-B1b+Rht-D1b* lines were 4.7 mm (NSD) less than *Rht-B1a+Rht-D1b* lines and 9.4 mm (SD) less than *Rht-B1b+Rht-D1a* lines. The fitted means of the *Rht-B1b+Rht-D1a* accessions were 4.7 (SD) mm greater than the fitted means of the *Rht-B1a+Rht-D1b* accessions.

6.3.6.4. *Rht-B1* and *Ppd-D1* association analysis with adjustment for population structure (AA-PS)

An association analysis that accounts for the effects of population structure (AA-PS) was performed using a kinship matrix based on genome-wide DArT and SSR markers (Table 6.9). In the AA-PS, the primary effect of the presence / absence of each allele (*Rht-B1b*; *Rht-B1a_160*; *Rht-B1a_197*; *Rht-D1b*; *Ppd-D1a*) on mean heading date, plant height, and GA treatment difference (GA expts. 1 and 2 only) was determined with a significance

threshold set at $p = 0.001$. For heading date, only *Ppd-D1a* was significantly associated. Plant height was found to be significantly associated with *Rht-B1b*, *Rht-D1b*, and *Ppd-D1a*, but not with *Rht-B1a_160* or *Rht-B1a_197*. Similarly, GA treatment difference was found to be associated with *Rht-B1b*, *Rht-D1b*, and *Ppd-D1a*, but not with *Rht-B1a_160* or *Rht-B1a_197*.

Table 6.9. Summary of the INRA BWCC association analysis with adjustment for population structure (AA-PS)

Trait[a]	Allele[b]	P value[c]	R^2[d]
Plant height	*Rht-B1b*	5.69E-22	0.228
	Rht-B1a_160	NS	
	Rht-B1a_197	NS	
	Rht-D1b	1.2E-23	0.234
	Ppd-D1a	1.1E-22	0.219
GA trt. diff.	*Rht-B1b*	6.5E-27	0.274
	Rht-B1a_160	NS	
	Rht-B1a_197	NS	
	Rht-D1b	2.5E-40	0.389
	Ppd-D1a	1.8E-10	0.108
Heading date	*Rht-B1b*	NS	
	Rht-B1a_160	NS	
	Rht-B1a_197	NS	
	Rht-D1b	NS	
	Ppd-D1a	1.1E-14	0.154

[a] Heading date and plant heights recorded in Cambridge, UK. GA treatment differences (GA trt. diff.) represent combined data from GA sensitivity expts 1 and 2.

[b] Association is based on presence/absence of the indicated allele

[c] NS = not significant at $p < 0.001$

[d] R^2 values are indicated only for associations with $p < 0.001$

6.3.7. Phenotype and genotype analyses of INRA BWCC accession types

To determine the effect of growth habit, varietal type (fixed or landrace), and year of registration of accessions on plant phenotype an additional REML analysis was performed and means separated using an LSD (probability threshold of 0.05) when more than two means were compared. Growth habit did not significantly affect height or GA treatment difference, but did have a significant effect on heading date (Table 6.10A). Accessions with a spring growth habit were associated with a 4.5 day delay (SD) relative to winter habit

accessions and an 8.5 day delay (SD) relative to facultative habit accessions. However, these differences may relate to the later planting date (February 2009) of the spring types relative to the winter and most facultative types (November 2008). Delays in heading date were reported for controls from the February 2009 sowing relative to the November 2008 sowing (section 6.3.4.) The frequencies of *Rht-B1b*, *Rht-D1b*, and *Ppd-D1a* do not differ largely between the spring and winter accessions while the *Rht-B1* insertion alleles are several-fold more frequent in the winter lines than in the spring lines.

Table 6.10. Allelic composition, plant heights, and heading dates of INRA BWCC accessions by growth habit (A), varietal type (B) and year of registration (C)

A) Growth Habit

Accessions		Days to heading[a]		Height (cm)		GA trt diff (mm)[b]		Allele Frequency[c]				
Habit	No.	mean	sem	mean	sem	mean	sem	160bp ins	197bp ins	*Rht-B1b*	*Rht-D1b*	*Ppd-D1a*
Facultative	10	135.4	1.4	134.7	9.9	22.1	4.5	0.00	0.20	0.30	0.00	0.40
Spring	115	143.9	1.6	130.3	7.9	24.7	1.1	0.04	0.05	0.10	0.14	0.23
Winter	227	139.4	1.4	134.6	9.1	23.4	0.8	0.23	0.15	0.13	0.13	0.26
Sig. Diff. ($p<.05$)[d]		S>I,W		NS		NS						

B) Varietal Type

Accessions		Days to heading		Height (cm)		GA trt diff (mm)		Allele Frequency				
Type	No.	mean	sem	mean	sem	mean	sem	160bp ins	197bp ins	*Rht-B1b*	*Rht-D1b*	*Ppd-D1a*
Fixed	322	140.4	1.6	129.3	8.4	22.9	0.7	0.17	0.13	0.14	0.14	0.28
Landrace	46	143.2	1.6	161.5	6.9	30.5	1.2	0.09	0.00	0.00	0.00	0.00
Sig.Diff. ($p<.05$)		L>F		L>F		L>F						

C) Year of Registration

Accessions		Days to heading		Height (cm)		GA trt diff (mm)		Allele Frequency				
Period	No.	mean	sem	mean	sem	mean	sem	160bp ins	197bp ins	*Rht-B1b*	*Rht-D1b*	*Ppd-D1a*
1) 1800-99	9	145.6	2.8	154.6	7.1	28.0	1.6	0.56	0.11	0.00	0.00	0.11
2) 1900-19	10	145.8	2.2	161.0	5.0	33.2	2.0	0.20	0.00	0.00	0.00	0.00
3) 1920-39	37	144.9	1.9	154.8	4.1	28.6	1.1	0.14	0.03	0.00	0.00	0.08
4) 1940-59	32	142.8	0.9	135.1	3.6	27.8	1.9	0.19	0.13	0.03	0.03	0.09
5) 1960-79	55	140.4	1.1	121.1	3.5	20.5	1.9	0.16	0.20	0.11	0.25	0.36
6) 1980-99	49	139.4	1.0	107.7	3.1	17.4	1.8	0.16	0.14	0.37	0.22	0.43
Sig.Diff. ($p<.05$)		1>6 2>6 3>5,6		1>4,5,6 2>4,5,6 3>4,5,6 4>5,6 5>6		1>6 2>5,6 3>5,6 4>5,6						

[a] Days from January 1. sem = standard error of the mean

[b] GA trt diff = Difference in STFL length between the GA+ and GA- treatments among accessions averaged across all three GA experiments.
[c] Allele frequency indicates proportion of lines that are homozygous for the allele shown.
[d] Any significant differences (Sig. Diff. $p \leq 0.05$) are shown for each phenotype or NS is indicated if no significant difference exists. In box A, S = Spring, I = Indeterminate, W= Winter. In box B, L = landrace and F = fixed. In box 3, the registration period is indicated by a number from 1 to 6 as assigned above.

The effects of varietal type were statistically significant for heading date, plant height, and GA treatment difference (Table 6.10B). The average days to heading were 143.2 for landraces and 140.4 days for fixed varieties. The reduction in days to heading in the fixed varieties relative to landraces coincides with an increased prevalence of the early-flowering *Ppd-D1a* allele, which occurs in 28% of the fixed lines, but none of the landraces. The mean height of the landraces was 161.5 cm while the fixed lines averaged only 129.3 cm. Reduced height in the fixed lines relative to landraces coincides with greater prevalence of *Rht-B1b* (0.14) and *Rht-D1b* (0.14) in fixed lines than in landraces where neither allele was detected. *Rht-B1a_197* bp was present in 13% of the fixed lines and was not detected in the landraces. *Rht-B1a_160* was present in 17% of the fixed lines and is the only allele among this group of five that was also detected in the landraces (freq. = 0.09).

For the 200 INRA BWCC accessions for which the year of varietal registration was available, REML analysis indicated that the period in which a variety was released had a significant effect on heading date, plant height, and GA treatment difference (Table 6.10C). A trend over time towards reduced days to heading, reduced plant height and reduced GA treatment difference was evident, along with an increased prevalence of *Rht-B1b*, *Rht-D1b*, and *Ppd-D1a* (Table 6.10). Mean heading dates of accessions released from 1980-1999 were 5.5 to 6.4 days earlier (SD) than those released in the time periods prior to 1940 (1800-99, 1900-19, and 1920-39). Mean heading date of the period 1960-1979 was also significantly earlier than 1920-39. Associated with time periods that have earlier heading dates is an increased prevalence of *Ppd-D1a*. In the periods 1960-79 and 1980-99, *Ppd-D1a* frequencies were 0.36 and 0.43, respectively, while in the time periods before 1940 *Ppd-D1a*

frequency ranged from 0 to 0.11. Mean plant height in the period 1980-1999 (107.7 cm) was significantly shorter than in any other time period. Similarly, mean plant height from 1960-79 (121.1 cm) was significantly shorter than in the preceding time periods, and mean plant height from 1940-1959 (135.1 cm) was also significantly reduced relative to preceding periods. GA treatment difference was significantly less in the periods 1960-1979 and 1980-99 relative to 1900-19, 1920-39, and 1940-59 and significantly less in 1980-99 relative to 1800-99. Reduced plant height and reduced GA treatment differences are associated with increased usage of the *Rht-B1b* and *Rht-D1b* alleles, which were not present in any variety released before 1940 and had increasing prevalence in the time periods following 1940. However, in the time frame 1940-59, only two of 32 accessions contained one of these alleles, yet plant heights were significantly shorter than in the preceding time periods. For the *Rht-B1* insertions, no clear trend in prevalence with time period of registration is apparent. The 160 bp insertion was most prevalent in the 1800's (five of nine lines, 0.56 frequency) and following that time frame, the frequency of lines containing this allele in any 20-year time span ranged from 0.14 to 0.20. The 197 bp insertion was most frequent (0.20) in accessions registered between 1960 and 1979 and least frequent (0.0) from 1900-1919.

6.3.8. Correlations among height, GA sensitivity, and ear emergences of the INRA BWCC

Correlation analyses among plant height, GA treatment difference, and heading date of accessions revealed the highest correlation among these three phenotypes is between plant height and GA treatment difference (R^2 = 0.338 (Figure 6.8). Short heights and small GA treatment differences are most frequently represented in accessions with the *Rht-B1b* or *Rht-D1b* allele (Figure 6.8A). In comparison, the correlations between heading date and GA treatment difference (R^2 = 0.010) (Figure 6.8B) and between heading date and plant height (R^2 = 0.033) (Figure 6.8C) were much lower.

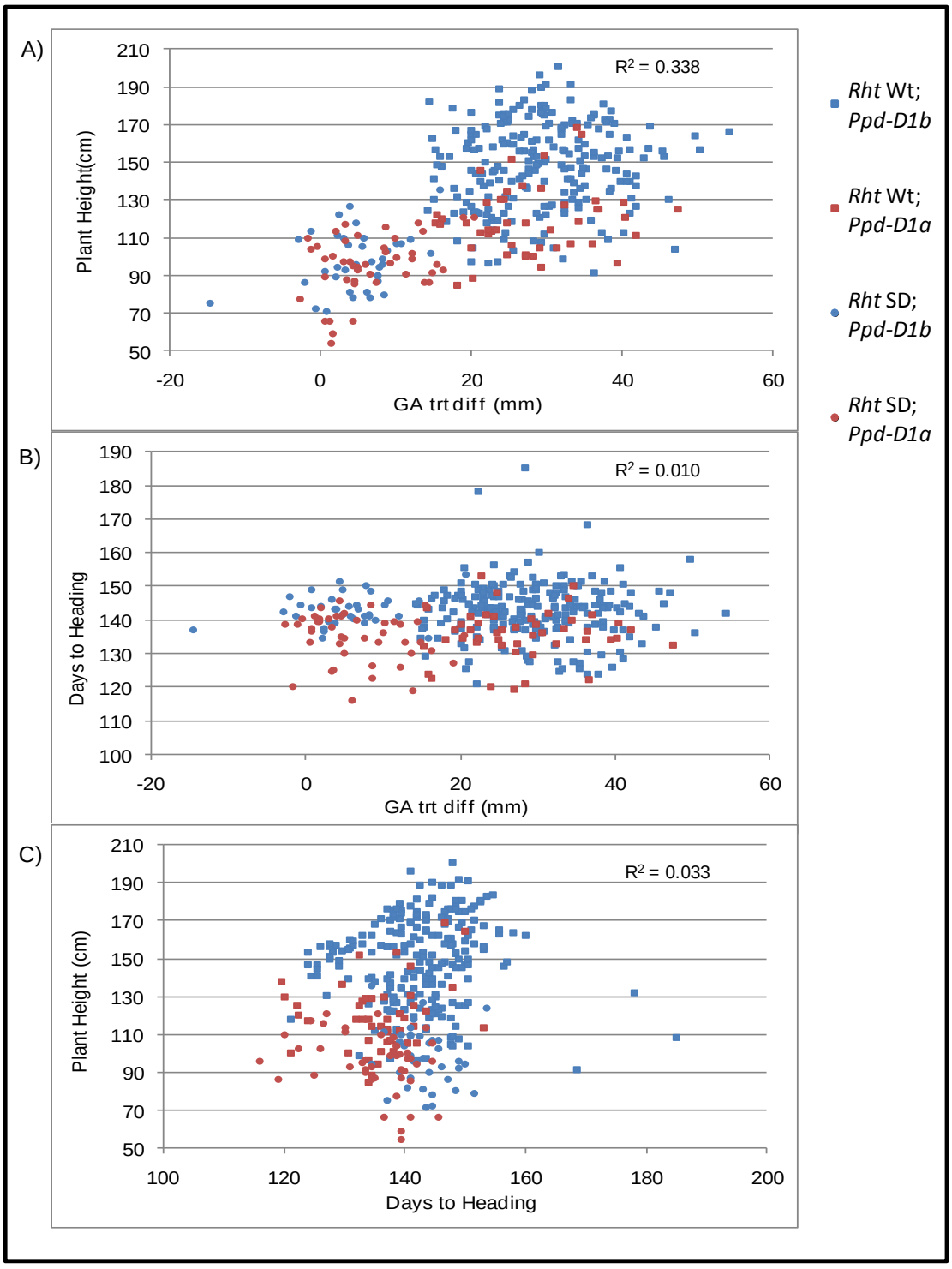


Figure 6.8. Correlations between plant phenotypes among INRA BWCC accessions. Correlations were made between plant height and GA treatment difference (A), days to heading and GA treatment difference (B), and plant height and days to heading (C) for the INRA BWCC accessions. Each point represents the mean of an accession. *Rht* WT = Accessions with the *Rht-B1a*+*Rht-D1a* genotype; *Rht* SD = accessions with a semi-dwarfing allele (*Rht-B1b* and/or *Rht-D1b*); *Ppd-D1b* = accessions containing the *Ppd-D1b* allele; *Ppd-D1a* = accessions containing the *Ppd-D1a* allele.

6.4. DISCUSSION

6.4.1. *Rht-1* and *Ppd-D1* allelic prevalence in the INRA BWCC

Approximately 23% of the 368 INRA BWCC accessions contained either *Rht-B1b* and/or *Rht-D1b* with *Rht-B1b* occurring in 12.2% and *Rht-D1b* occurring in 12.8% of the accessions (2.4% contained both alleles) (Table 6.2). In 67 wheat varieties from 20 countries held in the Institute of Field and Vegetable Crops, Novi Sad, core collection, *Rht-B1b* occurred in 58% of the accessions and *Rht-D1b* in 22% of the accessions (Tosovic-Maric, 2008). Other studies have examined prevalence of these alleles in geographically restricted sets of accessions, which vary widely in the occurrence of these alleles: in 220 autumn-sown Chinese wheats, *Rht-B1b* and *Rht-D1b* were present in 25% and 46% of the varieties, respectively (Zhang *et al.* 2006); in 57 Czech varieties, *Rht-B1b* and *Rht-D1b* were present in 11% and 25% of the varieties, respectively (Chrpova *et al.*, 2003); in 105 Serbian wheats, *Rht-B1b* and *Rht-D1b* were present in 29% and 22% of the germplasm, respectively (Tosovic-Maric, 2008); in 95 winter wheat varieties registered in Germany in 2004, *Rht-B1b* and *Rht-D1b* were present in 6% and 38% of varieties, respectively (Knopf *et al.*, 2008); in 216 CIMMYT wheats, *Rht-B1b* and *Rht-D1b* were present in 82% and 14% of the accessions, respectively (Dan *et al.*, 2009). The relatively low occurrence of *Rht-B1b* and *Rht-D1b* in the INRA BWCC is likely due to a high proportion of accessions that pre-date the widespread use of the *Rht-1* semi-dwarfing alleles (beginning in the 1960s). The increased usage of semi-dwarf alleles over time is evident in the collection as *Rht-B1b* and *Rht-D1b* were not present in any fixed line released before 1947 or in any landrace while nearly 60% of the INRA BWCC varieties released between 1980 and 1999 contained one of these alleles (Table 6.10). Although *Rht-B1b* and *Rht-D1b* were not present in INRA BWCC fixed lines released prior to 1947 or lNRA BWCC landraces, both alleles are present in Japanese landraces (Yamada 1990) and are supposedly derived from the Japanese landrace ‘Daruma’, which was present as early as 1894 (Kihara, 1983).

Among the INRA BWCC accessions, the frequencies of *Rht-B1a_160* (16.0%)

and *Rht-B1a_197* (11.7%) are similar to the frequencies of the *Rht-B1b* and *Rht-D1b* (Table 6.2). Both insertions are found in several geographical regions (Table 6.3) and can be found in wheat accessions registered prior to 1900 (Table 6.10). The 160 bp insertion is present in five of the nine fixed lines released prior to 1900 (the earliest being released in 1830) and in four landraces (three from France and one from Nepal). The 197 bp allele was found in a single fixed line released prior to 1900 and was not present in any landraces. However, as reported in Chapter 5, this insertion was present in accessions of *T. dicoccoides* and *T. dicoccum* and the sequence has high homology to collinear regions on the A and D genomes of bread wheat indicating that the presence of the 197 bp sequence is the ancestral condition.

The primer pairs used to screen for the *Rht-B1* and *Rht-D1* alleles did not clearly identify any alternative alleles in the 368 INRA BWCC accessions. However, a polymorphism would not be easily recognised unless it prevented primer annealing or was a large indel that could be visualised on an agarose gel. In the case of lines containing *Rht-B1c*, no product was amplified with either the *Rht-B1a* or *Rht-B1b* primer pair (Table 6.1). The *Rht-B1c* allele has recently been sequenced and a > 2 kb insertion is thought to exist in the DELLA domain (Wu *et al.*, 2011), which is in the region that would be amplified by the *Rht-B1a* or *Rht-B1b* primer pair. The increased product size is the likely cause of the PCR amplification failure as the 30 s extension time used may be insufficient to amplify a > 2 kb product. Not surprisingly, the dwarf alleles *Rht-B1d*, *Rht-B1e*, and *Rht-D1c* from the control set were not identified using the *Rht-B1a/b* or *Rht-D1a/b* primer pairs. The inability of primers designed to the *Rht-B1a/b* or *Rht-D1a/b* SNPs to identify these alleles was also reported by Pestsova *et al.* (2008) when using *Rht-B1a/b* and *Rht-D1a/b* primers from Ellis *et al.* (2002).

The photoperiod insensitive *Ppd-D1a* allele was present in 25.8% of the accessions (Table 6.2) and was found in geographical regions worldwide except for Northern European nations (Table 6.3). In an examination of 485 lines from the GEDIFLUX collection of European bread wheats released between 1940 and 2000, the *Ppd-D1a* allele was present in only 5% of the

accessions and was rarely found in Northern European wheats (Wilhelm, 2007). The *Ppd-D1a* allele is most suited to growing seasons that are shorter than those found in Northern Europe (Worland *et al.*, 1998), which explains its absence in this region. Looking only at accessions originating from France (a central / southern European nation), *Ppd-D1a* occurred in 24% of the INRA BWCC accessions, which is similar to the percentage (30%) reported for French accessions in the GEDIFLUX collection (Wilhelm, 2007). In the INRA BWCC, *Ppd-D1a* was most prevalent in the period 1980-1999 (43% of the varieties) (Table 6.10), but was also present in a variety (INRA_14011) registered as early as 1899.

In addition to the *Ppd-D1a* and *Ppd-D1b* alleles, which result in 297 bp and 414 bp products, respectively, with the *Ppd-D1* primers designed by Beales *et al.* (2007), an approximately 450 bp product was amplified from the synthetic line 'W7984' (INRA_13812). Guo *et al.* (2010) amplified a 453 bp product with the same *Ppd-D1* primers using DNA extracted from synthetic wheat and *Ae. tauschii* accessions. The similar product sizes, and *Ae. tauschii* origins of the two alleles, indicate that these are likely the same allele. The larger size of the 453 bp product relative to the 414 bp *Ppd-D1b* product results from 24 bp and 15 bp insertions that are separated by 105 bp (Guo *et al.*, 2010). For three fixed lines and three landraces from the INRA BWCC, no product was amplified using the *Ppd-D1* primers even though multiple DNA replicates known to amplify products with other primers were used. The data suggest a previously unidentified polymorphism upstream of *Ppd-D1*, which may have originated near the Black Sea as the landraces carrying this apparent allele were from Azerbaijan, Georgia, and Turkey.

6.4.2. Genotype-Phenotype associations in the INRA BWCC

For heading date, the *Ppd-D1* locus accounts for the majority of the genotype variation in the *Rht-B1.Rht-D1.Ppd-D1* REML analysis, but the effect of *Rht-B1* is also significant ($p = 0.023$). In the AA-PS, only *Ppd-D1* was associated with heading date (Table 6.9). Ear emergence of *Ppd-D1a* plants was an average of 6.6 days earlier than *Ppd-D1b* plants (Table 6.8). Similarly,

Worland *et al* (1988) reported that *Ppd-D1a* generally accelerates ear emergence by six to eight days in the UK. In the GEDIFLUX bread wheat collection, Wilhelm (2007) reported that heading date of *Ppd-D1a* accessions was an average of eight days earlier than lines containing *Ppd-D1b* when grown in the UK. At the *Rht-B1* locus, *Rht-B1a_160* accessions in the INRA BWCC had significantly later heading dates (2.9 days) than *Rht-B1a_0* accessions. The cause of this association is not clear, but may be related to population structure, which was not accounted for in the means comparisons. A lack of association of *Rht-B1b* and *Rht-D1b* with heading date agrees with previous observations (J. Flintham, pers. comm.).

Changes in plant height in the *Rht-B1.Rht-D1.Ppd-D1* REML mixed model were associated with the *Rht-B1*, *Rht-D1*, and *Ppd-D1* loci (F pr values for all loci < 0.001) with most of the variation associated with the *Rht-1* loci (Table 6.6B). All three loci were also found to be highly associated (*p* values both less than 5.7×10^{-22}) with changes in plant height in the AA-PS (Table 6.9). The greatest changes in mean plant height were associated with the *Rht-B1b* and *Rht-D1b* alleles (Table 6.8B). The mean plant heights of *Rht-B1a*+*Rht-D1b, Rht-B1b*+*Rht-D1a,* and *Rht-B1b*+*Rht-D1b* accessions were 74% (34.9 cm shorter), 75% (33.3 cm shorter), and 54% (61.1 cm shorter), respectively, that of the *Rht-B1a*+*Rht-D1a* accessions (Table 6.8B). The reductions in height due to the *Rht-1* semi-dwarf alleles is slightly greater than reported by Flintham *et al.* (1997) using NIL comparisons (% heights of *Rht-B1a*+*Rht-D1a*: *Rht-B1a*+*Rht-D1b*, 83%; *Rht-B1b*+*Rht-D1a*, 86%; *Rht-B1b*+*Rht-D1b*, 58%), but with the same rank. The larger effect of the semi-dwarf alleles in the INRA BWCC collection may be partially due to the inclusion of germplasm that pre-dates the widespread use of *Rht-B1b* and *Rht-D1b*. This older germplasm would be expected to contain fewer secondary height-reducing alleles than modern germplasm, thereby exaggerating the effects of these alleles. The presence of additional alleles affecting height in the more recently released INRA BWCC lines is suggested by the significantly shorter mean plant height in the years 1940-59 relative to preceding time periods even though only two of the 32 accessions released between 1940-59 contained *Rht-B1b* or *Rht-D1b*. Changes in plant height associated with the

Rht-B1 insertion alleles were less pronounced, but still present in the *Rht-B1* means analysis. Mean plant heights of *Rht-B1a_160* and *Rht-B1a_197* accessions were 92% (9.7 cm shorter) and 88% (14.7 cm shorter) the height of *Rht-B1a_0* accessions. The *Rht-B1a_197* height reduction was significant at $p < 0.05$ (Table 6.7B). In the AA-PS, the effects of these alleles were not significant (Table 6.9), although the probability threshold ($p < 0.001$) was greater. Also, the AA-PS did not include *Rht-B1b*, *Rht-D1b*, or *Ppd-D1* genotype information and the strong effects of these alleles may have masked weaker effects on plant height.

Accessions containing *Ppd-D1a* have plant heights that are 92% (9 cm shorter) that of *Ppd-D1b* accessions when averaged over the four *Rht-B1+Rht-D1* genotype groups (Table 6.8B). However, the *Rht-B1*Rht-D1*Ppd-D1* REML analysis indicates that a significant interaction of *Ppd-D1* with *Rht-B1* (F pr < 0.001) and a nearly significant interaction with *Rht-D1* (F pr = 0.084) are present (Table 6.6). Height changes associated with *Ppd-D1* are by far the greatest in the *Rht-B1a+Rht-D1a* background, where *Ppd-D1a* accessions are on average 80% the height (29.2 cm shorter) of *Ppd-D1b* accessions. In backgrounds containing *Rht-B1b* and/or *Rht-D1b, Ppd-D1a* accessions have mean plant heights that are 93% (5.5 cm reduction) to 103% (2.9 cm increase) the mean plant heights of *Ppd-D1b* accessions. The cause of the interaction is not clear and is further complicated by a close linkage of *Ppd-D1a* with the semi-dwarf allele *Rht8* (approximate 21-22 cM separation (Worland *et al.*, 1998; Gasperini, 2010)) and a shared ancestry, at least in European varieties, that is traced back to the Japanese variety Akakomugi (Giorgi *et al.*, 1982; Worland, 1999). Accessions with the *Ppd-D1a* allele (and therefore also likely to contain *Rht8*) in the *Rht-B1a+Rht-D1a* background had a greater height reduction here than in Cappelle-Desprez (*Rht-B1a+Rht-D1a* background)/Mara 2D substitution RILs where height reductions of 18 cm (Worland *et al.*, 1998) and 16 cm (15%) (Gasperini, 2010) were reported. The greater height reduction associated with *Ppd-D1a* in the *Rht-B1a+Rht-D1a* background reported here may, as described above, be caused by additional height genes present among INRA BWCC accessions or may result from population structure.

Changes in GA treatment difference (a measure of GA sensitivity) in the *Rht-B1*Rht-D1*Ppd-D1* REML were associated with *Rht-B1* and *Rht-D1*, but not with *Ppd-D1* (Table 6.6C). As with plant height, the greatest changes in GA treatment difference were associated with the *Rht-B1b* and *Rht-D1b* genotypes (Tables 6.7C; 6.8C). Both alleles were also associated with GA treatment difference in the AA-PS (Table 6.9). INRA BWCC accessions with the *Rht-B1a*+*Rht-D1a* alleles had a significantly ($p < 0.05$) greater mean GA treatment difference (28.5 mm) than accessions with the *Rht-B1b*+*Rht-D1a* (9.6 mm), *Rht-B1a*+*Rht-D1b* (4.7 mm), or *Rht-B1b*+*Rht-D1b* (-0.1 mm) genotype. The slight response to GA present in the *Rht-B1b*+*Rht-D1a* and *Rht-B1a*+*Rht-D1b* accessions was also reported by Yamada (1990) and indicates that varieties containing a single *Rht-1* semi-dwarf allele are partially sensitive to GA. Interestingly, the *Rht-B1b*+*Rht-D1a* mean GA treatment difference was significantly greater ($p < 0.05$) than that of *Rht-B1a*+*Rht-D1b* in the means analysis. A greater GA response in *Rht-B1b*+*Rht-D1a* wheat accessions relative to *Rht-B1a*+*Rht-D1b* accessions was also reported by Yamada (1990) in Japanese fixed lines and landraces. The increased GA sensitivity and plant height associated with *Rht-D1b* relative to *Rht-B1b* suggests that the effect associated with *Rht-B1b* may be slightly less potent than that associated with *Rht-D1b*. *Rht-B1a_160* and *Rht-B1a_197* were associated with a slight reduction (2.9 mm and 1.0 mm, respectively) in GA treatment difference relative to *Rht-B1a_0*. However, none of these differences were significant at $p < 0.05$ (Table 6.7C). Neither insertion allele was associated at $p < 0.001$ with GA treatment difference in the AA-PS (Table 6.9).

Although the variation in GA treatment difference associated with *Ppd-D1* was very small and not statistically significant in the REML analysis (Table 6.6), the effect was found to be highly significant (1.8×10^{-10}) in the AA-PS. This is surprising because accounting for population structure generally reduces the statistical significance of associations (I Mackay, NIAB, pers. comm.) and the *Ppd-D1* and *Rht8* loci are generally not associated with GA insensitivity. The direction (increase or decrease) of the effect of the *Ppd-D1a* allele on GA

sensitivity is not clear. In the *Rht-B1a+Rht-D1a* background, the mean GA treatment difference associated with *Ppd-D1a* is 1.4 mm less than that associated with *Ppd-D1b* while in backgrounds containing *Rht-B1b* and/or *Rht-D1b, Ppd-D1a* is associated with an increased GA treatment difference (0.5 to 5.0 mm) relative to *Ppd-D1b* (Table 6.8C). As discussed above, the AA-PS does not directly account for the *Rht-B1* or *Rht-D1* loci. This is likely to be an important consideration as 49% (44 of 90) of the *Ppd-D1a* accessions contain *Rht-B1b* and/or *Rht-D1b* whereas only 14% (36 of 262) of the *Ppd-D1b* accessions contain *Rht-B1b* and/or *Rht-D1b*.

In summary of the association analyses, *Rht-B1b* and *Rht-D1b* were significantly associated with reduced plant height, reduced GA sensitivity, and not associated with significant changes in heading date in either the *Rht-B1*Rht-D1*Ppd-D1* REML analysis or the AA-PS analysis. *Rht-B1a_160* and *Rht-B1a_197* were not associated with changes in height, GA sensitivity or heading date in the AA-PS. In the *Rht-B1*Rht-D1*Ppd-D1* REML analysis, however, *Rht-B1a_160* and *Rht-B1a_197* were associated with reduced plant height, reduced GA sensitivity, and later ear emergence relative to *Rht-B1a_0* when comparing fitted means. These effects were not significant at a probability threshold of 0.05 except for plant height (*Rht-B1a_197 > Rht-B1a_0*) and heading date (*Rht-B1a_160* was later than *Rht-B1a_0*), suggesting that if a real effect exists, it may be small. *Ppd-D1a* was significantly associated with earlier ear emergence and reduced height in the *Rht-B1*Rht-D1*Ppd-D1* REML and AA-PS analyses. An association of *Ppd-D1a* with GA sensitivity did not occur in the *Rht-B1*Rht-D1*Ppd-D1* REML analysis, but was present in the AA-PS analysis, although it is not clear if *Ppd-D1a* increased or decreased GA sensitivity.

6.4.3. Correlations among phenotypes in the INRA BWCC

The plant heights of the individual accessions ranged from 54 to 200.5 cm (mean of 133 cm) and heading dates ranged from 116 to 185 days (mean of 141 days). When the INRA BWCC accessions were grown in Clermont-Ferrand by Bordes *et al.* (2008), plant heights ranged from 49 to 150 cm

(mean of 94 cm) and ear emergence dates ranged from 127 to 160 days (mean of 140 days). UK heading dates were likely measured at an earlier stage than in France as ear emergence in France was recorded as the date (from January 1) when spikes had emerged from 50% of the tillers (Bordes *et al.*, 2008) and UK heading dates were recorded as the date when 50% of the primary spike was visible above the flag leaf. Nonetheless, the mean heading dates are fairly similar, while mean plant heights between the two locations are very different. Height difference could be attributed to differences in growth conditions as plants in the UK were grown in pots and irrigated while being grown in the soil without irrigation in France.

When correlations between plant phenotypes recorded in this study were estimated, not surprisingly the strongest correlation was between GA treatment difference and plant height (R^2 = 0.338; Figure 6.8). Correlations between plant height and heading date were low (R^2 = 0.033). In contrast, Bordes *et al* (2008) reported R^2 values of 0.33 between ear emergence and plant height when the INRA BWCC was grown in France. The differences may relate to the different growing conditions used as described above. In another study that looked at the correlation between plant height and flowering date in European winter wheat landraces and obsolete cultivars grown in Prague-Ruzyně, Czech Republic, Dotlacil *et al.* (2003) estimated an R^2 value of 0.036, similar to that reported here. UK heading date and GA treatment difference were only weakly correlated (R^2 = 0.010), suggesting that GA sensitivity has little effect on heading date in the UK.

6.4.4. GA sensitivities among INRA BWCC accessions

The MM procedure, which was based solely on the GA+ treatment, is similar to the scoring methods employed by Gale and Gregory (1977), which were used to classify GA-treated seedlings based on length distribution. For the *Rht-B1a*+*Rht-D1a* accessions, the MM procedure only identified one accession (INRA_03752, 'IAR W83-2') as GA insensitive (Table 6.5). This accession was classified as GA insensitive in GA expt. 2 due to a non-significant (p < 0.05) treatment difference, but in GA expt. 1 and 3, this line

was classified as GA sensitive as the treatment differences were much greater. It is not clear why the treatment differences varied between experiments for this accession. The LSD test, based on GA treatment differences, identified 29 *Rht-B1a+Rht-D1a* as NSTD (no significant treatment difference, an indicator of GA insensitivity) in GA expt. 1. However, GA expts. 2 and 3 showed 28 of these accessions to be GA sensitive based on the LSD test and the 29th accession was not re-tested due to poor germination.

Among INRA BWCC accessions containing *Rht-B1b* or *Rht-D1b*, the MM procedure correctly identified these as GA insensitive in the majority of cases (64% of the time across GA experiments) and more importantly, in only three instances of 142 (2%) incorrectly identified these accessions as GA sensitive (Table 6.5). For the LSD test, INRA BWCC accessions containing *Rht-B1b* or *Rht-D1b* were incorrectly identified as GA sensitive in 17 instances of 142 (12%) and classification of accessions as GA insensitive was not possible. These results indicate that the MM procedure had a greater success rate in correctly classifying GAI accessions than the LSD procedure. In addition, the MM procedure only requires the use of a GA+ treatment whereas the LSD requires a GA+ and GA- treatment. However, GAI accessions that have long STFL lengths in the GA+ treatment (due to genetic background) would be more difficult to detect with the MM.

These results indicate that there may not be additional GA insensitive alleles in the INRA BWCC, or if present they are more responsive to GA than *Rht-B1b*, *Rht-D1b*, or *Rht-B1c*. An example of a more responsive GAI allele is *Rht-B1d*, which when treated with GA has seedling lengths shorter than wild type and longer than *Rht-B1b* or *Rht-D1b* seedlings (Worland, 1986). Intermediate GA responders might be difficult to detect in this collection due to the heterogeneity of genetic backgrounds or due to experimental conditions. Based on mean values from the three GA expts., 42 *Rht-B1a+Rht-D1a* accessions fall within the range of GA treatment differences found among accessions with the *Rht-B1b* or *Rht-D1b* allele (20.8 mm or less; Appendix VI), suggesting that intermediate GA sensitivity alleles may exist at the *Rht-1* loci.

Part of the objective of the GA screen was to identify a GAI allele at the *Rht-A1* locus, at which no alleles affecting height are as of yet known. In contrast, several spontaneous GAI mutants exist at the *Rht-B1* and *Rht-D1* loci (listed in Table 1.2). The absence of GAI alleles at the *Rht-A1* locus in the BWCC and the low level of *Rht-A1* genetic diversity among the bread wheat accessions studied in Chapter 5, suggest that alterations at this locus may be detrimental to wheat survival.

6.4.5. Conclusions

Overall, although no novel GAI alleles were clearly detected in the INRA BWCC, valuable information was gained regarding GA sensitivity testing and analyses, which can be employed in searches of other germplasm sets for GAI alleles. Association analyses of the *Rht-B1* insertions indicates these alleles may have a minor effect on GA sensitivity and plant height, suggesting the need for further study of the insertion haplotypes. In addition, genotyping of the INRA BWCC accessions for the *Rht-B1* insertions, *Ppd-D1*, *Rht-B1*, and *Rht-D1* along with phenotyping for plant height, GA response, and flowering date increases knowledge of the INRA BWCC set, which may help identify intermediate GAI alleles and will be useful for utilising this collection in other genetic studies.

7. ORIGINS OF THE *RHT-B1B* AND *RHT-D1B* ALLELES

7.1 INTRODUCTION

The predominant sources of semi-dwarfism in modern wheat varieties are the *Rht-B1b* and *Rht-D1b* alleles with an estimated 70% of the wheat acreage planted with varieties carrying one of these alleles (Evans, 1998). The primary source of both of these mutant alleles is the line 'Norin 10' (Gale and Youssefian, 1985; Dalrymple, 1986), which originated in Southeast Asia. 'Norin 10' was introduced to the Western world by S.C. Salmon, a USDA advisor to the army in Japan, who noticed the stiff, short-stemmed variety and sent it to the USA (Reitz and Salmon, 1968). Orville Vogel, a breeder at Washington State University, crossed 'Norin 10' to a U.S. adapted variety 'Brevor'. Progeny of the 'Norin 10' × 'Brevor' cross, particularly selection 14, became the primary donors of semi-dwarf alleles in U.S. wheat varieties (Dalyrmple, 1986). Norman Borlaug at the International Maize and Wheat Improvement Centre (CIMMYT) in Mexico received selections of 'Norin 10/Brevor' and incorporated the semi-dwarf trait into Mexican wheat varieties, which were soon utilised globally as part of the 'green revolution'. In addition to 'Norin 10', relatively minor sources of semi-dwarfism have also been used, including 'Suweon (also referred to as 'Suwon') 92' and 'Seu Seun 27' (Allan *et al*., 1968). 'Seu Seun 27', was the apparent semi-dwarf donor for several U.S. varieties in the 1960s and 1970s and 'Suweon 92' had more limited use (Dalrymple 1980).

'Norin 10', 'Seu Seun 27', and 'Suweon 92' are thought to have in common the ancestor 'Daruma', believed to have been the donor of *Rht-B1b* and *Rht-D1b* (Dalrymple, 1980; Kihara, 1983; Dalrymple, 1986). The history of 'Daruma', a landrace (Worland, 1986; Yamada, 1990), dates back to as early 1894 where it was recorded as a control variety in wheat performance trials in Japan (Kihara, 1983). The origin of semi-dwarfism in 'Daruma' is not clear and may have arisen by spontaneous mutation in Japanese wheat populations or may have arisen in China or Korea (Kihara, 1983). It was suggested by Cho *et al*. (1980), that 'Daruma' is derived from the native

Korean wheat ‘Anzunbaengimil’ or ’Nanjangmil’ “crippled wheat”, existing in the 3rd or 4th century AD before being introduced to Japan during the 16th century Korean-Japanese War.

The lineage from ‘Daruma’ to ‘Norin 10’ as reported by Dalrymple (1980), Kihara (1983), and Dalrymple (1986) is shown in Figure 7.1. ‘Daruma’ or a white variant called ‘Shiro-Daruma’ was crossed with ‘Glassy Fultz’ (a variant of the American variety ‘Fultz’) in 1917 in Japan to produce ‘Fultz-Daruma’. In 1925, ‘Fultz Daruma’ was crossed with ‘Turkey Red’ (which was widely grown in the USA in the early 20th century and often referred to as ‘Turkey’) to produce ‘Tohuku’ selection number 34, better known as ‘Norin 10’, which was registered and released in 1935 in Japan. Figure 7.1 also shows the lineage from ‘Daruma’ to ‘Suweon 92’ and ‘Seu Seun 27’ as described by Dalrymple (1980), Kihara (1983), and Dalrymple (1986). A red variant of ‘Daruma’ (‘Aka-Daruma’) was crossed to ‘Glassy Fultz’ to produce the line ‘Aka-Daruma/Glassy Fultz’ in Japan with year unknown. This line was crossed to ‘Kanred’ (a line originally selected from ‘Crimean’, a strain of ‘Turkey Red’) in Japan and F_3 seeds exported to Korea where ‘Suweon 85’ was released in 1932. ‘Suweon 85’ was then crossed to ‘Suweon 13’ to produce ‘Suweon 92’ and ‘Suweon 90’, which were both released in 1934 in Korea. ‘Shiroboro’, a Japanese variety, was crossed to ‘Suweon 90’ to produce ‘Seu Seun 27’, which was used as a breeding line.

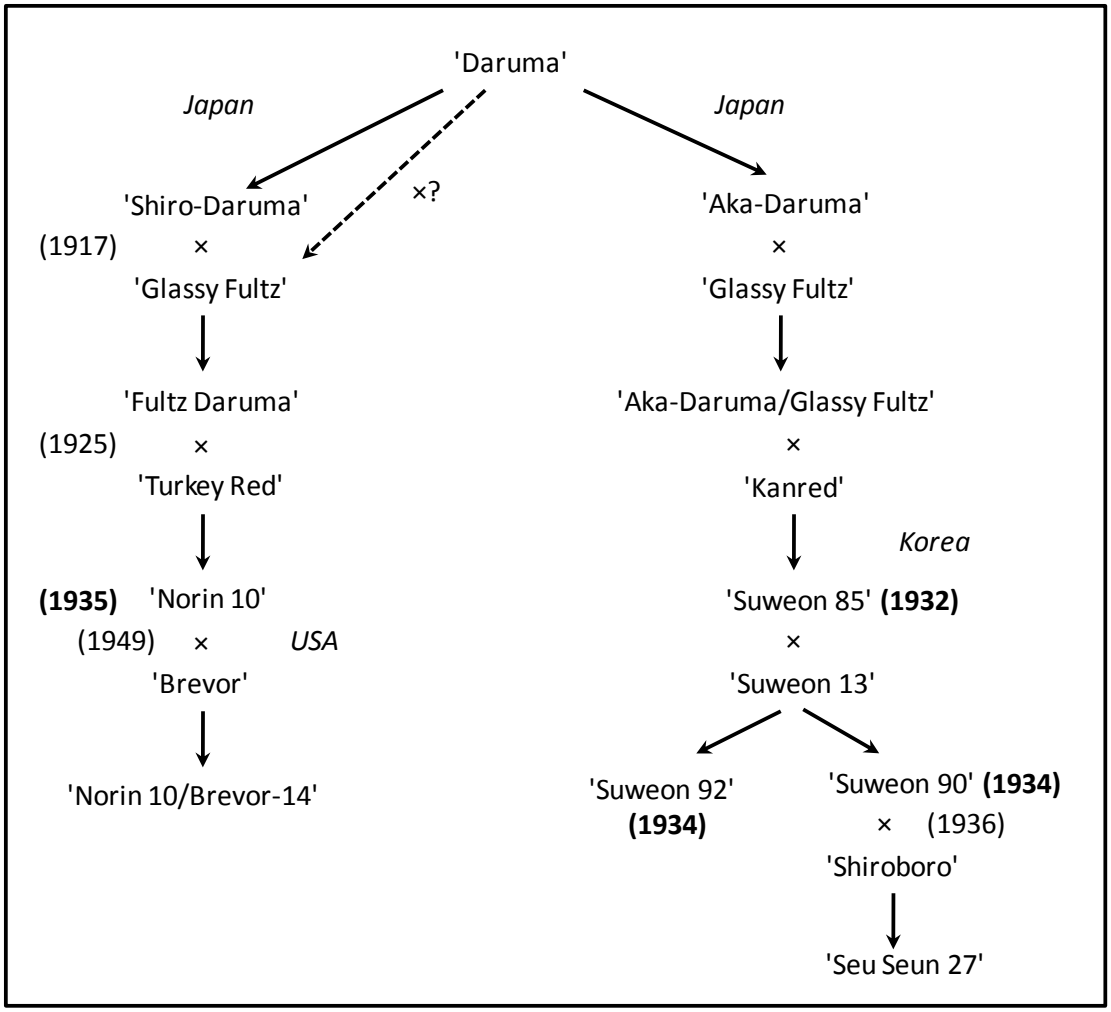


Figure 7.1. Origins of *Rht-B1b* and *Rht-D1b*. The genealogy from 'Daruma' to 'Norin 10/Brevor-14', 'Suweon 92', and 'Seu Seun 27' are shown according to Dalrymple (1986) and Kihara (1983). The dashed line (x?) indicates that 'Daruma', instead of 'Shiro-Daruma' may be a parent of 'Fultz Daruma'. The country where breeding was performed is shown in italics above that section of the chart. The year crosses were performed is shown in parenthesis and year of varietal release is shown in bold font if known.

The objective of this chapter is to use molecular techniques to confirm the donors of the *Rht-B1b* and *Rht-D1b* alleles present in 'Norin 10/Brevor-14' and to determine the *Rht-1* alleles present in 'Seu Seun 27' and 'Suweon 92' and the donors of these alleles. To accomplish this, the available accessions shown in the ancestral genealogy of these lines (Figure 7.1.) were grown and genotyped with markers specific to the *Rht-B1b* and *Rht-D1b* causative SNPs. Determining the ancestral lineage of the semi-dwarf alleles will aid in determining the origin of the alleles, which may also be useful for identifying alternative *Rht-B1b* and *Rht-D1b* sources.

7.2. MATERIALS AND METHODS

7.2.1. Germplasm sources

Seed of accessions used for *Rht-1* genotyping and plant phenotyping were provided by: M. Ambrose, JIC BBSRC cereals collection; H. Bockelman, USDA-ARS National Plant Germplasm System (NPGS); the NIAB DUS collection; T.Sasanuma, Kihara Institute of Biological Research (KIBR), Yokohama City University, Yokohama, Japan; Kyoto University, Kyoto, Japan; and CIMMYT. Accessions from Kyoto University and CIMMYT were obtained through M. Ambrose. Accessions from KIBR were ordered through the KOMUGI Wheat Genetics Resources Database (http://www.shigen.nig.ac.jp/wheat/komugi) (Table 7.1). Accession numbers in the text will be enclosed in square brackets [ ]. All germplasm was grown from original source material except [PI157599-1], which is a self-pollinated progenitor from an *Rht-B1b*+*Rht-D1b* homozygous [PI157599] individual.

Table 7.1. Summary of seed accessions and *Rht-B1* and *Rht-D1* genotype results

Variety[b]	Accession Number[c]	Seed Source[d]	Genotype Results[a]	
			No. plants assayed	*Rht-B1* and *Rht-D1* alleles
Aka-Daruma	PI325843	USDA-ARS	12	*B1a* ***D1b*** (8); *B1a*/***b*** ***D1b*** (2); *B1a D1a*/***b*** (1); *B1-* ***D1b*** (1)
Aka-Daruma	KT020-009	KIBR	8	*B1a* ***D1b*** (6); *B1a*/***b*** ***D1b*** (1); *B1a*/***b*** *D1a*/***b*** (1)
Aka-Daruma	KU-1206	Kyoto University	6	*B1a* ***D1b*** (5); *B1a D1a*/***b*** (1)
Aka-Daruma	CWI24999	CIMMYT		did not germinate
Brevor	CItr12385	USDA-ARS	8	*B1a D1a*
Daruma	KT020-110	KIBR	6	***B1b*** *D1a* (5), ***B1b*** *D1-* (1)
Daruma	CWI25102	CIMMYT		did not germinate
Fultz	W748	JIC	4	*B1a D1a*
Fultz	PI5493	USDA-ARS	8	*B1a D1a*
Fultz-Daruma	PI325844	USDA-ARS	4	*B1a D1a*
Kanred	CItr5146	USDA-ARS	8	*B1a D1a*
Kanred	PI90832	USDA-ARS	7	*B1a D1a*
Kanred	W741	JIC	4	*B1a D1a*
Norin 10	W204	JIC	4	***B1b D1b*** (3); *B1a*/***b D1b*** (1)
Norin 10	PI156641	USDA-ARS	7	***B1b D1b*** (6); ***B1b*** *D1a*/***b*** (1)
Norin 10/Brevor-14	W9743	JIC	4	***B1b D1b*** (3); *B1a*/***b*** *D1a* (1)
Norin 10/Brevor-14	CItr13253	USDA-ARS	22	***B1b D1b*** (18); ***B1b*** *D1a*/***b*** (2); *B1a*/***b*** *D1a*/***b*** (1); *B1-* ***D1b*** (1)

Table 7.1 continued. Summary of seed accessions and *Rht-B1* and *Rht-D1* genotype results

Variety[b]	Accession Number[c]	Seed Source[d]	Genotype Results[a]	
			No. plants assayed	*Rht-B1* and *Rht-D1* alleles
Seu Seun 27	PI157584	USDA-ARS	5	*B1a D1a*
Shiro-Daruma	PI191345	USDA-ARS	14	*B1a D1a* (13); *B1a/**b** D1a* (1)
Shiro-Daruma	KT020-016	KIBR	8	*B1a **D1b***
Shiro-Daruma	CWI25422	CIMMYT	8	*B1a D1a* (7); *B1a/**b** D1a* (1)
Shiro-Daruma	PI325845	USDA-ARS	8	*B1a **D1b***
Shiroboro 1	KT020-015	KIBR	8	*B1a D1a*
Suweon 85	PI157599	USDA-ARS	6	***B1b D1b*** (3); *B1a/**b D1b*** (3)
Suweon 85	PI157599-1	USDA-ARS	8	***B1b D1b***
Suweon 85	BW6431	CIMMYT	8	***B1b D1b***
Suweon 85	PI157600	USDA-ARS	8	***B1b** D1a*
Suweon 92	CItr13132	USDA-ARS	8	***B1b D1b***
Suweon 92	PI157603	USDA-ARS	8	*B1a D1a* (7); *B1a/**b** D1a* (1)
Turkey Red	PI565351	USDA-ARS	8	*B1a D1a*
Turkey Red	PI565343	USDA-ARS	4	*B1a D1a*

[a] *B1* and *D1* denote the genome; suffix '*a*' denotes wild type alleles; suffix *'b'* denotes the *Rht-B1b* or *Rht-D1b* alleles, which are shown in bold font. Where there is segregation at a locus, this is shown by a (*/*) between the genotypes. The numbers of plants of each genotype are indicated in parenthesis when segregants are present. A (-) represents no amplified product.

[b] 'Suweon' has also been described as 'Suwon'; 'Turkey Red' has also been described as 'Turkey'.

[c] PI5493 is also recorded as CItr1923; PI157603 also is recorded as CItr12666. PI157599-1 refers to progeny of a PI157599 (*Rht-B1b*+*Rht-D1b*) individual.

[d] USDA-ARS = United States Dept. of Agriculture - Agricultural Research Service, National Plant Germplasm System; KIBR = Kihara Institute of Biological Research; CIMMYT = International Maize and Wheat Improvement Center; JIC = John Innes Centre.

7.2.2. Growth conditions of accessions

Plants were grown in one of three glasshouse sowings: JIC 2008, NIAB 2010, or JIC 2010 (Table 7.2). Each sowing contained the lines Mercia (*Rht-B1a*+*Rht-D1a*) and 'Norin10/Brevor-14' (N10/B14) [CItr-13253] (*Rht-B1b*+*Rht-D1b*) for comparative purposes across sowings. Mercia is included only as a control whereas N10/B14 [CItr-13253] is also part of the 'Daruma' progeny tree. Seeds of all varieties were stratified for 3-5 days at 4°C on filter paper, and then placed at room temperature for 5-7 days before transplanting to 96-well trays containing compost. Following transplanting, all plants were vernalised at 4°C for 8-9 weeks and a minimum of six seedlings per accession

were transferred to 1 litre pots and grown in the glasshouse until maturity. Plants were treated with fungicide to control powdery mildew with care take to use fungicides that did not contain known plant growth regulators.

Table 7.2. Summary of plant phenotype scores

						Plant height (cm)[f]			50% heading (DAS)[g]		
Sow-ing[a]	Variety	Accession number[b]	*Rht-1* genotype[c]	Ind.[d]	Awns[e]	Mean	Range	CV	Mean	Range	CV
JIC 2008	Aka-Daruma	PI325843	*B1a* ***D1b***	3	Yes	59	55-61	6%	95	94-97	2%
	Fultz	W748	*B1a D1a*	4	No	110	101-115	6%	125	118-129	4%
	Fultz Daruma	PI325844	*B1a D1a*	4	Yes	93	90-98	4%	109	108-111	1%
	Kanred	W741	*B1a D1a*	4	Seg	95	85-109	12%	116	103-124	8%
	Mercia	W3685	*B1a D1a*	4	No	77	72-80	5%	121	120-122	1%
	Norin 10	W204	***B1b D1b***	3	Yes	43	40-49	12%	106	105-108	2%
	Norin 10	PI156641	***B1b D1b***	4	Yes	47	45-47	2%	107	106-107	0%
	N10/B14	W9743	***B1b D1b***	1	Yes	42	-	-	125	-	-
	N10/B14	Cltr13253	***B1b D1b***	4	Yes	52	50-57	6%	119	118-120	1%
	Seu Seun 27	PI157584	*B1a D1a*	4	Yes	82	78-87	4%	104	102-106	2%
	Shiro-Daruma	PI191345	*B1a D1a*	5	Yes	88	62-112	21%	104	102-109	3%
	Suweon 85	PI157599	***B1b D1b***	2	Yes	47	45-50	8%	96	95-97	1%
	Turkey Red	PI565343	*B1a D1a*	4	Yes	88	81-100	10%	116	115-118	1%
NIAB 2010	Aka-Daruma	PI325843	*B1a* ***D1b***	5	Yes	74	55-85	16%	118	115-123	3%
	Aka-Daruma	KU-1206	*B1a* ***D1b***	5	Yes	75	61-84	13%	117	113-121	3%
	Aka-Daruma	KT020-009	*B1a* ***D1b***	6	Yes	83	61-93	14%	118	115-121	2%
	Daruma	KT020-110	***B1b*** *D1a*	5	Yes	92	85-102	7%	124	123-125	1%
	Mercia	W3685	*B1a D1a*	8	No	86	70-97	12%	138	135-141	1%
	N10/B14	Cltr13253	***B1b D1b***	8	Yes	56	45-61	10%	139	137-141	1%
	Shiro-Daruma	KT020-016	*B1a* ***D1b***	8	Yes	65	50-79	16%	118	116-121	2%
	Shiro-Daruma	CWI25422	*B1a D1a*	7	Yes	146	114-166	11%	137	136-139	1%
	Shiro-Daruma	PI191345	*B1a D1a*	7	Yes	148	117-174	12%	138	134-141	2%
	Shiroboro 1	KT020-015	*B1a D1a*	8	Yes	97	80-113	13%	122	121-124	1%
	Suweon 85	PI157599-1	***B1b D1b***	8	Yes	71	62-78	7%	129	126-130	1%
	Suweon 85	BW6431	***B1b D1b***	8	Seg	72	64-81	8%	133	129-136	2%
	Suweon 92	Cltr13132	***B1b D1b***	8	Yes	71	55-86	16%	129	127-132	1%
JIC 2010	Brevor	Cltr12385	*B1a D1a*	7	No	76	72-81	5%	122	121-123	1%
	Fultz	PI5493	*B1a D1a*	8	No	112	103-122	6%	119	117-123	2%
	Kanred	PI90832	*B1a D1a*	7	No	98	92-105	5%	118	117-120	1%
	Kanred	Cltr5146	*B1a D1a*	8	Yes	120	113-127	4%	126	121-136	4%
	Mercia	W3685	*B1a D1a*	8	No	68	64-76	6%	124	122-127	1%
	N10/B14	Cltr13253	***B1b D1b***	6	Yes	48	43-56	9%	128	124-136	4%
	Shiro-Daruma	PI325845	*B1a* ***D1b***	8	Yes	59	56-62	5%	102	99-105	2%
	Suweon 85	PI157600	***B1b*** *D1a*	7	Yes	66	63-73	6%	111	108-113	2%
	Suweon 92	PI157603	*B1a D1a*	8	No	110	97-117	6%	119	117-122	2%
	Turkey Red	PI565351	*B1a D1a*	8	Yes	107	98-119	7%	127	124-132	2%

[a] Control varieties included in each sowing are shown in grey background.

[b] PI157599-1 refers to progeny of a PI157599 *Rht-B1b+Rht-D1b* individual.

[c] *Rht-1* genotype of plants that were phenotyped. '*a*' = wild type allele; '*b*' = *Rht-B1b* or *Rht-D1b* (dwarfing alleles shown in bold font).

[d] Number of individual plants phenotyped for awn presence, height, and 50% heading date.

[e] Seg indicates accessions segregating for awn presence/absence

[f] Height of the primary tiller from soil surface to the tip of the inflorescence, not including awns; CV = coefficient of variation.

[g] Days after sowing (DAS) until the primary tiller was 50% emerged from the flag leaf.

Seeds of the JIC 2008 planting were stratified beginning 29 July 2008. A total of 13 accessions were included (Table 7.2, JIC 2008). Plants were removed from vernalisation, transplanted, and placed in the JIC glasshouse with supplementary lighting (16 hr photoperiod; 22/20°C day/night) on 8 October. The NIAB 2010 planting consisted of 13 accessions (Table 7.2, NIAB 2010) that were stratified beginning 7 Jan 2010. Plants were transplanted on 19 March, and placed in the NIAB glasshouse under natural temperature and light conditions. The third planting, JIC 2010, consisted of 10 accessions (Table 7.2, JIC 2010) and stratification began 6 July 2010. Plants were transplanted and placed in the JIC glasshouse under supplementary artificial lighting (16 hr photoperiod; 22/15°C day/night) on 20 Sep 2010.

7.2.3. Genotyping and phenotyping of accessions

For genotyping, leaf tissue of individual seedlings was collected and DNA extracted using the method described in section 4.2.2. Accessions were genotyped for *Rht-B1a*, *Rht-B1b*, *Rht-D1a*, and *Rht-D1b* as described in section 5.2.3. Heading date was recorded as the number of days after sowing (DAS) until the inflorescence of the primary tiller was 50% emerged above the flag leaf. Plant height was measured at maturity as the distance from the soil surface to the tip of the inflorescence (not including awns) of the primary tiller. Awns were visually scored as absent or present following flowering. *Rht-1* segregants and phenotypic off-types were not included in the phenotypic analysis.

7.3. RESULTS

All but four varieties that comprise the 'Daruma' progeny tree (Figure 7.1) were obtained. The missing varieties were ‘Aka-Daruma/Glassy Fultz’, ‘Suweon 13’, ‘Suweon 90’, and ‘Glassy Fultz’. ‘Fultz’ was obtained as a near

substitute for ‘Glassy Fultz’. For each variety, from one to four different accessions were obtained. A minimum of four plants per accession were scored with the *Rht-B1a/b* and *Rht-D1a/b* markers with the exceptions of [CWI 24999] from variety ‘Daruma’, and [CWI-25102] from variety ‘Aka-Daruma’, which did not germinate (Table 7.1). Several accessions were not homozygous at the *Rht-B1* and *Rht-D1* loci and accessions within a variety in some cases did not have the same genotypes. Of the 29 accessions that were genotyped, nine segregated at the *Rht-B1* locus and five segregated at the *Rht-D1* locus, which includes four lines that segregated at both loci. The segregants represented 25% or less of the plants of any accession with the exception of ‘Suweon 85’ [PI157599] in which three individuals were *Rht-B1b/b* and three individuals were *Rht-B1a/b*. Multiple accessions were genotyped for nine of the varieties, the genotypes of the respective accessions were in agreement for six of the varieties, but this was not the case for ‘Shiro-Daruma’, ‘Suweon 85’, and ‘Suweon 92’ (described below).

The heading dates, flowering dates, and awn presence scores of each accession are shown in Table 7.2. The Table 7.2 phenotypes were only recorded on plants homozygous at the *Rht-B1* and *Rht-D1* loci. Among the three sowings, the ‘Mercia’ and ‘N10/B14’ [Cltr 13253] controls both have their greatest plant height in NIAB 2010 (mean = 71 cm) compared with JIC 2008 (mean = 65 cm) and JIC 2010 (mean = 58 cm). Similarly, among the two controls, the 50% heading date occurred latest in NIAB 2010 (mean = 139 DAS) compared with JIC 2010 (mean = 126 DAS) and JIC 2008 (mean = 120 DAS). The coefficient of variation (CV) for height across all accessions was greatest in the NIAB 2010 sowing (mean CV = 12%) compared with JIC 2008 (mean CV = 8%) and JIC 2010 (mean CV = 6%). The 50% heading date in each sowing had a mean CV of 2%.

In each sowing (with ‘Mercia’ excluded because it is not part of the ‘Daruma’ progeny tree), the plant heights of the double semi-dwarfs (*Rht-B1b*+*Rht-D1b*) were lowest, followed by plants carrying a single dwarfing allele (*Rht-B1b*+*Rht-D1a* or *Rht-B1a*+*Rht-D1b*), and the tallest lines were those carrying both wild type alleles (*Rht-B1a*+*Rht-D1a*). The only exception to this is ‘Shiro-

Daruma' [KT020-016] (*Rht-B1a*+*Rht-D1b*), which was only tested in the NIAB 2010 sowing and was shorter than three *Rht-B1b*+*Rht-D1b* accessions. The largest CV for height in any sowing date is within 4 percentage points of the mean of the CVs for that sowing date with the exception of 'Shiro-Daruma' [PI191345] in the JIC 2008 sowing, which is 13 percentage points higher than the mean sowing CV with plant heights ranging widely from 62 to 112 cm. Variation in flowering date was greatest in 'Kanred' [W741] from the JIC 2008 sowing, which had a CV of 8% and a 22-day spread in heading date whereas among all sowings, the next highest CV was 4% (three accessions) and the next highest range in heading date was 16 days ('Kanred' [Cltr5146], JIC 2010 sowing). 'Kanred' [W741] and 'Suweon 85' [BW6431] are the only two accessions that segregated for presence/absence of awns.

The 'N10/B14' pedigree tree is shown in Table 7.3 along with a summary of the results from the *Rht-1* genotyping and the plant phenotyping (from Tables 7.1 and 7.2, respectively). For comparative purposes among sowings, plant heights and heading dates are shown relative to the mean of the 'N10/B14' [Cltr 13253] plants grown in that sowing. In the 'N10/B14' pedigree tree, the only accessions with the *Rht-B1b*+*Rht-D1b* genotype are 'Norin 10' and 'N10/B14'. In contrast, the 'Norin 10' parental lines 'Fultz Daruma' and 'Turkey Red' do not contain *Rht-B1b* or *Rht-D1b* and plant heights are double that of 'Norin 10'. The 'Fultz Daruma' parental lines are 'Glassy Fultz', which could not be obtained, and 'Shiro-Daruma'. 'Fultz' was instead acquired as a near substitute for 'Glassy Fultz' and neither accession of this line contained *Rht-B1b* or *Rht-D1b*. Of the four 'Shiro-Daruma' accessions, two ([KT020-016] and [PI325845]) have an *Rht-B1a*+*Rht-D1b* genotype and two ([PI191345] and [CWI 25422]) have *Rht-B1a*+*Rht-D1a* as the predominant genotype. However, in [PI191345] one plant out of 14 and in [CWI 25422] one plant out of 8 was an *Rht-B1a*/*b* heterozygote (Table 7.1). The accessions of 'Shiro-Daruma' also differ greatly for days to heading with mean heading date ranging from 1 to 26 days before 'N10/B14'. Accession [PI191354] of 'Shiro-Daruma' was grown twice (JIC 2008 = flowering in the autumn under artificial lighting and NIAB 2010 = flowering in the spring under natural lighting) and found to differ by two weeks relative to the 'N10/B14'

control. 'Daruma', the progenitor of 'Shiro-Daruma', did not contain the *Rht-D1b* allele, but was homozygous for *Rht-B1b*+*Rht-D1a* (six seedlings tested).

Table 7.3. *Rht-1* genotypes and plant phenotypes of the 'Norin 10' ancestors.

Variety[a]	Accession Number	*Rht-B1*[b]	*Rht-D1*	Sowing	Awns	Plant ht. (% of N10/B14)[c]	Days to heading (diff vs. N10/B14)[d]
Daruma	KT020-110	***B1b***	*D1a*	NIAB '10	awned	166%	-15
↓							
Shiro-Daruma	PI191345	*B1a**	*D1a*	JIC '08	awned	168%	-15
				NIAB '10	awned	267%	-1
	KT020-016	*B1a*	***D1b***	NIAB '10	awned	117%	-21
	CWI25422	*B1a**	*D1a*	NIAB '10	awned	263%	-2
	PI325845	*B1a*	***D1b***	JIC '10	awned	123%	-26
×							
Glassy Fultz (not available)							
Fultz	W748	*B1a*	*D1a*	JIC '08	no awns	211%	6
	PI5493	*B1a*	*D1a*	JIC '10	no awns	234%	-9
↓							
Fultz Daruma	PI325844	*B1a*	*D1a*	JIC '08	awned	179%	-10
×							
Turkey Red	PI565343	*B1a*	*D1a*	JIC '08	awned	169%	-4
	PI565351	*B1a*	*D1a*	JIC '10	awned	224%	-1
↓							
Norin 10	W204	***B1b****	***D1b***	JIC '08	awned	83%	-13
	PI156641	***B1b***	***D1b****	JIC '08	awned	89%	-13
×							
Brevor	CItr12385	*B1a*	*D1a*	JIC '10	no awns	159%	-6
↓							
Norin 10/Brevor-14	W9743	***B1b***	***D1b****	JIC '08	awned	80%	6
	CItr13253	***B1b****	***D1b****	JIC '08	awned	100%	0
				NIAB '10	awned	100%	0
				JIC '10	awned	100%	0

[a] 'Norin 10' ancestry is duplicated from Fig. 7.1 except for the addition of 'Fultz', which was investigated due to the unavailability of 'Glassy Fultz'. An (×) indicates a genetic cross and arrows point to the resulting progeny. 'Daruma' instead of 'Shiro-Daruma' may have been crossed to 'Glassy Fultz' (Dalrymple, 1986).

[b] The predominant *Rht-B1* and *Rht-D1* genotypes are indicated with *Rht-B1b* or *Rht-D1b* alleles indicated by a (-*b*) and shown in bold font. Wild type (-*a*) alleles are in plain font. An (*) indicates that segregants at a particular locus were detected.

[c] Plant heights are given as a percentage of the height of 'Norin 10/Brevor-14' (N10/B14) plants grown in the same sowing.

[d] Days to heading are given as the difference in days relative to N10/B14 from the same sowing, with (-) indicating earlier heading.

The pedigree tree of ‘Suweon 92’ and ‘Seu Seun 27’ is shown in Table 7.4 along with a summary of the *Rht-B1* and *Rht-D1* genotypes identified and plant phenotypes (height and heading date relative to ‘N10/B14’ [Cltr13253]) obtained from Tables 7.1 and 7.2. The single accession of ‘Seu Seun 27’ did not contain a semi-dwarfing allele and was 158% the height of the ‘N10/B14’ control. The available parental line of ‘Seu Seun 27’ (‘Shiroboro 1’) also did not carry *Rht-B1b* or *Rht-D1b*. The two accessions of ‘Suweon 92’ differed in genotype and phenotype. [Cltr13132] was homozygous for *Rht-B1b*+*Rht-D1b* and was awned, whereas in [PI157603] *Rht-B1a*+*Rht-D1a* predominated, plants were awnless, and mean plant height was nearly double that of [Cltr13132]. Three original sources of ‘Suweon 85’, the only available parent of ‘Suweon 92’, were genotyped. One of these, [BW6431], was homozygous *Rht-B1b*+*Rht-D1b*. A second source, [PI157599], was homozygous *Rht-D1b* and segregated at the *Rht-B1* locus. The third ‘Suweon 85’ source [PI157600] had the genotype *Rht-B1b*+*Rht-D1a*. Three accessions of ‘Kanred’, a parent of ‘Suweon 85’ were tested and all three sources were homozygous for *Rht-B1a*+*Rht-D1a* and plants were relatively tall, but the accessions differed in awn phenotype: [PI 90832] had no awns, [Cltr 5146] was awned, and [W741] segregated for awn presence. Germplasm of the other parent of Suweon 85, ‘Aka-Daruma/Glassy Fultz’, was not available. Of the parents of ‘Aka-Daruma/Glassy Fultz’, ‘Glassy Fultz’ was not available and (as described above) the progenitor line ‘Fultz’ was homozygous *Rht-B1a*+*Rht-D1a*. Three accessions of ‘Aka-Daruma’ were tested and all three had the predominate genotype of *Rht-B1a*+*Rht-D1b*. Two of these accessions, [PI325843] and [KT020-009], had *Rht-B1a/b* segregants in 2 of 12 seeds and 2 of 8 seeds, respectively. ‘Daruma’, as described before, was homozygous for *Rht-B1b*+*Rht-D1a*.

Table 7.4. *Rht-1* genotypes and phenotypes of varieties in the 'Suweon 92' and 'Seu Seun 27' genealogy.

Variety[a]	Accession Number[b]	*Rht-B1*[c]	*Rht-D1*	Sowing	Awns	Plant ht. (% of N10/B14)[d]	Days to heading (diff vs. N10/B14)[e]
Daruma	KT020-110	***B1b***	*D1a*	NIAB '10	awned	166%	-15
↓							
Aka-Daruma	PI325843	*B1a**	***D1b****	JIC '08	awned	113%	-24
				NIAB '10	awned	134%	-21
	KT020-009	*B1a**	***D1b****	NIAB '10	awned	149%	-21
	KU-1206	*B1a*	***D1b****	NIAB '10	awned	134%	-22
×							
Glassy Fultz (not available)							
Fultz	W748	*B1a*	*D1a*	JIC '08	no awns	211%	6
	PI5493	*B1a*	*D1a*	JIC '10	no awns	234%	-9
↓							
Aka Daruma x Glassy Fultz	(not available)						
×							
Kanred	W741	*B1a*	*D1a*	JIC '08	seg	181%	-4
	Cltr5146	*B1a*	*D1a*	JIC '10	awned	251%	-3
	PI90832	*B1a*	*D1a*	JIC '10	no awns	205%	-10
↓							
Suweon 85	PI157599	***B1b****	***D1b***	JIC '08	awned	90%	-23
	PI157599-1	***B1b***	***D1b***	NIAB '10	awned	129%	-10
	BW6431	***B1b***	***D1b***	NIAB '10	seg	129%	-6
	PI157600	***B1b***	*D1a*	JIC '10	awned	138%	-17
×							
Suweon 13	(not available)						
Suweon 92	Cltr13132	***B1b***	***D1b***	NIAB '10	awned	129%	-10
	PI157603	*B1a**	*D1a*	JIC '10	no awns	231%	-9
Suweon 90	(not available)						
×							
Shiroboro 1	KT020-015	*B1a*	*D1a*	NIAB '10	awned	174%	-17
↓							
Seu Seun 27	PI157584	*B1a*	*D1a*	JIC '08	awned	158%	-16

[a] 'Norin 10' ancestry is duplicated from Fig. 7.1 except for the addition of 'Fultz', which was investigated due to the unavailability of 'Glassy Fultz'. An (×) indicates a genetic cross and arrows point to the resulting progeny.

[b] PI157599-1 represents progeny of an *Rht-B1b+Rht-D1b* individual from PI157599.

[c] The predominant *Rht-B1* and *Rht-D1* genotypes are indicated with *Rht-B1b* or *Rht-D1b* alleles indicated by a (-*b*) and shown in bold font. Wild type (-*a*) alleles are in plain font. An (*) indicates that segregants at a particular locus were detected.

[d] Plant heights are given as a percentage of the height of 'Norin 10/Brevor-14' (N10/B14) plants grown in the same sowing.

[e] Days to heading are given as the difference in days relative to N10/B14 from the same sowing, with (-) indicating earlier heading.

7.4. DISCUSSION

In this chapter, the origins of the *Rht-B1b* and *Rht-D1b* alleles utilised in wheat breeding in the Western world were investigated by assaying the semi-dwarf donor varieties 'Norin 10', 'Suweon 92', and 'Seu Seun 27' along with their progenitors (Figure 7.1) using allele-specific molecular markers and by measuring plant phenotypes. Two accessions of 'Norin 10' were examined and both had a primary genotype of *Rht-B1b*+*Rht-D1b* (Table 7.1) and were short statured (43 and 47 cm mean height; Table 7.2). The presence of *Rht-B1b* and *Rht-D1b* in 'Norin 10' was previously demonstrated by Gale *et al*. (1981) and Yamada (1989) by test crossing with *Rht-1* alleles and measuring GA response in F_2 plants and by Ellis *et al*. (2002) using genetic markers. The *Rht-B1b*+*Rht-D1b* genotype was also the predominant genotype of 'N10/B14' (the progeny of 'Norin 10' × 'Brevor') as previously reported by Gale *et al*. (1981) using *Rht-1* test-crosses and GA sensitivity testing. The absence of semi-dwarfing alleles in 'Brevor' demonstrates that 'Norin 10' is the source of both semi-dwarfing alleles in 'N10/B14', which was the primary semi-dwarf donor used in Orville Vogel's and Norman Borlaug's breeding programmes (Dalrymple, 1986). An accession of 'Suweon 92', [Cltr 13132], also was shown to have the *Rht-B1b*+*Rht-D1b* genotype and was short statured (129% of 'N10/B14'). The increase in height over 'N10/B14' may be the result of additional genes for tall culm height that are reported to exist in this variety (Allan *et al*., 1968). The second source of 'Suweon 92' tested, [PI 157603], carries neither *Rht-B1b* nor *Rht-D1b* and is much taller (231% of 'N10/B14) than the first source and, unlike the first source, has no awns. The second accession of 'Suweon 92' likely represents a seed misclassification because 'Suweon 92' is reported to be semi-dwarf and to carry both *Rht-1* mutant alleles based on *Rht-1* testcrosses and GA sensitivity testing (Allan *et al*., 1968). A single accession, [PI 157584], of 'Seu Seun 27' was acquired;

however, neither *Rht-B1b* nor *Rht-D1b* was detected. Most likely, this also represents a seed misclassification as the presence of a semi-dwarf allele had been previously reported for 'Seu Seun 27' [PI 157584] (Allan *et al*., 1968).

Although *Rht-B1b*+*Rht-D1b* is the predominant genotype of 'Norin 10', it is not clear where these alleles arose based on genotyping results (Table 7.3). The parental lines of 'Norin 10' are reported as 'Turkey Red' and 'Fultz-Daruma', however, none of the accessions of these varieties contained *Rht-B1b* or *Rht-D1b* (Table 7.1) and all were tall. The two 'Turkey Red' accessions were 169% and 224% the height of 'N10/B14' (Table 7.3). In agreement, Yamada (1990) reported that 'Turkey Red' was GA responsive (an indicator that the GA insensitive *Rht-B1b* and *Rht-D1b* alleles are absent) and tall with a culm length of 139 cm. U.S. historical records for 'Turkey Red' show that this variety was widely sown and "midtall" (short = 30 to 90 cm, midtall = 60 to 120 cm; tall = 90 to 150 cm under conditions in California) (Clark *et al*., 1922), giving further evidence that 'Turkey Red' is unlikely to contain *Rht-B1b* or *Rht-D1b*. The other 'Norin 10' parent, 'Fultz Daruma' is descended from the 'Daruma' lineage thought to be the source of semi-dwarfism in 'Norin 10' (Kihara, 1983; Dalrymple, 1986). However, the 'Fultz Daruma' accession that was assayed, [PI 325844], did not contain *Rht-B1b* or *Rht-D1b* and was tall (179% the height of N10/B14). In agreement, 'Fultz Daruma' was reported to be GA responsive and tall by Yamada (1990). In contrast, a photograph taken of 'Fultz Daruma' by Matsumoto (1968) showed the culm of 'Fultz Daruma' to be shorter than that of 'Norin 10'. This photograph suggests that the accession of 'Fultz Daruma' used to produce 'Norin 10' is not the same as the accession stored in the USDA-ARS NPGS collection and may contain *Rht-B1b* and *Rht-D1b*. Further, the USDA received seed of 'Fultz Daruma' in 1968, well after it would have been crossed to 'Turkey Red' (1925) and 'Fultz' is listed in the database (http://www.ars-grin.gov/npgs) as the parent of this accession instead of 'Glassy-Fultz', raising more doubt as to whether this accession is representative of the 'Fultz Daruma' that gave rise to 'Norin 10'.

On the Suweon side of the pedigree chart (Table 7.4), assay results show that the *Rht-B1b*+*Rht-D1b* alleles present in 'Suweon 92' are also the predominant

alleles present in its parental line, ‘Suweon 85’. The *Rht-B1b*+*Rht-D1b* homozygous ‘Suweon 85’ plants were 129% the height of ‘N10/B14’. The additional height may be the result of additional alleles for tall culm length that were detected in ‘Suweon 92’ by Allan *et al*. (1968). ‘Suweon 85’ is likely the source of both alleles in ‘Suweon 92’, but it is also possible that ‘Suweon 13’, which was not assayed, is a donor. Similar to ‘Norin 10’, the immediate source of the semi-dwarfing alleles in ‘Suweon 85’ is not clear. The parental line ‘Kanred’ is an unlikely donor because none of the accessions contained *Rht-B1b* or *Rht-D1b* and plant heights were 1.8 to 2.5 times the height of ‘N10/B14’. In addition, ‘Kanred’ is a selection from a strain of ‘Turkey Red’, which (as shown above) does not contain *Rht-B1b* or *Rht-D1b*, and ‘Kanred’ is described as “midtall” in height (Clark *et al*., 1922). The more likely immediate donor of *Rht-B1b* and *Rht-D1b* to ‘Suweon 85’ is ‘Aka-Daruma/Glassy Fultz’, but no accessions were available.

‘Daruma’ is thought to be the common ancestor of ‘Norin 10’, ‘Suweon 92’, and ‘Seu Suen 27’ and the source of semi-dwarfism in these lines (Kihara 1983; Dalrymple, 1986); however, ‘Daruma’ [KT020-110] had an *Rht-B1b*+*Rht-D1a* genotype with no segregants detected and a mean height (92 cm) that was 166% of ‘N10/B14’. Similarly, Yamada (1990) reported that ‘Daruma’ had an *Rht-B1b*+*Rht-D1a* genotype (based on test crosses and GA responsiveness of the F_2) and was 91 cm in height. Surprisingly, the two variants of ‘Daruma’, ‘Shiro-Daruma’ and ‘Aka-Daruma’, were shown to have predominant genotypes of *Rht-B1a*+*Rht-D1b*. Four sources of ‘Shiro-Daruma’ were assayed and two were homozygous *Rht-B1a*+*Rht-D1b* while the other two accessions were predominately *Rht-B1a*+*Rht-D1a*; however, importantly, an individual in each of the *Rht-B1a*+*Rht-D1a* sources was found to be heterozygous *Rht-B1a*/*b* (Table 7.1). Similarly, for ‘Aka-Daruma’, while *Rht-B1a*+*Rht-D1b* was the predominant genotype, two of the three sources assayed contained two seeds each that were heterozygous *Rht-B1a*/*b*. The presence of these alleles in low frequency could be the result of contamination of the original seed sources or the alleles may not have been fixed in the original populations. ‘Daruma’, ‘Aka-Daruma’, and ‘Shiro-Daruma’ are landraces according to Yamada (1990). ‘Daruma’ is also described as a

landrace by Worland (1986) and as a “native variety” by Kihara (1983). The *Rht-B1a*/*b* segregants found in ‘Shiro-Daruma’ and ‘Aka-Daruma’ may represent alleles that were segregating in low frequency in the original populations. Another possibility is that the alleles were not in low frequency, but were simply under-represented in the sample taken for curation. If these accessions were segregating at the *Rht-1* locus in curated stocks, the *Rht-B1b*+*Rht-D1b* plants may have been inadvertently selected against during seed grow-outs as the double-dwarf would appear to be an off-type. The presence of *Rht-B1b* in the parental line ‘Daruma’ also suggests that *Rht-B1b* was likely present in the original ‘Aka-Daruma’ and ‘Shiro-Daruma’ populations. In a similar manner, the accessions of ‘Daruma’ in seed banks may not be representative of native ‘Daruma’ populations. For ‘Daruma’, only six seeds were tested from a single source, so segregants in low frequency may not have been detected.

‘Shiro-Daruma’ and ‘Glassy Fultz’ are historically thought to be the parents of ‘Fultz-Daruma’. Dalrymple (1986), however, noted that the official records list ‘Daruma’ as the parent of ‘Fultz-Daruma’ and that the use of the white variant ‘Shiro-Daruma’ is suggested by Inazuka (1971) and Matsumoto (1968). ‘Aka-Daruma’ and ‘Glassy Fultz’ are thought to be the parents of ‘Aka-Daruma/Glassy Fultz’. Seed of ‘Glassy-Fultz’ could not be found in the germplasm banks that were searched, so its parent ‘Fultz’ was acquired instead. Neither of the ‘Fultz’ accessions contained a semi-dwarfing allele and both were over twice the height of ‘N10/B14’. Historically, ‘Fultz’ was widely grown in the USA and considered mid-tall (Clark *et al*., 1922), giving further evidence that ‘Fultz’ is unlikely to contain *Rht-B1b* or *Rht-D1b*. Because ‘Glassy Fultz’ is a variant of ‘Fultz’, these results reduce the likelihood that it would carry an *Rht-B1b* or *Rht-D1b*. Yamada (1990), however, identified an accession called ‘Shou Fultz’, and explained that “shou” corresponds to “glassy” in English. Yamada went on to report that ‘Shou Fultz’ contained the *Rht-B1b* allele (based on test crosses and F_2 GA responsiveness) and speculated that the *Rht-B1b* allele in this line may have resulted from a spontaneous mutation in ‘Fultz’. If ‘Shou Fultz’ does

correspond to the ‘Glassy Fultz’ used in these crosses, it raises the possibility of an alternative source of the *Rht-B1b* allele.

In summary, the *Rht-B1b* and *Rht-D1b* alleles are present in ‘Norin 10’, ‘Suweon 85, and ‘Suweon 92’, which are all descendants of ‘Daruma’. The source of these alleles is unclear as the parental lines do not have an *Rht-B1b*+*Rht-D1b* genotype. One explanation for this discrepancy is that the parentage of these lines (Figure 7.1) is in some way incorrect and/or that seed has been misclassified. A second explanation is that ‘Glassy Fultz’ (perhaps known in collections as ‘Shou Fultz’) contributed the *Rht-B1b* allele to these varieties and the *Rht-D1b* allele was contributed by ‘Shiro-Daruma’ or ‘Aka-Daruma’. However, the existence of *Rht-B1b* in ‘Daruma’ and the presence of *Rht-B1b* segregants in ‘Aka-Daruma’ and ‘Shiro-Daruma’ also suggest a third explanation. This explanation, which is not exclusive of the others, is that both semi-dwarfing alleles originally came from ‘Daruma’ as shown in Figure 7.1, but the seed bank accessions of ‘Daruma’, ‘Aka-Daruma’ and ‘Shiro-Daruma’, which are all landraces, is not representative of the populations used to make the original crosses.

8. GENERAL DISCUSSION

The introduction of the group IV semi-dwarf alleles *Rht-B1b* (formerly *Rht1*) and *Rht-D1b* (formerly *Rht2*) into bread wheat varieties beginning in the 1960s was a major contributor to the green revolution. These alleles are estimated to be present in 90% of modern semi-dwarf wheat varieties (Worland *et al*., 1998b) with a single variety, 'Norin 10', serving as the primary source of both alleles. *Rht-B1b* and *Rht-D1b*, along with the GA sensitive *Rht-B1a* and *Rht-D1a* alleles in CS, were cloned and sequenced over a decade ago (Peng *et al*., 1999). However, prior to this project, outside of the ORF sequences of these four alleles, little was known regarding the sequence diversity of the group IV *Rht* loci or the genetic composition of the contiguous sequence that was presumably introgressed into wheat varieties along with the semi-dwarfing alleles. Furthermore, while there were indications that the *Rht-A1* homoeologue existed (Peng *et al*., 1999), there was no published sequence and the locus had not been mapped.

Whilst *Rht-B1b* and *Rht-D1b* generally benefit wheat production, deleterious effects are also associated with these alleles. In several studies, *Rht-D1b* has been associated with increased susceptibility to Fusarium Head Blight (FHB), a major wheat disease (Hilton *et al.*, 1999; Draeger *et al.*, 2007; Holzapfel *et al*., 2008; Voss *et al*., 2008; Meidaner and Voss, 2008; Srinivasachary *et al*., 2008; 2009), and it has been suggested this is due to linkage (Srinivasachary *et al*., 2009). *Rht-B1b* in some studies has also been associated with changes in the level of resistance / susceptibility to FHB, although the effects were minor relative to *Rht-D1b* (Steiner *et al*., 2004; Medianer and Voss, 2008; Srinivasachary *et al.*, 2009). In addition, the yield advantage normally associated with *Rht-B1b* or *Rht-D1b* diminishes or disappears in low wheat production environments (Gale and Youssefian, 1985; Flintham *et al*., 1997; Chapman *et al*., 2007), and both alleles have a reduced coleoptile length and seedling vigour, which in dry soils can result in poor stand establishment (Rebetzke *et al*., 2001). In light of the agronomic issues associated with *Rht-B1b* and *Rht-D1b*, the primary objectives of this project were to gain a better understanding of the pre-existing genetic variation that exists at the *Rht-B1*,

Rht-D1, and *Rht-A1* loci, and to determine the genetic makeup of the contiguous region immediately surrounding these loci.

Screening of the CS BAC library (Chapter 2) was a necessary first step in order to obtain the homoeologous *Rht-1* sequences in a common genetic background. The screening process contained challenges in itself as the attempt to screen the French component of the library using probes designed to be *Rht-1* specific resulted in the identification of only two *Rht-1* containing clones although the probes had hybridised to several BAC clones. Screening of the UK component of the library using PCR with *Rht-1*-specific primers was much more effective. The screening was facilitated by the 3-D pooling of the BAC library (Febrer *et al*, 2009), which greatly reduced the number of PCR reactions required to identify *Rht-1* containing clones. The PCR screen identified 13 *Rht-1* containing BAC clones, of which three (one each from the A, B, and D genomes) were selected for full-length sequencing and assembly. From this PhD project, which was the first to use the pooled BAC library, it is clear that it represents a valuable resource to the wheat breeding community in the absence of a fully assembled genomic sequence. In addition, sequence of all three *Rht-1* homoeologues allows for primers to be designed for locus-specific amplification and for examining *Rht-1* homoeologue gene expression.

Analysis of the *Rht-1* containing BAC sequences (Chapter 3) revealed that most of the contiguous region was composed of transposable elements, but also revealed the existence of *Rht-1* on each BAC along with a *DUF6* (*Domain of Unknown Function 6*) family gene immediately upstream. The A and D genome BACs also contained a *ZnF* (*zinc finger*) family gene, which was not present on the B genome BAC, but may still be present upstream of the BAC sequence. The proportion of genic sequence comprised less than 3% of any CS BAC clone, similar to the findings of other studies of the wheat genome (Li *et al*., 2004; Devos *et al*, 2005; Paux *et al*., 2006), indicating this region is not particularly gene-rich or gene-poor. The conserved gene synteny among the three wheat homoeologues, rice, *Brachypodium*, sorghum, and maize is a positive indicator that synteny may extend further 5’

and 3'. Until a large number of ordered markers on the group IV chromosomes of wheat become available it will be difficult to understand how far this synteny extends. Currently available sequences of rice, *Brachypodium*, and the ongoing physical mapping and sequencing of the barley gene space by the International Barley Genome Sequencing Consortium (IBSC;http://barleygenome.org) will be useful for determining the extent of synteny and identifying genes linked to *Rht-B1b* or *Rht-D1b* that may be important in FHB resistance or other wheat agronomic characteristics. The region immediately 5' of the sequenced region proves to be interesting as *tb1* is the next gene upstream of the *ZnF* gene. *Tb1* is an important domestication gene in maize that controls branch number (Doebley *et al*., 2004) and the barley orthologue *Intermedium-C* (*Int-C*) is associated with lateral spikelet fertility and plant tillering (Ramsay *et al*., 2011). In contrast to the high level of gene synteny, transposable elements were not conserved amongst even the wheat homoeologues, similar to previous studies of homoeologues wheat regions (Gu *et al*., 2004; Chalupska *et al*., 2008; Ragupathy and Cloutier, 2008). This demonstrates how rapidly transposon sequences evolve relative to genes as the A, B, and D genome progenitors of wheat diverged only 2-4 MYA (Huang *et al*., 2002; Dvorak and Akhunov, 2005; Chalupska *et al*., 2008), whereas the *Poaceae* species analysed here diverged up to 60 MYA.

Analysis of the three genes discovered on the wheat BACs revealed a pattern of conservation among the *Poaceae* that mirrored previously established phylogenetic relationships (Paterson *et al*., 2009). In all cases, the barley ORF sequences were the most similar to wheat suggesting the potential usefulness of a fully-assembled barley sequence. After barley, *Brachypodium* sequences of the three genes were the most similar to wheat, suggesting a greater value of *Brachypodium* sequence for wheat genomics relative to rice, maize, and sorghum. Genetic comparisons amongst the *Poaceae* of the ORFs of the three genes revealed the *ZnF* gene was the most highly conserved and *DUF6* the least conserved. The high level of conservation among the *Poaceae ZnF* orthologues suggests that alterations to the amino acid sequence of the ZnF may be deleterious to the plant, thereby suggesting

an essential role of this gene. The *ZnF* gene is a specialised type of zinc finger known as a RING (Really Interesting New Gene) type protein. RING-type proteins are involved in protein:protein interactions including assembly of large protein complexes and the ubiqitination of proteins (Joazeiro and Weissman, 2000; Mathews and Sunde, 2002). The *Rht-1* gene was largely conserved amongst the three CS wheat homoeologues and there were no evident predicted stop codons or frameshift mutations. However, there was still variation amongst the homoeologues in the ORF and surrounding sequence that could potentially affect RNA expression or protein function. In the ORF, the predicted peptide lengths vary from 620 to 623 and non-conserved amino acids resulting from SNPs or indels occur at 1.9% to 2.6% of the sites when comparing any two homoeologues. *Rht-A1* and *Rht-D1* were the most similar homeologues. As expected, larger differences between *Rht-1* homoeologues are present just upstream and downstream of the coding region. The 3' region and 5' regions are still both highly conserved among the wheat homoeologues, suggesting that both regions could play important regulatory roles. Comparative analyses of the 5' and 3' wheat homoeologue sequences with the same regions in rice and *Brachypodium* revealed three areas of high conservation amongst the sequences. The most conserved sequence (~ 120 bp in length) occurred between 300 and 500 bp 5' of the ORF of *Rht-1* or its orthologue and two regions were identified 3' of the ORF, a ~100 bp sequence between 200 and 450 bp downstream and a ~300 bp sequence between1.6 and 2 kb downstream. Currently, little is known regarding the regulatory regions that affect *Rht-1* expression and the high level of conservation in these regions suggest these regions may be involved in cis-regulation of *Rht-1*. Sequence analysis of diverse wheat accessions revealed the presence of two 5' insertions (relative to CS) of 160 and 197 bp. The 160 bp insertion occurs 356 bp 5' of the ORF and in the middle of the most highly conserved region among wheat, rice and Brachypodium whilst the 197 bp insertion occurs 591 bp upstream. Homology to the 197 bp sequence was not present in region immediately upstream (1 to 10 000 bp) of the Rht orthologues in rice and *Brachypodium*. These insertions were investigated further and may affect plant height, GA sensitivity, and flowering time in

wheat, although no effect on RNA transcript abundance was detected in the stems and leaves of seedlings (discussed below).

In this project, the *Rht-A1* locus has been clearly identified based on a chromosome 4A location (Chapter 4), high nucleotide similarity to *Rht-B1* and *Rht-D1*, and a high degree of gene synteny in the *Rht-A1* region relative to the *Rht-B1* and *Rht-D1* homoeologous regions (Chapter 3). However, unlike *Rht-B1* and *Rht-D1*, which are on 4BS and 4DS, respectively, *Rht-A1* is located on 4AL. The location of *Rht-A1* on the long arm of 4A is not unexpected because the majority of the native short arm of 4A is located on the long arm of chromsome 4A in modern bread wheat cultivars due to a pericentric inversion thought to have occurred in tetraploid wheat (Miftahudin *et al*., 2004). *Rht-A1* appears to be near the centromere based on physical and genetic mapping; however this requires further investigation due to conflicting results between 4AL Kansas deletion lines, which precluded determining a more precise location on 4AL. Nonetheless, the molecular tools associated with *Rht-A1* developed as part of this project (an *Rht-A1* marker, *Rht-A1* sequences, and identification of linked SSR markers linked to *Rht-A1*) provides a resource to further investigation of this locus and the surrounding region and should be a useful resource for marker-based selection.

Interestingly, while *Rht-B1* appears to be located near the centromere based on previous physical telocentric F_2 mapping (McVittie *et al.*, 1978) and genetic mapping (Borner *et al.*, 1997; Ellis *et al*. 2002; Somers *et al.*, 2004), *Rht-D1* was mapped between breakpoint 0.82 and the telomere with the Kansas deletion lines in this project. The telomeric location agrees with telocentric F_2 mapping performed by Izumi *et al.* (1983), but differs from the more proximal location (15 cM from the centromere) reported by McVittie *et al.* (1978). Mapping *Rht-B1* with the Kansas deletion lines is possible, but complicated by the presence of a male sterility gene near the centromere, which precludes the production of homozygous 4BS deletion lines. Although a translocation near the centromere of 4B has been reported (Mickelson-Young *et al*., 1995), there are no reports of a translocation involving the telomeric region of 4BS or 4DS. The telomeric location of *Rht-D1* would suggest potentially greater

recombination at and near this locus relative to the more proximal *Rht-A1* or *Rht-B1*, as recombination is generally greater towards the telomere (Devos *et al.*, 1995; Akhunov *et al.*, 2003).

In this project, sequence diversity was present among bread wheat and tetraploid/diploid wheats at the *Rht-A1*, *Rht-B1*, and *Rht-D1* ORFs and in the flanking 5' (~1750 bp) and 3' (~450 bp) regions (Chapter 5). A large amount of genetic diversity at the *Rht-1* loci was shown to be lost between tetraploid/diploid wheat and bread wheat, similar to other reports of reduced genetic diversity in bread wheat relative to ancestral lines (Caldwell *et al*., 2004; Haudry *et al*., 2007). Focusing on bread wheat only, among the three homoeologous *Rht-1* regions, diversity was the greatest on the B genome and least on the D genome. Lack of diversity in the D genome relative to the A and B genomes has been reported in several previous studies of bread wheat (Bryan *et al.*, 1997; Huang *et al.*, 2002; Wang *et al.*, 2007; White *et al.*, 2008; Chao *et al.*, 2009). This likely is the result of the origin of bread wheat from just a small number of hexaploidisation events with the D genome donor *Ae. tauschii* (Talbert *et al*., 1998; Caldwell *et al*., 2004) along with little inter-mating between bread wheat and *Ae. tauschii* (Dvorak *et al*., 1998), whereas a substantial amount of inter-mating appears to have occurred between *T. aestivum* and tetraploid wheat (Dvorak *et al*., 2006; Dubocovsky and Dvorak 2007; Luo *et al*., 2007). The *Rht-B1* locus and flanking region of the accessions sequenced in this project had the greatest nucleotide and haplotype diversity. The polymorphisms included the three largest indels (197, 160, and 16 bp) and the largest number of predicted amino acid changes found among the *Rht-1* homoeoloci, including a previously unknown frameshift mutation. Several other studies have reported greater genetic diversity on the B genome relative to the A and D genomes (Huang *et al.*, 2002; Ravel *et al.*, 2006; Wang *et al.*, 2007; Haseneyer *et al.*, 2008; Li *et al.*, 2010). The closest known wild relative to the B genome is *Ae. speltoides* (Kilian *et al*., 2007) and its out-crossing nature may attribute to the higher level of genetic diversity often reported for the B genome. The *Rht-A1* locus, while containing more nucleotide diversity and more haplotypes than *Rht-D1* had the lowest haplotype diversity among the three genomes. This results

from the high proportion (~75%) of accessions that belong to a single haplotype, which includes all 12 “UK” accessions. The low haplotype diversity suggests there may have been selection at *Rht-A1* or a linked locus. If *Rht-A1* is centromeric, this may also reduce recombination at this locus.

There was no sequence variation amongst the *Rht-B1b* alleles or amongst the *Rht-D1b* alleles. This is not surprising considering the recent introgression of these alleles into bread wheat with the Japanese line Norin 10 serving as the primary source for both alleles (Dalrymple, 1986). Strong selection for *Rht-B1b* and *Rht-D1b* almost certainly has resulted in reduced haplotype diversity at these loci and at linked loci. The size of the linkage block, also known as a “selective sweep” is likely to be large for the reasons given above and likely affects a large number of genes. This is one reason why it is important to understand haplotype diversity at these loci. The haplotypes discovered in this project represent potentially useful sources of genetic variation for further research of important regulatory regions associated with *Rht* or for identifying beneficial variants of linked genes, such as genes potentially affecting FHB resistance, which could greatly benefit wheat breeding efforts.

Among the novel *Rht-1* alleles discovered in this project, the *Rht-B1* 160 bp and 197 bp indels were analysed further due to their large sizes and location in a potentially important regulatory region (discussed above). Sequencing of wheat tetraploid lines revealed the absence of the 160 bp insertion, indicating this is likely an insertion relative to the ancestral condition. This insertion dates back to at least 1830 as it was present in an accession released in that year (Line 03070 from the INRA BWCC; Balfourier *et al*., 2007). In contrast, the 197 bp sequence was present in the tetraploid lines and regions of high homology exist on the A and D genomes, indicating that the absence of the 197 bp sequence is representative of a deletion relative to the ancestral condition. Bioinformatic analysis revealed that neither insertion sequence has high homology to sequences in the NCBI (nr/nt) database or the TREP cereal repeat database. The insertions were then studied using qRT-PCR to estimate transcript abundance in stem and leaf tissue collected from two-week old wheat seedlings of accessions that contained no insertion, the 160

bp insertion, or the 197 bp insertion (Chapter 5). Significant ($p < 0.05$) changes in *Rht-B1* transcript levels were not consistently related to either of the insertions indicating that the insertions do not have a major effect on *Rht-B1* transcript level in leaf and stem tissues at this developmental stage. It is possible that changes in RNA transcript occur at a later developmental stage or in tissue types that were not measured in this study. To determine if the *Rht-B1* insertions affected plant height, GA sensitivity, or heading date in wheat, association analyses were performed that utilised genotypic and phenotypic data collected on the INRA BWCC as part of this project (Chapter 6). This revealed that accessions containing the 160 bp or 197 bp allele (*Rht-B1a_160* and *Rht-B1a_197*, respectively) had mean reductions in plant height and GA sensitivity, and a delay in mean ear emergence relative to *Rht-B1a* accessions with no insertion (*Rht-B1a_0)*. However, the height reductions were minor relative to the effects of *Rht-B1b* and only the height difference between *Rht-B1a_197* bp and *Rht-B1a_0* and heading date between *Rht-B1a_160 and Rht-B1a_0* were significant at a probability threshold of 0.05. The use of bi-parental populations (one with an insertion and one without) or examination of the insertions in a common background would be powerful tools to determine the effects of these alleles. Integration of these alleles into a common genetic background has now been initiated at NIAB.

In an attempt to discover novel GAI alleles at the *Rht-1* loci (particularly at *Rht-A1* where alleles affecting plant height or GA sensitivity are yet to be found), the INRA BWCC was screened by measuring response of seedlings to GA application. However, the experimental method and statistical analyses (mixed model or LSD analyses) employed did not clearly reveal the presence of any GAI alleles in the collection outside of *Rht-B1b*, *Rht-D1b*, or *Rht-B1c*, indicating that novel GAI alleles either do not exist in the collection or experimental conditions were insufficient to detect them. While the methods using in this study rarely incorrectly classified accessions containing *Rht-B1b* or *Rht-D1b* (both were genotyped in the collection as part of the PhD project) as GA sensitive, these alleles were only classified as GAI 64% of the time. The difficulty in classifying GAI alleles as GAI may be due to the effect of genetic background differences between the diverse accessions,

experimental design (for instance, too few plants screened per accession), and the slight GA response detected in accessions containing *Rht-B1b* or *Rht-D1b*, all of which would make detection of differences in seedling length between GA sensitive and insensitive lines more difficult. *Rht-1* alleles that have a GA response that is intermediate the *Rht-1b* and *Rht-1a* alleles would be even more difficult to detect. Intermediate GAI alleles (potentially including *Rht-B1a_160* and *Rht-B1a_197*) may exist in the collection as indicated by the large number *Rht-B1a*+*Rht-D1a* accessions (42) that fall within the range of GA treatment differences found among accessions with the *Rht-B1b* or *Rht-D1b* allele (20.8 mm or less; Appendix VI). The absence of a GAI allele at the *Rht-A1* locus, which is known to be expressed (Saville, 2011), relative to the *Rht-B1* and *Rht-D1* loci where several GAI alleles are known to exist, suggest that insensitivity at this locus may be detrimental to wheat. Even though no additional GAI alleles were discovered, knowledge was gained regarding the use of experimental and statistical methods and it appears that the mixed model method was more efficient at correctly classifying previously known *Rht-B1b* and *Rht-D1b* alleles than the LSD method, although this method would appear to be more likely to incorrectly classify GAI accessions that may have long seedling lengths due to other genes.

The origin of the *Rht-B1b* and *Rht-D1b* alleles in 'Norin 10' (the major donor of these alleles) and 'Suweon 85' (a minor donor) was investigated by genotyping accessions that make up the historical pedigree (Chapter 7) of these two lines (Figure 7.1). Historically, *Rht-B1b* and *Rht-D1b* are thought to be derived from 'Daruma', a landrace present in Japan as early as 1894 (Kihara, 1983). However, the genotyping of several accessions from the historical pedigree revealed that none of the progenitors of 'Norin 10' or 'Suweon 85' were homozygous for both alleles, including 'Daruma', which was homozygous for only *Rht-B1b*. However, the homozygous *Rht-D1b* genotype in the 'Daruma' variants 'Shiro-Daruma' and 'Aka-Daruma' and the presence of *Rht-B1a*/*Rht-B1b* heterozygotes in a small percentage of plants from these accessions suggests that the curated seed used in this study may not be representative of the original landraces, which may have been segregating at these loci. Hence, there is no clear evidence that contradicts the historical

pedigree, suggesting that ‘Daruma’ is still the most likely donor of *Rht-B1b* and *Rht-D1b* for ‘Norin 10’ and ‘Suweon 85’. Genotyping in this instance identified potential off-types in the accessions contained in seed banks, but has also strengthened confidence of the genotype of other accessions. For instance, the matching *Rht-1* sequences of SS7010073 and the putative *T. dicoccum* and *Ae. tauschii* donors supports the claim that these are the donors. In addition, genotyping for *Rht-B1b* and *Rht-D1b* was critical in the GAI and plant height screening. Hence, this project also emphasises the value of genotyping for quality assurance.

The *Rht-1* loci are agronomically important loci and in this project, I have increased knowledge of these loci and developed tools that can be further used for targeted breeding of improved wheat varieties. My PhD project is the first to determine all three *Rht-1* homoeologous sequences (including *Rht-A1*, previously unpublished) and the genetic makeup of the nearby surrounding region in a common genetic background in wheat. With the sequences, I developed locus-specific primers and for each homoeologue sequenced the entire *Rht-1* ORF and over two kb of flanking region in approximately 40 diverse wheat accessions to estimate genetic diversity. Included among the novel polymorphisms discovered were 160 bp and 197 bp insertions within 600 nucleotides of *Rht-B1* that I further characterised using *Rht-1* homoeologue specific expression analysis and by performing association analysis in a worldwide collection of bread wheat accessions. The *Rht-B1* haplotypes and other haplotypes discovered in this project require further characterisation, but represent potentially useful sources of *Rht-1* variation for wheat breeding efforts. Specifically, the discovery of novel haplotypes and the development of genetic tools associated with the *Rht-1* loci in this project represent a necessary starting point in characterising the *Rht-B1b* and *Rht-D1b* selective sweeps, which may be useful for identify linked genes with improved resistance to the disease Fusarium Head Blight. Overall, approaches similar to the one taken here could be applied for creation of tools for knowledge-based breeding at other wheat loci to improve wheat performance, which is especially critical in the face of climate change and current food insecurity.

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Appendix I. Primer sequences

Designation	Dir[b]	Sequence (5' to 3')
160-R1	R	CGG CCA GTT AAA TAT CGT CTG
197-R1	R	AAT TAA TTC TAG CGT CCA CTC G
414F-F10	F	ACG CCT CCC ACT ACA CTG
414F-R4	R	GTT GGT TCA AAC AGA GAG C
D-BAC_F1	F	CCG CTG TGC TCT GAA AAG AT
D-BAC_F2	F	AAT TCG TTA TCG GCG AAA AGT
D-BAC_F3	F	TCT GCC AAA TTG TCC TTG GT
D-BAC_F4	F	TGA CGG ATC CTA CTC CAA GG
D-BAC_F5	F	TTT TCG AGT GGC ATT TCC TC
D-BAC_R1	R	ATC GGT CAG TTT TGG TTG AA
D-BAC_R2	R	CTG ATG CGA TAG GCA AAC CT
D-BAC_R3	R	CCG GAT AAA TTA CGC TGG AA
D-BAC_R4	R	GTG AGA GGA CGA GGG ACA AG
D-BAC_R5	R	CGC CCA CCT GAG GTA TAA CT
DgR3	R	CAC TGG TGG TAG CTG AGA TT
PS-Rht-R2	R	GGA GGA AGA AGG AGG AAG AAT A
PS-Rht-R4	R	GGA GGA AGA AGG AGG AAG AAT G
Rht-11-R	R	CCT GTC GTC AGG GGC GGC GCC AGC
Rht-16-F	F	GGC ATG GGC TCG TCC GAG GAC AAG
Rht-22-F	F	GCC AGA GAT AGA TAG AGA GGC G
Rht-9-R	R	GAT CGG CCG CAG CGC GTA GAT GC
Rht-A-F2	F	CCC TCA ACA GTG CAA TAC CTT C
Rht-A-F3	F	GAT GCC GTC TCG CAA TCT
Rht-A-F4	F	TCC CAC AAA ATT GAG CCA AC
Rht-A-F5	F	TTG ACT TGT GCC TAC CTC TTT TT
Rht-A-R1	R	TTA CGG GTG ATC ATG GAG GT
Rht-A-R2	R	GGT TGG TGC AGT GTA AAG CTG
Rht-A-R3	R	TGC AAA GCC ATC ATG TTC ATA
Rht-ABD-F1	F	GGA CAC CGT GCA CTA CAA CC
Rht-ABD-F2	F	CAG GAG CTC TGT GGT GGA G
Rht-ABD-F3	F	TTC TAC GAG TCC TGC CCC TA
Rht-ABD-F4	F	GAA CCG AGG CAA GCA AAA G
Rht-ABD-F6	F	ACT CCT CCT GCA GCA CCT AC
Rht-ABD-F7	F	GCC CTG GAG AAG GTC CTG
Rht-ABD-F8	F	AGA AGC TGG AGC AGC TGG A
Rht-ABD-F9	F	AGA AGG AGG GCT GCC TGA C
Rht-ABD-R1	R	GAG AGG TTC TCC TGC TGC AC
Rht-ABD-R1a	R	CCA GCA AGG GTA TCT GCT TC
Rht-ABD-R1b	R	CAG CCT CCA CCA CAG AGC
Rht-ABD-R5	R	CGA CAC CAT CAT CTT GTC CT
Rht-ABD-R6	R	TGC ATC CCC TGC TTG ATG
Rht-ABD-R7	R	CAG GAA TGT GCC GGA GTT
Rht-ABD-R8	R	CCG GGG TTG TGT TGT CCT

Appendix I continued.

Designation	Dir[b]	Sequence (5' to 3')
Rht-ABD-R9	R	GAG GTC GGT GGG GTT GTA GT
Rht-ABD-R10	R	CAT AGC TAA TGC GAC ACA CG
Rht-B-F1	F	AGG CAA GCA AAA GCT TGA GA
Rht-B-F2	F	GAA ATG TTG GTT GTT ATA TCC TTG G
Rht-B-F4	F	CAC CAC CGA TCT CGA ACA A
Rht-B-F5	F	CAA TGA CAA AAT AAT AGC CAT TCT C
Rht-B-R1	R	CGG CAA AGG AAG CTA AGT TG
Rht-B-R3	R	AGC GGC AGC GTA GTA GTT GT
Rht-B1a-R2	R	CCA TGG CCA TCT CCA GAT G
Rht-B1b-R2	R	CCC ATG GCC ATC TCC AGA TA
Rht-D-F1	F	CGA GGC AAG CAA AAG CTT C
Rht-D-F2	F	CAG TGC AAT ACA CAG ATG CTT CA
Rht-D-F3	F	ATG GTC GCC TTT GTT TCT TG
Rht-D-F5	F	GCT CGT TCT CCT CCC AGT TC
Rht-D-F6	F	GGG TAC TTA TGT TAT TTG CTT GTT GG
Rht-D-R1	R	AAT TGC AAA GCC ACC ATT G
Rht-D1a-R2	R	ATG GCC ATC TCG AGC TGT TC
Rht-D1b-R2	R	CAT GGC CAT CTC GAG CTG TTA
Rht-F03	F	GAG TCT GAC GCA GCA GAG AG
Rht-F04	F	TTC AAT ATT AAT TTT AAT CAT CCC ACA
Rht-F06	F	AAC CGT GTG TCG CAT TAG C
Rht-F07	F	CCC GCT TTT CCC CTT TTG
Rht-F11	F	TTG TGA TTC CCA CCG GTT C
Rht-R01	R	ACC AGA CAA ACT TCG CCA TC
Rht-R02	R	CCC CGA CTC CCA CTT TAT TT
Rht-R03	R	ATC GGA GCT CTT ACG TTT TTC C
Rht-R04	R	CAA GTC TTT TAG ATC ATG TAC TTA TGC
Rht-R05	R	CCA GCA CGA ATA TTT ACC AAG G
Rht-R08	R	GGG GTG GCA CAA GAG GTG

[b] Direction (Dir) of primers were forward (F) or reverse (R)

Appendix III: Annotation of Chinese Spring BAC Clones 0224_M10 (A genome) (A), 1417-F16 (B genome) (B), and 0155_I24 (D genome) (C)

(A) DEFINITION: *Triticum aestivum* clone BAC 0224_M10 genomic sequence (164257 bp); ACCESSION: Chinese Spring *Rht-A1a*

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mobile element	complement(2189..3175) /note="TREP3160 Retrotransposon, LTR, Copia, RLC_WIS_A_consensus-1"
TSD	3605..3609 /note=" target site duplication of TREP3250 Retrotransposon, LTR, Gypsy, RLG_Sabrina_D_115G1-2"
LTR	3610..5184 /note=" long terminal repeat of TREP3250 Retrotransposon, LTR, Gypsy, RLG_Sabrina_D_115G1-2"
mobile element	5185..10278 /note="TREP3250 Retrotransposon, LTR, Gypsy, RLG_Sabrina_D_115G1-2"
LTR	10279..11842 /note=" long terminal repeat of TREP3250 Retrotransposon, LTR, Gypsy, RLG_Sabrina_D_115G1-2"
TSD	11843..11847 /note=" target site duplication of TREP3250 Retrotransposon, LTR, Gypsy, RLG_Sabrina_D_115G1-2"
mobile element	complement(12107..13426) /note="TREP3456 Retrotransposon, LTR, Copia, RLC_Barbara_consensus-1"
TSD	13442..13446 /note=" target site duplication of RLC_WIS_B_consensus-1, Retrotransposon, LTR, Copia, TREP3161"
LTR	13447..15175 /note="long terminal repeat of RLC_WIS_B_consensus-1, Retrotransposon, LTR, Copia, TREP3161"
mobile element	15175..20467 /note=RLC_WIS_B_consensus-1, Retrotransposon, LTR, Copia, TREP3161"
LTR	20468..22189 /note="long terminal repeat of RLC_WIS_B_consensus-1, Retrotransposon, LTR, Copia, TREP3161"
TSD	22190..22194 /note=" target site duplication of RLC_WIS_B_consensus-1, Retrotransposon, LTR, Copia, TREP3161"
mobile element	complement(22208..22409) /note="TREP3456 Retrotransposon, LTR, Copia, RLC_Barbara_consensus-1"
CDS	complement(24659..24737, 24835..24914, 25031..25111, 25242..25311, 25440..25514, 26833..27038, 27377..27626, 27704..27749, 29214..29283, 31686..31755, 33343..33427, 33551..33635, 33746..33836, 34419..34552) /gene /product = "putative zinc-finger protein gene"

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FQVLLCMRLEGTPPSARYIPIFALFSPLFILQGAGVLFSIGRLVEKVVLLLRNGPVS
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YPPEVVKKMPKKDLAEEVWRLQAALGEQSEITKSTQQEYERLQNEKVLCRICYE
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mobile element 28819..28983
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KDKTL

mobile element complement(49256..49419)
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TSD complement(61415..61419)
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LTR complement(61420..63133)
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Appendix III continued

mobile element complement(63134..68437)
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LTR complement(68438..70153)
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TSD complement(70154..70158)
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/note: "estimated sequence gap of 500 bp or less"

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MSEVYLGRQICNVVACEGAERTERHETLGQWRNRLGNAGFETVHLGSNAYKQA
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misc feature complement(84344..84464)
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mobile element join(87177..87259,87365..87569,87870..87972,88010..88854)
/note="TREP3530 Retrotransposon, LTR, Copia, RLC_Angela_B_consensus-1"

mobile element 90816..94499
/note="TREP2376 Retrotransposon, LTR, Copia, RLC_Bianca_509D2-1"

TSD 94500..94504
/note=" target site duplication of Retrotransposon, LTR, Gypsy, RLG_Carmilla_consensus-1", TREP3169"

LTR complement(94505..94996)
/note=" long terminal repeat of Retrotransposon, LTR, Gypsy, RLG_Carmilla_consensus-1", TREP3169"

mobile element complement(94997..107090)
/note="Retrotransposon, LTR, Gypsy, RLG_Carmilla_consensus-1", TREP3169"

LTR complement(107091..107576)
/note=" long terminal repeat of Retrotransposon, LTR, Gypsy, RLG_Carmilla_consensus-1", TREP3169"

TSD 107577..107581
/note=" target site duplication of Retrotransposon, LTR, Gypsy, RLG_Carmilla_consensus-1", TREP3169"

TSD 107838..107842

Appendix III continued

/note=“ target site duplication of Retrotransposon, LTR, Copia, RLC_WIS_A_consensus-1”

LTR 107843..109620
/note=“ long terminal repeat of Retrotransposon, LTR, Copia, RLC_WIS_A_consensus-1”

mobile element 109621..114681
/note=“Retrotransposon, LTR, Copia, RLC_WIS_A_consensus-1”

LTR 114682..116456
/note=“ long terminal repeat of Retrotransposon, LTR, Copia, RLC_WIS_A_consensus-1”

TSD 116457..116461
/note=“ target site duplication of Retrotransposon, LTR, Copia, RLC_WIS_A_consensus-1”

mobile element 116462..117037
/note=“TREP2376 Retrotransposon, LTR, Copia, RLC_Bianca_509D2-1”

misc feature 129602..130183
/predicted gene
//note=“homology with wEST CK209908 (homeobox protein DLX-2 related cluster) and CK209889 (Cluster related to UPI000037F185; COG3434: Predicted signal transduction protein containing EAL and modified HD-GYP domains)”

mobile element complement(131306..136997,138462..144668)
/note=“TREP3414 DNA transposon, TIR, CACTA, "DTC_Clifford_consensus-1"

gap 137254..137255
/note: “estimated sequence gap of 500 bp or less”

mobile element complement(145672..149405)
/note=“TREP3458 Retrotransposon, LTR, Gypsy, RLG_Jeli_consensus-1”

mobile element 149796..152280
/note=“TREP3529 Retrotransposon, LTR, Copia, RLC_Angela_A_consensus-1”

gap 150952..150953
/note: “estimated sequence gap of 500 bp or less”

mobile element 152282..153987
/note=“TREP3251 Retrotransposon, LTR, Gypsy, RLG_Sabrina_D_AY146588-2”

mobile element 154120..154372
/note=“TREP3245 Retrotransposon, LTR, Gypsy, RLG_Sabrina_B_AY368673-1”

TSD 154388..154392
/note=“target site duplication of TREP3529 Retrotransposon, LTR, Copia, RLC_Angela_A_consensus-1”

LTR 154393..156111
/note=“ long terminal repeat of "TREP3529 Retrotransposon, LTR, Copia, RLC_Angela_A_consensus-1”

mobile element 156112..161363
/note=“TREP3529 Retrotransposon, LTR, Copia, RLC_Angela_A_consensus-1"

LTR 161364..163084

Appendix III continued

/note=“ long terminal repeat of TREP3529 Retrotransposon, LTR, Copia, RLC_Angela_A_consensus-1"

TSD 163085..163089
/note=“target site duplication of TREP3529 Retrotransposon, LTR, Copia, RLC_Angela_A_consensus-1"

mobile element 163090..163481
/note=“TREP3245 Retrotransposon, LTR, Gypsy, RLG_Sabrina_B_AY368673-1"

mobile element 163573..164257
/note=“TREP3251 Retrotransposon, LTR, Gypsy, RLG_Sabrina_D_AY146588-2"

Appendix III continued

(B) DEFINITION: *Triticum aestivum* clone BAC 1417_F16 genomic sequence (187310 bp); ACCESSION: Chinese Spring *Rht-B1a*

repeat region 1..1327
/note="TREP3529 Retrotransposon, LTR, Copia, RLC_Angela_A_consensus-1"

LTR 1328..2396
/note="long terminal repeat of TREP3269 Retrotransposon, LTR, Gypsy, RLG_WHAM_consensus-1"

misc feature 4056..4057
/note: “estimated sequence gap of 500 bp or less”

mobile element 4130..4383
/note="TREP3269 Retrotransposon, LTR, Gypsy, RLG_WHAM_consensus-1"

repeat region complement(4410..6026)
/note="TREP3189 Retrotransposon, LTR, Gypsy, RLG_Fatima_consensus-1”

LTR 6401..7823
/note="long terminal repeat of TREP3269 Retrotransposon, LTR, Gypsy, RLG_WHAM_consensus-1"

LTR 6416..14539
/note="TREP3269 Retrotransposon, LTR, Gypsy, RLG_WHAM_consensus-1"

mobile element 8027..9008
/note="TREP3269 Retrotransposon, LTR, Gypsy, RLG_WHAM_consensus-1"

LTR 14540..15605
/note="TREP3269 Retrotransposon, LTR, Gypsy, RLG_WHAM_consensus-1"

mobile element complement(15949..17397)
/note="TREP3276 Retrotransposon, LTR, unknown, RLX_Gujog_10k23-3"

mobile element complement(17399..19953, 22509..22749)
/note="TREP1241 DNA transposon, TIR, CACTA, DTC_TAT2_231A16-1"

mobile element complement(22751..23280)
/note="TREP3276 Retrotransposon, LTR, unknown, RLX_Gujog_10k23-3"

LTR 23294..24251
/note="long terminal repeat of TREP3269 Retrotransposon, LTR, Gypsy, "RLG_WHAM_consensus-1"

mobile element 24252..32500
/note="TREP3269 Retrotransposon, LTR, Gypsy, RLG_WHAM_consensus-1"

LTR 29050..30121
/note="long terminal repeat of TREP3269 Retrotransposon, LTR, Gypsy, "RLG_WHAM_consensus-1"

mobile element 32545..32701
/note="TREP3173 Retrotransposon, LTR, Gypsy, RLG_Derami_AY368673-1"

TSD 33520..33524
/note=”target site duplication of TREP1527 Retrotransposon, LTR, Copia, "RLC_Inga_AY661558-2”

LTR 33525..35222
/note="long terminal repeat of TREP1527 Retrotransposon, LTR, Copia, RLC_Inga_AY661558-2”

mobile element 35223..44433
/note="TREP1527 Retrotransposon, LTR, Copia, "RLC_Inga_AY661558-2”

LTR 44434..46132
/note="long terminal repeat of TREP1527 Retrotransposon, LTR, Copia, RLC_Inga_AY661558-2”

TSD 46133..46137
/note=”target site duplication of TREP1527 Retrotransposon, LTR, Copia, RLC_Inga_AY661558-2”

mobile element 48715..50445
/note="TREP3173 Retrotransposon, LTR, Gypsy, RLG_Derami_AY368673-1"

CDS join(53952..54270, 54483..54724, 54815..54937, 55240..55356, 55461..55643, 55736..55877, 56030..56195, 56315..56333)
/gene
/product = “putative integral membrane protein DUF6 containing protein"
/translation=MASSVAPSSCALPLHPRVAAAAGPSCRVLLAFTAPRSAASVRRAGI
LAPLRCSPLEDPGATGREEGRKEEGDASKRVRGRPMWRRILFASKKTRSIMILN
ALTVIYASDIPVLKEVEALTEPAVFNMVRFVIAAIPFIPFVIRAFGDRRTRNGGLELG
LWVSLAYLAQAIGLITSEAGRASFIAAFTVIVVPLIDGIFGASIPMLTWFGAIVSIIGV
GLLECGGSPPCVGDVLNFLSAVFFGIHMLRTEQISRSTDKKKFMALLSFEVLVVA
FTSILWFLLKDVFAEVHDSSFESWTFGALWDSAASFPWIPALYTGVFSTGLCMW
AEMVAMAHVSATETAIVYGLEPVWGAAFAWFLLGERWDNAAWIGAALVLCGSL
TVQLFGSAPEKSQKVESCSGNTFESPLKRQDHLSLSAIPVDSSKNIGSQLERKDK
TL

mobile element join(64978..65210,65238..65413,65460..65511)
/note="TREP3189 Retrotransposon, LTR, Gypsy, RLG_Fatima_consensus-1"

mobile element complement(67476..69695)
/note="TREP1169 DNA transposon, TIR, HAT, DXX_George_AF521177-1"

CDS 77662..79527
/gene = “Rht-B1a”
/product = “DELLA protein”
/translation=MKREYQDAGGSGGGGGGMGSSEDKMMVSGSAAAGEGEEVDELL
AALGYKVRASDMADVAQKLEQLEMAMGMGGVGAGAAPDDSFATHLATDTVHY
NPTDLSSWVESMLSELNAPPPPLPPAPQLNASTSSTVTGGGYFDLPPSVDSSCS
TYALRPIPSPAVAPADLSADSVVRDPKRMRTGGSSTSSSSSSSSLGGGGARSSV
VEAAPPVAAAAGAPALPVVVVDTQEAGIRLVHALLACAEAVQQENFSAAEALVK
QIPLLAASQGGAMRKVAAYFGEALARRVFRFRPQPDSSLLDAAFADLLHAHFYE
SCPYLKFAHFTANQAILEAFAGCRRVHVVDFGIKQGMQWPALLQALALRPGGPP
SFRLTGVGPPQPDETDALQQVGWKLAQFAHTIRVDFQYRGLVAATLADLEPFML
QPEGEEDPNEEPEVIAVNSVFEMHRLLAQPGALEKVLGTVRAVRPRIVTVVEQE
ANHNSGTFLDRFTESLHYYSTMFDSLEGGSSGGPSEVSSGAAAAPAAAGTDQV
MSEVYLGRQICNVVACEGAERTERHETLGQWRNRLGNAGFETVHLGSNAYKQA
STLLALFAGGDGYKVEEKEGCLTLGWHTRPLIATSAWRLAAP

mobile element complement(83778..86302)
/note="TREP3162 Retrotransposon, LTR, Gypsy, RLG_BAGY2_consensus-1"

TSD complement(86302..86306)

/note=”target site duplication of TREP3198 Retrotransposon, LTR, Gypsy, RLG_Fatima_B_consensus-1"

LTR complement(86307..86776)
/note=”long terminal repeat of TREP3198 Retrotransposon, LTR, Gypsy, RLG_Fatima_B_consensus-1"

mobile element complement(86777..94987)
/note="TREP3198 Retrotransposon, LTR, Gypsy, RLG_Fatima_B_consensus-1"

LTR complement(94988..95457)
/note=”long terminal repeat of TREP3198 Retrotransposon, LTR, Gypsy, RLG_Fatima_B_consensus-1"

TSD complement(95458..95462)
/note=”target site duplication of TREP3198 Retrotransposon, LTR, Gypsy, RLG_Fatima_B_consensus-1"

mobile element complement(96203..101007)
/note="TREP3162 Retrotransposon, LTR, Gypsy, RLG_BAGY2_consensus-1”

mobile element 101008..105203
/note="TREP3174 Retrotransposon, LTR, Gypsy, RLG_Egug_EF067844-16"

TSD 105204..105208
/note=target site duplication of TREP3198 Retrotransposon, LTR, Gypsy, RLG_Fatima_B_consensus-1"

LTR 105209..105681
/note=long terminal repeat of TREP3198 Retrotransposon, LTR, Gypsy, RLG_Fatima_B_consensus-1"

mobile element 105682..113822
/note="TREP3198 Retrotransposon, LTR, Gypsy, RLG_Fatima_B_consensus-1"

LTR 113823..114295
/note=long terminal repeat of TREP3198 Retrotransposon, LTR, Gypsy, RLG_Fatima_B_consensus-1"

TSD 114296..114300
/note=target site duplication of TREP3198 Retrotransposon, LTR, Gypsy, RLG_Fatima_B_consensus-1"

mobile element complement(114924..116763)
/note="TREP3530 Retrotransposon, LTR, Copia, RLC_Angela_B_consensus-1"

mobile element 116802..117559
/note="TREP3239 Retrotransposon, LTR, Gypsy, RLG_Sabrina_consensus-1"

mobile element complement(117572..121727)
/note="TREP3530 Retrotransposon, LTR, Copia, RLC_Angela_B_consensus-1"

mobile element complement(122513..124825)
/note="TREP3202 Retrotransposon, LTR, Gypsy, RLG_Ifis_59e04-1"

mobile element 125058..128027
/note="TREP3026 DNA transposon, TIR, CACTA, DTC_Jorge_59e04-1"

misc feature complement(130587..130961)
/note= “similarity to EF396184.1: Triticum aestivum clone MML-002 HMW glutenin seed storage protein gene, promoter region"

Appendix III continued

mobile element complement(132413..133098)
/note="TREP2092 Retrotransposon, LTR, Copia, "RLC_TAR2_AY853252-1"

mobile element complement(134460..134642)
/note="TREP3445 DNA transposon, TIR, Harbinger, DTH_Kerberos_consensus-1"

mobile element complement(140571..141797)
/note="TREP3254 Retrotransposon, LTR, Gypsy, RLG_Sakura_10k23-4"

misc feature 141823..141824
/note: “estimated sequence gap of 500 bp or less”

mobile element 144536..148325
/note="TREP3026 DNA transposon, TIR, CACTA, DTC_Jorge_59e04-1"

LTR 148329..150041
/note="long terminal repeat ofTREP3161 Retrotransposon, LTR, Copia, "RLC_WIS_B_consensus-1"

mobile element 150042..155357
/note="TREP3161 Retrotransposon, LTR, Copia, RLC_WIS_B_consensus-1"

LTR 155358..157073
/note="long terminal repeat ofTREP3161 Retrotransposon, LTR, Copia, RLC_WIS_B_consensus-1"

mobile element 158258..160454
/note="TREP3026 DNA transposon, TIR, CACTA, DTC_Jorge_59e04-1"

mobile element complement(160627..161634)
/note="TREP1418 Retrotransposon, LTR, Gypsy, RLG_Ifis_AY368673-1"

mobile element complement(162142..163087)
/note="TREP3169 Retrotransposon, LTR, Gypsy, RLG_Carmilla_consensus-1”

mobile element complement(164294..164994)
/note="TREP1418 Retrotransposon, LTR, Gypsy, RLG_Ifis_AY368673-1"

mobile element 165214..166778
/note="TREP3254 Retrotransposon, LTR, Gypsy, RLG_Sakura_10k23-4"

mobile element complement(166779..171095)
/note="TREP3202 Retrotransposon, LTR, Gypsy, RLG_Ifis_59e04-1"

mobile element complement(179640..181027)
/note="TREP3202 Retrotransposon, LTR, Gypsy, RLG_Ifis_59e04-1"

mobile element complement(181409..183300)
/note="TREP3529 Retrotransposon, LTR, Copia, RLC_Angela_A_consensus-1"

mobile element 183301..184837
/note="TREP1438 Retrotransposon, LTR, Gypsy, RLG_Wilma_AY494981-2"

mobile element complement(184838..187310)
/note="TREP3529 Retrotransposon, LTR, Copia, RLC_Angela_A_consensus-1.

(D) DEFINITION: *Triticum aestivum* clone BAC 0155_I24 genomic sequence (213794 bp); ACCESSION: Chinese Spring *Rht-D1a*

mobile element 1..1989
/note="TREP3530 Retrotransposon, LTR, Copia, RLC_Angela_B_consensus-1"

misc_feature 3378..3490
/note="similarity to wEST CB307615 (HFIG600 Hessian fly infested cDNA library Triticum aestivum cDNA, mRNA sequence). Partial hit"

mobile element complement(4094..4810)
/note="TREP3067 DNA transposon, TIR, Mutator, DTM_Remus_consensus-1"

mobile element complement(5527..5681)
/note="TREP99 Retrotransposon, LTR, Gypsy, RLG_Cereba_AY040832-2"

CDS complement(6381..6459, 6557..6636, 6753..6833, 6944..7013, 7142..7216, 8483..8688, 9027..9276, 9354..9399, 9998..10067, 12454..12523, 13468..13552, 13643..13727, 13836..13926, 14545..14678)
/gene
/product = “putative zinc-finger protein gene”
/translation=MQRRRAQTWAGVGKTAQAAAAHAALFCFTLLLALRVDGRTDSSW
WIIFIPLWLFHGVAARGRFSMPAPSLPHGRHWAPCHSVVAAPLLIAFELLLCIYLE
SLRVKNHPAVDMKIVFLPLLTFEVIILVDNFRMCKALMPGDEESMSDEAIWETLPH
FWVAISMVFLIAATTFTLLKLSGDVGALGWWDLFINYGIAECFAFLVCTRWFNPMI
HRPPTHGEASSSSSAIRYRDWESGLVLPSLEDHEQERICGLPDIGGHLMKIPLVV
FQVLLCMRLEGTPPSARYIPIFALFSPLFILQGAGVLFSIGRLVEKLVLLLRNGPVS
PNYLTVSSKVRDCFAFLHHGSRLLGWWSIDEGSKEEQARLFYTESNGYNTFSG
YPPEVVKKMPKKDLAEEVWRLQAALGEQSEITKSTQQEYERLQNEKVLCRICYE
GEICMVILPCRHRTLCKSCAEKCKRCPICRNPIEERMAVYDV

mobile element complement(9595..9679)
/note="TREP3081 DNA transposon, TIR, Mariner, DTT_Athos_consensus-1"

mobile element complement(10410..10509)
/note="TREP3092 DNA transposon, TIR, Mariner, DTT_Icarus_consensus-1"

mobile element complement(12901..12991)
/note="TREP3100 DNA transposon, TIR, Mariner, DTT_Tantalos_42j2-1"

mobile element 16775..17025
/note="TREP1318 DNA transposon, TIR, CACTA, DTC_TAT1_AF459088-1"

mobile element complement(17197..18714)
/note="TREP3246 Retrotransposon, LTR, Gypsy, RLG_Sabrina_C_210J24-2

mobile element 18879..19283
/note="TREP3178 Retrotransposon, LTR, Gypsy, RLG_Erika_consensus-1"

gap 19776..19777
/note: “estimated sequence gap of 500 bp or less”

mobile element 20095..23277
/note="TREP3178 Retrotransposon, LTR, Gypsy, RLG_Erika_consensus-1."

mobile element complement(23283..23763)
/note="TREP3254 Retrotransposon, LTR, Gypsy, RLG_Sakura_10k23-4"

mobile element complement(23979..24584)

/note="TREP3269 Retrotransposon, LTR, Gypsy, RLG_WHAM_consensus-1"

mobile element complement(24744..25118)
/note="TREP3254 Retrotransposon, LTR, Gypsy, RLG_Sakura_10k23-4"

mobile element complement(25144..29355)
/note="TREP3246 Retrotransposon, LTR, Gypsy, RLG_Sabrina_C_210J24-2"

mobile element complement(29356..34389)
/note="TREP3530 Retrotransposon, LTR, Copia, RLC_Angela_B_consensus-1"

CDS join(40731..41061, 41431..41672, 41762..41884, 42195..42311, 42415..42597, 42693..42834, 42987..43152, 43275..43293)
/gene
/product = “putative integral membrane protein DUF6 containing protein"
/translation=MASSLAPASCALPLHPRVATAAAAAAGPSCRVLLAFTAPRSAASVR
RAGILAPLRCSPLEDPGATGREEGGKEKGGVSKRVHGRPMWRRILFASKKTRSI
MILNALTVIYASDIPVLKEVEALTEPAVFNMVRFVIAAIPFIPFVIRAFGDRRTRNGG
LELGVWVSLAYLAQAIGLITSEAGRASFIAAFTVIVVPLIDGIFGASIPMLTWFGAIV
SVIGVGLLECGGSPPCVGDVLNFLSAVFFGIHMLRTEQISRSTDKKKFMALLSFE
VLVVALTSIIWFLLKDAFVEVHDSSFESWTFGTLWDSAASFPWIPALYTGVFSTGL
CMWAEMVAMAHVSATETAIVYGLEPVWGATFAWFLLGERWDNAAWIGAALVLC
GSLTVQLFGSAPEKSQKVESRSGNTFESPLKRQERLSLSAIPVDSRKNIGSQLER
KDKTL

mobile element complement(41201..41363)
/note="TREP3107 DNA transposon, TIR, Mariner, DTT_Thalos_consensus-1; Stowaway"

mobile element 46153..49507
/note="TREP3322 Retrotransposon, LINE, unknown, RIX_Reina_EF540321-1"

mobile element complement(56211..57797)
/note="TREP3454 Retrotransposon, LINE, unknown, RIX_Karin_consensus-1"

mobile element complement(57798..60125)
/note="TREP1241 DNA transposon, TIR, CACTA, DTC_TAT2_231A16-1"

gap 61082..61083
/note: “estimated sequence gap of 500 bp or less”

mobile element complement(61496..63562)
/note="TREP3454 Retrotransposon, LINE, unknown, RIX_Karin_consensus-1"

TSD complement(64339..64343)
/note="target site duplication of TREP3160 Retrotransposon, LTR, Copia, RLC_WIS_A_consensus-1"

LTR complement(64344..66150)
/note="long terminal repeat of TREP3160 Retrotransposon, LTR, Copia, RLC_WIS_A_consensus-1"

mobile element complement(66151..67924)
/note="TREP3160 Retrotransposon, LTR, Copia, RLC_WIS_A_consensus-1"

LTR complement(67925..69464)
/note="long terminal repeat of TREP3223 Retrotransposon, LTR, Gypsy, RLG_Romani_10k23-3"

mobile element complement(69629..70930)
/note="TREP238 Retrotransposon, LINE, unknown, RIX_Isabelle_AF326781-1"

Appendix III continued

mobile element complement(70931..77539)
/note="TREP3223 Retrotransposon, LTR, Gypsy, RLG_Romani_10k23-3"

LTR complement(77540..79080)
/note="long terminal repeat of TREP3223 Retrotransposon, LTR, Gypsy, RLG_Romani_10k23-3"

mobile element complement(80254..83498)
/note="TREP3160 Retrotransposon, LTR, Copia, RLC_WIS_A_consensus-1"

LTR complement(83499..85284)
/note="long terminal repeat of TREP3160 Retrotransposon, LTR, Copia, RLC_WIS_A_consensus-1"

TSD complement(85285..85289)
/note="target site duplication of TREP3160 Retrotransposon, LTR, Copia, RLC_WIS_A_consensus-1"

mobile element 86879..86963
/note="TREP3081 DNA transposon, TIR, Mariner, DTT_Athos_consensus-1; Stowaway MITE "

mobile element complement(89479..89590)
/note="TREP3107 DNA transposon, TIR, Mariner, DTT_Thalos_consensus-1; Stowaway (162 total length)"

CDS 96445..98316
/gene = “Rht-D1a”
/product = “DELLA protein”
/translation=MKREYQDAGGSGGGGGGMGSSEDKMMVSAAAGEGEEVDELLAA
LGYKVRASDMADVAQKLEQLEMAMGMGGVGAGAAPDDSFATHLATDTVHYNP
TDLSSWVESMLSELNAPPPPLPPAPQLNASTSSTVTGSGGYFDLPPSVDSSSSI
YALRPIPSPAGATAPADLSADSVRDPKRMRTGGSSTSSSSSSSSLGGGARSSV
VEAAPPVAAAANATPALPVVVVDTQEAGIRLVHALLACAEAVQQENLSAAEALVK
QIPLLAASQGGAMRKVAAYFGEALARRVFRFRPQPDSSLLDAAFADLLHAHFYE
SCPYLKFAHFTANQAILEAFAGCRRVHVVDFGIKQGMQWPALLQALALRPGGPP
SFRLTGVGPPQPDETDALQQVGWKLAQFAHTIRVDFQYRGLVAATLADLEPFML
QPEGEEDPNEEPEVIAVNSVFEMHRLLAQPGALEKVLGTVRAVRPRIVTVVEQE
ANHNSGTFLDRFTESLHYYSTMFDSLEGGSSGGGPSEVSSGAAAAPAAAGTDQ
VMSEVYLGRQICNVVACEGAERTERHETLGQWRNRLGNAGFETVHLGSNAYKQ
ASTLLALFAGGDGYKVEEKEGCLTLGWHTRPLIATSAWRLAGP

mobile element 102395..103931
/note="TREP3173 Retrotransposon, LTR, Gypsy, RLG_Derami_AY368673-1"

mobile element complement(104930..108017)
/note="TREP3254 Retrotransposon, LTR, Gypsy, RLG_Sakura_10k23-4"

mobile element 108892..109755
/note="TREP3530 Retrotransposon, LTR, Copia, RLC_Angela_B_consensus-1"

mobile element 109820..112445
/note="TREP3026 DNA transposon, TIR, CACTA, DTC_Jorge_59e04-1"

mobile element 117082..118304
/note="TREP3245 Retrotransposon, LTR, Gypsy, RLG_Sabrina_B_AY368673-1"

gap 117199..117200
/note: “estimated sequence gap of 500 bp or less”

Appendix III continued

mobile element 120007..125187
/note="TREP766 DNA transposon, TIR, CACTA, DTC_Jorge_231A16-1"

mobile element 128763..142057
/note="TREP3169 Retrotransposon, LTR, Gypsy, RLG_Carmilla_consensus-1"

mobile element complement(143540..143957)
/note="TREP232 DNA transposon, TIR, Mutator, "DTM_Deimos_AF326781-1"

TSD 144835..144839
/note="target site duplication of TREP3254 Retrotransposon, LTR, Gypsy, RLG_Sakura_10k23-4"

LTR 144840..146387
/note="long terminal repeat of TREP3254 Retrotransposon, LTR, Gypsy, RLG_Sakura_10k23-4"

mobile element 146388..150379
/note="TREP3254 Retrotransposon, LTR, Gypsy, RLG_Sakura_10k23-4"

LTR 150380..151924
/note="long terminal repeat of TREP3254 Retrotransposon, LTR, Gypsy, RLG_Sakura_10k23-4"

TSD 151925..151929
/note="target site duplication of TREP3254 Retrotransposon, LTR, Gypsy, RLG_Sakura_10k23-4"

mobile element 152199..154346
/note="TREP766 DNA transposon, TIR, CACTA, DTC_Jorge_231A16-1"

mobile element 154347..164446
/note="TREP3529 Retrotransposon, LTR, Copia, RLC_Angela_A_consensus-1"

TSD complement(164447..164451)
/note="target site duplication of TREP2318 Retrotransposon, LTR, Gypsy, RLG_Nusif_AY494981-1"

LTR complement(164452..165358)
/note="long terminal repeat of TREP2318 Retrotransposon, LTR, Gypsy, RLG_Nusif_AY494981-1"

mobile element complement(165359..170921)
/note="TREP2318 Retrotransposon, LTR, Gypsy, RLG_Nusif_AY494981-1"

LTR complement(170922..171828)
/note="long terminal repeat of TREP2318 Retrotransposon, LTR, Gypsy, RLG_Nusif_AY494981-1"

TSD complement(171829..171833)
/note="target site duplication of TREP2318 Retrotransposon, LTR, Gypsy, RLG_Nusif_AY494981-1"

mobile element 172111..182151
/note="TREP3189 Retrotransposon, LTR, Gypsy, RLG_Fatima_consensus-1"

mobile element 182152..183849
/note="TREP3530 Retrotransposon, LTR, Copia, RLC_Angela_B_consensus-1"

mobile element 184035..185556
/note="TREP3250 Retrotransposon, LTR, Gypsy, RLG_Sabrina_D_115G1-2"

Appendix III continued

mobile element 185578..186361
/note="TREP3530 Retrotransposon, LTR, Copia, RLC_Angela_B_consensus-1"

mobile element 186885..191025
/note="TREP3250 Retrotransposon, LTR, Gypsy, RLG_Sabrina_D_115G1-2"

mobile element 191026..192740
/note="TREP3530 Retrotransposon, LTR, Copia, RLC_Angela_B_consensus-1"

mobile element 192907..194501
/note="TREP3250 Retrotransposon, LTR, Gypsy, RLG_Sabrina_D_115G1-2"

mobile element complement(194520..197112)
/note="TREP3269 Retrotransposon, LTR, Gypsy, RLG_WHAM_consensus-1"

mobile element 198781..200347
/note="TREP3250 Retrotransposon, LTR, Gypsy, RLG_Sabrina_D_115G1-2"

LTR 200367..200848
/note="long terminal repeat of TREP3254 Retrotransposon, LTR, Gypsy, RLG_Sakura_10k23-4"

mobile element 200849..206357
/note="TREP3254 Retrotransposon, LTR, Gypsy, RLG_Sakura_10k23-4"

LTR 206358..206839
/note="long terminal repeat of TREP3254 Retrotransposon, LTR, Gypsy, RLG_Sakura_10k23-4"

mobile element 207589..213064
/note="TREP3456 Retrotransposon, LTR, Copia, RLC_Barbara_consensus-1"

Appendix IV. *Poaceae* orthologues in the *Rht* region

Gene[a]	Genome[b]	Strand	Locus Name/Number[c]	Sequence location[d]	Start coord. relative *Rht-1*[e]	End coord. relative *Rht-1*	Exons (no.)	ORF (nt)[f]	ORF + Introns (nt)	Amino acids (no.)
Tb1	*B. distachyon*	plus	*Bradi1g11060*	Chr1:8129288..8130445	-56466	-55309	1	1158	1158	385
ZnF	*B. distachyon*	minus	*Bradi1g11070*	Chr1:8149832..8164731	-35921	-21022	14	1425	14900	474
DUF6	*B. distachyon*	plus	*Bradi1g11080*	Chr1:8168243..8171632	-17510	-14121	8	1320	3390	439
Rht-1	*B. distachyon*	plus	*Bradi1g11090*	Chr1:8185754..8187625	1	1872	1	1872	1872	623
Bradi1g11100	*B. distachyon*	plus	*Bradi1g11100*	Chr1:8201693..8203891	15939	18137	2	423	2199	140
Toc64	*B. distachyon*	minus	*Bradi1g11110*	Chr1:8215229..8225462	29475	39708	13	1767	10234	588
DUF6	*H. vulgare*		EST derived	NA	n/a	n/a	8	1308	n/a	435
Rht-1	*H. vulgare*		*AF460219 (SLN1)*	NA	n/a	n/a	1	1857	1857	618
ZnF	*H. vulgare*		EST derived	NA	n/a	n/a	14	1422	n/a	473
Tb1	*O. sativa*	plus	*Os03g49880*	Chr3:28422062..28423228	-83862	-82696	1	1167	1167	388
ZnF	*O. sativa*	minus	*Os03g49900*	Chr3:28449229..28442280	-63643	-56694	14	1422	6950	473
DUF6	*O. sativa*	plus	*Os03g49940*	Chr3:28463889..28466335	-42034	-39588	8	1365	2447	454
LOC_Os03g49960	*O. sativa*	minus	*Os03g49960*	Chr3:28475938..28467680	-29986	-38244	19	1620	8259	539
Rht-1	*O. sativa*	plus	*Os03g49990 (SLR1)*	Chr3:28505924..28507801	1	1878	1	1878	1878	625
Toc64	*O. sativa*	minus	*Os03g50010*	Chr3:28534130..28526495	20571	28206	13	1761	7636	586
Toc64	*S. bicolor*	plus	*Sb01g010650*	Chr1:9389669..9402483	-30546	-17732	13	1767	12815	588
Rht-1	*S. bicolor*	minus	*Sb01g010660*	Chr1:9420215..9422098	1	1884	1	1884	1884	627
DUF6	*S. bicolor*	minus	*Sb01g010670*	Chr1:9454427..9457360	34213	37146	8	1326	2934	441
ZnF	*S. bicolor*	plus	*Sb01g010680*	Chr1:9471685..9478938	51471	58724	14	1422	7254	473
Tb1	*S. bicolor*	minus	*Sb01g010690*	Chr1:9506057..9507199	85842	86984	1	1143	1143	380
ZnF	*T. urartu* A	minus	*ZnF*	BAC_T.urartu:19834..28865	-39576	-30545	14	1422	9032	473
DUF6	*T. urartu* A	plus	*DUF6*	BAC_T.urartu:42927..45500	-16483	-13910	8	1323	2574	440
Rht-1	*T. urartu* A	plus	*Rht-A1*	BAC_T.urartu:59411..61273	1	1863	1	1863	1863	620
ZnF	*T. aestivum* A (CS)	minus	*ZnF*	BAC_0224_M10:24659..34552	-55088	-45195	14	1422	9894	473
DUF6	*T. aestivum* A (CS)	plus	*DUF6*	BAC_0224_M10:48779..51352	-30968	-28395	8	1323	2574	440
Rht-1	*T. aestivum* A (CS)	plus	*Rht-A1*	BAC_0224_M10:79748..81610	1	1863	1	1863	1863	620
DUF6	*T. aestivum* B (CS)	plus	*DUF6*	BAC_1417_F16:53952..56333	-23709	-21328	8	1311	2382	436
Rht-1	*T. aestivum* B (CS)	plus	*Rht-B1*	BAC_1417_F16:77662..79527	1	1866	1	1866	1866	621

Appendix IV continued

Gene[a]	Genome[b]	Strand	Locus Name/Number[c]	Sequence location[d]	Start coord. relative *Rht-1*[e]	End coord. relative *Rht-1*	Exons (no.)	ORF (nt)[f]	ORF + Introns (nt)	Amino acids (no.)
ZnF	*T. aestivum* D (CS)	minus	*ZnF*	BAC_0155_I24:6381..14678	-90063	-81766	14	1422	8298	473
DUF6	*T. aestivum* D (CS)	plus	*DUF6*	BAC_0155_I24:40731..43293	-55713	-53151	8	1323	2563	440
Rht-1	*T. aestivum* D (CS)	plus	*Rht-D1*	BAC_0155_I24:96445..98316	1	1872	1	1872	1872	623
ZnF	*T. aestivum* D (Aibai/10*CS)	minus	*ZnF*	BAC_1J9:108486..116784	-90235	-81937	14	1422	8299	473
DUF6	*T. aestivum* D (Aibai/10*CS)	plus	*DUF6*	BAC_1J9:142474..145036	-56247	-53685	8	1323	2563	440
Rht-1	*T. aestivum* D (Aibai/10*CS)	plus	*Rht-D1*	BAC_1J9:198722..200593	1	1872	1	1872	1872	623
Tb1	*Z. mays* (B73)_chr1	minus	*AC233950.1_FG002*	Chr1:264847670..264848770	-351204	-350104	1	1101	1101	366
ZnF	*Z. mays* (B73)_chr1	minus	*GRMZM2G704032*	Chr1:265009869..265017820	-189004	-181053	14	1422	7952	473
DUF6	*Z. mays* (B73)_chr1	plus	*GRMZM2G093849*	Chr1:265182866..265186162	-16007	-12711	8	1344	3297	447
Rht-1	*Z. mays* (B73)_chr1	plus	*GRMZM2G144744 (D8)*	Chr1:265198874..265200766	1	1893	1	1893	1893	630
GRMZM2G086003	*Z. mays* (B73)_chr1	minus	*GRMZM2G086003*	Chr1:265398712..265402061	199838	203187	9	849	3350	282
GRMZM2G063355	*Z. mays* (B73)_chr5	minus	*GRMZM2G063355*	Chr5:11681303..11683108	-55634	-53829	5	702	1804	233
Rht-1	*Z. mays* (B73)_chr5	minus	*GRMZM2G024973 (D9)*	Chr5:11736937..11738814	1	1878	1	1878	1878	625
ZnF	*Z. mays* (B73)_chr5	plus	*GRMZM2G024690*	Chr5:11773272..11782144	36336	45208	14	1419	8873	472
Tb1	*Z. mays* (B73)_chr5	minus	*AC190734.2_FG003*	Chr5:11801898..11802977	64961	66040	1	1080	1080	359

[a] Orthologues of a given gene are grouped under a common gene name. *Rht* = *Reduced height*; *DUF6* = Domain of Unknown Function 6 family gene *ZnF* = Zinc finger family gene; *Tb1* = *Teosinte branched 1*; *Toc64* is a subunit of the pre-protein translocon of the outer envelope of chloroplasts. Other loci are named according to species' convention.

[b] For wheat species, genome from which the BAC sequence is derived is given after species name. For *T. aestivum*, clones were derived from Chinese Spring (CS) or Aibai/10*CS. *Z. mays* sequences are from the B73 inbred with chromosome number indicated

[c] *H. vulgare Rht-1* is from Genbank accession AF460219. *H. vulgare DUF6* is from the combined sequences of TA54310_4513 and GH226632, which do not cover the full length of the gene (~165 bp shortcoming). ORF, ORF+Introns, and amino acid numbers are estimated. *H. vulgare ZnF* is from combined sequences of TA38367_4513, TA38365_4513, and TA38366_4513. Orthologous gene names are given in parenthesis if available.

[d] Sequence location for BAC clones are relative to the beginning of the BAC sequence, otherwise chromosomal location is given if available

[e] Start and end coordinates are relative to the *Rht-1* orthologue.

[f] ORF - open reading frame

Appendix V. Marker scores of the 'SS7010073 x Paragon' F5 population.

Individual	*Xbarc 206-4A*	*Xgwm 397-4A*	*Xwmc 617-4A*	*Xwmc 48-4A*	*Xgwm 610-4A*	*Rht-A1*	*Xgpw 4545-4A*	*Xwmc 161-4A*	*Xwmc 89 -4A*	*Xbarc 70 -4A*	*Xwmc 420-4A*	*Xwmc 617-4A*	*Xgwm 44 -4A*	*Xwmc 497-4A*
1	P	-	P	P	P	P	P	P/S	-	S	S	P	S	S
2	P	-	P	P	P	P	P	P/S	-	-	-	P	S	P
3	S	S	S	S	S	S	S	S	P	P	-	S	P	P
4	S	S	S	S	S	S	S	S	S	P	P	P	P	S
5	P	S	S	S	S	S	S	S	P	P	S	S	P	S
6	P/S	S	S	S	S	S	S	S	P	P	P	S	S	P
7	P	-	S	S	S	S	S	S	P	-	P	S	P	P
8	P	-	P	P	P	-	-	P	P	P	S	P	P	P
9	P	S	S	S	S	S	-	S	P	P	P	S	S	S
10	P	S	S	S	S	S	S	S	S	P	P	S	S	S
11	P	P	P	P	P	P	S	P/S	P	S	S	P	P	P
12	P	P	P	P	P	P	P	P	P	P	S	P	S	S
13	P	S	S	S	S	S	S	S	P	P	S	S	P	S
14	S	S	S	S	S	S	S	S	S	P	P	S	P	P
15	P	P	P	P	P	P	P	P/S	P/S	P	S	P	P	P
16	P/S	S	S	P	P/S	P/S	P/S	-	P/S	S	P	S	S	P
17	-	-	P	P	P	P	-	P/S	P	P	S	P	P	P
18	-	S	S	P	P	P	P	P	P	P	S	S	S	P
19	-	S	S	S	S	S	S	S	P	-	P	S	S	S
20	-	S	S	S	S	S	S	S	P	-	P	S	P	P
21	-	P	P	P	P	P	P	P/S	P/S	P	S	P	S	P
22	-	S	S	S	S	S	S	S	S	P	P	S	S	S
23	-	-	P	P	P	P	P	P	P	S	S	P	S	P
24	-	S	S	P	P	P	P	P/S	P/S	P	S	S	S	P
25	P	S	S	S	S	S	S	S	-	P	-	S	S	P
26	P	S	P	P	P	P	P	P	-	-	P	P	P	P
27	P	P	P	P	P	P	P	P	P/S	-	P	P	S	P
28	P	-	S	P	P	P/S	P/S	S	P	P	P	S	P	S
29	P	P	P	P	P	P	P	P	P	-	P	P	S	P
30	P	P	P	P	P	P	P	P	P/S	-	P	P	S	S
31	P	S	S	S	S	S	S	S	S	-	S	S	P	P
32	P	P	P	P	P	P	P	P/S	P/S	P/S	S	P	S	S
33	S	S	S	S	S	S	S	S	P	P	S	S	S	P
34	S	S	S	P	S	S	P	P/S	P/S	S	P	S	P	P
35	P	P	P	P	P	P	P	-	P	-	S	P	S	S
36	P	-	P	P	P	P	P	P	P	-	-	P	S	S
37	P	P/S	S	S	S	S	-	S	S	P	P	S	S	S
38	S	S	S	S	S	S	S	S	P	-	P	S	S	P
39	P	S	P	P	P	P	P	P	P/S	P	P	P	S	P
40	P	-	P	P	P	P	P	P/S	P/S	P	-	P	P	P
41	P	P	P	P	P	P	P/S	P	-	P	P	P	S	P
42	S	S	S	S	S	S	S	S	P	P	S	S	S	S
43	P	S	S	S	S	S	S	S	P	P	P	S	S	P
44	P	S	S	S	S	S	S	S	P	P	S	S	P	S
45	-	P	P	P	P	P	P	-	P	P	S	P	P	S
46	P	P	P	P	P	P	P	P/S	P/S	-	P	P	P	P
47	S	-	S	P	P	P	S	S	P	P	-	S	S	P
48	P	P	P	-	P	P	P	P/S	P	P	S	P	P	S
49	P	-	P	P	P	P/S	P	P/S	-	-	-	S	S	P
50	P	S	S	S	S	S	S	S	S	-	S	S	P	P
51	P	-	P	P	P	P	P	P/S	P/S	P	P	P	S	P

Appendix V continued

	Marker[a]													
Individual	*Xbarc 206-4A*	*Xgwm 397-4A*	*Xwmc 617-4A*	*Xwmc 48-4A*	*Xgwm 610-4A*	*Rht-A1*	*Xgpw 4545-4A*	*Xwmc 161-4A*	*Xwmc 89 -4A*	*Xbarc 70 -4A*	*Xwmc 420-4A*	*Xwmc 617-4A*	*Xgwm 44 -4A*	*Xwmc 497-4A*
52	P	S	S	-	S	S	S	S	P	S	S	S	P	S
53	-	-	P	P	P	P	P/S	P/S	P/S	-	P	P	P	P
54	P	S	S	S	S	S	S	S	P	P	S	S	S	S
55	S	S	S	S	S	S	S	S	S	P/S	S	S	S	S
56	P	-	P	P	P	P	P	P/S	P/S	P	S	P	P	P
57	S	S	S	S	S	S	S	S	S	S	P	S	-	S
58	P	P	P	P	P/S	P/S	P/S	P/S	P/S	P	S	P	S	P
59	P	P	P	P	P	P	P	P	P	-	-	P	S	P
60	S	S	S	S	S	S	S	S	S	S	P	S	S	S
61	S	S	S	S	S	S	S	S	S	-	P	S	P	S
62	S	S	S	S	S	S	S	S	P	P	P	S	S	S
63	P	P	P	P	P	P	P	P/S	P	P	P	P	P	P
64	-	S	P	S	P	P	S	-	P	-	-	P	-	-
65	S	-	S	S	S	S	S	S	-	S	S	S	S	S
66	S	P	S	S	S	S	S	S	P	S	S	S	P	S
67	P	-	S	P	P	P	P	P	P	P	S	S	S	S
68	P	S	S	S	S	S	S	S	S	P	P	S	P	S
69	S	S	S	S	S	S	S	S	S	P	S	S	P	P
70	S	S	S	S	S	S	S	S	P	P	-	S	S	P
71	S	S	S	S	S	S	S	S	P	S	P	S	S	S
72	P	-	P	P	P	P	P	P	P	P	S	P	P	S
73	P	S	S	P	P/S	P/S	P	-	-	P	P	S	S	P
74	S	S	S	S	S	S	S	S	-	P	S	S	P	P
75	P	P	P	P	P	P	P	-	P	-	P	P	S	P
76	S	S	S	S	S	S	-	-	P	P	S	S	S	S
77	P	P	P	P	P	P	-	-	P/S	-	-	P	P	P/S
78	P	S	S	S	S	S	S	S	S	-	P	S	P	S
79	P	P	P	P	P	P	P	P/S	P	P	-	P	P	P
80	P	S	S	S	S	S	S	S	-	P	-	S	S	P/S
81	P	-	S	S	S	S	P	S	-	P/S	-	S	S	S
82	P	-	S	P	P/S	P/S	P	P/S	-	P	P	S	P	P
83	P	-	P	-	P	P	P	P/S	-	P/S	-	P	P	P
84	P	-	P	P	P	P	P	P	-	P	P	P	S	P
85	P	S	S	-	P	P	-	P	-	P/S	-	S	S	S
86	P/S	-	P	P	P	P	P	P	-	P	-	P	P	S
87	P	-	P	P	P	P	P	P	-	P	P	P	S	P
88	S	-	S	S	S	S	S	S	-	S	S	S	S	S
89	-	S	S	S	S	S	-	S	-	-	S	S	S	-
90	P	S	S	S	S	S	S	S	P	S	P	S	S	S
91	P	S	S	P	P	-	-	P	P	P	S	S	S	P
92	P	P	P	P	P	P	P	P/S	P	P	S	P	S	P
93	P	P	P	P	P	P	P	-	P	-	S	P	S	S
94	P	P	P	-	P	P	P/S	P	P	P	-	P	S	P
P	**58**	**23**	**41**	**47**	**47**	**43**	**39**	**20**	**45**	**52**	**38**	**41**	**37**	**50**
S	**21**	**46**	**53**	**42**	**43**	**43**	**40**	**43**	**14**	**13**	**38**	**53**	**55**	**40**
P/S	**3**	**1**	**0**	**0**	**4**	**6**	**6**	**22**	**16**	**5**	**0**	**0**	**0**	**2**
-	**12**	**24**	**0**	**5**	**0**	**2**	**9**	**9**	**19**	**24**	**18**	**0**	**2**	**2**

[a] P = Paragon allele (yellow background); S = SS7010073 allele (blue background); P/S = Paragon/SS7010073 heterozygote (grey background); the (-) in white background represents a result that was not scored. A count summary is shown in bold font at the bottom.

Appendix VI. Summary of INRA BWCC phenotype and genotype results

INRA BWCC accessions[a]							Genotype Results[b]			Phenotype Results[c]		
INRA BWCC number	Accession name	Origin[d]	Growth habit[e]	Year of reg.[f]	Type	Sowing Date	Rht-B1[g]	Rht-D1[h]	Ppd-D1[i]	Plant height (cm)	Days to heading[j]	GA trt diff (mm)
INRA_00007	(95-13*BEZOSTAIA)3-3	FRA	W	NA	fixed	Nov-08	B1a_197	D1a	D1a	104.5	139	18.1
INRA_00019	CH01193	CHE	W	NA	fixed	Nov-08	B1b	D1a	D1b	127	143	3.8
INRA_00092	11IWSWSN14	USA	W	NA	fixed	Nov-08	B1a_197	D1a	D1b	134	140	33.7
INRA_00177	DI15	FRA	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	162	146	28.7
INRA_00234	DI182-9	FRA	W	NA	fixed	Nov-08	B1a_160	D1a	D1b	137.5	144	35.2
INRA_00236	DI185	FRA	W	NA	fixed	Nov-08	B1a_0	D1a	D1a	127.5	133	32.3
INRA_00338	DI276	FRA	W	NA	fixed	Nov-08	B1a_0	D1a	D1a	120	123	16.2
INRA_00347	2838-39	BGR	W	NA	fixed	Nov-08	B1a_0	D1a	D1a	118	132	16.3
INRA_00386	DI330	FRA	W	NA	fixed	Nov-08	B1a_0	D1a	D1a	129	134	22.0
INRA_00419	DI37-12-2	FRA	W	NA	fixed	Nov-08	B1a_160	D1a	D1b	127	141	25.0
INRA_00421	3716-1	BGR	W	NA	fixed	Nov-08	B1a_0	D1a	D1a	114.5	136	30.5
INRA_00477	DI50-12	FRA	W	NA	fixed	Nov-08	B1a_160	D1a	D1a	130.5	141	24.3
INRA_00514	6-1-3	EGY	S	NA	fixed	Feb-09	B1a_0	D1a	D1b	135	138	19.8
INRA_00524	60293	NLD	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	123	140	32.3
INRA_00537	CH62022	CHE	W	NA	fixed	Nov-08	B1a_160	D1a	D1b	113.5	144	19.6
INRA_00546	664-258-18	BGR	W	NA	fixed	Nov-08	B1b	D1a	D1a	102	126	12.2
INRA_00748	A.4	AFG	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	160	131	25.7
INRA_00776	ACADEMIE DE PEKIN	CHN	F	NA	fixed	Feb-09	B1b	D1a	D1a	93	135	4.9
INRA_00794	ADMONTER	AUT	W	after 1934	fixed	Nov-08	B1a_0	D1a	D1b	172	144	33.5
INRA_00797	ADULAR	DEU	W	1986	fixed	Nov-08	B1a_197	D1a	D1b	118.5	144	25.3
INRA_00800	AFRICA MAYO	KEN	S	1960	fixed	Feb-09	B1a_0	D1a	D1b	123	140	24.3
INRA_00822	AIFENG-4	CHN	W	1971	fixed	Nov-08	B1a_197	D1b	D1a	86	119	13.9
INRA_00833	AKADARUMA	JPN	W	1924	fixed	Nov-08	B1a_0	D1a	D1a	125	133	47.5
INRA_00871	ALMA	FRA	W	1942	fixed	Nov-08	B1a_0	D1a	D1b	137.5	144	21.7
INRA_00901	AMIFORT	FRA	W	1991	fixed	Nov-08	B1a_160/197 het	D1b	D1a	89.5	134	0.6
INRA_00912	ANDES-56	COL	S	1956	fixed	Feb-09	B1a_0	D1a	D1a	121	139	40.5

Appendix VI continued

INRA BWCC accessions[a]							Genotype Results[b]			Phenotype Results[c]		
INRA BWCC number	Accession name	Origin[d]	Growth habit[e]	Year of reg.[f]	Type	Sowing Date	Rht-B1[g]	Rht-D1[h]	Ppd-D1[i]	Plant height (cm)	Days to heading[j]	GA trt diff (mm)
INRA_00933	APEX	GBR	S	1972	fixed	Feb-09	B1a_160	D1a	D1b	91.5	169	36.4
INRA_00957	ARAWA	NZL	W	1955	fixed	Nov-08	B1a_197	D1a	D1b	137	138	27.3
INRA_00964	ARCHE	FRA	W	1989	fixed	Nov-08	B1a_197	D1a	D1b	97.5	138	18.1
INRA_00983	ARGENT	GBR	W	1979	fixed	Nov-08	B1a_197	D1b	D1b	92	149	0.7
INRA_01005	ARKAS	DEU	S	1976	fixed	Nov-08	B1a_0	D1a	D1b	112	135	29.7
INRA_01032	ARROMANCHES	FRA	W	1962	fixed	Nov-08	B1a_197	D1a	D1b	119	144	36.0
INRA_01044	ARTOIS-DESPREZ	FRA	W	1957	fixed	Nov-08	B1a_0	D1a	D1b	121	145	29.2
INRA_01065	ATAKEDA-KOMUGI	JPN	W	NA	fixed	Nov-08	B1a_0	D1a	D1a	130	120	21.6
INRA_01080	ATUT-II	AUT	W	1974	fixed	Nov-08	B1a_0	D1a	D1b	119.5	146	23.0
INRA_01110	AURORE	AUS	S	1914	fixed	Feb-09	B1a_0	D1a	D1b	147	150	33.3
INRA_01113	AUSTRO BANKUT	AUT	S	1919	fixed	Feb-09	B1a_0	D1a	D1b	163	156	40.7
INRA_01177	BAHATANE-87	DZA	S	NA	fixed	Feb-09	B1a_0	D1a	D1b	148	147	16.2
INRA_01182	BAIONETTE I	ITA	W	after 1900	fixed	Nov-08	B1a_0	D1a	D1b	188.5	143	28.0
INRA_01192	BALKAN	YUG	W	1979	fixed	Nov-08	B1a_0	D1a	D1a	107	134	36.2
INRA_01217	BARANI-70	PAK	S	1970	fixed	Feb-09	B1a_0	D1b	D1a	97.5	141	4.0
INRA_01232	BARBU DU FINISTERE	FRA	W	NA	landrace	Nov-08	B1a_160	D1a	D1b	171.5	142	24.7
INRA_01236	BARBU DU TRONCHET	CHE	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	200.5	148	31.5
INRA_01244	BASS	AUS	S	1983	fixed	Feb-09	B1b	D1a	D1a	90.5	140	6.5
INRA_01249	BAULMES	CHE	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	181	149	37.5
INRA_01281	BEL ET BON	FRA	W	NA	fixed	Nov-08	B1a_197	D1a	D1b	114	149	23.0
INRA_01288	BELLYEI-590	HUN	W	NA	fixed	Nov-08	B1a_160	D1a	D1b	179	139	17.5
INRA_01313	BENCHUNG	NPL	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	153	124	36.3
INRA_01321	BENNI	USA	W	1980	fixed	Nov-08	B1a_197	D1a	D1a	136	130	29.3
INRA_01332	BERZATACA	FIN	W	NA	fixed	Nov-08	B1a_160	D1a	D1b	169.5	141	43.7
INRA_01357	BIRGITTA	SWE	W	1960	fixed	Nov-08	B1a_160	D1a	D1b	160.5	148	19.7
INRA_01400	BLANC PRECOCE	FRA	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	162	145	34.2

Appendix VI continued

INRA BWCC accessions[a]							Genotype Results[b]			Phenotype Results[c]		
INRA BWCC number	Accession name	Origin[d]	Growth habit[e]	Year of reg.[f]	Type	Sowing Date	Rht-B1[g]	Rht-D1[h]	Ppd-D1[i]	Plant height (cm)	Days to heading[j]	GA trt diff (mm)
INRA_01402	BLASON	FRA	W	1976	fixed	Nov-08	B1a_160	D1a	D1a	105	142	31.3
INRA_01417	BLE D'OR	FRA	W	NA	landrace	Nov-08	B1a_160	D1a	D1b	124	146	20.3
INRA_01422	BLE DE CRETE	FRA	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	171	144	39.0
INRA_01429	BLE DE HAIE	FRA	W	NA	landrace	Nov-08	B1a_160	D1a	D1b	165	151	20.0
INRA_01446	BLE DE MARAT BARBU	FRA	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	176	147	24.3
INRA_01498	BLE DU ROUSSILLON	FRA	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	176	137	24.3
INRA_01529	BLONDYNKA	POL	W	1920	fixed	Nov-08	B1a_0	D1a	D1b	180	153	29.3
INRA_01531	BLUEBOY	USA	W	1964	fixed	Nov-08	B1b	D1a	D1b	110	141	5.8
INRA_01542	BODELLIN	AUS	S	NA	fixed	Feb-09	B1a_0/197 het	D1a	D1a	105	141	29.2
INRA_01643	BT2277	TUN	S	after 1962	fixed	Feb-09	B1a/b het	D1a/b het	D1a	118	139	27.3
INRA_01647	BT2281	TUN	S	after 1963	fixed	Feb-09	B1a_0	D1b	D1a	99.5	139	10.2
INRA_01655	BT2296	TUN	W	after 1964	fixed	Nov-08	B1a_0	D1b	D1a	110	120	-1.7
INRA_01660	BUCK ATLANTICO	ARG	S	1952	fixed	Feb-09	B1a_0	D1a	D1b	130	145	46.3
INRA_01676	BUCKBUCK'S'	MEX	S	1980	fixed	Feb-09	B1a_0	D1b	D1a	94	142	5.0
INRA_01696	BUNG EPI BARIOLE	NPL	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	157	129	41.0
INRA_01697	BUNG EPI BLANC	NPL	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	157	136	50.3
INRA_01747	114/62	AUT	W	NA	fixed	Nov-08	B1b	D1a	D1b	106.5	145	10.2
INRA_01768	CANDEAL DE AREVALO	ESP	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	161.5	142	28.0
INRA_01774	CANUCK	CAN	S	1974	fixed	Feb-09	B1a_0	D1a	D1b	148.5	144	32.1
INRA_01885	CENAD 512	ROM	W	1958	fixed	Nov-08	B1a_0	D1a	D1b	156	137	27.0
INRA_01899	CEREALOR	FRA	W	1964	fixed	Nov-08	B1a_197	D1a	D1b	109	138	24.3
INRA_01957	CF3003-2-7-4-4-3	FRA	W	NA	fixed	Nov-08	B1a_197	D1b	D1b	113.5	141	-1.3
INRA_01974	CF4563-1-5-3-2-5	FRA	W	NA	fixed	Nov-08	B1b	D1a	D1b	94	135	2.2
INRA_02025	CH73052	CHE	W	NA	fixed	Nov-08	B1a_197	D1a	D1b	123	145	28.7
INRA_02072	CHANATE	MEX	S	1971	fixed	Feb-09	B1a_0	D1b	D1a	99	138	0.7
INRA_02135	CHINESE SPRING	CHN	S	NA	landrace	Feb-09	B1a_0	D1a	D1b	136	145	38.5

Appendix VI continued

INRA BWCC accessions[a]							Genotype Results[b]			Phenotype Results[c]		
INRA BWCC number	Accession name	Origin[d]	Growth habit[e]	Year of reg.[f]	Type	Sowing Date	Rht-B1[g]	Rht-D1[h]	Ppd-D1[i]	Plant height (cm)	Days to heading[j]	GA trt diff (mm)
INRA_02141	CHIRKUNG	NPL	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	156	128	32.5
INRA_02145	CHITLANG	NPL	W	NA	fixed	Nov-08	B1a_0	D1a	D1b/b het	148.5	127	47.0
INRA_02153	CHORTANDINKA	RUS	W	NA	fixed	Nov-08	B1a_160	D1a	D1b	145	150	22.8
INRA_02169	CHYAKSILA EPI NON VELU	NPL	W	NA	landrace	Nov-08	B1a_160	D1a	D1b	139.5	137	39.8
INRA_02171	CHYAMTANG	NPL	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	154	130	38.2
INRA_02289	COLLEGE ECLIPSE	AUS	S	1906	fixed	Feb-09	B1a_0	D1a	D1b	140	135	29.7
INRA_02301	COMET	AUS	S	1986	fixed	Feb-09	B1b	D1a	D1b	96	142	8.3
INRA_02308	COMPTON	USA	W	1983	fixed	Nov-08	B1a_0	D1b	D1b	122	138	2.4
INRA_02330	COPPADRA	TUR	S	NA	fixed	Feb-09	B1a_0	D1a	D1b	156.5	137	28.3
INRA_02337	CORIN	FRA	W	1976	fixed	Nov-08	B1a_197	D1a	D1a	94.5	136	29.3
INRA_02345	CORSODOR	FRA	W	1987	fixed	Nov-08	B1b	D1a	D1b	102	146	14.6
INRA_02353	COTIPORA	BRA	S	1965	fixed	Feb-09	B1a_197	D1a	D1a	164.5	150	34.7
INRA_02358	COURTOT	FRA	W	1974	fixed	Nov-08	B1b	D1b	D1b	75	137	-14.7
INRA_02364	CP4	FRA	W	NA	fixed	Nov-08	B1a_197	D1a	D1b	131	145	41.3
INRA_02399	D130-63	POL	W	NA	fixed	Nov-08	B1a_160	D1a	D1b	175	149	23.7
INRA_02411	DAERAAD	ZAF	S	1958	fixed	Feb-09	B1a_197	D1a	D1b	130	142	33.5
INRA_02424	DANUBIA	CZE	W	1984	fixed	Nov-08	B1b	D1a	D1a	95.5	145	15.5
INRA_02438	DAVIDOC	FRA	W	1986	fixed	Nov-08	B1a_160	D1a	D1a	111.5	137	42.0
INRA_02444	DAYKU	NPL	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	155.5	131	28.0
INRA_02475	DETENICKA CERVENA	CZE	W	1937	fixed	Nov-08	B1a_160	D1a	D1b	188.5	146	28.0
INRA_02481	DH8701	FRA	W	NA	fixed	Nov-08	B1a_160	D1a	D1a	101	138	27.2
INRA_02485	DHOJE	NPL	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	141	125	32.8
INRA_02489	DI6402-34-2-4	FRA	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	107.5	149	29.3
INRA_02491	DI6404-19-15	FRA	W	NA	fixed	Nov-08	B1a_197	D1a	D1a	112.5	137	21.3
INRA_02507	DI7003-1-12	FRA	W	NA	fixed	Nov-08	B1a_160	D1a	D1a	112	139	22.3
INRA_02508	DI7005-113-3	FRA	W	NA	fixed	Nov-08	B1b	D1b	D1b	81.5	141	6.3

Appendix VI continued

INRA BWCC accessions[a]							Genotype Results[b]			Phenotype Results[c]		
INRA BWCC number	Accession name	Origin[d]	Growth habit[e]	Year of reg.[f]	Type	Sowing Date	Rht-B1[g]	Rht-D1[h]	Ppd-D1[i]	Plant height (cm)	Days to heading[j]	GA trt diff (mm)
INRA_02526	DI7202-103	FRA	W	NA	fixed	Nov-08	B1b	D1b	D1b	71	144	0.7
INRA_02534	DI7210-15-11	FRA	W	NA	fixed	Nov-08	B1b	D1a	D1b	78.5	152	4.3
INRA_02536	DI7215-100	FRA	W	NA	fixed	Nov-08	B1a_0	D1a	D1a	118.5	140	34.3
INRA_02573	DIANA	POL	W	1966	fixed	Nov-08	B1a_160	D1a	D1b	146.5	151	27.7
INRA_02574	DIANA II	CZE	W	1967	fixed	Nov-08	B1a_197	D1a	D1b	123	145	21.0
INRA_02606	DNEPROVSKAIA ?	UKR	W	1980	fixed	Nov-08	B1a_0	D1a	D1a	129	135	40.2
INRA_02626	DONG FANG HONG NO.3	CHN	W	1979	fixed	Nov-08	B1a_0	D1a	D1b	130.5	127	35.3
INRA_02644	DRAGON	FRA	F	1967	fixed	Nov-08	B1a_197	D1a	D1b	123	146	17.8
INRA_02650	DRAVA	HRV	W	1980	fixed	Nov-08	B1a_160	D1a	D1a	96.5	134	39.5
INRA_02683	E108	FRA	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	166.5	142	54.3
INRA_02698	EBRO	ESP	W	1966	fixed	Nov-08	B1a_0	D1a	D1b	172	142	25.0
INRA_02709	EGYPCIO 208	ARG	S	NA	fixed	Feb-09	B1a_160	D1a	D1b	146.5	148	32.3
INRA_02759	EMU'S'	MEX	S	1979	fixed	Feb-09	B1a_0	D1b	D1a	97.5	141	3.0
INRA_02802	ESPOIR	FRA	W	1950	fixed	Nov-08	B1a_160	D1a	D1b	125	150	32.3
INRA_02810	ESTANZUELA-DORADO	URY	S	1981	fixed	Feb-09	B1a_0	D1a	D1b	136.5	144	34.5
INRA_02991	FERRUGINEUM	RUS	W	NA	fixed	Nov-08	B1a_160	D1a	D1b	178	149	28.2
INRA_03050	FLAMURA-85	ROM	W	1984	fixed	Nov-08	B1b	D1a	D1a	113.5	130	13.6
INRA_03070	FLINT	USA	W	1830	fixed	Nov-08	B1a_160	D1a	D1b	153	140	28.3
INRA_03165	FRONTANA 3671	BRA	S	NA	fixed	Feb-09	B1a_0	D1a	D1b	131.5	137	24.5
INRA_03170	FRONTHATCH	USA	S	1963	fixed	Feb-09	B1a_0	D1a	D1b	139.5	151	41.0
INRA_03176	FUKUHOKOMUGI	JPN	S	1979	fixed	Feb-09	B1b	D1a	D1a	91.5	134	14.8
INRA_03213	G66257	GRC	S	NA	fixed	Feb-09	B1a_0	D1a	D1a	153.5	139	29.7
INRA_03218	G7	NPL	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	170	138	32.3
INRA_03220	G72300	GRC	S	NA	fixed	Feb-09	B1a_0	D1a	D1a	146	141	21.3
INRA_03256	GAMENYA	AUS	S	1958	fixed	Feb-09	B1a_0	D1a	D1b	112.5	139	37.3
INRA_03267	GAU	NPL	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	157.5	133	43.5

Appendix VI continued

INRA BWCC accessions[a]							Genotype Results[b]			Phenotype Results[c]		
INRA BWCC number	Accession name	Origin[d]	Growth habit[e]	Year of reg.[f]	Type	Sowing Date	Rht-B1[g]	Rht-D1[h]	Ppd-D1[i]	Plant height (cm)	Days to heading[j]	GA trt diff (mm)
INRA_03278	GELPA	FRA	W	1967	fixed	Nov-08	B1a_0	D1a	D1b	104.5	148	18.6
INRA_03299	GH126	FRA	W	NA	fixed	Nov-08	B1a_0	D1a	D1a	118	137	17.7
INRA_03306	GHURKA	AUS	S	1924	fixed	Feb-09	B1a_0	D1a	D1a	113.5	153	22.7
INRA_03342	GK-SZÖKE	HUN	W	1985	fixed	Nov-08	B1b	D1a	D1a	108	138	3.3
INRA_03358	GLENLEA	CAN	S	1972	fixed	Feb-09	B1a_0	D1a	D1b	140	142	25.3
INRA_03366	GODOLLOI 15	HUN	W	1957	fixed	Nov-08	B1a_160	D1a	D1b	146.5	126	35.3
INRA_03402	GRANAROLO	ITA	F	1973	fixed	Nov-08	B1b	D1a	D1a	88	125	3.5
INRA_03406	GRANIT	RUS	W	1978	fixed	Nov-08	B1a_0	D1a	D1b	119	142	23.5
INRA_03414	GRENIER	FRA	W	1992	fixed	Nov-08	B1b	D1a	D1b	98.5	141	8.3
INRA_03437	GUDEL	NPL	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	153	128	28.7
INRA_03442	GULAR	AUS	S	1927	fixed	Feb-09	B1a_0	D1a	D1b	126.5	138	30.4
INRA_03463	H742A	ISR	S	NA	fixed	Feb-09	B1b	D1b	D1a	59	140	1.7
INRA_03485	H93-70	ESP	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	171.5	138	24.3
INRA_03617	HIVERNAL	FRA	W	1961	fixed	Nov-08	B1a_197	D1a	D1b	108	148	17.8
INRA_03645	HONGGAOAN	NPL	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	149.5	128	29.0
INRA_03665	HOROSHIRI KOMUGI	JPN	W	1974	fixed	Nov-08	B1a_0	D1b	D1a	110	136	10.0
INRA_03696	HYBRIDE 56 VILMORIN	FRA	S	1956	fixed	Feb-09	B1a_0	D1a	D1b	152	145	37.3
INRA_03752	IAR W83-2	ETH	W	NA	fixed	Nov-08	B1a_0	D1a	NP	143	134	34.7
INRA_03753	IAS 1	BRA	S	1955	fixed	Feb-09	B1a_0	D1a	D1b	145.5	145	39.2
INRA_03804	ICARDA 079	SYR	W	NA	fixed	Nov-08	B1b	D1a	D1a	95	133	4.4
INRA_03857	INIA-19	MEX	S	NA	fixed	Feb-09	B1b	D1b	D1a	66	146	4.3
INRA_03896	JANGO	FRA	W	NA	fixed	Nov-08	B1a_197	D1a	D1a	100.5	131	27.2
INRA_03912	JASZAJI TF	HUN	W	1973	fixed	Nov-08	B1a_160	D1a	D1b	175	142	36.3
INRA_03942	JO3045	FIN	S	NA	fixed	Feb-09	B1a_0	D1a	D1b	145.5	146	23.2
INRA_03965	JUBING	NPL	W	NA	fixed	Nov-08	B1a_0	D1a	D1a	151.5	133	25.3
INRA_03970	JUFY II	BEL	S	1954	fixed	Feb-09	B1a_160	D1a	D1b	127	151	29.7

Appendix VI continued

INRA BWCC accessions[a]							Genotype Results[b]			Phenotype Results[c]		
INRA BWCC number	Accession name	Origin[d]	Growth habit[e]	Year of reg.[f]	Type	Sowing Date	Rht-B1[g]	Rht-D1[h]	Ppd-D1[i]	Plant height (cm)	Days to heading[j]	GA trt diff (mm)
INRA_03975	JUNBESI	NPL	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	162	133	20.0
INRA_03991	K1898-9/L200-6	BGR	W	NA	fixed	Nov-08	B1a_0	D1a	D1a	125.5	142	37.0
INRA_04036	KATYIL	AUS	S	1982	fixed	Nov-08	B1a_0	D1a	D1b	128.5	137	19.3
INRA_04055	KE HAN NO.8	CHN	S	NA	fixed	Feb-09	B1a_0	D1a	D1b	141	147	26.3
INRA_04067	KENYA 350	KEN	S	1951	fixed	Feb-09	B1a_0	D1a	D1b	130	138	15.2
INRA_04105	KID	FRA	W	after 1946	fixed	Nov-08	B1a_160	D1a	D1b	97.5	144	23.5
INRA_04111	KIRAC 66	TUR	W	1970	fixed	Nov-08	B1a_0	D1a	D1b	139.5	131	40.7
INRA_04157	KOLBEN 3	SWE	W	1929	fixed	Nov-08	B1a_0	D1a	D1b	151	128	34.5
INRA_04187	KRAKA	DNK	W	1981	fixed	Nov-08	B1a_0	D1a	D1b	125	149	22.3
INRA_04194	KRELOF 3	FRA	W	NA	fixed	Nov-08	B1a_160	D1a	D1b	174.5	150	24.3
INRA_04207	KULUNG	NPL	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	157.5	128	21.0
INRA_04300	LESCZYNSKA WEZESNA	POL	W	1937	fixed	Nov-08	B1a_160	D1a	D1b	171	147	33.5
INRA_04324	LITTLE CLUB	USA	S	1878	fixed	Nov-08	B1a_197	D1a	D1b	158	141	31.0
INRA_04343	LONTOI	FIN	W	1971	fixed	Nov-08	B1a_0	D1a	D1b	171	139	34.0
INRA_04477	M45/66	ARG	S	NA	fixed	Feb-09	B1a_0	D1a	D1b	112.5	144	24.5
INRA_04482	M708//G25/N163	ISR	S	NA	fixed	Feb-09	B1a_0	D1a	D1b	139	134	22.4
INRA_04487	MACHA	JPN	S	NA	fixed	Feb-09	B1a_0	D1a	D1b	164	158	49.7
INRA_04492	MADIN PATEGA BARBU	NPL	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	146	129	28.5
INRA_04525	MALGORZATKA UDYCKA	POL	W	1958	fixed	Nov-08	B1a_160	D1a	D1b	169.5	147	21.8
INRA_04645	MARS DE SUEDE ROUGE BARBU	FRA	S	1922	fixed	Feb-09	B1a_0	D1a	D1b	183	154	33.3
INRA_04664	MASTER	GBR	W	1983	fixed	Nov-08	B1a_0/160 het	D1b	D1b	109	143	-2.9
INRA_04670	MATRADERECSKEI TF	HUN	W	1973	fixed	Nov-08	B1a_0	D1a	D1b	182.5	145	14.5
INRA_04698	MEDRA/AURORA//HOHEN TURM/DINA	POL	W	NA	fixed	Nov-08	B1a_197	D1a	D1a	122.5	144	14.2
INRA_04702	MEIRA	ESP	S	NA	fixed	Feb-09	B1a_0/197 het	D1a	D1b	154.5	153	33.0

Appendix VI continued

INRA BWCC accessions[a]							Genotype Results[b]			Phenotype Results[c]		
INRA BWCC number	Accession name	Origin[d]	Growth habit[e]	Year of reg.[f]	Type	Sowing Date	Rht-B1[g]	Rht-D1[h]	Ppd-D1[i]	Plant height (cm)	Days to heading[j]	GA trt diff (mm)
INRA_04706	MELCHIOR	NLD	S	1974	fixed	Feb-09	B1a_0	D1a	D1b	103.5	151	25.5
INRA_04712	MENFLO	ITA	S	1955	fixed	Feb-09	B1a_0	D1a	D1b	139.5	145	21.2
INRA_04776	MEXIQUE 58	MEX	S	1974	fixed	Feb-09	B1a_0	D1b	D1a	104	139	-1.2
INRA_04784	MEXIQUE 9	MEX	S	NA	fixed	Feb-09	B1b	D1a	D1b	109	141	12.0
INRA_04796	MICHE	FRA	W	1954	fixed	Nov-08	B1a_160	D1a	D1b	121	145	22.8
INRA_04838	MINTURK	USA	W	1919	fixed	Nov-08	B1a_0	D1a	D1b	164.5	144	38.3
INRA_04874	MISKAAGANI	LBN	S	NA	fixed	Feb-09	B1a_0	D1a	D1b	140.5	144	23.7
INRA_04901	MOCHO DE ESPIGA BIANCA	PRT	S	1928	fixed	Feb-09	B1a_197	D1a	D1b	135	140	38.3
INRA_04925	MONJO	NPL	W	NA	fixed	Nov-08	B1a_0	D1a	D1a	138	120	26.8
INRA_04947	MOTTIN	FRA	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	173	142	37.8
INRA_04991	MV-MA	HUN	W	1982	fixed	Nov-08	B1b	D1a	D1b	81	143	4.0
INRA_05088	N46	ISR	S	NA	fixed	Feb-09	B1b	D1b	D1a	66	141	1.2
INRA_05096	N67M2	ISR	S	NA	fixed	Feb-09	B1b	D1b	D1a	54	140	1.5
INRA_05102	NABU EPI BLANC	NPL	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	157	132	20.5
INRA_05108	NACHIPUNDO	NPL	S	NA	fixed	Feb-09	B1a_0	D1a	D1b	146	157	24.3
INRA_05115	NANG NANO 140	ESP	W	NA	fixed	Nov-08	B1a_0	D1a	D1a	125.5	122	36.7
INRA_05116	NANKING NO.25	CHN	S	NA	fixed	Feb-09	B1a_0	D1a	D1b	142.5	144	41.8
INRA_05120	NAPHAL	IND	W	1978	fixed	Nov-08	B1a_160	D1a	D1a	117.5	133	27.3
INRA_05166	NEPAL 84	NPL	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	159.5	132	25.3
INRA_05167	NEPAL 89	NPL	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	168	135	22.0
INRA_05219	NONG DA NO.141	CHN	F	1982	fixed	Nov-08	B1a_0	D1a	D1a	117	124	15.7
INRA_05250	NORIN 29	JPN	S	1949	fixed	Feb-09	B1a_160	D1a/b het	D1a	132.5	137	12.3
INRA_05260	NORIN 60	JPN	S	1965	fixed	Feb-09	B1a_0	D1b	D1a	100	141	1.7
INRA_05266	NORIN 64	JPN	S	after 1983	fixed	Feb-09	B1a/b het	D1a/b het	D1a	99	134	11.2
INRA_05293	NOUGAT	FRA	W	1985	fixed	Nov-08	B1a_160	D1a	D1b	98.5	133	32.2

Appendix VI continued

INRA BWCC accessions[a]							Genotype Results[b]			Phenotype Results[c]		
INRA BWCC number	Accession name	Origin[d]	Growth habit[e]	Year of reg.[f]	Type	Sowing Date	Rht-B1[g]	Rht-D1[h]	Ppd-D1[i]	Plant height (cm)	Days to heading[j]	GA trt diff (mm)
INRA_05308	NP120	IND	S	NA	fixed	Feb-09	B1a_0	D1a	D1b	127.5	137	30.7
INRA_05399	NYU BAY	JPN	S	1985	fixed	Feb-09	B1a_0	D1a	D1b	131.5	141	18.2
INRA_05401	NZ(81)P43	NZL	W	NA	fixed	Nov-08	B1a_0	D1b	D1a	102.5	123	8.7
INRA_05415	ODESSA EXP.STA.17413	SYR	S	NA	fixed	Feb-09	B1a_0	D1a	D1b	144	142	27.7
INRA_05419	ODESSA EXP.STA.19565	ETH	S	NA	fixed	Feb-09	B1a_197	D1a	D1b	158	148	23.0
INRA_05421	ODESSA EXP.STA.20722	PRT	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	162.5	135	14.8
INRA_05425	ODESSA EXP.STA.21821	PRT	S	NA	fixed	Feb-09	B1a_0	D1a	D1b	147.5	146	34.8
INRA_05438	ODESSKAIA-16	RUS	W	1953	fixed	Nov-08	B1a_0	D1b	D1a	113.5	144	2.0
INRA_05448	OGOSTA	BGR	F	1978	fixed	Nov-08	B1b	D1a	D1a	96.5	134	9.3
INRA_05486	OPAL	DEU	S	1959	fixed	Feb-09	B1a_0	D1a	D1b	128.5	145	34.3
INRA_05501	ORLANDI	ITA	W	1947	fixed	Nov-08	B1b	D1a	D1a	77.5	139	-2.8
INRA_05536	OULIANOWSKA	RUS	W	1974	fixed	Nov-08	B1a_0	D1a	D1b	152.5	149	39.3
INRA_05552	P. DE BROLLON	ESP	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	157	140	15.3
INRA_05558	P4523-80	AUT	W	NA	fixed	Nov-08	B1b	D1a	D1b	106.5	146	10.7
INRA_05636	PATO	ARG	S	1968	fixed	Feb-09	B1a_0	D1b	D1a	98.5	139	12.2
INRA_05702	PEYKRU INERME NON VELU	NPL	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	156	126	39.7
INRA_05748	PITIC 62	MEX	S	1962	fixed	Feb-09	B1a_0	D1b	D1a	105.5	141	-0.5
INRA_05773	POILU DU TARN	FRA	W	1986	fixed	Nov-08	B1a_0	D1a	D1b	165	144	32.1
INRA_05816	PRECOCE A BARBE BLANCHE	PRT	S	1955	fixed	Feb-09	B1a_197	D1a	D1b	153	143	15.8
INRA_05821	PRECOCE DU JAPON	JPN	S	1868	fixed	Feb-09	B1a_0	D1a	D1b	148	143	23.6
INRA_05897	PROMESSA	FRA	S	1991	fixed	Feb-09	B1a_0	D1b	D1b	94	150	7.8
INRA_06027	RECITAL	FRA	W	1986	fixed	Nov-08	B1b	D1a	D1a	93	131	16.3
INRA_06047	REDMAN	CAN	S	1946	fixed	Feb-09	B1a_0	D1a	D1b	136.5	144	17.7
INRA_06086	RENAN	FRA	W	1989	fixed	Nov-08	B1b	D1a	D1b	103.5	140	8.9
INRA_06170	RICHELLE	FRA	S	1926	fixed	Feb-09	B1a_0	D1a	D1b	165	156	20.6

Appendix VI continued

INRA BWCC accessions[a]							Genotype Results[b]			Phenotype Results[c]		
INRA BWCC number	Accession name	Origin[d]	Growth habit[e]	Year of reg.[f]	Type	Sowing Date	Rht-B1[g]	Rht-D1[h]	Ppd-D1[i]	Plant height (cm)	Days to heading[j]	GA trt diff (mm)
INRA_06191	RINGOT 2	FRA	W	NA	fixed	Nov-08	B1a_197	D1a	D1b	104	148	47.0
INRA_06198	RITAK	NPL	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	149	129	14.3
INRA_06308	ROUGE D'ALTKIRCH	FRA	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	180.5	150	29.7
INRA_06318	ROUGE DE MARCHISSY	FRA	W	1929	fixed	Nov-08	B1a_0	D1a	D1b	189	148	23.8
INRA_06396	S975-A4-A1	ZWE	S	NA	fixed	Feb-09	B1a_0	D1a	D1a	88.5	135	17.4
INRA_06522	SENTUNG	CHN	W	NA	fixed	Nov-08	B1a_0	D1b	D1a	117	125	3.3
INRA_06529	SEU SEUN 27	KOR	W	1936	fixed	Nov-08	B1a_0	D1a	D1b	144.5	126	18.3
INRA_06575	SIRAZAIAIBARIGI 2	JPN	S	NA	fixed	Feb-09	B1a_0	D1b	D1a	87	135	4.5
INRA_06605	SOANDRES LARACHA	ESP	W	NA	fixed	Nov-08	B1a_197	D1a	D1b	181.5	142	23.7
INRA_06740	STRUBES DICKKOPF	DEU	W	1880	fixed	Nov-08	B1a_160	D1a	D1b	145.5	149	20.0
INRA_06843	TAFERSTAT	FRA	S	NA	landrace	Feb-09	B1a_0	D1a	D1b	96.5	142	22.3
INRA_06846	TAHTI	FIN	S	1949	fixed	Feb-09	B1a_0	D1a	D1b	127	148	42.0
INRA_06922	TF6	FRA	W	NA	fixed	Nov-08	B1a_197	D1a	D1a	101	136	21.1
INRA_06986	TOM THUMB	USA	W	1972	fixed	Nov-08	B1a_197	D1a	D1b	55.5	150	-2.3
INRA_07011	TOUZELLE BLANCHE BARBUE	FRA	W	1936	fixed	Nov-08	B1a_0	D1a	D1b	178	141	25.5
INRA_07026	TRAMI~PUY DE DOME	FRA	S	1921	fixed	Feb-09	B1a_0	D1a	D1b	124	143	19.1
INRA_07040	TREMESINO MEIRA	ESP	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	155	131	36.3
INRA_07048	TRIGO DE MONTE	ESP	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	168	147	32.0
INRA_07085	TURDA 81-77	ROM	W	1984	fixed	Nov-08	B1a_0	D1a	D1a	114.5	135	20.3
INRA_07092	TYLER	USA	W	1980	fixed	Nov-08	B1b	D1a	D1b	135.5	135	15.9
INRA_07117	US(59)34	USA	F	1970	fixed	Nov-08	B1a_197	D1a	D1b	164	139	25.5
INRA_07166	US(62)P66	COL	S	NA	fixed	Nov-08	B1a_0	D1a	D1b	146.5	124	37.9
INRA_07276	VAKKA	FIN	W	1953	fixed	Nov-08	B1a_0	D1a	D1b	150	149	29.7
INRA_07279	VALD'OR	FRA	W	1956	fixed	Nov-08	B1a_197	D1a	D1b	136	146	27.5
INRA_07490	VPM V1-1-2-4R2-3-8-3-2	FRA	W	NA	fixed	Nov-08	B1a_0	D1a	D1b	144	143	21.5

Appendix VI continued

INRA BWCC accessions[a]							Genotype Results[b]			Phenotype Results[c]		
INRA BWCC number	Accession name	Origin[d]	Growth habit[e]	Year of reg.[f]	Type	Sowing Date	Rht-B1[g]	Rht-D1[h]	Ppd-D1[i]	Plant height (cm)	Days to heading[j]	GA trt diff (mm)
INRA_07585	WATTINES	FRA	W	1974	fixed	Nov-08	B1a_197	D1a	D1b	113	144	40.3
INRA_07848	RONGOTEA	NZL	W	1979	fixed	Nov-08	B1a_0	D1b	D1b	111.5	137	2.3
INRA_07965	BLANC DE CHENAN	FRA	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	154	150	35.0
INRA_07968	BLE DANOIS	FRA	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	168.5	141	32.0
INRA_07973	BORDEAUX 113	FRA	W	1936	fixed	Nov-08	B1a_0	D1a	D1a	168.5	147	34.0
INRA_07988	CREPIN A	FRA	W	NA	fixed	Nov-08	B1a_160	D1a	D1b	173	138	38.3
INRA_08011	INSTITUT 1802	FRA	W	NA	fixed	Nov-08	B1a_160	D1a	D1b	124.5	145	11.0
INRA_08048	RALET	FRA	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	190	145	29.3
INRA_08051	BLE BARBU DE MUROL	FRA	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	156	138	45.3
INRA_08058	ZANDA	BEL	W	1939	fixed	Nov-08	B1a_160	D1a	D1b	162	151	24.2
INRA_08073	CORONATION	CAN	W	1938	fixed	Nov-08	B1a_0	D1a	D1b	153	135	17.1
INRA_08079	KITCHENER	CAN	W	1911	fixed	Nov-08	B1a_0	D1a	D1b	175.5	140	29.5
INRA_08097	STANLEY	CAN	W	1890	fixed	Nov-08	B1a_0	D1a	D1b	171	136	30.3
INRA_08113	GP260	CAN	S	1983	fixed	Feb-09	B1a_0	D1a	D1a	129.5	137	36.5
INRA_08165	NAVARRO 150	ESP	W	NA	fixed	Nov-08	B1a_160	D1a	D1a	117.5	134	24.8
INRA_08170	WS-13 CARDENO 34/45	ESP	W	NA	fixed	Nov-08	B1b	D1b	D1a	111	130	5.0
INRA_08194	NEELKANT	SYR	W	1980	fixed	Nov-08	B1b	D1a	D1a	121	127	19.0
INRA_08197	SANUNU	SYR	W	NA	fixed	Nov-08	B1b	D1a	D1a	115.5	127	8.7
INRA_08227	NISHIKAZE KOMUGI	JPN	W	1986	fixed	Nov-08	B1b	D1a	D1a	96	116	5.9
INRA_08233	HOUMANA	NER	S	NA	fixed	Nov-08	B1a_0	D1a	D1b	117.5	121	22.1
INRA_08254	CADENZA	FRA	S	1993	fixed	Nov-08	B1a_0	D1a	D1b	105	141	31.0
INRA_08276	CARIBO	DEU	W	1968	fixed	Nov-08	B1a_160	D1a	D1b	118.5	148	14.6
INRA_08280	(V*C)5-3	FRA	W	NA	fixed	Nov-08	B1a_197	D1a	NP	115.5	150	35.3
INRA_08287	DC147U	FRA	W	NA	fixed	Nov-08	B1a_197	D1b	D1b	118	142	4.7
INRA_08289	TM7MB1-1	FRA	W	NA	fixed	Nov-08	B1a_160	D1b	D1b	109.5	140	3.0
INRA_08513	LIMPOPO	ZWE	S	1981	fixed	Feb-09	B1b	D1b	D1a	66	137	0.7
INRA_08578	GH 'S'	MEX	S	NA	fixed	Feb-09	B1b	D1a	D1a	105	145	8.4

Appendix VI continued

INRA BWCC accessions[a]							Genotype Results[b]			Phenotype Results[c]		
INRA BWCC number	Accession name	Origin[d]	Growth habit[e]	Year of reg.[f]	Type	Sowing Date	Rht-B1[g]	Rht-D1[h]	Ppd-D1[i]	Plant height (cm)	Days to heading[j]	GA trt diff (mm)
INRA_09024	GENESIS	FRA	W	1992	fixed	Nov-08	B1a_160	D1b	D1b	86	147	-2.0
INRA_09048	HOPEA	FIN	S	1936	fixed	Feb-09	B1a_0	D1a	D1b	108	185	28.3
INRA_09077	NON PLUS EXTRA	AUT	W	1919	fixed	Nov-08	B1a_160	D1a	D1b	148.5	157	28.7
INRA_09087	PRINCE-LEOPOLD	BEL	W	1936	fixed	Nov-08	B1a_160	D1a	D1b	191	151	29.8
INRA_13210	SOLARIS	CZE	W	1976	fixed	Nov-08	B1b	D1a	D1a	117.5	134	12.9
INRA_13282	ANATOLIE2	FRA	W	NA	fixed	Nov-08	B1a_197	D1a	D1a	114	142	23.3
INRA_13286	BAROOTA-WONDER	AUS	S	1895	fixed	Feb-09	B1a_0	D1a	D1b	162	160	30.3
INRA_13292	CONCURRENT	FRA	W	1905	fixed	Nov-08	B1a_0	D1a	D1b	151	143	27.7
INRA_13310	FRUH WEIZEN	DEU	W	NA	fixed	Nov-08	B1a_160	D1a	D1b	167	140	20.0
INRA_13436	FONDARD CRESPIN	FRA	W	1948	fixed	Nov-08	B1a_0	D1a	D1b	164.5	138	27.0
INRA_13440	PROVINS	FRA	W	1949	fixed	Nov-08	B1a_0	D1a	D1b	134	142	32.5
INRA_13445	VOLT	FRA	W	1994	fixed	Nov-08	B1b	D1a	D1a	91	140	11.3
INRA_13454	SPONSOR	FRA	W	1994	fixed	Nov-08	B1a_197	D1b	D1b	93	146	3.3
INRA_13461	BEHERT	FRA	W	1994	fixed	Nov-08	B1a_160	D1b	D1b	89.5	144	2.0
INRA_13471	ORNICAR	FRA	W	1998	fixed	Nov-08	B1b	D1a	D1b	90	139	7.7
INRA_13476	TALDOR	FRA	W	1998	fixed	Nov-08	B1b	D1a	D1a	86.5	140	14.4
INRA_13481	APACHE	FRA	W	1998	fixed	Nov-08	B1a_160	D1a	D1a	84.5	134	16.5
INRA_13494	BELLOVAC	FRA	W	NA	fixed	Nov-08	B1a_197	D1b	D1a	85.5	141	4.6
INRA_13500	ORFIELD	FRA	W	NA	fixed	Nov-08	B1a_197	D1b	D1b	95.5	149	4.7
INRA_13502	PALIO	FRA	W	NA	fixed	Nov-08	B1a_0	D1b	D1a	86	135	7.5
INRA_13642	AGATHA	CAN	S	1970	fixed	Feb-09	B1a_0	D1a	D1b	131	143	25.7
INRA_13781	TAI 911	CHN	W	NA	fixed	Nov-08	B1a_0	D1a	D1a	100.5	121	28.3
INRA_13792	CENTURK	USA	W	1971	fixed	Nov-08	B1a_160	D1a	D1b	137.5	135	41.8
INRA_13799	PAMUKALE	TUR	S	1997	fixed	Feb-09	B1a_0	D1a	D1a	134.5	148	24.7
INRA_13800	ZILVE	TUR	S	NA	fixed	Feb-09	B1a_0	D1a	D1b	157	148	32.3
INRA_13811	OPATA 85	MEX	S	1985	fixed	Feb-09	B1b	D1a	D1b	123.5	154	20.8
INRA_13812	W7984	MEX	S	NA	fixed	Feb-09	B1b	D1a	450bp	112	142	14.2
INRA_13861	AUGUSTE	FRA	W	1998	fixed	Nov-08	B1a_197	D1b	D1b	80	149	8.4

Appendix VI continued

INRA BWCC accessions[a]							Genotype Results[b]			Phenotype Results[c]		
INRA BWCC number	Accession name	Origin[d]	Growth habit[e]	Year of reg.[f]	Type	Sowing Date	Rht-B1[g]	Rht-D1[h]	Ppd-D1[i]	Plant height (cm)	Days to heading[j]	GA trt diff (mm)
INRA_13870	TALISMAN~SER	FRA	W	1998	fixed	Nov-08	B1a_197	D1b	D1b	97	143	6.8
INRA_13978	AXONA	NLD	S	1983	fixed	Feb-09	B1a_0	D1a	D1b	109	148	38.3
INRA_14000	ROKYCANSKA SAMETKA	CZE	W	1899	fixed	Nov-08	B1a_160	D1a	D1b	167.5	153	37.0
INRA_14011	HANA	CZE	W	1899	fixed	Nov-08	B1a_160	D1a	D1a	106	137	25.3
INRA_15606	BLE DE REDON BLANC BARBU 1 1	FRA	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	170.5	144	24.0
INRA_15652	BLE DE REDON BLANC COMPACT I 1	FRA	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	176.5	148	20.0
INRA_15658	BLE DE REDON BLANC 1/2 LACHE 1 1	FRA	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	177.5	152	38.7
INRA_15710	BLE DE REDON GLUMES VELUES 1	FRA	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	191.5	149	33.3
INRA_15809	BLE DE REDON ST-JUST Q21	FRA	W	NA	landrace	Nov-08	B1a_0	D1a	D1b	176.5	151	30.7
INRA_15950	AS68VM4-3-2/TJB636 13	FRA	W	NA	fixed	Nov-08	B1a_197	D1b	D1b	105.5	144	5.7
INRA_15954	ASVM4/BEAUCHAMP 81B13	FRA	W	1994	fixed	Nov-08	B1a_160	D1a	D1a	107	138	33.3
INRA_20074	MIRLEBEN	UKR	W	1993	fixed	Nov-08	B1a_0	D1b	D1b	106	139	3.7
INRA_20224	FANTAZIYA-ODESSKAYA	UKR	W	NA	fixed	Nov-08	B1a_0	D1b	D1a	120.5	136	20.4
INRA_20276	EQUINOX	GBR	W	1995	fixed	Nov-08	B1a_197	D1b	D1b	78	145	6.7
INRA_20384	DI9234-11-15	FRA	W	NA	fixed	Nov-08	B1b	D1a	D1b	87	141	7.6
INRA_20417	HAMAC	NLD	W	NA	fixed	Nov-08	B1a_160	D1b	D1b	72	145	-0.7
INRA_23891	LANDRACE	ARM	S	NA	landrace	Feb-09	B1a_0	D1a	D1b	141.5	138	15.0
INRA_23896	LANDRACE	TUR	S	NA	landrace	Feb-09	B1a_0	D1a	D1b	160.5	147	24.8
INRA_23902	LANDRACE	TUR	S	NA	landrace	Feb-09	B1a_0	D1a	NP	155.5	148	21.7
INRA_23909	LANDRACE	MAR	S	NA	landrace	Feb-09	B1a_0	D1a	D1b	144	143	40.7
INRA_23923	LANDRACE	ETH	S	1927	landrace	Feb-09	B1a_0	D1a	D1b	157.5	152	34.3
INRA_23933	PUNJAB TIPE 24	IND	S	1928	fixed	Feb-09	B1a_0	D1a	D1b	123	137	41.0
INRA_23934	PUNJAB TIPE 18	IND	S	1928	fixed	Feb-09	B1a_0	D1a	D1b	127	139	20.0

Appendix VI continued

INRA BWCC accessions[a]							Genotype Results[b]			Phenotype Results[c]		
INRA BWCC number	Accession name	Origin[d]	Growth habit[e]	Year of reg.[f]	Type	Sowing Date	Rht-B1[g]	Rht-D1[h]	Ppd-D1[i]	Plant height (cm)	Days to heading[j]	GA trt diff (mm)
INRA_23937	PUNJAB TIPE 15	IND	S	1928	fixed	Feb-09	B1a_0	D1a	D1b	122	138	22.0
INRA_23944	LANDRACE	CHN	S	NA	landrace	Feb-09	B1a_0	D1a	D1b	170	152	35.5
INRA_23945	LANDRACE	CHN	S	NA	landrace	Feb-09	B1a_0	D1a	D1b	169	150	29.8
INRA_23950	SARRUBRA	RUS	S	1931	fixed	Feb-09	B1a_160	D1a	D1b	160	143	24.4
INRA_23957	R 1	CHN	S	1932	fixed	Feb-09	B1a_0	D1a	D1b	132	178	22.3
INRA_23960	117-VAR.12/564	PAK	S	1934	fixed	Feb-09	B1a_0	D1a	D1b	143	141	26.8
INRA_23964	THORI 212-VAR.8/1	PAK	S	1934	fixed	Feb-09	B1a_0	D1a	D1b	128.5	138	32.5
INRA_23970	LANDRACE	TJK	S	NA	landrace	Feb-09	B1a_0	D1a	D1b	159.5	150	35.2
INRA_23971	LANDRACE	TJK	S	NA	landrace	Feb-09	B1a_0	D1a	D1b	175	147	29.7
INRA_23974	LANDRACE	TJK	S	NA	landrace	Feb-09	B1a_0	D1a	D1b	157.5	147	19.3
INRA_23977	LANDRACE	GEO	S	1931	landrace	Feb-09	B1a_0	D1a	D1b	167	149	17.9
INRA_23981	SOOR GHANUM	PAK	S	NA	landrace	Feb-09	B1a_0	D1a	D1b	122	138	29.8
INRA_23989	LANDRACE	GEO	S	1931	landrace	Feb-09	B1a_0	D1a	NP	171	150	21.3
INRA_23995	LANDRACE	RUS	S	1950	landrace	Feb-09	B1a_0	D1a	D1b	150	151	21.8
INRA_23996	GUISUISKAYA SYAO-BAI-MAI	CHN	S	1953	fixed	Feb-09	B1a_0	D1a	D1b	126	134	34.2
INRA_24003	KULDZHINSKAYA	CHN	S	1959	fixed	Feb-09	B1a_0	D1a	D1b	176	149	25.0
INRA_24006	U-MAN-SYAO-MAI	CHN	S	1954	fixed	Feb-09	B1a_0	D1a	D1b	152	142	43.0
INRA_24019	DALNEVOSTOCHNAYA 10	RUS	S	1978	fixed	Feb-09	B1a_160	D1a	D1b	144	143	34.7
INRA_24031	KRASNAYA	CAN	W	1914	fixed	Nov-08	B1a_160	D1a	D1b	179	144	30.0
INRA_24054	STEPNYACHKA	UKR	W	1969	fixed	Nov-08	B1a_0	D1a	D1b	163	140	26.7
INRA_24056	LANDRACE	TUR	F	NA	landrace	Nov-08	B1a_0	D1a	D1b	175.5	138	36.3
INRA_24058	SARY-BUGDA GULGERI	RUS	W	1928	fixed	Nov-08	B1a_0	D1a	D1b	174	140	27.7
INRA_24066	CROISEMENT 268	CHE	W	1929	fixed	Nov-08	B1a_0	D1a	D1b	156	153	26.3
INRA_24075	SPIN 121-VAR.12/536	PAK	F	1933	fixed	Nov-08	B1a_0	D1a	D1b	141	126	33.2
INRA_24080	DOLIS PURI	GEO	W	1933	fixed	Nov-08	B1a_0	D1a	D1b	176.5	139	29.3
INRA_24089	TAU-BUGDA	RUS	W	1955	fixed	Nov-08	B1a_0	D1a	NP	195.5	141	30.3
INRA_24108	ALBIDUM 12	RUS	W	1980	fixed	Nov-08	B1a_160	D1a	D1b	171.5	146	38.0

Appendix VI continued

INRA BWCC accessions[a]							Genotype Results[b]			Phenotype Results[c]		
INRA BWCC number	Accession name	Origin[d]	Growth habit[e]	Year of reg.[f]	Type	Sowing Date	Rht-B1[g]	Rht-D1[h]	Ppd-D1[i]	Plant height (cm)	Days to heading[j]	GA trt diff (mm)
INRA_24180	PALESTINSKAYA	PAL	S	1927	fixed	Feb-09	B1a_0	D1a	D1b	139	140	33.7
INRA_24184	LANDRACE	PAL	S	1927	landrace	Feb-09	B1a_0	D1a	D1b	183.5	155	27.0
INRA_24185	LANDRACE	TKM	S	NA	landrace	Feb-09	B1a_0	D1a	D1b	151.5	139	30.0
INRA_24186	SARY-BUGDA	AZE	F	1928	fixed	Nov-08	B1a_0	D1a	D1b	196	141	29.2
INRA_24193	LANDRACE	AZE	W	NA	landrace	Nov-08	B1a_0	D1a	NP	165.5	132	26.5
INRA_24196	ARAZBUGDASI	AZE	W	1934	fixed	Nov-08	B1a_0	D1a	D1b	173.5	144	35.8
INRA_24209	ARDITO	ITA	F	1916	fixed	Feb-09	B1a_0	D1a	D1b	153	149	45.7
INRA_24210	LAMMAS	GBR	W	1850	fixed	Nov-08	B1a_160	D1a	D1b	180.5	153	26.5

[a] Accession information on the INRA BWCC (Bread wheat core collection) received from F. Balfourier, INRA.

[b] Genotyping was performed using DNA from the INRA BWCC outdoor experiment, which consisted of two separate bulks of four plants for fixed lines and four individual plants for landraces. Heterozygotes (het) are shown with segregating alleles separated by a "/".

[c] Plant heights and days to heading were recorded in the INRA BWCC outdoor experiment, Cambridge (UK) and values represent the mean of two replicates. GA treatment differences (GA trt diffs) represent the average of GA exps. 1, 2, and 3 (Norwich, UK). GA trt diff = GA+ treatment seed-to-first-ligule (STFL) length minus GA- treatment STFL length. d Countries of origin are: (AFG Afghanistan; ALB Albania; ARG Argentina; ARM Armenia; AUS Australia; AUT Austria; AZE Azerbaijan; BEL Belgium; BGR Bulgaria; BLR Belarus; BRA Brazil; CAN Canada; CHE Switzerland; CHN China; COL Colombia; CSK Czech and Slovak Republics; DEU Germany; DNK Denmark; DZA Algeria; EGY Egypt; ESP Spain; ETH Ethiopia; FIN Finland; FRA France; GEO Georgia; GBR Great Britain; GRC Greece; GTM Guatemala; HUN Hungary; HRV Croatia; IND India; IRL Ireland; IRN Iran; IRQ Iraq; ISR Israel; ITA Italy; JPN Japan; KAZ Kazakhstan; KEN Kenya; KIR Kyrgyzstan; KOR Korea; KSM Kashmir; LBN Lebanon; MAD Macedonia; MAR Morocco; MEX Mexico; MNG Mongolia; NER Niger; NLD Netherlands; NOR Norway; NPL Nepal; NZL New Zealand; PAL Palestine; PAK Pakistan; POL Poland; POR Portugal; PER Peru; ROM Romania; RUS Russia; SYR Syria; SWE Sweden; TJK Tajikistan; TKM Turkenistan; TUN Tunisia; TUR Turkey; URY Uruguay; UKR Ukraine; USA United States; UZB Uzbekistan; YUG Yugoslavia; ZAF South Africa; ZWE Zimbabwe)

[e] S=spring, W=winter, F=facultative

[f] Year of reg = Year of registration; NA = not available

[g] *Rht-B1a* and *Rht-B1b* designations based on the *Rht-B1a/b* PCR assays. *Rht-B1a_0*, *Rht-B1a_160*, and *Rht-B1a_197* refer to accessions containing *Rht-B1a* with no insertion, a 160 bp insertion, or a 197 bp insertion, respectively. *Rht-B1_197* contains the 197 bp insertion but did not amplify a product associated with *Rht-B1a* or *Rht-B1b*.

[h] *Rht-D1* locus designations based on the *Rht-D1a/b* PCR assays.

[i] *Ppd-D1* locus designations based on the *Ppd-D1a/b* PCR assay (Beales *et al*., 2007). The ~450 bp does not correspond to the *Ppd-D1b* (414 bp product) or *Ppd-D1a* (297 bp product) allele. 'No product' indicates that a product was not obtained in either replicate.

[j] Days from January 1.

Appendix VII. Summary of GA sensitivity experiments 1, 2, and 3 for the INRA BWCC accessions and control lines.

Accession[a]	Genotype[b]			GA exp. 1					GA exp. 2					GA exp. 3[h]					Overall
INRA BWCC number / control line	Rht-B1	Rht-D1	Ppd-D1	GA+ length (mm)[c]	GA- length (mm)[d]	Trt Diff (mm)[e]	MM *p* GAI[f]	LSD *p* GA trt diff = 0[g]	GA+ length (mm)	GA- length (mm)	Trt Diff (mm)	MM *p* GAI	LSD *p* GA trt diff = 0	GA+ length (mm)	GA- length (mm)	Trt Diff (corr'd) mm[i]	Trt Diff t value (calc.)	LSD *p* GA trt diff = 0	Trt diff[j]
INRA_00007	B1a_197	D1a	D1a	61.0	54.5	6.5	0.73	0.45	82.3	56.0	26.3	0.02	0.00	54.2	26.8	21.4	8.80	0.00	18.1
INRA_00019	B1b	D1a	D1b	51.3	51.3	0.0	0.97	1.00	60.3	52.7	7.7	0.98	0.19						3.8
INRA_00092	B1a_197	D1a	D1b	88.0	54.3	33.7	0.00	0.00											33.7
INRA_00177	B1a_0	D1a	D1b	79.7	51.0	28.7	0.00	0.00											28.7
INRA_00234	B1a_160	D1a	D1b	81.0	51.0	30.0	0.00	0.00	92.7	52.3	40.3	0.00	0.00						35.2
INRA_00236	B1a_0	D1a	D1a	82.7	50.3	32.3	0.00	0.00											32.3
INRA_00338	B1a_0	D1a	D1a	77.5	61.3	16.2	0.01	0.04											16.2
INRA_00347	B1a_0	D1a	D1a	66.5	59.0	7.5	0.31	0.38	84.0	61.7	22.3	0.01	0.00	57.7	33.3	19.1	6.28	0.00	16.3
INRA_00386	B1a_0	D1a	D1a	65.3	50.0	15.3	0.40	0.05	81.7	53.0	28.7	0.03	0.00						22.0
INRA_00419	B1a_160	D1a	D1b	75.3	50.3	25.0	0.02	0.00											25.0
INRA_00421	B1a_0	D1a	D1a	80.0	49.5	30.5	0.00	0.00											30.5
INRA_00477	B1a_160	D1a	D1a	75.3	51.0	24.3	0.02	0.00											24.3
INRA_00514	B1a_0	D1a	D1b	77.5	63.5	14.0	0.01	0.10	91.3	65.7	25.7	0.00	0.00						19.8
INRA_00524	B1a_0	D1a	D1b	91.7	59.3	32.3	0.00	0.00											32.3
INRA_00537	B1a_160	D1a	D1b	76.5	59.7	16.8	0.01	0.03						63.5	35.0	22.3	9.60	0.00	19.6
INRA_00546	B1b	D1a	D1a	59.3	46.3	13.0	0.82	0.06	55.0	43.7	11.3	1.00	0.05						12.2
INRA_00748	B1a_0	D1a	D1b	96.0	70.3	25.7	0.00	0.00											25.7
INRA_00776	B1b	D1a	D1a	45.5	36.7	8.8	0.99	0.26	39.3	38.3	1.0	1.00	0.86						4.9
INRA_00794	B1a_0	D1a	D1b	100.0	58.0	42.0	0.00	0.00	96.0	71.0	25.0	0.00	0.00						33.5
INRA_00797	B1a_197	D1a	D1b	77.3	52.0	25.3	0.01	0.00											25.3
INRA_00800	B1a_0	D1a	D1b	76.7	52.3	24.3	0.01	0.00											24.3
INRA_00822	B1a_197	D1b	D1a	65.0	40.5	24.5	0.43	0.00	44.3	41.0	3.3	1.00	0.56						13.9
INRA_00833	B1a_0	D1a	D1a	95.5	48.0	47.5	0.00	0.00											47.5
INRA_00871	B1a_0	D1a	D1b	90.0	68.3	21.7	0.00	0.00											21.7
INRA_00901	B1a_160/ 197 het	D1b	D1a	48.0	49.5	-1.5	0.99	1.00	50.0	47.3	2.7	1.00	0.64						0.6

Appendix VII continued

Accession[a]	Genotype[b]			GA exp. 1					GA exp. 2					GA exp. 3[h]					Overall
INRA BWCC number / control line	Rht-B1	Rht-D1	Ppd-D1	GA+ length (mm)[c]	GA- length (mm)[d]	Trt Diff (mm)[e]	MM *p* GAI[f]	LSD *p* GA trt diff = 0[g]	GA+ length (mm)	GA- length (mm)	Trt Diff (mm)	MM *p* GAI	LSD *p* GA trt diff = 0	GA+ length (mm)	GA- length (mm)	Trt Diff (corr'd) mm[i]	Trt Diff t value (calc.)	LSD *p* GA trt diff = 0	Trt diff[j]
INRA_00912	B1a_0	D1a	D1a	81.0	40.5	40.5	0.00	0.00											40.5
INRA_00933	B1a_160	D1a	D1b	91.3	54.3	37.0	0.00	0.00	92.5	56.7	35.8	0.00	0.00						36.4
INRA_00957	B1a_197	D1a	D1b	78.0	50.7	27.3	0.00	0.00											27.3
INRA_00964	B1a_197	D1a	D1b	59.5	51.0	8.5	0.81	0.41	75.3	49.7	25.7	0.24	0.00	55.5	29.6	20.2	8.33	0.00	18.1
INRA_00983	B1a_197	D1b	D1b	54.7	54.0	0.7	0.94	0.93	52.7	52.0	0.7	1.00	0.91						0.7
INRA_01005	B1a_0	D1a	D1b	88.0	58.3	29.7	0.00	0.00											29.7
INRA_01032	B1a_197	D1a	D1b	90.3	54.3	36.0	0.00	0.00											36.0
INRA_01044	B1a_0	D1a	D1b	81.5	52.3	29.2	0.00	0.00											29.2
INRA_01065	B1a_0	D1a	D1a	71.0	50.0	21.0	0.08	0.05	73.3	54.3	19.0	0.39	0.00	62.0	30.3	24.8	8.80	0.00	21.6
INRA_01080	B1a_0	D1a	D1b	82.7	59.7	23.0	0.00	0.00											23.0
INRA_01110	B1a_0	D1a	D1b	109.0	70.0	39.0	0.00	0.00	103.3	75.7	27.7	0.00	0.00						33.3
INRA_01113	B1a_0	D1a	D1b	104.7	64.0	40.7	0.00	0.00											40.7
INRA_01177	B1a_0	D1a	D1b	78.7	62.5	16.2	0.00	0.04											16.2
INRA_01182	B1a_0	D1a	D1b	99.0	71.0	28.0	0.00	0.00											28.0
INRA_01192	B1a_0	D1a	D1a	87.5	51.3	36.2	0.00	0.00											36.2
INRA_01217	B1a_0	D1b	D1a	53.5	49.5	4.0	0.95	0.64											4.0
INRA_01232	B1a_160	D1a	D1b	83.0	58.3	24.7	0.00	0.00											24.7
INRA_01236	B1a_0	D1a	D1b	89.5	58.0	31.5	0.00	0.00											31.5
INRA_01244	B1b	D1a	D1a	47.7	41.7	6.0	0.99	0.39	48.7	41.7	7.0	1.00	0.23						6.5
INRA_01249	B1a_0	D1a	D1b	103.5	66.0	37.5	0.00	0.00											37.5
INRA_01281	B1a_197	D1a	D1b	76.7	53.7	23.0	0.01	0.00											23.0
INRA_01288	B1a_160	D1a	D1b	85.0	67.5	17.5	0.00	0.03											17.5
INRA_01313	B1a_0	D1a	D1b	110.0	73.7	36.3	0.00	0.00											36.3
INRA_01321	B1a_197	D1a	D1a	78.7	49.3	29.3	0.00	0.00											29.3
INRA_01332	B1a_160	D1a	D1b	99.3	55.7	43.7	0.00	0.00											43.7
INRA_01357	B1a_160	D1a	D1b	76.5	64.5	12.0	0.01	0.16	87.7	60.3	27.3	0.00	0.00						19.7

Appendix VII continued

Accession[a]	Genotype[b]			GA exp. 1					GA exp. 2					GA exp. 3[h]					Overall
INRA BWCC number / control line	Rht-B1	Rht-D1	Ppd-D1	GA+ length (mm)[c]	GA- length (mm)[d]	Trt Diff (mm)[e]	MM *p* GAI[f]	LSD *p* GA trt diff = 0[g]	GA+ length (mm)	GA- length (mm)	Trt Diff (mm)	MM *p* GAI	LSD *p* GA trt diff = 0	GA+ length (mm)	GA- length (mm)	Trt Diff (corr'd) mm[i]	Trt Diff t value (calc.)	LSD *p* GA trt diff = 0	Trt diff[j]
INRA_01400	B1a_0	D1a	D1b	98.7	64.5	34.2	0.00	0.00											34.2
INRA_01402	B1a_160	D1a	D1a	81.3	50.0	31.3	0.00	0.00											31.3
INRA_01417	B1a_160	D1a	D1b		52.5				75.0	54.7	20.3	0.26	0.00						20.3
INRA_01422	B1a_0	D1a	D1b	113.3	74.3	39.0	0.00	0.00											39.0
INRA_01429	B1a_160	D1a	D1b	87.3	67.3	20.0	0.00	0.00											20.0
INRA_01446	B1a_0	D1a	D1b	90.3	66.0	24.3	0.00	0.00											24.3
INRA_01498	B1a_0	D1a	D1b	80.0	62.3	17.7	0.00	0.07	95.3	64.3	31.0	0.00	0.00						24.3
INRA_01529	B1a_0	D1a	D1b	75.3	46.0	29.3	0.02	0.00											29.3
INRA_01531	B1b	D1a	D1b	49.0	40.5	8.5	0.98	0.32	50.3	47.3	3.0	1.00	0.60						5.8
INRA_01542	B1a_0/ 197 het	D1a	D1a	91.0	60.0	31.0	0.00	0.00	97.0	69.7	27.3	0.00	0.00						29.2
INRA_01643	B1a/b het	D1a/b het	D1a	84.0	60.5	23.5	0.00	0.00	89.3	58.3	31.0	0.00	0.00						27.3
INRA_01647	B1a_0	D1b	D1a	56.7	52.7	4.0	0.90	0.57	65.3	49.0	16.3	0.90	0.01						10.2
INRA_01655	B1a_0	D1b	D1a	46.7	48.3	-1.7	0.99	1.00											-1.7
INRA_01660	B1a_0	D1a	D1b	92.3	46.0	46.3	0.00	0.00											46.3
INRA_01676	B1a_0	D1b	D1a	47.0	42.0	5.0	0.99	0.56											5.0
INRA_01696	B1a_0	D1a	D1b	114.3	73.3	41.0	0.00	0.00											41.0
INRA_01697	B1a_0	D1a	D1b	107.3	57.0	50.3	0.00	0.00											50.3
INRA_01747	B1b	D1a	D1b	62.3	45.0	17.3	0.64	0.03	55.0	52.0	3.0	1.00	0.60						10.2
INRA_01768	B1a_0	D1a	D1b	105.5	77.5	28.0	0.00	0.00											28.0
INRA_01774	B1a_0	D1a	D1b	96.5	70.0	26.5	0.00	0.01	110.7	73.0	37.7	0.00	0.00						32.1
INRA_01885	B1a_0	D1a	D1b	85.0	58.0	27.0	0.00	0.00											27.0
INRA_01899	B1a_197	D1a	D1b	78.3	54.0	24.3	0.00	0.00											24.3
INRA_01957	B1a_197	D1b	D1b	55.3	60.0	-4.7	0.93	1.00	51.3	49.3	2.0	1.00	0.73						-1.3
INRA_01974	B1b	D1a	D1b	54.3	48.0	6.3	0.94	0.42	54.3	56.3	-2.0	1.00	1.00						2.2

Appendix VII continued

Accession[a]	Genotype[b]			GA exp. 1					GA exp. 2					GA exp. 3[h]					Overall
INRA BWCC number / control line	Rht-B1	Rht-D1	Ppd-D1	GA+ length (mm)[c]	GA- length (mm)[d]	Trt Diff (mm)[e]	MM *p* GAI[f]	LSD *p* GA trt diff = 0[g]	GA+ length (mm)	GA- length (mm)	Trt Diff (mm)	MM *p* GAI	LSD *p* GA trt diff = 0	GA+ length (mm)	GA- length (mm)	Trt Diff (corr'd) mm[i]	Trt Diff t value (calc.)	LSD *p* GA trt diff = 0	Trt diff[j]
INRA_02025	B1a_197	D1a	D1b	76.0	47.3	28.7	0.01	0.00											28.7
INRA_02072	B1a_0	D1b	D1a	53.3	52.7	0.7	0.96	0.92											0.7
INRA_02135	B1a_0	D1a	D1b	87.5	49.0	38.5	0.00	0.00											38.5
INRA_02141	B1a_0	D1a	D1b	103.0	70.5	32.5	0.00	0.00											32.5
INRA_02145	B1a_0	D1a	D1b/b het	105.3	58.3	47.0	0.00	0.00											47.0
INRA_02153	B1a_160	D1a	D1b	75.5	52.7	22.8	0.01	0.00											22.8
INRA_02169	B1a_160	D1a	D1b	102.0	61.0	41.0	0.00	0.00	107.0	68.3	38.7	0.00	0.00						39.8
INRA_02171	B1a_0	D1a	D1b	104.0	60.0	44.0	0.00	0.00	100.3	68.0	32.3	0.00	0.00						38.2
INRA_02289	B1a_0	D1a	D1b		56.3				93.7	64.0	29.7	0.00	0.00						29.7
INRA_02301	B1b	D1a	D1b	54.5	48.7	5.8	0.94	0.45	54.0	43.3	10.7	1.00	0.07						8.3
INRA_02308	B1a_0	D1b	D1b	43.7	40.5	3.2	0.99	0.68	48.7	47.0	1.7	1.00	0.77						2.4
INRA_02330	B1a_0	D1a	D1b	79.0	50.7	28.3	0.00	0.00											28.3
INRA_02337	B1a_197	D1a	D1a	77.3	48.0	29.3	0.01	0.00											29.3
INRA_02345	B1b	D1a	D1b	64.3	49.5	14.8	0.49	0.06	58.0	43.7	14.3	0.99	0.01						14.6
INRA_02353	B1a_197	D1a	D1a	114.7	80.0	34.7	0.00	0.00											34.7
INRA_02358	B1b	D1b	D1b	44.3	59.0	-14.7	0.99	1.00											-14.7
INRA_02364	B1a_197	D1a	D1b	96.0	54.7	41.3	0.00	0.00											41.3
INRA_02399	B1a_160	D1a	D1b	91.7	68.0	23.7	0.00	0.02											23.7
INRA_02411	B1a_197	D1a	D1b	97.0	57.0	40.0	0.00	0.00	98.7	71.7	27.0	0.00	0.00						33.5
INRA_02424	B1b	D1a	D1a	64.7	43.0	21.7	0.46	0.01	61.7	52.3	9.3	0.97	0.11						15.5
INRA_02438	B1a_160	D1a	D1a	89.7	47.7	42.0	0.00	0.00											42.0
INRA_02444	B1a_0	D1a	D1b	106.5	78.5	28.0	0.00	0.00											28.0
INRA_02475	B1a_160	D1a	D1b	77.0	49.0	28.0	0.01	0.00											28.0
INRA_02481	B1a_160	D1a	D1a	72.3	47.0	25.3	0.05	0.00	80.7	51.7	29.0	0.04	0.00						27.2
INRA_02485	B1a_0	D1a	D1b	104.3	71.5	32.8	0.00	0.00											32.8

Appendix VII continued

Accession[a]	Genotype[b]			GA exp. 1					GA exp. 2					GA exp. 3[h]					Overall
INRA BWCC number / control line	Rht-B1	Rht-D1	Ppd-D1	GA+ length (mm)[c]	GA- length (mm)[d]	Trt Diff (mm)[e]	MM *p* GAI[f]	LSD *p* GA trt diff = 0[g]	GA+ length (mm)	GA- length (mm)	Trt Diff (mm)	MM *p* GAI	LSD *p* GA trt diff = 0	GA+ length (mm)	GA- length (mm)	Trt Diff (corr'd) mm[i]	Trt Diff t value (calc.)	LSD *p* GA trt diff = 0	Trt diff[j]
INRA_02489	B1a_0	D1a	D1b	75.3	46.0	29.3	0.02	0.00											29.3
INRA_02491	B1a_197	D1a	D1a	76.7	55.3	21.3	0.01	0.00											21.3
INRA_02507	B1a_160	D1a	D1a	73.0	50.7	22.3	0.04	0.00											22.3
INRA_02508	B1b	D1b	D1b	47.5	37.3	10.2	0.99	0.19	38.0	35.7	2.3	1.00	0.69						6.3
INRA_02526	B1b	D1b	D1b	49.5	43.3	6.2	0.98	0.43	40.7	45.3	-4.7	1.00	1.00						0.7
INRA_02534	B1b	D1a	D1b	53.3	49.0	4.3	0.96	0.53											4.3
INRA_02536	B1a_0	D1a	D1a	86.0	51.7	34.3	0.00	0.00											34.3
INRA_02573	B1a_160	D1a	D1b	80.7	53.0	27.7	0.00	0.00											27.7
INRA_02574	B1a_197	D1a	D1b	78.0	57.0	21.0	0.00	0.01											21.0
INRA_02606	B1a_0	D1a	D1a	93.7	53.5	40.2	0.00	0.00											40.2
INRA_02626	B1a_0	D1a	D1b	83.3	48.0	35.3	0.00	0.00											35.3
INRA_02644	B1a_197	D1a	D1b	61.0	52.3	8.7	0.73	0.22	90.7	63.7	27.0	0.00	0.00						17.8
INRA_02650	B1a_160	D1a	D1a	87.0	38.0	49.0	0.00	0.00	81.7	51.7	30.0	0.03	0.00						39.5
INRA_02683	B1a_0	D1a	D1b	103.3	49.0	54.3	0.00	0.00											54.3
INRA_02698	B1a_0	D1a	D1b	93.0	68.0	25.0	0.00	0.00											25.0
INRA_02709	B1a_160	D1a	D1b	92.0	59.7	32.3	0.00	0.00											32.3
INRA_02759	B1a_0	D1b	D1a	59.7	55.0	4.7	0.80	0.55	60.0	58.7	1.3	0.98	0.82						3.0
INRA_02802	B1a_160	D1a	D1b	89.0	56.7	32.3	0.00	0.00											32.3
INRA_02810	B1a_0	D1a	D1b	89.0	54.5	34.5	0.00	0.00											34.5
INRA_02991	B1a_160	D1a	D1b	92.5	64.3	28.2	0.00	0.00											28.2
INRA_03050	B1b	D1a	D1a	70.3	53.5	16.8	0.10	0.03	66.0	55.7	10.3	0.88	0.08						13.6
INRA_03070	B1a_160	D1a	D1b	79.0	50.7	28.3	0.00	0.00											28.3
INRA_03165	B1a_0	D1a	D1b	88.5	64.0	24.5	0.00	0.00											24.5
INRA_03170	B1a_0	D1a	D1b		41.0				95.3	54.3	41.0	0.00	0.00						41.0
INRA_03176	B1b	D1a	D1a	53.0	27.5	25.5	0.96	0.00	49.5	45.3	4.2	1.00	0.52						14.8
INRA_03213	B1a_0	D1a	D1a	82.7	53.0	29.7	0.00	0.00											29.7

Appendix VII continued

Accession[a]	Genotype[b]			GA exp. 1					GA exp. 2					GA exp. 3[h]					Overall
INRA BWCC number / control line	Rht-B1	Rht-D1	Ppd-D1	GA+ length (mm)[c]	GA- length (mm)[d]	Trt Diff (mm)[e]	MM *p* GAI[f]	LSD *p* GA trt diff = 0[g]	GA+ length (mm)	GA- length (mm)	Trt Diff (mm)	MM *p* GAI	LSD *p* GA trt diff = 0	GA+ length (mm)	GA- length (mm)	Trt Diff (corr'd) mm[i]	Trt Diff t value (calc.)	LSD *p* GA trt diff = 0	Trt diff[j]
INRA_03218	B1a_0	D1a	D1b	95.7	63.3	32.3	0.00	0.00											32.3
INRA_03220	B1a_0	D1a	D1a	92.0	70.7	21.3	0.00	0.00											21.3
INRA_03256	B1a_0	D1a	D1b	87.3	51.0	36.3	0.00	0.00	99.0	60.7	38.3	0.00	0.00						37.3
INRA_03267	B1a_0	D1a	D1b	100.5	57.0	43.5	0.00	0.00											43.5
INRA_03278	B1a_0	D1a	D1b	68.0	44.3	23.7	0.21	0.00	73.0	58.0	15.0	0.41	0.01	50.8	29.0	17.1	7.35	0.00	18.6
INRA_03299	B1a_0	D1a	D1a	57.7	39.3	18.3	0.88	0.01	67.0	50.3	16.7	0.84	0.00	48.0	25.0	18.0	7.74	0.00	17.7
INRA_03306	B1a_0	D1a	D1a	83.7	61.0	22.7	0.00	0.00											22.7
INRA_03342	B1b	D1a	D1a	55.3	50.0	5.3	0.93	0.49	56.3	55.0	1.3	0.99	0.82						3.3
INRA_03358	B1a_0	D1a	D1b	77.0			0.01		99.0	73.7	25.3	0.00	0.00						25.3
INRA_03366	B1a_160	D1a	D1b	82.3	47.0	35.3	0.00	0.00											35.3
INRA_03402	B1b	D1a	D1a	51.3	46.3	5.0	0.97	0.47	48.0	46.0	2.0	1.00	0.73						3.5
INRA_03406	B1a_0	D1a	D1b	71.7	60.0	11.7	0.06	0.24	86.0	50.7	35.3	0.01	0.00						23.5
INRA_03414	B1b	D1a	D1b	59.7	47.5	12.2	0.80	0.12	60.3	56.0	4.3	0.98	0.45						8.3
INRA_03437	B1a_0	D1a	D1b	100.7	72.0	28.7	0.00	0.00											28.7
INRA_03442	B1a_0	D1a	D1b	70.3	60.5	9.8	0.10	0.21	101.0	50.0	51.0	0.00	0.00						30.4
INRA_03463	B1b	D1b	D1a	43.7	42.0	1.7	0.99	0.83											1.7
INRA_03485	B1a_0	D1a	D1b		74.3				113.3	89.0	24.3	0.00	0.00						24.3
INRA_03617	B1a_197	D1a	D1b	65.7	49.0	16.7	0.38	0.02	75.5	59.7	15.8	0.23	0.02	60.0	33.2	21.0	8.63	0.00	17.8
INRA_03645	B1a_0	D1a	D1b	93.3	64.3	29.0	0.00	0.00											29.0
INRA_03665	B1a_0	D1b	D1a	57.7	45.3	12.3	0.88	0.08	56.0	48.3	7.7	0.99	0.19						10.0
INRA_03696	B1a_0	D1a	D1b	99.0	61.7	37.3	0.00	0.00											37.3
INRA_03752	B1a_0	D1a	NP	110.0	41.0	69.0	0.00	0.00	53.0	48.3	4.7	1.00	0.42	72.5	33.5	30.5	7.71	0.00	34.7
INRA_03753	B1a_0	D1a	D1b	99.5	60.3	39.2	0.00	0.00											39.2
INRA_03804	B1b	D1a	D1a	65.0	58.3	6.7	0.43	0.39	63.7	61.5	2.2	0.94	0.74						4.4
INRA_03857	B1b	D1b	D1a	42.3	38.0	4.3	0.99	0.53											4.3
INRA_03896	B1a_197	D1a	D1a	77.0	49.0	28.0	0.01	0.00	78.3	52.0	26.3	0.10	0.00						27.2

Appendix VII continued

Accession[a]	Genotype[b]			GA exp. 1					GA exp. 2					GA exp. 3[h]					Overall
INRA BWCC number / control line	Rht-B1	Rht-D1	Ppd-D1	GA+ length (mm)[c]	GA- length (mm)[d]	Trt Diff (mm)[e]	MM *p* GAI[f]	LSD *p* GA trt diff = 0[g]	GA+ length (mm)	GA- length (mm)	Trt Diff (mm)	MM *p* GAI	LSD *p* GA trt diff = 0	GA+ length (mm)	GA- length (mm)	Trt Diff (corr'd) mm[i]	Trt Diff t value (calc.)	LSD *p* GA trt diff = 0	Trt diff[j]
INRA_03912	B1a_160	D1a	D1b	96.7	60.3	36.3	0.00	0.00											36.3
INRA_03942	B1a_0	D1a	D1b	88.0	66.0	22.0	0.00	0.03	94.0	69.7	24.3	0.00	0.00						23.2
INRA_03965	B1a_0	D1a	D1a	86.3	58.0	28.3	0.00	0.00	89.7	67.3	22.3	0.00	0.00						25.3
INRA_03970	B1a_160	D1a	D1b	85.7			0.00		95.2	65.5	29.7	0.00	0.00						29.7
INRA_03975	B1a_0	D1a	D1b	93.0	73.0	20.0	0.00	0.02											20.0
INRA_03991	B1a_0	D1a	D1a	82.0	45.0	37.0	0.00	0.00											37.0
INRA_04036	B1a_0	D1a	D1b		43.0				80.7	61.3	19.3	0.04	0.00						19.3
INRA_04055	B1a_0	D1a	D1b	94.3	68.0	26.3	0.00	0.00											26.3
INRA_04067	B1a_0	D1a	D1b	62.0	62.0	0.0	0.67	1.00	97.7	67.3	30.3	0.00	0.00						15.2
INRA_04105	B1a_160	D1a	D1b	85.0	70.0	15.0	0.00	0.15	90.3	58.3	32.0	0.00	0.00						23.5
INRA_04111	B1a_0	D1a	D1b	86.0	45.3	40.7	0.00	0.00											40.7
INRA_04157	B1a_0	D1a	D1b	96.5	62.0	34.5	0.00	0.00											34.5
INRA_04187	B1a_0	D1a	D1b	77.0	54.7	22.3	0.01	0.00											22.3
INRA_04194	B1a_160	D1a	D1b	92.3	68.0	24.3	0.00	0.00											24.3
INRA_04207	B1a_0	D1a	D1b	83.5	62.5	21.0	0.00	0.01											21.0
INRA_04300	B1a_160	D1a	D1b	94.0	60.5	33.5	0.00	0.00											33.5
INRA_04324	B1a_197	D1a	D1b	96.0	65.0	31.0	0.00	0.00											31.0
INRA_04343	B1a_0	D1a	D1b	104.0	70.0	34.0	0.00	0.00											34.0
INRA_04477	B1a_0	D1a	D1b	79.0	54.5	24.5	0.00	0.00											24.5
INRA_04482	B1a_0	D1a	D1b	90.5	61.0	29.5	0.00	0.01	92.3	77.0	15.3	0.00	0.01						22.4
INRA_04487	B1a_0	D1a	D1b						123.3	73.7	49.7	0.00	0.00						49.7
INRA_04492	B1a_0	D1a	D1b	84.0	55.5	28.5	0.00	0.00											28.5
INRA_04525	B1a_160	D1a	D1b	76.0	67.0	9.0	0.01	0.36	88.3	53.7	34.7	0.00	0.00						21.8
INRA_04645	B1a_0	D1a	D1b	111.0	77.7	33.3	0.00	0.00											33.3
INRA_04664	B1a_0/160 het	D1b	D1b	47.5	59.0	-11.5	0.99	1.00	58.0	52.3	5.7	0.99	0.38						-2.9

Appendix VII continued

Accession[a]	Genotype[b]			GA exp. 1					GA exp. 2					GA exp. 3[h]					Overall
INRA BWCC number / control line	Rht-B1	Rht-D1	Ppd-D1	GA+ length (mm)[c]	GA- length (mm)[d]	Trt Diff (mm)[e]	MM *p* GAI[f]	LSD *p* GA trt diff = 0[g]	GA+ length (mm)	GA- length (mm)	Trt Diff (mm)	MM *p* GAI	LSD *p* GA trt diff = 0	GA+ length (mm)	GA- length (mm)	Trt Diff (corr'd) mm[i]	Trt Diff t value (calc.)	LSD *p* GA trt diff = 0	Trt diff[j]
INRA_04670	B1a_0	D1a	D1b	77.5	63.0	14.5	0.01	0.09											14.5
INRA_04698	B1a_197	D1a	D1a	68.0	62.0	6.0	0.21	0.44	77.3	55.7	21.7	0.13	0.00	53.5	34.5	14.9	6.40	0.00	14.2
INRA_04702	B1a_0/19 7 het	D1a	D1b	91.7	58.7	33.0	0.00	0.00											33.0
INRA_04706	B1a_0	D1a	D1b	81.3	57.7	23.7	0.00	0.00	83.3	56.0	27.3	0.02	0.00						25.5
INRA_04712	B1a_0	D1a	D1b	86.5	65.3	21.2	0.00	0.01											21.2
INRA_04776	B1a_0	D1b	D1a	54.0	52.3	1.7	0.95	0.83	53.3	57.3	-4.0	1.00	1.00						-1.2
INRA_04784	B1b	D1a	D1b	60.0	47.0	13.0	0.78	0.10	57.3	46.3	11.0	0.99	0.06						12.0
INRA_04796	B1a_160	D1a	D1b	76.5	61.3	15.2	0.01	0.05	88.7	58.3	30.3	0.00	0.00						22.8
INRA_04838	B1a_0	D1a	D1b	99.0	60.7	38.3	0.00	0.00											38.3
INRA_04874	B1a_0	D1a	D1b	95.7	72.0	23.7	0.00	0.00											23.7
INRA_04901	B1a_197	D1a	D1b	108.0	69.7	38.3	0.00	0.00											38.3
INRA_04925	B1a_0	D1a	D1a	80.5	53.7	26.8	0.00	0.00											26.8
INRA_04947	B1a_0	D1a	D1b	101.3	63.5	37.8	0.00	0.00											37.8
INRA_04991	B1b	D1a	D1b	51.3	47.3	4.0	0.97	0.57											4.0
INRA_05088	B1b	D1b	D1a	44.5	43.3	1.2	0.99	0.88											1.2
INRA_05096	B1b	D1b	D1a	42.5	41.0	1.5	0.99	0.86											1.5
INRA_05102	B1a_0	D1a	D1b	92.5	72.0	20.5	0.00	0.01											20.5
INRA_05108	B1a_0	D1a	D1b	95.0	70.7	24.3	0.00	0.00											24.3
INRA_05115	B1a_0	D1a	D1a	79.0	42.3	36.7	0.00	0.00											36.7
INRA_05116	B1a_0	D1a	D1b	97.0	56.0	41.0	0.00	0.00	98.0	55.3	42.7	0.00	0.00						41.8
INRA_05120	B1a_160	D1a	D1a	75.0	47.7	27.3	0.02	0.00											27.3
INRA_05166	B1a_0	D1a	D1b	86.3	61.0	25.3	0.00	0.01											25.3
INRA_05167	B1a_0	D1a	D1b	92.3	70.3	22.0	0.00	0.00											22.0
INRA_05219	B1a_0	D1a	D1a		33.5				66.7	48.3	18.3	0.85	0.00	44.0	27.2	13.2	5.41	0.00	15.7

Appendix VII continued

Accession[a]	Genotype[b]			GA exp. 1					GA exp. 2					GA exp. 3[h]					Overall
INRA BWCC number / control line	Rht-B1	Rht-D1	Ppd-D1	GA+ length (mm)[c]	GA- length (mm)[d]	Trt Diff (mm)[e]	MM *p* GAI[f]	LSD *p* GA trt diff = 0[g]	GA+ length (mm)	GA- length (mm)	Trt Diff (mm)	MM *p* GAI	LSD *p* GA trt diff = 0	GA+ length (mm)	GA- length (mm)	Trt Diff (corr'd) mm[i]	Trt Diff t value (calc.)	LSD *p* GA trt diff = 0	Trt diff[j]
INRA_05250	B1a_160	D1a/b het	D1a	77.7	55.0	22.7	0.01	0.00	66.0	64.0	2.0	0.88	0.76						12.3
INRA_05260	B1a_0	D1b	D1a	40.0	38.3	1.7	1.00	0.81											1.7
INRA_05266	B1a/b het	D1a/b het	D1a	61.0	38.3	22.7	0.73	0.00	40.7	41.0	-0.3	1.00	1.00						11.2
INRA_05293	B1a_160	D1a	D1b	80.0	51.0	29.0	0.00	0.00	85.0	49.7	35.3	0.01	0.00						32.2
INRA_05308	B1a_0	D1a	D1b	91.0			0.00		103.7	73.0	30.7	0.00	0.00						30.7
INRA_05399	B1a_0	D1a	D1b	73.0	56.0	17.0	0.04	0.03	83.3	64.0	19.3	0.02	0.00						18.2
INRA_05401	B1a_0	D1b	D1a	49.3	39.7	9.7	0.98	0.17	52.0	44.3	7.7	1.00	0.19						8.7
INRA_05415	B1a_0	D1a	D1b	98.7	71.0	27.7	0.00	0.00											27.7
INRA_05419	B1a_197	D1a	D1b	91.0	68.0	23.0	0.00	0.01											23.0
INRA_05421	B1a_0	D1a	D1b	93.0	80.7	12.3	0.00	0.08	99.7	82.3	17.3	0.00	0.00						14.8
INRA_05425	B1a_0	D1a	D1b	95.5	60.7	34.8	0.00	0.00											34.8
INRA_05438	B1a_0	D1b	D1a	51.0	49.0	2.0	0.97	0.80											2.0
INRA_05448	B1b	D1a	D1a	55.0	47.0	8.0	0.93	0.44	65.3	54.7	10.7	0.90	0.07						9.3
INRA_05486	B1a_0	D1a	D1b	90.0	61.0	29.0	0.00	0.00	103.5	64.0	39.5	0.00	0.00						34.3
INRA_05501	B1b	D1a	D1a	52.0	54.5	-2.5	0.97	1.00	59.7	62.7	-3.0	0.98	1.00						-2.8
INRA_05536	B1a_0	D1a	D1b	98.7	59.3	39.3	0.00	0.00											39.3
INRA_05552	B1a_0	D1a	D1b	77.0	68.7	8.3	0.01	0.29	87.7	65.3	22.3	0.00	0.00						15.3
INRA_05558	B1b	D1a	D1b	63.0	52.7	10.3	0.59	0.14	62.3	51.3	11.0	0.96	0.06						10.7
INRA_05636	B1a_0	D1b	D1a	63.3	50.3	13.0	0.57	0.06	60.3	49.0	11.3	0.98	0.05						12.2
INRA_05702	B1a_0	D1a	D1b	99.7	60.0	39.7	0.00	0.00											39.7
INRA_05748	B1a_0	D1b	D1a	52.0	52.5	-0.5	0.97	1.00											-0.5
INRA_05773	B1a_0	D1a	D1b	96.5	66.0	30.5	0.00	0.00	107.3	73.7	33.7	0.00	0.00						32.1
INRA_05816	B1a_197	D1a	D1b	87.5	71.7	15.8	0.00	0.04											15.8
INRA_05821	B1a_0	D1a	D1b	78.5	60.0	18.5	0.00	0.08	84.3	55.7	28.7	0.01	0.00						23.6

Appendix VII continued

Accession[a]	Genotype[b]			GA exp. 1					GA exp. 2					GA exp. 3[h]					Overall
INRA BWCC number / control line	Rht-B1	Rht-D1	Ppd-D1	GA+ length (mm)[c]	GA- length (mm)[d]	Trt Diff (mm)[e]	MM *p* GAI[f]	LSD *p* GA trt diff = 0[g]	GA+ length (mm)	GA- length (mm)	Trt Diff (mm)	MM *p* GAI	LSD *p* GA trt diff = 0	GA+ length (mm)	GA- length (mm)	Trt Diff (corr'd) mm[i]	Trt Diff t value (calc.)	LSD *p* GA trt diff = 0	Trt diff[j]
INRA_05897	B1a_0	D1b	D1b	60.7	50.0	10.7	0.75	0.13	60.7	55.7	5.0	0.97	0.39						7.8
INRA_06027	B1b	D1a	D1a	60.0	41.0	19.0	0.78	0.07	61.7	48.0	13.7	0.97	0.02						16.3
INRA_06047	B1a_0	D1a	D1b	84.7	67.0	17.7	0.00	0.01											17.7
INRA_06086	B1b	D1a	D1b	60.7	48.5	12.2	0.75	0.12	53.0	47.3	5.7	1.00	0.33						8.9
INRA_06170	B1a_0	D1a	D1b	63.0	55.5	7.5	0.59	0.47	96.3	62.7	33.7	0.00	0.00						20.6
INRA_06191	B1a_197	D1a	D1b	92.0	45.0	47.0	0.00	0.00											47.0
INRA_06198	B1a_0	D1a	D1b	84.7	72.0	12.7	0.00	0.20	104.0	86.0	18.0	0.00	0.00	61.0	45.3	12.2	5.27	0.00	14.3
INRA_06308	B1a_0	D1a	D1b	89.3	59.7	29.7	0.00	0.00											29.7
INRA_06318	B1a_0	D1a	D1b	91.3	67.5	23.8	0.00	0.00											23.8
INRA_06396	B1a_0	D1a	D1a	65.0			0.43		77.7	57.0	20.7	0.12	0.00	54.3	36.2	14.2	5.83	0.00	17.4
INRA_06522	B1a_0	D1b	D1a	57.7	44.0	13.7	0.88	0.08	47.7	54.7	-7.0	1.00	1.00						3.3
INRA_06529	B1a_0	D1a	D1b	74.7	57.0	17.7	0.02	0.07	79.0	67.0	12.0	0.08	0.17	62.0	29.8	25.1	10.83	0.00	18.3
INRA_06575	B1a_0	D1b	D1a	41.0	36.5	4.5	1.00	0.56											4.5
INRA_06605	B1a_197	D1a	D1b	84.3	60.7	23.7	0.00	0.00											23.7
INRA_06740	B1a_160	D1a	D1b	70.7	55.7	15.0	0.09	0.03	88.0	63.0	25.0	0.00	0.00						20.0
INRA_06843	B1a_0	D1a	D1b	65.5	54.0	11.5	0.39	0.27	88.3	55.3	33.0	0.00	0.00						22.3
INRA_06846	B1a_0	D1a	D1b	105.0	63.0	42.0	0.00	0.00											42.0
INRA_06922	B1a_197	D1a	D1a	77.3	61.0	16.3	0.01	0.04						57.5	24.3	25.9	11.17	0.00	21.1
INRA_06986	B1_197	D1a	D1b	34.0	35.0	-1.0	1.00	1.00	27.3	31.0	-3.7	1.00	1.00						-2.3
INRA_07011	B1a_0	D1a	D1b	85.5	60.0	25.5	0.00	0.00											25.5
INRA_07026	B1a_0	D1a	D1b	66.3	55.5	10.8	0.33	0.17	90.7	63.3	27.3	0.00	0.00						19.1
INRA_07040	B1a_0	D1a	D1b	104.0	67.7	36.3	0.00	0.00											36.3
INRA_07048	B1a_0	D1a	D1b	89.0	57.0	32.0	0.00	0.00											32.0
INRA_07085	B1a_0	D1a	D1a	72.5	51.0	21.5	0.05	0.04	75.0	57.3	17.7	0.26	0.00	55.8	28.0	21.8	9.37	0.00	20.3
INRA_07092	B1b	D1a	D1b	64.5	41.7	22.8	0.47	0.00	62.7	53.7	9.0	0.95	0.12						15.9
INRA_07117	B1a_197	D1a	D1b	89.5	64.0	25.5	0.00	0.00											25.5

Appendix VII continued

Accession[a]	Genotype[b]			GA exp. 1					GA exp. 2					GA exp. 3[h]					Overall
INRA BWCC number / control line	Rht-B1	Rht-D1	Ppd-D1	GA+ length (mm)[c]	GA- length (mm)[d]	Trt Diff (mm)[e]	MM *p* GAI[f]	LSD *p* GA trt diff = 0[g]	GA+ length (mm)	GA- length (mm)	Trt Diff (mm)	MM *p* GAI	LSD *p* GA trt diff = 0	GA+ length (mm)	GA- length (mm)	Trt Diff (corr'd) mm[i]	Trt Diff t value (calc.)	LSD *p* GA trt diff = 0	Trt diff[j]
INRA_07166	B1a_0	D1a	D1b	102.0	62.5	39.5	0.00	0.00	99.3	63.0	36.3	0.00	0.00						37.9
INRA_07276	B1a_0	D1a	D1b	92.0	62.3	29.7	0.00	0.00											29.7
INRA_07279	B1a_197	D1a	D1b	82.5	55.0	27.5	0.00	0.00											27.5
INRA_07490	B1a_0	D1a	D1b	76.5	55.0	21.5	0.01	0.01											21.5
INRA_07585	B1a_197	D1a	D1b	96.0	55.7	40.3	0.00	0.00											40.3
INRA_07848	B1a_0	D1b	D1b	53.3	47.0	6.3	0.96	0.52	53.3	55.0	-1.7	1.00	1.00						2.3
INRA_07965	B1a_0	D1a	D1b	89.0	54.0	35.0	0.00	0.00											35.0
INRA_07968	B1a_0	D1a	D1b	101.0	69.0	32.0	0.00	0.00											32.0
INRA_07973	B1a_0	D1a	D1a	111.3	77.3	34.0	0.00	0.00											34.0
INRA_07988	B1a_160	D1a	D1b	97.0	61.0	36.0	0.00	0.00	110.0	69.3	40.7	0.00	0.00						38.3
INRA_08011	B1a_160	D1a	D1b	75.3	73.0	2.3	0.02	0.81						60.8	35.8	19.6	8.05	0.00	11.0
INRA_08048	B1a_0	D1a	D1b	89.7	60.3	29.3	0.00	0.00											29.3
INRA_08051	B1a_0	D1a	D1b	114.3	69.0	45.3	0.00	0.00											45.3
INRA_08058	B1a_160	D1a	D1b	92.0	60.0	32.0	0.00	0.00	82.7	66.3	16.3	0.02	0.01						24.2
INRA_08073	B1a_0	D1a	D1b	66.7	62.5	4.2	0.30	0.59	86.7	56.7	30.0	0.00	0.00						17.1
INRA_08079	B1a_0	D1a	D1b	84.0	54.5	29.5	0.00	0.00											29.5
INRA_08097	B1a_0	D1a	D1b	95.0	64.7	30.3	0.00	0.00											30.3
INRA_08113	B1a_0	D1a	D1a	100.5	64.0	36.5	0.00	0.00											36.5
INRA_08165	B1a_160	D1a	D1a	77.3	52.5	24.8	0.01	0.00											24.8
INRA_08170	B1b	D1b	D1a	53.7	46.7	7.0	0.95	0.32	49.7	46.7	3.0	1.00	0.60						5.0
INRA_08194	B1b	D1a	D1a	65.3	47.0	18.3	0.40	0.01	60.0	40.3	19.7	0.98	0.00						19.0
INRA_08197	B1b	D1a	D1a	70.3	57.7	12.7	0.10	0.07	67.7	63.0	4.7	0.81	0.42						8.7
INRA_08227	B1b	D1a	D1a	45.3	34.5	10.8	0.99	0.17	45.7	44.7	1.0	1.00	0.86						5.9
INRA_08233	B1a_0	D1a	D1b	71.7	55.7	16.0	0.06	0.02	87.5	59.3	28.2	0.00	0.00						22.1
INRA_08254	B1a_0	D1a	D1b	95.7	64.7	31.0	0.00	0.00											31.0
INRA_08276	B1a_160	D1a	D1b	76.3	62.3	14.0	0.01	0.05						54.8	35.5	15.1	6.51	0.00	14.6

Appendix VII continued

Accession[a]	Genotype[b]			GA exp. 1					GA exp. 2					GA exp. 3[h]					Overall
INRA BWCC number / control line	Rht-B1	Rht-D1	Ppd-D1	GA+ length (mm)[c]	GA- length (mm)[d]	Trt Diff (mm)[e]	MM *p* GAI[f]	LSD *p* GA trt diff = 0[g]	GA+ length (mm)	GA- length (mm)	Trt Diff (mm)	MM *p* GAI	LSD *p* GA trt diff = 0	GA+ length (mm)	GA- length (mm)	Trt Diff (corr'd) mm[i]	Trt Diff t value (calc.)	LSD *p* GA trt diff = 0	Trt diff[j]
INRA_08280	B1a_197	D1a	NP	92.3	57.0	35.3	0.00	0.00											35.3
INRA_08287	B1a_197	D1b	D1b	53.7	49.0	4.7	0.95	0.55											4.7
INRA_08289	B1a_160	D1b	D1b	49.7	46.7	3.0	0.98	0.67											3.0
INRA_08513	B1b	D1b	D1a	42.7	42.0	0.7	0.99	0.92											0.7
INRA_08578	B1b	D1a	D1a	71.5	59.7	11.8	0.07	0.13	77.0	72.0	5.0	0.15	0.56						8.4
INRA_09024	B1a_160	D1b	D1b	41.7	43.7	-2.0	0.99	1.00											-2.0
INRA_09048	B1a_0	D1a	D1b	82.0	61.5	20.5	0.00	0.05	90.0	54.0	36.0	0.00	0.00						28.3
INRA_09077	B1a_160	D1a	D1b		31.0				87.7	59.0	28.7	0.00	0.00						28.7
INRA_09087	B1a_160	D1a	D1b	87.3	57.5	29.8	0.00	0.00											29.8
INRA_13210	B1b	D1a	D1a	63.7	48.7	15.0	0.54	0.03	59.3	48.5	10.8	0.98	0.10						12.9
INRA_13282	B1a_197	D1a	D1a	70.3	53.7	16.7	0.10	0.02	82.7	52.7	30.0	0.02	0.00						23.3
INRA_13286	B1a_0	D1a	D1b	102.0	71.5	30.5	0.00	0.00	112.0	82.0	30.0	0.00	0.00						30.3
INRA_13292	B1a_0	D1a	D1b	94.0	69.0	25.0	0.00	0.02	103.3	73.0	30.3	0.00	0.00						27.7
INRA_13310	B1a_160	D1a	D1b	90.3	70.3	20.0	0.00	0.00											20.0
INRA_13436	B1a_0	D1a	D1b	90.7	63.7	27.0	0.00	0.00											27.0
INRA_13440	B1a_0	D1a	D1b	86.0	53.5	32.5	0.00	0.00											32.5
INRA_13445	B1b	D1a	D1a	60.0	48.7	11.3	0.78	0.11											11.3
INRA_13454	B1a_197	D1b	D1b	46.3	43.0	3.3	0.99	0.63											3.3
INRA_13461	B1a_160	D1b	D1b	52.0	50.0	2.0	0.97	0.80											2.0
INRA_13471	B1b	D1a	D1b	62.3	52.0	10.3	0.64	0.19	54.7	49.7	5.0	1.00	0.39						7.7
INRA_13476	B1b	D1a	D1a	64.5	45.0	19.5	0.47	0.01	58.3	49.0	9.3	0.99	0.11						14.4
INRA_13481	B1a_160	D1a	D1a	70.7	50.0	20.7	0.09	0.00	64.0	52.7	11.3	0.93	0.05	52.3	30.0	17.5	7.52	0.00	16.5
INRA_13494	B1a_197	D1b	D1a	43.5	37.3	6.2	0.99	0.43	44.3	41.3	3.0	1.00	0.60						4.6
INRA_13500	B1a_197	D1b	D1b	50.0	45.3	4.7	0.98	0.55											4.7
INRA_13502	B1a_0	D1b	D1a	45.7	37.0	8.7	0.99	0.27	53.0	46.7	6.3	1.00	0.27						7.5
INRA_13642	B1a_0	D1a	D1b	77.0	51.3	25.7	0.01	0.00											25.7

Appendix VII continued

Accession[a]	Genotype[b]			GA exp. 1					GA exp. 2					GA exp. 3[h]					Overall
INRA BWCC number / control line	Rht-B1	Rht-D1	Ppd-D1	GA+ length (mm)[c]	GA- length (mm)[d]	Trt Diff (mm)[e]	MM *p* GAI[f]	LSD *p* GA trt diff = 0[g]	GA+ length (mm)	GA- length (mm)	Trt Diff (mm)	MM *p* GAI	LSD *p* GA trt diff = 0	GA+ length (mm)	GA- length (mm)	Trt Diff (corr'd) mm[i]	Trt Diff t value (calc.)	LSD *p* GA trt diff = 0	Trt diff[j]
INRA_13781	B1a_0	D1a	D1a	89.0	60.0	29.0	0.00	0.00	88.3	60.7	27.7	0.00	0.00						28.3
INRA_13792	B1a_160	D1a	D1b	96.3	54.5	41.8	0.00	0.00											41.8
INRA_13799	B1a_0	D1a	D1a	72.7	48.0	24.7	0.04	0.00											24.7
INRA_13800	B1a_0	D1a	D1b	96.0	61.5	34.5	0.00	0.00	93.3	63.3	30.0	0.00	0.00						32.3
INRA_13811	B1b	D1a	D1b	72.5	48.0	24.5	0.05	0.00	71.3	54.3	17.0	0.55	0.00						20.8
INRA_13812	B1b	D1a	450bp	75.0	58.0	17.0	0.02	0.09	77.3	66.0	11.3	0.13	0.05						14.2
INRA_13861	B1a_197	D1b	D1b	45.7	37.5	8.2	0.99	0.29	48.7	40.0	8.7	1.00	0.13						8.4
INRA_13870	B1a_197	D1b	D1b	52.7	47.3	5.3	0.96	0.44	57.0	48.7	8.3	0.99	0.15						6.8
INRA_13978	B1a_0	D1a	D1b	82.3	31.0	51.3	0.00	0.00	97.5	72.3	25.2	0.00	0.00						38.3
INRA_14000	B1a_160	D1a	D1b	84.3	47.3	37.0	0.00	0.00											37.0
INRA_14011	B1a_160	D1a	D1a	69.7	42.0	27.7	0.13	0.00	78.3	55.3	23.0	0.10	0.00						25.3
INRA_15606	B1a_0	D1a	D1b	89.3	65.3	24.0	0.00	0.00											24.0
INRA_15652	B1a_0	D1a	D1b	84.7	64.7	20.0	0.00	0.00											20.0
INRA_15658	B1a_0	D1a	D1b	105.0	66.3	38.7	0.00	0.00											38.7
INRA_15710	B1a_0	D1a	D1b	91.7	58.3	33.3	0.00	0.00											33.3
INRA_15809	B1a_0	D1a	D1b	88.7	58.0	30.7	0.00	0.00											30.7
INRA_15950	B1a_197	D1b	D1b	44.3	37.7	6.7	0.99	0.34	44.7	40.0	4.7	1.00	0.42						5.7
INRA_15954	B1a_160	D1a	D1a	74.3	41.0	33.3	0.02	0.00											33.3
INRA_20074	B1a_0	D1b	D1b	54.3	48.7	5.7	0.94	0.42	51.0	49.3	1.7	1.00	0.77						3.7
INRA_20224	B1a_0	D1b	D1a	76.0	46.5	29.5	0.01	0.00	61.3	50.0	11.3	0.97	0.05						20.4
INRA_20276	B1a_197	D1b	D1b	59.0	44.0	15.0	0.83	0.06	49.0	50.7	-1.7	1.00	0.00						6.7
INRA_20384	B1b	D1a	D1b	55.7	48.5	7.2	0.92	0.36	58.3	50.3	8.0	0.99	0.17						7.6
INRA_20417	B1a_160	D1b	D1b	48.3	49.0	-0.7	0.98	1.00											-0.7
INRA_23891	B1a_0	D1a	D1b	78.0	63.0	15.0	0.00	0.03											15.0
INRA_23896	B1a_0	D1a	D1b	97.5	72.7	24.8	0.00	0.00											24.8
INRA_23902	B1a_0	D1a	NP	97.0	75.3	21.7	0.00	0.00											21.7

Appendix VII continued

Accession[a]	Genotype[b]			GA exp. 1					GA exp. 2					GA exp. 3[h]					Overall
INRA BWCC number / control line	Rht-B1	Rht-D1	Ppd-D1	GA+ length (mm)[c]	GA- length (mm)[d]	Trt Diff (mm)[e]	MM *p* GAI[f]	LSD *p* GA trt diff = 0[g]	GA+ length (mm)	GA- length (mm)	Trt Diff (mm)	MM *p* GAI	LSD *p* GA trt diff = 0	GA+ length (mm)	GA- length (mm)	Trt Diff (corr'd) mm[i]	Trt Diff t value (calc.)	LSD *p* GA trt diff = 0	Trt diff[j]
INRA_23909	B1a_0	D1a	D1b	97.0	56.3	40.7	0.00	0.00											40.7
INRA_23923	B1a_0	D1a	D1b	89.0	54.7	34.3	0.00	0.00											34.3
INRA_23933	B1a_0	D1a	D1b	114.0	81.0	33.0	0.00	0.01	113.8	64.8	49.0	0.00	0.00						41.0
INRA_23934	B1a_0	D1a	D1b	86.0	66.0	20.0	0.00	0.02											20.0
INRA_23937	B1a_0	D1a	D1b	98.5	76.5	22.0	0.00	0.01											22.0
INRA_23944	B1a_0	D1a	D1b	89.7	57.0	32.7	0.00	0.00	105.7	67.3	38.3	0.00	0.00						35.5
INRA_23945	B1a_0	D1a	D1b	95.3	65.5	29.8	0.00	0.00											29.8
INRA_23950	B1a_160	D1a	D1b	77.5	54.0	23.5	0.01	0.03	91.7	66.3	25.3	0.00	0.00						24.4
INRA_23957	B1a_0	D1a	D1b	87.3	65.0	22.3	0.00	0.00											22.3
INRA_23960	B1a_0	D1a	D1b	88.0	70.0	18.0	0.00	0.09	113.3	77.7	35.7	0.00	0.00						26.8
INRA_23964	B1a_0	D1a	D1b	97.5	65.0	32.5	0.00	0.00											32.5
INRA_23970	B1a_0	D1a	D1b		59.0				102.0	66.8	35.2	0.00	0.00						35.2
INRA_23971	B1a_0	D1a	D1b	113.3	83.7	29.7	0.00	0.00											29.7
INRA_23974	B1a_0	D1a	D1b	96.3	77.0	19.3	0.00	0.01											19.3
INRA_23977	B1a_0	D1a	D1b	84.0	71.5	12.5	0.00	0.14	94.7	71.3	23.3	0.00	0.00						17.9
INRA_23981	B1a_0	D1a	D1b	89.0	60.0	29.0	0.00	0.02	100.3	69.7	30.7	0.00	0.00						29.8
INRA_23989	B1a_0	D1a	NP	72.7	58.3	14.3	0.04	0.04	94.7	66.3	28.3	0.00	0.00						21.3
INRA_23995	B1a_0	D1a	D1b	94.5	72.7	21.8	0.00	0.01											21.8
INRA_23996	B1a_0	D1a	D1b	94.5	56.5	38.0	0.00	0.00	105.7	75.3	30.3	0.00	0.00						34.2
INRA_24003	B1a_0	D1a	D1b	88.0	63.0	25.0	0.00	0.00											25.0
INRA_24006	B1a_0	D1a	D1b	85.0	42.0	43.0	0.00	0.00											43.0
INRA_24019	B1a_160	D1a	D1b	103.3	68.0	35.3	0.00	0.00	107.7	73.7	34.0	0.00	0.00						34.7
INRA_24031	B1a_160	D1a	D1b	84.7	54.7	30.0	0.00	0.00											30.0
INRA_24054	B1a_0	D1a	D1b	80.0	53.3	26.7	0.00	0.00											26.7
INRA_24056	B1a_0	D1a	D1b	108.0	63.0	45.0	0.00	0.00	103.7	76.0	27.7	0.00	0.00						36.3
INRA_24058	B1a_0	D1a	D1b	85.3	57.7	27.7	0.00	0.00											27.7

Appendix VII continued

Accession[a]	Genotype[b]			GA exp. 1					GA exp. 2					GA exp. 3[h]					Overall
INRA BWCC number / control line	Rht-B1	Rht-D1	Ppd-D1	GA+ length (mm)[c]	GA- length (mm)[d]	Trt Diff (mm)[e]	MM *p* GAI[f]	LSD *p* GA trt diff = 0[g]	GA+ length (mm)	GA- length (mm)	Trt Diff (mm)	MM *p* GAI	LSD *p* GA trt diff = 0	GA+ length (mm)	GA- length (mm)	Trt Diff (corr'd) mm[i]	Trt Diff t value (calc.)	LSD *p* GA trt diff = 0	Trt diff[j]
INRA_24066	B1a_0	D1a	D1b	88.0	61.7	26.3	0.00	0.00											26.3
INRA_24075	B1a_0	D1a	D1b	89.7	67.0	22.7	0.00	0.02	112.7	69.0	43.7	0.00	0.00						33.2
INRA_24080	B1a_0	D1a	D1b	92.3	63.0	29.3	0.00	0.00											29.3
INRA_24089	B1a_0	D1a	NP	94.7	64.3	30.3	0.00	0.00											30.3
INRA_24108	B1a_160	D1a	D1b	81.0	43.0	38.0	0.00	0.00											38.0
INRA_24180	B1a_0	D1a	D1b	98.0	67.0	31.0	0.00	0.00	108.0	71.7	36.3	0.00	0.00						33.7
INRA_24184	B1a_0	D1a	D1b	88.0	61.0	27.0	0.00	0.00											27.0
INRA_24185	B1a_0	D1a	D1b	97.0	67.0	30.0	0.00	0.00											30.0
INRA_24186	B1a_0	D1a	D1b	90.5	61.3	29.2	0.00	0.00											29.2
INRA_24193	B1a_0	D1a	NP	78.0	51.5	26.5	0.00	0.00											26.5
INRA_24196	B1a_0	D1a	D1b	105.3	69.5	35.8	0.00	0.00											35.8
INRA_24209	B1a_0	D1a	D1b	122.7	77.0	45.7	0.00	0.00											45.7
INRA_24210	B1a_160	D1a	D1b	83.5	57.0	26.5	0.00	0.00											26.5
AB	B1a	D1a	D1b	88.1	57.3	30.8	0.00	0.00	93.9	63.7	30.2	0.00	0.00	61.0	36.5	19.2	4.84	0.00	26.7
AB_Rht-D1b	B1a	D1b	D1b	55.5	51.8	3.7	0.93	0.48	54.9	52.2	2.7	1.00	0.49	45.5	37.5	6.3	1.58	0.13	4.2
AB_Rht-B1c	B1_197	D1a	D1b	29.3	27.0	2.3	1.00	0.66	28.6	26.2	2.3	1.00	0.55	28.0	26.0	1.6	0.40	0.70	2.1
Bersee	B1a	D1a	D1b	94.1	64.6	29.4	0.00	0.00	95.6	69.8	25.8	0.00	0.00	72.5	36.0	28.5	7.21	0.00	27.9
Bersee_Rht-B1b	B1b	D1a	D1b	70.8	61.8	9.0	0.09	0.09	68.1	58.3	9.8	0.78	0.02	59.0	36.5	17.6	4.45	0.00	12.1
Mercia	B1a	D1a	D1b	81.6	57.4	24.2	0.00	0.00	90.4	60.7	29.8	0.00	0.00	60.0	29.5	23.8	6.03	0.00	26.0
Mercia_Rht-D1b	B1a	D1b	D1b	49.2	46.7	2.6	0.98	0.62	46.4	45.0	1.4	1.00	0.71	35.5	29.0	5.1	1.28	0.21	3.0

[a] Accession information on the INRA BWCC (Bread wheat core collection) received from F. Balfourier, INRA. Control lines are also included.
[b] Genotyping was performed using DNA from the INRA BWCC outdoor experiment, which consisted of two separate bulks of four plants for fixed lines and four individual plants for landraces. Heterozygotes (het) are shown with segregating alleles separated by a "/". NP = no product.
[c] GA+ length = Seed-to-first-ligule (STFL) length in the GA+ treatment
[d] GA- length = STFL length in the GA- treatment
[e] Treatment difference (Trt Diff) = GA+ length minus GA- length.

Appendix VII continued

[f] Mixed model (MM) probability of accessions belonging to the GA insensitive (pGAI) are shown with pGAI ≥ 0.95 shown in blue background and pGAI ≤ 0.05 is shown in orange background. Probability GAI ≤ 0.05 is equivalent to a probability of ≥ 0.95 of a GA sensitive classification.
[g] the probability of no treatment difference (p GA trt diff = 0) is shown for each accession. Probabilities ≤ 0.05 are shown in orange background, which is indicative of a GA sensitive accession.
[h] MM probabilities were not calculated for GA exp. 3 due to the lack of a bi-modal distribution of the GA+ STFL lengths.
[i] For GA exp 3, the mean GA treatment difference among controls was greater than in GA exp. 1 and 2. The GA trt diff value of each accession was multiplied by 0.7818 to allow for comparisons to GA exp. 1 and 2.
[j] Overall trt diffs are taken as the average of GA exp. 1 trt diff, GA exp. 2 trt diff, and GA exp. 3 trt diff (corr'd).