PHYSIOLOGICAL AND GENETIC ANALYSES OF POST-ANTHESIS HEAT TOLERANCE IN WINTER WHEAT (*TRITICUM AESTIVUM* L.)

by

KOLLURU VIJAYALAKSHMI

B.S., University of Delhi, India, 1995

M.S., University of Delhi, India, 1997

AN ABSTRACT OF A DISSERTATION

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Abstract

Post-anthesis high temperature stress in wheat (*Triticum aestivum* L.) is a major cause of yield reduction. This process results in the loss of viable leaf area and a decrease in green leaf duration ultimately causing a yield loss. The objectives of this study were to (i) phenotype a recombinant inbred line population for heat tolerance traits, (ii) understand the genetic basis of heat tolerance by mapping quantitative trait loci (QTL) linked to yield-related traits under high temperature, (iii) model stay-green under high temperature stress and map the QTL linked to stay-green parameters, and (iv) validate the markers linked to QTL under field conditions.

A filial$_{6:7}$ ($F_{6:7}$) recombinant inbred line (RIL) population was developed by crossing Ventnor, a heat-tolerant white winter wheat with Karl 92, a relatively heat susceptible hard red winter wheat. From 10 DAA to maturity, the treatments of optimum temperature or high temperature stress (30/25°C) were imposed on the RILs. The traits measured included grain filling duration (GFD), kernels per spike, thousand kernel weight (TKW), and grain filling rate (GFR). The stay-green traits calculated were: i) time between 75% and 25% green, ii) maximum rate of senescence, iii) time to maximum rate of senescence, and v) percent green at maximum senescence. Genetic characterization was performed using microsatellite (SSR), amplified fragment length polymorphism (AFLP) and a sequence tag site (STS) markers.

GFD was positively correlated with TKW and negatively with GFR and maximum rate of senescence. Principle component analysis (PCA) showed kernels per spike, maximum rate of senescence, and TKW accounted for 98% of total variability among the genotypes for heat tolerance.

The most significant QTL for yield traits co-localized with marker *Xgwm296* for TKW, *Xgwm356* for kernels per spike, and *Xksum61* for GFR. The QTL for stay-green traits co-localized with markers P41/M62-107 on Chromosome 2A, *Xbarc136* on Chromosome 2D, P58/MC84-146 on Chromosome 3B, P58/M77-343 on Chromosome 6A, and. P58/MC84-406 on Chromosome 6B. These results indicate that increased green leaf area duration has a positive effect on the grain yield under high temperature. Once the kernels per spike are established, GFD and TKW can be used as selection criteria for post-anthesis heat-tolerance.

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Approved by:

Co-Major Professor	Co-Major Professor	Co-Major Professor
Dr. Bikram S Gill	Dr. Allan K. Fritz	Dr. Gary M. Paulsen

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A filial$_{6:7}$ ($F_{6:7}$) recombinant inbred line (RIL) population was developed by crossing Ventnor, a heat-tolerant white winter wheat with Karl 92, a relatively heat susceptible hard red winter wheat. From 10 DAA to maturity, the treatments of optimum temperature or high temperature stress (30/25°C) were imposed on the RILs. The traits measured included grain filling duration (GFD), kernels per spike, thousand kernel weight (TKW), and grain filling rate (GFR). The stay-green traits calculated were: i) time between 75% and 25% green, ii) maximum rate of senescence, iii) time to maximum rate of senescence, and v) percent green at maximum senescence. Genetic characterization was performed using microsatellite (SSR), amplified fragment length polymorphism (AFLP) and a sequence tag site (STS) markers.

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Table of Contents

List of Figures

List of Tables

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CHAPTER 1 - Literature Review

Introduction

Wheat (*Triticum aestivum* L.) is a temperate cereal with an optimum temperature regimen of 15-18°C during the grain filling stage (Paulsen, 1994; Wardlaw and Wrigley, 1994; Porter and Gawith, 1999). Daily high temperature of 25-35°C or greater is common across much of the USA and many other regions of the world where wheat is grown. Stresses like heat and drought during the flowering, pollination, and grain filling stages reduces both production and productivity (Porch and Jahn, 2001). Terminal heat stress is a problem in 40% of the irrigated wheat-growing areas in developed and developing countries, especially the USA and Australia (Reynolds et al., 1994). It has been estimated that every 1°C rise in temperature above the optimum temperature reduces the yield per spike 3-4% (Wardlaw et al., 1989a, b) and from 2 to 3 Mg ha^{-1} under high-temperature stress condition as those prevailing in Great Plains compared to 7 Mg ha^{-1} under cooler conditions in western Europe (Paulsen, 1994). A four-fold difference in wheat yields were recorded when grown under heat stress compared to optimum conditions (Midmore et al., 1984; Shipler and Blum, 1986; Zhong-hu and Rajaram, 1994). The USDA has estimated crop loss of 21% due to drought and heat stress over a period of 50 years from 1948-2002 (USDA-NASS, 2004). Temperature above 20°C 10 d after anthesis (DAA) and 15 DAA reduced grain yield by 78% and 18% respectively (Gibson and Paulsen, 1999), and the grain number by 11% upon the rise in temperature from 21°C to 30°C 10 DAA (Tashiro and Wardlaw, 1990a). In other studies, a yield reduction of 23% was reported in response to high temperature above 32°C for as little as 4 d (Randall and Moss, 1990; Hawker and Jenner, 1993; Stone and

Nicolas, 1994). Temperature above 30°C limits productivity of the plant and injury may occur during vegetative or reproductive phases depending on the location and season (Kolderup, 1979; Rawson, 1986; Shipler and Blum, 1986).

Leaf temperature is determined by the energy balance budget, Qabs = R + C + LE + M, where Qabs is radiant energy absorbed by the leaf; R is the radiation emitted by the leaf (re-radiation); C is the energy exchange by convection (heat exchange) across the boundary layer depending on the difference between leaf and air temperature, leaf properties, and air movement; and LE is the latent energy of transpiration. The LE is the second most important regulator of leaf temperature, expending energy by evaporating moisture. Transpiration is determined by the difference in water vapor pressure between the mesophyll and the air surrounding the leaf and by resistance to diffusion of vapor to the surrounding air. The M is the metabolic energy consumed by biosynthetic processes or released by respiration; it contributes very little to the leaf temperature (Gates, 1968).

Roots have a lower temperature optimum than the shoots and are less adapted to temperature fluctuations (Nielsen, 1974). In nature, this is countered by the soil surrounding the root surface, which buffers the extreme variation in air temperature and causes a diurnal lag between minimum and maximum values. The greenness hormone cytokinin is synthesized in the root and transported to the shoot. In a study on maize, brief high-temperature exposure of the roots inhibited chlorophyll accumulation, chloroplast development, and photosynthetic activity in the shoot (Caers et al., 1985). High temperature also increases production of abscisic acid (ABA). There are two hypotheses regarding the involvement of ABA in heat tolerance. First, it may modify the water balance and provide thermotolerance to the plant (Daie and Campbell, 1981) or, alternatively, cause plant injury by lowering photosynthetic activity, leaf area duration, and

yield under high temperature (Lu et al., 1989). Apart from these changes, the activity of nitrate reductase enzyme is retarded due to high temperature, resulting in accumulation of nitrate in the root and reducing the supply of organic nitrogen to the shoot (Nielsen, 1974).

Agronomic traits

Yield and yield components

Yield components of wheat include plant density, tillers per plant, spikelets per spike, kernels per spikelet, and kernel mass (weight) (Przuli and Mladenov, 1999). Other traits like grain filling duration (GFD), grain filling rate (GFR) and stay-greenness of the plant also contribute to the final yield of a plant (Millet and Pinthus, 1983; van Sanford, 1985; Beiquan and Kronstad, 1994) since grain weight is a product of rate of grain filling and duration of the grain filling period (Gebeyehou et al., 1982). High temperature during grain-filling period decreases yield by decreasing kernel weight (Warrington et al., 1977; Tashiro and Wardlaw, 1990a; Stone and Nicolas, 1994). Kernel weight was decreased by 85% when the temperature rose from 20/16°C (day/night) to 36/31°C from 7 DAA until maturity (Tashiro and Wardlaw, 1989). In the hard red winter wheat Karl 92, which is adapted to Great Plains conditions, grain yield was reduced by 78%, kernel number by 63%, and kernel weight by 29% when a temperature regime of 35/20°C was imposed from 10 DAA until maturity (Gibson and Paulsen, 1999). Inheritance of most of the yield-related traits was polygenic and some, like GFD, had predominant additive

effect and maternal inheritance though epistasis involving dominant gene action was also noted (Przuli and Mladenov, 1999).

Chromosome 3A had genes affecting grain yield, yield components, grain volume weight, plant height and anthesis date (Shah et al., 1999). A recombinant inbred chromosome line population developed for Chromosome 3A indicated that anthesis date was controlled by a single gene, while all the other traits were polygenic (Shah et al., 1999). A monosomic analysis designed to detect QTL controlling thousand kernel weight (TKW) indicated the presence of QTL on eight chromosomes (1A, 1D, 2B, 4B, 5B, 6B, 7A and 7D); the short arm of chromosome 1A had QTL linked to marker *Xwmc333* accounting for 15% of the variation in grain weight (Varshney et al., 2000). Araki et al. (1999) found that the *Wx-B1* gene encoding the granule-bound starch synthase on Chromosome 4AL had a pleiotropic effect on spike emergence time and plant height but not yield or its components.

Carbohydrate translocation and starch synthesis

Wheat supplies nearly 55% of the carbohydrate consumed world-wide (Gupta et al., 1999).The grain-filling period starts from the day of fertilization. The grain-set period starts 3 DAA (Tashiro and Wardlaw, 1990b), followed by the grain-formation period, which last up to 7DAA (Tashiro and Wardlaw, 1990a). Grain deformation may occur if the kernels are exposed to high temperature during this period (Tashiro and Wardlaw, 1990b). High temperature 10DAA reduced grain mass but not the number of kernels (Kolderup, 1979; Bhuller and Jenner, 1985). Wardlaw (1994) found that high temperature (27/22°C) or low light (50%) during spike development (pre-anthesis) reduced the sensitivity of the developing grain to high temperature of 30°C after anthesis, while low light during grain filling (post-anthesis) increased the sensitivity

to high temperature and reduced kernel size. The effect was more prominent in a freely tillering plant than in a single-culm plant, probably due to a difference in light penetration of the canopy.

Acclimation to high temperature prevented drastic yield loss and the ability to acclimate differed among cultivars (Stone and Nicolas, 1995b). Increased temperature of both root and shoot had similar effects on the development and metabolism of the grain (Guedira and Paulsen, 2002). Transportation of metabolites to the grains depends on the source-sink relationship. High temperature causes rapid respiration in the spikes (sink) which, in turn, results in rapid mobilization of photosynthates from the vegetative parts (source) to the sink (Wardlaw et al., 1980). In a study of two wheat cultivars having contrasting tolerance to heat, the differences were expressed throughout the grain-filling period, with decreasing sensitivity as the grain filling period proceeds. Inspite of an increase in the GFR, shortening of grain filling period results in the reduction of kernel mass (Jenner, 1994; Stone and Nicolas, 1995a). In contrast Wardlaw and Moncur (1995), analyzed rate and duration of grain filling in seven wheat cultivars and found those that were most tolerant to high temperature were the ones in which the rate of grain filling was most enhanced by high temperature, indicating that increased rate compensated for reduced duration of grain filling. An ample supply of assimilates from the vegetative parts and high concentration of soluble sugars in reproductive parts suggested that sink capacity, not source capacity, limited grain filling (Wardlaw et al., 1980; Nicolas et al., 1984). Soluble starch synthase enzyme, which is responsible for the conversion of the sucrose to starch, was highly sensitive to elevated temperature. Genotypes with higher tolerance had higher efficiency in soluble starch synthase enzyme (Zahedi et al., 2003).

Natural senescence or induced senescence due to stress increased the proteolytic activity of the plant. Al-Khatib and Paulsen (1984), showed that specific proteolytic activity in wheat leaves

increased four-fold at 25°C and twenty-eight fold at 35°C. High proteolytic activity results in rapid breakdown of proteins and increased mobilization of nitrogen (N) to the grains, resulting in increased N concentration in the mature grains over carbohydrates (Bhullar and Jenner, 1985).

Heritability

Tolerance to high temperature exhibited transgressive segregation of the affected traits. Quantitative genetic study of plant hybrids derived from an interspecific cross point to the action of complementary gene action as the primary source of transgression (Rieseberg et al. 1999). In a study of membrane stability, relative injury in some of progeny was less than in the tolerant parent, suggesting that genes for high-temperature tolerance were contributed by both parents and the trait was not simply inherited (Saadalla et al., 1990). Screening of a diallele cross with chlorophyll fluorescence as a measure of heat tolerance indicated a high general combining ability (GCA) and maternal effect (Moffatt et al., 1990b). They also reported that specific reciprocal effects indicated the presence of both cytoplasmic and nuclear interaction in response to high temperature and suggested recurrent selection may be an appropriate method of accumulating genes that favor high-temperature tolerance.

Measurements of cell membrane viability in wheat at seedling and flowering stages by cell membrane thermostability (CMS) and tetrazolium chloride (TTC) assays showed that thermotolerance decreased from seedling to flowering stage (Fokar et al., 1998; Cekic and Paulsen, 2001). In a study of crosses between wheat cultivars V747 (heat-tolerant) and Barkaee (heat-susceptible), broad sense heritability, a ratio of genetic variance to total phenotypic variance, was determined as suggested by Allard (1960). The F1 hybrids from the crosses were

backcrossed twice with both parents, and F2 populations were generated. The broad sense heritability in the population was 89% based predominantly on additive genetic variance (Fokar et al., 1998).

The GFD of six spring wheat crosses had a narrow sense heritability ranging from 40 to 60% and additive genetic effect, although epistasis involving dominant gene action was also detected (Przuli and Mladenov, 1999). Narrow sense heritability of yield components in 12 hard red spring wheat crosses was high for grain protein (0.79), heading date (0.89) and test weight (0.79), intermediate for physiological maturity (0.64) and grain yield (0.59), and lowest for GFD (0.4) (Talbert et al., 2001). In sorghum, the physiological trait stay-green, correlating with GFD, had a broad sense heritability of 0.72 (Crasta et al., 1999). Yang et al. (2002b) estimated broad sense heritability for the trait GFD in F2 and F3 generation of Ventnor X Karl 92 crosses under controlled conditions to be 80%.

Physiological traits

Photosynthesis and its relation to yield

Under optimal conditions 80 to 90% of the carbohydrates translocated to the grain of wheat are assimilates from current photosynthesis and 10 to 20% from the plant's reserves (Spiertz and Vos, 1985). Yang et al. (2002a) found that up to 65% of the carbohydrates are provided to the grain at 30°C and either stable photosynthesis or high content of stem reserves are necessary for increasing tolerance to high temperature. Photosynthetic activity is sensitive to high temperature. High temperature increases the radiant energy absorbed by the leaf and, as a

consequence, the process of photosynthesis is affected (Krause and Santarius, 1975; Seeman et al., 1984).

Chloroplasts, the site of photosynthetic activity, have membranes carrying pigment molecules such as Chlorophyll *a* and *b* and accessory pigments (Emerson and Arnold, 1932; Hillier and Babcock, 2001). The end product of the photosynthetic reaction is CO_2 fixation in the form of sugars, which are the major source for grain growth (Evans et al., 1975; Al-Khatib and Paulsen, 1990). Any injury caused to the membranes carrying these molecules can be assessed by the change in fluorescence emitted by these pigments.

Light absorbed by the leaf excites these pigment molecules. Energy released during de-excitation results in photochemistry and heat dissipation. A small amount of the absorbed light, about 3 to 5% *in vivo*, is dissipated as red fluorescence. Damage to the chlorophyll pigments increases fluorescence, which can be measured with a fluorometer. A saturating flash of light (8000 µmol m^{-2} s^{-1} for 1 sec) raises the fluorescence from ground state value (Fo) to maximum value (Fm). In this condition, QA, the first electron acceptor of Photosystem II (PSII) is fully reduced. This allows the determination of maximum quantum efficiency of PSII, given by Fv/Fm = (Fm-Fo)/Fm. A lower value indicates that a proportion of PSII reaction centers are damaged by photoinhibition, which is often observed in plants under stress conditions (Fracheboud, et al., 1999). Moffatt et al. (1990a), in an experiment with six wheat cultivars subjected to controlled environment at 37/25°C and in field trials, found that Fv and grain yield were negatively correlated under controlled conditions and not significantly correlated under field conditions. Genotypes having higher Fv were also the ones having higher yield, indicating that chlorophyll fluorescence can be used in the screening for heat-tolerant genotypes. Hede et al. (1999) found a significant correlation between leaf chlorophyll content and kernel weight in 2,255 Mexican

landraces of wheat. Therefore, a visible trait such as leaf chlorophyll content can be used along with chlorophyll fluorescence for screening.

Temperate cereals are more susceptible to high temperature than tropical cereals. In temperate cereals like wheat, photosynthetic response to high temperature was associated with the ability of the reaction center, mainly PSII P700 molecules of the light reaction, to withstand heat stress (Al-Khatib and Paulsen, 1999). The P700 molecules accept electrons from the water-oxidation sites and transfer them to the plastoquinone to continue the electron transport chain. The threshold temperature for denaturation of PSII was found to be 35-41°C (Rekika et al., 1997). These temperatures are encountered during maturation of wheat, resulting in damage to PSII, but have little effect on PSI (Xu et al., 1995). Yamasaki et al. (2002) suggested that temperature dependence of the electron transport chain at the plastoquinone and water oxidation complexes is a plastic response and is modulated by the temperature at which the leaf developed

A study conducted by Al-Khatib and Paulsen (1990) on 10 wheat cultivars from different wheat-growing regions of the world showed that high temperature stress of 32/27°C for two weeks at the seedling stage and continual heat stress at the post-anthesis stage decreased the photosynthetic rate and visible fluorescence, ultimately affecting grain yield.

Chlorophyll biosynthesis is also affected by temperature regimen. In cucumber (*Cucumis sativus* L. cv poinsette) seedlings, chill-stress (7°C) completely inactivated all enzymes in the chlorophyll biosynthetic pathway and, heat stress of 42°C, partially inhibited chlorophyll biosynthesis up to 60% (Tewari and Tripathy, 1998). In the chloroplast, Ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco), one of the key enzymes in CO_2 fixation, is activated by light and the stromal enzyme called Rubisco activase. A moderately high

temperature inhibited light activation of Rubisco via a direct effect on Rubisco activase (Feller et al., 1998). These processes directly affect photosynthesis and, ultimately, yield.

High sugar levels in leaves repress expression of photosynthesis-associated genes via an end-product negative-feedback system (Jang et al., 1997; Dai et al., 1999). Loss of photosynthetic capability of the leaves results in senescence, which is also influenced by various environmental stresses (Nood`en et al., 1997; Buchanan-Wollaston, 1997; Chandler, 2001). Transcripts of several genes are upregulated during leaf senescence. These genes are referred to as senescence-associated genes (SAGs). Expression of a gene that is regulated specifically by senescence (SAG12) was repressed by exogenously supplied sugar in senescent Arabidopsis (*Arabidopsis thaliana* L Heynh.). leaves. In other words, sugars were a link between photosynthesis and senescence and helped in delaying senescence (Simpson and Dalling, 1981; Vicentini and Matile, 1993). Stay-green describes the delayed senescence during post-anthesis stages of plant development (Thomas and Howarth, 2000). Stay-green may act either by delaying onset of senescence or may slow the progress of senescence (Thomas and Smart, 1993), and influences the yield potential (Gentinetta et al., 1986; Evans, 1993; Thomas and Howarth, 2000). Genetically, delayed senescence in sorghum (*Sorghum bicolor* L. Moench.) (Borrell et al., 2000a; Borrell et al., 2000b), maize (*Zea mays* L.) (Baenziger et al., 1999) and durum wheat (*Triticum turgidum durum* Desf. Husn.) (Benbella and Paulsen, 1998; Hafsi et al., 2000) increased yields in water-stressed environments.

Biochemical traits

Hormones

Cytokinin hormones, mainly zeatin, dihydrozeatin and their respective ribosides, were detected in maturing grains of wheat (Banowetz et al., 1999). They also reported that kernel cytokinin content peaked within 3 days of anthesis and returned to baseline within 1-2 days after reaching the peak. High temperature stress reduced the kernel cytokinin content by 80% within one day of anthesis, but increasing cytokinin content alone did not increase thermotolerance of the plant. In transgenic tobacco (*Nicotiana tabacum* L.) with delayed senescence, Gan and Amasino, (1995) reported autoregulatory production of the cytokinin a senescence-inhibiting hormone increased seed production and biomass.

Ethylene, another phytohormone, hastened senescence and chlorophyll loss and induced expression of SAGs (senescence associated genes) in Arabidopsis with ethylene receptor mutant *etr1* having delayed senescence (Grbic and Bleecker, 1995). The dormancy hormone, abscisic acid (ABA), increased close to the cessation of growth. Physiologically, ABA acts as a sensor of osmotic stress and signals the ion channels leading to the stomatal movements (Luan, 2002). Exogenous application of ABA at high concentration inhibits the grain growth. This may be due several potential sites of action for ABA. The most important of these are, the sites of unloading assimilates from the sieve tubes, the sites of assimilate uptake by endosperm cells and the conversion of the sucrose taken up by the endosperm cells to form starch. Schussler et al., 1984 found reduced sucrose uptake at an increased concentration of ABA. Radley, 1976; Ahmadi and Baker, 1999 found a reduced conversion of sucrose to starch at high concentration of ABA, while low concentration did not seem to have any effect. The site of action in this respect

appeared to be more than one enzyme which included soluble starch synthase (SSS) and granule-bound starch synthase (GBSS).

Heat shock proteins (HSP)

Sudden exposure to heat stress causes the induction of certain proteins of molecular weight 15 to 30, 70 and 90 KDa. These proteins are implicated in thermotolerance, maintenance of cell and membrane integrity, prevention of protein denaturation, and protection of PSII in chloroplasts (Vierling, 1990). In spinach (*Spinacia oleracea* L.), Rokka et al. (2001) found that upon sudden exposure to high temperature Rubisco activase assumed the function of a chaperone and associated with the thylakoid-bound ribosomes to protect the protein synthesis machinery; however, Eckardt and Portis (1997) contradicted the report of Rokka et al. (2001), that Rubisco activase was more heat labile than Rubisco.

Burke and O'Mahony (2001) found that certain developmentally regulated HSPs are not involved in enhancing thermotolerance of cotton (*Gossypium hirsutum* L.) seedlings. The HSPs were present in the plant as a part of normal seed development and were lost within a few days of germination. They may last longer under stress than non-stress conditions.

Exposing wheat at the grain-filling stage to high temperature decreased the proportion of high molecular weight glutenin subunits needed for higher dough quality and increased the proportion of low molecular weight gliadin proteins. The later form of gluten proteins was assumed to have HSP properties, and their representative genes had multiple heat shock elements in the published sequences (Blumenthal et al., 1994; Wardlaw et al., 2002b).

Mature seeds of all species studied to date contained significant quantities of HSP-homologous proteins and their corresponding mRNAs, however, it is not known whether developmentally or heat stress-induced HSPs in developing seeds play a role in alleviating damage from high-temperature stress (Maestri et al., 2002).

Molecular markers

Wheat is allohexapliod (2n = 6x = 42) with three genomes, A, B, and D, and an extremely large genome size of 16 x 10^9 bp/1C (Bennett and Smith, 1976). Over 80% of the genome consists of repetitive DNA, and more than 85% of the genes are present in less than 10% of the genome (Li et al., 2004). A majority of these genes occur in clusters in small chromosomal regions that have high rates of recombination (Gill et al., 1996a; Gill et al., 1996b; Sandhu et al., 2001).

Various molecular markers have been developed and used for genome analysis and trait mapping. These molecular markers are especially useful to breeders for selecting quantitative trait loci (QTL), where traits have a polygenic inheritance and variable heritability. The most commonly used molecular markers for construction of the physical and genetic linkage maps are restriction fragment length polymorphisms (RFLPs) (Botstein et al., 1980), microsatellites (Röder et al., 1995), amplified fragment length polymorphisms (AFLPs) (Vos et al., 1995) and random amplified polymorphic DNA (RAPD) (Williams et al., 1990). Extensive genetic maps using molecular markers were initially prepared in tomato (*Lycopersicon esculentum* Mill.) Tanksley et al. (1992), and maize (*Zea mays* L.) by Helentjaris et al. (1986). Detailed genetic

linkage maps (Van Deynze et al., 1995; Nelson et al., 1995a, b, c; Marino et al., 1996) and physical maps (Mickelson-Young et al., 1995; Delaney et al., 1995; Gill et al., 1996) using RFLP markers have been published for all seven homoeologous chromosomes in wheat. The rate of polymorphism established using RFLP markers was less than 10% in wheat, while microsatellites, also called simple sequence repeats (SSRs), and AFLP markers had higher rates of polymorphism (Penner et al., 1998; Korzun et al., 1999). These markers were also ubiquitously distributed and chromosome-specific (Röder et al., 1998).

Traits related to yield and physiology have been mapped in various cereals and other important crops. In sorghum, QTL linked to yield and seed weight under drought conditions were detected on linkage group F (Tuinstra et al., 1998). Ribaut et al. (1997) found QTL linked to grain yield were on chromosomes 1 and 10 in maize, and on chromosomes 3, 4 and 8 in rice (Oryza sativa L.) (Lanceras et al., 2004)

Several important qualitative and quantitative traits in wheat have been mapped to date. These traits include grain protein content (Uauy et al., 2006), preharvest sprouting tolerance (Anderson et al., 1993), vernalization response (Dubcovsky et al., 1998; Snape et al., 1998), aluminum tolerance (Luo and Dvorak, 1996), kernel hardness (Sourdille et al., 1996), bread-making quality (D'Ovidio and Anderson, 1994), dwarfing genes (Korzun et al., 1997), red grain color (Nelson et al., 1995), flour color (Parker et al.,1997), amylose content (Araki et al., 1999), milling yield (Parker et al., 1997), and salt tolerance (Gao et al., 1998).

Groos et al. (2003) found that QTL affecting yield was located on chromosome 7 D and QTL affecting kernel weight were located on chromosomes 2B, 5B and 7A. Börner et al. (2002) reported 210 QTL controlling 20 morphological and physiological traits. Quarrie et al. (2005), in their study of yield QTL over 24 site x treatment x year combinations, which included nutrient

stress, drought stress and salt stress, found 17 clusters for yield QTL distributed around the genome with the strongest yield QTL effects on chromosomes 7AL and 7BL and two additional yield QTL on chromosomes 1DS/L and 5AS. Relatively little research has been done to identify chromosomal regions associated with heat tolerance in wheat. In an analysis of spring wheat populations for heat tolerance, loci on chromosomes 2B and 5B were most important (Byrne et al., 2002). Yang et al. (2002b) found QTL linked to GFD on chromosomes 1BS and 5AS. Interval mapping for heat tolerance in winter wheat has not been reported to date.

Another important physiological trait, called stay-green, has been studied extensively in sorghum. Xu et al. (2000) found that regions that contained the QTL for stay-green coincided with the genes for key photosynthetic enzymes, heat shock proteins, and ascorbic acid response. The QTL for stay-green in sorghum were on linkage groups A, D and E (Sanchez et al., 2002), linkage group A, E, and G by (Haussamann et al., 2002) , linkage group A, G and J (Kebede et al., 2001), linkage group B and I (Tao et al., 2000) , linkage group A, D, and J (Subudhi et al. 2000; Xu et al., 2000), linkage groups A, D and G (Crasta et al., 1999), and linkage groups B, F, I, G and H (Tuinstra et al., 1997). In a rice stay-green mutant, the phenotype was controlled by a single recessive nuclear gene symbolized as *sgr(t)*, mapping to the long arm of chromosome 9 (Cha et al., 2002). Jiang et al. (2004) found 46 main effects QTL distributed on all 12 rice chromosomes with individual QTL having small effects. Bertin and Gallais (2001) reported stay-green QTL on chromosome 10 of maize. The QTL influencing stay-green under high temperature stress in wheat have not been mapped to date.

Yield traits correlate positively with the stay-green character. In sorghum, stay-green QTL on linkage groups F and I had a strong pleiotropic effect on yield, and the QTL on linkage group H was associated with low GFR (Tuinstra et al., 1997; Tuinstra et al., 1998). In durum wheat, four

functional stay-green mutants with delayed leaf senescence were reported by Spano et al. (2003). These mutants had a longer photosynthetic competence than the parents and a higher kernel weight and grain yield. In a winter wheat cross, QTL linked to grain yield and green flag leaf area at 14 and 35 DAA were located on chromosomes 2B and 2D under optimum and drought-stressed conditions, respectively (Verma et al., 2004).

Wild relatives

Grasses that have the D genome are more thermotolerant than grasses with A and B(S) genomes (Ehdaie and Waines, 1992) and can be used as primary sources of resistant genes for wheat improvement (Fritz et al., 1995). Sun and Quick (1991) found that in a Langdon D-genome disomic substitution line with Chinese spring (D-genome donor), genes controlling membrane thermostability (a measure of heat tolerance) were on chromosomes 3A, 3B, 4A, 4B and 6A. *Aegilops geniculata* Roth (=*Ae. ovata* L.), a tetraploid with the MU genome constitution, was another good source for introgressing high-temperature and drought-tolerance genes introgression into bread wheat (Zaharieva et al., 2001).

Synthetic wheats derived from crosses between tetraploid wheat and *Ae*

. *tauschii* are good sources for introducing new genes for abiotic and biotic stresses into the bread wheat gene pool, and they have a higher level of AFLP diversity (39%) compared with hexaploid wheat with 12 to 21% (Lage et al., 2003). Yang et al. (2002c) subjected 30 synthetic hexaploids from durum wheat x *Aegilops tauschii* Cos. accessions and four octaploid amphiploids from Chinese spring wheat x different grasses (*Aegilops* spp.) to heat stress of 30/25°C. Some of the synthetic hexaploid and octaploid lines were tolerant to the high

temperature and could be used for wheat improvement. However, the octaploid lines would be less directly useful for wheat improvement because the kernel number was reduced greatly by unbalanced meiotic chromosomal segregation. Genetic stocks can be used as bridges for introducing alien genes into wheat cultivars (Siddiqui, 1976; Jiang et al., 1994; Rajaram et al., 1997).

Other related stresses

High temperature is often coupled with the other stresses, especially drought. Crops tend to maintain stable water relations regardless of the temperature when moisture is ample, but when water is limiting, heat stress strongly affects water status. This interaction of heat and drought stress affects plants by altering the soil water content, while it does not influence osmotic adjustment (Machado and Paulsen, 2001). If high temperature and drought occur concurrently after anthesis, there may be a degree of drought escape due to shortening of the grain filling period, though the rate of water use may increase due to high temperature (Wardlaw, 2002a).

The QTL with a major role in drought tolerance were located on group 7 chromosomes and those for salt tolerance on group 5 chromosomes (Cattivelli et al., 2002). Kirigwi (2005) found that QTL linked to grain yield, GFR, spike density, grains m^{-2}, biomass production, biomass production rate, and drought susceptibility index (DSI) under drought stress conditions in a spring wheat population were on the proximal region of chromosome 4AL.

By the end of 21st century, the mean temperature may rise on an average by 1.5 to 4.5 °C due to the global warming. This will be associated with an increase in the atmospheric CO_2 concentration (Wigley and Raper, 1992). The increase in temperature will reduce the grain filling period and eventually the grain yield. The increase in CO_2 concentration will increase CO_2 assimilation rate in-spite of high temperature, partially inactivating photosynthetic enzymes. The extra carbohydrates assimilated will increase grain yields at temperatures that do not cause floral abortion (Conroy et al., 1994). They also found increase in yield was due to increase in tiller number rather than kernel weight and number. This, however, did not compensate the yield loss due to the shortened grain filling period.

Bread making quality of flour produced from grains developed at high temperature is poor and that developed in the presence of high CO_2 may have low grain protein content (Conroy et al., 1994). Hossain et al. (1990) found that wheat cultivars with high kernel weight were more tolerant to chemical desiccation than cultivars with low kernel weight, and the high kernel weight was possible due to increased carbohydrate reserves and efficient translocation of those reserves to the grain.

Oxidative stress is often associated with abiotic or biotic stresses from the transfer of electron to molecular oxygen, resulting in the generation of reactive oxidative species such as hydrogen peroxide (Desikan et al., 2001), superoxides and hydroxyl radicals (Lascano et al., 2001). Antioxidant systems in plants such as superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase combat oxidative stress by scavenging the superoxide radical and hydrogen peroxide, thus preventing formation of highly toxic hydroxyl radicals (Foyer et al., 1994). In creeping bentgrass (*Agrostis palustris* Huds.), Huang et al. (2001) reported that high soil temperature caused more severe oxidative damage to leaves than high air temperature by limiting

antioxidant activities and inducing lipid peroxidation. The oxidative stress was associated with accelerated leaf senescence under high temperature conditions. Maintenance of antioxidant activities and low levels of lipid peroxidation was related to the better tolerance to high soil temperature stress imposed on roots or high air temperature on shoots.

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CHAPTER 2 - Phenotypic Characterization for High Temperature Stress Tolerance in a Recombinant Inbred Winter Wheat Population

Abstract

High temperature stress during post-anthesis is a major cause for reduction of wheat (*Triticum aestivum* L.) yields. Tolerant genotypes are needed to overcome the stress and increase the productivity of wheat. The objective of the experiment was to phenotypically characterize a recombinant inbred line population (RIL) developed from a cross between the heat-tolerant cultivar, 'Ventnor', and a relatively heat-susceptible cultivar, 'Karl 92' to evaluate the effect of heat stress on senescence rate and yield components. The filial$_{6:7}$ ($F_{6:7}$) lines and parents were grown under controlled conditions and maintained at 20/15°C (day/night) temperature up to 10 d after anthesis (DAA). At 10 DAA, a set of RILs were moved to a high-temperature regimen at 30/25°C that was imposed until maturity. Response of the RIL population for the traits grain filling duration (GFD), kernels per spike, thousand kernel weight (TKW), grain filling rate (GFR), and green leaf area duration measured as maximum rate of senescence were monitored. Significant differences between the lines were observed for all the traits. The GFD was positively correlated with TKW and negatively correlated with GFR and maximum rate of senescence, and TKW was positively correlated with GFR. The RIL population showed a normal distribution for the trait values and transgressive segregation, suggesting that heat tolerance

measured as variability in yield and stay-green is quantitative traits. Principle component analysis (PCA) indicated kernels per spike, TKW and maximum rate of senescence were the most important traits, accounting for up to 98% of variability. Broad sense heritability was highest for kernels per spike at 75.3%, intermediate for GFD and TKW at 59.5% and 56.4%, respectively, and low for GFR and maximum rate of senescence at 42% each. Longer GFD, higher TKW and lower senescence rate are associated with heat tolerance. Once the kernel number per spike is established, GFD and TKW can be used as selection criteria for selecting post-anthesis heat-tolerant genotypes in breeding programs. Kernels per spike can be used along with GFD and TKW for selection of tolerant genotypes under field conditions or when the kernel number is not established at the time that high temperature is imposed.

Introduction

High temperatures that often exceed 30°C limit the productivity of wheat. Injury may occur during the vegetative or reproductive phase (Kolderup, 1979; Rawson, 1986; Shpiler and Bulm, 1986). Terminal heat stress is a problem in 40% of the irrigated wheat areas in developing and developed countries, including the USA and Australia (Reynolds et al., 1994). Every 1°C rise in temperature above the optimum temperature of 15°C, reduces yield by 3-4% per spike (Wardlaw et al., 1989a, b).

Genotypes within a species have different levels of tolerance to heat stress. Wheat genotypes like Trigo 1 (Wardlaw et al., 2002b), Egret (Stone and Nicolas, 1994), Ventnor (Al-Khatib and Paulsen, 1990; Yang et al., 2002a) and others have been identified as heat-tolerant and used to

detect the effect of stress on yield, yield components, and other physiological traits. These genotypes have stable yield and perform better than relatively susceptible genotypes under heat-stress conditions (Al-Khatib and Paulsen, 1990; Stone and Nicolas, 1995a). Yield depends on several components, including tiller density, kernels per spike, and kernel weight. Kernel weight is the product of the rate of grain filling and its duration (Gebeyehou et al., 1982). Grain yield, grain volume weight, plant height, TKW, kernels per spike, and spike per square meter could not be separated into unequivocal groups, suggesting that they have a polygenic inheritance and are controlled either by several genes or by few genes with significant environmental influence (Shah et al., 1999). High heritability was found for grain protein content (0.92), heading date (0.89) and test weight (0.79), intermediate for physiological maturity (0.64) and grain yield (0.59), and low for GFD (0.4) in a study across 12 hard red spring wheat crosses by Talbert et al. (2001). Przulj and Maladenov (1999), in a study of six spring wheat crosses, found a prominent additive genetic effect and a narrow sense heritability of 40 to 60% for GFD, indicating that selection for heat tolerance in the breeding programs can be based on GFD.

High temperature (35°C) at 10 DAA reduced the grain yield by 78%, kernel number by 63% and kernel weight by 29% compared to 20°C (Gibson and Paulsen, 1999). Since starch alone accounts for 70% of the grain weight, reduction in grain weight is mainly due to the reduction in the deposition of starch (Bhullar and Jenner, 1985). Soluble starch synthase, one of the enzymes involved in the conversion of sucrose to starch, is most sensitive to high temperature stress (Denyer et al., 1994). Zahedi et al., (2003) reported that tolerant genotypes have soluble starch synthase that had higher efficiency at temperatures above 30°C. Wardlaw (1994) found that high temperature (27/22°C) or low light (50%) during spike development reduced the development of grain, while low light during grain filling increased the response to high temperature and reduced

the kernel size. The effect was more prominent in freely tillering plants compared to single culms. This was likely due to differences in light penetration of the canopy. Increased temperature of both root and shoot had similar effects on development and metabolism of the grain (Guedira and Paulsen, 2002). At elevated temperatures, the rate of translocation of assimilates to the grain was not affected, therefore reduction in grain growth was mainly due to effects on starch deposition. Temperatures above 34°C suppressed assimilation of current photosynthates and affected the grain weight by reducing the grain filling duration (Al-Khatib and Paulsen, 1984) and by inhibiting starch biosynthesis in the endosperm (Keeling et al., 1993; Jenner, 1994). Jenner (1994) and Stone and Nicolas (1995b) found kernel weight was reduced under heat stress as an increase in grain filling rate could not compensate for a shortened grain filling duration, while Wardlaw and Moncur (1995), found that the lines most tolerant to high temperature were those in which the rate of kernel-filling was most enhanced by high temperature, indicating that increased rate compensated for reduced duration of grain filling.

Under optimum conditions about 80 to 90% of the carbohydrates translocated to the grain are assimilates from current photosynthesis and 10 to 20% come from the plant's reserves (Spiertz and Vos, 1985). Photosynthesis is one of the most temperature sensitive processes. The plant photosynthetic rate declined when plants were stressed during their vegetative or reproductive phases (Grover et al., 1986). Rapid senescence of leaves accelerated a decline in the photosynthetic rate, resulting in less time for the plant to assimilate photosynthates. Yang et al. (2002a) found that only 65% of assimilates were provided to the grain by photosynthesis at temperatures above 30°C compared to 80% under optimum conditions. Fokar et al. (1998) found a significant and positive correlation between the rate of chlorophyll loss and photosynthetic stem reserves, indicating higher potential for utilization of stem reserves for the grain filling

associated with accelerated leaf senescence, while Verma et al. (2004) found a significant and positive correlation between percent green flag leaf area with yield, indicating possible mobilization of resources from the leaves to the sink. Either stable photosynthesis or high content of reserves were associated with low susceptibility of a genotype to stress (Yang et al., 2002a).

The objective of the experiment was to phenotypically characterize a RIL population developed from a cross between parents that are contrasting in their response to high temperature. Inheritance of heat-tolerant traits was studied, and the effect of heat stress on yield components and rate of senescence deduced.

Materials and Methods

Plant material

Two winter wheat cultivars that had contrasting response to heat stress were crossed to generate a RIL population. The cultivars used in the study were Ventnor, a hard white Australian wheat and Karl 92, a hard red wheat from the USA (Al-Khatib and Paulsen, 1990; Yang et al., 2002b). The pedigree of Ventnor is unknown, but is believed to be from a complex cross developed by Albert Pugsley, while Karl 92 is a F_{11} reselection from the cultivar Karl. The pedigree of Karl and Karl 92 is Plainsman V/3/Kaw/Atlas 50//Parker*5/Agent (Sears et al, 1997). The F_2 generation was advanced by the single seed descent (SSD) method in the green-house to generate a set of 101 $F_{6:7}$ RILs. Before planting the seedlings for each generation, five to

six seeds from each line were sown in a 7 x 8.5-cm pot filled with vermiculite. The pots were watered and kept at room temperature. After the seedlings attained a height of 2.5 cm, the pots were transferred to the vernalization chamber at 5°C for 6 weeks. The vernalized seedlings were then transferred to cones (3-cm diameter) containing soil and were grown at 20°C and 16-h photoperiod. The soil mix was silt loam consisting 1.7 g N, 0.11 g P, and 1.4 g K/kg soil, gypsum (4.0 g/kg soil), perlite (63.0 g/kg soil), and peat moss (400.0 g/kg soil). Minimal water and nutrients were provided to the plants to accelerate plant development. Seeds generated from F_6 generation (RIL) and the parents of the cross (Ventnor and Karl 92) were used for phenotyping. The phenotyping was conducted in controlled environment chambers (PGW-36, Conviron, Pembina, ND). Three replications for each RIL under normal and high temperature were planted in a split plot design. Each replicate was studied in sequential order. The experiment was blocked on time, with growth chambers as the experimental units for temperatures and pots as the experimental units for the RILs.

Vernalized seedlings in each replication were transplanted to vinyl pots (10x25-cm.) containing the soil mix mentioned above. Each pot held one seedling, and the RILs were maintained as single tillers up to maturity by clipping the secondary and tertiary tillers (Wardlaw, 2002a). One-half teaspoon of insecticide per pot (Marathon II, active ingredient: imidacloprid) was topically applied to the moist soil immediately after transplanting and a foliar spray of the fungicide Bayleton (active ingredient: triadimefon) at the rate of one-half teaspoon for 4 L of water was applied to prevent powdery mildew. The controlled chamber was set at an optimal temperature of 20/15°C, 50/70% relative humidity, 16-h photoperiod, and light intensity of 420 μmol m^{-2} s^{-1} as suggested by Yang et al. (2002a). Peters professional fertilizer was given to each pot once a month up till anthesis to supply 100 mg N, 43 mg P, and 87 mg K (Peters Professional Plant

Food, W.R. Grace & Co., Fogelsville, PA). Spikes were labeled when 50% of the heads reached anthesis. At 10 DAA, the replicates of RILs for high temperature were moved from the optimum temperature to a controlled chamber set at 30/25°C, with the other environmental conditions remained constant. This set of RILs experienced high temperature continuously until maturity. The plants were watered daily in the high temperature chamber and every other day in the optimum temperature treatment. The pots were randomized every 10 d to minimize spatial effects.

As a separate part of the experiment, five replicates of Ventnor and Karl 92 were compared on whole plant and single culm bases for the same traits. The design and conduct of the experiment was similar to the analysis of the RILs. All replicates of both parents were planted and analyzed at the same time.

Traits measured

Grain filling duration (GFD)

The grain filling duration is the period from anthesis to physiological maturity. In this experiment heat stress was imposed 10 DAA, therefore GFD was estimated as the duration between 10 DAA and physiological maturity. Physiological maturity was determined as the time when the glumes became chlorotic.

Kernels per spike

At maturity, the spikes were harvested and dried at 30°C in a growth chamber for one week. The dried spikes were threshed using a single-spike thresher (Precision Machine Co.,

Lincoln, NE) and cleaned using a micro-cleaner (Jim's Services and Specialties, Lincoln, NE). The kernels from each spike were packaged into individual bags and the number of kernels was determined using an electronic seed counter (SEEDBURO, 801 COUT-A-PAK, Chicago, IL). The number of kernels in each bag was equivalent to the number of kernels per spike, since all plants were maintained as single culm during their growth.

Kernel weight

Kernels per spike were weighed on a sensitive electronic balance (A-160, Denver Instrument Company, Denver, CO.). The weight of kernels in each bag was recorded and the TKW was determined.

Grain filling rate (GFR)

Grain filling rate is the rate at which assimilates are transported from the source to the sink. It was estimated as the ratio between kernel weight and GFD.

Green leaf area duration (Stay-green)

The greenness across all leaves of a plant was estimated visually and given a rating of 0 to 10. The maximum rating of 10 was given to fully intact and green leaves that retained all their color. As the leaves senesced, the rating descended from 10 to zero, with 0 being a fully senesced leaf. Senescence scores were recorded at 3-d intervals from 10 DAA after anthesis to physiological maturity. The green leaf area was estimated in terms of maximum rate of senescence. A non-linear regression curve was fitted on the recorded data using Gompertz model (Seber and Wild, 1989). The regression curve used to model senescence was:

$$Y = \alpha \{ 1- e^{-e^{[-b (time - c)]}} \}$$

Where Y is the response variable, α relates to the point where plants leaves are completely green, b relates to the degree of curvature of the curve between time to senescence and visual scale for green leaf area duration, and c relates to the point of time of maximum senescence. The maximum rate of senescence was estimated as the mid-point of the curve with maximum slope.

Heat susceptibility index (HSI) for the traits GFD and TKW for each of the recombinant inbred line was calculated as:

HSI = [(1-Y/Yp)/D], where Y = yield at 30/25°C, Yp = yield at 20/15°C, D = stress intensity = 1- X/Xp, X = mean of Y of all genotypes, and Xp = mean of Yp of all genotypes (Fischer and Maurer, 1978). Genotypes were categorized as tolerant and susceptible according to Khanna-Chopra and Viswanathan (1999). Genotypes having HSI ≤ 0.500 were considered to be highly tolerant, HSI > 0.500 to ≤ 1.000 moderately tolerant and those having HSI > 1.000 were susceptible.

Statistical procedures

Analysis of variance and least square means of all traits were estimated using the statistical procedure Proc. Mixed, and entry means were estimated using Proc. GLM (general linear model). Correlation for all the traits was performed using Pearson's correlation in the statistical procedure Proc. Corr., and principle component analysis was performed on the means data using procedure Proc. Princomp. (Jackson, 1991). Statistical software SAS Version 8.2 was used for all procedures (SAS Inst. Inc., 1990). The mean squares estimates for the analysis of

variance for genotype, genotype-by-environment interactions, and mean square errors were used to calculate broad sense heritability by the following equation: $\sigma^2_G / (\sigma^2_G + \sigma^2_{GE} + \sigma^2_E)$, where σ^2_G represents genotypic variance, σ^2_{GE} represents genotype x environmental variance, and σ^2_E represents error variance.

Results

Comparison of single culms to whole plants

Responses to high temperature for traits GFD, kernels per spike, and kernel weight in single culms and whole plants for the two cultivars Ventnor and Karl 92 differed in their magnitudes (Figures 1 and 2). The mean differences between Ventnor and Karl 92 for GFR were not significant under either situation. To further evaluate the single culm response with whole plant, correlation analysis was performed. The correlation analysis indicated that the GFD and kernel weight correlated significantly at $\alpha = 0.001$ and r^2 over 0.900 (Table 1). Kernels per spike and GFR were not correlated between culms and whole plants, probably due to a shift in source-sink relationship. The high correlation between single culm and whole plant for GFD and kernel weight, and a similar trend between single culms and whole plants for GFR, were the bases of screening the RILs as single culm. Maintaining the plant on a single culm basis eliminated the confounding effect of tiller number on plant responses under heat stress.

a) 14 days in heat stress (Single culms) b) 18 days in heat stress (Whole plants)

Figure 2-1. Performance of parents Ventnor (left) and Karl 92 (right) as single culms vs. whole plants under high temperature. Single culms and whole plants of Karl 92 mature faster than single culms or whole plants of Ventnor.

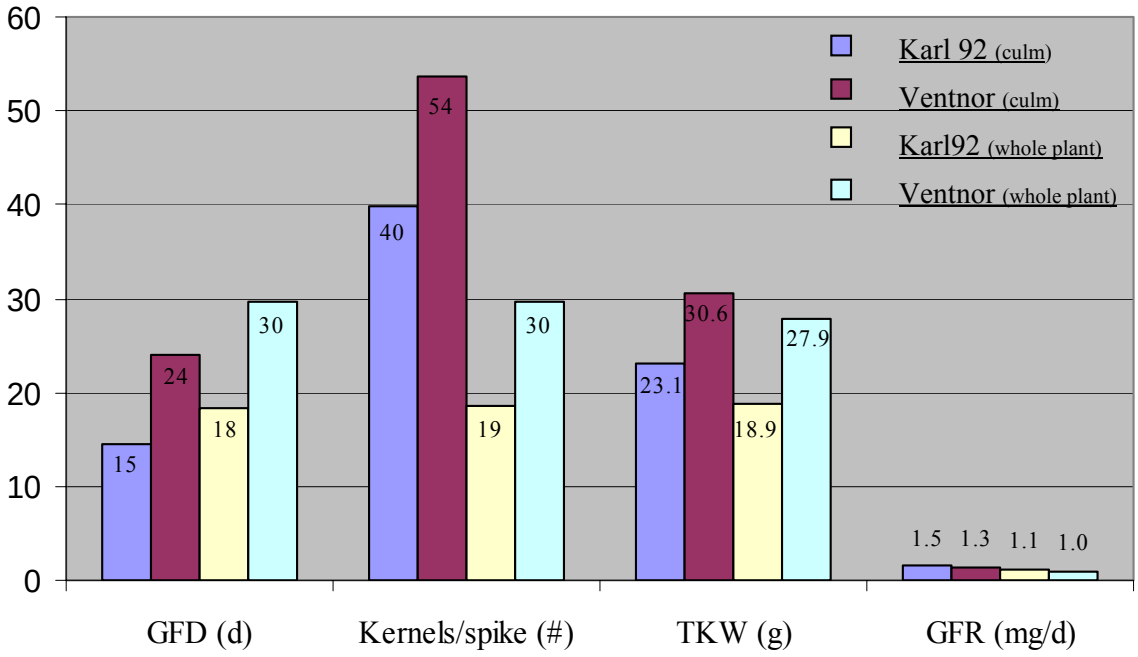


Figure 2-2. Comparison single culms and whole plants of Karl 92 and Ventnor, for the traits measured under high temperature. Grain filling duration (GFD), kernels per spike, thousand kernel weight (TKW), and grain filling rate (GFR) are represented on x-axis, and their respective units on y-axis. The mean for the traits under either situation are provided in the respective histogram bar. The least significant differences (LSDs) for GFD, kernels per spike, TKW and GFR were 4 d, 7, 2 g, and 0.2 mg/d respectively.

Table 2-1. Correlation of yield components, mainly grain filling duration (GFD), kernels per spike, thousand kernel weight (TKW), and grain filling rate (GFR) between single culms and whole plants under high temperature. GFD and TKW had high correlation among single culms and whole plants.

Trait	r^2	Probe F
GFD (d)	0.919	0.0012***
Kernels/spike (#)	0.266	0.521^{NS}
TKW (g)	0.929	0.0008***
GFR (mg/d)	0.302	0.465^{NS}

*** significant at $\alpha = 0.001$, NS = non significant.

Analysis of variance and means for yield traits and rate of senescence

Analysis of variance on the RILs indicated significant differences between the optimum and high temperature regimens for all traits (Table 2). The RILs differed from each other significantly at $\alpha = 0.001$ for all traits. The treatment X entry interaction was non-significant for kernels per spike, indicating that there was little effect on the performance of the lines under different temperature regimen. In the case of GFD, TKW, GFR and maximum rate of senescence, the treatment X entry interactions were significant at an $\alpha < 0.01$. The RILs differed significantly in performance within each treatment for all the traits except for GFR. Interaction plots for GFD, TKW, and maximum rate of senescence showed a non-crossover interaction (Figure 3), indicating that the lines which performed well under optimum conditions were also better performers under high temperature. From the analysis of variance, the heritability of kernels per spike was high at 75.3%, intermediate for GFD and TKW at 59.5% and 56.4%, respectively, and low for GFR and maximum rate of senescence at 42% for each trait.

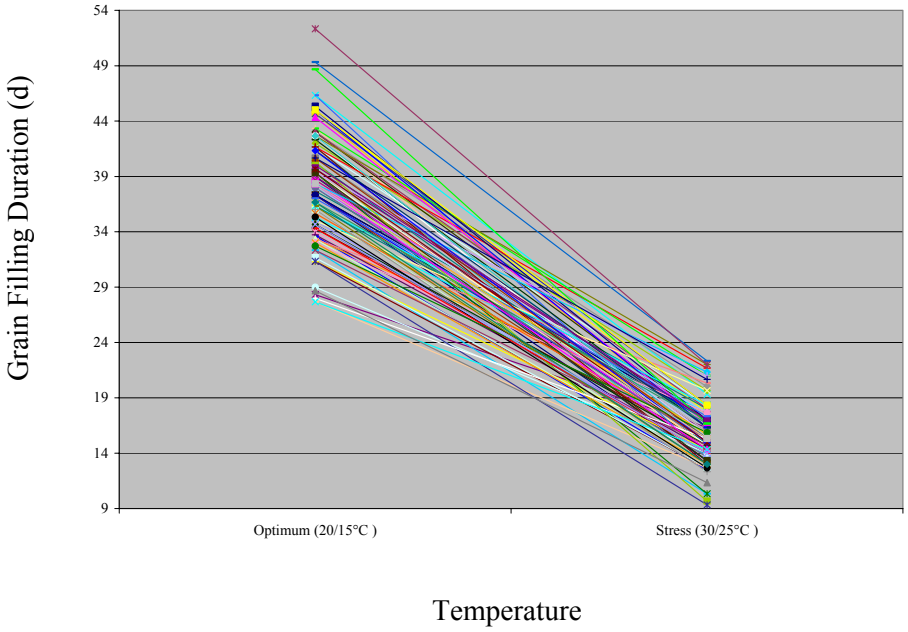
Means of RILs under optimum conditions (Table 3) were 38.3 d GFD, 60.2 kernels per spike, and 43.9 g TKW, which were with-in the range of the values of the parents. Values for GFD, kernels per spike, and TKW were higher in Ventnor than Karl 92, while the mean values for GFR in Ventnor, Karl 92, and the population were same. The mean rate of senescence was higher for the RIL population than either parent due to transgressive segregation of some lines in the population. Estimated population means for GFD, TKW, GFR, and maximum rate of senescence under high temperature were 15.8 d, 25.3 g, 1.2 mg/d and 17.3 respectively. These means were with-in the range of parental means (Table 4).

Three-dimensional plots between GFD, TKW and kernels per spike under high temperature had

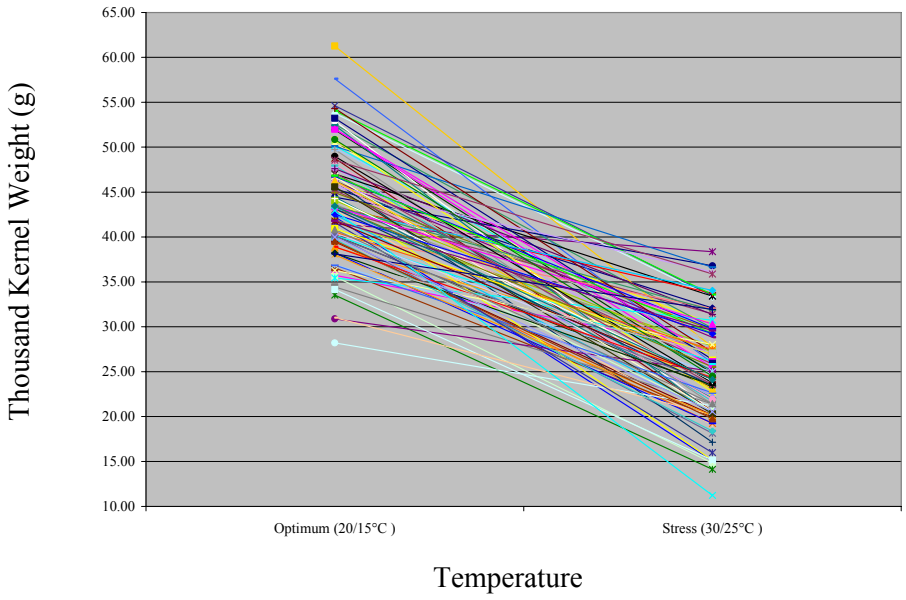
Table 2-2. Analysis of variance for grain filling duration (GFD), kernels per spike, thousand kernel weight (TKW), grain filling rate (GFR), and maximum rate of senescence (Max Sen.) in the RIL population derived from Ventnor X Karl 92 cross.

Effect	DF	GFD (d)	Kernels/spike (#)	TKW (g)	GFR (mg/d)	Max Sen.
Treatment (Optimum vs. Stress)	1	75571.744***	2547.030***	52325.167 ***	27.217***	601.416***
Replicates	2	116.840***	692.910***	1228.959***	4.050***	98.556 ^{NS}
Entry (RILs)	103	64.321***	529.733***	128.618 ***	0.203***	76.487***
Treatment*Entry	103	26.647***	82.226^{NS}	66.166 ***	0.123**	144.862 ***
Optimum	103	69.430***		106.325***	0.086^{NS}	22.026***
Stress	103	21.318***		89.955***	0.235***	300.467**
Error	404	17.060	91.108	33.199	0.080	97.046

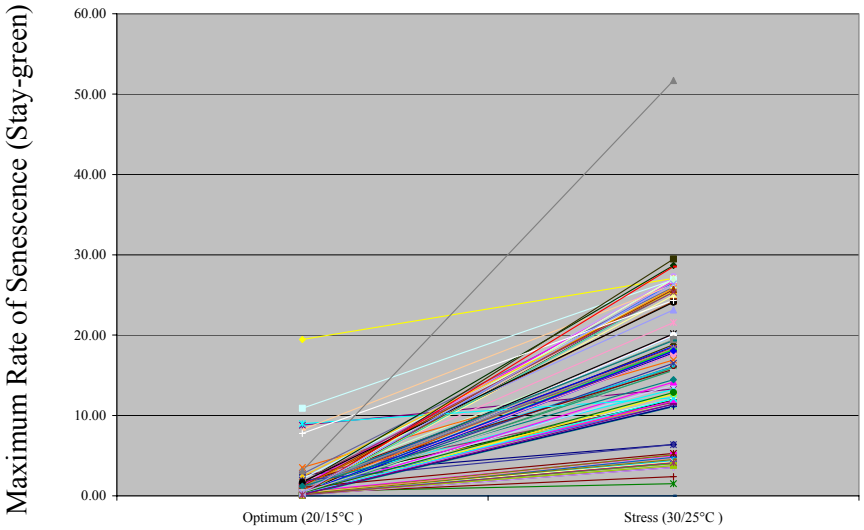
***, ** significant at $\alpha = 0.001$, and 0.01 respectively. NS = non significant.



a) Interaction plot of the performance of RILs for grain filling duration under optimum and heat stress conditions.



b) Interaction plot of the performance of RILs for thousand kernel weight under optimum and heat stress conditions.



c) Interaction plot of the performance of RILs for maximum rate of senescence under optimum and heat stress conditions.

Figure 2-3. Interaction plots (a), (b), and (c) showing a non-crossover, orderly interaction among the RILs for grain filling rate (GFD), thousand kernel weight (TKW), and maximum rate of senescence under optimum and high-temperature conditions.

Table 2-3. Mean grain filling duration (GFD), kernels per spike, thousand kernel weight (TKW), grain filling rate (GFR), and maximum rate of senescence (Max Sen.) of the RIL population and parents under optimum conditions.

Entry	Trait	Mean	Std. Dev.	Std.Er.	Minimum	Maximum	Range
RILs	GFD (d)	38.3	6.45	0.37	18.00	56.00	38.00
	Kernels/spike (#)	60.2	12.66	0.73	26.00	97.00	71.00
	TKW (g)	43.9	7.99	0.46	17.29	68.10	50.81
	GFR (mg/d)	1.6	0.07	0.01	0.42	3.47	3.05
	Max Sen.	1.3	3.50	0.20	0.04	23.82	23.78
PARENTS							
Ventnor	GFD (d)	52.3	3.22	1.86	50.00	56.00	6.00
	Kernels/spike (#)	72.0	9.00	5.20	63.00	81.00	18.00
	TKW (g)	48.6	4.07	2.35	44.17	52.22	8.06
	GFR (mg/d)	1.6	0.01	0.01	1.45	1.88	0.43
	Max Sen.	0.1	0.10	0.06	0.07	0.24	0.17
Karl92	GFD (d)	34.7	2.31	1.33	32.00	36.00	4.00
	Kernels/spike (#)	63.7	14.30	8.25	48.00	76.00	28.00

TKW (g)	40.0	4.04	2.33	35.33	42.52	7.20
GFR (mg/d)	1.5	0.02	0.01	1.29	1.73	0.44
Max Sen.	0.4	0.46	0.27	0.05	0.91	0.85

Table 2-4. Mean grain filling duration (GFD), kernels per spike, thousand kernel weight (TKW), grain filling rate (GFR), and maximum rate of senescence (Max Sen.) of the RIL population and parents under high-temperature.

Entry	Trait	Mean	Std. Dev.	Std.Er.	Minimum	Maximum	Range
RILs	GFD (d)	15.8	3.56	0.20	5.00	25.00	20.00
	Kernels/spike (#)	56.1	13.13	0.75	8.00	87.00	79.00
	TKW (g)	25.3	7.41	0.20	6.75	44.67	20.00
	GFR (mg/d)	1.2	0.04	0.00	0.58	2.47	1.90
	Max Sen.	17.3	15.00	0.85	0.07	62.54	62.47
PARENTS							
Ventnor	GFD (d)	22.0	1.73	1.00	21.00	24.00	3.00
	Kernels/spike (#)	69.0	4.58	2.65	65.00	74.00	9.00
	TKW (g)	36.0	3.13	1.81	33.60	39.54	5.94
	GFR (mg/d)	0.9	0.01	0.01	0.87	0.99	0.12
	Max Sen.	1.3	0.55	0.32	0.81	1.87	1.06
Karl92	GFD (d)	14.0	0.00	0.00	14.00	14.00	0.00
	Kernels/spike (#)	64.7	8.50	4.91	56.00	73.00	17.00

TKW (g)	20.5	3.23	1.86	18.05	24.15	6.10
GFR (mg/d)	1.2	0.02	0.01	1.10	1.18	0.08
Max sen.	23.5	0.49	0.28	22.93	23.84	0.91

normal distribution, indicating polygenic inheritance. The RILs showed transgressive segregation, suggesting that some genes contributing to each trait were provided by Ventnor and Karl 92 (Figure 4). Each arrow in the plot represents inbred line in the three dimensional space with GFD, TKW, and kernels per spike on x, y, and z-axis.

Heat susceptibility index estimates of GFD and TKW

The major agronomic traits affected by post-anthesis high temperature were GFD and TKW (Stone and Nicolas, 1995). The mean values of these traits under optimum and heat stress conditions were used to estimate the heat susceptibility index (HSI) (Table 5). The HSI ranged from 0.72 to 1.29 for GFD and 0.18 to 1.80 for TKW. According to the classification of Khanna-Chopra and Viswanathan (1999), the RILs can be broadly divided into two categories: moderately tolerant (HSI > 0.500 to ≤ 1.000) and susceptible (HSI > 1.000) for GFD and TKW. Under heat stress conditions, Ventnor was among the top nine lines for GFD, kernels per spike, and TKW, while Karl 92 was in the lower quartile for these traits. Though Ventnor's performance was better than Karl 92 under both optimum and heat-stress conditions, the HSI estimated for GFD and TKW for Ventnor was 0.99 and 0.60, respectively. Since the HSI is a ratio between optimum and heat-stress condition, higher trait values under both conditions make the ratio greater.

Correlations among yield traits and rate of senescence

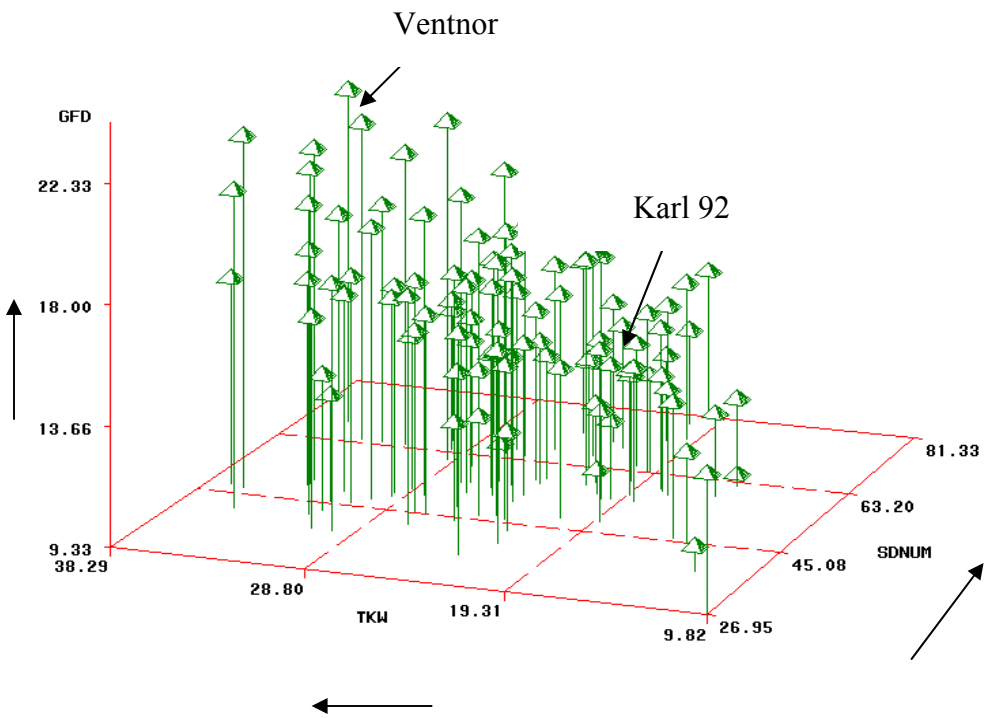


Figure 2-4. Three dimensional plot with thousand kernel weight (TKW) on x-axis, grain filling duration (GFD) on y-axis, and kernels per spike on z-axis in response to heat stress for the RILs. Each arrow in the plot represents inbred line in the three dimensional space. RIL population showed normal distribution for the traits, with Ventnor in upper and Karl 92 in lower quartile, and a transgressive segregation.

Table 2-5. Heat susceptibility indices (HSI) for grain filling duration (GFD) and thousand kernel weight (TKW) to estimate the relative performance of the RILs and their parents. The inbred lines having lower HSI for GFD and TKW than Ventnor, show transgressive segregation.

Entry	GFD	TKW	Entry	GFD	TKW
8	0.72	0.18	11	0.89	1.08
84	0.84	0.37	20	1.07	1.08
169	0.84	0.37	154	0.77	1.08
61	0.93	0.38	17	1.06	1.10
88	0.97	0.43	180	1.03	1.12
73	0.66	0.48	52	1.00	1.12
98	0.75	0.49	81	1.06	1.13
50	0.82	0.52	127	1.03	1.13
111	0.79	0.52	Karl92	1.01	1.14
4	0.95	0.52	67	1.02	1.14
103	0.75	0.53	9	0.96	1.15
Ventnor	0.99	0.60	150	0.93	1.16
137	0.93	0.63	125	1.01	1.16
74	0.93	0.64	101	1.04	1.17
68	0.95	0.66	148	1.06	1.17
45	0.93	0.66	80	1.06	1.18
178	1.02	0.66	92	1.17	1.18
171	0.98	0.69	56	1.04	1.19
2	1.09	0.70	94	0.91	1.19
30	0.80	0.71	168	0.88	1.20
162	1.07	0.71	13	1.16	1.20
159	1.08	0.74	37	1.04	1.21
70	0.88	0.75	153	1.09	1.21
57	0.85	0.76	31	1.02	1.22
128	0.97	0.78	109	0.79	1.24

6	1.03	0.78
72	0.98	0.79
28	0.90	0.80
161	1.01	0.81
117	0.92	0.81
62	0.82	0.82
108	0.91	0.83
96	1.08	0.83
66	1.02	0.83
158	1.12	0.87
119	0.90	0.87
51	0.88	0.88
33	0.91	0.88
121	1.29	0.88
63	1.00	0.89
14	0.95	0.90
157	1.01	0.91
44	1.00	0.92
7	0.99	0.93
141	0.98	0.94
65	1.01	0.94
76	1.06	0.95
129	1.03	0.95
170	1.09	0.98
41	0.99	0.99
38	1.03	1.00
174	1.10	1.02
35	1.03	1.02
18	0.99	1.04
167	1.06	1.04
136	1.03	1.06
40	1.05	1.07
19	0.98	1.07
24	1.14	1.26
120	1.09	1.27
23	1.20	1.27
29	1.08	1.28
32	1.03	1.29
82	1.10	1.30
69	1.05	1.31
100	1.02	1.31
143	1.12	1.32
22	1.14	1.33
130	1.12	1.33
59	1.01	1.35
16	1.10	1.40
64	1.07	1.41
46	1.12	1.44
152	1.19	1.45
34	1.12	1.44
60	1.25	1.47
12	1.07	1.48
53	0.92	1.55
163	0.82	1.80
Mean HSI	1.00	1.00

A significant and positive correlation of $\alpha = 0.001$ and 0.01 was observed between GFD and TKW, and between TKW and GFR, respectively, indicating that increased GFD and GFR increases TKW (Table 6). A significant negative correlation of $\alpha = 0.01$ between GFD and GFR, and of $\alpha = 0.001$ for maximum rate of senescence with GFD and TKW indicated that more rapid senescence is associated with decreased GFD. Decreased grain filling period lowered accumulation of photosynthetic assimilates. This in turn resulted in lower kernel weight.

Principle component analysis on yield traits and rate of senescence

To ascertain if the trait responses that were correlated can be reduced to a few principle components, principle component analysis (PCA) was performed on the least square means obtained for yield-related and physiological traits (Tables 7 and 8). The first three principle components accounted for 98% of total variability among the RILs for heat tolerance. The top two principle components accounted for 87% of total variability. Of the first three principle components, Principle Component 1 had a high correlation with kernels per spike and maximum rate of senescence; Principle Component 2 had highest positive correlation with kernels per spike and TKW and highly significant but negative correlation with maximum rate of senescence; and Principle Component 3 had the highest positive correlation with TKW.

Discussion

Responses of the whole plants and single culms under high temperature were comparable for

Table 2-6. Pearson's correlation coefficients among the grain filling duration (GFD), kernels per spike, thousand kernel weight (TKW), grain filling rate (GFR), and maximum rate of senescence (Max Sen.) under high temperature.

	GFD (d)	Kernel/spike (#)	TKW (g)	GFR (mg/d)	Max Sen.
GFD (d)	1.000	-0.136^{NS}	0.657***	-0.258**	-0.419***
Kernels/spike (#)		1.000	-0.053^{NS}	0.661***	0.197^{NS}
TKW (g)			1.000	0.331**	-0.414***
GFR (mg/d)				1.000	0.012^{NS}
Max Sen.					1.000

***, **, * significant at $\alpha = 0.001$, 0.01 and 0.05 respectively. NS = non significant.

Table 2-7. Principle component analysis (PCA) for grain filling duration (GFD), kernels per spike, thousand kernel weight (TKW), grain filling rate (GFR), and maximum rate of senescence (Max Sen.) measured under high temperature stress. The five principle components along with their Eigen values and cumulative variability showed the first three principle components accounted 98% of variability among the RILs for heat tolerance.

Principle Component	Eigen Value	Cumulative Variability
1	117.445	0.534
2	73.862	0.870
3	24.720	0.983
4	3.653	1.000

Table 2-8. Correlation among the principle components (Prin. 1, 2, 3, and 4) and grain filling duration (GFD), kernels per spike, thousand kernel weight (TKW), grain filling duration (GFR), and maximum rate of senescence (Max Sen.) measured under high temperature. Principle components 1, 2, and 3 showed highest association with kernels per spike, maximum rate of senescence, and TKW, respectively.

Traits	Prin. 1	Prin. 2	Prin. 3	Prin. 4
GFD	-0.083	0.147	0.272	0.947
Kernels/spike	0.889	0.455	-0.047	0.021
TKW	-0.137	0.372	0.861	-0.318
GFR	0.001	0.001	0.001	-0.009
Max Sen.	0.428	-0.796	0.426	0.038

GFD, kernels per spike, TKW, and GFR. Though GFR was not different in the parents, it followed the same trend under either situation. The GFD and TKW were correlated between single culms and whole plants. This was in accordance to the reports of Ford et al. (1976); Bhullar and Jenner (1983); and Bhullar and Jenner (1986), who suggested that high temperature stress had a direct effect on the developing kernels rather than an indirect effect through the remaining shoots. Due to the reduction in tiller number, the kernels in the terminal regions of the spike had a better chance of survival due to increased availability of nutrients to a single spike (Duggan et al., 2000). This resulted in non-significant correlations between single culms and whole plants for kernels per spike and GFR.

Previous studies found that Ventnor was photosynthetically more stable over other genotypes under high temperature stress and depended on current assimilates for grain growth (Al-Khatib and Paulsen, 1990; Yang et al., 2002a). Similar results were observed in this experiment. Maximum rate of senescence was very low in Ventnor compared to Karl 92. Genotypes with good tolerance to high temperature have a stable or long duration of photosynthetic activity (Al-Khatib and Paulsen, 1990). Increased green leaf area duration is associated with increased photosynthetic activity. Increased duration of synthesis of assimilates and the transport of assimilates to the kernel resulted in increased TKW.

The populations were advanced without selection to avoid favoring one genotype over another. The values for GFD, kernels per spike, TKW, and HSI for GFD and TKW exhibited transgressive segregation, which agreed with previous research (Yadav et al., 1998; Wu et al., 2003). The RIL population followed a normal distribution, indicating that tolerance to high temperature stress is a quantitative trait, as suggested by the earlier research of Shah et al. (1999).

The means of traits in the RIL population were with-in the range of the parental means, except for maximum rate of senescence, which had higher mean values than the parents under optimum conditions, and kernels per spike, which was lower than either parent under heat stress. Ventnor and some of the heat-tolerant lines had high TKW and kernels per spike under high temperature, but most lines that had high kernels per spike had relatively low TKW and vice versa. A kernel weight to kernel number compensation took place for efficient channeling of assimilates between the source and the sink (Davidonis et al., 2005; Shahinnia et al., 2005). It is possible that different sets of alleles were activated for kernels per spike and TKW under optimum and high temperature.

The post-anthesis stage is highly sensitive to high temperature. High temperature stress imposed at 10 DAA, decreased kernel mass rather than kernel number (Stone and Nicolas, 1995b). By 7 DAA, the kernel number is established, leaving the subsequent processes of cell enlargement and starch deposition to be affected by heat. Kernel weight is most affected by heat stress early in the grain-filling period and becomes less sensitive as the period progresses. According to Stone and Nicolas (1995b), the reduction in kernel weight is due to the shorter GFD rather than GFR.

It was observed that high temperature stress applied 10 DAA reduced performance of all agronomic and physiological traits. There was no treatment X entry interaction for kernels per spike and GFR, indicating the relative rank of the RILs was essentially the same in both temperature regimes. Due to the treatment x entry interaction for GFD, TKW, and maximum rate of senescence, there was a change in the relative ranking of the RILs. These traits were highly affected by heat stress after the kernel number was established. This observation was similar to that reported by Stone and Nicolas (1995b).

The GFD was positively correlated with TKW and negatively with GFR and maximum rate of senescence under heat stress, indicating that GFD had a positive influence on the former and a negative influence on the latter two traits, as reported by earlier researchers (Lu et al., 1989). Accelerated senescence causes cessation of vegetative and reproductive growth, deterioration of photosynthetic activities, and degradation of proteinaceous constituents (Al-Khatib and Paulsen, 1984). Grain filling rate was positively and significantly correlated with TKW, indicating that increased GFR is needed to increase kernel weight as suggested by Wardlaw and Moncur (1995). The negative correlation of GFR with GFD was consistent with earlier reports (Jenner, 1994; Stone and Nicolas, 1995a). Shortening GFD resulted in faster mobilization of assimilates and stored resources, causing an increase in the GFR. However, an overall increase in the GFR under stress conditions was not able to compensate for the shorter GFD. This may be due to decreased activity of starch synthase, which converts sucrose to starch in the grain and is highly heat labile. This would result in lower grain weight, as suggested by Bhullar and Jenner (1985) and Keeling et al. (1993).

Principle Components 1, 2 and 3 accounted for 98% total variability among the RILs for heat tolerance. Significant correlations of the principle components to traits were observed. Kernels per spike and TKW, correlated significantly with Principle Component 1 and 3, respectively, and maximum rate of senescence had a negative and significant correlation with Principle Component 2.

The RILs that had low to moderate rates of senescence usually had better kernel weight than those with high rates of senescence. The lines that had maximum rates of senescence equivalent to, or less than, Ventnor had kernel weight similar to, or higher than, Ventnor. There were exceptions, as some moderately tolerant lines defined by HSI, had higher senescence rates,

indicating those plants either depend on stored reserves, or as reported by Wardlaw and Moncur (1995); and Zahedi et al. (2003) had very efficient GFR coupled with efficient starch synthase to convert the sucrose into starch.

Susceptible lines with relatively low rates of senescence and low kernel weight might have had a highly sensitive soluble starch synthase (Zahedi et al., 2003). Above all, the genetic potential of the line is an important factor in determining the yield of a plant. Heritability is the direct estimate of the genetic contribution of the genotype to the trait. Yang et al. (2002b) estimated broad sense heritability for GFD under controlled conditions in an F_3 population derived from a cross between Ventnor and Karl 92 at 80%. In the present experiment, broad sense heritability for GFD in the same population in the $F_{6:7}$ RILs was estimated at 60%. High heritability of 75% was found for kernels per spike and an intermediate heritability of 56% for TKW. According to a report by Mohammadi et al. (2004), GFD had higher heritability than TKW in a spring wheat population under heat stress. Higher heritability ensures significant success in transfer of the genes to successive generations.

It can be concluded that genotypes having longer grain filling periods, productive tillers, lower rates of senescence, higher GFRs and efficient soluble starch synthase are ideal for stable yield under high temperature. Thousand kernel weight and kernels per spike are two important components of yield, with TKW being directly influenced by grain-filling duration. If post-anthesis stress is imposed after the kernels per spike are nearly fixed, selection for heat-tolerant genotypes can be based on GFD and TKW. If selections are made prior to this stage or under variable environmental conditions, kernels per spike can also be taken into consideration as a criteria for selection.

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CHAPTER 3 - QTL Mapping for Traits Linked to High-Temperature Tolerance in a Recombinant Inbred Winter Wheat Population

Abstract

High-temperature stress is one of the major constrains to wheat production world wide. Post-anthesis heat stress results in enormous reduction in the grain yield of wheat. Breeding for cultivars that are tolerant to post-anthesis high temperature stress is an effective strategy to overcome this problem. To hasten this process, traditional breeding, along with molecular markers, can be used. The objective of the experiment was to identify and map quantitative trait loci (QTL) linked to heat tolerance and establish their relationship with the stay-green trait of leaves. For this purpose, a recombinant inbred line (RIL) population was developed from a cross between heat-tolerant and relatively heat-susceptible cultivars, Ventnor and Karl 92, respectively. The population was characterized under controlled conditions for agronomic traits including grain filling duration (GFD), kernels per spike, thousand kernel weight (TKW), grain filling rate (GFR), and maximum rate of senescence to estimate the stay-greenness of the lines. Molecular markers, mainly microsatellites (SSR), amplified fragment length polymorphisms (AFLPs) and a sequence tag site (STS) were used to construct a linkage map. Composite interval mapping was used to identify QTL associated with agronomic traits under temperature stress. Results indicated the presence of heat tolerance QTL mainly on chromosomes 2, 4, and 6 of the A genome. Homoeologous group 2 chromosomes had QTL for all yield and stay-green trait

studied. Microsatellite marker *Xgwm356* was linked to kernels per spike, *Xksum20* to TKW explained 17.3% and 15.5% of the variability respectively, and *Xksum61* explained 12.9% of the variability for GFR. An AFLP marker, AGG.CTT-107 (P41/M62-107), on chromosome 2A was linked to GFD and maximum rate of senescence under heat stress, suggesting an association between yield traits and stay-green. Comparative mapping analysis of marker *Xgwm296* and *Xgwm356* on chromosome 2A, which were linked to yield traits, showed a synteny with the loci linked to QTL for yield in rice (*Oryza sativa* L.) and maize (*Zea mays* L.). Co-localization of stay-green and yield traits points to the strong association of these two traits. The SSR makers, especially *Xgwm296*, *Xgwm356*, *Xksum61*, and AGG.CTT-107 (P41/M62-107) which were linked to yield and stay-green, can be used in marker-assisted breeding programs.

Introduction

Wheat is a major cereal crop, supplying nearly 55% of the carbohydrates consumed world-wide (Gupta et al., 1999). It is an allohexapliod (2n = 6x = 42) with three genomes A, B, and D and an extremely large genome of 16 x 10^9 bp/1C (Bennett and Smith, 1976). A basic Triticeae genome contains over 90% repetitive DNA and genes constitute less than 3% of the genome (Li et al. 2004). However, up to 85% of the wheat genes are present in 10% of the entire genome on "gene islands" in distal chromosomal regions with high rates of recombination (Gill et al., 1996a; Gill et al., 1996b; Sandhu et al., 2001; Shah and Gill, 2001; Brook et al., 2002). Mapping for yield and yield-related agronomic traits is very important for modern day breeding, as it would aid marker-assisted breeding. It would also assist germplasm characterization, and

varietal development. Molecular markers are especially useful for breeders in selecting quantitative trait loci (QTL), where a trait has polygenic inheritance with variable heritability and needs to be selected in variable environments over generations. Extensive genetic maps using molecular markers were initially reported in tomato (*Lycopersicon esculentum* Mill.) by Tanksley et al. (1992), and maize (*Zea mays* L.) by Helentjaris et al. (1986). In wheat genetic linkage (Nelson et al., 1995a, b, c; Van Deynze et al., 1995; Marino et al., 1996) and physical maps (Delaney et al., 1995a, b; Mickelson-Yong et al., 1995; Gill et al., 1996a) using RFLP markers have been constructed for all seven homoeologous chromosomes. More recently deletion bin maps of ESTs anchored to the sequenced genome of rice have been constructed (Sorrells et al., 2003). RFLPs have low rates of polymorphism. Alternately, microsatellites, also called simple sequence repeats (SSRs) and AFLP markers, were found to be multiallelic, with higher rate of polymorphism (Penner et al., 1998; Korzun et al., 1999), high density (Myburg et al. 2002), and ubiquitous distribution. These markers are useful for genetic analysis of species, such as hexaploid wheat, with a narrow genetic base due to their recent origin (Powell et al., 1996; Röder et al., 1998).

Maps for identifying yield and yield-related QTL have been constructed in different species of the Poaceae family. In sorghum (*Sorghum bicolor* L. Moench), QTL linked to yield and seed weight under drought conditions were detected on linkage group F (Tuinstra et al., 1998). In maize, Ribaut et al. (1997) found QTL linked to grain yield on chromosomes 1 and 10, and in rice on chromosomes 3, 4, and 8 (Lanceras et al., 2004).

Several important qualitative and quantitative traits have been mapped in wheat. These include grain protein content (Uauy et al., 2006), preharvest sprouting tolerance (Anderson et al., 1993), kernel hardness (Sourdille et al., 1996), and amylose content (Araki et al., 1999). A mapping

study of yield and thousand kernel weight (TKW) by Groos et al. (2003) found a single QTL for yield on chromosome 7D and three QTL for TKW on chromosomes 2B, 5B, and 7A. In another detailed study, Börner et al. (2002) found 64 major QTL linked to morphologic, agronomic, and disease resistance traits. Quarrie et al. (2005), conducted a study looking for QTL over 24 site x treatment x year combinations that included nutrient stress, drought stress and salt stress. They found 17 clusters for yield QTL distributed around the genome with the strongest yield QTL effects on chromosomes 7AL and 7BL and two additional yield QTL on chromosomes 1DS/L and 5AS.

Grain yield has been shown to have a positive association with a low rate of leaf senescence. Four functional stay-green mutants with delayed leaf senescence were found in durum wheat (*Triticum turgidum durum* Desf. Husn) (Spano et al., 2003). These mutants had a longer photosynthetic competence, higher kernel weight and greater grain yield than the parents. In a winter wheat population, QTL linked to grain yield and green flag leaf area at 14 and 35 days after anthesis were located on the long arm of chromosome 2D between the interval *Xgwm 311* and *Xgwm 382*, and at loci *Xgwm 539* and *Xgwm 30* under unirrigated conditions (Verma et al., 2004). In sorghum, stay-green QTL located on linkage groups F and I had a strong pleiotropic effect on yield, and one on linkage group G had a weak effect. The QTL on linkage group H showed an association between stay-green and grain development, in which the stay-green trait was associated with a low rate of grain filling (Tuinstra et al., 1997; Tuinstra et al., 1998*).*

Few QTL mapping studies of heat tolerance have been conducted in wheat and other genomes. In the horticultural crop Chinese cabbage (*Brassica campestris* L. ssp. *Pekinensis*), 9 molecular markers linked with heat tolerance QTL were detected using single marker analysis in a RIL population (Zheng et al., 2004). Molecular mapping for heat tolerance genes in maize is

underway (Lee et al., 2001). In a spring wheat population, QTL for heat tolerance under hot and dry conditions were detected on chromosomes 2B and 5B (Byrne et al., 2002). Esten and Hays (2005) have initiated a project to integrate genotypic (QTL), phenotypic and transcript level data to identify genes controlling reproductive stage heat tolerance in a RIL population of spring wheat derived by crossing heat tolerant Halberd with the heat susceptible winter wheat Cutter. Evaluating for heat tolerance using single marker analysis in a winter wheat population, Yang et al. (2002b) found QTL linked to GFD on the short arms of chromosomes 1B and 5A. To date, interval mapping for heat tolerance in winter wheat has not been reported. The objective of this study was to characterize and map QTL linked to high-temperature tolerance and to establish a relationship between loci that were linked to yield and stay-green traits in winter wheat.

Materials and Methods

Population development

A recombinant inbred line (RIL) population in a filial (F_7) generation was derived from a cross between Ventnor (a heat-tolerant hard white Australian winter wheat) and Karl 92 (a hard red winter wheat from the USA). The seed for each of the RILs was germinated. After the seedlings reached 2.5 cm, they were vernalized at 5°C for 6 weeks. The vernalized seedlings were transplanted to pots (10x25-cm). Each pot contained one plant. The plants were grown as single culms under controlled conditions. The optimum temperature chambers were set at 20/15°C (day/night), 50/70% relative humidity, 16-h photoperiod, and light intensity of 420 μmol m^{-2} s^{-1} as suggested by Yang et al. (2002a). Spikes were labeled when 50% of them reached

anthesis. At 10 d after anthesis (DAA), the replicates of RILs were transferred to high temperature chambers and maintained in the same condition until harvest. The high temperature chambers were set at 30/25°C with all other conditions remaining the same as the optimum chambers. Peters professional fertilizer was applied to each pot once every month to supply 100 mg N, 43 mg P, and 87 mg K (Peters Professional Plant Food, W.R. Grace & Co., Fogelsville, PA). Plants were watered and randomized regularly. Three replicates of the inbred lines were studied in sequential order, and the experimental design used was a split plot. The experiment was blocked on time with growth chambers as the experimental units for temperature and pots as the experimental units for the RILs.

Traits studied for mapping

The traits measured for mapping were grain filling duration (GFD), kernels per spike, thousand kernel weight (TKW), grain filling rate (GFR), and green leaf area duration (stay-green). The GFD was estimated as the interval, in days, from 10 DAA to physiological maturity. After harvesting each of the RIL separately, kernels per spike were counted using a seed counter (SEEDBURO, 801 COUT-A-PAK, Chicago, IL) and weighed on sensitive electronic balance (A-160, Denver Instrument Company, Denver, CO.). The weight was extrapolated to get an estimate for TKW. The GFR was estimated as the ratio between kernel weight and GFD. Stay-green was measured on a visual scale of 0 to 10, and the maximum rate of senescence was estimated using a Gompertz growth curve statistical model (Seber and Wild, 1989). The growth curve model used to estimate maximum rate of senescence was:

$$Y = \alpha \{ 1- e^{-e^{[-b (time - c)]}} \}$$

Where Y is the response variable, α relates to the point where plants leaves are completely green, b relates to the degree of curvature of the curve between time to senescence and visual scale for green leaf area duration, and c relates to the point of time of maximum senescence. The maximum rate of senescence was estimated as the mid-point of the curve with maximum slope.

Molecular markers and map development

The DNA was extracted from RILs and the two parents using the CTAB extraction method (Saghai-Maroof et al., 1984). Molecular markers, mainly SSRs, AFLPs, and primers designed for STS that corresponded to the chloroplast elongation factor (EF-Tu) expressed under heat stress in maize, were used to construct a genetic linkage map. Microsatellites markers are tandem repeats of oligonulceotides like $(GA)_n$ or $(GT)_n$. The markers used in the experiment were comprised of GWM (Röder et al., 1998), BARC (Song et al., 2002), CFD and CFA (Sourdille et al., 2001), WMC (Gupta et al., 2002), GDM (Pestsova et al., 2000), and KSUM and KSM (Singh et al., 2000) primer sets.

The polymerase chain reaction (PCR) amplifications were done in an MJResearch Thermal Cycler (Bio-Rad formerly MJ Research, Hercules, CA). The PCR reaction mixture used for BARC markers had a total volume of 25 µL consisting of 150 ng genomic DNA, 2.6 µL 10X PCR buffer, 2.48 mM $MgCl_2$, 0.24 mM dNTPs, 1 unit *Taq* DNA polymerase and 20 ng primer (forward + reverse). The thermocycler program consisted of an initial denaturation step at 95°C for 3 min, followed by 34 cycles of 40 sec at 94°C, 40 sec at annealing temperature, and 1 min at

72°C followed by final extension temperature at 72°C for 10 min. For all other markers, the PCR reaction mixture contained a total 25 µL consisting of 150 ng genomic DNA, 2.5 µL 10X PCR buffer, 1.5 mM $MgCl_2$, 0.30 mM dNTPs, 1 unit *Taq* DNA polymerase, and 20 ng primer (forward + reverse). The program used consisted of an initial denaturation at 95°C for 3 min., and initial two cycles at an annealing temperature of 62°C, followed by 34 cycles of 1 min at 94°C, 1 min at annealing temperature and 2 min at 72°C followed by final extension at 72°C for 10 min. The SSR markers were run on either a 3% SFR agarose (Midwest Scientific, St. Louis, MO) gel at 70 volts and visualized by staining with ethidium bromide under ultra violet illumination or run at 80 watts on a 6% polyacrylamide denaturing gel modified with formamide solution and urea to enhance the resolution of the markers. The denaturing gel mixture contained 15 mL double-distilled water, 32 mL formamide, 33.6 g urea, 10 mL 10X Tris-boric acid-disodium ethylenediaminetetraacetic acid (TBE) solution, 17.5 mL Bis/acrylamide (19:1 polyacrylamide, 40%, ISC BioExpress). 1 mL 10% ammonium persulfate, and 40 µL N,N,N',N'-Tetramethylethylenediamine (TEMED) were mixed with the gel mix prior to pouring the gel to drive polyacrylamide polymerization. The gel was cast and run on a BioRad Sequi-Gen GT sequencing system (Bio-Rad, Hercules, CA) for 2 h after an initial pre-run of 20 min, and the bands were visualized by silver staining (Bassam et al., 1991). For the STS marker, the PCR reaction mixture and thermocycler program were the same as that for the BARC markers. The STS marker were run on a single stand conformational polymorphism (SSCP) gel at 3 watts for about 14 h (Martins-Lopes et al., 2001), and bands were visualized by silver staining. All the markers were scored as parental type (A or B), heterozygote (H), or missing data (-).

The AFLP reactions and DNA template preparation for *PstI/MseI* fragments were as described by Vos et al. (1995) with some modifications. In brief, *PstI* (six-base cutter methylation

sensitive) and *MseI* (four-base cutter) enzymes were added to 300 ng genomic DNA and incubated for 2 h at 37°C. The enzymes were subsequently heat inactivated at 70°C for 15 min. This was followed by the ligation of *PstI* (adapter 1, 5'-CTCGTAGACTGCGTACATGCA-'3; adapter 2, 5' -TGTACGCAGTCTAC-'3) and *MseI* (adapter1, 5' -GACGATGAGTCCTGAG-'3; adapter 2, 5'-TACTCAGGACTCAT- '3) overnight at 20°C. The samples were diluted 10-fold with distilled water. Pre-amplification of the diluted DNA template was then performed with AFLP primers having 0 selective nucleotides for *PstI* + 0 (5' –GACTGCGTACATGCAG- '3) in combination with *MseI* +0 (5' -GATGAGTCCTGAGTAA- '3). The PCR reaction mixture was diluted to 40 µL consisting of 10 µL diluted DNA template, 1x PCR buffer, 0.75 units *Taq* polymerase (Promega Corp., Madison, WI), 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.75 µL 100 ng/µl *PstI* pre-amplification primer (5' –GACTGCGTACATGCAG- '3), and 0.75µL 100 ng/µl *MseI* pre-amplification primer (5' –GATGAGTCCTGAGTAA- '3). Pre-amplification reaction was performed for 30 cycles of 30 sec at 94° C, 1 min at 56° C, and 1 min at 72° C. The pre-amplification product was diluted 10-fold to get a final DNA concentration of 25 pg/µL. The DNA was arrayed in a 64-well plate for use with a Li-Cor gel system (LI-COR Bioscience, Lincoln, NE).

Selective amplification reactions were performed using primers with three or four selective nucleotides (*6PstI* + NNN and *MseI* +NNN) resulting in 59 unique primer combinations. Infra-red dye (IRD)-labeled *PstI* primers were obtained from Li-Cor Inc. The final volume of the PCR reaction mixture for selective amplification was 10 µL consisting of 2µL diluted pre-amplification product, 1x PCR buffer, 0.2 units of *Taq* polymerase, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.35 µL 50 ng/µl *MseI* selective primer, and 0.4 µL 1pmole/µl IRD-*PstI* selective primer. Selective amplification was performed as follows: 2 min at 94° C followed by 13 cycles

of 30 sec at 94°C, 30 sec annealing at 65°C, and 1 min at 72°C, lowering the annealing temperature by 0.7°C after each cycle, followed by 23 cycles of 30 sec at 94°C, 30 sec at 56°C, 1 min at 72°C, and extension of 5 min at 72°C. Li-Cor loading buffer (5μL) was added to the PCR products and denatured for 3 min at 95°C. The AFLP product was analyzed with a Li-Cor model 4200L-2 dual-dye automated DNA sequencing system. Amplified product (1μL) was loaded on a KB 6.5% gel matrix (Li-Cor Inc.).The bands obtained were size-matched with a Li-Cor 50-700 base pair sizing marker labeled with 700 and 800 IRD dye and scored using SAGA-AFLP analysis soft ware (Li-Cor Inc.). All the markers were scored as parental type (A or B), heterozygote (H), or missing data (-).

Data analysis and map construction

The population was genotyped with 450 markers which included 259 AFLPs, 189 SSRs, and a STS. The AFLP markers were derived from 59 *PstI* and *MseI* primer combinations and generated a total of 259 dominant scorable loci (Table 1). Heterozygotes in the population were represented as missing data. Raw data were compiled in the Mapmaker/EXP format. Linkage analysis was conducted using Mapmaker/EXP for UNIX version 3 (Whitehead Institute, Cambridge, MA). The 'ri-self' setting was used. A Chi-square test was performed to test the markers for 1:1 segregation ratio, followed by two-point analysis of the markers. Twenty-one chromosomes (linkage groups) were defined with the 'make chromosome' command. Markers were anchored to these linkage groups. These linkage groups were compared with the microsatellite consensus map of Somers et al. (2004) to designate specific chromosomes. To assign the markers to the chromosomes, 'default linkage criteria' was set with a LOD grouping of 6 and a maximum recombination distance of 30 cM (Kosambi units) between the markers.

Table 3-1. List of *PstI* and *MseI* AFLP primers combinations (designated by their standard codes) used in the construction of wheat genetic linkage maps. Three or four selective bases were used for selective amplification reactions. A total of 259 scorable loci were obtained from the primer combinations.

AFLP Primer	Primer Combination	Standard Code	Scorable Loci
p-AGG/m-ACC	Pst1+AGG/Mse1+ACC	P41/M36	6
p-AGG/m-CGAT	Pst1+AGG/Mse1+CGAT	P41/MC66	1
p-AGG/m-CGTA	Pst1+AGG/Mse1+CGTA	P41/MC75	6
p-AGG/m-CTG	Pst1+AGG/Mse1+CTG	P41/M61	7
p-AGG/m-CTT	Pst1+AGG/Mse1+CTT	P41/M62	5
p-AGG/m-GCAG	Pst1+AGG/Mse1+GCAG	P41/MG49	6
p-AGG/m-GCAT	Pst1+AGG/Mse1+GCAT	P41/MG50	2
p-CAG/m-AGC	Pst1+CAG/Mse1+AGC	P49/M40	6
p-CAG/m-AGCT	Pst1+CAG/Mse1+AGCT	P49/MA70	3
p-CAG/m-CAG	Pst1+CAG/Mse1+CAG	P49/M49	1
p-CAG/m-CTC	Pst1+CAG/Mse1+CTC	P49/M60	1
p-CAG/m-GCG	Pst1+CAG/Mse1+GCG	P49/M69	6
p-CAG/m-GCAT	Pst1+CAG/Mse1+GCAT	P49/MG50	2
p-CGA/m-ACAG	Pst1+CGA/Mse1+ACAG	P55/MA49	6
p-CGA/m-ACAG	Pst1+CGA/Mse1+ACAG	P55/MA49	4
p-CGA/m-ACGC	Pst1+CGA/Mse1+ACGC	P55/MA56	3
p-CGA/m-AGAC	Pst1+CGA/Mse1+AGAC	P55/MA64	4
p-CGA/m-CAG	Pst1+CGA/Mse1+CAG	P55/M49	3
p-CGA/m-CAT	Pst1+CGA/Mse1+CAT	P55/M50	10
p-CGA/m-CGCT	Pst1+CGA/Mse1+CGCT	P55/MC70	11
p-CGA/m-CTC	Pst1+CGA/Mse1+CTC	P55/M60	2
p-CGA/m-GAC	Pst1+CGA/Mse1+GAC	P55/M64	10
p-CGA/m-GTG	Pst1+CGA/Mse1+GTG	P55/M77	7
p-CGT/m-ACGT	Pst1+CGT/Mse1+ACGT	P58/MA58	4
p-CGT/m-AGCT	Pst1+CGT/Mse1+AGCT	P58/MA70	11
p-CGT/m-ATGC	Pst1+CGT/Mse1+ATGC	P58/MA88	10
p-CGT/m-CAG	Pst1+CGT/Mse1+CAG	P58/M49	1

p-CGT/m-CAT	Pst1+CGT/Mse1+CAT	P58/M50	4
p-CGT/m-CGAT	Pst1+CGT/Mse1+CGAT	P58/MC66	1
p-CGT/m-CGTA	Pst1+CGT/Mse1+CGTA	P58/MC75	3
p-CGT/m-CTCG	Pst1+CGT/Mse1+CTCG	P58/MC84	4
p-CGT/m-CTG	Pst1+CGT/Mse1+CTG	P58/M61	7
p-CGT/m-CTGA	Pst1+CGT/Mse1+CTGA	P58/MC87	5
p-CGT/m-GCAG	Pst1+CGT/Mse1+GCAG	P58/MG49	2
p-CGT/m-GAC	Pst1+CGT/Mse1+GAC	P58/M64	7
p-CGT/m-GTG	Pst1+CGT/Mse1+GTG	P58/M77	6
p-CGT/m-TGCG	Pst1+CGT/Mse1+TGCG	P58/MT69	2
p-CTC/m-CTA	Pst1+CTC/Mse1+CTA	P60/M59	4
p-CTCG/m-ACC	Pst1+CTCG/Mse1+ACC	PC85/M36	9
p-CTCG/m-AGC	Pst1+CTCG/Mse1+AGC	PC85/M40	4
p-CTCG/m-CTG	Pst1+CTCG/Mse1+CTG	PC85/M61	3
p-CTCG/m-CTT	Pst1+CTCG/Mse1+CTT	PC85/M62	1
p-GCTG/m-CAG	Pst1+GCTG/Mse1+CAG	PG61/M49	1
p-GTG/m-ACAG	Pst1+GTG/Mse1+ACAG	P77/MA49	3
p-GTG/m-ACGC	Pst1+GTG/Mse1+ACGC	P77/MA56	3
p-GTG/m-ACGT	Pst1+GTG/Mse1+ACGT	P77/MA58	3
p-GTG/m-AGC	Pst1+GTG/Mse1+AGC	P77/M40	6
p-GTG/m-AGCT	Pst1+GTG/Mse1+AGCT	P77/MA70	7
p-GTG/m-ATGC	Pst1+GTG/Mse1+ATGC	P77/MA88	1
p-GTG/m-CGAC	Pst1+GTG/Mse1+CGAC	P77/MC64	6
p-GTG/m-CGCT	Pst1+GTG/Mse1+CGCT	P77/MC70	1
p-GTG/m-CTA	Pst1+GTG/Mse1+CTA	P77/M59	4
p-GTG/m-CTGA	Pst1+GTG/Mse1+CTGA	P77/MC87	1
p-GTG/m-TGCG	Pst1+GTG/Mse1+TGCG	P77/MT69	1
p-TGC/m-AGC	Pst1+TGC/Mse1+AGC	P88/M40	2
p-TGC/m-AGCT	Pst1+TGC/Mse1+AGCT	P88/MA70	7
p-TGC/m-CGAC	Pst1+TGC/Mse1+CGAC	P88/MC64	5
p-TGC/m-CTG	Pst1+TGC/Mse1+CTG	P88/M61	5
p-TGC/m-GCG	Pst1+TGC/Mse1+GCG	P88/M69	3
	Total	59	259

Markers with significant segregation distortion were excluded. to eliminate possibility of spurious linkage. Within the linkage group, commands 'compare', 'order' and 'ripple' were used. The initial anchored markers were determined based on published consensus maps and subsequently, other markers were assigned to linkage groups. These groups were then ordered and validated with the 'order' and 'ripple' commands. An order was accepted only when its likelihood was 100 times more than the likelihood of the best alternative order. The best order was designated as the framework for each linkage group. Other markers that were assigned to the group and not included in the framework map were placed on to the map in a decreasing order of informativeness. Map distances were compared with 'error detection on' and 'error detection off'. These procedures were repeated several times to decrease the chances of error. Finally, all the markers were assigned to the chromosomes using the 'place' command, and the placement markers were placed at odds between 1000:1 and 100:1.

QTL analysis

The QTL cartographer version 2.0 mapping program (Zeng, 1994; Basten et al., 2002) was used for QTL analysis. QTL linked to framework were detected using forward and backward regression options of composite interval mapping (CIM). The significance peak with the highest LOD score was recorded, as they indicate the presence of QTL at the given loci. Additive effects of the QTL and the variability accounted by the QTL were also estimated using the Cartographer program.

Comparative mapping

To cross-link different genomes of the grass species with respect to the traits studied, comparative genetic mapping was performed. Comparative mapping used the tools provided in the Gramene web-site (www.gramene.org/db/searches/browser), and synteny was established between wheat, sorghum, rice (*Oryza sativa* L.), and maize genomes.

Results

Molecular markers used in the map

The heterochromatic regions around the centromeres and tips of the chromosomes have a high degree of methylation and low recombination rate. Restriction enzymes like *PstI* are sensitive to methylation. Use of *PstI/MseI* primer increases the frequency of markers in the genetically active euchromatin regions, as reported by earlier workers (Young et al., 1999; Menz et al., 2002). The AFLP primer combinations p-CGA/m-CAT, p-CGA/m-CGCT, p-CGA/m-GAC, p-CGT/m-AGCT, p-CGT/m-ATGC and p-CTCG/m-ACC had the most scorable loci, with 10, 11, 10, 11, 10, and 9, respectively.

A total of 248 markers were used for mapping. The linkage maps were generated consisted of 172 framework markers and 76 placement markers (Table 2). The A genome had the most polymorphic markers (44%), followed by B genome (36%) and D genome by (20%). Markers coverage ranged from 27 on chromosomes 2Aand 6A to4 on chromosomes 3A, 3D, 5D and 7D. The map distances ranged from ≤ 0.1 cM for few markers on chromosomes 2A, 4B and 6A to ≥

Table 3-2. Distribution of markers over 21 chromosomes categorized as framework markers and markers placed in the region, and a comparison of their genetic lengths with the published consensus genetic linkage maps.

Linkage group	Framework	Region	Total	Length (cM)	Consensus Map* (cM)
1A	8	3	11	116.6	126
1B	10	3	13	101.1	111
1D	7	5	12	118.5	117
2A	11	16	27	142.0	143
2B	11	1	12	124.6	123
2D	5	3	8	94.7	107
3A	4	-	4	92.8	116
3B	9	2	11	113.0	148
3D	4	-	4	30.7	79
4A	10	5	15	182.5	88
4B	10	8	18	109.7	59
4D	8	4	12	113.3	91
5A	12	2	14	89.1	184
5B	10	1	11	107.1	173
5D	4	-	4	94.4	120
6A	14	13	27	152.6	156
6B	7	-	7	131.0	82
6D	6	-	6	56.2	110
7A	8	2	10	100.8	131
7B	10	8	18	142.1	151
7D	4	-	4	77.1	154
A genome	67	41	108	876.4	944
B genome	67	23	90	828.6	847
D genome	38	12	50	584.6	778

*Somers, D.J., 2004.

40 cM on chromosomes 2D, 3A, 5D and 6B. The colinearity and the genome lengths of the present maps were comparable with the consensus maps developed by Somers et al. (2004), but some of the chromosomes were smaller and incomplete, while chromosomes 4A, 4B, 4D, and 6B were longer than the published maps. The average distance between the framework markers was 13.3 cM. The placed markers reduced, the average distance between markers to 9.2 cM.

Genetic characterization of yield traits and rate of senescence

Genetic characterization of yield traits and maximum rate of senescence under optimum and heat stress conditions (Table 3 and 4) were done on a composite interval basis. The tables explain the QTL positions on the chromosomes, log of odds (LOD) scores, additive effects, and the variability accounted by each QTL. All markers with a LOD of 2.00 or greater have been listed. Additive effects of the traits are given as positive or negative values. Positive values indicate that the alleles for the traits in the RILs were contributed by the tolerant parent, and negative by the susceptible parent. The R^2 explains the fit of the regression model or the variability accounted by the trait. The smaller the variability of the residual values around the regression line relative to the overall variability, the better the fit. Total variability is the sum of all variabilities of a trait. Under optimum conditions, TKW and maximum rate of senescence had the highest variability at explained 65.4% and 66.2%, respectively. The GFD had a moderate variability at explained 50.8%, and kernels per spike and GFR had less variability explained at 20.8% and 27.2%, respectively. Under heat stress, maximum rate of senescence had the highest variability explained at 43.9%; GFD, TKW, and GFR had moderate variability explained at

Table 3-3. Genetic characterization of QTL linked to yield traits and maximum rate of senescence studied under optimum conditions. All markers having a LOD score of 2 and above are listed. The QTL were detected on composite interval bases. Additive effects, parent donating the allele and total variability, the sum of R^2 for the respective traits were also estimated.

Trait	Chrom	Marker	Position (cM)	LOD	Additive	Donor Parent	R^2	Total R^2 (%)
GFD	1B	Xbarc137	25.01	2.476	1.393	Ventnor	0.075	
GFD	1B	TGC.AGCT-481	80.70	2.252	1.196	Ventnor	0.054	
GFD	2A	Xgwm356	71.63	6.834	2.124	Ventnor	0.181	
GFD	2B	Xgwm55	8.01	2.868	1.664	Ventnor	0.116	
GFD	4A	GTG.CGCT-138	179.83	2.759	1.456	Ventnor	0.082	50.8
Kernels/spike	6A	CGA.CGCT-406	135.23	2.356	-2.999	Karl 92	0.080	
Kernels/spike	7A	Xbarc121	50.92	2.560	3.760	Ventnor	0.128	20.8
TKW	1B	Xbarc8	6.01	3.878	2.465	Ventnor	0.162	
TKW	1B	Xbarc137	23.01	5.512	2.679	Ventnor	0.192	
TKW	2B	CGT.ACGT-319	47.90	2.426	1.607	Ventnor	0.070	
TKW	3B	CGT.CTG-361	88.64	2.019	1.723	Ventnor	0.083	
TKW	5A	Xgwm293	8.66	3.014	-1.808	Karl 92	0.088	
TKW	7A	CGA.CGCT-272	45.80	2.840	-1.606	Karl 92	0.059	65.4
GFR	1A	Xbarc148	42.46	3.342	0.005	Ventnor	0.090	
GFR	2A	Xgwm356	73.63	2.609	-0.006	Karl 92	0.077	
GFR	6A	Xgwm334	12.01	2.058	0.005	Ventnor	0.105	27.2
Max Sen.	2A	GTG.ACGC-108	84.33	4.026	1.886	Ventnor	0.191	
Max Sen.	4A	GTG.CGCT-138	181.83	2.405	-0.838	Karl 92	0.092	
Max Sen.	5A	GTG.AGC-254	32.97	3.291	1.093	Ventnor	0.124	
Max Sen.	6A	CAG.AGC-101	70.86	6.392	-2.308	Karl 92	0.255	66.2

Table 3-4. Genetic characterization of QTL linked to yield traits and maximum rate of senescence under high temperature. All markers having a LOD score of 2 and above are listed. The QTL were detected on composite interval bases. Additive effects, parent donating allele and total variability, the sum of R^2 for the respective traits were also estimated.

Trait	Chrom	Marker	Position (cM)	LOD	Additive	Donor Parent	R^2	Total R^2 (%)
GFD	2A	AGG.CTT-107	76.48	5.927	1.174	Ventnor	0.174	
GFD	2B	AGG.ACC-240	122.24	2.447	-0.752	Karl 92	0.073	
GFD	4A	GTG.CGCT-138	171.83	2.522	0.773	Ventnor	0.078	32.5
Kernels/spike	2A	Xgwm356	73.63	5.016	-4.537	Karl 92	0.173	17.3
TKW	2A	Xgwm296	0.01	2.270	-1.856	Karl 92	0.099	
TKW	4A	Xksum20	167.26	3.827	2.263	Ventnor	0.155	
TKW	6A	CAG.AGC-101	72.86	2.292	1.569	Ventnor	0.073	32.7
GFR	1D	CGT.ATGC-297	85.74	2.291	-0.012	Karl 92	0.095	
GFR	2B	CGT.CGTA-189	124.24	2.686	0.008	Ventnor	0.078	
GFR	6A	Xksum61	144.19	4.323	-0.010	Karl 92	0.129	0.2
Max Sen.	2A	AGG.CTT-107	76.48	7.608	-4.316	Karl 92	0.235	
Max Sen.	2D	Xbarc136	56.76	4.110	-3.359	Karl 92	0.142	
Max Sen.	6A	Xgwm334	0.01	2.261	-2.179	Karl 92	0.062	43.9

32.5%, 32.7% and 30.2%, respectively; and kernels per spike had a low variability explained at 17.3%. Marker GTG.CGCT-138 (P77/MC70-138) on chromosome 4A was linked to GFD under both optimum and heat-stress conditions and accounted for 8.2 % variability under optimum conditions and 7.8% under heat stress. Under optimum conditions, the maximum variability explained for kernels per spike was at 12.8%, accounted for by marker *Xbarc121* on chromosome 7A, for TKW 19.2% was accounted for by marker *Xbarc137* on chromosome 1B, *Xgwm334* on chromosome 6A explained 10.5% of the variability for GFR, and 25.5% of variability for maximum rate of senescence was explained by marker CAG.AGC-101 (P49/M40-101) on chromosome 6A. The maximum amount of variability explained for GFD under optimum conditions was at 18.1% by *Xgwm356* on Chromosome 2A at 71.63 cM; under heat stress, 17.4% of variability was accounted for by AGG.CTT-107 (P41/M62-107) on chromosome 2A at 76.48 cM. This indicated that QTL for GFD under optimum and heat stress are located in the same region of chromosome 2A.

The linkage maps (Figure 1) indicated that chromosomes 2A, 4A, 6A, and 2B have at least two QTL linked to high temperature. On chromosome 2A, the interval between the makers *Xgwm356* at 71.6 cM and AGG.CTT-107 (P41/M62-107) at 76.4 cM has QTL linked to GFD, kernels per spike, and maximum rate of senescence. On chromosome 4A QTL for GFD and TKW are located between markers *Xksum20* at 153.8 cM and TGC.GCG-97 (P88/M69-97) at 182.5 cM. The QTL for maximum rate of senescence linked to marker *Xgwm334*, TKW linked to CAG.AGC-101 (P49/M40-101), and GFR linked to marker *Xksum61* were scattered on chromosome 6A.Chromosome 2B had QTL for GFD and GFR mapped to the interval between

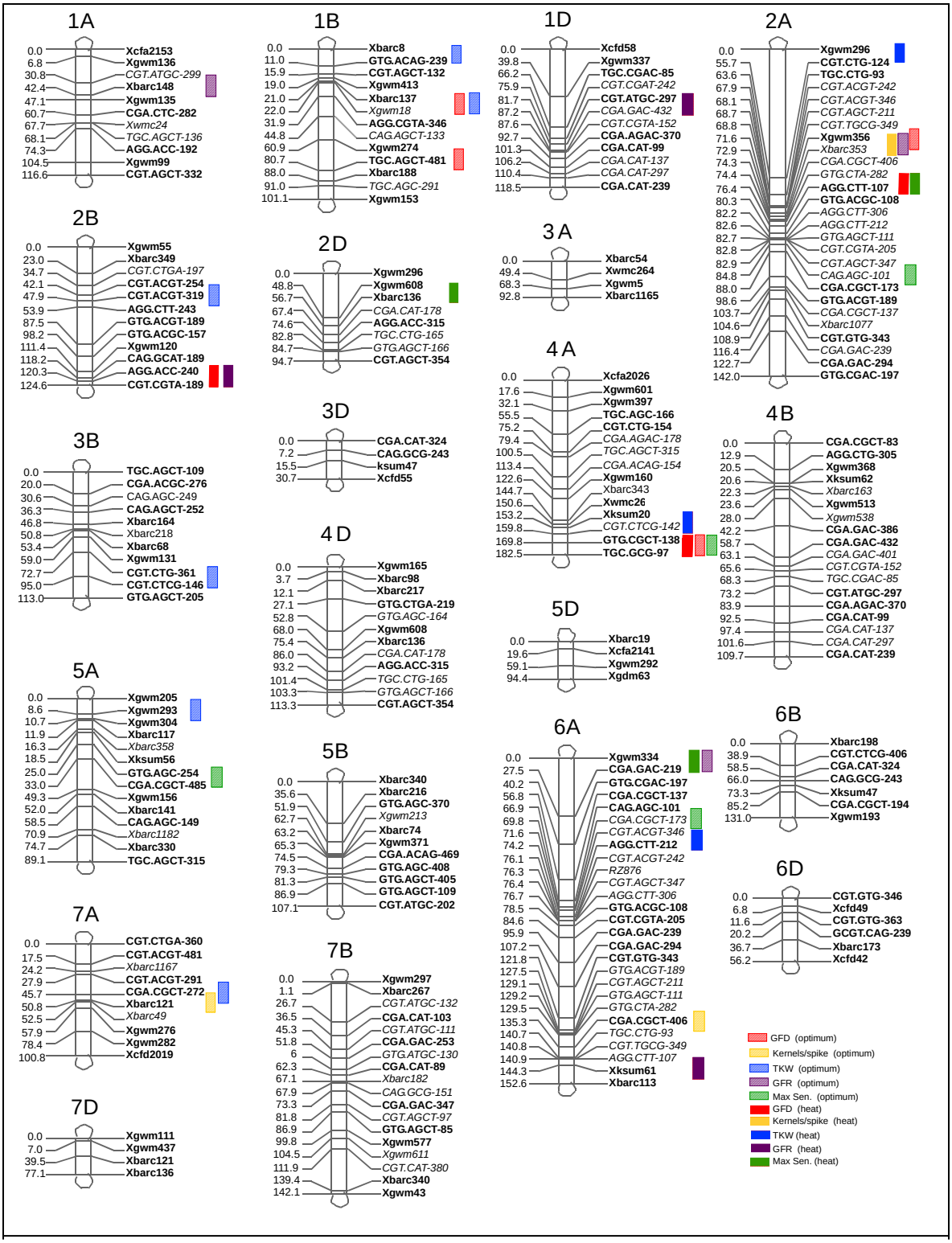
1A
0.0 Xcfa2153
6.8 Xgwm136
30.8 CGT.ATGC-299
42.4 Xbarc148
47.1 Xgwm135
60.7 CGA.CTC-282
67.7 Xwmc24
68.1 TGC.AGCT-136
74.3 AGG.ACC-192
104.5 Xgwm99
116.6 CGT.AGCT-332
1B
0.0 Xbarc8
11.0 GTG.ACAG-239
15.9 CGT.AGCT-132
19.0 Xgwm413
21.0 Xbarc137
22.0 Xgwm18
31.9 AGG.CGTA-346
44.8 CAG.AGCT-133
60.9 Xgwm274
80.7 TGC.AGCT-481
88.0 Xbarc188
91.0 TGC.AGC-291
101.1 Xgwm153
1D
0.0 Xcfd58
39.8 Xgwm337
66.2 TGC.CGAC-85
75.9 CGT.CGAT-242
81.7 CGT.ATGC-297
87.2 CGA.GAC-432
87.6 CGT.CGTA-152
92.7 CGA.AGAC-370
101.3 CGA.CAT-99
106.2 CGA.CAT-137
110.4 CGA.CAT-297
118.5 CGA.CAT-239
2A
0.0 Xgwm296
55.7 CGT.CTG-124
63.6 TGC.CTG-93
67.9 CGT.ACGT-242
68.1 CGT.ACGT-346
68.7 CGT.AGCT-211
68.8 CGT.TGCG-349
71.6 Xgwm356
72.9 Xbarc353
74.3 CGA.CGCT-406
74.4 GTG.CTA-282
76.4 AGG.CTT-107
80.3 GTG.ACGC-108
82.2 AGG.CTT-306
82.6 AGG.CTT-212
82.7 GTG.AGCT-111
82.8 CGT.CGTA-205
82.9 CGT.AGCT-347
84.8 CAG.AGC-101
88.0 CGA.CGCT-173
98.6 GTG.ACGT-189
103.7 CGA.CGCT-137
104.6 Xbarc1077
108.9 CGT.GTG-343
116.4 CGA.GAC-239
122.7 CGA.GAC-294
142.0 GTG.CGAC-197
2B
0.0 Xgwm55
23.0 Xbarc349
34.7 CGT.CTGA-197
42.1 CGT.ACGT-254
47.9 CGT.ACGT-319
53.9 AGG.CTT-243
87.5 GTG.ACGT-189
98.2 GTG.ACGC-157
111.4 Xgwm120
118.2 CAG.GCAT-189
120.3 AGG.ACC-240
124.6 CGT.CGTA-189
2D
0.0 Xgwm296
48.8 Xgwm608
56.7 Xbarc136
67.4 CGA.CAT-178
74.6 AGG.ACC-315
82.8 TGC.CTG-165
84.7 GTG.AGCT-166
94.7 CGT.AGCT-354
3A
0.0 Xbarc54
49.4 Xwmc264
68.3 Xgwm5
92.8 Xbarc1165
3B
0.0 TGC.AGCT-109
20.0 CGA.ACGC-276
30.6 CAG.AGC-249
36.3 CAG.AGCT-252
46.8 Xbarc164
50.8 Xbarc218
53.4 Xbarc68
59.0 Xgwm131
72.7 CGT.CTG-361
95.0 CGT.CTCG-146
113.0 GTG.AGCT-205
3D
0.0 CGA.CAT-324
7.2 CAG.GCG-243
15.5 ksum47
30.7 Xcfd55
4A
0.0 Xcfa2026
17.6 Xgwm601
32.1 Xgwm397
55.5 TGC.AGC-166
75.2 CGT.CTG-154
79.4 CGA.AGAC-178
100.5 TGC.AGCT-315
113.4 CGA.ACAG-154
122.6 Xgwm160
144.7 Xbarc343
150.6 Xwmc26
153.2 Xksum20
159.8 CGT.CTCG-142
169.8 GTG.CGCT-138
182.5 TGC.GCG-97
4B
0.0 CGA.CGCT-83
12.9 AGG.CTG-305
20.5 Xgwm368
20.6 Xksum62
22.3 Xbarc163
23.6 Xgwm513
28.0 Xgwm538
42.2 CGA.GAC-386
58.7 CGA.GAC-432
63.1 CGA.GAC-401
65.6 CGT.CGTA-152
68.3 TGC.CGAC-85
73.2 CGT.ATGC-297
83.9 CGA.AGAC-370
92.5 CGA.CAT-99
97.4 CGA.CAT-137
101.6 CGA.CAT-297
109.7 CGA.CAT-239
4D
0.0 Xgwm165
3.7 Xbarc98
12.1 Xbarc217
27.1 GTG.CTGA-219
52.8 GTG.AGC-164
68.0 Xgwm608
75.4 Xbarc136
86.0 CGA.CAT-178
93.2 AGG.ACC-315
101.4 TGC.CTG-165
103.3 GTG.AGCT-166
113.3 CGT.AGCT-354
5D
0.0 Xbarc19
19.6 Xcfa2141
59.1 Xgwm292
94.4 Xgdm63
5A
0.0 Xgwm205
8.6 Xgwm293
10.7 Xgwm304
11.9 Xbarc117
16.3 Xbarc358
18.5 Xksum56
25.0 GTG.AGC-254
33.0 CGA.CGCT-485
49.3 Xgwm156
52.0 Xbarc141
58.5 CAG.AGC-149
70.9 Xbarc1182
74.7 Xbarc330
89.1 TGC.AGCT-315
5B
0.0 Xbarc340
35.6 Xbarc216
51.9 GTG.AGC-370
62.7 Xgwm213
63.2 Xbarc74
65.3 Xgwm371
74.5 CGA.ACAG-469
79.3 GTG.AGC-408
81.3 GTG.AGCT-405
86.9 GTG.AGCT-109
107.1 CGT.ATGC-202
6A
0.0 Xgwm334
27.5 CGA.GAC-219
40.2 GTG.CGAC-197
56.8 CGA.CGCT-137
66.9 CAG.AGC-101
69.8 CGA.CGCT-173
71.6 CGT.ACGT-346
74.2 AGG.CTT-212
76.1 CGT.ACGT-242
76.3 RZ876
76.4 CGT.AGCT-347
76.7 AGG.CTT-306
78.5 GTG.ACGC-108
84.6 CGT.CGTA-205
95.9 CGA.GAC-239
107.2 CGA.GAC-294
121.8 CGT.GTG-343
127.5 GTG.ACGT-189
129.1 CGT.AGCT-211
129.2 GTG.AGCT-111
129.5 GTG.CTA-282
135.3 CGA.CGCT-406
140.7 TGC.CTG-93
140.8 CGT.TGCG-349
140.9 AGG.CTT-107
144.3 Xksum61
152.6 Xbarc113
6B
0.0 Xbarc198
38.9 CGT.CTCG-406
58.5 CGA.CAT-324
66.0 CAG.GCG-243
73.3 Xksum47
85.2 CGA.CGCT-194
131.0 Xgwm193
6D
0.0 CGT.GTG-346
6.8 Xcfd49
11.6 CGT.GTG-363
20.2 GCGT.CAG-239
36.7 Xbarc173
56.2 Xcfd42
7A
0.0 CGT.CTGA-360
17.5 CGT.ACGT-481
24.2 Xbarc1167
27.9 CGT.ACGT-291
45.7 CGA.CGCT-272
50.8 Xbarc121
52.5 Xbarc49
57.9 Xgwm276
78.4 Xgwm282
100.8 Xcfd2019
7B
0.0 Xgwm297
1.1 Xbarc267
26.7 CGT.ATGC-132
36.5 CGA.CAT-103
45.3 CGT.ATGC-111
51.8 CGA.GAC-253
6 GTG.ATGC-130
62.3 CGA.CAT-89
67.1 Xbarc182
67.9 CAG.GCG-151
73.3 CGA.GAC-347
81.8 CGT.AGCT-97
86.9 GTG.AGCT-85
99.8 Xgwm577
104.5 Xgwm611
111.9 CGT.CAT-380
139.4 Xbarc340
142.1 Xgwm43
7D
0.0 Xgwm111
7.0 Xgwm437
39.5 Xbarc121
77.1 Xbarc136
GFD (optimum)
Kernels/spike (optimum)
TKW (optimum)
GFR (optimum)
Max Sen. (optimum)
GFD (heat)
Kernels/spike (heat)
TKW (heat)
GFR (heat)
Max Sen. (heat)

Figure 3-1. Genetic linkage maps of 21 chromosomes of wheat. The population used was a set of RILs derived from Ventnor X Karl 92. The marker names are listed on the right of the chromosome, and map distances in centimorgans (cM) are listed on the left. Framework markers are represented in bold and placement markers are represented in italics. Markers beginning with "X" are SSR, RZ876 is a STS, and the others are AFLP markers denoted by their selective base combinations and size (bp) of the band. The QTL for yield traits, and maximum rate of senescence under optimum (striped boxes) and heat stress (soild boxes) have the same color coded.

AGG.ACC-240 (P41/M36-240) at 120.3 cM and CGT.CGTA-189 (P58/MC75-189) at 124.6 cM. Maximum variability explained for kernels per spike was 17.3% by *Xgwm356* on chromosome 2A, 15.5% for TKW by *Xksum20* on chromosome 4A, 12.9% for GFR by *Xksum61* on chromosome 6A, and 23.5% for maximum rate of senescence by AGG.CTT-107 (P41/M62-107) on chromosome 2A.

Under heat stress QTL for GFD was colocalized with AGG.CTT-107 (P41/M62-107) at 76.4 cM on chromosome 2A. A QTL for GFR was located 0.36 cM proximal from the marker CGT.CGTA-189 (P58/MC75-189) on 2B. Another QTL for GFR was located at 144.19 cM, which is 0.11 cM from the *Xksum61* on chromosome 6A, and a QTL for maximum rate of senescence co localized with CAG.GTT-107 (P41/M62-107) on chromosome 2A and *Xbarc136* on chromosome 2D.

The QTL identified under optimum and heat stress conditions and markers linked to those QTL were different, except for GTG.CGCT-138 (P77/MC70-138) which was linked to QTL for GFD. Based on the additive effects, under optimum conditions the favorable alleles for GFD were from Ventnor, while favorable alleles for all the other traits were from both Ventnor and Karl 92. Under heat stress, favorable alleles for all traits except maximum rate of senescence were from both Ventnor and Karl 92; for maximum rate of senescence, the alleles favoring senescence were from Karl 92 only. AGG.CTT-107 (P41/M62-107) had a positive additive effect of 1.174, and explained 17.4% of the variability for GFD, while the same marker had a negative additive effect of -4.316, and explained 23.5% of variability for maximum rate of senescence under high temperature. Using this marker, Ventnor type alleles can be selected for both the traits.

Figures 2 and 3 show the QTL position on the chromosomes and LOD scores for the markers linked to the traits, represented as the peaks in the graph. Under both optimum and heat stress

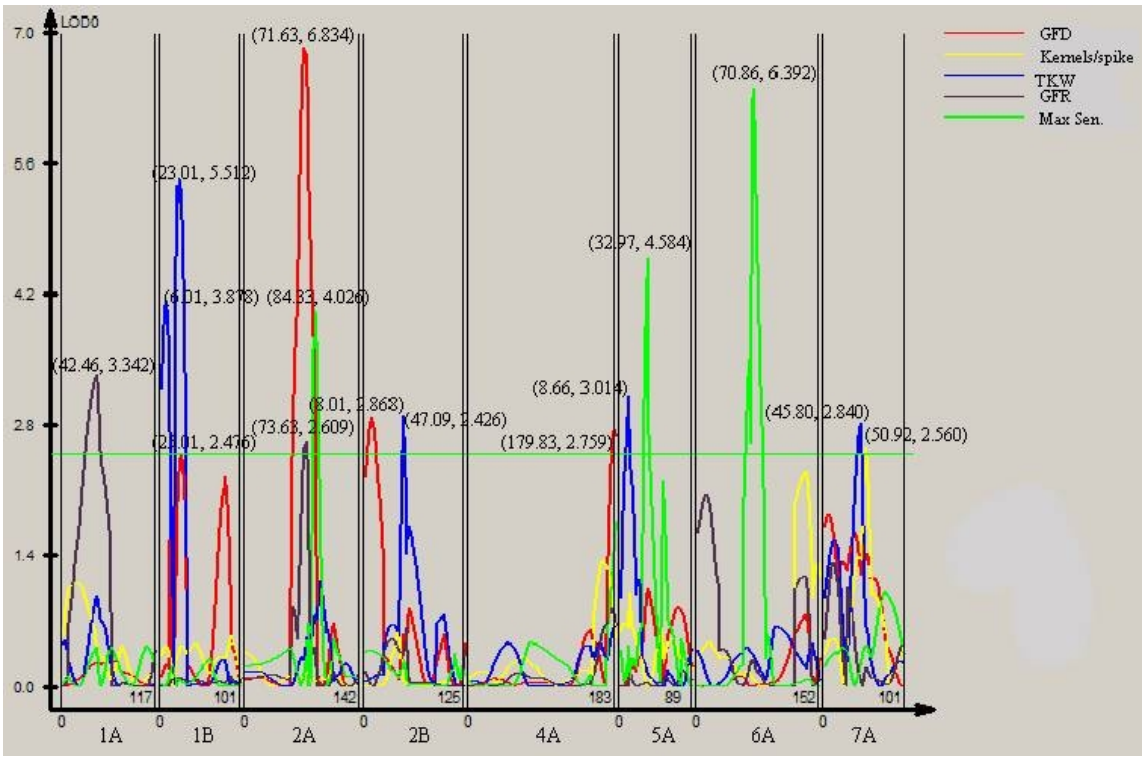

Figure 3-2. Composite interval mapping showing markers with a LOD score above 2.5 for yield traits and maximum rate of senescence under optimum conditions. Peaks in the plot indicate the presence of significant QTL. Positions of the QTL and LOD scores are provided inside the parenthesis.

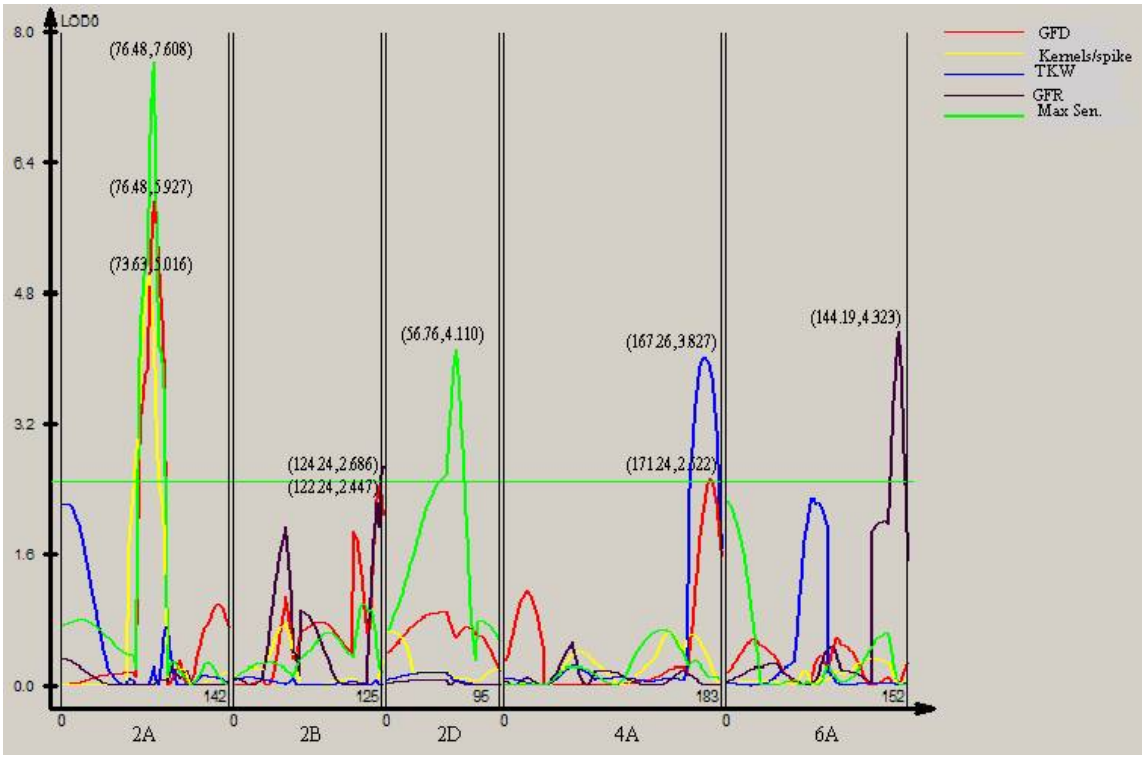

Figure 3-3. Composite interval mapping showing markers with a LOD score above 2.5 for yield traits and maximum rate of senescence under high temperature. Peaks in the plot indicate the presence of significant QTL. Positions of the QTL and LOD scores are provided inside the parenthesis.

conditions, the A genome appears to have the most QTL for yield traits and maximum rate of senescence. Chromosomes 2A, 2B, and 4A had QTL under both conditions. Under optimum conditions chromosome 2A had QTL for GFD with a LOD of 6.834; TKW on chromosomes 1B, 2B, 5A and 7A; GFR on chromosomes 1A; and maximum rate of senescence on chromosomes 2A, 5A, and 6A. Under heat stress, kernels per spike was found on chromosome 2A, TKW on chromosome 4A, GFR on chromosome 6A, and maximum rate of senescence on chromosomes 2A and 2D. Under optimum and heat stress conditions, chromosome 2A had QTL for GFD and maximum rate of senescence. Homoeologous group 2 chromosomes, in general, had QTL for yield traits and stay-green.

Comparative mapping for markers linked to yield traits

Under heat-stress conditions, microsatellite marker *Xgwm 356* at 71.6 cM on chromosome 2A was linked to kernels per spike, and a terminal marker *Xgwm 296* was linked to TKW. On the same chromosome, distal to *Xgwm356*, an AFLP marker AGG.CTT-107 (P41/M62-107) at 76.4 cM was linked with GFD. *Xgwm296* and *Xgwm356* were physically mapped to bins C-2AS5-0.78 and C-2AL1-0.85, respectively (Qi et al., 2003; Sourdille et al., 2004). Therefore Xgwm296 and the marker interval between Xgwm356 and AGG.CTT-107 (P41/M62-107) were used for comparative mapping. To establish the synteny, restriction fragment length polymorphism (RFLP) markers mapping to the same bins as those of makers *Xgwm296* and *Xgwm356* were used. The RFLP markers *Xbcd855* and *Xpsr388* were physically mapped to bins C-2AS5-0.78 and C-2AL1-0.85, respectively. Marker *Xbcd855* was located at 31.0 cM and 55.0 cM, and marker *Xpsr388* at 63.0 cM in the wheat genetic linkage map (Appels,

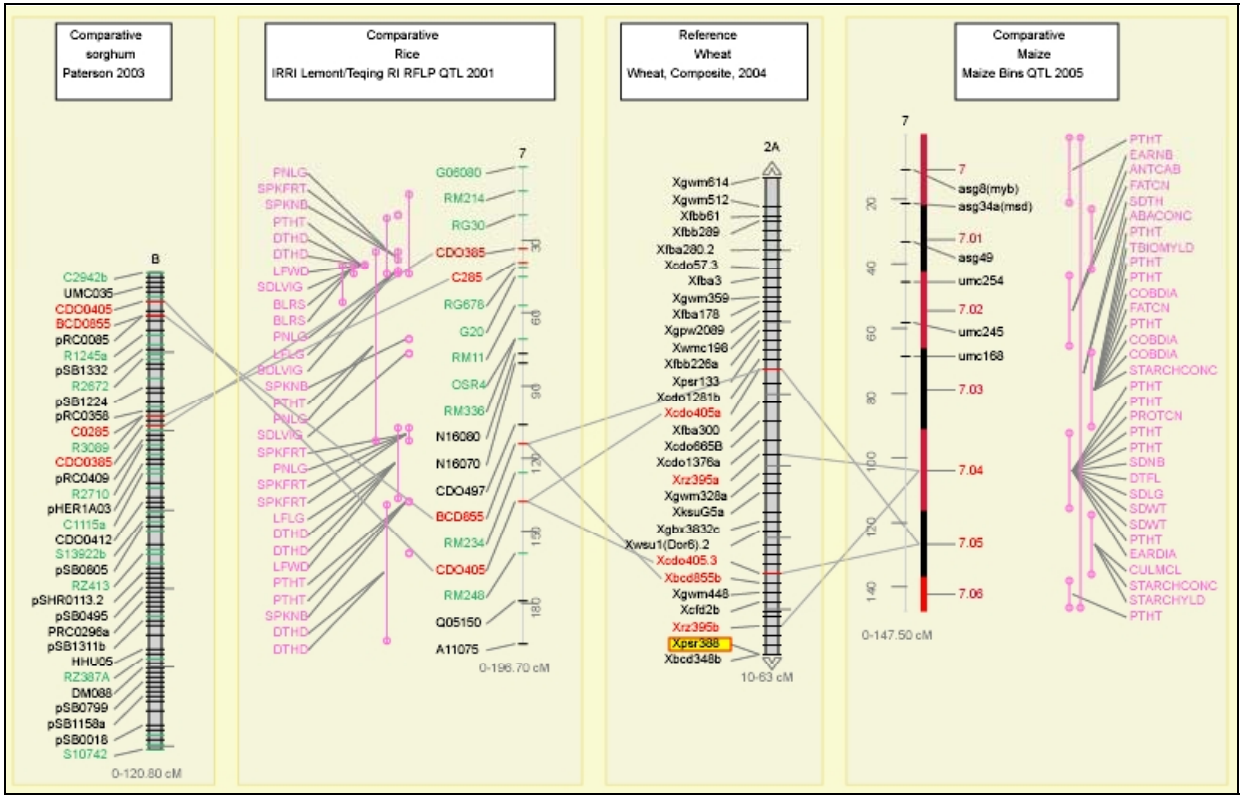

Figure 3-4. Comparative mapping for RFLP markers *Xbcd855* and *Xpsr388* (physically mapped to the same bins as SSR markers *Xgwm296* and *Xgwm356* in wheat) across wheat, rice, sorghum, and maize. These markers were present on chromosome 2A in wheat, chromosome 7 in rice and maize, and linkage group B in sorghum. In wheat, rice, and maize the regions containing these markers also contain yield related traits.

2004). In rice, *Xbcd855* was on chromosome 7 at 113.80 cM (Li et al., 2001) (Figure 4). Another marker, *Xcdo405* was at the same loci as *Xbcd855* at 31.0 cM and 54.0 cM in wheat and was also present at 137.30 cM in rice. The QTL linked to days to heading in rice was between 107.20 and 137.30 cM, and QTL linked to plant height, spikelet number, and spikelet fertility were at 137.30 cM.

Marker *Xrz395* in wheat was present at two loci, one between *Xbcd855* and *Xcdo405* at 41.0 cMand the other proximal to *Xpsr388* at 60.0 cM. This marker maps to bin 7.04 on chromosome 7 of maize, which spans a length of 91.50 to 116.30 cM (Gardiner, 1993). *Xcdo405* maps to maize bin 7.05, which spans a length of 116.30 to 137.50 cM. QTL for anthesis silking time, total biomass yield, plant height , kernel weight, seed length, starch yield, days to silk, ear diameter, grain yield, ear number, protein content, and starch concentration were in maize bins 7.04 and 7.05. Markers *Xcdo405* and *Xbcd855* were also mapped to linkage group B in the sorghum genetic map at 6.90 cM and 10.80 cM, respectively (Paterson, 2003).

Marker *Xksum20* at 153.2 cM on chromosome 4A had the highest LOD score and largest additive effect for TKW and was physically mapped to bin 4AL5-0.66-0.80 (Qi et al., 2003). RFLP marker *Xpsr115* was mapped to the same bin (Sourdille et al., 2004) at 105.00 cM (Appels, 2004). Another marker *Xcdo475* was mapped at 104.00 cM in wheat also mapped to chromosome 6 of rice at 1.90 cM (Figure not shown). In rice QTL linked to tiller number were present between 0.00 cM and 6.30 cM (Wang et al., 1994).

Discussion

Many of the important QTL for yield traits were reported to be present on the A genome. Shah et al. (1999) reported the presence of genes affecting grain yield, yield components, grain volume weight, plant height, and anthesis date on chromosome 3A, in a recombinant inbred chromosome line population. Varshney et al. (2000) reported QTL for grain weight on chromosome 1AS near *Xwmc333* and the long arm of chromosome 4A contains the granule-bound starch synthase gene that has pleiotropic effect on ear emergence time, and plant height (Araki et al., 1999). Kirigwi (2005) found that QTL linked to grain yield, GFR, spike density, grains m^{-2}, biomass production, biomass production rate, and drought susceptibility index (DSI) under drought stress conditions in a spring wheat population were associated with *Xwmc89* located proximal on chromosome 4AL.

The QTL for yield traits, were located on chromosomes 1, 2, 4, 5, 6, and 7 of the A genome under optimum conditions, and chromosomes 2A, 4A, and 6A under heat stress. *Xbarc148* on the short arm of chromosome 1A was linked to QTL for GFR. *Xgwm136* is distal to *Xbarc148* in the genetic linkage map and is located in bin 1AS-0.47-0.86 (Qi et al., 2003; Sourdille et al., 2004). Another RFLP marker, *Xbcd1072* which was synonymous to heat shock protein 70 (HSP 70), was in the same bin. Genetic lengths for most of the chromosomes were comparable to the previously published maps (Somers et al. 2004). Chromosomes 4A, 4B, 4D, and 6B were longer then the published maps, suggested the need to screen more markers on larger populations to detect recombination between the markers.

Under optimum conditions, marker *Xgwm293* on chromosome 5A mapped to the bin 5AS1-0.40-0.75 was linked to TKW. *Xbarc121* on chromosome 7A (bin C-7AL1-0.39) was linked to a QTL for kernels per spike. The same chromosome had QTL for yield under nutrition, drought and salt stress (Quarrie et al., 2005). Genome synteny of grasses shows that homoeologous group 2 is

syntenic to chromosomes 4 and 7 in rice; chromosomes 10, 7 and 2 in maize; and linkage groups B and F in sorghum (Devos and Gale, 1997; Sorrells et al., 2003). Verma et al. (2004) found that QTL linked to grain yield and green flag leaf area under drought stress in winter wheat on homoeologous group 2. Similarly, yield QTL in sorghum were found on linkage group F by Tuinstra et al. (1998), on chromosomes 1 and 10 in maize by Ribaut et al. (1997), and on chromosomes 3, 4, and 8 in rice by Lanceras et al. (2004).

In the present experiment, under high temperature stress *Xgwm356* was linked to kernels per spike, *Xgwm 296* was linked to TKW, and AFLP marker AGG.CTT-107 (P41/M62-107) was linked to GFD on chromosome 2A. On chromosome 2B, GFD and GFR were linked to markers AAG.ACC-240 (P41/M36-240) and CGT.CGTA-189 (P58/MC75-189), respectively, and marker *Xbarc136* on chromosome 2D was linked to maximum rate of senescence. AGG.CTT-107 (P41/M62-107) on chromosome 2A was linked to maximum rate of senescence under heat stress; this marker was also linked to GFD under the same conditions. Correlation analysis showed a negative correlation between maximum rate of senescence and the yield traits GFD and TKW (Chapter 2). These results are similar to the reports of earlier researchers (Tuinstra et al., 1998; Spano et al., 2003; Verma et al., 2004) in stay-green mutants and drought, where QTL for stay-greenness and yield traits were at the same loci, and decreased rate of senescence increased the GFD, and ultimately TKW. GTG.CGCT-138 (P77/MC70) was linked to GFD under optimum and heat stress conditions, suggesting the presence of genes related to stability of yield under high temperature, while other QTL detected only under heat stress would be presumed to contain genes for high-temperature tolerance, and could be used to enhance yield under heat stress. The QTL for GFD was co-localized with AGG.CTT-107 (P41/M62-107) on chromosome 2A, QTL for GFR with CGT.CGTA-189 (P58/MC75-189) and *Xksum61* on chromosomes 2B

and 6A, respectively, and the QTL for maximum rate of senescence with *Xbarc136* and AGG.CTT-107 (P41/M62-107) on chromosomes 2D and 2A, respectively.

Comparative genome analyses showed colinearity, or in other words, conserved gene orders in the genomes of different plant species. In plants, this is best documented in the grass family, where colinearity has been maintained over evolutionary periods as long as 60 million years (Devos and Gale, 1997). In the present experiment, RFLP markers in the same bin as microsatellite markers *Xgwm296* and *Xgwm356*, which were linked to yield QTL under heat stress, were used to determine synteny. Analysis showed synteny of *Xbcd855* and *Xcdo405* on chromosome 2A of wheat with linkage group B of sorghum, and chromosome 7 of both rice and maize genomes. These markers are colinear in sorghum, rice and wheat. In rice and maize QTL maps, the loci at which these markers are located have major QTL for grain yield.

Conclusions drawn from QTL mapping for agronomic traits under high temperature suggest that the A genome is the major contributor of QTL for the traits. Present study and previous reports on grain yield and related traits suggest that the A genome accessions can be further investigated to identify the donors of QTL associated with stress tolerance. The QTL for yield traits and maximum rate of senescence were mainly on chromosomes 2A, 4A, and 6A. The AFLP and microsatellite markers, especially AGG.CTT-107, *Xgwm20*, *Xgwm296*, *Xgwm356* and *Xksum61*, which were linked to yield and stay-green, can be used in marker-assisted breeding programs to select genotypes having tolerance to high temperature. Alleles with highest LOD scores and largest additive effects for GFD and TKW were from Ventnor. Alleles for kernels per spike and GFR were from Karl 92. All alleles linked to maximum rate of senescence with a significant LOD score were contributed by Karl 92. Although Ventnor has more heat tolerance than Karl 92 it is possible that Karl 92, a native cultivar has some level of heat tolerance. The AFLP marker

AGG.CTT-107 (P41/M62-107) on chromosome 2A can be converted to sequence tag site (STS) markers and used in selection, and the marker interval with QTL, which were flanked by markers about 2 cM on either side, can be enriched.

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CHAPTER 4 - Modeling and Mapping QTL for Stay-Green in a Recombinant Inbred Winter Wheat Population under High-Temperature Stress

Abstract

Senescence is a genetically programmed and environmentally influenced process resulting in the destruction of chlorophyll and remobilization of nutrients to younger or reproductive parts of plants. Delayed senescence, or stay-green, contributes to a long grain-filling period and stable yield under stress. Characterization of stay-green would facilitate development of cultivars with longer green leaf area duration. The objective of this study was to model the pattern of stay-green and map the quantitative trait loci (QTL) linked to the trait. The experiment was conducted on a recombinant inbred line (RIL) population at the filial-7 (F_7) generation that was derived from a cross between Ventnor, a heat-tolerant cultivar, and Karl 92, a relatively heat-susceptible cultivar. The RILs were grown under optimum temperature of 20/15°C (day/night) and were subjected to continuous heat stress of 30/25°C from 10 d after anthesis (DAA) until maturity. Visual observations of the green leaf area on a scale 0 to 10 were recorded every 3 days and statistically modeled to quantify percent greenness retained by the lines over the reproductive period. Chlorophyll content and fluorescence of leaves were also recorded at 10 and 16 DAA. Genetic characterization of stay-green was performed with microsatellite or simple sequence repeats (SSR), amplified fragment length polymorphism (AFLP), and a sequence tag site (STS) marker. Performance of RILs under heat stress for each of

the traits was significantly different compared to optimal conditions. Maximum rate of senescence was highly significantly and negatively correlated with yield traits. Based on the model, the stay-green traits were categorized into five groups. Most of the stay-green traits were mapped to chromosomes 2A and 6A under high temperature. Marker CGT.CTCG-146 (P58/MC84-146) was linked to time to 75% green under high temperature and time to maximum rate of senescence under optimum conditions. The QTL for different stay-green traits co-localized with markers CGT.GTG-343 (P58/M77-343), CGT.CTCG-146 (P58/MC84-146), AGG.CTT-107 (P41/M62-107), CGT.CTCG-406 (P58/MC84) and *Xbarc136*. The STS marker RZ876, which encodes for chloroplast elongation factor-Tu, was placed on 6AL of wheat (*Triticum aestivum* L.) between the markers AGG.CTT-212 (P41/M62-212) and GTG.ACGC-108 (P77/MA56). It showed synteny with the region of the maize genome linked to QTL for yield. It can be concluded that increased stay-greenness or decreased senescence will ultimately have a positive effect on grain yield under high temperature stress. A high correlation among the maximum rate of senescence, grain filling duration, and thousand kernel weight was also established. The maximum rate of senescence can be used as a secondary criterion for selecting genotypes under heat stress, and the markers linked to stay-green under high temperature can be used in breeding program.

Introduction

Senescence is internally programmed cell death and is affected by environmental factors like abiotic and biotic stresses (Buchanan-Wollaston, 1997; Noodén et al., 1997; Chandler, 2001). Senescence includes loss of chlorophyll content and a decline in photosynthetic capability

of the leaf. However, environmental factors can cause early loss of photosynthetic capacity in the life cycle of the plant and result in premature senescence. Abiotic stress, like heat during and after flowering, causes premature senescence, resulting in poor grain quality and loss of yield (Xu et al., 2000a; Jiang et al., 2004). Genotypes differ in their capacity to withstand stresses and retain their green leaf area. Stay-green refers to delayed senescence during post-anthesis stages of plant development (Thomas and Howarth, 2000). Stay-green may act either by delaying the onset of senescence or inducing a slower rate of senescence (Thomas and Smart, 1993). Photosynthesis in wheat contributes 80-90% of assimilates for grain filling under optimum temperature conditions (Evans et al., 1975). Therefore, premature senescence and the rate of senescence may be important factors determining plant yield potential (Gentinetta et al., 1986; Evans, 1993; Thomas and Howarth, 2000). In many crop species, stay-green plants have better quality of foliage, higher chlorophyll content, and greater resistance to pests and diseases (Ambler et al., 1987; Thomas and Smart, 1993; Xu et al., 2000a). Delayed senescence in sorghum (*Sorghum bicolor* L. Moench) (Borrell et al., 2000a; 2000b), maize (*Zea mays* L.) (Baenziger et al., 1999), and durum wheat (*Triticum turgidum durum* Desf. Husn) (Benbella and Paulsen, 1998; Hafsi et al., 2000) contributed to increased yields in water-stressed environments. Broad sense heritability was found to be 0.72 in sorghum by Crasta et al. (1999).

Most studies across plant species indicate that stay-green is a quantitative trait. Using a RIL population in sorghum, Xu et al. (2000b) found three major QTL for stay-green. In further analysis, they found that those regions contained genes for key photosynthetic enzymes, heat shock proteins, and ascorbic acid response. Mapping of the stay-green trait in sorghum indicated the presence of four QTL on linkage groups A, D, and E by Sanchez et al. (2002); on linkage groups A, E, and G by Haussamann et al. (2002); on linkage groups A, G, and J by Kebede et al.

(2001); on linkage groups B, and I by Tao et al. (2000); on linkage groups A, D, and J by Subudhi et al. (2000) and Xu et al. (2000b); on linkage groups A, D, and G by Crasta et al. (1999); and on linkage groups B, F, I, G, and H by Tuinstra et al. (1997). One of the stay-green QTL, *stg2*, showed a significant epistatic interaction with a region on linkage group C containing markers closely linked to chlorophyll content (Subudhi et al., 2000). Xu et al. (2000a) found that the QTL for chlorophyll content were present in the same region on linkage group A and D as those for stay-green. In a rice (*Oryza sativa* L.) stay-green mutant, the phenotype was controlled by a single recessive nuclear gene, *sgr (t)*. That gene was mapped to the long arm of chromosome 9 (Cha et al., 2002). Jiang et al. (2004) found 46 main effect QTL distributed on all 12 chromosomes of rice and individual QTL that had small effects. Bertin and Gallais, (2001) reported stay-green QTL on chromosome 10 of maize. In winter wheat, QTL associated with flag leaf senescence was detected on the long arms of chromosome 2D under drought stress and 2B under irrigated conditions (Verma et al., 2004). However, QTL influencing stay-green under high temperature in wheat and other grass genomes have not been mapped to date.

Interactions between different stress responses, along with the inability to evaluate stay-green until the plant matures, limits progress in traditional breeding (Xu et al., 2000b). Molecular markers increase breeding efficiency and reduce the time and cost of field trials (Tanksley, 1993). Comparative genomics can be used, along with regular mapping, to increase efficiency of mapping across genomes.

The objective of this study was to do a comprehensive study of the stay-green trait in response to heat stress, identify and map the QTL linked to the senescence related traits using a RIL population of winter wheat.

Materials and Methods

Plant material

A recombinant inbred line (RIL) population in a filial (F_7) generation was derived from a cross between Ventnor (a heat-tolerant hard white Australian winter wheat) and Karl 92 (a hard red winter wheat from the USA). The seed for each of the RILs was germinated. After the seedlings reached 2.5 cm, they were vernalized at 5°C for 6 weeks. The vernalized seedlings were transplanted to pots (10x25-cm). Each pot contained one plant. The plants were grown as single culms under controlled conditions. The optimum temperature chambers were set at 20/15°C (day/night), 50/70% relative humidity, 16-h photoperiod, and light intensity of 420 μmol m^{-2} s^{-1}, as suggested by Yang et al. (2002). Spikes were labeled when 50% reached anthesis. At 10 DAA, the replicates of RILs were transferred to high temperature chambers and maintained under those conditions until harvest. The high temperature chambers were set at 30/25°C, with all other conditions remaining the same as the optimum chambers. Peters professional fertilizer was applied to each pot once every month to supply 100 mg N, 43 mg P, and 87 mg K (Peters Professional Plant Food, W.R. Grace & Co., Fogelsville, PA). Plants were watered and randomized regularly. Three replicates of the inbred lines were studied in sequential order, and the experimental design used was split plot. The experiment was a blocked on time with growth chambers as the experimental units for temperature and pots as the experimental units for the RILs.

Traits measured

Green leaf area duration (Stay-green)

Stay-green was estimated visually and given a rating of 0 to 10, where 10 was 100% green leaf area and 0 was complete senescence. The plants were rated across all their leaves. Green leaf area scores were recorded at 3-d intervals starting from 10 DAA to physiological maturity. A non-linear regression curve was fitted on the recorded data using a Gompertz statistical model (Seber and Wild, 1989). The green leaf areas observed at different times were converted to percentage green using the model, and traits related to progression of senescence were estimated as: i) time interval between complete green and 75% green, ii) time interval between complete green and 25% green, iii) maximum rate of senescence, iv) time to maximum rate of senescence, v) time interval between 75% and 25% green, and vi) percent green at maximum senescence.

The model used in the analysis is as follows:

$$Y = \alpha \{ 1- e^{-e^{[-b (time - c)]}} \}$$

Where Y is the response variable, α relates to the point where plants leaves are completely green, b relates to the degree of curvature of the curve between time to senescence and visual scale for green leaf area duration, and c relates to the point of time of maximum senescence. The maximum rate of senescence was estimated as the mid-point of the curve with maximum slope.

The empirical graph plotted between time in days and the green leaf area duration measured as a visual observation on a 0 to 10 scale shows percentage greenness at different points of time (Figure 1). The curve was extrapolated to determine the initial point when the plant was 100% green; therefore, though observations started at 10 DAA, the time and condition of the plant

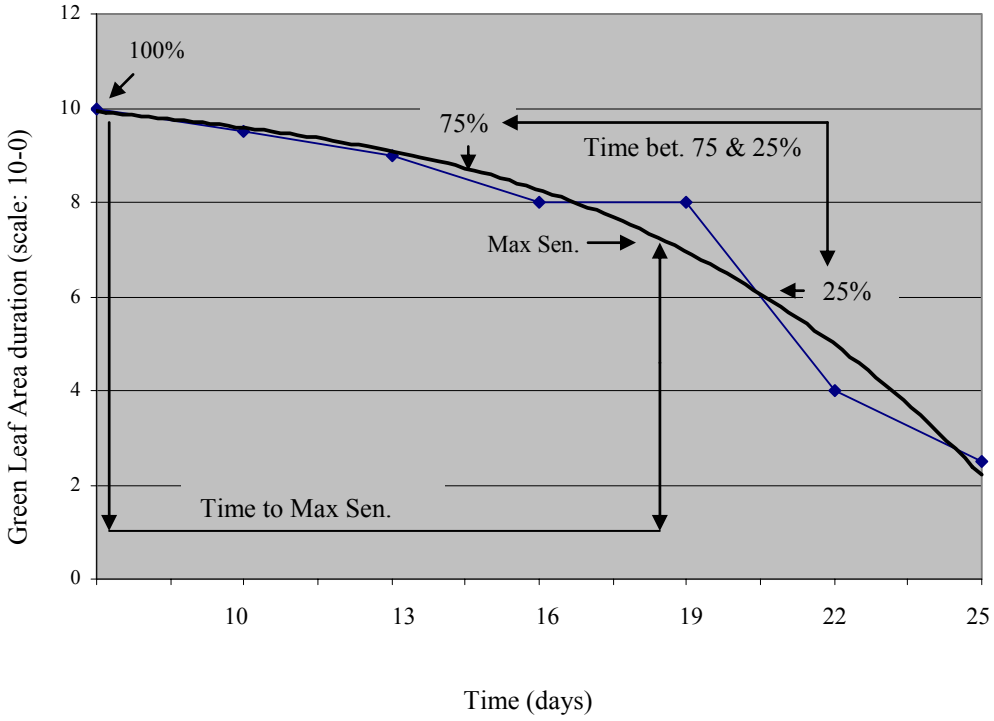

Figure 4-1. Non-linear regression model fitted over green leaf area duration of a wheat RIL population visualized on a scale of 0 to 10, and time in days obtained from the Gompertz model. Stay-green traits were estimated as: i) time interval between complete green and 75% green, ii) time interval between complete green and 25% green, iii) maximum rate of senescence, iv) time to maximum rate of senescence, v) time interval between 75% and 25% green, and vi) percent green at maximum senescence.

before 10 DAA were also deduced. For accuracy, only the central part of the curve was used.

Chlorophyll content (SDAP)

Chlorophyll content was estimated using a Model 502 SPAD meter (Minolta, Plainfield, IL). The readings were taken about 5 cm from the base of the abaxial surface of the flag leaf at two different times. The first time was 10 DAA, and the second 16 DAA. Data were recorded for both optimum and high-temperature conditions. The SPAD units at 10 DAA were subtracted from SPAD units at 16 DAA under both optimum and heat-stress conditions. Differences between SPAD units observed at the two dates were used in the analysis.

Chlorophyll fluorescence (Fv/Fm)

Chlorophyll fluorescence was estimated with a Chlorophyll fluorescence meter (Fluorescence Monitoring System, Hansatech Instruments, Norfolk, England). The readings were taken similar to the SPAD measurements at 10 and 16 DAA. Data were recorded for both optimum and high-temperature conditions. The Fv/Fm ratio observed at 10 DAA was subtracted from the ratio at 16 DAA under both. Differences between the ratios at the two dates were used in the analysis.

Statistical analysis

Analysis of variance and least square means for all the stay-green traits were estimated using Proc. Mixed, and entry means were estimated using Proc. GLM (general linear model). Correlations of all traits were performed by Pearson's correlation in the statistical procedure

Proc. Corr., and non-linear regressions were determined by the Gompertz model. Statistical software SAS Version 8.2 was used for all procedures (SAS Inst. Inc., 1990).

Molecular markers and map development

The DNA was extracted from RILs and the two parents using the CTAB extraction method (Saghai Maroof et al., 1984). Molecular markers, mainly SSRs, AFLPs, and primers designed for STS that corresponded to the chloroplast elongation factor (EF-Tu) expressed under heat stress in maize, were used to construct a genetic linkage map. Microsatellites markers are tandem repeats of oligonulceotides like $(GA)_n$ or $(GT)_n$. The markers used in the experiment were comprised of GWM (Röder et al., 1998), BARC (Song et al., 2002), CFD and CFA (Sourdille et al., 2001), WMC (Gupta et al., 2002), GDM (Pestsova et al., 2000), and KSUM and KSM (Singh et al., 2000) primer sets.

The polymerase chain reaction (PCR) amplifications were done in an MJResearch Thermal Cycler (Bio-Rad formerly MJ Research, Hercules, CA). The PCR reaction mixture used for BARC markers had a total volume of 25 µL consisting of 150 ng genomic DNA, 2.6 µL 10X PCR buffer, 2.48 mM $MgCl_2$, 0.24 mM dNTPs, 1 unit *Taq* DNA polymerase and 20 ng primer (forward + reverse). The thermocycler program consisted of an initial denaturation step at 95°C for 3 min, followed by 34 cycles of 40 sec at 94°C, 40 sec at annealing temperature, and 1 min at 72°C followed by final extension temperature at 72°C for 10 min. For all other markers, the PCR reaction mixture contained a total 25 µL consisting of 150 ng genomic DNA, 2.5 µL 10X PCR buffer, 1.5 mM $MgCl_2$, 0.30 mM dNTPs, 1 unit *Taq* DNA polymerase, and 20 ng primer (forward + reverse). The program used consisted of an initial denaturation at 95°C for 3 min., and

initial two cycles at an annealing temperature of 62°C, followed by 34 cycles of 1 min at 94°C, 1 min at annealing temperature and 2 min at 72°C followed by final extension at 72°C for 10 min. The SSR markers were run on either a 3% SFR agarose (Midwest Scientific, St. Louis, MO) gel at 70 volts and visualized by staining with ethidium bromide under ultra violet illumination or run at 80 watts on a 6% polyacrylamide denaturing gel modified with formamide solution and urea to enhance the resolution of the markers. The gel denaturing mixture contained 15 mL double-distilled water, 32 mL formamide, 33.6 g urea, 10 mL 10X Tris-boric acid-disodium ethylenediaminetetraacetic acid (TBE) solution, 17.5 mL Bis/acrylamide (19:1 polyacrylamide, 40%, ISC BioExpress). 1 mL 10% ammonium persulfate, and 40 µL N,N,N',N'-Tetramethylethylenediamine (TEMED) were mixed with the gel mix prior to pouring the gel to drive polyacrylamide polymerization. The gel was cast and run on a BioRad Sequi-Gen GT sequencing system (Bio-Rad, Hercules, CA) for 2 h after an initial pre-run of 20 min, and the bands were visualized by silver staining (Bassam et al., 1991). For the STS marker, the PCR reaction mixture and thermocycler program were the same as that for the BARC markers. The STS marker were run on a single stand conformational polymorphism (SSCP) gel at 3 watts for about 14 h (Martins-Lopes et al., 2001), and bands were visualized by silver staining. All the markers were scored as parental type (A or B), heterozygote (H), or missing data (-).

The AFLP reactions and DNA template preparation for *PstI/MseI* fragments were as described by Vos et al. (1995) with some modifications. In brief, *PstI* (six-base cutter methylation sensitive) and *MseI* (four-base cutter) enzymes were added to 300 ng genomic DNA and incubated for 2 h at 37°C. The enzymes were subsequently heat inactivated at 70°C for 15 min. This was followed by the ligation of *PstI* (adapter 1, 5'-CTCGTAGACTGCGTACATGCA-'3; adapter 2, 5' -TGTACGCAGTCTAC-'3) and *MseI* (adapter1, 5' -GACGATGAGTCCTGAG-

‘3; adapter 2, 5’-TACTCAGGACTCAT- ‘3) overnight at 20°C. The samples were diluted 10-fold with distilled water. Pre-amplification of the diluted DNA template was then performed with AFLP primers having 0 selective nucleotides for *PstI* + 0 (5’ –GACTGCGTACATGCAG- ‘3) in combination with *MseI* +0 (5’ -GATGAGTCCTGAGTAA- ‘3). The PCR reaction mixture was diluted to 40 µL consisting of 10 µL diluted DNA template, 1x PCR buffer, 0.75 units *Taq* polymerase (Promega Corp., Madison, WI), 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.75 µL 100 ng/µl *PstI* pre-amplification primer (5’ –GACTGCGTACATGCAG- ‘3), and 0.75µL 100 ng/µl *MseI* pre-amplification primer (5’ –GATGAGTCCTGAGTAA- ‘3). Pre-amplification reaction was performed for 30 cycles of 30 sec at 94° C, 1 min at 56° C, and 1 min at 72° C. The pre-amplification product was diluted 10-fold to get a final DNA concentration of 25 pg/µL. The DNA was arrayed in a 64-well plate for use with a Li-Cor gel system (LI-COR Bioscience, Lincoln, NE).

Selective amplification reactions were performed using primers with three or four selective nucleotides (*6PstI* + NNN and *MseI* +NNN) resulting in 59 unique primer combinations. Infra-red dye (IRD)-labeled *PstI* primers were obtained from Li-Cor Inc. The final volume of the PCR reaction mixture for selective amplification was 10 µL consisting of 2µL diluter pre-amplification product, 1x PCR buffer, 0.2 units of *Taq* polymerase, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.35 µL 50 ng/µl *MseI* selective primer, and 0.4 µL 1pmole/µl IRD-*PstI* selective primer. Selective amplification was performed as follows: 2 min at 94° C followed by 13 cycles of 30 sec at 94°C, 30 sec annealing at 65°C, and 1 min at 72°C, lowering the annealing temperature by 0.7°C after each cycle, followed by 23 cycles of 30 sec at 94°C, 30 sec at 56°C, 1 min at 72°C, and extension of 5 min at 72°C. Li-Cor loading buffer (5µL) was added to the PCR products and denatured for 3 min at 95°C. The AFLP product was analyzed with a Li-Cor model

4200L-2 dual-dye automated DNA sequencing system. Amplified product (1μL) was loaded on a KB 6.5% gel matrix (Li-Cor Inc.).The bands obtained were size-matched with a Li-Cor 50-700 base pair sizing marker labeled with 700 and 800 IRD dye and scored using SAGA-AFLP analysis soft ware (Li-Cor Inc.). All the markers were scored as parental type (A or B), heterozygote (H), or missing data (-).

Data analysis and map construction

The population was genotyped with 450 markers which included 259 AFLPs, 189 SSRs, and a STS. The AFLP markers consisted were derived from 59 *PstI* and *MseI* primer combinations, and generated a total of 259 dominant scorable loci (Table 1). Heterozygotes in the population were represented as missing. Raw data were compiled in the Mapmaker/EXP format. Linkage analysis was conducted using Mapmaker/EXP for UNIX version 3 (Whitehead Institute, Cambridge, MA). The 'ri-self' setting was used. A Chi-square test was performed to test the markers for 1:1 segregation ratio, followed by two-point analysis of the markers. Twenty-one chromosomes (linkage groups) were defined with the 'make chromosome' command. Markers were anchored to these linkage groups. These linkage groups were compared with the microsatellite consensus map of Somers et al. (2004) to designate specific chromosomes. To assign the markers to the chromosomes, 'default linage criteria' was set with a LOD grouping of 6 and a maximum recombination distance of 30 cM (Kosambi units) between the markers. Markers with significant segregation distortion were excluded. To eliminate possibility of spurious linkage. Within the linkage group, commands 'compare', 'order' and 'ripple' were used. The initial anchored markers were determined based on published consensus maps and subsequently, other markers were assigned to linkage groups. These groups were then

Table 4-1. List of *PstI* and *MseI* AFLP primers combinations (designated by their standard codes) used in the construction of wheat genetic linkage maps. Three or four selective bases were used for selective amplification reactions. 259 scorable loci were obtained from the primer combinations.

AFLP Primer	Primer Combination	Standard Code	Scorable Loci
p-AGG/m-ACC	Pst1+AGG/Mse1+ACC	P41/M36	6
p-AGG/m-CGAT	Pst1+AGG/Mse1+CGAT	P41/MC66	1
p-AGG/m-CGTA	Pst1+AGG/Mse1+CGTA	P41/MC75	6
p-AGG/m-CTG	Pst1+AGG/Mse1+CTG	P41/M61	7
p-AGG/m-CTT	Pst1+AGG/Mse1+CTT	P41/M62	5
p-AGG/m-GCAG	Pst1+AGG/Mse1+GCAG	P41/MG49	6
p-AGG/m-GCAT	Pst1+AGG/Mse1+GCAT	P41/MG50	2
p-CAG/m-AGC	Pst1+CAG/Mse1+AGC	P49/M40	6
p-CAG/m-AGCT	Pst1+CAG/Mse1+AGCT	P49/MA70	3
p-CAG/m-CAG	Pst1+CAG/Mse1+CAG	P49/M49	1
p-CAG/m-CTC	Pst1+CAG/Mse1+CTC	P49/M60	1
p-CAG/m-GCG	Pst1+CAG/Mse1+GCG	P49/M69	6
p-CAG/m-GCAT	Pst1+CAG/Mse1+GCAT	P49/MG50	2
p-CGA/m-ACAG	Pst1+CGA/Mse1+ACAG	P55/MA49	6
p-CGA/m-ACAG	Pst1+CGA/Mse1+ACAG	P55/MA49	4
p-CGA/m-ACGC	Pst1+CGA/Mse1+ACGC	P55/MA56	3
p-CGA/m-AGAC	Pst1+CGA/Mse1+AGAC	P55/MA64	4
p-CGA/m-CAG	Pst1+CGA/Mse1+CAG	P55/M49	3
p-CGA/m-CAT	Pst1+CGA/Mse1+CAT	P55/M50	10
p-CGA/m-CGCT	Pst1+CGA/Mse1+CGCT	P55/MC70	11
p-CGA/m-CTC	Pst1+CGA/Mse1+CTC	P55/M60	2
p-CGA/m-GAC	Pst1+CGA/Mse1+GAC	P55/M64	10
p-CGA/m-GTG	Pst1+CGA/Mse1+GTG	P55/M77	7
p-CGT/m-ACGT	Pst1+CGT/Mse1+ACGT	P58/MA58	4
p-CGT/m-AGCT	Pst1+CGT/Mse1+AGCT	P58/MA70	11
p-CGT/m-ATGC	Pst1+CGT/Mse1+ATGC	P58/MA88	10
p-CGT/m-CAG	Pst1+CGT/Mse1+CAG	P58/M49	1

p-CGT/m-CAT	Pst1+CGT/Mse1+CAT	P58/M50	4
p-CGT/m-CGAT	Pst1+CGT/Mse1+CGAT	P58/MC66	1
p-CGT/m-CGTA	Pst1+CGT/Mse1+CGTA	P58/MC75	3
p-CGT/m-CTCG	Pst1+CGT/Mse1+CTCG	P58/MC84	4
p-CGT/m-CTG	Pst1+CGT/Mse1+CTG	P58/M61	7
p-CGT/m-CTGA	Pst1+CGT/Mse1+CTGA	P58/MC87	5
p-CGT/m-GCAG	Pst1+CGT/Mse1+GCAG	P58/MG49	2
p-CGT/m-GAC	Pst1+CGT/Mse1+GAC	P58/M64	7
p-CGT/m-GTG	Pst1+CGT/Mse1+GTG	P58/M77	6
p-CGT/m-TGCG	Pst1+CGT/Mse1+TGCG	P58/MT69	2
p-CTC/m-CTA	Pst1+CTC/Mse1+CTA	P60/M59	4
p-CTCG/m-ACC	Pst1+CTCG/Mse1+ACC	PC85/M36	9
p-CTCG/m-AGC	Pst1+CTCG/Mse1+AGC	PC85/M40	4
p-CTCG/m-CTG	Pst1+CTCG/Mse1+CTG	PC85/M61	3
p-CTCG/m-CTT	Pst1+CTCG/Mse1+CTT	PC85/M62	1
p-GCTG/m-CAG	Pst1+GCTG/Mse1+CAG	PG61/M49	1
p-GTG/m-ACAG	Pst1+GTG/Mse1+ACAG	P77/MA49	3
p-GTG/m-ACGC	Pst1+GTG/Mse1+ACGC	P77/MA56	3
p-GTG/m-ACGT	Pst1+GTG/Mse1+ACGT	P77/MA58	3
p-GTG/m-AGC	Pst1+GTG/Mse1+AGC	P77/M40	6
p-GTG/m-AGCT	Pst1+GTG/Mse1+AGCT	P77/MA70	7
p-GTG/m-ATGC	Pst1+GTG/Mse1+ATGC	P77/MA88	1
p-GTG/m-CGAC	Pst1+GTG/Mse1+CGAC	P77/MC64	6
p-GTG/m-CGCT	Pst1+GTG/Mse1+CGCT	P77/MC70	1
p-GTG/m-CTA	Pst1+GTG/Mse1+CTA	P77/M59	4
p-GTG/m-CTGA	Pst1+GTG/Mse1+CTGA	P77/MC87	1
p-GTG/m-TGCG	Pst1+GTG/Mse1+TGCG	P77/MT69	1
p-TGC/m-AGC	Pst1+TGC/Mse1+AGC	P88/M40	2
p-TGC/m-AGCT	Pst1+TGC/Mse1+AGCT	P88/MA70	7
p-TGC/m-CGAC	Pst1+TGC/Mse1+CGAC	P88/MC64	5
p-TGC/m-CTG	Pst1+TGC/Mse1+CTG	P88/M61	5
p-TGC/m-GCG	Pst1+TGC/Mse1+GCG	P88/M69	3
	Total	59	259

ordered and validated with the 'order' and 'ripple' commands. An order was accepted only when its likelihood was 100 times more than the likelihood of the best alternative order. The best order was designated as the framework for each linkage group. Other markers that were assigned to the group and not included in the framework map were placed on to the map in a decreasing order of informativeness. Map distances were compared with 'error detection on' and 'error detection off'. These procedures were repeated several times to decrease the chances of error. Finally, all the markers were assigned to the chromosomes using the 'place' command, and the placement markers were placed at odds between 1000:1 and 100:1.

QTL analysis

The QTL cartographer version 2.0 mapping program (Zeng, 1994; Basten et al., 2002) was used for QTL analysis. QTL linked to the framework markers were detected using forward and backward regression options of composite interval mapping (CIM). The significance peak with the highest LOD score was recorded, as they indicate the presence of QTL at the given loci. Additive effects of the QTL and the variability accounted by the QTL were also estimated using the Cartographer program.

Comparative mapping

To cross-link different genomes of the grass species with respect to the traits studied, comparative genetic mapping was performed. Comparative mapping used the tools provided in

the Gramene web-site (www.gramene.org/db/searches/browser), and synteny was established between wheat, sorghum, rice, and maize genomes.

Results

Analysis of variance and means for stay-green traits

Analysis of variance indicated that stay-green traits differed for the regimens of 20/15°C vs. 30/25°C at $\alpha = 0.001$ (Table 2). The SPAD and Fv/Fm also differed significantly between temperature regimes. Differences between the replications were mostly non-significant. The RILs differed significantly at $\alpha \leq 0.05$ for all different stages of green leaf area duration, SPAD, and Fv/Fm. Treatment X entry interactions were significant at $\alpha < 0.05$ for maximum rate of senescence, time to maximum rate of senescence, time between 75% and 25% green, SPAD, and Fv/Fm. Performance of the RILs differed significantly within each treatment, except for SPAD and Fv/Fm. Interactions in each of the cases (not shown) except for SPAD were non-crossover type, indicating that the lines that retained greenness for longer time under optimum conditions did the same under high temperature. The interaction for SPAD appeared to be a crossover type, indicating that certain lines were better performers in heat than under optimum conditions.

The RILs were derived from parents with contrasting physiological characteristics. Ventnor, the tolerant parent, had broader and longer leaves than Karl 92 and remained green longer and matured later than Karl 92. The percent greenness of the leaves at maximum senescence was 64.7% for Ventnor, 56.4% for Karl 92, and 54.5% for the RILs. As stated in earlier reports,

Effect	DF	Time to75% green (d)	Time to 25% green (d)	Time bet.75 & 25% green (d)	Max Sen.	Time to Max Sen. (d)	Percent green at Max Sen.	SPAD	Fv/Fm
Treatment (Optimum vs. Stress)	1	25027.319***	483971.836***	288885.386***	38927.022***	72814.423***	76119.369***	756.678***	0.212***
Replicates	2	330.279**	2998.218^{NS}	1734.354^{NS}	97.123^{NS}	599.024*	353.685^{NS}	6.486^{NS}	0.476^{NS}
Entry RILs	103	76.100*	2113.539***	1797.203**	178.113***	203.538***	323.572***	29.914**	0.009*
Treatment*Entry	103	58.441^{NS}	1688.581^{NS}	1487.106**	145.695***	152.311*	171.507^{NS}	26.892*	0.009**
Optimum	103			3046.896**	20.860***	328.328**		5.258^{NS}	0.003^{NS}
Stress	103			213.797**	300.467***	24.174**		51.125**	0.014**
Error	404	55.306	1159.600	1069.486	97.973	108.678	155.530	19.006	0.007

Table 4-2. Analysis of variance for green leaf area duration at different stages of greenness, maximum rate of senescence (Max Sen.), percent green at maximum rate of senescence, chlorophyll content (SPAD), and chlorophyll fluorescence (Fv/Fm) measured in the recombinant inbred population derived from a cross between Ventnor and Karl 92.

Ventnor also had stable chlorophyll content (SPAD) and higher chlorophyll fluorescence (Fv/Fm) than Karl 92 (Al-Khatib and Paulsen, 1990). Under optimum conditions (Table 3) the maximum rates of senescence of 0.1 for Ventnor and 0.4 for Karl 92 indicated longer leaf area duration for Ventnor than Karl 92. Chlorophyll content and fluorescence was calculated as the change in SPAD and Fv/Fm from 10 to 16 DAA under optimum conditions, negative mean values indicated decrease in the trait value over an interval of 6 days. Chlorophyll content change from 10 to 16 DAA was 0.2 SPAD units for Ventnor and -0.1 SPAD units for Karl 92, and chlorophyll fluorescence was 0.004 for Ventnor and 0.017 for Karl 92, indicating that Ventnor had higher chlorophyll content and less damage to the pigment than Karl 92. In the population, some lines had higher chlorophyll fluorescence, while others had lower at 16DAA. Therefore the overall mean Fv/Fm value was negative.

Mean values for green leaf area duration at different stages, maximum rate of senescence, time to maximum rate of senescence, percent green at maximum senescence, chlorophyll content (SPAD), and chlorophyll fluorescence (Fv/Fm) in the inbred lines were intermediate between the values of parents under high temperature (Table 4). Mean SPAD units of -0.7 and -4.8 and fluorescence ratios of -0.026 and -0.113 for Ventnor and Karl 92, respectively, indicate that though values decreased in both parents under heat stress at 16 DAA, the change was greater in Karl 92 than Ventnor.

Least square means for different stages of green leaf area duration represented by a small sample of the RIL population indicated the trend in the rate of senescence. The sample population showed that senescence could be broadly categorized as slow, exemplified by RIL 111 or 92; moderate as in RIL 169 or 163; and very high as in RIL 180 (Table 5). Under high-temperature

Table 4-3. Mean green leaf area duration at different stages, maximum rate of senescence (Max Sen.), percent green at maximum senescence, chlorophyll content (SPAD), and chlorophyll fluorescence (Fv/Fm) of the recombinant inbred population and parents under optimum conditions.

Entry	Trait	Mean	Std. Dev.	Std.Er.	Minimum	Maximum	Range
RILs	Time to 75% green (d)	23.8	10.63	0.60	4.81	53.41	48.60
	Time to 25% green (d)	70.9	51.72	2.93	9.60	184.59	174.99
	Time bet. 75 & 25% green (d)	47.1	49.00	2.77	0.23	157.25	157.02
	Max Sen.	1.3	3.50	0.20	0.04	23.82	23.78
	Time to Max Sen (d)	33.6	15.81	0.90	9.42	60.00	50.58
	Percent green at Max Sen.	54.5	16.44	0.94	25.65	81.86	56.21
	SPAD	0.4	2.38	0.14	-14.00	8.60	22.60
	Fv / Fm	-0.001	0.06	0.00	-0.71	0.16	0.87
PARENTS							
Ventnor	Time to 75% green (d)	26.4	13.17	7.60	11.67	36.96	25.30
	Time to 25% green (d)	94.3	29.68	17.14	60.06	112.04	51.98
	Time bet. 75 & 25% green (d)	67.9	40.08	23.14	23.09	100.37	77.28

	Max Sen.	0.1	0.10	0.06	0.07	0.24	0.17
	Time to Max Sen. (d)	40.5	7.48	4.32	32.52	47.34	14.82
	Percent green at Max Sen.	64.7	15.46	8.92	47.85	78.23	30.38
	SPAD	0.2	0.49	0.28	-0.10	0.80	0.90
	Fv / Fm	0.004	0.02	0.01	-0.01	0.02	0.03
Karl92	Time to 75% green (d)	24.6	6.56	3.79	17.87	30.97	13.10
	Time to 25% green (d)	81.0	61.32	35.40	31.13	149.45	8.32
	Time bet. 75 & 25% green (d)	56.4	66.33	38.29	6.19	131.59	125.40
	Max Sen.	0.4	0.46	0. 27	0.05	0.91	0.85
	Time to Max Sen. (d)	36.3	9.54	5.51	26.23	45.20	18.97
	Percent green at Max Sen.	56.4	20.29	11.72	36.81	77.32	40.51
	SPAD	-0.1	2.81	1.62	-3.30	1.90	5.20
	Fv / Fm	0.017	0.02	0.01	-0.01	0.03	0.03

Table 4-4. Mean green leaf area duration at different stages, maximum rate of senescence (Max Sen.), percent green at maximum senescence, chlorophyll content (SPAD), and chlorophyll fluorescence (Fv/Fm) of the recombinant inbred population and parents under high temperature.

Entry	Trait	Mean	Std. Dev.	Std.Er.	Minimum	Maximum	Range
RILs	Time to 75% green (d)	11.1	2.56	0.15	2.12	25.46	23.34
	Time to 25% green (d)	14.8	13.06	0.74	9.23	92.30	83.06
	Time bet. 75 & 25% green (d)	3.7	11.96	0.68	0.09	78.63	78.53
	Max Sen.	17.3	14.97	0.85	0.07	62.54	62.47
	Time to Max Sen. (d)	11.9	4.13	0.23	5.59	31.76	26.16
	Percent green at Max Sen.	32.3	10.14	0.58	25.30	77.74	52.43
	SPAD	-1.8	6.28	0.36	-54.80	7.40	62.20
	Fv / Fm	-0.037	0.11	0.01	-0.68	0.22	0.89
PARENTS							
Ventnor	Time to 75% green (d)	14.3	1.57	0.91	12.68	15.81	3.13
	Time to 25% green (d)	19.5	1.93	1.11	17.94	21.64	3.69
	Time bet. 75 & 25% green (d)	5.1	2.07	1.20	3.01	7.15	4.14

	Max Sen.	1.3	0.55	0.32	0.81	1.87	1.06
	Time to Max Sen. (d)	15.4	1.42	0.82	13.77	16.44	2.67
	Percent green at Max Sen.	40.5	5.33	0.01	34.51	44.64	0.05
	SPAD	-0.7	1.45	0.84	-2.10	0.80	2.90
	Fv / Fm	-0.026	0.03	0.02	-0.05	0.01	0.06
Karl92	Time to 75% green (d)	9.4	0.00	0.00	9.37	9.38	0.01
	Time to25% green (d)	9.6	0.01	0.00	9.60	9.61	0.01
	Time bet. 75 & 25% green (d)	0.2	0.00	0.00	0.23	0.24	0.01
	Max Sen.	23.5	0.49	0.28	22.93	23.84	0.91
	Time to Max Sen.(d)	9.4	0.00	0.00	9.42	9.43	0.01
	Percent green at Max Sen.	26.5	0.02	3.08	26.44	26.48	10.13
	SPAD	-4.8	5.84	3.37	-11.50	-1.00	10.50
	Fv / Fm	-0.113	0.17	0.10	-0.31	0.01	0.32

Table 4-5. Least square means calculated over three replicates to estimate the maximum rate of senescence (Max Sen.), time to maximum rate of senescence, and percent green at maximum senescence as exemplified by a small sample of inbred lines in the RIL population under high temperature.

Lines	Max Sen.	Time to max Sen. (d)	Percent green at Max Sen.
92	2.4	10.7	35.1
111	3.5	16.1	48.7
163	12.1	10.2	33.3
169	11.1	20.9	47.4
180	51.7	10.1	25.7
Ventnor	4.1	15.4	40.7
Karl 92	26.4	9.4	26.4

conditions, Ventnor was a better performer with a lower rate of senescence and longer green leaf area duration than Karl 92 and any of the RILs.

Non-linear regression modeling using Gompertz analysis

The curves plotted for the parents under optimum conditions (Figure 2) show that though both parents gradually proceed towards senescence, Ventnor had longer leaf area duration than Karl 92. The curves under high temperature showed accelerated senescence in both parents due to heat stress (Figure 3). The rate of senescence was much faster with a steeper curve in Karl 92 than Ventnor. The performance of RILs was studied with similar graphs (not shown) plotted under optimum and high-temperature conditions.

Correlation analysis for stay-green traits

Pearson's correlation among the maximum rate of senescence and different stages of greenness and SPAD were significant but negative at $\alpha = 0.05$, indicating that with the increase in senescence the green leaf area and chlorophyll content decreased (Table 6). The reverse was true for the correlation between time to maximum rate of senescence and different stages of greenness. In healthy leaves, the Fv/Fm ratio is close to 0.8, upon the onset of senescence Fv/Fm and chlorophyll content decreases. Therefore the correlation between SPAD and Fv/Fm were positive and significant at $\alpha < 0.001$. However, no significant correlation was found among the stay-green traits and Fv/Fm, indicating the importance of other factors influencing stay-green over Fv/Fm.

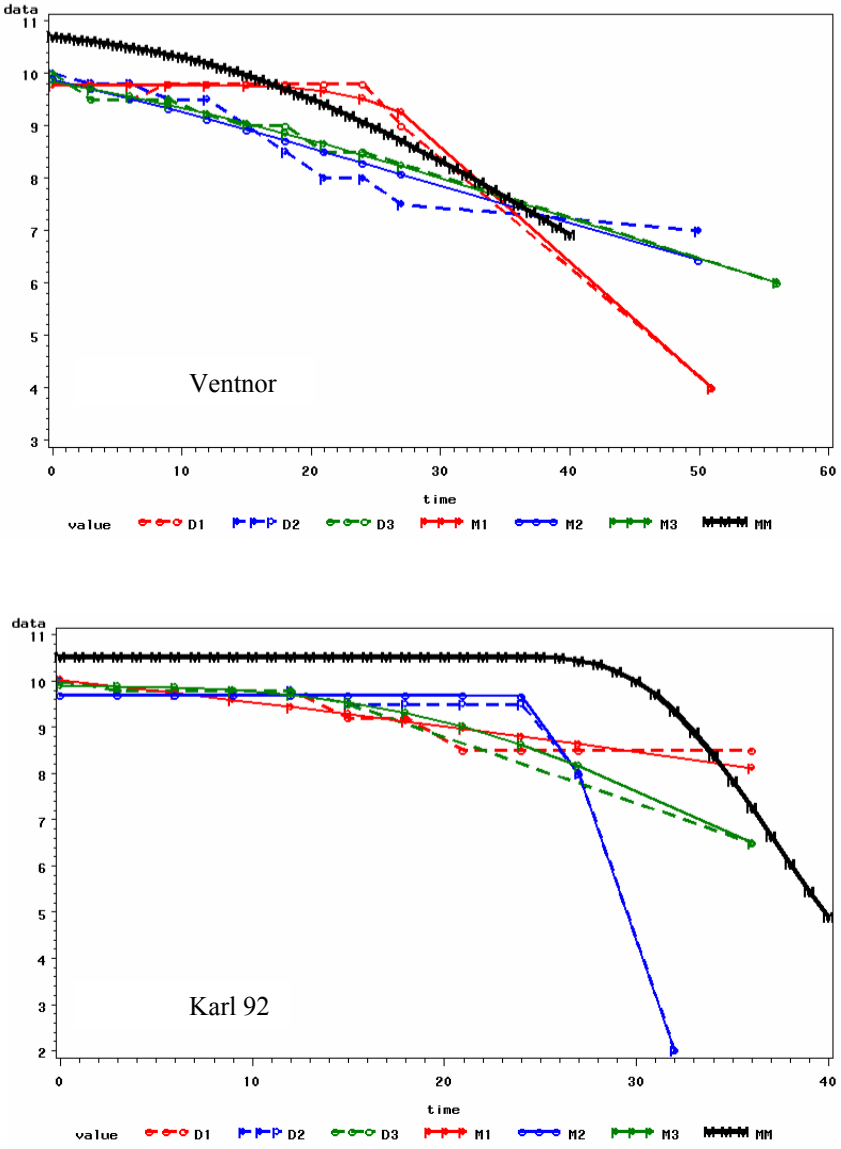

Figure 4-2. Non-linear regression curves fitted over the rating for stay-green (10-0) and time (days) in parents Ventnor and Karl 92 studied under optimum temperature. Time zero equals 10 DAA. Dotted lines (D1, D2, and D3) indicate the actual data points joined together, and the solid lines (M1, M2, and M3) in the same color represents the model fitted on the replicates. The solid black line represents the model fitted over the average of the three replicates.

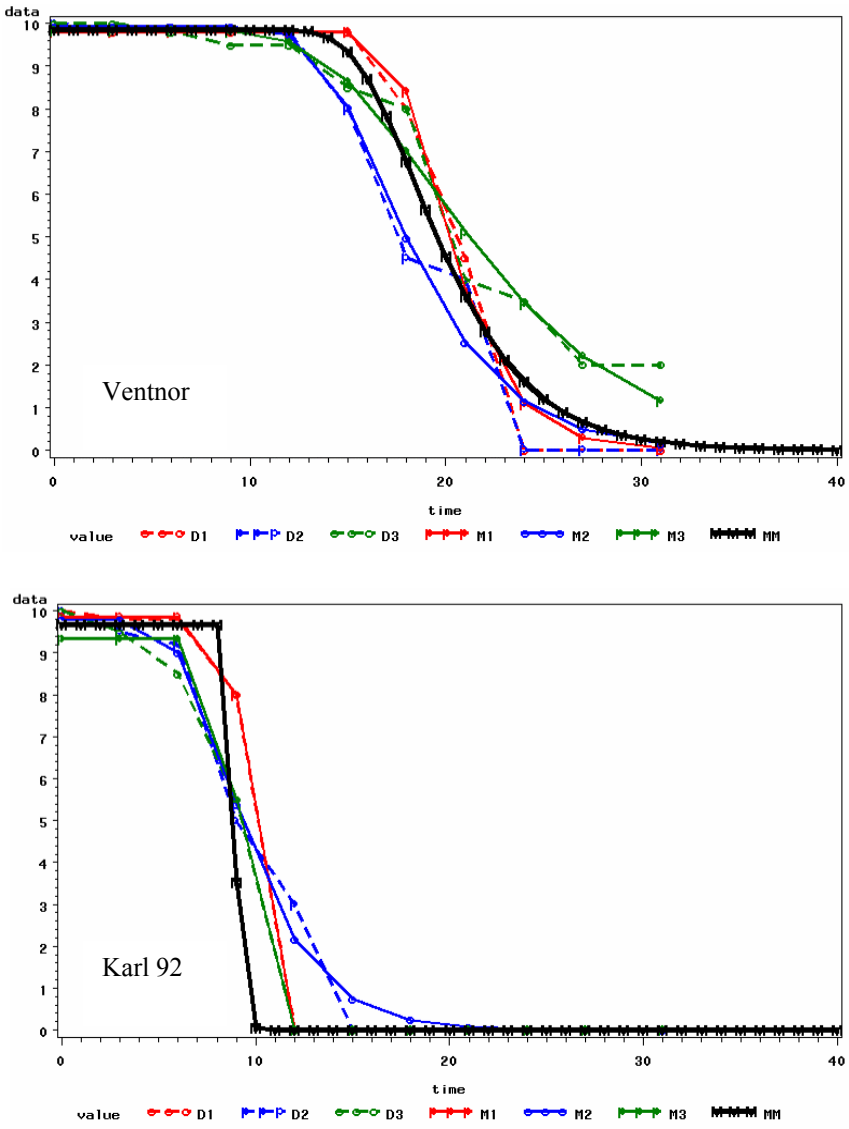

Figure 4-3. Non-linear regression curves fitted over the rating for stay-green (10-0) and time (days) in parents Ventnor and Karl 92 studied under high temperature. Time zero equals 10 DAA. Dotted lines (D1, D2, and D3) indicate the actual data points joined together, and the solid lines (M1, M2, and M3) in the same color represents the model fitted on the replicates. The solid black line represents the model fitted over the average of the three replicates.

Table 4-6. Pearson's correlation coefficients between maximum rate of senescence (Max Sen.), time to maximum rate of senescence, chlorophyll content (SPAD) and chlorophyll fluorescence (Fv/Fm) with green leaf area duration at different stages of greenness in RIL population under high temperature.

	Max Sen.	Time to Max Sen. (d)	SPAD	Fv / Fm
Time to 75% green (d)	-0.558***	0.810***	0.285**	0.180^{NS}
Time to 25% green (d)	-0.478***	0.903***	0.022^{NS}	-0.024^{NS}
Time bet. 75 & 25% green (d)	-0.402***	0.811***	-0.035^{NS}	-0.063^{NS}
Max Sen.	1.000	-0.592***	-0.226*	-0.090^{NS}
Time to Max Sen. (d)	-0.592***	1.000	0.154^{NS}	0.071^{NS}
Percent green at Max Sen.	-0.658***	0.832***	0.054^{NS}	-0.048^{NS}
SPAD	-0.226*	0.154^{NS}	1.000	0.439***
Fv / Fm	-0.090^{NS}	0.071^{NS}	0.439***	1.000

***, **, * significant at $\alpha = 0.001$, 0.01 and 0.05 respectively. NS = non significant.

Genetic characterization of stay-green traits

Genetic characterization of stay-green traits under optimum and heat stress (Table 7 and 8) was performed on a composite interval bases. All markers with a LOD score of 2.000 and above are listed. Total variability explained under optimum conditions for time to 75% green and maximum rate of senescence was to 66.9% and 66.2%, respectively. Time to 25% green and time to maximum rate of senescence had 41.7% and 49.1% of the total variability explained. For time between 75 and 25% green and percent green at maximum senescence, variabilities explained were 29.2% and 26.4%, respectively. The SPAD had 8.7%. of the variability explained. Under heat stress, total variabilities explained were 59.8% and 58.6% for time between 75 and 25% green and time to maximum rate of senescence, respectively. Maximum rate of senescence, percent green at maximum senescence, time to 75% green, time to 25% green and Fv/Fm, had 43.9%, 36.4%, 35.7%, 20.2%, and 11.2% variabilities explained, respectively. Most of the markers were linked to multiple stay-green traits.

Under optimum conditions, marker *Xgwm111* on chromosome 7D was linked to time to 25% green, time between 75 and 25% green, and time to maximum senescence, while marker *Xbarc121* on chromosome 7A was linked to time to 75% green, 25% green, and time to maximum senescence. Under heat stress, marker AGG.CTT-107 (P41/M62-107) on chromosome 2A was linked to time between 75 and 25% and time to maximum rate of senescence. Marker CGT.CTCG-146 (P58/MC84-146) on chromosome 3B was linked to time to maximum rate of senescence under optimum condition and to 75% green under heat stress. The traits 75% green and maximum rate of senescence had greatest amount of variability explained under optimum conditions, but only moderate levels of variability explained under high temperature. Time

Table 4-7. Genetic characterization of QTL linked to stay-green in RIL population under optimum conditions. The QTL linked to green leaf area duration at different stages, maximum rate of senescence (Max Sen.), percent green at maximum senescence, and chlorophyll content (SPAD) were detected by composite interval mapping. All markers having a LOD score of 2 and above are listed.

Trait	Chrom.	Marker	Position (cM)	LOD	Additive	Donor Parent	R^2	Total R^2 (%)
Time to 75% green	1B	Xbarc188	100.09	2.030	-1.937	Karl 92	0.077	
Time to 75% green	5A	CGA.CGCT-485	36.99	4.799	-4.368	Karl 92	0.302	
Time to 75% green	5B	Xbarc340	8.01	2.327	-3.046	Karl 92	0.217	
Time to 75% green	7A	Xbarc121	50.92	2.268	1.770	Ventnor	0.073	66.9
Time to 25% green	7A	Xbarc121	52.92	2.125	10.464	Ventnor	0.087	
Time to 25% green	7B	Xgwm577	131.79	3.203	-15.901	Karl 92	0.208	
Time to 25% green	7D	Xgwm111	2.01	2.520	-12.266	Karl 92	0.122	41.7
Time bet.75 & 25% green	4B	Xgwm368	20.53	3.092	-10.407	Karl 92	0.103	
Time bet.75 & 25% green	5D	Xgwm292	93.05	2.518	9.973	Ventnor	0.094	
Time bet.75 & 25% green	7D	Xgwm111	2.01	2.192	-10.043	Karl 92	0.095	29.2

Max Sen.	2A	GTG.ACGC-108	84.33	4.026	1.886	Karl 92	0.191	
Max Sen.	4A	GTG.CGCT-138	181.83	2.405	-0.838	Karl 92	0.092	
Max Sen.	5A	GTG.AGC-254	32.97	4.584	1.093	Ventnor	0.124	
Max Sen.	6A	CAG.AGC-101	70.86	6.392	-2.308	Karl 92	0.255	66.2
Time to Max Sen.	3B	CGT.CTCG-146	102.95	2.387	4.490	Karl 92	0.179	
Time to Max Sen.	7A	Xbarc121	50.92	2.887	3.173	Ventnor	0.085	
Time to Max Sen.	7B	Xbarc340	139.34	3.319	-3.729	Karl 92	0.123	
Time to Max Sen.	7D	Xgwm111	0.01	3.060	-3.460	Karl 92	0.104	49.1
Percent green at Max Sen.	4B	Xgwm368	20.53	4.658	-5.849	Karl 92	0.166	
Percent green at Max Sen.	5D	Xgwm292	93.05	2.465	3.634	Ventnor	0.098	26.4
SPAD	7B	CGA.GAC-347	73.49	2.600	0.433	Ventnor	0.087	8.7

Table 4-8. Genetic characterization of QTL linked to stay-green in RIL population under high temperature. The QTL linked to green leaf area duration (days) at different stages, maximum rate of senescence (Max Sen.), percent green at maximum senescence, and chlorophyll fluorescence (Fv/Fm) were detected by composite interval mapping. All markers having a LOD score of 2 and above are listed.

Trait	Chrom.	Marker	Position (cM)	LOD	Additive	Donor Parent	R^2	Total R^2 (%)
Time to 75% green	2A	AGG.CTT-107	76.48	8.670	0.922	Ventnor	0.260	
Time to 75% green	3B	CGT.CTCG-146	94.95	3.542	0.559	Ventnor	0.097	35.7
Time to 25% green	2A	AGG.CTT-107	76.48	4.149	3.491	Ventnor	0.135	
Time to 25% green	6B	CGT.CTCG-406	38.86	2.072	-2.517	Karl 92	0.067	20.2
Time bet.75 & 25% green	2A	AGG.CTT-107	76.48	2.624	2.624	Ventnor	0.088	
Time bet.75 & 25% green	6A	CGT.GTG-343	125.71	14.397	-17.094	Karl 92	0.510	59.8
Max Sen.	2A	AGG.CTT-107	76.48	7.608	-4.316	Karl 92	0.235	
Max Sen.	2D	Xbarc136	56.76	4.110	-3.359	Karl 92	0.142	
Max Sen.	6A	Xgwm334	0.01	2.261	-2.179	Karl 92	0.062	43.9
Time to Max Sen.	2A	AGG.CTT-107	76.48	7.025	1.345	Ventnor	0.212	
Time to Max Sen.	6A	CGT.GTG-343	121.71	2.910	-2.488	Karl 92	0.297	

Time to Max Sen.	6B	CGT.CTCG-406	38.86	2.791	-0.840	Karl 92	0.077	58.6
Percent green at Max Sen.	2A	AGG.CTT-107	76.48	6.001	4.552	Ventnor	0.192	
Percent green at Max Sen.	3A	Xgwm5	68.38	2.535	2.837	Ventnor	0.075	
Percent green at Max Sen.	6B	Xbarc198	36.01	2.443	-3.381	Karl 92	0.100	36.4
Fv/Fm	7A	CGA.CGCT-272	49.80	2.817	0.024	Ventnor	0.112	11.2

between 75 and 25% green, and time to maximum rate of senescence had the most variability explained under heat stress and moderate variability explained under optimum conditions. Additive effects of the traits are given as positive or negative values. Positive values indicate that the alleles for the traits in the RILs were contributed by the Ventnor, and negative by the Karl 92. All traits, except SPAD (with positive additive effect), had both positive and negative additive effects under optimum conditions, indicated that alleles for the trait were contributed by both parents.

Under heat stress, time to 75% green had a completely positive additive effect and maximum rate of senescence had a completely negative additive effect. An AFLP marker AGG.CTT-107 (P41/M62-107) had a positive additive effect on all the stay-green traits, and a negative additive effect on maximum rate of senescence. Therefore, while using this marker for selection Ventnor type of alleles can be selected for all the traits.

From the linkage maps (Figure 4), it was noted that chromosomes 2A, 6A, and 6B had at least two QTL linked to heat stress. QTL for time to 75% green, time to 25% green, time between 75 and 25% green, maximum rate of senescence, time to maximum rate of senescence, and percent green at maximum senescence under heat stress co-localized with marker AGG.CTT-107 (P41/M62-107) at 76.4 cM on chromosome 2A. QTL for time to 25% green and time to maximum senescence co-localized with marker CGT.CTCG-406 (P58/MC84-406) at 38.9 cM on chromosome 6B. QTL for time between 75 and 25% green was in the interval of marker CGT.GTG-343 (P58/M77-343) at 121.8 cM and CGA.CGCT-406 (P55/MC70-406) at 135 cM on chromosome 6A, and time to maximum senescence co-localized with marker CGT.GTG-343 (P58/M77-343) at 121.8 cM on chromosome 6A. Similarly, QTL for time to 75% green co-localized with CGT.CTCG-146 (P58/MC84-146) at 95.0 cM on chromosome 3B, maximum rate

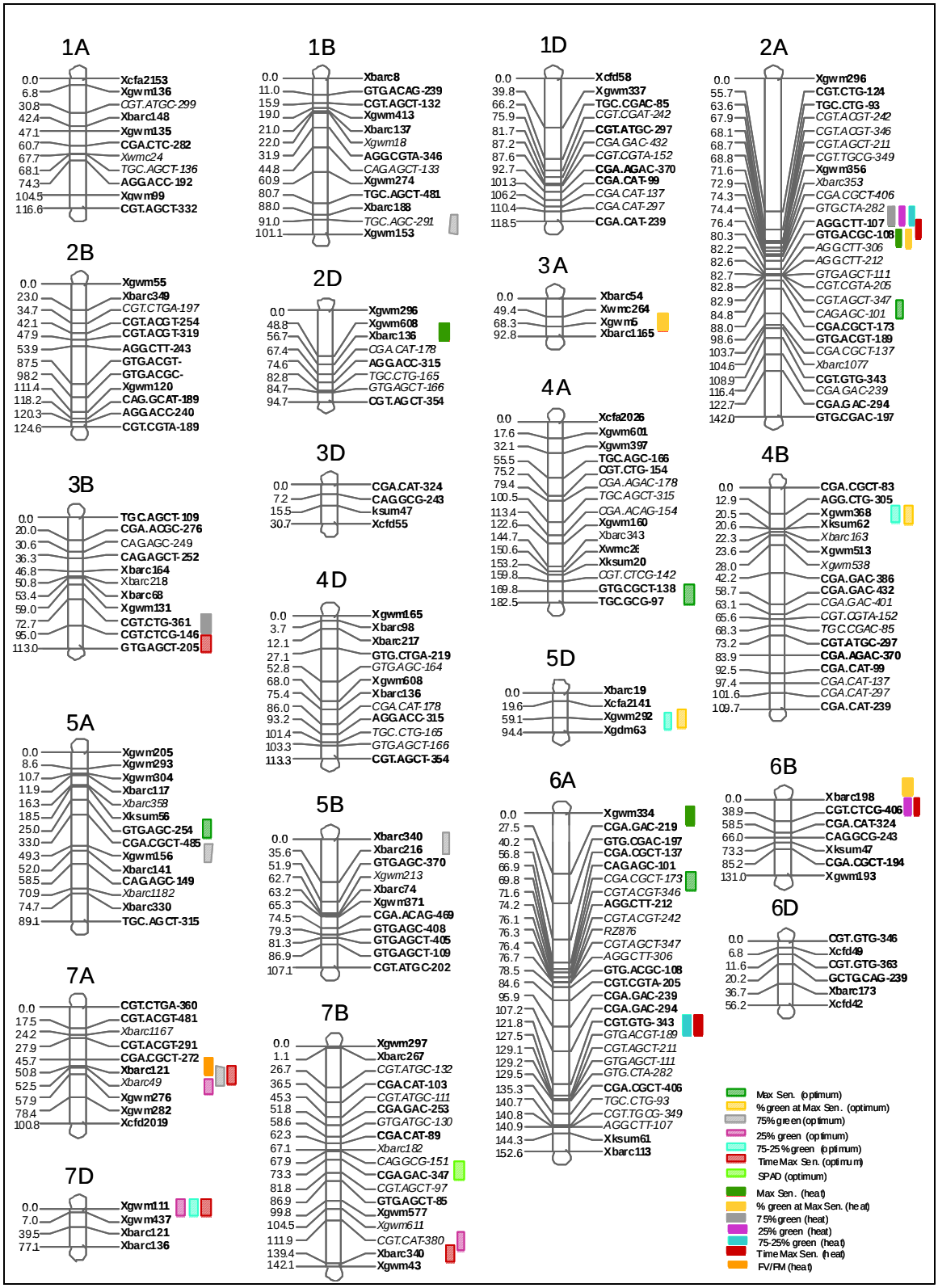
1A
0.0 Xcfa2153
6.8 Xgwm136
30.8 CGT.ATGC-299
42.4 Xbarc148
47.1 Xgwm135
60.7 CGA.CTC-282
67.7 Xwmc24
68.1 TGC.AGCT-136
74.3 AGG.ACC-192
104.5 Xgwm99
116.6 CGT.AGCT-332
2B
0.0 Xgwm55
23.0 Xbarc349
34.7 CGT.CTGA-197
42.1 CGT.ACGT-254
47.9 CGT.ACGT-319
53.9 AGG.CTT-243
87.5 GTG.ACGT-
98.2 GTG.ACGC-
111.4 Xgwm120
118.2 CAG.GCAT-189
120.3 AGG.ACC-240
124.6 CGT.CGTA-189
3B
0.0 TGC.AGCT-109
20.0 CGA.ACGC-276
30.6 CAG.AGC-249
36.3 CAG.AGCT-252
46.8 Xbarc164
50.8 Xbarc218
53.4 Xbarc68
59.0 Xgwm131
72.7 CGT.CTG-361
95.0 CGT.CTCG-146
113.0 GTG.AGCT-205
5A
0.0 Xgwm205
8.6 Xgwm293
10.7 Xgwm304
11.9 Xbarc117
16.3 Xbarc358
18.5 Xksum56
25.0 GTG.AGC-254
33.0 CGA.CGCT-485
49.3 Xgwm156
52.0 Xbarc141
58.5 CAG.AGC-149
70.9 Xbarc1182
74.7 Xbarc330
89.1 TGC.AGCT-315
7A
0.0 CGT.CTGA-360
17.5 CGT.ACGT-481
24.2 Xbarc1167
27.9 CGT.ACGT-291
45.7 CGA.CGCT-272
50.8 Xbarc121
52.5 Xbarc49
57.9 Xgwm276
78.4 Xgwm282
100.8 Xcfd2019
7D
0.0 Xgwm111
7.0 Xgwm437
39.5 Xbarc121
77.1 Xbarc136
1B
0.0 Xbarc8
11.0 GTG.ACAG-239
15.9 CGT.AGCT-132
19.0 Xgwm413
21.0 Xbarc137
22.0 Xgwm18
31.9 AGG.CGTA-346
44.8 CAG.AGCT-133
60.9 Xgwm274
80.7 TGC.AGCT-481
88.0 Xbarc188
91.0 TGC.AGC-291
101.1 Xgwm153
2D
0.0 Xgwm296
48.8 Xgwm608
56.7 Xbarc136
67.4 CGA.CAT-178
74.6 AGG.ACC-315
82.8 TGC.CTG-165
84.7 GTG.AGCT-166
94.7 CGT.AGCT-354
3D
0.0 CGA.CAT-324
7.2 CAG.GCG-243
15.5 ksum47
30.7 Xcfd55
4D
0.0 Xgwm165
3.7 Xbarc98
12.1 Xbarc217
27.1 GTG.CTGA-219
52.8 GTG.AGC-164
68.0 Xgwm608
75.4 Xbarc136
86.0 CGA.CAT-178
93.2 AGG.ACC-315
101.4 TGC.CTG-165
103.3 GTG.AGCT-166
113.3 CGT.AGCT-354
5B
0.0 Xbarc340
35.6 Xbarc216
51.9 GTG.AGC-370
62.7 Xgwm213
63.2 Xbarc74
65.3 Xgwm871
74.5 CGA.ACAG-469
79.3 GTG.AGC-408
81.3 GTG.AGCT-405
86.9 GTG.AGCT-109
107.1 CGT.ATGC-202
7B
0.0 Xgwm297
1.1 Xbarc267
26.7 CGT.ATGC-132
36.5 CGA.CAT-103
45.3 CGT.ATGC-111
51.8 CGA.GAC-253
58.6 GTG.ATGC-130
62.3 CGA.CAT-89
67.1 Xbarc182
67.9 CAG.GCG-151
73.3 CGA.GAC-347
81.8 CGT.AGCT-97
86.9 GTG.AGCT-85
99.8 Xgwm577
104.5 Xgwm611
111.9 CGT.CAT-380
139.4 Xbarc340
142.1 Xgwm43
1D
0.0 Xcfd58
39.8 Xgwm337
66.2 TGC.CGAC-85
75.9 CGT.CGAT-242
81.7 CGT.ATGC-297
87.2 CGA.GAC-432
87.6 CGT.CGTA-152
92.7 CGA.AGAC-370
101.3 CGA.CAT-99
106.2 CGA.CAT-137
110.4 CGA.CAT-297
118.5 CGA.CAT-239
3A
0.0 Xbarc54
49.4 Xwmc264
68.3 Xgwm5
92.8 Xbarc1165
4A
0.0 Xcfa2026
17.6 Xgwm601
32.1 Xgwm397
55.5 TGC.AGC-166
75.2 CGT.CTG-154
79.4 CGA.AGAC-178
100.5 TGC.AGCT-315
113.4 CGA.ACAG-154
122.6 Xgwm160
144.7 Xbarc343
150.6 Xwmc26
153.2 Xksum20
159.8 CGT.CTCG-142
169.8 GTG.CGCT-138
182.5 TGC.GCG-97
5D
0.0 Xbarc19
19.6 Xcfa2141
59.1 Xgwm292
94.4 Xgdm63
6A
0.0 Xgwm334
27.5 CGA.GAC-219
40.2 GTG.CGAC-197
56.8 CGA.CGCT-137
66.9 CAG.AGC-101
69.8 CGA.CGCT-173
71.6 CGT.ACGT-346
74.2 AGG.CTT-212
76.1 CGT.ACGT-242
76.3 RZ876
76.4 CGT.AGCT-347
76.7 AGG.CTT-306
78.5 GTG.ACGC-108
84.6 CGT.CGTA-205
95.9 CGA.GAC-239
107.2 CGA.GAC-294
121.8 CGT.GTG-343
127.5 GTG.ACGT-189
129.1 CGT.AGCT-211
129.2 GTG.AGCT-111
129.5 GTG.CTA-282
135.3 CGA.CGCT-406
140.7 TGC.CTG-93
140.8 CGT.TGCG-349
140.9 AGG.CTT-107
144.3 Xksum61
152.6 Xbarc113
2A
0.0 Xgwm296
55.7 CGT.CTG-124
63.6 TGC.CTG-93
67.9 CGT.ACGT-242
68.1 CGT.ACGT-346
68.7 CGT.AGCT-211
68.8 CGT.TGCG-349
71.6 Xgwm356
72.9 Xbarc353
74.3 CGA.CGCT-406
74.4 GTG.CTA-282
76.4 AGG.CTT-107
80.3 GTG.ACGC-108
82.2 AGG.CTT-306
82.6 AGG.CTT-212
82.7 GTG.AGCT-111
82.8 CGT.CGTA-205
82.9 CGT.AGCT-347
84.8 CAG.AGC-101
88.0 CGA.CGCT-173
98.6 GTG.ACGT-189
103.7 CGA.CGCT-137
104.6 Xbarc1077
108.9 CGT.GTG-343
116.4 CGA.GAC-239
122.7 CGA.GAC-294
142.0 GTG.CGAC-197
4B
0.0 CGA.CGCT-83
12.9 AGG.CTG-305
20.5 Xgwm368
20.6 Xksum62
22.3 Xbarc163
23.6 Xgwm513
28.0 Xgwm538
42.2 CGA.GAC-386
58.7 CGA.GAC-432
63.1 CGA.GAC-401
65.6 CGT.CGTA-152
68.3 TGC.CGAC-85
73.2 CGT.ATGC-297
83.9 CGA.AGAC-370
92.5 CGA.CAT-99
97.4 CGA.CAT-137
101.6 CGA.CAT-297
109.7 CGA.CAT-239
6B
0.0 Xbarc198
38.9 CGT.CTCG-406
58.5 CGA.CAT-324
66.0 CAG.GCG-243
73.3 Xksum47
85.2 CGA.CGCT-194
131.0 Xgwm193
6D
0.0 CGT.GTG-346
6.8 Xcfd49
11.6 CGT.GTG-363
20.2 GCTG.CAG-239
36.7 Xbarc173
56.2 Xcfd42
Max Sen. (optimum)
% green at Max Sen. (optimum)
75% green (optimum)
25% green (optimum)
75-25% green (optimum)
Time Max Sen. (optimum)
SPAD (optimum)
Max Sen. (heat)
% green at Max Sen. (heat)
75% green (heat)
25% green (heat)
75-25% green (heat)
Time Max Sen. (heat)
FV/FM (heat)

Figure 4-4. Genetic linkage maps of 21 chromosomes of wheat. The population used was a set of RILs derived from Ventnor X Karl 92. The marker names are listed on the right of the chromosome, and map distances in centimorgans (cM) between the markers are listed on the left. Framework markers are represented in bold and placement markers are represented in italics. Markers beginning with "X" are SSR, RZ876 is a STS, and the others are AFLP markers denoted by their selective base combinations and size (bp) of the band. The QTL for green leaf area duration at different stages, maximum rate of senescence (Max Sen.), percent green at maximum senescence, chlorophyll content (SPAD), and chlorophyll fluorescence (Fv/Fm) under optimum conditions (striped boxes) and heat stress (solid boxes) have the same color code.

of senescence co-localized with *Xbarc136* at 56.7 cM on chromosome 2D, and QTL for percent green at maximum senescence co-localized with marker *Xgwm5* at 68.3 cM on chromosome 3A. The AFLP markers that co-localized with stay-green can be converted to STS markers and can be used for selecting genotypes with longer green leaf area duration under high temperature. The peaks from composite interval mapping (Figures 5 and 6) represent QTL at the marker loci on the chromosomes. Chromosomes 2A, 6A, and 7A had an abundance of stay-green QTL under optimum and heat-stress conditions. Under optimum conditions, QTL for time between 75 and 25% green and percent green at maximum senescence on chromosome 4B; time to 75% green and maximum rate of senescence on chromosome 5A; time to maximum senescence and time to 25% green on chromosome 7B; and time to maximum senescence and time between 75 and 25% greenness on chromosome 7D overlapped. Under heat stress, QTL for all the traits could be found, except SPAD, on chromosome 2A. Other chromosomes that had QTL with prominent effects under optimum conditions were chromosomes 2A and 6A for maximum rate of senescence, chromosome 5D for time between 75 and 25% green, chromosome 7A for time to maximum rate of senescence, and chromosome7B for chlorophyll content (SPAD). QTL for maximum rate of senescence were on chromosome 2D, percent green at maximum senescence on chromosome 3A, time to 75% green on chromosome 3B, time to maximum senescence on chromosome 6B, and Fv/Fm on chromosome 7A. Marker CGT.GTG-343 (P58/M77-343) on chromosome 6A was linked to time between 75 and 25% green with the highest LOD score of 14.397 and a huge negative additive effect of -17.094.

Comparative mapping for the markers linked to stay-green traits

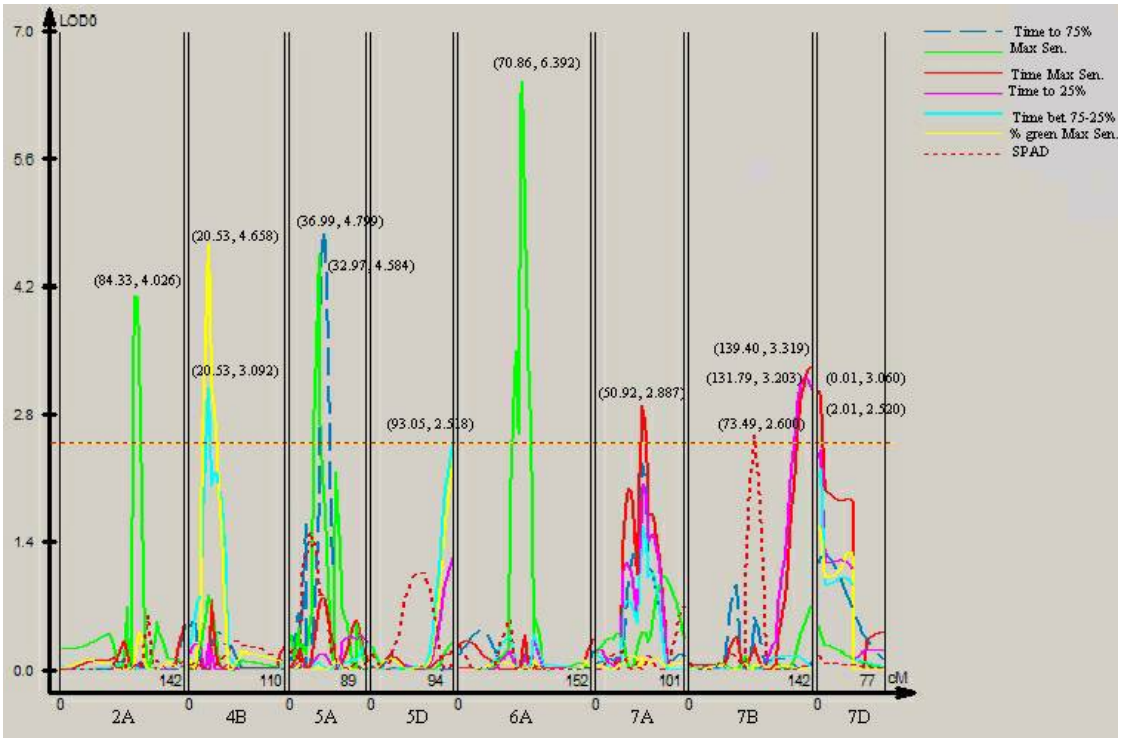

Figure 4-5. Composite interval map for different stages of green leaf area duration, maximum rate of senescence (Max Sen.), percent green at maximum senescence, and chlorophyll content (SPAD) linked to the markers under optimum conditions. Peaks on the graph that exceed the orange dotted line indicative of a LOD 2.5 denote the presence of a significant QTL. Position of the QTL and LOD scores are inside the parenthesis.

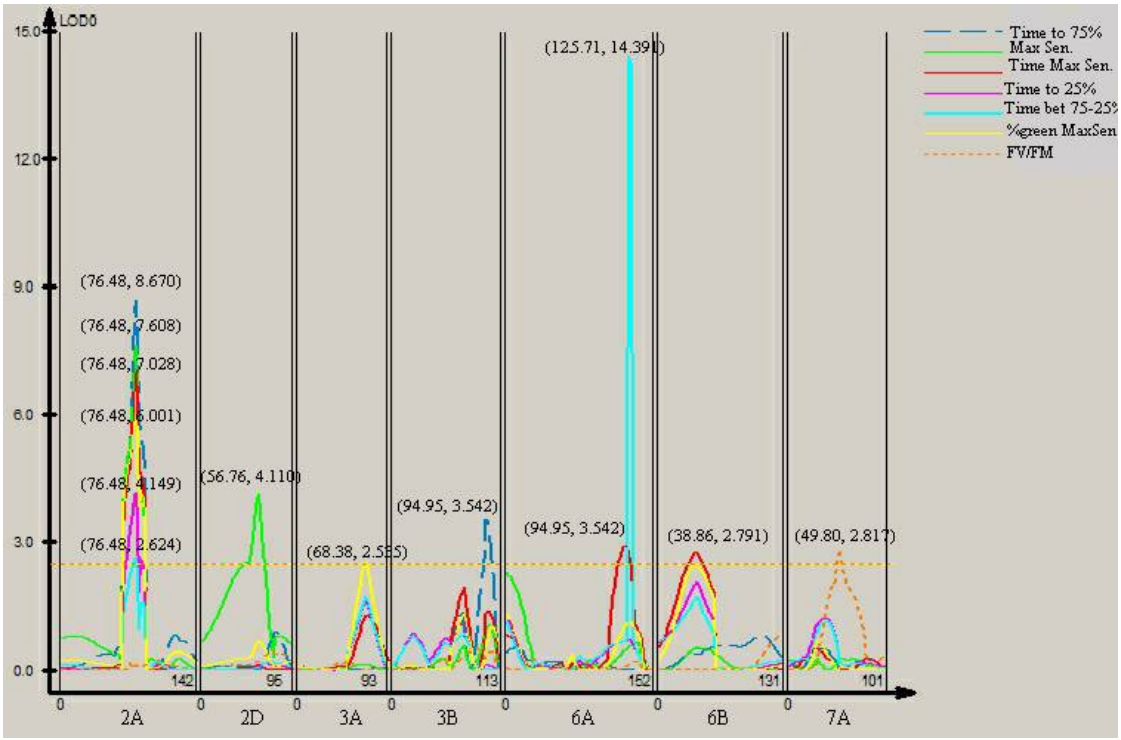

Figure 4-6. Composite interval map for different stages of green leaf area duration, maximum rate of senescence (Max Sen.), percent green at maximum senescence, and chlorophyll fluorescence (Fv/Fm) linked to the markers under heat stress. Peaks on the graph that exceed the orange dotted line indicative of a LOD 2.5 denote the presence of a significant QTL. Position of the QTL and LOD scores are inside the parenthesis.

A framework marker CGT.GTG-343 (P58/M77-343) present on chromosome 6AL was strongly linked to stay-green trait, time between 75% and 25% green under heat stress. It had the highest LOD of 14.397 and largest additive effect of -17.094. CGT.GTG-343 (P58/M77-343) is located at 121.8 cM and is distal to a STS marker RZ876 mapped at 76.3 cM. According to Bhadula et al (2001) heat stress induces an enhanced synthesis of chloroplast elongation factor (EF-Tu) protein and plays an important role in thermotolerance. In GenBank (National Center for Biotechnology Information) the BAC clone AP004023 on the physical map of rice chromosome 2 mapped between 23.050 Mb and 23.146 Mb had 16 putative genes (TIGR Rice Genome Annotation). One of those genes was a chloroplast elongation factor Tu (EF-Tu) spanning between 23.160 to 23.108 Mb on the BAC clone. This gene corresponded to the RFLP probe RZ876 that mapped at 91.10 cM in the rice genetic linkage map (http://www.tigr.org/tigr-scripts/osa1_web/gbrowse/rice/?name=Marker:Cornell_92) (Cheng et al. 2001). The PCR primers designed from the EF-Tu sequence corresponding to RZ876 was used on the RIL population. This STS marker for RZ876 was not a framework marker and hence was analyzed with single marker analysis. Single marker analysis for RZ876 showed significant linkage to time between 75% and 25% green, and explained a variability of 6.9%.

In rice the RFLP marker CDO204 was at the same locus (91.10 cM) as RZ876 (Cheng et al. 2001) (Figure 7). The marker CDO204 mapped at 101.00 cM on chromosome 6A in wheat (Rudi, 2004) and linkage group F at 68.80 cM in sorghum (Paterson, 2003). In sorghum it was flanked by marker UMC156 at 37.70 cM and marker CSU39 at 75.40 cM; both these markers were also present on chromosome 4 in maize at82.00 cM and 144.00 cM, respectively (Ribaut et al. 1996). In maize QTL for ear num- ber (82.00 cM) and grain yield (114.00 cM) were present

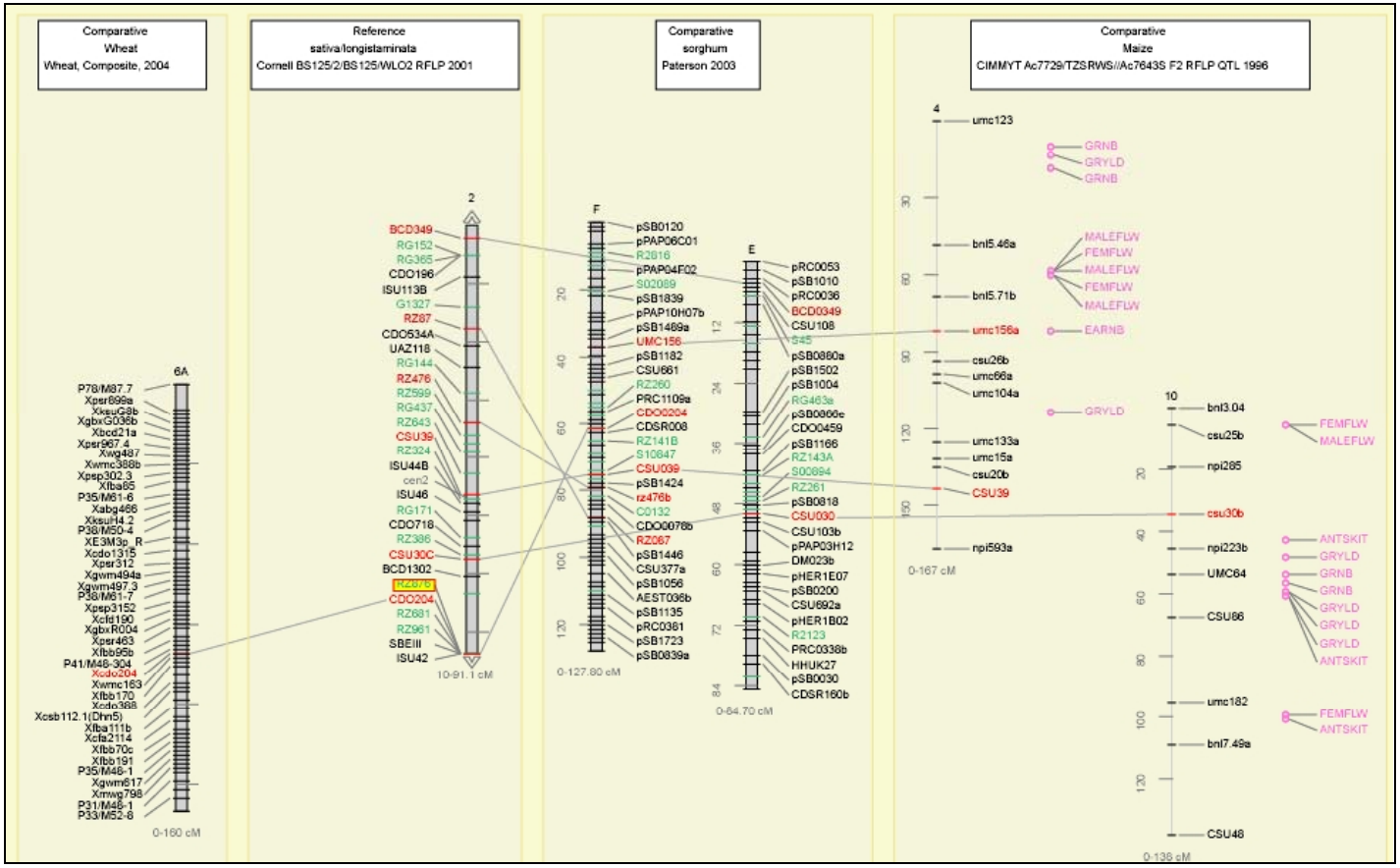

Figure 4-7. Comparative mapping for RFLP marker CDO204 present at the same locus as RZ876 on rice chromosome 2. The marker was present on chromosome 6A in wheat and linkage group F in sorghum. Markers UMC156 and CSU39 were flanking marker CDO204 in sorghum, both the markers were present on chromosome 4 in maize and were linked yield traits.

in the marker interval of UMC156 and CSU39. Marker CSU39 was present on chromosome 2 of rice at 60.90 cM. In rice another marker CSU30 proximal to marker CDO204 at 73.30 cM was also present on linkage group E at 50.10 cM in sorghum and on chromosome 10 at 34.00 cM in maize. The QTL for anthesis silking interval, grain yield, and grain number were between the interval 43.00 to 61.00 cM close to the marker CSU30 in maize.

Discussion

The recombinant inbred lines derived from parents that differed in their response to high temperature also show a differential response to the varying temperature regimes (Chen et al., 2002; Mohammadi et al., 2004). Ventnor, the tolerant parent, had longer duration of photosynthetic activity, higher chlorophyll content and lower chlorophyll fluorescence under both optimum and high temperature conditions. This was in accordance to the reports of Al-Khatib and Paulsen (1990) and Yang et al. (2002). The RILs in the experiment differed significantly for stay-green under optimum and high temperature regime.

Observations of green leaf area duration started 10 DAA, after which the plants were subjected to continuous heat stress. Senescence, which is a degenerative process, should have already set in to remobilize nutrients to the growing kernels. The visible manifestation of this process was breakdown of chlorophyll, which typically starts from the leaf margins and progresses towards the interior of the leaf blade. Imposition of heat stress accelerates senescence. Gompertz's analysis was performed to analyze the senescence pattern in the RILs. The initial parameter in the Gompertz's analysis was set to statistically determine the 100% green stage in the lines to make a uniform starting point for the curve. The patterns of senescence can be broadly separated

into five groups that were typically characterized by a set of RILs: (i) RIL 92 with a slow rate of leaf senescence at 2.4 reached maximum senescence in a shorter time (10.7 days) and had a moderate level of greenness at 35.1% at maximum senescence. (ii) RIL 111 had a slow rate of leaf senescence at 3.5, took longer (16.1 days) to reach maximum senescence, and had a high percentage of greenness at 48.7% at maximum senescence. (iii) RIL 163 had a moderate rate of leaf senescence at 12.1, took a shorter time (10.2 days) to reach maximum senescence, and had a moderate level of greenness at 33.3% at maximum senescence. (iv) RIL 169 had a moderate rate of leaf senescence at 11.1, took longer time (20.9 days) to reach maximum senescence, and had a moderate level of greenness at 47.4% at maximum senescence. (v) RIL 180 had a fast rate of senescence at 51.7, a shorter time (10.1 days) to reach maximum senescence, and had a low greenness at 25.7% at maximum senescence. The categorization used the parents as checks. Ventnor had values of 4.1, 15.4 days and 40.7%; and Karl 92 had values of 26.4, 9.4 days, and 26.4% for maximum rate of senescence, time to maximum rate of senescence, and percent green at maximum rate of senescence, respectively.

The SPAD chlorophyll readings had significant, negative correlations with maximum rate of senescence, indicating that with longer green leaf duration, the chlorophyll content and photosynthetic ability of the plant were maintained longer. This was similar to the report of Spano et al. (2003) for functional stay-green mutants in durum wheat, which had delayed leaf senescence and longer photosynthetic competence. The chlorophyll fluorescence and chlorophyll content correlated positively between one another. These traits also had significant, positive correlation with agronomic traits such as grain filling duration (GFD) and thousand kernel weight (TKW) under high temperature (data not shown). Similar results were observed in wheat genotypes subjected to heat stress by Al-Khatib and Paulsen (1990) and in maize under

differential water conditions by O′Neill et al. (2006). Therefore, SPAD and FV/FM can be used as secondary criteria for measuring heat tolerance of a genotype, as suggested by Moffatt et al. (1990).

According to the genome synteny by Devos and Gale (1997) and Sorrells et al. (2003), homoeologous wheat chromosome 2 is syntenic to chromosomes 4 and 7 in rice, chromosomes 10, 7, and 2 in maize, and linkage groups B and F in sorghum. Similarly, homoeologous wheat chromosome 6 is syntenic to chromosome 2 in rice, chromosomes 4 and 5 in maize, and linkage group D in sorghum. Composite interval mapping in the RIL population showed that markers linked to the stay-green traits under optimum conditions were distributed on most of the chromosomes, while under heat stress the markers were mostly on chromosomes 2A and 6A. Markers linked to stay-green under optimum and heat stress conditions were mostly different, except CGT.CTCG-146 (P58/MC84-146). The QTL linked to marker CGT.CTCG-146 (P58/MC84-146) possibly contains genes that influence the trait *per se*, while the QTL linked to markers like CGT.GTG-343 (P58/M77-343), and AGG.CTT-107 (P41/M62-107) which are prominent under heat stress possibly contains genes for enhanced stay-green. Marker AGG.CTT-107 (P41/M62-107), which was strongly linked to stay-green under heat stress, was also strongly linked to grain filling duration (GFD) under the same conditions (Chapter 3), indicating that the QTL activated under heat stress sustained green leaf area of the RIL and ultimately enhanced plant yield. The results were similar to those of Tuinstra et al. (1998) in sorghum; Spano et al. (2003), and Verma et al. (2004) in wheat, who found positive associations between stay-green at a given point of time and grain yield under stress. Although stay-green rating at a given point of time is useful, getting a rating of senescence related traits over a period of crop development from flowering to physiological maturity can be more useful in characterizing behavior of lines

under stress. In this regard the method we used was to model visual rating of stay-green over the reproductive growth phase and map traits related to senescence that can more quantitatively characterize stay-green.

Comparative mapping of marker interval CGT.GTG-343 (P58/M77-343) and RZ876 on chromosome 6A showed a synteny with chromosome 2 in rice, linkage group E and F in sorghum, and chromosomes 4 and 10 in maize. It also showed a synteny with regions of the maize genome linked to QTL for yield. Similarly another prominent marker interval of AGG.CTT-107 (P41/M62-107) and *Xgwm356* on chromosome 2A, show a synteny with maize bins on chromosome 7 containing QTL for yield related traits (Chapter 3). The syntenies observed, strongly suggested a relationship between stay-green and yield traits.

Leaf senescence is a complex process, and the rate differs among genotypes. The modeling of stay-green over the reproductive period of wheat, helped better characterize stay-green and senescence related traits in a quantitative manner. Stay-green was divided into different stages, among which maximum rate of senescence and time to maximum rate of senescence are key. Time between 75% and 25% green had a large additive effect and explained a large variability for the trait. The stay-green traits were mainly on chromosomes 2A and 6A of wheat and correlated positively with the yield traits. Markers co-localized with QTL for stay-green under heat stress, especially CGT.GTG-343 (P58/M77-343), CGT.CTCG-146 (P58/MC84-146), AGG.CTT-107 (P41/M62-107), CGT.CTCG-406 (P58/MC84-406), and *Xbarc136*, can be converted to STS markers and used in marker-assisted selection.

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CHAPTER 5 - Field Evaluation and Validation of Markers Linked to QTL for Heat Tolerance in a Winter Wheat Population

Abstract

High-temperature stress is a major factor in the loss of wheat (*Triticum aestivum* L.) yield in the Great Plains and other regions where the crop faces stress during the post-anthesis stage. One of the major challenges to breeders is making selections under variable environmental conditions for cultivars that are tolerant to heat stress. To overcome this challenge, traditional breeding complemented with molecular markers will prove useful. The objective of this experiment was to analyze a population derived from a cross of a heat-tolerant cultivar 'Ventnor' by a heat-susceptible cultivar 'Karl 92' and to validate the markers linked to the heat-tolerant quantitative trait loci (QTL) in an independently derived population. Twenty-five tolerant and 25 susceptible filial$_{3:5}$ ($F_{3:5}$) lines were analyzed in multilocation replicated field trials for their performance under two planting dates. The traits analyzed were grain filling duration (GFD), kernels per spike, thousand kernel weight (TKW), grain filling rate (GFR), and yield. The lines were genotyped using the molecular markers *Xgwm296*, *Xgwm356*, and *Xksum61* that were linked to the QTL for heat tolerance. Treatment X environment interactions were highly significant for all traits. The lines segregated transgressively for the traits, and genes for heat tolerance were contributed by both Ventnor and Karl 92 alleles. Correlations were positive among yield, TKW, and kernels per spike and between GFD and TKW. Heritability of the traits ranged from 46 to 60%. The observations in the field were similar to those under controlled

conditions. The $F_{3:5}$ lines 27, 29, 40, and 48 were the top lines with respect to GFD, kernels per spike, GFR, and TKW, respectively, under field conditions and had genotypically superior alleles. The traits TKW and kernels per spike can be used as primary criteria and GFD as a secondary criterion for selecting heat-tolerant genotypes. Markers *Xgwm296*, *Xgwm356*, and *Xksum61* validated under field conditions can be used in marker-assisted breeding program to identify genotypes that are tolerant to high temperature stress.

Introduction

Wheat in the Great Plains experiences temperatures over 30°C during grain growth, resulting in yields that average 2 to 3 Mg ha^{-1} compared with 7 Mg ha^{-1} in cooler regions (Paulsen, 1994). In a comparison between favorable and high-temperature field environments, a four-fold difference was reported in wheat yields (Midmore et al., 1984; Shipler and Blum, 1986; Zhong-hu and Rajaram, 1994). In a survey of wheat crop losses from 1948 to 2002 from abiotic and biotic stresses (USDA-NASS, 2004), 21% was accounted by heat and drought alone, 10% by hail, 11% by precipitation, 1% by cold, 4% by floods, 10% by wind, 3% by insects, 8% by diseases, and 6% by all other stresses.

The duration to heading of a genotype is determined by its response to photoperiod and temperature responses (Slafer and Rawson, 1996). Two important yield components, spikelet number per spike and grain number per spike, are formed during this phase (Przuli and Mladenov, 1999). The phase from flowering to physiological maturity is the grain filling duration (GFD). The kernel number in spikes is established by one week after anthesis, and the

rapid phase of grain filling starts (Stone and Nicolas, 1995). Once the plant reaches physiological maturity, grain filling stops and the kernels begin to lose moisture.

Post-anthesis high-temperature stress during grain fill reduces yield by decreasing the kernel weight (Warrington et al., 1977; Tashiro and Wardlaw, 1990; Stone and Nicolas, 1994). A decrease in kernel weight up to 85% was recorded when the temperature increased from to 20/16°C to 36/31°C from 7 d after anthesis (DAA) until maturity (Tashiro and Wardlaw, 1989). In the hard red winter wheat Karl 92, which is adapted to Great Plains conditions, grain yield decreased 78%, kernel number 63%, and kernel weight 29% at 35/20°C from 10 DAA until maturity. High temperature from 15 or 20 DAA reduced kernel weight by 18%, but did not affect kernel number (Gibson and Paulsen, 1999).

In addition to GFD, grain filling rate (GFR) also plays a significant role in the final yield of wheat (Millet and Pinthus, 1983; van Sanford, 1985; Beiquan and Kronstad, 1994). Both the GFD and the GFR were positively associated with final grain weight (Wardlaw, 1970; Bhatt, 1972; Wiegand and Cuellar, 1981). Most of the yield-related traits had a polygenic inheritance and some, like GFD, had a predominantly additive genetic effect and maternal inheritance, though epistasis involving dominant gene action was also noted (Przuli and Mladenov, 1999).

Variation from one season to another in response to high temperature is a concern for breeders for developing tolerant cultivars, where selection takes place over a number of generations and under variable conditions (Wardlaw, 1994). Molecular markers will hasten selection for heat-tolerant cultivars and reduce the cost and labor of field trials.

The objective of the experiment was to analyze a population derived from a cross between heat-tolerant and heat-susceptible cultivars under field conditions. To compare those results with previous experiments on recombinant inbred line (RIL) population of the same cross studied

under controlled conditions, and to genotypically analyze the inbred lines using molecular markers linked to the QTL for high-temperature tolerance.

Materials and Methods

Population development

Two winter wheat cultivars, Ventnor, a hard white wheat from Australia that is tolerant to heat stress, and Karl 92, a hard red wheat from Kansas, were crossed to produce an inbred line population. The F_1 and F_2 generations were advanced in the greenhouse. Nine-hundred head rows of F_3s were planted in the field at Ashland Bottom in Manhattan, Kansas, in autumn of 2001. Since Karl 92 is adapted to Central Plains conditions, lines similar to it in agronomic type and time of anthesis were selected in spring 2002. The selected head rows were harvested by hand and threshed and seed obtained from each row was bulked. Two replicates of 222 $F_{3:4}$ lines, including the parents, were grown under irrigated conditions in Manhattan during 2002-2003. Normal and delayed plantings was done to ensure high temperature stress at post-anthesis stage. These lines were evaluated for agronomic traits, and based on heat susceptibility indices (HSI) for GFD and TKW, tails of the population were selected that comprised of 25 tolerant and 25 susceptible lines. Fifty $F_{3:5}$ lines along with the parents were grown in four replicates at three locations under normal and delayed plantings during 2003-2004. Two locations were at Ashland Bottoms in Manhattan; one was the same irrigated site used in 2003, and the other was a non-irrigated site. The third location was a non-irrigated site of Hutchinson, Kansas. Combine harvester was used to harvest and thresh the inbred lines of $F_{3:4}$ and $F_{3:5}$ populations.

Experimental design

The optimum winter wheat planting date in the Great Plains is in the month of October. The seedlings overwinter and the following spring, they come to flowering, by second week of May. Delaying planting by three to four weeks in autumn results in a two to three week delay in flowering in the spring. Delayed flowering coincides with the rising temperature, ensuring increased high-temperature stress during the post-anthesis grain-filling period (Witt, 1996). Planting for the experiment was done on two different dates (Figure 1). "Optimum" planting dates were 6 November 2003 and 12 October 2004. Late plantings were on 10 December 2003 and 20 November 2004. Before planting, fertilizers were applied in August for both years at rates of 67 kg/ha N and 22 kg/ha P in Manhattan (Reading silt loam soil) and Hutchinson (Ost silt loam soil). A balanced incomplete block design with strips was used for the experiment. The design consisted of one treatment with two levels, which were the normal and late planting dates. Experimental units for the strips were the differential planting dates and for rows it was inbred line. The site had four blocks comprising a total of four replicates. Plots were 1.5 x 0.67 m with 20 cm between rows and three rows per plot. In 2003 and 2004 wheat seeds were planted at a rate of 36 kg/ha. In both the years, a second top-dressing of urea to supply 16 kg/ha N was applied in early spring, and QUILT™ fungicide(azoxystrobin and propiconazole active ingredients) at 1 kg/ha at flowering and during grain filling to control fungal disease. Temperature at each location was recorded daily by HOBO (Onset Computer Corp., Bourne, MA) temperature-recorder-monitoring devices placed in the center of the field.

Traits measured

a) The field site during early spring with strips of normal and late plantings.

b) Wheat plots during maturity showing the contrast between normal and late plantings.

Figure 5-1. Inbred lines derived from Ventnor by Karl 92 cross, at different stages of plant growth during normal and late planting. A differential maturity was noted due to planting date.

Grain filling duration (GFD)

Anthesis was recorded when 50% of the main culms extruded anthers from their inflorescences. Similarly, physiological maturity was recorded when 50% of the main culms in a given plot lost chlorophyll and turned golden in color. The GFD was calculated as the days between anthesis and physiological maturity for a given plot.

Kernels per spike

Twenty spikes were randomly picked from each plot and, threshed, and the kernels were counted. Number of kernels per spike was estimated.

Thousand Kernel Weight (TKW) and Yield

The plots were harvested and threshed using a combine harvester. The grain from each plot was stored in separate bag. Thousand kernels from each harvested plot were counted using an electronic seed counter (SEEDBURO, 801 COUT-A-PAK, Chicago, IL) and weighed. For estimating the yield per plot, weight of the all the kernels harvested from the plot was determined.

Grain Filling Rate (GFR)

GFR was estimated as a ratio between kernel weight and GFD.

HSI for GFD and TKW of each inbred line was calculated as: HSI = [(1-Y/Yp)/D], where Y = yield in late planting, Yp = yield in normal planting, D = stress intensity = 1- X/Xp, X = mean of Y of all genotypes, and Xp = mean of Yp of all genotypes (Fischer and Maurer, 1978). Genotypes were categorized as tolerant and susceptible according to Khanna-Chopra and

Viswanathan (1999). Genotypes having HSI ≤ 0.500 were highly tolerant, HSI > 0.500 to ≤ 1.000 were moderately tolerant, and HSI > 1.000 were susceptible.

Statistical analysis

Each location in 2003 and 2004 was considered a separate environment. There were four environments in total; Environment 1 at Manhattan in 2002-2003; Environments 2, 3, and 4 in the crop year 2003-2004. Environments 2 and 3, were the irrigated and non-irrigated sites at Manhattan; and Environment 4 was at Hutchinson.

Proc. Mixed was used for ANOVA, and entry means were estimated by Proc. GLM (general linear model). Correlations for all the traits were calculated by Pearson's method using Proc. Corr. Statistical software SAS Version 8.2 was used for all procedures (SAS Inst. Inc., 1990). The mean squares estimates for the analysis of variance for genotype, genotype-by-environment interactions, and mean square errors were used to calculate broad sense heritability by the following equation: $\sigma^2_G / (\sigma^2_G + \sigma^2_{GE} + \sigma^2_E)$, where σ^2_G represents genotypic variance, σ^2_{GE} represents genotype x environmental variance, and σ^2_E represents error variance.

Molecular markers

The DNA was extracted from the 50 experimental lines and two parents by the CTAB method (Saghai-Maroof et al., 1984). Molecular markers were the microsatellite or simple sequence repeats (SSR) which co-localized with the agronomic traits in earlier experiments (Chapter 3). The microsatellite markers consisted of *Xgwm296*, *Xgwm356* (Röder et al., 1998),

and *Xksum61*(Singh et al., 2000). *Xgwm356* and *Xgwm296* were amplified at an annealing temperature of 55°C and the annealing temperature for *Xksum61* was 60°C. The polymerase chain reaction (PCR) amplifications were done in an MJResearch Thermal Cycler (Bio-Rad formerly MJ Research, Hercules, CA) as described by Röder et al. (1998). The PCR reaction mixture contained a total 25 µL consisting of 150 ng genomic DNA, 2.5 µL 10X PCR buffer, 1.5 mM $MgCl_2$, 0.30 mM dNTPs, 1 unit *Taq* DNA polymerase, and 20 ng primer (forward + reverse). The program consisted of an initial denaturation at 95°C for 3 min., and two initial cycles at an annealing temperature of 62°C, followed by 34 cycles of 1 min at 94°C, 1 min at annealing temperature and 2 min at 72°C followed by final extension at 72°C for 10 min. Markers *Xgwm356* and *Xksm61* were run on a 3% SFR agarose (Midwest Scientific, St. Louis, MO) gel at 70 volts and visualized by staining with ethidium bromide under ultra violet illumination .

Marker *Xgwm296* was run at 80 watts on a 6% polyacrylamide denaturing gel modified with formamide solution and urea to enhance the resolution of the markers. The gel mixture contained 15 mL double-distilled water, 32 mL formamide, 33.6 g urea, 10 mL 10X Tris-boric acid-disodium ethylenediaminetetraacetic acid (TBE) solution, 17.5 mL Bis/acrylamide (19:1 polyacrylamide, 40%, ISC BioExpress). 1 mL 10% ammonium persulfate, and 40 µL N,N,N',N'-Tetramethylethylenediamine (TEMED) were added to the gel mix prior to pouring the gel to drive polyacrylamide polymerization. The gel was cast and run on a BioRad Sequi-Gen GT sequencing system (Bio-Rad, Hercules, CA) for 2 h after an initial pre-run of 20 min. The bands were visualized by silver staining (Bassam et al., 1991). All markers were scored as parental type (A or B), heterozygote (H), or missing data (-).

Data from the molecular markers were analyzed with Graphical GenoType (GGT) software developed by Ralph van Berloo, Laboratory of Plant Breeding, Wageningen University (Van

Berloo, 1999), to select the ideal genotype. The output of this analysis was an illustrated representation of the genotypic data, which simplified identification of possible heat-tolerant lines.

Results

Environmental conditions and plant development

For Environment 1 at Manhattan, anthesis of the normal planting occurred between 10 to15 May 2003 and physiological maturity from 17 to 22 June 2003. For the late planting, anthesis was from 23 to 28 May 2003 and physiological maturity was from 25 June to 1 July 2003. For Environments 2 and 3 at Manhattan and Environment 4 at Hutchinson (2003-2004), anthesis of the normal planting was from 7 to 11 May 2004 and, 5 to 8 May 2004 respectively, and physiological maturity was 8 to 14 June 2004, and 7 to 11 June 2004, respectively. In the late planting at Manhattan and Hutchinson in 2004, anthesis was from 11 to 15 May 2004, and 9 to 11 May 2004, respectively, and physiological maturity was from 12 to 18 June 2004, and 7 to 16 June 2004, respectively. Environment 1 had a lower temperature than Environments 2, 3, and 4 (Table 1). Maximum temperature was higher at Hutchinson than at the other two environments in 2004, though the mean temperatures were nearly similar during maturation for the three environments.

Analysis of variance for yield traits

Table 5-1. Weekly mean high and low temperature and maximum high temperatures at Manhattan during 2003 and 2004 and at Hutchinson during 2004. The temperatures were monitored using HOBO temperature-recorder-monitoring devices placed in the center of the field.

Time	Manhattan						Hutchinson		
	2003			2004			2004		
	High	Low	Max.High	High	Low	Max.High	High	Low	Max.High
	……. °C……								
May 6-13	22.6	16.0	26.1	29.6	15.4	34.9	29.1	14.7	31.8
May 14-20	22.0	11.2	27.4	25.2	13.0	31.3	25.9	13.8	33.2
May 21-27	23.5	9.6	26.4	28.8	16.6	32.9	30.2	16.0	34.1
May28- June 3	25.8	13.6	33.8	28.8	12.7	32.2	28.6	12.3	33.4
June 4-10	24.3	12.0	30.0	28.5	18.5	31.7	29.0	18.3	32.9
June 11-17	30.0	15.7	31.1	30.6	17.8	32.5	32.6	18.9	35.8
June 18-24	30.6	18.6	33.0	26.3	14.5	30.2	27.5	15.3	32.5
June 25- July 1	29.5	16.0	32.4	25.4	13.6	29.9	27.9	16.0	30.1

The traits GFD, kernels per spike, TKW, yield and GFR differed at $\alpha < 0.01$ between normal and late plantings (Table 2). The entries and environments also differed significantly at $\alpha < 0.001$ for the same traits. Three-way interactions among treatments, environments, and entries were highly significant at $\alpha < 0.001$ for GFD and at $\alpha < 0.05$ for yield. Performance of the entries differed significantly between the treatments across different environments. Response of the lines to yield differed significantly at $\alpha < 0.001$ under normal planting in Environments 3, and between Environments 1 and 2 under late planting. Response of the lines to GFD was highly significant under both plantings in all environments, except normal planting in Environment 4. Environment being a random effect, the three-way interaction was partitioned into two-way interactions. The treatment X entry interaction was highly significant at $\alpha < 0.001$ for GFD and nonsignificant for the other traits. Lines differed significantly in performance within each treatment. There was a noncrossover interaction between GFD and planting date (Figure 2). Most of the lines decreased in GFD in the late planting compared to normal planting, except for eight lines in which GFD was statistically similar in the late planting and the normal planting. The treatment X environment interaction was highly significant for all traits. The treatments differed significantly within and between environments. Interaction plots (not shown) for each trait indicated that there was a noncrossover interaction between treatments and environments. Except for kernels per spike, all values were lower under late planting than normal planting. Maximum difference in response for the treatments was observes in Environment 1 for GFD, Environment 1 and 3 for kernels per spike, Environment 1 and 4 for TKW, and Environment 4 for both yield, and GFR. Environment X entry interactions were highly significant at $\alpha < 0.01$ only in the case of GFD, indicating that the overall performance of lines was affected by the environments.

Table 5-2. Analysis of variance for grain filling duration (GFD), kernels per spike, thousand kernel weight (TKW), yield, and grain filling rate (GFR) over four environments in the RIL population derived from Ventnor X Karl 92 cross under normal and late planting conditions.

Effects	DF	GFD (d)	Kernels/spike (#)	TKW (g)	Yield (ton/ha)	GFR (mg/d)
Treatment (Normal vs. Late)	1	338.808***	1558.650***	4864.155*	230.080***	2.574***
Entry (Lines)	51	18.899***	44.963***	142.132**	3.227***	0.177***
Treatment*Entry	51	5.839***	17.561^{NS}	55.448^{NS}	1.204^{NS}	0.052^{NS}
Normal	51	11.011***				
Late	51	13.725***				
Environments (1, 2, 3, 4)	3	762.751***	267.710***	3518.357***	166.148***	6.316***
Treatment*Environment	3	312.711***	332.262***	493.349***	69.147***	0.517***
Normal	3	989.545***	59.471*	1936.926***	140.110***	4.539***
Late	3	86.007***	541.870***	2076.196***	94.796***	2.289***
Environment*Entry	153	6.689***	19.756^{NS}	67.999^{NS}	1.454^{NS}	0.086^{NS}
1	51	7.774***				
2	51	10.014***				
3	51	9.731***				
4	51	13.930***				
Treatment*environment*Entry	153	6.174***	16.581^{NS}	65.850^{NS}	1.735*	0.064^{NS}
Normal (1)	51	9.480***			1.651^{NS}	
Normal (2)	51	6.488**			0.733^{NS}	
Normal (3)	51	4.904*			3.727***	

Normal (4)	51	4.040^{NS}			1.308^{NS}	
Late (1)	51	8.634***			2.326***	
Late (2)	51	7.057***			2.209***	
Late (3)	51	10.717***			1.009^{NS}	
Late (4)	51	13.838***			0.945^{NS}	
Error	1037	3.764	23.520	97.877	1.354	0.109
R-Square		0.632	0.341	0.339	0.557	0.364
CV %		5.908	22.923	25.427	39.502	27.708

***, **, * significant at α = 0.001, 0.01 and 0.05 respectively. NS = non significant.

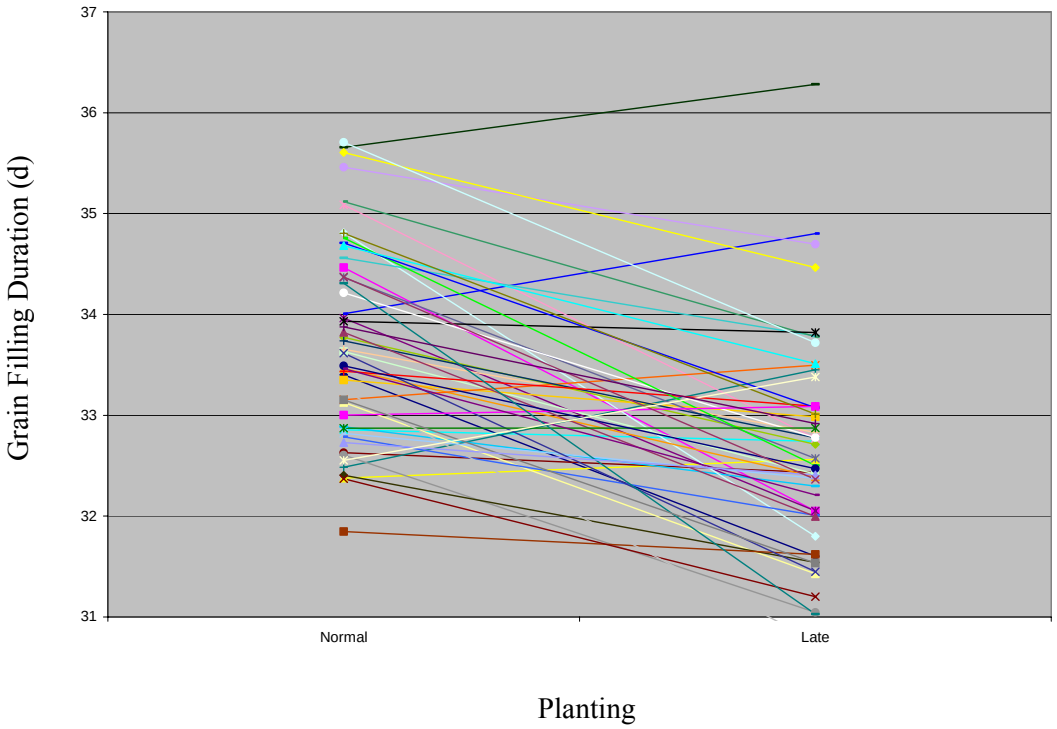

Figure 5-2. Interaction plot showing a noncrossover interaction among the fifty inbred lines for grain filling duration under normal and late planting. Most of the lines decreased in GFD in the late planting compared to normal planting, except for eight lines in which GFD was statistically similar at a least significant difference (LSD) of 0.98 in the late planting and the normal planting.

Trait means for inbred lines under late planting

The mean GFD for Ventnor calculated over all the environments was greater than Karl 92 (Table 3). For all other traits, Karl 92 had greater values than Ventnor. Based on the least significant differences (LSD) mean values for the traits in $F_{3:5}$ lines were different from the mean values of the parents. In the inbred lines mean GFD of 32.6 d, was lower than that of the parents. Kernels per spike at 22.1 was intermediate between the parents, and mean TKW of 37.1 g, mean yields of 2.5 ton/ha, and mean GFR at 1.2 mg/d were higher than both parents. Heritability estimated for GFD, kernels per spike, TKW, yield, and GFR was 64.4%, 51.0%, 46.2%, 53.5%, and 47.6%, respectively.

Heat susceptibility index (HSI) estimate for GFD and TKW

Karl 92 had lower HSI for GFD and TKW than Ventnor (Table 4). The HSI was negative in eight lines for GFD, two lines for TKW, indicating that the trait values were higher under late planting than normal planting. The inbred lines displayed a range of tolerance to heat stress, with some lines performing better than the parents. From the three-dimensional plot of GFD, yield, and kernels per spike (Figure 3) for the population, Ventnor's performance under field condition appeared to be lower than Karl 92. There were many lines performing better than both parents.

Correlation among yield traits under late planting condition

Table 5-3. Means for grain filling duration (GFD), kernels per spike, thousand kernel weight (TKW), yield, and grain filling rate (GFR) estimated over all four environments under late planting. The least significant differences (LSD) for GFD, kernels per spike, TKW, yield, and GFR were 0.184, 0.459, 0.400, 0.096, and 0.014 respectively.

Entry	Trait	Mean	Std. Dev.	Std.Er.	Minimum	Maximum	Range
Lines	GFD (d)	32.6	2.53	0.09	20.00	41.00	21.00
	Kernels/spike (#)	22.1	6.30	0.23	6.90	148.48	141.58
	TKW (g)	37.1	5.52	0.20	3.05	99.28	96.23
	Yield (ton/ha)	2.5	1.32	0.05	0.36	21.75	21.39
	GFR (mg/d)	1.2	0.19	0.01	0.10	3.10	3.00
PARENTS							
Ventnor	GFD (d)	33.9	2.85	0.76	27.00	38.00	11.00
	Kernels/spike (#)	19.8	5.61	1.50	11.50	26.55	15.05
	TKW (g)	35.2	4.77	1.28	28.19	42.00	13.80
	Yield (ton/ha)	1.8	1.10	0.29	0.43	3.57	3.14
	GFR (mg/d)	1.0	0.13	0.03	0.82	1.20	0.38
Karl92	GFD (d)	33.3	2.95	0.79	29.00	38.00	9.00
	Kernels/spike (#)	24.4	2.61	0.70	21.05	28.84	7.79

TKW (g)	36.4	5.15	1.38	26.40	48.75	22.35
Yield (ton/ha)	2.4	1.16	0.31	1.32	4.75	3.43
GFR (mg/d)	1.1	0.21	0.06	0.90	1.68	0.78

Table 5-4. Heat susceptibility indices (HSI) for grain filling duration (GFD) and thousand kernel weight (TKW) to estimate the relative performance of the inbred lines and their parents over four environments.

Entry	GFD	TKW
3	-0.188	-0.312
22	-0.334	-0.135
44	1.214	0.020
40	1.172	0.144
48	0.318	0.146
8	-0.758	0.187
16	0.795	0.209
18	0.725	0.285
36	1.526	0.401
28	0.865	0.430
29	0.231	0.510
9	0.580	0.557
49	1.894	0.579
34	0.338	0.590
12	1.663	0.627
50	-0.824	0.639
41	-0.001	0.659
32	0.106	0.669
47	1.582	0.759
38	-0.085	0.809
7	-0.965	0.825
27	-0.570	0.830
24	1.551	0.838
35	2.103	0.844
6	0.196	0.853
23	1.693	0.855
20	0.355	0.948
43	1.670	0.973
5	1.825	1.539
15	0.697	1.545
46	2.428	1.592
19	1.019	1.705
10	2.803	1.745
11	1.171	1.764
37	1.040	1.771
13	0.441	2.925
17	0.770	3.058
Mean HSI	1.000	1.000

45	3.101	1.034
26	1.236	1.058
30	1.750	1.058
Karl 92	0.917	1.080
14	2.137	1.081
42	0.987	1.097
31	2.090	1.111
4	0.115	0.145
21	1.042	1.157
25	0.924	1.264
39	1.095	1.264
1	1.747	1.335
2	2.271	1.495
Ventnor	1.806	1.511
33	1.361	1.520

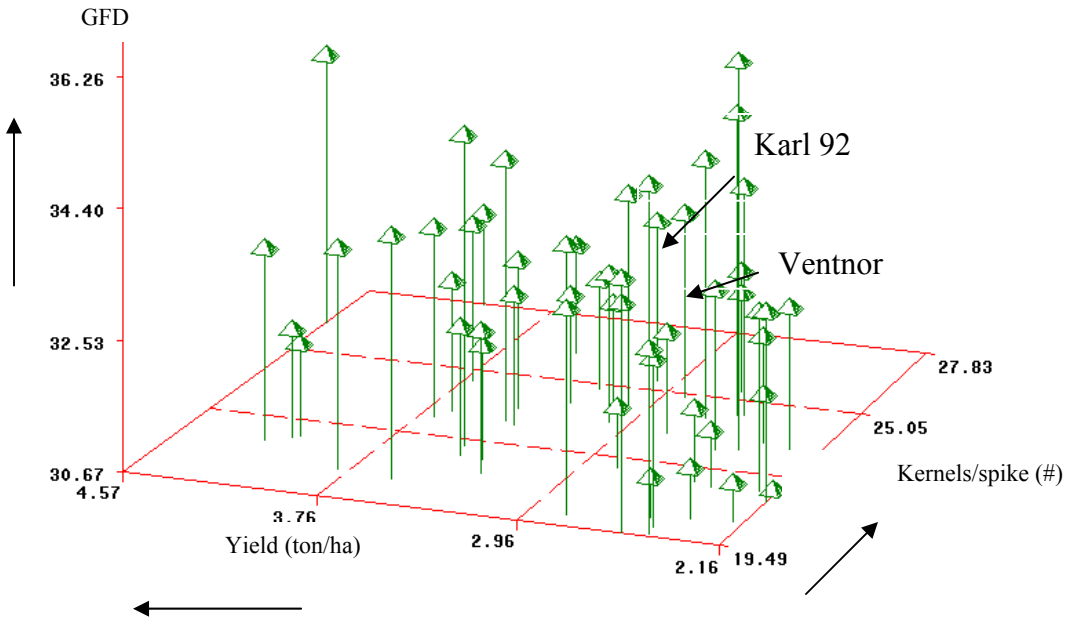

Figure 5-3. Three dimensional plot with yield on x-axis, grain filling duration (GFD) on y-axis, and kernels per spike on z-axis under late planting for the $F_{3:5}$ lines. Each arrow in the plot represents inbred line in the three dimensional space. The population shows normal distribution for the traits and transgressive segregation

The GFD and TKW correlated positively, indicating that an increase in GFD increased the kernel weight (Table 5). Yield correlated positively with two of its main components, kernel per spike and TKW. The GFR correlated positively with TKW and yield, indicating more grain filling increased kernel weight and yield.

Graphical GenoType (GGT) for markers linked to yield traits

The F3:5 inbred line population was genotypically analyzed using the GGT. Markers *Xgwm356*, *Xgwm296*, and *Xksum61* which were co-localized with kernels per spike, TKW, and GFR under late planting conditions were used. The variability explained by these markers for the trait was significantly high (Chapter 3). The parental alleles linked to the trait were color-coded in the analysis (Figure 4). Red represented Ventnor allele and green represented alleles for Karl 92. From the left first 5 bars represent the selected inbred lines which represented extremes of the population, the solid red and solid green bars represent the parental genotypes Ventnor and Karl 92, respectively. The last three bars on the right represented parental alleles linked to kernels per spike, TKW, and GFR. To determine the effect of favorable alleles linked to the traits, composite interval mapping of QTL was performed on a recombinant inbred line population derived from the same cross and screened under the controlled conditions.

Performance of the lines based on the least square means (Table 6) estimate indicated that Line 48 the highest TKW, GFR, and yield at 41.4 g, 1.3 mg/d, and 3.6 ton/ha, respectively. Kernels per spike in this line was moderate. Line 29 had the highest kernels per spike at 27.2 and was among the top 9 lines for yield. It had a moderate TKW and GFR. Line 27 had a moderate TKW and kernels per spike, but had low GFR and yield. Line 37 and 42 were among the susceptible

Table 5-5. Pearson's correlation coefficients among grain filling duration (GFD), kernels per spike, thousand kernel weight (TKW), yield, and grain filling rate (GFR) over four environments under late planting.

	GFD (d)	Kernels/spike (#)	TKW (g)	Yield (ton/ha)	GFR (mg/d)
GFD (d)	1.000	0.054^{NS}	0.364**	0.178^{NS}	-0.018^{NS}
Kernels/spike (#)		1.000	0.023^{NS}	0.269*	0.018^{NS}
TKW (g)			1.000	0.449***	0.924***
Yield (ton/ha)				1.000	0.417**
GFR (mg/d)					1.000

***, **, * significant at $\alpha = 0.001$, 0.01 and 0.05 respectively. NS = non significant

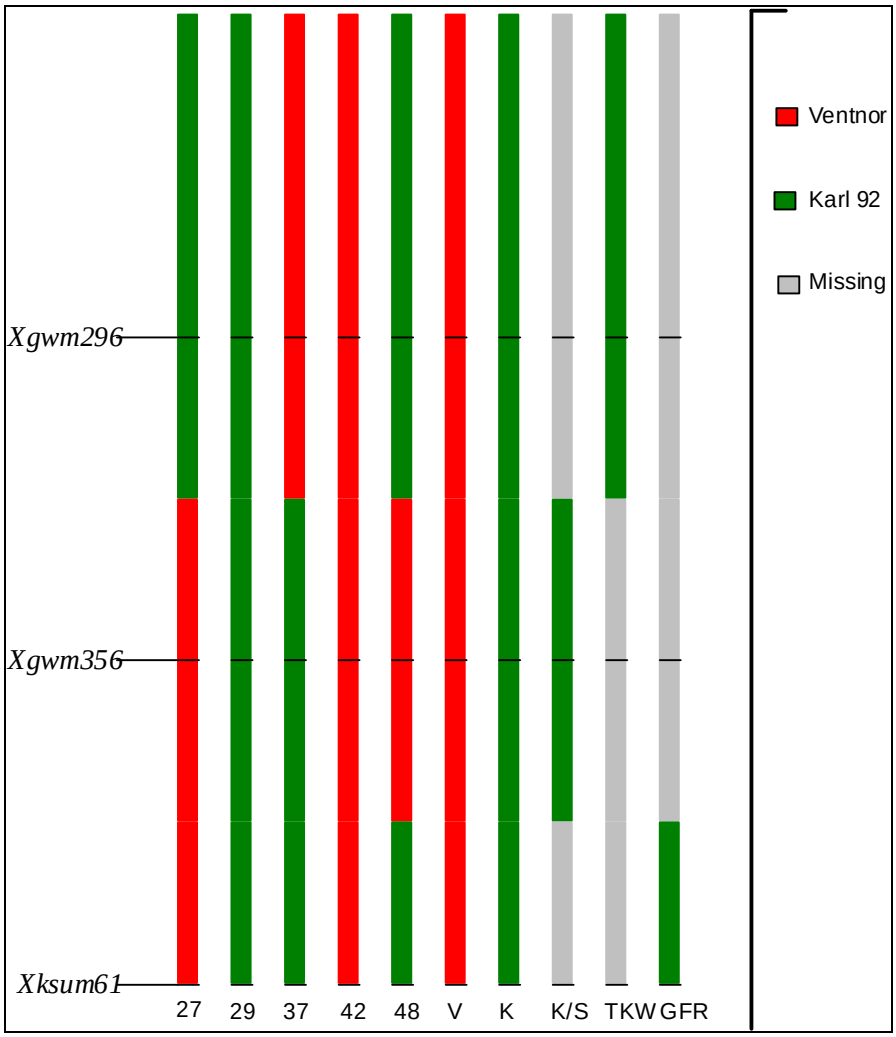

Figure 5-4. Graphical display of genotypic analysis on $F_{3:5}$ population with markers Xgwm296, Xgwm356, and Xksum61. Red codes for Ventnor allele, green for Karl 92 allele, and gray for missing data. The solid red bar (V) and green bar (K) are the parental genotypes Ventnor and Karl 92, respectively. The three bars from right represent alleles for kernels per spike (K/S) linked to Xgwm356, TKW linked to Xgwm296, and Xksum61 linked to GFR.

Table 5-6. Least square means (LSM) and least significant differences (LSD) for kernels per spike, thousand kernel weight (TKW), yield, and grain filling rate (GFR) in selected F3:5 lines, studies under late planting conditions. The lines represent extremes of the inbred population.

Lines	Kernels/spike (#)	TKW (g)	Yield (ton/ha)	GFR (mg/d)
27	21.5	35.6	2.0	1.0
29	27.2	34.6	3.0	1.1
37	22.3	30.5	2.8	0.9
42	19.9	34.0	2.4	1.0
48	21.9	41.4	3.6	1.3
Ventnor	20.2	30.1	1.9	1.0
Karl 92	24.5	35.5	2.5	1.2
$LSD_{0.05}$	2.5	5.2	0.6	0.2

lines with either a moderate TKW or kernels per spike, but had a low GFR and yield. The trait values in Ventnor were similar to susceptible lines, and were lower than Karl 92.

Discussion

The first week after anthesis is crucial for the establishment of kernel number, after which rapid grain filling takes place (Stone and Nicolas, 1995). Anthesis under normal planting in Environment 1 was about two weeks later than anthesis in Environments 2, 3, and 4. Environment 1 had a relatively lower temperature than Environments 2, 3, and 4. Therefore, rapid grain filling in Environment 1 experienced better conditions than the others, though all environments experienced post-anthesis heat stress. Under late planting, anthesis and maturity occurred at similar times under Environments 2, 3, and 4, while plants under Environment 1 flowered and matured later than in the other environments. Temperatures in Environment 1 were low at anthesis compared with the other environments, but all environments had similar temperature regimes for the rest of the grain-filling period.

The treatment X entry interactions for kernels per spike, TKW, and GFR were nonsignificant, indicating performance of the lines for those traits under differential planting was stable. Under controlled conditions RILs derived from the same cross had a significant treatment X entry interaction for TKW and a non significant interaction for kernels per spike and GFR (Chapter 2). The variable conditions experienced by the plants in the field compared to continuous high of 30°C and low of 25°C under control conditions probably affected the kernel weight. Yield followed a similar trend as the above mentioned traits, except three-way interaction was significant. The GFD had a significant three-way interaction with the environment and the planting dates, indicating that the performance of the lines for GFD was affected by planting

dates across all the environments. The treatment X environment interaction was highly significant for all traits, indicating environment had an effect of on performance of differential planting dates. Environments 1 and 4 had a greater effect on GFD. Environment 1 had lower temperature during most of the grain-filling period compared to other environments, while Environment 4, had maximum temperature throughout the grain-filling period.

The inbred lines were derived from a cross between parents that differed in responses to high temperature. The inbred lines exhibited transgressive segregation for the traits, indicated that genes for heat tolerance were contributed by both Ventnor and Karl 92. Though Ventnor was reported to be more heat-tolerant than Karl 92 (Al-Khatib and Paulsen, 1990; Yang et al., 2002; Chapter 2), its field performance was either almost comparable or lower. Winter wheat in Kansas is planted during October. They overwinter, vernilize, and flower during early spring. Ventnor has poor winter hardiness and little resistance to leaf rust (*Puccinia recondita* f.sp. tritici) compared with Karl 92. Even upon the application of fungicide, the disease pressure additional affected the performance of Ventnor under field condition.

Under both normal and delayed planting conditions Ventnor had a longer GFD at 35 d and 34 d respectively, than Karl 92 at 33 d and 33 d. Under normal planting conditions Ventnor had a higher GFR and TKW than Karl 92, while kernels per spike were statistically similar for the two cultivars. Inspite of this under normal planting conditions, the overall yield of Ventnor was lower than Karl 92. Under late planting Karl 92 had a higher mean values for kernels per spike, TKW, GFR and yield than Ventnor. It was hard to evaluate heat tolerance of Ventnor under field conditions due to its poor adaptation to the environment, and its higher susceptibility to leaf rust. Another factor which may have affected Ventnor's performance is its lower winter hardiness than Karl 92, which affected the plant density during spring.

The HSI for GFD and TKW was higher in Ventnor than Karl 92. Since the HSI is a ratio between values under late and normal plantings, higher trait values under late planting impact HIS. The negative HSI values for GFD and TKW in some of the inbred lines were due to nonsignificant increase in the traits in late plantings over normal plantings, indicating that performance of those lines was stable. In most of the lines, values for GFD and TKW were higher, but there was no corresponding increase in the yield. In some lines, where the values for the GFD and TKW were moderate, the yields were high. The difference in yield of the inbred lines which have Ventnor as one of the parent might possibly due to the difference in stand establishment which affects the plant density. The other factor effecting yield may be the delayed planting, which reduces the tiller number per plant and hence number of kernels per plant. Often, a seed number to seed weight compensation takes place to supply the resources more efficiently to the developing grains (Davidonis et al., 2005; Shahinnia et al., 2005). Since both TKW and kernels per spike are major components of yield, it is essential to have a high value for both traits in order to have a high yield.

The correlations of traits under field conditions were similar to those under controlled conditions (Chapter 2). The GFD was correlated with TKW, and TKW correlated with GFR. Yield correlated significantly and positively with both TKW and kernels per spike, but did not show correlation with yield. Though yield of a line is affected by GFD, it is primarily determined by TKW and kernel number. The GFR under controlled conditions had a significant and negative correlation with GFD, but under field conditions the correlation was non significant. This was possibly due to the impact of the environment on the trait and the fact that the field experiments were conducted on the whole plant rather than the single culm (chapter 2). In a complete plant, the presence of tillers may have buffered the grain filling rate. The genetic potential of the line,

apart from the environmental effect, determine the yield. Heritability is the direct estimate of the genetic contribution of the genotype to the trait. A heritability estimate of yield-related traits was highest for GFD at 64%, and TKW and kernels per spike had heritabilities at 46 and 51%, respectively. In spring wheat population under heat stress Mohammadi et al. 2004 reported, high heritability for GFD, and low heritability for TKW. Heritabilities of GFD, TKW, and kernels per spike under field conditions were less than under controlled condition (Chapter 2).

Graphical GenoType analysis is an effective illustrative tool to study the genotype profiles. $F_{3:5}$ lines 48 and 29 were among the best performing heat tolerant lines. Line 27 performed moderately, while lines 37 and 47 were among the heat susceptible lines. Often lines possessing the favorable allele were observed to have better performance for the trait. In these lines kernel weight to kernel number compensation was quite evident. For most genotypic profiles of the lines, the GGT analysis agreed with its field performance.

From the field evaluation of the inbred line population, it can be concluded that genes for heat tolerance were contributed by both Ventnor and Karl 92. Yield correlated positively with TKW and kernels per spike, and GFD correlated with TKW. Under field conditions, TKW and GFD can be used as a criterion for selection. Since the environmental conditions in the field variable with respect to onset of heat, kernels per spike can also be taken into consideration as criteria for selection. Markers *Xgwm296*, *Xgwm356*, and *Xksum61*, which were validated for heat tolerance under field conditions, can be used in marker-assisted breeding programs. Alleles that co-localized with the QTL for kernels per spike, TKW, and GFR were from Karl 92. Although Ventnor has more heat tolerance than Karl 92 it is possible that Karl 92, a cultivar adapter to the Great Plains has some level of heat tolerance. The top $F_{3:5}$ lines 29 and 48 identified under field

conditions and genotypically proved to have superior alleles can be further used in the breeding programs.

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