Understanding the developmental basis of grain yield potential in bread wheat

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Abstract

Grain yield potential in wheat is a complex trait controlled by sub-traits like grain number, grain size, and assimilate partitioning. To achieve further understanding of grain yield potential, this thesis combines physiological and genetic dissection of wheat development. In particular, how the length of developmental phases can be optimized in favour of increased partitioning of assimilates to the spike, in order to reduce abortion rates of florets.

The physiological section of the thesis (Chapter 2 and 3) comprises the in depth study of the CIMCOG panel (CIMMYT Core Germplasm). In this section the variation in patterns of floret and phenological development was determined, ascertaining how these differences affect the number of fertile florets. The differences in floret development were clear in the intermediate florets (floret primordia 3, 4, and 5 from the rachis). Floret survival was found to be positively related to the length of the period of floret development. Also fruiting efficiency, using the frontier concept, show a positive relationship with the stem elongation period.

The genetic section (Chapter 4 and 5) involves the use of quantitative trait locus (QTL) analysis with a segregating population to determine the chromosomal locations affecting key developmental traits. A Buster x Charger doubled haploid population provided a crucial contrast between similar genetic background and differences in length of phenological phases. The results show that a QTL on chromosome 7A has an effect on the time to terminal spikelet phase, i.e. the onset of stem elongation, and QTL affecting time to heading was found on chromosomes 2D and 4A. Furthermore, this population also varies in lodging resistance. A major QTL was found on chromosome 2D affecting height and providing lodging resistance.

These studies provide the basis to optimise wheat developmental patterns and, therefore, maximize spike fertility.

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1. Introduction

Wheat, the most widely grown crop, is an essential component of global food security. It provides one-fifth of the total calories consumed by the world's population. Keeping up with the demand has not been an easy task. Since the 1960s, increases in productivity have been achieved by the adoption of technologies, better agronomic practices, and genetic gains developed during the Green Revolution (Evenson and Gollin 2003; Fischer and Stockman 1986; Sayre et al. 1997; Shearman et al. 2005). The challenge of increasing wheat yield is still substantial; with an estimate world population of 9 billion expected to be reached by year 2050, on top of the effects of climate change (Lobell et al. 2008).

There is no doubt that increasing wheat yield is of major importance for global food security, and in that same level of socio-economical and political complexity as is food security, sits yield. The yield harvested by farmers is rarely identical to the yield reported by research centres; i.e. agronomical practices known to improve yield, like optimum crop rotation, are rarely applied as market demand forces them to focus on the most profitable sub set of species. Smaller farmers would minimize or avoid the use of irrigation systems and fertilization due to the increased cost of production compared to the potential income. On the other hand, bigger farmers or consortiums tend to reduce cost by reducing human workforce in favour of bigger and heavier agricultural machinery that increase soil compaction, creating a penalty in yield. So, in all agricultural systems the agronomy applied is a compromise based on economic considerations of which final crop yield is just one.

Historically, the biggest improvement in wheat production happened during the first half of the 20th century; attributed to an increase in harvested area. Soon after, when further increases in agricultural land became less possible, the green revolution provided the massive increase in yield needed to match the increased demand from a population growing faster than ever (saving countless lives from famine). That success in raising wheat production was due to both: genetic gains (yield potential and tolerance to stress) and agronomic gains (mainly associated with a much higher use of fertilisers and pesticides) in similar proportions (e.g. Slafer and Andrade, 1991; Evans and Fischer, 1999). Although the increase in use of agrochemicals was relevant to increase yields in the past, it also brought about environmental problems and it seems largely unlikely that future gains in yield will be possible from further increasing the use of resources. In this scenario, to match the still growing demand, genetic gains -that were critical in the green revolution- will be even more critical as

the growing area cannot increase very much and crop management is expected to be implemented considering sustainability and environmental issues (Connor and Mínguez 2012; Albajes et al. 2013). In other words, 'future agricultural growth will be more reliant than ever on raising yields' (Fischer et al. 2011). Increases in yield potential were critical in the past (across a huge range of yielding regions), and will be critical in the future, because it is the (i) only alternative for regions where actual yields are close to the potential, and (ii) a relevant alternative for other regions in which the crop is subjected to stresses and exhibit noticeable yield gaps (as there is evidence indicating that increasing yield potential would concomitantly increase actual yields under a very wide range of non-potential growing conditions; (Calderini and Slafer 1999; Richards et al. 2002; Araus et al. 2002; Reynolds and Borlaug 2006). The reason for the observed parallelism between potential and actual yield trends (Abeledo et al. 2003) may be due to the fact that cultivars with improved yield potential would be more efficient in acquiring/using available resources and consequently they regularly outyield their predecessors at the same level of inputs (Calderini and Slafer 1999; Van Wart et al. 2013). In terms of farm economics, the cost of wheat seed is very low, so any gains in yield resulting from better genetics carries little or no penalty in terms of extra investment required, in contrast to most agronomic measures- extra agrochemicals, more labour, new equipment.

1.1. Project description

The International Maize and Wheat Improvement Centre (CIMMYT) consulted crop experts regarding food security and wheat improvement challenges. In November 2009 the first Wheat Yield Consortium (WYC) meeting occurred, leading to synergies of ongoing research with the common aim of raising wheat yield potential (Reynolds et al. 2011a). Without a doubt every crop land in the world will need to increase the current yield level in order to feed future generations. The common aim allowed the creation of a structured list of subprojects (see Table 1). This thesis is the result of the work conducted under SP2.2.

Yield potential, by definition, is the yield of a cultivar when grown with no stress (e.g. nutrient, water, pests, diseases, weeds, lodging, etc.) in an environment to which it is adapted (Evans and Fischer 1999b). Improvements in yield potential are a significant component for increasing yield as this represent a relevant benchmark for crop systems (van Ittersum et al. 2013).

Crop management also plays a pivotal role in feeding the world (Reynolds et al. 2009). Some regions have a low farm yield average leading to yield gap. To tackle this, research programs like conservation agriculture lead by Dr. Bram Goaverts work in combination with the WYC under the Sustainable Modernization of Traditional Agriculture program (MasAgro) intend to help farmers by obtaining higher and more stable yields in wheat and maize, using the genetic resources of CIMMYT (see http://www.masagro.mx). The combination of efforts from experts around the world to increase yield potential and transfer of technology makes the project as a whole an unprecedented effort from industries, governments, and scientific community to fight current and future global hunger.

Table 1. List of subprojects (SP) structure of the Wheat Yield Consortium from Reynolds et al. (2011a).

Theme 1. Increasing photosynthetic capacity and efficiency	
SP1.1	Phenotypic selection for photosynthetic capacity and efficiency
SP1.2	Capturing the photosynthetic potential of spikes
SP1.3	Optimizing canopy photosynthesis and photosynthetic duration
SP1.4	Chloroplast CO_2 pumps
SP1.5	Optimizing RuBP regeneration
SP1.6	Improving the thermal stability of Rubisco activase
SP1.7	Replacement of large subunit Rubisco
Theme 2. Optimizing partitioning to grain while maintaining lodging resistance	
SP2.1	Optimizing harvest index through increasing partitioning to spike growth and maximizing grain number
SP2.2	Optimizing developmental pattern to maximize spike fertility
SP2.3	Improving spike fertility through modifying its sensitivity to environmental cues
SP2.4	Improving grain filling and potential grain size
SP2.5	Identifying traits and developing genetic sources for lodging resistance
SP2.6	Modelling optimal combinations of, and trade-offs between, traits
Theme 3. Breeding to accumulate yield potential traits	
SP3.1	Trait- and marker-based breeding
SP3.2	Wide crossing to enhance photosynthetic capacity
SP3.3	Genomic selection to increase breeding efficiency
SP3.4	Germplasm evaluation and delivery

1.1.1. Theme 1. Increasing photosynthetic capacity and efficiency

The work of Ainsworth and Long (2005) showed that, at least, crops like wheat and rice have the potential to increase crop yields by increasing their photosynthetic capacity and efficiency; They demonstrated this carrying CO2-enrichment field trial, where yield increases were obtained. Current efforts under Theme 1 have resulted in the identification of wheat germplasm with variation in photosynthetic capacity and efficiency, and establishing genetic variation in elite wheat germplasm (Furbank et al. 2013).

1.1.2. Theme 2. Optimizing partitioning to grain while maintaining lodging resistance

Photosynthetic improvement (Theme 1) need an optimization of the assimilates partitioning to convey increased in yield (Foulkes et al. 2011a). Theme 2 main focus is on partitioning traits, this are being analysed with emphasis in dry matter allocation and fruiting efficiency (see Chapter 3). To further clarify partitioning of assimilates, prior understanding of developmental patterns determining spike fertility is needed (see Chapter 2). Furthermore, increases in grain number and grain size will need an optimization of crop structural strength to avoid lodging (Piñera-Chavez et al. 2016a; Piñera-Chavez et al. 2016b).

1.1.3. Theme 3. Breeding to accumulate yield potential traits

Breeding efforts, under Theme 3, are focused on an integrated approach towards yield potential. Main objectives are (i)strategic hybridization to combine traits associated with radiation-use-efficiency and partitioning, (ii)high throughput phenotyping and molecular marker assisted breeding that includes a whole genome selection approach for efficient deployment of yield traits-linked markers, and (iii)use of exotic germplasm to enrich conventional genepools (Reynolds et al. 2011b).

1.2. Diversity on plant material

Part of the experiments for this thesis were focused on elite spring bread wheat lines. The CIMMYT Mexico Core Germplasm panel (CIMCOG), established by CIMMYT breeding programs, contains 60 wheat elite lines (58 *T. aestivum* and 2 *T. turgidum* var. *durum*). These genotypes were selected because they (i)represent historical genetic gains, (ii)are genotypes derived from most recent selections in CIMMYT since

1999, (iii) it includes synthetic wheat material, and (iv)two durum wheats as controls (Table 2).

The CIMCOG panel is potentially useful in practical breeding programs aiming to further raising yield potential and for that reason is the main germplasm studied so far by the Wheat Yield Consortium (Reynolds et al. 2011a; Parry et al. 2011; Foulkes et al. 2011b).

Table 2. Entry number, year of release, cross name, pedigree and selection history from CIMCOG trial (n=60). In bold text are the two durum wheat lines. A comprehensive list that includes CIMMYT's crossing, seed and genotype identification numbers is provided in A1.

Ref	Year	Cross Name	Pedigree	Selection History
1	1966	SIETE CERROS T66	PJ62/GB55	II8156-1M-2R-4M-0Y
2	1976	PAVON F 76	VCM//CNO67/7C/3/KAL/BB	CM8399-D-4M-3Y-1M-1Y-1M-0Y-0MEX
3	1982	SERI M 82	KVZ/BUHO//KAL/BB	CM33027-F-15M-500Y-0M-87B-0Y-0MEX
4	1988	BACANORA T 88	JUP/BJY//URES	CM67458-4Y-1M-3Y-1M-5Y-0B-0MEX
5	1990	ATTILA (PBW 343)	ND/VG9144//KAL/BB/3/YACO/4/VEE#5	CM85836-50Y-0M-0Y-3M-0Y
6	1992	BAVIACORA M 92	BOW/NAC//VEE/3/BJY/COC	CM92066-J-0Y-0M-0Y-4M-0Y-0MEX
7	1999	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*KAUZ	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*KAUZ	CMSS93B01824M-040Y-73Y-010M-010Y-010M-1Y-0M-0KBY
8	2000	ATTILA*2/PBW65	ATTILA*2/PBW65	CGSS96B00123F-099M-037Y-099M-26Y-0B-0SY
9	2001	Navojoa M2007	ATTILA/PASTOR	CMSS97Y04045S-040Y-050M-040SY-030M-14SY-010M-0Y-0MEX
10	2002	MILAN/KAUZ//PRINIA/3/BAV92	MILAN/KAUZ//PRINIA/3/BAV92	CMSS97M02941T-040Y-020Y-030M-040Y-020M-1Y-0M
11	2003	OASIS/5*BORL95/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI	OASIS/5*BORL95/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI	CMSS98Y04800S-020Y-030M-020Y-040M-31Y-1M-0Y
12	2003	ATTILA//PGO/SERI/3/PASTOR	ATTILA//PGO/SERI/3/PASTOR	CMSS98Y03455T-040M-0100M-040Y-020M-040SY-21M-0Y-0SY
13	2003	RL6043/4*NAC//2*PASTOR	RL6043/4*NAC//2*PASTOR	CMSS98M00790M-040Y-0100M-040Y-020M-040SY-15M-0Y-0SY
14	2004	MISR 1	OASIS/SKAUZ//4*BCN/3/2*PASTOR	CMSS00Y01881T-050M-030Y-030M-030WGY-33M-0Y
15	2005	**CIRNO C 2008 (durum)**	SOOTY_9/RASCON_37//CAMAYO	CGSS02Y00004S-2F1-6Y-0B-1Y-0B
16	2005	WBLL1*2/KIRITATI (BECARD)	WBLL1*2/KIRITATI	CGSS01B00063T-099Y-099M-099M-099Y-099M-27Y
17	2005	PASTOR/3/URES/JUN//KAUZ/4/WBLL1	PASTOR/3/URES/JUN//KAUZ/4/WBLL1	CMSA00Y00865T-040M-0P0Y-040M-040SY-030M-6ZTM-0ZTY-0M-0SY
18	2005	CROC_1/AE.SQUARROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2	CROC_1/AE.SQUARROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2	CMSA00Y00817T-040M-0P0Y-040M-040SY-030M-8ZTM-0ZTY-0M-0SY
19	2005	CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC/5/PFAU/WEAVER	CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC/5/PFAU/WEAVER	CMSA00M00102S-040P0M-040Y-030M-030ZTM-12ZTY-0M-0SY
20	2005	SUPER 152	PFAU/SERI.1B//AMAD/3/WAXWING	CGSS02Y00153S-099M-099Y-099M-46Y-0B
21	2005	BRBT1*2/KIRITATI	BRBT1*2/KIRITATI	CGSS01B00072T-099Y-099M-099M-099Y-099M-30Y-0B
22	2005	BECARD	WBLL1*2/KIRITATI	CGSS01B00063T-099Y-099M-099M-099Y-099M-9Y-0B

23	2006	MUNAL #1	WAXWING*2/KIRITATI	CGSS01B00054T-099Y-099M-099M-099Y-099M-13Y-0B
24	2006	CHWL86/6/FILIN/IRENA/5/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER	CHWL86/6/FILIN/IRENA/5/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER	CMSA00M00291S-040P0M-040Y-030M-040SY-2M-0Y-0SY
25	2006	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA(TAUS)/4/OCI/5/PASTOR/6/TEMPORALERA M 87/ROMO96	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/OCI/5/PASTOR/6/TEMPORALERA M 87/ROMO96	CMSA01Y00167S-040P0Y-040M-040SY-040M-15Y-0M-0SY
26	2006	BABAX/LR42//BABAX/3/ER2000	BABAX/LR42//BABAX/3/ER2000	CMSA01Y00176S-040P0Y-040M-030ZTM-040SY-24M-0Y-0SY
27	2006	TC870344/GUI//TEMPORALERA M 87/AGR/3/2*WBLL1	TC870344/GUI//TEMPORALERA M 87/AGR/3/2*WBLL1	CMSA01Y00725T-040M-040P0Y-040M-030ZTM-040SY-10M-0Y-0SY
28	2006	W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1	W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1	PTSS02B00102T-0TOPY-0B-0Y-0B-11Y-0M-0SY
29	2007	**ARMENT//2*SOOTY_9/RASCON_37/4/CNDO/PRIMADUR//HAI-OU_17/3/SNITAN (durum)**	ARMENT//2*SOOTY_9/RASCON_37/4/CNDO/PRIMADUR//HAI-OU_17/3/SNITAN	CDSS02B00643S-0Y-0M-1Y-4M-04Y-0B
30	2007	WHEAR/SOKOLL	WHEAR/SOKOLL	CMSS04Y00201S-099Y-099ZTM-099Y-099M-11WGY-0B
31	2008	TRCH/SRTU//KACHU	TRCH/SRTU//KACHU	CGSS05B00189T-099TOPY-099M-099NJ-099NJ-7WGY-0B
32	2008	TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON/5/KACHU #1	TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON/5/KACHU #1	CMSS05B00160S-099Y-099M-099Y-099ZTM-21WGY-0B
33	2008	C80.1/3*BATAVIA//2*WBLL1/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	C80.1/3*BATAVIA//2*WBLL1/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	CMSS05B00722S-099Y-099M-099Y-099ZTM-4WGY-0B
34	2008	BABAX/LR42//BABAX/3/VORB	BABAX/LR42//BABAX/3/VORB	CMSA05M00103S-040ZTM-040ZTY-13ZTM-03Y-0B
35	2008	SOKOLL//PBW343*2/KUKUNA/3/NAVJ07	SOKOLL//PBW343*2/KUKUNA/3/NAVJ07	CMSA05Y01188T-040M-040ZTP0Y-040ZTM-040SY-17ZTM-01Y-0B
36	2008	SOKOLL*2/3/BABAX/LR42//BABAX	SOKOLL*2/3/BABAX/LR42//BABAX	CMSA05Y01225T-040M-040ZTP0Y-040ZTM-040SY-12ZTM-01Y-0B
37	2008	GK ARON/AG SECO 7846//2180/4/2*MILAN/KAUZ//PRINIA/3/BAV92	GK ARON/AG SECO 7846//2180/4/2*MILAN/KAUZ//PRINIA/3/BAV92	CMSA05Y00954T-040M-040ZTP0Y-040ZTM-040SY-12ZTM-01Y-0B
38	2008	MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN	MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN	PTSS02B00132T-0TOPY-0B-0Y-0B-25Y-0M-0SY-0B-0Y-0Y
39	2008	BCN/WBLL1	BCN/WBLL1	PTSS02GH00001S-0Y-0B-040M-040Y-9M-0Y-0Y-0Y
40	2008	BCN/RIALTO	BCN/RIALTO	PTSW02B00137S-31DHB-0GHB-0Y-0Y-0M-0Y-0Y
41	2009	CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/6/RIALTO	CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/6/RIALTO	PTSW02B00139S-65DHB-0GHB-0Y-0Y-0Y-099Y
42	2009	BECARD/KACHU	BECARD/5/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	CMSS06B00169S-0Y-099ZTM-099Y-099M-21WGY-0B
43	2009	QUAIU #3//MILAN/AMSEL	QUAIU #3//MILAN/AMSEL	CMSS06B00640S-0Y-099ZTM-099Y-099M-7WGY-0B
44	2009	ROLF07*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	ROLF07*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	CMSS06B00704T-099TOPY-099ZTM-099Y-099M-23WGY-0B
45	2009	TACUPETO F2001/BRAMBLING*2//KACHU	TACUPETO F2001/BRAMBLING*2//KACHU	CMSS06B00707T-099TOPY-099ZTM-099Y-099M-2WGY-0B
46	2009	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA (498)/5/LINE1073/6/KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ/7/KRONSTAD F2004/8/KAUZ/PASTOR//PBW343	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA (498)/5/LINE 1073/6/KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ/7/KRONSTAD F2004/8/KAUZ/PASTOR//PBW343	CMSS06B00762T-099TOPY-099ZTM-099Y-099M-11RGY-0B

47	2009	ATTILA*2/PBW65*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	ATTILA*2/PBW65*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	CMSS06B00786T-099TOPY-099ZTM-099Y-099M-1RGY-0B
48	2009	WBLL1*2/TUKURU*2/4/CROC_1/AE.SQUARROSA (205)//BORL95/3/2*MILAN	WBLL1*2/TUKURU*2/4/CROC_1/AE.SQUARROSA (205)//BORL95/3/2*MILAN	CMSS06Y00627T-099TOPM-099Y-099ZTM-099Y-099M-15WGY-0B
49	2009	UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/MILAN/KAUZ//CHIL/CHUM18/6/UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/MILAN/KAUZ//CHIL/CHUM18/6/UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	CMSS06Y00859T-099TOPM-099Y-099ZTM-099Y-099M-35WGY-0B
50	2009	WBLL1*2/KURUKU*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	WBLL1*2/KURUKU*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	CMSS06Y00933T-099TOPM-099Y-099ZTM-099Y-099M-1WGY-0B
51	2009	SAUAL/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/ATTILA/5/SAUAL	SAUAL/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/ATTILA/5/SAUAL	CMSS06Y01021T-099TOPM-099Y-099ZTM-099Y-099M-15WGY-0B
52	2009	PANDORA//WBLL1*2/BRAMBLING	PANDORA//WBLL1*2/BRAMBLING	CMSS06B00229S-0Y-099ZTM-099Y-099M-12RGY-0B
53	2009	KINGBIRD #1//INQALAB 91*2/TUKURU	KINGBIRD #1//INQALAB 91*2/TUKURU	CMSS06B00485S-0Y-099ZTM-099NJ-099NJ-6WGY-0B
54	2009	CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/FH6-1-7	CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/FH6-1-7	CMSS06Y00707T-099TOPM-099Y-099ZTM-099NJ-099NJ-5WGY-0B
55	2009	SAUAL/WHEAR//SAUAL	SAUAL/WHEAR//SAUAL	CMSS06Y01284T-099TOPM-099Y-099ZTM-099Y-099M-6WGY-0B
56	2009	TACUPETO F2001/SAUAL//BLOUK #1	TACUPETO F2001/SAUAL//BLOUK #1	CMSS06B00700T-099TOPY-099ZTM-099NJ-099NJ-2WGY-0B
57	2009	OASIS/SKAUZ//4*BCN/3/2*PASTOR/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/6/SAUAL #1	OASIS/SKAUZ//4*BCN/3/2*PASTOR/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/6/SAUAL #1	CMSS06B00959T-099TOPY-099ZTM-099NJ-099NJ-6WGY-0B
58	2009	PBW343*2/KUKUNA*2//FRTL/PIFED	PBW343*2/KUKUNA*2//FRTL/PIFED	CMSS06Y00831T-099TOPM-099Y-099ZTM-099NJ-099NJ-5WGY-0B
59	2009	WBLL1*2/4/BABAX/LR42//BABAX/3/BABAX/LR42//BABAX	WBLL1*2/4/BABAX/LR42//BABAX/3/BABAX/LR42//BABAX	CMSS06Y00885T-099TOPM-099Y-099ZTM-099NJ-099NJ-24WGY-0B
60	2009	KFA/3/PFAU/WEAVER//BRAMBLING/4/PFAU/WEAVER*2//BRAMBLING	KFA/3/PFAU/WEAVER//BRAMBLING/4/PFAU/WEAVER*2//BRAMBLING	CMSS06B01006T-099TOPY-099ZTM-099Y-099M-1RGY-0B

1.3. Wheat physiology

In recent decades, the genetic gain in yield potential for wheat worldwide has been approximately 1% annually (Gaju et al. 2009). To further increase wheat yield potential, it is critical to better understand the physiological and genetic bases of wheat yield, in order to increase the likelihood of success in breeding for complex traits, such as yield, either through traditional or molecular biology approaches (Slafer 2003; Reynolds and Tuberosa 2008). It has frequently been reported that wheat yield is most often limited by the strength of the sink (the capacity of the grains to accumulate assimilates) during post-anthesis (Calderini and Reynolds 2000; Reynolds et al. 2005), and therefore to improve yield we must further increase either the number of grains per m^2 (Miralles and Slafer 2007; Fischer 2007, 2011; Foulkes

et al. 2011b) or the potential size of grains (Calderini et al. 2001). This assertion applies even though the final average weight of individual grains is frequently reported to be negatively correlated to the number of grains per m^2 (Slafer et al. 2006), as in many cases the nature of the negative relationship is not competition among growing grains for limited assimilates (Acreche and Slafer 2006).

To continually increase the number of grains per m^2 is not an easy prospect. The introduction of dwarfing genes improved partitioning to the juvenile spikes, reduced the rate of degeneration of floret primordia, and increased the number of fertile florets that could produce grains (Foulkes et al. 2011b). However, modern wheat varieties have reached an apparent optimum height of ~70-100 cm (Fischer 2011), so it is therefore important to look for alternatives to increase grain number without affecting plant height.

1.3.1. Phasic development

Wheat phenology studies the presence of physiological events regulated by genetic factors that are determined by the interaction with abiotic factors. The ontogenesis of cereals is separated into three main phases: vegetative, reproductive and grain-filling. These stages outline production, development and degeneration at specific moments of the growth cycle (Figure 1).

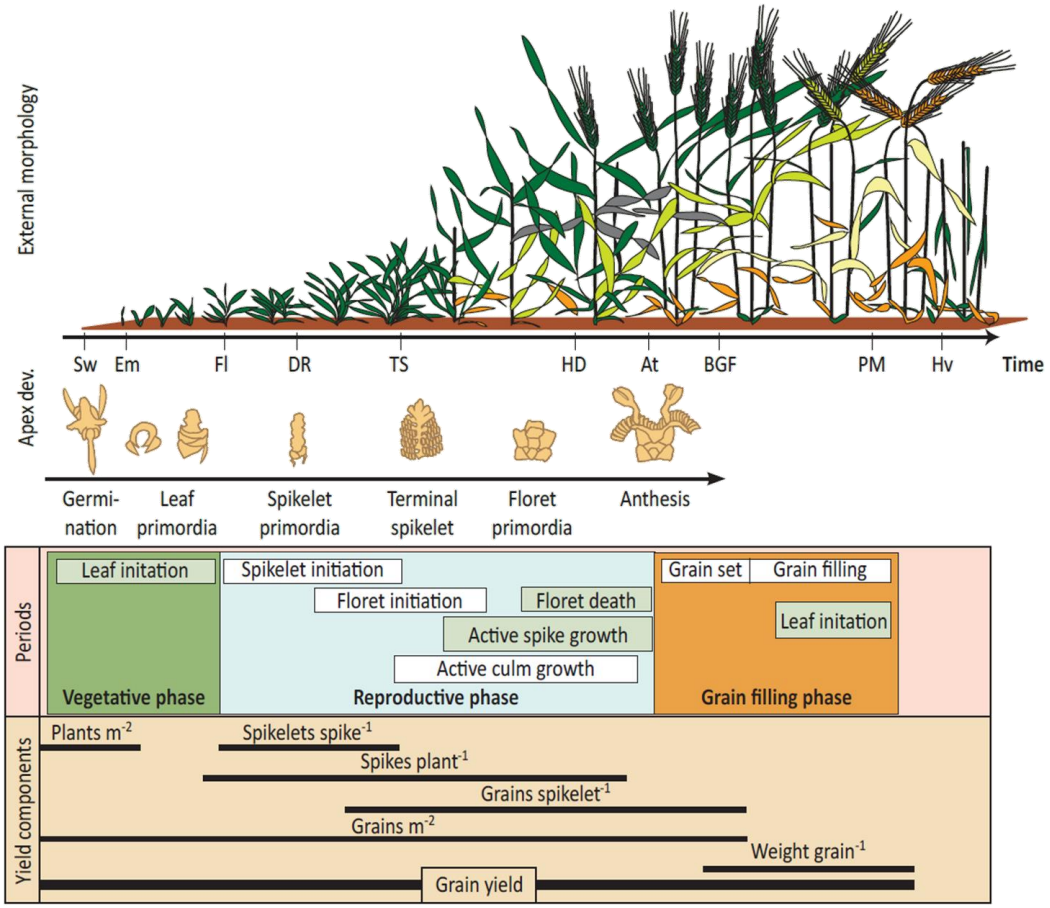

Figure 1. Schematic diagram of wheat growth and development adapted from Slafer and Rawson (1994), showing the stages of sowing (Sw), emergence (Em), floral initiation (FI), first double ridge appearance (DR), terminal spikelet initiation (TS), heading (HD), anthesis (At), beginning of the grain-filling period (BGF), physiological maturity (PM), and harvest (Hv). Apex development is shown under each phenological stage. The periods of initiation or growth of specific organs and those of when different components of grain yield are produced, are represented in the bottom boxes (from Slafer (2012a)).

Although sub-components of the number of grains per unit land area are developed during the entire period from sowing to anthesis (Slafer and Rawson 1994), it has been repeatedly shown that the late reproductive phase of stem elongation, occurring a few weeks before anthesis (from terminal spikelet initiation to anthesis), is of paramount importance in determining the number of grains, as this is the period when spikes actively grow (Bancal 2009; Ferrante et al. 2010; Whitechurch et al. 2007). This is probably because wheat is a cleistogamous plant and therefore most of the

fertile florets at anthesis become grains afterwards. Floret development, the process which determines the progress of floret primordia differentiation, and therefore the number of fertile florets reaching anthesis, takes place mainly during the stem elongation phase (Kirby 1988).

1.3.2. Grain number

Spike dry weight per unit land area has been positively associated with grain number (Slafer et al. 2005). This relationship held during the Green Revolution as the increase in partitioning to the juvenile spikes led to an increase in spike dry weight at anthesis, causing improvements in grain number (Miralles et al. 1998). It seems unlikely that we could further increase dry matter partitioning to the spike, but improvements in grain number may be achieved through increases in fruiting efficiency, that is, the efficiency by which dry matter allocated to the spikes at anthesis is used to set grains (Pedro et al. 2011).

It has been proposed that extending the stem elongation phase could lead to increases in the number of grains per unit land area, by raising the number of floret primordia reaching the fertile floret stage at anthesis (Slafer and Andrade 1991; González et al. 2003a, 2005a). The variability in this phenological trait has been studied in screenings of cultivars (Whitechurch et al. 2007), but it has not been determined within populations that may be relevant for the genetic improvement of bread wheat.

The interrelationship between the duration of the stem elongation phase, fruiting efficiency, and grain number production were only exceptionally considered so far (González et al. 2011b). Analysing these relationships within a population of genotypes selected for good agronomic performance may provide realistic opportunities for improving these traits.

1.4. Wheat genetics

Wheat has a polyploid genome (2n=6x=42) with somatic cells containing three closely-related ancestral diploid genomes (AABBDD) in the nucleus. The alleles obtained from the parent's gametes (haploid) are alternative forms of the same gene, producing a different effect. Most genes are present in at least three copies, one per genome. This complicates genetic mapping and molecular analysis (Paux et al. 2012)

A sporophyte, the product of fertilization between male and female gametes, contains a set of chromosomes from each parent. The law of segregation states that when the

gametes are formed the allelic genes segregate and pass to different gametes, hence the gametophytes each carry half the sporophytic complement of chromosomes (Forster et al. 2007). Under a heterozygous assumption, each gametophyte contains an alternative form or allele that will be passed randomly to its offspring. The many alleles comprising an individual are called its genotype.

1.4.1. Mendelian genetics

Mendel's experiments always showed a 3:1 ratio between dominant and recessive phenotypes, a variation of physical and behavioural attributes, as he mixed one trait. When he started performing a dihybrid cross (mixing two traits) the ratio become 9:3:3:1, showing that each of the two alleles is independently inherited with a 3:1 ratio; the law of independent assortment states that separate genes for separate traits are passed independently of one another.

The above ratios were possible because the alleles were located on separate chromosomes. If any two of Mendel's traits had been found on the same pair of chromosome, they would have shown linkage effects which would have produced unusual ratios in the F2 generation.

During meiosis, more specifically prophase I, chromatids are in tight formation allowing homologous sites to exchange genetic material, producing new combinations of alleles. Conventional breeding achieves increments in yield by recombining alleles, mainly from elite materials, and selecting among thousands of progeny per cross for expression of appropriate agronomic traits (Reynolds et al. 2012). Genes that are located close to each other on a chromosome (whose loci are nearer) are less likely to be separated during the chromosomal crossover, and are said to be genetically linked.

Quantitative genetics is used to study traits that have a continuous variation, i.e. no discrete phenotypes. The basic principle is that the phenotype value is considered as the result of the total genetic variation and the environmental effect.

1.4.2. Recombination fraction

Recombination fractions are used to infer the presence of certain genes by building linkage maps. These are genetic maps that show the position of known genes or genetic markers relative to each other in terms of recombination frequency, not by a specific physical distance along the chromosome. The recombination frequency, also

known as recombination fraction (r), is defined as the ratio of recombined gametes to the total number of gametes produced. This is:

$$0 \leq r \leq 0.5$$

Where r = 0 indicates a perfect linkage, and r = 0.5 indicates complete independence.

The linkage between markers is usually calculated using odds ratios (i.e. the ratio of linkage versus no linkage), more conveniently expressed as the logarithm of odds (LOD). While using a LOD value >3 to build the linkage maps, it is inferred that a linkage is 1000:1 more likely than no linkage (Collard et al. 2005). Recombination frequency is not linearly related; therefore, a mapping function is required to convert recombination fractions into centiMorgans (cM). A cM is the distance between two loci that recombine with a frequency of 1%. When a map distance is <10 cM it equals the recombination frequency. There are two commonly used mapping functions:

- Kosambi: assumes that recombination events influence the occurrence of adjacent recombination events.
- Haldane: assumes no interference between chromosomal crossover events.

1.4.3. Linkage disequilibrium

Linkage disequilibrium is the non-random association of alleles at different loci, this is an important parameter when, using single nucleotide polymorphisms (SNP), determining the power of genomic analyses (Cleveland and Deeb 2012). The idea is to find markers that are close together in linkage equilibrium, meaning that are not associated, so that the quantitative trait locus is associated with one marker only. Linkage is an important component of breeding, with the use of techniques like marker assisted selection (MAS), where a desirable QTL allele tightly linked to a marker can be used to create new germplasm. Genetic maps are based on linkage information. As described in the above section, recombination fractions are used to produce a linkage map; as markers that are close together tend to remain close during meiosis.

1.4.4. Quantitative trait locus

Quantitative traits, are traits that have a variation because of the segregation of several polymorphic genes, and the genomic regions with genes associated to these quantitative traits is known as quantitative trait loci (QTLs). Collard et al. (2005) use the metaphor of finding the proverbial needle in a haystack, when describing the process of finding of a gene. QTLs analysis can be used to divide such a haystack

into manageable piles and systematically search them. QTL analysis relies on detecting an association between phenotype and genotype through their linkage. This means that QTL requires three main sets of data: (i)molecular markers, (ii)phenotype, and (iii)linkage map.

The strategy behind QTL, as described by Tanksley (1993), is to fundamentally assume the presence of linkage disequilibrium between alleles at the locus of interest. This is because the most common cause of linkage disequilibrium (others can be selection, genetic drift, etc.), in segregating populations, is physical linkage of loci; allowing the creation of linkage maps.

The identification of quantitative trait loci (QTLs) allows the further finding of genes, therefore the task of using the right phenotypic measurements in a crop that is nothing but a series of complex traits, one after another, becomes of paramount importance. A significant difference between phenotypic means is a must in order to locate the QTL controlling the trait in question.

The diagram (Figure 2) presented in the review of Jones et al. (1997) summarized perfectly the basics of mapping a QTL. First a mapping population is developed by crossing two parent lines that differ in the quantitative trait of interest. The alleles and QTLs will segregate in the progeny (Figure 2a). Then in Figure 2b four different examples are given for the single QTL shown:

- Locus 1: locus *a* is tightly linked to the QTL, which can be seen as two separate distributions of the phenotype.
- Locus 2: locus *b* is closely linked to the QTL, this can be seen as a small overlap in the distribution of the phenotype.
- Locus 3: locus *c* is distantly linked to the QTL, creating a bigger overlap.

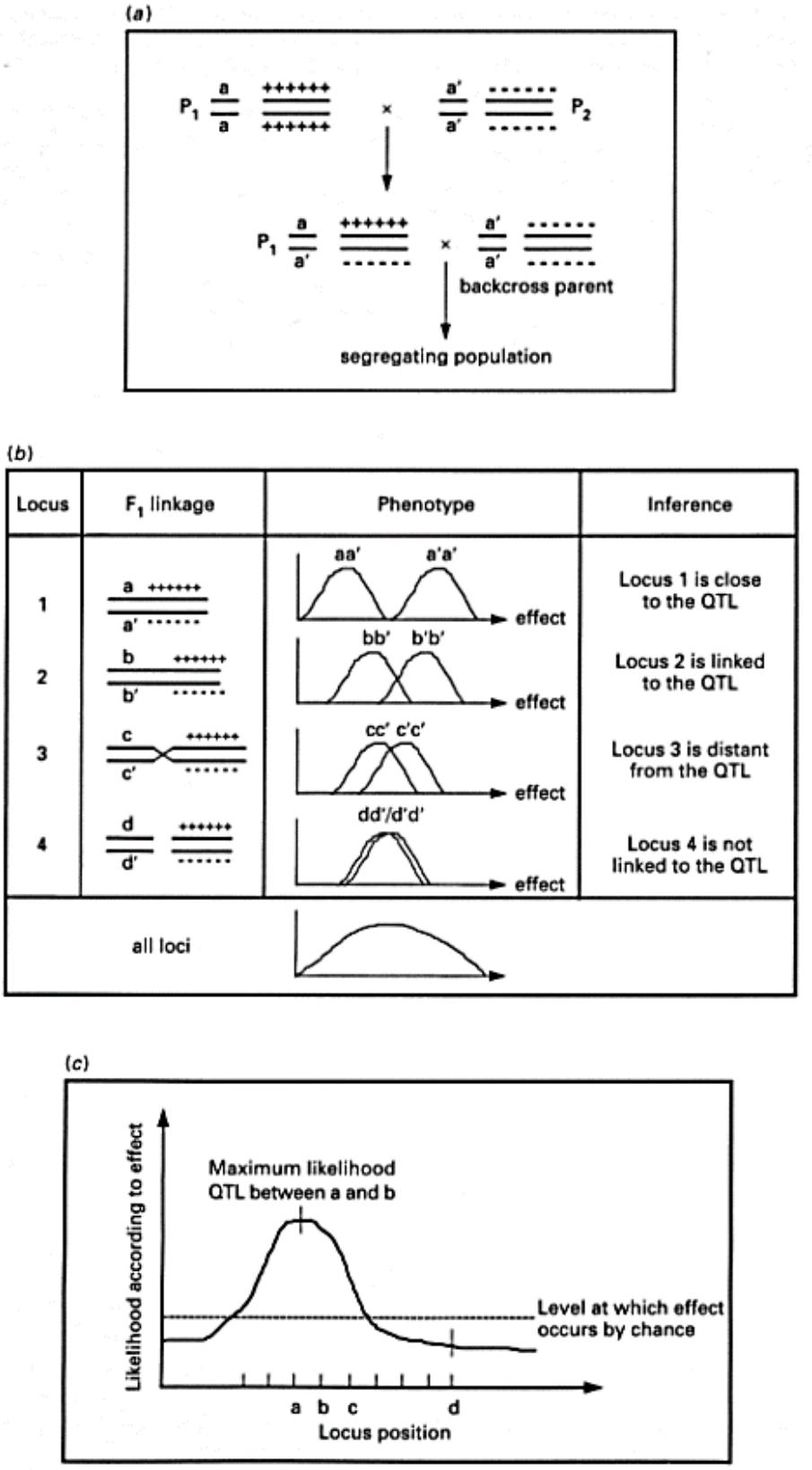

Figure 2. Panel with diagrams of the process of mapping a quantitative trait locus. (a) mapping population different markers for the quantitative trait. Heterozygous F1 is backcrossed to P2 to produce a segregating population. (b) Linkage between QTL and marker loci can be established by the phenotype distribution patterns and its association with the alleles segregation. (c) Map of the QTL determined as the maximum likelihood (or logarithm of odds). Diagrams from Jones et al. (1997).

- Locus 4: locus *d* is unlinked to the QTL, the phenotypic value distribution is shown as a single curve

Then the QTL is mapped in relation to the nearest markers, allowing the dissection of the trait and the possibility of using the markers for selection in breeding (Figure 2c).

1.5. Aims

The aim of this work is to clarify the physiological basis of spike fertility, and identify genetic bases associated

with phenological patterns influencing grain yield potential, thus identifying novel genetic locations useful for the optimization of developmental patterns to maximize grain yield potential.

2. Floret development determining spike fertility

2.1. Introduction

Due to the increasing global population together with a growing demand for meat and dairy products (implying a growing amount of grains should be used to produce animal food at a low rate of conversion), a substantial increase of grain production in the next decades is critical. This is particularly challenging as the basic manageable resources for crop growth and yield (water, nutrients) will not increase (Connor and Mínguez 2012), and the land available for crop production is likely to decline (Albajes et al. 2013 and references quoted therein). These challenges together with the need to make future production of crops more sustainable amount to a 'perfect storm' (Godfray et al. 2010; Fischer et al. 2014). Among the major crops, wheat is one of the most critical for nourishment: it is the most widely grown crop globally and is one of the primary sources of protein for the world population, representing c. 20% of the daily intake for developing countries (Braun et al. 2010). In order to maintain balance between demand and supply, alternative ways and means to further raise wheat yield must be found (Chand 2009). A major way to navigate this 'perfect storm', facing the restrictions mentioned above, is through re-gaining high rates of genetic gains in yield. However, this may not be easily achieved as there is mounting evidence that genetic gains in yield have recently been much lower than required (Reynolds et al. 2012; Fischer et al. 2014). The likelihood of accelerating breeding progress would increase with knowledge of the genetic variation available for traits determining yield (Slafer 2003; Reynolds et al. 2009; Reynolds and Borlaug 2006), meaning that it is of great benefit for breeding programs to be able to generate tools (e.g. genetic markers) that are associated, or linked, to traits that potentially can increase yield.

Yield in wheat is generally more related to grain number than to the average weight of the grains (Fischer 2008, 2011), as the number of grains is far more plastic than the size of the grains (Sadras and Slafer 2012). Consequently, genetic gains in wheat yield have been more related to improvements in the number than in the size of the grains (e.g. Canevara et al., 1994, Calderini et al., 1995, Sayre et al., 1997, Shearman et al., 2005, Peltonen-Sanio et al., 2007 and Acreche et al., 2008). As even in modern cultivars grain growth seems not strongly limited by the source (Borrás et al. 2004; Pedro et al. 2011), it seems likely that further increases in yield potential may require additional improvements in grain number (Reynolds et al. 2001; Reynolds et al. 2005;

Acreche and Slafer 2009; González et al. 2014), despite the well-known negative relationship between grain number and grain size (further discussed in section 3.4). The identification of potential traits to increase grain number is of great interest to ensure that increased photosynthetic potential is fully utilized by matching it with adequate sink demand (Reynolds et al. 2012; Slafer et al. 2014). To achieve this aim, it would be useful to understand the degree of variation of physiological drivers of grain number within elite lines. Grain number is largely determined during the stem elongation (SE) phase (Fischer 1985; Slafer and Rawson 1994). Therefore improvements of traits determined during SE would be required to further increase grain number (Slafer et al. 2005).

Beyond increasing crop growth rate and further improving biomass partitioning before anthesis, it may also be relevant to optimize the developmental attributes to maximize spike fertility (Foulkes et al. 2011b; Reynolds et al. 2012). This involves two different aspects of development: (i) the pattern of partitioning of time to anthesis into different phases (Slafer et al. 2001), as lengthening the duration of the SE phase may increase yield (Slafer 2003; Miralles and Slafer 2007); and (ii) the dynamics of floret development (Kirby 1988), as grain number is the consequence of the developmental process of floret generation/degeneration resulting in a certain number of fertile florets (González et al. 2011a).

Looking for variation in dynamics of floret development within modern elite cultivars, could contribute to the elucidation of mechanisms which may identify sources for an increase in grain number. Floret development in wheat has been long studied (Stockman et al. 1983; Sibony and Pinthus 1988; Miralles et al. 1998; Wang et al. 2001; González et al. 2003a; Bancal 2008; Shitsukawa et al. 2009; Dreccer et al. 2014), especially its response to nitrogen applications (Holmes 1973; Langer and Hanif 1973; Ferrante et al. 2010). However, it seems that due to the difficulties involved with the developmental analysis of spike morphogenesis there is an absence of research describing variation for this trait among elite wheat cultivars.

The objective of the present study was to determine the degree of variation within elite germplasm of wheat in patterns of floret development responsible for differences in number of fertile florets, and to further understand the differences in generation of fertile florets among genotypes with differing yield components. The specific aims are:

- Quantify differences in dynamics of floret development in elite wheat germplasm.

- Determine if this differences are responsible for variations in number of fertile florets.
- Evaluate the dynamics of floret initiation and survival as determinants of the numbers of fertile florets.

2.2. Materials and methods

2.2.1. General conditions

Two field experiments (i.e. trials over two years) were conducted in the Mexican Phenotyping Platform (MEXPLAT) established at the research station "Centro Experimental Norman E. Borlaug" (CENEB), near Ciudad Obregón, Sonora, Mexico (27°33′ N, 109°09′ W, 38 masl), with conditions that represent the high-yield potential environments of short season and full irrigated spring wheat (Braun et al. 2010). The soil is a Chromic Haplotorrert (Vertisol Calcaric Chromic), low in organic matter (<1%), and slightly alkaline (pH = 7.7). A summary of climatic data key parameters is presented in Table 3 and full meteorological data is provided in A2 and A3, for seasons 2010-11 and 2011-12 respectively. Water available was estimated considering the water from irrigation and the total accumulated rain during crop cycle.

Table 3. Growing conditions of the crop cycles 2010-11 and 2011-12. Average emergence date, average daily temperature during preanthesis (PreA) and postanthesis (PostA), solar radiation, mean evapotranspiration (ETo) and water available during each crop cycle.

Crop Cycle	Emergence	Temp PreA Mean (°C)	Temp PostA Mean (°C)	Radiation (MJ m^{-2} day^{-1})	ETo (mm)	Water available (mm)
2010-11	15-Dec-10	14.9 (5.6 – 24.2)	19.7 (9.2 – 30.1)	21.4	4.4	573
2011-12	16-Dec-11	15.2 (5.7 – 24.8)	19.3 (8.5 – 30.0)	21.4	4.4	592

2.2.2. Treatments and experimental design

Experiments were sown on 06 December 2010 and 09 December 2011, within the optimal sowing period for the winter–spring cycle of cereals in the region. Sowing density was 101.5 and 108.8 kg ha^{-1} respectively, and 200 units of N fertilizer (urea) were applied. Weeds were removed by hand throughout the growing season and diseases and insects controlled by the application of recommended fungicides and insecticides at the doses suggested by their manufacturers.

The treatments consisted of the ten wheat genotypes (Table 1), all elite material belonging to the CIMMYT Mexico Core Germplasm Panel (CIMCOG) with good agronomic adaptation. The full set of 60 genotypes of the CIMCOG panel are

potentially useful in practical breeding programmes aiming to further raise yield potential, and for that reason it is the main germplasm studied so far by the Wheat Yield Consortium (Reynolds et al. 2011a). For this particular study, the number of genotypes was restricted to ten because of the detailed measurements required, particularly regarding floret development (Table 4). The criteria used for selecting the 10 genotypes out of the whole panel was with regard of the known main trait that allows each genotype to reach the status of elite material, therefore capturing the variation in yield components traits while keeping the number of genotypes manageable. The selected genotypes do represent fairly well the whole CIMCOG panel in terms of yield and its major determinants both considering average values as well as range of variation (Table 5).

The experiment was designed in randomized complete blocks with two replicates, where plots were assigned to genotypes. In season 2010–2011 plots were 5 m long and 3.2 m wide, consisting of four raised beds 0.80 m wide, with two rows per bed (0.24 m apart), and in season 2011–2012 plots were 8.5 m long and 1.84 m wide, consisting of two raised beds 0.80 m wide, with two rows per bed (0.24 m apart) (Figure 3, left panel).

Table 4. Subset selected from the CIMCOG panel. For each entry, the name of the cultivar or cross is indicated, as well as the main trait for which the genotype was selected to be part of the CIMCOG.

Entry	Name	Trait
1	BACANORA T88	high grains/m^2
2	BCN/RIALTO	late development
3	BRBT1*2/KIRITATI	large grains
4	CROC_1/AE.SQUARROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2	high floret number
5	ATTILA/PASTOR	high floret number; late development
6	PFAU/SERI.1B//AMAD/3/WAXWING	early development
7	SERI M 82	wide adaptation
8	SIETE CERROS T66	Benchmark
9	TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON/5/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	wide adaptation
10	WHEAR/SOKOLL	wide adaptation

Table 5. Comparison of yield and its determinants between the CIMCOG panel and the subset of ten genotypes. Data are the adjusted means from a combined analysis of the wheat genotypes grown during the 2010-2011 and 2012 at CENEB, near Ciudad Obregon, Mexico.

Trait	Average		CIMCOG		Subset	
	CIMCOG	Subset	Range	$LSD_{0.05}$	Range	$LSD_{0.05}$
Yield (Mg ha^{-1})	6.42	6.40	4.99 – 7.63	0.7	6.13 – 6.61	0.7
Biomass (Mg ha^{-1})	14.12	13.97	11.73 – 15.76	1.5	13.23 – 14.72	1.5
Harvest index	0.46	0.46	0.41 – 0.52	0.02	0.43 – 0.49	0.03
Number of grains (m^2)	15072	16554	11626 – 21769	1848	13752 – 21950	2639
Number of grains ($spike^{-1}$)	50	50	41 – 63	8.3	45 – 56	9.1
Grain weight (mg $grain^{-1}$)	43	39	30 – 52	3.1	30 – 45	4.4
Days to anthesis	87	87	78 – 95	2.5	80 – 95	1.2

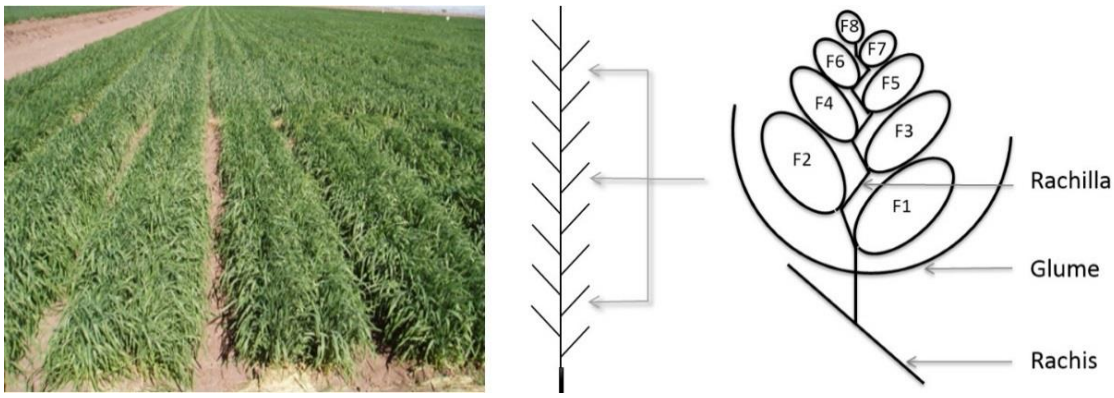

Figure 3. The 60 CIMCOG lines were grown under raised beds (left panel); and schematic diagram illustrating spikelet positions within the spike as well as the position of florets within the spikelet that were used in this study to characterize floret development in CIMCOG (right panel).

2.2.3. Measurements and analyses

Plots were inspected periodically (twice per week) and one plant per plot regularly sampled and dissected under binocular microscope (Carl Zeiss, Germany) to detect the timing of initiation of the terminal spikelet in each case. From then on until a week

after anthesis, one plant per plot, that fairly represent the whole plot, was randomly sampled twice or thrice weekly. In spite of the fact that one plant is sampled per plot, the genotypic variation is being captured by the two replicate plots per genotype, and then by two crop cycles; this results in a dynamic of development that evidence meaningful genotypic variation. The samples were then taken to the lab and the apex of the main shoot dissected under binocular microscope. On the dissected juvenile spikes, the total number of floret primordia was counted in each of the analysed spikelets. In addition, the stage of development of each of the florets within particular spikelets was determined. Together these measurements represent the variability expected in the spikes, in developmental terms. To determine the stage of development of the floret primordia, we followed the scale of Waddington et al. (1983). This scale is based on gynoecium development from floret primordia present (W3.5), to styles curved outwards and stigmatic branches spread wide with pollen grains on well-developed stigmatic hairs (W10), which are considered fertile florets (see A5).

The analysed spikelets were those on the apical (fourth spikelet from the top of the spike), central (middle spikelet of the spike), and basal (fourth spikelet from the bottom of the spike) positions of the spike (Figure 3, right panel). Naming of florets within the spikelets followed the same system described by González et al. (2003a); that is, from F1 to the last developed floret depending on their position with respect to the rachis (F1 was the floret most proximal to the rachis and the most distal floret primordia was F6–F8, depending on the specific spikelet and genotype analysed; Figure 3, right panel).

To analyse the dynamics of development we plotted the developmental score of the particular florets against thermal time (°C d) (as seen in Figure 8, 9, and 10). Thermal time was calculated daily assuming, as it is standard, that the mean temperature was the average of the maximum and minimum values and the base temperature was 0 °C for all genotypes and stages of development (e.g. if the maximum temperature was 35 and the minimum was 15, then the mean temperature, 25, is recorded and used in the accumulation of °C d). Then, for each sampling date we calculated the number of floret primordia which were alive and developing normally; the timing when floret primordia were considered not developing normally any longer was that when the maximum stage of development of a particular floret primordium was reached. The number of floret primordia was plotted against thermal time around anthesis for each particular genotype and experiment. For this analysis we considered a primordia to be a floret when it reached at least stage 3.5 in the scale of Waddington et al. (1983).

The data were subjected to analysis of variance (ANOVA), and the relationships between variables were determined by regression analysis (SAS statistics program, 2002). The adjusted means across the 2 years were obtained by using the PROC MIXED procedure of the SAS statistical package (SAS statistics program, 2002). All the effects, years, replications within years, blocks within years and replications, and genotype by year interaction (G × E) were considered as random effects and only the genotypes were considered as fixed effects.

2.3. Results

The year and position effects were very large (which confirms what is known in wheat: the year affects yield largely and the main component affected is grain number, tightly linked to the number of fertile florets in a cleistogamous species). And that even though all genotypes analysed were elite germplasm, the genotypic effect was statistically significant using 10% threshold with an 8% probability whilst the GxY interaction was not significant at this level (P>0.16). While these are small differences I was keen to understand trends within the data set to shed light on this important trait. This analysis confirms that there is a sufficiently high level of consistency in the genotypic differences observed across two contrasting growing seasons to put forward these inferences

There were significant differences in number of fertile florets per spikelet in each of the two experiments, and these differences were reasonably consistent between years with the unique exception of line 2 (Figure 4). Line 8 was among the lines exhibiting the highest levels of spike fertility in both experiments, and line 9 was among those exhibiting the lowest values (Figure 4).

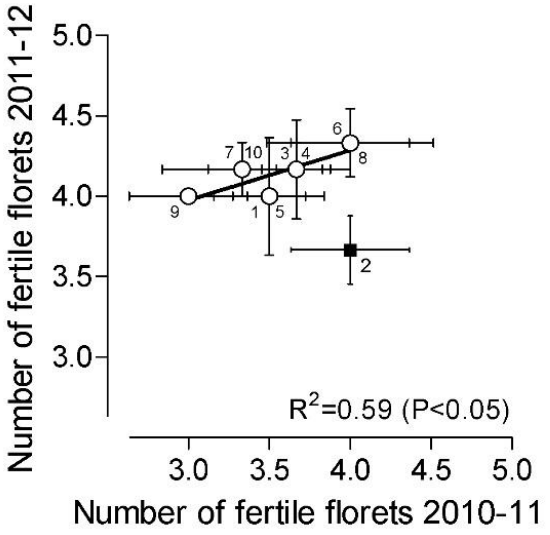

Figure 4. Fertile florets per spikelet in both experiments for the subset of the CIMCOG panel. Bars on each data-point show the standard error of the mean. Genotypes were labelled as in Table 1. Genotype 2 was the exception, not behaving consistently between the two years, and genotypes 8 and 9 were those having respectively the highest and the lowest number of fertile florets per spikelet of the lines analysed consistently between years. Data points of some genotypes are overlapped.

There was significant variation in both components of the number of fertile florets: the maximum number of floret primordia initiated and the proportion of primordia surviving to become fertile florets at anthesis. However, the number of fertile florets was much more strongly related to the survival of floret primordia than to the maximum number florets initiated (Figure 5).

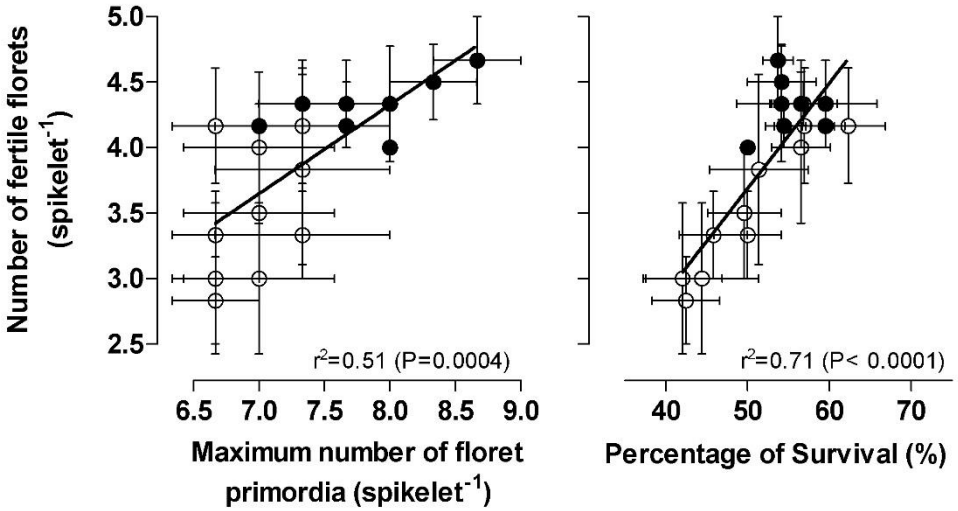

Figure 5. Number of fertile florets per spikelet related to either the maximum number of floret primordia initiated (left panel) or the percentage of these primordia which developed normally surviving to produce fertile florets at anthesis (right panel). Open circles represent season 2010-11 and closed circles season 2011-12.

To further understand the processes involved in the genotypic differences within the CIMCOG panel we studied the dynamics of generation and survival of floret primordia in apical, central and basal spikelets. The general dynamics were similar in all cases (genotypes × spikelet positions): during stem elongation the number of floret primordia first increased rapidly, reaching a peak representing the maximum number of floret primordia and finally decreased sharply until a certain number of fertile florets was established as the balance of the generation and degeneration process (Table 6). Cultivars varied in the dynamics of generation/degeneration of floret primordia determining the number of fertile florets per spikelet at different spikelet positions (Figure 6). To illustrate these genotypic differences, we compared the dynamics of floret generation/degeneration for the apical, central and basal spikelets of the two genotypes exhibiting the extreme cases of floret fertility (Figure 4): lines 8 and 9 representing high and low spike fertilities, respectively. Both genotypes had a similar maximum number of floret primordia initiated in the apical and central spikelets, whilst genotype 9 had a slightly lower maximum number of florets initiated in the basal spikelets than genotype 8 (Figure 7). On the other hand, in all spikelets the decrease in number of floret primordia (floret mortality) was more noticeable in genotype 9 than in 8 and 6 (Figure 7). Interestingly, it seemed that in all spikelet positions genotype 9 reached the maximum number of floret primordia closer to anthesis than genotype 8 and 6, implying that the time for floret survival was consistently shorter in the genotype with lowest final number of fertile florets at anthesis (Figure 7).

Position	Entry	Maximum number of floret primordia				Number of fertile florets			Floret mortality rate			
		Floret Primordia	TT (before anthesis)	Waddington Scale of F1	SE	Fertile florets	SE	TT (before anthesis)	Primordia ˚C d^{-1} (x100)	r^2	*P*	
Apical	1	6.5	250	7.6	±0.31	2.75	±0.25	0	-1.536 ± 0.1060	0.977	< 0.0001	***
	2	5.75	394	5.9	±0.55	3	±0	145.5	-1.055 ± 0.2146	0.829	0.0044	**
	3	6.5	391.5	7.2	±0.25	3.25	±0.25	0	-0.911 ± 0.0995	0.903	< 0.0001	***
	4	5.5	88	9.3	±0.42	3.5	±0.289	0	-2.318 ± 0.3824	0.974	0.1041	ns
	5	6.75	159.5	8.3	±0.37	3.5	±0.289	0	-1.802 ± 0.4563	0.839	0.029	*
	6	6.5	104.5	8.8	±0.42	3.5	±0.289	0	-2.708 ± 0.4124	0.956	0.0224	*
	7	6	236.5	7.5	±0.35	3	±0.577	0	-1.070 ± 0.1264	0.935	0.0004	***
	8	6	290	7.6	±0.51	3.25	±0.479	0	-0.652 ± 0.1182	0.813	0.0009	***
	9	7	194.5	8.1	±0.23	3.25	±0.479	0	-1.182 ± 0.3567	0.687	0.0211	*
	10	6.5	141.5	8.8	±0.37	3.5	±0.25	0	-1.398 ± 0.3334	0.854	0.0247	*
Central	1	8	250	8.2	±0.27	4.5	±0.289	0	-1.403 ± 0.0998	0.975	< 0.0001	***
	2	7	394	7	±0.35	4.5	±0.289	0	-0.614 ± 0.0832	0.872	< 0.0001	***
	3	7.25	432	7.5	±0.35	4.25	±0.25	0	-0.695 ± 0.0914	0.853	< 0.0001	***
	4	7	123.5	9.3	±0.27	4.5	±0.289	0	-1.785 ± 0.7602	0.734	0.1433	ns
	5	7.5	312	7.7	±0.43	4	±0	0	-0.965 ± 0.1405	0.871	0.0002	***
	6	7.5	334	8.3	±0.37	4.5	±0.289	0	-0.802 ± 0.1218	0.844	0.0002	***
	7	7.25	269.5	8	±0.32	4.5	±0.25	0	-1.148 ± 0.0730	0.976	< 0.0001	***
	8	7.75	318.5	7.3	±0.55	4.5	±0.289	0	-0.800 ± 0.1220	0.843	0.0002	***
	9	7.25	194.5	8.5	±0.17	4	±0	0	-1.355 ± 0.2463	0.858	0.0027	**
	10	7.25	260	8.3	±0.23	4.25	±0.25	0	-1.054 ± 0.1124	0.936	< 0.0001	***
Basal	1	7.25	288	7.6	±0.31	4	±0	0	-1.182 ± 0.0847	0.970	< 0.0001	***
	2	7	235.5	7.5	±0.20	4	±0	0	-1.208 ± 0.1999	0.901	0.0038	**
	3	7.25	315	8.1	±0.24	4.25	±0.25	0	-0.918 ± 0.0672	0.964	0.0001	***
	4	7	391.5	7	±0.61	3.75	±0.25	0	-0.718 ± 0.0587	0.943	< 0.0001	***
	5	6.75	312	7.2	±0.43	3.75	±0.25	0	-0.969 ± 0.1770	0.811	0.0009	***
	6	7.5	334	7.7	±0.25	4.5	±0.289	0	-0.849 ± 0.1226	0.857	0.0001	***
	7	6.5	200.5	8.1	±0.27	4	±0.408	0	-1.269 ± 0.1534	0.945	0.0012	**
	8	7.25	318.5	7.25	±0.32	4.75	±0.25	0	-0.762 ± 0.0830	0.913	< 0.0001	***
	9	6.75	278	7.8	±0.31	3.25	±0.479	0	-0.988 ± 0.1540	0.855	0.0004	***
	10	7.25	260	8.1	±0.23	3.5	±0.289	0	-1.036 ± 0.2817	0.693	0.0103	*

Table 6. Floret mortality rate as a linear model from the maximum number of floret primordia vs. the number of fertile florets for the mean of season 2010-11 and 2011-12. It includes the spike position analysed, entry number (genotype), the number of maximum floret primordia, the thermal time this was reaches, the developmental stage of F1 at this moment in time, the standard error, the number of fertile florets with its standard error, and the thermal time at which the final number of fertile florets was reached. The floret mortality is presented as number of primordia (x100) per thermal time (negative values reflect the floret mortality), the coefficient of correlation, and their p-values. SE = Standar Error of the Mean. * <0.05, **<0.01, ***<0.001.

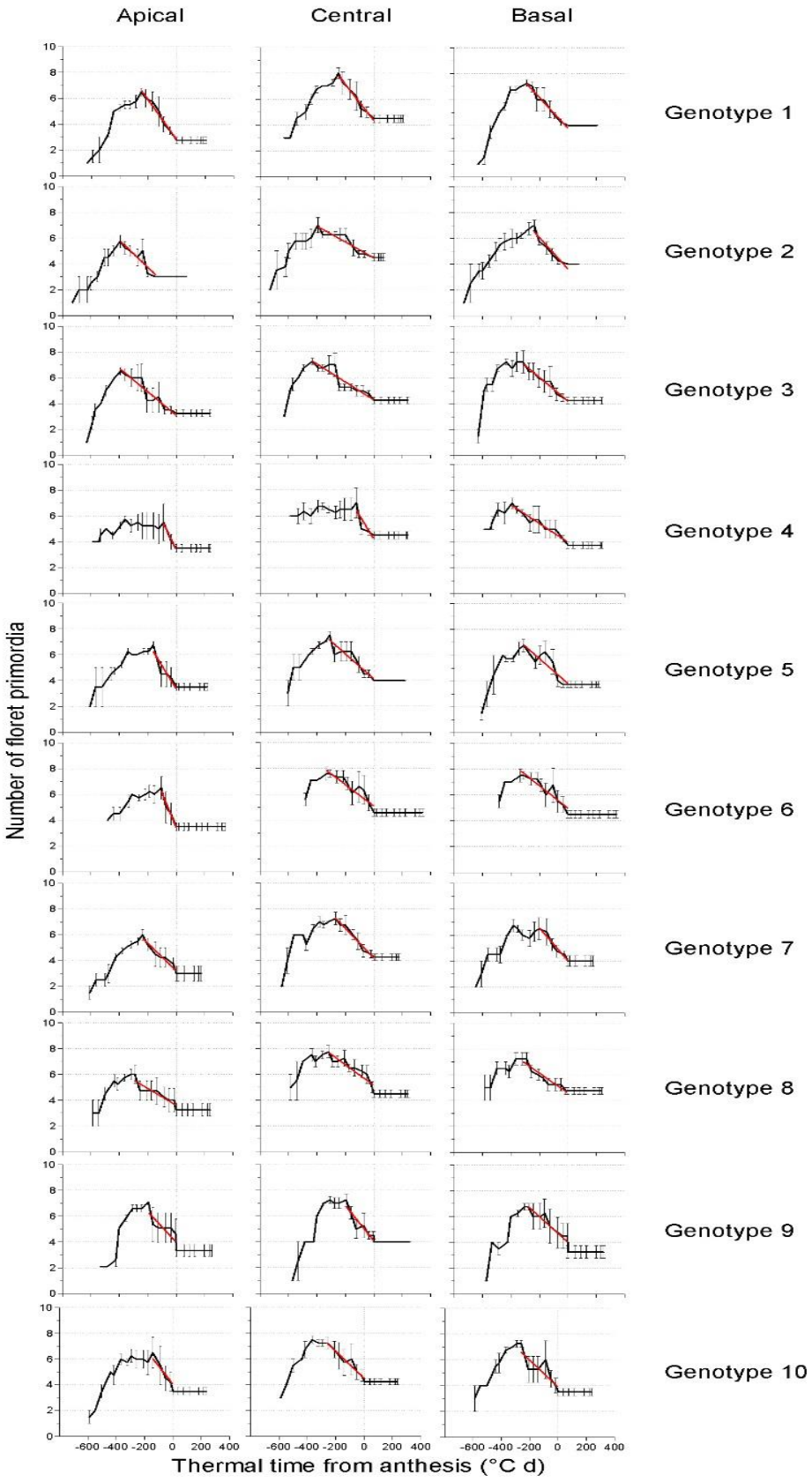

Figure 6. Dynamics of the number of living floret primordia from the onset of stem elongation onwards, plotted against thermal time from anthesis, in the apical (left panel) central (middle panel) and basal spikelets (right panel). The red line represent the linear model fitted between the maximum number of living floret primordia and the number of living floret primordia at anthesis.

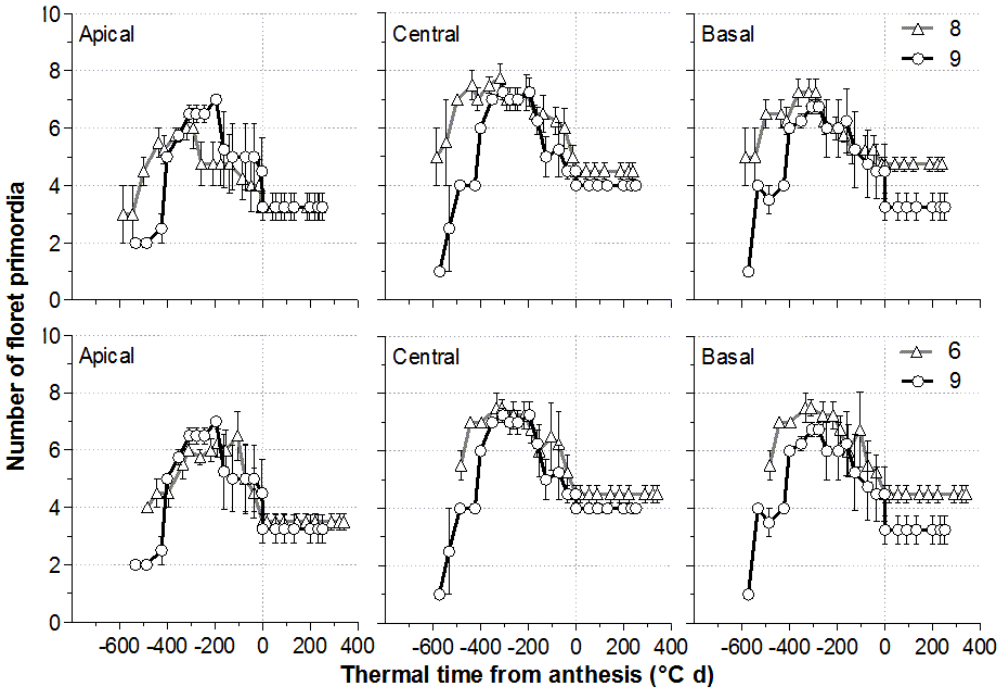

Figure 7. Dynamics of the number of living floret primordia (those developing normally at the time of measurement) from the onset of stem elongation onwards, plotted against thermal time from anthesis in genotypes 8 and 9, which consistently had high and low spike fertility, respectively, within the subset analysed from the CIMCOG panel in the apical (left panel) central (middle panel) and basal spikelets (right panel).

When analysing the development of the individual florets it was clear that florets 1 and 2 developed normally and always reached the stage of fertile florets: in all spikelets and all genotypes (Figure 8). Thus, none of the differences between genotypes in spike fertility were related to the fate of the two most proximal florets. Similarly, none of the genotypic differences in spike fertility were related to the fate of florets 6, 7 and 8; as none of these florets developed normally to reach the stage of fertile florets ever (Figure 10). Therefore, genotypic differences in the developmental patterns of intermediate florets (3, 4 and 5) were critical for establishing the genotypic variation in spike fertility. Focusing on these particular florets it became clear that:

(i) floret 3 developed normally, achieving the stage of fertile florets, in the two genotypes and in all the spikelets: even when the difference in spike fertility was not due to the fate of floret 3, a difference in developmental rates was noticeable: it seemed that floret 3 in genotype 9 developed with some delay compared to that in genotype 8 (Figure 9, left panels).

(ii) floret 4 in the central spikelets did also develop normally achieving the stage of fertile florets in both genotypes, though again it seemed that this

floret started its development in genotype 9 with some delay respect to the timing of development initiation in genotype 8 (Figure 9, central panel).

(iii) floret 4 in the basal and apical spikelets developed normally to become fertile only in genotype 8 (in the apical spikelets only in some of the plants analysed) but was never fertile in apical and basal spikelets of genotype 9 (Figure 9, top and bottom of the central panels).

(iv) floret 5 was never fertile in the apical spikelets of any of the two genotypes (Figure 9, top-right panel), while in the central and basal spikelets it was fertile in some of the plants of genotype 8 and in none of the plants of genotype 9 (Figure 9, central- and bottom-right panels).

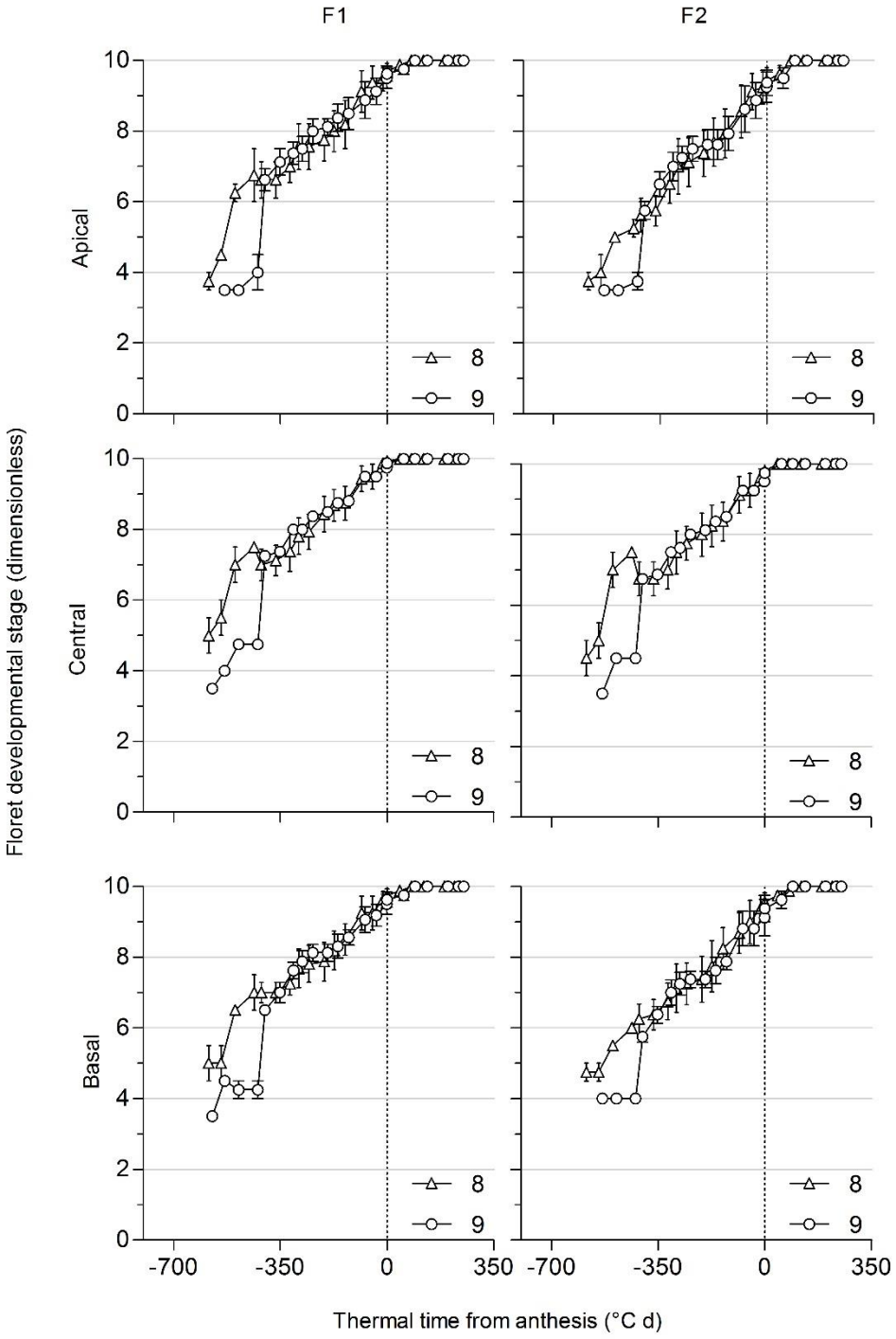

Figure 8. Developmental progress of floret primordia 1 and 2 in apical, central and basal spikelets (from top to bottom panels) from the onset of stem elongation onwards, plotted against thermal time from anthesis.

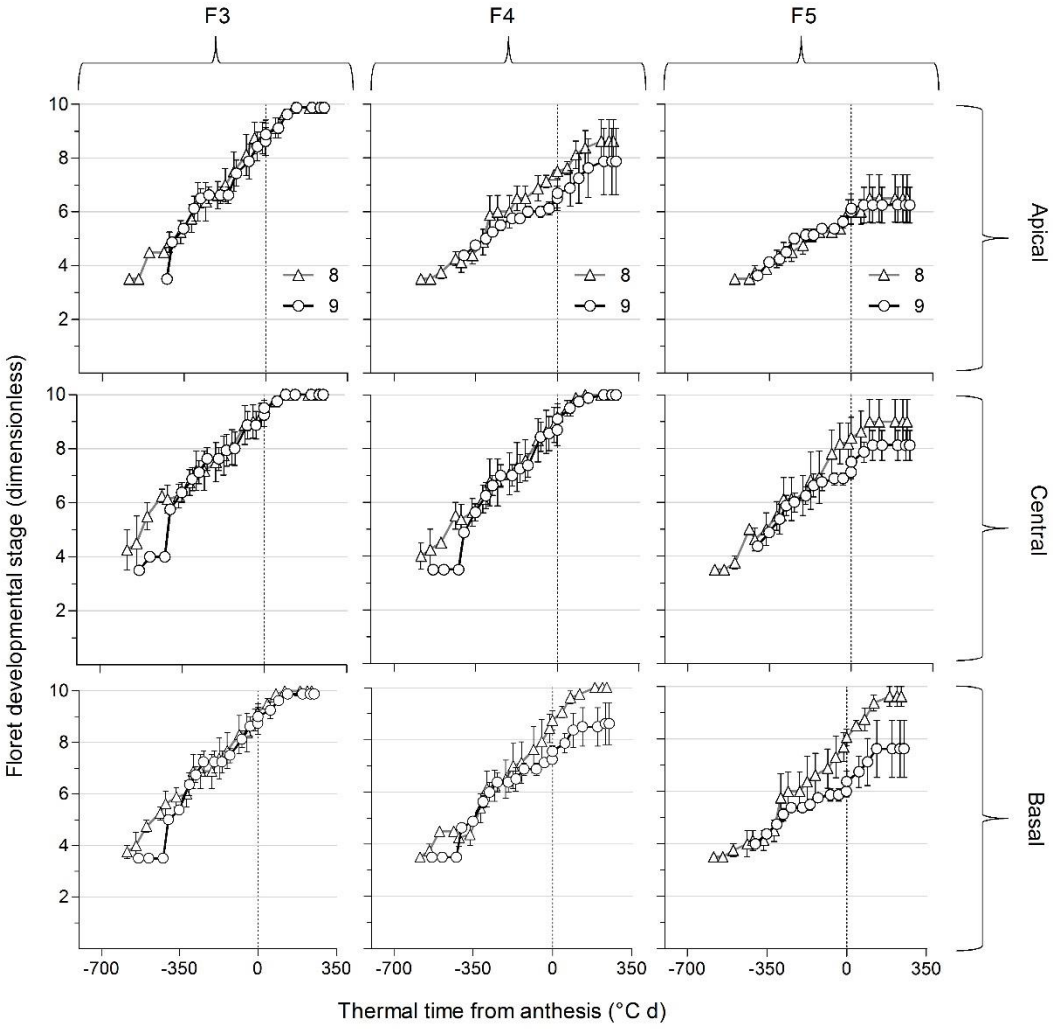

Figure 9. Developmental progress of floret primordia 3, 4 and 5 (from left to right panels) in apical, central and basal spikelets (from top to bottom panels) from the onset of stem elongation onwards, plotted against thermal time from anthesis in genotypes 8 and 9 of the subset analysed from the CIMCOG panel. The florets are fertile when achieving stage 10 in the scale developed by Waddington et al. (1983).

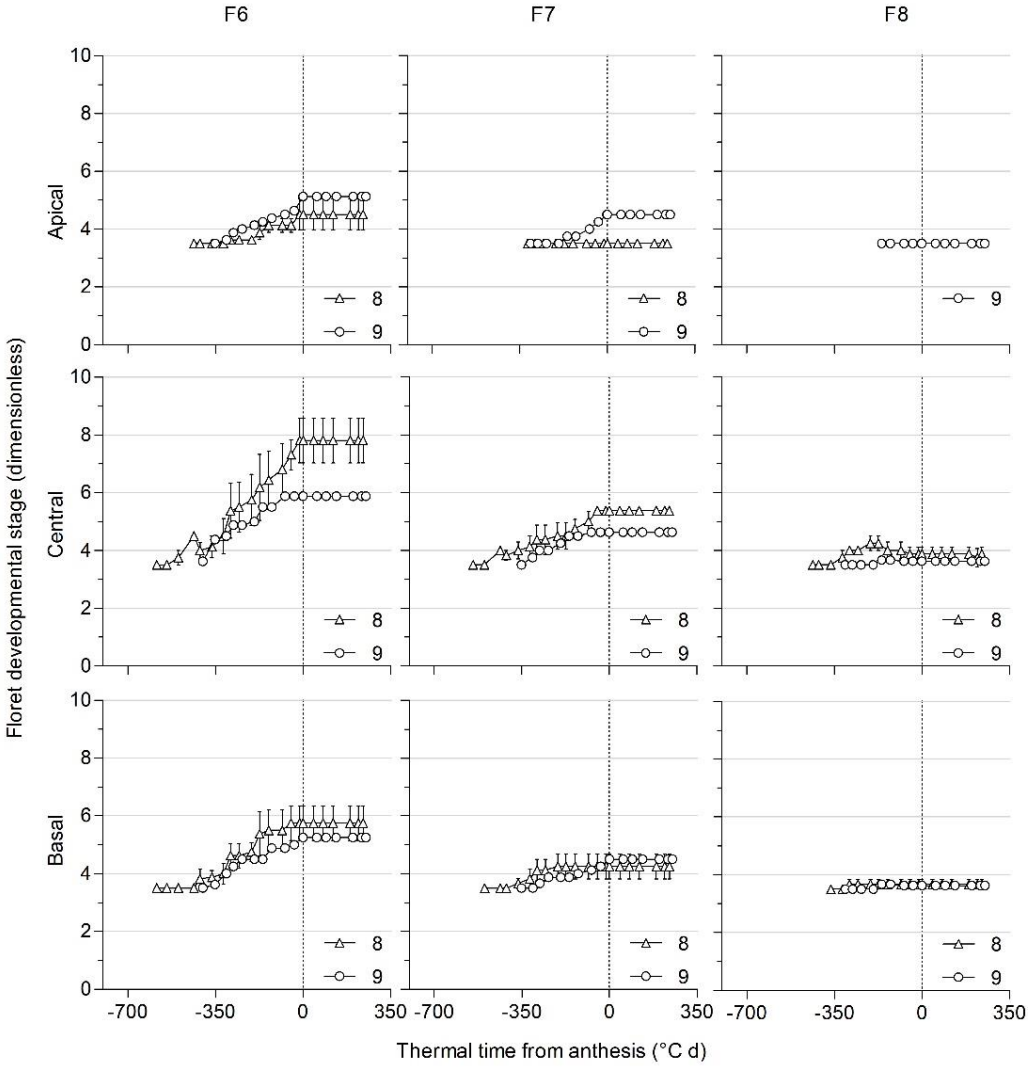

Figure 10. Developmental progress of floret primordia 6, 7 and 8 (from left to right panels) in apical, central and basal spikelets (from top to bottom panels) from the onset of stem elongation onwards, plotted against thermal time from anthesis.

Even in the case of the floret × spikelet positions in which primordia did not continue developing normally to achieve the stage of fertile florets, there was a clear trend, though with few exceptions, for the floret primordia of genotype 8 to have developed more than the equivalent florets of genotype 9 (Figure 9 and Figure 10).

2.4. Discussion

Future wheat breeding needs to be extremely efficient as the land allocated to wheat (and most other major food crops) is unlikely to increase significantly, and the use of inputs cannot increase at similar rates as they have in the last half-century (Chand 2009; Reynolds et al. 2012; Hall and Richards 2013). Although farm yields may be

much lower than yield potential, they seem to be related (Slafer and Araus 2007; Fischer and Edmeades 2010) and therefore there is agreement that genetic gains in yield potential need to be accelerated (Reynolds et al. 2009). To identify opportunities for major improvements in crop photosynthesis is essential (Reynolds et al. 2000; Parry et al. 2011), but will not translate in yield gains without further gains in sink strength, the major determinant of which is grain number. In fact, genotypic differences in yield are most frequently associated with those in grains per m^2 (Slafer et al. 2014), and genetic gains in yield have been mostly explained by improvements in this component (Calderini et al., 1999 and references quoted therein). Further improving grain number would require the identification of variation in its physiological determinants within high-yielding, well adapted populations for breeding. As wheat is a cleistogamous plant, a major determinant of grain number is the number of fertile florets produced. Unfortunately, studies on the dynamics of floret primordia generation/degeneration, which ultimately determines spike fertility, are rather rare, likely because of the intrinsic difficulties of determining these dynamics.

Most of the relatively few studies on floret development dynamics were focused on the effects of environmental factors affecting grain number. In these cases, it was consistently revealed that floret survival was more critical than the initiation of primordia for most environmental factors affecting the number of fertile florets at anthesis. Examples of this include cases in which spike growth during pre-anthesis was altered by shading (Fischer and Stockman, 1980), nitrogen availability (Sibony and Pinthus 1988; Ferrante et al. 2010), photoperiod condition (González et al. 2003b) and combinations of some of these environmental treatments (Langer and Hanif 1973; Whingwiri and Stern 1982; González et al. 2003b, 2005a). Regarding genotypic variation, which is key for genetically improving a trait, there have only been reports based on the introgression of semi-dwarfing genes. Miralles et al. (1998) reported that Rht1 and Rht2 alleles (further described in Chapter 5) increased the likelihood of relatively distal floret primordia successfully progressing to the production of fertile florets, and attributed this to an improved assimilate allocation of resources to the growing spike before anthesis (Siddique et al. 1989; Slafer and Andrade 1991). As opportunities to further increase partitioning to the juvenile spike in respect of most modern cultivars are restricted, variation in floret development and spike fertility within elite germplasm must be identified. In the present study we reported variation in the dynamics of floret primordia in a panel assembled for its potential relevance for breeding to further raise yield potential. The genotypic variation in maximum number of florets initiated was marginal, whereas variation in floret

primordia survival was found to be the main determinant of the genotypic variation in the number of fertile florets at anthesis. The fact that the final number of fertile florets was related to floret primordia survival, and independent of the maximum number of florets initiated, is in agreement with results reported with a comparison of four modern durum wheats by Ferrante et al., 2010 and Ferrante et al., 2013a. Thus, it seems that the differences between elite genotypes in spike fertility are based on similar processes responsible for differences in spike fertility when plants are grown under contrasting environmental conditions.

The model hypothetically applicable is that wheat (and all other cereals) may produce an excessive number of floret primordia without penalties as it is energetically inexpensive. However, when progressing to later developmental stages, growth of these primordia requires increasing amounts of resources, so the plant adjusts the number of primordia that become fertile florets (Sadras and Slafer 2012). This adjustment would be quantitatively related with the availability of resources for the growing juvenile spike before anthesis. This is further reinforced by evidence that the triggers for floret primordia death are not purely developmental processes (Ferrante et al. 2013b), but likely resource-driven (González et al. 2011a). Bancal (2009) suggested that floret death starts when the first floret of the central position reaches a Waddington scale of 7–8; which in the panel of elite lines analysed is not true for all the cultivars (e.g., the onset of floret death in genotype 4 is when the proximal floret at the central position scores 9.3 in the Waddington scale (Table 6)).

Many of the differences between the genotypes analysed from the CIMCOG panel, in terms of spike fertility, were associated with differences in floret survival that can be traced back to the processes of floret development. Comparing the two extreme genotypes of this study (in terms of fertile florets produced per spikelet), it seemed clear that the cultivar maximizing floret survival has a consistently longer period of floret development. Thus, it seemed possible to speculate that advancing development progress of labile florets increases the likelihood of floret primordia becoming fertile florets. For instance growing a particular genotype under relatively shorter photoperiods during the period of floret development (and spike growth) before anthesis normally brings about significant increases in floret primordia survival (González et al. 2003b; Serrago et al. 2008). It seems consistent with this that genotypes having slightly longer periods of floret development may increase the number of fertile florets through reducing the proportion of primordia dying, in line with the earlier hypothesis that lengthening the stem elongation phase would bring about increases in the number of grains per m^2 (Slafer et al. 2001).

2.5. Conclusion

In this chapter is shown that within elite wheat germplasm, which could be used directly in breeding programmes, there is variation in developmental dynamics of the florets which are ultimately responsible for differences in spike fertility. Genotypes with more fertile spikes exhibited an improved survival of floret primordia related to a longer period of floret mortality: the longer the period the more time (and resources) will be available for allowing labile primordia to continue developing normally therefore reducing floret mortality. Selecting lines exhibiting this property as prospective parents may help in further raising yield potential in wheat. Novelty of this chapter is based on the following highlights which lead to the publication in a peer-reviewed journal (see A6):

- Final number of fertile florets is related to survival of floret primordia.
- Critical developmental differences occurred in florets 3, 4 and 5.
- Longer period of floret development maximizes floret survival.

3. Variation in developmental patterns among elite wheat lines and relationship with spike fertility

3.1. Introduction

A substantial increase of wheat yield potential is required within the next decades (Reynolds et al. 2012; Fischer et al. 2014). This yield increase seems difficult to achieve, considering that rates of genetic gains in yield have been slowing down noticeably and are currently well below the level required to match the projected cereal demand (Reynolds et al. 2012; Hall and Richards 2013). When aiming to increase yield potential or other complex traits, breeders need to pyramid traits and are reluctant to use materials that are not within their elite pools. Thus, quantifying the degree of genetic variation within elite germplasm in traits which may contribute to increase yield potential would be critical, at least to design strategic crosses (Slafer 2003; Foulkes et al. 2011b; Reynolds et al. 2012).

Yield can be analysed in terms of the number of grains and their average weight. The capacity of the canopy to provide assimilates to fill the grains does not appear to limit grain growth in a wide range of background growing conditions and genotypes (Borrás et al. 2004; Serrago et al. 2013), even within elite high-yielding material (Pedro et al. 2011; González et al. 2014; Sanchez-Bragado et al. 2014). Consequently, grain number is more plastic than grain weight (Peltonen-Sainio et al. 2007; Sadras 2007; Sadras and Slafer 2012), and yield is far more commonly related to grain number than to the average weight of grains (Fischer 2011; Slafer et al. 2014). Thus to achieve relevant genetic gains in yield potential it is important to identify traits responsible for the determination of grain number (Slafer et al. 2014).

3.1.1. Duration of late reproductive phase and grain number

Grain number in wheat is largely determined during the stem elongation phase (Fischer 1985; Slafer and Rawson 1994), when the juvenile spikes grow whilst floret developmental processes determine the survival of floret primordia (Kirby 1988). As wheat is a cleistogamous plant, most fertile florets become grains and therefore the process of floret survival and spike growth before anthesis is critical for determining grain number (González et al. 2011a; Ferrante et al. 2013b); which is behind the widely reported positive relationship between grain number and spike dry weight at anthesis firstly by Fischer (1985) and later validated in a wide range of cases (Slafer

et al. 2005 and references quoted therein), disregarding whether variations are produced by manipulations in growing conditions (Fischer 1985; Savin and Slafer 1991; Abbate et al. 1995; Fischer 1993; Demotes-Mainard and Jeuffroy 2004; Prystupa et al. 2004; Acreche and Slafer 2011; Ferrante et al. 2012; Marti and Slafer 2014) or genetically through altering dry matter partitioning to the spikes (*e.g.* Siddique et al. 1989; Slafer and Andrade 1993; Miralles and Slafer 1995; Flintham et al. 1997; Miralles et al. 1998; Reynolds et al. 2001; Reynolds et al. 2005) during stem elongation.

Therefore, it has been proposed that the duration of the late reproductive phase, from the initiation of terminal spikelet to anthesis (Slafer 2012b), may influence the number of grains produced by the crop. The rationale behind this proposition is that a longer phase when florets are developing and grains are then set, may influence the likelihood of a floret primordia becoming a fertile floret, and then to set a grain (Miralles and Slafer 2007). Empirical support for this proposition has been provided through artificially manipulating the duration of the late reproductive phase (through changing photoperiod conditions exclusively during stem elongation), producing parallel changes in duration of the late reproductive phase and grain number (Miralles et al. 2000; González et al. 2003a, 2005a; Serrago et al. 2008). This in turn may be due to two alternative, non-exclusive possible mechanisms: a longer period of stem elongation may (i) bring about increases in accumulated growth increasing the resource availability for the juvenile spike where florets are developing (and then the increase in fertility would be associated with increases in spike dry weight at anthesis), or (ii) allow floret primordia which would not, normally, progress to produce a fertile floret a longer period for development and eventually to be able to reach the stage of fertile floret (and then the increase in fertility would be associated with increases in fruiting efficiency).

3.1.2. Lengthening the late reproductive phase

As flowering time is critical for crop adaptation (Richards 1991; Worland 1996; Slafer et al. 2015) modern, high-yielding wheat have a flowering time that has been largely optimised in most regions. Thus, optimizing the developmental pattern through changing the partitioning of developmental time to anthesis into different duration of phases occurring earlier or later than the initiation of the terminal spikelet may contribute to increasing spike fertility (Slafer et al. 2001; Miralles and Slafer 2007; Foulkes et al. 2011b; Reynolds et al. 2012). The likelihood of breeding being able to lengthen the late reproductive phase is proportional to the existence of genetic

variation on the partitioning of time to anthesis between phases occurring before and after the initiation of the terminal spikelet.

It has been shown that the durations of the different pre-anthesis phases may be independent (Whitechurch et al. 2007; Borràs et al. 2009; García et al. 2014; González et al. 2014), which is consistent with the fact that different phases vary in sensitivity to vernalisation, photoperiod, and temperature (Slafer and Rawson 1994; Miralles and Richards 2000; González et al. 2002; Slafer and Rawson 1995; Slafer and Rawson 1996). The existence of genetic variation is relevant for the appropriateness of a trait to become a candidate for designing strategic crosses. But as breeders combine favourable genes in order to achieve genetic progress in yield (and other complex traits), they are enthusiastic to consider potential parents from a selected group of genotypes that can be considered elite. CIMMYT has gathered a special population for studying opportunities for improvements in photosynthesis and biomass simultaneously with maintaining high levels (or even increasing further) partitioning while avoiding losses due to lodging, the CIMMYT Mexico Core Germplasm (CIMCOG). It includes advanced hexaploid wheat lines that have the potential to bring together traits required for producing step changes in yield gains, as well as few historical cultivars and high-yielding durum wheats. CIMCOG was the focal panel used by the Wheat Yield Consortium to study alternatives for further raising yield potential (Reynolds et al. 2011a).

3.1.3. Objective

The objective of the present study was to determine the degree of variation in patterns of phenological development within the elite germplasm of the CIMCOG population, ascertaining whether the differences were related to traits determining spike fertility within the population.

3.2. Materials and methods

Four field experiments (two of which are mentioned in Chapter 2) were conducted in the Mexican Phenotyping Platform (MEXPLAT) established at the research station “Centro Experimental Norman E. Borlaug” (CENEB), near Ciudad Obregon, Sonora, Mexico (27°33’N, 109°09’W, 38 masl), with conditions that represent the high-yield potential wheat mega-environment 1 (Braun et al. 2010). The soil was a Chromic Haplotorret (Vertisol Calcaric Chromic), low in organic matter (<1%), and slightly alkaline (pH=7.7).

3.2.1. Plot information

Experiment 1 and 2 were conducted in 2010-11, experiment 3 in 2011-12, and experiment 4 in 2012-13. Plots in experiments 1, 3, and 4 were carried out in raised beds while experiment 2 had flat (conventional) plots, and in all cases plots were large (17.7-30 m^2) and sown within the optimal period in the region and with optimal sowing densities (Table 7) Full meteorological data can be found in A2, A3 and A4 for season 2010-11, 2011-12, and 2012-13, respectively.

All plots were grown under optimal conditions: they were fertilised and irrigated to avoid N and water stress, and biotic stresses were prevented or controlled (weeds were removed by hand throughout the growing season and diseases and insects prevented by applying recommended fungicides and insecticides at the doses suggested by their manufacturers).

Environment		Sowing	Plot size	Available water	Average temperature (˚C)		Average daily radiation (MJ m^{-2} d^{-1})
					E-A	A-M	
Exp.1 raised beds		06 Dec 2010 101 kg_{seeds} ha^{-1}	5m long and 4.16m wide (4 raised beds 0.80m wide, with 2 rows per bed, 0.24m apart)	573mm	14.9	19.7	21.8
Exp.2 flat beds		06 Dec 2010 101 kg_{seeds} ha^{-1}	5m long and 6m wide (8 rows, 0.2m apart)	573mm	14.9	19.7	21.8
Exp.3 raised beds		09 Dec 2011 108 kg_{seeds} ha^{-1}	8.5m long and 2.08m wide (3 raised beds 0.80m wide, with 2 rows per bed, 0.24m apart)	592mm	15.2	19.3	21.6
Exp.4 raised beds		25 Nov 2012 110 kg_{seeds} ha^{-1}	8.5m long and 2.08m wide (3 raised beds 0.80m wide, with 2 rows per bed, 0.24m apart)	600mm	15.2	18.4	19.5

Table 7. Description of environment, sowing, field trial setup, and meteorological data for the four experiments. Environment (4 experiments sowed in three years under irrigated conditions), Sowing (date of sowing and seed density), Plot size (long, wide, and setup of the plots), Available water (millimetres of rain throughout the crop cycle), Average temperature (mean daily temperature for the period between emergence to anthesis (E-A), and anthesis to maturity (A-M)), and Average daily radiation (mean solar radiation).

3.2.2. Treatments

The treatments analysed in this study consisted of a subset of 27 genotypes (comprised of 22 elite lines, 4 historic lines, and 1 *T. durum*) that were grown through the 4 field experiments. The original panel of 60 genotypes (A1) was only grown and measured in experiments 1 and 2. In experiment 3 half of these lines were selected to represent fairly the complete panel (see Figure 11) to progress on the work (based on results of the first two experiments). In experiment 4 three of the 30 lines grown in experiment 3 were not grown (Table 7). All four experiments were designed in randomized complete blocks with two replicates on experiment 2 and three replicates on experiments 1, 3, and 4.

3.2.3. Determination of key phenology stages

Plots were inspected twice per week after sowing to determine the growth stage (Zadoks et al. 1974). Seedling emergence was determined when half of the seedlings of the plot had the tip of the first leaf emerged from the coleoptile. From then on, one plant per plot (two or three per genotype depending on each experiment) was sampled (once a fortnight at the beginning and then increasing the frequency as the plot was approaching to terminal spikelet initiation, around late January, to up to thrice a week) and dissected under binocular microscope (Carl Zeiss, Germany) to detect the stage of development of the apex to determine the timing of initiation of the terminal spikelet with reasonable accuracy. Thereafter the plots were regularly inspected to determine the timing of anthesis when half of the spikes of the plot had anthers extruded.

3.2.4. Sampling and determinations

A sample of 0.5 m of two rows was taken seven days after anthesis, in which above-ground biomass was determined dividing it into spikes and the rest of the canopy. In experiment 4, a sub-sample was taken in which all the starting-to-grow grains were removed from the spikes and therefore the non-grain spike dry weight at a week after anthesis was measured. Eliminating the weight of the grains is relevant as they may represent a sizeable, and variable among genotypes, part of the spike dry weight at that stage and would overestimate the bulk of resources that were available to allow a certain number of grains to be set (Fischer (2011) and references therein). With these values we estimated the proportion of grain and non-grain spike dry weight at a week after anthesis for each genotype to estimate the non-grain spike dry weight in all cases.

At maturity yield was determined from harvesting the plot (excluding the extreme 50 cm to avoid border effects) using standard protocols (Pask et al. 2012). Before that 100 fertile culms were sampled, dried, weighed and threshed to allow calculation of yield components.

With the measurements of grain number at maturity and non-grain spike dry weight at a week after anthesis we estimated fruiting efficiency; i.e. the efficiency by which dry matter allocated to the spikes at anthesis is used to determine the survival of floret primordia and set grains (Ferrante et al. 2012; García et al. 2014)).

3.2.5. Analyses

The analysis of variance (ANOVA) and the principal component analysis (PCA) were performed using R 3.0.2 (R Development Core Team), the latter was plotted with ggbiplot package from R. The linear model fitting and figures were produced using GraphPad Prism 5 (2007). For the relationship between fruiting efficiency and duration of the phase from terminal spikelet to anthesis we also fitted a boundary function for establishing an upper threshold (a line edging the upper limit of the data-cloud; Casanova et al., 1999) describing the highest fruiting efficiencies observed over the range of durations of this phase measured; a procedure commonly used to establish upper limits in ecology (e.g. Cade and Noon, 2003) and agronomy (e.g. Sadras and Angus, 2006). To derive the boundary function, we subdivided the phase duration data in intervals of 2 days (from 36 to 48 d) and fitted a regression considering the maximum values of fruiting efficiency within each interval.

3.3. Results

3.3.1. Representativeness of the subsets

The 27 lines selected to represent the CIMCOG population in the 4 studies were shown to be representative of the whole population. Considering either the duration from seedling emergence to anthesis or the number of grains per unit land area (the most integrative phenological and yielding traits in which this chapter is focused), the average and the ranges explored in the complete CIMCOG panel (60 lines) and the subset of 27 lines studied in the 4 experiments were quite similar in the two experiments in which the complete panel was grown (Figure 11).

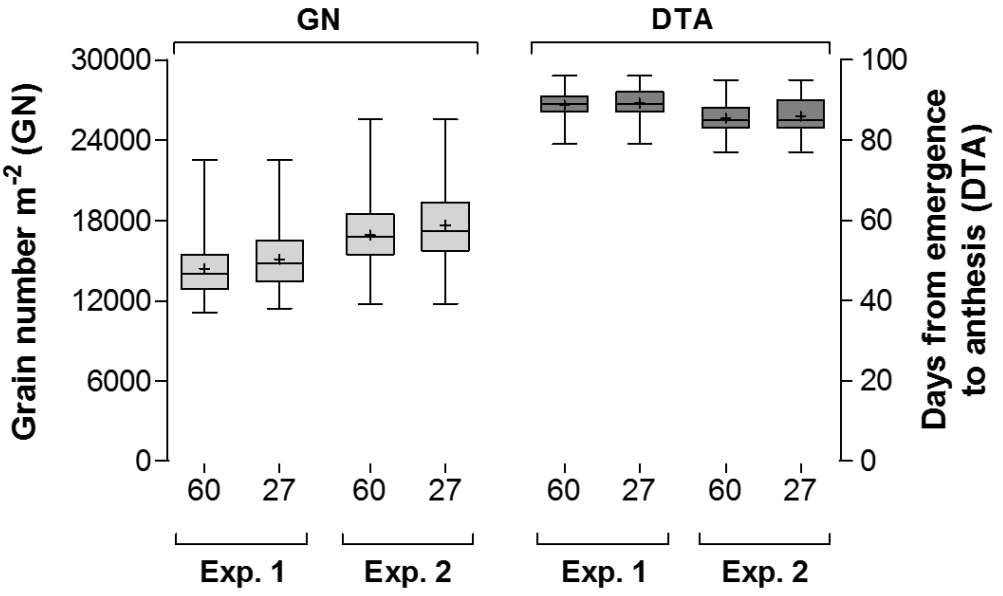

Figure 11. Boxplot of season 2010-11 for the CIMCOG panel (60) and the subset used across the 4 environments (27); for grain number (GN, left side) and days to anthesis (DTA, right side). The different population sizes (60 and 27) are grouped by experiment (1 and 2). The asterisk inside the boxes stands for the mean (while the line dividing the box is the median).

Even though the CIMCOG is a special panel selected to be integrated mainly by elite material (i.e. well adapted and high-yielding), there seemed to have been a wealth of variation within the panel even when analysing the most integrative phenological and yielding traits considered here. Variation in grain number might be somehow expected as some lines may be high-yielding due to possessing large grains and then the variation in yield components may be larger than expected. However, the variation in time to anthesis within the CIMCOG was much larger than expected for a population of well adapted material.

In the rest of this Results section all the analyses will be shown considering both the whole subset of 27 lines representing well the whole CIMCOG population as well as restricting the variability to the 22 lines of this subset which are exclusively elite hexaploid lines (disregarding the four historical cultivars and the durum wheat). Therefore, any differences in results from analysing the 27 or the 22 lines would be the influence of the historic lines and/or the tetraploid wheat (*T. durum*) in the overall analysis.

3.3.2. Time to anthesis and duration of the stem elongation period

The subset of 27 genotypes analysed throughout this chapter, varied noticeably in time to anthesis (Figure 12). The variation was not due to the inclusion of the historic cultivars or due to the durum wheat cultivar, it was actually evident within the 22 lines of elite hexaploid wheat as well (Figure 12).

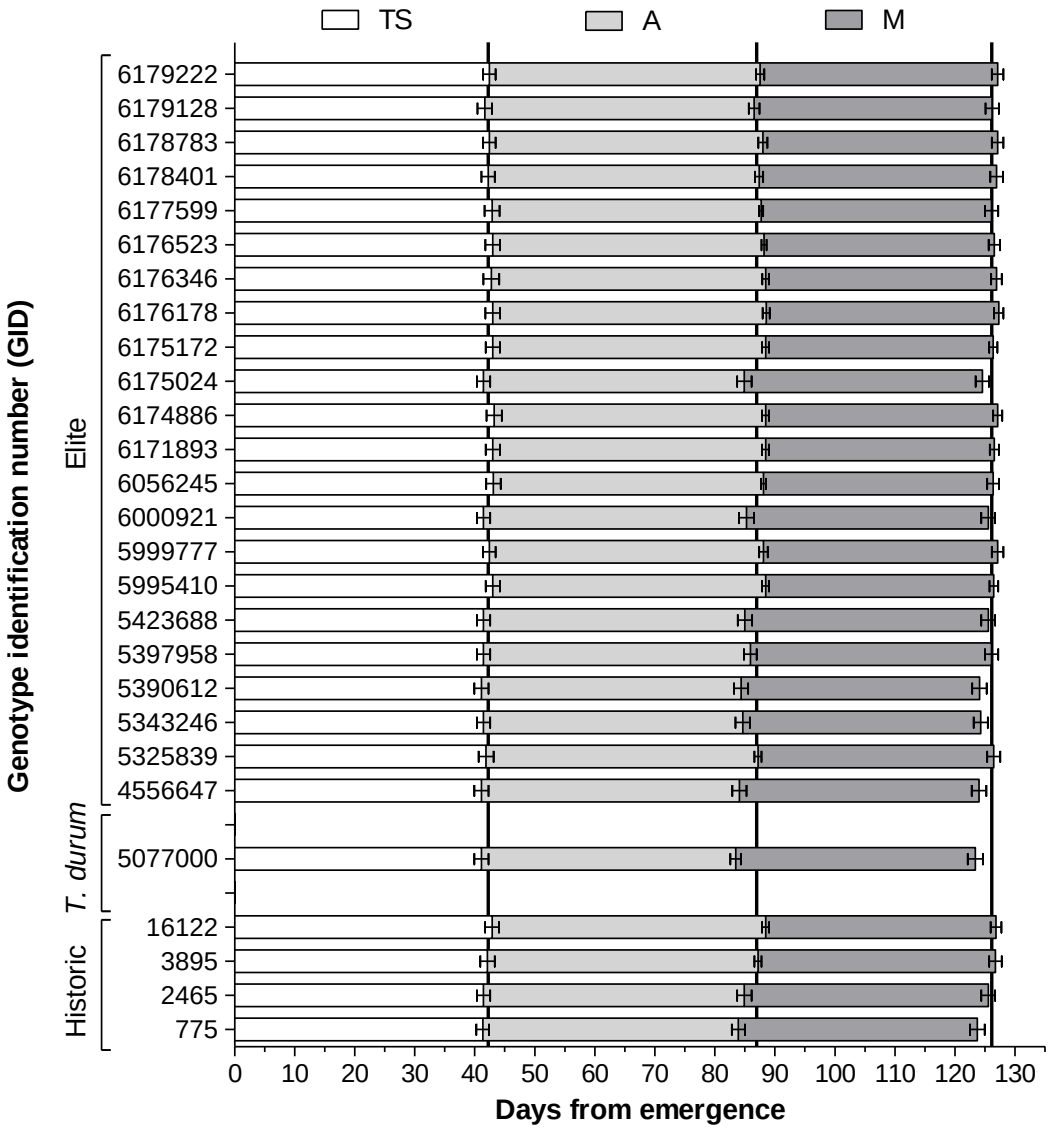

Figure 12. Duration of phasic development in days from emergence to terminal spikelet (TS), from then to anthesis (A), and from then to maturity (M). The genotypes are grouped in 22 elite cultivars, 1 *T. durum*, and 4 historic lines. Each bar is the average of four experiments and the segments stand for the standard error of the means.

The variation in time to anthesis was due to variation in the durations of both the time from seedling emergence to terminal spikelet and the time from then to anthesis (Figure 12).

Variation in grain filling duration was less clear (Figure 12), as the time to maturity more or less mimicked the pattern of genotypic variation in time to anthesis (Figure 12). In fact, the relationship between time to maturity and time to anthesis (in both cases from seedling emergence) was extremely high (r^2=$0.97_{27lines}$ and $0.98_{22lines}$), the slope very close to 1 (0.9 in both cases), and the intercepts (reflecting the overall average duration of grain filling) exhibited little variation ($49.8\pm2.6_{27lines}$ days - $49.7\pm2.6_{22lines}$ days).

In general, the variation found in phenology and the relationships between the durations of different phases were quite similar (both in terms of ranges explored and in degree of association between phases in the regressions) when analysing the whole subset of 27 lines or restricting it to 22 elite hexaploid lines disregarding the 4 historic cultivars and the *T. durum* (Figure 13).

Time from seedling emergence to anthesis was similarly and highly correlated with the duration of its two component phases: time from emergence to terminal spikelet (Figure 13a,d) and time from terminal spikelet to anthesis (Figure 13b,e). Despite the similar relationships, it seemed that the duration of the late reproductive phase was more relevant than that of the period from emergence to terminal spikelet in determining variation in total time to anthesis. This is not only because the coefficients of determination were slightly higher for the relationship with the duration of the late reproductive phase (r^2=0.77-0.80) than with the time to terminal spikelet (r^2=0.71-0.73), but also because the range of variation in the former (abscissa in Figure 13b,e) was noticeably larger than the latter (abscissa in Figure 13a,b).

More importantly, the length of the two phases constituting time to anthesis were largely independent of each other: they were significantly positively related but the proportion of the duration of time to terminal spikelet related to the duration of the late reproductive phase was only c. 25% (Figure 13c,f), which indicates that cultivars may combine contrasting durations of these two phases. This shows that even in a restricted range of well adapted elite lines there may be a large number of possible combinations for reaching a particular aim regarding phenology. For instance, a particular duration of the stem elongation phase (any of the isolines in Figure 13a,c) could be combined with different durations of the phase to terminal spikelet and therefore changes in time to anthesis may be achieved by exclusively modifying the

duration of phenological phases when leaf and spikelet primordia are being formed. The contrary is also true, and a particular duration of the period to terminal spikelet (any of the isolines in Figure 13b,e) could be combined with different durations of the late reproductive phase, and therefore changes in time to anthesis may be achieved by exclusively modifying the duration of phenological phases when floret primordia are being formed. Or a similar time to anthesis (isolines in Figure 13c,e) may well be achieved by combining a relatively short phase to terminal spikelet and a relatively long stem elongation period and *vice-versa* (pairs of genotypes with the same duration to anthesis but differing in how this developmental time is partitioned between phases occurring before or after the initiation of the terminal spikelet, can easily be identified (Figure 13c,f and Figure 12)).

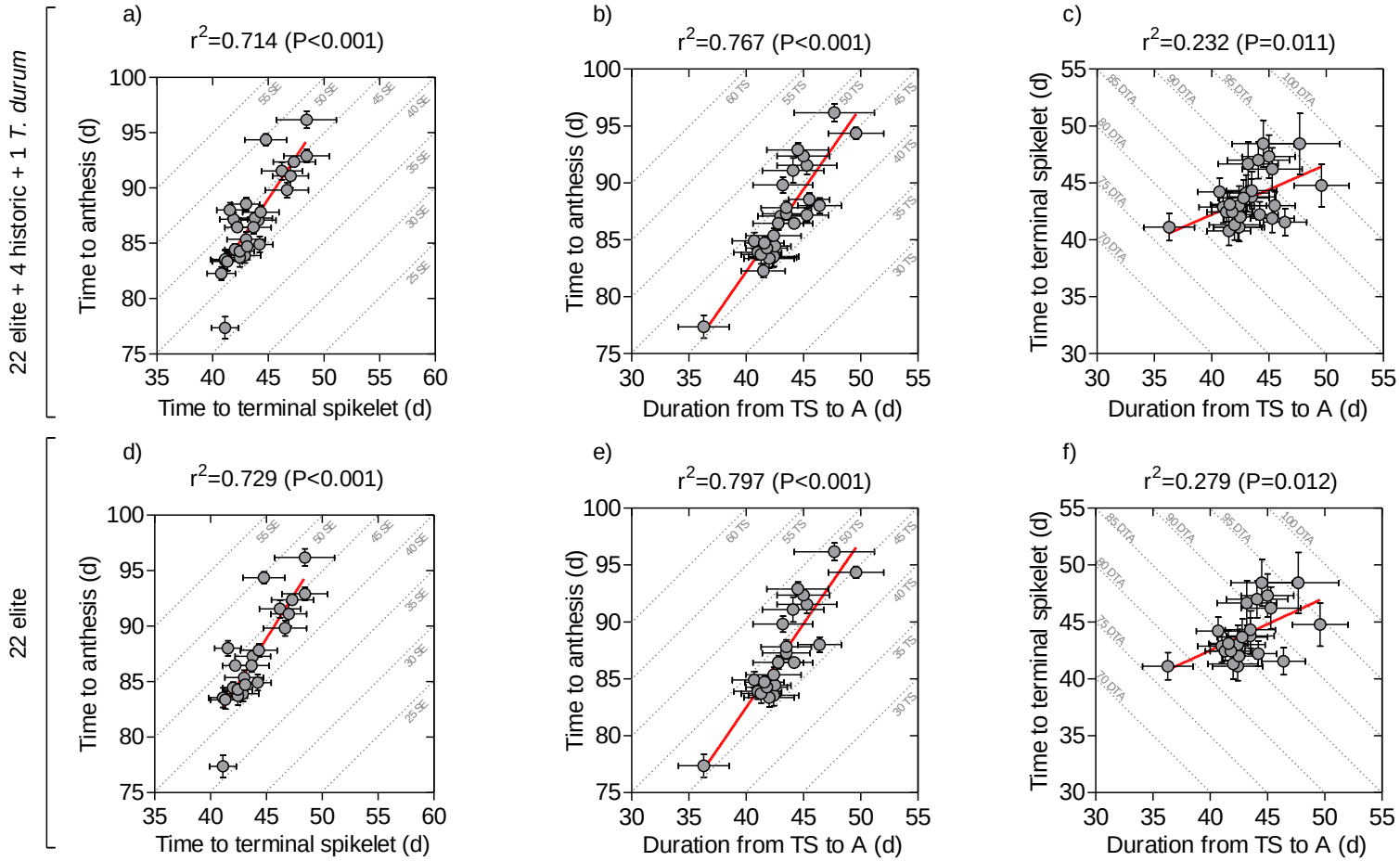

Figure 13. Relationships between the duration of the periods from seedling emergence to anthesis and its two components time from emergence to terminal spikelet (a,d) and from then to anthesis (b,e) as well as the relationship between these component phases (c,f) considering the whole subset of 27 genotypes (top panels) or only the 22 elite hexaploid genotypes (bottom panels). Within each of the panels, isolines for the same duration of complementary phases were drawn. They were (the stem elongation period (SE) in panels a and d, the time to terminal spikelet (TS) in panels b and e, and the time to anthesis (DTA) in panels c and f. Each data-point is the average across the 4 environments and segments stand for the standard error of the means (not seen when smaller than the sizer of the symbol).

3.3.3. Yield and yield components

Yield showed a range of more than 2 Mg ha^{-1} (from c. 5.5 to almost 8 Mg ha^{-1}) when the whole subset was analysed while it was lowered to c. 1 Mg ha^{-1} when considering only the 22 elite lines (Figure 14 on ordinates).

The difference between considering the whole subset or only the 22 elite lines was noticeable when considering the relationships between yield and its components. Considering the whole subset, yield was completely unrelated to the number of grains per unit land area (Figure 14a) and significantly related to the average weight of the grains, even though the coefficient of determination was low (Figure 14b). However, it seems clear that the relationship was strongly dependent on two of the 27 data-points, those exhibiting the highest and the lowest yield, the former also having the highest thousand grain weight and the later having one of the lowest thousand grain weight (Figure 14b). As these two cases correspond to the durum wheat line that outyielded the hexaploid wheats and to one of the historic cultivars; when restricting the analysis to the 22 elite lines the relationship between yield and thousand grain weight was completely lost (Figure 14d) and an incipient linear trend, though not statistically significant, with grain number became apparent mainly because the actual significant relationship was quadratic ($r=0.527$, $P<0.01$), implying that yield tended to increase with increases of grain number until intermediate values of this component and further increases in grain number tended to reduce yield (Figure 14c). Essentially it could be seen that within the CIMCOG panel yield differences between genotypes were determined by particular combinations of grain number and grain weight of the different genotypes, that yield was not strongly related to any of them particularly (Figure 14), and there was a clear negative relationship between these components (Figure 15a,d). This negative relationship was more pronounced when considering the 22 elite lines (Figure 15d) than when the whole subset was taken into account (Figure 15a). Within the 22 elite lines, yield was maximised when cultivars presented intermediate values of grains per unit land area (when data-points crossed over the curves representing iso-yields; Figure 15d): if compared with the lines with the lowest number of grains, these intermediate ones had smaller grains but not small enough to compensate for the increase in grain number, while when genotypes increased grain number further the reduction in grain size was more than compensating the increase in grain number (Figure 15d), generating the quadratic relationship between yield and grains per unit land area (Figure 14c).

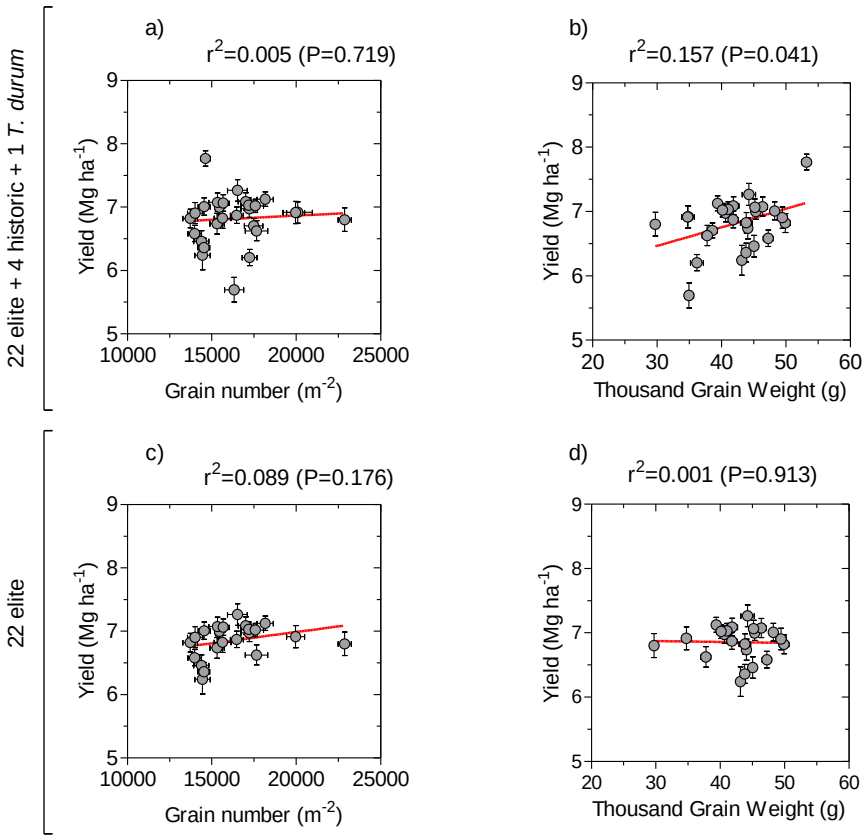

Figure 14. Relationships between yield and its two components: grains per unit land area (a,c) and the average weight of grains estimated as thousand grain weight (b,d) considering the whole subset of 27 genotypes (top panels) or only the 22 elite hexaploid genotypes (bottom panels). Each data-point is the average across the 4 environments and segments stand for the standard error of the means (not seen when smaller than the sizer of the symbol).

Fruiting efficiency was the trait which most strongly explained both yield components: the relationship was positive with grain number (Figure 15b,e) and negative with grain weight (Figure 15c,f), which would be the functional cause of the partial compensation between both yield components (Figure 5a,d). The relationships mentioned between yield components and fruiting efficiency held for both the whole subset of 27 genotypes (Figure 15b,c) and for the analysis restricted to the 22 elite hexaploid lines (Figure 15e,f), but they were sharper when restricting the analysis to the 22 elite hexaploid lines. Although there seemed to be an outlier in which fruiting efficiency was distinctly higher than in the rest of the population, the correlations coefficients would have been still significant if the analysis were made disregarding that particular genotype, particularly so for the analysis restricted to the 22 elite hexaploid lines (as the correlation coefficients between fruiting efficiency and either grain number [$r=+0.77_{27lines}$ $P<0.001$ and $+0.77_{22lines}$ $P<0.001$] or grain weight [$r=-0.59_{27lines}$ $P<0.001$ and $-0.76_{22lines}$ $P<0.001$] remained significant excluding that genotype of highest fruiting efficiency).

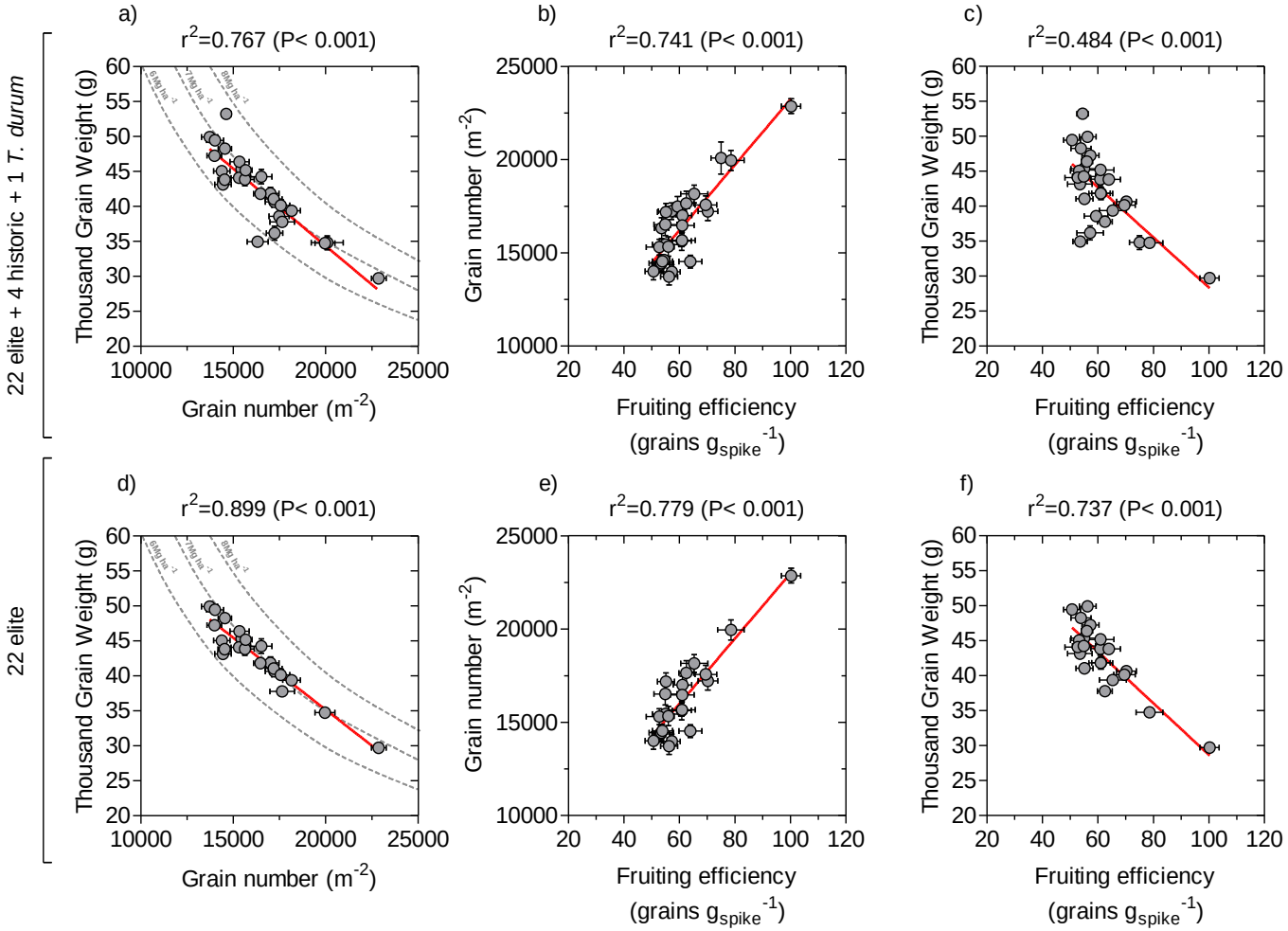

Figure 15. Relationships between the two major yield components (a,d) and between each of them (grains per unit land area [b,e]; average weight of grains estimated as thousand grain weight [c,f]) and fruiting efficiency considering the whole subset of 27 genotypes (top panels) or only the 22 elite hexaploid genotypes (bottom panels). Within panels a and d, isolines for the yields of 6, 7 and 8 Mg ha^{-1} were drawn. Each data-point is the average across the 4 environments and segments stand for the standard error of the means (not seen when smaller than the sizer of the symbol).

3.3.4. Duration of phases and yield components

The duration of the late reproductive phase tended to be positively related to the number of grains per unit land area (Figure 16a,c), and negatively to the average weight of the grains (Figure 16b,d). The relationships were similar when considering the whole subset (Figure 16, top panels) or only the 22 elite genotypes (Figure 16, bottom panels). But in all cases the relationships were rather weak.

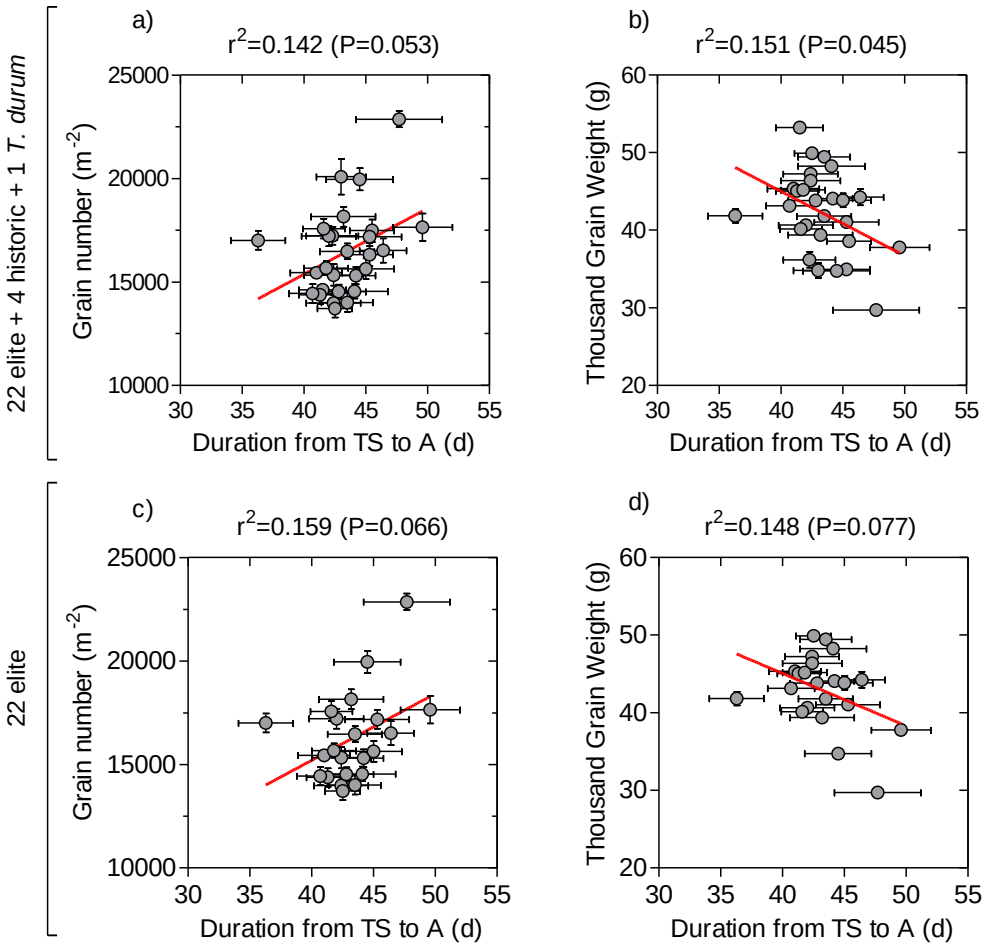

Figure 16. Relationships between either the number of grains per unit land area (a,c) or the average weight of grains estimated as thousand grain weight (b,d) and the duration of the late reproductive phase from terminal spikelet (TS) and anthesis (A) considering the whole subset of 27 genotypes (top panels) or only the 22 elite hexaploid genotypes (bottom panels). Each data-point is the average across the 4 environments and segments stand for the standard error of the means (not seen when smaller than the sizer of the symbol).

In the case of the relationship between grain weight and duration of the late reproductive phase (Figure 16b,d), the fact that the length of the period from terminal spikelet to anthesis was the main determinant of time to anthesis (see above, and Figure 13) could bring about the interpretation that the longer the late reproductive phase the later the grain filling condition and the smaller the grains. However this

explanation would be hardly plausible as the duration of the period from anthesis to maturity was very similar among all lines (see above and Figure 12); and differences in thousand grain weight were chiefly determined by differences in the rate of grain filling ($r=0.99_{27lines}$ $P<0.001$ and $0.98_{22lines}$ $P<0.001$).

Regarding the weakness of the relationship between grain number and duration of the late reproductive phase (Figure 16a,c), it implies that the main driving force for the genotypic differences in grain number was not the differences in spike dry weight at anthesis (the correlation between grain number and non-grain spike dry weight at 7 days after anthesis was extremely low; $r=-0.09_{27lines}$ $P=0.62$ and $-0.17_{22lines}$ $P=0.45$). As the difference in grain number between lines was largely explained by their differences in fruiting efficiency (Figure 15b,e) there might be room for a subtle effect of the duration of the late reproductive phase on fruiting efficiency.

Analysing the relationship between fruiting efficiency and the length of the late reproductive phase produced a positive, though not significant, trend (Figure 17). As the likely effect would be subtle it was not expected to find a highly significant degree of association between them. Using a frontier analysis for the relationship there was a rather strong positive relationship both for the whole subset and for the 22 elite hexaploid genotypes (Figure 17), implying that the length of the late reproductive phase might set an upper threshold for fruiting efficiency.

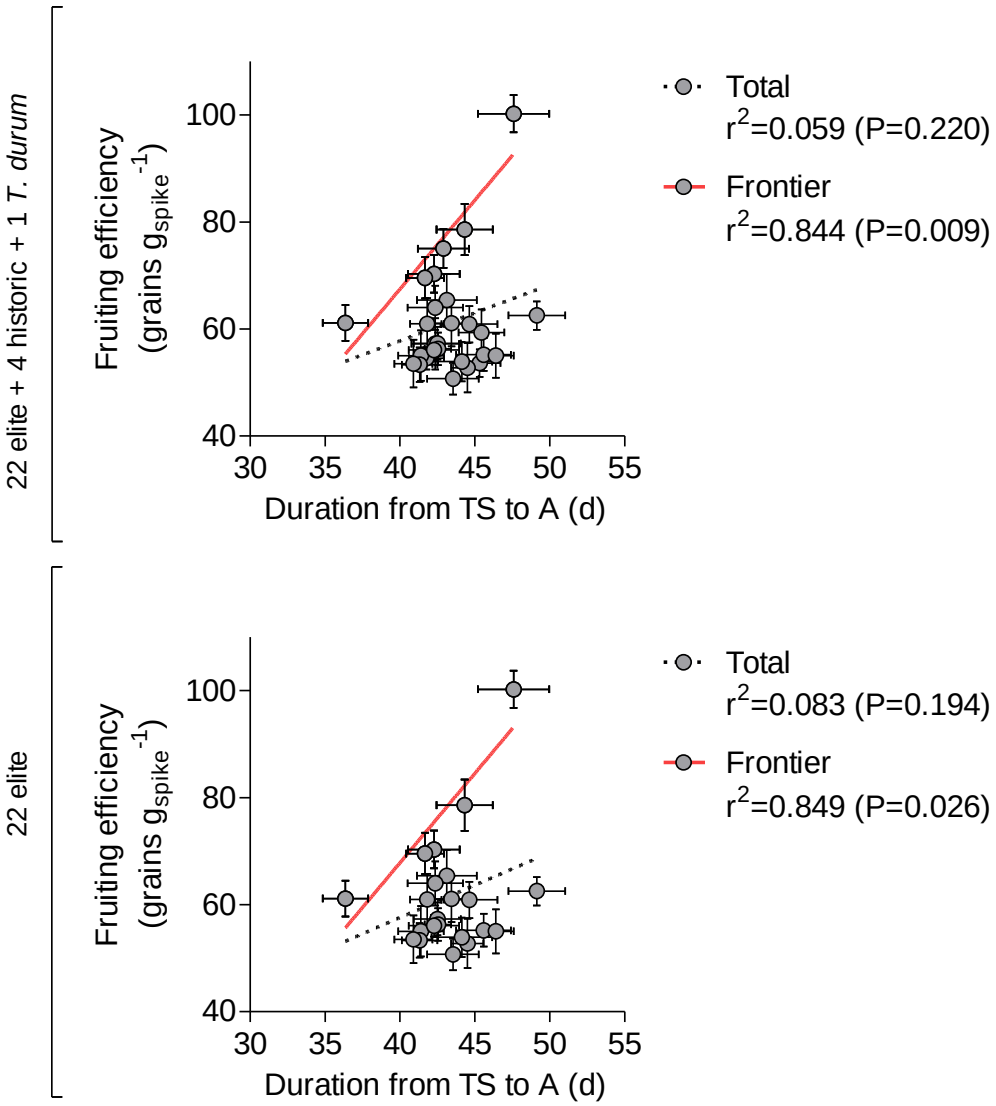

Figure 17. Relationships between fruiting efficiency and the duration of the late reproductive phase from terminal spikelet (TS) and anthesis (A) considering the whole subset of 27 genotypes (top panel) or only the 22 elite hexaploid genotypes (bottom panel). Each data-point is the average across the 4 environments and segments stand for the standard error of the means. The solid line shows the linear regression using the French and Schultz (1984a) frontier concept. The dashed line represents the linear regression for all the data-points.

Overall relationships through principal component analysis

Over all of the G x E conditions, differences in yield considering the whole subset of 27 genotypes were virtually unrelated to increases in either grain number or grain weight (Figure 18a). On the other hand, when analysing the subset of 22 elite hexaploid genotypes, the scenario changes dramatically: yield seemed quite well and positively related to grain number per unit land area, while it was negatively related to thousand grain weight (Figure 18b). Thus across the G x E interaction, the view is reinforced that when the analysis is restricted to the 22 elite hexaploid lines,

genotypes outyielding others were those able to increase grain number, even though there was a partial compensation in the average weight of grains.

In the biplots of the whole subset as well as in that of the 22 elite hexaploid lines there was a clear positive relationship between grain number and fruiting efficiency (and no relationship with spike dry weight at anthesis), and a strong negative relationship between fruiting efficiency and grain weight (Figure 18a,b). It seemed that the main attribute responsible for major differences in grain number was in turn responsible for the grains set to be smaller in average.

The effect of the differences in duration of the late reproductive phase on grain number generation seemed to have been virtually irrelevant for either grain number or fruiting efficiency (Figure 18a), but part of the lack of relationship between the length of the late reproductive phase and both the number of grains per unit land area and fruiting efficiency seemed to have been due to a positive relationship of the duration of this phase with the number of spikes per unit land area (Figure 18).

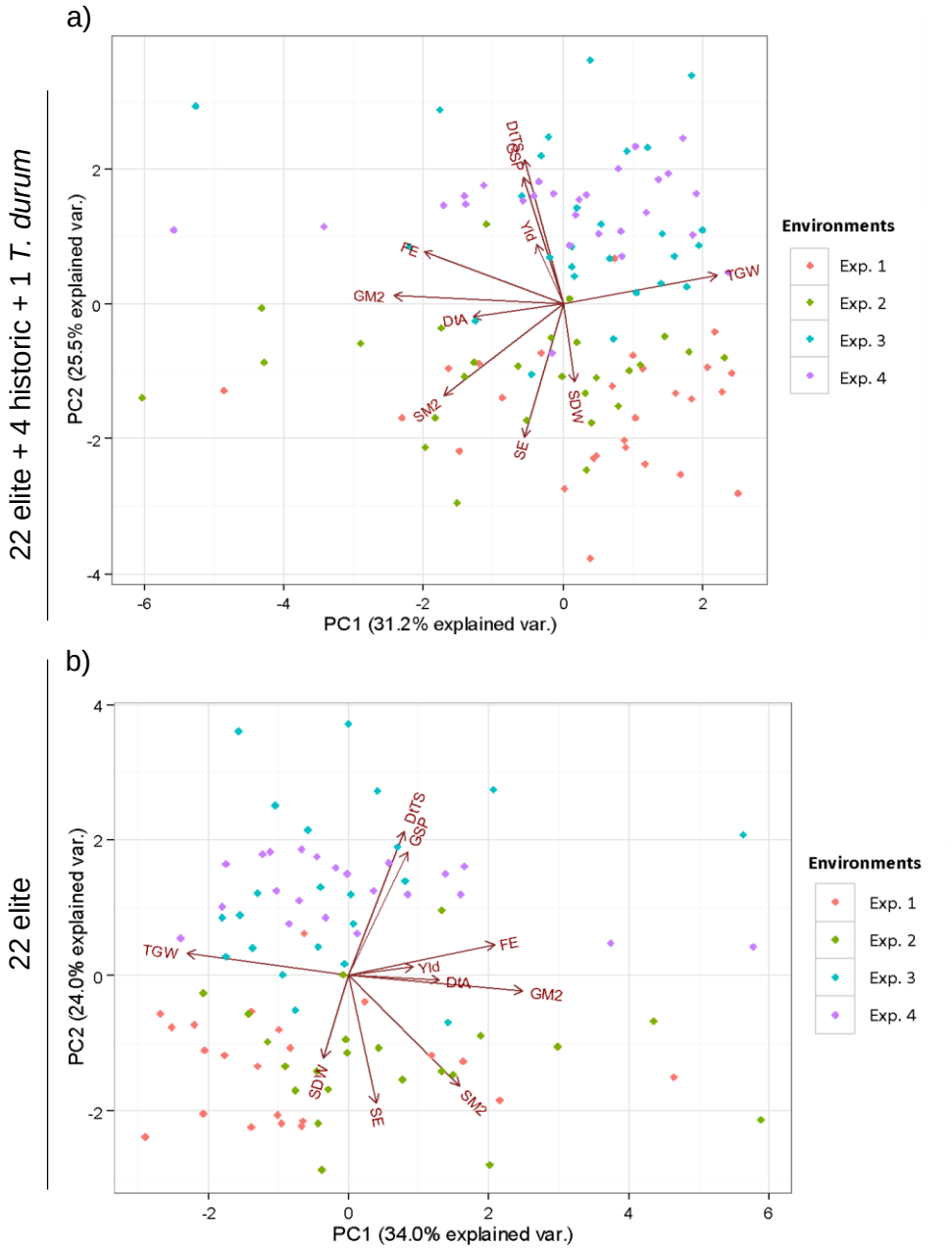

Figure 18. Biplot of principal components analysis considering the whole subset of the 27 genotypes (a) or only the 22 elite hexaploid genotypes (b) grown across 4 experiments (Table 7). Variables considered were Yld: grain yield, TGW: thousand grain weight, GM2: grains per square meter, SM2: spikes per square meter, GSP: grains per spikelet, SDW: non-grain spike dry weight at 7 d after anthesis, DtTS: days from emergence to terminal spikelet, DtA: days from emergence to anthesis, SE: days of the stem elongation period (from DtTS to DtA), FE: fruiting efficiency.

3.4. Discussion

This chapter examines the degree of variation of the duration of the stem elongation phase among elite wheat lines, as well as the bases for the differences in yielding capacity among them, using a subset that includes the genetic variation contained in the whole panel with a minimum of repetitiveness.

Genetic variation is key to further improvements independent of the use of GMO technologies. As breeders pyramid yielding genes the truly relevant variation is that present within elite materials breeders would be keen to cross in their programs. Although searching for genetic variation in modern elite cultivars might be like looking for the needle in the haystack (Able et al. 2007), several studies are far more enthusiastic suggesting that the genetic diversity within elite lines may still provide useful tools towards improving yield potential (Dreisigacker et al. 2004; Soleimani et al. 2002).

In the present study not only was there variation in the duration of phenological phases, but also their durations seemed to be independent of each other. This was in agreement with studies carried out with other populations (Halloran and Pennell 1982; Whitechurch et al. 2007; Miralles and Richards 2000; González et al. 2002) and collectively supports the idea of fine-tuning the developmental phases as a tool for improving not only adaptation but also yield potential (Slafer et al. 2001; Miralles and Slafer 2007).

The lack of strong correlations between yield and yield components imply that among the 27 genotypes, as well as for the 22 elite genotypes, there is more than one way to reach a high yield. Some high yielding genotypes have high grain number (Gonzalez-Navarro et al. 2015) while others have high grain weight (Quintero et al. 2014). Besides this, further improvements must be focused on grain number (Foulkes et al. 2011b) as the plasticity of grain number is much larger than that of grain weight (Sadras and Slafer 2012) and consequently any large increase in yield must require improvements in grain number (Slafer et al. 2014).

An increased stem elongation period could provide further allocation of biomass to the spike (i.e. a greater spike dry weight) at anthesis (Slafer et al. 2001; Miralles and Slafer 2007; González et al. 2005b; González et al. 2011b). By providing more photo-assimilates to the spike through an extended stem elongation period, there could be an improvement in floret primordia survival (Ferrante et al. 2013b), consequently increasing the number of fertile florets. However, crosses with this purpose between the elite lines analysed in this study with this aim might be risky as there was no relationship between the length of the stem elongation phase and spike dry weight at anthesis in the panel analysed. This means that lines possessing longer stem elongation phases in this panel may have also possess lower rates of canopy growth, and/or lower levels of dry matter partitioning to the juvenile spikes, compensating the expected advantage of longer late reproductive phase on grain number.

On the other hand, there was a subtle relationship between the duration of the late reproductive phase and fruiting efficiency, which is relevant as the latter had a strong correlation with grain number, which supports the idea of using fruiting efficiency as an alternative trait to further increase grain yield (Slafer et al. 2015). Even though both fruiting efficiency and grain number had a highly significant negative correlation towards grain weight, fruiting efficiency is shown to have a weaker association to grain weight than grain number. Similar results from González et al. (2014) provide some reassurance on using fruiting efficiency as a tool for the potential improvement of grain yield.

The relationship between the duration of stem elongation and fruiting efficiency was analysed with a frontier approach, which has been successfully used in other studies of complex traits like water-use efficiency (Sadras and Angus 2006; French and Schultz 1984a, b; Sadras and McDonald 2012; Hunt and Kirkegaard 2012). This approach showed how an increased stem elongation period could be instrumental in increasing the upper threshold in fruiting efficiency, and therefore in grain number.

3.5. Conclusion

In this chapter, the relationship between stem elongation and fruiting efficiency was investigated in a panel of modern elite genotypes. Substantial variation was found in partitioning of developmental time between phases occurring before and after terminal spikelet, and the length of these phases seemed largely independent. Extending the stem elongation period could raise the upper threshold for fruiting efficiency, which was the most relevant trait determining differences in grain number between elite lines; this in turn could provide benefits towards increasing grain yield potential. Novelty of this chapter is based on the following highlights which lead to the publication in a peer-reviewed journal (see A7):

- Time to terminal spikelet and from then to anthesis were largely independent.
- The length of the stem elongation phase was slightly but positively related to grains per m2.
- Fruiting efficiency was critical for determining grain number, but it was also negatively related to grain weight.
- The length of the stem elongation phase seems to have imposed an upper threshold for fruiting efficiency.

4. Quantitative trait locus for stem elongation landmarks in Buster x Charger DH population.

4.1. Introduction

Food security has never been more important. With a world that passed the 7 billion people mark and a predicted food shortage of 70 to nearly 100 percent of the current food production by 2050 (Bruinsma 2009), ensuring that main sources of food can keep up with the forthcoming overpopulated world is a must, hence the focus on increasing yield in cereals. Wheat sustains 1.2 billion wheat dependant poor, who live on less than US$ 2 per day. It provides 21% of food calories and 20% of protein intake for more than 4.5 billion people in 94 countries (Braun et al. 2010).

For almost 100 years, efforts in understanding the genetic variation controlling crop adaptation have resulted in a vast accumulation of knowledge of flowering time and flowering time related genes (Figure 19) (Milec et al. 2014). There are three key groups of genes responsible for controlling crop responsiveness to (i)the period of time the crop receive day light, photoperiod genes (*Ppd*); (ii)the period of cold, vernalisation (*Vrn*); and (iii) developmental rates after vernalisation and photoperiod are fully satisfied, known as eraliness *per se* (Eps) (Snape et al. 2001). This third group is believed to be involved in a more specialized adaptation that can also be called fine-tuning (Griffiths et al. 2009).

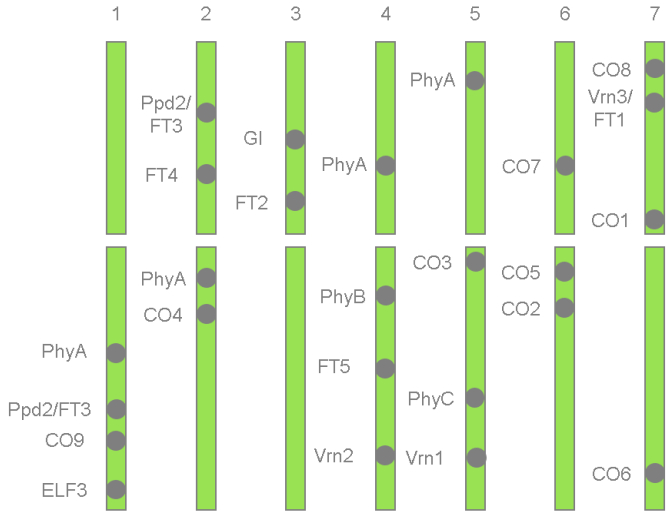

Figure 19. Chromosomal locations of flowering time genes in the *Triticeae*, where *FLOWERING LOCUS T* (*FT*), phytochrome genes (*PhyA, PhyB,* and *PhyC*), *CONSTANS* genes (*CO1, CO2, CO3, CO4, CO5, CO6, CO7, CO8*, AND *CO9*), *GIGANTEA* (*GI*), vernalization genes (*Vrn*), and photoperiod genes (*Ppd*). Adapted from: https://www.jic.ac.uk/adaptawheat/ADAPTAWHEAT_common_slides.pdf

4.1.1. Photoperiod genetic control

The “Green Revolution”, which provided a shorter stem and an increased partitioning to the spike, was also characterized by the adaptation of hexaploid wheat by insensitive alleles of *Ppd-1*. Therefore translating into cultivars that performed well from Canada to Argentina (Borlaug 1983). Insensitivity happen due to a deletion or mutation in *Ppd-1*, this means that cultivars with insensitive *Ppd-1* allele reach flowering time earlier in shorter days, i.e. less than 10 hours of daylight. On the other hand, the sensitive cultivars will delay flowering under the same conditions (Beales et al. 2007).

The three main genes controlling photopriod sensitivity are locates in chromosome 2D (i.e. *Ppd-A1*, *Ppd-B1*, and *Ppd-D1*, in chromosome 2A, 2B, and 2D respectively), with Ppd-D1 being the gene with the stronger effect (Guo et al. 2010), it is studied as a key gene to address drought resistance in UK winter wheat (Foulkes et al. 2004). As described by Worland (1996), this genes played an important role in the adaptability of winter wheat across Europe.

4.1.2. Vernalization genetic control

Wheat is often refer to as “winter” or “spring” type, referring to the crops requierement of a certain amount of cold hours. The sensitivity to vernalisation is given by three homoeologous copies of *Vrn-1* genes in hexaploid wheat, which are mainly found in chromosome 5 (Loukoianov et al. 2005). The low or absence of vernalisation requirement is often dominant (Snape et al. 2001). Thus, a dominant Vrn gene in any of these locations will provide spring phenology growth (Stelmakh 1987). Nevertheless, it is reported there is a link between photoperiod and vernalization; where Ppd-D1 simulates long days photoperiod responses while causing Vrn-2 to express abnormaly, in short days (Turner et al. 2013).

This group of genes are of great importance in adaptability terms. Europe is predominantly winter type (i.e. vernalization sensitive), this allows the crop to stand severe winters conditions after autumn sowing; while in southern regions of Europe sping types of wheat are necessary for flowering to happen before the extreme temperatures of summer arrives, despite the mild winters (Worland 1996).

4.1.3. Earlines *per se* genetic control

Differences in developmental rate after the photoperiod and vernalization requierements are met, are considered to be an effect of this third group of genes known as earlines *per se* genes (*Eps*). Whereas the two previous groups are well documented, current efforts are focused on finding *Eps*. One major quantitative trait locus for *Eps* is reported in chromosome 1D by Zikhali et al. (2014).

Subtle changes in adaptation are also known to happen with gene duplication, or also known as copy number variations. This mutations “tinker”, as Dubcovsky and Dvorak (2007) mention, with the genes, having a subtle dosage effect that seems to provide adaptability traits, as fine-tuning.

Orthologue genes, mainly from Arabidopsis (*Arabidopsis thaliana*) and rice (*Oryza sativa*), have proven useful in the search of flowering time genes as described by Amasino and Michaels (2010). This orthology is what allowed the development of flowering time genetic pathways, by using Brachypodium (*Brachypodium distachyon*) genome, a model for temperate grasses. Higgins et al. (2010) put toghether a comparative genetic pathway (Figure 20) where differences, like the absence in Arabidopsis of four flowering time genes found in rice.

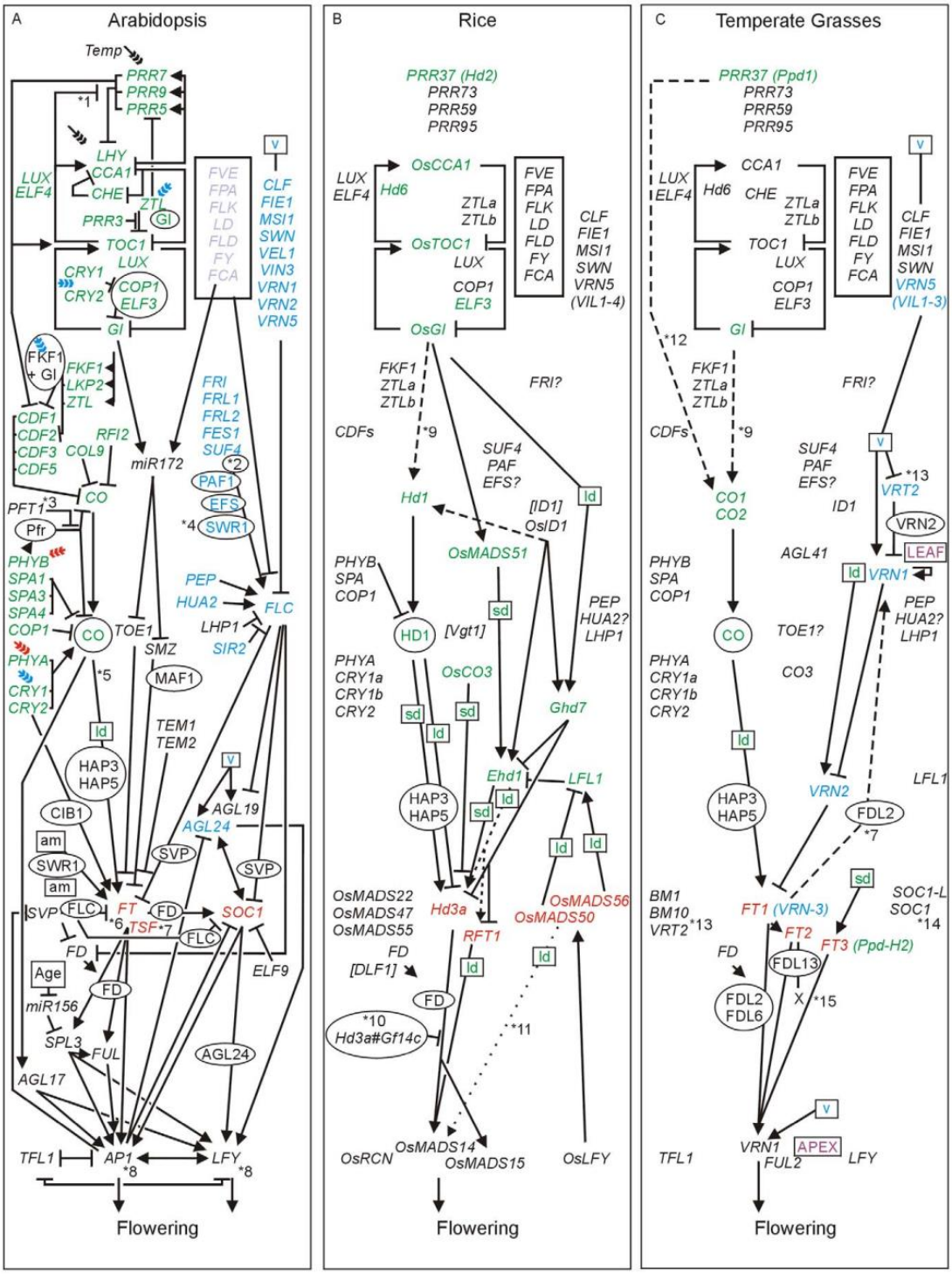

Figure 20. Genetic pathways controlling flowering in Arabidopsis, rice and temperate grasses. Arrows show promoting effects, T-bars show repressing effects, v is extended cold (vernalization); ld is long days; sd is short days; am is ambient (non-vernalizing) temperature. Genes are shown in italics and proteins in non-italics in ovals. Adapted from Higgins et al. (2010).

Embracing the task of matching crop production with demand requires a deeper understanding and association of yield component traits with their genes. Thus, an understanding of the complex traits in wheat enables an accurate and effective search

in a well-designed mapping population. Truly indept adaptation can potentially provide region specific cultivars far beyond the gross adaptation requirements provided by photoperiod and vernalization requieremnts. This is why projects like ADAPTAWHEAT are being supported by the European Comission under the Environment Theme of the 7th Framework Programme for Research and thecnological Development. The aim of this project relies on prospective genetic factors for flowering time adaptation in wheat (https://www.jic.ac.uk/adaptawheat/).

Doubled haploids (DH) lines, homozygous offspring from haploid individuals that went through a chromosome doubling, allows a direct fixation of the genetic content. Once the heterozygosity is taken from the equation, it becomes a relatively easy task to identify genomic loci that explain quantitative variation of a trait that segregates throughout the lines derived from distinct parental cultivars.

The aim of the present study is to identify QTL responsible for developmental length in a DH population derived from two winter wheat cultivars with a known difference in flowering time.

4.2. Materials and methods

4.2.1. General conditions

In previous work a DH population of 109 lines was developed from the cross between *T.aestivum* elite winter lines well adapted to UK's environment: Buster (Brimstone x Parade) a cultivar developed by Nickerson Seeds Ltd., and Charger (Fresco 'Sib' x Mandate) developed by Plant Breeding International Cambridge Ltd (see **Error! R eference source not found.**). Both, Buster and Charger, belonged to the UK's recommended list to represent bread-making, cake/biscuit-making, and feed wheat until 2001 (Budge and Henry 2002).

The design of this population had as its main objective the segregation of critical developmental phases for adaptation (e.g. flowering time). Both cultivars share common backgrounds, hence a similar set of major genes affecting the developmental phases (i.e. *Ppd-1* and *Vrn-1*) for a full diagram of the pedigree of Buster and Charger see Figure A8. They are both day length sensitive winter wheat varieties, and locally (Norfolk's Farmers Weekly news: http://www.fwi.co.uk/news/early-drill-may-suit-right-winter-wheat-varieties.htm) not recommended for early autum drilling due to their rapid development.

4.2.2. Experimental design

Experiments were carried out over three seasons with a randomized complete block design and three replicates per experiment. Field trials were drilled in two locations: John Innes Centre Church Farm (Norwich, Norfolk, UK) and Limagrain Ltd. (Woolpit, Suffolk, UK) in the first season, 2011-2012. Due to being the first experiments after the development of this population, the trial in Chruch Farm for this season only, consisted of one replicate per genotype in a $1m^2$ plot. The following seasons were drilled only in Church Farm, see Table 8. The lines were grown following standard agronomic practices.

Location	Coordinates	Plot size (m)	Harvest year	Total rainfall (mm)	Avg. daily temperature (°C)	Avg. daily radiation (MJ m^{-2} d^{-1})	Environment
Norwich	52°63' N, 01°30' E, 14 m a.s.l.	1x1	2012	414	7.6	13.7	CF12
		1.5x4	2013	501	6.1	12.8	CF13
		1.5x4	2014	434	8.1	14.2	CF14
Woolpit	52°11' N, 00°51' E, 80 m a.s.l.	1.2x4.5	2013	497	6.1	12.8	WP13

Table 8. Field trials data for Buster x Charger DH including location, coordinates, plot size, harvest year, and meterological data for average for each environment: total rainfall, daily temperature, and daily radiation.

4.2.3. Measurements and analyses

4.2.3.1. Phenology

Days to ear emergence was recorded as the time when 50% of the plot presented half of the spike emerged from the flag leaf. Terminal spikelet on the other hand, is a more demanding trait to be scored. At first instance, for environment CF12, three main tillers were dissected in the field and if two or more plants had reached terminal spikelet stage, then that moment in time was scored as terminal spikelet for that plot. For a more accurate analysis of the terminal spikelet initiation phase, for CF13 and CF14, 5 random samples at one moment in time were taken consisting of the main tiller's spike, which was then stored in FAA (water 50%, ethanol 35%, acetic acid 10%, and formaldehyde 5%, v/v) (Bancal 2008) for further scoring and determination of organs primordia present. The method developed and used to back-score samples is shown in Figure 21. The images represent the range of developmental growth stages surrounding the moment where terminal spikelet (represented by TS) is set.

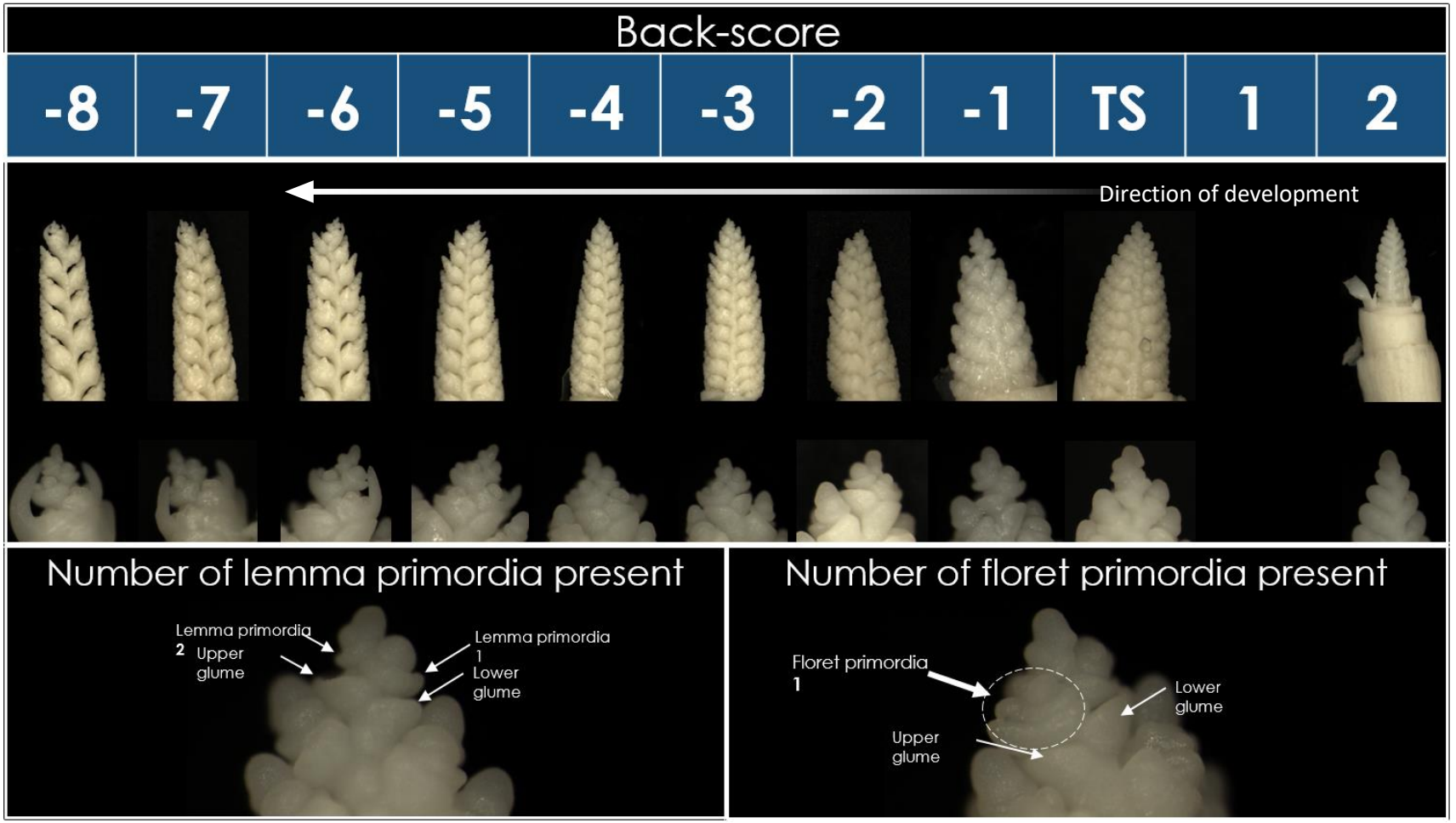

Figure 21. Top; images of developmental range surrounding terminal spikelet phase used for the back-scoring method. There is no photograph available for 1 in the back-score scale.

4.2.3.2. DNA extraction

Four seeds of each line of the population were positioned in a petri dish, on top of a water-soaked filter paper. Of those seeds which emerged, one of the resulting plants was sown in a plastic tray approximately 1.5cm deep. Between one and two weeks later in the glasshouse, the plants had developed 3-5 leaves from where the samples were taken. With clean scissors a 1 inch segment was dissected and collected in a Qiagen 96 well box (Collection microtube, cat. No. 19560), placed on ice and stored at -80°C prior to extraction. The DNA was then extracted following the protocol of Qiagen DNeasy 96 plant kit (cat. No. 69181). The extracted DNA was quantified using a Nanodrop spectrophotometer, measuring absorbance at 260nm and 280nm for quality (absorbance ratio) and concentration (compared to a standard curve), normalised to 50ng/ul and sent on dry ice to TraitGenetics (Am Schwabeplan 1b, Stadt Seeland OT Gatersleben, D-06466, Germany) to be analysed with the 90K iSelect chip (Wang et al. 2014).

4.2.3.3. Genetic map construction

In the first instance, a genetic map was provided with 224 markers. This served as initial genetic map, which I further improved using SNP markers developed by Bristol University and available via CerealsDB (www.cerealsdb.com). LGC KASP reagents were used as per LGC's protocols (www.lgcgroup.com). Each reaction containing 2µl water, 2 µl KASP mix (2x), 0.0625µl assay mix (12µl FAM/VIC SNP specific primer [100µM], 30µl common primer [100µM], 46µl water), with 2µl DNA [5-10ng/µl] dried down into the PCR plate. An activation time (94°C, 15 min) was followed by 20 cycles of [94°C for 10 sec; 57°C for 5 sec; 72°C for 10 sec] followed by 24 cycles of [94°C for 10 sec; 57°C for 20 sec; 72°C for 40 sec]. Fluorescence was read as an end point reading at 25°C using a BMG Pherastar plate reader (BMG LABTECH GmbH, Germany). This improvement provided a genetic map based on 264 markers from which 250 formed linkage groups, using the software MapDisto v. 1.7 (Lorieux 2007).

By May 2015, I sent DNA to Traitgenetics, Germany. Here the population (109 lines and 2 parents) was subjected to illumina infinium 90k wheat chip array genotyping. This chip analysed 81587 single nucleotide polymorphisms (SNP) on infimum ultra HD chip with 24 samples each. The genetic map was generated by Traitgenetics using three different software packages (JoinMap 4.0, Map Manager QTXb20, and MapChart 2.2).

5,665 SNP markers formed 21 linkage groups, 7 per genome (A, B, and D). The three genomes were well covered with markers, however markers for the D genome were

significantly underrepresented. In addition, some chromosomes showed large gaps as a result of the lack of polymorphisms between the parents. A total of 695 loci were mapped with an average distance between them of 7.3 cM with a combined map length of 3740.3 cM (Table 9).

linkage group	length (cM)	number of marker loci	number of map loci	mean distance between loci (cM)	max. distance between loci (cM)
1A	99.1	286	22	4.7	25.6
1B	176.7	367	45	4.0	28.6
1D	90.3	131	19	5.0	25.6
2A	209.1	475	65	3.3	20.1
2B	318.7	847	48	6.8	101.7
2D	147.2	75	25	6.1	35.2
3A	235.5	341	38	6.4	45.3
3B	267.0	373	58	4.7	28.6
3D	127.2	29	12	11.6	37.0
4A	102.9	123	19	5.7	18.8
4B	92.7	153	20	4.9	20.1
4D	117.5	5	5	29.4	101.7
5A	298.9	320	59	5.2	35.2
5B	221.7	528	43	5.3	40.9
5D	179.9	180	22	8.6	47.7
6A	139.5	250	26	5.5	43.0
6B	115.5	226	24	5.0	17.5
6D	180.3	87	21	9.0	68.0
7A	286.3	499	74	3.9	35.2
7B	171.8	328	36	4.9	24.2
7D	162.5	42	14	12.5	45.3
Total	3740.3	5665	695	7.3	

Table 9. Genetic map statistics with the genetic distance as centimorgan (cM).

The heatmap of pairwise recombination frequencies shown in Figure 22 is interesting because it clearly shows the presence of the 5B/7B translocation, which is known to be in the variety Charger (Badaeva et al. 2007). The translocation can be seen through its effect on linkage during the process of making genetic maps using any commonly used mapping software. The 5B and 7B markers fuse together into one large coalesced linkage group. In this study this did not prove to be an issue as no QTL were found on either of the effected chromosomes.

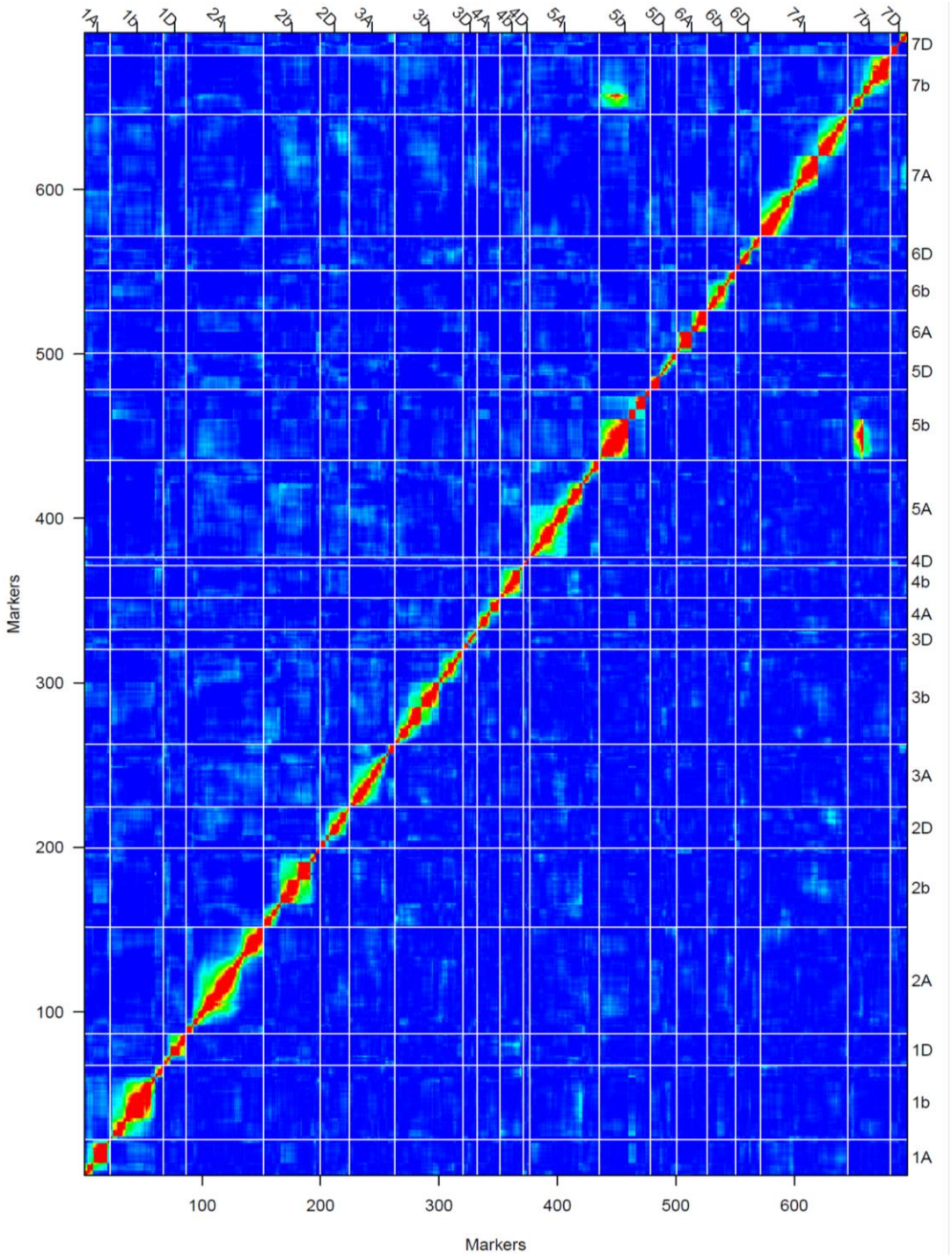

Figure 22. Heatmap of pairwise recombination fractions of BxC DH genetic map. Red stands for 1, and blue for 0.

4.2.4. Traits

Table 10 shows the traits scored for each of the four experiments. Restriction on the traits analysed was due to distance limitations, i.e. travelling to field trials in Woolpit, Suffolk, UK. And also detailed analysis and dissection of spikelets was extremely labour intensive.

Code	Trait	Unit	CF12	WP12	CF13	CF14
yield	Grain yield	t / ha		X	X	X
tgw	Thousand grain weight	g			X	X
gm2	Grains per m^2	No. of grains / m^2			X	X
gps	Grains per spike	No. of grains / spike			X	X
sm2	Spikes per m^2	No. of spike / m^2			X	X
spks	Spikelets per spike	No. of spikelet / spike			X	X
gspk	Grains per spikelet	No. of grains / spikelet			X	X
dy_ts	Days from sowing to terminal spikelet	Days	X		X	X
dy_b	Days from sowing to booting	Days				X
dy_ib	Days from sowing to initiation of booting	Days				X
dy_ee	Days from sowing to heading	Days	X		X	X
dy_se	Days of stem elongation period (TS to EE)	Days	X		X	X
tt_ts	Thermal time to terminal spikelet	°C d	X		X	X
tt_b	Thermal time to booting	°C d				X
tt_ib	Thermal time to initiation of booting	°C d				X
tt_ee	Thermal time to heading	°C d	X		X	X
tt_se	Thermal time of stem elongation period	°C d	X		X	X
floretp	Floret primordia present (W3.5)	No. of primordia			X	X
lemmap	Lemma primordia present (W3.25)	No. of primordia			X	X

Table 10. Traits subjected to QTL analysis and execution per experiment.

4.2.5. Statistical analysis

Statistical analysis was performed using a combination of Genstat 17th (for heritability) and R software (for the rest of analysis). The significance of differences in traits between lines was assessed using a one-way analysis of variance.

In order to obtain a good indication of the different genomic regions contributing to the variations in phasic development, QTL mapping was done with R statistical software and R/qtl package (Broman et al. 2003), where in a first step a single-QTL genome scan is performed with a normal model calculated on QTL genotype probabilities. In a second step, all QTLs for one trait above a significance threshold in step one are statistically tested in a multi-QTL model. This second step detects ghost QTL, as they do not reach statistical significance, and detects the true LOD scores for each QTL.

The QTL is considered when the logarithm of the odds (LOD) score exceed the threshold calculated using a permutation level of 1000 and a significance level at 0.2 is used initially to determine a first group of QTL's that will be used as cofactors, then reanalysed at a significance level of 0.05. Interval mapping was calculated using the Haley-Knott regression method.

4.2.6. Heritability

Heritability was estimated using a mixed model, where environment, replicates within environment and checks (parents) within environment are considered as fixed factors; rows of plots within environment, column within environment, genotype, and genotype x environment interaction are set as random factors. The formula used is:

$$h^2 = \sigma^2_g / [\sigma^2_g + (\sigma^2_{ge} / e) + (\sigma^2_r / re)]$$

Where σ^2_g is the variance of the genotype, σ^2_{ge} is the variance of the genotype x environment interaction, σ^2_r is the residual, e is the number of environments, and r is the number of replicates.

The figures were produced using GraphPad Prism 5 (2007), except for the principal component analysis which was done with ggbiplot package using R.

4.3. Results

4.3.1. General phenotypic description

4.3.1.1. Phenotypic ranges

The histograms for yield and the two major yield components, grain number and grain weight, show unimodal skewedness to some extent (Figure 23). The yield histogram for WP12 provided a normal distribution, while CF13 had a slightly positive skew, and CF14 had a negative skew. Grain number showed to some degree a negative skew

in both CF13 and CF14. As for grain weight, CF13 showed a positive skew while CF14 showed a distribution suggesting negative skew but possibly affected by a number of genotypes in the given interval; which in order to keep the panel up to scale, intervals were set in order to have no more than 30 genotypes represented in each bar.

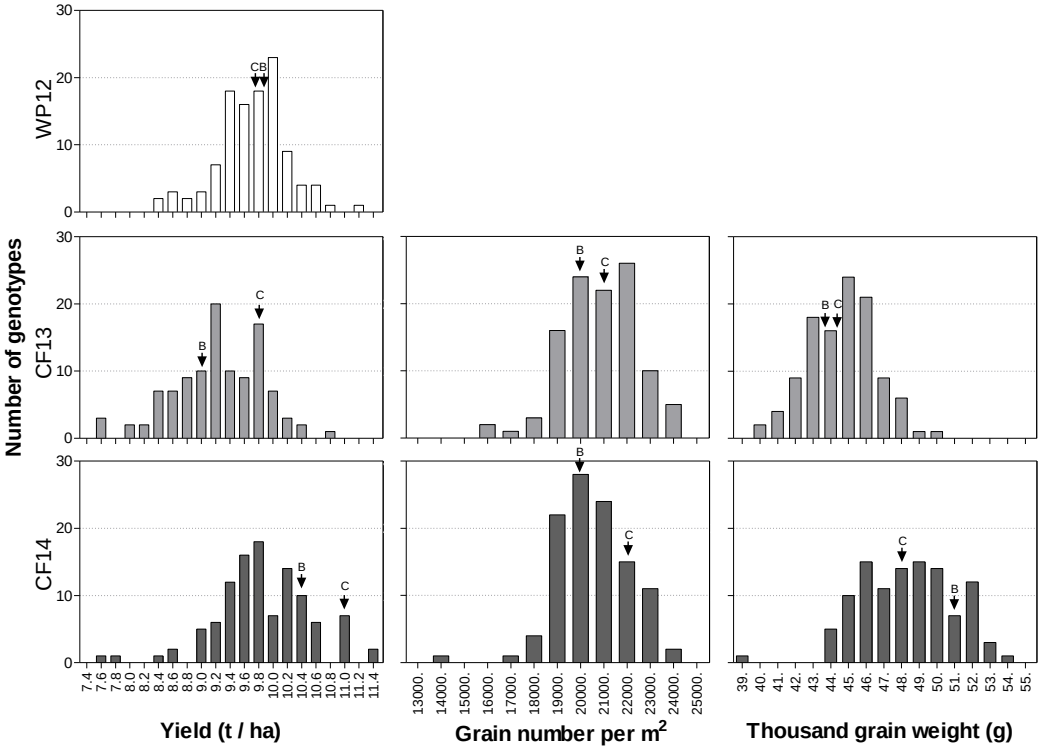

Figure 23. Histograms of Buster x Charger DH numerical yield components for WP12, CF13, and CF14. The mean value for Buster (B) and Charger (C) are represented by the arrows.

Yield and it's major components, grain number and grain weight, had a similar behaviour across environments. All trait phenotypes of the doubled haploid population were outside the range of the parental lines mean values.

4.3.1.2. Year to year variation

As can be seen in Table 11, the parental lines higher number of grains per spike from environment CF14 (63 and 62.9 from CF14 compared to 52.6 and 47.9 from CF13, for Buster and Charger each respectively) seems to be due to an increased number of spikelets per spike (20.5 and 20.6 from CF13 compared to 19.7 and 19.5 from CF14, also for Buster and Charger respectively for each environment.

Environment	Trait	Parents				DH population		
		Buster	SD	Charger	SD	Mean	Range	SD
WP12	yield	9.9	0.5	9.9	0.8	9.7	8.3 - 11.2	0.5
CF12	dy_ts	197	-	187	-	193.1	187 - 203	4.0
	dy_ee	245	-	239	-	241.9	236 - 247	2.5
	dy_se	48	-	52	-	48.8	37 - 55	3.8
CF13	yield	9.1	1.1	9.8	1.1	9.2	7.5 - 10.7	0.6
	tgw	45.4	3.1	45.1	3.1	44.6	40 - 49.7	1.9
	gm2	20494	2832	21970	2435	20830	15859 - 24498	1618
	gps	52.6	3.6	47.9	6.6	50.5	41.8 - 61.4	3.8
	sm2	389.6	43.3	466.2	83.7	412.5	335.8 - 597.8	42.9
	spks	20.5	1.2	20.6	1.3	20.2	18.3 - 22.1	0.8
	gspk	2.6	0.3	2.3	0.4	2.5	2.0 - 2.9	0.2
	dy_ts	203.9	1.1	203.6	-	203.7	200.4 - 207.2	1.3
	dy_ee	246.6	0.5	244.8	1.2	245.1	241.3 - 249.0	1.8
	dy_se	42.4	0.6	40.4	-	41.5	37.9 - 44.3	1.4
	floretp	1.9	0.3	1.8	-	1.9	0.1 - 3.3	0.8
	lemmap	4.4	0.9	4.4	-	4.3	2.2 - 6.4	0.9
CF14	yield	10.4	0.4	11.1	0.6	9.8	7.5 - 11.4	0.7
	tgw	48.6	2.9	47.1	2.7	48.2	39.5 - 54.2	2.6
	gm2	20202	1062	22692	1262	20462	13868 - 23807	1591.7
	gps	63	5.0	62.9	3.8	67.0	52.7 - 85.8	6.1
	sm2	322.3	30.8	361.8	29.7	309.2	226.3 - 428.0	32.1
	spks	19.7	0.7	19.5	0.9	20.7	18.0 - 24.0	1.3
	gspk	3.2	0.2	3.2	0.2	3.3	2.6 - 3.9	0.3
	dy_ts	172	0.7	171.3	0.8	172	170.0 - 174.7	0.9
	dy_ib	211.1	1.5	209.4	0.9	210.5	207.3 - 213.0	1.3
	dy_ee	217.7	0.5	215.2	1.0	216.4	213.0 - 219.0	1.5
	dy_se	45.7	0.8	43.9	1.2	44.4	40.9 - 46.5	1.2
	floretp	0	0.0	0	0.0	0.01	0.0 - 0.1	0.0
	lemmap	1.3	0.7	1.8	0.7	1.4	0.0 - 2.9	0.7

Table 11. Mean phenotypic values of Buster, Charger and DH population.

The effect of genetic diversity over the phenotypes was determined as the heritability (h^2). The highest heritability values correspond to developmental traits (e.g. days to ear emergence and days to terminal spikelet; see Table 12). Some unusually low heritability values were obtained for traits like thousand grain weight, spikes per unit land area, and floret primordia developed, this might be the result of a strong environmental effect in the variation observed, rather than a genetic factor. Possible the most unusual one would be thousand grain weight as in literature heritability values range from 86-99 (Sun et al. 2009; Ali et al. 2008). Despite this, heritability values of time to ear emergence was high, suggesting that most of the variation observed can be explained by genetic factors. Similarly, the rest of the phenological stages where also fairly high which can translate into genetic gains.

	Variance parameters						
Trait	σ^2 *G*	s.e.	σ^2 *(G x E)*	s.e.	σ^2 *E*	s.e.	h^2
yield	0.10	0.02	0.13	0.02	0.20	0.01	0.61
tgw	0.30	0.37	0.21	0.52	9.32	0.66	0.15
gm2	643843	244939	777274	252467	2361895	180720	0.45
gps	4.24	2.37	11.46	2.76	21.82	1.58	0.31
sm2	90	119	342	164	1885	145	0.16
spks	0.51	0.12	0.15	0.10	1.36	0.10	0.63
gspk	0.01	0.00	0.01	0.01	0.06	0.00	0.39
dy_ts	1.24	0.31	1.95	0.29	0.54	0.04	0.64
dy_ib	1.29	0.25	0.35	na	0.35	0.04	0.74
dy_ee	2.64	0.40	0.32	0.09	0.89	0.06	0.93
dy_se	1.47	0.34	1.61	0.30	1.17	0.10	0.69
floretp	0.005	0.027	0.217	0.037	0.094	0.008	0.04
lemmap	0.39	0.07	0.09	0.03	0.26	0.02	0.81

Table 12. Variance parameters and heritability for Buster x Charger DH population. σ^2 *G* (genotypic variance), σ^2 *(G x E)* (variance of the genotype-environment interaction), σ^2 *E* (environmental viarance), h^2 (narrow sense heritability), and s.e. (standard error).

4.3.1.3. Principal component analysis

Bi-plots showing the principal component analysis of yield and yield components using the unadjusted arithmetic mean for CF13 (Figure 24) and CF14 (Figure 25) were plotted, explaining 69.9% and 67.7% of the total variation respectively. Traits that present less than 90° angles have a positive correlation, while angles higher than 90° are negatively correlated.

Principal component analysis for CF13 showed a strong association between yield and grain number (gm2), supported by a positive correlation (r^2=0.826). Also is shown a negative association between grain number and grain weight (tgw) supported by a correlation of r^2=-0.478.

Grain number was better explained by the number of spikes per unit land area (sm2) with a correlation of r2=0.663, despite the fact that there is a strong negative correlation between sm2 and the number of grains per spike (gps) of r^2=-0.596.

There seems to be a significant effect from days to ear emergence (dy_ee) on yield with a r^2=0.196, especially over gm2 r^2=0.162, thus supporting the hypothesis from chapter 1 of a positive effect on grain number from extending the developmental length (Table 13).

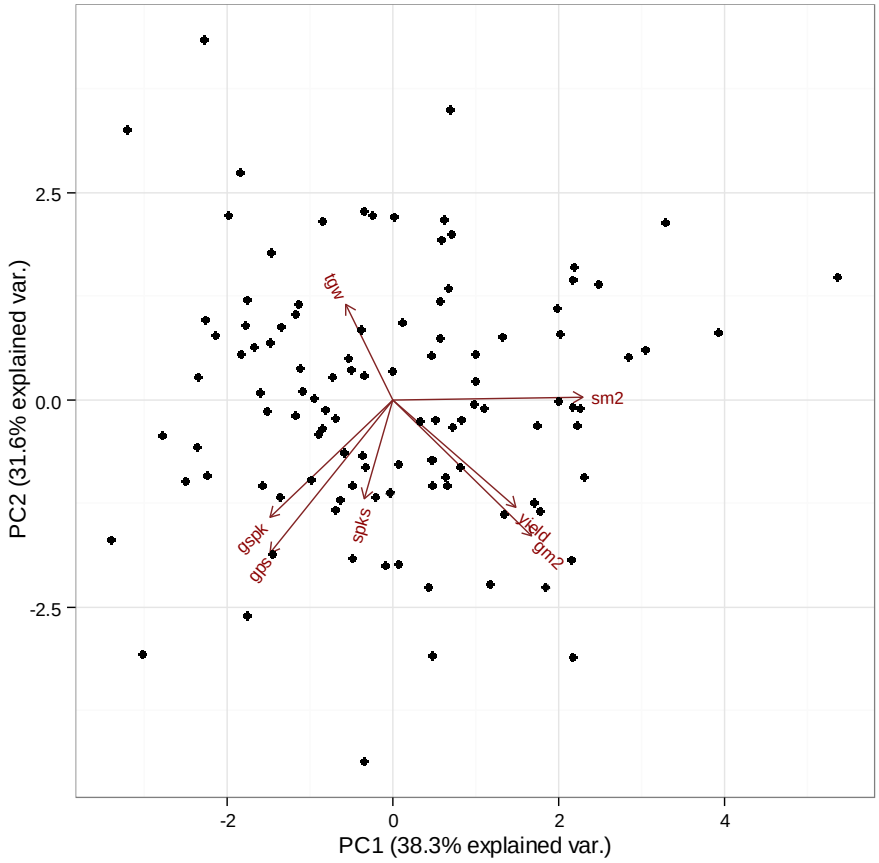

Figure 24. Bi-plot of the principal component analysis for CF13.

	tgw	gm2	gps	sm2	spks	gspk	dy_ts	dy_ee	dy_se
yield	-0.008	**0.826*****	0.048	**0.528*****	0.140	-0.017	0.031	0.196*	0.083
tgw		**-0.478*****	-0.183	-0.271**	-0.086	-0.152	-0.065	-0.032	0.079
gm2			0.091	**0.663*****	0.174	0.006	0.118	0.162	-0.034
gps				**-0.596*****	**0.488*****	**0.857*****	-0.150	0.047	0.064
sm2					-0.189*	**-0.569*****	0.123	0.050	-0.041
spks						-0.025	0.073	0.081	-0.007
gspk							-0.230*	-0.002	0.081
dy_ts								**0.578*****	-0.245*
dy_ee									**0.649*****

Table 13. Pearson correlation coefficient for the unadjusted arithmetic mean in CF13. Values in bold have a P<0.001.

Matching results between the two environments were obtained. The principal component analysis for environment CF14 (Figure 25) showed a similar strong association between yield and grain number, this time supported by a positive correlation of r^2=0.734, reaffirming the importance of the grain number as a component of yield. The trade-off between grain number and grain weight had an even higher negative correlation (r^2=0.569) than the previous environment (Table 14).

Some correlations between traits became clearer in the data collected from the CF14 environment. The effect from days to ear emergence with yield is highly significant with a positive correlation of 0.431. Also a significant positive correlation was found between days to terminal spikelet and yield (r^2=0.310). Thus denoting the importance of phasic development upon yield, even with relatively minor changes. Furthermore, these two phases of development that encompass the stem elongation period, correlate significantly to grain number; with an r^2=0.390 for days to terminal spikelet and an r^2=0.304 for days to ear emergence.

The number of spikelets per spike is correlated to days to terminal spikelet (r^2=0.480) suggesting that a delay on the time to terminal spikelet initiation provided more time for the development of spikelets. This happens during the early reproductive phase, before the stem elongation onset.

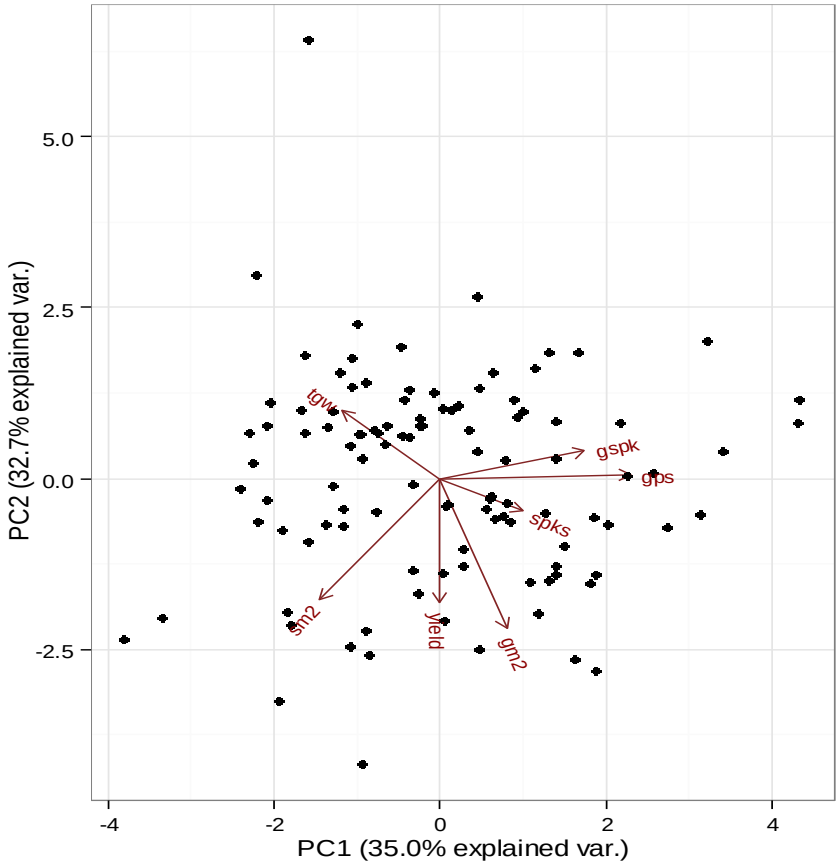

Figure 25. Bi-plot of the principal component analysis for CF14.

	tgw	gm2	gps	sm2	spks	gspk	dy_ts	dy_ee	dy_se
yield	0.131	**0.734*****	0.066	**0.496*****	0.039	0.052	0.310**	**0.431*****	0.305**
tgw		**-0.569*****	**-0.364*****	-0.131	-0.285**	-0.182	-0.185	0.076	0.230*
gm2			0.303**	**0.500*****	0.246*	0.155	**0.390*****	0.304**	0.088
gps				**-0.650*****	**0.469*****	**0.724*****	0.301**	0.232*	0.064
sm2					-0.203*	**-0.544*****	0.024	0.023	0.011
spks						-0.266**	**0.480*****	-0.023	**-0.381*****
gspk							-0.031	0.276**	**0.363*****
dy_ts								**0.586*****	-0.011
dy_ee									**0.803*****

Table 14. Pearson correlation coefficient for the unadjusted arithmetic mean in CF14. Values in bold have a P<0.001.

4.3.2. Identification of quantitative trait loci

All QTL identified in the study are shown in Table 15 and their location on the Buster x Charger DH map in Figure 33. Detailed descriptions are given below.

4.3.2.1. Yield

A yield QTL from WP12 was detected on chromosome 2D with a LOD of 4.367 explaining 19.8% of the variation, the increasing effect of 0.207 tons/ha was provided by Buster (Figure 26).

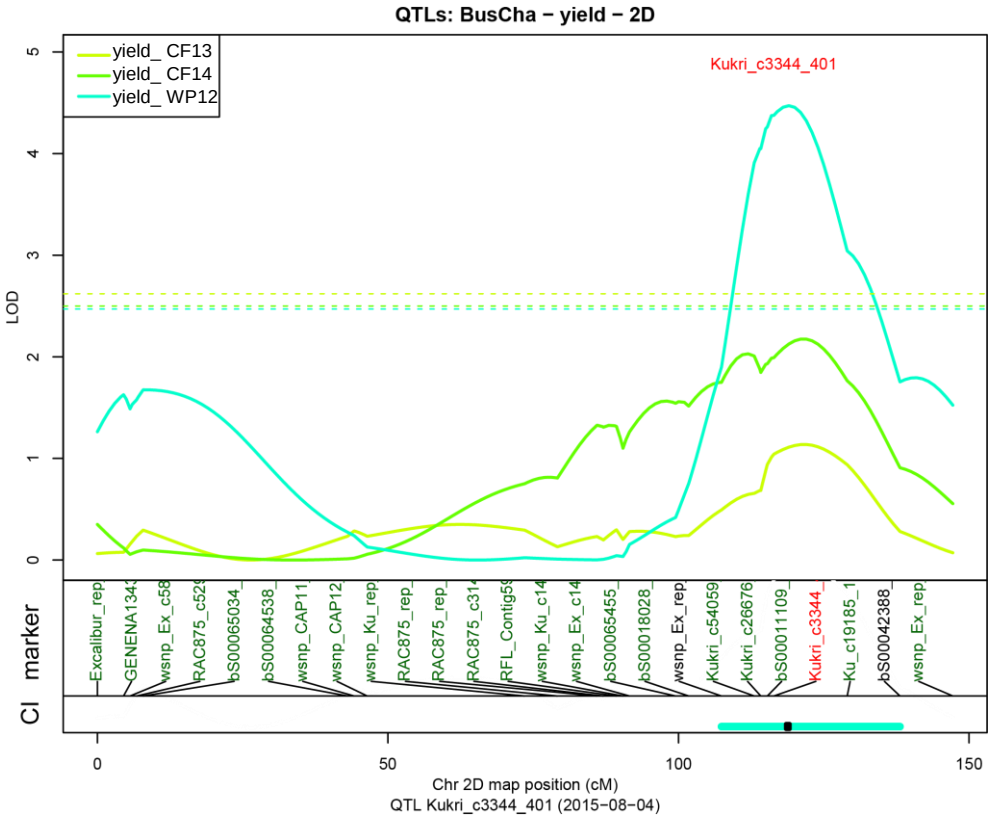

Figure 26. QTL for yield on chromosome 2D.

4.3.2.2. Grain number

Chromosomal locations affecting grain number components were identified. A locus for the number of grains per spike of CF14 was found on chromosome 4A at 102.9 cM. LOD score of 5 and a positive additive effect of 2.964 grains per spike from Charger, explaining 23% of the variation. The number of spikelets per spike showed two loci on chromosome 7A (similar locus as for TS), for CF13 and CF14, at 203.2 and 202 cM respectively. The CF13 effect had a LOD of 2.961 and an additive effect from the Charger allele of 0.295 spikelets per spike that explained 13.9% of the variation. In CF14 a stronger LOD of 18.5 was found which explained 59.7% of the variation with an additive effect of 1.036 spikelets per spike from the Charger allele (Figure 27). Further to the 7A locus, a minor locus on 5D was located for CF14, with a LOD of 1.8 that explained 3.6% of the variation with an additive effect of 0.462 spikelets per spike from the Buster Allele.

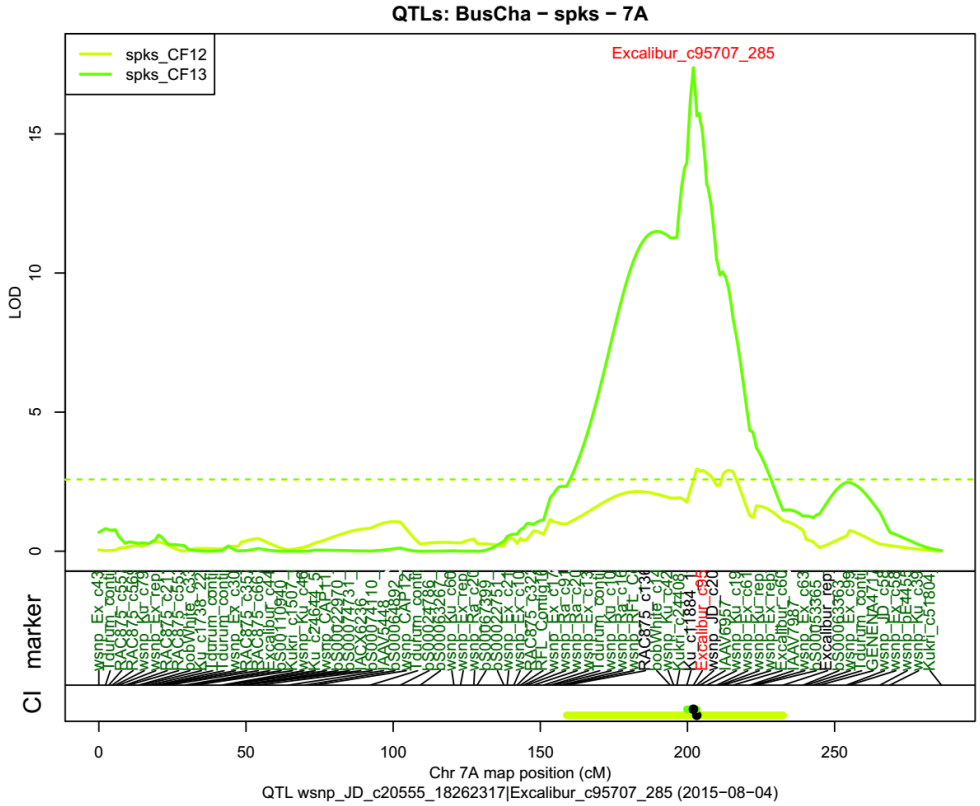

Figure 27. QTL for spikelets per spike on chromosome 7A.

Besides the loci for number of grains per spike and number of spikelets per spike, a locus on 4A for the number of grains per spikelet was identified at the same position as the locus for grains per spikelet, 102.9 cM, with a LOD of 4.65 explaining 21.6% of the variation with an additive effect of 0.123 grains per spikelet from Charger allele.

4.3.2.3. Terminal spikelet

Time to terminal spikelet was detected in 7A with both (i) days and (ii) thermal time, in two of the three seasons with an additive effect of 0.409 days for CF13, 0.446 days for CF14, 3.306 ˚C d for CF13, and 4.172 ˚C d for CF14. The increasing allele came from Charger, meaning Charger allele makes it late for terminal spikelet. This locus explain 12.2%, 22.6%, 14.1%, and 20.8% of the variation, respectively (Figure 28).

Alongside the analysis of time to TS, the number of florets (W3.5) and lemma (W3.25) primordia present in the terminal spikelet were counted as a supplementary method to score development. This resulted in the identification of QTL for 7A for both traits in both seasons analysed. The positive additive effect is 0.314 floret primordia developed for CF13 and 0.017 floret primordia developed for CF14, with the increasing allele coming from Buster; the percentage explaining the variation is 15.6% and 20% respectively. Also a region was identified on chromosome 1B only for CF13, this has and additive effect of 0.293 floret primordia developed, explaining 10.8% of the variation. As for the lemma primordia presence of a 7A region showed, in both seasons, an additive effect of 0.31 and 0.251 lemma primordia developed provided by Buster's allele, and explaining 12.6% and 20% of the variation.

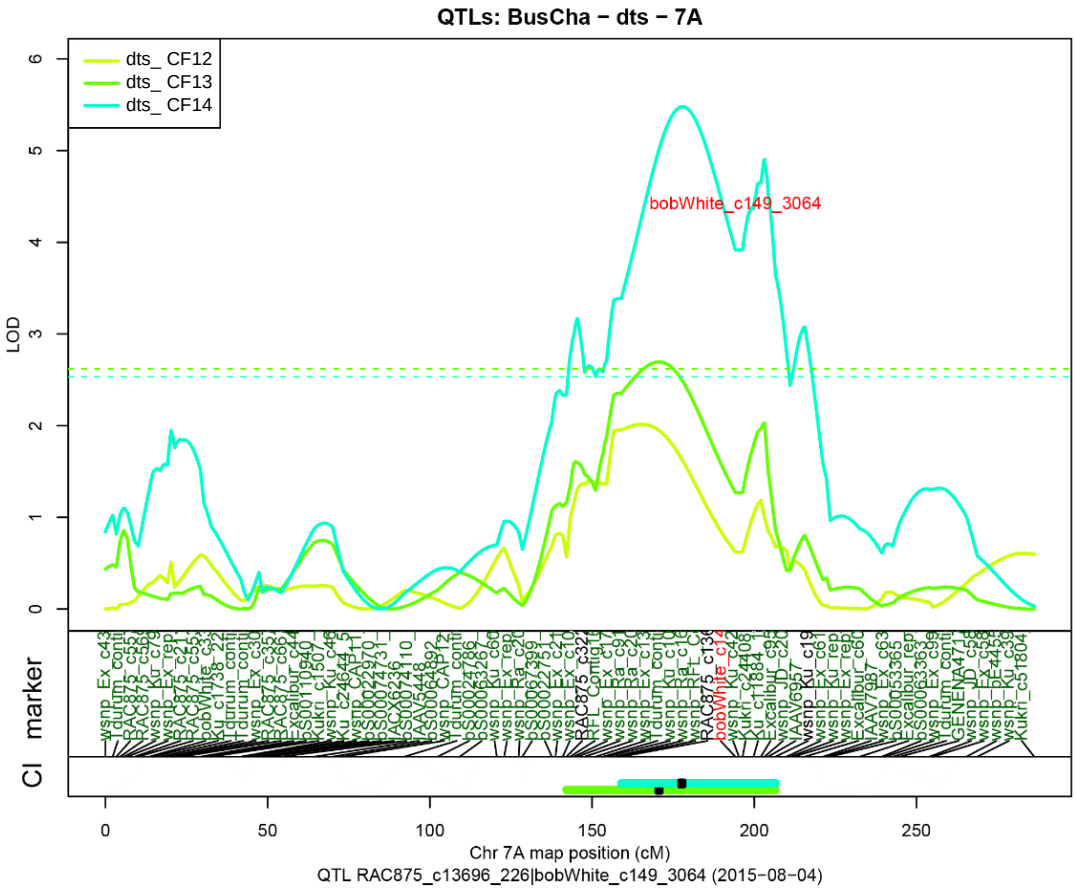

Figure 28. QTL for days to terminal spikelet on chromosome 7A.

A region on chromosome 7A was found for the number of spikelets per spike; an additive effect of 0.295 spikelets per spike for CF13, and 1.036 spikelets per spike for CF14 explaining 13.9% and 59.7%, respectively, was due to the Charger allele. This means that it seems possible the more spikelets can be achieved through deployment of Charger's 7A locus.

4.3.2.4. Ear emergence

Time to ear emergence QTL were identified on chromosomes 2D and 4A for CF12, CF13, and CF14; with the 2D locus having a LOD score of 4.6, 2.8, and 2.8 respectively for each experiment; explaining 16.1, 9.9, and 11.4 percent of the variation with a positive additive effect from Buster allele of 1.15, 0.79, and 0.61 days to heading respectively for each experiment. This means that Buster confers lateness, in terms of days to ear emergence.

The 4A QTL had a LOD score of 5.5, 2.7, and 2.7; with a positive additive effect from Charger allele of 1.2, 0.65, and 0.6 days to heading. These explain 19.9, 9.4, and 11 percentage of the variation (Figure 29).

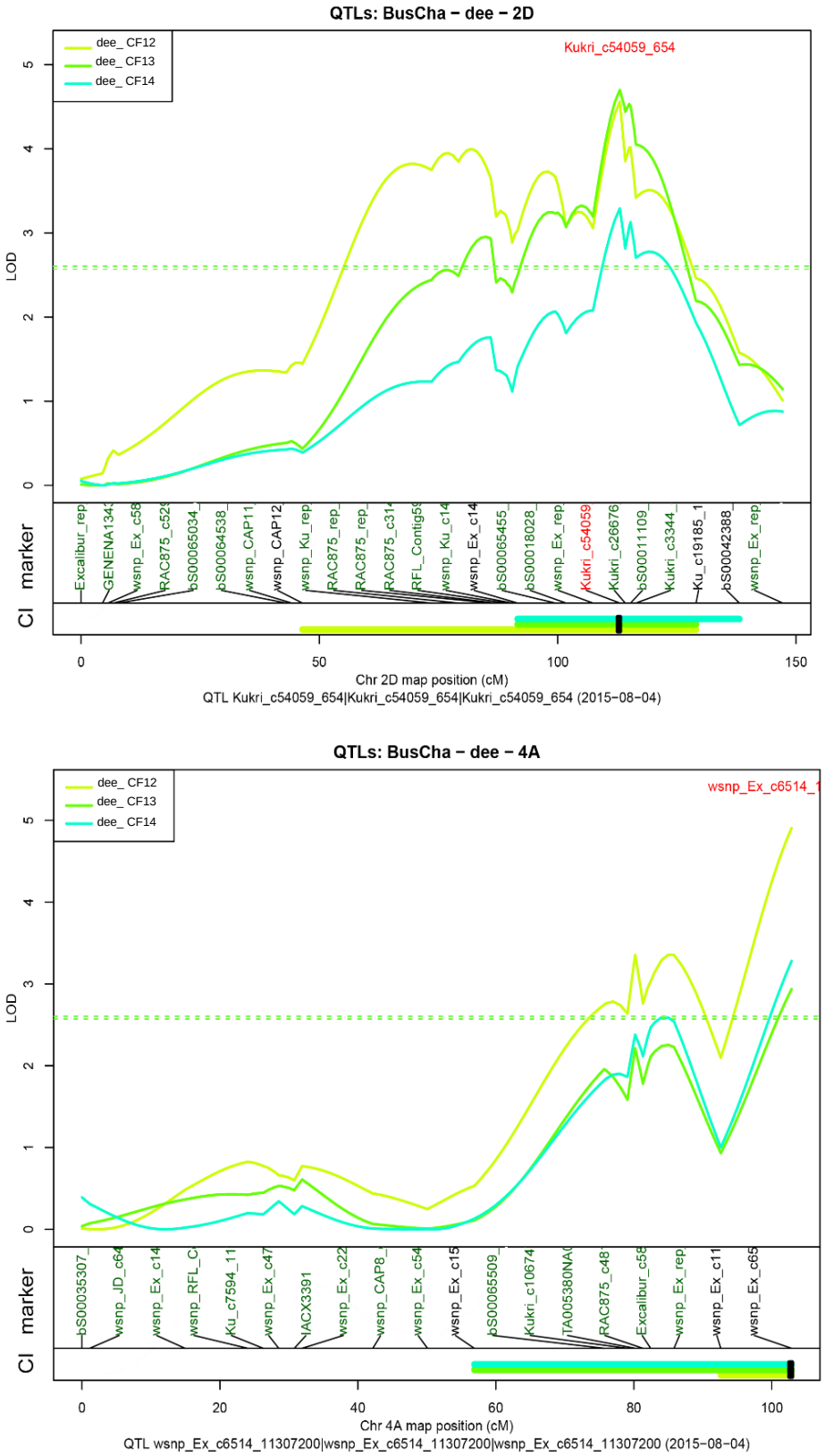

Figure 29. QTL for days to ear emergence on chromosome 2D (top) and chromosome 4A (bottom).

Also a QTL on chromosome 2B was found in CF12 environment with a LOD score of 1.6 providing an additive effect of 0.63 days to heading by the Buster allele. This explained 5.4 percent of the observed variation.

4.3.2.5. Stem elongation

The majority of the QTLs for stem elongation were found in the same locations as for days to terminal spikelet and days to ear emergence. A QTL on chromosome 2B was located for two of the environments, CF13 and CF14. QTL from the third environment analysed didn't reach the threshold, but it is possible to see a trend in that chromosomal location (Figure 30). The first QTL for environment CF13 have a LOD score of 3.4, it explains 13.7% of the variation with a positive additive effect of 0.512 days from Buster allele. The second QTL had a LOD score of 2.9, and explains 8.8% of the variation with a positive additive effect of 0.489, also from Buster allele.

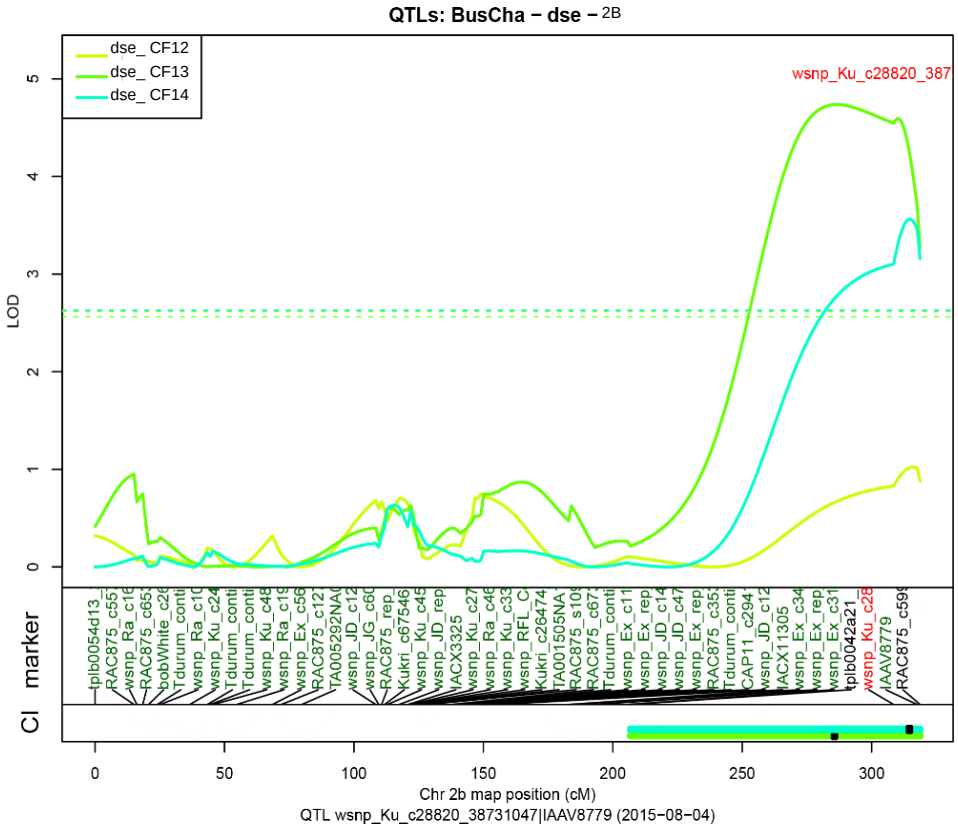

Figure 30. QTL for stem elongation period on chromosome 2B

A locus on 2D was also found, the threshold was only reached by the environment CF14, but a trend is visible for the other two environments analysed. The QTL have a LOD score of 2.8 and it explains 2.8% of the variation with a positive additive effect of 0.524 days from Buster allele (Figure 31). Thus the Buster allele in this locus increases the length of the stem elongation period.

On chromosome 4A a QTL for stem elongation period from environment CF14 was also found. With a LOD score of 4.2, it explains 13% of the variation with a positive additive effect of 0.444 days from Charger allele (Figure 32).

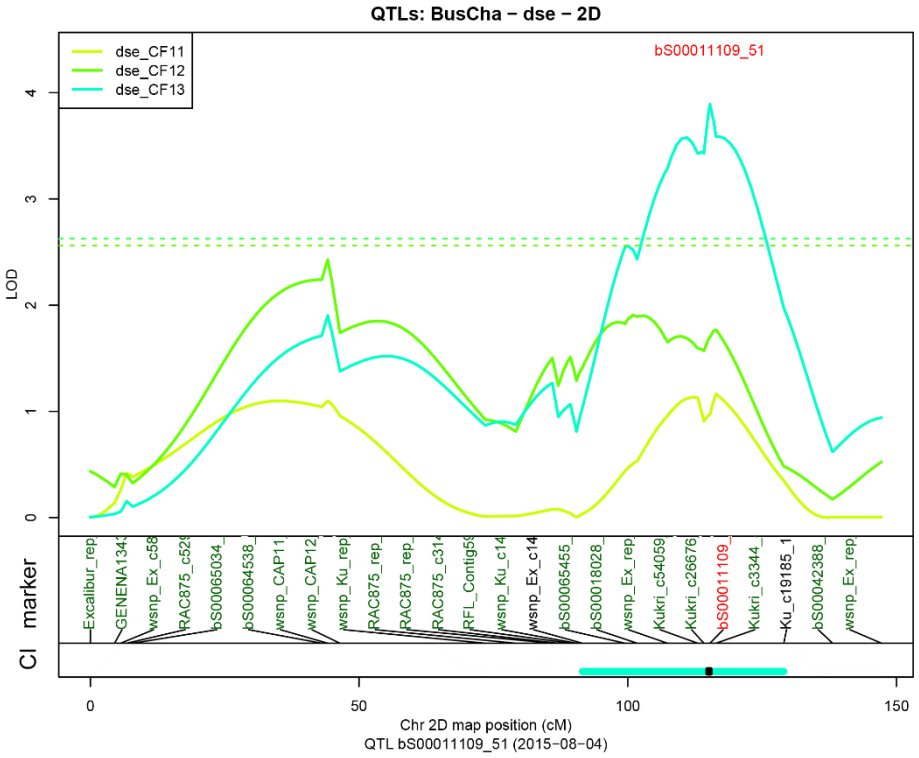

Figure 31. QTL for stem elongation period on chromosome 2D

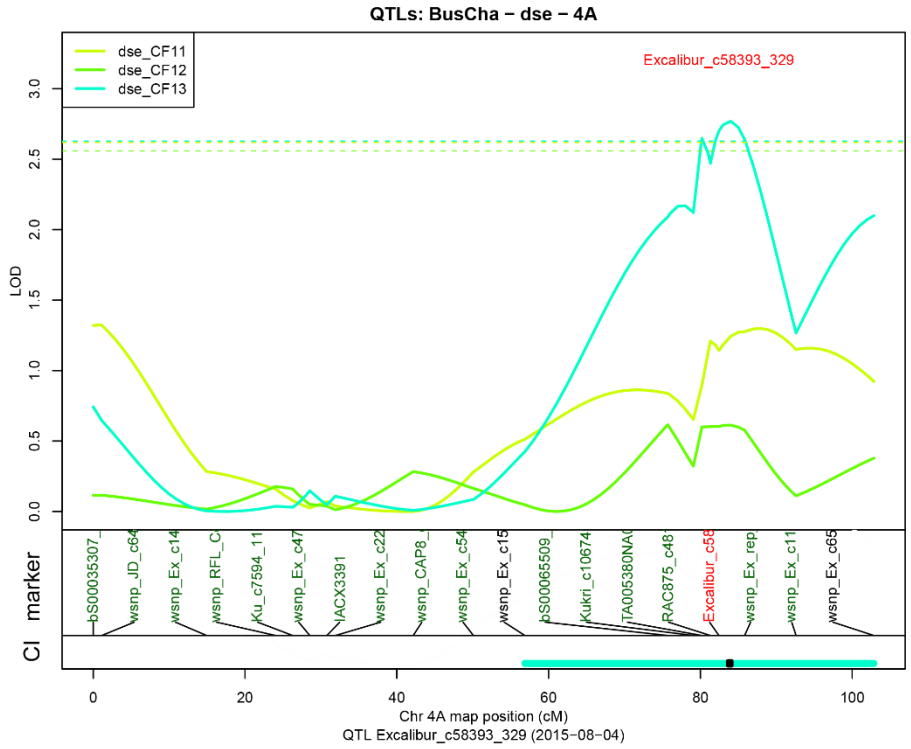

Figure 32. QTL of stem elongation period on chromosome 4A

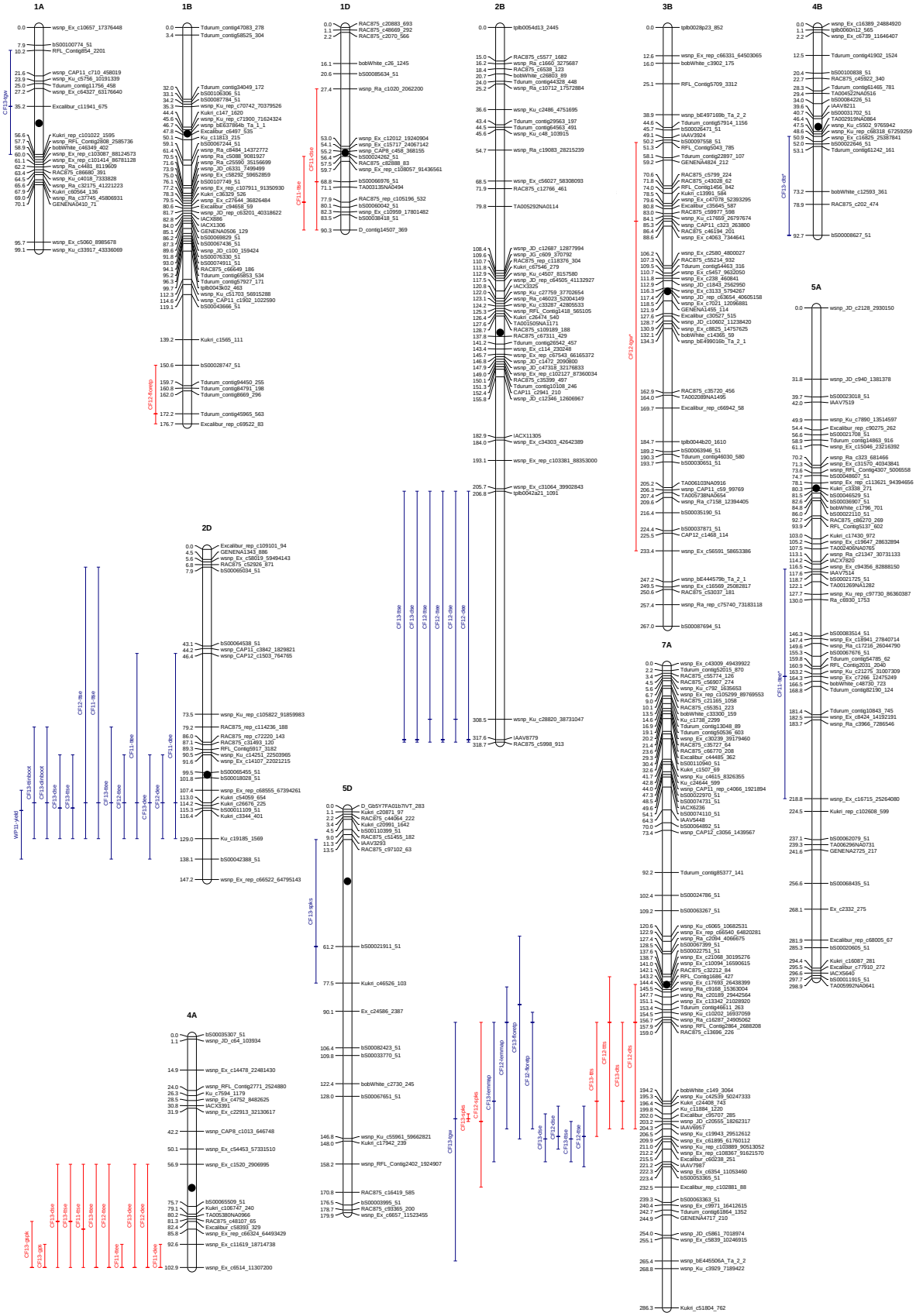

Figure 33. Diagrams of 11 wheat chromosomes (1A, 1B, 1D, 2B, 2D, 3D, 4A, 4B, 6A, 6D, 7A) with QTL locations. Markers are shown to the right of the chromosomes. QTL confidence intervals are shown as vertical lines to the left of the chromosomes; colour indicates the parent providing the positive effect: blue for Buster and red for Charger. Centromeres are indicated by a black circle in each chromosome.

Table 15. Description of QTL identified in Buster x Charger DH population and references to QTLs in similar location (same arm chromosome). Positive additive effects coming from Charger and negative from Buster.

Trait code	Environment	Chromosome	Position	LOD	%variation	additive effect	Nearest marker	Position of nearest marker	QTLs in literature
dee	CF13	2B	316.0	1.6	5.4	-0.635	IAAV8779	317.6	Maccaferri et al. (2008); Wang et al. (2009)
	CF12	2D	113.0	4.6	16.1	-1.154	Kukri_c54059_654	113.0	Kulwal et al. (2003)
	CF13	2D	113.0	2.8	9.9	-0.795	Kukri_c54059_654	113.0	
	CF14	2D	113.0	2.8	11.4	-0.611	Kukri_c54059_654	113.0	
	CF12	4A	102.9	5.5	19.9	1.202	wsnp_Ex_c6514_11307200	102.9	Kirigwi et al. (2007)
	CF13	4A	102.9	2.7	9.4	0.65	wsnp_Ex_c6514_11307200	102.9	
	CF14	4A	102.9	2.7	11	0.606	wsnp_Ex_c6514_11307200	102.9	
dinboot	CF14	2D	113.0	2.97	14.425	-0.506	Kukri_c54059_654	113.0	Kulwal et al. (2003)
dse	CF12	1D	67.0	3.17	14.862	1.407	bS00066976_51	68.8	Borràs-Gelonch et al. (2012)
	CF13	2B	286.0	3.4	13.7	-0.512	wsnp_Ku_c28820_38731047	308.5	-
	CF14	2B	315.0	2.9	8.8	-0.489	IAAV8779	317.6	-
	CF14	2D	115.3	1	2.8	-0.524	bS00011109_51	115.3	Borràs-Gelonch et al. (2012)
	CF14	4A	84.0	4.2	13	0.444	Excalibur_c58393_329	82.4	Borràs-Gelonch et al. (2012)
	CF13	7A	209.0	2.5	9.9	-0.607	wsnp_Ex_c61895_61760112	209.9	Borràs-Gelonch et al. (2012)
	CF14	7A	211.0	4.9	15.4	-0.595	wsnp_Ku_rep_c103889_90513052	211.0	
dts	CF13	7A	171.0	2.33	12.168	0.409	RAC875_c13696_226	159.0	Borràs-Gelonch et al. (2012)
	CF14	7A	178.0	4.90	22.635	0.446	bobWhite_c149_3064	194.2	
floretp	CF13	1B	172.2	2.6	10.8	0.293	Tdurum_contig45965_563	172.2	-
	CF13	7A	174.0	3.6	15.6	-0.314	RAC875_c13696_226	159.0	-
	CF14	7A	151.1	2.76	20	-0.017	wsnp_Ex_c13342_21028920	151.1	
gps	CF14	4A	102.9	5.00	23.028	2.964	wsnp_Ex_c6514_11307200	102.9	Quarrie et al. (2005)
gspk	CF14	4A	102.9	4.65	21.601	0.123	wsnp_Ex_c6514_11307200	102.9	Quarrie et al. (2005)
lemmap	CF13	7A	170.0	2.41	12.549	-0.31	RAC875_c13696_226	159.0	-
	CF14	7A	180.0	4.21	19.977	-0.251	bobWhite_c149_3064	194.2	
spks	CF13	7A	203.2	2.96	13.916	0.295	wsnp_JD_c20555_18262317	203.2	Quarrie et al. (2005)

	CF14	7A	202.0	18.5	59.7	1.036	Excalibur_c95707_285	202.0	
	CF14	5D	51.0	1.8	3.6	-0.462	bS00021911_51	61.2	Quarrie et al. (2005)
tgw	CF14	1A	35.2	2.5	10.8	-1.053	Excalibur_c11941_675	35.2	Wang et al. (2009)
	CF13	3B	86.4	2.565	12.174	0.632	RAC875_c46194_201	86.4	Wang et al. (2009)
	CF14	7A	202.0	2.2	9.2	-0.98	Excalibur_c95707_285	202.0	-
ttee	CF13	2B	317.0	1.5	5	-9.344	IAAV8779	317.6	Maccaferri et al. (2008); Wang et al. (2009)
	CF12	2D	113.0	4.6	15	-13.553	Kukri_c54059_654	113.0	Kulwal et al. (2003)
	CF13	2D	113.0	2.9	10.4	-11.842	Kukri_c54059_654	113.0	
	CF14	2D	113.0	2.6	10.7	-8.583	Kukri_c54059_654	113.0	
	CF12	4A	102.9	4.1	13.1	13.98	wsnp_Ex_c6514_11307200	102.9	Kirigwi et al. (2007)
	CF13	4A	102.9	2.6	9.1	9.469	wsnp_Ex_c6514_11307200	102.9	
	CF14	4A	102.9	2.8	11.4	8.747	wsnp_Ex_c6514_11307200	102.9	
	CF12	5A	164.3	2	6	-10.803	wsnp_Ex_c7266_12475249	164.3	-
ttinboot	CF14	2D	113.0	2.97	14.421	-7.563	Kukri_c54059_654	113.0	Kulwal et al. (2003)
ttse	CF12	1D	76.0	3.7	13.7	13.062	RAC875_rep_c105196_532	77.9	Borràs-Gelonch et al. (2012)
	CF13	2B	313.0	4	16.4	-9.055	wsnp_Ku_c28820_38731047	308.5	-
	CF14	2B	315.0	2.8	8.9	-7.129	IAAV8779	317.6	
	CF12	2D	113.0	2.2	7.9	-11.678	Kukri_c54059_654	113.0	Borràs-Gelonch et al. (2012)
	CF13	2D	113.0	1.7	6.8	-7.804	Kukri_c54059_654	113.0	
	CF14	2D	115.3	0.7	2.1	-7.61	bS00011109_51	115.3	
	CF12	4A	85.8	2.3	8	11.416	wsnp_Ex_rep_c66324_64493429	85.8	Borràs-Gelonch et al. (2012)
	CF14	4A	84.0	4	12.9	6.594	Excalibur_c58393_329	82.4	
	CF13	7A	209.9	1.6	6.1	-7.885	wsnp_Ex_c61895_61760112	209.9	Borràs-Gelonch et al. (2012)
	CF14	7A	211.0	3.3	10.7	-7.637	wsnp_Ku_rep_c103889_90513052	211.0	
ttts	CF13	7A	170.0	2.74	14.113	3.306	RAC875_c13696_226	159.0	Borràs-Gelonch et al. (2012)
	CF14	7A	178.0	4.46	20.844	4.172	bobWhite_c149_3064	194.2	
yield	WP12	2D	119.0	4.36	19.829	-0.207	Kukri_c3344_401	116.4	-

4.4. Discussion

By examining the histograms in Figure 23 which show the distribution of yield components, we can clearly see the presence of lines within the population with phenotypes that vary widely from either parent. This suggests transgressive segregation; which is frequently observed among plants (Rieseberg et al. 1999) and is the basis of genetic gain in breeding.

Stem elongation initiation is usually determined indirectly by inferring the establishment of terminal spikelet. This is done by scoring the moment the first detectable node reaches 1cm above ground level (GS31). In this study to enable a more accurate QTL analysis, main tillers were dissected and terminal spikelet initiation was precisely scored.

For most of the traits, as shown in Table 11, the parents tend to be close to the population mean and roughly centred from the extremes. But terminal spikelet seems to provide a more positive effect. Meaning that Charger, the parent with the lowest average number of days from sowing to terminal spikelet (in all three environments analysed) was closer to the lowest of the population's range of days to terminal spikelet.

Genetic factors determining the phenotypic variances were analysed as heritability. While most traits have heritability similar to those reported in some studies (Aycicek and Yildirim 2006), others like thousand grain weight, spikes per unit land area, and floret primordia developed had a very low heritability. In the case of thousand grain weight the data collected from environment CF14 was very different from environment CF13 (see Figure 23), possibly affecting the calculated heritability and inflating the variation due to genotype-environment interaction.

The heritability of floret primordia developed was extremely low. This was possibly caused by time of sample collection for the CF14 environment. These samples were collected slightly earlier, when most of the plants were at terminal spikelet initiation phase, therefore the range only varied 0.0 to 0.1 (see Table 11). At the moment of collection not enough floret primordia were present, although lemmas primordia were developed. For this reason, I suggest that the organ's primordia developed actually has a higher heritability, than seen in these experiments. Especially as it is closely linked to lemma primordia development which shows high levels of heritability (Table 12).

The importance of grain number is reflected in both environments analysed for yield components, with a high correlation to yield (CF13 and CF14. See Table 13 and Table 14, respectively). In both environments the number of spikes per unit land area verify a strong correlation to grain number.

There was a clear effect on yield and yield components by the length of the stem elongation period, from the data collected from environment CF14. An increase in the stem elongation period resulted in an increased yield, mostly through grain weight rather than grain number. This is possibly because it allows more time for the ovaries to develop (as this will affect all grains, no matter what their position in the spike or spikelet). The effect of ovary size influences to the final grain size, hence its weight (Calderini et al. 2001; Ugarte et al. 2007).

Stem elongation duration has well defined loci on chromosomes 2D and 5A, strongly affecting its duration due to *Ppd-1* and *Vrn-1* respectively (Chen et al. 2009; Chen et al. 2010). The relatively small time difference in days for the initiation and culmination of stem elongation was expected due to a similar set of major flowering time genes that are shared between Buster and Charger. As both parents carry the photoperiod sensitive alleles of *Ppd-D1*, this allows us to look beyond the gene which is known to bring forward the time of terminal spikelet (Snape et al. 2001).

Independent loci for terminal spikelet and ear emergence were identified by QTL analysis. These offer the possibility of providing fine-tuning of adaptive capabilities and even extending the stem elongation period, a key element for increasing the likelihood of floret primordia becoming fertile florets (as explained in Chapter 2).

Stem elongation QTLs fall under the terminal spikelet and ear emergence loci to form clusters of QTLs (Figure 33), with the exception of the stem elongation QTL on chromosome 1D. In the three environments analysed the threshold for this QTL was only reached in environment CF12; this is the experiment with less accurate measurements for days to terminal spikelet. For this reason, I suspect that this QTL may be an artefact of the experiment rather than a real effect. The long arm of chromosome 1D has an earliness *per se* gene controlling flowering time that might explain the observed effect (Zikhali et al. 2014).

Another interesting ear emergence QTL is located on chromosome 2B. It was only found significant for environment CF13. This is present in the same location as the stem elongation QTLs for environments CF13 and CF14. The locus with a Buster allele delays ear emergence by half a day, about the same delay in the stem

elongation period. Therefore, indicating a direct effect on extending the duration of the stem elongation period.

Further experiments would focus on developing near isogenic lines (NIL) in order to test and validate the QTLs found that have an effect on the stem elongation landmarks.

4.5. Conclusion

In this Chapter the landmarks of stem elongation, comprising ear emergence and terminal spikelet were scored. Wheat is phasic crop, with physiological events that mark when a developmental phase starts and ends. Some of these events are easy to determine, such as ear emergence. Others are more difficult due to the organs in which they take place, which can be difficult to access; requiring a destructive process, such as measuring terminal spikelet. Therefore, this stage is usually scored by association to a different physiological event. In this study where precision is a must, the precise moment in which terminal spikelet occurs was scored directly using dissection, for the first time in a segregating population. Using this unique and precise data, the analysis revealed the presence of QTLs for both terminal spikelet and ear emergence on chromosome 7A for the former, and 2D and 4A for the latter. These QTLs were found to be independent, so can be used for fine-tuning stem elongation by adding or removing them separately, and which could, in future, provide a novel way to increase yield.

5. Serendipity: Identification of a major lodging QTL on chromosome 2D.

5.1. Introduction

Yield in wheat is severely affected by lodging, which can account for up to 80% decreases, and is defined as two types: (i) stem lodging, the permanent displacement of the stem from the vertical due to plastic failure, or (ii) root lodging, anchorage system failure (Foulkes et al. 2011b). During the summer of 2014 (grain filling stage during experiment CF14), a clear and interesting trait was observed in the Buster x Charger mapping population that could be definitively scored. As shown in Figure 34 some plots experienced root lodging, either of the whole plot, or a percentage of it.

There was therefore an opportunity to investigate lodging, a trait of international relevance that affect all cereals, and that in UK it has an incidence of 10% per year (Griffin 1998). The other major effect of lodging is that it reduces the end use quality of the harvested grain mainly by reducing Hagberg falling number (HFN). Low HFN is associated with low bread making quality (Baker et al. 1998). A difficulty with research into lodging is the intermittent occurrence of the trait, which makes experiments hard to plan. This opportunity to investigate lodging could not be considered during previous seasons due to the non-appearance of lodging or the lack of abiotic conditions required for the occurrence of lodging. It has high relevance to the high level aims of this work, as increasing lodging resistance will act to protect any future yield gains achieved through altered phenology.

Figure 34. Buster x Charger DH population during summer 2014

Lodging can be caused either by root or stem failure or both and is most likely to happen in the last one or two months before harvesting, when the mass of grain in the wheat spike is increasing rapidly. Plant architecture, rain, wind and soil are all

important factors. Wind can displace the soil, or exert force on the stem which might bend or break the stem base. Rain can increase the weight of the ear, and reduce root anchorage. Both root and stem lodging have been reported as predominant reasons for loses in winter wheat (Crook and Ennos 1993; Neenan and Spencer-Smith 1975).

Stem and anchorage strengths are well characterised in both Buster and Charger, and are detailed in the HGCA project report No.305 for the UK recommended list for wheat (Spink et al. 2003). Charger is known to have a low resistance to root lodging, with a root failure wind speed of 8.53m/s, and is among the lower standing power cultivars introduced into commercial practice in the UK between 1986 and 1999; on the other hand Buster has one of the highest standing powers with a root failure wind speed of 11.81m/s (Berry et al. 2003).

Some researchers argue that the new green revolution should focus on the root systems (Lynch 2007; Den Herder et al. 2010), the idea being that crops with specific root architectural traits like root spread and adventitious root development, will be well adapted to low soil fertility, while providing structural aid to the above-ground biomass. The relationships between yield, lodging, root system and height is complex, with genes on almost all the chromosomes having an effect in determining plant height (Keller et al. 1999).

Breeding has selected against varieties prone to lodging and reduced height (without yield penalty) but this still remains an important target in breeding. An example of this is the experiment by Verma et al. (2005) in which they used a doubled haploid population that segregates for plant height due to different alleles of *Rht-B1* and *Rht-D1*; with three main groups: (i)semi-dwarfs, (ii)doubled tall, and (iii) doubled dwarf (However selecting for stiff stems results in an increased force transmitted to the roots(Ennos 1991b)).

There is no doubt that the introduction of semi-dwarf wheat cultivars during the Green Revolution provided an increase in the overall yield which was accompanied, in many cases, by a reduction in crop height. But a shorter stem, as necessary as it is, is not enough for an adequate lodging resistance The Buster x Charger doubled haploid population results in a suitable segregating population for study, due to its similar background.

5.2. Materials and methods

The same trial described in section 4.2, with a mapping population of 109 double-haploid lines from Buster x Charger was used for this study.

5.2.1. Trait, measurements and analyses

Traits known to be related to lodging were considered for this chapter. As the experiment was not planned, some measurements of the developing crop relevant to lodging but that occurred prior to the lodging event were not measured. A list of the traits analysed are shown in Table 16.

Code	Trait	Unit	CF12	WP12	CF13	CF14
colint	Distance between root crown and the furthest nodal root	centimetres	X		X	X
col05	Percentage of samples with colint greater than or equal to 0.5 cm.	percentage	X		X	X
col1	Percentage of samples with colint greater than or equal to 1 cm.	percentage	X		X	X
area	Area of the spike	cm^2			X	X
width	Width of the spike	centimetres			X	X
length	Length of the spike	centimetres			X	X
LodS	Lodging score	na				X
LodR	Percentage of the plot affected by lodging	percentage				X
LodA	Angle of stem from the vertical	degrees				X
height	Total plant height above ground	centimetres	X	X		X
Ped	Peduncle length	centimetres	X			
Int1	First internode below the peduncle	centimetres	X			
Int2	Second internode below the peduncle	centimetres	X			
Int3	Third internode below the peduncle	centimetres	X			
Int4	Fourth internode below the peduncle	centimetres	X			
Int5	Fifth internode below the peduncle	centimetres	X			

Table 16. Abbreviations of trait data, with units of measurements and which environment the measurement was taken. Lodging occurred in CF14.

All traits, except for the LodS, LodR, and LodA, were carried out in samples taken from the field before harvesting. A few plants (no less than 15) were recovered and taken to the laboratory. Once on site, each bunch of tillers was carefully spread on a table and 10 were sampled randomly. The spikes were saved in a paper bag for further analysis and threshing. Length was measured using a ruler with metric units. With the exception of the measurements for spike length, width, and area; which were done using the MARVIN grain analyser (GTA Sensorik, Germany).

In response to the lodging that occurred in the environment CF14, I decided to score it with the following formula, described by Fischer and Stapper (1987):

Lodging score = (% plot area lodged) x (angle of lodging from the vertical)

Where the '% plot area lodged' accounts for the proportion of stems within the plot affected by lodging, using a scale from 0 (0%) to 10 (100%). The 'angle of lodging from the vertical' was measured using a scale from 1 to 5; where 1 represents 0˚ from the vertical and 5, 90˚ (see Figure 35).

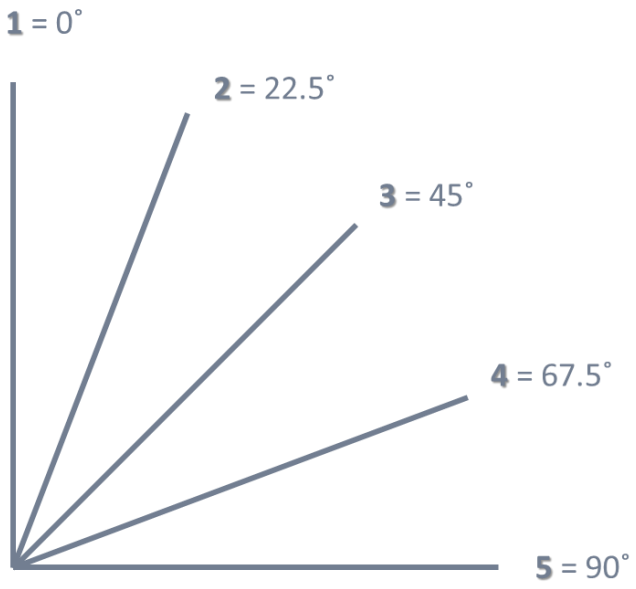

Figure 35. Scale used in determining the angle of lodging.

Spike morphometric measurements were acquired by analysing the 10 mature spikes saved in the paper bag using the MARVIN grain analyser (GTA Sensorik, Germany), using the "brassica pods" setup (Figure 36). This provided average measurements for length, width, and area (area as a 2 dimensional face of the spike) of the 10 spikes.

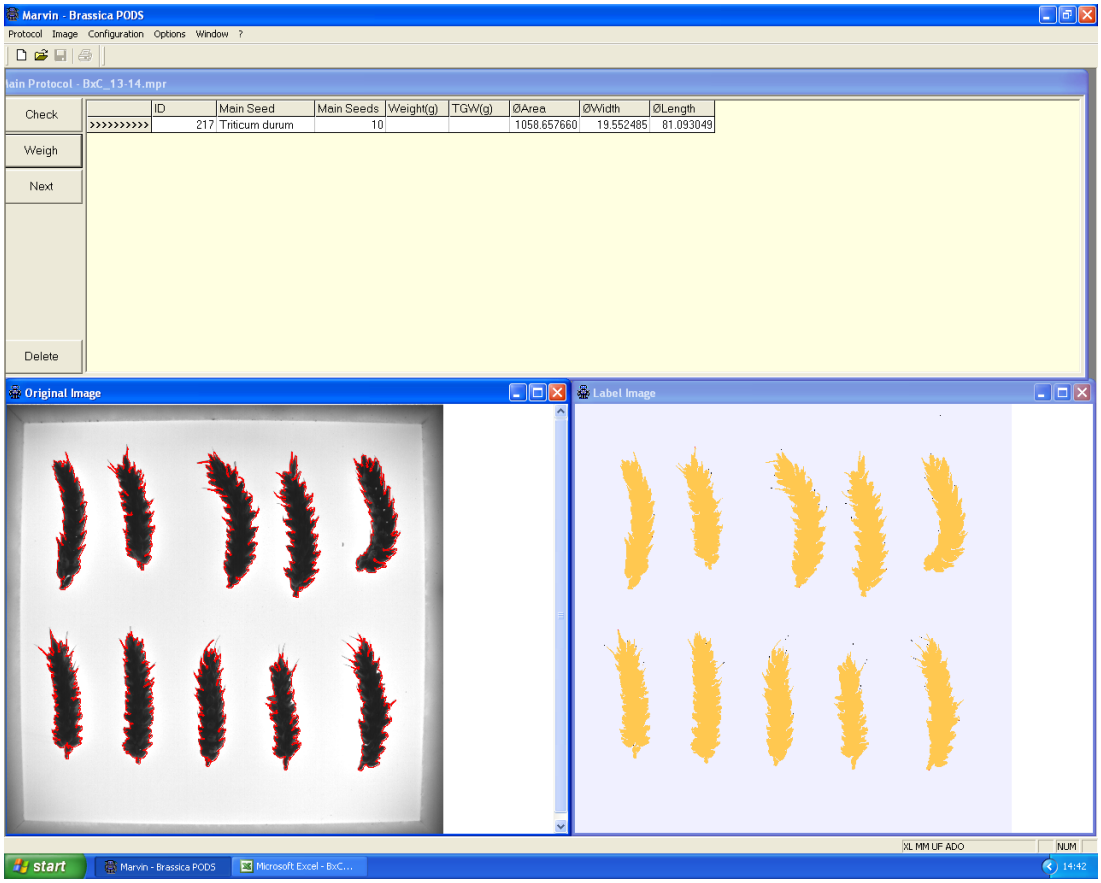

Figure 36. Printscreen of MARVIN grain analyzer software determining morphological parameters in 10 samples (spikes).

Descriptive statistics and normality tests on quantitative data were performed using R 3.0.2 (R Development Core Team). Library lme4 (Bates et al. 2012) was used for model fitting. The figures were produced using GraphPad Prism 5 (2007), except for the principal component analysis which was done with ggbiplot package using R.

The same procedure for the QTL analysis in section 4 were used in this section. This includes the same genetic map provided by TraitGenetics (Am Schwabeplan 1b, Stadt Seeland OT Gatersleben, D-06466, Germany).

5.3. Results

One of the first tasks after the discovery of root lodging in this trial was to unearth root systems, in order to compare plots with the highest lodging score and those that remained unaffected (Figure 37). At that moment in the screening it was possible to suppose that the presence of nodal roots closer to the crown seems to provide less anchorage to the plant, therefore an increased susceptibility to root failure wind speed.

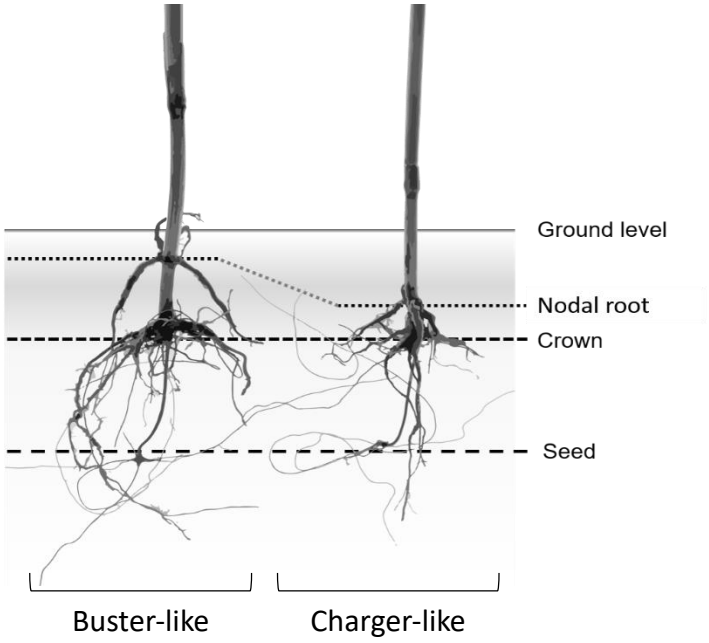

Figure 37. Diagram with differences in nodal root between lodged and non-lodged plots.

The histograms of the distance between the crown and the furthest nodal root show transgressive segregation across the three environments sampled, (CF12, CF13, and CF14) they also show a trend of Charger having closer nodal roots to the crown than Buster (Figure 38).

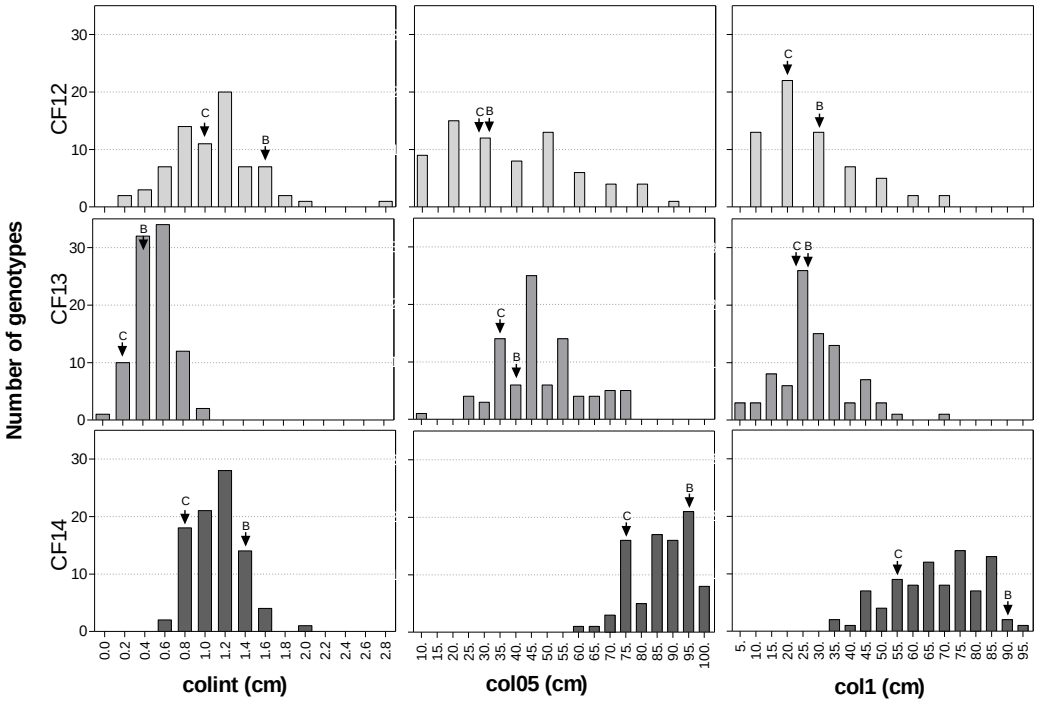

Figure 38. Histograms of Buster x Charger DH distance between nodal root and crown (colint), percentage of colint greater than 0.5cm (col05), and greater than 1cm (col1) for CF12, CF13, and CF14. The mean value for Buster (B) and Charger (C) are represented by the arrows.

5.3.1. Plant height

WP12 and CF12 Buster is shorter than Charger. However, in CF14 Charger is ~6cm shorter than Buster. This shows that genotype x environment interaction is occurring for this trait. In each year height segregation ranges within ~20cm. In 2012 WP has the greatest mean height for the whole population compared to CF. The population was tallest in CF14. Transgressive segregation for height is seen in all three environments with genotypes taller and shorter than either parent. In 2014, the year lodging was observed, 20 lines where taller than Buster.

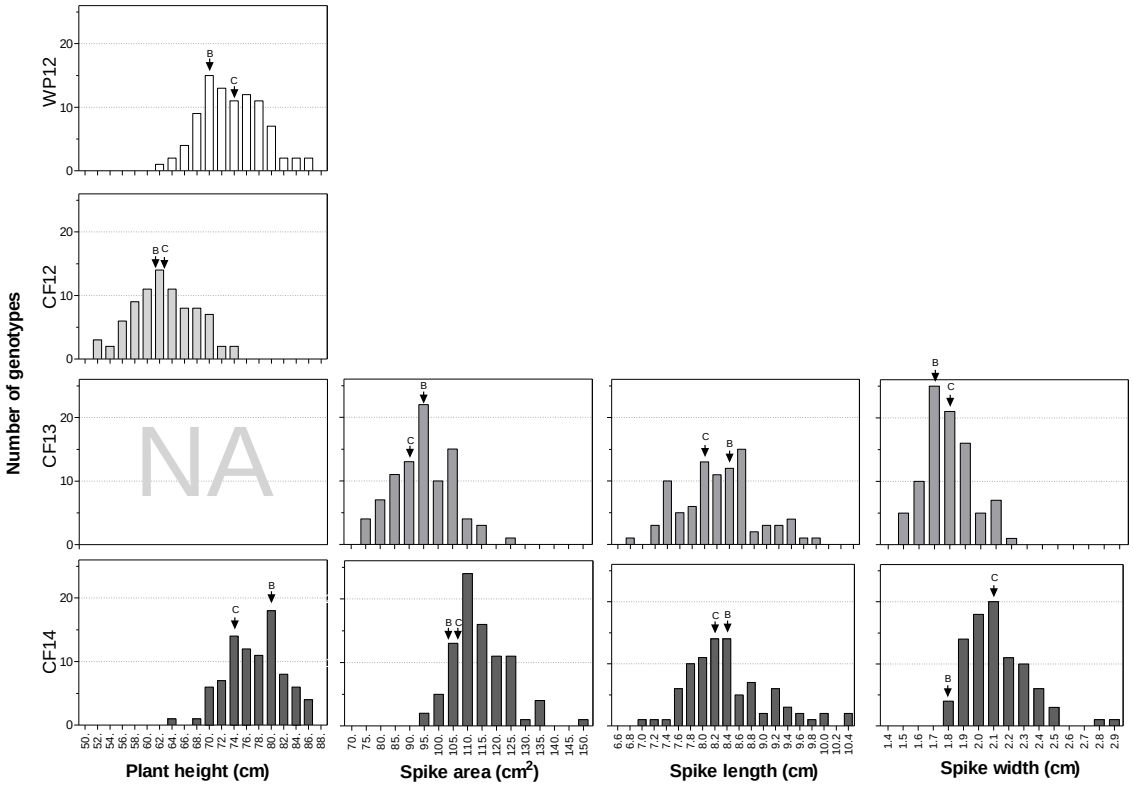

Figure 39. Histograms of Buster x Charger DH plant height, spike area, spike length, and spike width for WP12, CF12, CF13, and CF14. The mean value for Buster (B) and Charger (C) are represented by the arrows.

Lodging score data collected in CF14 had a uniform distribution (see Figure 40a). The QTL analysis assumes a normal distribution; therefore, the data was transformed as the inverse of the cumulative normal distribution function (see Figure 40b). The use of functions to force data to display a normal distribution is a practice that has to be done carefully as it might hide the genotypic-phenotypic interaction.

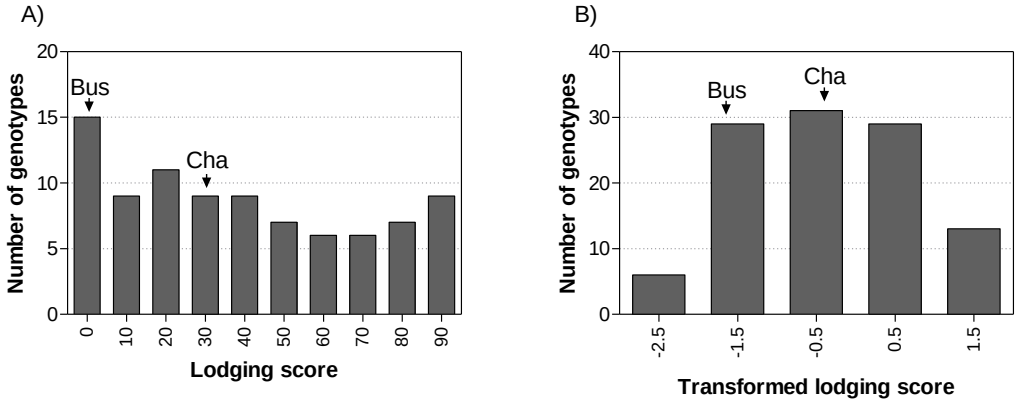

Figure 40. Histograms of Buster x Charger DH lodging score for CF14, untransformed (A) and tranformes (B). The mean value for Buster (Bus) and Charger (Cha) are represented by the arrows.

Fifteen lines displayed the same standing power as Buster in the CF14 trial. In accordance with existing standing power data for Charger was relatively poor with a LodS of 30. The majority of the population exhibited even worse performance than Charger, with nine of them left almost completely lodged in all three replicates.

Lodging score was strongly correlated to plant height (r^2=0.720), this seems to suggest that lodging is being caused by different alleles of reduced height (*Rht*) genes. From the lodging score components, the percentage of plot affected regardless of the lodging angle, have a slightly higher correlation with height (r^2=0.748). While the lodging angle was not too far lower (r^2=0.718). Lodging score also was positively correlated to the spike area (r^2=0.377), especially with the length of the spike (r^2=0.634) (Table 17).

On the other hand, the distance between the crown and the nodal root, or the percentage of plants within the plot with a distance greater than 0.5cm and greater than 1cm, was not correlated with lodging.

	col05	col1	area	width	length	LodR	LodA	height	LodS
colint	**0.745*** **	**0.857*** **	-0.122	-0.084	-0.081	-0.060	-0.075	0.086	-0.091
col05		**0.764*** **	0.039	0.163	-0.010	-0.045	-0.067	0.053	-0.056
col1			-0.063	0.014	-0.069	-0.076	-0.082	0.068	-0.086
area				**0.593*** **	**0.830*** **	0.281**	0.323**	0.245*	**0.377*** **
width					0.170	-0.169	-0.185	-0.277	-0.119
length						**0.562*** **	**0.602*** **	**0.548*** **	**0.634*** **
LodR							**0.936*** **	**0.748*** **	**0.957*** **
LodA								**0.718*** **	**0.965*** **
height									**0.720*** **

Table 17. Pearson correlation coefficient for the unadjusted arithmetic mean of lodging components in CF14. Values in bold have a P<0.001.

5.3.2. Identification of lodging QTL

At first instance, one major locus for lodging score on chromosome 2D was found, which even after subjecting the data to a transformation to display a normal distribution, the single QTL in 2D was still found (Figure 41). After obtaining this results, further analysis in the Buster x Charger doubled haploid population were performed to include traits related to lodging (e.g. height and coleoptile length).

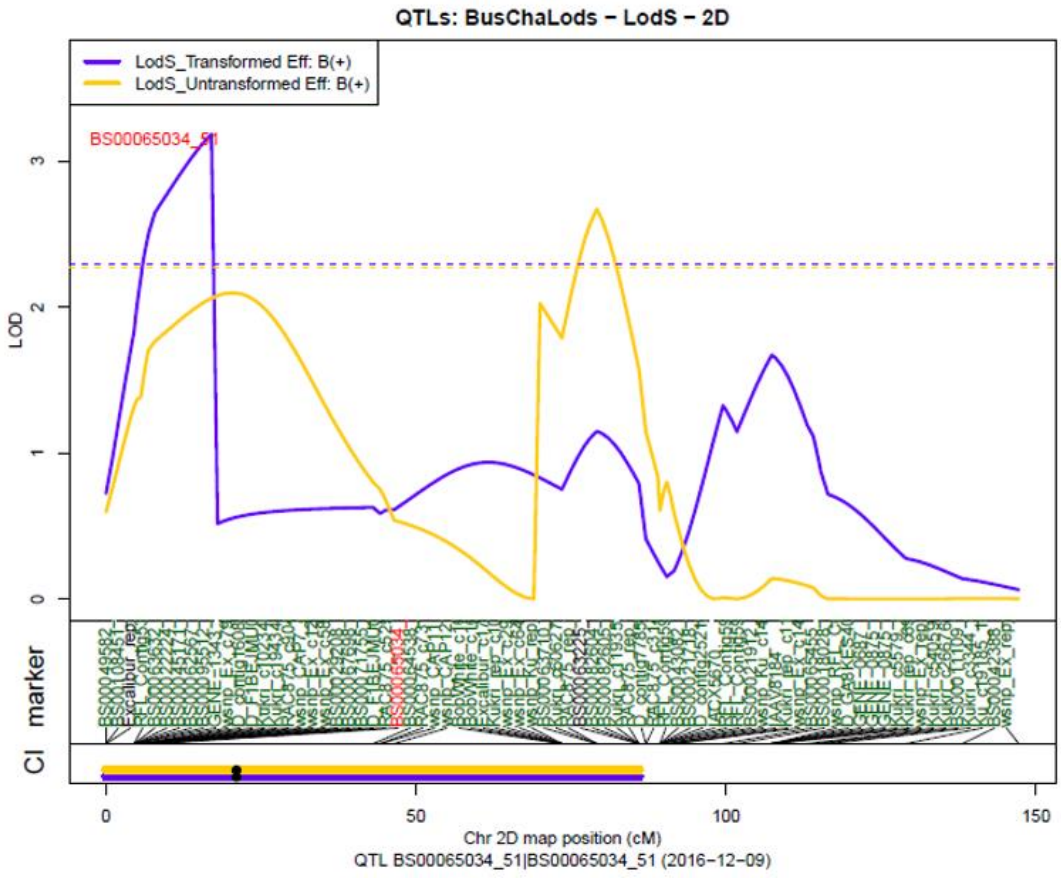

Figure 41. Lodging score QTL on chromosome 2D with both analysis, for transformed (blue) and untransformed (yellow) values.

In-depth QTL analysis using the R/qtl package within R software, provided 24 locations across 12 chromosomes for the 16 traits related to plant morphometries and lodging (Figure 42). Besides the set of loci involved, it becomes clear that chromosome 2D emerged as an interesting and important locus, with QTLs for Lodging score, height, and spike area.

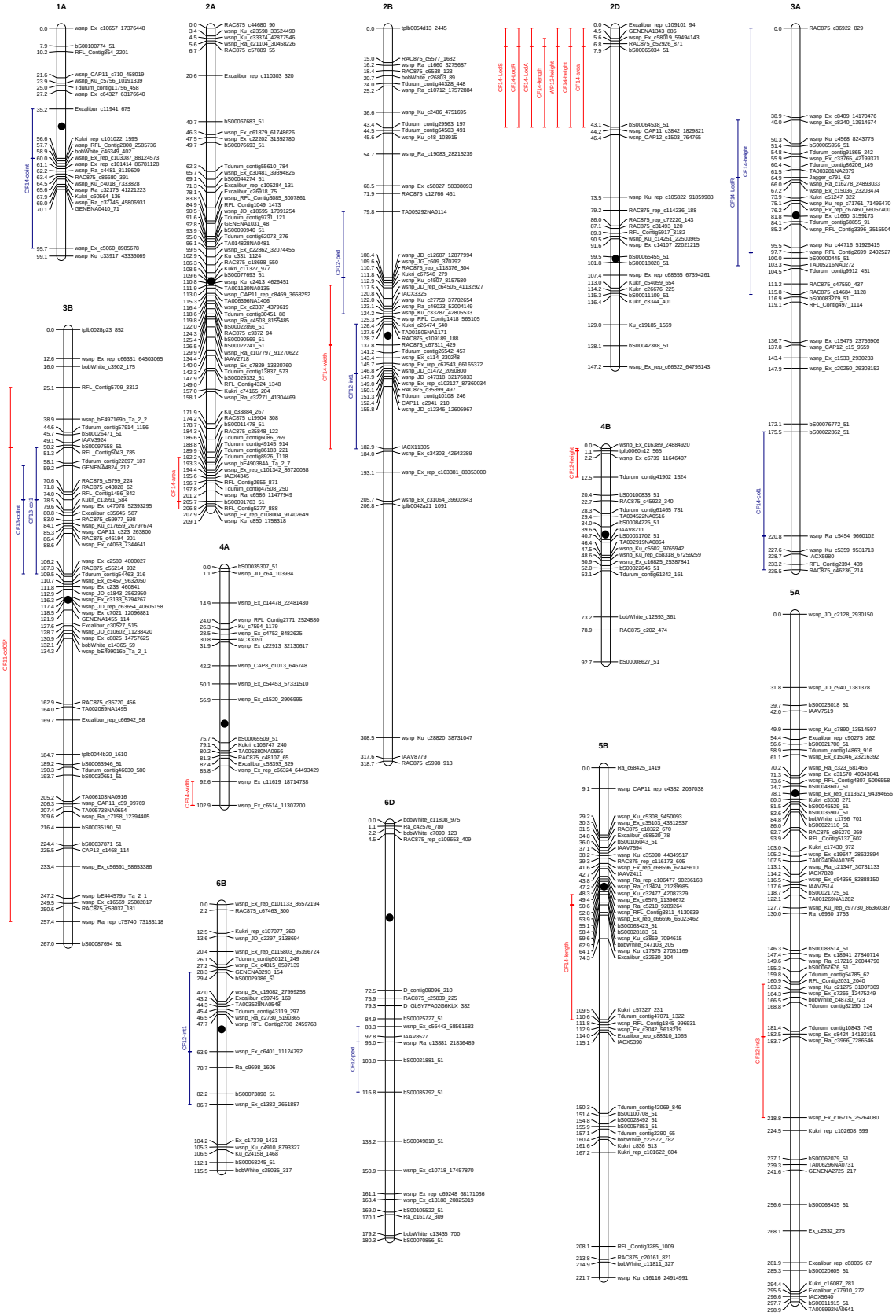

Figure 42. Diagrams of 12 wheat chromosomes (1A, 2A, 2B, 2D, 3A, 3B, 4A, 4B, 5A, 5B, 6B, 6D) with QTL locations. Markers are shown to the right of the chromosomes. QTL confidence intervals are shown as vertical lines to the left of the chromosomes; colour indicates the parent providing the positive effect: blue for Buster and red for Charger. Centromeres are indicated by a black circle in each chromosome. Trait name abbreviations are given in Table 11.

Trait code	Environment	Chromosome	Position	LOD	%variation	additive effect	nearest marker	pos nearest marker
area	CF14	2A	204	2.4	10.2	-3.507	bS00091763_51	205.687
area	CF14	2D	14	2.1	9.2	3.495	bS00065034_51	7.878
col05	CF12	3B	52	2.82	16.506	8.196	RFL_Contig5043_785	51.298
col1	CF14	3A	223	2.1	8.8	-4.97	wsnp_Ra_c5454_9660102	220.801
col1	CF14	3B	76	2.9	12.4	-5.573	RFL_Contig1456_842	74.009
colint	CF14	1A	56.6	3	12.5	-0.095	Kukri_rep_c101022_1595	56.614
colint	CF14	3B	74	3.1	12.8	-0.095	RFL_Contig1456_842	74.009
height	CF14	2D	9	4.6	18.2	2.009	bS00065034_51	7.878
height	WP12	2D	14	6.502	28.039	2.483	bS00065034_51	7.878
height	CF14	3A	98	3.4	13.3	-1.738	wsnp_RFL_Contig2699_2402527	97.713
height	CF12	4B	1.1	3.305	16.753	2.123	tplb0060n12_565	1.124
int1	CF12	2B	150	3.1	13.8	-0.618	RAC875_c35399_497	150.149
int1	CF12	6B	59	2.5	10.8	-0.541	wsnp_Ex_c6401_11124792	63.945
int3	CF12	5A	189	4.018	19.982	0.426	wsnp_Ra_c3966_7286546	183.651
length	CF14	2D	19	4.9	19.3	0.281	bS00065034_51	7.878
length	CF14	5B	61	2.6	9.9	0.252	wsnp_Ku_c3869_7094615	59.567
LodA	CF14	2D	19	3.425	16.408	9.926	bS00065034_51	7.878
LodR	CF14	2D	16	3.9	16.1	0.138	bS00065034_51	7.878
LodR	CF14	3A	64	2.2	8.6	-0.121	Jagger_c791_62	64.919
LodSU	CF14	2D	24	2.842	15.631	0.769	Kukri_c60627_74	79.172
LodST	CF14	2D	21	2.637	14.593	25.783	BS00065034_51	7.878
ped	CF12	2B	108.5	6.9	23.8	-1.269	wsnp_JD_c12687_12877994	108.449
ped	CF12	6D	95	7.8	27.6	-1.426	wsnp_Ra_c13881_21836489	95.024
width	CF14	2B	138	2.8	11.7	0.076	RAC875_c67311_429	137.781
width	CF14	4A	102.9	2.6	10.9	0.074	wsnp_Ex_c6514_11307200	102.852

Table 18. Description of QTL identified in Buster x Charger DH population. Positive additive effects indicate that the increasing allele is from Charger and negative from Buster.

It becomes clear that LodS, LodA, LodR, height, length, and area are all closely related and linked traits. This is illustrated by these six traits falling in the same confidence interval at chromosome 2D in Figure 42.

Height was scored in three environments, of which only two (CF14 and WP12) had a significant LOD score. However, it is possible to see a trend in the third environment (CF12) despite the fact that it did not meet the significance threshold level (Figure 43, lower panel).

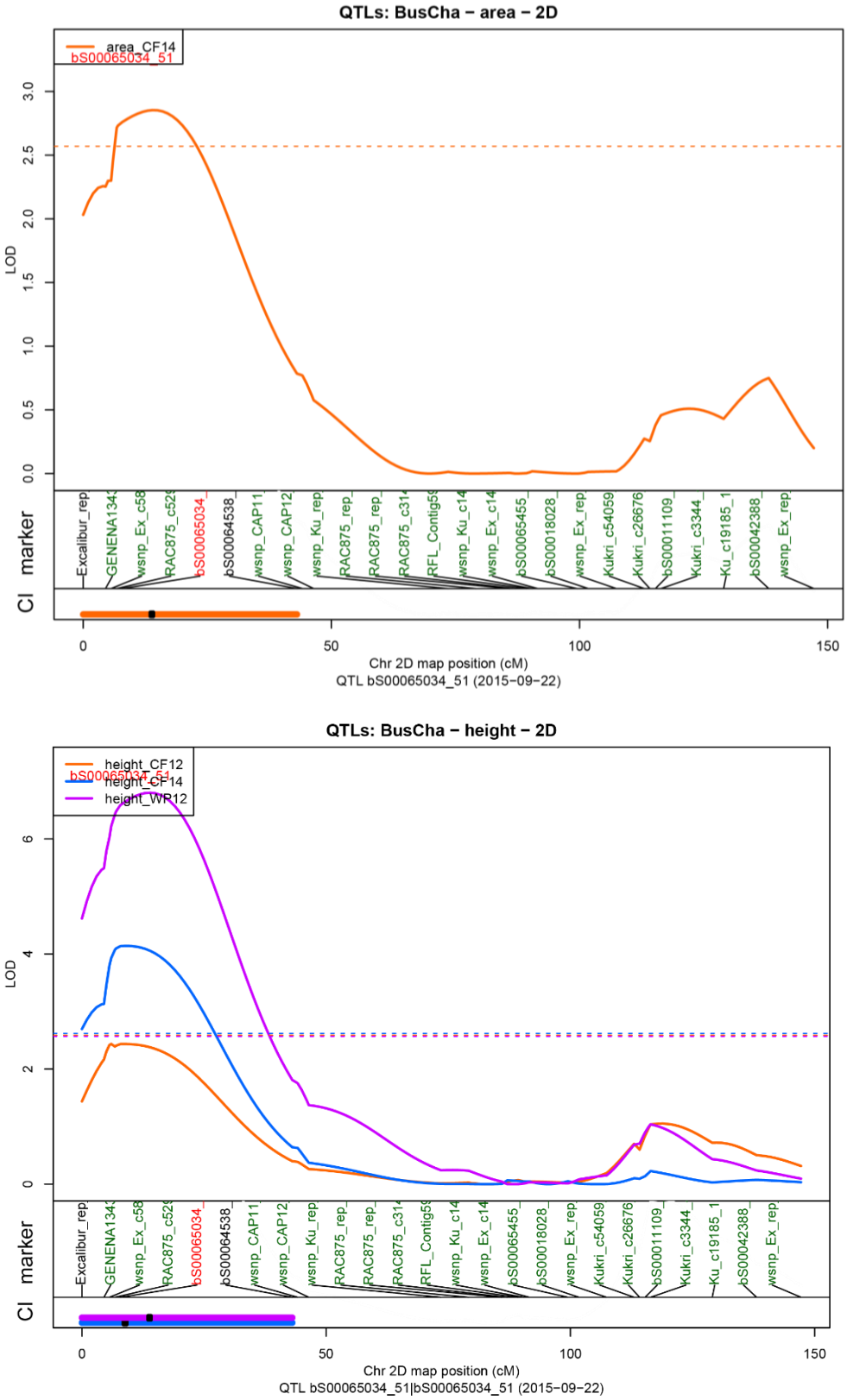

Figure 43. QTL on chromosome 2D for top panel; spike area data from Churchfarm, UK, season 2013-14. Bottom panel; for plant height data from Churchfarm UK, seasons 2011-12 (orange), and 2013-14(blue), and for Woolpit Uk, season 2011-12 (purple).

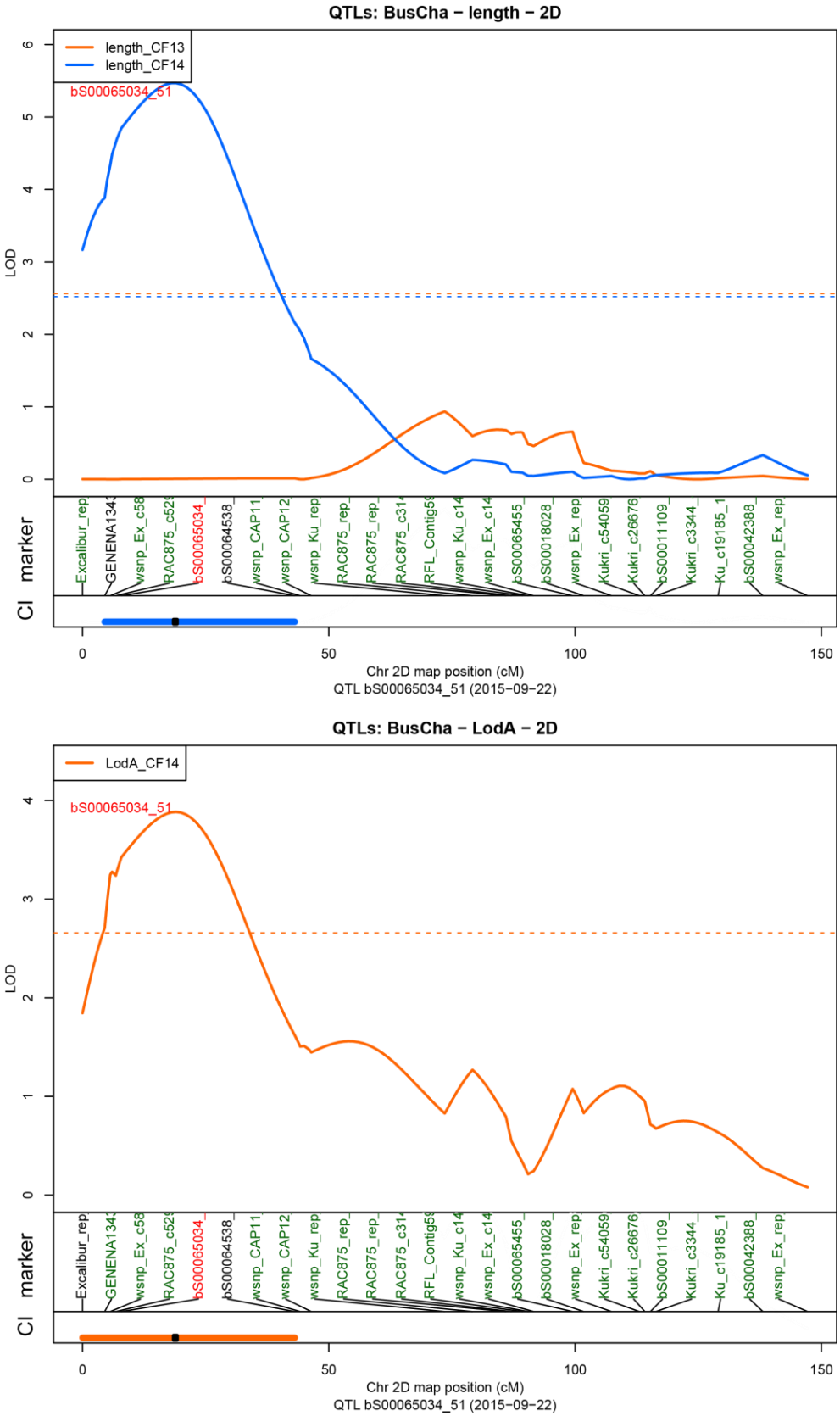

Figure 44. QTL on chromosome 2D for top panel; spike length from Churchfarm, UK, season 2012-13 (orange), and 2013-14 (blue); and for the bottom panel; angle of stem from the vertical from Churchfarm, UK, season 2013-14.

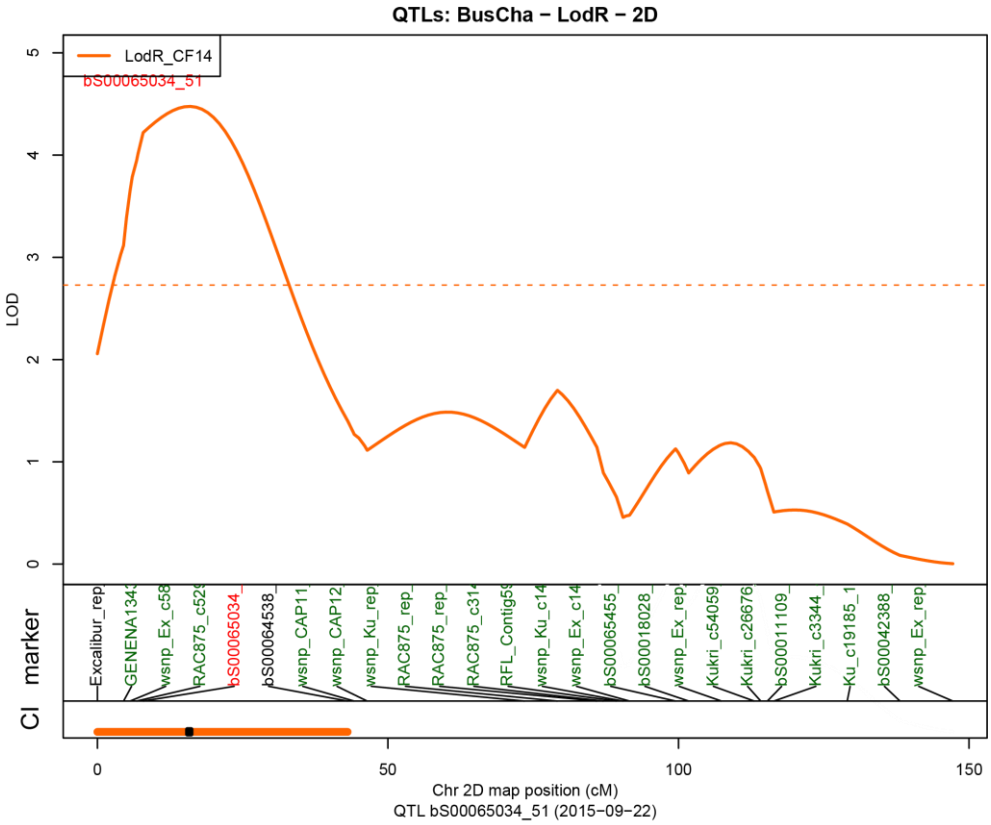

Figure 45. QTL on chromosome 2D for the percentage of the plot affected by lodging from Churchfarm, UK, season 2013-14.

5.4. Discussion

When the lodging event occurred in the summer of 2014 in environment CF14, it gave me the opportunity to analyse it. It is a mapping population of two elite winter lines, with similar genetic background; both parental lines have the wild-type allele *Rht-B1a* and the semi-dwarf allele *Rht-D1b*. Both of these dwarfing genes are widely used by plant breeders to reduce the risk of lodging by reducing the height of cultivars (Berry et al. 2007). The fact that Buster x Charger is genetically fixed at these loci meant that any variation observed is not due variation at *Rht1*.

The segregation of lodging across the doubled haploid population was well spread across the field trial site, with some plots standing perfectly perpendicular to the ground while others, in the immediate vicinity, were completely flat (Figure 34). Moreover, the storm appeared to treat my plots with a very even hand with the LodS for genotypes between replicated blocks very well correlated. Initial screening across the population indicated root lodging, as the stem integrity of the plants had not been compromised. Further inspection of root architecture showed a pattern. A sample of those plots with no lodging had plants which nodal roots were farther distant to the root crown, possibly providing a better resistance to lodging, than in plots with nodal

roots closer to the crown. However, measurement of this trait in the complete experiment showed that my initial observations were misleading and there appears to be no increase in lodging resistance from lines carrying the Buster type adventitious root arrangement, it was crop height that was best correlated with lodging, together with the area of ear presented to the wind and rain.

The risk of lodging is increased by wet, weak soil and windy environmental conditions (Berry et al. 2003). These conditions enable the displacement of the stem by reducing the plants anchorage to the ground. The root architecture in wheat that provides anchorage consists of a cone of lignified coronal roots (Verma et al. 2005). Lignin is responsible for providing the structural bending capability of the roots (Ennos 1991b, a). CF14 was the trial in which the population grew the tallest. The combination of this overall susceptibility with the weather conditions enable the lodging events.

The phenotypic diversity and high heritability of the trait observed was sufficient to allow QTL analysis to be performed (Figure 38 and Figure 39). The histogram for lodging score presented a fairly uniform distribution (Figure 40).

The results show a strong correlation between lodging prevalence and increased plant height (Table 17). Although this is not a historical population, it is congruent with the general consensus of the target ideotype with shorter stem (Berry et al. 2007; Kelbert et al. 2004).

The initial thought was that the lodging resistance observed was due to an increased distance between the nodal roots and the crown, therefore providing extra anchorage. This was most clearly seen in the parental lines (Figure 37), with Buster having a greater nodal root length and therefore providing the lodging resistance. However, further analysis proved this idea to be wrong. No correlation was found between the distances of the nodal roots from the crown and lodging score (Table 14), this trait was neither providing lodging resistance, nor causing lodging.

One of the multiplicands, (i.e. LodR) used to calculate lodging score is the percentage of affected plants. In order to also take into account a percentage in the measurements of distance between crown and nodal roots, I calculated the percentage of samples (plants) that had a length from nodal root to crown longer than 0.5 cm and 1 cm. These calculated values did not provide evidence that extended nodal root length from the crown were providing extra anchorage. Instead, I found that there was a high correlation between lodging score and plant height (Table 17).

In the first instance, a single QTL analysis for lodging score was carried out, this showed a major QTL on the short arm of chromosome 2D (Figure 41). After collecting more data related to lodging, additional phenotypic traits were also found to be affected by this locus (i.e. lodging score and its multiplicands, spike area, spike length, and plant height.), shown in Figure 42. It is noteworthy that this is the same region in which I identified a yield and heading date QTL (section 4). Increased spike weight associated with increased yield could explain the tendency of Charger alleles to induce lodging at this locus. However, the yield increasing allele at this locus is actually from Buster. So, height reduction and yield increase are conferred together.

As stated, the lodging tolerance appears to be provided by the Buster allele, similar to the original hypothesis related to root phenotypic differences. However, root lodging resistance for this population is correlated with the height of the crop, even though the parental lines are both modern semi-dwarf elite cultivars with the same *RhtB1* and *RhtD1* alleles. This is not to say that the increased height of the Charger allele is causative. This height effect accounts for between 3.3 and 6.5 % of the variation observed in the BxC population, so any pleiotropic or linked effects of the QTL could contribute, some of these, such as deeper root phenotypes are effectively invisible to us in this trial. Others, such as spike area, increased by the Charger allele, might increase lodging by acting as a sail against the wind and absorbing more surface water to increase the leverage imposed from the top of the step to the root plate.

The locus of interest on chromosome 2D overlaps with an alternative semi dwarfing gene called *Rht8* gene (Worland et al. 1998). This is a semi-dwarfing gene that improves lodging resistance through shorter stems, without having a negative effect on yield (Gasperini et al. 2012). Compared to *RhtB1* and *RhtD1*, *Rht8* does not confer insensitivity to external sources of gibberellic acid (Korzun et al. 1998).

Interestingly by examining records from the archive of screening carried out in Simon Griffith's group, 174bp fragment is present in both parental lines Buster and Charger (unpublished data). In accordance with the findings of Ellis et al. (2007), that *gwm261* is not always diagnostic for *Rht8* this does not tell us for sure whether alleles of *Rht8* are segregating in Buster x Charger, but this information might be useful to compare varieties within related pedigrees. Another, very strong height QTL identified in the Avalon x Cadenza DH population (Griffiths et al. 2012) also occupies this space. It is highly possible that *Rht8* is the gene associated with the peak observed; as it coincides with a marker linked to this gene. In that case Cadenza carried the tall allele

and a yield increasing effect (Ma et al. 2015). Here, the yield increase is with height reduction. So the putative relationship of these 2D loci is not clear but well worth further study as the ability to select for reduced height, reduced lodging, and increased yield, without trade off based on this locus could make an important contribution to wheat breeding.

5.5. Conclusion

This Chapter was the result of a serendipitous event, where we were able to analyse the lodging resistance in this population. The QTL experiments in this analysis can only be seen in certain conditions, such as were found in Church Farm in 2013-14.

The initial hypothesis was that the increased distance between nodal roots and the crown in the Buster-like plants would translate to an increased anchorage, and therefore conferring lodging resistance. However, further analysis showed that this phenotypic measurement did not correlate with lodging score.

The lodging QTLs found suggest that the lodging in this population was due to height differences between the two parents. The QTL results indicate that Charger increases lodging. In addition, Charger causes a bigger plant with a greater spike area, mostly through a longer spike.

This population appears to have the same *Rht* genes. The similar background between the parents allowed us to look beyond the known *Rht* genes used to develop semi-dwarf cultivars, to potentially find novel variation for lodging.

6. Summary and outlook

6.1. General summary

Grain yield potential is somewhat related to biomass accumulation in the spikes (Fischer 2011; Miralles and Slafer 2007; Reynolds et al. 2009). Improvements can be focused towards providing a longer period of stem elongation, which happens parallel to the spike growth period (Slafer et al. 2001; Slafer et al. 1996). Studies suggest that competition for assimilates during this critical phase of development have an effect on the final number of grains; during this phase the demand for the assimilates (building blocks) is high, with the stem rapidly growing, as well as the spike, while floret primordia are being generated and the number of fertile florets is being determined (Kirby 1988; Brooking and Kirby 1981).

To understand the relationship between phenotypic development and grain yield potential, I started by determining the differences in generation and degeneration of florets in elite germplasm. This was done by dissecting and scoring the development of the spikes throughout the stem elongation period. This two-year experiment provides further insight of the floret dynamics for the three main positions of the spike, i.e. apical, central, and basal position. The results show that the variations in development seem to be associated to an extended growth period (Chapter 2).

The extended growth period is shown to have a positive effect on the final number of fertile florets, and as these are set to become grains, I focussed on this in Chapter 3. Determining the variation of fruiting efficiency and developmental phases in elite germplasm, and their relationship across 4 environments provided interesting results on the degree of improvement that the stem elongation period supplies.

By assessing elite germplasm we can get a snapshot of the current genetic diversity that is ready to be implemented by breeders. It is assumed that years of domestication, and a relatively short number of landraces involved in the production of modern wheat, have reduced the genetic diversity of current cultivars (Frankel 1970; Reif et al. 2005). But current genetic diversity is still of great use (Dreisigacker et al. 2004).

The possibility of manipulating grain yield potential seems clearer when looking at the stem elongation period. To deeply acknowledge the contribution of developmental phases, it is necessary to determine the genetic locations involved. In Chapter 4 I looked for the chromosomal location affecting the stem elongation period, specifically

the terminal spikelet phase and flowering time. The QTL analysis was comprised of data from three seasons, which provided repeatedly two loci for flowering time and a single locus for terminal spikelet phase, all of which were located in different chromosomes. These findings provide substantial instruments to further develop markers that, under marker assisted selection, could contribute to current breeding programs in the search of increased grain yield potential. Another possibility, which could be included in an adaptation framework, that looks into fine-tuning phenotypic development to secure the optimal seasonal growth; this is of great importance in the context of global climate change and targeting environments.

The serendipitous presence of lodging in the doubled haploid population Buster x Charger in 2014, is the focus of Chapter 5. Reynolds et al. (2011a) stated that “any comprehensive strategy to improve wheat yield potential should include lodging resistance”.

Initially a screening of three genotypes totally lodged and three genotypes unaffected by lodging, showed a trend that pointed towards root architecture and its anchorage properties. However, further analysis showed that the trait most related to lodging was plant height and spike area. A major QTL was found on chromosome 2D, in a similar location to the Rht8 gene.

6.2. General outlook

Increasing yield potential is not a trivial task. It has been stated that it is of great importance to further understand the physiological and genetic control of how the developmental phenology affects spike fertility and therefore grain number (Reynolds et al. 2009). The work described in this thesis compiles the first steps towards achieving a better understanding of the relationship between length of phenological phases and grain number. The expected culmination of this line of research is the development of useful markers that provide enhanced control over the crop development. This has the potential to translate into an optimization of the time that the plant invests in producing and developing florets, supplying the increases in sink strength needed in modern cultivars.

The time constraints of one crop cycle per year and the time spent in detailed phenotyping, translate into a work that successfully achieved (i)the physiological understanding of the relationship between phenological phases and spike fertility, and (ii)the chromosomal location of the developmental phases that determine the stem

elongation phase, as well as determining the additive effect provided by each of these regions.

Future work under the scope of this research should focus on narrowing the QTL with the addition of markers in the area of interest, and then the development of Near Isogenic Lines (NILs) to test the QTLs found, leading to the development of markers that can be used by breeders.

Field phenotyping is labour-intensive and costly. In 2011 during the 1st Wheat Yield Consortium meeting a consensus of opinion was that the bottleneck in the research was cost effective fast genotyping. Interestingly by the end my postgraduate studies the story has flipped, the limiting factor is becoming the phenotyping. When the results are to come from the interflow of phenotypic and genotypic data, it is of major importance that both data outputs keep up with the pace.

When I first started my studies, the John Innes Centre has just acquired the tools to carry genotypic analysis using KASPar; a competitive allele-specific polymerase chain reaction. This new genotyping tool simplifies the process as it is a system based on fluorescence that requires no post-reaction electrophoresis step. Large amounts of data can be quickly generated and collected digitally. KASP has now been superseded by SNP wheat arrays made by various companies like Axiom. Fast affordable generation of data is now available to all.

Also during my studies, improvements in next-generation sequencing provided the tools needed for the identification of SNPs, that together with Illumina's iSelect array, produce a genotypic tool that increased ~180x the genetic map that I was using on early stages of my studies for QTL mapping.

Phenotypic advances are starting to appear, with platforms for ultra-high-throughput plant phenotyping like the ones develop by LemnaTec and Phenospex. As with every new technology, prices are high and therefore the adoption of phenotyping platforms has a slow start. During my studies, and not as a part of it, a colleague (Ricardo H. Ramirez-Gonzalez) and I developed a more affordable tool to acquire ~100 height measurements per second, using 2 ultrasonic sensors (£1 each) attached to an Arduino UNO microcontroller system (£4) and a Bluetooth module (£4) to send the raw data, and the processed mean, to a mobile phone (https://github.com/homonecloco/arduino-distance). Once in a phone we used a free data capture system (FieldScorer app) to match the measurements with field trial (Figure 46).

Figure 46. Deployment of the height sensor in Churchfarm Farm, UK. Picture taken 12 May 2015.

Future studies of floret development need to address the problem of being a destructive measurement. So far, all studies involving floret development rely on different plants to build the graph with the developmental dynamic. We tried to tackle this problem by looking for new technologies. On June 2013 I travelled to ThorLabs (Luebeck, Germany) to test a promising new product based on Optical Coherence Tomography (OCT) imaging. We hoped that this imaging technique would allow us to determine the floret development in early stages of the spike, but the device was only able to penetrate two folds of a leaf, and did not provide a clear image to determine early stages of development like the terminal spikelet stage.

I recently came across a borescope only .35mm in diameter (Figure 47). It seems possible to insert the thin fibre scope with minimal damage to the plant, therefore allowing us to follow the floret and spike development of the same plant.

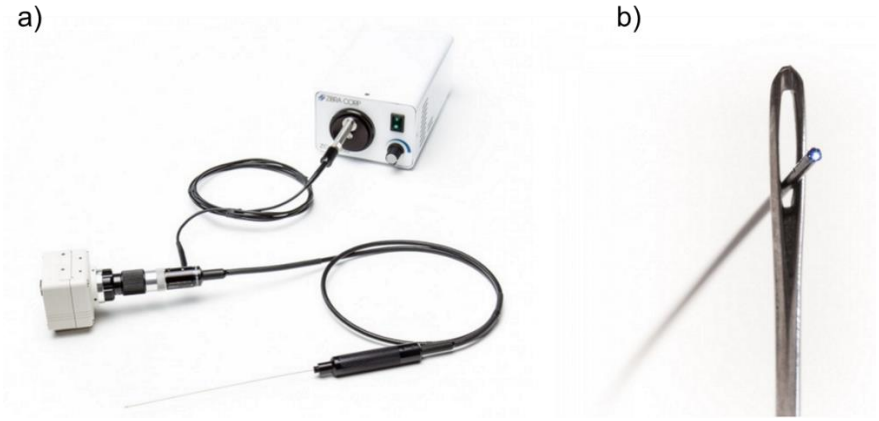

Figure 47. Borescope by Advanced Inspection Technologies (ATI). (a) The device and (b) the .35mm fibre scope.

It would be more informative to follow the development of florets in the same plant throughout the whole crop cycle. Specially analysing the response in abortion rates while undergoing abiotic stress treatments. Where the resilience of number of fertile florets can be precisely determined across cultivars.

7. General conclusions

From this thesis we can draw the conclusion that the stem elongation period is of great importance for grain yield potential. Extending this developmental period allows for an increased number of fertile florets in the spike, which afterwards translates into an increased number of grains.

Chromosomal locations for the phenological stages that delimit the stem elongation period were found. With a locus on 7A for terminal spikelet and two loci on 2D and 4A for ear emergence. These new loci have the potential to provide useful markers to be used in breeding programs under marker assisted selection, where the phenological phases are to be optimized for grain yield potential and for adaptation requirements; in terms of fine-tuning critical phenological phases. The development of NILs with these chromosomal locations are the next step in order to confirm the effect of these loci which due to time limitation were not part of the objectives of this thesis.

On top of the findings for grain yield potential, the basis for further studies on lodging are set. The segregating population of Buster x Charger DH, previously used for its segregation in developmental phases, have also proven useful also for mapping lodging. A locus was found on chromosome 2D affecting the resistance to lodging, total plant height, and spike area. Further studies are required to work within the QTL gap.

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Appendix

A1. List of the 60 genotypes of the CIMCOG panel.

Entry 2010-11	CID	SID	GID	Year of release	Specie	Cross Name	Pedigree	Selection History
45	6831	33	775	1966	T. aestivum	SIETE CERROS T66	PJ62/GB55	II8156-1M-2R-4M-0Y
37	7624	7	2465	1976	T. aestivum	PAVON F 76	VCM//CNO67/7C/3/KAL/BB	CM8399-D-4M-3Y-1M-1Y-1M-0Y-0MEX
44	7691	50	3895	1982	T. aestivum	SERI M 82	KVZ/BUHO//KAL/BB	CM33027-F-15M-500Y-0M-87B-0Y-0MEX
7	7896	254	16122	1988	T. aestivum	BACANORA T 88	JUP/BJY//URES	CM67458-4Y-1M-3Y-1M-5Y-0B-0MEX
1	8890	34	41948	1990	T. aestivum	ATTILA (PBW 343)	ND/VG9144//KAL/BB/3/YACO/4/VEE#5	CM85836-50Y-0M-0Y-3M-0Y
8	8626	465	447647	1992	T. aestivum	BAVIACORA M 92	BOW/NAC//VEE/3/BJY/COC	CM92066-J-0Y-0M-0Y-4M-0Y-0MEX
22	135029	263	3585839	1999	T. aestivum	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*KAUZ	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*KAUZ	CMSS93B01824M-040Y-73Y-010M-010Y-010M-1Y-0M-0KBY
2	313200	45	3686333	2000	T. aestivum	ATTILA*2/PBW65	ATTILA*2/PBW65	CGSS96B00123F-099M-037Y-099M-26Y-0B-0SY
31	325362	63	4313703	2001	T. aestivum	Navojoa M2007	ATTILA/PASTOR	CMSS97Y04045S-040Y-050M-040SY-030M-14SY-010M-0Y-0MEX
29	342454	62	4556647	2002	T. aestivum	MILAN/KAUZ//PRINIA/3/BAV92	MILAN/KAUZ//PRINIA/3/BAV92	CMSS97M02941T-040Y-020Y-030M-040Y-020M-1Y-0M
32	366109	122	4774392	2003	T. aestivum	OASIS/5*BORL95/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI	OASIS/5*BORL95/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI	CMSS98Y04800S-020Y-030M-020Y-040M-31Y-1M-0Y
4	363214	50	4883021	2003	T. aestivum	ATTILA//PGO/SERI/3/PASTOR	ATTILA//PGO/SERI/3/PASTOR	CMSS98Y03455T-040M-0100M-040Y-020M-040SY-21M-0Y-0SY
42	373334	61	4885594	2003	T. aestivum	RL6043/4*NAC//2*PASTOR	RL6043/4*NAC//2*PASTOR	CMSS98M00790M-040Y-0100M-040Y-020M-040SY-15M-0Y-0SY
33	414815	55	4902859	2004	T. aestivum	MISR 1	OASIS/SKAUZ//4*BCN/3/2*PASTOR	CMSS00Y01881T-050M-030Y-030M-030WGY-33M-0Y
20	459206	190	5077000	2005	T. durum	CIRNO C 2008 (durum)	SOOTY_9/RASCON_37//CAMAYO	CGSS02Y00004S-2F1-6Y-0B-1Y-0B
55	448409	32	5325839	2005	T. aestivum	WBLL1*2/KIRITATI (BECARD)	WBLL1*2/KIRITATI	CGSS01B00063T-099Y-099M-099M-099Y-099M-27Y
36	428648	78	5342983	2005	T. aestivum	PASTOR/3/URES/JUN//KAUZ/4/WBLL1	PASTOR/3/URES/JUN//KAUZ/4/WBLL1	CMSA00Y00865T-040M-0P0Y-040M-040SY-030M-6ZTM-0ZTY-0M-0SY
24	428600	39	5343246	2005	T. aestivum	CROC_1/AE.SQUARROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2	CROC_1/AE.SQUARROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2	CMSA00Y00817T-040M-0P0Y-040M-040SY-030M-8ZTM-0ZTY-0M-0SY
17	425852	42	5344432	2005	T. aestivum	CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC/5/PFAU/WEAVER	CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC/5/PFAU/WEAVER	CMSA00M00102S-040P0M-040Y-030M-030ZTM-12ZTY-0M-0SY
39	459355	73	5390612	2005	T. aestivum	SUPER 152	PFAU/SERI.1B//AMAD/3/WAXWING	CGSS02Y00153S-099M-099Y-099M-46Y-0B
13	448418	52	5397958	2005	T. aestivum	BRBT1*2/KIRITATI	BRBT1*2/KIRITATI	CGSS01B00072T-099Y-099M-099M-099Y-099M-30Y-0B
11	448409	101	5398160	2005	T. aestivum	BECARD	WBLL1*2/KIRITATI	CGSS01B00063T-099Y-099M-099M-099Y-099M-9Y-0B
30	448400	62	5398530	2006	T. aestivum	MUNAL #1	WAXWING*2/KIRITATI	CGSS01B00054T-099Y-099M-099M-099Y-099M-13Y-0B
18	426041	54	5422787	2006	T. aestivum	CHWL86/6/FILIN/IRENA/5/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER	CHWL86/6/FILIN/IRENA/5/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER	CMSA00M00291S-040P0M-040Y-030M-040SY-2M-0Y-0SY

21	435266	116	5423297	2006	T. aestivum	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA(TAUS)/4/OCI/5/PASTOR/6/TEMPORALERA M 87/ROMO96	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/OCI/5/PASTOR/6/TEMPORALERA M 87/ROMO96	CMSA01Y00167S-040P0Y-040M-040SY-040M-15Y-0M-0SY
5	435275	67	5423326	2006	T. aestivum	BABAX/LR42//BABAX/3/ER2000	BABAX/LR42//BABAX/3/ER2000	CMSA01Y00176S-040P0Y-040M-030ZTM-040SY-24M-0Y-0SY
50	437242	76	5423688	2006	T. aestivum	TC870344/GUI//TEMPORALERA M 87/AGR/3/2*WBLL1	TC870344/GUI//TEMPORALERA M 87/AGR/3/2*WBLL1	CMSA01Y00725T-040M-040P0Y-040M-030ZTM-040SY-10M-0Y-0SY
54	473251	30	5435924	2006	T. aestivum	W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1	W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1	PTSS02B00102T-0TOPY-0B-0Y-0B-11Y-0M-0SY
40	477630	14	5546111	2007	T. durum	ARMENT//2*SOOTY_9/RASCON_37/4/CNDO/PRIMADUR//HAI-OU_17/3/SNITAN (durum)	ARMENT//2*SOOTY_9/RASCON_37/4/CNDO/PRIMADUR//HAI-OU_17/3/SNITAN	CDSS02B00643S-0Y-0M-1Y-4M-04Y-0B
59	487192	32	5794687	2007	T. aestivum	WHEAR/SOKOLL	WHEAR/SOKOLL	CMSS04Y00201S-099Y-099ZTM-099Y-099M-11WGY-0B
52	510201	41	5993957	2008	T. aestivum	TRCH/SRTU//KACHU	TRCH/SRTU//KACHU	CGSS05B00189T-099TOPY-099M-099NJ-099NJ-7WGY-0B
51	509138	32	5995410	2008	T. aestivum	TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON/5/KACHU #1	TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON/5/KACHU #1	CMSS05B00160S-099Y-099M-099Y-099ZTM-21WGY-0B
14	509700	20	5995735	2008	T. aestivum	C80.1/3*BATAVIA//2*WBLL1/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	C80.1/3*BATAVIA//2*WBLL1/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	CMSS05B00722S-099Y-099M-099Y-099ZTM-4WGY-0B
6	512111	79	5999777	2008	T. aestivum	BABAX/LR42//BABAX/3/VORB	BABAX/LR42//BABAX/3/VORB	CMSA05M00103S-040ZTM-040ZTY-13ZTM-03Y-0B
47	507064	90	6000921	2008	T. aestivum	SOKOLL//PBW343*2/KUKUNA/3/NAVJ07	SOKOLL//PBW343*2/KUKUNA/3/NAVJ07	CMSA05Y01188T-040M-040ZTP0Y-040ZTM-040SY-17ZTM-01Y-0B
46	507101	61	6000966	2008	T. aestivum	SOKOLL*2/3/BABAX/LR42//BABAX	SOKOLL*2/3/BABAX/LR42//BABAX	CMSA05Y01225T-040M-040ZTP0Y-040ZTM-040SY-12ZTM-01Y-0B
25	506830	99	6001014	2008	T. aestivum	GK ARON/AG SECO 7846//2180/4/2*MILAN/KAUZ//PRINIA/3/BAV92	GK ARON/AG SECO 7846//2180/4/2*MILAN/KAUZ//PRINIA/3/BAV92	CMSA05Y00954T-040M-040ZTP0Y-040ZTM-040SY-12ZTM-01Y-0B
28	473281	85	6056086	2008	T. aestivum	MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN	MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN	PTSS02B00132T-0TOPY-0B-0Y-0B-25Y-0M-0SY-0B-0Y-0Y
10	467519	358	6056196	2008	T. aestivum	BCN/WBLL1	BCN/WBLL1	PTSS02GH00001S-0Y-0B-040M-040Y-9M-0Y-0Y-0Y
9	485002	407	6056245	2008	T. aestivum	BCN/RIALTO	BCN/RIALTO	PTSW02B00137S-31DHB-0GHB-0Y-0Y-0M-0Y-0Y
19	485004	542	6171893	2009	T. aestivum	CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/6/RIALTO	CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/6/RIALTO	PTSW02B00139S-65DHB-0GHB-0Y-0Y-0Y-099Y
12	520227	43	6174886	2009	T. aestivum	BECARD/KACHU	BECARD/5/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	CMSS06B00169S-0Y-099ZTM-099Y-099M-21WGY-0B
41	520698	31	6174949	2009	T. aestivum	QUAIU #3//MILAN/AMSEL	QUAIU #3//MILAN/AMSEL	CMSS06B00640S-0Y-099ZTM-099Y-099M-7WGY-0B
43	520762	43	6175022	2009	T. aestivum	ROLF07*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	ROLF07*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	CMSS06B00704T-099TOPY-099ZTM-099Y-099M-23WGY-0B
49	520765	33	6175024	2009	T. aestivum	TACUPETO F2001/BRAMBLING*2//KACHU	TACUPETO F2001/BRAMBLING*2//KACHU	CMSS06B00707T-099TOPY-099ZTM-099Y-099M-2WGY-0B
60	520820	43	6175172	2009	T. aestivum	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA (498)/5/LINE1073/6/KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ/7/KRONSTAD F2004/8/KAUZ/PASTOR//PBW343	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA (498)/5/LINE1073/6/KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ/7/KRONSTAD F2004/8/KAUZ/PASTOR//PBW343	CMSS06B00762T-099TOPY-099ZTM-099Y-099M-11RGY-0B
3	520844	25	6175208	2009	T. aestivum	ATTILA*2/PBW65*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	ATTILA*2/PBW65*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	CMSS06B00786T-099TOPY-099ZTM-099Y-099M-1RGY-0B

58	516383	29	6176054	2009	T. aestivum	WBLL1*2/TUKURU*2/4/CROC_1/AE.SQUARROSA (205)//BORL95/3/2*MILAN	WBLL1*2/TUKURU*2/4/CROC_1/AE.SQUARROSA (205)//BORL95/3/2*MILAN	CMSS06Y00627T-099TOPM-099Y-099ZTM-099Y-099M-15WGY-0B
53	516615	84	6176178	2009	T. aestivum	UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/MILAN/KAUZ//CHIL/CHUM18/6/UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/MILAN/KAUZ//CHIL/CHUM18/6/UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	CMSS06Y00859T-099TOPM-099Y-099ZTM-099Y-099M-35WGY-0B
57	516689	38	6176346	2009	T. aestivum	WBLL1*2/KURUKU*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	WBLL1*2/KURUKU*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	CMSS06Y00933T-099TOPM-099Y-099ZTM-099Y-099M-1WGY-0B
15	516777	76	6176523	2009	T. aestivum	SAUAL/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/ATTILA/5/SAUAL	SAUAL/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/ATTILA/5/SAUAL	CMSS06Y01021T-099TOPM-099Y-099ZTM-099Y-099M-15WGY-0B
35	520287	53	6177058	2009	T. aestivum	PANDORA//WBLL1*2/BRAMBLING	PANDORA//WBLL1*2/BRAMBLING	CMSS06B00229S-0Y-099ZTM-099Y-099M-12RGY-0B
26	520543	41	6177599	2009	T. aestivum	KINGBIRD #1//INQALAB 91*2/TUKURU	KINGBIRD #1//INQALAB 91*2/TUKURU	CMSS06B00485S-0Y-099ZTM-099NJ-099NJ-6WGY-0B
23	516463	88	6178401	2009	T. aestivum	CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/FH6-1-7	CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/FH6-1-7	CMSS06Y00707T-099TOPM-099Y-099ZTM-099NJ-099NJ-5WGY-0B
16	517040	33	6178783	2009	T. aestivum	SAUAL/WHEAR//SAUAL	SAUAL/WHEAR//SAUAL	CMSS06Y01284T-099TOPM-099Y-099ZTM-099Y-099M-6WGY-0B
48	520758	38	6179128	2009	T. aestivum	TACUPETO F2001/SAUAL//BLOUK #1	TACUPETO F2001/SAUAL//BLOUK #1	CMSS06B00700T-099TOPY-099ZTM-099NJ-099NJ-2WGY-0B
34	521017	12	6179185	2009	T. aestivum	OASIS/SKAUZ//4*BCN/3/2*PASTOR/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/6/SAUAL #1	OASIS/SKAUZ//4*BCN/3/2*PASTOR/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/6/SAUAL #1	CMSS06B00959T-099TOPY-099ZTM-099NJ-099NJ-6WGY-0B
38	516587	41	6179222	2009	T. aestivum	PBW343*2/KUKUNA*2//FRTL/PIFED	PBW343*2/KUKUNA*2//FRTL/PIFED	CMSS06Y00831T-099TOPM-099Y-099ZTM-099NJ-099NJ-5WGY-0B
56	516641	60	6179253	2009	T. aestivum	WBLL1*2/4/BABAX/LR42//BABAX/3/BABAX/LR42//BABAX	WBLL1*2/4/BABAX/LR42//BABAX/3/BABAX/LR42//BABAX	CMSS06Y00885T-099TOPM-099Y-099ZTM-099NJ-099NJ-24WGY-0B
27	521064	14	6179559	2009	T. aestivum	KFA/3/PFAU/WEAVER//BRAMBLING/4/PFAU/WEAVER*2//BRAMBLING	KFA/3/PFAU/WEAVER//BRAMBLING/4/PFAU/WEAVER*2//BRAMBLING	CMSS06B01006T-099TOPY-099ZTM-099Y-099M-1RGY-0B

A2. Meteorological data for field trials season 2010-11 at CENEB. DAE (days after emergence), Month, Date, T Max (maximum temperature), T Min (minimum temperature), Rainfall, Tmean (average between T Max and T Min), Thermal Time (accumulation of Tmean from emergence), Rad Solar (solar radiation).

DAE	Month	Date	T Max (°C)	T Min (°C)	Rainfall (mm)	Tmean °C	Thermal Time (°C day)	Rad Solar (MJ/m^{-2})
1	Dec	15/12/10	26.9	9.4	0	18.2	18.15	15.4
2	Dec	16/12/10	24.6	12.6	0	18.6	36.75	14.9
3	Dec	17/12/10	23.7	10.2	0	17.0	53.7	13.6
4	Dec	18/12/10	26.6	7	0	16.8	70.5	15.2
5	Dec	19/12/10	28.1	7.2	0	17.7	88.15	15.4
6	Dec	20/12/10	26.2	10	0	18.1	106.25	15.1
7	Dec	21/12/10	25.8	10.2	0	18.0	124.25	15.2
8	Dec	22/12/10	23.7	9.5	0	16.6	140.85	8.9
9	Dec	23/12/10	24.5	11.5	0	18.0	158.85	14.7
10	Dec	24/12/10	25.9	8.4	0	17.2	176	15.8
11	Dec	25/12/10	26.6	6.2	0	16.4	192.4	15.6
12	Dec	26/12/10	24.5	8.3	0	16.4	208.8	14.2
13	Dec	27/12/10	22.1	7.5	0	14.8	223.6	11.5
14	Dec	28/12/10	22.6	4.9	0	13.8	237.35	15.7
15	Dec	29/12/10	21.9	7	0	14.5	251.8	13.7
16	Dec	30/12/10	21.2	14	0	17.6	269.4	12.2
17	Dec	31/12/10	19.7	8.8	0.2	14.3	283.65	12.8
18	Jan	01/01/11	21.3	6.4	0	13.9	297.5	14.3
19	Jan	02/01/11	21.1	6.7	0	13.9	311.4	11.1
20	Jan	03/01/11	23.1	4.5	0	13.8	325.2	16.4
21	Jan	04/01/11	24.5	2.9	0	13.7	338.9	15.4
22	Jan	05/01/11	21.7	8.4	0	15.1	353.95	9.2
23	Jan	06/01/11	23.9	11.5	0	17.7	371.65	10.3
24	Jan	07/01/11	25.4	11.6	0	18.5	390.15	14.9
25	Jan	08/01/11	22	6.1	0	14.1	404.2	16.4
26	Jan	09/01/11	23.2	5.2	0	14.2	418.4	16.7
27	Jan	10/01/11	24.5	3.1	0	13.8	432.2	16.9
28	Jan	11/01/11	25.7	2.6	0	14.2	446.35	17.0
29	Jan	12/01/11	25.7	3.2	0	14.5	460.8	17.0
30	Jan	13/01/11	27	5	0	16.0	476.8	17.1
31	Jan	14/01/11	26.5	3.2	0	14.9	491.65	17.3
32	Jan	15/01/11	26.3	3.9	0	15.1	506.75	16.9
33	Jan	16/01/11	26.8	5.8	0	16.3	523.05	16.8
34	Jan	17/01/11	26.3	5.2	0	15.8	538.8	16.8
35	Jan	18/01/11	25.7	7.4	0	16.6	555.35	17.1
36	Jan	19/01/11	24.6	7.2	0	15.9	571.25	14.9
37	Jan	20/01/11	25.2	7.4	0	16.3	587.55	16.8
38	Jan	21/01/11	26.1	6.9	0	16.5	604.05	16.9
39	Jan	22/01/11	25.5	6.9	0	16.2	620.25	17.6
40	Jan	23/01/11	23.2	5.2	0	14.2	634.45	17.5
41	Jan	24/01/11	23.5	4.4	0	14.0	648.4	17.8
42	Jan	25/01/11	24.6	3.8	0	14.2	662.6	18.0

43	Jan	26/01/11	23.4	4.6	0	14.0	676.6	14.8
44	Jan	27/01/11	21.9	4.1	0	13.0	689.6	11.8
45	Jan	28/01/11	24.4	5.5	0	15.0	704.55	17.2
46	Jan	29/01/11	24.5	9.9	0.2	17.2	721.75	17.5
47	Jan	30/01/11	25.7	6.5	0	16.1	737.85	18.9
48	Jan	31/01/11	21.9	5.2	0	13.6	751.4	18.6
49	Feb	01/02/11	22.4	5.9	0	14.2	765.55	19.4
50	Feb	02/02/11	21	4.1	0	12.6	778.1	18.7
51	Feb	03/02/11	11	-2.2	0	4.4	782.5	19.5
52	Feb	04/02/11	20.1	-1.8	0	9.2	791.65	20.0
53	Feb	05/02/11	22.4	-1.2	0	10.6	802.25	19.7
54	Feb	06/02/11	25.3	2.1	0	13.7	815.95	19.6
55	Feb	07/02/11	27.7	3.1	0	15.4	831.35	20.1
56	Feb	08/02/11	25.2	5.4	0	15.3	846.65	19.4
57	Feb	09/02/11	24.7	8.4	0	16.6	863.2	19.7
58	Feb	10/02/11	24.1	4.3	0	14.2	877.4	20.0
59	Feb	11/02/11	24.3	3.9	0	14.1	891.5	20.5
60	Feb	12/02/11	27.8	3.1	0	15.5	906.95	20.6
61	Feb	13/02/11	28.1	4	0	16.1	923	20.6
62	Feb	14/02/11	29.6	6.1	0	17.9	940.85	20.6
63	Feb	15/02/11	30.2	9.9	0	20.1	960.9	20.7
64	Feb	16/02/11	28.9	6.5	0	17.7	978.6	20.6
65	Feb	17/02/11	28.2	8.7	0	18.5	997.05	17.5
66	Feb	18/02/11	29	8	0	18.5	1015.55	20.5
67	Feb	19/02/11	26.2	9.5	0	17.9	1033.4	20.9
68	Feb	20/02/11	24.5	7.8	0	16.2	1049.55	20.7
69	Feb	21/02/11	23.3	4.6	0	14.0	1063.5	20.3
70	Feb	22/02/11	22.7	4.3	0	13.5	1077	21.3
71	Feb	23/02/11	22.4	4.9	0	13.7	1090.65	21.7
72	Feb	24/02/11	23.8	4	0	13.9	1104.55	22.2
73	Feb	25/02/11	26.6	5	0	15.8	1120.35	22.5
74	Feb	26/02/11	23.5	4.8	0	14.2	1134.5	22.6
75	Feb	27/02/11	21.2	7.5	0	14.4	1148.85	22.1
76	Feb	28/02/11	23	6.2	0	14.6	1163.45	21.6
77	Mar	01/03/11	25.7	5.6	0	15.7	1179.1	22.8
78	Mar	02/03/11	28.7	5.5	0	17.1	1196.2	22.8
79	Mar	03/03/11	29.9	9.2	0	19.6	1215.75	22.3
80	Mar	04/03/11	30.3	10	0	20.2	1235.9	22.8
81	Mar	05/03/11	31	6.6	0	18.8	1254.7	23.1
82	Mar	06/03/11	27.7	6.1	0	16.9	1271.6	22.8
83	Mar	07/03/11	26.5	10.1	0	18.3	1289.9	22.9
84	Mar	08/03/11	24.7	8.4	0	16.6	1306.45	20.3
85	Mar	09/03/11	28.9	7.3	0	18.1	1324.55	23.2
86	Mar	10/03/11	31.1	8.3	0	19.7	1344.25	22.9
87	Mar	11/03/11	29.5	6.8	0	18.2	1362.4	23.8
88	Mar	12/03/11	27.4	9	0	18.2	1380.6	22.6
89	Mar	13/03/11	29.9	8.9	0	19.4	1400	24.0

90	Mar	14/03/11	32.4	9.4	0	20.9	1420.9	24.2
91	Mar	15/03/11	30.8	9.4	0	20.1	1441	24.0
92	Mar	16/03/11	29.9	9.1	0	19.5	1460.5	24.4
93	Mar	17/03/11	30.5	8.3	0	19.4	1479.9	24.2
94	Mar	18/03/11	31.6	11.1	0	21.4	1501.25	23.9
95	Mar	19/03/11	31.6	8.9	0	20.3	1521.5	24.9
96	Mar	20/03/11	29.4	8.1	0	18.8	1540.25	24.6
97	Mar	21/03/11	30.3	13.3	0	21.8	1562.05	23.9
98	Mar	22/03/11	26.4	9.6	0	18.0	1580.05	24.7
99	Mar	23/03/11	27.8	6.6	0	17.2	1597.25	25.4
100	Mar	24/03/11	29.4	7.3	0	18.4	1615.6	24.9
101	Mar	25/03/11	28.9	8	0	18.5	1634.05	23.5
102	Mar	26/03/11	28.8	9.4	0	19.1	1653.15	25.7
103	Mar	27/03/11	28.7	6.2	0	17.5	1670.6	25.6
104	Mar	28/03/11	29.9	7.4	0	18.7	1689.25	25.3
105	Mar	29/03/11	29.6	9.2	0	19.4	1708.65	25.3
106	Mar	30/03/11	29.9	9.7	0	19.8	1728.45	40.3
107	Mar	31/03/11	32.3	10.6	0	21.5	1749.9	56.2
108	Apr	01/04/11	33	6.8	0	19.9	1769.8	35.2
109	Apr	02/04/11	30.7	12.6	0	21.7	1791.45	25.7
110	Apr	03/04/11	30.5	10.7	0	20.6	1812.05	25.3
111	Apr	04/04/11	32.6	12.2	0	22.4	1834.45	23.8
112	Apr	05/04/11	33.7	12.1	0	22.9	1857.35	26.2
113	Apr	06/04/11	27.7	12.3	0	20.0	1877.35	21.8
114	Apr	07/04/11	28.7	13.8	0	21.3	1898.6	26.0
115	Apr	08/04/11	26	13.1	0	19.6	1918.15	25.2
116	Apr	09/04/11	24.9	12.7	0	18.8	1936.95	24.4
117	Apr	10/04/11	23.6	9.5	0	16.6	1953.5	27.0
118	Apr	11/04/11	29	7	0	18.0	1971.5	27.0
119	Apr	12/04/11	28.3	9	0	18.7	1990.15	26.6
120	Apr	13/04/11	30.5	7.8	0	19.2	2009.3	25.6
121	Apr	14/04/11	33.2	8.1	0	20.7	2029.95	27.5
122	Apr	15/04/11	32.3	10.9	0	21.6	2051.55	27.4
123	Apr	16/04/11	30.5	11.7	0	21.1	2072.65	27.0
124	Apr	17/04/11	30.6	12.5	0	21.6	2094.2	27.4
125	Apr	18/04/11	30.5	12.6	0	21.6	2115.75	25.5
126	Apr	19/04/11	29.6	10.9	0	20.3	2136	27.7
127	Apr	20/04/11	30.7	9	0	19.9	2155.85	27.9
128	Apr	21/04/11	32	10.7	0	21.4	2177.2	28.0
129	Apr	22/04/11	32.3	11.4	0	21.9	2199.05	28.3
130	Apr	23/04/11	31.1	15.3	0	23.2	2222.25	23.8

A3. Meteorological data for field trials season 2011-12 at CENEB. DAE (days after emergence), Month, Date, T Max (maximum temperature), T Min (minimum temperature), Rainfall, Tmean (average between T Max and T Min), Thermal Time (accumulation of Tmean from emergence), Rad Solar (solar radiation).

DAE	Month	Date	T Max (°C)	T Min (°C)	Rainfall (mm)	Tmean °C	Thermal Time (°C day)	Rad Solar (MJ/m^{-2})
1	Dec	16/12/11	22.1	2.4	0	12.2	12.2	15.8
2	Dec	17/12/11	23.4	8.3	0	15.9	28.1	15.6
3	Dec	18/12/11	21.1	7.9	0	14.5	42.7	12.5
4	Dec	19/12/11	21.2	4.6	0	12.9	55.6	6.8
5	Dec	20/12/11	21.3	7.9	0	14.6	70.2	15.4
6	Dec	21/12/11	20.5	7.2	0	13.9	84.1	10.4
7	Dec	22/12/11	23.7	4.7	0	14.2	98.3	13.4
8	Dec	23/12/11	18.8	4.6	0	11.7	110.0	15.6
9	Dec	24/12/11	22.9	3.2	0	13.1	123.1	16.3
10	Dec	25/12/11	25.5	1.3	0.25	13.4	136.5	16.2
11	Dec	26/12/11	26.1	1.4	0	13.8	150.3	15.8
12	Dec	27/12/11	27.1	2.7	0	14.9	165.2	15.4
13	Dec	28/12/11	27.9	7.9	0	17.9	183.1	15.6
14	Dec	29/12/11	27.8	4.3	0	16.0	199.1	14.9
15	Dec	30/12/11	27.7	3.8	0	15.7	214.8	15.7
16	Dec	31/12/11	28.0	4.0	0	16.0	230.8	15.4
17	Jan	01/01/12	30.4	4.6	0	17.5	248.3	15.5
18	Jan	02/01/12	32.9	6.3	0	19.6	267.8	15.1
19	Jan	03/01/12	31.7	7.2	0	19.4	287.3	15.2
20	Jan	04/01/12	31.5	9.5	0	20.5	307.8	14.9
21	Jan	05/01/12	32.2	9.7	0	20.9	328.7	15.4
22	Jan	06/01/12	27.8	7.4	0	17.6	346.3	15.7
23	Jan	07/01/12	25.5	7.2	0	16.4	362.7	15.3
24	Jan	08/01/12	22.4	6.9	0	14.7	377.3	10.5
25	Jan	09/01/12	24.0	3.9	0	14.0	391.3	10.6
26	Jan	10/01/12	25.4	3.0	0	14.2	405.5	16.0
27	Jan	11/01/12	25.4	3.1	0	14.3	419.8	16.1
28	Jan	12/01/12	24.6	5.6	0	15.1	434.9	14.6
29	Jan	13/01/12	23.8	3.8	0	13.8	448.7	13.5
30	Jan	14/01/12	26.2	6.8	0	16.5	465.2	12.2
31	Jan	15/01/12	29.4	6.3	0	17.8	483.1	13.2
32	Jan	16/01/12	28.8	9.8	0	19.3	502.3	15.3
33	Jan	17/01/12	28.2	9.9	0	19.0	521.3	15.0
34	Jan	18/01/12	27.8	7.1	0	17.4	538.8	15.4
35	Jan	19/01/12	25.5	4.8	0	15.2	553.9	16.4
36	Jan	20/01/12	21.4	6.6	0	14.0	568.0	13.9
37	Jan	21/01/12	26.7	5.0	0	15.9	583.8	9.6
38	Jan	22/01/12	27.1	6.3	0	16.7	600.5	12.6
39	Jan	23/01/12	27.8	4.6	0	16.2	616.7	14.5
40	Jan	24/01/12	23.1	5.2	0	14.2	630.9	17.2
41	Jan	25/01/12	25.1	6.4	0	15.8	646.7	16.8
42	Jan	26/01/12	27.8	3.9	0	15.8	662.5	17.2

43	Jan	27/01/12	27.8	5.4	0	16.6	679.0	17.5
44	Jan	28/01/12	26.3	4.8	0	15.6	694.6	17.4
45	Jan	29/01/12	28.6	6.2	0	17.4	712.0	17.2
46	Jan	30/01/12	28.2	7.4	0	17.8	729.8	17.1
47	Jan	31/01/12	27.9	7.7	0	17.8	747.6	16.9
48	Feb	01/02/12	27.7	7.5	0	17.6	765.2	17.4
49	Feb	02/02/12	27.5	5.6	0	16.5	781.7	17.8
50	Feb	03/02/12	22.6	4.8	0	13.7	795.4	17.5
51	Feb	04/02/12	24.5	6.7	0	15.6	811.1	11.7
52	Feb	05/02/12	25.7	3.0	0	14.4	825.4	16.8
53	Feb	06/02/12	26.5	4.7	0	15.6	841.0	18.5
54	Feb	07/02/12	25.9	7.5	0	16.7	857.8	18.3
55	Feb	08/02/12	17.9	10.7	0	14.3	872.1	17.2
56	Feb	09/02/12	27.4	11.3	3.55	19.3	891.4	5.6
57	Feb	10/02/12	27.9	9.7	0	18.8	910.2	19.0
58	Feb	11/02/12	25.0	10.0	0	17.5	927.7	19.1
59	Feb	12/02/12	22.5	11.0	0	16.8	944.4	19.3
60	Feb	13/02/12	22.0	9.9	0	16.0	960.4	19.4
61	Feb	14/02/12	22.2	8.2	0	15.2	975.6	16.8
62	Feb	15/02/12	23.7	4.8	0.25	14.3	989.9	16.7
63	Feb	16/02/12	18.7	6.5	0	12.6	1002.5	20.2
64	Feb	17/02/12	19.6	7.8	0	13.7	1016.3	14.2
65	Feb	18/02/12	22.7	4.3	9.9	13.5	1029.8	20.8
66	Feb	19/02/12	25.1	5.6	0	15.4	1045.2	20.6
67	Feb	20/02/12	25.4	4.7	0	15.1	1060.2	21.0
68	Feb	21/02/12	25.2	5.4	0	15.3	1075.5	21.5
69	Feb	22/02/12	26.9	4.9	0	15.9	1091.5	21.0
70	Feb	23/02/12	25.9	8.0	0	17.0	1108.5	21.3
71	Feb	24/02/12	25.9	10.0	0	18.0	1126.4	18.4
72	Feb	25/02/12	28.4	8.1	0	18.2	1144.7	20.8
73	Feb	26/02/12	27.7	8.4	0	18.1	1162.7	21.5
74	Feb	27/02/12	21.3	10.8	0	16.1	1178.8	22.0
75	Feb	28/02/12	24.5	9.1	0	16.8	1195.6	16.7
76	Feb	29/02/12	24.5	7.4	0	16.0	1211.6	21.8
77	Mar	01/03/12	26.3	6.5	0	16.4	1228.0	21.0
78	Mar	02/03/12	26.2	6.3	0	16.3	1244.3	22.6
79	Mar	03/03/12	28.9	5.9	0	17.4	1261.7	22.3
80	Mar	04/03/12	27.4	6.2	0	16.8	1278.5	23.1
81	Mar	05/03/12	24.0	7.5	0	15.8	1294.2	23.6
82	Mar	06/03/12	26.5	10.5	0	18.5	1312.8	20.1
83	Mar	07/03/12	25.8	10.3	0	18.1	1330.8	21.8
84	Mar	08/03/12	22.6	6.5	0	14.6	1345.4	23.6
85	Mar	09/03/12	26.0	3.1	0	14.6	1360.0	24.0
86	Mar	10/03/12	26.7	6.3	0	16.5	1376.5	24.0
87	Mar	11/03/12	29.5	6.2	0	17.8	1394.3	23.6
88	Mar	12/03/12	28.9	6.5	0	17.7	1412.0	24.4
89	Mar	13/03/12	29.5	6.7	0	18.1	1430.1	21.4

90	Mar	14/03/12	28.1	8.4	0	18.3	1448.4	20.8
91	Mar	15/03/12	26.6	9.2	0	17.9	1466.3	18.8
92	Mar	16/03/12	32.2	8.7	0	20.4	1486.7	17.8
93	Mar	17/03/12	26.2	9.5	0	17.9	1504.5	23.7
94	Mar	18/03/12	22.3	13.4	0	17.9	1522.4	21.7
95	Mar	19/03/12	20.1	10.0	0	15.1	1537.5	13.7
96	Mar	20/03/12	23.8	10.0	0	16.9	1554.4	20.3
97	Mar	21/03/12	25.6	5.1	0	15.4	1569.7	24.8
98	Mar	22/03/12	26.6	6.8	0	16.7	1586.5	25.8
99	Mar	23/03/12	27.8	7.5	0	17.6	1604.1	25.0
100	Mar	24/03/12	28.6	6.8	0	17.7	1621.8	25.7
101	Mar	25/03/12	25.6	9.2	0	17.4	1639.2	25.6
102	Mar	26/03/12	27.6	9.4	0	18.5	1657.7	23.6
103	Mar	27/03/12	28.8	8.1	0	18.4	1676.1	25.2
104	Mar	28/03/12	29.1	9.2	0	19.1	1695.2	25.6
105	Mar	29/03/12	29.7	10.3	0	20.0	1715.2	25.5
106	Mar	30/03/12	30.5	11.4	0	20.9	1736.1	25.5
107	Mar	31/03/12	31.4	12.3	0	21.8	1758.0	25.6
108	Apr	01/04/12	29.1	12.1	0	20.6	1778.5	25.8
109	Apr	02/04/12	27.2	9.8	0	18.5	1797.0	26.1
110	Apr	03/04/12	28.3	8.3	0	18.3	1815.3	26.2
111	Apr	04/04/12	29.4	7.2	0	18.3	1833.6	26.4
112	Apr	05/04/12	29.2	8.3	0	18.8	1852.3	26.7
113	Apr	06/04/12	31.0	9.9	0	20.4	1872.8	26.6
114	Apr	07/04/12	30.4	10.4	0	20.4	1893.1	26.8
115	Apr	08/04/12	33.3	9.3	0	21.3	1914.4	26.7
116	Apr	09/04/12	31.4	9.9	0	20.6	1935.0	26.5
117	Apr	10/04/12	30.5	10.6	0	20.5	1955.5	26.9
118	Apr	11/04/12	27.3	13.4	0	20.3	1975.9	24.1
119	Apr	12/04/12	28.0	9.3	0	18.6	1994.5	25.8
120	Apr	13/04/12	27.6	9.6	0.25	18.6	2013.0	26.9
121	Apr	14/04/12	25.3	10.3	0	17.8	2030.9	27.0
122	Apr	15/04/12	25.4	7.3	0	16.4	2047.2	26.8
123	Apr	16/04/12	30.1	6.9	0	18.5	2065.7	27.5
124	Apr	17/04/12	32.0	9.4	0	20.7	2086.4	27.5
125	Apr	18/04/12	31.6	11.1	0	21.3	2107.7	27.3
126	Apr	19/04/12	31.5	9.8	0	20.6	2128.3	27.7
127	Apr	20/04/12	32.2	10.7	0	21.4	2149.8	27.8
128	Apr	21/04/12	33.1	12.7	0	22.9	2172.7	27.4
129	Apr	22/04/12	36.2	14.5	0	25.4	2198.0	26.5
130	Apr	23/04/12	36.4	13.3	0	24.9	2222.9	26.3
131	Apr	24/04/12	35.1	13.5	0	24.3	2247.2	27.2
132	Apr	25/04/12	32.3	11.2	0	21.7	2268.9	26.7
133	Apr	26/04/12	33.9	16.7	0	25.3	2294.2	22.5
134	Apr	27/04/12	32.7	14.5	0	23.6	2317.8	15.9
135	Apr	28/04/12	31.6	12.0	0	21.8	2339.6	27.2
136	Apr	29/04/12	29.7	14.7	0	22.2	2361.8	27.7

A4. Meteorological data for field trials season 2012-13 at CENEB. DAE (days after emergence), Month, Date, T Max (maximum temperature), T Min (minimum temperature), Rainfall, Tmean (average between T Max and T Min), Thermal Time (accumulation of Tmean from emergence), Rad Solar (solar radiation).

DAE	Month	Date	T Max (°C)	T Min (°C)	Rainfall (mm)	Tmean °C	Thermal Time (°C day)	Rad Solar (MJ/m^{-2})
1	Dec	02/12/12	29.2	11.5	0	20.4	20.4	14.3
2	Dec	03/12/12	30.1	10.8	0.25	20.5	40.8	14.4
3	Dec	04/12/12	31.3	10.3	0.25	20.8	61.6	14.3
4	Dec	05/12/12	31.2	10.2	0.25	20.7	82.3	14.2
5	Dec	06/12/12	27.3	11.8	0	19.6	101.9	13.4
6	Dec	07/12/12	26.9	14	0.5	20.5	122.3	12.5
7	Dec	08/12/12	25.7	12.4	0.25	19.1	141.4	10.9
8	Dec	09/12/12	25.6	11.8	0.25	18.7	160.1	12.9
9	Dec	10/12/12	25.3	10.8	0	18.1	178.1	11.9
10	Dec	11/12/12	25.4	7.4	0	16.4	194.5	13.7
11	Dec	12/12/12	26.2	6.8	0	16.5	211.0	13.9
12	Dec	13/12/12	23.2	10.6	5.32	16.9	227.9	4.8
13	Dec	14/12/12	21.4	11.3	3.3	16.4	244.3	12.5
14	Dec	15/12/12	20.6	10.4	0	15.5	259.8	8.8
15	Dec	16/12/12	23.4	9.8	0	16.6	276.4	14.7
16	Dec	17/12/12	24.8	9.1	0	17.0	293.3	15.6
17	Dec	18/12/12	25.9	4.7	0	15.3	308.6	15.3
18	Dec	19/12/12	22.9	11.3	0	17.1	325.7	7.1
19	Dec	20/12/12	25.6	11.1	0	18.4	344.1	8.4
20	Dec	21/12/12	24.1	8	0	16.1	360.1	11.1
21	Dec	22/12/12	26.3	10.9	0	18.6	378.7	11.8
22	Dec	23/12/12	22.6	10.9	0	16.8	395.5	7.8
23	Dec	24/12/12	25.5	7.9	0	16.7	412.2	15.1
24	Dec	25/12/12	26.9	9	0	18.0	430.1	15.8
25	Dec	26/12/12	24.7	4.8	0	14.8	444.9	15.6
26	Dec	27/12/12	22.6	6.9	0	14.8	459.6	14.4
27	Dec	28/12/12	22.2	7.1	0	14.7	474.3	14.8
28	Dec	29/12/12	23.8	7.8	0	15.8	490.1	12.5
29	Dec	30/12/12	18.8	9	0	13.9	504.0	4.8
30	Dec	31/12/12	20.8	8.9	0.25	14.9	518.8	14.8
31	Jan	01/01/13	22.5	6.4	0	14.5	533.3	13.4
32	Jan	02/01/13	23.3	4.4	0	13.9	547.1	15.1
33	Jan	03/01/13	21.1	5.9	0	13.5	560.6	13.2
34	Jan	04/01/13	24.2	5.7	0	15.0	575.6	15.8
35	Jan	05/01/13	25.4	5.5	0	15.5	591.0	15.7
36	Jan	06/01/13	25.3	5	0	15.2	606.2	14.5
37	Jan	07/01/13	22.7	5	0	13.9	620.0	15.6
38	Jan	08/01/13	20.9	6.3	0	13.6	633.6	15.2
39	Jan	09/01/13	24	6.6	0	15.3	648.9	15.1
40	Jan	10/01/13	24.4	5.9	0	15.2	664.1	13.9
41	Jan	11/01/13	17.1	7.6	0	12.4	676.4	4.6
42	Jan	12/01/13	17.9	2.3	0	10.1	686.5	15.9
43	Jan	13/01/13	17.3	2.7	0	10.0	696.5	16.1
44	Jan	14/01/13	17.9	1.3	0	9.6	706.1	16.3

45	Jan	15/01/13	17.1	0.3	0	8.7	714.8	16.6
46	Jan	16/01/13	23.2	4.2	0	13.7	728.5	16.9
47	Jan	17/01/13	26.1	0.7	0	13.4	741.9	16.3
48	Jan	18/01/13	25.2	5	0	15.1	757.0	13.2
49	Jan	19/01/13	27.4	7.6	0	17.5	774.5	15.5
50	Jan	20/01/13	28.2	8.7	0	18.5	793.0	16.0
51	Jan	21/01/13	27.7	8.2	0	18.0	810.9	15.9
52	Jan	22/01/13	28.7	8	0	18.4	829.3	16.2
53	Jan	23/01/13	28.4	9.2	0	18.8	848.1	14.5
54	Jan	24/01/13	27.9	10.8	0	19.4	867.4	11.8
55	Jan	25/01/13	30	13.6	0	21.8	889.2	13.9
56	Jan	26/01/13	27.7	15.6	1.26	21.7	910.9	12.3
57	Jan	27/01/13	24.9	10.4	0.25	17.7	928.5	16.7
58	Jan	28/01/13	22.6	8.3	0.25	15.5	944.0	15.0
59	Jan	29/01/13	19.3	6.2	0	12.8	956.7	17.7
60	Jan	30/01/13	22.6	7.7	0	15.2	971.9	16.6
61	Jan	31/01/13	22.3	7.6	0	15.0	986.8	12.6
62	Feb	01/02/13	25.3	4.9	0	15.1	1001.9	15.3
63	Feb	02/02/13	27.1	7.9	0	17.5	1019.4	14.6
64	Feb	03/02/13	22.1	11.2	0	16.7	1036.1	6.8
65	Feb	04/02/13	26.6	8.6	0	17.6	1053.7	13.3
66	Feb	05/02/13	25.6	9	0	17.3	1071.0	15.7
67	Feb	06/02/13	25.8	8.3	0	17.1	1088.0	18.5
68	Feb	07/02/13	26.1	7.5	0	16.8	1104.8	18.9
69	Feb	08/02/13	21.9	6.1	0	14.0	1118.8	11.2
70	Feb	09/02/13	21.7	9.6	0	15.7	1134.5	17.8
71	Feb	10/02/13	22.1	4.3	0	13.2	1147.7	19.6
72	Feb	11/02/13	20.8	3.6	0	12.2	1159.9	15.3
73	Feb	12/02/13	19.3	3.6	0	11.5	1171.3	19.3
74	Feb	13/02/13	22.3	1.5	0	11.9	1183.2	19.3
75	Feb	14/02/13	24.1	2.2	0	13.2	1196.4	19.5
76	Feb	15/02/13	24.4	5.5	0	15.0	1211.3	14.1
77	Feb	16/02/13	29.7	6.2	0	18.0	1229.3	19.5
78	Feb	17/02/13	26.9	5.7	0	16.3	1245.6	18.9
79	Feb	18/02/13	27	7.7	0	17.4	1262.9	19.5
80	Feb	19/02/13	22.3	9.9	0	16.1	1279.0	6.4
81	Feb	20/02/13	20.1	7.9	0.25	14.0	1293.0	17.5
82	Feb	21/02/13	20.7	4.6	0	12.7	1305.7	19.9
83	Feb	22/02/13	22.6	2.5	0	12.6	1318.2	20.0
84	Feb	23/02/13	24.3	3.6	0.25	14.0	1332.2	20.4
85	Feb	24/02/13	22.5	5.8	0	14.2	1346.3	19.4
86	Feb	25/02/13	23.4	4.7	0	14.1	1360.4	19.9
87	Feb	26/02/13	21.2	4.2	0	12.7	1373.1	14.0
88	Feb	27/02/13	25.6	2.5	0	14.1	1387.1	20.0
89	Feb	28/02/13	25.4	4.9	0	15.2	1402.3	19.0
90	Mar	01/03/13	30.4	6.4	0	18.4	1420.7	21.3
91	Mar	02/03/13	31.7	4.7	0	18.2	1438.9	21.3
92	Mar	03/03/13	31.3	5.4	0	18.4	1457.2	21.3
93	Mar	04/03/13	28.2	6.1	0	17.2	1474.4	19.7

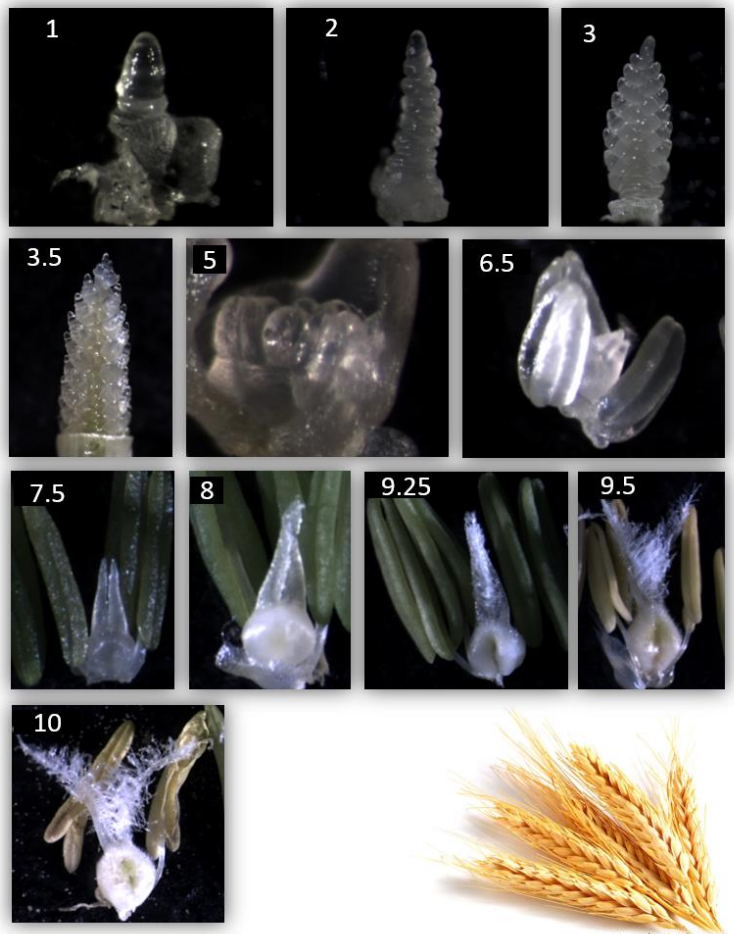
94	Mar	05/03/13	28.3	10.4	0	19.4	1493.7	17.2
95	Mar	06/03/13	28.6	12.3	0	20.5	1514.2	17.2
96	Mar	07/03/13	22.9	10.8	0	16.9	1531.0	8.2
97	Mar	08/03/13	25.9	12.7	0.25	19.3	1550.3	14.7
98	Mar	09/03/13	21.3	8.4	0	14.9	1565.2	21.5
99	Mar	10/03/13	24.1	6.2	0	15.2	1580.3	22.2
100	Mar	11/03/13	27.9	5.1	0.25	16.5	1596.8	21.9
101	Mar	12/03/13	29.8	4.5	0	17.2	1614.0	22.2
102	Mar	13/03/13	31.9	7.4	0	19.7	1633.6	21.9
103	Mar	14/03/13	32.1	8.8	0	20.5	1654.1	21.9
104	Mar	15/03/13	31.4	9.6	0	20.5	1674.6	21.3
105	Mar	16/03/13	29.4	9.7	0	19.6	1694.1	21.7
106	Mar	17/03/13	28.3	12.6	0	20.5	1714.6	21.6
107	Mar	18/03/13	27.8	8.7	0.25	18.3	1732.8	22.0
108	Mar	19/03/13	29.8	8.2	0.25	19.0	1751.8	22.0
109	Mar	20/03/13	32.7	8.6	0	20.7	1772.5	21.6
110	Mar	21/03/13	28.3	11.1	0	19.7	1792.2	19.8
111	Mar	22/03/13	27.1	13.7	0	20.4	1812.6	22.2
112	Mar	23/03/13	25.7	11.7	0.25	18.7	1831.3	19.8
113	Mar	24/03/13	29.4	8	0.25	18.7	1850.0	23.0
114	Mar	25/03/13	30.2	9.8	0	20.0	1870.0	22.0
115	Mar	26/03/13	31.3	8.3	0	19.8	1889.8	22.8
116	Mar	27/03/13	30.2	11.6	0	20.9	1910.7	22.5
117	Mar	28/03/13	30.1	12.9	0	21.5	1932.2	20.7
118	Mar	29/03/13	31.7	13.4	0	22.6	1954.7	23.2
119	Mar	30/03/13	31.8	12.2	0	22.0	1976.7	19.0
120	Mar	31/03/13	32.6	14.3	0	23.5	2000.2	21.0
121	Apr	01/04/13	32.3	11.2	0	21.8	2021.9	23.4
122	Apr	02/04/13	29.6	10.3	0	20.0	2041.9	23.5
123	Apr	03/04/13	30.6	9.9	0	20.3	2062.1	23.6
124	Apr	04/04/13	32	10.6	0	21.3	2083.4	22.2
125	Apr	05/04/13	31.9	13.6	0	22.8	2106.2	23.1
126	Apr	06/04/13	32.1	11	0	21.6	2127.7	23.7
127	Apr	07/04/13	29.4	11.7	0	20.6	2148.3	23.4
128	Apr	08/04/13	27.3	14.3	0	20.8	2169.1	21.8
129	Apr	09/04/13	24.9	12.4	0.25	18.7	2187.7	22.8
130	Apr	10/04/13	28.1	8	0	18.1	2205.8	23.1
131	Apr	11/04/13	30.4	9.4	0	19.9	2225.7	24.1
132	Apr	12/04/13	29	7.1	0	18.1	2243.7	24.1
133	Apr	13/04/13	27.8	12.3	0	20.1	2263.8	23.8
134	Apr	14/04/13	29.6	11.3	0.25	20.5	2284.2	23.8
135	Apr	15/04/13	28.5	11.4	0	20.0	2304.2	23.7
136	Apr	16/04/13	27.9	12.5	0.25	20.2	2324.4	24.7
137	Apr	17/04/13	26.6	13.7	0.25	20.2	2344.5	23.2
138	Apr	18/04/13	29.9	10.2	0	20.1	2364.6	26.2

A5. Poster with the different spike developmental stages, their descriptions and pictures. I created this poster, as a guide for consistent scoring, in early 2011 with photos provided by Dr. Ariel Ferrante.

CIMMYT MR
Wheat Physiology

Spike Development

Developmental score	Description
1.5	Transition apex
2	Early double ridge stage
2.5	Double ridge stage
3	Glume primordium present
3.25	Lemma primordium present
3.5	Floret primordium present
4	Stamen primordium present
4.25	Pistil primordium present
4.5	Carpel primordium present
5	Carpel extending round three sides of ovule
5.5	Stylar canal closing; ovarian cavity enclosed on all sides but still open above
6	Stylar canal remaining as a narrow opening; two short round style primordia present
6.5	Style begin elongating
7	Stigmatic branches just differentiating as swollen cell on styles
7.5	Unicellular hairs just differentiating on ovary wall; stigmatic branches elongating
8	Stigmatic branches and hairs on ovary wall elongating
8.5	Stigmatic branches and hairs on ovary wall continue to elongate; stigmatic branches form a tangled mass
9	Styles and stigmatic branches erect; stigmatic hairs differentiating
9.5	Styles and stigmatic branches spreading outwards. Stigmatic hairs well developed
10	Styles curved outwards and stigmatic branches spread wide; pollen grains on well-developed stigmatic hairs



Waddington *et al.*, 1983

Pictures by: Ariel Ferrante

A6. Publication based on the findings of Chapter 2.

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Dynamics of floret development determining differences in spike fertility in an elite population of wheat

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ABSTRACT

Further increases in wheat yield potential could be achieved through a better understanding of the dynamics of floret primordia generation/degeneration, a process which has received little attention. We quantified genotypic variation among elite genotypes of the CIMCOG panel assembled by CIMMYT for its usefulness for wheat breeding. Ten genotypes, representing the range of variation for yield and its components of the whole panel, were grown under high-yielding conditions in NW Mexico for two growing seasons. The stage of development of floret primordia was determined 2–3 times weekly during stem elongation for apical, central and basal spikelets within the spike. The dynamics of floret initiation/death, and the resulting number of fertile florets, were determined for each spikelet position. We found that the variation in number of fertile florets within this elite germplasm was much more related to the survival of floret primordia than to the maximum number of florets initiated. As the two floret primordia most proximal to the rachis were almost always fertile and most distal florets (florets 6–8) were never fertile, the differences in number of fertile florets were clearly attributed to the differential developmental patterns of intermediate florets (floret primordia 3, 4 and 5, counted from the rachis, depending on the spikelet position). We found significant differences among elite germplasm in dynamics of floret development. Differences in floret survival seemed positively related to those in the length of the period of floret development: the longer the duration of floret development the higher the likelihood of that floret becoming fertile. It is proposed that this type of study may be instrumental for identifying prospective parents for further raising yield potential wheat breeding programmes.

1. Introduction

Due to the increasing global population together with a growing demand for meat and dairy products (implying a growing amount of grains should be used to produce animal food at a low rate of conversion), a substantial increase of grain production in the next decades is critical. This is particularly challenging as the basic manageable resources for crop growth and yield (water, nutrients) will not increase (Connor and Mínguez, 2012) and the land available for crop production is likely to decline (Albajes et al., 2013 and references quoted therein). These challenges together with the need of making future production of crops more sustainable amount to a 'perfect storm' (Godfray et al., 2010; Fischer et al., 2014). Among the major crops, wheat is one of the most critical for warranting human nourishment: it is the most widely crop grown globally and is the primary source of protein for the world population, representing c. 20% of the daily intake for developing countries (Braun et al., 2010). In order to maintain balance between demand and supply alternative ways and means to further raise wheat yield must be found (Chand, 2009). A major way to navigate this 'perfect storm', facing the restrictions mentioned above, is through re-gaining high rates of genetic gains in yield. However, this may not be easily achieved as there is mounting evidence that genetic gains in yield have recently been much lower than what it would be required (Reynolds et al., 2012; Fischer et al., 2014). The likelihood of accelerating breeding progress would increase with knowledge of genetic variation available for traits putatively determining yield (Slafer, 2003; Reynolds and Borlaug, 2006; Reynolds et al., 2009).

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Yield in wheat is generally more related to grain number than to the average weight of the grains (Fischer, 2008, 2011) as the number of grains is far more plastic than the size of the grains (Sadras and Slafer, 2012). Consequently, genetic gains in wheat yield have been more related to improvements in the number than in the size of the grains (e.g. Canevara et al., 1994; Calderini et al., 1995; Sayre et al., 1997; Shearman et al., 2005; Acreche et al., 2008). As even in modern cultivars grain growth seems not strongly limited by the source (Borrás et al., 2004; Pedro et al., 2011), it seems likely that further increases in yield potential may require additional improvements in grain number (Reynolds et al., 2001, 2005; Acreche and Slafer, 2009; González et al., 2014). The identification of potential traits to increase grain number is of great interest to ensure that increased photosynthetic potential is fully utilized by matching it with adequate sink demand (Reynolds et al., 2012; Slafer et al., 2014). To achieve this aim, it would be useful to understand the degree of variation of physiological drivers of grain number within elite lines. Grain number is largely determined during the stem elongation (SE) phase (Fischer, 1985; Slafer and Rawson, 1994). Therefore improvements of traits determined during SE would be required to further increase grain number (Slafer et al., 2005).

Beyond increasing crop growth rate and further improving biomass partitioning before anthesis, it may also be relevant to optimize the developmental attributes to maximize spike fertility (Foulkes et al., 2011; Reynolds et al., 2012). This involves two different aspects of development: [i] the pattern of partitioning of time to anthesis into different phases (Slafer et al., 2001), as lengthening the duration of the SE phase may increase yield (Slafer, 2003; Miralles and Slafer, 2007); and [ii] the dynamics of floret development (Kirby, 1988), as grain number is the consequence of the developmental process of floret generation/degeneration resulting in a certain number of fertile florets (González et al., 2011).

Looking for variation in dynamics of floret development within modern elite cultivars, could contribute to the elucidation of the mechanisms which are most likely to provide opportunities to identify sources for a potential increase in grain number. Floret development in wheat has been long studied (Stockman et al., 1983; Sibony and Pinthus, 1988; Miralles et al., 1998; Wang et al., 2001; González et al., 2003a; Bancal, 2008; Shitsukawa et al., 2009; Dreccer et al., 2014), especially its response to nitrogen applications (Holmes, 1973; Langer and Hanif, 1973; Ferrante et al., 2010). It seems that due to the difficulties involved with the developmental analysis of spike morphogenesis there is an absence of research describing variation for this trait among elite wheat cultivars.

The objective of the present study was to determine the degree of variation within elite germplasm of wheat in patterns of floret development responsible for differences in number of fertile florets, and to further understand the differences in generation of fertile florets among genotypes differing in yield components.

2. Materials and methods

2.1. General conditions

Two field experiments were conducted in the Mexican Phenotyping Platform (MEXPLAT) established at the research station "Centro Experimental Norman E. Borlaug" (CENEB), near Ciudad Obregón, Sonora, Mexico (27°33′ N, 109°09′ W, 38 masl), with conditions that represent the high-yielding environments of wheat worldwide (Braun et al., 2010). The soil is a Chromic Haplotorrert (Vertisol Calcaric Chromic), low in organic matter (<1%), and slightly alkaline (pH = 7.7).

2.2. Treatments and experimental design

Experiments were sown on 06 December 2010 and 09 December 2011, within the optimal sowing period for the winter–spring cycle of cereals in the region. Sowing density was 101.5 and 108.8 kg ha^{-1} respectively, and 200 units of N fertilizer (urea) were applied. Weeds were removed by hand throughout the growing season and diseases and insects prevented by applying recommended fungicides and insecticides at the doses suggested by their manufacturers.

Table 1
Subset selected from the CIMCOG panel. For each entry, the name of the cultivar or cross is indicated, as well as the main trait for which the genotype was selected to be part of the CIMCOG.

Entry	Name	Trait
1	BACANORA T88	High grains/m^2
2	BCN/RIALTO	Late development
3	BRBT1*2/KIRITATI	Large grains
4	CROC_1/AE.SQUARROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2	High floret number
5	ATTILA/PASTOR	High floret number; late development
6	PFAU/SERI.1B//AMAD/3/WAXWING	Early development
7	SERI M 82	Wide adaptation
8	SIETE CERROS T66	Benchmark
9	TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/ RAYON/5/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	Wide adaptation
10	WHEAR/SOKOLL	Wide adaptation

The treatments consisted of the ten wheat genotypes (Table 1), all elite material belonging to the CIMMYT Mexico Core Germplam Panel (CIMCOG) with good agronomic adaptation. The full set of 60 genotypes of the CIMCOG panel are potentially useful in practical breeding programmes aiming to further raising yield potential and for that reason is the main germplasm studied so far by the Wheat Yield Consortium (Reynolds et al., 2011). For this particular study, the number of genotypes had to be restricted to ten because of the detailed measurements required, particularly regarding floret development (see below). However, it is worth noting that the selected genotypes do represent fairly well the whole CIMCOG panel in terms of yield and its major determinants both considering average values as well as range of variation (Table 2).

The experiment was designed in randomized complete blocks with two replicates, where plots were assigned to genotypes. In season 2010–2011 plots were 5 m long and 3.2 m wide, consisting of four raised beds 0.80 m wide, with two rows per bed (0.24 m apart), and in season 2011–2012 plots were 8.5 m long and 1.84 m wide, consisting of two raised beds 0.80 m wide, with two rows per bed (0.24 m apart) (Fig. 1, left panel).

2.3. Measurements and analyses

Plots were inspected periodically and one plant per plot regularly sampled and dissected under binocular microscope (Carl Zeiss, Germany) to detect the timing of initiation of the terminal spikelet in each case. From then on until a week after anthesis, one plant per plot was randomly sampled twice or thrice weekly. The samples were taken to the lab and the apex of the main shoot dissected under binocular microscope. On the dissected juvenile spikes the total number of floret primordia was counted in each of the analysed spikelets. In addition the stage of development of each of the florets within particular spikelets was determined. Together these measurements represent the variability expected in the spikes, in developmental terms (see below). To determine the stage of development of the floret primordia, we followed the scale of Waddington et al. (1983). This scale is based on

Table 2
Comparison of yield and its determinants between the CIMCOG panel and the subset of ten genotypes. Data are the adjusted means from a combined analysis of the wheat genotypes grown during the 2010–2011 and 2012 at CENEB, near Ciudad Obregon, Mexico.

Trait	Average		CIMCOG		Subset	
	CIMCOG	Subset	Range	$LSD_{0.05}$	Range	$LSD_{0.05}$
Yield (Mg ha^{-1})	6.42	6.40	4.99–7.63	0.7	6.13–6.61	0.7
Biomass (Mg ha^{-1})	14.12	13.97	11.73–15.76	1.5	13.23–14.72	1.5
Harvest index	0.46	0.46	0.41–0.52	0.02	0.43–0.49	0.03
Number of grains (m^2)	15,072	16,554	11,626–21,769	1848	13,752–21,950	2639
Number of grains (spike^{-1})	50	50	41–63	8.3	45–56	9.1
Grain weight (mg grain^{-1})	43	39	30–52	3.1	30–45	4.4
Days to anthesis	87	87	78–95	2.5	80–95	1.2

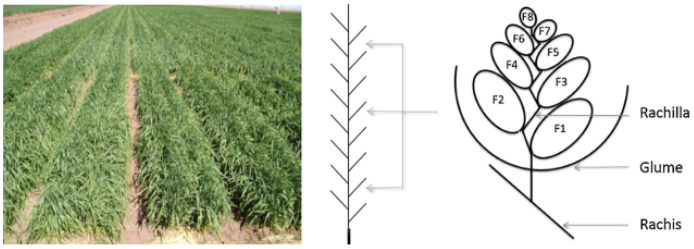

Fig. 1. The 60 CIMCOG lines were grown under raised beds (left panel); and schematic diagram illustrating spikelet positions within the spike as well as the position of florets within the spikelet that were used in this study to characterize floret development in CIMCOG (right panel).

gynoecium development from floret primordia present (W3.5), to styles curved outwards and stigmatic branches spread wide with pollen grains on well-developed stigmatic hairs (W10), which are considered fertile florets (for details see Fig. 1 in Ferrante et al., 2013a).

The analysed spikelets were those on the apical (fourth spikelet from the top of the spike), central (middle spikelet of the spike), and basal (fourth spikelet from the bottom of the spike) positions of the spike (Fig. 1, right panel). Naming of florets within the spikelets followed the same system described by González et al. (2003a); that is, from F1 to the last developed floret depending on their position with respect to the rachis (F1 was the floret most proximal to the rachis and the most distal floret primordia was F6–F8, depending on the specific spikelet and genotype analysed; Fig. 1, right panel).

To analyse the dynamics of development we plotted the developmental score of the particular florets against thermal time (°C d), which was calculated daily assuming, as it is standard, that the mean temperature was the average of the maximum and minimum values and the base temperature was 0 °C for all genotypes and stages of development. Then, for each sampling date we calculated the number of floret primordia which were alive and developing normally; the timing when floret primordia were considered not developing normally any longer was that when the maximum stage of development of a particular floret primordium was reached. Then the number of floret primordia was plotted against thermal time around anthesis for each particular genotype and experiment. For this analysis we considered a primordia to be a floret when it reached at least the stage 3.5 in the scale of Waddington et al. (1983).

The data were subjected to analysis of variance (ANOVA), and the relationships between variables were determined by regression analysis (SAS statistics program, 2002). The adjusted means across the 2 years were obtained by using PROC MIXED procedure of the SAS statistical package (SAS statistics program, 2002). All the effects, years, replications within years, blocks within years and replications, and genotype by year interaction (G × E) were considered as random effects and only the genotypes were considered as fixed effects.

3. Results

There were significant differences in number of fertile florets per spikelet in each of the two experiments, and in addition these differences were reasonably consistent between years, with the unique exception of line 2 (Fig. 2). Line 8 was within the lines exhibiting the highest levels of spike fertility in both experiments, and line 9 was within those exhibiting the lowest values (Fig. 2).

There was significant variation in both components of the number of fertile florets: the maximum number of floret primordia initiated and the proportion of primordia surviving to become fertile florets at anthesis. However, the number of fertile florets was much more strongly related to the survival of floret primordia than to the maximum number florets initiated (Fig. 3).

To further understand the processes involved in the genotypic differences within the CIMCOG panel we studied the dynamics of generation and survival of floret primordia in apical, central and basal spikelets. The general dynamics was similar in all cases (genotypes × spikelet positions): during stem elongation the number of floret primordia firstly increased rapidly, reaching a

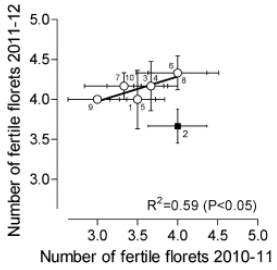

Fig. 2. Fertile florets per spikelet in both experiments for the subset of the CIMCOG panel. Bars on each data-point show the standard error of the mean. Genotypes were labelled as in Table 1. Genotype 2 was the exception, not behaving consistently between the 2 years, and genotypes 8 and 9 were those having respectively the highest and the lowest number of fertile florets per spikelet of the lines analysed consistently between years. Data points of some genotypes are overlapped.

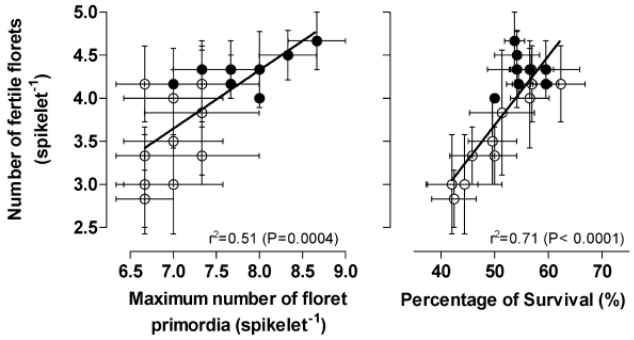

Fig. 3. Number of fertile florets per spikelet related to either the maximum number of floret primordia initiated (left panel) or the percentage of these primordia which developed normally surviving to produce fertile florets at anthesis (right panel). Open circles represent season 2010–11 and closed circles season 2011–12.

peak representing the maximum number of floret primordia and finally decreased sharply until a certain number of fertile florets is established as the balance of the generation and degeneration process (Table A.1). Cultivars varied in the dynamics of generation/degeneration of floret primordia determining the number of fertile florets per spikelet at different spikelet positions (Fig. A.1). To illustrate these genotypic differences we compared this dynamics of floret generation/degeneration for the apical, central and basal spikelets of the two genotypes exhibiting the extreme cases of floret fertility (Fig. 2): lines 8 and 9 representing high and low spike fertilities, respectively. Both genotypes had a similar maximum number of floret primordia initiated in the apical and central spikelets, whilst genotype 9 had a slightly lower maximum number of florets initiated in the basal spikelets than genotype 8 (Fig. 4). On the other hand, in all spikelets the decrease in number of floret primordia (floret mortality) was more noticeable in genotype 9 than in 8 (Fig. 4). Interestingly it seemed that in all spikelet positions genotype 9 reached the maximum number of floret primordia closer to anthesis than genotype 8, implying that the time for floret survival was consistently shorter in the genotype with lowest final number of fertile florets at anthesis (Fig. 4).

When analysing the development of the individual florets it was clear that florets 1 and 2 developed normally and always reached the stage of fertile florets: in all spikelets and all genotypes (Fig. A.2). Thus, none of the differences between genotypes in spike fertility were related to the fate of the two most proximal florets. Similarly, none of the genotypic differences in spike fertility were related to the fate of florets 6, 7 and 8; as none of these florets developed normally to reach the stage of fertile florets ever (Fig. A.3). Therefore, genotypic differences in the developmental patterns of intermediate florets (3, 4 and 5) were critical for establishing the genotypic variation in spike fertility. Focusing on these particular florets it became clear that:

(i) floret 3 developed normally, achieving the stage of fertile florets, in the two genotypes and in all the spikelets: even when the difference in spike fertility was not due to the fate of floret 3, a difference in developmental rates was noticeable: it seemed that floret 3 in genotype 9 developed with some delay compared to that in genotype 8 (Fig. 5, left panels).
(ii) floret 4 in the central spikelets did also develop normally achieving the stage of fertile florets in both genotypes, though again it seemed that this floret started its development in genotype 9 with some delay respect to the timing of development initiation in genotype 8 (Fig. 5, central panel).
(iii) floret 4 in the basal and apical spikelets developed normally to become fertile only in genotype 8 (in the apical spikelets only in some of the plants analysed) but was never fertile in apical and basal spikelets of genotype 9 (Fig. 5, top and bottom of the central panels).
(iv) floret 5 was never fertile in the apical spikelets of any of the two genotypes (Fig. 5, top-right panel), while in the central and basal spikelets it was fertile in some of the plants of genotype 8 and in none of the plants of genotype 9 (Fig. 5, central- and bottom-right panels).

Even in the case of the floret × spikelet positions in which primordia did not continue developing normally to achieve the stage of fertile florets, there was a clear trend, though with few

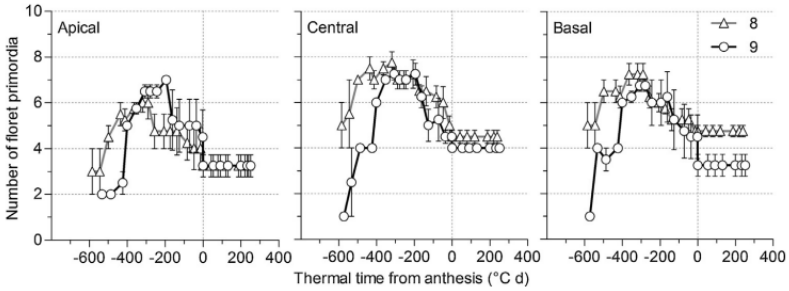

Fig. 4. Dynamics of the number of living floret primordia (those developing normally at the time of measurement) from the onset of stem elongation onwards, plotted against thermal time from anthesis in genotypes 8 and 9, which consistently had high and low spike fertility, respectively, within the subset analysed from the CIMCOG panel in the apical (left panel), central (middle panel) and basal spikelets (right panel).

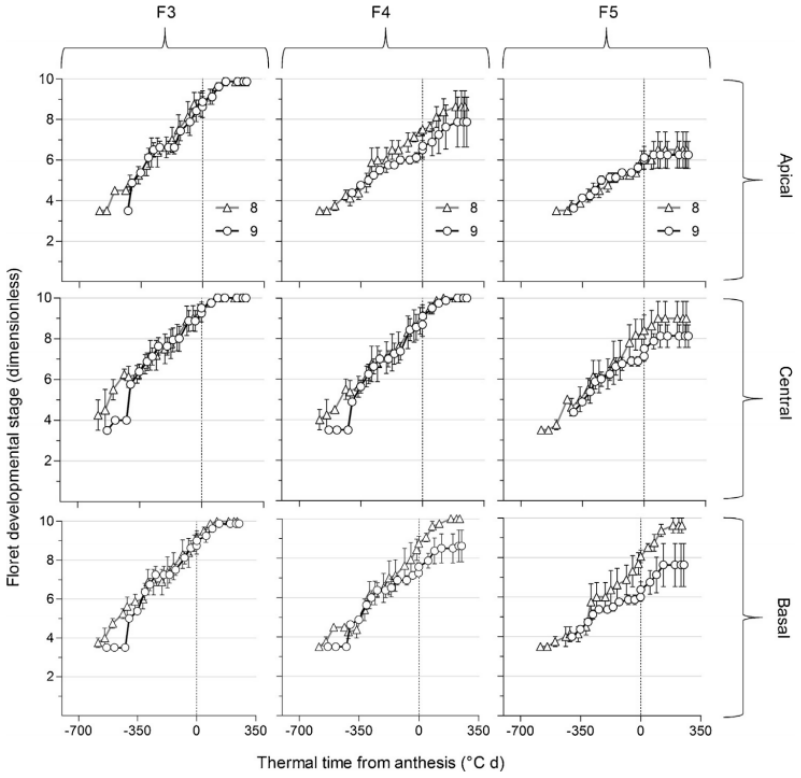

Fig. 5. Developmental progress of floret primordia 3, 4 and 5 (from left to right panels) in apical, central and basal spikelets (from top to bottom panels) from the onset of stem elongation onwards, plotted against thermal time from anthesis in genotypes 8 and 9 of the subset analysed from the CIMCOG panel. The florets are fertile when achieving the stage 10 in the scale developed by Waddington et al. (1983).

exceptions, for the floret primordia of genotype 8 to have developed more than the equivalent florets of genotype 9 (Figs. 5 and A.3).

4. Discussion

Future wheat breeding needs to be extremely efficient as the land allocated to wheat (and most other major food crops) is unlikely to increase significantly, and the use of inputs cannot increase at similar rates as they have in the last half-century (Chand, 2009; Reynolds et al., 2012; Hall and Richards, 2013). Although farm yields may be much lower than yield potential, they seem to be related (Slafer and Araus, 2007; Fischer and Edmeades, 2010) and therefore there is agreement that genetic gains in yield potential will need to be accelerated (Reynolds et al., 2009). To identify opportunities for major improvements in crop photosynthesis is essential (Reynolds et al., 2000; Parry et al., 2011), but will not translate in yield gains without further gains in sink strength, the major determinant of which is grain number. In fact, genotypic differences in yield are most frequently associated with those in grains per m^2 (Slafer et al., 2014) and genetic gains in yield have been mostly explained by improvements in this component (Calderini et al., 1999 and references quoted therein). Further improving grain number would require the identification of variation in its physiological determinants within high-yielding, well adapted populations for breeding. As wheat is a cleistogamous plant, a major determinant of grain number is the number of fertile florets produced. Unfortunately, studies on the dynamics of floret primordia generation/degeneration, which ultimately determines spike fertility, are rather rare, likely because the intrinsic difficulties of determining these dynamics.

Most of the relatively few studies on floret development dynamics were focused on the effects of environmental factors affecting grain number. In these cases, it was consistently revealed that floret survival was more critical than the initiation of primordia for most environmental factors affecting the number of fertile florets at anthesis. Examples of this include cases in which spike growth during pre-anthesis was altered by shading (Fischer and Stockman, 1980), nitrogen availability (Sibony and Pinthus, 1988; Ferrante et al., 2010), photoperiod condition (González et al., 2003b) and combinations of some of these environmental treatments (Langer and Hanif, 1973; Whingwiri and Stern, 1982; González et al., 2003b, 2005). Regarding genotypic variation, which is key for genetically improving a trait, there have been reports only based on the introgression of semi-dwarfing genes. Miralles et al. (1998) reported that Rht1 and Rht2 alleles increased the likelihood of relatively distal floret primordia to successfully progress to the production of fertile florets and attributed this to an improved assimilate allocation of resources to the growing spike before anthesis (Siddique et al., 1989; Slafer and Andrade, 1993). As opportunities to further increase partitioning to the juvenile spike in respect of most modern cultivars are restricted, variation in floret development and spike fertility within elite germplasm must be identified. In the present study we reported variation in the dynamics of floret primordia in a panel assembled for its potential relevance for breeding to further raise yield potential. The genotypic variation in maximum number of florets initiated was marginal whereas variation

in floret primordia survival was found to be the main determinant of the genotypic variation in the number of fertile florets at anthesis. The fact that final number of fertile florets was related to floret primordia survival and rather independent of the maximum number of florets initiated is in agreement with results reported with a comparison of four modern durum wheats by Ferrante et al. (2010, 2013a). Thus, it seems that the differences between elite genotypes in spike fertility are based on similar processes responsible for differences in spike fertility when plants are grown under contrasting environmental conditions.

The model hypothetically applicable is that wheat (and all other cereals) may produce an excessive number of floret primordia without penalties as it is energetically inexpensive. However, when progressing to later developmental stages, growth of these primordia requires increasing amounts of resources, so the plant adjusts the number of primordia that become fertile florets (Sadras and Slafer, 2012). This adjustment would be quantitatively related with the availability of resources for the growing juvenile spike before anthesis. This is further reinforced by evidence that the triggers for floret primordia death are not purely developmental processes (Ferrante et al., 2013b) but likely resource-driven (González et al., 2011). Bancal (2009) suggested that floret death starts when the first floret of the central position reaches a Waddington scale of 7–8; which in the panel of elite lines analysed is not true for all the cultivars (e.g., the onset of floret death in genotype 7 is when the proximal floret at the central position scores 9.3 in the Waddington scale (Table A.1)).

Much of the differences between the set of genotypes analysed from the CIMCOG panel, in terms of spike fertility, were associated with differences in floret survival that can be traced back to the processes of floret development. Comparing the two extreme genotypes of this study (in terms of fertile florets produced per spikelet), it seemed clear that the cultivar maximizing floret survival has a consistently longer period of floret development. Thus, it seemed possible to speculate that advancing development progress of labile florets increases the likelihood of a floret primordia becoming fertile floret. For instance growing a particular genotype under relatively shorter photoperiods during the period of floret development (and spike growth) before anthesis normally brings about significant increases in floret primordia survival (González et al., 2003b; Serrago et al., 2008). It seems consistent with this that genotypes having slightly longer periods of floret development may increase the number of fertile florets through reducing the proportion of primordia dying, in line with the earlier hypothesis that lengthening the stem elongation phase would bring about increases in the number of grains per m^2 (Slafer et al., 2001).

5. Conclusion

We concluded that within elite wheat germplasm, which could be used directly in breeding programmes, there is variation in developmental dynamics of the florets which are ultimately responsible for differences in spike fertility. Genotypes with more fertile spikes exhibited an improved survival of floret primordia related to a longer period of floret mortality: the longer the period the more time (and resources) will be available for allowing labile primordia to continue developing normally therefore reducing floret mortality. Selecting lines exhibiting this property as prospective parents may help in further raising yield potential in wheat.

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Appendix A. Appendix

See Fig. A.1.
See Fig. A.2.
See Fig. A.3.
See Table A.1.

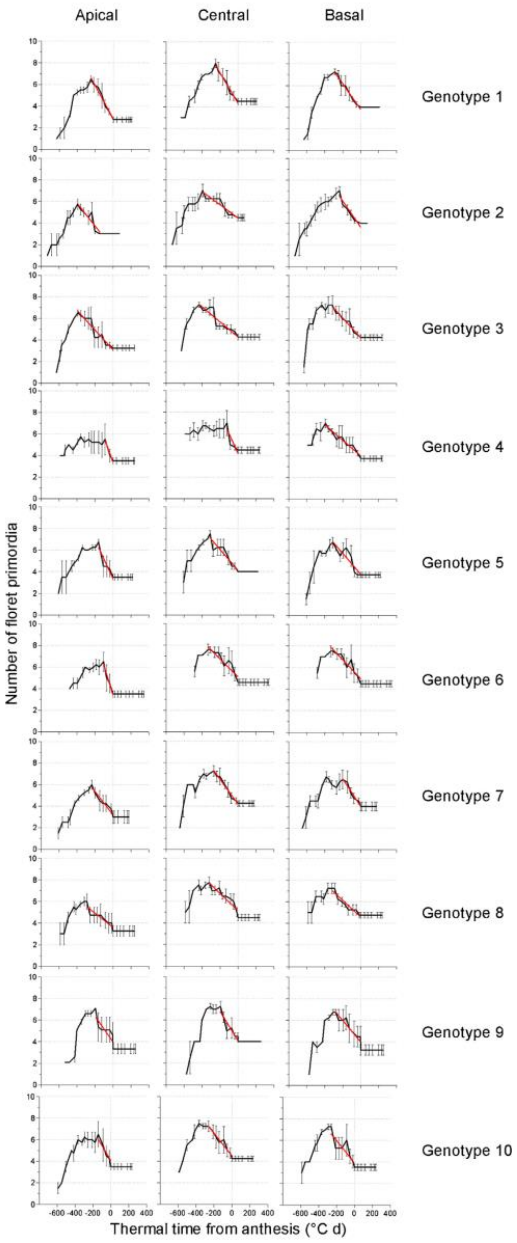

Fig. A.1. Dynamics of the number of living floret primordia from the onset of stem elongation onwards, plotted against thermal time from anthesis, in the apical (left panel), central (middle panel) and basal spikelets (right panel).

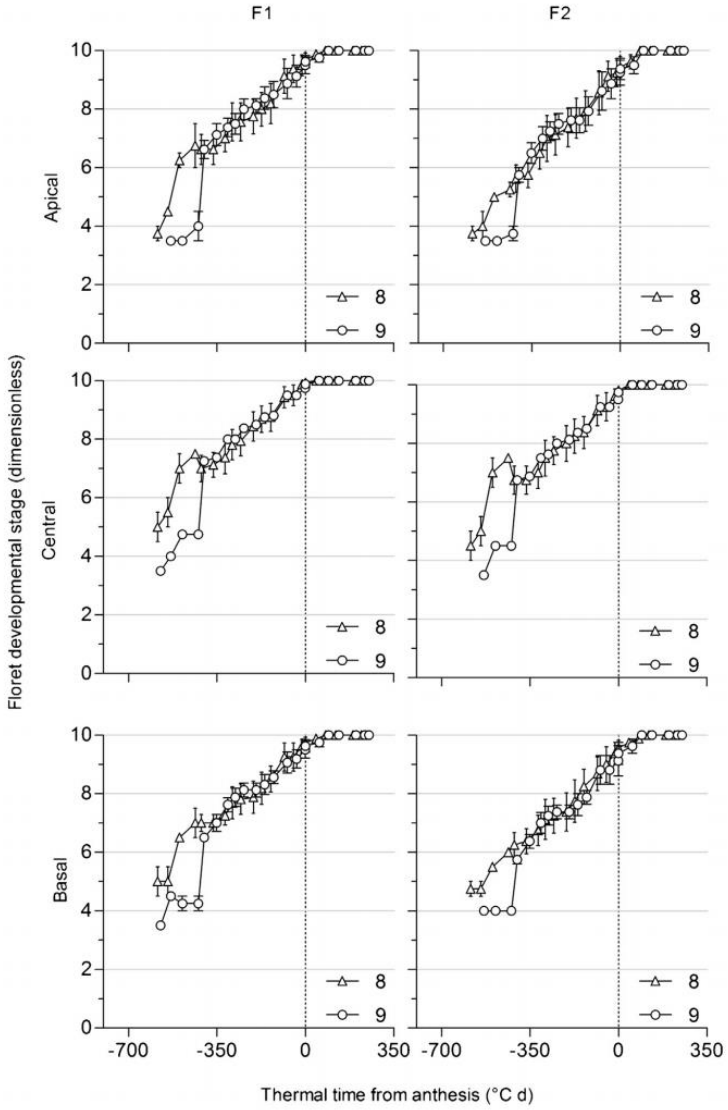

Fig. A.2. Developmental progress of floret primordia 1 and 2 in apical, central and basal spikelets (from top to bottom panels) from the onset of stem elongation onwards, plotted against thermal time from anthesis.

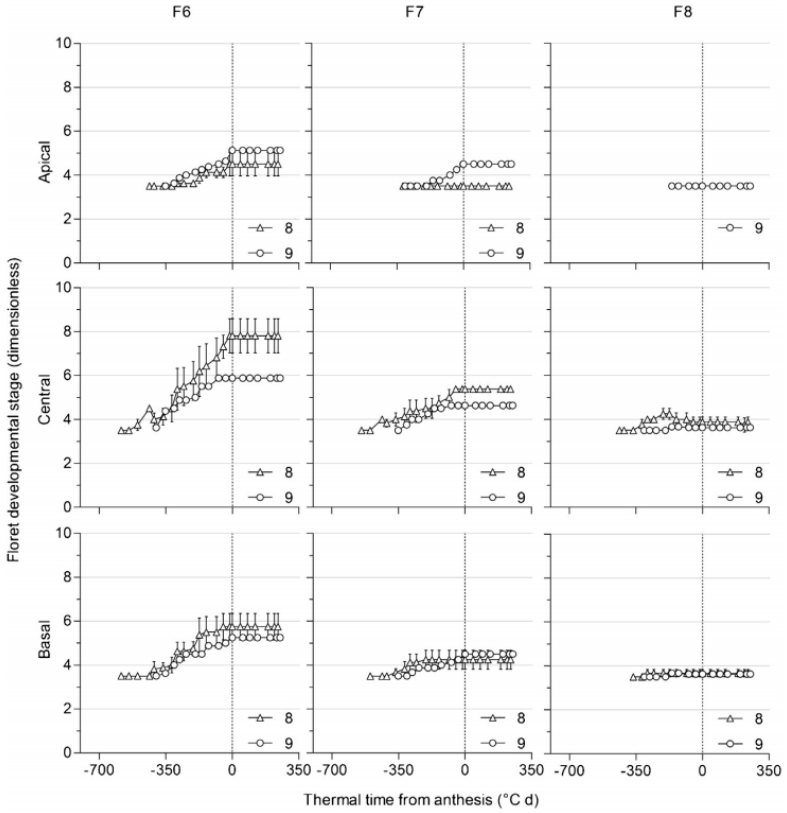

Fig. A.3. Developmental progress of floret primordia 6, 7 and 8 (from left to right panels) in apical, central and basal spikelets (from top to bottom panels) from the onset of stem elongation onwards, plotted against thermal time from anthesis.

Table A.1
Floret mortality rate as a linear model from the maximum number of floret primordia vs. the number of fertile florets for the mean of season 2010–11 and 2011–12.

Position	Entry	Maximum number of floret primordia				Number of fertile florets			Floret mortality rate		
		Floret primordia	TT (before anthesis)	Waddington Scale of F1	SE	Fertile florets	SE	TT (before anthesis)	Primordia °C d^{-1} (×100)	r^2	P
Apical	1	6.5	250	7.6	±0.31	2.75	±0.25	0	−1.536 ± 0.1060	0.977	<0.0001***
	2	5.75	394	5.9	±0.55	3	±0	145.5	−1.055 ± 0.2146	0.829	0.0044**
	3	6.5	391.5	7.2	±0.25	3.25	±0.25	0	−0.911 ± 0.0995	0.903	<0.0001***
	4	5.5	88	9.3	±0.42	3.5	±0.289	0	−2.318 ± 0.3824	0.974	0.1041ns
	5	6.75	159.5	8.3	±0.37	3.5	±0.289	0	−1.802 ± 0.4563	0.839	0.029*
	6	6.5	104.5	8.8	±0.42	3.5	±0.289	0	−2.708 ± 0.4124	0.956	0.0224*
	7	6	236.5	7.5	±0.35	3	±0.577	0	−1.070 ± 0.1264	0.935	0.0004***
	8	6	290	7.6	±0.51	3.25	±0.479	0	−0.652 ± 0.1182	0.813	0.0009***
	9	7	194.5	8.1	±0.23	3.25	±0.479	0	−1.182 ± 0.3567	0.687	0.0211*
	10	6.5	141.5	8.8	±0.37	3.5	±0.25	0	−1.398 ± 0.3334	0.854	0.0247*
Central	1	8	250	8.2	±0.27	4.5	±0.289	0	−1.403 ± 0.0998	0.975	<0.0001***
	2	7	394	7	±0.35	4.5	±0.289	0	−0.614 ± 0.0832	0.872	<0.0001***
	3	7.25	432	7.5	±0.35	4.25	±0.25	0	−0.695 ± 0.0914	0.853	<0.0001***
	4	7	123.5	9.3	±0.27	4.5	±0.289	0	−1.785 ± 0.7602	0.734	0.1433ns
	5	7.5	312	7.7	±0.43	4	±0	0	−0.965 ± 0.1405	0.871	0.0002***
	6	7.5	334	8.3	±0.37	4.5	±0.289	0	−0.802 ± 0.1218	0.844	0.0002***
	7	7.25	269.5	8	±0.32	4.5	±0.25	0	−1.148 ± 0.0730	0.976	<0.0001***
	8	7.75	318.5	7.3	±0.55	4.5	±0.289	0	−0.800 ± 0.1220	0.843	0.0002***
	9	7.25	194.5	8.5	±0.17	4	±0	0	−1.355 ± 0.2463	0.858	0.0027**
	10	7.25	260	8.3	±0.23	4.25	±0.25	0	−1.054 ± 0.1124	0.936	<0.0001***
Basal	1	7.25	288	7.6	±0.31	4	±0	0	−1.182 ± 0.0847	0.970	<0.0001***
	2	7	235.5	7.5	±0.20	4	±0	0	−1.208 ± 0.1999	0.901	0.0038**
	3	7.25	315	8.1	±0.24	4.25	±0.25	0	−0.918 ± 0.0672	0.964	0.0001***
	4	7	391.5	7	±0.61	3.75	±0.25	0	−0.718 ± 0.0587	0.943	<0.0001***
	5	6.75	312	7.2	±0.43	3.75	±0.25	0	−0.969 ± 0.1770	0.811	0.0009***
	6	7.5	334	7.7	±0.25	4.5	±0.289	0	−0.849 ± 0.1226	0.857	0.0001***
	7	6.5	200.5	8.1	±0.27	4	±0.408	0	−1.269 ± 0.1534	0.945	0.0012**
	8	7.25	318.5	7.25	±0.32	4.75	±0.25	0	−0.762 ± 0.0830	0.913	<0.0001***
	9	6.75	278	7.8	±0.31	3.25	±0.479	0	−0.988 ± 0.1540	0.855	0.0004***
	10	7.25	260	8.1	±0.23	3.5	±0.289	0	−1.036 ± 0.2817	0.693	0.0103*

SE = standard error of the mean.
* <0.05.
** <0.01.
*** <0.001.

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Variation in developmental patterns among elite wheat lines and relationships with yield, yield components and spike fertility

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ABSTRACT

Developmental patterns strongly influence spike fertility and grain number, which are primarily determined during the stem elongation period (i.e. time between terminal spikelet phase and anthesis). It has been proposed that the length of the stem elongation phase may, to an extent, affect grain number; thus it would be beneficial to identify genetic variation for the duration of this phase in elite germplasm. Variation in these developmental patterns was studied using 27 elite wheat lines in four experiments across three growing seasons. The results showed that the length of the stem elongation phase was (i) only slightly related to the period from seedling emergence to terminal spikelet, and (ii) more relevant than it for determining time to anthesis. Thus, phenological phases were largely independent and any particular time to anthesis may be reached with different combinations of component phases. Yield components were largely explained by fruiting efficiency of the elite lines used: the relationships were strongly positive and strongly negative with grain number and with grain weight, respectively. Although fruiting efficiency showed a positive trend with the duration of stem elongation that was not significant, a boundary function (which was highly significant) suggests that the length of this phase may impose an upper threshold for fruiting efficiency and grain number, and that maximum values of fruiting efficiency may require a relatively long stem elongation phase.

1. Introduction

A substantial increase in wheat yield potential is required in the coming decades, but rates of genetic gain are currently well below the level required to match the projected cereal demand (Reynolds et al., 2012; Hall and Richards, 2013; Fischer et al., 2014). Quantifying the degree of genetic variation within elite germplasm in traits which may contribute to increased yield potential is critical to the design of strategic crosses (Slafer, 2003; Foulkes et al., 2011; Reynolds et al., 2012).

Yield can be analysed in terms of the number of grains and their average weight. The capacity of the canopy to provide assimilates to fill the grains does not appear to limit grain growth in a wide range of background growing conditions and genotypes (Borrás et al., 2004; Serrago et al., 2013), even within elite high-yielding material (Pedro et al., 2011; González et al., 2014; Sanchez-Bragado et al., 2014). As grain number is strongly source-limited and highly responsive to changes in availability of assimilates (see below), grain number is more plastic than grain weight (Peltonen-Sainio et al., 2007; Sadras, 2007; Sadras and Slafer, 2012) and yield is far more commonly related to grain number than to the average weight of grains (Fischer, 2011; Slafer et al., 2014). Thus to achieve relevant genetic gains in yield potential it is important to identify traits responsible for the determination of grain number (Slafer et al., 2014).

Grain number in wheat is largely determined during the stem elongation phase (Fischer, 1985; Slafer and Rawson, 1994), when the juvenile spikes grow whilst floret developmental processes determine the survival of floret primordia (Kirby, 1988). As wheat is a cleistogamous plant, most fertile florets become grains and

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therefore the process of floret survival and spike growth before anthesis is critical for determining grain number (González et al., 2011a; Ferrante et al., 2013). This underlies the widely reported positive relationship between grain number and spike dry weight at anthesis, first proposed by Fischer (1985) and later validated in a wide range of cases (Slafer et al., 2005 and references quoted therein), irrespective of whether variations are produced by manipulations in growing conditions (Fischer, 1985; Savin and Slafer, 1991; Fischer, 1993; Abbate et al., 1995; Demotes-Mainard and Jeuffroy, 2004; Prystupa et al., 2004; Acreche and Slafer, 2011; Ferrante et al., 2012; Marti and Slafer, 2014) or genetically through altering dry matter partitioning to the spikes (*e.g.* Siddique et al., 1989; Slafer and Andrade 1993; Miralles and Slafer 1995; Flintham et al., 1997; Miralles et al., 1998; Reynolds et al., 2001; Reynolds et al., 2005) during stem elongation.

Therefore, it has been proposed that the duration of the late reproductive phase, from the initiation of terminal spikelet to anthesis (Slafer, 2012), may influence the number of grains produced by the crop. The rationale behind this proposition is that a longer phase when florets are developing may influence the likelihood of floret primordia become fertile florets, and then to set a grain (Miralles and Slafer, 2007). Empirical support to this proposition has been provided through manipulating the duration of the late reproductive phase (through changing photoperiod conditions during stem elongation) producing parallel changes in duration of the late reproductive phase and grain number (Miralles et al., 2000; González et al., 2003, 2005a; Serrago et al., 2008). This in turn may be due to two alternative, non-exclusive mechanisms: a longer period of stem elongation could (i) bring about increases in accumulated growth enhancing the resource availability for the juvenile spike in which florets are developing (the increase in fertility would then be associated with increased spike dry weight at anthesis), or (ii) allow floret primordia which would not, normally, progress to produce a fertile floret a longer period of development and eventually to be able to reach the stage of fertile floret (and then the increase in fertility would be associated with increases in fruiting efficiency; the efficiency with which resources allocated to the spike by anthesis are used to set grains).

As time to anthesis is critical for crop adaptation (Richards, 1991; Worland, 1996; Slafer et al., 2015) modern, high-yielding wheat have a flowering time that has been largely optimised in most regions. Thus, optimizing the developmental pattern through changing the partitioning of developmental time to anthesis into different duration of phases occurring earlier or later than the initiation of the terminal spikelet may contribute to increasing spike fertility (Slafer et al., 2001; Miralles and Slafer, 2007; Foulkes et al., 2011; Reynolds et al., 2012). The ability of breeders to increase the duration of the late reproductive phase depends on genetic variation for this trait.

It has been shown that the duration of the different pre-anthesis phases may be independent (Whitechurch et al., 2007; Borràs et al., 2009; García et al., 2014; González et al., 2014), which is consistent with the fact that different phases vary in sensitivity to vernalisation, photoperiod, and temperature (Slafer and Rawson, 1994, 1995; Slafer and Rawson, 1996; Miralles and Richards, 2000; González et al., 2002). The existence of genetic variation is key to the design of strategic crosses. As breeders combine favourable alleles in order to achieve genetic progress for yield (and other complex traits), they are enthusiastic to consider potential parents from a selected group of genotypes that can be considered elite. CIMMYT has gathered a special population for studying opportunities for improvements in photosynthesis and biomass simultaneously while maintaining high levels of harvest index, namely the CIMMYT Mexico Core Germplasm (CIMCOG). It includes advanced hexaploid wheat lines that have the potential to bring together traits required for producing step changes in yield gains, as well as historical cultivars and high-yielding durum wheats for reference. CIMCOG was the focal panel used by the Wheat Yield Consortium to studying alternatives for further raising yield potential (Reynolds et al., 2011).

The objective of the present study was to determine the degree of variation in patterns of phenological development within the elite germplasm of the CIMCOG population, ascertaining whether the differences were related to traits determining spike fertility within the population.

2. Materials and methods

Four field experiments were conducted at the Mexican Phenotyping Platform (MEXPLAT) established at the research station "Centro Experimental Norman E. Borlaug" (CENEB), near Ciudad Obregon, Sonora, Mexico (27°33′N, 109°09′W, 38 masl), with conditions that represent the high-yield potential wheat mega-environment 1 (Braun et al., 2010). The soil was a Chromic Haplotorret (Vertisol Calcaric Chromic), low in organic matter (<1%), and slightly alkaline (pH = 7.7).

2.1. Plot information

Experiments 1 and 2, differing in the sowing system, were conducted in 2010/11, experiment 3 in 2011/12, and experiment 4 in 2012/13. Plots in experiments 1, 3, and 4 were carried out in raised beds while experiment 2 had flat (conventional) plots, and in all cases plots were large (17.7–30 m^2) and sown within the optimal period in the region and with optimal sowing densities (Table 1).

All plots were grown under optimal conditions: they were fertilised and irrigated to avoid N and water stress, and biotic stresses were prevented or controlled (weeds were removed by hand throughout the growing season and diseases and insects prevented by applying recommended fungicides and insecticides at the doses suggested by their manufacturers).

2.2. Treatments

The treatments analysed in this study consisted of a subset of the CIMCOG panel of 27 genotypes (comprised of 22 elite lines, 4 historic lines, and 1 *T. durum*) that were grown throughout the 4 field experiments. The original CIMCOG panel of 60 genotypes was only grown and measured in experiments 1 and 2. In experiments 3 and 4 the subset of 27 lines were selected to represent fairly the complete panel (based on results of the first two experiments). All four experiments were designed in randomized complete blocks with two replicates on experiment 2 and three replicates on experiments 1, 3, and 4.

2.3. Determination of key phenology stages

Plots were inspected periodically after sowing. Seedling emergence was determined when half of the seedlings in the plot reached the point when the tip of the first leaf emerged from the coleoptile. From then on, one plant per plot (two or three per genotype depending on each experiment) was sampled (once a fortnight at the beginning and then increasing the frequency as the plot was approaching terminal spikelet initiation, around late January, to up to three times a week) and dissected under a binocular microscope (Carl Zeiss, Germany) to record the stage of development of the apex and so determine the timing of initiation of the terminal spikelet with accuracy. Thereafter the plots were regularly inspected to determine the timing of anthesis when half of the spikes of the plot had anthers extruded.

*

Table 1
Description of environment, sowing, field trial setup, and meteorological data for the four experiments. Environment (4 experiments sowed in three years under irrigated conditions), Sowing (date of sowing and seed density), Plot size (long, wide, and setup of the plots), Available water (millimetres of rain throughout the crop cycle), Average temperature (mean daily temperature for the period between emergence to anthesis (E-A), and anthesis to maturity (A-M)), and average daily radiation (mean solar radiation).

Environment		Sowing	Plot size	Available water	Average temperature (°C)		Average daily radiation ($MJ\ m^{-2}\ d^{-1}$)
					E-A	A-M	
Exp.1Raised beds		06 Dec 2010 101 $kg_{seeds}\ ha^{-1}$	5 m long and 4.16 m wide (4 raised beds 0.80 m wide, with 2 rows per bed, 0.24 m apart)	573 mm	14.9	19.7	21.8
Exp.2 flat beds		06 Dec 2010 101 $kg_{seeds}\ ha^{-1}$	5 m long and 6 m wide (8 rows, 0.2 m apart)	573 mm	14.9	19.7	21.8
Exp.3 raised beds		09 Dec 2011 108 $kg_{seeds}\ ha^{-1}$	8.5 m long and 2.08 m wide (3 raised beds 0.80 m wide, with 2 rows per bed, 0.24 m apart)	592 mm	15.2	19.3	21.6
Exp.4 raised beds		25 Nov 2012 110 $kg_{seeds}\ ha^{-1}$	8.5 m long and 2.08 m wide (3 raised beds 0.80 m wide, with 2 rows per bed, 0.24 m apart)	600 mm	15.2	18.4	19.5

2.4. Sampling and determinations

A sample of 0.5 m of two rows was taken seven days after anthesis, in which above-ground biomass was determined dividing it into spikes and the rest of the canopy. In experiment 4, a sub-sample was taken in which all of the immature grains were removed from the spikes so that the non-grain spike dry weight at a week after anthesis could be obtained. The elimination of the weight of the grains is relevant as they may represent a sizeable, and genotypically variable, portion of the spike dry weight at that stage (7d after anthesis). Their inclusion would overestimate the bulk of resources that were available for the set grains(Fischer, 2011 and references therein). With these values we estimated the proportion of grain and non-grain spike dry weight at a week after anthesis for each genotype to estimate the non-grain spike dry weight in all previous experiments. The reported spike dry weight at anthesis is the value of spike dry weight 7 days after anthesis multiplied by each genotypic factor obtained from experiment 4. The rate of grain filling was determined by calculating a linear model for the relationship between time from anthesis to maturity (grain filling period) and grain weight.

At maturity, yield was determined from harvesting the plot (excluding the extreme 50 cm to avoid border effects) using standard protocols (Pask et al., 2012). Before that, 100 fertile culms were sampled, dried, weighed and threshed to allow calculation of yield components.

With the measurements of grain number at maturity and non-grain spike dry weight one week after anthesis we estimated fruiting efficiency; i.e. the efficiency by which dry matter allocated to the spikes at anthesis is used to determine the survival of floret primordia and set grains (Ferrante et al., 2012; García et al., 2014).

2.5. Analyses

Analyses of variance (ANOVA) and of principal components (PCA) were performed using R 3.0.2 (R Development Core Team). PCA was plotted with the ggbiplot package from R. Regression analysis was conducted to establish the correlation between traits, and figures were produced, using GraphPad Prism 5 (2007). For the relationship between fruiting efficiency and duration of the phase from terminal spikelet to anthesis we also fitted a boundary function for establishing an upper threshold (a line edging the upper limit of the data-cloud; Casanova et al., 1999) describing the highest fruiting efficiencies observed over the range of durations of this phase measured; a procedure commonly used to establish upper limits in ecology (e.g. Cade and Noon, 2003) and agronomy (e.g. Sadras and Angus, 2006). To derive the boundary function we subdivided the phase duration data in intervals of 2 days (from 36 to 48 d) and fitted a regression considering the maximum values of fruiting efficiency within each interval.

3. Results

3.1. Representativeness of the subset

The 27 lines selected to represent the CIMCOG population in the 4 studies were shown to be representative of the whole population. The duration from seedling emergence to anthesis and the number of grains per unit land area (the two most integrative traits considered in this study), for the complete CIMCOG panel (60 lines) and the subset of 27 lines studied here show similar variability (Fig. 1).

Although the genotype by environment interaction (GxE) was statistically significant in most of the traits analysed in this study, in all cases the mean squares of GxE were much smaller than those of the genotypes. For instance, the magnitude of the genotypic effects was 19-fold greater than that of the GxE for the number of grains per m^2 (and it was 56-fold greater for the average grain weight). The genotypic effects were also much greater than the GxE interaction for the duration of the two phenological phases considered here, from sowing to terminal spikelet (5-fold greater) and from then to anthesis (4-fold greater). Finally, the two physiological determinants of grain number also showed larger mean squares for genotypes than for the GxE (Table 2). Therefore, even though the GxE interaction was statistically significant the genotypic effects can be reliably considered across environments. For simplicity in most of the Results section of this paper we showed the

Table 2
Means, least significant difference (LSD α=0.05), coefficient of variation (CV), and mean squares of genotype (G) by environment (E) interaction (GxE) for yield components and main phenological traits of subset of 27 lines and four experiments.

Source of variation	Trait						
	Yld	GN	TGW	TS	SE	FE	SDW
Mean squares							
Environment	69632	72364612	106.89	1149.44	1539.8	1925.77	53545
Genotype	17015	50152422	320.18	47.97	64.23	1207.44	9113
GxE interaction	2669	2620653	5.73	8.76	16.87	144.05	6471
Residual	1356	1353401	3.95	2.94	4.71	134	7948
F-values							
Environment	51.35***	53.47***	27.06***	390.97**	326.92***	14.37***	6.74***
Genotype	12.55***	37.06***	81.06***	16.32***	13.64***	9.01***	1.15^{ns}
GxE Interaction	1.97***	1.94***	1.45*	2.98***	3.58***	1.08^{ns}	0.81^{ns}

Yld: grain yield (g m^{-2}), GN grains (m^{-2}), TGW: thousand grain weight (g), TS: days from emergence to terminal spikelet, SE: stem elongation period (days from TS to anthesis), FE: fruiting efficiency (grains g_{spike}^{-1}), SDWa: non-grain spike dry weight at anthesis.
Significance: *** P < 0.001, ** P < 0.01, * P < 0.05, and ns not significant.

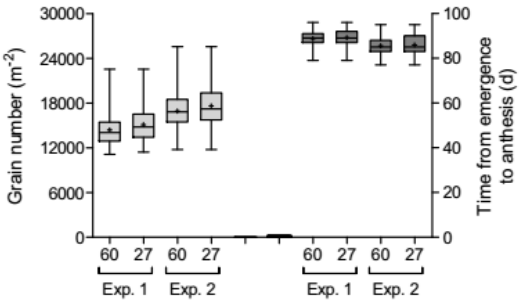

Fig. 1. Boxplot of grain number (left side) and days to anthesis (right side) considering either the complete CIMCOG panel of 60 lines (60) or its subset of 27 lines grown throughout the four experiments (27) for each of the two experiments in which they were grown.

averages across environments for each genotype, but in the last part we offered a principal component analysis which the GxE interaction is unavoidably expressed (and correspondence of conclusions derived from both analyses reinforce the usefulness of genotypic means in this study).

In the rest of this Results section all the analyses will be shown considering both the whole subset of 27 lines representing the whole CIMCOG population as well as restricting the variability to the 22 lines of this subset which are exclusively elite hexaploid lines (disregarding the four historical cultivars and the durum wheat). Therefore, any differences in results from analysing the 27 or the 22 lines would be the influence of the historic lines and/or the tetraploid wheat (*T. durum*) in the overall analysis.

3.2. Phenology

The subset of 27 genotypes analysed throughout this chapter, varied noticeably in time to anthesis (Fig. 2). The variation was not due to the inclusion of the historic cultivars or due to the durum wheat cultivar, it was actually evident within the 22 lines of elite hexaploid wheat as well (Fig. 2a).

Variation in the duration of grain filling was much lower (Fig. 2a), as the time to maturity was strongly correlated with time to anthesis (Fig. 2b). In fact, the relationship between time to maturity and time to anthesis (in both cases from seedling emergence) was extremely high (r^2 = $0.97_{27lines}$ and $0.98_{22lines}$), the slope very close to 1 (0.9 in both cases), and the intercepts (reflecting the overall average duration of grain filling) exhibited little variation ($49.8 \pm 2.6_{27lines}$ days and $49.7 \pm 2.6_{22lines}$ days) (Fig. 2b).

In general, the variation found in phenology and the relationships between the durations of different phases were quite similar (both in terms of ranges explored and in degree of association between phases in the regressions) when analysing the whole subset of 27 lines or restricting it to 22 elite hexaploid lines disregarding the 4 historic cultivars and the *T. durum* (Fig. 3).

Time from seedling emergence to anthesis was also highly correlated with the duration of its two component phases: time from emergence to terminal spikelet (Fig. 3a) and time from terminal spikelet to anthesis (Fig. 3b). Despite the similar relationships, it seemed that the duration of the late reproductive phase was more relevant than that of the period from emergence to terminal spikelet in determining variation in total time to anthesis. This is not only because the coefficients of determination were slightly higher for the relationship with the duration of the late reproductive phase (r^2 = 0.77–0.80) than with the time until terminal spikelet (r^2 = 0.71–0.73), but also because the range of variation in the former (abscissa in Fig. 3b) was noticeably larger than the latter (abscissa in Fig. 3a).

More importantly, the length of either of the two phases constituting time to anthesis showed a level of independence from the other: they were significantly positively related but the proportion of the duration of time to terminal spikelet related to the duration of the late reproductive phase was only c. 25% (Fig. 3c), which indicates that cultivars may combine contrasting durations of these two phases. This shows that even within a restricted range of well adapted elite lines, there may be a large number of possible phenological combinations for reaching the same time to anthesis. For instance, a particular duration of the stem elongation phase (any of the isolines in Fig. 3a) could be combined with different durations of the phase to terminal spikelet and therefore changes in time to anthesis may be achieved by modifying exclusively the duration of phenological phases when leaf and spikelet primordia are being formed. The contrary is also true and a particular duration of the period to terminal spikelet (any of the isolines in Fig. 3b) could be combined with different durations of the late reproductive phase and therefore changes in time to anthesis may be achieved by only modifying the duration of phenological phases when floret primordia are being formed. Or a similar time to anthesis (isolines in Fig. 3c) may well be achieved combining a relatively short phase to terminal spikelet and a relatively long stem elongation phase and *vice-versa* (pairs of genotypes with the same duration to anthesis but differing in how this developmental time was partitioned between phases occurring before or after the initiation of the terminal spikelet, can easily be identified (arrowed data points in Fig. 3c and Fig. 3d).

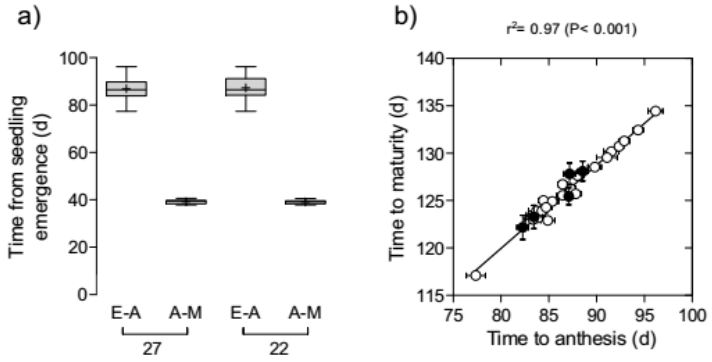

Fig. 2. Boxplot for time from seedling emergence (E) to anthesis (A), and from then to maturity (M) considering the whole subset of 27 lines or restricting the variation to the 22 elite hexaploid lines (i.e. excluding the 4 historical and the *T. durum* cultivars) (a); and relationship between time from seedling emergence to either anthesis or maturity (b). Open circles represent the 22 elite hexaploid lines and closed circles represent the 4 historical and the *T. durum* cultivars.

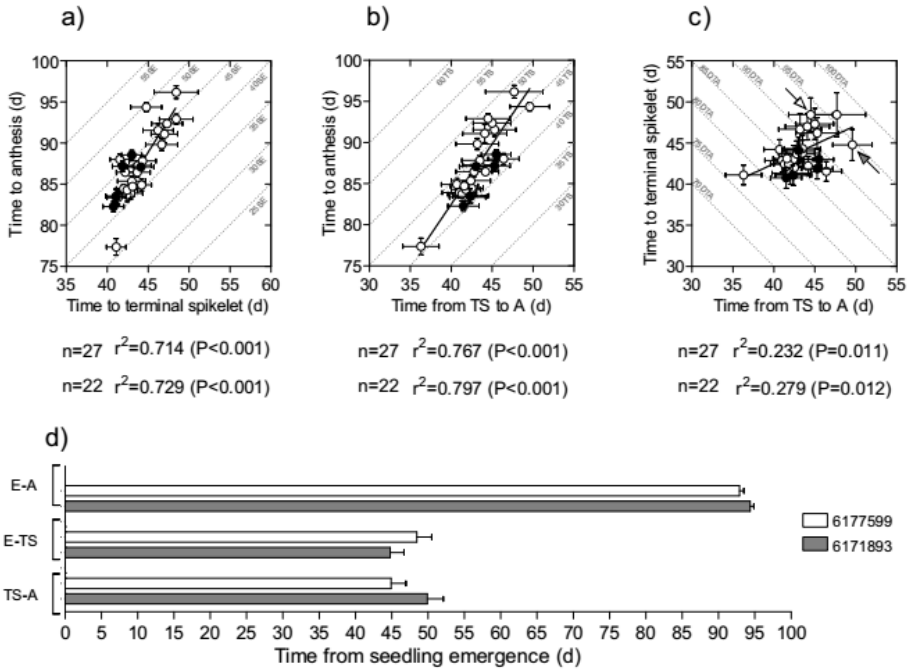

Fig. 3. Relationships between time to anthesis and either time to terminal spikelet (a), or time from then to anthesis (b), and relationship between these two component phases (c). Within each of the panels, isolines for the same duration of complementary phases were drawn. They were (the stem elongation period (SE) in panel a, the time to terminal spikelet (TS) in panel b, and the time to anthesis (DTA) in panel c. Each data-point is the average across the 4 environments and segments stand for the standard error of the means (not seen when smaller than the size of the symbol). Open circles represent the 22 elite hexaploid lines and closed circles represent the 4 historical and the *T. durum* cultivars. The arrows in panel c point the genotypes 6177599 (open arrow head), and 6171893 (closed arrow head) illustrating a pair of genotypes with similar time to anthesis but different developmental partitioning.

3.3. Yield and yield components

Yield showed a range of more than 2 Mg ha^{-1} (from c. 5.5 to almost 8 Mg ha^{-1}) when the whole subset was analysed while it was lowered to c. 1 Mg ha^{-1} when considering only the 22 elite lines (Fig. 4 on ordinates).

The difference between the consideration of the whole subset or only the 22 elite lines was noticeable in the relationships between yield and its components. For the whole subset, yield was completely unrelated to the number of grains per unit land area (Fig. 4a) and significantly related to the average weight of the grains, even though the coefficient of determination was low (Fig. 4b). However, it seems clear that the relationship was strongly dependent on two of the 27 data-points, those exhibiting the highest and the lowest yield, the former also having the highest thousand grain weight and the later having one of the lowest thousand grain weight

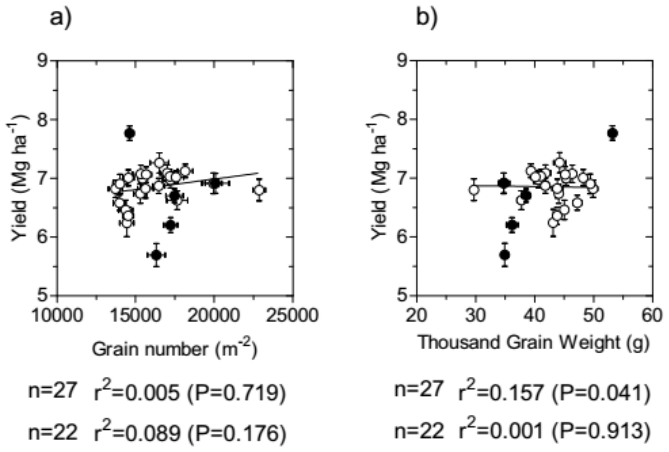

Fig. 4. Relationships between yield and its two components: grains per unit land area (a) and the average weight of grains estimated as thousand grain weight (b). Each data-point is the average across the 4 environments and segments stand for the standard error of the means (not seen when smaller than the size of the symbol). Open circles represent the 22 elite hexaploid lines and closed circles represent the 4 historical and the *T. durum* cultivars.

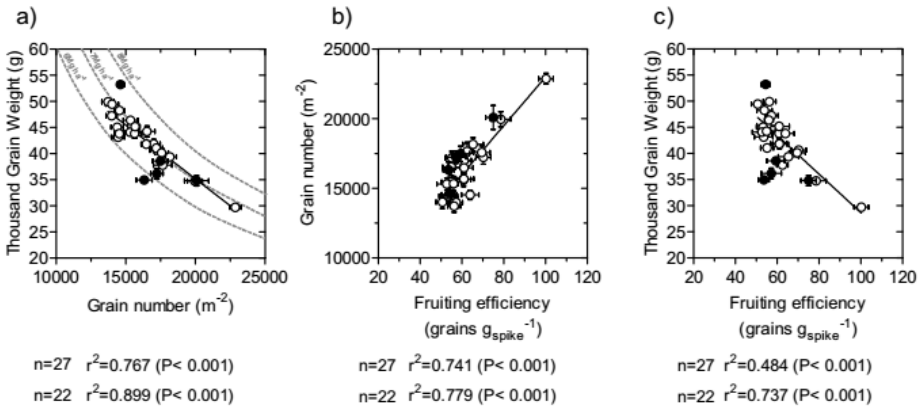

Fig. 5. Relationships between the two major yield components (a) and between each of them (grains per unit land area [b]; average weight of grains estimated as thousand grain weight [c]) and fruiting efficiency. For panel a, isolines for the yields of 6, 7 and 8 Mg ha^{-1} were drawn. Segments stand for the standard error of the means (not seen when smaller than the sizer of the symbol). Open circles represent the 22 elite hexaploid lines and closed circles represent the 4 historical and the *T. durum* cultivars.

(Fig. 4b). As these two cases correspond to the durum wheat line that produced higher yield than the hexaploid wheats and to one of the historic cultivars; when restricting the analysis to the 22 elite lines the relationship between yield and thousand grain weight was completely removed (Fig. 4b) and an incipient linear trend, though not statistically significant, with grain number became apparent. This was mainly because the actual significant relationship was quadratic ($r = 0.527$, $P < 0.01$), implying that within this population of 22 elite hexaploid lines yield tended to increase with increases of grain number until intermediate values of this component and further increases in grain number tended to reduce yield (Fig. 4a). Essentially it could be seen that within the CIMCOG panel yield differences between genotypes were determined by particular combinations of grain number and grain weight of the different genotypes and then yield was not strongly related to any particularly numerical component (Fig. 4). There was a clear negative relationship between these major yield components (Fig. 5a). This negative relationship was stronger when considering the 22 elite lines than when the whole subset was taken into account (Fig. 5a). Due to the quadratic relationship between yield and grain number within the 22 elite lines (Fig. 4c) data-points crossed over the curves representing iso-yields at intermediate values of grain number: if compared with the lines with the lowest number of grains, the cultivars displaying intermediate values had smaller grains but not small enough to compensate for the increase in grain number, while, when genotypes increased grain number further the reduction in grain size was more than compensating the increase in grain number (Fig. 5a).

Fruiting efficiency was the trait most strongly explaining both yield components: the relationship was positive with grain number (Fig. 5b) and negative with grain weight (Fig. 5c), which would be the functional cause of the partial compensation between both yield components (Fig. 5a). The relationships mentioned between yield components and fruiting efficiency held for both the whole

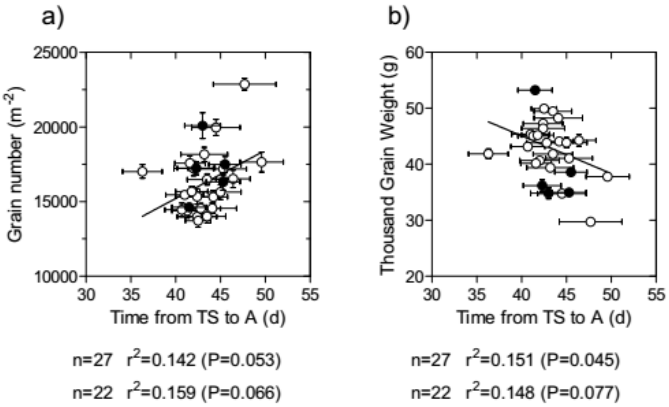

Fig. 6. Relationships between either the number of grains per unit land area (a) or the average weight of grains estimated as thousand grain weight (b) and the duration of the late reproductive phase from terminal spikelet (TS) to anthesis (A). Each data-point is the average across the 4 environments and segments stand for the standard error of the means (not seen when smaller than the sizer of the symbol). Open circles represent the 22 elite hexaploid lines and closed circles represent the 4 historical and the *T. durum* cultivars.

subset of 27 genotypes and for the analysis restricted to the 22 elite hexaploid lines (Fig. 5b,c), but they were stronger when restricting the analysis to the 22 elite hexaploid lines. Although there seemed to be an outlier in which fruiting efficiency was distinctly higher than for the rest of the population, the correlations coefficients would have been still significant if the analysis were made disregarding that particular genotype, particularly so for the analysis restricted to the 22 elite hexaploid lines (as after excluding that genotype of highest fruiting efficiency the correlation coefficients between fruiting efficiency and either grain number [$r = +0.77_{27lines}$ $P < 0.001$ and $+0.77_{22lines}$ $P < 0.001$] or grain weight [$r = -0.59_{27lines}$ $P < 0.001$ and $-0.76_{22lines}$ $P < 0.001$] remained highly significant).

3.4. Duration of phases and yield components

The duration of the late reproductive phase tended to be related positively with the number of grains per unit land area (Fig. 6a) and negatively with the average weight of the grains (Fig. 6b). The relationships were similar when considering the whole subset or only the 22 elite genotypes. But in all cases the relationships were rather weak.

In the case of the relationship between grain weight and duration of the late reproductive phase (Fig. 6b) the fact that the length of the period from terminal spikelet to anthesis was the main determinant of time to anthesis (see above, and Fig. 3) could bring about the interpretation that the longer the late reproductive phase the later the grain filling condition and the smaller the grains. However, this explanation would be hardly plausible as the duration of the period from anthesis to maturity was very similar among all lines (see above and Fig. 2); and differences in thousand grain weight were chiefly determined by differences in the rate of grain filling ($r = 0.99_{27lines}$ $P < 0.001$ and $0.98_{22lines}$ $P < 0.001$).

Regarding the weakness of the relationship between grain number and duration of the late reproductive phase (Fig. 6a), it implies that the main driving force for the genotypic differences in grain number was not the differences in spike dry weight at anthesis (the correlation between grain number and non-grain spike dry weight at 7 days after anthesis was extremely low; $r = -0.09_{27lines}$ $P = 0.62$ and $-0.17_{22lines}$ $P = 0.45$). As the difference in grain number among lines was largely explained by their differences in fruiting efficiency (Fig. 5b) there might be room for a subtle effect of the duration of the late reproductive phase on fruiting efficiency.

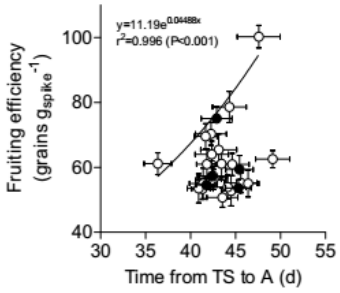

Fig. 7. Relationships between fruiting efficiency and the duration of the late reproductive phase from terminal spikelet (TS) to anthesis (A). The solid line shows the boundary function (the equation and coefficient of determination were also included). Open circles represent the 22 elite hexaploid lines and closed circles represent the 4 historical and the *T. durum* cultivars. Each data-point is the average across the 4 environments and segments stand for the standard error of the means. See Table A.1 for more information in the genotype identification.

Analysing the relationship between fruiting efficiency and the length of the late reproductive phase produced a positive, though not significant, trend (Fig. 7). As the likely effect would be subtle it was not expected to find a highly significant degree of association between them. When analysing the relationship with a boundary function there was a rather strong positive relationship both for the whole subset and for the 22 elite hexaploid genotypes (Fig. 7), implying that the length of the late reproductive phase might set an upper threshold for fruiting efficiency.

3.5. Overall relationships through principal component analysis

The principal component analysis showed a greater variation of the yield determinants than yield itself, providing evidence that current elite material reach high yields by different sets of yield components. The variation across the four experiments is fairly captured, in both the whole 27 genotypes and 22 elite genotypes, by the two dimensions obtained from the analysis. Differences in yield considering the whole subset of 27 genotypes were virtually unrelated to increases in either grain number or grain weight (Fig. 8a).

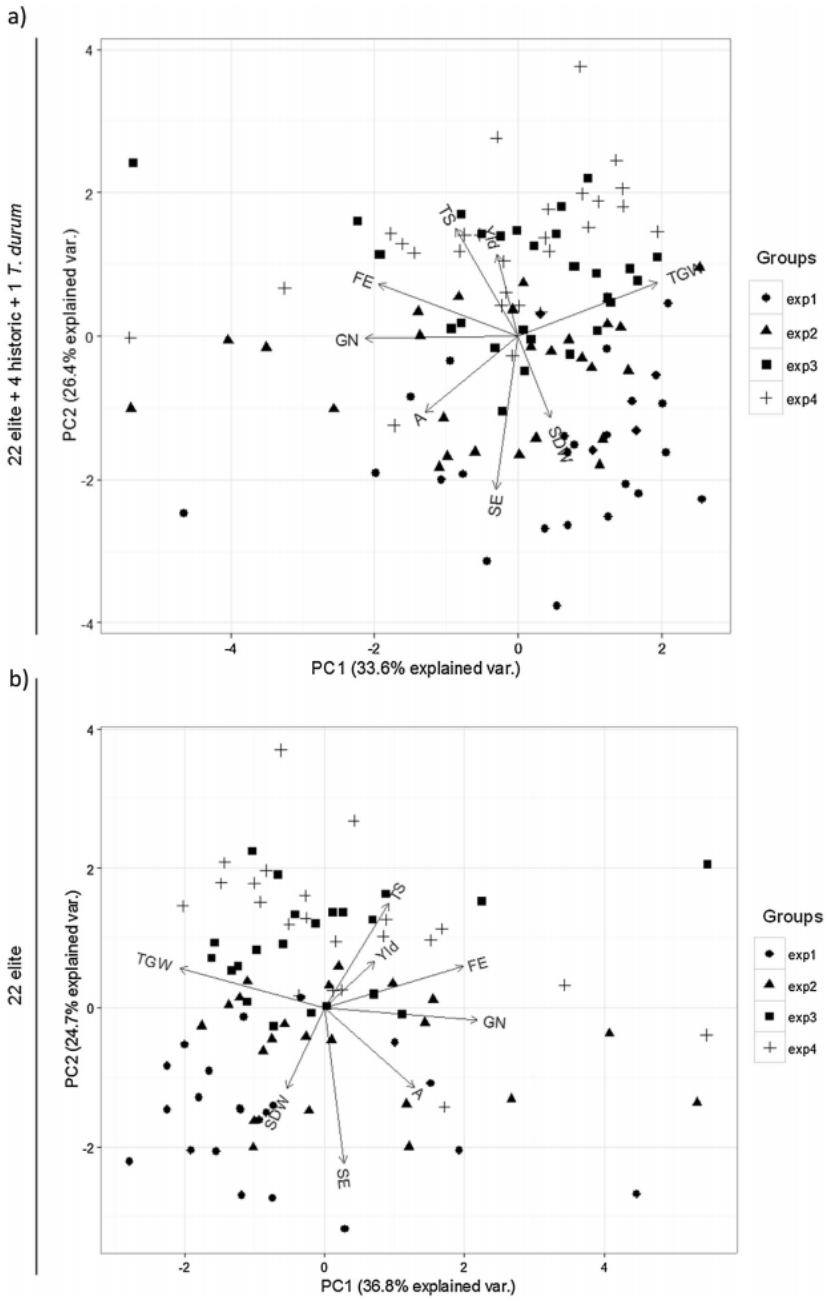

Fig. 8. Biplot of principal components analysis considering the whole subset of the 27 genotypes (a) or only the 22 elite hexaploid genotypes (b) grown across 4 experiments (Table 1). Variables considered were Yld: grain yield, TGW: thousand grain weight, GN: grains per square meter, SDW: non-grain spike dry weight at 7 d after anthesis, TS: days from emergence to terminal spikelet, A: days from emergence to anthesis, SE: stem elongation period (days from TS to A), FE: fruiting efficiency.

On the other hand, when analysing the subset of 22 elite hexaploid genotypes the scenario changes dramatically: yield seemed positively related to grain number per unit land area, while it was negatively related to thousand grain weight (Fig. 8b). Thus, across the G × E interaction for the analysis of the 22 elite hexaploid lines, the highest yielding genotypes were those able to increase grain number, even though there was a partial compensation in the average weight of grains.

In the biplots of the whole subset as well as in that of the 22 elite hexaploid lines there was a clear positive relationship between grain number and fruiting efficiency (and no relationship with spike dry weight at anthesis) and a strong negative relationship between fruiting efficiency and grain weight (Fig. 8a,b). It seemed that the main attribute responsible for major differences in grain number was in turn responsible for the grains set to be smaller.

4. Discussion

Native trait variation is key to further improvements independent of the use of GMO technologies. As breeders pyramid yield genes the most accessible variation is present within elite materials. Although searching for genetic variation in modern elite cultivars might be considered as 'looking for the needle in the haystack' (Able et al., 2007), several studies are far more enthusiastic suggesting that the genetic diversity within elite lines may still provide useful tools towards yield potential (Soleimani et al., 2002; Dreisigacker et al., 2004).

In the present study not only was there variation in the duration of phenological phases but also their durations seemed to be quite independent of each other. This was in agreement with studies carried out with other populations (Halloran and Pennell, 1982; Miralles and Richards, 2000; González et al., 2002; Whitechurch et al., 2007) Even though the CIMCOG is a panel selected mainly of elite material (i.e. well adapted and high-yielding), the wealth of variation within the panel is not surprising given that (i) CIMMYT germplasm is typically highly diverse with pedigrees incorporating landraces and products of interspecific variation including synthetics, and (ii) breeding programs generally do not assess or deliberately select for detailed phenology beyond heading and maturity date. Collectively the results support the idea of fine-tuning the developmental phases as a tool for improving not only adaptation but also yield potential (Slafer et al., 2001; Miralles and Slafer, 2007).

The lack of strong correlations between yield and yield components, imply that among the 27 genotypes, as well as for the 22 elite genotypes, there is more than one way to reach a high yield. Some high yielding genotypes had high grain number (Gonzalez-Navarro et al., 2015) while others have high grain weight (Quintero et al., 2014). Besides this, further improvements must be focused on grain number (Foulkes et al., 2011) as the plasticity of grain number is much larger than that of grain weight (Sadras and Slafer, 2012) and consequently any large increase in yield must require improvements in grain number (Slafer et al., 2014).

An increased stem elongation period could provide further allocation of biomass to the spike (i.e. a greater spike dry weight) at anthesis (Slafer et al., 2001; González et al., 2005b; Miralles and Slafer, 2007; González et al., 2011b). By providing more photoassimilates to the spike through an extended stem elongation period, there could be an improvement in floret primordia survival (Ferrante et al., 2013) consequently increasing the number of fertile florets. However, making crosses for this purpose using the elite lines in the current study might be risky as there was no relationship between the length of the stem elongation phase and spike dry weight at anthesis. This means that lines possessing longer stem elongation phases in this panel may have also possess lower rates of canopy growth and/or lower levels of dry matter partitioning to the juvenile spikes compensating the expected advantage of longer late reproductive phase on spike dry weight at anthesis.

On the other hand, there was a subtle relationship between the duration of the late reproductive phase and fruiting efficiency, which is relevant, as the latter had a strong correlation with grain number. This supports the idea of using fruiting efficiency as an alternative trait to further increase grain yield (Slafer et al., 2015). In part, the relationship was only subtle because of the unexpected variation within the panel on time to anthesis. It would be likely that in another panel–varying less in time to anthesis-differences in duration of stem elongation phase may be more evident. At least this has been proven for individual genotypes when the duration of their stem elongation phase were modified artificially (Stockman et al., 1983; Savin and Slafer, 1991). Even though both fruiting efficiency and grain number had a highly significant negative correlation with grain weight, fruiting efficiency is shown to have a weaker association to grain weight than grain number. Similar results from González et al. (2014) provide some reassurance on using fruiting efficiency as a tool for the potential improvement of grain yield; notwithstanding potential drawbacks (Slafer et al., 2015). However, it is also true that the negative relationship between grain weight and fruiting efficiency may well represent a trade-off. Depending on the nature of the negative relationship, it might make improvements in fruiting efficiency either relevant or of little value to improve yield. Although data available from the present study does not allow us to elaborate further on the likely reason for the negative relationship, results from other studies suggest that the decrease in the average grain weight in high fruiting efficiency cultivars does not imply a constitutive effect on all grains (Ferrante et al., 2015; Elía et al., 2016), but rather evince the likely increased presence of more distal grains of smaller size potential reducing the average weight (Acreche and Slafer, 2006; Ferrante et al., 2015).

The relationship between the duration of stem elongation and fruiting efficiency was analysed with a boundary approach, which has been successfully used in other studies of complex traits (French and Schultz, 1984a; French and Schultz, 1984b; Sadras and Angus, 2006; Hunt and Kirkegaard, 2012a,b; Sadras and McDonald, 2012). Although unfeasible for direct selection in breeding programs due to the complexity of manipulating phenological phases, there is an increased use of marker assisted selection techniques that allow breeding programs to incorporate further improvement from complex traits (e.g. fruiting efficiency) to current elite lines (Dreisigacker et al., 2016). The analysis of the relationship showed that within this set of elite lines, traits other than duration of stem elongation phase were determining fruiting efficiency, but that the maximum possible value of fruiting efficiency would be only achievable with relatively long periods of stem elongation.

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Appendix A.

Table A.1
Genotype identification (GID), year of release, cross name, and pedigree from the subset of 27 CIMCOG genotypes. In bold are the 4 historic lines and 1*T. durum*.

GID	Year	Cross Name	Pedigree
775	1966	SIETE CERROS T66 (historic)	PJ62/GB55
2465	1976	PAVON F 76 (historic)	VCM//CNO67/7C/3/KAL/BB
3895	1982	SERI M 82 (historic)	KVZ/BUHO//KAL/BB
16122	1988	BACANORA T 88 (historic)	JUP/BJY//URES
4556647	2002	MILAN/KAUZ//PRINIA/3/BAV92	MILAN/KAUZ//PRINIA/3/BAV92
5077000	2005	CIRNO C 2008 (*T. durum*)	SOOTY_9/RASCON_37//CAMAYO
6176346	2005	WBLL1*2/KIRITATI (BECARD)	WBLL1*2/KIRITATI
5343246	2005	CROC_1/AE-SQUARROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2	CROC_1/AE-SQUARROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2
5390612	2005	SUPER 152	PFAU/SERI.1B//AMAD/3/WAXWING
5397958	2005	BRBT1*2/KIRITATI	BRBT1*2/KIRITATI
5423688	2006	TC870344/GUI//TEMPORALERA M 87/AGR/3/2*WBLL1	TC870344/GUI//TEMPORALERA M 87/AGR/3/2*WBLL1
5995410	2008	TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON/5/KACHU #1	TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON/5/KACHU #1
5999777	2008	BABAX/LR42//BABAX/3/VORB	BABAX/LR42//BABAX/3/VORB
6000921	2008	SOKOLL//PBW343*2/KUKUNA/3/NAVJ07	SOKOLL//PBW343*2/KUKUNA/3/NAVJ07
6056245	2008	BCN/RIALTO	BCN/RIALTO
6171893	2009	CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/6/RIALTO	CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/6/RIALTO
6174886	2009	BECARD/KACHU	BECARD/5/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES
6175024	2009	TACUPETO F2001/BRAMBLING*2//KACHU	TACUPETO F2001/BRAMBLING*2//KACHU
6175172	2009	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE·SQUARROSA (498)/5/LINE 1073/6/KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ/7/KRONSTAD F2004/8/KAUZ/PASTOR//PBW343	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE·SQUARROSA (498)/5/LINE 1073/6/KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ/7/KRONSTAD F2004/8/KAUZ/PASTOR//PBW343
6176178	2009	UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/MILAN/KAUZ//CHIL/ CHUM18/6/UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/MILAN/KAUZ//CHIL/ CHUM18/6/UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
6176346	2009	WBLL1*2/KURUKU*2/5/REH/HARE//2*BCN/3/ CROC_1/AE·SQUARROSA (213)//PGO/4/HUITES	WBLL1*2/KURUKU*2/5/REH/HARE//2*BCN/3/ CROC_1/AE·SQUARROSA (213)//PGO/4/HUITES
6176523	2009	SAUAL/4/CROC_1/AE·SQUARROSA (205)//KAUZ/3/ATTILA/5/SAUAL	SAUAL/4/CROC_1/AE·SQUARROSA (205)//KAUZ/3/ATTILA/5/SAUAL
6177599	2009	KINGBIRD #1//INQALAB 91*2/TUKURU	KINGBIRD #1//INQALAB 91*2/TUKURU
6178401	2009	CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/FH6-1-7	CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/FH6-1-7
6178783	2009	SAUAL/WHEAR//SAUAL	SAUAL/WHEAR//SAUAL
6179128	2009	TACUPETO F2001/SAUAL//BLOUK #1	TACUPETO F2001/SAUAL//BLOUK #1
6179222	2009	PBW343*2/KUKUNA*2//FRTL/PIFED	PBW343*2/KUKUNA*2//FRTL/PIFED

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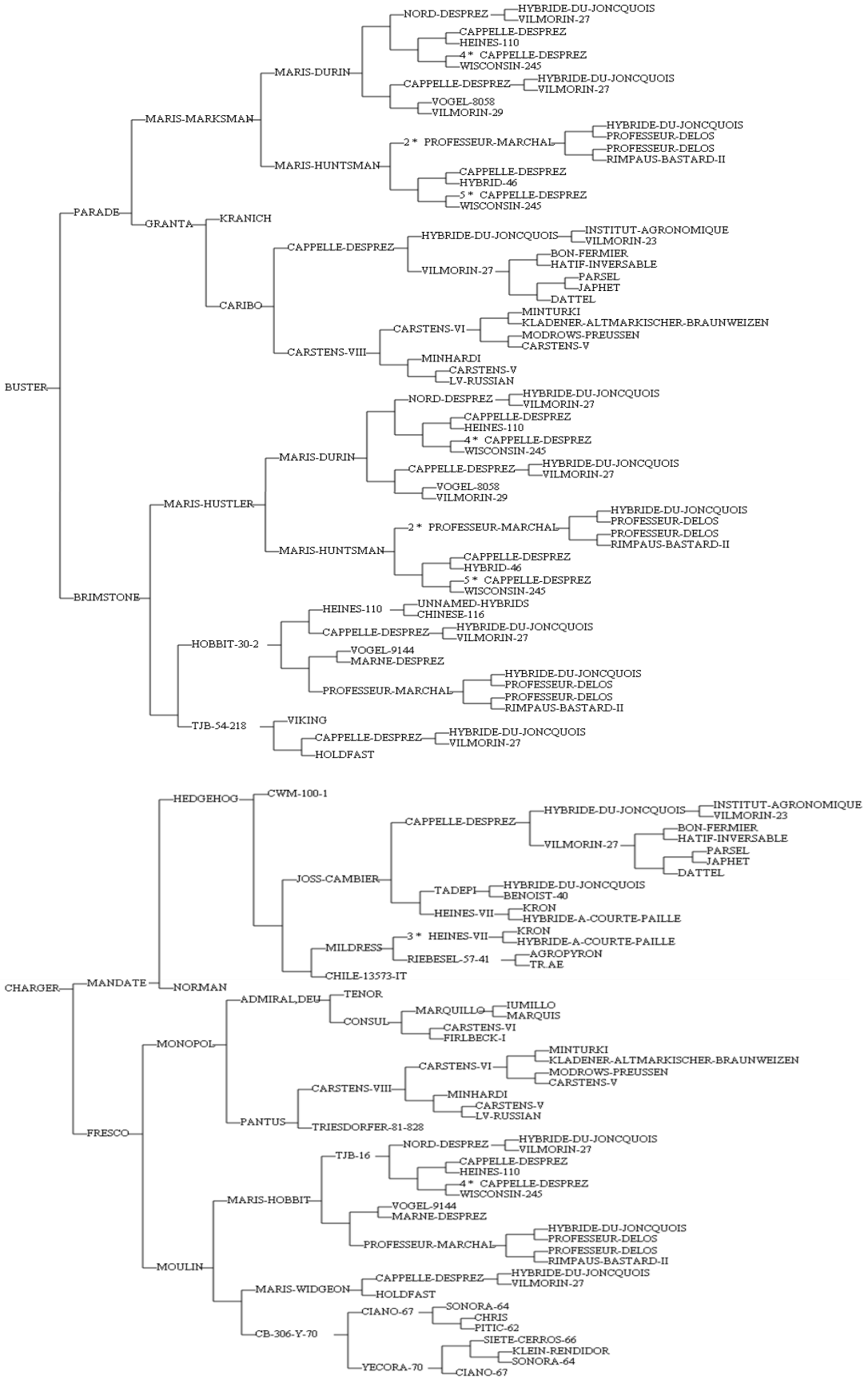
A8. Diagram of the pedigree of Buster and Charger wheat cultivars.



A9. Meteorological data for 2011-2012 season at Churchfarm, UK.

day	date	Tmax	Tmin	Avg	TT
0	06/10/2011	15.1	10.4	12.75	12.75
1	07/10/2011	16	5.8	10.9	23.65
2	08/10/2011	13.1	7	10.05	33.7
3	09/10/2011	19.5	10.4	14.95	48.65
4	10/10/2011	20.5	15	17.75	66.4
5	11/10/2011	19.2	15.3	17.25	83.65
6	12/10/2011	14.4	10.6	12.5	96.15
7	13/10/2011	14.3	10.7	12.5	108.65
8	14/10/2011	15	4.6	9.8	118.45
9	15/10/2011	15.6	-0.3	7.65	126.1
10	16/10/2011	17.4	-1.6	7.9	134
11	17/10/2011	15.5	3.8	9.65	143.65
12	18/10/2011	12.5	5.7	9.1	152.75
13	19/10/2011	11.5	3.5	7.5	160.25
14	20/10/2011	11.4	-0.9	5.25	165.5
15	21/10/2011	15.1	-0.5	7.3	172.8
16	22/10/2011	14	4.1	9.05	181.85
17	23/10/2011	18.4	7.2	12.8	194.65
18	24/10/2011	14.9	10.3	12.6	207.25
19	25/10/2011	14.3	11.3	12.8	220.05
20	26/10/2011	14.4	5.7	10.05	230.1
21	27/10/2011	13.4	6.5	9.95	240.05
22	28/10/2011	14.9	4.7	9.8	249.85
23	29/10/2011	15.5	4.6	10.05	259.9
24	30/10/2011	17.9	11.2	14.55	274.45
25	31/10/2011	16.7	13.4	15.05	289.5
26	01/11/2011	14.8	10.5	12.65	302.15
27	02/11/2011	14	1.6	7.8	309.95
28	03/11/2011	17.2	13.1	15.15	325.1
29	04/11/2011	16.2	12.2	14.2	339.3
30	05/11/2011	14.2	6	10.1	349.4
31	06/11/2011	12.1	11.2	11.65	361.05
32	07/11/2011	12.3	10.7	11.5	372.55
33	08/11/2011	11.5	10.5	11	383.55
34	09/11/2011	13.9	9.2	11.55	395.1
35	10/11/2011	14.8	4	9.4	404.5
36	11/11/2011	10.7	8.8	9.75	414.25
37	12/11/2011	13.7	10.7	12.2	426.45
38	13/11/2011	14.8	9.2	12	438.45
39	14/11/2011	9.7	4.2	6.95	445.4
40	15/11/2011	11.9	3	7.45	452.85
41	16/11/2011	10.3	-1.5	4.4	457.25
42	17/11/2011	12.8	6.2	9.5	466.75
43	18/11/2011	13.7	4.4	9.05	475.8
44	19/11/2011	13.4	0.3	6.85	482.65
45	20/11/2011	9.9	1.8	5.85	488.5
46	21/11/2011	9.5	0.5	5	493.5
47	22/11/2011	10.6	1	5.8	499.3
48	23/11/2011	8.5	-0.3	4.1	503.4
49	24/11/2011	13	7.1	10.05	513.45
50	25/11/2011	11.8	7.9	9.85	523.3
51	26/11/2011	11.8	4.1	7.95	531.25
52	27/11/2011	13	10.6	11.8	543.05
53	28/11/2011	8.9	-2.9	3	546.05
54	29/11/2011	12.9	9.2	11.05	557.1
55	30/11/2011	11.5	5.2	8.35	565.45
56	01/12/2011	10.9	8.8	9.85	575.3
57	02/12/2011	6.8	-0.4	3.2	578.5

58	03/12/2011	10.2	5.7	7.95	586.45
59	04/12/2011	7.9	5.5	6.7	593.15
60	05/12/2011	5.4	0.5	2.95	596.1
61	06/12/2011	5.3	1.1	3.2	599.3
62	07/12/2011	7.3	3.2	5.25	604.55
63	08/12/2011	12.1	2	7.05	611.6
64	09/12/2011	6.7	2.4	4.55	616.15
65	10/12/2011	4.1	-1.8	1.15	617.3
66	11/12/2011	7.3	0.6	3.95	621.25
67	12/12/2011	7.2	1.6	4.4	625.65
68	13/12/2011	7.2	4.7	5.95	631.6
69	14/12/2011	6.3	2.2	4.25	635.85
70	15/12/2011	5.3	-0.3	2.5	638.35
71	16/12/2011	3.5	1.7	2.6	640.95
72	17/12/2011	3.5	-1.7	0.9	641.85
73	18/12/2011	2.7	-1.8	0.45	642.3
74	19/12/2011	5	-4.6	0.2	642.5
75	20/12/2011	5.6	3.8	4.7	647.2
76	21/12/2011	10.2	1.3	5.75	652.95
77	22/12/2011	11.4	7.9	9.65	662.6
78	23/12/2011	11.4	6.8	9.1	671.7
79	24/12/2011	7.5	1.3	4.4	676.1
80	25/12/2011	11.9	7.5	9.7	685.8
81	26/12/2011	13.7	10.9	12.3	698.1
82	27/12/2011	10.2	6.5	8.35	706.45
83	28/12/2011	10.7	7	8.85	715.3
84	29/12/2011	8.1	4.6	6.35	721.65
85	30/12/2011	5.7	1.8	3.75	725.4
86	31/12/2011	12	4.6	8.3	733.7
87	01/01/2012	12.3	10.3	11.3	745
88	02/01/2012	6.6	3.5	5.05	750.05
89	03/01/2012	13	4.8	8.9	758.95
90	04/01/2012	10.2	3.5	6.85	765.8
91	05/01/2012	7.5	5.7	6.6	772.4
92	06/01/2012	7.2	3	5.1	777.5
93	07/01/2012	9.1	6.8	7.95	785.45
94	08/01/2012	9.5	4.6	7.05	792.5
95	09/01/2012	10.7	6.4	8.55	801.05
96	10/01/2012	9.6	-0.5	4.55	805.6
97	11/01/2012	11.5	5	8.25	813.85
98	12/01/2012	11	7	9	822.85
99	13/01/2012	6.2	-0.4	2.9	825.75
100	14/01/2012	5.2	0.1	2.65	828.4
101	15/01/2012	5	-5.6	-0.3	828.4
102	16/01/2012	6.6	-5.8	0.4	828.8
103	17/01/2012	6.1	-6.5	-0.2	828.8
104	18/01/2012	10.4	-2.8	3.8	832.6
105	19/01/2012	9.1	5.5	7.3	839.9
106	20/01/2012	5.8	2.6	4.2	844.1
107	21/01/2012	10.4	4.2	7.3	851.4
108	22/01/2012	10.6	3.7	7.15	858.55
109	23/01/2012	8	0.6	4.3	862.85
110	24/01/2012	4.9	-3	0.95	863.8
111	25/01/2012	9.2	4.9	7.05	870.85
112	26/01/2012	8.4	5.8	7.1	877.95
113	27/01/2012	7.9	-0.6	3.65	881.6
114	28/01/2012	7.1	-0.7	3.2	884.8
115	29/01/2012	4.2	-2.8	0.7	885.5
116	30/01/2012	3.1	0.8	1.95	887.45
117	31/01/2012	2.5	-1.2	0.65	888.1
118	01/02/2012	2.9	-0.5	1.2	889.3

119	02/02/2012	1.3	-0.3	0.5	889.8
120	03/02/2012	3.2	-6	-1.4	889.8
121	04/02/2012	0.5	-2.8	-1.15	889.8
122	05/02/2012	-0.2	-2.7	-1.45	889.8
123	06/02/2012	2.7	-1.5	0.6	890.4
124	07/02/2012	1	-2.9	-0.95	890.4
125	08/02/2012	0.6	-1.5	-0.45	890.4
126	09/02/2012	1.4	-0.5	0.45	890.85
127	10/02/2012	1.2	-3.1	-0.95	890.85
128	11/02/2012	2.8	-10.9	-4.05	890.85
129	12/02/2012	6.7	-7.6	-0.45	890.85
130	13/02/2012	6.2	0.2	3.2	894.05
131	14/02/2012	7.2	4.4	5.8	899.85
132	15/02/2012	8.9	6.1	7.5	907.35
133	16/02/2012	10.2	4.9	7.55	914.9
134	17/02/2012	11.2	3.5	7.35	922.25
135	18/02/2012	10.2	7.5	8.85	931.1
136	19/02/2012	5.2	-0.8	2.2	933.3
137	20/02/2012	7.9	-5	1.45	934.75
138	21/02/2012	11.9	5.1	8.5	943.25
139	22/02/2012	10.7	5.6	8.15	951.4
140	23/02/2012	18.3	7.9	13.1	964.5
141	24/02/2012	14.3	9.2	11.75	976.25
142	25/02/2012	9.8	-0.4	4.7	980.95
143	26/02/2012	13	0.6	6.8	987.75
144	27/02/2012	11.5	2.5	7	994.75
145	28/02/2012	14.1	9.6	11.85	1006.6
146	29/02/2012	12.5	8.5	10.5	1017.1
147	01/03/2012	14.9	2	8.45	1025.55
148	02/03/2012	9.5	-1	4.25	1029.8
149	03/03/2012	12.9	3.8	8.35	1038.15
150	04/03/2012	8.7	2.4	5.55	1043.7
151	05/03/2012	5.9	2.7	4.3	1048
152	06/03/2012	8.8	4	6.4	1054.4
153	07/03/2012	9.2	3.2	6.2	1060.6
154	08/03/2012	11.6	-0.8	5.4	1066
155	09/03/2012	13.1	3.8	8.45	1074.45
156	10/03/2012	15.4	6.7	11.05	1085.5
157	11/03/2012	14.9	2	8.45	1093.95
158	12/03/2012	10.5	1.8	6.15	1100.1
159	13/03/2012	8.1	6	7.05	1107.15
160	14/03/2012	7.5	5.7	6.6	1113.75
161	15/03/2012	17.5	-1.4	8.05	1121.8
162	16/03/2012	11.2	6.5	8.85	1130.65
163	17/03/2012	10.7	3.8	7.25	1137.9
164	18/03/2012	8.4	5.7	7.05	1144.95
165	19/03/2012	12.2	-2.3	4.95	1149.9
166	20/03/2012	16.1	0	8.05	1157.95
167	21/03/2012	14.6	0.5	7.55	1165.5
168	22/03/2012	14.8	0.2	7.5	1173
169	23/03/2012	16.2	2.6	9.4	1182.4
170	24/03/2012	14.2	0.2	7.2	1189.6
171	25/03/2012	11.3	3.2	7.25	1196.85
172	26/03/2012	17	-1.8	7.6	1204.45
173	27/03/2012	17.8	-2.3	7.75	1212.2
174	28/03/2012	20.7	-1.5	9.6	1221.8
175	29/03/2012	16	0.8	8.4	1230.2
176	30/03/2012	14.9	2.6	8.75	1238.95
177	31/03/2012	9.9	3.2	6.55	1245.5
178	01/04/2012	13.1	-0.2	6.45	1251.95
179	02/04/2012	13.8	-0.5	6.65	1258.6

180	03/04/2012	13.3	3.5	8.4	1267
181	04/04/2012	7.1	6.3	6.7	1273.7
182	05/04/2012	8.9	5.3	7.1	1280.8
183	06/04/2012	11.2	-4.7	3.25	1284.05
184	07/04/2012	8.6	5.5	7.05	1291.1
185	08/04/2012	12.1	-1.5	5.3	1296.4
186	09/04/2012	11.6	7.4	9.5	1305.9
187	10/04/2012	13.5	5.7	9.6	1315.5
188	11/04/2012	13.7	-0.9	6.4	1321.9
189	12/04/2012	12.1	1.7	6.9	1328.8
190	13/04/2012	10	-1.8	4.1	1332.9
191	14/04/2012	10.2	-2.6	3.8	1336.7
192	15/04/2012	9.6	1.8	5.7	1342.4
193	16/04/2012	9.7	-2.4	3.65	1346.05
194	17/04/2012	12.3	3.3	7.8	1353.85
195	18/04/2012	9.7	2.8	6.25	1360.1
196	19/04/2012	10.8	6.6	8.7	1368.8
197	20/04/2012	15	5.6	10.3	1379.1
198	21/04/2012	10.8	2.7	6.75	1385.85
199	22/04/2012	14.5	3.5	9	1394.85
200	23/04/2012	13.2	2.3	7.75	1402.6
201	24/04/2012	9.7	6.6	8.15	1410.75
202	25/04/2012	10.1	0.2	5.15	1415.9
203	26/04/2012	15.1	8.7	11.9	1427.8
204	27/04/2012	10.8	8.5	9.65	1437.45
205	28/04/2012	8.8	7.7	8.25	1445.7
206	29/04/2012	12.1	5.5	8.8	1454.5
207	30/04/2012	17.5	2.2	9.85	1464.35
208	01/05/2012	12.4	9.8	11.1	1475.45
209	02/05/2012	13.2	8.2	10.7	1486.15
210	03/05/2012	8.6	6.6	7.6	1493.75
211	04/05/2012	9.3	6.8	8.05	1501.8
212	05/05/2012	9.9	2.8	6.35	1508.15
213	06/05/2012	8.5	4.2	6.35	1514.5
214	07/05/2012	12.8	3	7.9	1522.4
215	08/05/2012	17.6	8.7	13.15	1535.55
216	09/05/2012	14.6	10.2	12.4	1547.95
217	10/05/2012	21.2	13.2	17.2	1565.15
218	11/05/2012	16.2	12	14.1	1579.25
219	12/05/2012	12.7	5.6	9.15	1588.4
220	13/05/2012	16.7	1.7	9.2	1597.6
221	14/05/2012	12.3	7.3	9.8	1607.4
222	15/05/2012	10.5	3.2	6.85	1614.25
223	16/05/2012	14	1.1	7.55	1621.8
224	17/05/2012	12.1	2.5	7.3	1629.1
225	18/05/2012	15.7	7.7	11.7	1640.8
226	19/05/2012	15.8	10.7	13.25	1654.05
227	20/05/2012	12	8.3	10.15	1664.2
228	21/05/2012	11.9	8.6	10.25	1674.45
229	22/05/2012	17.3	9.2	13.25	1687.7
230	23/05/2012	22.2	9.2	15.7	1703.4
231	24/05/2012	22.7	8.8	15.75	1719.15
232	25/05/2012	21.4	8.1	14.75	1733.9
233	26/05/2012	21.2	10.5	15.85	1749.75
234	27/05/2012	23.1	8.1	15.6	1765.35
235	28/05/2012	25.1	7.2	16.15	1781.5
236	29/05/2012	15.1	10.3	12.7	1794.2
237	30/05/2012	23.1	8	15.55	1809.75
238	31/05/2012	18.5	11.1	14.8	1824.55
239	01/06/2012	13.9	9.6	11.75	1836.3
240	02/06/2012	16.4	5.7	11.05	1847.35

241	03/06/2012	10.7	9.1	9.9	1857.25
242	04/06/2012	14.1	6.7	10.4	1867.65
243	05/06/2012	16.5	1	8.75	1876.4
244	06/06/2012	20.4	10.3	15.35	1891.75
245	07/06/2012	18	9.2	13.6	1905.35
246	08/06/2012	14.9	11.6	13.25	1918.6
247	09/06/2012	16.6	10	13.3	1931.9
248	10/06/2012	18.2	5	11.6	1943.5
249	11/06/2012	12.8	10.8	11.8	1955.3
250	12/06/2012	14.2	10.4	12.3	1967.6
251	13/06/2012	15.6	3.2	9.4	1977
252	14/06/2012	16.1	9	12.55	1989.55
253	15/06/2012	19.4	10.6	15	2004.55
254	16/06/2012	19.6	10.6	15.1	2019.65
255	17/06/2012	20.1	12.3	16.2	2035.85
256	18/06/2012	17.4	11.6	14.5	2050.35
257	19/06/2012	20.6	6.6	13.6	2063.95
258	20/06/2012	20	7	13.5	2077.45
259	21/06/2012	21.6	8.5	15.05	2092.5
260	22/06/2012	16.6	10.2	13.4	2105.9
261	23/06/2012	18.9	11.2	15.05	2120.95
262	24/06/2012	18.7	10.4	14.55	2135.5
263	25/06/2012	17.5	8.7	13.1	2148.6
264	26/06/2012	21.6	5.8	13.7	2162.3
265	27/06/2012	23.9	15.6	19.75	2182.05
266	28/06/2012	25.7	13.2	19.45	2201.5
267	29/06/2012	22.2	11.5	16.85	2218.35
268	30/06/2012	22.4	14	18.2	2236.55
269	01/07/2012	19.7	10.6	15.15	2251.7
270	02/07/2012	20.1	9.3	14.7	2266.4
271	03/07/2012	21.4	14.7	18.05	2284.45
272	04/07/2012	24.1	15.4	19.75	2304.2
273	05/07/2012	24	12.2	18.1	2322.3
274	06/07/2012	21.2	14.2	17.7	2340
275	07/07/2012	21.2	10.1	15.65	2355.65
276	08/07/2012	17.3	13.6	15.45	2371.1
277	09/07/2012	20	11.1	15.55	2386.65
278	10/07/2012	18	13.3	15.65	2402.3
279	11/07/2012	18.3	10.7	14.5	2416.8
280	12/07/2012	19.6	6.7	13.15	2429.95
281	13/07/2012	16.5	12.8	14.65	2444.6
282	14/07/2012	17.5	12.9	15.2	2459.8
283	15/07/2012	18.8	11.1	14.95	2474.75
284	16/07/2012	16.9	8.5	12.7	2487.45
285	17/07/2012	21.3	14.2	17.75	2505.2
286	18/07/2012	19.4	15.7	17.55	2522.75
287	19/07/2012	16.4	12.6	14.5	2537.25
288	20/07/2012	16.7	10.7	13.7	2550.95
289	21/07/2012	18.7	10.3	14.5	2565.45
290	22/07/2012	23	9.3	16.15	2581.6
291	23/07/2012	27.5	11.8	19.65	2601.25
292	24/07/2012	28.6	11.4	20	2621.25
293	25/07/2012	25.9	10.5	18.2	2639.45
294	26/07/2012	21.7	12.8	17.25	2656.7
295	27/07/2012	22.3	9.8	16.05	2672.75
296	28/07/2012	20	9	14.5	2687.25
297	29/07/2012	20.1	6.2	13.15	2700.4
298	30/07/2012	18.7	7.6	13.15	2713.55
299	31/07/2012	17.6	6.9	12.25	2725.8
300	01/08/2012	29.2	16.3	22.75	2748.55
301	02/08/2012	23.3	16.7	20	2768.55

302	03/08/2012	23.9	15.3	19.6	2788.15
303	04/08/2012	24.4	11.1	17.75	2805.9
304	05/08/2012	26.2	16.2	21.2	2827.1
305	06/08/2012	20.2	11.6	15.9	2843
306	07/08/2012	18.8	7.7	13.25	2856.25
307	08/08/2012	21.3	9.2	15.25	2871.5
308	09/08/2012	22.1	14.2	18.15	2889.65
309	10/08/2012	20.1	12	16.05	2905.7
310	11/08/2012	22	8.3	15.15	2920.85
311	12/08/2012	21.1	9.3	15.2	2936.05
312	13/08/2012	20.7	8	14.35	2950.4
313	14/08/2012	22.9	7.2	15.05	2965.45
314	15/08/2012	25	16.4	20.7	2986.15
315	16/08/2012	21.5	16.6	19.05	3005.2
316	17/08/2012	22.1	9	15.55	3020.75
317	18/08/2012	23.5	16.1	19.8	3040.55
318	19/08/2012	20.7	9	14.85	3055.4
319	20/08/2012	23.7	11.2	17.45	3072.85
320	21/08/2012	25.4	8.5	16.95	3089.8
321	22/08/2012	24	13.2	18.6	3108.4

A10. Meteorological data for 2012-2013 season at Churchfarm, UK.

day	date	Tmax	Tmin	Avg	TT
0	10/10/2012	14.6	-1.6	6.5	6.5
1	11/10/2012	14.7	1.1	7.9	14.4
2	12/10/2012	13.6	11.7	12.65	27.05
3	13/10/2012	12.7	1.3	7	34.05
4	14/10/2012	11	3	7	41.05
5	15/10/2012	11.7	1.5	6.6	47.65
6	16/10/2012	14.1	4.1	9.1	56.75
7	17/10/2012	17.1	2	9.55	66.3
8	18/10/2012	15.7	11.6	13.65	79.95
9	19/10/2012	13.5	11.2	12.35	92.3
10	20/10/2012	13.1	8.6	10.85	103.15
11	21/10/2012	12.3	8.3	10.3	113.45
12	22/10/2012	14.3	12	13.15	126.6
13	23/10/2012	16.2	11.9	14.05	140.65
14	24/10/2012	14.4	12.7	13.55	154.2
15	25/10/2012	11.5	10.5	11	165.2
16	26/10/2012	8.7	6.5	7.6	172.8
17	27/10/2012	8.5	2	5.25	178.05
18	28/10/2012	7.7	0.3	4	182.05
19	29/10/2012	11.5	6.3	8.9	190.95
20	30/10/2012	9.9	3.2	6.55	197.5
21	31/10/2012	12.3	4.5	8.4	205.9
22	01/11/2012	8.7	6.3	7.5	213.4
23	02/11/2012	9.4	2.9	6.15	219.55
24	03/11/2012	8.4	-2	3.2	222.75
25	04/11/2012	6.6	-1.8	2.4	225.15
26	05/11/2012	9.9	0.4	5.15	230.3
27	06/11/2012	9.2	-0.8	4.2	234.5
28	07/11/2012	10.3	1.5	5.9	240.4
29	08/11/2012	12.2	7.7	9.95	250.35
30	09/11/2012	12.3	7.5	9.9	260.25
31	10/11/2012	9.6	6.7	8.15	268.4
32	11/11/2012	9.5	0	4.75	273.15
33	12/11/2012	10.6	-0.8	4.9	278.05
34	13/11/2012	15.7	9.2	12.45	290.5
35	14/11/2012	12.7	10.7	11.7	302.2
36	15/11/2012	12.3	0.6	6.45	308.65
37	16/11/2012	7	4.9	5.95	314.6
38	17/11/2012	11.9	6.9	9.4	324
39	18/11/2012	8.9	-1.9	3.5	327.5
40	19/11/2012	11.1	-2	4.55	332.05
41	20/11/2012	12.5	9.4	10.95	343
42	21/11/2012	9.4	9.5	9.45	352.45
43	22/11/2012	13.2	4.5	8.85	361.3
44	23/11/2012	10	5.3	7.65	368.95
45	24/11/2012	9.2	-1.8	3.7	372.65
46	25/11/2012	9.4	7.2	8.3	380.95
47	26/11/2012	10.2	7.9	9.05	390
48	27/11/2012	8.5	5.5	7	397
49	28/11/2012	8.4	2.7	5.55	402.55
50	29/11/2012	7.4	1.3	4.35	406.9
51	30/11/2012	5.3	-0.8	2.25	409.15
52	01/12/2012	3.8	-3	0.4	409.55
53	02/12/2012	4.1	-1.5	1.3	410.85
54	03/12/2012	7.6	-4.4	1.6	412.45
55	04/12/2012	6.1	1.4	3.75	416.2
56	05/12/2012	3.7	0	1.85	418.05
57	06/12/2012	3.2	-3.3	-0.05	418.05

58	07/12/2012	5.8	0.9	3.35	421.4
59	08/12/2012	5.4	0.1	2.75	424.15
60	09/12/2012	8.7	4.4	6.55	430.7
61	10/12/2012	4.8	3.2	4	434.7
62	11/12/2012	4.7	-2.5	1.1	435.8
63	12/12/2012	1.2	-2.4	-0.6	435.8
64	13/12/2012	3	-6.9	-1.95	435.8
65	14/12/2012	9.7	-3.3	3.2	439
66	15/12/2012	10	5.9	7.95	446.95
67	16/12/2012	8.3	0.7	4.5	451.45
68	17/12/2012	6.4	0	3.2	454.65
69	18/12/2012	7.6	3.2	5.4	460.05
70	19/12/2012	7.1	2	4.55	464.6
71	20/12/2012	6.2	2.4	4.3	468.9
72	21/12/2012	8.2	6.2	7.2	476.1
73	22/12/2012	12.1	3.6	7.85	483.95
74	23/12/2012	11.8	11.8	11.8	495.75
75	24/12/2012	11.3	3.8	7.55	503.3
76	25/12/2012	8.3	5.6	6.95	510.25
77	26/12/2012	8.9	3.9	6.4	516.65
78	27/12/2012	6.5	3.7	5.1	521.75
79	28/12/2012	11.3	2.5	6.9	528.65
80	29/12/2012	11	10.6	10.8	539.45
81	30/12/2012	8.2	4.6	6.4	545.85
82	31/12/2012	11.1	7.8	9.45	555.3
83	01/01/2013	6.5	1.7	4.1	559.4
84	02/01/2013	9.30	-0.40	4.45	563.85
85	03/01/2013	11.50	8.10	9.8	573.65
86	04/01/2013	9.8	7.7	8.75	582.4
87	05/01/2013	10	6.8	8.4	590.8
88	06/01/2013	9.8	1.8	5.8	596.6
89	07/01/2013	8.8	6.8	7.8	604.4
90	08/01/2013	10.5	5.1	7.8	612.2
91	09/01/2013	6.8	4.2	5.5	617.7
92	10/01/2013	2.4	-1	0.7	618.4
93	11/01/2013	5.6	2.1	3.85	622.25
94	12/01/2013	5	-1.9	1.55	623.8
95	13/01/2013	3.5	0.5	2	625.8
96	14/01/2013	1.4	-2	-0.3	625.8
97	15/01/2013	3.3	-2	0.65	626.45
98	16/01/2013	1.4	-13.2	-5.9	626.45
99	17/01/2013	1.4	-9	-3.8	626.45
100	18/01/2013	1	-6.8	-2.9	626.45
101	19/01/2013	0.9	-0.1	0.4	626.85
102	20/01/2013	1.2	-0.9	0.15	627
103	21/01/2013	0.3	-1.7	-0.7	627
104	22/01/2013	3	-1.2	0.9	627.9
105	23/01/2013	1.2	0	0.6	628.5
106	24/01/2013	1.6	-0.4	0.6	629.1
107	25/01/2013	0.2	-7.4	-3.6	629.1
108	26/01/2013	4.8	-0.8	2	631.1
109	27/01/2013	9.4	4.6	7	638.1
110	28/01/2013	9.8	1.9	5.85	643.95
111	29/01/2013	13.8	8.2	11	654.95
112	30/01/2013	11.5	8.6	10.05	665
113	31/01/2013	10.5	5.5	8	673
114	01/02/2013	7.2	5.9	6.55	679.55
115	02/02/2013	5.3	1.2	3.25	682.8
116	03/02/2013	8.3	-0.8	3.75	686.55
117	04/02/2013	10.2	7.7	8.95	695.5
118	05/02/2013	5.6	0.8	3.2	698.7

119	06/02/2013	5.5	1.9	3.7	702.4
120	07/02/2013	5	0.5	2.75	705.15
121	08/02/2013	3.5	-0.3	1.6	706.75
122	09/02/2013	6.7	-0.8	2.95	709.7
123	10/02/2013	3	-1.8	0.6	710.3
124	11/02/2013	2	1	1.5	711.8
125	12/02/2013	1.7	1	1.35	713.15
126	13/02/2013	2.2	-1	0.6	713.75
127	14/02/2013	8.8	0.1	4.45	718.2
128	15/02/2013	8.8	1	4.9	723.1
129	16/02/2013	9.7	0.5	5.1	728.2
130	17/02/2013	5	-3.3	0.85	729.05
131	18/02/2013	6.1	-0.5	2.8	731.85
132	19/02/2013	7.9	-3.5	2.2	734.05
133	20/02/2013	2.4	2.2	2.3	736.35
134	21/02/2013	1.6	0.7	1.15	737.5
135	22/02/2013	1.2	0.4	0.8	738.3
136	23/02/2013	2.2	0.1	1.15	739.45
137	24/02/2013	2.5	0.5	1.5	740.95
138	25/02/2013	4	2	3	743.95
139	26/02/2013	4.5	2.9	3.7	747.65
140	27/02/2013	6.3	2.2	4.25	751.9
141	28/02/2013	6.6	0	3.3	755.2
142	01/03/2013	6.6	2.5	4.55	759.75
143	02/03/2013	5.8	2.1	3.95	763.7
144	03/03/2013	8.6	-1.8	3.4	767.1
145	04/03/2013	7.9	-2.8	2.55	769.65
146	05/03/2013	14.5	-3	5.75	775.4
147	06/03/2013	12	-0.1	5.95	781.35
148	07/03/2013	8	4.7	6.35	787.7
149	08/03/2013	5.6	5.4	5.5	793.2
150	09/03/2013	3.8	3.7	3.75	796.95
151	10/03/2013	2	0.6	1.3	798.25
152	11/03/2013	-0.1	-2	-1.05	798.25
153	12/03/2013	3.5	-2.4	0.55	798.8
154	13/03/2013	5.2	-0.5	2.35	801.15
155	14/03/2013	6.7	-4.1	1.3	802.45
156	15/03/2013	9	1.2	5.1	807.55
157	16/03/2013	7.1	3.6	5.35	812.9
158	17/03/2013	7.2	4.7	5.95	818.85
159	18/03/2013	8	2	5	823.85
160	19/03/2013	4.7	-2	1.35	825.2
161	20/03/2013	2.7	1.2	1.95	827.15
162	21/03/2013	4.3	-1.8	1.25	828.4
163	22/03/2013	2.7	1.2	1.95	830.35
164	23/03/2013	2.4	0.7	1.55	831.9
165	24/03/2013	2.6	-0.6	1	832.9
166	25/03/2013	2.4	-0.1	1.15	834.05
167	26/03/2013	2.8	0.2	1.5	835.55
168	27/03/2013	3.6	0.1	1.85	837.4
169	28/03/2013	4.1	-0.5	1.8	839.2
170	29/03/2013	4.8	-2	1.4	840.6
171	30/03/2013	5.1	0.6	2.85	843.45
172	31/03/2013	4.3	-2.1	1.1	844.55
173	01/04/2013	5.2	0.4	2.8	847.35
174	02/04/2013	7.1	-2	2.55	849.9
175	03/04/2013	6.1	0.5	3.3	853.2
176	04/04/2013	5.1	1.8	3.45	856.65
177	05/04/2013	7	2.1	4.55	861.2
178	06/04/2013	8	-3.8	2.1	863.3
179	07/04/2013	11.2	-5.6	2.8	866.1

180	08/04/2013	6.6	-0.7	2.95	869.05
181	09/04/2013	8.7	-1.3	3.7	872.75
182	10/04/2013	11.2	0.7	5.95	878.7
183	11/04/2013	7	3.8	5.4	884.1
184	12/04/2013	9.7	4.5	7.1	891.2
185	13/04/2013	15	1.8	8.4	899.6
186	14/04/2013	20.8	9.6	15.2	914.8
187	15/04/2013	18.2	10.5	14.35	929.15
188	16/04/2013	17	9.3	13.15	942.3
189	17/04/2013	16.9	4	10.45	952.75
190	18/04/2013	14.4	9.1	11.75	964.5
191	19/04/2013	10.6	7	8.8	973.3
192	20/04/2013	12	-2.8	4.6	977.9
193	21/04/2013	14.6	-3.1	5.75	983.65
194	22/04/2013	15.6	2.3	8.95	992.6
195	23/04/2013	19.6	7.9	13.75	1006.35
196	24/04/2013	21.2	4.6	12.9	1019.25
197	25/04/2013	22.8	10.2	16.5	1035.75
198	26/04/2013	12.1	10.2	11.15	1046.9
199	27/04/2013	9.6	0.7	5.15	1052.05
200	28/04/2013	12.6	-2	5.3	1057.35
201	29/04/2013	14.1	5.7	9.9	1067.25
202	30/04/2013	10.2	1.4	5.8	1073.05
203	01/05/2013	13.2	-2.8	5.2	1078.25
204	02/05/2013	12.9	-1.2	5.85	1084.1
205	03/05/2013	19.7	-1.7	9	1093.1
206	04/05/2013	17.5	7.5	12.5	1105.6
207	05/05/2013	19.9	2.2	11.05	1116.65
208	06/05/2013	20	7	13.5	1130.15
209	07/05/2013	18.7	3.2	10.95	1141.1
210	08/05/2013	20.3	4.6	12.45	1153.55
211	09/05/2013	15.8	6.3	11.05	1164.6
212	10/05/2013	16.8	9.9	13.35	1177.95
213	11/05/2013	14.5	7.5	11	1188.95
214	12/05/2013	14.6	4.2	9.4	1198.35
215	13/05/2013	14.3	9.5	11.9	1210.25
216	14/05/2013	13	4.1	8.55	1218.8
217	15/05/2013	10.2	7.5	8.85	1227.65
218	16/05/2013	14.6	2.8	8.7	1236.35
219	17/05/2013	9.9	7.7	8.8	1245.15
220	18/05/2013	13.3	7.7	10.5	1255.65
221	19/05/2013	15.9	3.3	9.6	1265.25
222	20/05/2013	15.9	9	12.45	1277.7
223	21/05/2013	12.3	8.6	10.45	1288.15
224	22/05/2013	13.3	3.7	8.5	1296.65
225	23/05/2013	10.1	3.5	6.8	1303.45
226	24/05/2013	9.2	3.8	6.5	1309.95
227	25/05/2013	11.4	1.3	6.35	1316.3
228	26/05/2013	16.9	6.1	11.5	1327.8
229	27/05/2013	18.6	4	11.3	1339.1
230	28/05/2013	16.9	6.3	11.6	1350.7
231	29/05/2013	12.6	9	10.8	1361.5
232	30/05/2013	12.2	9.1	10.65	1372.15
233	31/05/2013	17.7	9	13.35	1385.5
234	01/06/2013	13	6.9	9.95	1395.45
235	02/06/2013	15.6	5	10.3	1405.75
236	03/06/2013	15	2.1	8.55	1414.3
237	04/06/2013	15	2.7	8.85	1423.15
238	05/06/2013	14.5	8.5	11.5	1434.65
239	06/06/2013	14.5	9.3	11.9	1446.55
240	07/06/2013	15	4.7	9.85	1456.4

241	08/06/2013	12.5	6.6	9.55	1465.95
242	09/06/2013	13	9.4	11.2	1477.15
243	10/06/2013	14	9.5	11.75	1488.9
244	11/06/2013	20.5	6.3	13.4	1502.3
245	12/06/2013	21.4	14.2	17.8	1520.1
246	13/06/2013	18	14.3	16.15	1536.25
247	14/06/2013	18.7	9.4	14.05	1550.3
248	15/06/2013	17.1	11.6	14.35	1564.65
249	16/06/2013	19.4	11.1	15.25	1579.9
250	17/06/2013	18.2	6.3	12.25	1592.15
251	18/06/2013	20.6	11.2	15.9	1608.05
252	19/06/2013	22.8	13.1	17.95	1626
253	20/06/2013	17.8	12.6	15.2	1641.2
254	21/06/2013	19.5	14	16.75	1657.95
255	22/06/2013	18.8	11.6	15.2	1673.15
256	23/06/2013	17.7	12.2	14.95	1688.1
257	24/06/2013	14.2	12	13.1	1701.2
258	25/06/2013	18.4	7.6	13	1714.2
259	26/06/2013	20.2	7	13.6	1727.8
260	27/06/2013	19.2	8.1	13.65	1741.45
261	28/06/2013	18.1	12.4	15.25	1756.7
262	29/06/2013	19.1	12.1	15.6	1772.3
263	30/06/2013	24.2	12.1	18.15	1790.45
264	01/07/2013	18.9	11.6	15.25	1805.7
265	02/07/2013	18.5	6.7	12.6	1818.3
266	03/07/2013	19.8	13.8	16.8	1835.1
267	04/07/2013	24.1	9.2	16.65	1851.75
268	05/07/2013	22.1	9.2	15.65	1867.4
269	06/07/2013	25.2	7.2	16.2	1883.6
270	07/07/2013	24.2	8.5	16.35	1899.95
271	08/07/2013	18.8	10.7	14.75	1914.7
272	09/07/2013	19.8	11	15.4	1930.1
273	10/07/2013	17.8	9.1	13.45	1943.55
274	11/07/2013	16.6	12.2	14.4	1957.95
275	12/07/2013	22.3	12.6	17.45	1975.4
276	13/07/2013	26.1	10.5	18.3	1993.7
277	14/07/2013	23.1	14.2	18.65	2012.35
278	15/07/2013	25	7.8	16.4	2028.75
279	16/07/2013	25.1	11.3	18.2	2046.95
280	17/07/2013	27.6	11.5	19.55	2066.5
281	18/07/2013	23.7	13	18.35	2084.85
282	19/07/2013	24.4	9.2	16.8	2101.65
283	20/07/2013	19	14.2	16.6	2118.25
284	21/07/2013	17.5	15.8	16.65	2134.9
285	22/07/2013	28.2	13.7	20.95	2155.85
286	23/07/2013	24.1	14.9	19.5	2175.35
287	24/07/2013	27	15.1	21.05	2196.4
288	25/07/2013	26.5	12.2	19.35	2215.75
289	26/07/2013	26.9	12.6	19.75	2235.5
290	27/07/2013	23.4	11.2	17.3	2252.8
291	28/07/2013	24.8	16.4	20.6	2273.4
292	29/07/2013	24.5	14.8	19.65	2293.05
293	30/07/2013	20.7	13.6	17.15	2310.2
294	31/07/2013	19.6	12	15.8	2326
295	01/08/2013	29.2	16.3	22.75	2348.75
296	02/08/2013	23.3	16.7	20	2368.75
297	03/08/2013	23.9	15.3	19.6	2388.35
298	04/08/2013	24.4	11.1	17.75	2406.1
299	05/08/2013	26.2	16.2	21.2	2427.3
300	06/08/2013	20.2	11.6	15.9	2443.2
301	07/08/2013	18.8	7.7	13.25	2456.45

302	08/08/2013	21.3	9.2	15.25	2471.7
303	09/08/2013	22.1	14.2	18.15	2489.85
304	10/08/2013	20.1	12	16.05	2505.9
305	11/08/2013	22	8.3	15.15	2521.05
306	12/08/2013	21.1	9.3	15.2	2536.25
307	13/08/2013	20.7	8	14.35	2550.6
308	14/08/2013	22.9	7.2	15.05	2565.65
309	15/08/2013	25	16.4	20.7	2586.35
310	16/08/2013	21.5	16.6	19.05	2605.4
311	17/08/2013	22.1	9	15.55	2620.95
312	18/08/2013	23.5	16.1	19.8	2640.75
313	19/08/2013	20.7	9	14.85	2655.6
314	20/08/2013	23.7	11.2	17.45	2673.05
315	21/08/2013	25.4	8.5	16.95	2690
316	22/08/2013	24	13.2	18.6	2708.6
317	23/08/2013	18.7	15.7	17.2	2725.8
318	24/08/2013	24.4	16.8	20.6	2746.4
319	25/08/2013	21.8	15.7	18.75	2765.15
320	26/08/2013	22.2	13.7	17.95	2783.1
321	27/08/2013	20.8	8.7	14.75	2797.85

A11. Meteorological data for 2013-2014 season at Churchfarm, UK.

day	date	Tmax	Tmin	Avg	TT
0	18/10/2013	16.2	5.1	10.65	10.65
1	19/10/2013	16.5	12.5	14.5	25.15
2	20/10/2013	17.8	11	14.4	39.55
3	21/10/2013	17.4	10.8	14.1	53.65
4	22/10/2013	17.7	14.7	16.2	69.85
5	23/10/2013	16.7	12.7	14.7	84.55
6	24/10/2013	16.9	4.7	10.8	95.35
7	25/10/2013	18.2	8.8	13.5	108.85
8	26/10/2013	17.9	13	15.45	124.3
9	27/10/2013	15.7	10.7	13.2	137.5
10	28/10/2013	13.2	10.7	11.95	149.45
11	29/10/2013	11.9	7.2	9.55	159
12	30/10/2013	13.3	3.8	8.55	167.55
13	31/10/2013	13.1	8.5	10.8	178.35
14	01/11/2013	14.7	8.9	11.8	190.15
15	02/11/2013	13.3	9.2	11.25	201.4
16	03/11/2013	10.9	6.8	8.85	210.25
17	04/11/2013	9.8	5.5	7.65	217.9
18	05/11/2013	9.5	1.4	5.45	223.35
19	06/11/2013	13.3	5.2	9.25	232.6
20	07/11/2013	10.6	5.5	8.05	240.65
21	08/11/2013	10.5	1.7	6.1	246.75
22	09/11/2013	7.1	2.4	4.75	251.5
23	10/11/2013	8.8	2.3	5.55	257.05
24	11/11/2013	10.6	-2.4	4.1	261.15
25	12/11/2013	12.2	8.5	10.35	271.5
26	13/11/2013	10	-0.4	4.8	276.3
27	14/11/2013	9.3	5.3	7.3	283.6
28	15/11/2013	10.9	6.4	8.65	292.25
29	16/11/2013	8.6	0	4.3	296.55
30	17/11/2013	10.8	2.2	6.5	303.05
31	18/11/2013	9	7.5	8.25	311.3
32	19/11/2013	6.1	0.2	3.15	314.45
33	20/11/2013	5.5	-1.4	2.05	316.5
34	21/11/2013	8.7	2	5.35	321.85
35	22/11/2013	9	2.1	5.55	327.4
36	23/11/2013	8	1.1	4.55	331.95
37	24/11/2013	8.3	5.4	6.85	338.8
38	25/11/2013	8	1.8	4.9	343.7
39	26/11/2013	7.2	1.7	4.45	348.15
40	27/11/2013	10.1	1.2	5.65	353.8
41	28/11/2013	9.5	1.8	5.65	359.45
42	29/11/2013	9.4	6.2	7.8	367.25
43	30/11/2013	9.1	5.1	7.1	374.35
44	01/12/2013	8.6	-1.3	3.65	378
45	02/12/2013	7.5	-0.2	3.65	381.65
46	03/12/2013	7.1	0.2	3.65	385.3
47	04/12/2013	7.5	2.9	5.2	390.5
48	05/12/2013	9	0.2	4.6	395.1
49	06/12/2013	4.5	1.9	3.2	398.3
50	07/12/2013	9.5	1.2	5.35	403.65
51	08/12/2013	10	5.2	7.6	411.25
52	09/12/2013	9.1	6.8	7.95	419.2
53	10/12/2013	8	1.2	4.6	423.8
54	11/12/2013	8.5	0.3	4.4	428.2
55	12/12/2013	7.6	-2.3	2.65	430.85
56	13/12/2013	10.7	6.6	8.65	439.5
57	14/12/2013	10.1	2.6	6.35	445.85

58	15/12/2013	12.3	6.6	9.45	455.3
59	16/12/2013	13.1	11.9	12.5	467.8
60	17/12/2013	7.4	3.2	5.3	473.1
61	18/12/2013	9.3	4.6	6.95	480.05
62	19/12/2013	7.7	4.2	5.95	486
63	20/12/2013	8.5	1.5	5	491
64	21/12/2013	12	8.3	10.15	501.15
65	22/12/2013	8.5	6	7.25	508.4
66	23/12/2013	10.7	2.1	6.4	514.8
67	24/12/2013	9	7	8	522.8
68	25/12/2013	7	2.2	4.6	527.4
69	26/12/2013	5.4	-2.4	1.5	528.9
70	27/12/2013	7	2.4	4.7	533.6
71	28/12/2013	7.8	3	5.4	539
72	29/12/2013	6.2	-0.2	3	542
73	30/12/2013	9	2.9	5.95	547.95
74	31/12/2013	9.8	4.2	7	554.95
75	01/01/2014	10.2	4.1	7.15	562.1
76	02/01/2014	10	6	8	570.1
77	03/01/2014	10.3	7.3	8.8	578.9
78	04/01/2014	9.7	5.7	7.7	586.6
79	05/01/2014	8.6	1.5	5.05	591.65
80	06/01/2014	12.2	8.3	10.25	601.9
81	07/01/2014	10.2	8.7	9.45	611.35
82	08/01/2014	11	7.2	9.1	620.45
83	09/01/2014	8.7	8.1	8.4	628.85
84	10/01/2014	8.5	2.2	5.35	634.2
85	11/01/2014	7.8	5.1	6.45	640.65
86	12/01/2014	6.6	-4.2	1.2	641.85
87	13/01/2014	9.1	4.9	7	648.85
88	14/01/2014	6.4	0.9	3.65	652.5
89	15/01/2014	10.2	2.5	6.35	658.85
90	16/01/2014	10.1	7.2	8.65	667.5
91	17/01/2014	9.9	6.9	8.4	675.9
92	18/01/2014	10.1	3.7	6.9	682.8
93	19/01/2014	9.5	5	7.25	690.05
94	20/01/2014	4.8	-2.9	0.95	691
95	21/01/2014	3.3	-1.5	0.9	691.9
96	22/01/2014	6.6	1.1	3.85	695.75
97	23/01/2014	6.2	2.7	4.45	700.2
98	24/01/2014	5.7	-3.1	1.3	701.5
99	25/01/2014	9.2	4.6	6.9	708.4
100	26/01/2014	7.3	1	4.15	712.55
101	27/01/2014	6.4	1.2	3.8	716.35
102	28/01/2014	8.8	2.3	5.55	721.9
103	29/01/2014	6.2	5.2	5.7	727.6
104	30/01/2014	5.5	3.1	4.3	731.9
105	31/01/2014	7	5.2	6.1	738
106	01/02/2014	8.2	5.4	6.8	744.8
107	02/02/2014	9.9	5.2	7.55	752.35
108	03/02/2014	8.8	1.3	5.05	757.4
109	04/02/2014	9.4	3.3	6.35	763.75
110	05/02/2014	9.7	5	7.35	771.1
111	06/02/2014	8.7	6	7.35	778.45
112	07/02/2014	8.7	4.8	6.75	785.2
113	08/02/2014	9.5	5.3	7.4	792.6
114	09/02/2014	8.2	5.6	6.9	799.5
115	10/02/2014	6.6	0.8	3.7	803.2
116	11/02/2014	6.6	0.7	3.65	806.85
117	12/02/2014	8	2.2	5.1	811.95
118	13/02/2014	8.5	2.6	5.55	817.5

119	14/02/2014	10.1	1	5.55	823.05
120	15/02/2014	11.2	8.7	9.95	833
121	16/02/2014	10	3	6.5	839.5
122	17/02/2014	10.3	-2	4.15	843.65
123	18/02/2014	11.3	6.3	8.8	852.45
124	19/02/2014	11.4	2.9	7.15	859.6
125	20/02/2014	11.9	6	8.95	868.55
126	21/02/2014	10	1.9	5.95	874.5
127	22/02/2014	11.4	4.4	7.9	882.4
128	23/02/2014	12.5	5.6	9.05	891.45
129	24/02/2014	14.4	7.6	11	902.45
130	25/02/2014	12.8	8.8	10.8	913.25
131	26/02/2014	12.2	2.5	7.35	920.6
132	27/02/2014	10.6	4.4	7.5	928.1
133	28/02/2014	7.2	-2.1	2.55	930.65
134	01/03/2014	7.2	1.4	4.3	934.95
135	02/03/2014	10.7	-3	3.85	938.8
136	03/03/2014	11	4.7	7.85	946.65
137	04/03/2014	11.7	-1.8	4.95	951.6
138	05/03/2014	13.5	-2.2	5.65	957.25
139	06/03/2014	13.8	6.2	10	967.25
140	07/03/2014	16.1	7.2	11.65	978.9
141	08/03/2014	14.6	-1.2	6.7	985.6
142	09/03/2014	19.3	4.7	12	997.6
143	10/03/2014	10	1.3	5.65	1003.25
144	11/03/2014	10.4	4.5	7.45	1010.7
145	12/03/2014	13.6	-0.1	6.75	1017.45
146	13/03/2014	16	-1.3	7.35	1024.8
147	14/03/2014	15.5	1.1	8.3	1033.1
148	15/03/2014	14.1	7.1	10.6	1043.7
149	16/03/2014	17.1	6.7	11.9	1055.6
150	17/03/2014	15.1	8.2	11.65	1067.25
151	18/03/2014	13.4	4.2	8.8	1076.05
152	19/03/2014	13.4	7.2	10.3	1086.35
153	20/03/2014	16.8	5.6	11.2	1097.55
154	21/03/2014	13	5	9	1106.55
155	22/03/2014	11.5	4.7	8.1	1114.65
156	23/03/2014	9	1.2	5.1	1119.75
157	24/03/2014	11.3	-3	4.15	1123.9
158	25/03/2014	10.9	1.3	6.1	1130
159	26/03/2014	7.6	-0.5	3.55	1133.55
160	27/03/2014	9.5	-2.7	3.4	1136.95
161	28/03/2014	11.2	2.2	6.7	1143.65
162	29/03/2014	14.9	0.5	7.7	1151.35
163	30/03/2014	17.3	3.2	10.25	1161.6
164	31/03/2014	18.5	7.4	12.95	1174.55
165	01/04/2014	17.7	5.7	11.7	1186.25
166	02/04/2014	12.6	3.9	8.25	1194.5
167	03/04/2014	13.3	7.5	10.4	1204.9
168	04/04/2014	12.8	8.8	10.8	1215.7
169	05/04/2014	18	2.4	10.2	1225.9
170	06/04/2014	18.1	12.3	15.2	1241.1
171	07/04/2014	15.2	12.5	13.85	1254.95
172	08/04/2014	12.4	6.2	9.3	1264.25
173	09/04/2014	17.2	2.4	9.8	1274.05
174	10/04/2014	15.9	7.1	11.5	1285.55
175	11/04/2014	13.7	8.2	10.95	1296.5
176	12/04/2014	12.7	1.7	7.2	1303.7
177	13/04/2014	15	2.3	8.65	1312.35
178	14/04/2014	12.2	6.2	9.2	1321.55
179	15/04/2014	11.1	-0.5	5.3	1326.85

180	16/04/2014	14.7	-2.3	6.2	1333.05
181	17/04/2014	16.4	3.7	10.05	1343.1
182	18/04/2014	10.2	2.9	6.55	1349.65
183	19/04/2014	11.8	6.1	8.95	1358.6
184	20/04/2014	15.3	6.3	10.8	1369.4
185	21/04/2014	15.3	5.3	10.3	1379.7
186	22/04/2014	15.4	9.8	12.6	1392.3
187	23/04/2014	18	3.8	10.9	1403.2
188	24/04/2014	15.4	9.2	12.3	1415.5
189	25/04/2014	13	7.5	10.25	1425.75
190	26/04/2014	12.9	5.7	9.3	1435.05
191	27/04/2014	15.7	5	10.35	1445.4
192	28/04/2014	12.1	7.8	9.95	1455.35
193	29/04/2014	13.2	8.8	11	1466.35
194	30/04/2014	17.2	8.2	12.7	1479.05
195	01/05/2014	14.5	8.1	11.3	1490.35
196	02/05/2014	11.4	8.2	9.8	1500.15
197	03/05/2014	10.7	3.4	7.05	1507.2
198	04/05/2014	16.8	-1.2	7.8	1515
199	05/05/2014	18.4	4	11.2	1526.2
200	06/05/2014	17.9	10.5	14.2	1540.4
201	07/05/2014	16.2	6.7	11.45	1551.85
202	08/05/2014	14.3	10.9	12.6	1564.45
203	09/05/2014	13.2	11	12.1	1576.55
204	10/05/2014	18.3	6	12.15	1588.7
205	11/05/2014	14.8	9.2	12	1600.7
206	12/05/2014	14.8	9	11.9	1612.6
207	13/05/2014	14.2	6.9	10.55	1623.15
208	14/05/2014	15	3.2	9.1	1632.25
209	15/05/2014	18.7	2.5	10.6	1642.85
210	16/05/2014	21.3	7.5	14.4	1657.25
211	17/05/2014	22.8	9.5	16.15	1673.4
212	18/05/2014	22.3	9.5	15.9	1689.3
213	19/05/2014	22.6	7.5	15.05	1704.35
214	20/05/2014	23.3	10	16.65	1721
215	21/05/2014	19.4	12.4	15.9	1736.9
216	22/05/2014	18.7	12	15.35	1752.25
217	23/05/2014	18.5	8.8	13.65	1765.9
218	24/05/2014	18	7	12.5	1778.4
219	25/05/2014	18.8	6.7	12.75	1791.15
220	26/05/2014	18.2	8.8	13.5	1804.65
221	27/05/2014	13.3	12.5	12.9	1817.55
222	28/05/2014	13	12.2	12.6	1830.15
223	29/05/2014	12.7	11.8	12.25	1842.4
224	30/05/2014	16.5	7.3	11.9	1854.3
225	31/05/2014	17.1	2.8	9.95	1864.25
226	01/06/2014	18.7	6.1	12.4	1876.65
227	02/06/2014	19.1	6	12.55	1889.2
228	03/06/2014	18.3	13.2	15.75	1904.95
229	04/06/2014	15.7	11	13.35	1918.3
230	05/06/2014	17.8	6.2	12	1930.3
231	06/06/2014	21.2	3.9	12.55	1942.85
232	07/06/2014	23.2	10.1	16.65	1959.5
233	08/06/2014	24.2	8.7	16.45	1975.95
234	09/06/2014	23.7	9.2	16.45	1992.4
235	10/06/2014	23.5	14.1	18.8	2011.2
236	11/06/2014	21.7	8.2	14.95	2026.15
237	12/06/2014	22.7	8	15.35	2041.5
238	13/06/2014	22.5	7.9	15.2	2056.7
239	14/06/2014	17.4	13	15.2	2071.9
240	15/06/2014	16.8	12.9	14.85	2086.75

241	16/06/2014	16.9	12.9	14.9	2101.65
242	17/06/2014	17	12.3	14.65	2116.3
243	18/06/2014	16.5	13	14.75	2131.05
244	19/06/2014	16.9	5.3	11.1	2142.15
245	20/06/2014	20	6.4	13.2	2155.35
246	21/06/2014	21.2	7.1	14.15	2169.5
247	22/06/2014	20.8	7.7	14.25	2183.75
248	23/06/2014	24	10.3	17.15	2200.9
249	24/06/2014	19.8	9.3	14.55	2215.45
250	25/06/2014	17.5	6.8	12.15	2227.6
251	26/06/2014	18.2	5.3	11.75	2239.35
252	27/06/2014	17.7	11	14.35	2253.7
253	28/06/2014	17.9	8.6	13.25	2266.95
254	29/06/2014	17.3	8.1	12.7	2279.65
255	30/06/2014	18.7	7	12.85	2292.5
256	01/07/2014	19	6	12.5	2305
257	02/07/2014	22.4	4	13.2	2318.2
258	03/07/2014	25	13.2	19.1	2337.3
259	04/07/2014	27.2	11.2	19.2	2356.5
260	05/07/2014	21.2	16.7	18.95	2375.45
261	06/07/2014	19.8	15.4	17.6	2393.05
262	07/07/2014	21.8	7.7	14.75	2407.8
263	08/07/2014	21.2	8.1	14.65	2422.45
264	09/07/2014	17.3	11.7	14.5	2436.95
265	10/07/2014	15.4	14.3	14.85	2451.8
266	11/07/2014	16.7	14	15.35	2467.15
267	12/07/2014	25.5	14.8	20.15	2487.3
268	13/07/2014	24	15.6	19.8	2507.1
269	14/07/2014	22.8	12.6	17.7	2524.8
270	15/07/2014	21.6	16.7	19.15	2543.95
271	16/07/2014	26.2	8.1	17.15	2561.1
272	17/07/2014	23.6	14.5	19.05	2580.15
273	18/07/2014	25.6	13.7	19.65	2599.8
274	19/07/2014	26.3	16.1	21.2	2621
275	20/07/2014	24.9	17.3	21.1	2642.1
276	21/07/2014	23.4	14.7	19.05	2661.15
277	22/07/2014	19.3	15.2	17.25	2678.4
278	23/07/2014	24.3	14.2	19.25	2697.65
279	24/07/2014	25	14.5	19.75	2717.4
280	25/07/2014	24.8	15.6	20.2	2737.6
281	26/07/2014	24.9	16	20.45	2758.05
282	27/07/2014	22.6	13	17.8	2775.85
283	28/07/2014	19.6	13.1	16.35	2792.2
284	29/07/2014	23.5	12.2	17.85	2810.05
285	30/07/2014	23.3	12.7	18	2828.05
286	31/07/2014	24.9	10.7	17.8	2845.85
287	01/08/2014	25	11.6	18.3	2864.15
288	02/08/2014	24.1	12.5	18.3	2882.45
289	03/08/2014	23.2	10.5	16.85	2899.3
290	04/08/2014	23.3	9.3	16.3	2915.6
291	05/08/2014	26	10.7	18.35	2933.95
292	06/08/2014	24.4	16.7	20.55	2954.5
293	07/08/2014	22.6	12.1	17.35	2971.85
294	08/08/2014	21.2	13	17.1	2988.95