GENETICS OF PARTIAL RESISTANCE OF WHEAT TO SEPTORIA TRITICI BLOTCH

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Abstract

Septoria tritici blotch (STB), caused by the fungus *Mycosphaerella graminicola*, is the major foliar disease of wheat in many countries. Most resistance in wheat cultivars is partial resistance, which is polygenic, or oligogenic and non-specific to particular pathogen genotypes, and hence is durable. Selection for partial resistance to STB may be restricted if that trait has a significant cost, for example reduced yield, which is the most important target for many wheat breeders. The first aim of the research reported here was to investigate if partial resistance to STB in wheat reduces yield, and if so, which yield components are affected. The second aim was to identify quantitative trait loci which account for this correlation. The data were obtained from a F1-doubled-haploid population of Senat x Savannah (Eriksen *et al.* 2003, TAG 107:515-527). Quantitative trait loci (QTL) controlling STB were located on four chromosomes. Here, genes increasing STB scores were closely linked in coupling to genes increasing yield components, including thousand grain weight and grains m^{-2}, and to genes that increase the qualitative trait grain protein content. Hence, there is a yield penalty of resistance to STB but it should be possible for breeders to select high-yielding resistant varieties by selecting genes which improve resistance but do not depress yield. The last part of this work refers to the location of a susceptible gene in the double haploid population Hobbit sib x Hobbit sib (Bezostaya 1 5BS-7BS). A QTL controlling susceptibility to STB was detected on a segment of chromosome 5B in this population. This QTL for susceptibility was closely linked with a QTL for heading date.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Disease importance

Mycosphaerella graminicola (anamorph *Septoria tritici*) is the ascomycete fungus that causes the disease Septoria tritici leaf blotch (STB) on bread and durum wheat, *Triticum aestivum* and *T. Turgidum*, respectively. Septoria tritici blotch may cause yield losses up to 50 % (King *et al*., 1983a) and more than 60% of the grain yield (Shipton, 1971; van Ginkel and S**c**haren, 1988a; Cornish, 1990) in highly susceptible cultivars. It has been the most damaging foliar disease of wheat in recent years in the U.K. (Bayles *et al*., 1985; Polley and Thomas, 1991; Cook *et al*., 1999; Hardwick, *et al*., 2001; Fraaije *et al*., 2005). Since the introduction of highly susceptible semi-dwarf, early-maturing varieties in the early 1970's, there has been a sustained increase in STB (Shipton, 1971; Danon *et al*., 1982; Shaner *et al*., 1975; Eyal *et al*., 1987; Austin *et al*., 1980; Angus, 2001; Hardwick *et al*., 2001). The success of these varieties is based on their good characteristics as highest yielding, resistant to lodging (Austin *et al*., 1980; Angus, 2001). Some of them are considered adequately hardy for coldest winters, or yield higher grain protein content and a good bread-making quality (Angus, 2001). Besides, the introduction of these cultivars very conveniently coincided with the use of fungicides, plant growth regulators and high quantities of nitrogen fertilizers (Shipton, 1971; Austin *et al*., 1980; Angus, 2001).

Although the control of the disease depends mainly on the application of fungicides, this activity has the inconvenience of being of high financial costs (Polley and Thomas, 1991), besides the fact that these pesticides have to be applied two or three times during the growing season (Cook *et al*., 1991; Hardwick *et al*., 2001; Robert *et al*., 2004). More recently, resistance of *M. graminicola* to the azole fungicides, sterol demethylation inhibitors (DMIs) has been reported by Cools and Fraaije (2006), Fraaije *et al*. (2007), and McCartney *et al*. (2007). They found that

mutations in the target enzyme, sterol 14α-demethylase (CYP51 gene) of *M. graminicola*, confer the resistance to these fungicides. In addition, resistance to strobilurin fungicides, quinone outside inhibitors (QoI), has also been identified (Fraaije *et al*., 2005; McCartney *et al*., 2007). Resistance to QoI fungicides is attributed to a mutation in the mitochondrial cytochrome b gene, target gene (G143A) (Fraaije *et al*., 2005; Fraaije *et al*., 2007). It has been shown, at least for QoI fungicides, that successive applications trigger the resistance of strains within populations through selection and asexual multiplication in a single season (Fraaije *et al*., 2005).

Resistance to STB is another major control measure, though resistance to Septoria tritici blotch has been broken down in the cultivar Gene of the USA, only five years after its commercial introduction, due to a fast evolution of local strains; it was allowed by favourable environmental conditions, wherein the frequency of virulence increased in the fungal population in the region where this cultivar was grown (Cowger *et al*., 2000).

1.2 Disease symptoms

Leaves inoculated with the disease remain green during the first eight to nine days after inoculation (Eyal *et al*., 1985; Kema *et al*., 1996a). The first symptoms appear as irregular rectangular chlorotic lesions that emerge 14 to 21 days after the inoculation, though this depends on the environmental conditions and cultivar (Eyal *et al*., 1985). Next, there appear necrotic lesions developing at the chlorotic sites of leaves of some cultivars. Necrosis is usually distinguished by its straw-, or straw-reddish- to greyish black- colour, with few and abundant pycnidia produced usually 15 days after the necrosis developed on the necrotic area (Kema *et al*., 1996a, 1996b). Pycnidia can also be found in green tissues of susceptible plants (Kema *et*

al., 1996b), and different isolates cause different symptoms in the host (Eyal, 1999). Although the genotype of the cultivar predisposes the symptoms (Lovell *et al*., 2004a), the amount of disease is also influenced by the morphological traits as plant height, canopy growth and architecture (Shaw and Royle, 1993; Lovell *et al*., 1997, 2004a). STB symptoms development is also influenced by light (Keon *et al*., 2007).

1.3 The pathogen

1.3.1 Taxonomy

The taxonomic name of the fungi involved in STB of wheat, in its sexual state (teleomorph), is *Mycosphaerella graminicola* (Fückel) J. Schröt in Cohn, while in its asexual state (anamorph), it is *Septoria tritici* Roberge in Desmaz. *Mycosphaerella graminicola* is classified in the kingdom Fungi (produce mycelium); Phylum *Ascomycota* (ascomycetes, the sac fungi, produce sexual spores called ascospores); Class *Ascomycete* (group of fungi that produces an ascus containing ascospores); Subclass *Loculoascomycetes* (the ascocarp -pseudothecia- is perithecioid in shape with an opening at the top, the ascus is bitunucate); Order *Dothideales* (the asci are developed in a stroma within perithecial cavities which lack a definitive wall); Family Dothideaceae; Genus *Mycosphaerella.* The asexual state, *Septoria tritici* is classified in the *Deuteromycetes* (imperfect or asexual fungi); Order *Sphaeropsidales* (produces pycnidiospores, in semi-closed fruiting bodies called pycnidia).

1.3.2 Reproduction and dispersal

Pycnidiospores, the asexual spores, can be present as macropycnidiospores or micropycnidiospores. Macropycnidiospores are 35-98 μm long by 1-3 μm wide, with three to five septa. Micropycnidiospores are 10.5 μm long by 0.8-1 μm wide without septa (Eyal *et al*., 1987), though there is variability in spore size (King *et al*., 1983a).

The former probably are generated from primary lesions and the latter from secondary lesions as a result of crowding (Shaw and Royle, 1993). Ascospores consist of two cells of unequal size and they are 10-15 μm long by 2-3 μm wide (Eyal *et al*., 1987). The bipolar heterothallic mating system (Kema *et al*., 1996c) results in a wide genetic variability (McDonald and Martinez, 1990).

The sexual form of the disease has been identified in Australia, the United Kingdom, the United States of America, the Netherlands, South America and Australia (Eyal *et al*., 1987), France (Halama, 1996) and Denmark (Eriksen and Munk, 2003). *M. graminicola* can complete several sexual cycles during the growing season (Kema *et al*., 1996a; Hunter *et al*., 1998). However, it seems that the number of sexual cycles completed in a season depends on cultivar susceptibility, and pseudothecia are only produced at the end of the growing season (Zhang *et al*., 1998; Cowger *et al*., 2002; Eriksen and Munk, 2003), and are developed on dead leaf tissue (Halama, 1996; Hunter *et al*., 1998; Eriksen and Munk, 2003). After working for three years with cultivars that differ in resistance to *M. graminicola*, Cowger *et al*. (2002) found a positive correlation between the susceptibility of the host cultivar and the frequency of sexual reproduction, as well as between the intensity of epidemics and the frequency of sexual fruiting.

Primary infections in new crops are due to airborne ascospores (Shaw and Royle, 1989a; McDonald and Martinez, 1990; Shaw and Royle, 1993; Eyal, 1999; Eriksen and Munk, 2003), probably from the remains of the crop from the previous year (Shaw and Royle, 1993; Eriksen and Munk, 2003). Infection of lower leaves and stem can occur as soon as they emerge after sowing (Shaw and Royle, 1989a, 1993). Subsequent conidia dissemination occurs due to rain splash from lower to higher up leaves to leaves in the canopy or to the surrounding leaves (Shaw and

Royle, 1989a). It has also been proposed that there is transmission by contact between different leaves with similar height or between emerging healthy leaves and infected ones (Shaw and Royle, 1993; Lovell, 1997, 2004a). Spatial distribution of STB is presented at random at the start of the infection in a crop; slowly, the disease spreads to infect the entire crop as it matures (Shaw and Royle, 1993).

Periods of rainfall are a key factor in the release and dispersal of spores, as well as in the initiation and development of the epidemics of STB in a crop (Eyal *et al*., 1987; Eyal, 1999; Thomas *et al*., 1989; Shaw and Royle, 1993; Lovell *et al*., 1997, 2004b). Rain storms move spores in the area around the crop and by splash on leaf layers on a plant. Windblown ascospores are another source of epidemics (Eyal *et al*., 1987; Shaw and Royle, 1989a; Eyal, 1999).

1.4 Wheat-*Septoria tritici* interaction

1.4.1 Infection process

Septoria tritici is a hemibiotrophic pathogen with a long symptomless phase considered as biotrophic, followed by the necrotrophic phase. This phase conducts to a tissue collapse which further results in chlorosis and necrosis development on the leaf, and pycnidia formation (Kema *et al*., 1996d; Dancer *et al*., 1999; Shetty *et al*., 2003; Shetty *et al*., 2007).

The infection process has been reported to appear with a relatively high frequency, through germination of ascospores and conidium on the surface of leaves (Kema *et al*., 1999). Germination of spores occurs by elongation or by budding (Eyal *et al*., 1987; Eyal, 1999) giving place to the formation of branched Germ tubes or clusters of hyphae (Kema *et al*., 1996d), sometimes forming even appressorium-like structures (Kema *et al*., 1996d; Shetty *et al*., 2003). After penetration takes place

through the stomata, (Cohen and Eyal, 1993; Eyal, 1999; Kema *et al*., 1996d, 1999; Duncan and Howard, 2000; Shetty *et al*., 2003) or in anticlinal cell wall grooves (Cohen and Eyal, 1993; Dancer *et al*., 1999), the mycelium is formed in the stomatal cavity. Hyphae colonize then the mesophyll intercellularly and continue growing and expanding (Kema *et al.*, 1996d; Shetty *et al*., 2003). From a single penetration site, STB invades the apoplast and the neighbouring substomatal cavities establishing multiple stomata infections (Duncan and Howard, 2000).

During colonization, a latent period exists where no visual symptoms are present (Kema *et al*., 1996d; Dancer *et al.*, 1999; Shetty *et al.*, 2003; Shetty *et al*., 2007). The latent period could last for 11 to 36 days, depending on the temperature (Lovell *et al*., 2004b). In this period, no mesophyll cells are penetrated or damaged and it is supposed that the pathogen survives on available nutrients present in the apoplast (Dancer *et al.*, 1999). The cell mesophyll walls will eventually become wrinkled, collapse and finally die (Kema *et al.*, 1996d; Dancer *et al.*, 1999; Palmer and Skinner, 2002). With cell death, mycelia proliferation takes place (Kema *et al*., 1996d; Dancer *et al*., 1999; Jørgensen and Smedegaard-Peterson, 1999).

Mature pycnidia form in the substomatal cavities and are of subglobose (not entirely spherical) shape, with the ostioles confined by the stomatal openings, usually with occupancy of one per substomatal cavity (Eyal *et al*., 1987; Kema *et al*., 1996d). The conidiophores on the pycnidium wall form aseptate conidia and they have two layer walls in their mature state, and are exuded through the ostiole in a cirrhus (Kema *et al*., 1996d). The number of pycnidiospores liberated per pycnidium has been reported to be of the order of $5\text{-}10\times10^3$ (Eyal, 1999).

Infection success reaches its highest levels on rainy cloudy days with temperatures between 20-25°C and with high relative humidity (Magboul *et al*.,

1992; Kema *et al*., 1996d; Dancer *et al.*, 1999). Temperature and relative humidity (leaf wetness) are also important environmental factors for subsequent disease development (Magboul *et al*., 1992). Jørgensen and Smedegaard-Petersen (1999) mentioned that if one environmental factor is under the optimum, it is possible that another parameter may compensate the former during the STB process of infection (also in *Septoria nodorum*) and disease development. This is explained by Magboul *et al*. (1992), who studied the interaction of leaf wetness period and temperature on the infection process. He found that the temperature range at which infection occurs increases when the leaf wetness period is prolonged; thus, for example, with a leaf wetness period of 25 hours after inoculation (hai), the effective temperature range was 13-23ºC, but when the leaf wetness period was greater than 80 hai, the effective temperature range increased to 9–25ºC.

The absence of an interaction or incompatible response can be explained by the lower colonization of the host tissues and no visible effects on the mesophyll cells (Kema *et al*., 1996d). Although hyphae can be observed in the vicinity of the stomata cavities and occasionally between the mesophyll cells (Shetty *et al*., 2003), no visible detrimental effects are caused. In such an incompatible interaction, the fungus is limited in colonization and often fails to form pycnidia (Kema *et al.*, 1996d; Shetty *et al*., 2003), or the number of pycnidia is low (King *et al*., 1983a; Cohen and Eyal, 1993).

1.4.2 Wheat responses: metabolic processes

The signal perception and signal transduction systems to activate defence responses by wheat against STB are not known; the pathogen molecules that result in plant susceptibility are not known either. Defence–related genes have been identified during the first hours after inoculation, as well as a second gene induction that begins

18-24 days after inoculation (dai) in resistant cultivars (Ray *et al*., 2003; Adhikari *et al*., 2007). In addition, reactive oxygen species (superoxide (O_2^-) and hydrogen peroxide (H_2O_2)) have been detected during the early and late gene-induction (Shetty *et al*., 2003, 2007; Keon *et al*., 2005, 2007). Features of hypersensitive response and host programmed cell death have also been investigated (Keon *et al*., 2007; Rudd *et al*., 2008).

1.4.2.1 Early gene-induction

Ray *et al.* (2003) identified genes that are differentially expressed by the plant during the resistance response to *M. graminicola*. While working with resistant and susceptible cultivars, Tadinia and Yecora Rojo, they detected: the putative defence response genes; a protein disulfide isomerase (PDI) gene; three defence response genes, the pathogenesis related proteins: PR-1, PR-2 (β-1,3-endoglucanase) and PR-5 (a thaumatin-like protein); and the WCI-2 gene, a lipoxygenase (LOX). Transcripts of LOX were detected at 0 hai in both susceptible and resistant cultivars, though in the resistant cultivar a maximum peak was detected at 3 hai with a sudden decreased; whereas in the susceptible cultivar the maximum peak was found at 6 hai, with a more continuous expression than that shown with the resistant cultivar. Ray *et al*. (2003) also found that PDI-transcripts were present at 0 hai with a maximum expression in the susceptible cultivar at 6 hai, remaining in this condition until 12 hai; later, the levels of transcripts declined. Yet, in the resistant cultivar the maximum peak was at 12 hai, two times higher than in the susceptible cultivar. Ray and co-workers also detected that PR-transcripts began to accumulate at 3 hai, with a maximum expression at 12 hai, followed by a sharp decline in both, the resistant and the susceptible cultivars, though the expression was ten times higher in the resistant cultivar.

Early recognition (0 hai) of *S. tritici* by wheat is due probably to compounds delivered by the fungus that trigger the activation of defence related transcription genes by the plant (Ray *et al*., 2003). The precise role of these genes in the interaction wheat-*S. tritici* is unknown. Ray *et al*. (2003) suggest that PDI could have a regulatory role in the plant disease signalling pathway by preventing potential cell damage by reactive oxygen species (ROS). LOX could help in the establishment of *S. tritici* in the plant; thus, its expression is brief in resistant cultivars. It is known that the expression of the PR gene family confers important disease resistance responses. In agreement with the proposal function of PDI, Apel and Hirt (2004) pointed out that PDI is an antioxidant defence protein that accumulated during oxidative stress in *Arabidopsis*. Nevertheless, LOX has been found to be a chloroplastic enzyme involved in the first step of the jasmonates biosynthesis, signalling compounds that induce host defence, as well as its production of O_2^- (Vidhyasekaran, 2008). PR genes as PR-1, PR-2, and PR-5 encode protective proteins against pathogens. Thus, it has been suggested that PR-1 proteins might be involved in cell wall thickening and may offer resistance to the spread of pathogens in the apoplast. PR-5 alters the permeability of fungal membranes. PR-2, an extracellular enzyme constitutively expressed in several plants (Vidhyasekaran, 2008), acts releasing β-1,3 glucans, which are components of fungal pathogens cell walls that have been characterized as elicitors that trigger other plant defence responses (Ray *et al*., 2003; Vidhyasekaran, 2008).

In addition, Adhikari *et al*. (2007) when they were working with the resistant cultivars Tadinia and W7984 and the susceptible cultivars Yecora Rojo and Opata 85 also found early induction (3 hai) of four defence genes: phenylalanine ammonia lyase (Pal), chitinase (Chit), peroxidase (Per) and PR-1 (a gene found also by Ray *et*

al., 2003). The time and magnitude of expression of these four genes differ among cultivars. Pal was induced at three hai in the resistant cultivars, but its expression was eight-fold over the mock in Tadinia and persisted until 6 hai, then dropped to two-fold until 2 dai, while in W7984 the induction was only two-fold over the mock at 3 hai and then disappeared at 12 hai. Chit reached a maximum peak at 3 hai in Tadinia, whereas in W7984 at 1 dai, 40-fold and 60-fold over the mock (control), respectively; the expression of this gene disappeared at 3 dai in both cultivars. Per had a maximum expression at 12 hai in Tadinia but only 10-fold over the mock, while in W7984 the maximum peak was reached at 1 dai and 50 times over the mock, then dropped and disappeared at 6 dai. In agreement with Ray *et al*. (2003), PR-1 presented a maximum expression at 12 hai in Tadinia, but at 1 dai in W7984; the magnitude of expression was similar in both cultivars, around 10-times over the mock.

Adhikari *et al*. (2007) found that transcription of these four genes in the susceptible cultivars was very slight and that Pal was involved in the synthesis of antimicrobial compounds, of antioxidant protectans (as flavonoid compounds), and of precursors of lignin. Vidhyasekaran (2008) indicated that Pal was the key enzyme in the synthesis of the following compounds: 1) phenolics; constitutive secondary metabolites with antifungal properties which are implicated in disease resistant response and some of which are highly toxic to pathogens and have been found in wheat; 2) phytoalexins; compounds synthesized in response to infection as flavonoids, and; 3) salicylic acid, a signal molecule which induces several defence-related genes as PR-1, PR-2 and PR-5 proteins.

Chitinases have also been reported to be secreted extracellularly by plants when pathogen penetrates host tissues (Vidhyasekaran, 2008); as they degrade the

fungal cell wall and release the elicitor chitin Per and PR-9 proteins and are associated with the generation of superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (Apel and Hirt, 2004; Vidhyasekaran, 2008).

Pathogen elicitors that can induce defensive responses by both host and non host plants (Heath, 2000; Nürnberger and Brunner, 2002) are called general elicitors (Nürnberger and Brunner, 2002). Two of these have been identified as chitin and ergosterol from fungi (Zipfel *et al*., 2004). The cultivar-specific resistance expressed during the gene-for-gene interactions, is genetically determined by the complementary avirulence (Avr) genes and the resistance (R) genes. Each avirulence gene encodes a specific elicitor that is recognized by the product of the R gene, and specific defence responses from the host are triggered (Flor, 1971; Heath, 2000; Dangl and Jones, 2001; Cohn *et al*., 2001; Nürnberger and Brunner, 2002; Zipfel *et al*., 2004; Vidhyasekaran, 2008). In the non-cultivar-specific host resistance (non-host plants), inducible defence responses (Heath, 2000) or basal defences (Dangl and Jones, 2001: Zipfel *et al*., 2004), or innate defence mechanisms (Nürnberger and Brunner, 2002) are also triggered without the activation of R genes and without compromising the entire resistance according to Heath (2000). Dangl and Jones (2001), and Heath (2000) pointed out that defence mechanisms are shared by both cultivar-specific and non-cultivar-specific systems (genetic overlap).

1.4.2.2 Late gene-induction

Adhikari *et al*. (2007) found that late gene response in resistant cultivars took place when the fungal biomass increased in susceptible cultivars (first lesions were visible at 16 or 18 dai in Opata 85 and Yecora Rojo, respectively). Resistant cultivars on the other hand seemed to recognize the change from biotrophic to necrotrophic phase of STB (no significant necrosis was observed in Tadinia and slight infection in W7984

at 24 dai). Three defence-related genes were detected during the late response induction to STB: ADP-glucose pyrophosphorylase (Agp), ATP synthase (ATPase) and brassinosteroid-6-oxidase (Brox). Maximum peak of expression of Agp at 24 dai took place in the resistant cultivars, though in Tadinia the magnitude of expression was 1400-fold over the mock, while W7984 an 800-fold over the mock was observed. The ATPase was expressed at 18 dai in Tadinia and at 24 dai in W7984, with a magnitude of 550-fold and 200-fold over the mock, respectively. Conversely, maximum expression of Brox in Tadinia was observed at 24 dai while in W7984 at 18 dai, this corresponded to 300-fold and 400-fold over the mock, respectively.

Differential expression of other six genes was also found in the resistant cultivars: 40S ribosomal protein (40Srp), protease inhibitor Bsi1 (Bsi), methionine sulfoxide reductase (Msr), peptidylprolyl isomerase (Ppi), RNase S-like protein precursor (RNase) and peroxidase 2 (Per2), at different times and magnitudes. A lower expression of these nine genes was found in the susceptible cultivars. Bimodal patterns of these genes were clear in the resistant cultivars, the first one at 1-3 dai and the second one (the strongest expression) at 12-24 dai, with expressions ranging from 200 to 1400-fold higher than the mock. Only two of the nine genes have been associated with the induction of defence response, Bsi and Per2, though the function of Bsi is not known (Adhikari *et al.*, 2007).

Differences in signal perception and signal transduction systems may be leading to resistance or susceptibility (Vidhyasekaran, 2008). Several of these differences have been identified and could partly explain the different expression in the genes identified in the interaction wheat-STB. For example, signalling pathways are expressed faster in the resistant cultivar than in the susceptible one. This performance gives rise to a delayed expression on pathways that trigger the

appearance of defence genes. Therefore, if the accumulation of defence compounds is delayed, the disease develops. Some elicitors are less active in the susceptible cultivar inducing fewer defence compounds than in the resistant cultivars (Vidhyasekaran, 2008).

1.4.2.3 Reactive oxygen species accumulation

During the germination, penetration, and colonization of the mesophyll by *Septoria tritici*, an accumulation of hydrogen peroxide (H_2O_2) was detected at 3 and 6 hai in outer epidermal cell walls, in mesophyll cells, and especially around the substomatal cavities, both in susceptible and resistant cultivars (Shetty *et al*., 2003). However, they observed that the accumulation of hydrogen peroxide was faster in the resistant cultivar, occurring particularly around the substomatal cavities where penetration took place, and in the mesophyll cell walls it was associated with the arrest of fungal growth in this cultivar. In contrast, in the susceptible cultivar a slight accumulation of hydrogen peroxide took place at the beginning of the infection (Shetty *et al*., 2003). An accumulation of superoxide ions, O_2^-, and hydrogen peroxide was detected at the chlorotic areas, "water-soaked areas", just before the necrosis initiates (Keon *et al*., 2007). A massive accumulation of H_2O_2 (Shetty *et al*., 2003, 2007) and O_2^- (Keon *et al*., 2005, 2007) throughout the mesophyll and the substomatal cavities coincides with sporulation of the fungus (13 dai), and it is associated with cell collapse and visual symptoms e.g. necrosis (Shetty *et al*., 2003, 2007; Keon *et al*., 2005, 2007).

Shetty *et al*. (2003, 2007) proposed that the accumulation of hydrogen peroxide at the beginning of the infection, also during the sporulation of *S. tritici*, was a defence reaction of the plant. Their arguments were based, firstly, on the roles suggested in the defence reaction of active oxygen species [*e.g*. superoxide (O_2^-), hydroxyl radical (OH·) and hydrogen peroxide (H_2O_2)], the "oxidative burst", such

as cell wall modifications, lipid peroxidation, phytoalexin production, and activation of defence related genes. Secondly, their arguments also rely on tests in which wheat leaves infiltrated with catalase detoxifying H_2O_2 in the first and late phases of infection result in an increased penetration, colonization and fungal biomass, and the latent period is reduced, while infiltration with H_2O_2 resulted in reduced colonization and an increased latent period.

Keon *et al*. (2005) suggested that the latest accumulation of reactive oxygen species (ROS) was probably originated from the fungus itself because the leaf at such stage is totally senesced. Keon *et al*. (2007) pointed out that it has to be defined if ROS is generated by the plant or by the fungus. It has been found that necrotrophic pathogens often promote susceptibility-related host cell death through the generation of ROS (Vidhyasekaran, 2008).

1.4.2.4 Hypersensitive response (HR) and programmed cell death (PCD) in the interaction STB-wheat

With reference to the HR and PCD, Keon *et al*. (2007) and Rudd *et al*. (2008) found, working with susceptible cultivars, that as disease symptoms develop, the following processes take place: 1) a loss of membrane integrity restricted to the mesophyll cells (9 dai onwards, beginning of symptoms); 2) an accumulation of cytochrome c in the cytosol (10 to 14 dai); 3) a host DNA laddering response {cleavage of genomic DNA into internucleosomal 180 bp fragments (Tada *et al*., 2001) (9 dai onwards); 4) a disappearance of chloroplastic rRNA species (14 dai, pycnidial initials). All these features were associated with apoptosis (Vidhyasekaran, 2008). They have proposed that the development of disease symptoms in STB-susceptible wheat has characteristics of hypersensitive response (HR)-like programmed cell death (PCD). However, the hypersensitive response (HR) is a

feature of a resistance expression to host and less commonly to non host pathogens (Heath, 2000; Vidhyasekaran, 2008). This is also called hypersensitive cell death or HR-related cell death, and it is induced by host signals (endoelicitors) that trigger resistance gene-encoded proteins. Thus, HR is also called host-induced cell death, and it is a form of PCD, a genetically controlled cellular suicide (Vidhyasekaran, 2008).

The HR consists of a rapid cell death at the site of the infection that is associated with a pathogen limitation as well as with the defence gene activation (Heath, 2000; Cohn *et al*., 2001; Vidhyasekaran, 2008). Susceptible-related cell death or normosensitive cell death is found in susceptible interactions that seem to confer susceptibility to necrotrophic pathogens. In this case, the induction of the cell death may be by virulence factors from the pathogen, as toxins and cell wall degrading enzymes, or through generation of toxic levels of ROS; sometimes PCD is observed (Vidhyasekaran, 2008). Although both types of cell death could show similar characteristics, the function could be different. HR-cell death restricts the pathogen invasion and proliferation within the host, depraving it from water or nutrients and triggering other defence responses (Cohn *et al*., 2001; Vidhyasekaran, 2008). In biotrophic/ necrotrophic pathogens, susceptible-related cell death supplies nutrients to the fungus and enhances the disease development (Vidhyasekaran, 2008). Thus, though there is not an identified host elicitor that promotes PCD, the mesophyll cell death during the interaction STB-wheat seems to be a susceptible-related cell death, not a HR-like PCD.

Putting all together and keeping in mind that timing depends both, on environmental factors and on cultivar, at least during the first hours of the *S. tritici* infection and during its sporulation. Several events occur in both resistant and susceptible

cultivars. Early events that occurred were: (i) at 0 hai, transcripts of PDI and LOX began to accumulate in both cultivars; (ii) at 2 hai, the germination of spores took place; (iii) at 3 hai, in the resistant cultivar, transients of LOX reached a peak, and slow accumulation of H_2O_2 was detected; maximum expression of Pal was detected in the resistant cultivars Tadinia and W7984, as well as Chit in Tadinia; (iv) at six hai, maximum expression of LOX and PDI were detected in the susceptible cultivar and H_2O_2 was present; then there was a decline in LOX transient but PDI sustained its expression; (v) at 12 hai, hyphae began to penetrate the substomal cavities and there was maximal expression of the defence response genes PDI, PR-1, PR-2 and PR-5, as well as for Per2 in the resistant cultivar Tadinia; (vi) at 24 hai, maximum peak of the defence response genes Chit, Per2 and PR-1 is detected in cultivar W7984. Later events that occurred were: (vii) at 9 dai, starting with the appearance of the first lesions onwards, there was a loss of membrane integrity which was restricted to the mesophyll cells, a disappearance of chloroplastic rRNA species, and a host DNA laddering response, as well as an accumulation of cytochrome c in the cytosol, in addition to the maximum expression of the ATPase reached in Tadinia and Brox in W7984 genes; (viii) at 13 dai, the sporulation of the fungus took place in an enriched superoxide and hydrogen peroxide environment, and a maximum expression of Agp was observed in both resistant cultivars. A maximum expression of ATPase in W7984 and Brox in Tadinia was also obtained.

1.4.3 Crop physiology of the disease plants

Infection with *M. graminicola* was reported in the past to cause a considerably reduction of the yield, characterized by the production of shrivelled grains in the susceptible cultivars tested. For the more tolerant cultivars the loss of yield and quality from severe infection was relatively low (Ziv and Eyal, 1976, 1978; King *et*

al., 1983a). In the literature, it has been widely documented that the STB colonization and dispersal in the crop is associated, firstly, with a reduction in the photosynthetic activity of the infected leaves after the lesion formation, mainly due to the green leaf area reduction (Shaw and Royle, 1989a; Thomas *et al*, 1989; Cornish *et al*., 1990; Magboul *et al*., 1992; Leitch and Jenkins, 1995; Zuckerman *et al*., 1997; Parker *et al*., 2004; Robert *et al*., 2006; Foulkes *et al*., 2006). Secondly, this phenomenon has also been associated with the earlier senescence of leaves (Shaw and Royle, 1989b; Cornish *et al*., 1990; Leitch and Jenkins, 1995; Zuckerman *et al*., 1997; Lovell *et al*., 1997; Parker *et al*., 2004), and with the induction of apical senescence, as reported by King *et al*. (1983b), Magboul *et al*. (1992) and Robert *et al*. (2006).

According to Robert *et al*. (2006), during the symptomless phase of the infection, STB has no significant effect on net photosynthesis and respiration but, with the expression of symptoms, both become significantly altered. In the period of the chlorotic symptoms, there is a decrease in the leaf photosynthesis and although this process persists in the chlorotic areas (Robert *et al*., 2006), a reduction in chlorophyll content (Zuckerman *et al*., 1997) is possible, lowering the effect on the net photosynthetic rate. Necrotic lesions however, decreased the net photosynthesis, the leaf photosynthetic capacity and, at the same time, it enhanced leaf respiration up to three times those found in diseased leaves (Robert *et al*., 2006). The increase in respiration of typical plants infected by virus, bacteria and fungi, has been related to the induction of host defence mechanisms, and/or to fungus respiration (Lucas, 1998; Robert *et al*., 2006). The consequence of all these is reflected on the assimilate supply alteration, due to the disrupted photosynthetic apparatus (Dimmock and Gooding, 2002b). The authors suggest that plant metabolism performances

differently, this in correspondence to the form of nutrition of STB, as biotrophic or necrotrophic fungus. As referred by them the biotrophic state consists in the ability of STB to redirect and retain N in infected tissues, whereas during the necrotrophic state STB reduces carbon assimilation due to the damage produce on the photosynthetic capacity. Furthermore, as a biotrophic fungus, STB reduces both nitrogen uptake and partitioning of N into the grain, decreasing the protein concentration (McCabe *et al*., 2001; Ruske *et al*., 2001; Ruske *et al*., 2003). As a necrotroph, STB probably has a greater effect on carbon accumulation, as has been detected in *S. nodorum* (S**c**haren *et al*., 1975).

Environmental factors are of great importance in the development cause-effects of the disease. Magboul *et al*. (1992) found, in the susceptible spring cultivar Anza in controlled conditions, that temperature and leaf wetness periods were critical for auguring the disease severity (percentage of infected area) and the senescence development (percentage of senescent area) of the flag leaf. These authors found, that the development rate of disease severity reached its maximum at 18ºC, ranging from 3.0 to 9.7% per day, this when testing different temperatures and misting periods (leaf wetness periods). Magboul and co-workers (1992) concluded the more the leaf wetness period increased, the more severe the disease was. Higher temperatures and long periods of leaf wetness tended to increase the maximum rate of senescence. Thus, maximum senescence (2% per day) was presented at 20ºC and 96 h of wetness leaf period.

The effects of the senescence induced by STB have been investigated by the analysis of the green flag leaf duration (delayed senescence) after application of fungicides on plots, as compared with control plots of diseased plants. Senescence decreased the rate of grain filling, with the consequent effect on the diminution of

grain yield, grain weight, (Gooding *et al.*, 1994, 2000; Ruske *et al*., 2001; Dimmock and Gooding, 2002a), specific weight (Gooding *et al*., 1994; Ruske *et al*., 2001; Dimmock and Gooding, 2002a), and grain size in some cultivars (Dimmock and Gooding, 2002a). However, Dimmock and Gooding (2002a) suggested that these effects probably overestimated the favourable physiological reactions the plant experienced (*e.g*., the delayed senescence) under the application of fungicides (Ruske *et al*., 2001; McCabe *et al*., 2001; Ruske *et al*., 2003; McCartney *et al*., 2007). As a consequence of this, grain yield improved (Gooding *et al*., 1994; Cook *et al*., 1999; Gooding *et al*., 2000; Dimmock and Gooding, 2002a, 2002b; Ruske *et al*., 2001, 2003; McCartney *et al*., 2007), at least with strobilurin fungicides, increase triggered by a shift in the hormonal balance, producing the inhibition of ethylene biosynthesis, which in turn provokes a decrease in chlorophyll catabolism, and the maintenance or increase of cytokinin contents, inducing in turn a chlorophyll and thylakoid formation, and hence an increase in CO_2 uptake (Grossmann and Retzlaff, 1997).

Analyses of the disease plants have showed a reduction in: leaf area by up to 40 %; green area index by 23 %; leaf area index by 69 %; and dry matter by 20% according to Cornish *et al*. (1990), and a harvest index reduction of 40% has also been reported in susceptible cultivars by Zuckerman *et al*. (1997). Furthermore, the effect of STB on yield and yield components depended on the severity of the epidemic at a given developmental stage of the crop at the time of the infection (Ziv and Eyal, 1976; Cornish *et al*., 1990; Shaw and Royle, 1993; Simón *et al*., 2002). Infection of wheat by STB at the seedling stage reduced both, root development (31.4-61.1%), and according to King *et al*. (1983a), foliage (12.5-19.4%). Although on one hand early infections can reduce the number of ears per square metre, on the other, late infections can diminish the grains per ear or the thousand grains weight

(Leitch and Jenkins, 1995; Simón *et al*., 2002). Yield losses occur usually when the flag leaf, specially, was severely infected, as well as the second and third leaves (Shaner *et al*., 1975; Ziv and Eyal, 1978; King *et al*., 1983a; Thomas *et al*., 1989; Shaw and Royle, 1989a, 1989b, and 1993; Paveley, 1999). These leaves are mainly the responsible of providing photo-assimilates for the developing grain.

Thus, in general, the damage that STB caused to the crop depended on: 1) the crop stage at the time of infection; 2) the developmental rate of infection; 3) the duration of the symptomless phase of the infection which could vary among cultivars (Shaw, 1990); 4) the development rate of disease severity; 5) the percentage of infection and severity on the flag leaf; 6) the development rate of senescence, and 7) the environmental conditions in which the diseased crop develops.

1.5 Factors that affect the risk and progression of STB on crop

Disease response is a highly complex reaction of the plant to fungal attack. Resistance expression of plants to pathogens, though genetically inherent, may be increased or decreased by changes in plant metabolism and physiology through growth pattern, plant morphology and anatomy and by changes in chemical composition. Apparent host resistance known as "escape of attack" or "disease escape" has relatively small effects in highly susceptible or highly resistant cultivars, but considerable responses can be achieved in moderately susceptible and partially resistant cultivars (Marschner, 1990). The mechanisms of disease escape can vary, and are usually determined by environmental conditions which intricately affect plant metabolism to give such response. The performance of a genotype could change in different environments. Thus, environmental variables of a "macro" nature

have to be considered, as well as other possible differences in the environment — "micro" ones that affect individual plants (Brown and Caligari, 2008).

1.5.1 Agronomic practices

Nitrogen (N) fertilization is a major factor affecting the morphology of leaf, shoot and root, as well as the yield and the yield components of wheat. However, there are controversial results about the effect of increasing the amount of N fertilizer in the development of STB. High quantities of applied N fertilizer promoted disease development (Howard *et al*., 1994; Leitch and Jenkins, 1995; Lovell *et al*., 1997; Simón *et al*., 2003; Olesen *et al*., 2003a and b) irrespective of the timing of its application (Leitch and Jenkins, 1995). Differences in severity could be either not statistically significant, until a few weeks after the first symptoms appear (Lovell *et al*., 1997), or they were not relatively high (Howard *et al*., 1994; Simón *et al*., 2003). The severity has been reported to be different for each year of experiment (Howard *et al*., 1994; Simón *et al*., 2003). The effect of N rate applied could diminish the severity with later application (Leitch and Jenkins, 1995; Olesen *et al*., 2003a and b). Conversely, no effect has been reported of high N fertilizer rate on the development of STB (Johnston *et al*., 1979; Tompkins, *et al*., 1993; Olesen *et al*., 2000; Olesen *et al*., 2003a). Furthermore, Olesen *et al.* (2003b) found no significant correlations between STB and leaf N concentration in two of the three seasons of experiments. These authors also found that splitting N-fertilizer application the disease was less severe whereas the severity increased under single applications of N.

Dense canopy structures with higher levels of N applied allowed favourable microclimatic conditions for STB development (Shaw and Royle, 1993 Leitch and Jenkins, 1995; Lovell *et al*., 1997). Some of the reasons suggested to explain the increasing severity of STB at higher rates of N fertilizer were the nutritional status of

the host (Leitch and Jenkins, 1995), the time of infection in relation to the emergence of the leaf layer (Leitch and Jenkins, 1995; Lovell *et al*., 1997), the type of N fertilizer applied (Simón *et al*., 2003), and the high N-leaf concentration and reduction of phenolic compounds (Olesen *et al*., 2003b). In contrast, in order to offer a sound explanation to the non-effect of N rates on the STB increase in plants, Olesen *et al*. (2000) and Johnston *et al*. (1979) referred to possible N fertilizer indirect effects on spore dispersal (higher plants present less risk of infection on upper leaves). Tompkins *et al*. (1993) stated that this response might be due to the environmental factors affecting the infection process.

Therefore, as stated before, it is concluded that contradictory performance and complexity of the plant metabolism and STB interaction with the environment, as applied to plant response as affected by N application may still not be sufficiently clear, needing more research. The studies exposed above, were realized using different rates and types of N fertilizers. N- ammonium nitrate was applied (*i.e*. Howard *et al*., 1994; Leitch and Jenkins, 1995), or urea (i.e. Simón *et al*., 2003) alone, or in combination with phosphorus (P) and potassium (K) fertilizers (*i.e*. Johnston *et al*., 1979; Howard *et al*., 1994; Tompkins *et al*., 1993; Leitch and Jenkins, 1995; Simón *et al*., 2003; Olesen *et al*., 2000, 2003a and b), and manganese (Mn) (*i.e*. Olesen *et al*., 2000). Applying N alone to the plant provokes a nutrient imbalance (Marschner, 1990; Tompkins *et al*., 1993), which may produce more disease-susceptible plants (Marschner, 1990). High rates of N fertilizer enhanced the susceptibility to biotrophic pathogens. The effect in necrotrophic pathogens was opposite (Marschner, 1990). Thus, it is possible that STB, as a hemibiotrophic pathogen, incited two different responses in wheat: the increase of the disease during the biotrophic state (*i.e*. Leitch and Jenkins, 1995; Lovell *et al*., 1997; Simón *et al*.,

2003; Olesen *et al*., 2003a and b); and reducing it with later application, as necrotrophic fungus (*i.e*. Leitch and Jenkins, 1995; Simón *et al*., 2003; Olesen *et al*., 2003a). High rates of N application depress the enzymes of phenol metabolism and consequently diminishing the concentration of phenolics (Marschner, 1990; Olesen *et al*., 2003b). Coincidently, in those experiments in which not only N, but also P and K fertilizer and/or Mn were applied to the soil, no significant STB severity increase or diverse responses to the increase of N were observed. Potassium increases the resistance to pathogens in plants; its effect has been related to the metabolic functions as: enzyme activation, synthesis of proteins, photosynthesis, and osmoregulation (Marschner, 1990). Manganese may be directly involved in phenolic synthesis in the cell wall, and it has been reported to play a role as inducer of phenylalanine ammonia-lyase (PAL), an enzyme involved in the biosynthesis of several defence compounds (Vidhyasekaran, 2008).

Incorporating wheat straw into the soil can reduce the severity of STB (Rodgers-Gray and Shaw, 2000). They suggested that straw incorporation results in changes in the availability of soil nutrients, probably altering the pH which may increase concentration of silica (Si) in leaves of plants grown in straw-treated plots. Relatively high Si contents (23 % of ashes) in leaves and sheaths of wheat straw have been reported (Hess *et al*., 2003). It has been found that Si is rapidly absorbed and transported by wheat (Rafi and Epstein, 1999). Also, a large deposition of phenolic compounds has been reported in infected epidermal cells in plants treated with Si solutions (Bélanger *et al*., 2003).

High tillering capacity or agronomic practices that promote it may increase the effect of STB on yield losses of susceptible varieties (Ziv and Eyal, 1976), perhaps as a result of green area increase. Reduced plant density in the field

increased nutrient availability aggravating the disease (Rodgers-Gray and Shaw, 2000), and increasing the dispersal ability of STB spores by rain-splash (Shaw and Royle, 1993).

Crops sown at early times pose greater risks of infection (Shaner *et al*., 1975; Thomas *et al*., 1989; Shaw and Royle, 1993). These plantings have shown a considerable higher severity of STB than those made in late sowing dates (Shaner *et al*., 1975; Shaw and Royle, 1986a, 1993). Early sown plants produced more leaves (Shaw and Royle, 1986a, 1993), and resulting in greater presence of inoculums (Shaner *et al*., 1975; Shaw and Royle, 1986a, 1993); stem extension was also slower, which give more time for the infection to move from older to younger leaves (Shaw and Royle, 1993; Lovell *et al*., 1997, 2004b). Time to maturation was longer too, giving rise to another STB cycle of multiplication in diseased plants (Shaw and Royle, 1993). However, leaves in late-sown crops emerged lower than those of an early-sown crops and therefore, closer to basal inoculum sources (Lovell *et al*., 1997), though the rapid stem extension and reduced vegetative growth diminished the risk of STB disease diminished too through the reduced inoculums production and restricted opportunity for the inoculum to be transferred to the upper leaves (Shaw and Royle, 1993).

1.5.2 Disease escape

In wheat, disease escape prevents or reduces contact between pathogen spores and the upper canopy (Paveley *et al*., 2005), as the damage is mostly related to the disease on the top leaves whose effect is the reduction of yield (Shaner *et al*., 1975; Ziv and Eyal, 1978; King *et al*., 1983a; Thomas *et al*., 1989; Shaw and Royle, 1989a, b, and 1993; Paveley 1999; Paveley *et al*., 2005).

Late maturity and tall plants were associated with resistance to STB. Due to the fact that plants combining short stature and earliness with resistance have been difficult to obtain, it has been proposed that both traits could be genetically linked or present pleiotropy with resistance (Danon *et al*., 1982; Eyal and Talpaz, 1990; van Beuningen and Kohli, 1990). Negative and no significant correlations have been found between plant height and STB (Danon *et al*., 1982). Also negative significant associations have been reported (van Beuningen and Kohli, 1990; Camacho-Casas *et al*., 1995; Chartrain *et al*., 2004b; Simón *et al*., 2004b, 2005; Arraiano *et al*., 2006), probably because tall plants present a physical barrier to the spread of STB spores upwards (van Beuningen and Kohli, 1990; Fraaije *et al*., 2002). However, Arraiano *et al*. (2006) found a positive correlation between distance from flag leaf to leaf 2 and STB, suggesting that there was more disease as the distance between leaf 2 and the flag leaf increased. Eriksen *et al*. (2003) found a quantitative trait locus (QTL) with a resistant effect to STB on chromosome 3A of Senat cultivar, probably the resistance gene *Stb6* (Brading *et al*., 2002; Chartrain *et al*., 2005b), and a QTL with an effect on plant height on the same chromosome. Also, they found a QTL with a resistant effect on chromosome 2B and, on the same chromosome, a QTL with an effect on height. Thus, a genetic linkage between plant height and resistance to STB seems to exist.

The height encoded by *Rht* genes is a major trait that confers disease escape (Fraaije *et al*., 2002). In the semi-dwarf varieties, *Rht* genes and susceptibility are not *per se* genetically linked. The susceptibility is related to the height because the pathogen has easier access to the upper leaves (van Ginkel and Rajaram, 1993). However, associations between each *Rht* gene and resistance have been found. Plants carrying the *Rht-D1b* (formerly *Rht1*) gene were more susceptible than those with

Rht-B1 (formerly *Rht2)* (Baltazar *et al*., 1990). Plants carrying *Rht3* and *Rht12* were more diseased than those with *Rht1* and *Rht2* (Simón *et al*., 2004b). Similar results were found by Paveley *et al*. (2005), where stronger dwarfing genes (*Rht3*) increase the disease.

Apparent resistance was found in later-heading wheat plants where lower levels of STB were present than on earlier-heading ones (Shaner *et al*., 1975; Danon *et al*., 1982; Shaw and Royle, 1989a, b, 1993; Eyal and Talpaz, 1990; van Beuningen and Kohli, 1990; Arraiano *et al*., 2007a). It is considered as a mechanism of disease escape, most likely conferred by environmental factors (Shaner *et al*., 1975; Eyal and Talpaz, 1990; van Beuningen and Kohli, 1990), as lines × years interaction reveals differences that are explained by different environmental conditions (Eyal and Talpaz, 1990; Simón *et al*., 2004b; Simón *et al*., 2005). Thus, genotype × environment interaction and variation in pathogenicity have been found among locations (van Beuningen and Kohli, 1990; Simón *et al*., 2004b, 2005). Therefore, no genetic association between resistance and heading date has been proposed (Arama *et al*., 1999; Simón *et al*., 2004b, 2005), due to the fact that, when late and early maturating cultivars were exposed to similar weather conditions, the disease began at the same development stage. The rate of epidemic development was also similar (Arama *et al*., 1999). Associations between heading date and susceptibility, positive or negative, depend on conditions, specially temperature, precipitation, relative humidity (Simón *et al*., 2004b, 2005), and radiation (Simón *et al*., 2005).

Manipulation of some microenvironment variables, in order to increase yield, are usually applied, although they can frequently increase the risk of STB disease in the crop. As a complement of disease control, these practices can be improved in order to avoid or to diminish the damage caused by STB. Therefore, taking into account: 1)

the rate of each element applied (usually, N-P-K) and its equilibrium according with the soil characteristics; 2) addition of Mn fertilizer in adequate doses; 3) the time and system of fertilizer application; 4) the plant density and other practices related to the increase of vegetative parts; 5) the sowing date; 6) the cultivar, and 7) the addition of some organic matter as straw, could improve the yield, and at the same time, diminish the risk of STB disease. Plant height and heading date are directly related to the cultivar traits. Thus, cultivars that allow disease escape due to macro environmental factors should be an objective for breeding; in this case, "breeding for apparent resistance".

1.6 Breeding for disease resistance

Resistance in the wheat -*M. graminicola* system has been found to be expressed as a restriction of the pathogen growth within the host (Eyal *et al*., 1973) and, as fungal incapability to form pycnidia on leaves. It is not yet clear whether the mycelia were equally spread in the leaf tissues of susceptible and resistant hosts (Pnini-Cohen *et al*., 2000). However, no complete resistance has been observed (Van Ginkel and S**c**haren, 1988; Kema *et al*., 1996a and b; Kema and van Silfhout, 1997**;** Eyal, 1999).

In wheat, there are essentially two types of resistance to septoria tritici blotch: specific resistance and partial resistance. Specific resistance is effective against some isolates of the septoria fungus *Mycosphaerella graminicola*, but not against others, and it is controlled by single genes (SR-genes) of large effect. It follows a gene-for-gene relationship (Brading *et al*., 2002). As in rust and mildew (Parlevliet, 1993), partial resistance to Septoria tritici blotch is incomplete, polygenic and isolate non-specific (Jlibene *et al*., 1994; Simón and Cordo, 1998; Zhang *et al*., 2001; Eriksen *et al*., 2003; Chartrain *et al*., 2004b).

1.6.1 Resistance

Specificity of wheat cultivars-*M. Graminicola* isolates has been demonstrated in adult plants under field conditions (Kema and van Silfhout, 1996b and c, 1997; Brown *et al*., 2001). Specific expression of some resistant genes to specific isolates of STB at seedling stage, but not in adult plants, has been detected. Also, genes that confer resistance at seedling stage were also expressed in the adult stage of wheat (Kema and van Silfhout, 1997; Arraiano *et al*., 2001a).

Several resistance genes have been identified in past. Rillo and Caldwell (1966) identified a single dominant gene *Stb1* that controls resistance in Bulgaria 88 variety. This gene has been recently mapped in the resistance line P881072-75-1 on the 5B chromosome (Adhikari *et al*., 2004a). Wilson (1979, 1985) reported the resistance genes *Stb2* and *Stb3* which control the resistance in Veranopolis and Israel 493 varieties, respectively, but just recently been mapped (Adhikari *et al*., 2004b). Whereas; *Stb2* gene is located on chromosome 3B, *Stb3 found* on chromosome 6D. *Stb4* is a resistant gene identified in the variety Tadinia (Somasco *et al*., 1996), recently mapped near the centromere of chromosome 7D (Adhikari *et al*., 2004c).

The *Stb5* gene was found in the short arm of chromosome 7D in the synthetic hexaploid 'Synthetic 6x' (*Triticum dicoccoides* × *T. tauschii*). It was the first gene located on a chromosome and mapped (Arraiano *et al*., 2001b). The *Stb5* gene confers specific resistance to the *M. graminicola* isolate IPO94269. Brading *et al*. (2002) found, in cultivars (*cvs.)* Flame and Hereward, the gene *Stb6* in the short arm of chromosome 3A; it confers specific resistance to isolate IPO323. At the same time, Brading *et al*. (2002) found in IPO323 a gene for specific avirulence on *cv*. Flame. It was the first time a gene-for-gene interaction between wheat and *M. graminicola* was tested and demonstrated genetically. Chartrain *et al*. (2005b)

showed that *Stb6* was present in both European and Chinese landraces, suggesting that this is an old gene probably dating from the mid-Neolithic period. M**c**Cartney *et al*. (2003) mapped *Stb7* gene on chromosome 4AL — it confers resistance in the line ST6 to isolate MG2 from Manitoba. In the synthetic hexaploid wheat W7984, a gene for resistance to the field isolate (IN95-Lafayette-1196-WW 1-4) was also mapped on chromosome 7BL, *Stb8* (Adhikari *et al*., 2003).

The gene *Stb9*, located on the long arm of chromosome 2B, confers resistance to varieties Courtot and Tonic to isolate IPO89011 (Chartrain *et al*., 2009). *Stb10* and *Stb12* genes were found in Kavkaz-K 4500 variety (Chartrain *et al*., 2005a). The *Stb12* and *Stb10* genes were determined on chromosome 4A and 1D respectively; the first one confers resistance to isolate ISR398 and the second one to IPO94269 and ISR8036. A newly identified gene, *Stb11*, was detected on chromosome 1BS in the variety TE 9111 (Nabão), gene that confers resistance to isolate IPO90012 (Chartrain *et al*., 2005c). Recently, a new gene of resistance to STB has also been identified in the wheat cultivar Arina and Riband, *Stb15*. It was located on chromosome 6AS and it confers resistance to the Ethiopian isolate IPO88004 (Arraiano *et al*., 2007a).

Although specific resistance is not durable, `pyramiding´ single isolate-specific resistance genes would be efficient when much of the pathogen population overcome the combined resistance (Brown *et al*., 2001; Chartrain *et al*., 2004a). Thus this combination of genes reduces the rate of evolution of the pathogen (Burdon, 1993).

Kema *et al*., (1996b) pointed that *M. graminicola* is a pathogen that is characterized by quantitative and qualitative aspects of resistance and virulence. However, the loss of resistance can be fast when optimal conditions for evolution of the pathogen are encountered, as presented by Cowger *et al*. (2000).

1.6.2 Partial resistance

Partial resistance is a term associated with the control of the disease by a number of genes of a rather small individual effect (Johnson, 1984, 1993). It is expressed phenotypically in traits in the form of reduced infectivity and longer latency periods than those occurring on more susceptible cultivars (Burdon, 1993).

Different genes confer partial resistance in seedlings and adult stages (Eriksen *et al*., 2003; Chartrain *et al*., 2004; Simón *et al*., 2004a). The segregation of partial resistance sometimes makes it difficult to identify specific resistant genes (Chartrain *et al*., 2005b).

In wheat, evidence was found about the partial resistance to STB, but it is only recently that this kind of resistance has begun to be understood. Eriksen *et al*. (2003) have located five QTLs in the variety Senat on chromosomes 3A, 6B, 2B and 7B, for resistance to the *M. graminicola* isolates Risø97-86 and IPO323, at seedling and adult stages. Chartrain *et al*. (2004b) have contributed with the identification of a quantitative trait locus on chromosome 6B for partial resistance in the susceptible variety Riband to the *M. graminicola* isolates IPO94269, IPO89011 and IPO290. Simón *et al*. (2004a) located, in seedlings of the synthetic hexaploid wheat `W7984`, three resistance loci in the short arms of chromosomes 1D, 2D and 6B, and two resistance QTLs for an adult stage of *M. graminicola* isolates IPO92067 and IPO93014.

Several sources of partial resistance to Septoria tritici blotch have been detected in cultivars and breeding lines from Brazil, Portugal, France, Switzerland, the Czech Republic, Germany, USA, UK and The Netherlands (Brown *et al*., 2001).

Partial resistance has also been reported in several host-pathogen systems (Parlevliet, 1993). Its durability in wheat to stripe rust (*Puccinia striiformis*) has been

mentioned by Zhang (1995) in old cultivars of China, where the cultivars Bai Qimai, Hong Huomai, Hong Qimai, Qing Shoumai and Yu Zhonghong maintained their quantitative resistance over substantial areas for at least 60 years. Another example of this is the cultivar Hope from the U.S.A., which has shown resistance to stem rust (*Puccinia graminis* f. sp. *tritici*) after 60 years (van Ginkel and Rajaram, 1993). However, some pathogens have the ability to adapt to such polygenic characters, though the lost of resistance will be slow (Burdon, 1993).

Quantitative inheritance research of resistance, at seedling stage (Zhang *et al*., 1998) and adult plant stage (Jlibene *et al*., 1994; Camacho-Casas *et al*., 1995; Simón and Cordo, 1998) in the pathosystem wheat-STB, has been conducted in the diallel experiments. In diallel experiments (parental lines in cross combination), the estimation of general combining ability (GCA) and specific combining ability (SCA), as well as the reciprocal effects (parents used as female and male), have been tested in *Triticum aestivum* cultivars. A general combining ability and SCA, but not reciprocal effects, have been estimated in *Triticum durum* cultivars. General combining ability estimates the additive action of the genes, and it is also an inherent test for a particular line in hybrid combination with several other lines. The specific combining ability indicates mainly a dominant gene action, and the test measures the special combination of a specific cross (Brown and Caligari, 2008). Negative values (below the general mean) of GCA and SCA were associated with resistance to STB, and positive values (above the general mean) were associated to susceptibility. Estimation of GCA and SCA was made with crosses among resistant, moderate and susceptible cultivars (Jlibene *et al*., 1994; Simón and Cordo, 1998; Zhang *et al*., 2001), as well as with the test of reciprocals (Jlibene *et al*., 1994; Zhang *et al*., 2001).

General combining ability effects account for the largest portion of the phenotypic variation. Therefore, additive gene effects were the most important in the inheritance of resistance to STB (Jlibene *et al*., 1994; Camacho-Casas *et al*., 1995; Simón and Cordo, 1998; Zhang *et al*., 2001). General combining ability effects were generally in agreement with parental performance in such a way that a negative value of GCA indicated that the corresponding parent was resistant, while a positive value was associated with susceptible parents (Jlibene *et al*., 1994; Simón and Cordo, 1998; Zhang *et al*., 2001). General combining ability was significant for the resistant components incubation period, latent period, pycnidial coverage, and maturation period (Simón and Cordo, 1998). The F_1 hybrids from crosses between susceptible cultivars were highly susceptible to STB (Zhang *et al*., 2001). Negative SCA effects were found only when one of the parents was resistant, and positive SCA effects when parents with medium resistance were involved (Jlibene *et al*., 1994). In general, SCA was significant only in some F_1 combinations (Jlibene *et al*., 1994; Simón and Cordo, 1998; Zhang *et al*., 2001). Similarly, diallel analysis in *Triticum durum* showed a major role of GCA in the inheritance of resistance to STB, while SCA was significant only in particular crosses (van Ginkel and S**c**haren, 1988b).

Reciprocal effects for enhanced resistance to STB were observed in a few combinations. When resistant cultivars were used as female, hybrids had a significantly lower disease score than those of the reciprocal crosses (Jlibene *et al*., 1994; Zhang *et al*., 2001). This indicated a possible maternal effect (Jlibene *et al*., 1994). A cytoplasmic resistance was found by Mazouz *et al*. (2002) when evaluating effects in the enhanced resistance of *Triticum aestivum* genotypes to STB. Resistant genotype parents were crossed with susceptible ones, using the resistant cultivar as a male providing nuclear inheritance, and as a female providing nuclear and

cytoplasmic inheritance (F_1's). Additionally, backcrosses with resistant (BC_1P_1) and susceptible (BC_2P_2) parents were tested as well as the F_2. Cytoplasmic resistance was detected in two of eight parents, and the resistance was specific to few of seven STB isolates. Thus, nuclear genetic information and extra nuclear information, mitochondria and/or chloroplast genomes, seemed to be involved in the resistance expression of some wheat genotypes.

1.6.3 Susceptibility

Arraiano *et al*. (2007b) have been trying to identify the chromosomal locations of the genes controlling resistance and susceptibility to STB in a study of the substitution lines series of Cappelle Desprez (CD) and Hobbit sib (Dwarf A) (Hs) with chromosomes of Bezostaya 1 (Bez). A substitution line set consisted of 21 monosomic plants (41 chromosomes), in which one of the chromosomes comes from a "donor" variety and the background (40 chromosomes) comes from a "recipient" variety. In this case, the recipient varieties were CD and Hs, while the donor variety was Bez. Thus, a complete set of substitution lines comprises, in the CD and Hs series: CD (Bez1A), CD (Bez1B), CD (Bez1D),…, CD (Bez7D), and Hs (Bez1A), Hs (Bez1B) Hs (1D),…, Hs (Bez7D) (Law and Worland, 1972; Law *et al*.,1987). CD and Hs have a reciprocal translocation with respect to Bez karyotypes involving chromosomes 5B and 7B. Thus, the substitution line Hobbit sib (Bez 5BS-7BS) has a chromosome 5B consisting of Hobbit sib 5BL and Bezostaya 5BS, and a chromosome 7B with Hobbit sib 7BL and Bezostaya 7BS (Johnson, 1992; Law and Worland, 1996).

Arraiano *et al*. (2007b) found that Hs (Bez5BS-7BS) was significantly more resistant to STB than the euploid line with isolates IPO323 and IPO94269 in adult trials, but not in a seedling test. CD (Bez5BS-7BS) had a similar disease score to

those on euploid CD with both isolates, moderately resistant in adult and seedling tests. CD series have been almost completely correctly substituted by Bez, except for a small telomeric part of 5BS. As the Bez 7BS chromosome arm does not confer resistance to STB in CD (Bez 5BS-7BS), and this arm has been substituted correctly into Hs (5BS-7BS), it does not explain the resistance of the Hs (Bez5BS-7BS) substitution line. However, Hs arm 5BS was not substituted by the homologous Bezostaya arm in the 5BS segment. The 5BS arm included a large segment of Chinese Spring material with a small segment of Hs in the telomeric region, possibly because the Hobbit sib monosomic series was developed from the original Chinese Spring monosomic series. Chinese Spring chromosome arm 5B did not confer resistance to Hobbit sib (5BS-7BS) line. Euploid CS was susceptible to IPO94269 isolate, though it was resistant to IPO323, because 3A chromosome carries the resistant gene *Stb6* (Chartrain *et al*., 2005b). Thus, either, it is possible that Hobbit sib carries a gene or genes for susceptibility to STB on chromosome 5BS, and its removal increases the resistance of the Hobbit sib (Bez5BS-7BS) substitution line; or there might be a gene which suppresses resistance in Hobbit sib. Thus, the substitution of chromosome arm 5BS of Hs by Chinese Spring would nullify the effect of the suppressor allowing expression of a previously inhibited resistance gene. However, Hs does not have a known resistance gene to STB (Arraiano *et al*., 2007b).

As a summary, resistance of wheat to STB is presented as: 1) specific resistance which is controlled by genes of large effect and is isolate-specific, and 2) partial resistance, conferred by genes that have partial effects and are isolate-non-specific. In addition, enhanced resistance could be obtained through the expression of cytoplasmic resistance, present in some cultivars to specific STB isolates. In contrast,

a gene (or genes) that suppressed resistance or increase susceptibility to STB seems to be present in wheat cultivars.

1.7 Cost of resistance

Cost of resistance has been defined as a reduction of fitness in the absence of the targeted pest (Simms and Rausher, 1987; Korves and Bergelson, 2004). Korves and Bergelson (2004) defined net cost of infection as a 'fitness advantage of infected, susceptible plants over infected, resistant plants'. Active defence processes mean that the plant increases its biological activities as synthesis of salicylic acid, pathogenesis-related (PR) proteins, molecules that have toxic effects even to the plant itself, phytoalexins and cell wall material as callose and lignin (Smedegaard-Petersen and Tolstrup, 1985; Heil and Baldwin, 2002; Vidhyasekaran, 2008).

Defence may incur in costs to the plants in such a way that better protected plants probably have lower fitness than susceptible plants (Heil, 2001). Costs of resistance can also be important in the absence of attack (Heil *et al*., 2000; Korves and Bergelson, 2004). Active defence processes have a cost for the plant because of the diversion of energy, processes involved in plant growth, and synthesis of defence compounds (Smedegaard-Petersen and Tolstrup, 1985; Heil *et al*., 2000). They may be a limiting factor in plant growth and yield (Smedegaard-Petersen and Tolstrup, 1985). Evidence of resistance cost has been investigated in wheat. On 7D chromosome from *Aegilops ventricosa* (relative of wheat) has been transferred to the variety VPM1. This chromosome has gene *Pch1* that confers resistance to eyespot (*Pseudocercosporella herpotrichoides*), but the presence of this chromosome lead to a reduction in yield of around 8% (Worland *et al*., 1989). Leaf rust (*Puccinia recondita*) resistant near isogenic lines (NIL) of wheat, carrying the rust resistant gene *Lr9* were compared to the susceptible recurrent parent Arina. When no leaf rust

was present, NIL had a 12% lower grain yield than Arina, due to the reduction of the grain number per square meter and of the mean grain weight (Ortelli *et al*., 1996).

Indirect costs have been described by Brown (2002) associated with mechanisms of disease escape such as plant architecture, rate of development, and date of crop maturity, when the development or spreading of the disease was permitted. In the case of partial resistance there were no clear examples of cost of resistance, but Brown (2002) discussed the possible costs. This was based on the fact that resistance could be lost if selection is not frequently carried out, as in the case of the loss of partial resistance by the selection of R genes for long time in potato against the blight disease caused by *Phytophthora infenstans* (the Vertifolia effect), and the lack of partial resistance showed against tropical rust (*Puccinia polysora*) of maize in Africa, when this disease arrived and losses were severe.

Both resistance and tolerance are different stages of a single process; resistance limits the amount of fungus on the leaf, tolerance increases growth and reproduction of the plant at a given level of infection (Brown and Handley, 2006). Little is known about the cost of resistance in the wheat-*M. graminicola* interaction, and its effect on yield, but both aspects are relevant in breeding programs.

1.8 Conclusion

It has been suggested that partial resistance is costly and probably genetically based. The main purpose of this project was to find phenotypic relationships between agronomic traits and STB and how this might affect the reduction in yield and yield components. Also, the genetic basis of the reduction in yield and yield components due to partial resistance are investigated. To achieve this goal, the Senat x Savannah (SESA) doubled haploid population was studied. In this population, Senat presents good levels of partial resistance, while Savannah is a susceptible cultivar.

1.9 Outline of the thesis

In chapter II yield penalty due to STB partial resistance has been studied through the analysis of eleven agronomic traits where phenotypic relationships have been established. Two specific traits, heading date and grain protein content, were detected as major factors related with disease and yield.

In Chapter III, the genotypic analysis of the SESA population included the detection of quantitative trait loci (QTL) for agronomic traits and STB resistance. The genotypic screening allowed the detection of putative QTL for agronomic traits linked with QTL for STB resistance. Also, this analysis gives a new approach to understand how partial resistance allows a small loss of yield through maintenance of the TGW and the specific weight, due to equilibrium between protein content and starch content in the grain.

Finally, in Chapter IV a susceptible gene was detected in the double haploid population Hobbit sib x Hobbit sib (5BS-7BS). This should allow breeders to identify cultivars or breeding lines carrying this susceptible gene and to remove it from their breeding programs.

CHAPTER 2

Phenotypic relationships among agronomic traits and Septoria tritici blotch (*Mycosphaerella graminicola*) of wheat

2.1 Introduction

The phenotype, that is, the observable or discernible characteristics of a crop, is usually analyzed through few traits. This is due to the economic and human resources it implies. In wheat, yield and yield components have been the focus of extensive phenotypic and genotypic analysis and they are the most important traits for plant breeders. Grain quality traits are the second group in which more effort has been applied. A great effort has also been applied to study the adaptative traits high to the flag leaf and heading date. All these traits have been cited mostly individually as traits affected by the disease Septoria tritici blotch.

In wheat, as in other cereals, compensatory effects among yield components are commonly found (Evans and Wardlaw, 1976, 1996; Evans, 1993). This is due to the fact that, during plant development, the components are determined successively (Evans and Wardlaw, 1976, 1996; Evans, 1993; Simane *et al*., 1993; García del Moral *et al*. 2003). Adverse conditions can limit the early formed yield components but they can be compensated by late ones (Evans and Wardlaw, 1976, 1996; Evans, 1993). This performance of wheat may be the result of competition for limited resources (Evans and Wardlaw, 1976, 1996; Simane *et al*., 1993). The yield components interact in this way especially under stressed environments (Blum, 1983; Fischer, 1985; Simane *et al*., 1993). However, it seems that the negative association between grains m^{-2} and grain weight is independent of competition for assimilates, due to the fact that, when one or the other increase, the yield increases (Slafer, 2007).

The mature grain of wheat is typically mainly composed of starch and proteins. Starch makes up about 64-74 % (Bechtel *et al*., 1990) or even 75-85 % (Jenner *et al*., 1991) of the total grain dry weight, and protein between 10-14 % in UK winter wheat (Blanco *et al*., 1996) and durum wheat (Blanco *et al*., 2006).

Basically, yield is dependent on the deposition of endosperm starch (Herzog, 1986; Jenner *et al*., 1991), but the quality of the grain is conferred by the protein content (Cox *et al*., 1985; Herzog, 1986; Panozzo and Eagles 1999; Panozzo *et al*., 2001; Monaghan *et al*., 2001 and others). Another grain quality character is the specific weight (Bayles, 1977a; Hook, 1984; Gaines *et al*., 1997; Clarke *et al*., 2004; Atkinson *et al*., 2005). It indicates grain density and packing properties (Bayles, 1977b). Grain density indicates the plumpness of the grain, *i.e.* how well it has been filled (Atkinson *et al*., 2005). Low specific weight has been related to shrivelling grains, that is, reduction of total endosperm in the grain (Gaines *et al*., 1997; Clarke *et al*., 2004). Values of specific weight in UK cultivars are between 67.9 to 78.1 kg hl^{-1} (Hook, 1984). Specific weight generally helps to distinguish poorly or well filled grains, but not to distinguish grains with intermediate quality (Bayles, 1977b). As a trait of the grain, specific weight is related to yield and its components, especially with thousand grain weight and its major compounds, starch and proteins.

The determination of the height of the plant and the heading date marks the final events of the vegetative and reproductive phase of the life cycle of the plant, respectively. The vegetative phase is characterized by the initiation and growth of leaves, tillers, and roots. The reproductive period begins when floral initiation occurs (double ridge); during the phase of floral initiation and anthesis the height, the number of potential ears per unit area and number of grains ear^{-1} is defined. The late reproductive phase is of great importance because it is then the yield components, ear number, grains ear^{-1}, and grain m^{-2}, are mainly established and hence the potential yield (Kirby, 1988; Slafer and Rawson, 1994; Slafer, 2007). Heading is the visible change from the reproductive to the grain filling period (Slafer and Rawson, 1994). At heading, when ears and stem are growing at the most rapid rate and the peduncle

is also growing rapidly, there is still a considerable production of florets, but it is also the time for floret death (Kirby, 1980; Herzog, 1986). Height and heading seemed to be related at this time of crop development.

The purpose of this research was to investigate the influence of partial resistance to Septoria tritici blotch of wheat on yield, yield components, quality grain traits, and plant adaptative traits (heading date and height). A Senat × Savannah (SESA) doubled haploid population was chosen as a suitable study population as the Senat parent has shown good levels of partial resistance, while the Savannah parent is susceptible towards STB. Segregation data on partial resistance to STB for the SESA DH population at two sites, NørreAaby (NrAaby02) and Sejet (Sejet02) Denmark was provided by Eriksen *et al*. (2003).

The first approach was to understand the partial resistance responses of wheat to STB as part of the physiology of the whole plant (Brown, 2002). This describes the phenotypic variation of eleven agronomic traits in three different environments. The phenotypic relationship of partial resistance to yield and yield components, yield and other traits were treated as interconnected traits and their relationships with Septoria tritici blotch severity is investigated.

2.2 Materials and methods

2.2.1 Plant material

A Senat × Savannah double haploid population (SESA) of 106 lines was used, which was developed using the wheat × maize technique from F_1 generation at Sejet Planteforædling, Denmark (Eriksen *et al*., 2003). Savannah is a winter wheat cultivar highly susceptible to Septoria tritici blotch, which is grown in the UK since 1999

(Angus, 2001), while Senat is a Danish variety that expresses high partial resistance to *M. graminicola* isolates (Eriksen *et al*., 2003).

2.2.2 Field experiments and disease data

Phenotypic data of 104 DH lines and their parents stemmed from field experiments in the seasons 2004/2005, 2005/2006 at two sites -Nickerson, UK (52º N, 0.96º E) and Sejet, Denmark (55º N, 9º E)- named Nickerson 2005, Nickerson 2006, Sejet 2005, and Sejet 2006, respectively. Each experimental layout was a lattice design with two replicates of 11 miniblocks with 10 entries per miniblock, 110 total entries. The single plot size was 1.5 × 5 m. Appropriate doses of fertilizer (120 kg ha^{-1}), fungicide (2.5 l ha^{-1} Opus Team; 0.3 l ha^{-1} Stereo; and, 0.4 l ha^{-1} Comet) and growth regulators (1 l ha^{-1} Terpal) were applied after sowing at appropriate stages of growth.

Segregation data on partial resistance to Septoria tritici blotch for the SESA population from previous field trials was provided by Sejet (Eriksen *et al*., 2003). Disease was not scored at Nickerson trials. Septoria tritici blotch severity data were collected at two sites Sejet02 (55° N, 9° E) and NrAaby02 (55° N, 9° E) which were about 38 km apart. These trials were inoculated twice with a mixture of 11 *M. graminicola* isolates. The first inoculation was performed after the flag leaves were fully expanded and the second one was performed 12 days after (Eriksen *et al*., 2003).

The area under the disease progress curve (AUDPC) on flag leaf (Shaner and Finney, 1977) was calculated as a measurement of STB severity. The AUDPC was calculated from the combined scores (Shaner and Finney, 1977). The maximum AUDPC was calculated assuming a score of 100 % on every date the test was scored with the formula:

$$\text{AUDPC} = \sum_{i=1}^{n} [Y_{i+n1} + Y_i)/2]\,[X_{i+1} - X_i]$$

where:

Y_i is the Stb severity (per unit) at the *ith* observation; X_i is the time (days) at the ith observation; *n* is the total number of observations.

Afterwards, data were transformed into logit area under the disease progress curve (lgtAUDPC) as a proportion of the maximum area under the disease progress curve. The logit (p) takes the logit transformation: log $(p/(100-p))$ of the percentages p (0 < p 100 %). The mean score for each line was calculated using generalized linear mixed modelling (GLMM) (GenStat 9.1).

2.2.3 Data collection

The crop phenotypic data set included the following traits: heading date (days from the 1st of January onwards), height to the flag leaf (HFL) (cm), canopy maturity classification (maturity), grains per ear (grains ear^{-1}), a thousand grain weight (TGW), grains per square meter (grains m^{-2}), ears per square metre (ears m^{-2}), yield (t ha^{-1}), specific weight (SW), grain protein content (GPC) (% DM) and starch content (% DM). Canopy maturity was taken at Sejet 2005 on the 22nd of July, at Nickerson 2006 on the 19th of July, and at Sejet 2006 on the 21st of July. The classification of the canopy maturity was made according to the following groups: 1- green flags & straw; 3-flags half ripe; 5 straw half ripe, nodes green; 7- straw ripe, nodes green; 9- straw ripe, nodes ripe. Before harvest, the number of fertile tillers was counted in a 2 m (2005) or 0.5 m (2006) row from two locations in the plot. The number of ears per square metre was calculated from these counts. Twenty ears were sampled from each plot. These were threshed and the cleaned grains weighed. Grains per ear, a thousand grain weight (gr) and grains per square metre were calculated from these measurements. Yield (t ha^{-1}) was calculated from plot yield. The specific weight (kg hl^{-1}), grain protein content (% DM) and grain starch content (% DM) were measured,

at the Sejet trials, on the combined harvested material on a FOSS infratec NIT instrument with a specific weight module. At Nickerson, a NIR instrument BRAN + LUEBBE Infra Analyzer 260 was used to obtain the grain protein content (% DM), and an apparatus for determining the mass by the hectolitre specific weight.

The Sejet trials data sets were in general complete. The Nickerson 2005 data had only one replicate of the traits HD, TGW, SW and yield. This environmental data were thus excluded from the analysis. The Nickerson 2006 data were complete, only lacking the grain starch content. Data for six lines were discarded due to errors at sowing, impure seed or because they were present in one environment only.

Data on rainfall and temperature were obtained from nearby weather stations, Wattisham, UK (52º N, 0.96º E) for the Nickerson trial and Aarhus Sid, Denmark (56º N, 10º E) for the Sejet trials.

2.2.4 Statistical analysis

Analysis of variance (ANOVA) was conducted for each trait at each trial. A Generalized Linear Models (GLM) analysis from the statistical package GenStat® 9.1 (GenStat committee, 2006) was conducted to test for differences among trials for each trait. Mean values were calculated from replicates of each line for each trait for the different trials. Test of X^2 goodness of fit was applied to investigate if there was normality performance of each trait at each trial. Pearson correlation coefficients (r) among each trial for each agronomic trait were calculated. In addition, Pearson correlation coefficients were calculated for all possible comparisons among the traits for each trial, as well as correlation coefficients among the traits of each trial *versus* (*vs*.) STB severity.

2.3 Results

2.3.1 Analysis of variance

The analysis of variance for each trial showed highly statistical significant differences among lines for most of the traits (P<0.001) in the three environments tested. However, differences among lines were not highly significant for grains m^{-2} (Appendix A, Tables A.1, A.2, and A.3). Additionally, for the trait ears m^{-2} highly significant differences were only found at Sejet 2005 but not at Nickerson 2006 and Sejet 2006 (Appendix A, Table A.2).

The analysis of variance to detect differences among trials showed highly significant statistical differences ($P \leq 0.001$) among trials and lines for all the traits (Table 2.1). Also, the analysis of variance for each trait showed a significant trial × line interaction except for ears m^{-2} (Table 2.1). However, trials × line effects were all smaller than the main effects of the lines. These results suggest that the site-environment influence on the performance of the DH population; it was probably due to the genotype × environment interaction, except for the trait ears m^{-2}.

Table 2.1 Analysis of variance for agronomic traits of Senat × Savannah double haploid population and parents at different environments

		Ears/m^2	**Grains/m^2**	**Grains/ear**	**TGW**	**Yield**	**GPC**
Source	df	MS	MS	MS	MS	MS	MS
Trial	2	2283468***	2.760E+09***	16607.28***	3055.36***	94.99***	346.10***
Line	97	10565***	3.435E+07***	72.47***	37.04***	0.72***	0.90***
Trial. Line	194	7153	2.529E+07*	16.74*	5.73***	0.18***	0.16***
Residual	215	6260	2.014E+07	11.67	3.04	0.07	0.09
Total	587	15883	4.105E+07	99.33	21.31	0.61	1.52

		SW	**HD**	**HFL**	**Maturity**			**Starch**
Source	df	MS	MS	MS	MS	Source	df	MS
Trial	2	964.91***	2769.20***	1614648***	141.81***	Trial	1	167.48***
Line	97	14.58***	12.99***	132.40***	5.60***	Line	97	2.16***
Trial.Line	194	2.12***	1.00**	8.11**	1.41***	Trial. Line	97	0.33***
Residual	215	0.99	0.43	5.45	0.76	Residual	154	0.12
Total	587	7.54	12.51	37.28	2.40	Total	391	1.29

***Significant at $P \leq 0.001$; **Significant at P = 0.005; *Significant at P = 0.05

TGW, thousand grain weight: Protein, grain protein content; SW, specific weight; HD, heading date; HFL, height to the flag leaf; Maturity, canopy maturity; Starch, grain protein starch

The test of X^2 goodness of fit for each agronomic trait showed normality of the data (X^2 $\alpha_{=0.001}$) for all the traits at each site (Appendix B). This performance was expected for quantitative traits as yield, height and other traits. Qualitative traits (or quasi-quantitative traits) as heading date and canopy maturity could not show normality if the size of the sample was not large enough, however, the trend of all the distributions tends towards normality when the sample is large, as in this study. Although the hypothesis of normality of the data (null hypothesis) could be accepted with a $P < 0.05$, the test showed a higher probability to be acceptable at $P < 0.001$ for all the traits.

For most of the traits, the means measurements of the DH lines were between the means of the parents (Table 2.2, and Appendix C), except for ears m^{-2} and grains m^{-2} in Nickerson 2006 data.

The overall trait means for each trait of the double haploid population showed significant differences at the three environments tested, except for grains m^{-2} and canopy maturity (Table 2.2). The DH population exhibited transgressive segregation with respect to the parents for the agronomic traits (Appendix C).

Kearsey and Pooni (1998) stated that DH lines produced from the F_1 were equivalent to F_∞ lines in their mean and variance. For a trait exhibiting heterosis, although the mean declined, the variance among lines increased reaching a maximum at F_∞; they also showed that there may be lines which might show transgressive segregation.

Table 2.2 Means and standard deviations of Senat × Savannah DH lines and parents showing differences among environments

Trait	Genotype	Nickerson 2006	Sejet 2005	Sejet 2006	Significance [a]
Ears m^{-2}	Senat	479	571	783	
	Savannah	560	458	588	
	DH lines	451	512	661	*
	SD	76	57	72	
Grains m^{-2}	Senat	34409	25654	37324	
	Savannah	34126	22047	28371	
	DH lines	27690	23504	30929	NS
	SD	6073	2352	3378	
Grains ear^{-1}	Senat	72	45	48	
	Savannah	61	48	48	
	DH lines	61	46	47	**
	SD	7	4	4	
TGW (gr)	Senat	47.96	38.36	38.13	
	Savannah	54.56	48.52	46.97	
	DH lines	51.78	44.98	44.56	**
	SD	2.80	2.81	3.37	
Yield (ton ha^{-1})	Senat	10.14	8.44	9.15	
	Savannah	10.76	9.18	9.16	
	DH lines	10.52	9.20	9.48	**
	SD	0.42	0.53	0.48	
Protein (% DM)	Senat	13.33	10.31	12.60	
	Savannah	11.69	9.21	11.29	
	DH lines	12.28	9.89	12.08	**
	SD	0.54	0.40	0.51	
Starch (% DM)	Senat	_	68.56	67.20	
	Savannah	_	69.26	68.01	
	DH lines	_	68.98	67.68	**
	SD	_	0.70	0.99	
Specific weight	Senat	78.08	74.84	72.28	
(kg hl^{-1})	Savannah	78.62	73.93	74.20	
	DH lines	78.41	74.71	74.37	**
	SD	1.60	1.81	2.33	
Heading date	Senat	160	167	166	
(days 1/1)	Savannah	155	162	163	
	DH lines	158	164	164	**
	SD	2	2	1	
Height to the flag leaf	Senat	60.89	55.74	63.65	
(cm)	Savannah	59.81	58.34	64.51	
	DH lines	61.37	59.74	65.23	*
	SD	4.97	5.73	5.47	
Canopy maturity	Senat	4	3	3	
	Savannah	6	3	5	
	DH lines	5	3	3	NS
	SD	1	1	1	

[a] H_0: $\bar{X}1 = \overline{X}2 = \overline{X}3$, where $\bar{X}1$ is the trait mean of the double haploid population at Nickerson 2005; $\overline{X}2$ is the trait mean of the double haploid population at Sejet 2005; and, $\overline{X}3$ is the trait mean of the double haploid population at Sejet 2006

*Tukey $_{\alpha = 0.05}$; **Tukey $_{\alpha = 0.01}$; NS_ not significant

Analysis of variance for STB severity indicates highly significant differences between the trials and lines, also trial × line interaction ($P < 0.001$) is suggested. However, main effects were higher than the interaction effect (Table 2.3).

Differences between the means are supported by the analysis of variance at NrAaby02 and Sejet02 trials. The means for site, cultivar, and for the double haploid population are presented in Table 2.4.

Table 2.3 ANOVA for STB severity at NrAaby02 and Sejet02 trials

		STB
Source	df	MS
Trial	1	6.56***
Line	97	76.57***
Trial. Line	97	2.21***
Residual	3884	0.51
Total	4079	2.36

H_0: $\bar{X}1 = \overline{X}2$, where $\bar{X}1$ is the Stb severity mean at NrAaby02 and $\overline{X}2$ is the Stb severity mean at Sejet02
*** Significant at $P \leq 0.001$

Table 2.4 Means of Senat and Savannah parents and DH lines under STB disease pressure trials at NrAaby02 and Sejet02 trials

	Genotype	NrAaby02	Sejet02
STB (lgtAUDPC)	Senat	-4.22	-5.08
	Savannah	1.15	0.19
	DH lines	-1.55	-2.37

2.3.2 Correlation analysis

2.3.2.1 Correlation among agronomic traits and trials

According with the analysis of variance presented in Table 2.1, Pearson correlation coefficients were calculated among the three trials for ten traits (Nickerson 2006, Sejet 2005, and Sejet 2006), and in two trials (Sejet 2005 and Sejet 2006) for starch content in the Senat × Savannah DH population (Table 2.5). The correlation coefficients were positive and highly significant among the three environments for each agronomic trait. However, ears m^{-2} and grains m^{-2} were not associated in the comparisons of Nickerson 2006 vs. Sejet 2005 and Nickerson 2006 vs. Sejet 2006 trials.

Table 2.5 Correlation coefficients amongst three trials for eleven agronomic traits of the Senat × Savannah double haploid population

Trait	Site	Sejet 2005	Sejet 2006
Ears m^{-2}			
	Nickerson 2006	- 0.03	0.02
	Sejet 2005		0.65***
Grains m^{-2}			
	Nickerson 2006	0.03	0.07
	Sejet 2005		0.38***
Grains ear^{-1}			
	Nickerson 2006	0.42***	0.42***
	Sejet 2005		0.61***
TGW			
	Nickerson 2006	0.55***	0.59***
	Sejet 2005		0.67***
Yield			
	Nickerson 2006	0.29***	0.54***
	Sejet 2005		0.27**
Protein (DM %)	Nickerson 2006	0.51***	0.51***
	Sejet 2005		0.46***
Starch (DM %)	Sejet 2005	----	0.74***
Specific Weight			
	Nickerson 2006	0.69***	0.61***
	Sejet 2005		0.73***
Heading date			
	Nickerson 2006	0.83***	0.79***
	Sejet 2005		0.87***
Height to the flag leaf (cm)	Nickerson 2006	0.79***	0.78***
	Sejet 2005		0.89***
Maturity			
	Nickerson 2006	0.43***	0.54***
	Sejet 2005		0.41***

*** Significance at $P \leq 0.001$

2.3.2.2 Correlations between STB severity and agronomic traits

Septoria tritici blotch severity showed highly positive significant association (r = 0.95) between both sites analyzed, NrAaby02 and Sejet02 (Table 2.6).

The correlation coefficients were calculated from the Nickerson 2006, Sejet 2005, and Sejet 2006 data sets and the segregation population data for STB resistance-susceptibility were obtained at NrAaby02 and Sejet02. In all three environments tested, a negative correlation coefficient was obtained between heading

date and STB severity. This means that in early heading lines the disease severity was higher than in late lines. This trend was more evident at Nickerson 2006 and Sejet 2005, where the association was highly significant for both STB trials (Table 2.6). Maturity was positively associated to disease severity at Nickerson 2006 and Sejet 2006. This result suggests that in these environments STB severity was higher in mature tissues (straw, flag leaf and nodes) than in green tissues (Table 2.6). These results are in agreement with the findings of Ziv and Eyal (1976), Cornish *et al*., (1990), Shaw and Royle (1993), and Simón *et al*. (2002) who reported that the effect of STB on yield and yield components depended on the severity of the epidemic at a given developmental stage of the crop at the time of the infection.

The yield components were significantly correlated to STB severity in NrAaby02 and Sejet02 trials (Table 2.6). The most remarkable association between STB severity and a yield component was to TGW in the three environments considered. A statistically significant positive association was found, suggesting that when STB severity increased, TGW did not decrease, especially for Sejet 2005 data, where the correlation coefficient was high (Table 2.6). Conversely, and similarly important, the association between grains m^{-2} and STB severity was negative at all the environments (Table 2.6). It suggests that, as STB severity increased, the number of grains m^{-2} decreased. The yield component ears m^{-2} was also negatively associated with STB severity at Sejet 2005 and Sejet 2006. The grains ear^{-1} was associated with STB only at Nickerson 2006. In contrast to the performance of ears m^{-2}, a slightly negative association was obtained between grains per ear and STB severity at Nickerson 2006. Contradictory results were obtained by Leitch and Jenkins (1995) and Simón *et al.* (2002); they found that with early infections the number of ears m^{-2}

is diminish by STB while with late infections the grains ear^{-1} or the thousand grain weight can be reduced.

The negative association detected between STB severity and ears m^{-2} and grains ear^{-1} suggest that in these environments an increase of STB severity resulted in a decrease in these two yield components. For the DH population, contrasting performance was found for the association between yield and STB severity at Sejet 2005 and 2006. At Sejet 2005, this association was positive. The slight correlation coefficient suggests that severity of disease increased in parallel with yield. However, at Sejet 2006 this association was negative. Thus, STB severity increased and yield decreased (Table 2.6). Several authors (Shaner *et al.*, 1975; Ziv and Eyal, 1978; King *et al.*, 1983a; Thomas *et al.*, 1989; Shaw and Royle, 1989a, b, and 1993; Paveley, 1999) have found that yield losses were present when the flag leaf is infected, and also the second and third leaves. However, in tolerant cultivars the yield is not severely affected (Ziv and Eyal, 1976, 1978; Ziv *et al.*, 1981; Zilberstein *et al.*, 1985; Cornish *et al.*, 1990; Zuckerman *et al.*, 1997; Simón *et al.*, 2002).

The correlation coefficient between disease severity and grain protein content was negative; suggesting that STB increased as grain protein content decreased. This negative correlation coefficient was highly significant at Nickerson 2006 and moderately significant at Sejet 2005, while at Sejet 2006 it was slightly and only significant with the STB severity data from Sejet02.

Table 2.6. Correlation coefficients amongst STB at two environments and eleven agronomic traits at three environments for the Senat x Savannah DH population

Trait/ Environment	NrAaby02	Sejet02	Ears m^{-2}	Grains m^{-2}	HD	HFL	Grains ear^{-1}	Maturity	Protein %	SW	TGW	Yield
StbSejet02	0.95***											
Ears m^{-2}												
Nickerson 2006	- 0.11	- 0.13										
Sejet 2005	- 0.34***	- 0.33***										
Sejet 2006	- 0.28*	- 0.26*										
Grains m^{-2}												
Nickerson 2006	- 0.19*	- 0.23*	0.85***									
Sejet 2005	- 0.26*	- 0.24*	0.62***									
Sejet 2006	- 0.32**	- 0.29**	0.64***									
HD												
Nickerson 2006	- 0.36***	- 0.39***	0.00	- 0.06								
Sejet 2005	- 0.43***	- 0.43***	0.33***	0.05								
Sejet 2006	- 0.24*	- 0.23*	0.35***	0.27*								
HFL												
Nickerson 2006	0.03	0.05	0.12	0.07	- 0.27*							
Sejet 2005	- 0.09	- 0.05	- 0.31**	- 0.35***	- 0.11							
Sejet 2006	- 0.08	- 0.06	- 0.25*	- 0.37***	- 0.24*							
Grains ear^{-1}												
Nickerson 2006	- 0.21*	- 0.25*	0.12	0.61***	- 0.13	- 0.01						
Sejet 2005	0.11	0.12	- 0.52***	0.34***	- 0.33***	- 0.01						
Sejet 2006	- 0.05	- 0.05	- 0.42***	0.42***	- 0.09	- 0.14						
Maturity												
Nickerson 2006	0.20*	0.19*	0.09	0.12	- 0.53***	0.13	0.11					
Sejet 2005	- 0.02	- 0.03	- 0.17	0.08	- 0.38***	0.12	0.28**					
Sejet 2006	0.23*	0.25*	- 0.20*	- 0.22*	- 0.57***	0.05	- 0.03					
Protein %												
Nickerson 2006	- 0.53***	- 0.55***	0.05	0.10	0.43***	- 0.04	0.12	- 0.38***				
Sejet 2005	- 0.28*	- 0.30**	0.02	0.12	0.38***	- 0.20*	0.10	- 0.01				
Sejet 2006	- 0.13	- 0.20*	0.24*	- 0.02	0.43***	- 0.04	- 0.33***	- 0.21*				
SW												
Nickerson 2006	- 0.14	- 0.06	- 0.05	- 0.16	- 0.25*	0.45***	- 0.22*	- 0.08	- 0.01			
Sejet 2005	- 0.16	- 0.13	- 0.18*	- 0.29**	- 0.24*	0.61***	- 0.12	0.10	- 0.23*			
Sejet 2006	- 0.19*	- 0.16	- 0.13	- 0.32**	- 0.19*	0.50***	- 0.22*	- 0.13	- 0.08			
TGW												
Nickerson 2006	0.30**	0.29**	- 0.05	- 0.25*	- 0.12	0.25*	- 0.34***	- 0.08	- 0.08	0.25*		
Sejet 2005	0.35***	0.37***	- 0.40***	- 0.44***	- 0.34***	0.45***	0.00	0.15	- 0.40***	0.34***		
Sejet 2006	0.29**	0.29**	- 0.25*	- 0.51***	- 0.27*	0.37***	- 0.27*	- 0.06	- 0.07	0.36***		
Yield												
Nickerson 2006	0.02	0.02	0.11	0.17*	- 0.46***	0.43***	0.21*	0.17*	-0.25*	0.30**	0.18*	
Sejet 2005	0.27*	0.34***	- 0.10	- 0.11	- 0.59***	0.21*	0.01	0.10	- 0.50***	0.16	0.42***	
Sejet 2006	- 0.21*	- 0.21*	- 0.31**	- 0.25*	- 0.33***	0.44***	0.07	- 0.15	- 0.21*	0.54***	0.47***	
Starch %												
Sejet 2005	- 0.02	0.00	- 0.11	- 0.23*	- 0.40***	0.40***	- 0.12	0.11	- 0.71***	0.67***	0.42***	0.43***
Sejet 2006	- 0.06	- 0.03	- 0.12	- 0.13	- 0.56***	0.21*	0.01	0.20*	- 0.65***	0.46***	0.20*	0.39***

HD, heading date; HFL, height to the flag leaf; SW, specific weight; TGW, thousand grain weight

* Significant at $0.01 \leq P \geq 0.09$; ** Significant at $0.009 \leq P \geq 0.001$; *** Significant at $P \leq 0.001$

2.3.2.3 Correlation between different traits

There were 131 Pearson significant correlation coefficients detected among eleven traits and between STB severity and agronomic traits in the phenotypic analysis. Thus, the more important associations are summarized in this section.

2.3.2.3.1 Plant adaptation traits and yield

The association between HD and yield was negative and highly significant in all the environments, suggesting that early lines yielded more than the late ones. Similarly, the association between HD and the yield components grains ear^{-1} and TGW was significant and negative at Sejet 2005 and 2006. These results suggest that, in early lines, the grains reached more weight than in later ones. However, the association between HD and ears m^{-2} was positive at Sejet 2005 and 2006. Also, the association between HD *vs.* grains m^{-2} was slightly positive only at Sejet 2006. Heading date and TGW were negatively associated at the Sejet trials. Also, factors related to TGW were associated to HD. The association between HD and SW, and between HD and starch %, was negative in the environments where they were assessed, meaning that early lines reached more SW and starch % than late ones. Conversely, the association between HD and GPC was positive, so that early lines have less GPC than late lines in all environments. Another highly negative association was found between HD and maturity at the three environments tested (Table 2.6).

The adaptative traits, HD and height to the flag leaf, were slightly negative associated at Nickerson 2006 and Sejet 2006, suggesting that early lines were taller than late lines. The height (HFL) was positively associated to TGW, SW, starch % and yield, meaning that in taller lines, the grains reached higher weight, SW, and starch % giving as result superior yield. The HFL was negatively associated to ears

m^{-2} and grains m^{-2} at the Sejet trials, suggesting that, in these environments, taller lines had less grains m^{-2} and ears m^{-2} (Table 2.6).

2.3.2.3.2 Yield and yield components

Of the yield components, only TGW was positively associated to yield in all the environments. The trait grains ear^{-1} was also positively associated to yield only at Nickerson 2006. At Sejet 2006, the yield and ears m^{-2} were negatively correlated. Only at Nickerson 2006, the association between yield *vs.* grains m^{-2} was positive. The yield components ears m^{-2} and grains m^{-2}, and grains ear^{-1} and grains m^{-2} were positively associated in all environments. Conversely, negative correlations were found between ears m^{-2} and grains ear^{-1} at Sejet 2005 and 2006. A TGW and grains m^{-2} were in a significant way negatively associated at all the environments. Similarly, the association between TGW and ears m^{-2} was negative at Sejet 2005 and 2006. Also, the association between TGW vs. grains ear^{-1} was negative at Nickerson 2006 and Sejet 2006.

2.3.2.3.3 Quality grain traits and yield

The traits GPC, SW and percentage of starch are traits of the grain and thus it was expected they should be associated to yield and TGW. The associations yield-starch %, yield-SW, yield-TGW, TGW-starch %, TGW-SW, SW-starch %, were positive at all the environments, indicating that, as the starch % increased, TGW, SW and yield also increased (grain starch content was not measured at Nickerson 2006). Conversely, grain protein content was negatively associated to starch %, SW, TGW and yield, especially at Sejet 2005. However, SW *vs*. GPC and TGW *vs*. GPC did not show statistical significance association at Nickerson 2006 and Sejet 2006 (Table

2.6). These results suggested that, as GPC increased the starch %, SW and yield decrease (Table 2.6).

The association between grains m^{-2} and SW was negatively significant at Sejet 2005 and 2006. Also, the association between grains m^{-2} and starch % was negative at Sejet 2005. For the grains m^{-2} factors, the association between ears m^{-2} and GPC was positive only at Sejet 2006. The association between ears m^{-2} and SW was negative only at Sejet 2005. The association between grains ear^{-1} and GPC was negative at Sejet 2006. The association between grains ear^{-1} and SW was negative at Nickerson 2006 and Sejet 2006 (Table 2.6).

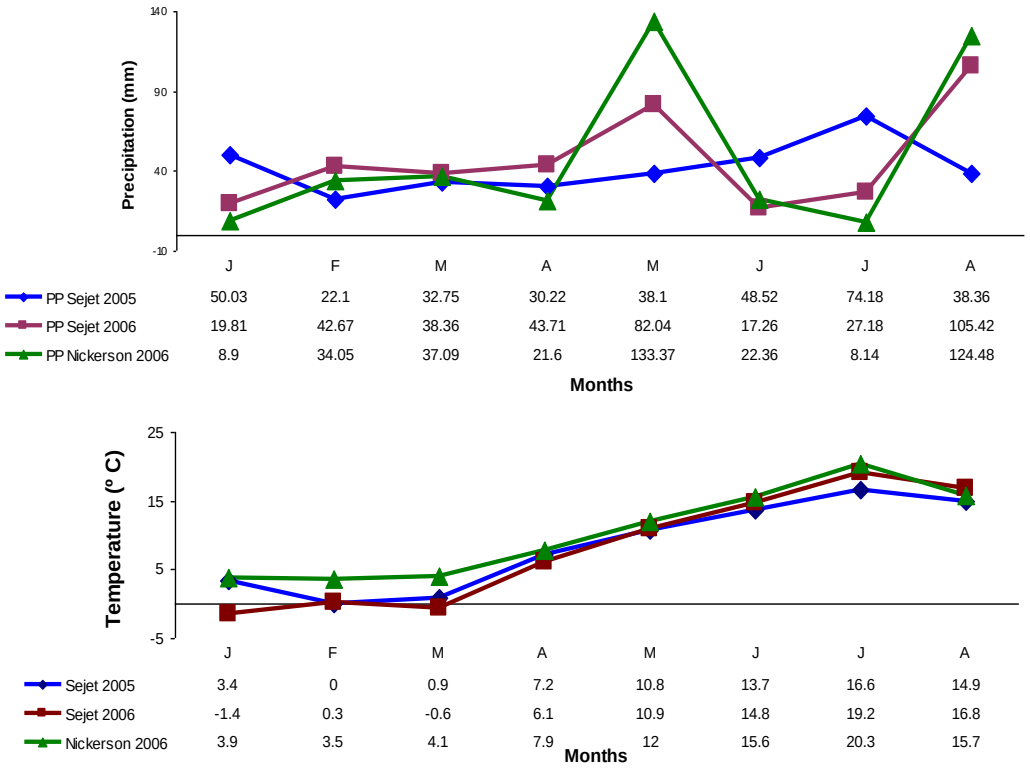
2.3.3 Environmental factors

Environmental factors are important in the performance of the DH population at each environment. Figure 2.1 shows precipitation and temperature during the crop development of the Savannah x Senat DH population at Nickerson 2006, Sejet 2005, and Sejet 2006. This figure intended to give a general idea of the climatic differences among the trials analysed.

At Nickerson 2006, during most of the vegetative period the temperatures registered were around 3ºC higher than at the Sejet environments. Temperatures up to 14ºC were registered before heading and early grain filling period. There was a period of moisture stress during heading date and early grain filling period, after which the precipitation increased during the last month of the grain filling period. The total precipitation from January to August was 389.99 mm.

At Sejet 2005 and 2006, lower temperatures were registered during the vegetative period. At Sejet 2005, the total amount of rainfall was the lowest (334.26 mm) but its distribution was steady during the vegetative phase, and reached it its maximum during the heading, anthesis and grain-filling period.

Figure 2.1 Environmental factors: temperature and precipitation at Nickerson 2006, Sejet 2005 and Sejet 2006 from January to August



At Sejet 2006, there was a period of scarce precipitation where temperature was up to 15ºC days before heading and during anthesis and early grain development. At the middle of the grain development, the precipitation increased. The total precipitation from January to August was 376.45 mm.

2.4 Discussion

The analysis of variance that compares the three trials reveals that there was much phenotypic variation in the Senat × Savannah DH population, as there were differences for almost all the traits. No significant trial × line interaction was found

for ears m^{-2}, however there was no association found between Nickerson 2006 vs. Sejet 2005 and Nickerson 2006 vs. Sejet 2006 for this trait.

These results clearly indicate that the DH population performed differently in the three environments. The differences become more evident throughout the comparison of the means among the trials and the correlation coefficients among the agronomic traits. Additionally, the associations between each agronomic trait and STB severity were different under each environment. The yield components TGW, grains m^{-2}, ears m^{-2} and grains ear^{-1} were associated to STB severity, as well as the yield itself. Furthermore, other traits like SW, HD, maturity and grain protein content were also associated to STB severity.

These associations are presented as subsystems to allow a better understanding of the relationships among the agronomic traits and the agronomic traits and Septoria tritici blotch.

Phenotypic association of plant adaptation traits and STB

In wheat, disease escape prevents or reduces contact between pathogen spores and the upper canopy (Paveley *et al*., 2005), as yield reduction is mostly related to the disease of the top leaves (Shaner *et al*., 1975; Ziv and Eyal, 1978; King *et al*., 1983a; Thomas *et al*., 1989; Shaw and Royle, 1989a, b, and 1993; Paveley, 1999, 2005). The heading date and the plant height have been considered factors that confer 'disease escape' to STB severity, but its mechanisms may differ and are determined by the environment.

Differences in time to heading in the Senat × Savannah DH population among the three environments may be due to sensitivity to temperature during the vegetative and generative phases. Heading date was found to be negatively associated to yield in a highly significantly way in all the environments (Fig. 2.2a, b, and c).

Figure 2.2 Associations of plant adaptation traits (shown by arrows) with yield and yield components and associations of heading date and yield components with STB severity at three environments (only significant associations are shown).

STB association with an agronomic trait; Trait that contributes to increase another trait; Inversely associated traits; normal number, correlation coefficient at NrAaby02; **bold** number, correlation coefficient at Sejet02

Fig. 2.2a Nickerson 2006; Fig. 2.2b Sejet 2005; Fig. 2.2c Sejet 2006.

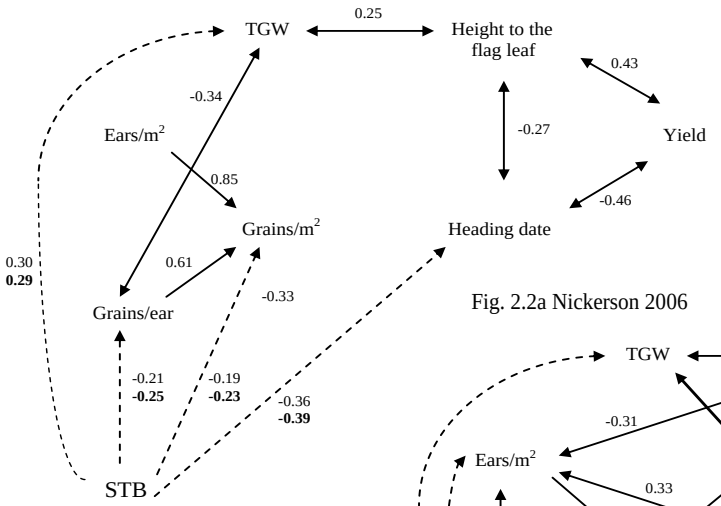


Fig. 2.2a Nickerson 2006

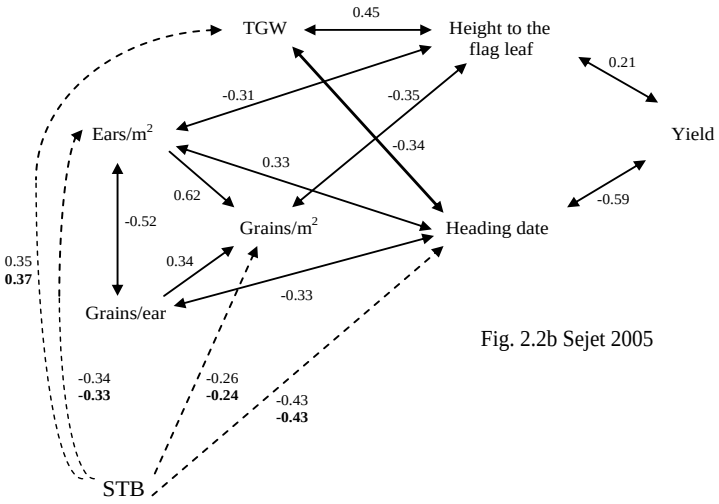


Fig. 2.2b Sejet 2005

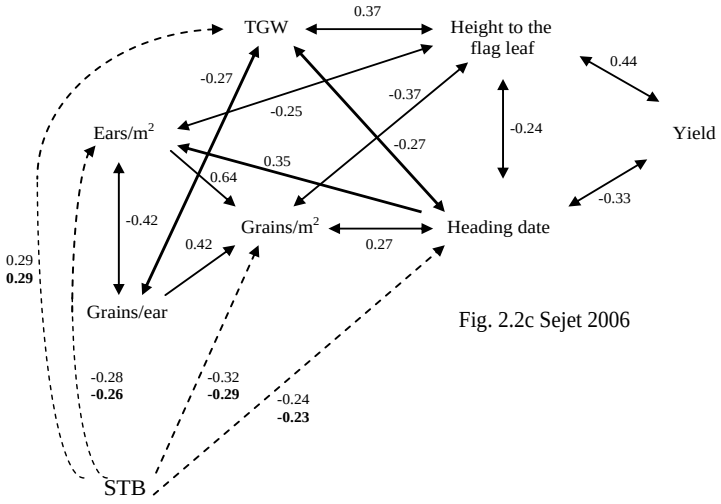


Fig. 2.2c Sejet 2006

These results agree with those found by Zhong-hu and Rajaram (1994) under late planting. Also, HD was negatively associated to TGW at Sejet 2005 and 2006. According to Sofield *et al*. (1977b), Angus and Moncur (1977), Evans (1993) and Beltrano *et al*. (2006), this may be due to the short grain growth period that limits the accumulation of dry matter in the grain. There was highly positive association between HD and ears m^{-2} at the Sejet trials (Fig. 2.2). A positive association was found between HD and grains m^{-2} only at Sejet 2006. A highly significant negative association between HD and grains ear^{-1} was calculated only at Sejet 2005 (Fig. 2.2b), suggesting that HD was a limiting factor for the increase of grains numbers.

According to Kirby (1988) and Herzog (1986), these associations between the HD and the yield components may be related to the time to heading. At this stage, when ears and stem were growing at the most rapid rate and peduncle was also growing rapidly, there was still a considerable production of florets, but it was also the time for floret death.

The late reproductive phase is of great importance because the yield components ears m^{-2}, grains ear^{-1} and grain m^{-2} are mainly established and hence, the potential yield (Kirby, 1988; Slafer and Rawson, 1994; Slafer, 2007). Competition for assimilates during the reproductive stage is mainly between the elongating stems and the ears development (Evans and Wardlaw, 1976; Kirby, 1988). However, stem growth is probably the principal sink before anthesis (Evans and Wardlaw, 1996). Under favourable conditions, competition is not significant (Herzog, 1986). Thus, height and heading seemed to be related at this time of the crop development. Slightly significantly negative associations were calculated between these traits at Nickerson 2006 and Sejet 2006, where the means for height were superior (Table 2.2, Fig. 2.2a and c), though there were not statistically significant differences

between them. Nevertheless, there was no significant correlation between height and HD at Sejet 2005 (Fig. 2.2b), suggesting independent development between these traits at this environment.

A highly negative significant association was found between heading date and STB at Nickerson 2006 and at Sejet 2005. This result agrees with several reports of associations between incidence of STB and earliness (Danon *et al*., 1982; Camacho-Casas *et al*., 1995; Arama *et al*., 1999; Simón *et al*., 2002 and 2004; Chartrain *et al.*, 2004b; Arraiano *et al*., 2006). The negative association between HD and STB severity was slightly significant at Sejet 2006 trial (Table 2.6 and Fig. 2.2). This result agrees with those reported for late heading (Shaner *et al*., 1975; Danon *et al*., 1982; Shaw and Royle, 1989a, b, 1993; Eyal and Talpaz, 1990; van Beuningen and Kohli, 1990; Arraiano *et al*., 2007a).

At Nickerson 2006 there was no association between height and yield components, except with TGW (Fig. 2.2a). However, at Sejet 2005 and 2006 there were negative associations between height and grains m^{-2} and height and ears m^{-2} (Fig. 2.2b and c), suggesting competition for assimilates between stem elongation and ears and grains survival. The association between height and TGW and between height and yield was positive in all the environments. Similar results have been found by several authors (Ziv *et al*., 1981; Slafer and Andrade, 1991; Zhon-hu and Rajaram, 1994). Slafer and Andrade (1991) suggested that this positive association is based on the better light distribution within the leaf canopy of taller cultivars. Thus, the highly positive significant association between specific weight and height is important. This association is also related to the positive associations between height and starch content, between height and TGW, and between height and yield and

among these traits where positive associations were also found in all the environments (Fig. 2.2, Table 2.6).

In this work, the height to the flag leaf did not show association with disease severity in the environments tested. Similar results were found by Eriksen *et al*. (2003) when they were working with the Savannah × Senat DH population. Danon *et al*. (1982) have also reported no significant associations between STB severity and height. However, several other authors have reported negatively significant associations (van Beuningen and Kohli, 1990; Camacho-Casas *et al.*, 1995; Chartrain *et al*., 2004b; Simón *et al*., 2004, 2005; Arraiano *et al*., 2006).

Phenotypic association between yield components and STB

The yield components ears m^{-2}, grains ear^{-1}, grains m^{-2} and TGW are physiologically associated to the final yield, and these associations can be explained by the equation:

Yield = grains m^{-2} × grains weight

Grains m^{-2} = grains ear^{-1} × ears m^{-2}

which involves the major yield components (Slafer, 2007). These yield components were correlated with STB severity in a different way in each environment (Fig.2.3).

At Nickerson 2006, the positive associations between the yield components indicate that compensatory effects were almost absent (Figure 2.3a), which agrees with the results found by García del Moral *et al*. (2003) in *Triticum turgidum.* In their work, under favourable conditions during the development process of the crop, with sufficient amount of water, nitrogen and moderate temperature, there were no compensatory effects or they were not significant. The significantly high yield in this environment (Table 2.2) proves there were better conditions for the development of the DH population than at the Sejet trials.

Figure 2.3 Associations between yield components with yield and STB severity at three environments and their relationships (shown by arrows. Only significant associations are shown).

STB association with an agronomic trait; Trait that contributes to increase another trait; Inversely associated traits; normal number, correlation coefficient at NrAaby02; **bold** number, correlation coefficient at Sejet02

Fig. 2.3a Nickerson 2006; Fig. 2.3b Sejet 2005; Fig. 2.3c Sejet 2006.

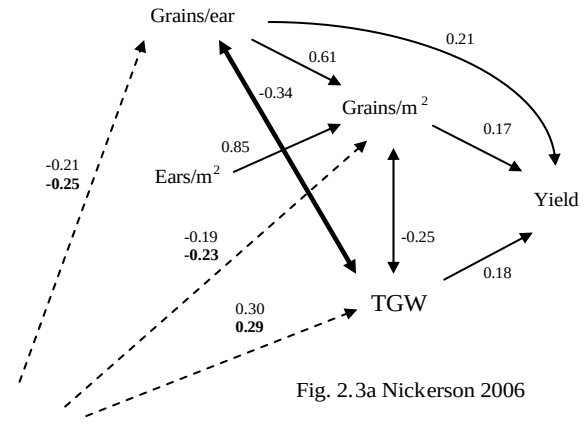


Fig. 2.3a Nickerson 2006

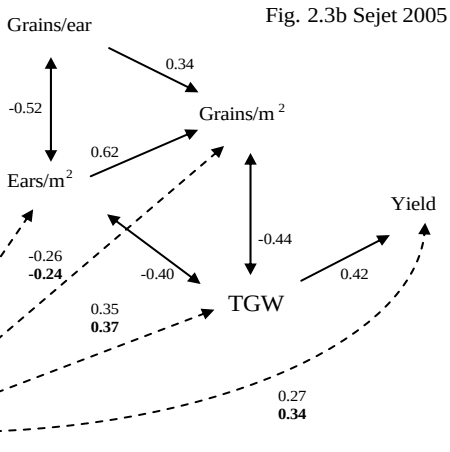
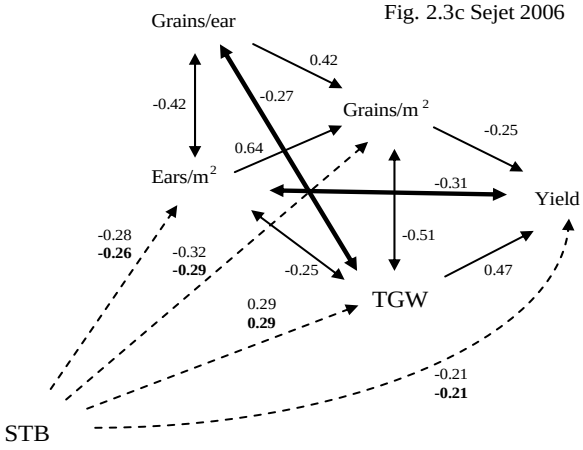


Fig. 2.3b Sejet 2005

Fig. 2.3c Sejet 2006



Nevertheless, a strong negative association was observed between grains ear^{-1} and TGW. A similar association has also been found by García del Moral *et al.*

(2003) and Huang *et al*. (2004). Early heading was registered at this environment (Table 2.2). In wheat, this performance is caused by its sensitivity to high temperatures (Herzog, 1986; Slafer and Rawson, 1994). Slafer and Rawson (1994) indicate that, although there were differences among genotypes, the response to temperature was stronger in the vegetative phase than in the spikelet initiation or stem elongation phases.

The response to a short vegetative period is usually manifested as a reduction in ears m^{-2} formation, according to Herzog (1986). Values were lower in Nickerson 2006, however, the higher amount of grains ear^{-1} was reached in this environment (Table 2.2), which in terms of the results reported by Evans & Wardlaw (1976) and Blum & Pnuel (1990), seemed to occur as a compensation response for the reduction in ear number; this in order to maintain yield. Both characteristics, low number of ears m^{-2} and high amount of grains ear^{-1}, represent no competition for assimilates between these traits, which conducts towards a better filling of grains (Evans and Wardlaw, 1976; Giunta *et al*., 1993), most probably this was the reason a thousand grain weight was significantly higher in Nickerson 2006 than in the Sejet trials (Table 2.2). The grains ear^{-1} contributed directly and positively to the yield (Fig. 2.3a), as it has been found in *Triticum turgidum* (Gebeyehou *et al*., 1982; Simane *et al*., 1993; García del Moral *et al*., 2003) and *T. aestivum* wheat cultivars (Evans, 1993; Zhong-hu and Rajaram, 1994; Denčić *et al*., 2000) under drought stress. A thousand grain weight also contributed positively with final yield (García del Moral *et al*., 2003). However, the positive associations of all these traits grains m^{-2}, grains ear^{-1} and TGW to yield were individually slight (Figure 2.3a).

Conversely, at Sejet 2005 and 2006, late heading of the DH population was present and the amount of ears m^{-2} was higher than at Nickerson 2006 (Table 2.2).

These characteristics have been found when the temperature was low during the vegetative period (Herzog, 1986). Also at Sejet 2005 and 2006, the number of grains ear^{-1} was significantly lower than at Nickerson 2006 (Table 2.2). Mainly, reduction in grains per ear was related to limited precipitation during the differentiation of spikelets and florets (Brocklehurst *et al*., 1978; Giunta *et al*., 1993; Simane *et al*., 1993; Denčić *et al*., 2000; García del Moral *et al*., 2003; Beltrano *et al*., 2006). The association ears m^{-2} and TGW was negative at the Sejet environments, suggesting competition for assimilates between the growth of the surviving ears per plant and the grain growth (Fig. 2.3b and 2.3c). Similar results have been found by Gebeyehou *et al*. (1982), indicating that with an increase of ears m^{-2}, the number of grains ear^{-1} and the TGW diminished (Table 2.2). Compensatory effects at Sejet 2005 and 2006 were indicated by the negative association between ears m^{-2} and grains ear^{-1} at both environments. Also, there was no positive contribution of grains m^{-2} to the yield (Fig. 2.3b and 2.3c). The association between grains m^{-2} and TGW was highly negative in these two environments. The high positive association between yield and TGW indicates that this trait determined the final yield mostly in these environments. This result agrees with the findings of García del Moral *et al*. (2003) and Gross *et al*. (2003). García del Moral *et al.* (2003) reported a highly positive correlation coefficient between grain yield and grain weight in cooler and warmer environments and two moisture regimes (irrigated and rain fed). However, the lowest TGW and yield were reached at the Sejet trials (Table 2.2).

Compensatory effects among all the yield components were mainly found at Sejet 2006 (Figure 2.3c). This may be due to the higher number of ears m^{-2} which increased competition for assimilates. There was a highly negative association between ears m^{-2} and yield. Also, the association between ears m^{-2} and grains ear^{-1},

grains m^{-2} and TGW were highly significant negative. These results suggest a strong competition for assimilates between ears surviving against grains development. Several authors like Fischer and Maurer (1978), Giunta *et al*. (1993) and Denčić *et al*. (2000) have assumed that this response is due to the limited amount of water during the early grain-filling period.

Yield and yield components data from the trials analyzed were correlated with the segregation data for STB severity at NrAaby02 and Sejet02 sites, giving the following results. At Nickerson 2006, the trait grains ear^{-1} was slightly negatively associated to STB severity at NrAaby02 and Sejet02. Reductions in grains ear^{-1} have been found as a result of early infections as reported by Ziv and Eyal (1976, 1978) and Simón *et al*. (2002). The trait ears m^{-2} was highly negatively associated to STB severity at Sejet 2005, while it was slightly negatively associated to STB severity at Sejet 2006. A reduction of the amount of ears m^{-2} by STB in some tolerant wheat cultivars has also been found by Simón *et al*. (2002) and Ziv and Eyal (1976), the latter suggesting it might be due to the high amount of tillers. Shaw and Royle (1989a) explained the dissemination of conidia from lower to higher up leaves in the canopy or to the surrounding leaves by rain splash. Also, Shaw and Royle (1993) and Lovell (1997, 2004a) indicated the transmission of septoria tritici by contact between different leaves with similar height may occur, both ways to infect healthy plants are easy allowed by the high amount of tillers.

A thousand grain weight was positively associated with STB severity at Nickerson 2006, Sejet 2005 and Sejet 2006. This result agrees very well with those found by Ziv and Eyal (1976, 1978), Ziv *et al*. (1981), Zilberstein *et al*. (1985), Cornish *et al*. (1990), Zuckerman *et al*. (1997) and Simón *et al*. (2002) in tolerant cultivars. The positive association between these traits indicates the maintaining of a

TGW under STB severity stress. Conversely, in susceptible varieties TGW can be particularly reduced by STB disease (Ziv and Eyal, 1978; Zilverstein *et al*., 1985; Cornish *et al*., 1990; Leith and Jenkins, 1995; Simón *et al*., 2002).

The yield component grains m^{-2} was negatively associated to STB severity. This negative association has not been reported before. Three points have to be clarified here with respect to grains m^{-2}. Firstly, grains m^{-2} showed a negative association with TGW in the three environments. According to Evans and Wardlaw (1976), this trait tends to vary inversely with grain weight from up to 25,000 grains m^{-2} in wheat. The negative association between these traits indicate that the total amount of available assimilates for grain growth is not large enough to satisfy the demand of the grains to be completely filled (Slafer and Andrade, 1991), or grain set took place in distal positions within the central spikelets and/or in extreme spikelets of the ear, positions where grains have a lower weight potential (Slafer and Andrade, 1991; Evans and Wardlaw, 1996). Secondly, but linked with the last point, we can mention the negative association between grains m^{-2} and yield at Sejet 2006 and the lack of association between these traits at Sejet 2005, it signals not only to the reduction of yield when there are many ears per unit area, but also to the existence of a further biological interpretation, the physiological limitation of potential yield (Evans and Fischer, 1999). To this respect, in terms of the arguments presented by Fischer and Wood (1979), the amount of ears m^{-2} was in super optimal conditions to reach the maximum yield. Thirdly, with reference to the association between grains m^{-2} and STB, since grains m^{-2} has not been explicitly reported in the literature as an independent trait affected by STB disease, it might be just because grains m^{-2} is the arithmetic product of ears m^{-2} and grains ear^{-1}. However, it is not just the product of these traits; it is also the interaction between them and with the environment.

At Sejet 2005, yield was positively associated to STB severity. However, at Sejet 2006, yield was negatively associated to STB severity (Figure 2.3b and 2.3c). Yield is the integrated end product of a great variety of processes (Evans, 1993); it is the action and the interactions among different traits more than a trait itself, determined by particular genes (Slafer, 2003 and 2007). Thus, it is not easy to explain the positive association between yield and STB severity at Sejet 2005, and the negative association between these traits at Sejet 2006, although these associations were negligible. Maybe at Sejet 2005 the positive association would be a resemblance of the relation between TGW and STB severity (highly significant), since TGW is dependent on the deposition of starch in the grain, which mainly contributes to the weight of the grain (Jenner *et al*., 1991). Also, starch percentage was positively associated to yield. Interestingly to note that, the correlation coefficients between TGW and starch %, TGW and yield, and starch percentage and yield were highly positive and similar at this environment (Fig. 2.3b). These associations suggest that at Sejet 2005, the grain starch percentage was mainly determined the TGW and yield. On the other hand, at Sejet 2006, where TGW and STB severity association was moderately high, the association between TGW and starch percentage was negligible (Fig. 2.3c). Although grain starch percentage was positively and highly significantly associated to yield, this suggests that the TGW and yield do not only depend on starch percentage, but also on other compounds, at this environment.

Association of grain protein content with STB

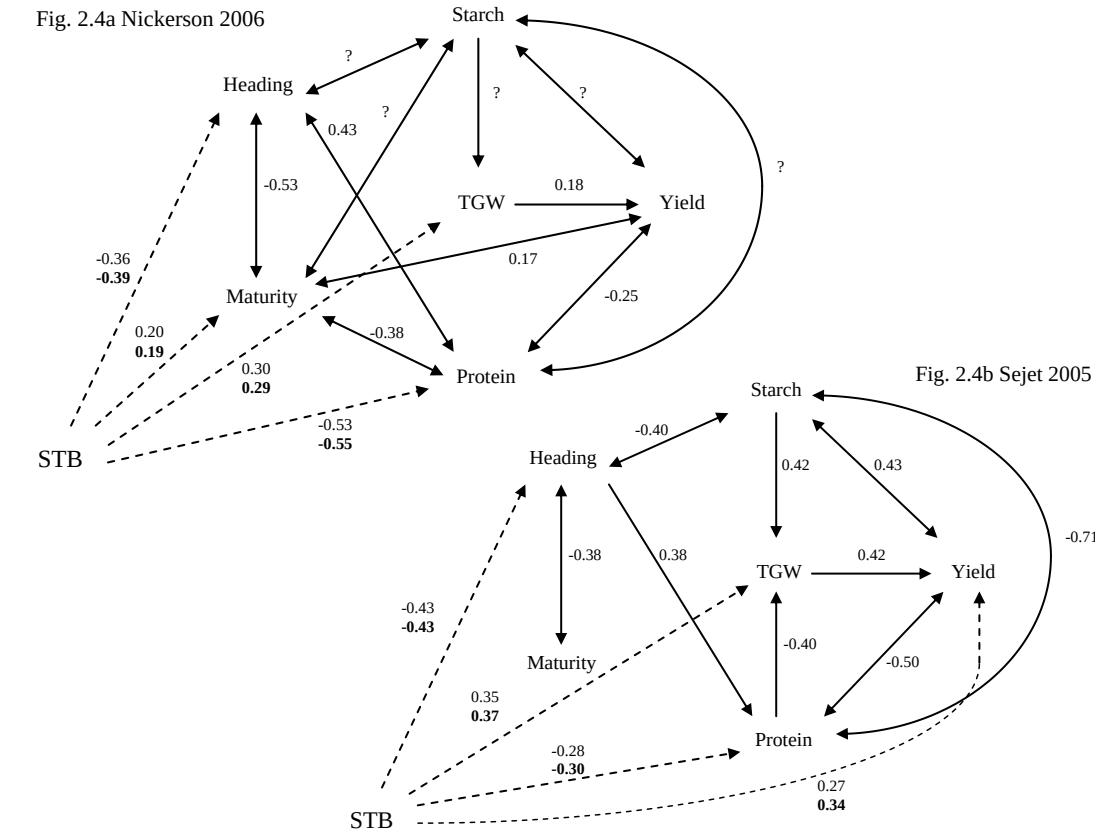
Grain protein content was significantly negatively correlated with STB severity at the three environments tested. The extent of this association was different at each

environment. At Nickerson 2006, a highly significant association was found, while at Sejet 2005 it was moderately high, and slightly significant at Sejet 2006 (Figure 2.4).

Figure 2.4 Associations between grain protein content and grain starch content with agronomic traits that determine grain composition and their association with STB severity (shown as arrows). Also, associations between grain protein content and grain starch content with TGW and yield and their association with STB severity at three environments (only significant associations are shown).

STB association with an agronomic trait; Trait that contributes to increase another trait; Inversely associated traits; normal number, correlation coefficient at NrAaby02; **bold** number, correlation coefficient at Sejet02

Fig. 2.4a Nickerson 2006; Fig. 2.4b Sejet 2005; Fig. 2.4c Sejet 2006.



Grain protein content was negatively associated to yield in all the environments (Figure 2.4). This correlation agrees with those found by Evans and Wardlaw (1976), Cox *et al*. (1985), Herzog (1986), Jenner *et al*. (1991), Stone and Nicholas (1994), Monaghan *et al*. (2001), Groos *et al*. (2003), Brown *et al*. (2005) and many others. The relationship between GPC and yield actually depends on the available nitrogen according to Jenner *et al*. (1991), Monaghan *et al*. (2001), Brown *et al*. (2005) and Kichey *et al*. (2007). However, Evans and Wardlaw (1976) stated that a negative correlation between these traits is often found. They indicated that this performance is influenced by the available amount of nitrogen during grain development but also by other environmental factors as drought stress and high temperatures.

Brown *et al*. (2005) found a positive correlation between GPC and yield at several locations if GPC protein was lower than 12.5%. These results suggest that available N at these sites was not sufficient for high yield. In contrast, a negative correlation between GPC and yield was found at other sites where GPC exceeded 12.5%, meaning that N was adequate for maximizing yield. Conversely, at Sejet 2005, GPC was less than 11.17 % in all the lines, but still a negative correlation between GPC and yield was found. The controversial results between Brown *et al.* (2005) and those found in this work suggest that the association between these traits was not a function of N availability only, but that other factors might be involved as climatic factors, management practices, soil conditions and/or further physiological relationships, as it was explained before. Thus, more investigation about the physiological and biochemical relationships between grain protein content and yield in wheat has to be developed.

At Nickerson 2006 and Sejet 2006, GPC means of the lines were high, i.e., 12.27 % and 12.08 %, respectively, and a slight significant negative association of GPC and yield was found. These results indicate that GPC was adequate to reach the maximum yield (Fig. 2.4a and 2.4c). However, no significant association was found between GPC and TGW under these environments. Similar results were found by Kichey *et al*. (2007), when analysing two different levels of N fertilizer. Also, Pomeranz *et al*. (1985) have reported no association between GPC and TGW in several wheat varieties. At Sejet 2005, where the mean for GPC was inferior (9.89 %) than at Nickerson 2006 (12.28 %) and Sejet 2006 (12.08 %), a highly significant negative association between yield and GPC was found. Cox *et al*. (1985) reported a similar association between these traits. In addition, GPC was highly negatively associated to TGW at this environment only, suggesting higher grain weight when the protein content was lower (Table 2.6 and Fig. 2.4b).

At Sejet 2005 and 2006, starch percentage was found highly positively associated to yield and also positively correlated with TGW. The association of starch content and TGW was negligible at Sejet 2006, while it was highly significant at Sejet 2005, but the means for the yield were similar at both trials (Table 2.2 and Fig. 2.4). In addition, the association between GPC and starch content was negative under these environments (Figure 2.3b and 2.3c). A negative correlation between these traits has been reported before (Herzog, 1986; Jenner *et al*., 1991). Jenner *et al*. (1991) conducted a physiological analysis to find an explanation for this inverse relationship but failed. The authors concluded that their results were not enough to explain this association.

At Nickerson 2006, where a statistically higher amount of grains ear^{-1} was obtained, also the TGW was superior. However, at Sejet 2005 and 2006 where a

lower amount of grains ear^{-1} was reached, the lower TGW was also found (see table 2.2). At Sejet 2005, a slight negative correlation coefficient was calculated between starch content and grains m^{-2}, suggesting that the last trait limited the accumulation of starch in the grains. At Sejet 2006, a significant negative association was found between GPC and grains ear^{-1}. Conversely, a slight positive correlation coefficient was detected between GPC *vs*. ears m^{-2}, suggesting the supra-optimal condition of ears m^{-2} increased protein content (Table 2.6). The contradictory results exposed above among GPC, grain starch content, TGW, yield and yield components could be explained by source and sink events, and the process of accumulation of protein and starch in the grains.

There is certain synchronization, but not always, in the accumulation of both N and starch in the grain, though there is independence between these events (Herzog, 1986; Jenner *et al*., 1991), and the rate and duration of their deposition are determined by source and sink events (Herzog, 1986; Jenner *et al*., 1991; Evans and Wardlaw, 1996). If sink demand is reduced during the grain filling period, that is, the number of grains per ear is decreased, the deposition of assimilates in the grain is lowered (Herzog, 1986; Jenner *et al*., 1991; Evans, 1993; Evans and Wardlaw, 1996). Under these circumstances, the single-grain weight is also lowered (Jenner *et al*., 1991), whereas with a greater sink, higher TGW has been found (Evans and Wardlaw, 1996). Fischer (1985) found that the 30 days before anthesis are critical in the onset of ears per unit area and grains ear^{-1}. This effect may be related to source and sink relationships and assimilate supply. These last findings could explain some of the data obtained in this work. At Nickerson 2006, where more grains ear^{-1} were found (high sink demand) the TGW was superior. However, at Sejet 2005 and 2006

where lower number of grains ear^{-1} was found (low sink demand), also the lower TGW were found (Table 2.2).

The GPC and starch content during grain filling can be modified by the environment. Radiation, temperature (Fischer, 1985) and drought stress (Fischer and Wood, 1979) are the elements of the environment which affect mostly these traits. Environmental differences between the three trials during the reproductive period were basically in the precipitation distribution and temperature (Fig. 2.1). At Nickerson 2006, an increase in the temperature during the vegetative period compared with Sejet 2005 and 2006, probably was the reason why the heading was appeared 6 days before that found at Sejet trials as stated by Slafer and Rawson (1994) too; thus, the grain filling period was probably extended (Evans, 1993). At Sejet 2005, the precipitation was steady although at the late grain filling period it declined; the temperature was around 15ºC, considered the maximum for an optimum grain development and photosynthesis by Herzog (1986), Wardlaw *et al*. (1989) and Evans and Wardlaw (1996). Although at Sejet 2006 as well as at Nickerson 2006, a scarce water availability period was present before heading, the precipitation increased during anthesis and the early grain-filling phases (around a month). Besides that, when a drought stress is present, usually the temperature rises (Blum, 1998), as in these two trials, where the mean temperature was *ca*. 15ºC with a maximum of 25ºC (26.6ºC at Nickerson 2006) during the moisture stress. Under drought conditions during the grain growth, lower rates of accumulation of carbohydrates have been detected due to reduced remobilization (Brocklehurst *et al*., 1978; Panozzo and Eagles, 1999; Beltrano *et al*., 2006). This performance is primary responsible for the "apparent" increase of GPC as Evans and Warland (1976) and Jenner *et al*. (1991) stated. Possibly this effect took place at Sejet 2006, where the

grain starch content of the DH population was lower than at Sejet 2005 (67.68 % and 68.98 %, respectively), but the mean for GPC (12.28 %) was similar to that at Nickerson 2006 (12.08 %), but with better environmental conditions.

Water deficit also provoked loss of photosynthetic capacity (Evans and Wardlaw, 1976), premature induction of maturity (Brocklehurst *et al*., 1978; Clarke, 1983; Beltrano *et al*., 1999; Beltrano *et al*., 2006), and shortened grain growth period (Brooks *et al*., 1982; Panozzo and Eagles, 1999; Beltrano *et al*., 2006) while the grain dry weight is reduced (Brocklehurst *et al*., 1978; Beltrano *et al*., 2006). According to Brooks *et al*. (1982), Panozzo *et al*. (1999, 2001), Beltrano *et al*. (1999) and Beltrano *et al*. (2006) protein accumulation does not seem to be severely affected by water stress. However, Panozzo *et al*. (1999, 2001) found a relatively short duration of N synthesis in dry environments and Brocklehurst *et al*. (1978) reported a reduction in N content in grain, but Brooks (1982) found an increase in amino acid concentration.

Water deficit and high temperatures are environmental factors that may produce moderate stress, causing a reduction in the crop cycle as sustained by Angus (1977). Blum and Pnuel (1990) described that yield depends on water regimen, thus a positive correlation between yield and total precipitation in several trials can be presumed. Similar results were registered in the present work, since lowest yields were reached under low precipitation environments. This is the case of Sejet 2005 with a precipitation of 334 mm, whereas highest yield was obtained at environments with maximum precipitation during the crop cycle. This latter case was found – at Nickerson 2006, with a precipitation of 390 mm (see Fig. 2.1, Table 2 .2).

Jenner *et al*. (1991), and Stone and Nicolas (1994) declared that effects of high temperature are similar to those of drought stress, and the deposition of starch is

more sensitive than that of proteins. Stress by temperature effects provokes reduction in the duration of grain growth (Sofield *et al*., 1977a; Bhullard and Jenner, 1985; Herzog, 1986; Blum *et al*., 1994; Zhon-hu and Rajaram, 1994; Slafer and Rawson, 1994; Blum, 1998; Evans and Wardlaw, 1996; Panozzo and Eagles, 1999) or even its ending (Bhullard and Jenner, 1985). High temperatures also accelerated the rate of deposition of starch (Jenner *et al*., 1991) and N compounds (Bhullard and Jenner, 1985; Panozzo and Eagles, 1999; Beltrano *et al*., 1999, 2006; Brown *et al*., 2005), but their amounts could be less causing even, an early termination of their deposition, as reported by Evans and Wardlaw (1976), Bhullard and Jenner (1985), Jenner *et al*. (1991) and Beltrano *et al*. (2006), with the consequent reduction of the grain weight and yield (Bhullard and Jenner, 1985; Tashiro and Wardlaw, 1990; Jenner *et al*., 1991; Shi *et al*., 1994; Blum *et al*., 1994; Stone and Nicolas, 1994; Evans and Wardlaw, 1976, 1996; Beltrano *et al*., 2006). The decline of photosynthetic activity and accelerated leaf senescence could also be present (Sofield *et al*., 1977a; Herzog, 1986; Blum *et al*., 1994). On the other hand, low temperatures during the vegetative period delayed the heading date, and then the grain filling period becomes short (Evans, 1993) even with a low degree of senescent tissues of the plant (Sofield *et al*., 1977a; Evans and Wardlaw, 1976; Jenner *et al*., 1991; Kichey *et al*., 2007). It is well known that the poor yield potential of late cultivars (Evans, 1993) is closely related to the amount of starch deposition. This last characteristic may have limited the yield potential in Sejet 2005 and Sejet 2006, where lateness of the Savannah × Senat DH population was found.

Heading date seems to have a central position in the determination of grain final composition as it was positively correlated with grain protein content at all the environments tested. Conversely, HD was negatively correlated with grain starch

content at Sejet 2005 and 2006 (Table 2.6 and Figure 2.3). Canopy maturity also seemed to be involved in the determination of protein and starch deposition in the grains. Canopy maturity was highly significantly negatively correlated with HD in all the environments (Table 2.6 and Fig. 2.4). The performance of canopy maturity with GPC was completely opposite to that of HD with this grain trait at Nickerson 2006 and Sejet 2006. Thus, canopy maturity was negatively associated to GPC. A negative association between canopy maturity and GPC has also been found by Cox *et al*. (1985) and Kichey *et al*. (2007). Conversely, a positive association between canopy maturity and grain starch content was found at Sejet 2006 (Figure 2.3c). At Nickerson 2006 canopy maturity was positively associated to yield (Fig. 2.4a). However, a negative correlation coefficient between leaf senescence and grain yield was found by Kichey *et al*. (2007). They proposed that extended metabolic activity influenced grain productivity. Thus, low degree of senescence of the plant tissues at maturity indicates the photosynthesis process is occurring (Sofield *et al*., 1977a; Evans and Wardlaw, 1976; Jenner *et al*., 1991; Kichey *et al*., 2007). It is suggested from the data that the character 'greenness' is present in the Senat × Savannah DH population. The means for canopy maturity classification were 5 (straw half mature, nodes green) at Nickerson 2006 and 3 (flag leaf half mature) at the Sejet trials (Table 2.2).

Zilberstein *et al*. (1985), Cornish *et al*. (1990), Gaunt (1995) and Blum (1998) offered a set of possible explanations for the small effect of STB on yield in tolerant cultivars of wheat. First, a high capacity for stem reserves utilization during grain filling; Second, due to a high rate of CO_2 fixation of residual photosynthetic tissue (Zuckerman *et al*., 1997); and third, by a longer green area duration (Shaner *et al*., 1975; Cornish *et al*., 1990; Gaunt, 1995).

The relationships found between the data for STB severity and the trait GPC and GPC and grain starch content from the Savannah × Senat DH population are presented in Figure 2.4. Septoria tritici blotch severity showed a negative correlation coefficient with GPC at Nickerson 2006, Sejet 2005 and Sejet 2006 (highly, moderately and negligibly associated, respectively). Several authors have reported the change in plant N metabolism due to STB (MacCabe *et al*. 2001; Ruske *et al*., 2001; Dimmock and Gooding, 2002; Ruske *et al*., 2003). *Septoria tritici*, as a biotrophic fungus, reduces both nitrogen uptake and partitioning of N to the grain, decreasing grain protein content (MacCabe *et al*. 2001; Ruske *et al*., 2001; Ruske *et al*., 2003).

Canopy maturity was slightly positively correlated to STB severity at Nickerson 2006 and Sejet 2006 (Fig. 2.4a and 2.4c). Septoria tritici blotch provokes earlier senescence of leaves (Shaw and Royle, 1989b; Cornish *et al*., 1990; Leitch and Jenkings, 1995; Zuckerman *et al*., 1997; Lovell *et al*., 1997; Parker *et al*., 2004) and the induction of apical senescence (King *et al*., 1983b; Magboul *et al*., 1992; Robert *et al*., 2006).

Association between specific weight and STB

The specific weight was associated positively to yield (Table 2.6). A moderately high correlation was found at Nickerson 2006. However at Sejet 2006, this association was highly significant, while at Sejet 2005 there was no significant association between these traits. The specific weight at Nickerson 2006 had the highest mean of the three environments (Table 2.2). According to reported values (Hook, 1984), the grains were well filled. The means of SW and TGW at Sejet 2005 and 2006 were significantly lower than at Nickerson 2006, suggesting that a considerable percentage

of grains at the Sejet trials may be shrivelled and/or softer (Pomeranz *et al*., 1985; Gaines *et al*., 1997).

Figure 2.5 Significant associations between specific weight, yield components and yield at three environments (shown as arrows). Also, significant associations of these traits with STB severity at three environments (only significant associations are shown).

STB association with an agronomic trait; Trait that contributes to increase another trait; Inversely associated traits; normal number, correlation coefficient at NrAaby02; **bold** number, correlation coefficient at Sejet02

Fig. 2.5a Nickerson 2006; Fig. 2.5b Sejet 2005; Fig. 2.5c Sejet 2006

Fig. 2.5a Nickerson 2006

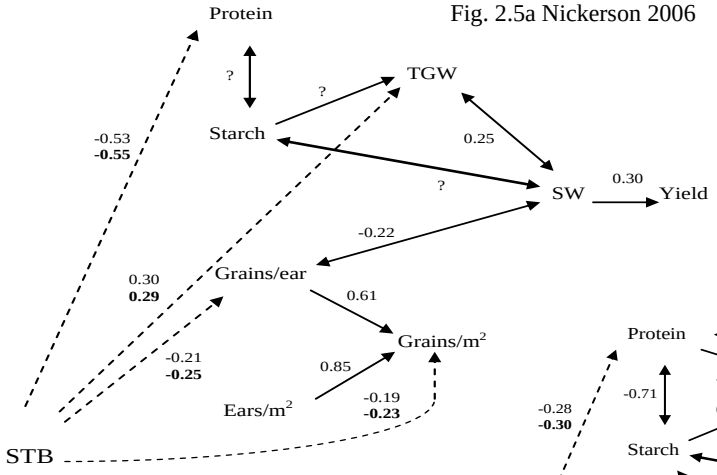


Fig. 2.5b Sejet 2005

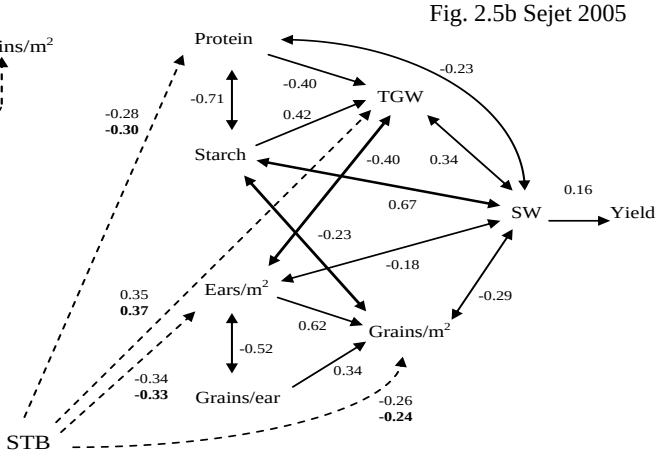
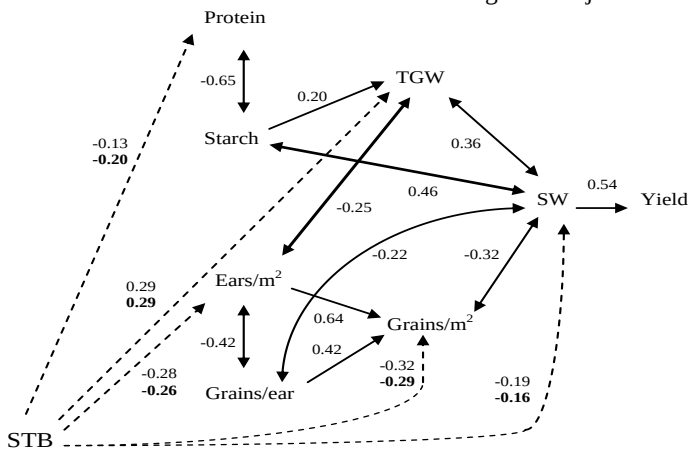


Fig. 2.5c Sejet 2006



The association between SW and TGW was positive (Table 2.6 and Fig. 2.5). At Sejet 2005 and 2006 it was moderately high and positive, while at Nickerson 2006 it was negligible. According to Gaines *et al*. (1997), TGW can depend on the size of the grain, SW of small grains can be as high as that of larger grains, all depending on the texture properties (softness) and on the degree of grain filling (packing) during the grain growth period. The specific weight was negatively associated to grains m^{-2} at the Sejet trials. It was also negatively associated to ears m^2 in Sejet 2005. Also, slightly negative associations were found between SW and grains ear^{-1} at Nickerson 2006 and Sejet 2006 (Fig. 2.5).

Specific weight, as well as TGW, depends on the deposition of starch and proteins in the grain. Thus, it has been found that the cessation of starch accumulation in the grain is normally characterized by the incapacity of the endosperm to convert sucrose into starch (Jenner and Rathjen, 1975). Internal grain factors may be involved in the ending of starch and N accumulation in the grain (Jenner and Rathjen, 1975). Brooks *et al*. (1982) suggest that similar factors control grain maturation under optimal conditions as well as under stress. Stress just accelerates the production or action of these factors. Small grains with a reduced grain filling period do not fill out well (Brocklehurst *et al*., 1978; Gaines *et al*., 1997). Also, secondary grains have not enough time to develop properly, thus they become shrivelled and softer. This is related to the form of deposition of starch granules and proteins, the amount of them accumulated in the grain, and the environmental conditions at each specific environment.

At optimum grain-growth temperatures (18ºC), protein covers the starch granules in the grain, giving place to a strong interaction. However, at 25ºC (and higher) starch granules seemed not to be covered completely by proteins (Shi *et al*.,

1994). At this temperature, the protein matrix between starch granules seems weaker than under optimal conditions (Shi *et al*., 1994). High temperatures weaken the interaction of starch and protein, which results in a decrease of grain hardness and reducing the number of starch granules (Shi *et al*., 1994).

The SW seems to be affected by STB severity with NrAaby02 data only at Sejet 2006 (Fig. 2.5). McKendry *et al*. (1995) and Simón *et al*. (2002) have reported a reduction in SW by STB. McKendry *et al*. (1995) found, for resistant and susceptible cultivars that, even when the infection did not reach the flag leaf, SW was reduced. In their work, disease severity increased flour protein content, which indicates shrivelled grains. This was consistently related to the loss of photosynthetic area. Simón *et al*. (2002) also found a reduction in SW by STB only in one year of a two year trial of fertilization treatments, where SW did not change on fertilization. Also, reductions in specific weight have been observed by several authors in trials comparing the application of fungicide plots with control plots of diseased plants (Gooding *et al*., 1994; Ruske *et al*., 2001; Dimmock and Gooding, 2002a).

Severity of Septoria tritici blotch and traits performance

The trend of STB severity against each agronomic trait was very similar at both sites NrAaby02 and Sejet02. The significant correlations were mostly similar even though severity was about 10 % higher at NrAaby02 (Table 2.4). An exception to this was the grain protein content. The correlation at Sejet 2005 was moderately significant with STB at Sejet02 (r = -0.30), and just significant (r = -0.28) at NrAaby02, the site with higher STB severity (Fig. 2.5). Another exception is the association of STB severity vs. yield, which was highly significant at Sejet02 (r = 0.34) but negligibly significant at NrAaby02 (r = 0.27) also at Sejet 2005. This performance suggests that a high STB severity, the yield would not be maintained (Fig. 2.5). In Sejet 2006, the

association between STB severity and SW was not significant with Sejet02 data, but it was significant at NrAaby02 (Fig. 2.5). These associations suggest that a higher severity, the specific weight diminishes and consequently the grain quality.

2.5 Conclusion

The results are clear; STB severity was associated to yield and yield components and with quality traits in the Savannah x Senat double haploid population. The traits affected are those cited in several papers. The yield components ears m^{-2} and grains ear^{-1} were negatively correlated to STB. Also, grains m^{-2} was negatively correlated to STB, a trait that has not been cited as affected by this disease. Conversely, a thousand grain weight was positively associated to STB, suggesting the maintaining of TGW, a trait closely linked with yield. This performance has been mentioned in tolerant cultivars, where the grain weight is the yield component less affected by STB. In opposition, in susceptible cultivars, the TGW is the trait that is mostly diminished by Septoria tritici blotch. Thus, the Senat x Savannah double haploid population seems to performance as a tolerant population to the Septoria tritici blotch disease, proving to be a partial resistance population which, although it is affected by STB, the yield losses are smaller than in susceptible cultivars.

Grain protein content and heading date were also negatively associated to STB severity. Interestingly, the association was stronger when earliness of the double haploid population was present (Nickerson 2006), suggesting a physiological relationship among STB, earliness and grain protein content.

CHAPTER 3

Mapping quantitative trait loci for Septoria tritici blotch (*Mycosphaerella graminicola*) and agronomic characters in winter wheat

3.1 Introduction

Mycosphaerella graminicola (Fückel) Schrot (anamorph *Septoria tritici*) causes the disease Septoria tritici leaf blotch (STB). This disease has caused yield losses up to 60% of the grain yield in highly susceptible cultivars (Shipton, 1971; van Ginkel and Scharen, 1988; Cornish, 1990). Recent investigations on wheat have lead breeders to look for other options as partial resistance, that although less effective than resistant genes, could be durable because of polygenic inheritance (Brown, 2002). In wheat, although there has been found evidence of partial resistance to STB, it is only recently that this kind of resistance has begun to be understood (Eriksen *et al.*, 2003; Chartrain *et al.*, 2004; Simón *et al.*, 2004). Quantitative or partial resistance to STB is incomplete and polygenic (Jlibene *et al.*, 1994; Simon and Cordo, 1998; Zhang *et al.*, 2001).

Partial resistance is also costly (Brown, 2002). The effect of STB on yield and yield components although depends on the severity of the epidemic at a given developmental stage of the crop at the time of infection (Ziv and Eyal, 1976; Cornish *et al.*, 1990; Shaw and Royle, 1993; Simón *et al.*, 2002), if it develops at an early stage infections can reduce the number of ears m^{-2}. Late infections can diminish the grains ear^{-1} or the thousand grains weight (TGW) (Leitch and Jenkins, 1995; Simón *et al.*, 2002). In addition, yield losses occur usually when the flag leaf, specially, is severely infected, as well as the second and third leaves (Shaner *et al.*, 1975; Ziv and Eyal, 1978; King *et al.*, 1983a; Thomas *et al.*, 1989; Shaw and Royle, 1989a, b, and 1993; Paveley, 1999). In tolerant cultivars, there is a relatively small loss of yield and quality from severe infection (Ziv and Eyal, 1976, 1978; King *et al.*, 1983a). Genetics for partial resistance, where several chromosomes seemed to be implicated in resistance to STB (Worland *et al.*, 1995/1996), has also been investigated and

recently, QTL, which improve the levels of resistance to STB, have been reported (Simón *et al.*, 2001; Eriksen *et al.*, 2003; Simón *et al.*, 2004; Chartrain *et al.*, 2004b).

Compensatory effects among yield components are commonly found in cereals, mainly because adverse conditions can limit the early formed yield components, but they can be compensated by late ones (Evans and Wardlaw, 1976, 1996; Evans, 1993). It seems that under stress, biotic or abiotic, the best equilibrium between the yield components is tried to be reached between the major yield components and its factors, in order to achieve the highest yield under those unfavourable circumstances. However, compensatory effects were present among the yield and yield components, and they were mostly determined before heading. They also involve readjustments among quality traits of the grain determined after the heading date, as found in this work i.e. GPC and grain starch content (see Chapter 2). In terms of research, Kang (2002) expressed that some genotype characteristics, for instance efficiency and tolerance, can be identified and investigated only under stress based on the fact that genome responds by selectively regulating (increasing or decreasing) the expression of specific genes.

It has been demonstrated that a significant correlation coefficients between two agronomic traits (mostly yield and yield components) implies a high probability that the QTL for both traits are linked as demonstrated by Kato *et al.* 2000; Campbell *et al.*, 2003; Huang *et al.*, 2003 and 2004 and McCartney *et al.*, 2005, among others. Though there was no indication that the degree of significance in this association (slight, moderately high or highly significant) was due to the number of linked QTL for both traits.

Conversely, it is suggested that linked QTL on the same chromosome do not imply any phenotypic association (Chapter 2). This last finding also suggested that

physiological events that determine the traits may occur at different times. Because of this, perhaps a phenotypic association would not be detected**.** Affirming the words of Kato *et al.* (2000), trait correlations may reflect patterns of plant growth and development.

In this work, the performance of the Senat × Savannah double haploid population was investigated under three different environments, where compensatory effects were detected differently. The genotype is analysed in order to establish their association with the Septoria tritici blotch disease and to detect how the attributes of partial resistance, conferred by the Senat cultivar, worked in order to provide tolerance to STB. The phenotype is analysed through Pearson correlation coefficients (see Chapter 2). The genotype is analysed by screening for QTL. In addition, this study incorporates those phenotypic associations related with QTL for STB resistance and those QTL for agronomic traits that were not linked with QTL for STB resistance. Finally, the response of the plant to a stress is a process that involves the whole system.

3.2 Material and methods

3.2.1 Plant material

A Senat × Savannah (SESA) double haploid population of 106 lines was used as described in Chapter 2.

3.2.2 Field trials and data collection.

Field trials, agronomic practices and data collection, as well as segregation data on partial resistance of the Senat × Savannah double haploid population are described in Chapter 2.

3.2.3 Map construction and QTL analysis

The genetic map of the Senat × Savannah DH population already existed, but it has gaps that were filled with genetic markers generated through Diversity Array Technology (DArT) (Triticarte Pty Ltd, 2007). Three types of genetic markers were used for the first map construction according to Eriksen *et al.* (2003), AFLPs (Amplified Fragment Length Polymorphism) markers, which show a high level of polymorphism and reproducibility (Vos *et al.*, 1995). Simple sequence repeat markers (SSR), which are stable, abundantly dispersed throughout the wheat genome and are locus-specific (Röder *et al*., 1995). The resistant gene *Stb6* (Brading *et al*., 2002) was also used as a genetic marker. The new genetic mapping was carried out using JoinMap® 3.0 as presented by Jansen and van Ooijen (2004). Linked groups were formed at a log-likelihood (LOD) threshold of 5. Recombination fractions were converted to map distances using the Kosambi (1944) mapping function.

According with Jansen and van Ooijen (2004), a genetic linkage map is a representation of the relative positions of genetic loci (genes and genetic markers) on the chromosomes, determined on the basis of linkage (inherited together) or become separated by genetic recombination. Distances are measured in genetic map units called centiMorgans. The map units are derived from recombination frequencies. A linked group is a set of genes and/or markers that, by evidence, are linked. They are supposed to reside on the same chromosome. As a role, if genes are linked (that is, on the same chromosome) the recombinants will arise when crossing over occurs among them and their frequency will be < 0.5. Briefly, the likelihood (L) is defined as the probability of the data given a model that considered the recombination frequencies.

$$L \alpha (1 - r)^{N-R} r^{R}$$

where:

α denotes proportionality, r is the recombination frequency, R is the observed number of recombinant individuals, N total number of individuals observed.

The maximum likelihood is a statistical method to estimate parameters. The parameter that maximizes the likelihood function (*L*) will be a function of the unknown parameter *r, L(r)* is differentiated with respect to *r,* equated to zero, and the resulting equation is solved for *r*. The natural logarithm (ln (*L*(*r*)) and the maximum *L*(*r*) are determined by the same value of *r*. The log-likelihood, ℓ = ln (*L*(*r*)), is easier to differentiate than *L*(*r*) then usually log-likelihood is employed. The maximum estimator of *r* becomes:

$$\hat{r} = (\frac{R}{N})$$

The significance of linkage between two markers is tested by the statistic likelihood ratio, *LR*, defined as the ratio of the maximum likelihood under the assumption 'no linkage' with r = 0.5. That is, the statistical test for determining the null hypothesis H_O: r = 5 *versus* the alternative hypothesis H_1: r < 0.5 is based on the likelihood ratio. The formula is:

$$LR = 2^N(1 - \frac{R}{N})^{N-R} (\frac{R}{N})^R$$

The LOD or logarithm of odds, term used in genetics is defined as the 10-base logarithm of the likelihood ratio:

$$LOD = LR\ ^{10}\log (e)$$

A mapping function describes the relationship between the recombination frequency (*r*) and the map distance (*d*): *distance = mapping function* (*r*). The Kosambi mapping function is an empirical mapping function that describes crossover

interference, that is, the presence of one crossover reduces the probability of another crossover in the area.

Thus, steps in linkage analysis implies to create a segregating population (the segregating population in this work was the Senat × Savannah double haploid population); determine the genotypes of the loci; estimate recombination frequencies between all pair of loci; establish the linkage groups; and, finally, determine the order of loci and their distances per linkage group.

Output from JoinMap was converted to a graphical format using the program MapChart (Vorrips, 2006). Komugi composite wheat map (2008), Sommers *et al.* (2004) consensus map, Triticarte wheat map alignment version 1.2 (2004), and Eriksen *et al.* (2003) Senat × Savannah DH population maps were consulted to confirm the linkage groups and alignment of markers assigned to chromosomes.

Quantitative trait loci analysis for each trait at each environment, and also unified analysis of QTL across sites for each trait were done by interval mapping (Jansen, 1993), and MQM mapping was used to detect any possible secondary QTL (Jansen, 1993; Jansen and Stam, 1994) using the MAPQTL® 5 program (van Ooijen, 2004). A QTL was declared significant at a 5% ($P \leq 0.05$) threshold LOD score for genome wide identification of a QTL (van Ooijen 1999).

Kearsey and Pooni (1998) defined a quantitative trait as a 'trait for which the observed variation is due to the segregation of several to many naturally occurring polymorphic genes, for each of which the effects of the allelic differences on the phenotype are generally small compared with the effects of the environment'. Quantitative trait loci (QTL or QTLs) analysis is called to the location of genes (or alleles) that affect a trait that is measure on a quantitative scale.

The steps in the QTL analysis according with Jansen and van Ooijen (2005) are: 1) create a segregation population; 2) determine the genotypes of all loci; 3) determine the phenotypes of the quantitative trait (s) of interest; 4) estimate the linkage map; and, 5) estimate the number and positions of the segregating QTLs (for each trait). Briefly, this statistically complex analysis works looking for associations among markers (that show a genotype and a recombination frequency), individuals (lines) and the mean trait of each individual. Many positions on the linkage map are statistically tested and calculated through the LOD score, a likelihood ratio statistic. The LOD thresholds will depend on the genome size of the specie analyzed (the number and length of the chromosomes) and on the number of markers on the chromosome (van Ooijen, 1999).

3.2.4 Statistical analysis

Additive Main effects and the Multiplicative Interaction (AMMI) model from Gauch (1988) were calculated for each trait in order to test whether the genotype × environment interaction was present among the trials. A balanced design is necessary for the AMMI analysis (Gauch, 1988), thus 96 lines of the DH population were considered. The AMMI analysis combines analysis of variance for the genotype and environment main effects with principal components analysis of the genotype × environment interaction into a single model with additive and multiplicative parameters. The AMMI model equation is:

$$Y_{ger} = \mu + \alpha_{g+} \beta_e + \Sigma_n \lambda_n \gamma_{gn} \delta_{en} + \rho_{ge} + \varepsilon_{ger}$$

where:

Y_{ger} is the observed yield of genotype g in environment e for replicate r

The additive parameters are:

μ the grand mean; α_g the deviation of genotype g from the grand mean; β_e the deviation of environment e

The multiplicative parameters are:

λ_n the singular value for interaction principal component axis (IPCA) n; γ_{gn} the genotype eigenvectors for axis n; δ_{en} the environment eigenvector

The eigenvectors are scaled as unit vectors and are unitless, whereas λ has the units of yield (in this work, the units for eleven agronomic traits). A scaling for the multiplicative parameter is $\lambda^{0.5} \gamma_g$ and $\lambda^{0.5} \delta_e$, termed the 'genotype IPCA scores' and 'environment IPCA scores', because their products give the expected interaction value directly without need of further multiplication by the singular value. There are at least two axes (G-1, E-1), but usually the number of axes N retained in the model is smaller, producing a reduced model, denoted in the present work AMMI2, due to the fact that it was retaining 2 IPCAs. A reduced model leaves residuals, ρ_{ge}. Finally, if the experiment is replicated, there is also the error term ε_{ger}. The last squares solution is found for balanced data by analysis of variance followed by PCA. The AMMI model is useful for understanding complex Genotype x Environment interactions. The results can be graphed in a very informative biplot that shows both main and interaction effects for both genotypes and environments. In addition, AMMI can partition the data into a pattern rich model and discard noise rich residual to gain accuracy.

The segregation data for STB disease were taken from two locations, Sejet02 and NrAaby02, with two randomised replicates per location. There were ten replicates per plot for each line and more than one plot of Savannah and Senat per replicate. The data represents the percent coverage on flag leaves —10 leaves were assessed per plot. The average of leaf 1 was used as plot score for the calculation of

AUDPC per plot (Eriksen *et al.*, 2002). A difference was found between the means for STB severity at NrAaby02 and Sejet02 (see Chapter 2), thus the t-Student test for means of the lines comparison, and the Mann-Whitney test were applied to the STB severity datum to prove if the difference was due to a difference in the location of the distribution (Conover, 1980). The statistical analysis was done using GenStat® 9.1 (GenStat committee, 2006).

Special attention is given in this Chapter to the data of HFL and HD related to STB, given to the fact that these agronomic traits have been considered to provide disease escape from STB (For height: van Beuningen and Kohli, 1990; Fraaije *et al.*, 2002. For HD: Shaner *et al.*, 1975; Eyal and Talpaz, 1990; van Beuningen and Kohli, 1990; Jlibene *et al.*, 1994; Simón *et al.*, 2004, 2005). The test of standardised residuals was applied in order to detect possible outliers (Montgomery and Peck, 1982). The ordinary residuals from regression analysis are not independent as in general, they do not show the same variance. Standardised residuals are a transformed version of the ordinary residuals. The standardised residuals have zero mean and unit variance. They are found using an unbiased estimator of the standard deviation (σ). The *i*th standardised residual is found by substituting the standard deviation of the *i*th residual (σ_i) in a transformed version ($\hat{\sigma}$) of the ordinary residuals in the formula:

$$f(e_i, \sigma_j) = e_i / \sigma_i$$

where:

σ_i is the standard deviation of the *i*th residual; e_i is the ordinary least squares residuals

The standardised residuals were obtained using the General Model in the statistical package GenStat® 9.1 (GenStat committee, 2006). Quantitative trait locus

analysis of the standardised residuals with data of NrAaby02 and Sejet02 was conducted for these traits and STB in all the environments tested. The analysis of residuals for HFL and HD with respect to STB intensity of disease is due to the fact that the residual variance consists of environmental and unexplained genetic variances that, together with the trait variance induced by the QTL (the ratio), gave place to the power of detection of a QTL (Jansen, 1996).

3.3 Results

3.3.1 Additive Main effects and Multiplicative Interaction (AMMI) analysis

Differences among the trials detected by the Analysis of Variance for each agronomic trait (Chapter 2, Table 2.1) were also confirmed with the AMMI analysis, where all the agronomic traits showed highly significant environmental differences (Table 3.1). Similarly, differences among the genotypes for all agronomic traits detected in the ANOVA analysis (Table 2.1, Chapter 2, this Thesis) were also confirmed for HD, HFL, maturity, GPC, SW, TGW and yield. However, differences among genotypes were not detected with the AMMI analysis for ears m^{-2}, grains m^{-2}; grains ear^{-1} and starch content (Table 3.1). Also, interaction trial x line was suggested for all the traits except ears m^{-2} with the ANOVA analysis (Table 2.1, Chapter 2 this Thesis). The AMMI analysis showed highly significant genotype × environment interaction (G x E) for the heading date, specific weight, thousand grain weight and yield ($P < 0.001$) traits. Also, slightly significant G x E interaction was calculated for the traits grains m^{-2}, HFL, grains ear^{-1}, maturity and GPC, while there was not a G × E interaction for the ears m^{-2} and grain starch content traits (Table 3.1).

Table 3.1 AMMI analysis for ten agronomic traits at three environments and for starch content at two environments for the Senat × Savannah double haploid population.

		Ears m^{-2}	Grains m^{-2}	Grains ear^{-1}	TGW	Yield	GPC
Source	df	MS	MS	MS	MS	MS	MS
Treatments	293	23427***	52666200***	160.0***	38.7***	1.1***	2.7***
Genotypes	97	7015	32611152	45.0	39.4***	0.8***	0.4 *
Environments	2	2283468***	2759951408***	16607.0***	3055.4***	95.0***	346.1***
Block	3	21300*	53214641	0.0	6.9	0.8***	1.8**
Interactions	194	8333	34783567*	47.0*	7.2***	0.3***	0.4*
IPCA	98	9733	56619812***	70.0***	7.9***	0.4***	0.5**
IPCA	96	6904	12492401	24.0	6.6***	0.2**	0.3
Error	291	8232	29232438	40.0	4.0	0.1	0.3
Total	587	15883	41051925	99.0	21.3	0.6	1.5

		SW	HD	HFL	Maturity			Starch
Source	df	MS	MS	MS	MS	Source	df	MS
Treatments	293	13.9***	24.6***	47.6***	3.3***	Treatments	195	1.6***
Genotypes	97	17.1***	14.7***	43.3***	2.9***	Genotypes	97	0.7
Environments	2	964.9***	2769.2***	1614.6***	141.8***	Environments	1	167.5***
Block	3	5.6**	1.6*	26.0	1.1	Block	2	0.4
Interactions	194	2.5***	1.2***	33.7*	2.1*	Interactions	97	0.8
IPCA	98	3.3***	1.4***	35.9*	2.3*	IPCA	97	0.8
IPCA	96	1.6*	1.0***	31.4	1.8	IPCA	95	0.0
Error	291	1.2	0.5	27.0	1.6	Error	194	1.0
Total	587	7.5	12.5	37.3	2.4	Total	391	1.3

TGW, thousand grain weight; GPC, grain protein content; SW, specific weight; HD, heading date; HFL, height to the flag leaf; Maturity, canopy maturity; Starch, grain starch content

* Significant at $0.10 < P \geq 0.01$; ** Significant at $0.009 \leq P \geq 0.001$; *** Significant at $P < 0.001$

Differences among the environments were highly significant for all the traits and the genotype × environment interaction; although negligible for some traits, it was significant. Thus, the QTL analysis was done individually for each trait at each environment. However, QTL analysis was also carried out for the mean data set for each trait.

3.2.2 t-Student test and Mann-Whitney test

The Senat cultivar was more resistant (-4.22 and -5.08 lgtAUDPC at NrAaby02 and Sejet02, respectively) than the Savannah cultivar (1.15 and 0.19 lgtAUDPC at NrAaby02 and Sejet02, respectively) which was the susceptible parent. The t-Student test for means comparison of lines was applied with original data (maximum percentage of the area under the disease progress curve (AUDPC). The t-Student test showed 56 lines with similar disease severity at both locations (NrAaby02 and

Sejet02), including the parents. Ten lines were significantly more resistant (averaged 6 % less flag leaf area diseased) at NrAaby02 than at Sejet02, but nine lines were significantly more susceptible (averaged 10 % more flag leaf area diseased) at NrAaby02 than at Sejet02 ($P < 0.01$). In addition, the t-Student test between each line data of predicted means at each trial, showed a significant difference ($P < 0.001$) for some lines. Thus, the lines predicted means from both NrAaby02 and Sejet02 were evaluated through the Mann-Whitney test Assumption 5, where 'if there was a difference between population distribution functions, that difference was a difference in the location of the distribution' (Conover, 1980). NrAaby02 population was assigned as F(x), while the population at Sejet02 was assigned as G(x). The hypothesis in terms of the means of X and Y was,

H_0: $E(X) \geq E(Y)$

where:

$E(X)$ = -1.55 logitAUDPC, denotes the mean sample population at NrAaby02 (X_i)

$E(Y)$ = -2.37 logitAUDPC, denotes the mean sample population at Sejet02 (Y_i)

Value of T = 5931

Value of w_p = 3176

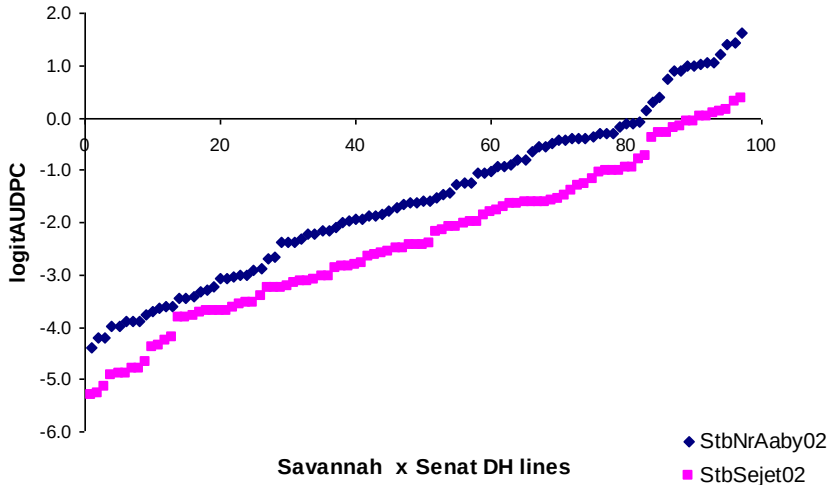
Normal approximation: 3.29 ($P = 0.001$)

Thus H_0 is accepted, as the population NrAaby02 severity of disease presented a higher mean than that shown for the population at Sejet 02. In other words, the STB severity at NrAaby02 was higher than at Sejet02 (Fig. 3.1). It implies a statistically significant difference in the percentage of diseased leaves for

the SESA double haploid population at each location, probably due to environmental differences. The next part of the assumption 'If F(x) is not identical to G(x), then F(x) is identical to G(x + c), where c is some constant' being this value at the same time, the confidence interval (Conover, 1980). Thus F(x) – G(x) = 0.83, which represents around 10% of the difference between the AUDPC between NrAaby02 and Sejet02. Therefore, when c was applied to the G(x) sample of population Sejet02, with c = 0.83, , similar means at both sites resulted, that is:

$$E(X) = -1.55,\ E(Y + c) = -1.55$$

Figure 3.1 Distribution of STB severity among the Senat × Savannah DH population at two different trials, NrAaby02 and Sejet02



3.3.3 Map construction

The bread wheat is a hexaploid (2n = 6, x = 42) organism with three (A, B, D) genomes originated from natural hybridizations. The first hybridization occurring between *T. urartu* (AA), and an unknown species (BB), related with *T. speltoides*, giving place to durum wheat *T. turgidum* (AABB). *Triticum turgidum* hybridized

with *T. tauschii* (DD) to generate the hexaploid wheat *T. aestivum* (Zohary *et al*., 1969).

The genetic map determined into 37 linkage groups (see Figs. 3.2, 3.3, and 3.4), where groups 1D and 5D were represented by SSRs and DarT isolated markers. In comparison with the Eriksen *et al.* (2003) map, new AFLPs were anchored by SSRs and DarT markers, thus the map was constructed by 216 AFLPs, 79 SSRs, 43 DarT markers and the resistant Stb*6* gene locus IPO323 (Brading *et al.*, 2002; Eriksen *et al.*, 2003). These markers covered a map distance of 1683 cM. The Linkage group IX from Eriksen *et al.* (2003) was reassigned into chromosome 6B with a LOD score of 7 (JoinMap® 3.0). The marker wmc175b on chromosome 2Bb (Eriksen *et al.*, 2003) was relocated to chromosome 2Db by JoinMap; this linkage group of five markers was formed at LOD score of 7. This suggests there is genetic similarity between the homoeologous chromosomes 2B and 2D in this population as it has been found in hexaploid wheat by Röder et al. (1995)**.**

Linked groups are showed only when one or more QTL for the agronomic traits and/or QTL for STB resistance were detected. The QTL analysis for STB severity at NrAaby02 and Sejet02 data was performed again instead of using the Eriksen *et al.* (2003) QTL analysis, due to the fact that more markers were incorporated in this work.

3.3.4 Genotypic analysis

The quantitative trait loci analysis for STB severity and for the eleven traits analysed is discussed here. The unified analysis of QTL across sites is shown in Appendix E.

3.3.4.1 Quantitative trait loci for resistance to STB severity

Quantitative trait loci for resistance to STB were detected at Sejet02 on chromosomes 2D, 3A and 6B (Table 3.2). The QTL on chromosome 2D was located near the marker wmc175b, the same marker reported by Eriksen *et al.* (2003) on chromosome 2B. The resistant to STB severity QTL at wmc388a marker position on chromosome 3A described by Eriksen *et al.* (2003) was also found in this work. A third QTL for STB resistance was located on chromosome 6B near the marker wmc397. It explained 49 % of the variance at this trial with a LOD score of 26.72 (Table 3.2). This QTL has also been reported by Eriksen *et al.* (2003) between the same markers.

At NrAaby02, five QTL for resistance to STB were detected (Table 3.2). A QTL was also detected on chromosome 2D at position 14.48 cM, and it was associated with the marker wmc144. On chromosome 3A, a QTL was located at position 31.5 cM, and it was associated with the marker wmc388a (Table 3.2). This QTL was also found by Eriksen *et al.* (2003) associated with the same marker. Two QTL were detected on chromosome 6B. The first QTL on chromosome 6B was located closely linked with marker M49/P14_428. The second QTL on chromosome 6B was also found with the Sejet02 data (Table 3.2). These two QTL for resistance on chromosome 6B have also been reported by Eriksen *et al.* (2003), one of them closely linked with the AFLP marker M48/P32_112, probably the same one reported here associated with the marker M49/P14_428. The second QTL was also flanked by the markers wmc397 and wmc341. Finally, the QTL on chromosome 7B was located on the AFLP marker M49/P11_229 at position 0.0 cM (Table 3.2). Also reported by Eriksen *et al.* (2003), this QTL on chromosome 7B was associated with the same marker that is described here, but at different position.

All the alleles that confer resistance to STB detected within the Senat × Savannah DH population came from the Senat cultivar, as Eriksen *et al.* (2003) also found.

Table 3.2 Quantitative trait loci for STB resistance in Senat × Savannah double haploid population at NrAaby02 and Sejet02 trials

Chromosome	NrAaby02 Markers	Sejet02 Markers	LOD	Position cM	% Exp	Additive value*
2D	wmc144		3.8	14.5	5.6	0.38
		wmc175b	8.7	43.6	11.1	0.46
3A	wmc388a		3.9	31.5	3.7	0.30
		wmc388a	6.4	31.5	7.1	0.39
6B	wmc341		13.5	49.4	17.1	0.83
		wmc397	26.7	48.4	48.6	1.04
	M49/P14_428		7.0	27.7	7.0	0.53
7B	M49/P11_229		3.7	0.0	3.6	0.30

LOD, log-likelihood threshold scores ($P \leq 0.05$); cM, centiMorgans
*Positive additive value indicate that the resistant allele is from Senat cultivar
% Exp, percent of the variation explained

3.3.4.2 Residuals Analysis

Quantitative trait loci analysis for standardised residuals was performed in order to detect hidden effects of HFL and HD for STB resistance. Normality of the residuals was indicated with the data for both traits at the three trials tested (Appendix D). No outliers were detected; all the residuals were calculated between less than three standard deviations showing the normality assumption (Montgomery and Peck, 1982). Though some more resistant lines had large negative standardised residuals, and the more susceptible line presented a large positive standardised residuals in the plots for both traits, in general the same lines shown this performance for HD and HFL (Appendix D).

3.3.4.2.1 Heading date effects

According to the putative QTL detected for the residuals of STB-HD at Nickerson 2006, Sejet 2005, and Sejet 2006, a strong effect of heading date was found. The putative QTL for the standardised residuals showed that those QTL on chromosome 2D and 7B just disappeared with the NrAaby02 data in all the environments. At Sejet 2006 the residual QTL on 7B chromosome showed a LOD score below the threshold of detection, there was still an indication of its presence (Table 3.3). The strong effect of the two QTL for STB resistance on chromosome 6B decreased with analysis of residuals at the three environments analysed, but there was still a significant effect (Table 3.3).

The heading effects for the QTL for STB resistance with the Sejet02 data were particularly remarkable at Sejet 2005, where there simply were no putative QTL (Table 3.3). With the NrAaby02 data at the same environment, just three QTL were detected, those on chromosomes 3A and 6B. On the contrary, at Nickerson 2006 and Sejet 2006 new putative QTL were also detected. At Nickerson 2006, with the NrAaby02 data, a putative QTL was detected on chromosome 3A, associated with the marker wmc264. During the analysis of QTL for STB resistance, the presence of this QTL for STB resistance on chromosome 3A (the allele also from the Senat cultivar) was suspected, but it was under the critical threshold. Also, with the Sejet02 data at Nickerson, the putative QTL for STB resistance on chromosome 6B associated with the marker M49/P14_428 was significant. This last putative QTL was also significant at Sejet 2006 together with other putative QTL on chromosome 2B, both with the Sejet02 data (Table 3.3).

Table 3.3 Quantitative trait loci for STB resistance and putative QTL for residuals of STB and heading date, and residuals of STB and height to the flag leaf with data from two trials with differing STB severity

Chromosome	NrAaby02	Sejet02	LOD*	LOD STB-	LOD STB-	LOD STB-	LOD STB-	LOD STB-	LOD STB-
2D	wmc144		3.8	0.7	0.3	1.5	3.5	3.3	3.3
		wmc175b	8.7	3.0	0.0	4.8	6.4	8.2	8.3
2B		M48/P40_248	-	-	-	3.2	-	-	-
		M48/P40_248	-	-	-	-	3.2	-	-
3A	wmc388a		3.9	0.6	4.2	5.3	4.4	2.5	2.9
		wmc388a	6.4	5.8	0.5	9.0	9.2	5.4	5.5
	wmc264		-	4.2	-	-	-	-	-
6B	wmc341		13.5	8.0	6.8	8.7	11.4	14.33	13.8
		wmc397	26.7	9.6	0.4	13.2	14.3	26.7	27.3
	M49/P14_428		7.0	8.9	6.5	8.0	7.5	5.8	6.1
7B	M49/P11_229		3.7	1.6	0.8	2.4	3.4	4.3	3.9

LOD* Original QTL for Septoria tritici blotch (STB) resistance, log-likelihood threshold scores ($P \leq 0.05$) (LOD)
LOD STB-HD, log-likelihood threshold ($P \leq 0.05$) for residuals between Septoria tritici blotch and heading date
LOD STB-HFL, log-likelihood threshold ($P \leq 0.05$) for residuals between Septoria tritici blotch and height to the flag leaf
Environments: N-06, Nickerson 2006; S-05, Sejet 2005; S-06 Sejet 2006

These results suggest that variation in STB resistance is accounted for by variation in HD, specifically at Sejet 2005. It is also suggested that in partial resistance —Savannah × Senat DH population — there may be a physiological effect of HD on STB resistance at NrAaby02 and Sejet02 severity of the disease. This supports the fact that there were negatively significant correlation coefficients between HD and STB severity in the phenotypic analysis at Nickerson 2006, Sejet 2005, and Sejet 2006 (see Chapter 2).

3.3.4.2.2 Height to the flag leaf effects

The QTL analysis of standardised residuals HFL-STB showed no significant differences from the original QTL for STB resistance in general. At Nickerson 2006, two new putative QTL on chromosome 2B and 6B were significant with the Sejet02 data. The new QTL on chromosome 6B was associated with the marker M49/P14_428, the same marker where an original QTL for STB resistance was detected at NrAaby02. During the analysis of QTL for STB resistance, the presence of a QTL on chromosome 2B (the allele also from the Senat cultivar) was suspected, but it was under the critical threshold. The QTL analysis for standardised residuals uncovered this QTL for STB on chromosome 2B. Only at Sejet 2005 and 2006, the QTL on chromosome 3A near the marker wmc388a was under the threshold of confidence with the NrAaby02 data, but there was still a sign of its presence (Table 3.3).

Thus, these results suggested that in partial resistance, Senat × Savannah DH population, there was not a physiological effect of HFL on resistance to STB at NrAaby02 and Sejet02 intensity of the disease. This supported the fact that there were no significant correlation coefficients between these traits in the phenotypic analysis.

3.3.4.3 Quantitative trait loci for agronomic traits

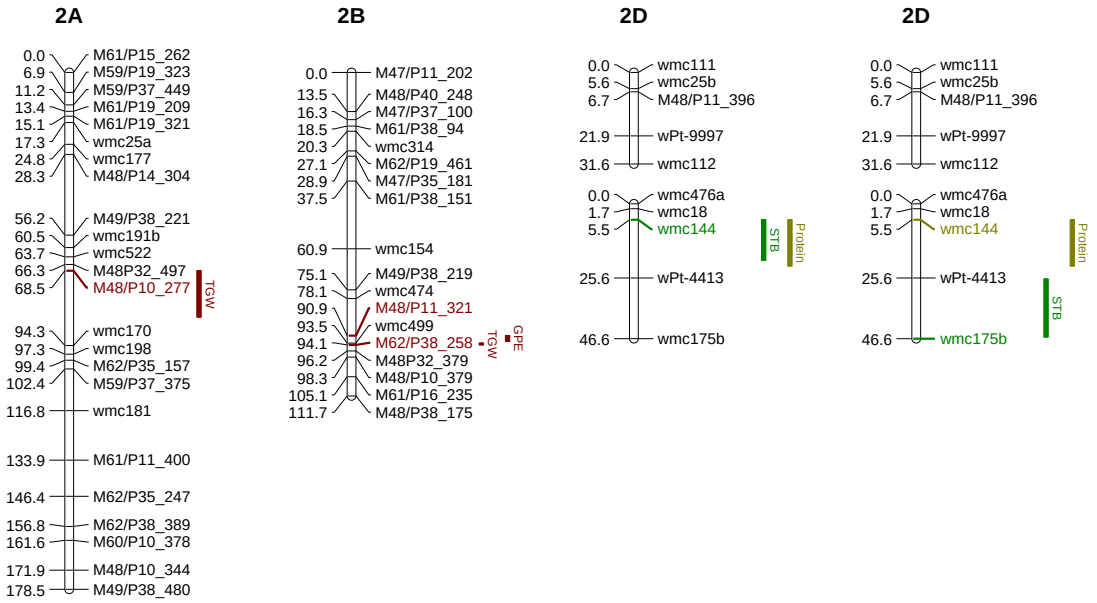
A total of 59 QTL were detected for agronomic traits at the three environments analyzed. However, with the unified analysis of QTL across sites, most of QTL disappeared, in general those with little effect (this effect has been found for several authors), and only 21 QTL remained significant.

3.3.4.3.1 Quantitative trait loci for agronomic traits not linked with QTL for STB resistance

Nickerson 2006

Quantitative trait loci for the yield component TGW was detected on chromosome 2A, which explained 17 % of the phenotypic variance, the allele from the Senat cultivar. A second QTL for TGW was also detected on chromosome 2B, the allele from the Savannah cultivar (Table 3.4). In the same region of chromosome 2B, a QTL for grains ear^{-1} was detected, which alone explained 25 % of the phenotypic variance. On chromosome 4D, a QTL for GPC was found on the short arm of this chromosome (Fig. 3.2).

Figure 3.2 Linkage maps of chromosomes showing QTL for agronomic traits for the Savannah × Senat DH population of wheat and QTL for partial resistance to STB at Nickerson 2006 environment



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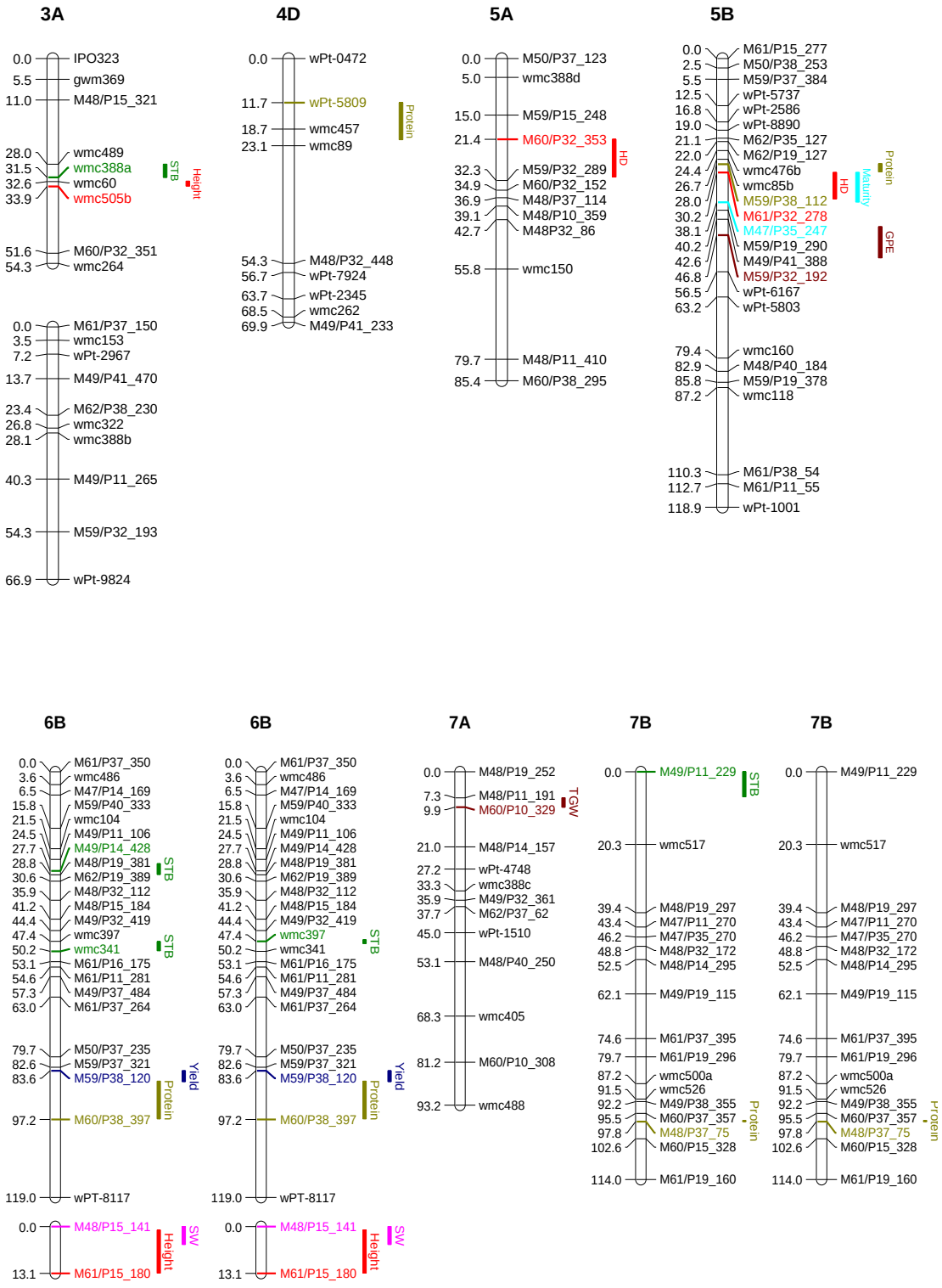


First chromosomes 2D, 6B and 7B show QTL for STB resistance with NrAaby02 data set
Second chromosomes 2D, 6B and 7B show QTL for STB resistance with Sejet02 data set

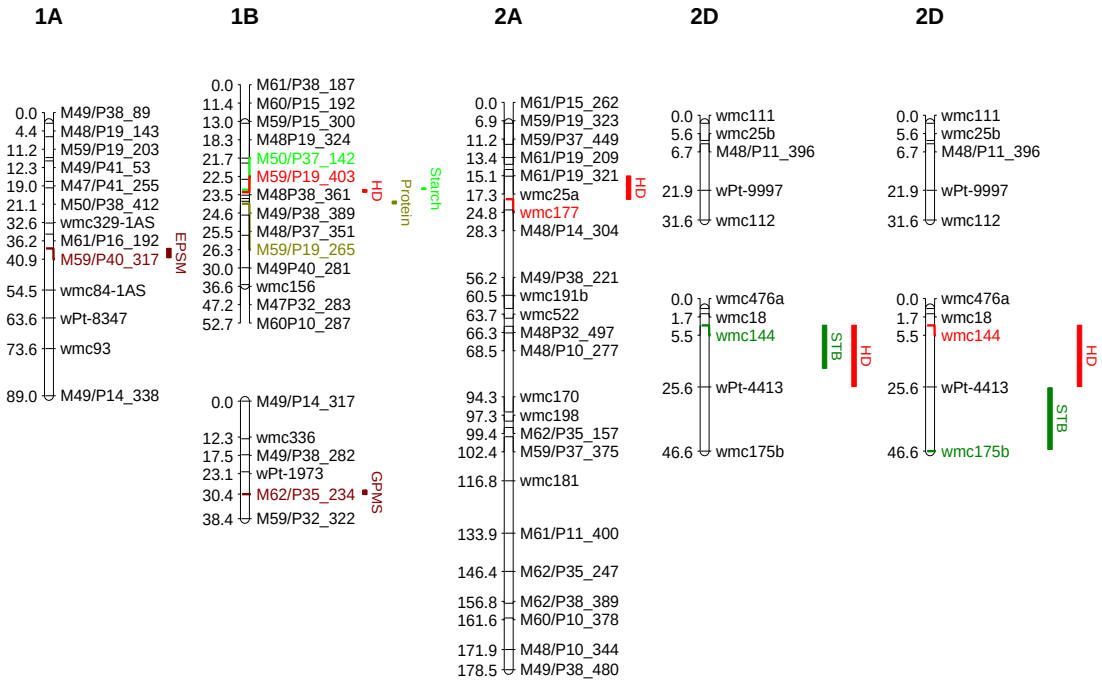
Height, height to the flag leaf; STB, Septoria tritici blotch; HD, heading date; Protein, grain protein content; Maturity, canopy maturity; GPE, grains ear^{-1}; Yield; SW, specific weight; TGW, thousand grain weight

For the trait HD, a QTL on chromosome 5A was detected, which explained 23 % of the phenotypic variance (Table 3.4). Four QTL were detected on chromosome 5B, one for each of the following traits, HD, canopy maturity, GPC and grains per ear, all of them in a same region (Table 3.4). A QTL for TGW was distinguished on the short arm of chromosome 7A (Fig. 3.2).

Sejet 2005

For the yield component ears m^{-2} a QTL was found on chromosome 1A, which explained 20 % of the phenotypic variance. In contrast, four QTL were distinguished on chromosome 1B; three of them were located in a similar region of the short arm (Fig. 3.3), one for each of the traits HD, GPC and starch content. The fourth QTL was for grains m^{-2}, located on the segment of chromosome 1Bb. The QTL for starch content explained 43 % of the phenotypic variance, suggesting it is a major gene (Table 3.4).

Figure 3.3 Linkage maps of chromosomes showing QTL for agronomic traits for the Senat × Savannah DH population of wheat and QTL for partial resistance to STB at Sejet 2005 environment



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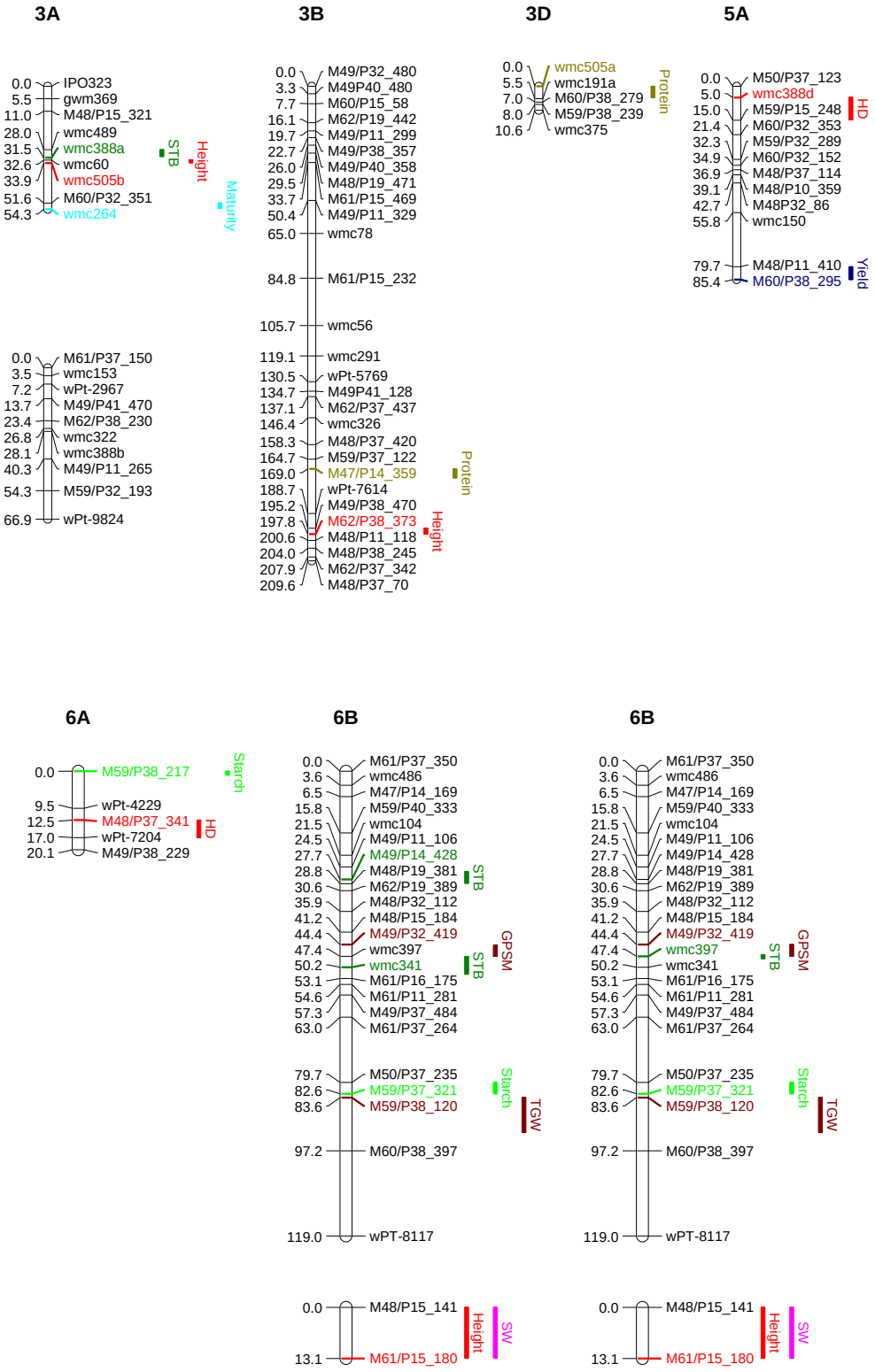
3A
0.0 IPO323
5.5 gwm369
11.0 M48/P15_321
28.0 wmc489
31.5 wmc388a
32.6 wmc60
33.9 wmc505b
51.6 M60/P32_351
54.3 wmc264
STB
Height
Maturity
0.0 M61/P37_150
3.5 wmc153
7.2 wPt-2967
13.7 M49/P41_470
23.4 M62/P38_230
26.8 wmc322
28.1 wmc388b
40.3 M49/P11_265
54.3 M59/P32_193
66.9 wPt-9824
3B
0.0 M49/P32_480
3.3 M49P40_480
7.7 M60/P15_58
16.1 M62/P19_442
19.7 M49/P11_299
22.7 M49/P38_357
26.0 M49/P40_358
29.5 M48/P19_471
33.7 M61/P15_469
50.4 M49/P11_329
65.0 wmc78
84.8 M61/P15_232
105.7 wmc56
119.1 wmc291
130.5 wPt-5769
134.7 M49P41_128
137.1 M62/P37_437
146.4 wmc326
158.3 M48/P37_420
164.7 M59/P37_122
169.0 M47/P14_359
188.7 wPt-7614
195.2 M49/P38_470
197.8 M62/P38_373
200.6 M48/P11_118
204.0 M48/P38_245
207.9 M62/P37_342
209.6 M48/P37_70
Protein
Height
3D
0.0 wmc505a
5.5 wmc191a
7.0 M60/P38_279
8.0 M59/P38_239
10.6 wmc375
Protein
5A
0.0 M50/P37_123
5.0 wmc388d
15.0 M59/P15_248
21.4 M60/P32_353
32.3 M59/P32_289
34.9 M60/P32_152
36.9 M48/P37_114
39.1 M48/P10_359
42.7 M48P32_86
55.8 wmc150
79.7 M48/P11_410
85.4 M60/P38_295
HD
Yield
6A
0.0 M59/P38_217
9.5 wPt-4229
12.5 M48/P37_341
17.0 wPt-7204
20.1 M49/P38_229
Starch
HD
6B
0.0 M61/P37_350
3.6 wmc486
6.5 M47/P14_169
15.8 M59/P40_333
21.5 wmc104
24.5 M49/P11_106
27.7 M49/P14_428
28.8 M48/P19_381
30.6 M62/P19_389
35.9 M48/P32_112
41.2 M48/P15_184
44.4 M49/P32_419
47.4 wmc397
50.2 wmc341
53.1 M61/P16_175
54.6 M61/P11_281
57.3 M49/P37_484
63.0 M61/P37_264
79.7 M50/P37_235
82.6 M59/P37_321
83.6 M59/P38_120
97.2 M60/P38_397
119.0 wPT-8117
0.0 M48/P15_141
13.1 M61/P15_180
STB
GPSM
STB
Starch
TGW
Height
SW
6B
0.0 M61/P37_350
3.6 wmc486
6.5 M47/P14_169
15.8 M59/P40_333
21.5 wmc104
24.5 M49/P11_106
27.7 M49/P14_428
28.8 M48/P19_381
30.6 M62/P19_389
35.9 M48/P32_112
41.2 M48/P15_184
44.4 M49/P32_419
47.4 wmc397
50.2 wmc341
53.1 M61/P16_175
54.6 M61/P11_281
57.3 M49/P37_484
63.0 M61/P37_264
79.7 M50/P37_235
82.6 M59/P37_321
83.6 M59/P38_120
97.2 M60/P38_397
119.0 wPT-8117
0.0 M48/P15_141
13.1 M61/P15_180
STB
GPSM
Starch
TGW
Height
SW

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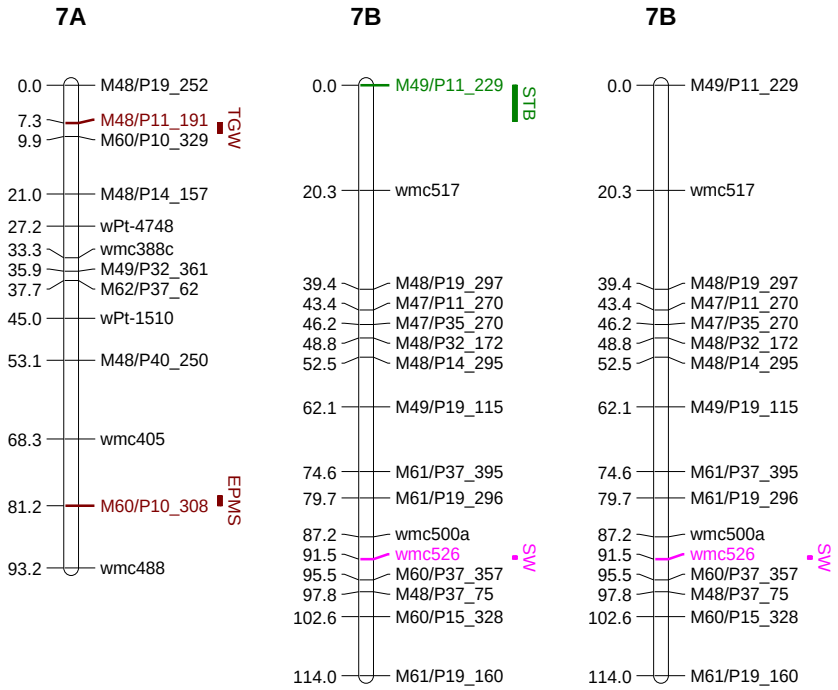


First chromosomes 2D, 6B and 7B show QTL for STB resistance with NrAaby02 data set
Second chromosomes 2D, 6B and 7B show QTL for STB resistance with Sejet02 data set

EPMS, Ears m^{-2}; Starch, grain starch content; HD, heading date; GPSM, grains m^{-2}; STB, Septoria tritici blotch; Height, height to the flag leaf; Maturity, canopy maturity; Protein, grain protein content; Yield; TGW, thousand grain weight; SW, specific weight

On the short arm of chromosome 2A, a QTL for HD was distinguished. Neighbouring QTL for height to the flag leaf and GPC were located on chromosome 3B (Fig. 3.3). Also for GPC, a QTL was detected on the small segment of chromosome 3D (Fig. 3.3).

For the traits HD and yield, a QTL for each were found on chromosome 5A. The QTL for yield explained 16 % of the phenotypic variance (Table 3.4). Two QTL were detected on the short segment of chromosome 6A, one for HD and the other for starch content. Also, two QTL were distinguished on chromosome 7A one of each for the yield components TGW and ears m^{-2} (Fig. 3.3).

Sejet 2006

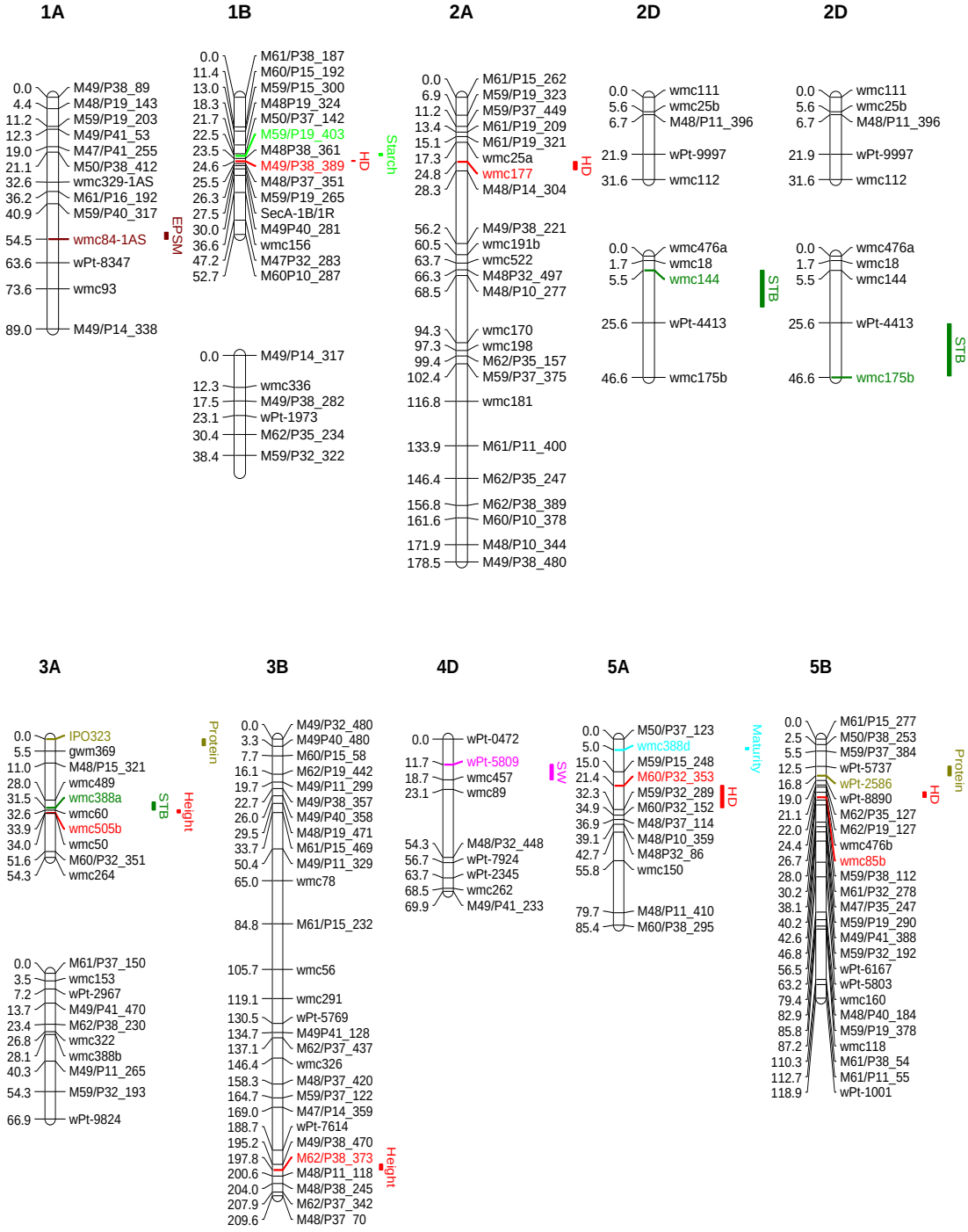
On chromosome 1A a QTL for ears m^{-2} was detected, probably the same QTL for this trait was detected at Sejet 2005. Both were located between the markers M59/P40_317 and wmc84-1AS (Fig. 3.4), and with a large effect (33 % of the phenotypic variance at Sejet 2006). Also, the QTL detected on chromosome 1B for HD and starch content seemed to be the same ones detected at Sejet 2005 (Table 3.4).

The QTL for starch content were detected between the markers M50/P37_142 and M59/P19_403, and the QTL for HD was associated with the same marker, M59/P19_403. These QTL are either very close, or pleiotropic effects of the locus gave place to both traits (Fig. 3.4 and Table 3.4). Newly, the QTL for HD on chromosome 2A was associated to the same marker that the QTL for HD on the same chromosome at Sejet 2005 (Table 3.4). A similar result was found for the QTL for HFL on chromosome 3B, which was associated with the same marker at the Sejet trials (Table 3.4).

On chromosome 4D, a QTL for SW was detected, associated with the same marker where a QTL for GPC was found at Nickerson 2006. Thus, possibly GPC and SW traits are tightly linked, or pleiotropic effects are produced by this locus. Two QTL were linked on chromosome 5A, one for HD and another for canopy maturity. The QTL for HD was associated with the same marker than at Nickerson 2006 (M60/P32_353). The QTL for canopy maturity is only suggested, due to the fact that it was just below the threshold of detection (LOD 3.2, $P \leq 0.05$). Finally, two QTL were detected on chromosome 5B, one for HD and the other for GPC. The QTL for HD appeared to be associated with the same marker (wmc85b) than the one at

Nickerson 2006, suggesting it is the same QTL. However, the QTL for GPC seemed to be different to that QTL detected on the same chromosome at Nickerson 2006.

Figure 3.4 Linkage maps of chromosomes showing QTL for agronomic traits for the Senat × Savannah DH population of wheat and QTL for partial resistance to STB at Sejet 2006 environment



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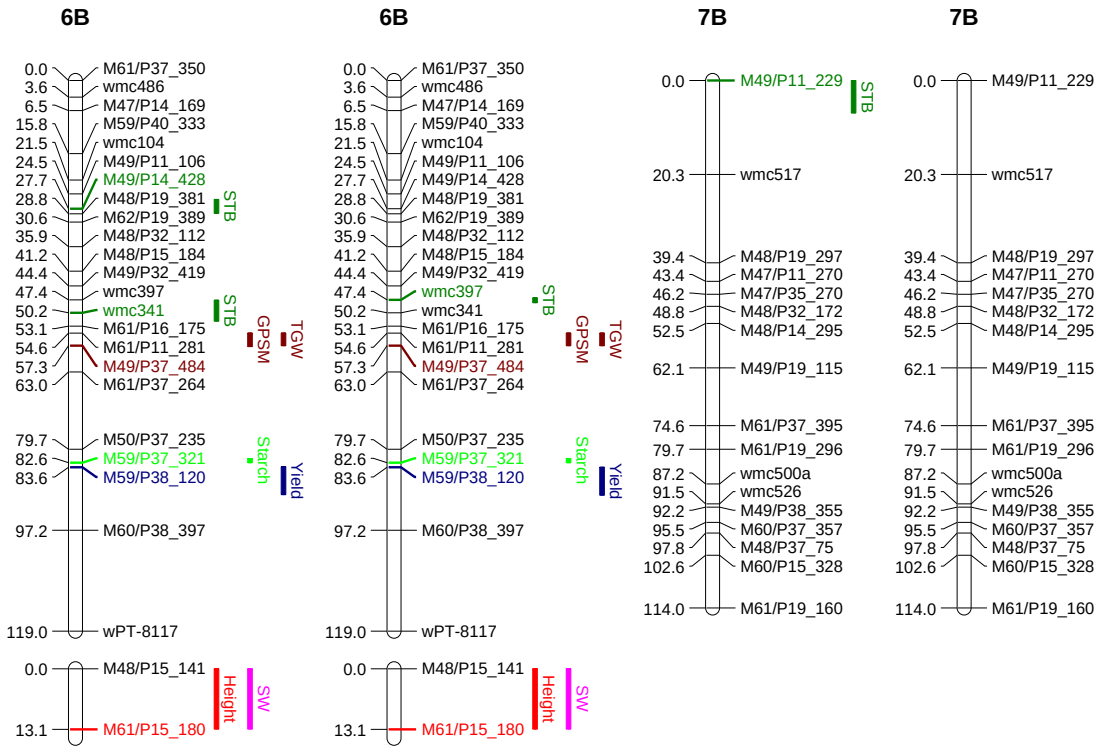


First chromosomes 2D, 6B and 7B show QTL for STB resistance with NrAaby02 data set
Second chromosomes 2D, 6B and 7B show QTL for STB resistance with Sejet02 data set

EPSM, Ears m^{-2}; Starch, grain starch content; HD, heading date; STB, Septoria tritici blotch; Protein, grain protein content; Height, height to the flag leaf; SW, specific weight; Maturity, canopy maturity; Yield; TGW, thousand grain weight; GPSM, grains m^{-2}

Table 3.4 Quantitative trait loci for ten agronomic traits at three environments and for grain starch content at two environments for the Senat × Savannah DH population

		N06	S05	S06				
Trait	Chromosome	Marker	Marker	Marker	Position cM	LOD	% Exp	Additive effect*
				::::				
	1Ba		M59/P19_403	M59/P19_403	22.5	5.7	12	0.7
					22.5	5.7	13	0.5
	2A		wmc177	wmc177	24.3	6.3	14	-0.8
					25.8	4.2	11	-0.5
	2Db		wmc144		15.5	4.2	10	-0.7
HD	5Aa	M60/P32_353			22.4	6.9	23	-0.8
			wmc388d		8.0	4.8	11	-0.7
				M60/P32_353 ::	24.4	6.0	16	-0.5
	5B	wmc85b		wmc85b	26.7	7.7	26	-0.8
					27.7	6.1	15	-0.5
	6A		M48/P37_341		13.5	4.9	10	-0.7
	3A	wmc505b			33.8	5.3	18	-2.1
			wmc505b		33.8	10.4	28	-3.0
				wmc505b	33.8	11.0	28	-3.0
HFL	3B		M62/P38_373		197.8	5.4	13	1.9
				M62/P38_373	197.2	4.6	21	2.1
	6Bb	M61/P15_180			13.0	4.3	14	1.9
			M61/P15_180		13.0	6.9	17	2.3
				M61/P15_180	13.0	8.7	10	2.5
	3A		wmc264		54.3	3.8	15	-0.5
Maturity	5A			wmc388d	4.0	3.0	13	0.5
	5B	M47/P35_247			35.2	3.4	15	0.4
	1A		M59/P40_317		45.9	5.4	20	-27.3
Ears m^{-2}				wmc84-1AS	51.9	7.6	33	-42.1
	7A		M60/P10_308		79.3	3.3	12	-19.9
	1Bb		M62/P35_234		29.1	4.8	18	-1055.7
Grains m^{-2}	6B		M49/P32_419		44.4	3.9	13	-874.8
				M49/P37_484	56,6	4.6	19	-1587.6
Grains ear^{-1}	2B	M48/P11_321			90.9	6.9	25	-3.6
	5B	M59/P32_192			49.8	3.5	14	2.7
	2A	M48/P10_277			70.5	5.1	17	-1.3
	2B	M62/P38_258			94.1	4.8	14	1.0
	6B		M59/P38_120		83.6	4.9	18	1.3
TGW				M49/P37_484	56.6	3.5	15	1.3
	7A	M60/P10_329			9.3	5.1	16	1.1
			M48/P11_191		8.3	3.4	13	1.0
	5Aa		M60/P38_295		85.4	4.0	16	0.2
Yield	6B	M59/P38_120			83.6	4.1	17	0.2
				M59/P38_120	83.6	4.7	19	0.2
	1Ba		M59/P19_265		26.3	3.9	12	0.2
	2D	wmc144			9.5	5.0	12	-0.19
	3A			IPO323	0.0	4.0	14	-0.2
	3B		M47/P14_359		173.0	3.3	12	-0.2
	3D		wmc505a		3.0	3.7	12	-0.2
GPC	4D	wPt-5809			15.7	6.0	13	-0.19
	5B	M59/P38_112			29.0	3.9	8.0	-0.15
				wPt-2586	16.8	4.7	17	-0.2
	6B	M60/P38_397			97.2	3.6	6	-0.14
	7B	M48/P37_75			97.7	3.5	6	-0.14
	1Ba		M50/P37_142		21.7	16.9	43	-0.5
				M59/P19_403	22.5	12.8	41	-0.6
Starch %	6A		M59/P38_217		0.0	3.2	7	0.2
	6B		M59/P37_321		82.6	3.5	8	0.2
				M59/P37_321	82.6	3.5	9	0.3
	4D			wPt-5809	14.7	4.6	17	-1.0
	6Bb	M48/P15_141			5.0	3.9	18	0.7
SW			M61/P15_180		9.0	5.6	22	0.8
				M61/P15_180	11.0	5.9	22	1.1
	7B		wmc526		91.5	3.8	13	-0.7

* Negative additive value indicate that the allele is from Senat cultivar; Marker, nearest marker associated with the QTL;
LOD, log-likelihood threshold scores ($P \leq 0.05$); cM, centiMorgans; % Exp, percent of the variation explained
NO6_Nickerson 2006; SO5_Sejet 2005; S06_Sejet 2006
HD, heading date; HFL, height to the flag leaf; Maturity, canopy maturity; TGW, thousand grain weight; GPC, grain protein content; Starch %, grain starch content; SW, specific weight

3.3.4.3.2 Quantitative trait loci for agronomic traits linked with Quantitative trait loci for STB resistance

Table 3.3 shows the QTL for STB resistance detected in the Senat × Savannah double haploid population at NrAaby02 and Sejet02, while Figures 3.2, 3.3 and 3.4 show the QTL for agronomic traits linked with QTL for STB resistance at Nickerson 2006, Sejet 2005 and Sejet 2006, respectively. Quantitative trait loci in wheat associated with QTL for STB resistance were detected for the yield, grains m^{-2}, TGW, HD, maturity, SW, grain protein content, grain starch content and height to the flag leaf traits.

Chromosome 2D

At Nickerson 2006 with the Sejet02 data, the QTL for STB resistance on chromosome 2Db appeared to be linked with a QTL for GPC while, with NrAaby02 data, the QTL for STB resistance was tightly linked, or pleiotropic effects exist (Fig. 3.2). Senat alleles increased STB resistance and protein percentage. Similarly, at Sejet 2005 with the Sejet02 data, the QTL for STB resistance was linked with a QTL for HD while, at NrAaby02, the QTL for STB resistance was tightly linked, or this allele showed pleiotropy for both traits (Fig. 3.3).

Chromosome 3A

The QTL for STB resistance was associated with the marker wmc388a on this chromosome at Sejet02 and NrAaby02. This QTL appeared to be linked with an invariable QTL for height associated with the marker wmc505b at the three environments (Table 3.3, Figs. 3.2, 3.3, and 3.4). In addition, at Sejet 2005 this QTL for STB resistance appeared to be linked with a QTL for maturity (Fig. 3.3). At Sejet

2006, a QTL for grain protein content was associated with the marker IPO323, a QTL for STB resistance (Brading *et al.*, 2002) (Fig. 3.4).

Chromosome 6B

At Nickerson 2006, QTL for each of the traits HFL, grain protein content, SW and yield were detected on this chromosome. They were linked with a QTL for STB resistance at Sejet02. Similarly, both QTL for STB resistance detected at NrAaby02 were linked with these agronomic traits (Figs. 3.2). At Sejet 2005, quantitative trait loci for the HFL, SW, starch content, grains m^{-2} and TGW traits were also linked with QTL for STB resistance (Sejet02 and NrAaby02) (Figs. 3.3). At Sejet 2006, quantitative trait loci for the HFL, grain protein content, starch content, SW, grains m^{-2}, TGW, and yield traits were linked with QTL for STB resistance at Sejet02 and NrAaby02 (Figs. 3.4).

Chromosome 7B

The QTL for STB resistance at NrAaby02 was linked with a QTL for protein content at Nickerson 2006 (Fig. 3.2). At Sejet 2005, this QTL for STB resistance was found to be linked with a QTL for SW (Fig. 3.3).

3.3.4.3.3 Unified analysis of QTL for agronomic traits

Quantitative trait loci analysis was performed using the means of each agronomic trait. This analysis across sites identified 21 QTL. Additional QTL identified previously were no longer present, in general those with little effect, ‘minor genes’. From these QTL, few could be considered stable genes, usually the ‘major genes’. Actually, the QTL for height to the flag leaf on 3A chromosome and a second one on 6Bb chromosome were stable. They were found at Nickerson 2006 and Sejet 2005

and 2006 environments (Table 3.5). Also, a QTL on 6Bb chromosome for SW was calculated at the three environments showing stability. For yield, the QTL on 6B chromosome seemed to be a stable QTL; it was detected at Nickerson 2006 and Sejet 2006 for this trait, however it was also detected at Sejet 2005 for the yield component TGW. It suggests that this QTL is stable for the trait yield (Table 3.5). For the trait starch content, two QTL appeared stable at the Sejet trials, the first one on 1Ba chromosome and the second on 6B chromosome, that in the combined analysis appeared very close to the marker where the QTL for yield was detected on the same chromosome (Table 3.5).

Table 3.5 Quantitative trait loci for the means of ten agronomic traits at three environments and for means of grain starch content at two environments for the Senat × Savannah DH population

Trait	Chromosome	Marker	Position	LOD	% Exp	Additive
HD	1Ba	M59/P19_403	22.5	3.3	9	0.45
	5Aa	M60/P32_353	23.4	7.2	23	-0.74
	5B	wmc85b	26.7	8.1	24	
HFL	3A	wmc505b	33.8	11.9	30	-2.93
	3B	M62/P38_373	33.8	4.9	11	1.73
	6B	M61/P15_180	13.0	8.3	20	2.29
Maturity	-	-	-	-	-	-
Ears m^{-2}	-	-	-	-	-	-
Grains m^{-2}	2B	wmc154	63.9	3.7	15	-1019.9
	6B	M49/P37_484	57.3	4.7	16	-1114.1
Grains ear^{-1}	2B	M48/P11_321	91.9	5.0	18	-1.70
	5B	M59/P32_192	48.8	4.2	16	1.61
TGW	2B	M62/P38_258	94.1	3.6	9	0.80
	6B	M61/P15_180	8.0	5.7	19	1.11
	7A	M60/P10_329	9.3	5.5	17	1.08
Yield	6B	M59/P38_120	83.5	6.8	26	0.20
GPC	3B	M47/P14_359	174.0	3.4	13	-0.16
	3D	wmc505a	1.0	3.3	10	-0.14
	5B	M61/P32_278	30.2	4.4	13	-0.16
Starch %	1Ba	M59/P19_403	22.5	17.0	48	-0.59
	6A	M59/P38_217	0.0	3.1	6	0.22
	6B	M59/P38_120	83.56	4.4	9	0.26
SW	6B	M61/P15_180	8.0	5.3	25	0.87

* Negative additive value indicate that the allele is from Senat cultivar
LOD, log-likelihood threshold scores ($P \leq 0.05$); cM, centiMorgans; % Exp, percent of the variation explained
HD, heading date; HFL, height to the flag leaf; Maturity, canopy maturity; TGW, thousand grain weight; GPC, grain protein content; Starch %, grain starch content; SW, specific weight

3.4 Discussion

The representation of chromosome maps (shown below), especially of the genomes D (15 %) and genome A (37 %), limited in part the detection of QTL for the agronomic traits analysed in this work, mostly due to the lower polymorphism detected in the Senat × Savannah double haploid population, results also found by Eriksen *et al.* (2003). Similar results have also been found by others authors (e.g. Plaschke *et al*. (1996) reported 45 % of loci found in the B genome, 30 % in the A genome and 25 % in the D genome, working with nullisomic-tetrasomic and ditelosomic lines of Chinese Spring cultivar). In addition, QTL may remain under the threshold of detection (50% of occasions) due to environmental variation (Kearsey, 2002) or modified by it (Shah *et al.*, 1999a). Thus, QTL were not totally detected, especially for complex traits as the yield. Therefore, several of the phenotypic associations found in the Senat × Savannah double haploid population through the Pearson correlation analysis (see Chapter 2) could not be confirmed by the genotype screening in the course of the QTL analysis (Appendix E).

At Nickerson 2006, 17 QTL were detected for the agronomic traits, and six traits were associated with QTL for STB resistance. At this environment, the Senat × Savannah DH population confronted the best environmental conditions for its development, giving the almost insignificant compensatory effects among the yield components. At Sejet 2005, 24 QTL were identified and also six agronomic traits were associated with QTL for STB resistance. This environment was possibly the most stressing for the DH population, receiving the lowest precipitation (see Chapter 2). At Sejet 2006, 18 QTL were detected; eight QTL for agronomic traits were associated with QTL for STB resistance.

Septoria tritici blotch severity at NrAaby02 and Sejet02

The severity of septoria tritici blotch was different at NrAaby02 and Sejet02 sites. The flag leaf was 10 % more affected (0.8247 logitAUDPC) at NrAaby02 than at Sejet02. These results suggest that environmental conditions were more favourable to the development of STB at NrAaby02 than at Sejet02. If similar environmental conditions at both sites are assumed, although there are minimal differences in latitude, longitude and altitude, microclimatic differences could allow a faster development of the disease at NrAaby02, due to the quantitative nature of partial resistance. Thus, the expression of QTL for STB resistance could differ. However, the difference in severity may be due to differences in the time the assessment was made at each site. Though the inoculation was carried out at the same date and at the same plant developmental stage at each trial, when the flag leaves were fully expanded, the disease assessment was not carried out simultaneously at both sites. The STB severity assessment at Sejet02 was made on June 20th and 26th, and on July 3rd 2002, while at NrAaby02 the assessment was made on June 25th and on July 1st and 7th 2002 (Eriksen *et al.*, 2003). According to Magboul *et al.* (1992), the minimum development rate of disease severity was 3% day^{-1} in chamber conditions, indicating that four days would make a difference of 12% in severity, very similar to the 10% of difference found between NrAaby02 and Sejet02 data sites in the field**.**

Quantitative trait loci for STB resistance

Quantitative trait loci which improve the levels of resistance to STB have been reported (Eriksen *et al.*, 2003; Simón *et al.*, 2001; Simón *et al.*, 2004; Chartrain *et al.*, 2004b). Different numbers of QTL for STB resistance at the adult stage were detected according to the intensity of STB severity in the Senat × Savannah double

haploid population. At Sejet02, where the STB severity was lower than at NrAaby02, one QTL for STB resistance was detected on each of the chromosomes 2D, 3A and 6B. Five QTL for STB resistance were detected at NrAaby02, one on each of on chromosomes 2D, 3A and 7B, and two QTL for STB resistance on chromosome 6B. Little is known about the mechanisms throughout partial resistance operate, however possibly with a major increase of the disease, another locus or other loci are "switched on" by the plant in order to respond to the disease severity.

The QTL for STB resistance detected on chromosome 2D, associated with different markers, —NrAaby02 (wmc144 marker) and Sejet02 (wmc175b marker) — may be the same one (Table 3.1). Walsh (2002) indicates that a QTL of large effect could be initially located to a region of around 20 to 50 cM. The QTL for STB resistance on chromosome 2D are separated by 29 cM. However, the alleles may be different for STB resistance. Quantitative trait loci for disease resistance have been found to be tightly linked (Hammond-Kosack and Jones, 1997). A QTL on chromosome 2D was found by Simón *et al.,* (2004) at seedling stage. However, this QTL may not be the same reported in this work, firstly because it was not detected at seedling stage in the Senat × Savannah DH population (Eriksen *et al.,* 2003). Secondly, they cannot be compared because the markers used were different and the position was different.

The QTL for STB resistance on chromosome 3A was detected at similar positions and was associated with the same marker (wmc388a) for both NrAaby02 and Sejet02 data. The resistant gene *Stb6* has been detected on chromosome 3A on the marker IPO323 (Brading *et al.,* 2002), and its presence has been confirmed in the Senat cultivar at seedling stage (Eriksen *et al.,* 2003; Chartrain *et al.,* 2005b). However, this allele was not detected in the QTL analysis at adult stage in the

Savannah × Senat DH population in this work nor in Eriksen *et al.*'s study (2003). At field trials, the inoculum consisted of eleven *M. graminicola* isolates. Thus, non-specific 'background resistance' (Brown *et al.*, 2001) could diminish the effect of IPO323; that is, genes that confer partial resistance could modify the expression or mask the effects (Chartrain *et al.*, 2005c) of *Stb6*. Also, environmental elements could inhibit the effect of resistant alleles (Kema and van Silfhout, 1997). The specific expression of some resistant genes to specific isolates of STB at seedling stage, but not in adult plants, has been detected. (Kema and van Silfhout, 1997; Arraiano *et al.*, 2001a).

The QTL for STB resistance detected between the markers wmc397 and wmc341 on chromosome 6Ba may be a major gene for STB resistance, due to the fact that the LOD score was higher than other QTL for STB resistance (Chartrain *et al.*, 2004b), and also because it explained a high percentage of the phenotypic variance. Chartrain *et al.* (2004b) detected a minor QTL for partial resistance to STB on chromosome 6B in an F1 double haploid population from the cross between Arina and Riband cultivars. This QTL was detected at adult stage, and the allele that confers resistance was from Riband. However, this QTL can not be compared with the QTL found in this work, as the markers used were different. In addition, the allele on chromosome 6B for resistance to STB was from the Senat cultivar. The second QTL for STB resistance on chromosome 6Ba, and the QTL for STB resistance on chromosome 7B, were detected only at NrAaby02 site. The resistant gene *Stb8* to *M. graminicola* was detected on chromosome 7B (Adhikari *et al.*, 2003). However, the QTL for STB resistance detected on chromosome 7B in this work may not be the same, due to the fact that the position was different and the variance explained was low, suggesting it is a minor gene.

Quantitative trait loci for plant adaptation traits

In the Senat × Savannah DH population, seven different QTL for heading date were detected in the three environments tested (Table 3.4). At Nickerson 2006, two QTL for HD were found, five QTL for HD were detected at Sejet 2005, and four QTL for HD at Sejet 2006. From these seven QTL for HD, three were detected in the chromosomes 5 group, two on chromosome 5A and one on chromosome 5B. The first QTL on chromosome 5A was found at Nickerson 2006 and Sejet 2006 associated with the same marker (M60/P32_353). On chromosome 5A, one QTL for earliness *per se* that possibly corresponds to that detected in this analysis has been detected near the centromere, as it was also found by Sourdille *et al.* (2000). The QTL for HD found on chromosome 5B also at Nickerson 2006 and Sejet 2006 environments are located in similar position. Tóth *et al.* (2003) mapped an *eps* locus on chromosome 5B near the marker wmc73 (27.8 cM). In the Senat × Savannah DH population, the wmc73 marker was at 24.2 cM, in a similar region. The QTL for HD on chromosome 5A might be homoeologous to those found on chromosome 5B. They were detected in a similar region (22.4- 27.7 cM) and, in such a case, they would be *Eps* alleles.

The QTL for HD on chromosome 2A, associated with the wmc177 marker at Sejet 2005 and Sejet 2006 (24.3 and 25.8 cM, respectively), and the QTL on chromosome 2D at Sejet 2005 (15.5 cM), probably correspond to *eps* alleles. They explain 11-14 % and 10 % of the phenotypic variance, respectively. Kuchel *et al.* (2006) and Huang *et al.* (2003) found a QTL for HD on the short arm of chromosome 2A. Some other authors have also detected *eps* alleles on chromosomes 2B and 2D, close to the *Ppd-B1* gene (Shindo *et al.*, 2003; Hanocq *et al.*, 2004; Sourdille *et al.*, 2003; Kuchel *et al.*, 2006) and the *Ppd-D1* gene (Hanocq *et al.*,

2004; Börner *et al.*, 2002; Huang *et al.*, 2003, 2004; Xu *et al.*, 2005). Hanocq *et al.* (2004) propose that e*ps* genes exist close to the *Ppd* genes, or that pleiotropic effects of *Ppd* genes give place to the *eps* alleles (Worland, 1996; Börner *et al.*, 2002), or that there is confusion between *Ppd* genes and e*ps* alleles in the homoeologous group 2.

The QTL for HD detected on chromosome 1B (McEwan and Kaltsikas, 1970; Law *et al.*, 1981; Hoogendoorn, 1985), chromosome 5A and chromosome 6A (Huang *et al.*, 2003) may be related with those alleles for 'earliness *per se*'. Snape *et al.* (2001a) have predicted alleles for photoperiod response (on chromosomes group 1 and 6), vernalization response (on chromosomes group 1 and 4), and earliness *per se* (on chromosomes groups 3, 4, 5, 6 and 7) in wheat from comparative analysis with barley.

The cultivar Savannah (Riband × Brigadier) presumably possesses the semidwarf *Rht-D1b* (formerly *Rht1*) allele because it is present in both parents (Sherman *et al.*, 2005), however, this gene was not detected in this work (chromosome 4D), which is also consistent with the findings of Eriksen *et al.* (2003), presumably by the under representation of this chromosome in the linked group. In the Senat × Savannah DH population only three QTL for HFL were detected, one on each chromosome 3A, 3B and 6B. The QTL on chromosome 3A was detected in all the environments tested, associated with the marker wmc505b, and it is comparable with that found by Eriksen *et al.* (2003). A quantitative trait locus for height on the short arm of chromosome 3A, which possibly correspond with the QTL in the SESA population, has been cited by several authors (Shah *et al.*, 1999 b; Campbell *et al.*, 2003; Huang *et al.*, 2004; Dilbirligi *et al.*, 2006). On chromosome 3B, a QTL for HFL was also detected at the Sejet trials. Chromosome 3B did not account for height

variation, but interactions between the homoeologous chromosomes (3A and 3B) were found to be of negative type, although not significant, when using single chromosome substitution lines of Capelle Desprez into Chinese Spring cultivars (Snape *et al.*, 1977). Eriksen *et al.* (2003) also detected a QTL for height on chromosome 3B, that in the new map is assigned to the long arm of chromosome 3B. Huang *et al.* (2004) and Marza *et al.* (2006) detected a QTL for height on the long arm of chromosome 3B that possibly corresponds to the QTL found in this work.

At Nickerson 2006, Sejet 2005 and Sejet 2006, the third QTL for HFL on chromosome 6Bb was associated with the same marker (M61/P15_180). Snape *et al.* (1977) found that this chromosome has a significant additive effect and it is involved in interactions with other chromosomes. Eriksen *et al.* (2003) detected a QTL on group IX, assigned to chromosome 6B in the map constructed in this work. Cadalen *et al.* (1998) also found a QTL for plant height on chromosome 6BL that possibly corresponds to the QTL in the SESA population.

The cultivar Savannah presumably carried the 1BL.1RS translocation probably inherited by Brigadier (Sherman *et al.*, 2005). Cultivars with the translocation 1BL.1RS, where the 1BS arm is replaced by the homoeologous rye (*Secale cereale* L.) 1RS arm, confer longer green leaf (at least 2 days) and yield benefits (Villarreal, *et al.*, 1998; Foulkes *et al.*, 2006). However the background effects are large and genotype × environment interactions could affect the expression of agronomic traits in wheats with the translocation (Villarreal, *et al.*, 1998). No QTL was detected on this chromosome for canopy maturity. In the Senat x Savannah DH population, three QTL for canopy maturity were detected, one on each chromosome, 3A, 5A and 5B. Marza *et al.* (2006) detected a QTL for maturity date on chromosome 3AS.

Quantitative trait loci for yield and yield components

One QTL for yield on chromosome 5AL was detected in the Sejet 2005 dataset, position 85.4 cM, where the allele with additive effects was from Savannah cultivar (Fig. 4.2). A QTL on the long arm of chromosome 5A (position around 85 cM), that possibly corresponds with the QTL found here, has been described by several authors (Kato *et al.*, 2000; Gross *et al.*, 2003; Quarrie *et al.*, 2005; Huang *et al.*, 2006). The second QTL for yield was found on chromosome 6B, detected at 83.6 cM position, at Nickerson 2006 and Sejet 2006. Huang *et al.* (2004) and Marza *et al.* (2006) have described a QTL for yield on chromosome 6B, but in a different region than that for the QTL found in the SESA DH population.

Grain weight is a trait under polygenic control, where QTL with major effects are few, while QTL with minor effects are many (Kumar *et al.* 2006). For the yield component TGW, five QTL were detected on chromosomes 2A, 2B, 6B and 7A. Varshney *et al.* (2000) mentioned that chromosomes 2B and 7A carried alleles for high grain weight, while chromosome 6B carried alleles for low grain weight. On chromosome 2A, a QTL for TGW was found at 70.5 cM, position comparable with that QTL found by McCartney *et al.* (2005) also on chromosome 2A. On chromosome 2B, a second QTL for TGW was detected in the SESA DH population, at 94.1 cM position. This QTL is possibly the same one cited by several authors (Gross *et al.*,2003; Kumar *et al.*, 2006; Hai *et al.*, 2008), as they located it in interval 68 to 90 cM. For chromosome 6B, two QTL were detected at position 56.6 cM, at Sejet 2005, and the second one at 83.6 cM, at Sejet 2006. These QTL possibly correspond with those ones cited by Börner *et al.* (2002), as supported intervals included the regions where the two QTL for TGW were found for the SESA DH population. Marza *et al.* (2006) reported a QTL for spike weight on chromosome 6B

(position around 97 cM). The QTL for TGW on chromosome 7A was detected between the markers M48/P11_191 and M60/P10_329 (interval position 7.3 to 9.9 cM). Huang *et al.* (2004) mapped a QTL on the distal arm of chromosome 7AS, in the same region the QTL was found in this work.

Three QTL for the yield component grains m^{-2} were detected in the SESA DH population. One QTL on chromosome 1B and another on chromosome 6B were detected at Sejet 2005. A second QTL for grains m^{-2} on chromosome 6B was detected at Sejet 2006. However, in the literature considered, there were no QTL reported for this trait that was coincident with those QTL cited here. Nevertheless, for the grains m^{-2} factors, ears m^{-2} and grains ear^{-1}, several QTL have been mapped. Two QTL for the trait ears m^{-2} were detected one on 1A chromosome and a second one on 7A chromosome. The first QTL on chromosome 1A was located at Sejet 2005 (position 40.9-54.5 cM) and Sejet 2006. Kumar *et al.* (2007) reported a QTL for tiller number on this chromosome. However, they located the QTL at position 145.11 cM, very far from the QTL found in this work. Also, Li *et al.* (2007) detected a QTL for spike number on chromosome 1A, at position around 21 cM**.** It was difficult to make any comparisons in detail, because different markers and different maps were used. The second QTL detected only at Sejet 2005, on chromosome 7A, was located at position 79.3 cM. Huang *et al.* (2003, 2004) and Kumar *et al.* (2007) mapped a QTL for tiller number m^{-2} on the short arm of this chromosome, near the centromere, which possibly corresponds to the QTL detected in this work. For the trait grains ear^{-1}, two QTL were detected only at Nickerson 2006, one on each chromosome, 2B (position 90.9 cM) and 5B (position 49.8 cM). The first QTL (on chromosome 2B) was detected in a region described by Hai *et al.* (2008), where yield parameters have been detected. Kumar *et al.* (2007) and Hai *et al.* (2008) mapped two QTL on

chromosome 2B for grains per ear, the first one may correspond with the QTL described in the SESA DH population, but the markers used where different. On chromosome 5B, Quarrie *et al.* (2005) reported a QTL for grains per ear.

Quantitative trait loci for quality grain traits

Grain protein content is a complex trait determined by many genes distributed in different chromosomes of hexaploid wheat and, as a quantitative character, strongly influenced by the environment as studied by several authors (Joppa *et al.*, 1997; Chee *et al.*, 2001; Prasad *et al.*, 2003; Blanco *et al.*, 2002; Gross *et al.*, 2003; Prasad *et al.*, 2003; Turner *et al.*, 2004). In the SESA DH population, nine chromosomes that determined the trait grain protein content on chromosomes 1B, 2D, 3A, 3B, 3D, 4D, 5B, 6B and 7B were detected. No QTL for GPC were coincident in the three environments analysed. From the nine QTL, five were located at Nickerson 2006 (on chromosomes 2D, 4D, 5B, 6B and 7B), three at Sejet 2005 (on chromosomes 1B, 3B, and 3D), and only two at Sejet 2006 (on chromosomes 3A and 5B). The QTL on chromosome 1B was found at position 26.3 cM. Sourdille *et al.* (2003) also detected on this chromosome a QTL for GPC with a confidence interval of 39.0-103 cM. Prasad *et al.* (1999, 2003) and Börner *et al.* (2002) detected one QTL for GPC on the long arm of chromosome 2DL and another on the short arm of the same chromosome 2DS, respectively. In the homoeologous group 3, one QTL on each chromosome was detected, two of them were detected at Sejet 2005; they appeared on an homoeologous region on chromosomes 3A and 3D, as they were located at 0.0 and 3.0 cM position, respectively. The QTL on chromosome 3A was detected at Sejet 2006 on a similar position (0.0 cM) to that found by Gross *et al.* (2003) on the same chromosome. On chromosome 3B, also Gross *et al.* (2003) reported a QTL for GPC in the confidence interval 19-175 cM, that possibly corresponds to that one found in

the SESA population on this chromosome at position 173.0 cM, at Sejet 2005. Prasad *et al.* (2003) reported a QTL on chromosome 3D near the marker gwm456 at position 1.8 cM; possibly, it corresponds with the one detected in this work. Huang *et al.* (2006) and McCartney *et al.* (2006) detected a region on chromosome 4D between 0.0 to 30 cM (in the same position than the one found by Gross *et al.*, 2003), where not only a QTL for GPC, but also several QTL for agronomic and quality grain traits were found, respectively. This QTL is comparable to that found in the SESA DH population. For chromosome 5B, two QTL were detected, one at Nickerson 2006 and the second one at Sejet 2006, that differ in position (29.0 and 16.8 cM, respectively). Gross *et al.* (2003) detected a QTL for GPC only in one location of six in their research. It was detected in the confidence interval 27-128 cM, thus possibly, it corresponds with the QTL found at Nickerson 2006. It has been described that a QTL for high GPC on 6B chromosome accounted for a high percentage of the total phenotypic variation, the allele from *Triticum turgidum*, in a variety *dicoccoides* accession (Joppa *et al.*, 1997; Chee *et al.*, 2001; Blanco *et al.*, 2002), donor of A and B genomes of common wheat. Turner *et al.* (2004) also detected a QTL on chromosome 6B in the Avalon x Hobbit Sib RIL population (*Triticum aestivum*), differing in location in two years data (76.5 and 86.5 cM), which possibly correspond with the QTL for GPC found in the SESA DH population (97.2 cM). The QTL for GPC detected on 7B chromosome (position 97.7 cM) possibly corresponds to the QTL for this trait detected by Blanco *et al.* (2006) on 7BL chromosome, the allele from an accession of variety *dicoccoides*. Huang *et al.* (2006) reported a QTL for GPC also on chromosome 7B in *Triticum aestivum*, difficult to compare with the one found here.

Three QTL for specific weight were located in the SESA DH population, one in the short arm of chromosome 4D at Sejet 2006, the second one in the short segment of chromosome 6Bb at Nickerson 2006, Sejet 2005 and Sejet 2006. The third QTL for SW was detected at Sejet 2005 on chromosome 7B. Huang *et al.* (2006 and McCartney *et al.* (2006) detected a QTL for SW on the short arm chromosome 4D, in the same segment where several QTL for agronomic and grain quality traits have been detected. McCartney *et al.* (2006) associated this QTL for SW with reduced height, low grain yield, low TGW and longer time to maturity. These characteristics associated with the dwarfing gene *Rht-D1*, are presumably present in the Savannah cultivar. However, the dwarfing gene was not detected on this chromosome in the SESA DH population, and neither was the QTL for TGW, yield or maturity. Nonetheless, the QTL for GPC and another for SW were detected (at Nickerson 2006 and Sejet 2006, respectively), associated with the same marker (wPt-5809); the decrease of these traits was due to the Savannah cultivar. McCartney *et al.* (2006) also detected a QTL for SW on chromosome 6B, but it cannot be compared with the QTL found for this trait in the SESA SH population.

Three QTL for starch content were detected in the SESA DH population. On chromosome 1B, a QTL was detected at Sejet 2005 and Sejet 2006, between the markers M50/P37_142 to M59/P19_403 (position 21.7 cM and 22.5 cM, respectively). This QTL was comparable with the QTL detected by McCartney *et al.* (2006) in *Triticum aestivum*, where a QTL for starch content was located on the short arm of chromosome 1B (position 19 cM).

Phenotypic associations and genotypic associations

There were 131 significant correlation coefficients (Chapter 2, this Thesis), and 57 of them were confirmed to be linked to at least one chromosome during the genotypic

screening, that is, one QTL for each trait was detected on the same chromosome (Appendix E). These results suggested that physiological relationships between two (or more) traits took place at the same time. On the contrary, traits where QTL were detected on the same chromosome (even in the same region), appeared not to be associated (no significant correlation coefficient), during the phenotypic analysis, i.e. GPC and grains ear^{-1} on 5B chromosome, at Nickerson 2006. These results suggest that physiological events for each trait took place at different times.

Plant adaptation traits

Associations between HD and yield components were found at Sejet 2005 and Sejet 2006. Heading date was also associated with yield and HFL. Only the high negative association HD vs. yield was confirmed by the genotypic analysis at Sejet 2005 (Appendix E). These associations are most likely due to the series of genes involved in the duration of the life cycle and consequently with the time to heading and the floral development. These genes control the vernalization requirement (*Vrn* genes) on group 5 chromosomes, photoperiod response (*Ppd* genes) on group 2 chromosomes, and developmental rate, 'earliness *per se'* genes (*Eps*), on several chromosomes (Snape *et al.*, 2001a and b). The *Vrn* genes reduce the vernalization requirements (decrease sensitivity to vernalization), giving place to a faster rate of primordia production, resulting in early flowering (Sourdille *et al.*, 2000; Snape *et al.*, 2001a). For chromosomes 5A and 5B, the *Vrn* genes have also been related with the duration of the phenological phases: emergence to floral initiation, and terminal spikelet to heading. They are also related with the increase of number of spikelets and rate of generation (Whitechurch and Snape, 2003). The *Ppd* genes affect the timing of terminal spikelet production and stem elongation. These genes participate in accelerating the days to flowering (Scarth and Law, 1983; Hoogendoorn, 1985;

Börner *et al.*, 1993; Worland and Sayers, 1996; Snape *et al.*, 2001a) and interact with the *Vrn* genes. The *Ppd* genes are also associated with the reduction in plant height, reduced tiller number (Worland *et al.*, 1998) and fewer numbers of spikelets per ear (Worland *et al.*, 1994, 1998). However, the grain setting increases, implying that spikelet fertility improves (Worland *et al.*, 1994, 1996; Börner *et al.*, 1993). The same authors found an increase in some European areas of 5% to 30 % in yield, due to the *Ppd-D1* gene, depending on environmental conditions. The 'earliness *per se*' (*Eps*) genes effects, detected in this work, have been described as differences in growth and development, independent of photoperiod and vernalization stimuli (Hoogendoorn, 1985; Worland *et al.*, 1994; Worland and Sayers, 1996; Snape *et al.*, 2001a). Also, the *Eps* genes have been associated with fewer spikelets (Hoogendoorn, 1985) and improved yield (Worland *et al.*, 1994). They determine the duration of phenological phases until flowering (Whitechurch and Snape, 2003).

The phenotypic analysis showed that HD was negatively and significantly associated with STB resistance at all the environments (Chapter 2, this Thesis). Only at Sejet 2005 was the phenotypic association confirmed by the genotypic analysis (Appendix E). Accordingly, a physiological relationship was suggested between these traits, at least at Sejet 2005. In addition, the results for heading date and STB residuals suggest that variation in STB resistance is accounted for by variation in HD. These results also suggest that in partial resistance, Savannah × Senat DH population, there may be a genetic linkage and a physiological effect of HD on STB resistance. The results presented in this research agree with several reports of associations between incidence of STB and earliness, as it has been cited in Chapter 2, this Thesis.

Height to the flag leaf was associated with the yield and the yield components. Some of these associations were confirmed by the genotypic analysis (Appendix E). The *Rht* genes (*Rht1* and *Rht2*) promoted the reduction in height approximately 20 % and increased spikelet fertility (Kuchel *et al.*, 2007). On the other hand, Börner *et al.*, 1993 state that the number of grains per ear increase, but that there are negative effects on grain weight and reduction in tiller number. Yet, a higher number of grains m^{-2} is associated with small grain size (Kuchel *et al.*, 2007). They also increase yield, subjected to temperature stress at the period between flag leaf to ear emergence (Worland and Law, 1985). *Rht* genes are important in the determination of plant height, but they do not explain all the variation for this character, thus the final plant height is under polygenic control (Snape *et al.*, 1977; Cadalen *et al.*, 1998), where seventeen chromosomes accounted for genetic variation, and interactions between these chromosomes are an important component of the final height (Snape *et al.*, 1977).

The results of the analysis of residuals between HFL and STB suggest that, although both chromosomes 3A and 6B carry alleles for STB resistance and HFL, this last trait does not account for variation in STB resistance. Thus, these results also suggest that in partial resistance, Savannah x Senat DH population, there is not a physiological effect of HFL on resistance to STB at NrAaby02 and Sejet02 severity of the disease. This supports the fact that there were no significant correlation coefficients between height to the flag leaf and STB severity in the phenotypic analysis (see Chapter 2) — results also found by Danon *et al.* (1982) and Eriksen *et al.* (2003).

Canopy maturity was negative, associated with HD at all the trials. At Nickerson 2006, a QTL for each trait was linked on chromosome 5B, while at Sejet

2006, a QTL for each trait was linked on chromosome 5A (Appendix E). At Nickerson 2006, one QTL for maturity and one QTL for GPC were linked on chromosome 5B, confirming the negative association found in the phenotypic analysis (Appendix E).

Yield and yield components

The yield and the yield components were associated at all the environments differently (Chapter 2, this Thesis), suggesting different physiologic relationships. However, few of these associations were confirmed by the genotypic screening (Appendix E). Nevertheless, yield and the yield components TGW and grains m^{-2} were linked at Sejet 2006 by the chromosome 6B, confirming their strong association and the central physiological relationship among them (Fig. 3.4). The QTL for TGW and the QTL for grains m^{-2} were found associated with the same marker (M49/P37_484), suggesting opposite pleiotropy gene effects or loci tightly linked for these factors of the final yield (Fig. 3.4). In the phenotypic analysis, the highest negative association between TGW and grains m^{-2} was found at this trial. Also, TGW was positively associated with yield, while grains m^{-2} was negative associated with it (see Chapter 2).

The QTL for yield distinguished on 6B chromosome in Nickerson 2006 and Sejet 2006 environments possibly indicates a major gene. Additionally, this QTL for yield on chromosome 6B was associated with the marker M59/P38_120. However, the trait TGW was also associated with the same marker at Sejet 2005 (Table 3.4, Fig. 3.3). It suggests a tight physiological relationship between yield and TGW at the last environment, possibly a pleiotropy effect where TGW 'substitutes' the yield. In the phenotypic analysis at this trial, TGW was the trait that mainly contributed with the yield, since no association between yield vs. grains m^{-2} was found at this trial.

Quantitative trait loci for STB resistance is related with TGW and grains m^{-2}. They were linked with chromosome 6B at Sejet 2005 and Sejet 2006, as the phenotypic analysis suggested (Fig. 3.3 and 3.4). Thus, physiological relationships are suggested between TGW, grains m^{-2} and STB resistance.

A stable QTL for yield and TGW was found on 6B chromosome, which presented a moderately high effect across the three environments. The stability shown by this QTL is unusual, given the fact that yield is a character environmentally dependent (Quarrie *et al.*, 2005). The allele was conferred by the Savannah cultivar thus; this QTL is a good candidate to be considered for plans on plant breeding.

Quality grain traits

Quality grain traits were associated between them and with yield and yield components. Furthermore, they were also associated with the plant adaptation traits. Newly, these associations differ in each environment and not all of them were confirmed by the genotypic analysis.

The phenotypic analysis showed that GPC and yield were negatively associated at the three environments. This association was confirmed by the genotypic analysis only at Nickerson 2006 (Table 3.4 Appendix E). One QTL for GPC was co-located with a QTL for yield on chromosome 6B, suggesting a physiological relationship between GPC and yield (Table 3.4, Fig. 3.2).

Starch percentage was significant and positively associated with TGW and yield at the Sejet trials. This association was confirmed by the genotypic analysis only at Sejet 2006. One QTL for each trait was detected on chromosome 6B, linked by close markers (Table 3.4, Fig. 3.4). Thus, a strong physiological relationship is suggested among yield, TGW and grain starch content. Physiological relationships

were suggested between starch % and GPC in the phenotypic analysis at Sejet 2005 and 2006, where negative associations were found between these traits. A QTL for each trait was nearly linked on 1B chromosome at Sejet 2005 (Fig. 3.3). At this environment, the highest percentage of starch and the lowest content of grain protein were found (see Chapter 2).

The phenotypic analysis indicated a positive association between HD and GPC at the three environments analysed. Also, a QTL for each trait was co-located on 5B chromosome at Nickerson 2006 and Sejet 2006 (Fig. 3.2 and 3.4). Also, at Sejet 2005, a QTL for each trait was linked by chromosome 1B (Fig. 3.3). The fact that different chromosomes were involved in the association HD and GPC at all the trials, suggests that different environmental signals turned on the QTL implicated in these responses. In addition, it has to be noted that the alleles for HD and GPC at Nickerson 2006 and Sejet 2006 were conferred by the Senat cultivar, while those at Sejet 2005 for the same traits were conferred by the Savannah cultivar.

In opposition, highly negative associations were detected between HD and grain starch content in the phenotypic analysis at Sejet 2005 and Sejet 2006. This association was also confirmed by the genotypic analysis at both trials (Appendix E). At Sejet 2005, two chromosomes showed to have linked QTL for both traits on chromosome 1B and 6A. At Sejet 2006, a QTL for each trait was also detected on chromosome 1B. Thus, physiological relationships are suggested among GPC and HD (Fig. 3.3 and 3.4).

The data from the trials tested and the segregation data for STB in the Savannah x Senat population indicated that grain protein content was negatively associated with STB severity at all the trials. At Nickerson 2006, from five QTL detected for GPC, three were linked with QTL for STB resistance on chromosomes

2D, 6B and 7B (Fig. 3.2). At Sejet 2006, a QTL for GPC was linked with a QTL for STB resistance on chromosome 3A. Interestingly, this QTL for GPC was associated with the same marker where the resistant gene *Stb6* is located, IPO323 (Brading *et al.*, 2002), suggesting pleiotropic effects or alleles for each trait that are tightly linked.

The phenotypic analysis showed association between SW and yield at Nickerson 2006 and Sejet 2006 (Chapter 2, this Thesis). A QTL for SW and a QTL for yield were detected on chromosome 6B at both environments (Fig. 3.2 and Fig.3.4).

From segregation data for STB at NrAaby02 and the data of the Savannah x Senat DH population, Septoria tritici blotch was negatively associated with SW at Sejet 2006 (Chapter 2, this Thesis). A QTL for SW was detected on chromosome 6B, where two QTL for STB resistance have been cited above. Thus, this result may be suggesting a physiological relationship between SW and STB resistance. The QTL for SW on chromosome 6B showed a strong effect at the three trials tested. The results suggest it is a major gene. The allele that increased the trait was from the Savannah cultivar.

3.5 Conclusion

Putting all together and keeping in mind this is a projection of the performance of the Savannah × Senat DH population associated with segregation data of STB from the same population. The detection of QTL for GPC, where most of the alleles that increase this trait were conferred by the Senat cultivar, gave place to a new sight not considered in the phenotypic analysis: In partial resistance, with the decrease of GPC by STB, different responses could be achieved. That is, if GPC is diminished under

disease stress, the protein matrix is reduced avoiding shrivelled and/or soft grains when the deposition of starch in the grain is not completed. This performance allowed the preservation of SW, although the TGW could be diminished by the small size of the grains (Gaines *et al.*, 1997). It has been reported that STB is associated with reduction of photosynthetic activity by infected leaves, early senescence of leaves and apical senescence (Chapter 1). Since the leaves are the principal source of carbohydrates to the growing grain, this suggests the decrease of the photosynthetic capacity of the plant, due to STB that has a greater effect on carbon accumulation. Thus, the maintaining of TGW and SW in partial resistance cultivars (Senat cultivar) may be due to equilibrium between GPC-starch granules; in other words, equilibrium in the interaction starch granules-protein matrix (Chapter 2).

In addition, the alleles that increase the GPC were mostly conferred by the Senat cultivar in the Senat × Savannah DH population. These results suggest that, in partial resistance, there may be a trade-off: Losing grain protein content by STB attack, but maintaining TGW and specific weight, due to equilibrium between protein content and starch content in the grain that, at the same time, avoid the reduction in yield.

CHAPTER 4

A gene for susceptibility to septoria tritici blotch

4.1 Introduction

Hobbit sib is an old UK cultivar which is susceptible to Septoria tritici blotch. Hobbit sib has shown to be susceptible to the *Mycosphaerella graminicola* isolates IPO323 and IPO94269 in detached leaf test, at seedling stage, and at adult stage in polytunnel trials (Arraiano, 2001a; Arraiano *et al.*, 2007b). Susceptibility has also been shown in the same isolates in field experiments (Brown *et al.*, 2001). Hobbit sib has a reciprocal chromosome translocation 5BS-7BS and 5BL-7BL with respect to Chinese Spring, which is considered to have a primitive chromosome structure (Riley *et al.*, 1967). The cultivar Cappelle Desprez, which also carries the reciprocal translocation 5BS-7BS (Riley *et al.*, 1967; Law and Worland, 1996 and 1997), has shown moderate levels of resistance to the same *M. graminicola* isolates in field trials (Brown *et al.*, 2001). However, Cappelle Desprez has been susceptible in polytunnel trials to these isolates (Arraiano, 2001a; Arraiano *et al.*, 2007b). The Russian cultivar Bezostaya 1 has shown to be resistant to IPO323 isolate, but susceptible to IPO94269 isolate (Arraiano, 2001a; Arraiano *et al.*, 2007b). This cultivar lacks the 5BS-7BS translocation (Law and Worland, 1996 and 1997).

In experiments conducted to identify the chromosomal location for specific resistance of cultivar Bezostaya 1 to the *M. graminicola* isolate IPO323, two series of substitution lines of chromosomes from Bezostaya 1 in Hobbit sib and in Cappelle Desprez cultivars were used. The results of these experiments indicate that the substitution line Hobbit sib (Bez 5BS-7BS) was resistant not only to isolate IPO323, but also to isolate IPO94269. Conversely, the Cappelle Desprez (Bez 5BS-7BS) substitution line was susceptible to both isolates (Arraiano, 2001a).

In further tests to confirm the presence of the translocated chromosome 5BS-7BS of Bezostaya 1 into the Hobbit sib and Cappelle Desprez substitution lines, it

has been shown that no Bezostaya 1 DNA is present in the 5BS arm of Hobbit sib (Bez 5BS-7BS) substitution line. Chinese Spring DNA is the other material present, and a small segment of Hobbit sib DNA was found in the telomeric region of this arm (Arraiano *et al.,* 2007b). The development of the Hobbit sib monosomic series originated from the Chinese Spring monosomic series (Law *et al.*, 1987). It was confirmed that the 7BS arm from Bezostaya 1 was correctly substituted in the Hobbit sib (Bez 5BS-7BS) substitution line. The 5BS-7BS chromosome in the substitution line Cappelle Desprez (Bez 5BS-7BS) was also correctly substituted (Korzun *et al.*, 1997). These results suggested that Hobbit sib carries a gene or genes for susceptibility to STB on 5BS, and its removal increases the resistance of Hobbit sib (Bez 5BS-7BS) substitution line. Alternatively, there is a gene in the Hobbit sib euploid 5BS-7BS chromosome that suppresses resistance in this cultivar, and its removal nullifies the effect of this suppressor, allowing the expression of an inhibited resistance gene (Arraiano *et al.*, 2007).

Wheat chromosomal substitution lines, as well as varieties carrying a reciprocal translocation have been used before with the purpose of locating important disease related genes on specific chromosomes (Johnson and Law, 1975; Pink *et al.*, 1983; Law *et al.*, 1987; Law and Worland, 1991, 1996). The varieties Besoztaya 1, Hobbit Sib, Cappelle Desprez, and the inter-varietal chromosome substitution lines Hobbit Sib (Bezostaya 5BS-7BS) and Cappelle Desprez (Bezostaya 5BS-7BS) have been studied in order to determine genes that promote resistance to yellow rust and mildew in wheat (Law and Worland, 1991, 1996, and 1997), and Septoria tritici blotch of wheat (Arraiano, 2001; Arraiano *et al.* 2001b; Arraiano *et al.*, 2007b).

The aim of this work was to investigate if a gene for susceptibility is present in the double haploid population Hobbit sib × Hobbit sib (5BS-7BS) and if so, to identify and map this gene.

4.2 Materials and methods

4.2.1 Plant Material

Forty lines of Hobbit sib (5BS-7BS) × Hobbit sib double haploid population (Arraiano, John Innes Centre) were tested in a polytunnel trial at adult stage at the John Innes Centre in 2006 in the first year of experiments. Also the following cultivars and lines were considered:

- Hobbit sib
- Bezostaya 1 (Bez)
- Chinese Spring (CS)
- The substitution lines Hobbit sib (Bez 5BS-7BS) and Cappelle Desprez (Bez 5BS-7BS), the line Hobbit sib nullisomic for 5BS-7BS (Law and Worland, 1996), and the Chinese Spring nullitetrasomics N5A T5B, N5B T5A, N5B T5D and N5D T5B (Sears, 1954)

In a second year of polytunnel trials at the same institute in 2007, the same lines and cultivars were tested. In addition, the following lines and varieties were also used:

- The F_1 lines (created in 2006)
- The Chinese Spring nullitetrasomics: N5A T5D, N5D T5A
- Hobbit sib ditelosomic 5BS
- Hobbit sib ditelosomic 7BL
- Hobbit sib (Hope 5BS-7BS)

- Hobbit sib (Fiorello 5BS-7BS)
- Hobbit sib (Bersee 5BS-7BS)
- Hobbit sib (*Triticum macha* 5BS-7BS)
- Hobbit sib (Mara 5BS-7BS)
- Cappelle Desprez (Vilmorin 27 5BS-7BS)
- Cappelle Desprez (Desprez 80 5BS-7BS) Duplicate A and Duplicate B
- Cappelle Desprez
- Hope
- Fiorello
- Bersee
- *Triticum macha*
- Mara
- Vilmorin 27
- Desprez 80

4.2.2 Disease tests

The *M. graminicola* isolates IPO323 and IPO94269 were separated by the Institute of Plant Protection (IPO) Wageningen, The Netherlands. The IPO323 isolate was isolated in 1981 from the commercial cultivar Arminda (Kema and van Silfhout, 1997). It is highly specific to a range of wheat cultivars at adult plant stage (Kema and Van Silfhout, 1997; Brown *et al.*, 2001). The IPO94269 isolate was derived from a single ascospore. It is highly virulent to a range of commercial wheat cultivars (Kema *et al.*, 1996).

The isolates from stock were allowed to grow on potato dextrose agar plates (PDA) for six days. From the initial culture, spore suspension was used to inoculate yeast-glucose liquid medium (glucose 10g lt^{-1}, yeast extract 30g lt^{-1}) in Erlenmeyer flasks, where isolates were allowed to grow for 6 days. Spore concentration was adjusted to 3.78×10^6 spores ml^{-1} and Tween 20 (polyoxyethilene-sorbitan monolaurate) was added to 0.15 % as a surfactant when spores were applied using a knapsack sprayer at a rate of 3.38×10^9 spores m^{-2}.

Inoculations were carried out twice with an interval of seven days after 50 % of the plants had reached the heading stage. Disease of leaves was scored as the percentage of leaf area covered by lesions bearing pycnidia. This assessment was undertaken three times between 15 and 27 days after the second inoculation, in both years. The area under the disease progress curve was calculated from the combined scores (Shaner and Finney, 1977). Afterwards, data were transformed into logit area under the disease progress curve (lgtAUDPC) as a proportion of the maximum area under AUDPC. The maximum AUDPC was calculated assuming a score of 100 % on every date the test was scored. The mean score from each line and each isolate was calculated using generalized linear mixed modelling (GLMM) (Genstat 9.1 for Windows, 2005). The heading date (HD) was assessed when 50 % of the plants reached this stage. The height to the flag leaf (HFL) was measured from the soil surface to the base of the spike on the main tiller. Spring during 2007 was warmer than usual, and it caused earliness of the lines. In addition, significant plot and block effects in the polytunnel affected the reliability of data. Thus, the data presented here only relate to experiments conducted in the first year of the polytunnel trial (2006).

4.2.3 Molecular mapping

The microsatellite marker map of chromosome 5BS-7BS of the Hobbit sib × Hobbit sib (Bez 5BS-7BS) double haploid population has been developed using microsatellite markers by Arraiano *et al.* (2007b). However, some regions of the 5BS arm were not completely covered by markers. Four further microsatellite markers were employed to obtain more information of markers in that region. The microsatellite markers were Xbarc004, Xbarc072, Xbarc176 and Xbarc267 (US Wheat and Barley Scab Initiative). The marker Xbarc176 is located on 5B arm at position 36.5 cM in the Avalon × Cadenza progeny and at position 32.5 cM in the Spark × Rialto progeny (TriticartewhtmapalingVI-2(1)). However, this marker has also been found on the long arm of 7B chromosome at position 51.33 cM in the Komugi composite wheat map (2008). The marker Xbarc267 located on 7BS chromosome (Komugi composite wheat map, 2008), has also been reported on chromosome 5B at position 41.7 cM in the Arina × NK93604 progeny (TriticartewhtmapalingVI-2(1)). According to Arraiano *et al.*, (2007b) Xbarc004 and Xbarc072 markers were located on chromosome 5BS at position 44.99 cM, near positions in the TriticartewhtmapalingVI-2(1)), and at position 42.00 cM on chromosome 7BS, respectively.

DNA from the Hobbit sib × Hobbit sib (Bez 5BS-7BS) double haploid population was extracted from two week old seedlings of each line and parents. Polymerase chain reaction (PCR) was conducted in a volume of 15 µl in order to generate 20 ng of DNA. Reactions consisted of 2 µl of 2 µM of the appropriate primer pair, 2.5 mM each of dATP, dCTP, dGTP and dTTP, and 0.07 µl of *Taq* DNA polymerase (Roche) in 10 mM Tris-HCL (pH 8.3), 1.5 mM $MgCl_2$, 50 Mm KCl, 100 µg ml^{-1} gelatine, 0.05 % (w/v) Tween 20, and Nonidet 40. Water was added to a final

reaction volume of 15 µl total. Samples of the PCR product were checked by gel electrophoresis and detected by silver staining (de Vienne, 2003). To do so, an aliquot of 5 µl of each PCR product was added to 5 µl of formamide dye loading buffer (10 ml formamide, 10 mg xylene cyanol FF, 10 mg bromophenol blue and 200 µl of 0.5 M EDTA (pH 8.0)), it was denatured for five minutes at 95°C and cooled on ice 5 µl. Then it was loaded into the gel.

4.2.4 Map development and QTL analysis

A linkage map segment for chromosome 5BS-7BS was calculated using JoinMap 3.0 (van Ooijen and Vorrips, 2001). The linked segment was formed at a log-likelihood (LOD) threshold of 4. Recombination fractions were converted to map distances using the Kosambi (1944) mapping function. The output from JoinMap was converted to a graphical format using the program MapChart (Vorrips, 2006). Quantitative trait loci analysis was performed for the separated data of STB severity for IPO323 and IPO94269 isolates. Also, QTL analysis for traits HFL and HD were conducted. MQM mapping was used to detect QTL (Jansen, 1993; Jansen and Stam, 1994). The threshold LOD score for genome-wide significant identification of QTL was selected (P = 0.05) (van Ooijen 1999).

4.2.5 Statistical analysis

The trials were conducted in a randomised complete blocks layout generated with the Experimental Design Generator and Randomiser (EDGAR) (Brown, 2000). In each trial there were two blocks per isolate and five randomised plots per block. Each plant line was present once in each plot. Mean values were calculated from replicates of each line for each trait for the different trials. A test of X^2 goodness of fit was applied to investigate if each trait at each trial was normally distributed. Data were

analysed using a generalised linear mixed modelling of binomial proportions (GLMM) in GenStat® 9.1 (VSN International, Oxford, UK). Line, isolate and the interaction line × isolate were considered as fixed effects. Blocks were considered as random effects. Pearson correlations coefficients were calculated between heading date versus STB isolates severity data and height to the flag leaf versus STB isolates severity data.

4.3 Results

4.3.1 Generalised linear mixed modelling of binomial proportions

The line term in the GLMM analysis was large and significant, indicating that there was variation in the mean responses of lines to infection by *M. graminicola* IPO323 and IPO94269 isolates. There was also a large and highly significant effect of line × isolate interaction, suggesting that isolate-specific resistance or susceptibility accounted for much of the variation in the data. When the lines Bezostaya 1, Chinese Spring, Chinese Spring nullitetrasomics, Hobbit sib nullisomic for 5BS-7BS and Cappelle Desprez (Bez 5BS-7BS) were removed from the analysis, the isolate main effect was not significant anymore, suggesting that there was no significant variation in the mean response of the DH population lines to both IPO323 and IPO94269 isolates**.** Also, there was no significant difference in the line × isolate interaction to any further extent, meaning there were not specific responses of the lines to a particular isolate (Table 4.1).

Table 4.1 Generalised linear mixed modelling of percentage leaf area covered by lesions bearing pycnidia of the *Mycosphaerella graminicola* IPO323 and IPO04269 isolates in the Hobbit sib (5BS-7BS) × Hobbit sib double haploid population in a polytunnel trial

Term	d.f.	Wald statistic	Deviance ratio	X^2 Pr
Line	41	1036.21	25.27	***
Isolate	1	1.06	1.06	NS
Line. Isolate	41	44.73	1.09	NS

*** Significant at P < 0.001; NS not significant

4.3.2 Pearson correlation coefficients

The correlation coefficient of height to the flag leaf versus *M. graminicola* isolate severity was not significant. However, there was a significant negative association between HD and STB disease for both IPO323 and IPO94269 isolates. Also, a positive significant correlation coefficient was found for IPO323 vs. IPO94269 isolates severity (Table 4.2).

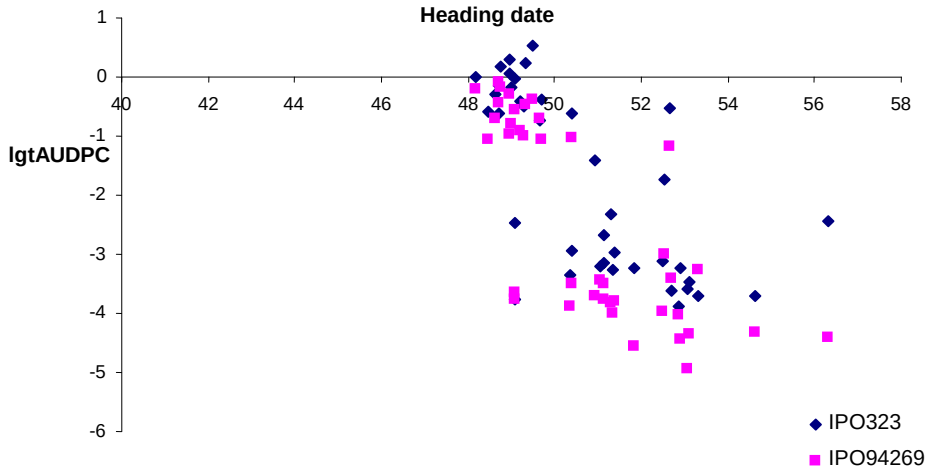
Table 4.2 Pearson correlation coefficients for heading date, height to the flag leaf and isolates IPO323 and IPO94269 severity of disease

	Height	Heading date	IPO323	IPO94269
Height	1			
Heading date	-0.21	1		
IPO323	-0.01	-0.69***	1	
IPO94269	0.06	-0.75***	0.94***	1

*** Significant at P < 0.001

Figure 4.1 shows there exists an association between heading date and susceptibility/resistance to Septoria tritici blotch. The scatter diagram represents the susceptibility of Hobbit sib to both isolates and the characteristic heading date from Hobbit sib (Bez5BS-7BS).

Figure 4.1 lgtAUDPC vs heading date association between septoria tritici blotch severity and heading date for the Hobbit sib × Hobbit sib (Bez 5BS-7BL) DH population



The difference in time to heading associated with the difference in severity of the disease very strongly suggests that a gene determining heading date must be closely linked with a gene for susceptibility (or resistance) which is an important contribution of this research that contradict the statement made by Arama *et al*. (1999) and Simón *et al*. (2004b, 2005) who mentioned that there is no genetic association between resistance and heading date.

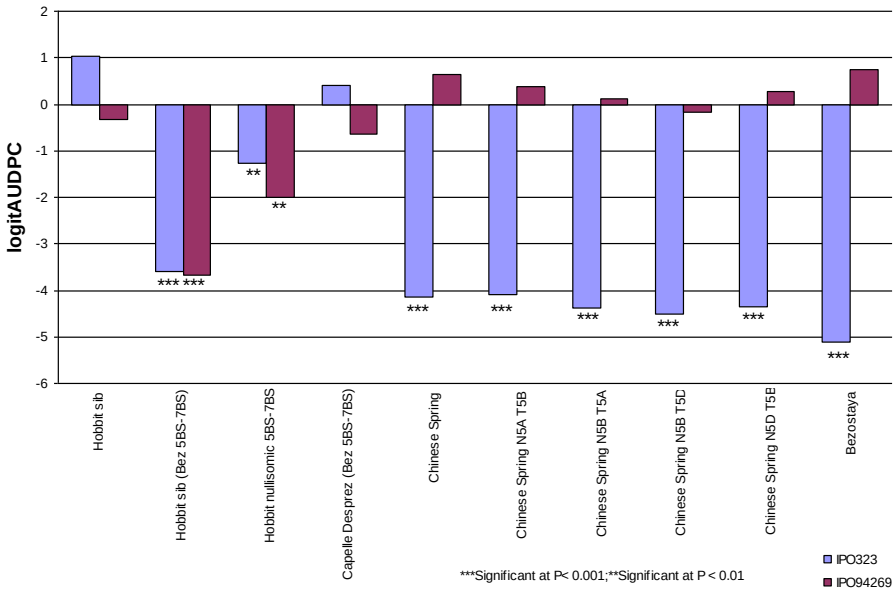
4.3.3 Line performance

In the polytunnel trial, Hobbit sib was found to be susceptible to both *M. graminicola* isolates IPO323 and IPO94269. Bezostaya 1 and Chinese Spring —they both lacking the 5BS-7BS translocation— were significantly resistant to the *M. graminicola* IPO323 isolate. However, both cultivars were significantly more susceptible to IPO94269 isolate as compared with Hobbit sib (Fig. 4.2). This difference in resistance was also reflected in the response of the Chinese Spring nullitetrasomics

lines with IPO94269 isolate of *M. graminicola* in this experiment; all these lines were as susceptible as CS (Fig. 4.2).

The Cappelle Desprez (Bez 5BS-7BS) line was as susceptible as Hobbit sib to both isolates. This line was found in other polytunnel trials to be as susceptible as the Cappelle Desprez euploid. Unfortunately, the Cappelle Desprez cultivar performance could not be confirmed in the present work due to the fact that during the second year of experiments the results were not convinced.

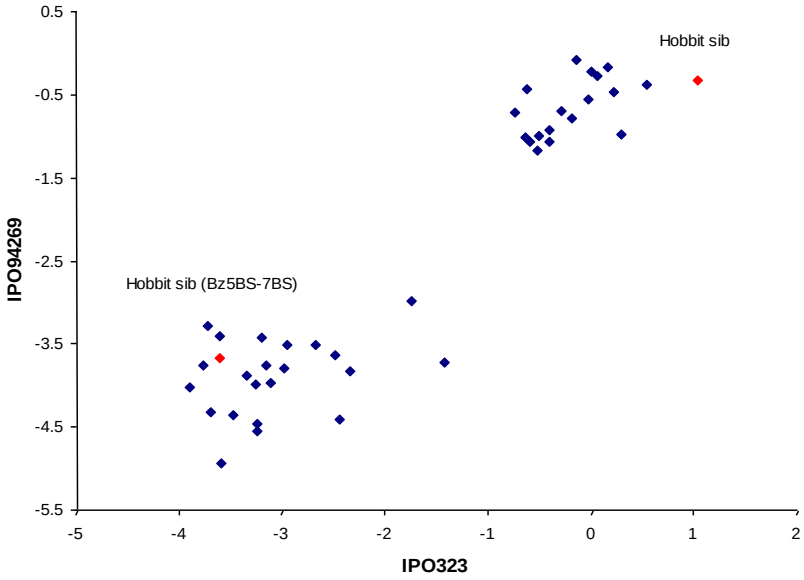
Figure 4.2 Visual sympthom severity of various cultivars and lines under disease stress caused by IPO323 and IPO94269 isolates of *Mycosphaerella graminicola*



Hobbit sib (Bez 5BS-7BS) line showed to be resistant to both isolates which confirms previous results in polytunnel trials (Arraiano, 2001; Arraiano *et al.*, 2007b). Hobbit sib nullisomic for 5BS-7BS line results showed a moderate resistance to both isolates (22 % of leaf area covered by lesions bearing pycnidia with IPO323 isolate and 12 % with IPO04269 isolate). This also suggests that there exist background resistant effects on Hobbit sib to *M. graminicola* IPO323 and IPO94269 isolates.

Figure 4.3 shows the logit area under the disease progress curve (lgtAUDP) of the flag leaf for each isolate from which the severity disease caused by *M. graminicola* isolates IPO323 and IPO94269 in the Hobbit sib × Hobbit sib (Bz 5Bs-7Bs) DH population in 2006 can be inferred.

Figure 4.3 Resistance and susceptibility response (lgtAUDPC) of the lines of the Hobbit sib × Hobbit sib (Bez 5BS-7BS) DH population when inoculated with isolates IPO323 and IPO94269 of *Mycosphaerella graminicola*

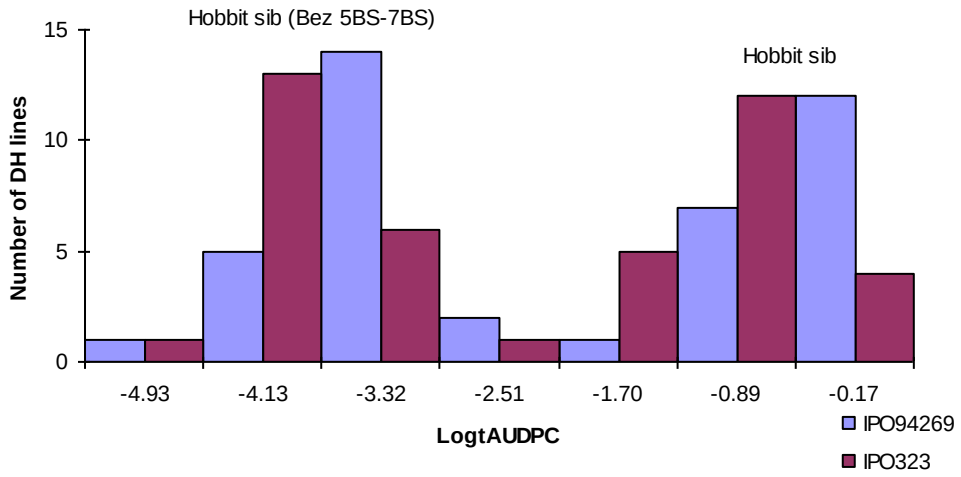


Based on the fact that positive values indicate susceptibility and negative values indicate resistance the response to STB severity of the 40 DH lines can be classified into two different groups. The first one being, the susceptible group, Hobbit sib and 18 lines were both susceptible to IPO323 isolate, with more than 32 % of the leaf area covered by pycnidia (mean value of -1.79 for lgtAUDPC). And IPO94269 isolate with more than 23 % of the leaf area covered by pycnidia (mean-value of -1.8 for lgtAUDPC). The heading date mean-value for this group was 49 days.

In the second group, we include the resistant lines, Hobbit sib (Bz 5BS-7BS) substitution line and 22 lines more showed resistance to both isolates (Fig. 4.3). The means lgtAUDPC values were -3.17 and -3.92 for IPO323 and IPO94269, respectively. For this last group, the mean heading date value was 53 days.

There was no significant difference from the segregation ratio 1:1 resistant and susceptible lines for each isolate ($X^2 = 0.4$ both isolates, $P < 0.001$). In Figure 4.4 the bimodal distribution performance of the DH population for both isolates indicates that resistance and/or susceptibility might be controlled by a single gene. Data very strongly suggest that a single gene or a tightly linked group of genes segregate from this population. This is the first time this is demonstrated for susceptibility/resistance studies.

Figure 4.4 Segregation responses to IPO323 and IPO94269 isolates of *Mycosphaerella graminicola* in the Hobbit sib × Hobbit sib (Bez 5BS-7BS) double haploid population

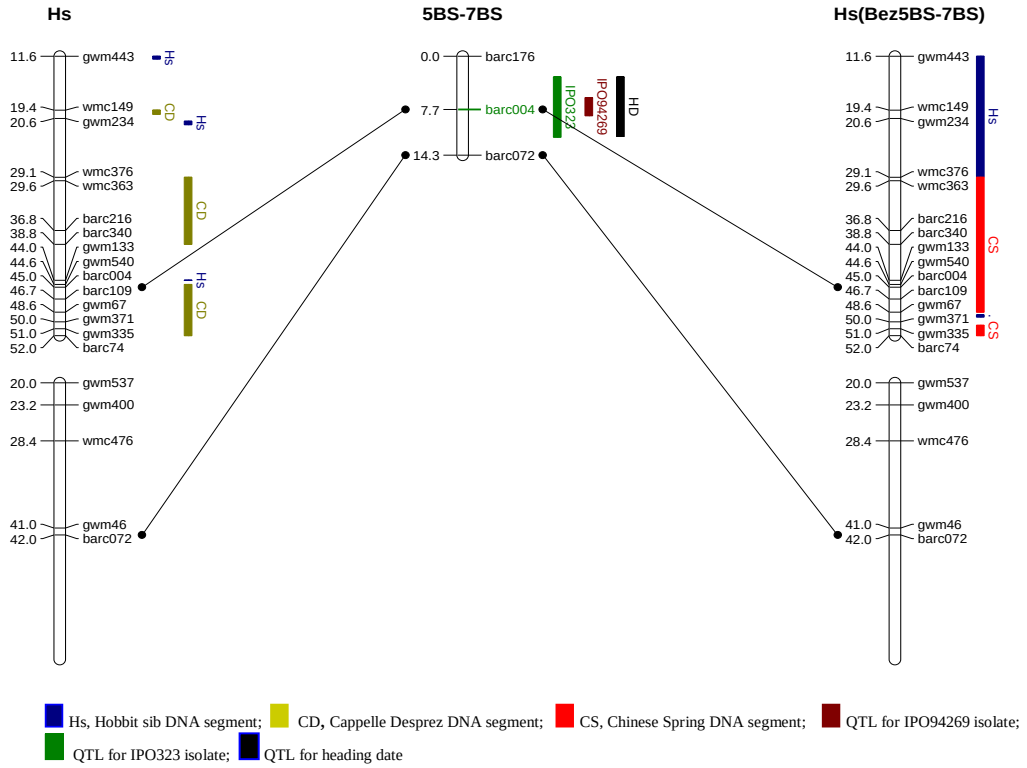


4.3.4 Mapping a gene for Septoria tritici blotch

Figure 4.5 shows the screening of three out of the four microsatellite markers that appeared to be linked building a segment of 14.3 cM, in the Hobbit sib × Hobbit sib (Bez 5BS-7BS) DH population. Only two markers, Xbarc176 and Xbarc004, of the

three linked markers, were assigned to the chromosome segment 5BS (TriticartewhtmapalingVI-2(1) and Arraiano *et al.*, 2007b). The marker Xbarc072 was assigned to 7BS (Fig. 4.5). The Xbarc267 marker was not linked with this small segment of the 5BS-7BS chromosome.

Figure 4.5 Segments of chromosome 5BS-7BS of Hobbit sib (Hs), Hobbit sib × Hobbit sib (5BS-7BS) DH population, and Hobbit sib (Bez5BS-7BS) substitution line (Hs (Bez5BS-7BS)), respectively



Left. The segment of chromosome 5BS-7BS of Hobbit sib cultivar is shown where segments of Hobbit sib DNA and Cappelle Desprez DNA were detected (Arraiano *et al.*, 2007b).
Centre. Quantitative trait loci detected for the *M. graminicola* isolates IPO323 and IPO94269 response on a segment of the 5BS-7BS chromosome of Hobbit sib x Hobbit sib DH population. Also, a QTL for heading date distinguished tightly associated to the QTL for susceptibility/resistance to STB disease in the same population.
Right. The segment of chromosome 5BS-7BS of Hobbit sib (Bez 5BS-7BS) substitution line is shown where segments of Hobbit sib DNA and Chinese Spring were detected (Arraiano *et al.*, 2007b)

When conducting the QTL analysis for susceptibility to the IPO323 isolate specific QTL associated with the marker barc004 was detected with a LOD score of 14.96, explaining 87 % of the variance. The additive value was -1.42 and the line that decreased resistance the most was Hobbit sib.

Similar results were found for the IPO94269 isolate. In this case, the QTL associates with the same marker (barc004) with a high LOD score of 19.3, explaining 93 % of the variance. The additive value was -1.61 and the susceptibility was also ascribed to the Hobbit sib line.

The QTL for HD was also associated with the same marker barc004 in the chromosome segment 5BS (LOD score of 8, explaining 67 % of the variance). The allele was conferred by the Hobbit sib (Bez 5BS-7BS) substitution line, with an additive value of 1.66 (Fig. 4.5).

4.4 Discussion

The experimental data collected demonstrate that the susceptibility of Hobbit sib wheat cultivar to IPO323 and IPO94269 isolates of *M. graminicola* is partly controlled by its chromosome 5BS-7BS. The results of these studies confirm Brown *et al.*, (2001) and Arraiano 2001; Arraiano *et al.*, 2007b studies as Hobbit sib was found to be susceptible to IPO323 and IPO94269 in field trials and polytunnel trials respectively, at an adult plant stage. In correspondence with these, segments of Hobbit sib and Cappelle Desprez in the Hobbit sib 5BS-7BS chromosome were reported by Arraiano *et al.* (2007b), but not surprisingly as Cappelle Desprez is one of the complex lines used as parents of the Hobbit sib cultivar (Annual report, 1975). Also, Cappelle Desprez has shown to have a similar level of susceptibility to Hobbit sib in polytunnel trials (Arraiano *et al.*, 2007b).

The Hobbit sib (Bez 5BS-7BS) substitution line showed resistance to IPO323 and IPO94269 isolates in polytunnel trials, which demonstrates that the 5BS arm of this line does not derive from Bezostaya 1 DNA. Instead, the arm consists of a large segment of the Chinese Spring material and, in the telomeric region, of a small

segment from Hobbit sib chromosome. The 7BS arm was, as assumed, substituted by the Bezostaya 1 arm, as also has been supported by Arraiano *et al.* (2007b).

The cultivar Chinese Spring was found to be specifically resistant to IPO323 isolate and susceptible to IPO94269 isolate at an adult stage in the field and in polytunnel trials as reported by Brown *et al.* (2001) and Arraiano *et al.* (2007b), respectively. In this study, the performance of this cultivar was confirmed with the isolates tested in adult plants growing in a polytunnel trial. Chinese Spring has the gene *Stb6* that confers resistance to IPO323 isolate (Chartrain *et al.*, 2005b). The Chinese Spring nulli tetrasomic lines showed resistance to IPO323 too, because they also have this *Stb6* resistant gene. These lines were also as susceptible as Chinese Spring to IPO94269 isolate. In contrast with this, the Chinese Spring nullitetrasomic N5B T5D showed significantly less disease severity than the Chinese Spring euploid to this isolate (Fig. 4.2). Similar allelic variations in the homoeologous chromosomes group 5 were detected by Pink *et al.* (1983) when they investigated the resistance to yellow rust and powdery mildew in Chinese Spring. Nevertheless, the resistance of Hobbit sib (Bez 5BS-7BS) to IPO94269 isolate seemed not to be conferred by Chinese Spring, as supported by Pink *et al.* (1983) studies and this work.

Bezostaya 1 was reported by Brown *et al.* (2001) and Arraiano *et al.* (2007b) to be resistant to IPO323 at an adult plant stage, results that are confirmed by the polytunnel research conducted as part of this study. Susceptibility of Bezostaya 1 to IPO94269 at an adult plant stage reported in Brown *et al.* (2001) and Arraiano *et al.* (2007b) studies was also confirmed by these research studies.

The Cappelle Desprez (Bez 5BS-7BS) substitution line has almost completely substituted by the chromosome 5BS-7BS from Bezostaya 1, according to Korzun *et al.* (1997). This substitution line on our studies showed to be as

susceptible as Hobbit sib to IPO323 and IPO94269 isolates, confirming also previous reports (Arraiano *et al.*, 2007b). Based on all this, it can be concluded the 5BS-7BS chromosome of Bezostaya 1 does not confer resistance to IPO94269 isolate.

Although the moderate resistance of Hobbit sib nullisomic for 5BS-7BS chromosome line to the *M. graminicola* isolates IPO323 and IPO94269 indicates the existence of a background resistance, the background chromosomes resistance effects are nullified by the chromosome 5BS-7BS of the Hobbit sib euploid. Thus, it is suggested that the background chromosome effects of Hobbit sib, the "Bezostaya 1 chromosome 5BS-7BS" effects, and the interaction between both gave place to the Hobbit sib (5BS-7BS) substitution line resistance to IPO323 and IPO94269 isolates. Figure 4.2 shows the 5BS-7BS chromosome strong effect on the Hobbit sib background. Law and Worland (1997) found resistance to yellow rust to be caused by the chromosome 5BS-7BS of Cappelle Desprez cultivar, but not by its background. Conversely with the results found in this work, the susceptibility to yellow rust of two varieties (Hybride du Joncquois and Nord Desprez) occurred due to their backgrounds, but not by their 5BS-7BS chromosome. These results point to the importance of the interaction between the background and an individual locus on one chromosome.

Figure 4.5 shows that a QTL for resistance/susceptibility on the 5BS segment from chromosome 5BS-7BS of Hobbit sib × Hobbit sib (Bez 5BS-7BS) DH population associates with marker barc004, through disease data of each *M. graminicola* isolates. This QTL was found on the segment which came from Cappelle Desprez into the Hobbit sib cultivar and from Chinese Spring in the Hobbit sib (Bez 5BS-7BS) substituted line (Fig. 4.5). This QTL therefore signals an allele for susceptibility to the *M. graminicola* isolates IPO323 and IPO94269, the coming

from the translocated chromosome 5BS-7BS of the Hobbit sib cultivar. These results also very clearly suggest there is a gene for resistance to the *M. graminicola* isolates IPO323 and IPO94269 on the short arm of the Chinese Spring chromosome 5B segment of the Hobbit sib (Bez 5BS-7BS) line. This QTL seems to be due to a major gene present at this position as the LOD score was high for both isolates.

Pink *et al.* (1983) suggested that the Chinese Spring 5BS must carry a gene for resistance to yellow rust and powdery mildew. In addition, it has also been suggested that Hobbit sib (Law and Worland, 1991), Cappelle Desprez and Chinese Spring carry genes promoting resistance to yellow rust on their chromosome 5BS (Law and Worland, 1997). Conversely, susceptibility is promoted by their chromosomes 5BL.

The identification of a QTL for HD, also associated with marker barc004, suggests that this QTL for HD is most tightly linked to the QTL for susceptibility. The substitution line Hobbit sib (Bez 5BS-7BS) contributes the allele that delays days to heading. Thoth *et al.* (2003) detected a QTL for heading date (earliness *per se* locus) near to marker Xgwm371 on 5BL chromosome in the Hobbit sib × Hobbit sib (Chinese Spring 5BL) population. Also, Hanocq *et al.* (2004) detected a QTL for earliness *per se* on chromosome 5B, associated with the same marker that in the Renan × Recital population. This marker (Xgwm371) is located on the long arm of chromosome 5B at position 50 cM in the Komugi composite wheat map (2008). It has also been cited in Hobbit sib and Hobbit sib (Bez 5BS-7BS) lines, and its position appeared to be 5cM away from marker barc004 (Arraiano *et al.*, 2007b). Thus, the QTL for HD and the QTL for susceptibility to IPO323 and IPO94269 are closely located on the 5B segment. The closely linked QTL for HD was also clearly shown by the significant correlation coefficient found between heading date and both

isolates severity data shown in Table 4.1 and the almost undetectable recombination events that took place between these traits, as shown in Figure 4.1. This association was also confirmed by the genotypic analysis.

Further marker information has to be developed in order to obtain a dense map of the 5BS chromosome of the Hobbit sib × Hobbit sib (5BS-7BS) double haploid population to improve the localisation of the susceptibility gene.

4.5 Conclusion

The results of this experiment indicate that the 5BS-7BS chromosome of Hobbit sib is responsible for the susceptibility or suppression of resistance to *M. graminicola* isolates IPO323 and IPO94269 in this cultivar. The resistance of Hobbit sib nullisomic for 5BS-7BS to these isolates proved it. However, the Hobbit sib background cannot alone confer the high levels of resistance of the Hobbit sib (Bez 5BS-7BS) line to these isolates. Thus, the segment of Chinese Spring 5BS chromosome must carry a gene for resistance, suggesting also that its 5BL chromosome carries a gene or genes that confer susceptibility to IPO94269 isolate. Septoria tritici blotch severity scores between Hobbit sib nullisomic for 5BS-7BS and Hobbit sib (Bez 5BS-7BS) showed a highly significant difference ($P \leq 0.001$) between both lines.

Important implications for plant breeding originate from the discovery that Hobbit sib carries a gene for susceptibility to STB. Hobbit sib is a cultivar which was present during the 1970´s as a high yield variety in the National Institute of Agricultural Botany (NIAB) recommended list of wheat varieties, although it showed susceptibility to several diseases (Annual report, 1976). It was used as a parent of other U.K. varieties delivered during the 1980s such as Sentry, Norman, Fenman,

Galahad and Longbow. Relatives of Hobbit sib, such as the Riband and Savannah varieties (Angus, 2001), also susceptible to STB, have also been used as parents during the 1990s. The susceptible gene seems to have been selected and spread into different varieties. This may be due to the tight linkage of the susceptibility gene to a gene or genes related to yield or yield components. Hobbit sib has been a source of susceptibility, and then the identification of this susceptible gene will give breeders the chance to remove it from their breeding programs.

CHAPTER 5.

GENERAL CONCLUSION

The first purpose of this work was to find phenotypic relationships between agronomic traits from the partial resistance cultivar Senat and Septoria tritici blotch disease. The Senat × Savannah doubled haploid population, in which Savannah is a susceptible cultivar, was studied. Phenotypic associations between agronomic traits and STB disease detected have been described by several authors. However, the detection of a negative association between the yield component grains m^{-2} and STB severity has not been described before. Conversely, a thousand grain weight was positively associated to STB severity, suggesting that TGW is maintained under disease pressure (Chapter 2, section 2.4 Discussion, Table 2.6, and Figure 2.3). Grain protein content and heading date were also associated with STB; in this case negatively (Chapter 2, section 2.4 Discussion, Table 2.6, and Figures 2.2 and 2.4). According with Kato *et al*. (2000), "trait correlations may reflect a consequence of patterns of plant growth and development", thus the associations detected in the three trials analysed strongly suggest there exist close physiological relationships between STB resistance and the following agronomic traits: grains m^{-2}, thousand grain weight, heading date and grain protein content. In addition, these associations were confirmed by the QTL analysis.

The second aim of this thesis was to investigate the genetic basis of partial resistance in wheat to STB resistance and its genetic association with agronomic traits, due to the fact that a yield penalty is always found (Chapter 3). It was found that QTL for the agronomic traits thousand grain weight, grains m^{-2}, heading date and grain protein content were linked with QTL for STB resistance. A QTL for heading date was localized on the same chromosome for STB resistance (2D); QTL for grains m^{-2} and thousand grain weight were localized on chromosome 6B, where QTL for STB resistance were also localized. A remarkable finding was the three

QTL for grain protein content on chromosomes 3A, 2D, 6B and 7B, where also QTL for STB resistance were found; those on chromosomes 3A and 2D closely linked or the allele showed a pleiotropic effect for these traits. (see Chapter 3, Table 3.4, Figures 3.2, 3.3, 3.4, and Appendix F). In Chapter 3, it is also described how the QTL increasing grain protein content showed mostly to be conferred by the partial resistant cultivar Senat. It is also discussed the possible physiological bases for the preservation of thousand grain weight, the specific weight and consequently, the yield (Chapter 3, 3.5 Conclusions). Thus, the results of this research suggest that in partial resistance there may be a trade-off between the lost of grain protein content by STB attack, but the preservation of the TGW, the specific weight and the yield, due to equilibrium between protein content and starch content in the grain. In other words, STB damage is associated with the reduction of photosynthetic activity by infected leaves, early senescence of leaves and apical senescence (Chapter 1, section 1.4.3 Crop physiology of the disease plants). The consequence of leaf senescence is the decrease of the photosynthetic capacity of green tissues, thus the source of carbohydrates to the growing grain is affected. However, STB severity also affects the deposition of protein in the grains. These interactions allow the equilibrium between the protein matrix that involves the starch granules, avoiding shrivelled grains (Chapter 2, 2.4 Discussion, Association between specific weight and STB).

The findings of this work contradict the statement made by Arama *et al*. (1999) and Simón *et al*. (2004b, 2005) who mentioned that there is no genetic association between resistance and heading date. Hence, the results of this work suggest that in partial resistance, Senat × Savannah DH population, the heading date, a trait that confers disease escape, is genetically linked to STB (Chapter 1, 1.5.2

Disease escape; Chapter 3, Table 3.3 and 3.4) and it is suggested variation in STB severity is accounted for by variation in HD.

On the other hand, the results suggest that in partial resistance, Senat x Savannah DH population, there is not a physiological effect of high to the flag leaf on resistance to STB at NrAaby02 and Sejet02 intensity of the disease. This supported the fact that there were no significant correlation coefficients between these traits in the phenotypic analysis (Chapter 2, 2.4 Discussion, Phenotypic association of plant adaptation traits and STB) and the residual analysis (Chapter 3, 3.3.4.2.2 Height to the flag leaf effects).

Finally, the third objective of this thesis was to detect the gene that confers susceptibility to specific isolates of *M. graminicola* in the cultivar Hobbit sib (Chapter 5). Indeed, there was found a gene for susceptibility near the centromere of chromosome 5BS-7BS of Hobbit sib cultivar; its detection will certainly allow breeders to avoid this source of susceptibility from their breeding programs in wheat. Furthermore, the presence of a gene for resistance to *M. graminicola* on chromosome 5BS of Chinese Spring cultivar is also important for plant breeding for disease resistance (Chapter 5).

In the Hobbit sib (5BS-7BS) × Hobbit sib double haploid population, as in the case of the Senat x Savannah DH population, the heading date was associated with STB severity and also a QTL for both traits were found tightly linked (Chapter 5).

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APPENDICES

APPENDIX A. Analysis of variance for agronomic traits of the Senat × Savannah double haploid population and parents at Nickerson 2006, Sejet 2005 and Sejet 2006.

Table A.1 Analysis of variance for agronomic traits of the Senat × Savannah double haploid population and parents at Nickerson 2006

Source	df	Ears/m^2 MS	Grains/m^2 MS	HD MS	HFL MS	Grains/ear MS
Block	1	4834	2.677E+07	3.19	51.02	0.03
iBlock	18	11325	1.856E+08***	3.71***	66.13***	505.13***
Line	97	10720	6.178E+07	4.45***	40.37***	52.92***
Residual	79	11184	4.251E+07	0.43	6.92	19.18
Total	195	10934	6.522E+07	2.75	29.25	80.72

Source	df	Maturity MS	Protein MS	SW MS	TGW MS	Yield MS
Block	1	0.41	1.14	0.13	1.11	0.01
iBlock	18	1.60***	0.21	4.75***	21.39***	0.70***
Line	97	2.26***	0.59***	4.56***	12.89***	0.28***
Residual	79	0.34	0.13	0.90	3.65	0.04
Total	195	1.41	0.37	3.07	9.87	0.22

*** Significant at $P \leq 0.001$

HD, heading date; HFL, height to the flag leaf; SW, specific weight; TGW, thousand grain weight

Table A.2 Analysis of variance for agronomic traits of the Senat × Savannah double haploid population and parents at Sejet 2005

Source	df	Ears/m^2 MS	Grains/m^2 MS	HD MS	HFL MS	Grains/ear MS	Maturity MS
Block	1	156	3159270	1.30	10.33	0.42	2.47
iBlock	10	15627***	7067099	3.70***	18.24***	66.92***	2.05***
Line	97	5472***	11049664	8.27***	63.17***	29.07***	3.31***
Residual	87	2906	6669886	0.69	2.55	10.83	0.33
Total	195	4821	8850911	4.62	33.55	22.73	1.91

Source	df	Protein MS	SW MS	TGW MS	Yield MS	Starch MS
Block	1	2.45***	0.96	19.41	0.94	0.68
iBlock	10	0.44***	6.24***	3.82	0.85***	0.86***
Line	97	0.32***	5.83***	16.87***	0.50***	0.91***
Residual	87	0.09	0.66	2.58	0.13	0.12
Total	195	0.23	3.52	9.84	0.35	0.55

HD, heading date; HFL, height to the flag leaf; SW, specific weight; TGW, thousand grain weight

*** Significant at $P \leq 0.001$

Table A.3 Analysis of variance for agronomic traits of the Senat × Savannah double haploid population and parents at Sejet 2006

Source	df	Ears/m^2 MS	Grains/m^2 MS	HD MS	HFL MS	Grains/ear MS	Maturity MS
Block	1	58910	129712164	0.41	16.58	0.02	0.33
iBlock	10	7724	57196015***	1.88***	27.62***	93.97***	4.41
Line	97	10146	20131539	3.36***	56.75***	32.27***	3.27***
Residual	87	6486	16999288	0.29	7.02	9.78	1.55
Total	195	8639	21196767	1.90	32.86	25.24	2.54

Source	df	Protein MS	SW MS	TGW MS	Yield MS	Starch MS
Block	1	1.77	15.66	0.21	1.47***	0.03
iBlock	10	1.55***	10.00***	24.51***	0.62***	1.51***
Line	97	0.47***	9.82***	20.75***	0.42***	1.86***
Residual	87	0.25	1.66	3.41	0.09	0.40
Total	195	0.43	6.22	13.10	0.29	1.18

HD, heading date; HFL, height to the flag leaf; SW, specific weight; TGW, thousand grain weight
*** Significant at $P \leq 0.001$

APPENDIX B. Test of X^2 goodness of fit for eleven agronomic traits of the Senat × Savannah double haploid population and parents

Table B.1 Test of X^2 Goodness of fit for eleven agronomic traits at three different environments

Site	Ears/m^2 X^{2a}	Grains/m^2 X^{2a}	HD X^{2b}	HFL X^{2a}	Grains/ear X^{2a}	Maturity X^{2b}
Nickerson 2006	4.97	7.64	6.16	7.63	3.87	12.45
Sejet 2005	14.56	11.22	5.74	7.04	4.26	12.99
Sejet 2006	5.75	4.72	14.79	6.90	3.40	9.85

	Protein X^{2a}	SW X^{2a}	TGW X^{2a}	Yield X^{2a}	Starch X^{2a}
Nickerson 2006	6.48	11.86	5.98	5.50	-
Sejet 2005	2.55	2.66	6.66	4.90	4.19
Sejet 2006	8.81	5.90	0.87	5.00	11.32

HD, heading date; HFL, height to the flag leaf; SW, specific weight; TGW, thousand grain weight

[a] DF = 5, $X^2_{\alpha=0.001} = 21$

[b] DF = 4, $X^2_{\alpha=0.001} = 18.47$

APPENDIX C. Phenotypic distribution for eleven agronomic traits and septoria tritici blotch resistance-susceptibility of the Senat × Savannah double haploid population

Figure C.2 Phenotypic distribution of the DH lines for ears m^{-2} at Nickerson 2006, Sejet 2005 and Sejet 2006 environments

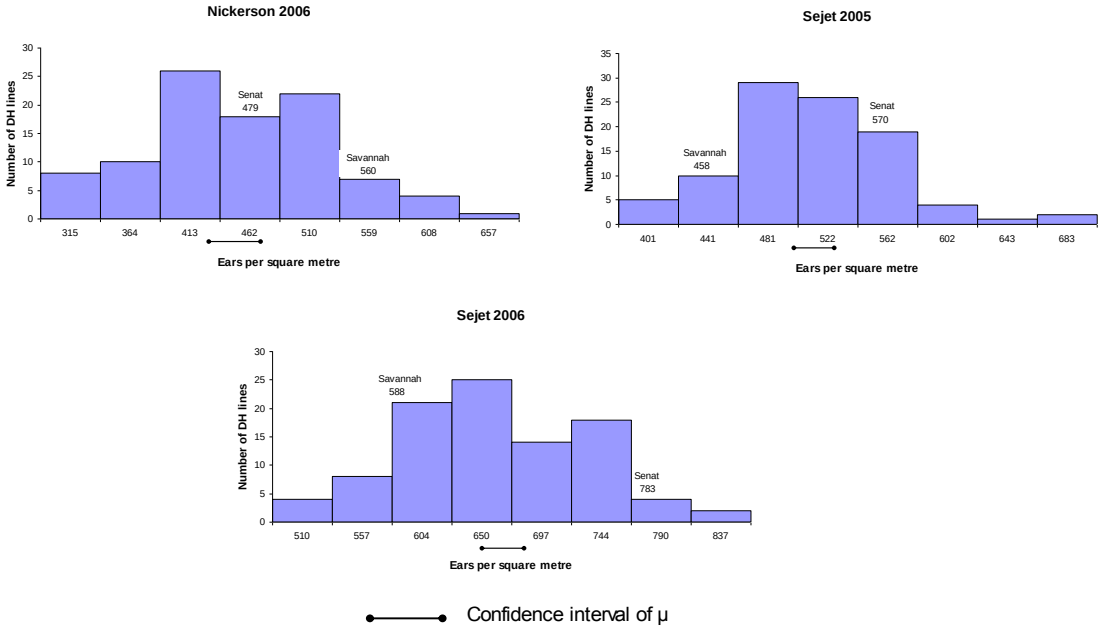


Figure C.2 Phenotypic distribution of the DH lines for grains m^{-2} at Nickerson 2006, Sejet 2005 and Sejet 2006 environments

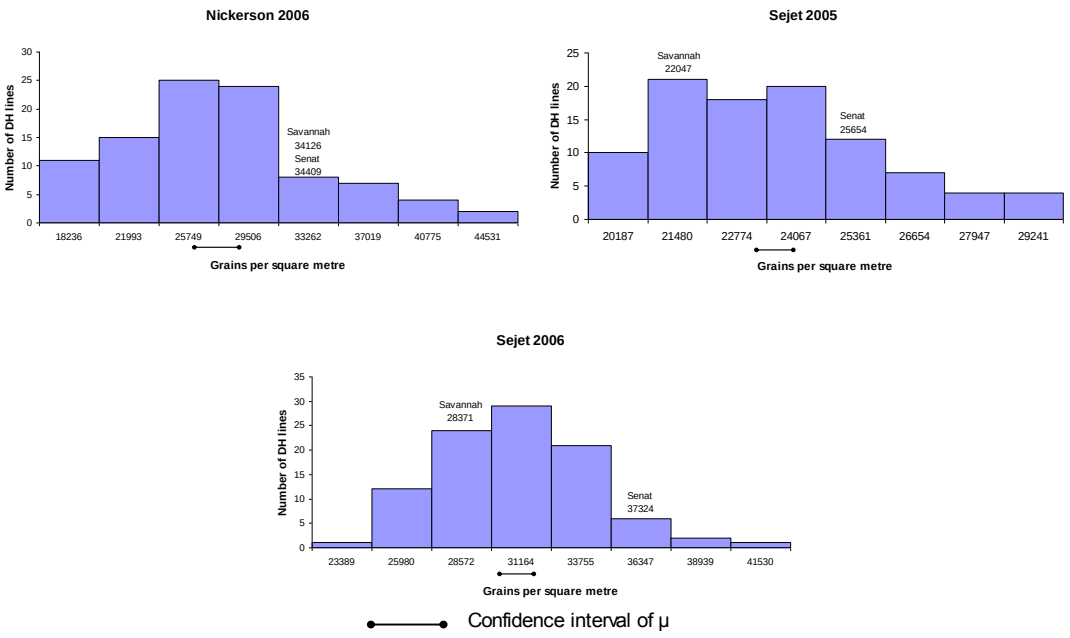


Figure C.3 Phenotypic distribution of the DH lines for heading date (days 1/1) at Nickerson2006, Sejet 2005 and Sejet 2006 environments

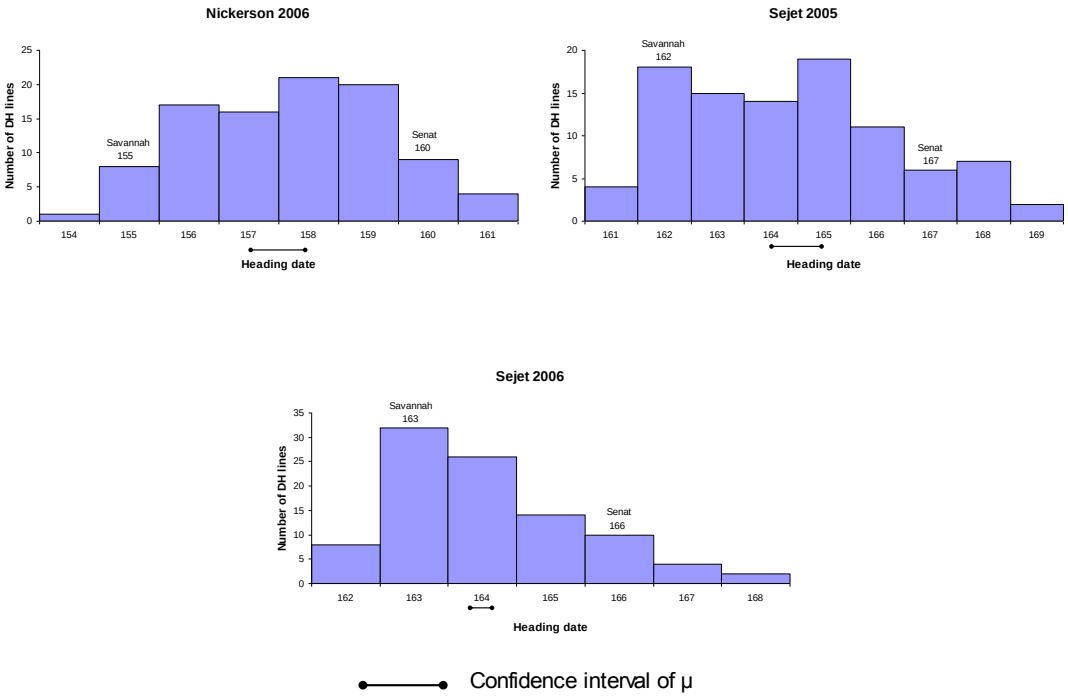


Figure C.4 Phenotypic distribution of the DH lines for height to the flag leaf (cm) at Nickerson 2006, Sejet 2005 and Sejet 2006 environments

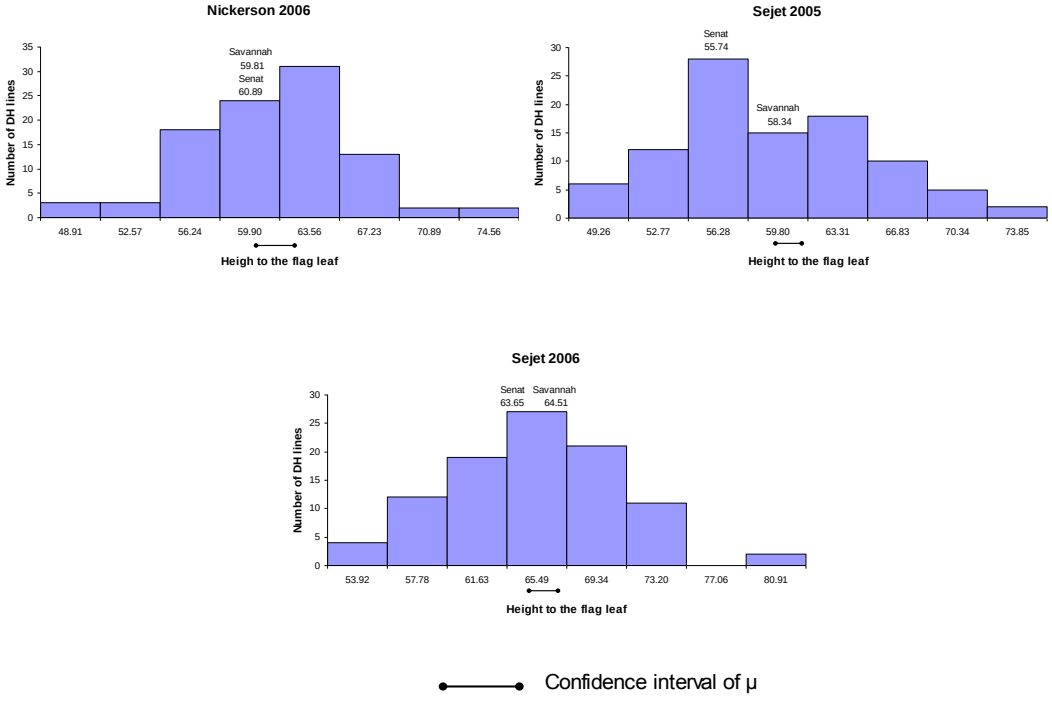


Figure C.5 Phenotypic distribution of the DH lines for grains ear^{-1} at Nickerson 2006, Sejet 2005 and Sejet 2006 environments

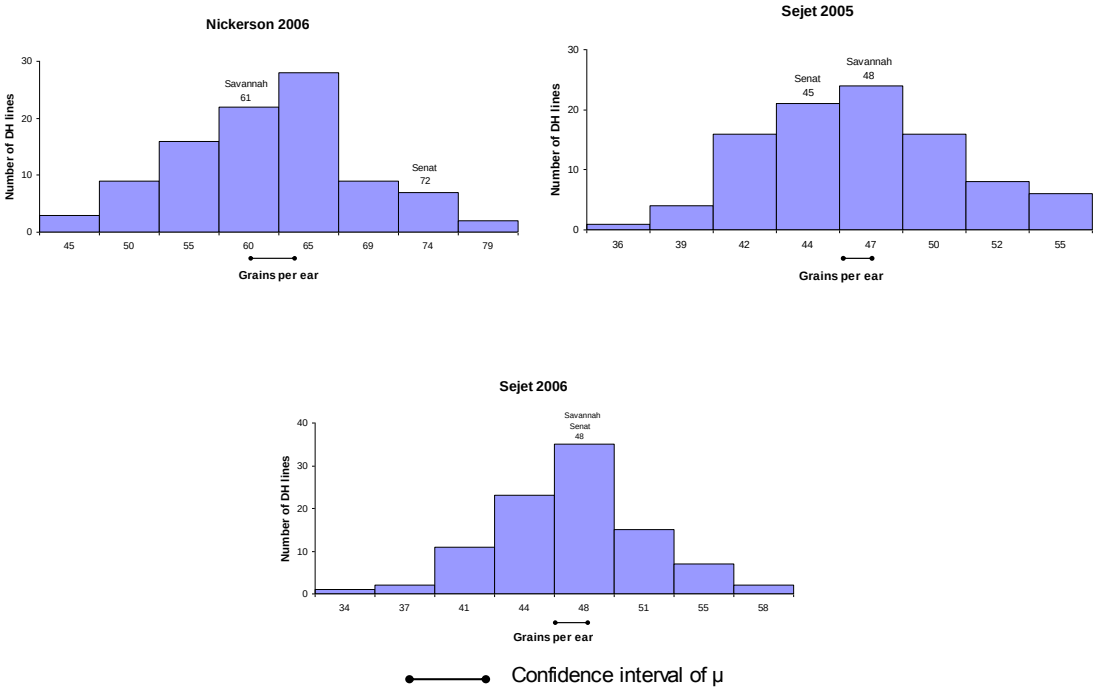


Figure C.6 Phenotypic distribution of the DH lines for canopy maturity (classification 1 to 9) at Nickerson 2006, Sejet 2005 and Sejet 2006 environments

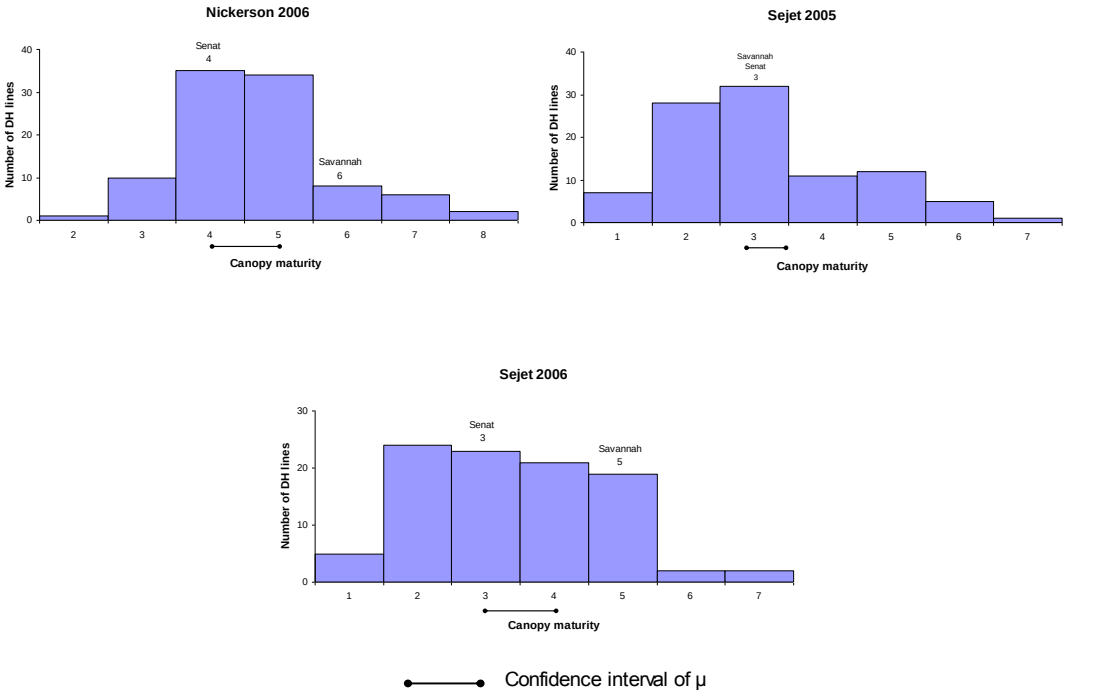


Figure C.7 Phenotypic distribution of the DH lines for protein content (% DM) at Nickerson 2006, Sejet 2005 and Sejet 2006 environments

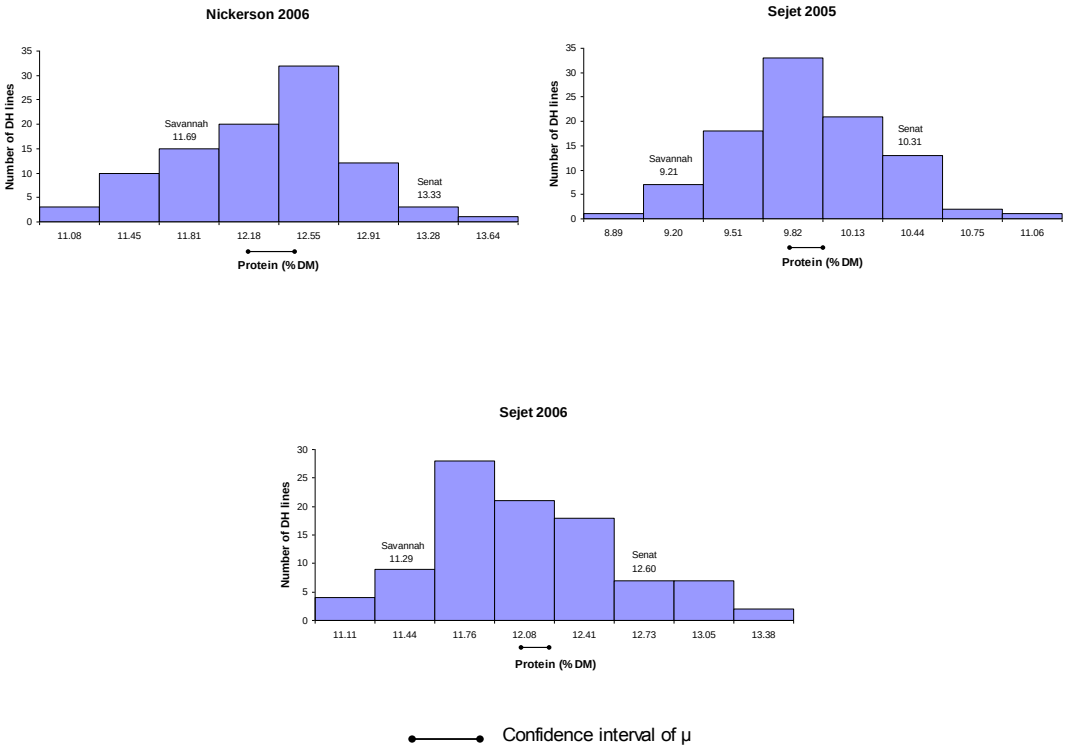


Figure C.8 Phenotypic distribution of the DH lines for specific weight (kg hl^{-1}) at Nickerson 2006, Sejet 2005 and Sejet 2006 environments

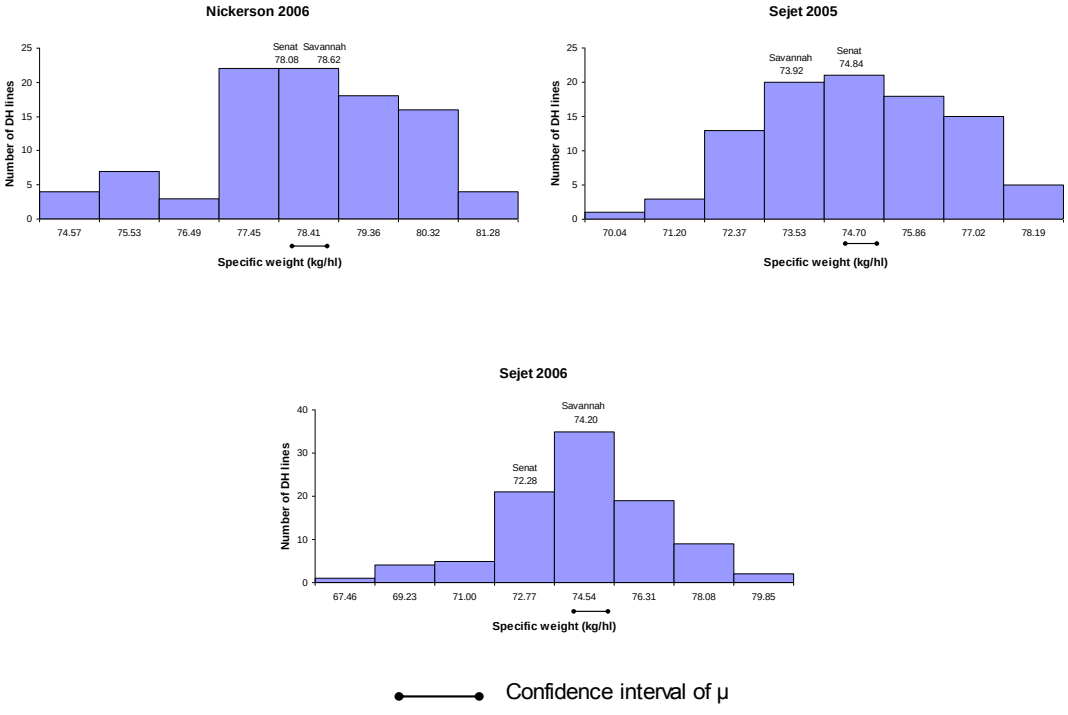


Figure C.9 Phenotypic distribution of the DH lines for thousand grain weight (gr) at Nickerson 2006, Sejet 2005 and Sejet 2006 environments

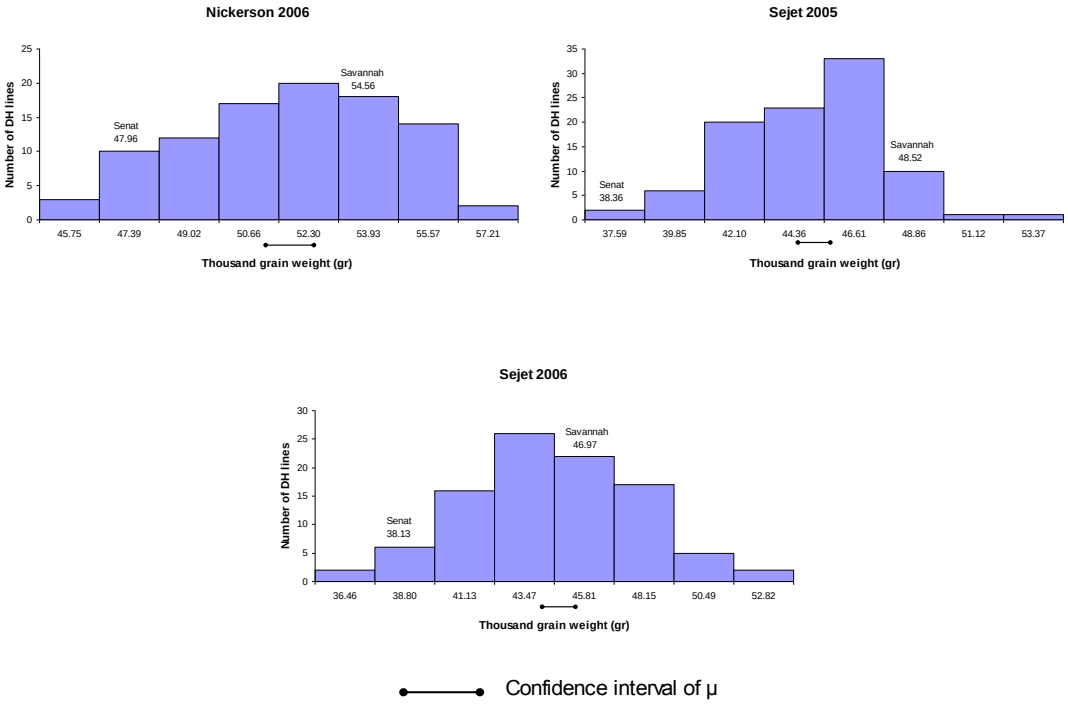


Figure C.10 Phenotypic distribution of the DH lines for yield (ton ha^{-1}) at Nickerson 2006, Sejet 2005 and Sejet 2006 environments

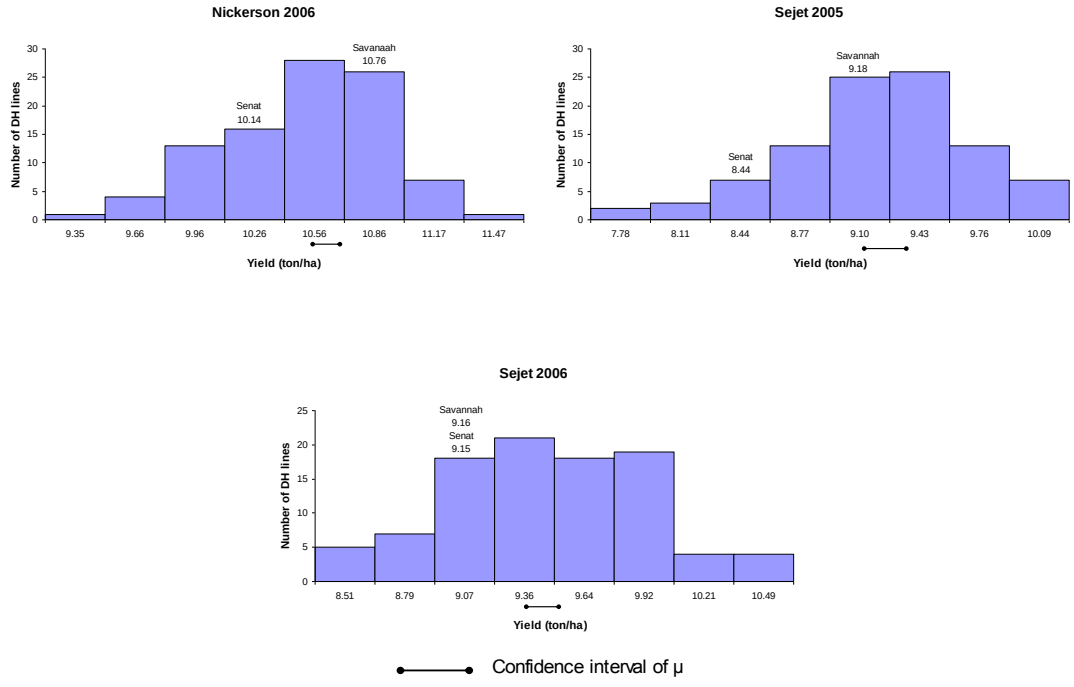


Figure C.11 Phenotypic distribution of the DH lines for starch content (% DM) at Sejet 2005 and Sejet 2006 environments

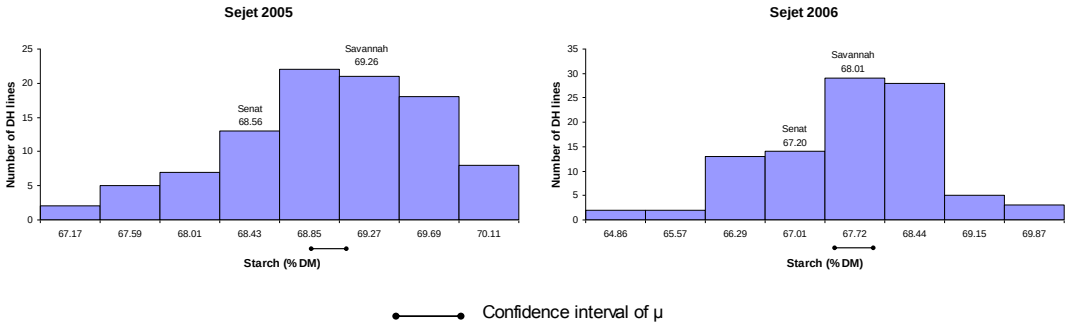
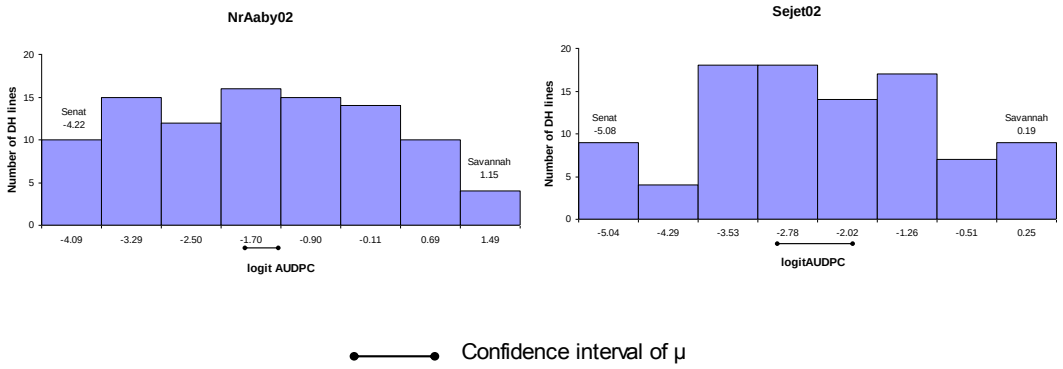


Figure C.12 Phenotypic distribution of the DH lines for resistance-susceptibility to septoria tritici blotch logitAUDPC (logit area under the disease progress curve) at NrAaby02 and Sejet02 environments



APPENDIX D. Normality of standardized residuals. Heading date and STB severity, and height to the flag leaf and STB severity, both at three environments and two STB severity data

Figure D.1 Normality of Standardised residuals for heading date and STB severity

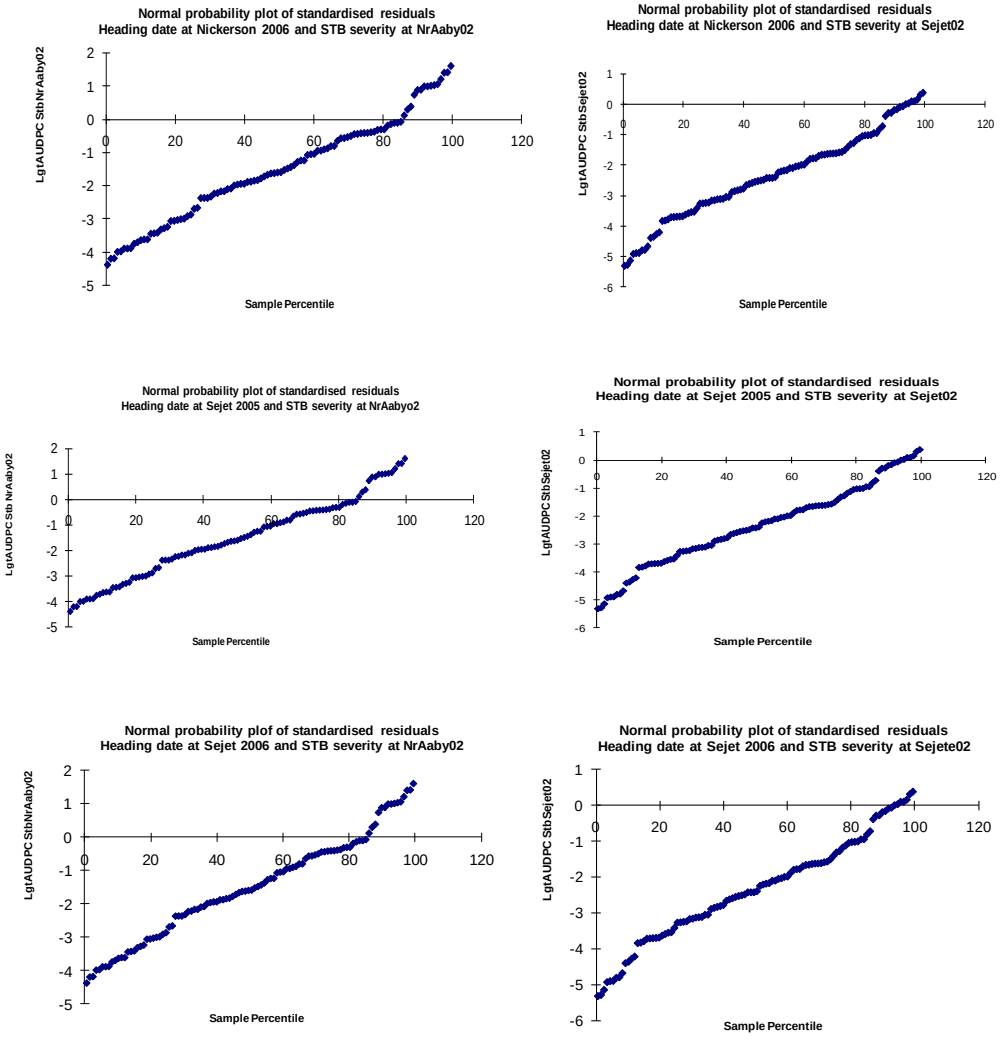
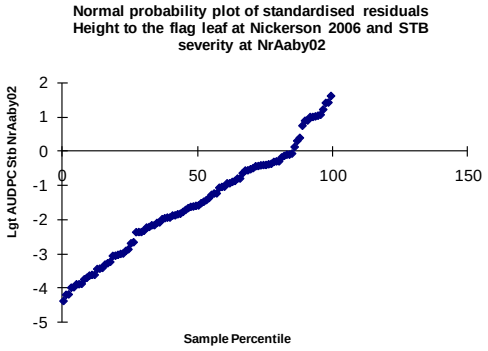
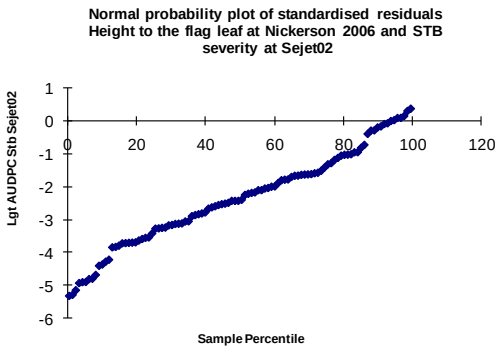
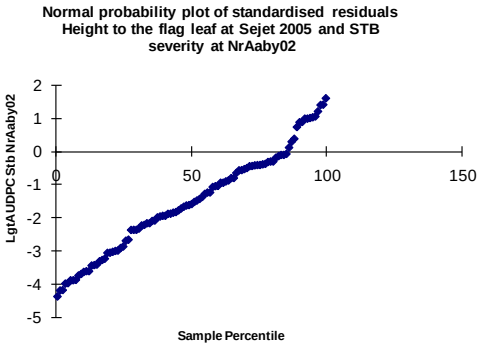
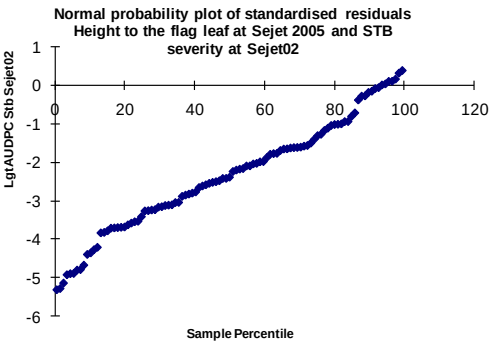


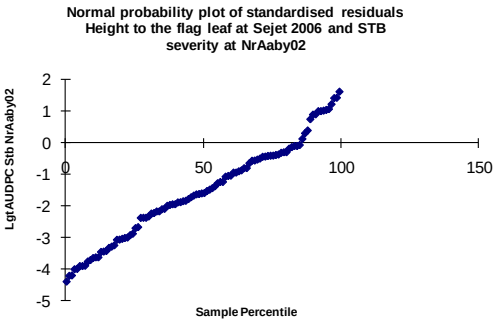
Figure D.2 Normality of Standardised residuals for height to the flag leaf and STB severity

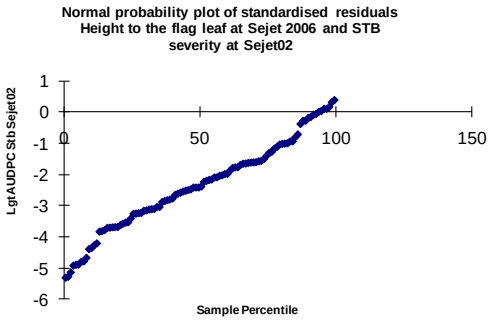












APPENDIX E. Phenotypic associations among agronomic traits. The chromosome or chromosomes where both traits were linked by QTL, detected at Nickerson 2006, Sejet 2005 and Sejet 2006 are signalled.

Figure E.1 Associations of plant adaptation traits (shown by arrows) with yield and yield components and associations of heading date and yield components with STB severity at three environments (only significant associations are shown).

STB association with an agronomic trait; Trait that contributes to increase another trait;
Inversely associated traits; normal number, correlation coefficient at NrAaby02; **bold** number, correlation coefficient at Sejet02; Linked traits in chromosomes are showed in blue

Fig. E.1a Nickerson 2006; Fig.E.1b Sejet 2005; Fig. E.1c Sejet 2006.

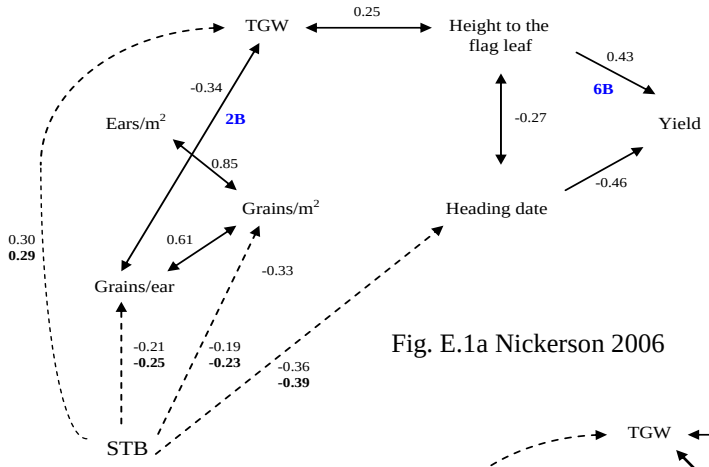


Fig. E.1a Nickerson 2006

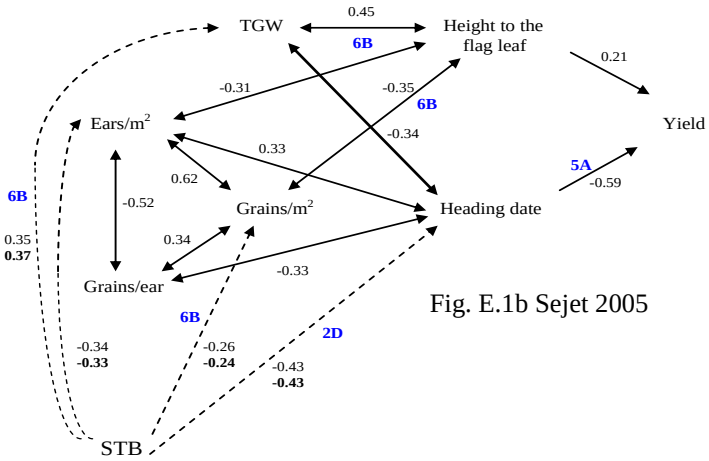


Fig. E.1b Sejet 2005

Fig. E.1c Sejet 2006

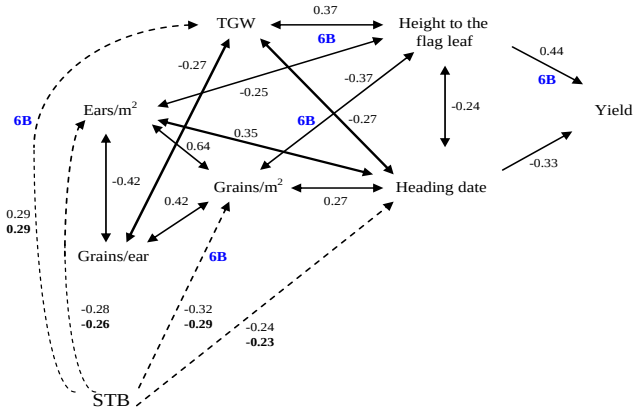


Figure E.2 Associations between yield components with yield and STB severity at three environments and their relationships (shown by arrows; only significant associations are shown).

STB association with an agronomic trait; Trait that contributes to increase another trait; Inversely associated traits; normal number, correlation coefficient at NrAaby02; **bold** number, correlation coefficient at Sejet02; Linked traits in chromosomes are showed in blue

Fig. E.2a Nickerson 2006; Fig. E.2b Sejet 2005; Fig. E.2c Sejet 2006.

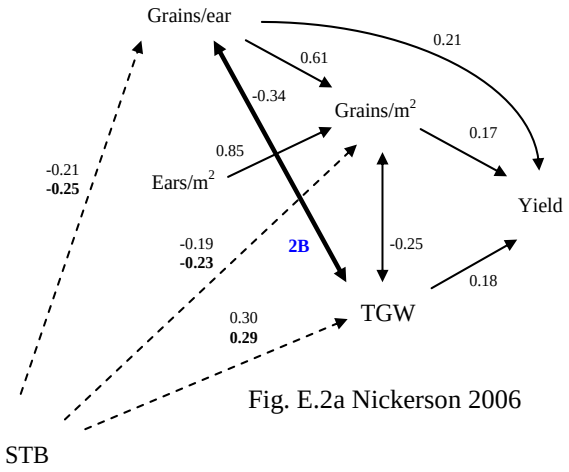


Fig. E.2a Nickerson 2006

Fig. E.2b Sejet 2005

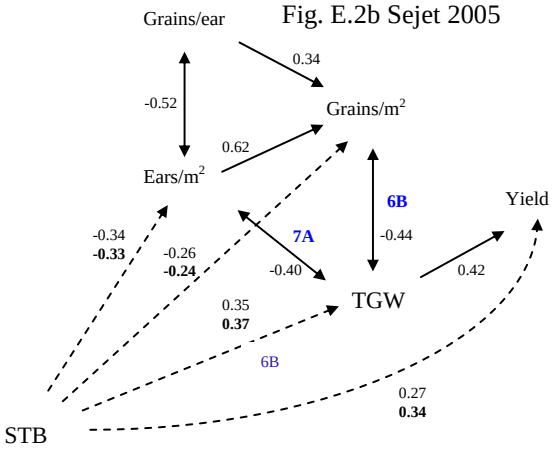


Fig. E.2c Sejet 2006

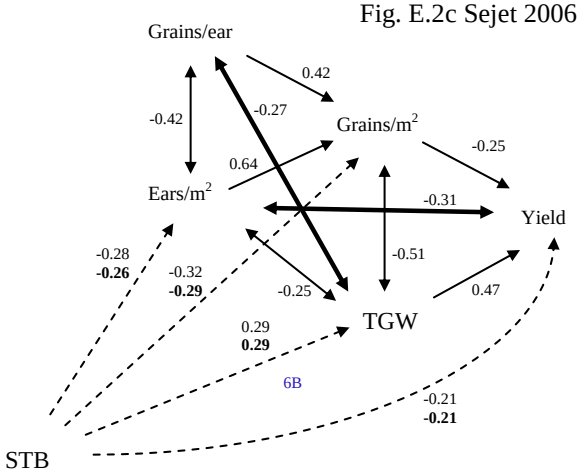


Figure E.3 Associations between grain protein content and grain starch content with agronomic traits that determine grain composition and their association with STB severity (shown as arrows). Also, associations between grain protein content and grain starch content with TGW and yield and their association with STB severity at three environments (only significant associations are shown).

STB association with an agronomic trait; Trait that contributes to increase another trait; Inversely associated traits; normal number, correlation coefficient at NrAaby02; **bold** number, correlation coefficient at Sejet02; Linked traits in chromosomes are showed in blue

Fig. E.3a Nickerson 2006; Fig. E.3b Sejet 2005; Fig. E.3c Sejet 2006.

Fig. E.3a Nickerson 2006

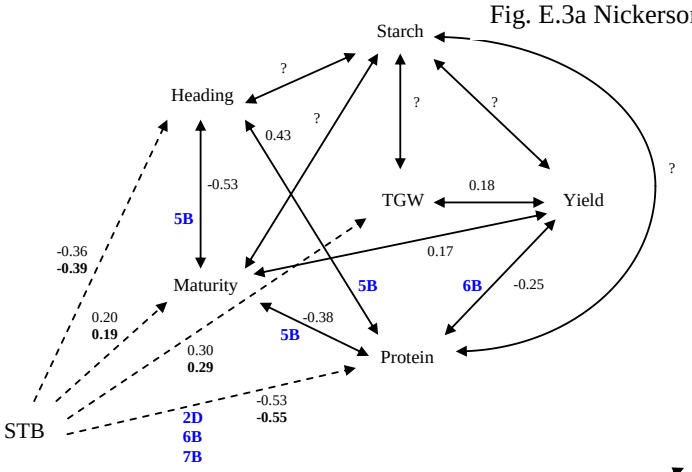


Fig. E.3b Sejet 2005

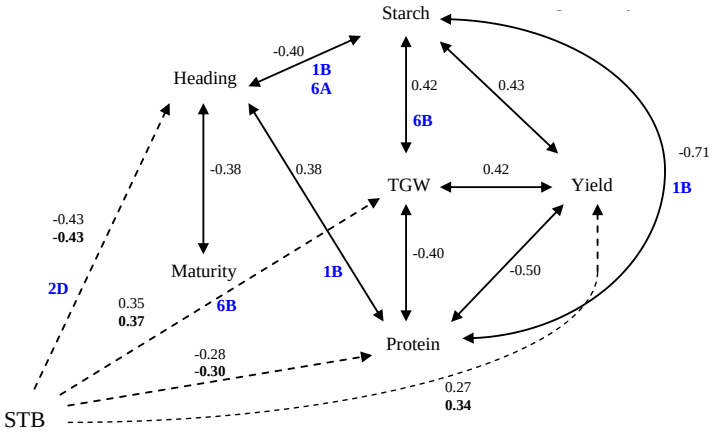


Fig. E.3c Sejet 2006

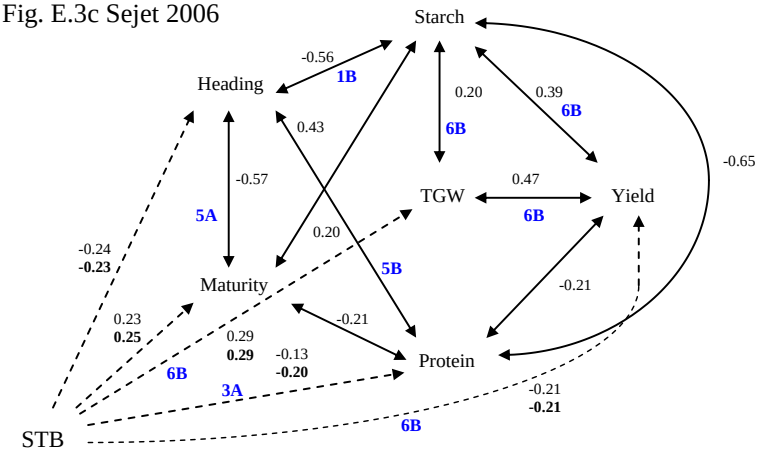


Figure E.4 Significant associations between specific weight, yield components and yield at three environments (shown as arrows). Also, significant associations of these traits with STB severity at three environments (only significant associations are shown).

STB association with an agronomic trait; Trait that contributes to increase another trait; Inversely associated traits; normal number, correlation coefficient at NrAaby02; **bold** number, correlation coefficient at Sejet02; Linked traits in chromosomes are showed in blue

Fig. E.4a Nickerson 2006; Fig. E.4b Sejet 2005; Fig. E.4c Sejet 2006

Fig. E.4a Nickerson 2006

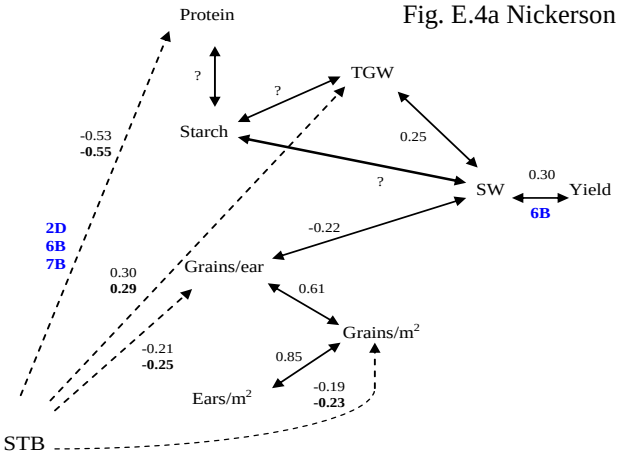


Fig. E.4b Sejet 2005

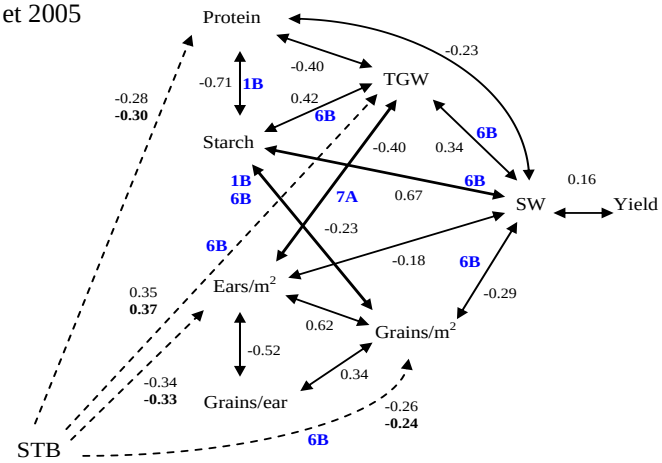


Fig. E.4c Sejet 2006