MAPPING OF DROUGHT TOLERANCE AND LEAF RUST RESISTANCE IN WHEAT

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Abstract

Water availability is commonly the most limiting factor to crop production, especially in drought prone areas like the Midwest. This study was conducted to map quantitative trait loci (QTL) involved in drought tolerance in wheat (*Triticum aestivum* L.) to enable their use for marker assisted selection (MAS) in breeding. A population of 122 F_7 derived recombinant inbred lines from a cross between Dharwar Dry and Sitta, spring wheat lines with contrasting drought tolerances, was analyzed using the amplified fragment length polymorphism (AFLP) technique and Diversity Array Technology (DArT) markers to create a QTL map. Of the 256 AFLP primer combinations evaluated, 151 were found to be polymorphic between the parents and were used to screen the population. A linkage map of 48 groups was created from the combined DArT markers, AFLP data, and SSR markers. This was used to create a QTL map which identified QTL in 24 of these groups. Using these markers for MAS in a breeding program could overcome the difficulties of selecting for drought tolerance.

Another serious limitation to wheat production is leaf rust caused by the pathogen *Puccinia triticina*. Leaf rust causes between 1% and 20% yield loss on average and tends to be the worst in years with high yield potential. PI 289824 contains a single, dominant gene for seedling resistance mapping to chromosome 5BS and thought to be different from *Lr52*. An F_2 mapping population from a cross between PI 289824 and Jagger was used to try to identify markers very closely linked to the gene and therefore useful for MAS. The population presented some mapping challenges, but with the use of SSR and

EST-STS markers, the gene was flanked. However, the markers were at too a great distance to be useful for mapping.

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CHAPTER 1 - Review of Literature

Drought

Drought and Past Breeding Efforts in Wheat

Droughts are a worldwide problem and are becoming even more prevalent in some areas of the world. Since 1960, rainfall has been decreasing in Morocco where droughts used to occur once in ten years, but now are occurring every other year (Forester et al., 2004). Seasonal rainfall for winter annuals in Serbia has decreased from 511 mm to 453 mm from 1981-1995 (Quarrie et al., 1999). A computer model has shown that continued warming over the Pacific Ocean could cause a period of drought in the Western United States. This drought was compared to past droughts, estimated from tree ring data, with the Palmer Drought Severity Index and it was determined that the model prediction would be the most severe (Cook et al., 2004). To alleviate the impacts of these drought periods, irrigation is widely used. However, the Food and Agriculture Organization of the United Nations has predicted a 14% increase in water use for irrigation in developing countries between 2002 and 2030 that will lead to water shortages in one out of five developing countries (FAO, 2002). Irrigation water also adds salts to the soil and can lead to salinity problems for crop production. Increasing fuel costs have also raised the cost of irrigation. For these reasons, it will be more important in the future to have crops that can tolerate drought stress.

A study of the economic impact of drought on farmers was conducted in the Ballia district of Eastern Uttar Pradesh, India. Farms were spilt into groups by size with

marginal farms having less than one hectare, small farms having one to two hectares, medium farms having two to four hectares, and large farms having more than four hectares. Years of drought were reported to increase the money spent by farmers on inputs such as seed and irrigation and decrease yields leading to huge reductions in income for farms of all sizes. Marginal farms experienced a 441% decrease in total income from a normal year to a drought year. The decrease was 156% for small farms, 119% for medium farms, and 128% for large farms (Kumar, 2002). Farmers in this study were growing paddy rice (*Oryza sativa* L.), sugarcane (*Saccharum officinarum*), arhar (*Cajanus cajan*), and maize (*Zea mays* L.), but the nature of the impact, if not the reported values, are probably representative of all crops. While the impact may be worse for subsistence farmers, all farmers were negatively impacted by drought and so all farmers can benefit from drought tolerant cultivars.

Water is the most limiting factor in crop production. According to Boyer (1982), 44.9% of U.S. soils have low water availability and 40.8% of all crop insurance payments from 1935 to 1978 were for drought. Boyer reports an 87% yield reduction in wheat with diseases, insects, and weeds causing 2.7%, 1.1%, and 2.0% of this loss respectively. Thus, 94% of the loss is due to environmental factors with drought being the largest contributor to environment.

Droughts can occur at different stages of crop development and affect the plant in different ways. In some environments, crops are grown completely or almost completely on residual moisture. In these environments the drought stress occurs later in development and worsens as the season progresses. In other environments droughts occur more sporadically and can affect plants early in development, but precipitation later

in the season can relieve the water stress. García del Moral et al. (2003) reviewed yield component compensation under stress in durum wheat. Drought stress very early during planting limits germination and thus affects stand establishment and later during vegetative stages it affects tillering. As the plant transitions from vegetative to reproductive growth during the jointing stage, the size and number of spikes is decreased. During anthesis the number of kernels per spike is reduced due to the loss of pollen or stigma viability, which then influences fertilization. Stress during grain fill can cause seed abortion and lower the kernel weight because it affects grain fill rate and duration (García del Moral et al., 2003). Genes that confer tolerance at one stage may not be effective at another stage so it is important to select for drought tolerance in an environment that is very similar to the one in which the cultivars will be grown.

Wheat and Selection for Tolerance

Rice, maize, and wheat provide 60% of the energy consumed as food in the world making them the most important food crops (FAO, 1995). The world consumption of wheat was 687 million tons in 2005 (Lawrance, 2006). World trade of wheat is the largest of cereal crops in terms of U.S. dollars, accounting for 40% and 41% of cereal import and export dollars respectively in 2004 (FAO, 2006). Wheat accounted for 19% of total production among major cereal crops. This was exceeded only by coarse grains and maize (FAO, 2006). Breeders have been trying to increase drought tolerance in wheat for many years. In Australia, drought tolerance became a major concern in the late 1800's when wheat production began to move inland to more arid regions and the commonly used cultivars from England were not well adapted to the new environment. Breeders began to visually select for traits such as earliness, short straw, and less tillering to

improve yield under drought conditions (Marshall, 1987). Earliness is used as a drought avoidance mechanism so the plant can complete its life cycle before the major period of stress (Barnabas et al., 2008). Less leaf area due to reduced height and tillering can limit transpiration (Barnabas et al., 2008). However, these traits limit the amount of vegetative biomass produced and biomass has been shown to be correlated with yield (Babu et al., 2003). Therefore the yield potential under more ideal non-stress conditions is also reduced. This is an example of why drought tolerance mechanisms are more attractive to breeders than drought avoidance mechanisms.

There is debate about the best ways to breed for drought tolerance. Are selections more effective for developing stress adapted cultivars when made in stressed or unstressed conditions? Research has been conducted to try to answer this question, but opinions are divided on the issue (Srivastava, 1987). The quantitative nature of abiotic stress tolerance, low heritability, high gene by environment interaction, and variation and unpredictability of environmental conditions have hindered breeders' efforts to select for drought tolerance.

Non-Visual Selection

Many traits have been considered for drought tolerance screening that could eliminate the need for field testing in unpredictable environments. Some of these traits include osmotic adjustment, relative water content, water loss rate, and water use efficiency (Rampino et al., 2006; Gorny, 1999) all of which influence the plant's ability to maintain high turgor and normal growth with less water available. Osmotic adjustment through the accumulation of solutes in the cytosol lowers the osmotic potential of the cell and therefore increase the amount of water held in the cell. Relative water content is a

measure of the amount of water held in the leaves relative to full turgor. Maintaining high water content allows normal growth to occur as water becomes scarce. Relative water content is calculated as (Fresh Weight – Dry Weight)/(Turgid Weight – Dry Weight) (Barrs and Weatherley, 1962). One way a plant can stay closer to full turgor is to lose water at a slower rate by closing stomata or accumulating insulating wax layers. Water loss rate is calculated as (Fresh Weight – $Weight_{24}$)/(Dry Weight*24) where $Weight_{24}$ is the weight of the leaf after twenty four hours on filter paper (Suprunova et al., 2004). Water use efficiency is grain yield divided by the water used by the plant. However, the aim of breeding drought tolerance is to develop cultivars that have high yield potential under drought conditions instead of merely being able to survive them. Unfortunately, these traits are not always associated with economic yield. For example, Gorny (1999) used durum addition lines from Chinese Spring to study water use. With the exception of 1D, the increased efficiency was only seen in vegetative tissues. Only the addition of 1D to the durum line showed increased efficiency when considering water use efficiency.

Another trait commonly considered as a measure of drought tolerance is cell membrane stability (CMS). The technique for measuring CMS includes washing leaf samples to remove surface solutes, stress induction with a polyethylene glycol (PEG) solution, rehydrating in deionized water, measuring the electrical conductivity of the solution, and finally killing the tissue by autoclaving and measuring the electrical conductivity again. This technique was developed in grain sorghum (*Sorghum bicolor;* L.Sullivan and Ross, 1979) and is useful in many other crop species however, results vary greatly with the experimental conditions such as the duration of rehydration and

concentration of PEG. Quilambo (2004) evaluated the use of CMS in peanut (*Arachis hypogaea* L.) and found it to be effected by drought tolerance of the cultivar and growth stage and that the amount of injury did not show a constant relationship with tolerance at different growth stages. Cultivars with increased cell injury also did not show a decrease in yield. Bajji et al. (2001) evaluated CMS in durum wheat and reported a relationship between CMS and relative growth rate in shoots. If increased relative growth rate can be assumed to increase biomass and therefore yield, this may be a very useful evaluation technique. However, CMS was measured in seedlings only and final yield was not considered in the study by Bajji et al. (2001).

Traits of Drought Tolerance

Drought tolerance traits have been mapped in many plants such as Arabidopsis (*Arabidopsis thaliana*), rice, barley (*Hordeum vulgare* L.), maize, and pearl millet (*Pennisetum glaucum* L.). Studies of these plants can provide information useful for wheat as well. Rice, barley, maize, sorghum, foxtail millet (*Setaria italica* L. Beauv.), sugarcane, and wheat genome comparisons have led to the hypothesis that these cereals share a single ancestral chromosome (Moore et al., 1995) and so, genes and gene clusters found in one species can provide an idea of where similar genes may be found in another species (Moore et al., 1995). However, the synteny of the genomes is not complete, so genes identified in one species cannot be sure to map to the homologous position in all other species (Moore et al., 1997). Summary of some quantitative trail loci (QTL) mapping experiments in species with known synteny to wheat are given below. The QTL identified in these studies may also exist in wheat and so the regions influencing drought

tolerance in the other grass species may be supporting evidence for drought tolerance genes identified in syntenic regions of wheat.

Root Traits

Roots traits are commonly considered drought tolerance traits because the ability of a plant to reach and extract water from the soil should impact its ability to continue normal growth during periods of low moisture. Increased root biomass and root:shoot ratio have been reported under drought stress (Blum et al., 1983). However, in a physiological study of Mexican landraces under drought stress, Reynolds et al. (2007a) reported only a modest increase in either root mass or root:shoot ratio for drought adapted landraces. Instead, significant differences in root distribution were seen. More root mass appeared in the lower portion of the soil profile in the adapted landraces leading to increased water use by these lines (Reynolds et al., 2007a).

Zhang et al. (2001) studied root traits in a rice population derived from a cross between the upland cultivar CT9993-5-10-1-M and the lowland cultivar IR62266-42-6-2 differing in root morphology. Traits considered included penetrated root number, root thickness, root pulling force, and root dry weight. All phenotypic data was collected under well watered conditions. A wax-petroleum layer was used to simulate soil compaction. Penetrated root number was the number of roots that had penetrated this layer after 50 days. Root pulling force was measured as the force required to pull the plant from the soil as reported by O'Toole and Soemartono (1981). This measure was correlated with root weight, root branching, and thick root number (O'Toole and Soemartono, 1981) and so is a measure of how extensive the root system is. Thirty-six QTL were detected for these root traits with at least one appearing on each of the twelve

chromosomes. Chromosome 4 had the largest number of QTL which were concentrated in the region around the marker *RG476*. Traits affected by QTL in this cluster were root penetration index, basal root thickness, penetrated root thickness, and penetrated root dry weight. The authors suggested this QTL is most likely a single gene with pleiotropic effects.

A significant problem with considering any secondary trait for drought avoidance or tolerance is that they may not contribute to a greater economic yield under stress conditions as there may be compensation in other areas. It has even been suggested that vigorous root growth in general may have a negative impact on grain yield (Bruce et al., 2002) as the increased underground biomass takes resources away from production of above ground biomass and so can limit photosynthesis. However, Babu et al. (2003) report that grain yield in rice is positively correlated with root penetration index, basal root thickness, root pulling force, deep root thickness, deep root dry weight, and root depth. They also report the overlap of production and root trait QTL as evidence of their association. Reynolds et al. (2007a) found that lines with increased water use had lower water use efficiency, but they went on to suggest that the linkage between these traits may be broken through breeding and so, be of value. An additional concern with measuring any trait is the difficulty in extrapolating the response that will be seen in an environment different from the one in which the trait is measured. In a study involving both well watered and drought stress treatments, no QTL for root traits was consistent across both treatments (Hemamalini et al., 2000). This implies that regions identified under ideal conditions may not be effective under stress.

Relative Water Content

Relative water content (RWC) measures the percent of water in a leaf relative to full turgor and thus represents the water stress being experienced by the plant (Barrs and Weatherley, 1962). Plants that are able to maintain high levels of RWC under drought stress should be less affected by the stress and be able to maintain more normal growth and yield. Teulat et al. (2001) mapped six QTL for RWC to chromosomes 2H (1 QTL), 6H (1 QTL), and 7H (4 QTL) of barley in a growth chamber study. Three of these were detected under well watered conditions and three were detected under stress. Only one stress QTL, on the long arm of chromosome 7H, is located close to a non-stress QTL. A field study in Mediterranean environments in Europe and North Africa tested the consistency of QTL across environments (Teulat et al., 2003). The QTL on chromosomes 2H and 6H in the 2001 study were still detected, but only 6H was consistent across environments in 2003. There was an additional RWC QTL detected across multiple environments on chromosome 4H in 2003 where a QTL for water soluble carbohydrates at full turgor under well watered conditions was identified in the 2001 study (Teulat et al. 2003). There was also a QTL detected in multiple environments on chromosome 7H that was not detected for any water status trait in the 2001 study (Teulat et al. 2003). QTL for RWC were also mapped in upland rice by Price et al. (2002). They identified QTL on chromosomes 1, 3, 4, 5, 6, 8, 9, 10, and 11. The QTL on chromosomes 4 and 5 are in homologous locations to those showing QTL by environment interaction on 2H and 1H, respectively, identified by Teulat et al. (2003). Additionally, the QTL on chromosome 9 is homologous to the QTL on 5H which was detected in one environment by Teulat et al. (2003). Regions conserved across some grass species are likely to be present in other grass species as well and so could be

important regions for drought tolerance in wheat (Moore, 1995). The differences in QTL detected between moisture regimes and across environments suggest that different genes are expressed under stress conditions than are under normal conditions and that the nature of the stress determines the genes involved. It is therefore important that QTL be identified under conditions similar to those in the region a breeding program is targeting.

Yield and its Components

As yield is the goal of any producer, directly identifying QTL for maintained yield under drought stress is the most efficient way to make progress in a breeding program. However, yield is a trait with low heritability so it is difficult to successfully select for. Both Zou et al. (2005) and Yue et al. (2006) mapped QTL for yield and yield components in rice. Both studies used RIL populations derived from Zhenshan 97, a lowland rice cultivar, and IRAT109, an upland rice cultivar. Six QTL were identified by both studies. These QTL were on chromosomes 2 and 3 (two each) and 10 and 12 (one each). However, only one of the QTL on chromosome 2 and the one on chromosome 10 were for the same trait, yield, in both studies. Yue et al. (2006) used the ratio of drought stressed to control measures for all QTL analysis. This measure is not drought susceptibility index in the strict sense, but it does give an idea of percent loss in the specific trait due to drought stress. Zou et al. (2005) detected the QTL on chromosome 2 under drought conditions and that on chromosome 10 under well watered conditions. This implies that QTL for drought tolerance may be genes with pleiotropic effects under different conditions.

Yield QTL were mapped in hexaploid wheat by Quarrie (2005). QTL appearing under drought conditions were located on chromosomes 1A, 1B, 2A, 2B, 2D, 3D, 5A,

5B, 7A, and 7B. The QTL at end of the long arm of 5B is homologous to that at the end of the short arm of rice chromosome 3, QTL near the centromere of 1A and 1B are homologous to that on chromosome 10 in rice, and the QTL near the centromere of chromosome 5A and 5B are homologous to that at the end of the long arm of chromosome 12 in rice. All of these rice QTL were detected under well watered conditions. Additionally, QTL detected under well watered conditions in wheat on 4B and 4D were homologous to QTL on the long arm of rice chromosome 3. One of these rice QTL was detected under stress and the other under well watered conditions. These regions with QTL conserved across species are possible targets for breeding programs. However, they must be evaluated for effect under stress in wheat since QTL were usually detected in the opposite conditions in the rice.

Additional Traits

A recent study by Reynolds et al. (2007b) evaluated diverse spring wheat germplasm under drought and heat stress to determine which traits had the largest potential to contribute to improved tolerance. The increased yield potential was calculated for each trait individually, but principle component analysis suggests that while the traits are all associated with yield and biomass, most are not associated with each other and so could probably give cumulative improvements in yield (Reynolds et al., 2007b). The traits thought to give the largest yield improvement under drought stress were canopy temperature at 10%, carbon isotope discrimination at 9%, and stem carbohydrates for remobilization at 6% (Reynolds et al., 2007b). Canopy temperature is influenced by the ability to extract water from the soil as increased transpiration leads to evaporative cooling of the leaves. Carbon isotope discrimination is a measure of

transpiration efficiency (the amount of dry matter produced per unit of water transpired) and has been shown to be highly heritable (Condon and Richards, 1992) thus making it an attractive trait for selection. The accumulation of carbohydrates in the stems during vegetative growth provides a resource to be remobilized and used during grain filling if environmental conditions prevent maximum photosynthesis at that time (Blum, 1998). Additional study of and breeding for these traits may greatly increase drought tolerance in wheat.

Candidate Genes for Drought Tolerance

Some molecular studies have been done to identify specific genes and proteins involved in drought tolerance. Much work has been done on dehydrin proteins, but the expression of many other genes is altered by drought conditions as well. Candidate genes can be involved in drought tolerance at many levels including, water stress sensing and signaling, cell protection, and cell recovery. Some drought candidate gene studies are reviewed below.

Drought Stress Signaling

Signaling of drought stress has been well studied in Arabidopsis. Abscisic acid (ABA) is a plant hormone that influences germination, maintenance of seed dormancy, control of stomatal closure, and response to abiotic stresses. ABA synthesis, catabolism, and regulatory actions influence the plant's ability to respond to drought stress so these pathways present some candidate genes for use in drought tolerance breeding. Abscisic acid-responsive element binding protein1 (AREB1) is a regulatory protein that induces the expression of ABA responsive genes such as late embryogenesis abundant (LEA) proteins. A study was conducted in Arabidopsis to identify the impact of AREB1 on

drought tolerance. Mutants that constitutively express an active form of ARBE1 had increased drought tolerance that was not due to stomatal closure. Almost all transgenic plants were able to recover from a twelve day period of drought stress applied two to three weeks after germination where as almost all wild type plants died. Loss of function mutants for AREB1 all died during a drought period that some wild type and most constitutive mutants were able to survive (Fujita et al., 2005). Little was discussed about the other plant characteristics affected by this transformation.

Another way to improve drought tolerance is to increase the amount of ABA in a plant and therefore, its response to the stress. CYP707A3 is an ABA 8'-hydroxylase that breaks down ABA in the plant. There are three other CYP707A enzymes with ABA catabolic functions, but CYP707A3 was the most highly expressed under dehydration and rehydration (Umezawa et al., 2006). It is abundant in vegetative tissues and loss of function mutants showed no differences in seed germination. However, the loss of function mutants maintained a higher level of ABA especially under dehydration stress and so, had higher drought survival rates than wild type plants (Umezawa et al., 2006).

Calcium is an important secondary messenger for signaling in plant cells. Knight et al. (1997) showed that calcium plays a role in signaling and regulation of gene expression in response to drought and salt stress. Drought stress in six to seven day old Arabidopsis seedlings, caused by placement in mannitol solution, caused short lived elevated levels of calcium in the cells and increased expression of three genes, P5CS, l*ti78,* and *rab18,* known to be upregulated under drought stress. P5CS codes for the first enzyme for the formation of proline which is believed to be the rate limiting step. Proline is a major solute used by plants for osmotic adjustment and so can help maintain cell

turgor. *Lti78* is expressed independently of ABA where as *rab18* is dependent on ABA. The functions of these genes are unknown. Pretreatment with calcium channel blockers and calcium chelators, which act primarily on extracellular calcium, were seen to reduce calcium concentration and gene expression relative to the normal stress treatments, but they did not prevent the reaction, indicating that intracellular calcium, most likely coming from the vacuole, also plays a role in the signaling (Knight et al., 1997).

An example of improved drought tolerance through transformation with genes from another species is the transcription factor DREB1A identified in Arabidopsis and transformed into tobacco (*Nicotiana tobacum*; Kasuga et al., 2004). Transgenic plants over-expressed the transcription factor and the downstream genes that it regulates. Transgenic plants survived and recovered from a two week drought stress better than control plants. These plants also had significantly lower electrolyte leakage after drought stress. When the DREB1A gene was under the control of a constitutively expressed promoter, it caused a large reduction in growth, but under the control of a stress induced promoter, it caused only a small reduction in growth (Kasuga et al., 2004). The DREB1A gene was also transformed into wheat under a stress induced promoter with positive effects on drought survival and yield under drought (Pellegrineschi et al., 2004). Water was withheld from plants at the five to six leaf stage for ten to 15 days. Transgenic wheat plants showed no symptoms of drought stress until the fifteenth day without water where as control plants showed symptoms after ten days and all leaf tissue was dead by the fifteenth day. Higher total head number, better head development, and more branched root development were associated with the DREB1A gene. Transgenic

lines did show delayed and nonuniform germination, but no other differences in growth or morphology were observed (Pellegrineschi et al., 2004).

Genes Responding to the Signals

When the signal that a stress is occurring is received by the cells, other genes are expressed in response to help protect the cell and maintain growth as normally as possible. In a study of rice by Vinod et al. (2006), sequences from the National Center for Biotechnology Information (NCBI) database were used to design primers for putative gene candidates for drought tolerance, specifically elements such as MYB, MYC, and abscisic acid responsive elements which have sequence similarities across species. Of the 21 primers originally designed, two were found to be associated with possible drought tolerance traits under both well watered and stress conditions through single marker analysis. These were *EXP15* and *EXP13* which are cell elongation proteins. *EXP15* was found to affect root number and *EXP13* was found to affect silicon content in the stem (Vinod et al., 2006). Root elongation is a highly water sensitive process, so it is logical that these genes would be responsive to drought. Silicon has been shown to have positive effects under abiotic stresses such as metal toxicities and salinity. It is likely it also plays a role in plant water relations, but studies have not found this conclusively (Epstein, 1999), so increasing silicon content in the plant could increase tolerance.

A study of barley identified two candidate genes co-segregating with drought tolerance QTL, both on chromosome 7H (Diab et al., 2004). The *Acl3* gene codes for barley acyl carrier protein III and co-segregated with a QTL for RWC and another for water soluble carbohydrate at full turgor. Both QTL were identified under stress conditions. The role of this protein has not been explained, but the authors suggest it

might protect membranes and membrane fluidity as it is involved in fatty-acyl chain synthesis (Diab et al., 2004). The *bSS1B* gene codes for sucrose synthase and co-segregated with a QTL for RWC under well watered conditions (Diab et al., 2004). Osmotic adjustment throught the accumulation of solutes, such as sucrose, can decrease solute potential in a cell and so increase the amount of water. This could help maintain cell turgor, but would need to be shown to function under stress conditions as well, before it is used in breeding.

Another example of osmotic adjustment through concentration of solutes is transgenic rice accumulating glycinebetaine which is accumulated in dicotyledonous plants and in some grass species, such as barley, wheat, and rye (Kishitani et al., 1994). Rice lacks the two enzymes used by higher plants to produce glycinebetaine from choline. However, *Arthrobacter globiformis* uses choline oxidase to convert choline to glycinebetaine in a single step (Sawahel, 2004.). When this gene is transformed into rice plants, their drought tolerance was greatly enhanced and growth continued normally. When three week old plants were drought stressed by withholding water for two weeks, 70.8% of transgenic plants survived where as only 0.9% of control plants did (Sawahel, 2004). Transgenic plants also maintained higher water potential than control plants.

Mannitol is also accumulated by some plants as an osmoprotectant, however this is not true of wheat (Abebe et al., 2003). The *E. coli* gene *mtlD,* which codes for mannitol-1-phosphate dehydrogenase and reversibly converts fru-6-phosphate to mannitol-1-phosphate, was transformed into wheat (Abebe et al., 2003). Control plants showed leaf rolling and wilting on the second or third day of stress three weeks post germination, but these symptoms were delayed until the fourth day in transgenic plants.

While both control and transgenic plants showed decreased growth under water stress, the decrease was much smaller for transgenic plants. However, the increase in mannitol accumulation was very small and the differences in osmotic adjustment between transgenic and control plants was not significant. Therefore, the authors hypothesized that better performance under drought stress must be due to some other protective effect of mannitol such as hydroxyl radical scavenging or stabilization of macromolecules (Abebe et al., 2003).

Dehydrin

Dehydrins belong to the LEA class of proteins and have been shown to accumulate under dehydrative stresses such as drought, freezing, salinity, and with the application of ABA (Choi et al., 1999). Dehydrins are identified by a K segment as defined by Close (1996). The K segment is high in lysine content and forms an amphipathic α-helix so it is hydrophobic and able to interact with lipid membranes (Close, 1996). Dehydrins are further divided into classes by the number of Y and S segments they contain. Between these segments the proteins are rich in hydrophilic amino acids so they can interact with water molecules (Brini, 2007).

QTL have been detected in barley close to known dehydrin gene locations. They are between *dhn1* and *dhn9* on chromosome 5H, distal of *dhn5* and *dhn4* of chromosome 6H (Teulat et al., 2003) and *dhn2* on chromosome 7H (Teulat et al., 2001). All of these dehydrin genes have been shown to be upregulated in barley under stress with no expression observed in well watered plants (Choi et al., 1999). The genes *dhn2* and *dhn6* were seen to have large increases in expression under polyethylene glycol stress in sorghum. The expression was greatest after three hours of stress and had declined after

twenty seven hours although the levels were still much higher than control (Buchanan et al., 2005).

Spring Wheat Drought Tolerance

Kirigwi et al. (2007) attempted to map drought tolerance traits of importance to wheat breeders using a mapping population of 127 recombinant inbred lines from a cross between Dharwar Dry and Sitta. Dharwar Dry is an extremely drought tolerant spring wheat variety collected in India. Sitta is a spring wheat developed at CIMMYT in Mexico and is well adapted to conditions there, but relatively drought susceptible when compared to Dharwar Dry. The population was selected at each generation for plant vigor, tillering, spike number, and spike fertility. This presents some problems when mapping, but because Dharwar Dry has an extremely undesirable plant type, this population represents the useful variability available to breeders. Kirigwi et al. (2007) used SSR and EST-STS markers to map the population and focused on one particular QTL that mapped to chromosome 4A. This QTL affects drought susceptibility index (DSI), grain yield, biomass, spikes per square meter, grains per square meter, grain fill rate, and biomass production rate under stress conditions as well as days to heading under well watered conditions. Markers associated with this QTL were *XBE637912*, *Xwmc89,* and *Xwmc420*. These markers explain 15, 18, and 20% of the variation in yield and 29, 32, and 30% of the variation in biomass, respectively. Lower than expected levels of polymorphism were seen in the population. The pedigree of Dharwar Dry is unknown so it is possible that it might be related to Sitta because there is much CIMMYT material is grown in India. Use of a different kind of marker with higher frequency in the genome and higher polymorphism might allow better mapping of the population.

Leaf Rust

Disease Characteristics

Leaf rust is a fungal disease of wheat that is caused by the pathogen *Puccinia triticina.* The fungus undergoes a sexual cycle on meadow rue (*Thalictrum* spp.) in Eurasia, but this does not occur in North America. This means that North American leaf rust races do not cross with each other and so adaptations occur only through genetic mutations (Bowden, 2000). The disease spreads as windborne urediniospores. When the spores are deposited on a leaf with adequate moisture from rain or dew and the weather is favorable, infection can occur in as little as four hours (Bowden, 2000). Spores travel through the "*Puccinia* Pathway" blowing up from South Texas and Mexico in the spring to establish infections in the north and then back south in the fall to reestablish infections there (Eversmeyer and Kramer, 2000.) However, the disease can also over winter as dormant mycelium in the fall planted wheat crop as far north as Central Nebraska in seven out of ten years (Eversmeyer and Kramer, 1996.) This presents a large problem as infections begin much earlier in the spring and allow more cycles of spore production to occur, making epidemics worse. For example, around Manhattan, Kansas, the first pustules were found in fields where leaf rust over wintered around March 1 where as fields without over wintering mycelium did not have pustules until around April 25. (Eversmeyer and Kramer, 2000).

Leaf rust causes small orange pustules on young leaves and black pustules on older leaves. The pustules may be surrounded by a narrow halo which is either yellow or white in color (Bowden, 2000). Pustules occur on leaf blades with the majority of yield reduction being due to the infection on the flag leaf (Eversmeyer and Kramer, 2000).

The disease develops most rapidly with 20-25°C days and 15-20°C nights when enough moisture is present to form dew (CDL, 2006). These conditions are also ideal for wheat growth which means that the greatest losses from leaf rust occur in years with the greatest yield potential (Eversmeyer and Kramer, 2000). Statewide yield loss due to leaf rust for Kansas from 1980-1999 was 4.6% with losses of 11.3% and 11.0% in 1992 and 1993 respectively (Eversmeyer and Kramer, 2000). Losses in individual fields can be as much as 40% (Bowden, 2000). Leaf rust reduces yield by causing premature death of infected leaves (Bowden, 2000). This decreases the leaf area available to intercept light and so reduces photosynthesis. The earlier the infection occurs, the more severe the losses will be (Bowden, 2000). Losses will likely increase as the amount of primary inoculum in the field is increased by practices such as minimum or no tillage and shorter crop rotations (Eversmeyer and Kramer, 2000).

Leaf Rust Resistance

Currently there are 59 leaf rust resistance genes with permanent gene designations (CDL, 2008). There is thought to be a gene-for-gene relationship between the leaf rust pathogen and the wheat host meaning that for a single resistance gene in wheat there is a single gene in the pathogen that makes it either virulent or avirulent on the host (Kolmer, 1996). Leaf rust resistance in wheat is due to major genes, which can provide adult or seedling resistance, and minor genes. Seedling resistance genes are effective for the whole lifecycle of the plant (Sawhney, 1995). Adult plant resistance genes are effective only in adult plants, but have been shown to be an important part of durable leaf rust resistance (Singh et al., 2001). Minor genes have effects that are too small to identify individually, but are thought to be more durable than the major genes. They result in

fewer, smaller pustules and longer latent periods (Kolmer, 1996). Leaf rust resistance is commonly scored by the Stakman scale as described by McIntosh et al. (1995). This scale uses numbers from zero to four to describe the reaction with zero being immune and four being susceptible. A ; is used to describe a very resistant hypersensitive reaction and X, Y, and Z are used to describe heterogeneous reactions. Plus and minus signs can also be used to modify the classes (McIntosh et al., 1995.)

The prevalent race of the pathogen can shift very quickly to overcome the resistance imparted by a single gene. For example, *Lr3ka*, *Lr11*, *Lr24*, *Lr24, Lr26* have all been released in Kansas with effective leaf rust control at first, but within one to two years virulent races had been selected for and greatly increased in the Great Plains limiting the effectiveness of the genes (Kolmer, 1996). Kolmer (1996) also stated that of nine resistance genes common in cultivars of Kansas and Nebraska, only two, *Lr9* and *Lr16*, would have provided effective levels of control in North America in 1996. Because of the frequent shifts in pathogen races, breeders would like to pyramid genes so that multiple genes are present in the same cultivar. Leaf rust races virulent on *Lr13* have been present in Australia since 1984, but as of 1997, the combination of *Lr13* and *Lr1* had remained effective against all races of leaf rust in Australia (Singh et al., 2001). However, pyramiding genes is difficult to do with phenotypic selection because of the lack of pathotypes with the desired virulence to select for gene combinations (Nocente et al., 2007). For this reason, markers closely linked to the genes of interest to allow their selection by marker assisted selection are highly desirable. Nocente et al. (2007) evaluated the usefulness of markers reported for *Lr1, Lr9, Lr24,* and *Lr47* for marker assisted selection. They were unable to select for *Lr1* with the STS marker reported as it

was not polymorphic between the resistant and susceptible materials used, but the other genes showed 100% cosegregation with the markers (Nocente et al., 2007).

Objective

The overall objectives of these two research projects were to create linkage maps for the populations and through QTL mapping or leaf rust screening to identify markers that may be used for marker assisted selection to aid in breeding for drought tolerance and leaf rust resistance respectively.

CHAPTER 2 - Amplified Fragment Length Polymorphism Mapping and QTL Analysis of Drought Tolerance in a Spring Wheat Population

Introduction

Drought tolerance is an important area for improvement in crop species because drought is the largest factor limiting production (Boyer, 1982). Irrigation is commonly used to alleviate drought stress, but increased irrigation will lead to water shortages (FAO, 2002) and can increase the salinity of the soil which will cause further problems. For these reasons, increasing drought tolerance of the crops is important to allow sustained yields with less water use. This is especially important for staple crops such as wheat which, together with rice and maize, provide 60% of the world's food energy (FAO, 1995).

Breeding for drought tolerance has occurred for many years. The first selections were visual for traits such as earliness, short straw, and reduced tillering (Marshall, 1987). Then field traits such as root architecture and canopy temperature (Reynolds et al., 2007a; Reynolds et al., 2007b) and traits tested for in the laboratory such as osmotic adjustment, relative water content, water use efficiency, cell membrane stability, stem carbohydrates, and carbon isotope discrimination were selected for (Rampino et al., 2006; Gorny, 1999; Sullivan and Ross, 1979; Reynolds et al., 2007b). There is debate among breeders about the best environment in which to select for drought tolerance. Srivastava (1987) reviewed several studies aimed at determining the most efficient environment for drought improvement and reported mixed results from the studies. Some studies

recommended selection under intermediate conditions where as others reported that lines selected in intermediate conditions did not yield well in stress environments and so, recommended selection under stress at all generations (Srivastava, 1987). Yield has low heritability and there is high gene by environment interaction for drought tolerance traits (Quarrie et al., 2005), making selection very difficult. Marker assisted selection (MAS) is a proposed solution to this problem. Cattivelli et al. (2008) reviewed progress of breeding for drought tolerance and suggested that markers tightly linked to traits conferring drought tolerance could improve breeding efficiency. Quantitative trait loci (QTL) analysis can be performed to statistically analyze the association between markers and traits of interest. This identifies regions of the chromosomes that influence these traits. QTL analysis of yield and yield components can break these complex traits down into components which can be selected for collectively to improve the heritability (Quarrie et al., 2005).

QTL maps have been made for traits thought to be involved in drought tolerance in many species including rice, barley, and wheat (Zhang et al., 2001; Teulat et al., 2001; Teulat et al., 2003; Quarrie et al., 2005). However, since drought is a complex trait and tolerance is hard to measure, many of these studies focus on secondary traits, such as root characteristics and relative water content, under stress. These traits definitely influence drought survival, but the goal of any producer is yield. Most studies do not consider the final yield of the plant under stress and so the mapped traits may not be of interest in breeding programs. Identifying QTL for yield directly or for traits correlated with yield could be an efficient way to incorporate drought tolerance into a breeding program.

Kirigwi et al. (2007) attempted to map genome wide QTL for yield and yield components under drought stress in a spring wheat population of recombinant inbred lines from a cross between Dharwar Dry and Sitta. A QTL on 4AL was identified that explained large portions of the variation in grain yield (R^2= 0.32) and biomass (R^2=0.20). There was lower than expected polymorphism between these parents with the SSR and EST-STS markers used resulting in a map that was not as complete as desired. The addition of markers providing higher levels of polymorphism could identify additional important drought tolerance QTL that would be of benefit to wheat breeders.

Objective

The objective of this study was to saturate a linkage map of the population of spring wheat used by Kirigwi et al. (2007) differing in drought tolerance using the amplified fragment length polymorphism technique (Haen et al., 2004) and Diversity Arrays Technology (DArT) markers (Akbari et al., 2006). Then, to QTL map yield and correlated traits of interest to identify regions that affect these traits.

Materials and Methods

Experimental Lines

The population used for this study was developed by Drs. Sanjaya Rajaram and Maartin van Ginkel at the International Center for Maize and Wheat Improvement (CIMMYT) in Mexico. Dharwar Dry is a line of unknown parentage that was collected in India. Sitta is a line developed at CIMMYT that is reasonably well adapted to growing conditions in Mexico, but compared to Dharwar Dry, is relatively drought susceptible. Both are spring wheats. The population consists of 122 F_7 derived recombinant inbred

lines that were selected at each generation for plant vigor, tillering, spike number, and spike fertility.

The phenotypes were characterized under both stress and control conditions in the 1998-99 and 1999-2000 growing seasons in Sonora, Mexico. Stress plots received only one irrigation, which occurred prior to planting. Control plots received three irrigations in 1998-98 and two in 1999-00. The number of irrigations was reduced due to high levels of lodging in the first season. Traits measured on the population were heading date (days), days to maturity (days), height (cm), green leaf index (proportion of total possible green leaf rating), harvest index (ratio), biomass (tons/ha), biomass production rate (kg/ha/day), spike number (per m^2), grains per spike (number), grains per meter (per m^2), kernel weight (mg), grain fill rate (kg/ha/day), and grain yield (tons/ha). Days to heading was determined by the date at which 50% of the spikes had emerged from the boot. Days to maturity was determined by the date at which 50% of the peduncles had turned yellow. Height was measured from the soil surface to the top of the spikes excluding the awns. Green leaf index was calculated as described by Cox et al. (1995). Fifty tillers were randomly harvested from each plot to measure yield components. The remainder of the plot was harvested with a small plot combine. Grain yield was adjusted to 12% moisture content. A drought susceptibility index was also calculated for each trait using the method of Fischer and Maurer (1978) and expressed by the relationship DSI=[1-$(Y_{s1})/(Y_{p1})$]/SI where Y_{s1} and Y_{p1} are the yields of a line under stress and non-stress conditions, respectively, and SI is the stress intensity estimated as [1- $(Y_{s2})/(Y_{p2})$], where Y_{s2} and Y_{p2} are the mean yield of all genotypes under stress and non-stress conditions, respectively.

DNA Isolation

Seeds of each line were germinated in Petri dishes lined with blot paper. Leaf tissue was collected from seedlings at the two leaf stage. Tissue was immediately frozen with liquid nitrogen and ground to a fine powder. DNA was extracted with a procedure very similar to Allen et al. (2006) using 2X CTAB extraction buffer (1.4 M NaCl, 100mM Tris pH 8.0, 2% hexadecyltrimethylammonium bromide, 2.0 mM EDTA, 0.5% sodium bisulfide, and 1% 2-mercaptoethanol). Extraction buffer (700 µL) was added to each sample and vortexed. The samples were incubated at 65°C for one hour vortexing three times during the incubation. Chloroform: isoamylalcohol (24:1 v/v; 500 µL) was added and the samples were incubated at room temperature for 15 minutes on a rotary shaker. Samples were then centrifuged at 5000 x *g* for five minutes and the supernatant was transfer to a clean tube. Two µL RNase A (10mg/mL; Sigma, St. Louis, MO) was added to each sample which was then incubabted at 37°C for one hour. Phenol:chloroform:isoamyl alcohol (25:24:1 v/v/v, 500 µL) was added to the samples. They were mixed by inversion for five minutes, centrifuged at 5000 x *g* for five minutes, and the supernatant was transferred to a clean tube. Chloroform: isoamylalcohol (24:1 v/v, 500 µL) was added and the samples were again mixed by inversion for five minute, centrifuged at 5000 x *g* for five minutes, and the supernatant transfer to a clean tube. DNA was precipitated by adding cold isopropyl alcohol in a volume approximately equal to the sample. Tubes were mixed by inversion and incubated at room temperature for 15 minutes. The DNA was pelleted by centrifugation at 5000 x *g* for five minutes. The pellet was washed twice in 70% ethanol centrifuging at 5000 x *g* for five minutes after each wash and then allowed to air dry. The DNA was resuspended in 1XTE (Tris-

ethylene diamine tetraacetic acid) overnight and then the concentration in each sample was quantified using a spectrophotometer.

Amplified Fragment Length Polymorphism Analysis

The amplified fragment length polymorphism (AFLP) protocol was modified similar to that used by Haen et al. (2004), with the following modifications. One microgram of genomic DNA was digested with the restriction enzymes *Mse*I and *Pst*I (New England BioLabs, Ipswich, MA). Adaptors, designed to correspond to the restriction site and add a known sequence to the fragments, were simultaneously annealed to the restriction sites (see Appendix A for adaptor sequences). The reaction volume was 50 µL. Reaction mix included 5 units *Pst*I, 1 unit *Mse*I, 5 pmoles Mse adaptor, 50 pmoles Pst adaptor, 1 µL 10 mM ATP (New England BioLabs, Ipswich, MA), 1 unit T4 DNA ligase (Invitrogen, Carlsbad, CA), 0.5 µL 100x BSA (bovine serum albumin; New England BioLabs, Ipswich, MA), 5µL One-Phor-All Buffer Plus (GE Healthcare, Buckinghamshire, England), and water to bring to volume. Reactions were incubated at 37°C for two hours and then brought to 70°C for 15 minutes to denature the enzymes.

A 10X dilution was made of the digested and ligated DNA samples and used as the template DNA for the pre-amplification PCR reaction. Primers for the reaction corresponded to the sequence of the adaptor plus one additional base, a C on the *Mse*I primer and an A on the *Pst*I primer (see Appendix A for sequences). The pre-amplification reaction mixture contained 5 µL diluted DNA template, 75 ng MsePre primer, 75 ng PstPre primer, 4 µL 10 mM dNTPs (Sigma, St. Louis, MO), 5 µL 10x PCR buffer (Sigma, St. Louis, MO), 5 µL 25 mM $MgCl_2$, 1.5 units Taq DNA Polymerase (Sigma, St. Louis, MO), and water to bring volume to 50 µL. Reaction conditions were

94°C for one minute, 56°C for two minutes, and 72°C for two minutes repeated 19 times, then 72°C for ten minutes.

A 50X dilution was made of the pre-amplified reactions and used as template DNA for the selective amplifications. Primers for the selective amplification correspond to the pre-amplification primers, with the addition of two bases, making 16 primers for each enzyme and 256 primer combinations. The reaction mix consisted of 2.5 µL DNA template, 75 ng Mse selective primer, 75 ng Pst selective primer, 2.5 µL 10x PCR buffer (Sigma, St. Louis, MO), 2.5 µL 25 mM $MgCl_2$, 2.5 µL 10 mM dNTPs (Sigma, St. Louis, MO), 1 unit Taq DNA polymerase (Sigma, St. Louis, MO), and water to bring the volume to 25 µL. The selective amplification reaction conditions were 92°C for one minute, 65°C for one minute decreasing by 0.7°C per cycle, and 72°C for two minutes repeated eleven times. Then 92°C for one minute, 56°C for one minute, and 72°C for two minutes repeated 22 times, and finally 72°C for ten minutes. All 256 primer combinations were scored on the parents of the population and the polymorphic combinations were used to screen the population. AFLP markers were named with the two selective bases of the *Mse*I primer, then the *Pst*I primer, and then the size of the fragment.

DNA Fragment Separation

The parental screening was carried out on 7% AccuGel 19:1 acrylamide:bisacrylamide (National Diagnostics, Atlanta, GA) denaturing gels run for 2.5 hours in 1% TBE buffer. Bands were visualized using silver staining by fixing with 10% glacial acetic acid for five minutes, washing three times for two minutes each in distilled water, soaking in a 2% silver nitrate solution (2 L distilled water, 2 g silver nitrate, 3 mL

formaldehyde) for 25 minutes, developing until bands were visible (2 L distilled water, 60 g sodium carbonate, 400 µL sodium thiosulfate, 3 mL formaldehyde), stopping with 10% glacial acetic acid for five minutes, and rinsing three more times for two minute each in distilled water. The polymorphic primer combinations were noted for future use. The population screening used fluorescently labeled *Pst*I primers for the selective amplification and capillary fragment analysis was conducted on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). GeneMarker 1.51 software (Soft Genetics, State College, PA) was used to visualize and score the results.

DArT Marker Analysis

Genomic DNA of the lines was sent to Diversity Arrays Technology (Yarralumla ACT, Australia) for analysis with their hybridization based markers. Their technology involves reducing the complexity of the sample by cutting with restriction enzymes and annealing adaptors. Then fragments are amplified from the adaptors. The fragments are labeled and hybridized to a microarray of variable fragments representing the diversity within the species. See the Diversity Arrays website at www.diversityarrays.com for more information.

Linkage and QTL Mapping

Marker data from the AFLPs and DArTs was combined with the SSR and EST data from Kirigwi et al. (2007) and mapped using the CarthaGene software (INRA, Paris, France). Linkage groups were formed at an LOD score of three with a maximum recombination fraction of 0.2. The LOD score was raised on the extremely large groups to separate them. The 'mrkdouble' command was used to identify markers with potentially identical scoring patterns. These were then merged using the 'mrkmerge'

command. Maps were formed using the 'Nicemapl' command and improved with 'flips' and 'polish' as well as the annealing, taboo, and genetic algorithms. Maps with the highest likelihood were selected and the Kosambi mapping function was used to determine map distances.

QTL analysis was carried out using the WinQTL Cartographer software (NCSU, Raleigh, NC). Maps were constructed using the composite interval mapping function. The LOD score for significance of the QTL was determined by 1,000 permutations and so varied for each trait. Multiple interval mapping was used to find a cumulative R^2 value for each trait. Markers not incorporated into linkage groups were analyzed by single marker analysis.

Results

Of the 256 AFLP primer combinations screened on the parents, 151 were determined to be polymorphic (see Table 2.1). These were screened on the population and 234 polymorphic markers were identified from them. There were also 208 DArT markers polymorphic between the parents returned by Diversity Arrays Technology. These markers, together with the 201 SSR and EST-STS markers from Kirigwi et al. (2007), were mapped. Overall, 58 AFLP, 86 DArT, eleven SSR, and five EST-STS markers were merged into other markers due to highly similar scoring patterns. Grouping in CarthaGene resulted in 370 markers in 48 linkage groups with 113 markers remaining unlinked. The markers cover a total of 1,382 centiMorgans (cM) of the genome with an average of 3.7 cM between markers. Published map positions of SSR markers and probable locations of the DArT markers allowed the chromosomal location of some groups to be identified. Other groups were made entirely of AFLP markers or contained

markers reported to map in conflicting locations and so their location is unknown. Groups containing only one DArT marker along with AFLPs were also considered to be of unknown location since the chromosomal assignments of DArT markers are tentative.

QTL were found in 24 of the 48 groups. Groups that could be located from SSR or DArT markers were on chromosomes 1D, 2B, 3A, 3B, 4A, 4B, 5A, 5B, 6A, 6D, 7A, and 7B. Two separate linkage groups were identified on 3B, 4B, and 5B and three on 4A. Seven of the groups containing QTL could not be located reliably from the markers included. QTL were mapped under both full and reduced irrigation for all traits except grains per meter and spike number, which were mapped under reduced irrigation only, and grains per spike which was mapped only under full irrigation. QTL maps for linkage groups with unknown locations are given in Figure 2.1. QTL identified in chromosome groups 1-3 are found in Figure 2.2, groups 4-5A are in Figure 2.3, and 5B-7 are in Figure 2.4. The identified QTL explained between 1% and 34% of observed variation in their respective trait x year combination. R^2 values for each QTL are given in Table 2.2. Cumulative R^2 values for all traits are given in Table 2.3.

Cloning and sequencing was undertaken on eight AFLP fragments of interest identified from QTL analysis. However, with the difficulty of isolating a band identified from capillary fragment analysis on an acrylamide gel, only two of the correct fragments were cloned after several attempts. STS markers were designed from the sequences using MacVector v9.5 (MacVector, Inc., Cary, NC), but the amplified fragments were monomorphic in the parents. Details of the cloning undertaken in this study are given in Appendix B.

Eight markers not included in a linkage group were significantly associated with several traits and so were selected to be of interest for marker assisted selection. These were six SSRs, one AFLP, and one DArT marker. The markers' reported locations and associated traits are given in Table 2.4.

Discussion

The wheat genome consists of approximately 2,600 cM of DNA. If a marker every ten cM on average is desirable for mapping, then 260 markers are required for wheat. Combining the data from the SSRs, ESTs, AFLPs, and DArTs gave 370 markers and so genome coverage should be adequate to map. However, since only 1,382 cM of the genome were covered by the markers incorporated into linkage groups it appears that there are still large parts of the genome that were unable to be mapped. This is possibly due to the population being selected, the lower than expected polymorphism between the parents, and the tendency of AFLP markers to cluster.

Using a selected population presents some challenges for mapping as there will be little or no variation in some regions of the genome. This means there will appear to be no recombination between markers falling in this area of the chromosomes because the area represents only the DNA contributed by one parent. This compresses the map and leads to deletion of markers. Markers in a selected population do not segregate in predictable ratios so it is more difficult to detect segregation distortion (R.W. Doerge, personal communications, April 28, 2007). This population is not ideal for mapping, but the objective of the study was to develop markers for marker assisted selection in a breeding program. The population represents the variation available to a breeder after

material with very poor agronomic traits has been removed. Therefore, valuable markers should still be identified.

The QTL explaining the most variation in traits by far was the 4AL QTL reported by Kirigwi et al. (2007) which is 4A-3 in this study. It will not be discussed here as it has previously been published on. However, additional QTL of interested were identified in this study. Cloning the AFLP fragments in these groups to design diagnostic primers could allow them to be used for marker assisted selection and for deletion mapping to solidify their location on the chromosome arms. Primers could also be designed for the DArT markers if the sequences can be obtained for Diversity Arrays Technology.

The AFLP marker *XGCGT210*, which was in a linkage group of unknown location designated D in Figure 2.1, also explained relatively large portions of variation with R^2 values of 0.09 for biomass, 0.15 for biomass production rate, 0.16 for grain fill rate, 0.18 for grains per meter, 0.11 for spike number, and 0.11 for yield under reduced irrigation and so could be interesting for drought tolerance selections. The desirable allele seems to come from Sitta as the lines with the Sitta genotype have significantly higher mean values for each of the traits mentioned above as well as drought susceptibility indexes calculated from two different year combinations. It is not unexpected to have Sitta contributing some desirable alleles because it is a cultivar well adapted to a dry area and is just relatively drought susceptible when compared with Dharwar Dry. Means for each genotype and P-values for the difference of means for all QTL are given in Table 2.5. Because the chromosomal location of this linkage group is unknown, the QTL cannot be compared to those reported in other studies. However, if this fragment could be successfully cloned and sequenced and a polymorphic diagnostic

primer designed, it could be deletion mapped and then assigned to a chromosomal location.

Another marker of interest for selection is *XCGCG157* in linkage group 5A. It has R^2 values of 0.17 for biomass, 0.09 for biomass production rate, 0.08 for grain fill rate, and 0.05 for yield. This linkage group falls somewhere on the short arm of chromosome 5A. The desirable allele, again, seems to be contributed by Sitta based on the differences in genotype means. *Xgdm68,* an SSR marker in the same linkage group, but 55 cM from *XCGCG157,* was linked to a QTL for biomass under full irrigation with an R^2 value of 0.09. The desirable allele here was contributed by Dharwar Dry so retention of both the desirable alleles from the respective parent may help increase stability across environments. QTL for thousand grain weight and grains per spike, both under drought stress conditions were reported on 5AS by Quarrie et al. (2005). These traits were not affected by the 5A QTL in this study, but grain fill rate could impact kernel weight. There were also QTL detected under salt and nitrogen stress in the same location (Quarrie et al., 2005). No drought tolerance QTL were reported on the short arm of group 5 chromosomes by Cattivelli et al. (2002) in their summary of QTL and gene locations in *Triticeae.* Bernier et al. (2007) mapped QTL for yield and yield components in rice. Wheat and rice genetic maps were compared (www.gramene.org/cmap) to determine if similar results had been reported between the two species. The region of chromosome 5A containing this QTL may be syntenic to the region of rice chromosome 12 containing a QTL for grain yield, biomass yield, harvest index, days to flowering, height at maturity, panicle number, flowering delay, and drought response index, all under drought stress, in rice (Bernier et al., 2007).

The AFLP marker *XAAAA278* in group 3B-1 is of general interest for breeding as it had large effects on biomass, biomass production rate, grain fill rate, and yield with R^2 values of 0.17, 0.12, 0.12, and 0.17 respectively. However, these were all detected only under full irrigation. Incorporation of this QTL would probably not be effective in a drought breeding program, but may be useful in breeding lines for more favorable environments. Interestingly, the desirable allele is contributed by Dharwar Dry. This could allow easier selection for yield stability if this QTL were selected for at the same time as the 4A-3 QTL which is also contributed by Dharwar Dry and affects a large number of traits under reduced irrigation. Maintaining these two alleles in a population during backcrossing could improve biomass and yield under both drought and ideal conditions. The 3B-1 group was difficult to assign to a chromosomal location by comparing the SSR markers in linkage groups with the DArT markers provided by Diversity Arrays Technology with the Somers et al. (2004) map. However, it appears that the group is near the centromere. No QTL were reported here by Quarrie et al. (2005) or Cattivelli et al. (2002). There were also no QTL reported in the regions of rice believed to be syntenic to this area of wheat chromosome 3B (Bernier et al., 2007).

Linkage group 7B is located somewhere on the long arm of chromosome 7B and is also of interest for drought tolerance breeding. The group could not be placed in a definite position on the chromosome arm due to the lack of SSR markers that appear in both the Diversity Arrays Technology maps and the Somers et al. (2004) map. This group contains multiple QTL for harvest index under drought with R^2 values of 0.12, 0.11, and 0.10. There were also multiple QTL for drought susceptibility index calculated from spike number with R^2 values of 0.12 and 0.10 and a QTL for drought susceptibility

index calculated from yield (R^2=0.11) and overall drought susceptibility index comparing reduced irrigation in 1999 to the average of full irrigation from 1998 and 1999 (R^2=0.06) as well as QTL for maturity under full irrigation with R^2 values of 0.10 and 0.09. The desirable allele is contributed by Dharwar Dry. Cattivelli et al. (2002) report several areas affecting drought tolerance on the long arm of the group 7 chromosomes. Quarrie et al. (2005) also report QTL on the distal portion of 7AL for grains per spike and thousand grain weight under both drought and control conditions as well as grains per spike under nitrogen stress. This region is thought to be syntenic to rice chromosome 6 where a QTL for biomass yield and panicle number under drought stress were reported (Bernier et al., 2007).

Eight unlinked markers were identified by single marker analysis. These were significantly associated with multiple traits under reduced irrigation. Many were highly significant including three that were significant at the α=.0001 level. These were *XGTGT526* for grains per meter and *Xcfd31* for spike number under reduced irrigation in 1999, and *Xgwm46* for drought susceptibility index. The mean of the associated traits for each genotype and the P-values for the differences in means are given in Table 2.6. The six SSRs identified by single marker analysis can be used directly for marker assisted selection. The AFLP fragment identified would need to be cloned and sequenced so that STS primers could be designed. Sequence for the DArT marker would need to be obtained from Diversity Array Technology, but then it too could be used for marker assisted selection.

The alleles linked to *Xbarc204* contributed by each parent are desirable for some of the traits affected. The majority of traits were significantly affected under both

reduced and full irrigation and the same allele was desirable under both conditions. The Dharwar Dry allele was better for grain fill duration, heading date, and kernel weight under both full and reduced irrigation where as the Sitta allele was better for biomass production rate, grain fill rate, grains per meter, and spike number. There were additional traits associated with the locus only under full irrigation of these, the Dharwar Dry allele was better for biomass in 1999 and maturity where as the Sitta allele was better for biomass in 1998, grains per spike, and yield. Sitta was also better for drought susceptibility index calculated from green leaf index, heading date, and kernel weight. These groups of traits would need to be evaluated to determine which was more important before selection at this marker would be beneficial, but Sitta is probably the best choice.

At the marker *Xbarc280*, the Sitta allele is desirable for all traits affected except for height. These traits were biomass, biomass production rate, grain fill rate, and yield all under reduced irrigation in 1999. The Sitta allele also contributed improved drought susceptibility indexes calculated from grain fill rate, grains per meter, yield, and five different year comparisons. As with *Xbarc204,* the majority of traits linked to *Xcfd31* were affected under both reduced and full irrigation. However, the Dharwar Dry allele was more conclusively beneficial as it was better for biomass, biomass production rate, and grains per meter under both conditions as well as grain fill rate and spike number under reduced irrigation. The association with spike number was also the strongest association between a trait and this marker with significance at α=0.0001. The Sitta allele was better for kernel weight under both conditions and grain fill duration and harvest index under reduced irrigation, but probably provides less benefit overall. Sitta

provides the desirable allele at the *Xgwm3* locus as it affects grain fill duration and harvest index under reduced irrigation, height and kernel weight under full irrigation and heading date under both conditions. The Dharwar Dry allele was more desirable for biomass production rate under reduced irrigation and grains per meter under full irrigation, but would not be the best for selection. At the *Xgwm46* locus, the most desirable allele was contributed by Sitta. Dharwar Dry was beneficial for yield under full irrigation and drought susceptibility index calculated from harvest index. The Sitta allele was beneficial for grain fill duration, grain fill rate, grains per meter, grains per spike, harvest index, kernel weight, and yield under reduced irrigation and heading date and maturity under both conditions as well as many drought susceptibility indexes. Dharwar Dry is the best donor parent for the *Xgwm160* locus. This allele affected biomass, biomass production rate, grain fill rate, grains per meter, spike number, and yield under reduced irrigation and drought susceptibility index where as Sitta affected only heading date. Most of the Dharwar Dry affected traits are also relatively strongly associated with the marker at α values of 0.01 and less. At the *XGTGT526* locus the desirable parent is Sitta which is beneficial for biomass, grain fill rate, grains per meter, grains per spike, spike number, and yield under reduced irrigation as well as height under full irrigation and heading date and maturity under both conditions. Sitta also contributed the better drought susceptibility indexes. Dharwar Dry's allele is desirable only for kernel under reduced irrigation. Lastly, the desirable allele at the *XwPt-4487* locus is donated by Dharwar Dry. While Sitta is better only in kernel weight under both conditions, Dharwar was better for biomass, biomass production rate, grain fill rate, spike number, and yield under reduced irrigation, maturity under full irrigation, and grains per meter under both

conditions as well as drought susceptibility index. So, the Dharwar Dry genotype should be selected for at the markers *Xcfd31, Xgwm160,* and *XwPt-4487* while the Sitta genotype should be selected for at the markers *Xbarc280, Xgwm3, Xgwm46, XGTGT526,* and probably *Xbarc204*.

In conclusion, this study mapped QTL to twelve of the 21 wheat chromosomes. Some of these QTL were confirmed by other studies. Others have not been reported before. Eight unlinked markers were identified by single marker analysis, six of which are immediately available for marker assisted selection, as are *Xwmc89, Xwmc48*, and *Xwmc420*, which are linked to the 4AL QTL reported by Kirigwi et al. (2007). Three AFLP fragments were identified from QTL analysis and one from single marker analysis that could be very useful if successful STS markers could be designed. Future efforts should focus on isolation and cloning of these fragments.

Table 2.1 AFLP primer combinations polymorphic between Dharwar Dry and Sitta.

	P-AAA	P-AAT	P-AAG	P-AAC	P-ATA	P-ATT	P-ATG	P-ATC	P-AGA	P-AGT	P-AGG	P-AGC	P-ACA	P-ACT	P-ACG	P-ACC
M-CAA	X	X				X		X	X	X	X	X	X	X	X	X
M-CAT						X			X	X				X	X	
M-CAG	X															
M-CAC									X			X			X	
M-CTA	X	X	X			X				X						
M-CTT		X	X	X		X	X	X		X	X	X	X	X		X
M-CTG		X			X	X			X	X			X	X	X	X
M-CTC	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X
M-CGA					X	X			X	X	X	X	X	X	X	X
M-CGT	X	X			X	X		X	X	X	X	X	X	X	X	X
M-CGG	X	X	X	X	X	X	X		X	X	X	X		X	X	X
M-CGC	X		X		X	X		X	X	X			X			
M-CCA	X			X			X				X		X			X
M-CCT	X	X	X	X	X	X	X		X	X		X	X	X	X	X
M-CCG	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
M-CCC		X						X	X	X	X	X		X	X	X

Figure 2.1 QTL maps of linkage groups with unknown chromosomal locations.

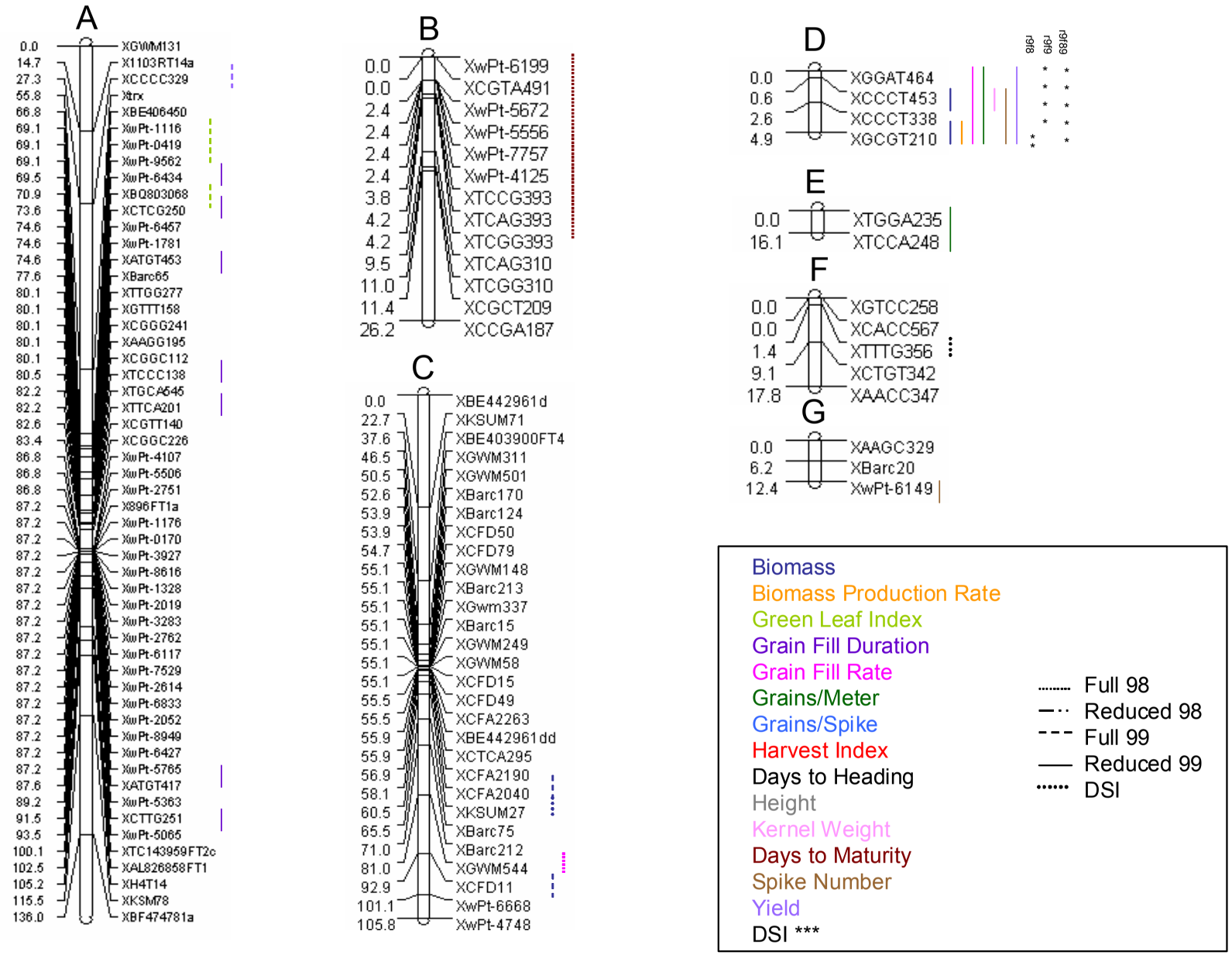


Figure 2.2 QTL maps for linkage groups mapping to homeologous groups 1-3.

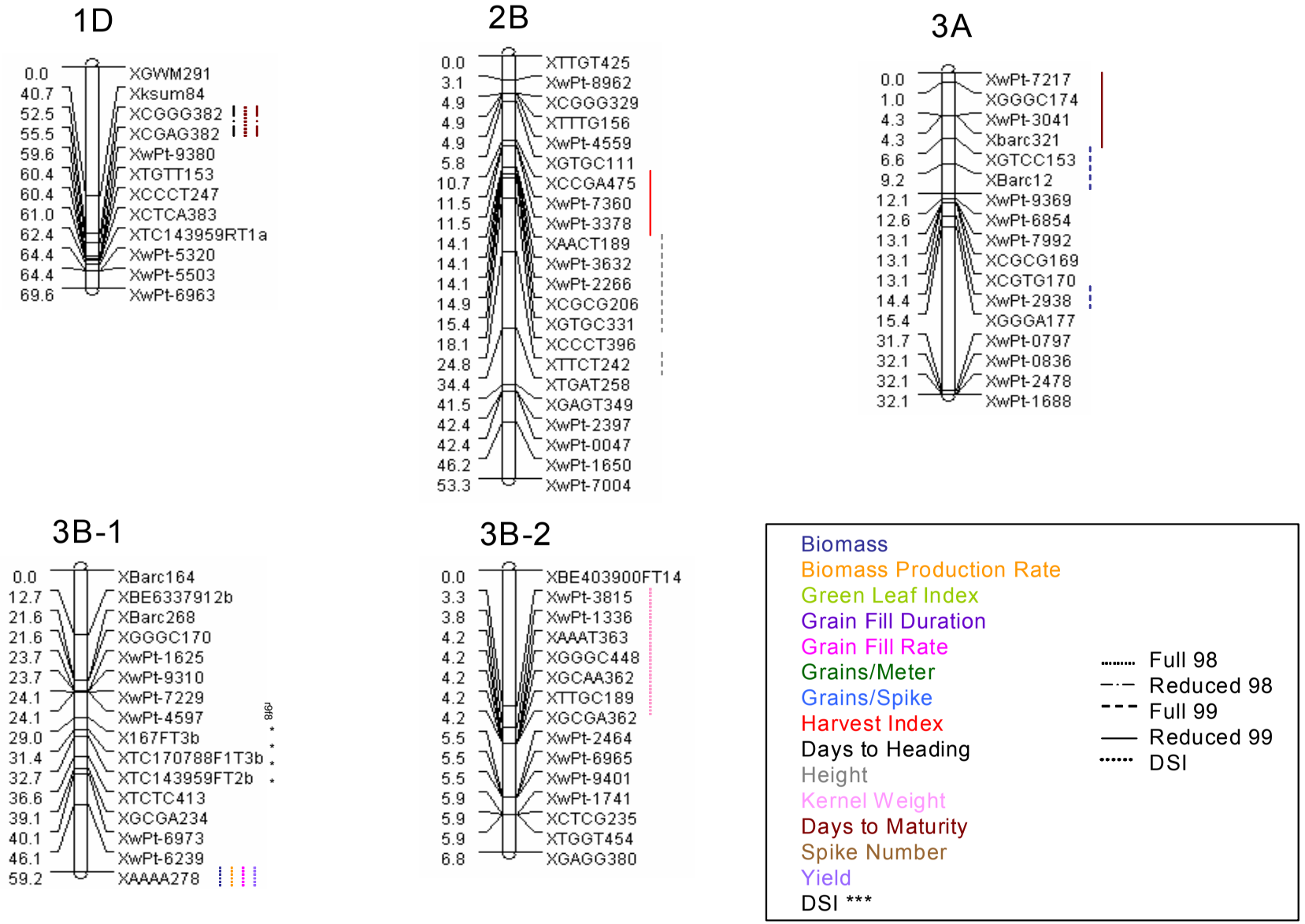


Figure 2.3 QTL maps for linkage groups mapping to homeologous group 4 and 5A.

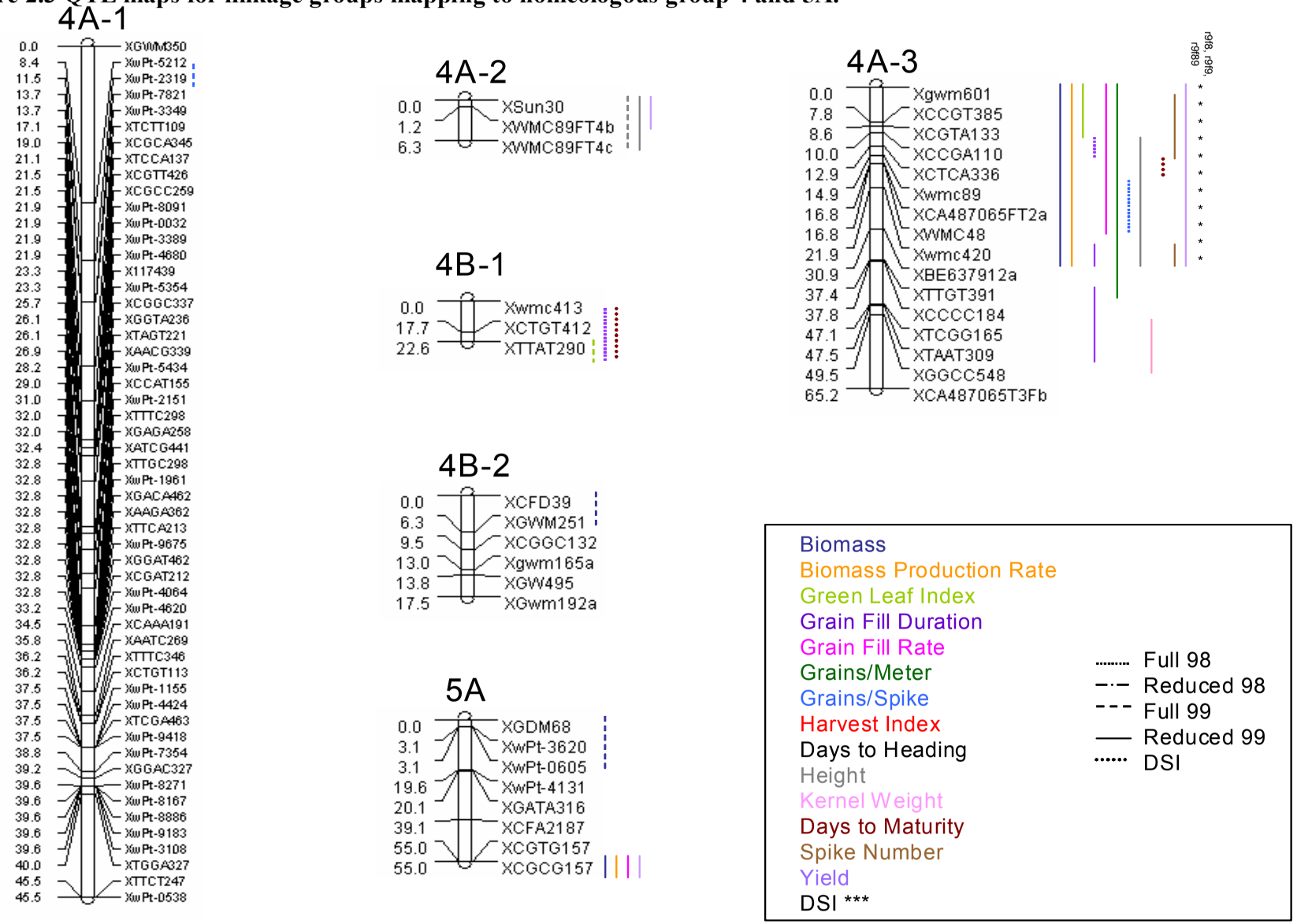


Figure 2.4 QTL maps for linkage groups mapping to 5B and homeologous groups 6 and 7.

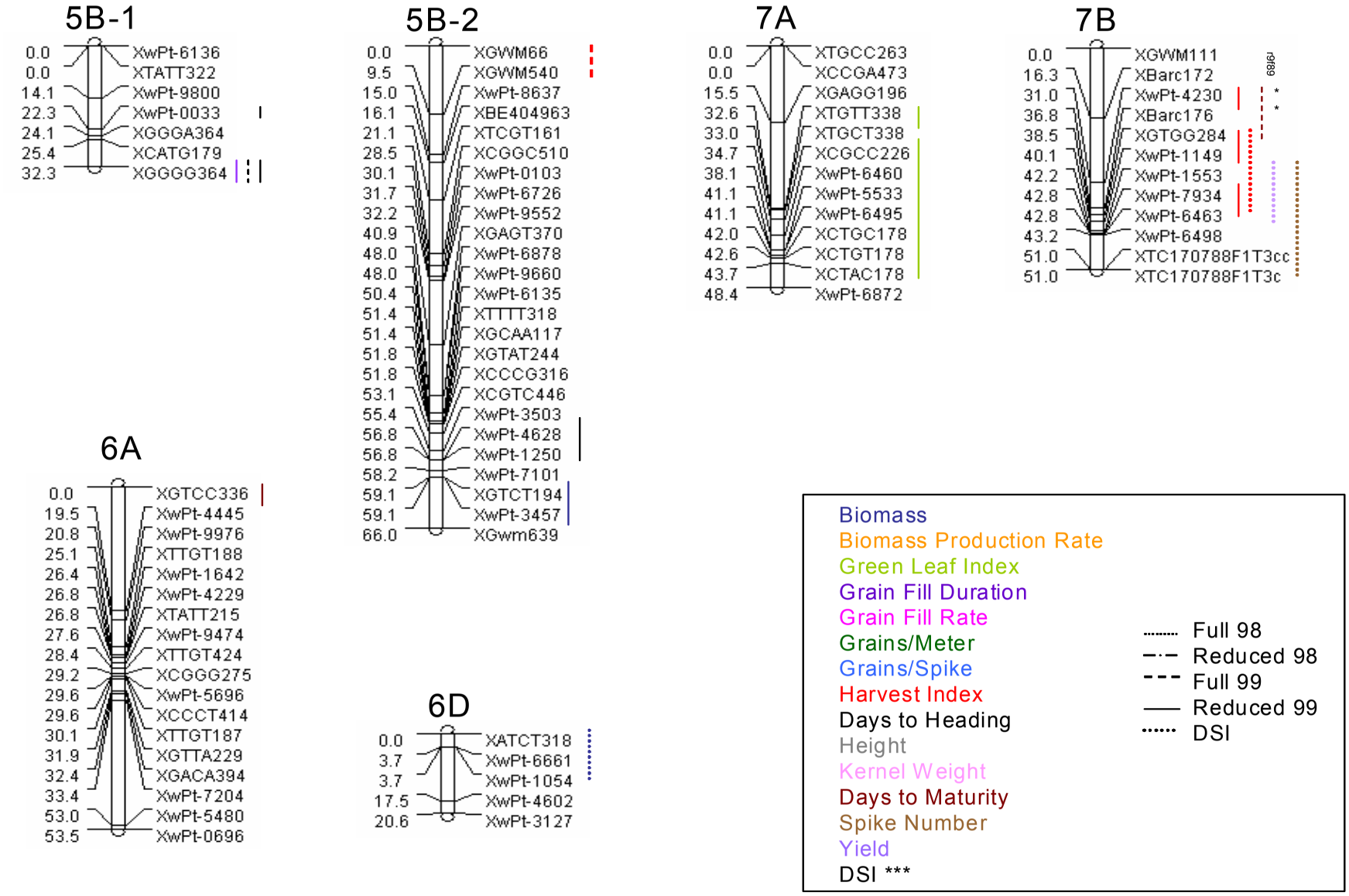


Table 2.2 Individual R^2 values for QTL.

The marker with the highest R^2 value in each QTL is given. If a value is given in a cell merged between two markers the QTL occurred between the markers with neither marker having an R^2 value comparable to the area between. Due to the varying sizes of charts, not all groups appear in order.

Unknown A	GLI	GFD	Yield
	F9	R9	F9
XGWM131			
XGTCC187			
X1103RT14a			
XwPt-9857			
XCCCC329			0.094
XCFD48			
Xtrx			
XBF474781b			
XBE406450			
XCGTG239			
XCTCC158	0.089		
XwPt-6434		0.095	
XBQ803068	0.112		
X167RT10		0.099	
XCTCG250			
XATGT453		0.106	
XCTAC187			
XBarc65			
XCTCG399			
XACCG350			
XTCCC138		0.135	
XCCGT386			
XTTCA201		0.098	
XCTTG410			
XCGGC226			
XwPt-7833			
XwPt-2751			
XCTGT186			
XATGT417		0.095	
XwPt-2725			
XwPt-5363			
XwPt-9977		0.099	
XTC143959RT1b			
XwPt-5065			
XTC170788F1T3a			
XBE438268FT14			
XAL826858FT1			
XH4T14			
XBE442811RT14			
XKSM78			
XBE518424RT5			
XBF474781a			
XBE443710FT14			

Unknown B	Maturity
	F8
XwPt-6199	
XCGTA491	
XwPt-5672	
XwPt-5556	
XwPt-7757	
XwPt-4125	
XTCCG393	0.122
XTCAG393	
XTCGG393	
XTCAG310	
XTCGG310	
XCGCT209	
XCCGA187	

Unknown C	Biomass		GFR
	F9	DSI	F8
XBE442961d			
XKSUM71			
XBE403900FT4			
XGWM311			
XGWM501			
XBarc170			
XBarc124			
XCFD50			
XCFD79			
XGWM148			
XBarc213			
XGWM337			
XBarc15			
XGWM249			
XGWM58			
XCFD15			
XCFD49			
XCFA2263			
XBE442961dd			
XCTCA295			
XCFA2190			
XCFA2040	0.171		
XKSUM27		0.095	
XBarc75			
XBarc212			
XGWM544			0.093
XCFD11	0.076		
XwPt-6668			
XwPt-4748			

Unknown D	Biomass	BioRate	GFR	GpM	KW	Spike #	Yield	DSI		
	R9	R9	R9	R9	R9	R9	R9	r9f8	r9f9	r9f89
XGGAT464			0.113	0.188			0.073		0.072	0.094
XCCCT453	0.060		0.121	0.175	0.083	0.080	0.080		0.077	0.103
XCCCT338			0.116	0.172		0.078	0.085		0.077	0.102
XGCGT210	0.076	0.123	0.154	0.182		0.103	0.108	0.077		0.119

Unknown E	Grains per Meter
	R9
XTGGA235	0.133
XTCCA248	

1D	Heading Date	Maturity	
	R8	F8	R8
XGWM291			
Xksum84			
XCGGG382			
XCGAG382	0.127	0.147	0.143
XwPt-9380			
XTGTT153			
XCCCT247			
XCTCA383			
XTC143959RT1a	0.105		
XwPt-5320			
XwPt-5503			
XwPt-6963			

2B	HI	Height
	R9	F9
XTTGT425		
XwPt-8962		
XCGGG329		
XTTTG156		
XwPt-4559		
XGTGC111		
XCCGA475		
XwPt-7360		
XwPt-3378	0.088	
XwPt-2266		0.121
XCGCG206		
XGTGC331		
XCCCT396		0.141
XTTCT242		
XTGAT258		
XGAGT349		
XwPt-2397		
XwPt-0047		
XwPt-1650		
XwPt-7004		

1D	Heading Date	Maturity	
	R8	F8	R8
XGWM291			
Xksum84			
XCGGG382			
XCGAG382	0.127	0.147	0.143
XwPt-9380			
XTGTT153			
XCCCT247			
XCTCA383			
XTC143959RT1a	0.105		
XwPt-5320			
XwPt-5503			
XwPt-6963			

Unknown G	Spike #
	R9
XAAGC329	
XBarc20	
XwPt-6149	0.083

3A	Biomass	Maturity
	F9	R9
XwPt-7217		
XGGGC174		
XwPt-3041		
Xbarc321		0.082
XGTCC153		
XBarc12	0.111	
XwPt-9369		
XwPt-6854		
XwPt-7992		
XCGCG169		
XCGTG170		
XwPt-2938	0.081	
XGGGA177		
XwPt-0797		
XwPt-0836		
XwPt-2478		
XwPt-1688		

3B-1	Biomass	BioRate	GFR	Yield	DSI
	F8	F8	F8	F8	r9f8
XBarc164					
XBE6337912b					
XBarc268					
XGGGC170					
XwPt-1625					
XwPt-9310					
XwPt-7229					
XwPt-4597					
X167FT3b					0.104
XTC170788F1T3b					
XTC143959FT2b					
XTCTC413					
XGCGA234					
XwPt-6973					
XwPt-6239					
XAAAA278	0.169	0.118	0.117	0.165	

4A-1	Grains per Spike
	F9
XGWM350	
XwPt-5212	
XwPt-2319	0.091
XwPt-7821	
XwPt-3349	
XTCTT109	
XCGCA345	
XTCCA137	
XCGTT426	
XCGCC259	
XwPt-8091	
XwPt-0032	
XwPt-3389	
XwPt-4680	
X117439	
XwPt-5354	
XCGGC337	
XGGTA236	
XTAGT221	
XAACG339	
XwPt-5434	
XCCAT155	
XwPt-2151	
XTTTC298	
XGAGA258	
XATCG441	
XTTGC298	
XwPt-1961	
XGACA462	
XAAGA362	
XTTCA213	
XwPt-9675	
XGGAT462	
XCGAT212	
XwPt-4064	
XwPt-4620	
XCAAA191	
XAATC269	
XTTTC346	
XCTGT113	
XwPt-1155	
XwPt-4424	
XTCGA463	
XwPt-9418	
XwPt-7354	
XGGAC327	
XwPt-8271	
XwPt-8167	
XwPt-8886	
XwPt-9183	
XwPt-3108	
XTGGA327	
XTTCT247	
XwPt-0538	

3B-2	Kernel Weight
	F8
XBE403900FT14	
XwPt-3815	0.130
XwPt-1336	
XAAAT363	
XGGGC448	
XGCAA362	
XTTGC189	
XGCGA362	
XwPt-2464	
XwPt-6965	
XwPt-9401	
XwPt-1741	
XCTCG235	
XTGGT454	
XGAGG380	

4A-2	Height		Yield
	F9	R9	R9
XSun30	0.130	0.109	0.053
XWMC89FT4b	0.127	0.118	0.056
XWMC89FT4c	0.131	0.120	0.066

4B-1	GLI	GFD	Maturity
	F9	F8	DSI
Xwmc413		0.059	0.123
XCTGT412		0.084	
XTTAT290	0.147		

5A	Biomass		BioRate	GFR	Yield
	F9	R9	R9	R9	R9
XGDM68	0.087				
XwPt-3620					
XwPt-0605					
XwPt-4131					
XGATA316					
XCFA2187					
XCGTG157					
XCGCG157		0.066	0.081	0.07	0.054

4B-2	Biomass
	F9
XCFD39	0.092
XGWM251	0.110
XCGGC132	
Xgwm165a	
XGWM495	
XGwm192a	

5B-1	GFD	Heading Date	
	R9	F9	R9
XwPt-6136			
XTATT322			
XwPt-9800			
XwPt-0033			0.096
XGGGA364			
XCATG179			
XGGGG364	0.085	0.118	0.137

5B-2	Biomass	HD
	R9	R9
XGWM66		
XGWM540		
XwPt-8637		
XBE404963		
XTCGT161		
XCGGC510		
XwPt-0103		
XwPt-6726		
XwPt-7A9552		
XGAGT370		
XwPt-6878		
XwPt-9660		
XwPt-6135		
XGCAA117		
XGTAT244		
XCCCG316		
XCGTC446		
XwPt-3503		
XwPt-4628		
XwPt-1250		0.089
XwPt-7101		
XGTCT194		
XwPt-3457	0.058	
XGwm639		

4A-3	Biomass	BioRate	GLI	GFD		GFR	GpM	GpS	Height	KW	Maturity	Spike #	Yield	DSI		
	R9	R9	R9	R8	R9	R9	R9	F8	R9	R9	DSI	R9	R9	r9f8	r9f9	r9f89
xgwm601			0.081													
XCCGT385			0.090				0.134									
XCGTA133	0.116	0.012										0.098				
XCCGA110			0.077	0.012		0.278							0.399			
XCTCA336	0.119						0.127		0.157		0.081			0.195	0.240	0.247
Xwmc89								0.120								
XCA487065T2a	0.077	0.143				0.189							0.311	0.173	0.226	0.226
XWMC48	0.077	0.143				0.189							0.311	0.173	0.226	0.226
Xwmc420	0.089	0.164			0.092	0.219	0.105	0.156	0.172			0.093	0.339	0.268	0.229	0.276
XBE637912a	0.063				0.066		0.073									
XTTGT391							0.071		0.094							
XCCCC184					0.137											
XTCGG165					0.089					0.125						
XTAAT309																
XGGCC548																
XCA487065T3Fb																

6A	Maturity
	R9
XGTCC336	0.126
XwPt-4445	
XwPt-9976	
XTTGT188	
XwPt-1642	
XwPt-4229	
XTATT215	
XwPt-9474	
XTTGT424	
XCGGG275	
XwPt-5696	
XCCCT414	
XTTGT187	
XGTTA229	
XGACA394	
XwPt-7204	
XwPt-5480	
XwPt-0696	

6D	Biomass
	DSI
XATCT318	0.112
XwPt-6661	
XwPt-1054	
XwPt-4602	
XwPt-3127	

7A	GLI
	R9
XTGCC263	
XCCGA473	
XGAGG196	
XTGTT338	
XTGCT338	0.087
XCGCC226	
XwPt-6460	
XwPt-5533	
XwPt-6495	
XCTGC178	0.100
XCTGT178	
XCTAC178	0.096
XwPt-6872	

	HI		Maturity	Spike #	Yield	DSI
7B	R9	DSI	F9	DSI	DSI	r9f89
XGWM111						
XBarc172	0.119					
XwPt-4230			0.099			0.057
XBarc176			0.086			
XGTGG284	0.107					
XwPt-1149		0.104				
XwPt-1553				0.119	0.112	
XwPt-7934						
XwPt-6463	0.095					
XwPt-6498				0.096		
XTC170788F1T3cc						
XTC170788F1T3c						

Table 2.3 Cumulative R^2 values for trait and environment combinations.

	Biomass	Biomass Production Rate	Green Leaf Index	Grain Fill Duration	Grain Fill Rate	Grains per Meter	Grains per Spike	Harvest Index	Days to Heading	Height	Kernel Weight	Days to Maturity	Spike Number	Yield
Full irrigation '98	0.070	0.062	-	0.218	0.148	-	0.047	-	-	-	0.126	0.195	-	0.074
Reduced irrigation '98	-	-	-	-	-	-	-	-	0.077	-	-	0.115	-	-
Full irrigation '99	0.411	-	0.215	-	-	-	0.075	0.216	0.085	0.011	-	0.124	-	0.084
Reduced irrigation '99	0.594	0.440	0.177	0.207	0.553	0.485	-	0.172	0.124	0.034	0.175	0.258	0.332	0.647
drought susceptibility index	0.140	-	-	-	-	-	-	0.113	0.044	-	-	0.094	0.094	0.120

	DSI
reduced 98 v full 99	-
reduced 98 v full 98	-
reduced 98 v full 98 and 99	-
reduced 99 v full 99	0.4573
reduced 99 v full 98	0.6205
reduced 99 v full 98 and 99	0.5343
reduced 99 and 98 v full 99	-
reduced 99 and 98 v full 98	-
full 99 and 98 v full 99 and 98	-

Table 2.4 Single marker analysis results of selected unlinked markers.

Significance at the α=.05, α=.01, α =.001, and α=.0001 levels are represented by *, **, ***, and **** respectively.

		Associated Traits																												
		Biomass					BM Prod. Rate					Green Leaf Index					Grain Fill Duration					Grain Fill Rate					Grains/Meter				
Marker	Location	F8	R8	F9	R9	dsi	F8	R8	F9	R9	dsi	F8	R8	F9	R9	dsi	F8	R8	F9	R9	dsi	F8	R8	F9	R9	dsi	F8	R8	F9	R9	dsi
Xbarc204	1A 6AD	*		*			**			**						***	*			**		**			*		**		*	**	
Xbarc280					**					**															**	**					*
Xcfd31	4A 7AD	*			**		*			***										*					**				**	**	
Xgwm3	2D 3D									**										**							*				
Xgwm46	7B																			*	*				*	**				*	**
Xgwm160	4A				***					**															**					*	
XGTGT526	?				*																				**					****	*
XwPt-4487	4A				**					**															**				*	***	

		Associated Traits																												
		Grains/Spike					Harvest Index					Day to Heading					Height					Kernel Weight					Days to Maturity				
Marker	Location	F8	R8	F9	R9	dsi	F8	R8	F9	R9	dsi	F8	R8	F9	R9	dsi	F8	R8	F9	R9	dsi	F8	R8	F9	R9	dsi	F8	R8	F9	R9	dsi
Xbarc204	1A 6AD			**										**	**	*								**	*	*			*		
Xbarc280																				**											
Xcfd31	4A 7AD									*														**	**						
Xgwm3	2D 3D									***	*			*	**				**					*							
Xgwm46	7B				*					**	***			*	**						*		*						***	**	
Xgwm160	4A												*																		
XGTGT526	?				*									**	**				**		*				***				***	***	
XwPt-4487	4A																							*	*		*				

		Associated Traits																		
		Spike Number					Yield					Drought Susecptiblity Index								
Marker	Location	F8	R8	F9	R9	dsi	F8	R8	F9	R9	dsi	r8f9	r8f8	r8f89	r9f9	r9f8	r9f89	r89f9	r89f8	r89f89
Xbarc204	1A 6AD	**			*		*													
Xbarc280										**	*				**	**	***		*	*
Xcfd31	4A 7AD				****															
Xgwm3	2D 3D																			
Xgwm46	7B					*			*	**	**				****	**	***	*	*	*
Xgwm160	4A				*					**					**		*			
XGTGT526	?				**					*					**	*	**			
XwPt-4487	4A				**					**					*	*	*			

Table 2.5 Genotype means, differences of means, and P-values for markers associated with QTL.

The desirable value for each trait is highlighted in yellow. P-values were calculated with two sample t tests in Minitab 15 (Minitab Inc., State College, PA)

	Marker	Trait	DD Mean	ST Mean	Difference of Means	P-value
Unknown A	XCCCC329	Yield F9	411.05	435.29	-24.24	0.004
	XCTCC158	Green Leaf Index F9	0.39	0.44	-0.05	0.001
	XwPt-6434	Grain Fill Duration R9	41.10	39.87	1.23	0.010
	XBQ803068	Green Leaf Index F9	0.37	0.44	-0.07	0.002
	X167RT10	Grain Fill Duration R9	41.60	39.58	2.02	0.035
	XATGT453	Grain Fill Duration R9	41.08	39.90	1.18	0.011
	XTCCC138	Grain Fill Duration R9	41.16	39.87	1.29	0.005
	XTTCA201	Grain Fill Duration R9	41.12	40.06	1.06	0.018
	XATGT417	Grain Fill Duration R9	41.04	39.98	1.06	0.028
	XwPt-9977	Grain Fill Duration R9	41.06	40.02	1.04	0.030
B	XTCCG393	Maturity F8	131.18	129.77	1.42	0.006
Unknown C	Xcfa2040	Biomass F9	12.38	9.97	2.41	0.000
	Xksum27	Biomass DSI	0.69	0.99	-0.30	0.007
	Xgwm544	Grain Fill Rate F8	0.93	0.98	-0.05	0.456
	Xcfd11	Biomass F9	9.34	10.12	-0.79	0.003
Unknown D	XGGAT464	Grain Fill Rate R9	69.90	80.46	-10.56	0.000
		Grains/Meter R9	7942.28	9479.28	-1537.00	0.000
		Yield R9	283.88	317.79	-33.92	0.000
		r9f9	1.15	0.87	0.28	0.006
		r9f89	1.09	0.93	0.17	0.001
	XCCCT453	Biomass R9	6.02	6.62	-0.59	0.001
		Grain Fill Rate R9	70.08	79.69	-9.61	0.000
		Grains/Meter R9	7902.39	9376.14	-1473.75	0.000
		Kernel Weight R9	36.01	34.05	1.96	0.001
		Spike Number R9	212.26	234.73	-22.48	0.001
		Yield R9	283.07	314.87	-31.80	0.000
		r9f9	1.17	0.89	0.28	0.004
		r9f89	1.11	0.94	0.16	0.002
	XCCCT338	Grain Fill Rate R9	70.41	79.33	-8.92	0.000
		Grains/Meter R9	7942.39	9323.10	-1380.71	0.000
		Spike Number R9	211.64	234.28	-22.65	0.001
		Yield R9	285.40	313.76	-28.35	0.002
		r9f9	1.15	0.91	0.24	0.012
		r9f89	1.09	0.95	0.14	0.007
	XGCGT210	Biomass R9	5.97	6.64	-0.67	0.000
		BM Prod. Rate R9	144.77	163.68	-18.91	0.000
		Grain Fill Rate R9	69.27	79.97	-10.69	0.000
		Grains/Meter R9	7912.30	9404.12	-1491.82	0.000
		Spike Number R9	209.00	236.66	-27.66	0.000
		Yield R9	281.76	315.38	-33.62	0.000
		r9f8	1.07	0.95	0.12	0.006
		r9f89	1.11	0.94	0.16	0.002
E	XTGGA235	Grains/Meter R9	9432.04	8188.27	1243.76	0.000
	XTCCA248	Grains/Meter R9	9348.67	8402.46	946.20	0.001
F	XTTTG356	Heading Date DSI	1.07	0.92	0.15	0.028
G	XwPt-6149	Spike Number R9	244.50	218.38	26.12	0.000

	Marker	Trait	DD Mean	ST Mean	Difference of Means	P-value
1D	XCGAG382	Heading Date R8	81.68	80.15	1.531	0.004
		Maturity F8	131.49	129.87	1.622	0.001
		Maturity R8	118.15	116.29	1.865	0.000
	XTC143959RT1a	Heading Date R8	80.84	79.45	1.392	0.073
2B	XwPt-3378	Harvest Index R9	0.47	0.48	-0.013	0.034
	XwPt-2266	Height F9	101.46	99.64	1.820	0.117
	XCCCT396	Height F9	101.56	99.94	1.618	0.165
	XTTCT242	Height F9	101.08	99.93	1.151	0.328
3A	Xbarc321	Maturity R9	119.48	120.71	-1.231	0.019
	XBarc12	Biomass F9	9.78	10.41	-0.631	0.001
	XwPt-2938	Biomass F9	9.84	10.43	-0.585	0.004
3B-1	X167FT3b	r9f8	1.01	0.95	0.066	0.335
	XAAAA278	Biomass F8	15.70	14.35	1.353	0.005
		BM Prod. Rate F8	294.39	269.51	24.885	0.006
		Grain Fill Rate F8	119.15	110.29	8.854	0.009
		Yield F8	536.17	496.00	40.164	0.006
3B-2	XwPt-3815	Kernel Weight F8	37.28	40.78	-3.499	0.000
4A-1	XwPt-2319	Grains/Spike F9	39.20	36.08	3.118	0.005
4A-2	XSun30	Height F9	98.44	102.60	-4.167	0.000
		Height R9	78.41	82.00	-3.591	0.000
		Yield R9	303.27	306.29	-3.022	0.704
	Xwmc89FT4b	Height F9	98.56	100.78	-2.224	0.272
		Height R9	77.30	79.60	-2.300	0.182
		Yield R9	295.97	298.08	-2.108	0.842
	Xwmc89FT4c	Height F9	99.71	99.91	-0.200	0.926
		Height R9	78.11	78.89	-0.786	0.647
		Yield R9	295.64	297.95	-2.305	0.822
4B-1	Xwmc413	Grain Fill Duration F8	53.88	53.10	0.776	0.026
		Maturity DSI	1.01	0.98	0.033	0.191
	XCTGT412	Grain Fill Duration F8	53.99	52.86	1.131	0.001
		Maturity DSI	1.02	0.96	0.061	0.010
	XTTAT290	Green Leaf Index F9	0.38	0.42	-0.046	0.000
4B-2	Xcfd39	Biomass F9	10.54	9.83	0.708	0.002
	Xgwm251	Biomass F9	10.42	9.94	0.477	0.012

	Marker	Trait	DD Mean	ST Mean	Difference of Means	P-value
4A-3	Xgwm601	Green Leaf Index R9	0.30	0.27	0.033	0.011
	XCCGT385	Green Leaf Index R9	0.31	0.27	0.039	0.002
		Grains/Meter R9	9667.56	8211.53	1456.028	0.000
	XCGTA133	Biomass R9	7.02	5.93	1.097	0.000
		BM Prod. Rate R9	170.20	146.86	23.340	0.000
		Spike Number R9	241.03	215.83	25.196	0.000
	XCCGA110	Green Leaf Index R9	0.31	0.27	0.037	0.003
		Grain Fill Duration R8	46.52	46.08	0.441	0.188
		Grain Fill Rate R9	83.86	70.49	13.369	0.000
		Yield R9	335.72	279.12	56.600	0.000
	XCTCA336	Biomass R9	6.99	5.92	1.070	0.000
		Grains/Meter R9	9628.95	8280.93	1348.026	0.000
		Height R9	81.88	78.20	3.687	0.001
		Maturity DSI	0.96	1.02	-0.059	0.025
		r9f8	0.86	1.07	-0.211	0.000
		r9f9	0.71	1.24	-0.528	0.000
		r9f89	0.83	1.13	-0.305	0.000
	Xwmc89	Grains/Spike F8	43.62	46.48	-2.856	0.010
	XCA487065FT2a	Biomass R9	6.72	5.91	0.815	0.001
		BM Prod. Rate R9	160.68	144.41	16.268	0.015
		Grain Fill Rate R9	79.89	69.95	9.941	0.001
		Yield R9	326.33	280.39	45.944	0.000
		r9f8	0.85	1.04	-0.190	0.010
		r9f9	0.84	1.24	-0.405	0.000
		r9f89	0.85	1.11	-0.259	0.001
	Xwmc48	Biomass R9	6.97	5.98	0.990	0.000
		BM Prod. Rate R9	168.27	148.86	19.407	0.000
		Grain Fill Rate R9	82.89	71.44	11.452	0.000
		Yield R9	333.23	281.77	51.467	0.000
		r9f8	0.87	1.08	-0.214	0.000
		r9f9	0.69	1.22	-0.533	0.000
		r9f89	0.83	1.13	-0.307	0.000
	Xwmc420	Biomass R9	7.00	6.00	1.003	0.000
		BM Prod. Rate R9	169.24	148.75	20.498	0.000
		Grain Fill Duration R9	41.52	40.38	1.143	0.003
		Grain Fill Rate R9	83.01	71.77	11.239	0.000
		Grains/Meter R9	9627.11	8388.13	1238.980	0.000
		Grains/Spike F8	43.66	46.88	-3.211	0.003
		Height R9	82.38	78.70	3.674	0.001
		Spike Number R9	240.14	218.73	21.414	0.002
		Yield R9	333.00	283.70	49.295	0.000
		r9f8	0.88	1.06	-0.186	0.000
		r9f9	0.70	1.19	-0.495	0.000
		r9f89	0.84	1.11	-0.276	0.000
	XBE637912a	Biomass R9	6.86	6.01	0.851	0.000
		Grain Fill Duration R9	41.57	40.41	1.162	0.002
		Grains/Meter R9	9293.59	8407.03	886.559	0.003
		r9f8	0.87	1.08	-0.211	0.000
		r9f9	0.74	1.19	-0.450	0.000
		r9f89	0.84	1.13	-0.283	0.000
	XTTGT391	Grains/Meter R9	9415.64	8553.80	861.838	0.003
		Height R9	81.50	79.43	2.070	0.053
	XCCCC184	Grain Fill Duration R9	41.66	40.28	1.385	0.000
	XTCGG165	Grain Fill Duration R9	41.77	40.45	1.323	0.001
		Kernel Weight R9	36.31	33.96	2.353	0.001

	Marker	Trait	DD Mean	ST Mean	Difference of Means	P-value
5A	Xgdm68	Biomass F9	10.37	9.88	0.495	0.019
	XCGCG157	Biomass R9	6.14	6.71	-0.573	0.001
		BM Prod. Rate R9	149.90	165.04	-15.142	0.000
		Grain Fill Rate R9	72.98	80.12	-7.143	0.001
		Yield R9	293.13	316.81	-23.680	0.003
5B-1	XwPt-0033	Heading Date R9	78.73	80.13	-1.394	0.020
	XGGGG364	Grain Fill Duration R9	41.39	40.63	0.760	0.030
		Heading Date F9	78.29	66.78	11.515	0.002
		Heading Date R9	78.50	80.00	-1.500	0.006
5B-2	XwPt-1250	Heading Date R9	80.03	78.72	1.310	0.019
	XwPt-3457	Biomass R9	6.66	6.21	0.453	0.010
6A	XGTCC336	Maturity R9	121.16	119.42	1.735	0.000
6D	XATCT318	Biomass DSI	0.91	1.03	-0.120	0.010
7A	XTGCT338	Green Leaf Index R9	0.28	0.31	-0.030	0.016
	XCTGT178	Green Leaf Index R9	0.27	0.31	-0.040	0.002
	XCTAC178	Green Leaf Index R9	0.27	0.31	-0.041	0.010
7B	Xbarc172	Harvest Index R9	0.49	0.47	0.014	0.027
	XwPt-4230	Harvest Index R9	0.49	0.47	0.017	0.004
		Maturity F9	125.19	127.21	-2.026	0.000
		r9f89	0.87	1.07	-0.202	0.000
	Xbarc176	Maturity F9	125.44	127.26	-1.820	0.001
	XGTGG284	Harvest Index R9	0.49	0.47	0.019	0.001
	XwPt-1149	Harvest Index DSI	1.46	0.82	0.644	0.001
	XwPt-1553	Spike Number DSI	0.73	1.06	-0.325	0.002
		Yield DSI	0.85	1.05	-0.200	0.000
	XwPt-6463	Harvest Index R9	0.49	0.47	0.018	0.004
	XwPt-6498	Spike Number R9	238.12	224.07	14.053	0.061

Table 2.6 Genotype means, differences of means, and P-values for single markers.

The desirable value for each trait is highlighted in yellow. P-values were calculated with two sample t tests in Minitab 15 (Minitab Inc., State College, PA)

Marker	Trait	DD mean	ST mean	Difference of Means	P-value
Xbarc204	Biomass F8	14.252	15.274	-1.022	0.017
	Biomass F9	10.252	9.836	0.416	0.026
	BM Prod. Rate F8	265.448	287.944	-22.496	0.006
	BM Prod. Rate R9	151.619	163.433	-11.814	0.006
	Green Leaf Index dsi	1.111	0.859	0.252	0.001
	Grain Fill Duration F8	53.784	53.063	0.721	0.028
	Grain Fill Duration R9	41.440	40.310	1.130	0.001
	Grain Fill Rate F8	108.344	116.923	-8.579	0.003
	Grain Fill Rate R9	73.991	79.183	-5.192	0.016
	Grains/Meter F8	12539.509	13861.063	-1321.554	0.003
	Grains/Meter F9	10831.180	11713.032	-881.852	0.014
	Grains/Meter R9	8521.957	9277.857	-755.900	0.010
	Grains/Spike F9	36.422	39.103	-2.681	0.010
	Heading Date F9	78.569	80.063	-1.494	0.009
	Heading Date R9	78.552	80.317	-1.765	0.001
	Heading Date dsi	1.051	0.943	0.108	0.101
	Kernel Weight F9	39.170	36.349	2.821	0.002
	Kernel Weight R9	35.474	33.988	1.486	0.023
	Kernel Weight dsi	1.365	0.807	0.558	0.028
	Maturity F9	125.810	127.048	-1.238	0.017
	Spike Number F8	280.353	310.321	-29.968	0.004
	Spike Number R9	219.138	235.627	-16.489	0.010
	Yield F8	491.961	522.957	-30.996	0.011
Xbarc280	Biomass R9	5.953	6.661	-0.708	0.004
	BM Prod. Rate R9	144.846	160.089	-15.243	0.023
	Grain Fill Rate R9	70.834	78.700	-7.866	0.009
	Grain Fill Rate dsi	1.112	0.798	0.314	0.010
	Grains/Meter dsi	1.038	0.771	0.267	0.017
	Height R9	77.086	81.454	-4.368	0.031
	Yield R9	284.690	320.115	-35.425	0.001
	Yield dsi	1.064	0.856	0.208	0.017
	r9f9	1.212	0.857	0.355	0.001
	r9f8	1.058	0.824	0.234	0.001
	r9f89	1.114	0.835	0.279	0.000
	r89f8	1.026	0.839	0.187	0.017
	r89f89	1.063	0.855	0.208	0.016
Xcfd31	Biomass F8	15.424	14.424	1.000	0.021
	Biomass R9	6.752	6.253	0.499	0.005
	BM Prod. Rate F8	289.655	270.172	19.483	0.019
	BM Prod. Rate R9	167.457	152.104	15.353	0.000
	Grain Fill Duration R9	40.344	41.175	-0.831	0.030
	Grain Fill Rate R9	80.363	74.552	5.811	0.009
	Grains/Meter F9	12026.433	10859.390	1167.043	0.003
	Grains/Meter R9	9523.244	8560.604	962.640	0.003
	Harvest Index R9	0.468	0.482	-0.014	0.030
	Kernel Weight F9	36.111	38.677	-2.566	0.010
	Kernel Weight R9	33.556	35.390	-1.834	0.009
	Spike Number R9	245.067	217.396	27.671	0.000

Marker	Trait	DD mean	ST mean	Difference of Means	P-value
Xgwm3	BM Prod. Rate R9	163.430	151.411	12.019	0.006
	Grain Fill Duration R9	40.348	41.464	-1.116	0.002
	Grains/Meter F8	13651.038	12734.773	916.265	0.040
	Harvest Index R9	0.468	0.488	-0.020	0.000
	Harvest Index dsi	0.894	1.343	-0.449	0.015
	Heading Date F9	80.038	78.573	1.465	0.011
	Heading Date R9	80.265	78.573	1.692	0.002
	Height F9	102.083	98.855	3.228	0.004
	Kernel Weight F9	36.677	38.909	-2.232	0.014
Xgwm46	Grain Fill Duration R9	40.574	41.283	-0.709	0.051
	Grain Fill Duration dsi	1.005	0.921	0.084	0.023
	Grain Fill Rate R9	74.726	80.064	-5.338	0.019
	Grain Fill Rate dsi	1.061	0.836	0.225	0.003
	Grains/Meter R9	8634.108	9397.272	-763.164	0.014
	Grains/Meter dsi	1.007	0.846	0.161	0.015
	Grains/Spike R9	38.547	40.630	-2.083	0.019
	Harvest Index R9	0.470	0.488	-0.018	0.002
	Harvest Index dsi	0.838	1.486	-0.648	0.001
	Heading Date F9	79.926	78.511	1.415	0.018
	Heading Date R9	80.149	78.478	1.671	0.004
	Height dsi	1.025	0.954	0.071	0.040
	Kernel Weight R8	36.791	38.571	-1.780	0.017
	Maturity F9	127.209	125.228	1.981	0.000
	Maturity R9	120.723	119.761	0.962	0.013
	Spike Number dsi	1.023	0.782	0.241	0.022
	Yield F9	423.439	408.924	14.515	0.035
	Yield R9	296.284	320.076	-23.792	0.004
	Yield dsi	1.040	0.874	0.166	0.003
	r9f9	1.104	0.777	0.327	0.000
	r9f8	1.032	0.908	0.124	0.002
	r9f89	1.063	0.881	0.182	0.000
	r89f9	1.054	0.811	0.243	0.026
	r89f8	1.016	0.916	0.100	0.049
	r89f89	1.038	0.895	0.143	0.026
Xgwm160	Biomass R9	6.757	6.152	0.605	0.001
	BM Prod. Rate R9	165.197	151.213	13.984	0.002
	Grain Fill Rate R9	79.887	73.777	6.110	0.007
	Grains/Meter R9	9309.750	8567.057	742.693	0.016
	Heading Date R8	81.375	80.262	1.113	0.051
	Spike Number R9	235.010	220.680	14.330	0.036
	Yield R9	318.010	293.770	24.240	0.003
	r9f9	0.849	1.105	-0.256	0.003
	r9f89	0.946	1.044	-0.098	0.048

Marker	Trait	DD mean	ST mean	Difference of Means	P-value
XGTGT526	Biomass R9	6.279	6.640	-0.361	0.050
	Grain Fill Rate R9	73.909	79.912	-6.003	0.009
	Grains/Meter R9	8325.661	9608.443	-1282.782	0.000
	Grains/Meter dsi	1.008	0.852	0.156	0.017
	Grains/Spike R9	38.714	40.764	-2.050	0.022
	Heading Date F9	80.196	78.177	2.019	0.003
	Heading Date R9	80.196	78.660	1.536	0.010
	Height F9	101.991	98.877	3.114	0.008
	Height dsi	1.034	0.951	0.083	0.015
	Kernel Weight R9	35.870	33.467	2.403	0.001
	Maturity F9	127.330	125.509	1.821	0.001
	Maturity R9	121.018	119.679	1.339	0.001
	Spike Number R9	216.679	237.377	-20.698	0.003
	Yield R9	296.027	317.320	-21.293	0.013
	r9f9	1.093	0.828	0.265	0.002
	r9f8	1.019	0.928	0.091	0.035
	r9f89	1.050	0.910	0.140	0.006
XwPt-4487	Biomass R9	6.597	6.020	0.577	0.004
	BM Prod. Rate R9	161.665	146.468	15.197	0.002
	Grain Fill Rate R9	78.360	71.201	7.159	0.003
	Grains/Meter F9	11552.262	10577.342	974.920	0.012
	Grains/Meter R9	9210.805	7977.871	1232.934	0.000
	Kernel Weight F9	37.226	39.447	-2.221	0.021
	Kernel Weight R9	34.299	36.214	-1.915	0.003
	Maturity F8	130.207	131.329	-1.122	0.056
	Spike Number R9	231.671	210.614	21.057	0.001
	Yield R9	311.652	287.671	23.981	0.009
	r9f9	0.931	1.136	-0.205	0.020
	r9f8	0.959	1.062	-0.103	0.018
	r9f89	0.961	1.096	-0.135	0.008

CHAPTER 3 - Mapping a Seedling Leaf Rust Resistance Gene Identified in PI 289824

Introduction

Leaf rust is a fungal disease of wheat caused by the pathogen *Puccinia triticina*. It occurs throughout the world in all growing seasons and caused an average of 4.6% yield loss annually in Kansas from 1980-1999 (Eversmeyer and Kramer, 2000). Favorable conditions for wheat growth, mild temperatures and plentiful moisture, also favor fungal growth so the greatest losses tend to occur in years with the highest yield potential (Eversmeyer and Kramer, 2000). Leaf rust is best controlled by planting resistant cultivars as fungicides are often not cost effective. There are currently 59 leaf rust resistance genes identified in wheat (CDL, 2008). However frequent shifts in pathogen races quickly overcome many resistance genes and so new sources of resistance must be identified.

Pyramiding multiple resistance genes into a single cultivar can increase the length of time a new cultivar is effective in the field. However, it is difficult to screen for the presence of multiple genes in a single line by inoculation, so markers closely linked to each gene for marker assisted selection are necessary to make pyramiding genes practical (Nocente et al., 2007). Detailed mapping is also necessary to ensure that genes from new sources are truly novel genes.

Obert et al. (2005) identified a land race from Iran, PI 289824, with a high level of seedling resistance to field populations of leaf rust in Texas and a wide variety of races

tested under laboratory conditions. They determined that the resistance was controlled by a single dominant gene and mapped it to chromosome 5BS using a mapping population derived from PI 289824 and T112, an experimental line from Trio Research (Valley Center, KS). They designed an STS marker, *XTXW200*, from an AFLP fragment that is 2.3 cM proximal to the resistance gene. The gene was also 16.7 cM proximal to the SSR marker *Xgwm443*, suggesting that it is unique from *Lr52* which was mapped 16.5 cM distal to *Xgwm443* (Hiebert et al., 2005).

For a marker to be useful for marker assisted selection it must be very closely linked to the gene of interest as hundreds of lines will be scored in a breeding program. To be able to effectively pyramid the gene from PI 289824, a more closely linked marker must be identified. This may be possible through mapping with populations derived from crossing the donor parent to other adapted material.

Objective

The objective of this study was to map a population of winter wheat lines from a cross between Jagger and PI 289824 in order to find a marker more closely linked to the leaf rust resistance gene from PI 289824 than the AFLP based STS marker designed by Obert et al. (2005). This would allow marker assisted selection for this gene facilitating its incorporation into breeding programs especially to pyramid resistance genes.

Materials and Methods

Experimental Lines

The mapping population for this study was an F_2 population from a cross between Jagger (PI 245386), a common winter wheat grown in Kansas susceptible to the leaf rust race used in this study, and PI 289824, a land race from Iran with resistance to a broad

range of races of leaf rust. Individual seeds were germinated in small pots filled with Metromix 360 (Sun Gro Horticulture, Vancouver, Canada) and grown in a greenhouse until leaf rust screening was possible.

Leaf Rust Screening

At the three leaf stage, seedlings were inoculated with spores of leaf rust race PRTUS6. The spores were suspended in Soltrol 170 (Phillips Petroleum, Bartlesville, OK) and sprayed on to the leaves. Plants were then placed in a mist chamber overnight at 18°C to allow spore germination and successful infection. The following day, seedlings were moved to a growth chamber with 20°C days and 18°C nights and a day length of 16 hour to allow symptoms to develop. After twelve days, seedlings were scored using the Stakman scale (Stakman et al., 1962). After scoring, tissue samples were taken from each seedling for genetic analysis.

DNA Extraction

Tissue samples collected from seedlings were frozen in liquid nitrogen and ground to a fine powder. DNA was extracted with a protocol similar to Allen et al. (2006) using 2X CTAB extraction buffer (1.4 M NaCl, 100mM Tris pH 8.0, 2% hexadecyltrimethylammonium bromide, 2.0 mM EDTA, 0.5% sodium bisulfide, and 1% 2-mercaptoethanol). Extraction buffer (700 µL) was added to each sample and vortexed. The samples were incubated at 65°C for one hour vortexing three times during the incubation. Chloroform: isoamylalcohol (24:1 v/v, 500 µL) was added and the samples were incubated at room temperature for 15 minutes on a rotary shaker. Samples were then centrifuged at 5000 x *g* for five minutes and the supernatant was transfer to a clean tube. Two µL RNase A (Sigma, St. Louis, MO) was added to each tube and

incubated for one hour at 37°C. The chloroform:isoamylalcohol extraction was repeated, then the DNA was precipitated by adding isopropyl alcohol in a volume approximately equal to the sample. The tubes were mixed by inversion and incubated at room temperature for 15 minutes. The DNA was pelleted by centrifugation at 5000 x *g* for five minutes. The pellet was washed twice in 70% ethanol centrifuging at 5000 x *g* for five minutes after each wash and then allowed to air dry. The DNA was resuspended in 1X TE (Tris-ethaline diamine tetraacetic acid) and the concentration was checked with a spectrophotometer. A 10x dilution of each sample was made to use as a template for PCR reactions.

Marker Analysis

SSR Markers

All of the SSR markers shown to map to the short arm of chromosome 5B by Somers et al. (2004) were screened on Jagger and PI 289824 to determine which were polymorphic. The polymorphic SSR markers and the STS marker designed by Obert et al. (2005) were mapped in 94 lines of the population. The eight markers that appeared to surround the gene and the STS marker were mapped on an additional 94 lines. The STS marker was amplified as described by Obert et al. (2005) and was scored by 1% agarose gel electrophoresis and ethidium bromide staining. SSR PCR reactions included 1 µL DNA, 5 pmoles reverse primer, 1 pmole forward primer, 5 pmoles m13 labeled primer, 2.5 µL 10x PCR buffer (Sigma, St. Louis, MO), 2.5 µL 25 mM $MgCl_2$ (Sigma, St. Louis, MO), 2.5 µL 10 mM dNTPs (Sigma, St. Louis, MO), 1 unit Taq DNA polymerase (Sigma, St. Louis, MO), and water to bring the volume to 25 µL. Reaction conditions were 95°C for five minutes, then 95°C for 45 seconds, 68°C for five minutes decreasing

2°C per cycle, and 72°C for one minute repeated four times. Then, 95°C for 45 seconds, 58°C for two minutes decreasing by 2° per cycle, and 72°C for one minute repeated four times. Followed by 95°C for 45 seconds, 50°C for two minutes, and 72°C for one minute repeated 25 times and finally, 72°C for five minutes. Samples were run on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) for capillary fragment analysis. These results were visualized using the GeneMarker 1.51 software (Soft Genetics, State College, PA).

EST Markers

After preliminary linkage analysis with the SSR markers, the gene was tentatively assigned to a deletion bin. Expressed sequence tags (ESTs) in this deletion bin from the U.S. Wheat EST Project (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi) were aligned against the RiceBLAST database (http://riceblast.dna.affrc.go.jp/cgi-bin/robo-blast/blast2.cgi?dbname=all) for sequence similarity. ESTs were assigned to groups based on the chromosomal location of the best hit in rice. Six EST-STS primers were designed using MacVector v9.5 (MacVector, Inc., Cary, NC) for the ESTs corresponding to unique rice BAC clones in the largest group as well as two with hits to disease resistance proteins. These were screened on the eight lines with the highest observed levels of leaf rust resistance and the eight lines with the highest susceptibility to leaf rust. Primers were also designed from 22 other ESTs in the wheat deletion bin and were screened on the second set of 94 lines used to screen the SSR markers. PCR reactions for all EST primers included 1 µL DNA, 5 pmoles forward primer, 5 pmoles reverse primer, 2.5 µL 10x PCR buffer (Sigma, St. Louis, MO), 2.5 µL 25 mM $MgCl_2$ (Sigma, St. Louis, MO, St. Louis, MO), 2.5 µL 10 mM dNTPs (Sigma, St. Louis, MO), 1 unit Taq

polymerase (Sigma, St. Louis, MO), and water to bring the volume to 25 µL. Reaction conditions were 92°C for three minutes, then 92°C for one minute, the appropriate annealing temperature for one minute, and 72°C for two minutes repeated 34 times, and finally 72°C for ten minutes. All primer sequences and annealing temperatures are given in Appendix C. The amplified fragments were separated on SSCP gels made with MDE gel solution (Cambrex, East Rutherford, NJ) for 16 hours at four watts. The bands were visualized by silver staining by fixing with 10% glacial acetic acid for five minutes, washing three times for two minutes each in distilled water, soaking in a 2% silver nitrate solution (2 L distilled water, 2 g silver nitrate, 3 mL formaldehyde) for 25 minutes, developing until bands were visible (2 L distilled water, 60 g sodium carbonate, 400 µL sodium thiosulfate, 3 mL formaldehyde), stopping with 10% glacial acetic acid for five minutes, and rinsing three more time for two minutes each in distilled water.

Linkage Mapping

The marker data was analyzed to construct a linkage map using CarthaGene software (INRA, Paris, France). Markers were grouped at an LOD of 3 with a maximum recombination fraction of 0.5. Markers with extremely similar scoring patterns were identified with the 'mrkdouble' command and then merged in order of highest likelihood of being identical with the 'mrkmerge' command. A map was built with markers linked to the gene using the 'Nicemapl' command and then improved with the 'flips' and 'polish' commands as well as the annealing, taboo, and genetic algorithms. The Kosambi mapping function was used to determine distances. WinQTL Cartographer (NCSU, Raleigh, NC) was used to create a visual representation of the linkage group.

Results

Twenty-three of the 35 SSR markers screened were polymorphic between the parents and are highlighted in the Somers map in Figure 3.2. When these 23 SSR markers and *XTXW200* were mapped on the population, *XTXW200* was the closest marker to the leaf rust gene, temporarily designated *LrAF*, at a distance of 18.2 cM followed by *Xgwm234* at a distance of 39 cM from *LrAF*. The markers distal to *Xgwm234* according to the Somers map were each located in a unique linkage group and so *LrAF* was not able to be flanked by this method. The eight SSR markers that should have surrounded *LrAF* based on its location relative to *Xgwm234* were screened on additional lines to determine if the lack of linkage was due to the population or to experimental error. These markers were *Xcfd60, Xwmc47, Xwmc728, Xgwm234, Xcfa2121, Xwmc740, Xgwm133*, and *Xbarc4*. The addition of these lines confirmed the result of distal markers being unlinked.

EST-STS markers were then introduced. Deletion bin mapping was attempted with *XTXW200*, but it does not amplify in Chinese Spring so this was not possible. ESTs were therefore chosen from the deletion bin 5BS5-0.71-0.81, which contains *Xgwm234*. Sequence alignment against the rice database and grouping of the highest quality hits by rice chromosomal location identified rice chromosome 12, containing eight high quality hits, as the most similar. Only three of the primers designed from the ESTs mapping to unique rice BAC clones on rice chromosome 12 and the two with similarity to known disease resistance sequences showed polymorphism between the parents. These were BE498930 (which hit on a barley stem rust resistance protein), BF146083, and BF202632. When these markers were screened on the eight most tolerant and eight most

susceptible lines nothing was seen to cosegregate with resistance so these primers were discarded.

The 22 other EST-STS primers were run on a subset of the population consisting of 94 plants resulted in 17 polymorphic markers. Linkage analysis of these markers plus the original 23 SSRs and the STS marker showed that three of the EST-STS markers were in the linkage group with *LrAF*. These were two markers from the EST-STS primer for BM134523 and one from BE499458. *LrAF* was now flanked, but still at a great distance. *XBM134523a*, the closest EST-STS marker, was 34.3 cM distal to the leaf rust gene and *XTXW200* was 18.5 cM proximal. See Figure 3.2 for the complete linkage map.

Discussion

Though *LrAF* was able to be flanked, the markers are much too far away from the gene to be used for marker assisted selection and in fact are not linked to the gene at all if the linkage criteria for mapping is strengthened to LOD 3 and maximum recombination fraction of 0.2. The lack of linkage between the markers distal to *Xgwm234* led to the idea that a chromosomal rearrangement may have occurred on chromosome 5B of PI 289824. The chromosome structure of the line was checked by Giemsa C-banding with the technique described by Gill et al. (1991) and all chromosomes appeared normal (B. Friebe, personal communications, March 12, 2008). However, there does appear to be a small rearrangement of markers close to the centromere (*Xwmc386, Xbarc4,* and *Xgwm133*). A rearrangement this small would most likely not be detectable by Giesma C-banding and so it is also possible that other small rearrangements could have occurred on the chromosome arm resulting in the lack of linkage between the far distal markers.

The position of *XTXW200* and *Xgwm234* on the same side of *LrAF* is different from that reported by Obert et al. (2005) in which they flanked the gene. The only other marker mapped in both studies was *Xgwm544*. This marker was approximately twice as far from *XTXW200* in this study than reported previously. It is difficult to compare the location of the leaf rust score to that reported by Obert et al. (2005) and that of *Lr52* as very few markers are common to this study and either of the others. The markers that are shared, such as *Xgwm133* for this study and *Lr52*, are far from the genes and do not give any detailed information about their locations. *Xgwm443* that served as evidence of a gene in PI 289824 different from *Lr52* was not polymorphic in this population and so could not be used to confirm or refute the conclusion.

The large distances between the flanking markers and the leaf rust gene may have caused ESTs to be selected from an incorrect deletion bin to be linked to the gene. However, since the EST-STS markers were mapped on the opposite side of *LrAF* from an SSR marker also in the bin, it seems that deletion bin choice was correct. These EST-STS markers therefore should be the closest markers available from this technique. BM134523 had its highest quality blast hit on rice BAC AC079736, which is on chromosome 3 (E value 2×10^{-13}). The other three hits for this EST were on BACs from rice chromosome 12 and had E values ranging from 5×10^{-11} to 2×10^{-4}. The only hit for BE499458 was on rice chromosome 9, but was of poor quality (E value .071).

When the linkage map from this study is compared with that of Somers et al. (2004) the linkage distances are seen to be greatly expanded. These two maps are compared in Figure 3.2. The expanded recombination distances could be due to the small size of the mapping population used in this study. Markers were scored on either 94 or

188 F_2 lines in this study where as four mapping populations of 68 recombinant inbred lines, 91 doubled haploid lines, 93 double haploid lines, and 186 double haploid lines were used to generate the Somers map. The higher number of lines and the consensus between multiple populations leads to a more precise map. The markers polymorphic in this study should have given coverage of the entire chromosome arm, but due to the lack of linkage between far distal markers, only the proximal portion of the chromosome is represented in the map. Mapping in this population did not improve the accuracy of tracking the leaf rust gene from PI 289824. However, mapping in crosses between this donor parent and other adapted material may still be useful.

Figure 3.1 Maps from the Leaf Rust Studies by Obert et al. (2005) and Hiebert et al. (2005).

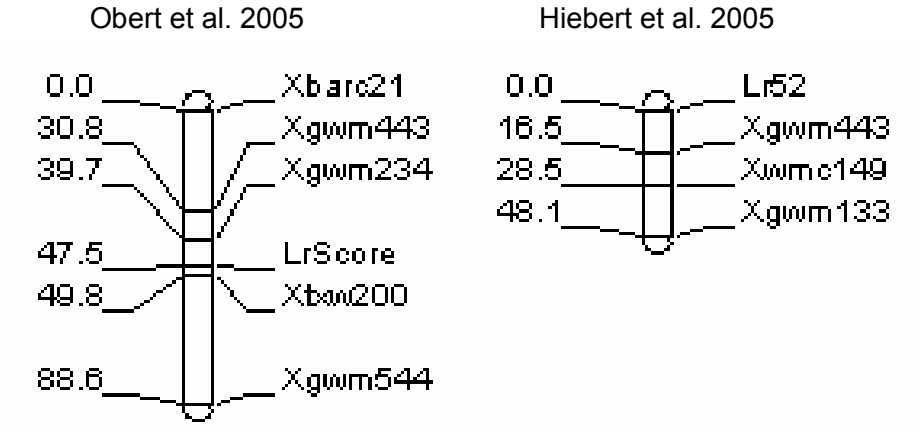
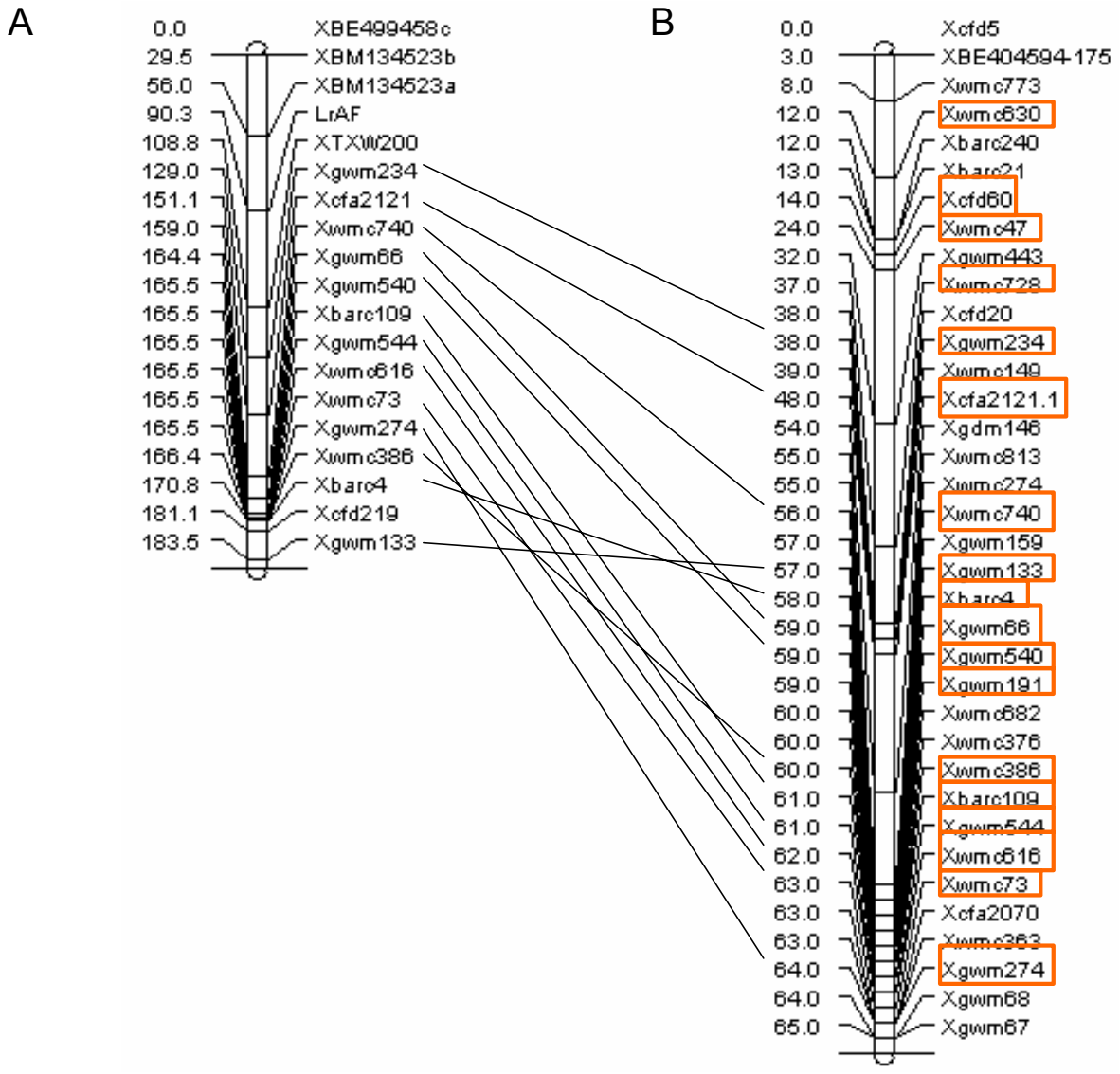


Figure 3.2 Linkage maps of chromosome 5BS.

A- The linkage group from this study
B- The complete chromosome arm map from Somers et al. (2004) with markers polymorphic in this study highlighted in orange.

Maps are not drawn to scale. Markers appearing in both maps are connected.



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Appendix A - AFLP Primer and Adaptor Sequences

All oligos were synthesized by IDT, Coralville, IA.

Mse adaptor oligos

M-Ad1	GAC GAT GAG TCC TGA G
M-Ad2	TAC TCA GGA CTC AT

Pst adaptor oligos

P-Ad1	CTC GTA GAC TGC GTA CAT GCA
P-Ad2	TGT ACG CAG TCT AC

PreAmp Pimers

MsePre	GAT GAG TCC TGA GTA AC
PstPre	GAC TGC GTA CAT GCA GA

Mse Selective Primers

M-CAA	GAT GAG TCC TGA GTA ACA A
M-CAT	GAT GAG TCC TGA GTA ACA T
M-CAG	GAT GAG TCC TGA GTA ACA G
M-CAC	GAT GAG TCC TGA GTA ACA C
M-CTA	GAT GAG TCC TGA GTA ACT A
M-CTT	GAT GAG TCC TGA GTA ACT T
M-CTG	GAT GAG TCC TGA GTA ACT G
M-CTC	GAT GAG TCC TGA GTA ACT C
M-CGA	GAT GAG TCC TGA GTA ACG A
M-CGT	GAT GAG TCC TGA GTA ACG T
M-CGG	GAT GAG TCC TGA GTA ACG G
M-CGC	GAT GAG TCC TGA GTA ACG C
M-CCA	GAT GAG TCC TGA GTA ACC A
M-CCT	GAT GAG TCC TGA GTA ACC T
M-CCG	GAT GAG TCC TGA GTA ACC G
M-CCC	GAT GAG TCC TGA GTA ACC C

Pst Selective Primers

P-AAA	GAC TGC GTA CAT GCA GAA A
P-AAT	GAC TGC GTA CAT GCA GAA T
P-AAG	GAC TGC GTA CAT GCA GAA G
P-AAC	GAC TGC GTA CAT GCA GAA C
P-ATA	GAC TGC GTA CAT GCA GAT A
P-ATT	GAC TGC GTA CAT GCA GAT T
P-ATG	GAC TGC GTA CAT GCA GAT G
P-ATC	GAC TGC GTA CAT GCA GAT C
P-AGA	GAC TGC GTA CAT CGA GAG A
P-AGT	GAC TGC GTA CAT CGA GAG T
P-AGG	GAC TGC GTA CAT CGA GAG G
P-AGC	GAC TGC GTA CAT CGA GAG C
P-ACA	GAC TGC GTA CAT CGA GAC A
P-ACT	GAC TGC GTA CAT CGA GAC T
P-ACG	GAC TGC GTA CAT CGA GAC G
P-ACC	GAC TGC GTA CAT CGA GAC C

Appendix B - Attempted Cloning of AFLP Fragments

AFLP markers determined to be of interest from QTL analysis conducted prior to the addition of the DArT markers were selected for cloning. Because these were not the final results presented in this paper, some of the fragments selected are no longer of much interest and other fragments should have been included. The selected fragments were CGTA133 and CTCA336 located in the QTL on 4AL reported by Kirigwi et al. (2007), and CCCC329, TCCC284, TCTA185, and TCTC413 which were selected from other linkage groups.

The selectively amplified DNA of the parents from primer combinations containing bands of interest was run out on a 7% acrylamide denaturing at 90W for approximately two hours, depending on the size of the fragment desired. The band of interest was determined from the size standard and then cut from the gel and placed into a 0.5mL microcentrifuge tube. Fifteen μL of water was added to each band and then the tubes were incubated at 50ºC for 15 minutes. One μL of this solution was used as template to reamplify the band. The selective amplification protocol for master mix and reaction conditions was used.

The reamplified band was ligated into the pCR2.1 vector (Invitrogen, Carlsbad, CA) and transformed into chemically competent cells following the recommendations provided by the manufacturer with the TA cloning kit (Invitrogen, Carlsbad, CA). Antibiotic and blue/white colony selection were used to pick colonies likely to contain an insert. These colonies were grown in liquid LB media containing ampicillin to select only bacteria containing the pCR2.1 plasmid. Plasmid DNA was extracted from the

bacteria using the Miniprep Spin Kit (Qiagen, Valencia, CA). Extracted DNA was digested with the *Eco*RI restriction enzyme (Invitrogen, Carlsbad, CA) and then run on a 1% agarose gel. Samples containing what appeared to be the correct fragment were purified using Qiagen spin columns (Qiagen, Valencia, CA) and sequenced at the Kansas State University Sequencing Facility. Sequences were blasted against the National Center for Biotechnology Information (NCBI) database. Results of sequencing and blast searches are given below.

Primer Design

Insert sequences that were close to the desired length of the AFLP fragment from which they were cloned were used to design STS primers. MacVector v9.5 (MacVector, Inc., Cary, NC) was used to determine the best primer pairs for each fragment. Three primer pairs were designed for TCCC284, two for CGTA133, one for CTCA336, one for TCTA185, and one forward primer with two reverse primers for CCCC329 due to a poly G in the sequence. Sequences and annealing temperatures for these primers are given in Table B.1. Primers were tested with 35 PCR cycles at the recommended annealing temperature for the primer and separated on 2.3% agarose and if necessary, 7% acrylamide gels. However, none of these primers were able to produce a diagnostic band for their respective fragment.

Sequencing and Blast Results

Results from sequencing and blast searching are given below. The colonies were assigned a number as they were selected. Primes were added to the numbers for each additional cloning attempt. The letters ‘a’ and ‘b’ were used if two bands for the same target fragment were cut from the same gel and cloned simultaneously. The numbers in

parenthesis after the name represent the number of base pairs in the sequence and the number of base pairs desired from the fragment length. Blast results are given in bold below the sequence. Sequences marked with an asterisk were used to design primers.

CCCC 5 (236/329)

GACTGCGTACATGCAGACCCGAATGGGAAGCCGGAACCATGACTCCATCTTTGAAGATCCC
TCACATTTGGGGAGGATTCCAGGACACTGCTATGACAATCTAAGGCATGTGAAGATCACTA
GGTTCTTCTCCACAAAGCTTCTGGTTGAGCTCACATGCCATATTCTTGAGAATGCACCATCA
CTCGAGTGCCTCACACTGGACATAACTGATGGTGGGTTACTCAGGACTCATC

cDNA from *Triticum aestivum* (3), Rice chromosome 5 (2)

CCCC 6 (235/329)

ACTGCGTACATGCAGACCCGAATGGGAAGCCGGAACCATGACTCCATCTTTGAAGATCCCT
CACATTTGGGGAGGATTCCAGGACACTGCTATGACAATCTAAGGCATGTGAAGATCACTAG
GTTCTTCTCCACAAAGCTTCTGGTTGAGCTCACATGCCATATTCTTGAGAATGCACCATCAC
TCGAGTGCCTCACACTGGACATAACTGATGGTGGGTTACTCAGGACTCATC

Same sequence as CCCC5

CCCC 8 (218/329)

GATGAGTCCTGAGTAACCCGTTGTCCGCGATAAATTCGCTGACATAGTCATTCTATCCGAAT
CTCATCCGCATGCAGAGTTGAACACCTATATTTGACTTTCTGCTATTTTGCAACTAATCTCC
GACTATTGTGGTGTGCGGGGCCAATATGCTGAGCCAGTATGTTCTGTCGCAGATGAATCTT
GCGTTTGCGAAATCCACCAAGTGGTGGGAAAAG

Cold treated wheat cDNA, No good hits in nucleotide

CCCC 9 (218/329)

GATGAGTCCTGAGTAACCCGTTGTCCGCGATAAATTCGCTGACATAGTCATTCTATCCGAAT
CTCATCCGCATGCAGAGTTGAACACCTATATTTGACTTTCTGCTATTTTGCAACTAATCTCC
GACTATTGTGGTGTGCGGGGCCAATATGCTGAGCCAGTATGTTCTGTCGCAGATGAATCTT
GCGTTTGCGAAATCCACCAAGTGGTGGGAAAAG

Same sequence as CCCC8

CCCC'-3 (365/329)

GATGAGTCCTGAGTAACCCCCCAGGGCCAGTGCTCCTCTGAGTGTTGGTCCGAACTGAGCT
GCCTGCGGGGCCACCTTGGGGAAACTTGTGGGTTGGTTTTACTCGTAGCTAGTCTCATCTGG
TGTTTCCCTGAGAACGAGGTACGTGCGAGTCCTATCGGGATTTGTCGGCACATCGGGTGGC
TTTGCTGGTCTTGTTTTGCCATTGTCGAAATGTCTTGTAACCGGGATTCTGAGCCTGATCGG
GTCTTCCTGGGAGAAGGAATATCCTTCGTTGACCGTGAGAGCTTGTGATGGGCTAAGTTGG
GACTGCAGGGTTTTAGATGGACCGTGAGACTGTGAGAGCTTGTGATCGGGTCTGCATG

Fusarium infected spike and etiolated seedling cDNA, *Triticum* clones from several species

CCCC'-6 (374/329)

GACTGCGTACATGCAGACCTTGAGCTCCCACCGCTTGGCCGACTTGCTCCTGGACGTAGCT
GGGCCAAGAGAGGCGAGGTAGTCGGCGTAGGCTTGCTGCCACATAGAAGTCGCATCCACC
AACTGATGGCTATCGTTGATTCAAAGAAAAGAGACGAAGAAAAGTGAGGTCTTGAGCAGA
TCAGAAGAATAATGCAACAAAAATTCTCCAAACAAAACCGCTTTACATTGTGATTCCGGTA
CTTGCAGAAACGCTCGTCGGCGTTCTGCCCCTGCCGAGCTATCTTGATTTCGACTTGGGGAT
CGATTGAAACATCGGCTAGGGTTTCTCTTCTCTCAATTTGGGGATGGATTGGGGTTACTCAG
GACTCATC

No good hits in ESTs, or nucleotides

CCCC''-2 (421/329)

GATGAGTCCTGAGTAACCCCAAATAAAAAAGAGCTCATTCATCAGTGCACTAGCTAGTGAT
TTAGATCTGCATCCATTACTGGTCTGCATGTACGCAGTCGACTGCGTACATGCAGACCGGAT
TTACTATGTACGATCCTCCAAGTTGGGTAAGTGAACAACTGCGCTTGCCCGTTTGTTTTGTA
CTCCCACGGTTAGATGTGTAGTCTAACGACTTCAATGCAGGAACTGCGACAGGTGGTAAAG
GATATAAATAGCCGCCCTCCACAACCCGAGGACCCAGAACGGTCCCTCGATCCAGACTCCA
AAGAGGATCCGGACATATCGATGGAGCTTATCGACGGGGTGTTCCATCAGCTAAGCAAGG
ACAATACATTGGTAGCCATCACGGCTGATTACCCAGGGTTACTCAGGACTCATC

***Triticum aestivum* cDNA clone, *Triticum monococcum, turgidum*, and *urartu* BAC clones**

***CCCC''-3 (353/329)**

GACTGCGTACATGCAGACCTCGTCGCGTGGACTAGCTTCGCCAGCGTCCACTTGATG
GGATGACCCATTGGTGATCTTGTTTATGCTTTCTCTTATGTTTTCTGGGCGTGCTTGT
GACGTGGCCGCACCAGACTATCTATGTTTGTATGTTGCAACTCGATGTTAGAACGGT
TGGTTGCTTCATTTATAAAGCAGGATGAAAGTTTGTTCTGAGAAACCACATAAAATA
GATTTGGGGGGGGGGGGGTGATTTGTCCGTATTTGTAATGTTTAGGGGGCTAGATA
CCCTATTTCACAGTTGAGGGGGTTACTCAGGACTCATCATGTACATATAATATATGT
ACATATATGT

No good hits in ESTs or nucleotides

***CCCC'''-3 (sequenced from m13R) (324/329)**

ACATATATGTACATATATTATATGTACATGATGAGTCCTGAGTAA**CCC**CCTCAACTG
TGAAATAGGGTATCTAGCCCCCTAAACATTACAAATACGGACAAATCACCCCCCCCCC
CCCCCAAATCTATTTTATGTGGTTTCTCAGAACAAACTTTCATCCTGCTTTATAAATG
AAGCAACCAACCGTTCTAACATCGAGTTGCAACATACAAACATAGATAGTCTGGTG
CGGCCACGTCACAAGCACGCCCAGAAAACATAAGAGAAAGCATAAACAAGATCAC
CAATGGGTCATCCCATCAAGTGGACGCTGGCGAAGCTAGTCCACGCGACGAGGTCT
GCATGTACGCAGTC

No good hits in ESTs or nucleotides

CGTA1 (222/133)

GATGAGTCCTGAGTAACCGTAGTTGATGTCGACAACTATGTTAGGGGTGCCATCCAAAGTT
GCCGGCGAGCCAAATGCATCTAACAGATCAGGCAATGTCATTTTCAGATGGTGCATGCATC
TCCAAATCATTTTCAATCATGGTTTCCATCAGCAGATTGATCCTATAGCTACAAGGTTGACA
CAGTCTAAGGCCATTAGGCATTTGTATCTGCATGTACG

No good hits on EST or nucleotide blasts

CGTA2 (260/133)

AGTCCTGAGTAACCGGCAACATCTACTAATGCATCCATGGTTAGCACATTTCTCAACGTGCT
TACTGAAAGCGAGAGATGTAACTGTGAAAGGGATGTAAAAGATGAAGTTGTGACCATAAA
AAGGGCATGTTACTTTCAGTCCTACTGATATCTGCATGTACGCAGTCGACTGCGTACATGCA

GATACGTGGAAGGTGGTGAGGACAATACATGATCTCGAATACCTAGTCTAGAGCACCCGGT
TACTCAGGACTCATC

No good hits in EST or nucleotide blasts

CGTA4 (230/133)

GATGAGTCCTGAGTAACCGAAAGAATAATACATGCCATCCTCGGGAGTACTTGCCTTTTTCT
TTCTATCTGCATGTACGCAGTCGACTGCGTACATGCAGATAGCTGACACATCTGCACTTGTT
CTAAAATTTATCTTTTTTGTGTACAATGCACATATGGTTCACTTCAGAAACAACATTATGCA
AGCACATATTCTAACATCTTGGCGTCGGTTACTCAGGACTCATC

No good hits in EST or nucleotide blasts

CGTA7 (252/133)

CGTACATGCAGATAAAATACATGCACTAATGAGTGCATATTTGACAATGCATATGGCTATT
CATATGAAAATGGAAGCCAACCGATATGGCACCAGGTGTGCTAACCACTCTGTGCATGAAA
AGATAATCAGAAATGACAATGAATGGAACACTCTTTATTCGATGATCTTATATGTGATCAG
TATTTTCTGTTGGAACGAAGGAAGGACCGTAAGAGAAAAGGTTTCAATTCTGTAGCGGTTA
CTCAGGAC

No good hits in EST, rice chromosome 3 sequence from BAC and genomic DNA

CGTA9 (263/133)

GACTGCGTACATGCAGATACTAAGTTATCCAGGTGTGGTTGAATTGACAACTCAACTGCTA
ATACTTGAGAATATTGTTTGGCTCCCCTTGTGTCGAATCAATAAATTTGGGTTGAATACTCT
ACCCTCGAAAACTGTTGCGATCCCCTATACTTCTGGGTTATCAGTAGTCCTTCCCATCGAAG
GTAGGACACCCAACCGAGACTTTGATCATACCTGTGGTCGACATAACTAAAACTCCAGGCG
GTTACTCAGGACTCATC

***Triticum aestivum* cDNA clone, retrotransposon**

CGTA'a-4 (273/133)

GATGAGTCCTGAGTAACCGTCATTTTGATGGCAATCCTCCTATCCACGTCCAAGCCGACTTG
TGCTATTTTTGAAATCTCTGAGCTTCAATTCGAAACTCTACACAGTTGTCTAGTGGCTATCT
GCATGTACGCAGTCGACTGCGTACATGCAGATAAAATGACATAAATGGTGGATATGAACGC

CGACAGTAAATATGTCCTTGTTGCTCAATTTCTACCAGCAGTTTTTCTGCCTAGAAGGACTT
TTGCCGGCGGTTACTCAGGACTCATC

No good hits for ESTs or nucleotide collection

CGTA''-1 (104/133)

GACTGCGTACATGCAGATAGCCACTAGACAACTGTGTAGAGTTTCGAATTGAAGTTCAGAG
ATTTCAAAAATAGCACAAGTCGGCTTGGACGTGGATAGGAGGA

No Good hits in EST or nucleotide collection

***CGTA''-2 (138/133)**

GACTGCGTACATGCAGATAGCCACTAGACAACTGTGTAGAGTTTCGAATTGAAGTTCAGAG
ATTTCAAAAATAGCACAAGTCGGCTTGGACGTGGATAGGAGGATTGCCATCAAAATGACG
GTTACTCAGGACTCATC

Like CGTA''-1, but longer

***CGTA''-3 (135/133)**

GATGAGTCCTGAGTAACCGCCGGCAAAAGTCCTTCTAGGCAGAAAAACTGCTGGTAGAAAT
TGAGCAACAAGGACATATTTACTGTCGGCGTTCATATCCACCATTTATGTCATTTTATCTGC
ATGTACGCAGTC

No good hits in EST or nucleotide collection

CGTA''-4 (134/133)

GATGAGTCCTGAGTAACCGCCGGCAAAAGTCCTTCTAGGCAGAAAAACTGCTGGTAGAAAT
TGAGCAACAAGGACATATTTACTGTCGGCGTTCATATCCACCATTTATGTCATTTTATCTGC
ATGTACGCAGT

Like CGTA''-3

CTCA4 (513/336)

GATGAGTCCTGAGTAACCTACTTTGTTTGTTTTTGTCTCATTACTAACTTATCTGGTACACTA
ATCTACAAGTTCAAACTTTCAGGATGCTATGGAACAAATGATTGAACAACCACAACCACCT
GAAGGTGACGAGGCTACTACAGGCACAATGTCACCTCTTGCTACAGTGCGTCAGTACCTCT

CCACTAACAGTGCAAAAAGCACCTTCCTGCGTAATACTGGGTTGGTTGTCCAGGTAACCTC
GTCCAAATCGCCTACTGAACAAAATCTTCCTGCTAGACAGAGTGATATATCTGTGCTCCAG
ACACAAGTCCAATCCCTAATGGACGTTGTTTGGGAAACAAGAATAGTGGTTGAGAAATGTC
GTCAAGATATGAATGGTTTTGAAACCAGACTATCAGTCATTCGCTTCGTTGTTCAAGAGCA
ATGGCGGAAAAACTGTGGAGATCGTGCTGCTCCATCAGATTCTACAGCCTGAAACATTAGC
ACTCAGGTTACTCAGGACTCATC

Triticum aestivum* cDNA, *Triticum turgidum

CTCA6 (incomplete sequence) (315/336)
GACTGCGTACATGCAGACACTAAGTTATCCAGGTGTGGTTGAATTGACAACTCAACTTCTA
ATACTCAAGTATATTCTTTGGCCCCCCTTGTGTCAAATCAATAAATTTGGGTTGTACTTCAC
CCTCGAAAGCTGTTGCGATCCCCTACACTTGTGGGTTATCAAAACTAATTTCTGGTGCCGTT
GCCGGGGAGCATAGCTCTATTCTCTGAGTCACTTGGGATTTATATCTGTTGATCACTATAAG
GAATTTGAAAGACAACAAAACCAAGATCTATCCCTCTACTATGAGGGGGGGGGGGGGTAA
GGAACTGC
***Triticum aestivum* cDNA clone, *Aegilops tauschii* transposons**

CTCA8 (no signal)

CTCA10 (515/336)
GATGAGTCCTGAGTAACCTGGCACAGAGAAAGAGGAAGAAACACAGAATGGCAGGGAAT
GAGAAGCATTAGAAGGTCAAAGTCCAGCAGAAAAATCTACAAACAGGGCATCCCATGCTA
CACGAGCTGCAAAAAAAGCCAGACAGAAACAAACAGCTGCCACCAAACAAGCAGAGACA
GCCCTAGTTCTTGCAACACCTTCCACAGTACTACCAGCTGCACGAGTTACTCGTGCGTCAAC
TGAGCTAGCAGCACATTCCCAAGCTCCCTTGCCACCTGACACTACTTCCCAAGCACTCTTGA
CACAAGACGAGTTGGCGATATTAGATGAACCTTGTGAGCTTCAAAAGCAAGAAGGTTGTTG
CTCGAGTCAAGTTACAGTTGCATGCTTCTTTATATGTAGTCTCACAGTTTGATGTACACTAA
CACTTCCTATGTTCGTTGTTGGACTAGCACCTAGGCGCAAGAGGAAACAAACATCAGGGAT
AATGCTCGATAGGTTACTCAGGACTCATC
Triticum aestivum* cDNA clone, *Triticum turgidum

CTCA'a-1 (353/336)

ACAAGTCATACGTTACAAGTCCTCCTATTCATATTCAGGTCAGCAATCTTATACGTTACAAG
TGAATCATCCTCCTATTCATATCATATTCAGGTCAGCAATCTGGTTCCTCTGCTTCTGCACTG
CACGTATTTTCTTGATCTGATCGAAAATGTCTTGAACACCCAATTTTCTCACTGTTATTACTT
TGGAATTTATTTATTTGGCCTGTTGCAATTACCATGTATAGAAGAAATCAGAGGAAAGCTG
CTGACACATAAGGTAACACCAGGGGATGATGATGATCTGACAGAAGAAACCAGAGGGATG
ATGACAGAGGTTACTCAGGACTCATC

No good hits for ESTs or nucleotide collection

CTCA'a-2 (352/336)

GACTGCGTACATGCAGACAGCGGCTCGGTGACTGGATCTAACACGTGGATATGGATTATGA
AGCTCTTACACACAATTTCATTAGAAAAATGAACACAGTTTTGTAGAGAGCTTTAGATTGC
CTTTTTCCTGATAAACATTGTATATTCAGGATGGGAATGGGCCGGGTCGCTTTCCCAACCCA
AATATTCGAGGCTAGTGAGTCATGTGGGTTGGTCTTCCGATCAAGATTCTTTTGGCAAGGA
GATAGCGAGACAAAGAAATATCGGCTGGCTAAATGGAGTGTGGTTTGCCGTTCCAAGGACC
AAGGTGGGTTAGGCATTCATGACCTTGAGGTTACTCAGGACTCATC

***Triticum aestivum* FGAS, some rice sequence in nucleotide**

CTCA'a-3 (vector sequence after EcoRI site was missing)(352/336)

GACTGCGTACATGCAGACAGCGGCTCGGTGACTGGATCTAACACGTGGATATGGATTATGA
AGCTCTTACACACAATTTCATTAGAAAAATGAACACAGTTTTGTAGAGAGCTTTAGATTGC
CTTTTTCCTGATAAACATTGTATATTCAGGATGGGAATGGGCCGGGTCGCTTTCCCAACCCA
AATATTCGAGGCTAGTGAGTCATGTGGGTTGGTCTTCCGATCAAGATTCTTTTGGCAAGGA
GATAGCGAGACAAAGAAGTATCGGCTGGCTAAATGGAGTGTGGTTTGCCGTTCCAAGGACC
AAGGTGGGTTAGGCATTCATGACCTTGAGGTTACTCAGGACTCATC

Same sequence as CTCA'a-2

CTCA'a-5 (350/336)

GATGAGTCCTGAGTAACCTTCCCAAGGACCGGGCGTAGCCACACTCGGTTCAACTAAAGTT
GGAGAAACTGACACCCGCCAGCCACCTATGTGCAAAGCACGTCGGTAGAACCAGTCTCGC
GTAAGCGTACGCGTAATGTCGGTCCGGGCCGTTTCATCCAACAATACCGCCGAACCAAAGT
ATGACATTGATAGCCCACAGGTGTAGGGGATCGCAACGGCTTTCGACGGTAGAGTATTCAA
CCAAAATTTATTGATTCGACACAAGGGGAGTCAAAGAATATTACTGAGTATTAGCAGTTGA
GTTGTCAATTCAACCACACCTGGATAACTTAGTGTCTGCATGTACG

cDNA from fusarium infested spike, *Triticum tugidum/Triticum aestivum*

CTCA'b-1 (352/336)

GACTGCGTACATGCAGACAGCGGCTCGGTGACTGGATCTAACACGTGGATATGGATTATGA
AGCTCTTACACACAATTTCATTAGAAAAATGAACACAGATTTGTAGAGAGCTTTAGATTGC

CTTTTTCCTGATAAACATTGTATATTCAGGATGGGAATGGGCCGGGTCGCTTTCCCAACCCA
AATATTCGAGGCTAGTGAGTCATGTGGGTTGGTCTTCCGATCAAGATTCTTTTGGCAAGGA
GATAGCGAGACAAAGAAATATCGGCTGGCTATATGGAGTGTGGTTTGCCGTTCCAAGGACC
AAGGTGGGTTAGGCATTCATGACCTTGAGGTTACTCAGGACTCATC

Same as CTCA'a-2 and CTCA'a-3

CTCA'b-4 (352/336)

GACTGCGTACATGCAGACAGCGGCTCGGTGACTGGATCTAACACGTGGATATGGATTATGA
AGCTCTTACACACAATTTCATTAGAAAAATGAACACAGTTTTGTAGAGAGCTTTAGATTGC
CTTTTTCCTGATAAACATTGTATATTCAGGATGGGAATGGGCCGGGTCGCTTTCCCAACCCA
AATATTCGAGGCTAGTGAGTCATGTGGGTTGGTCTTCCGATCAAGATTCTTTTGGCAAGGA
GATAGCGAGACAAAGAAATATCGGCTGGCTAAATGGAGTGTGGTTTGCCGTTCCAAGGGCC
AAGGTGGGTTAGGCATTCATGACCTTGAGGTTACTCAGGACTCATC

Same as CTCA'b-1

CTCA'b-7 (393/336)

GATGAGTCCTGAGTAACCTCAAGGTCATGAATGCCTAACCCACCTTGGTCCTTGGAACGGC
AAACCACACTCCATTTAGCCAGCCGATATTTCTTTGTCTCGCTATCTCCTTGCCAAAAGAAT
CTTGATCGGAAGACCAACCTACATGACTCACTAGCCTCGAATATTTGGGTTGGGAAAGCGA
CCCGGCCCATTCCCATCCTGAATATACAATGTTTATCAGGAAAAAGGCAATCTAAAGCTCT
CTACAAAACTGTGTTCATTTTTCTAATGAAATTGTGTGTAAGAGCTTCATAATCCATATCCA
CGTGTTAGATCCAGTCACCGAGCCGCTGTCTGCATGTACGCAGTCGACTGCGTACATGCAG
ACAAGAAGGTTACTCAGGACTCATC

Reverse compliment CTCA'b-1

CTCA''-3 (323/336)

CATGCAGACATGAGCAAGGTACCGTCAGCCTATTGCAAGTCAGTTCCCGAAACTGAAAACT
TAAGCTTGATCGGGGCTAGTGGCAGAGTCGTGATCAAGATAAGAAAGACATGAACTGGGA
AAGTATAGTTGTATCTCCGACTTGGGTATAACTTTAGATTTATTAGTACAACAAGAAACTTT
CTCAGGGCTGTTGCGGAGCCGTCAGCTAGATACAAAGATGCGAACCAACAAAAGACATAG

GTGTGCCCTCGACTTGGGTAGAGCTTCTGGAAGCATGAAGGAAGATCTAATATTGTGGATC
AGGTTACTCAGGACTCATC

No good hits in EST or nucleotide collection

***CTCA'''-1 (320/336)**

CGTACATGCAGACAGAGAGGGCAAATGCCATTCACAGGGGTGCCAAAGTTGACACAAATA
TTGAGGGAAGAGATATGGAGGGATCAGAGGAAGACGAAGATGAAGTATATGATTATGCAC
AAGTAGAATCAGAAGAAAGAGAGCCATTGTGAGTGGCGGTTGTGATGTTCATTATGCTTCT
ATGATAATGTGACCTAAAGAACAAGTGGCAAGGAGATTGGGTGTTTTGACATATTCGAATG
TGATCATTAGGCGGCCAGTAAGAAGATGGCTTAGTCAGCAGGTAATAAACAATAGTACGA
GGTTACTCAGGACTCATC

***Hordeum vulgare* cDNA clones, no good hits in nucleotides**

CTCA'''-2 (223/336)

GATGAGTCCTGAGTAACCTTTCGTAATAGTTGTCCCACAGCTGGCGGACGTAATATATCTA
AGTGCTACCAGCTGGCTGGTGTCTTCAACTCTTACGTGAAGGCTCCATCGATGGGCTAAGTC
ATTGAGTTTCTTAGTAAGGACCTCGATCCAAGTCTAAGTCCTGTTTTGCAGAGTCTACGTTT
ATTTGCTGTTTCATATCCTGACCTTGTCTGCATGTACG

No good hits in ESTs or nucleotides

***TCCC 2 (283/284)**

GATGAGTCCTGAGTAACTCCCAGCTGAGTCAAGTGGTTATGCAACCCAGACATAGTGAGAA
TGTGCTTATCAAGAACTTGTTGGAGACTTGATATCTCTCGACCCGGGCATGAGCTTGGAAA
ACCATTTTCAGCTCTTCGAACATCTCATATGCTCCGTGTCTCTCAAAACGCTTTTGGAGCCC
CGGCTCTAAGCTGTAAAGCATGCCGCACTGAACGAGGGAGTAGTCATCGGTACGTGCCTGC
CAAGCATTCATAACATCTTGGTCTGCATGTACGCAGTC

Triticum aestivum (2) and etiolated durum seedling, Triticum turgidum A genome HMW glutenin A gene locus sequence

***TCCC 3 (282/284)**

GATGAGTCCTGAGTAACTCTACCTGGAATGTTGGACTGGAACTTTTGTGATTGGGTGCATGT
AAAAACTATGAACACCGACCTCTTTTTGATTATGATCGGGCGCTATGAACACCACACTCTA
ATTTGCTCATATTGCTATGAATTGTGCTATTTTATAAGAATTGTTTTCAGTGCTGCGGAGGG
TCGGGCGTTTACAGGACATGATTGGATGGCCAGCTCCCGTATCAGTAGTGCGTTGACAGAT
GATGTGTTCATTTTAGTGGTCTGCATGTACGCAGTC

No good hits in EST, No good hits in nucleotide

***TCCC 4 (280/284)**

CGTACATGCAGACCGGCAGGGGCCTCCTGGGGTCCGACCAGGTGCTGTACACGGATGAGA
GGTCCCGCGGCACCGTGGACTTCTACGCGGCCAACCAGGGCACCTTCTTCTCCGACTTCGTC
ATTGCCATGACGAAGCTCGGCAGGGTTGGGGTCAAGACGGCCGCTGACGGCGAGATACGC
CGTGACTGTCAGTACCCAAACTAAGGCTAGTCGACCCGTGCAGCTGCACAGGCTAGGTGTA
TTGGAAAAAATAAATGAAGAGTTACTCAGGACTCATC

Wheat heat stressed spike and many other stresses across grass species, corn and rice sequences

TCCC 8

GACTGCGTACATGCAGACCACTAAAATGAACACATCATCTGTCAACGCACTACTGATACGG
GAGCTGGCCATCCAATCATGTCCTGTAAACGCCCGACCCTCCGCAGCACTGAAAACAATTC
TTATAAAATAGCACAATTCATAGCAATATGAGCAAATTAGAGTGTGGTGTTCATAGCGCCC
GATCATAATCAAAAAGAGGTCGGTGTTCATAGTTTTTACATGCACCCAACCACAAAAGTTC
CAGTCCAACATTCCAGGTAGAGTTACTCAGGACTCATC

Reverse compliment of TCCC3

TCCC 10 (end restriction site was funny, may not be right) **(218/284)**

GATGAGTCCTGAGTAACTCATTACTTGTGGGAGAAAAGAGCTAGACACGAAACATGCCTTC
TATCTCGATCTGTATGTTTGTGCAACGAAATTTCTCTGCACACGACAAGTTGGAAGACGACC
TTGTCATCGATGGCTACAGCCCACATGAACTGAGGTGATCGATGGCTGTTGTATCTTGTCAT
CCAGAGATTGGGCACACGTCGTTTGTGCAGCAGTTCTCTTGTGCAATCACCACAAAAGCAC
GCCACCACGAGTTGG

No good hits in EST, No good hits in nucleotide

TCTA 2 (257/185)

GATGAGTCCTGAGTAACTCTCAGCGCCATAGTATCCTTCCCACACTATCTGCATGTACGCAG
TCATGATGAGTCCTGAGTAACTCACCCTGCTGATAACCCACAAGTATAGGGGACCGCAACA
GCTTTCGAGGGTAGAGTATTCAACCCAAATTTATTGATTCAACACAAGGGGAGCCAAAGAA
TATTCTTGAGTATTAGCAATTGAGTTGTCAATTCAACCCCACATGGATAACTTAGTATCTGC
ATGTACGCAGT

Triticum aestivum **(many) one is fusarium infected spike, retrotransposon**

TCTA' -2 (Vector sequence was missing after first restriction site) **(135/185)**

GACTGCGTACATGCAGATAGAACCATAAGAAGAGGACTGGTCCTCTGATCATTGTAGGACT
TCAACGCCCATTTCGGAATTAGGACACACATGCTATGTTTTGTTACCAGCCGGTGGAGTTAC
TCAGGACTCATC

No good hits in ESTs or nucleotide

TCTA'-4 (Vector was missing after first restriction site) **(135/185)**

GACTGCGTACATGCAGATAAAACCATAAGAAGAGGACTGGTCCTCTGATCATTGTAGGACT
TCAACGCCCATTTCGGAATTAGGACACACATGCTATGTTTTGTTACCAGCTGGTGGAGTTAC
TCAGGACTCATC

Only one base substitution from TCTA'-2

TCTA'-7 (Vector was missing after first restriction site) **(135/185)**

GACTGCGTACATGCAGATAGAACCATAAGAAGAGGACTGGTCCTCTGATCATTGTAGGACT
TCAACGCCCATTTCGGAATTAGGACACACATGCTATGTTTTGTTACCAGCTGGTGGAGTTAC
TCAGGACTCATC

Same sequence as TCTA'-2

TCTAa''-1 (Second restriction site missing) **(163/185)**

GCACGTGCACGTGCAAGTCCAATTCCAACCCACTGGCGGCCGTTGCTGGTGGATCCGGGCT
CGGTACCGAGCTTGGCGTAGTCATGGTCATAGCTGGTTCCTGTGTGAAATTGTTATCCGATC
ACAAATCCACACAAAATACGAGCCGGAAGCATAAAGTGTA

Pooled ***Arabidopsis thaliana*** **cDNA, cloning vector sequences**

***TCTAa''-2 (190/185)**

ACTGCGTACATGCAGATAATCCCAGAAGAAGTTGTGCGGCGTTTGAAAGGTGAGATCCCAG
GAGAGATCAAGCTAGAAACCCGAAATGGTTACAGTCATACTATTGTGGTTGCCAAGAACCA
AGAAAAGTGTTAGAGTACGTAATGGGCCTAATGGACCCCGTTAGTCTTAGAGTTACTCAGG
ACTCATC

***Brachypodium distachyon* cDNA clone, no good hits in nucleotides**

TCTAb''-4 (Vector sequence missing after first restriction site) **(172/185)**

GATGAGTCCTGAGTAACTCTATTCCTTTACTCTATACAGGTGCTTTCAGCAACATCCTATAC
AACTTAGAATAAACTCTCCTAATCGGTGCTTTTCAAGCTCCCTTTTTTGGCCAGAAAGTGCT
TTTCATGTTACAGTAATTTATTTCTTCAGATCATTATCTGCATGTACG

No good hits in ESTs or nucleotides

TCTAb''-5 (174/185)

GACTGCGTACATGCAGATAATCCCGGGGTGAATCTGGAGGGCGGAAGAGATGATGACTCG
CATCTCAAAGGGCAGCTAAAGGCTGGCGAGCGCATTCTCTCGCAAGTTCGGCAGCATAAGA
ACAGTCTTCAAGATGCCATGTCCGGCTGGACGTAGAGTTACTCAGGACTCATC

No good hits in EST or nucleotides

TCTC 2 (264/413)

GATGAGTCCTGAGTAACTCTACCTGGAATGTTGGACTGGAACTTTTGTGATTGGGTGCATGT
AAAAACTATGAACACCGACCTCTTTTTGATTATGATCGGGCGCTATGAACACTACACTCTA
ATTTGCTCATATTGCTATGAATTGTGCTATTTTATAAGAATTGTTTTCAGTGCTGCGGAGGG
TCGGGCGTTTACAGGACATGATTGGATGGCCAGCTCCCGTATCAGTAGTGCGTTGACAGAT
GATGTGTTCATTTTAGTG

No good hits in EST, No good hits in nucleotide

TCTC 5 (271/413)

CTGCGTACATGCAGATCAGAATAAAATAAGATTATAAACACGTGCCATCACAGCCACGTAA
AGCTACCTTGATGAGTGATGGCCCATTGTGGTTTTGGGTGGCTCAATCAATTGGCCATGGCC

CCTTTGTGGTGAACCGTGAACGTGATTTCACAGCGACAGTGGAGAGAGAGTAGGGTGTCGC
AGCCTCGATCGCGGAGGTGACAACGTGGCCGTCCAAAGCGACATCCACCTGCCGCGAGTA
ATACCTCCGAGTTACTCAGGACTCATC

No good hits in EST, No good hits in nucleotide

TCTC'-1 (343'413)

GATGAGTCCTGAGTAACTCAATACATTTTTTCCTATGCCTAATAAGAAATAAACACAGGTA
TGAGGACAGTTGTAGTATTTGAAGAAGCCCAAGCAGTACTTCACACAAGAGCTATGAGTCC
TTGTGTGTGACAGAAACGCATCCATAAATACTCTTCTTCGCTCTTTGTTGGGACTATTAGTC
GTTGGCATACTTCTGCGGAAGTGCTACACTATCATGTTTGATCTGCATGTACGCAGTCCATG
CAGATCTCTGGAGTAAGCAAGAATAGCTGACTGTTGTTTCTTATTTTTCTTCATAATTTGTTT
CTTATTTTTCTTTAGGAGTTACTCAGGACTCATC

No good hits is ESTs or nucleotide collection

TCTC''-2 (277/413)

GATGAGTCCTGAGTAACTCAAGTATCCCTCTTCTAACAACATTTTGGGCATCAATATGAGAT
CAAATGAAAATGATGCAATGGAATGAAATGATGTACTCTCTGAGATGAACATCTTGATATA
CTATATGCTCAAATCGGAGTTACGGATGCAAAGTTGTGATGCGATGAACATGGCAACTTGG
ATCTGCATGTACGCAGTCGACTGCGTACATGCAGATCCCCGATGGAGACCTCGAGGAGACG
GGCATGTTCCAAGGA
GTTACTCAGGACTCATC

Etiolated durum seedling cDNA, triticum BAC clones

TCTC''-3 (vector sequence wrong before 2nd EcoRI site) **(202/413)**

GATGAGTCCTGAGTAACTCAAGTAGCCCTCTTCTAACAGCATTTCGGGCATCAAGATGAGC
TCAAATGAAAATGATGCAATGGAATGAAATGATGTACTCTCTGAGTTGAACATTTTGATAT
ATTATGTGCCCAAATCGGAGCTACGGAAGCAAAGTTACGGAGCCACAAATAGAGGCATTT
GGATCTGCATGTACGCAGTC

Like TCTC''-2, but shorter

TCTC''-6 (236/413)

GATGAGTCCTGAGTAACTCTCAGCATCAAAGTATTCCTCCCCCCAGACTCTCTGCAGGTTTT
CATGTACCAATCATGTAATCTTCGCATCATCGTTGTTAGAGATCTTTCATCGTTGACGAGAG
GCTTCCCGTAATGGTATTTGTGTTCGTCCACCTCCAAGATTTCATAATGTACATCATCGGGC
AGGTAATCTCCAAGATTGCTACAACCGGGAACCATCCTCGGATCTGCATG

***Triticum aestivum* cDNA clones, *Aegilops* and *Triticum turgidum* sequences**

TCTC'''a-1 (556/413)

GATGAGTCCTGAGTAACTCCTATCCAGTTTTTATGCGTGTAAGTTGTTGTGCTTCACCTATTC
ATGGGTGTGGAAAGTGTTTTTTGTCAAGATGCGTAGCTTGTTCAATTTGGCTCAATATAGCT
AGAATTTGCAGGATGAAACCTGACAAAATCTGGCCGAAGCTGAACATTCATGCCAGTTATA
TTAGTGAAACATGCCAATGTTCATGGGTTGCGACATGCCACGGGTTCAGTTCTGTGGTATGT
GCATGTTCCCTCATTCAGAGCTGCAAAGATGCAGTCTAGTTGTATTTTGCATGGAAGAAATC
TGGCGTCATTGTATATTGAGCCTACCGGTGATCGCCGATAGAGATTGAGCTGTTTTCATTGA
GGATCTGAAAATGTATAGACCAGCATCTTCAACTGCAAGGGTGTTAGAAAAACAAGAAAA
CAAGACGATTCAGGAAGCTACTAGGCTGAGAGTGGTGGAGAGTAACAGAAAAAGTAATGA
AGTTGCACACATTCTAGTTTAGTAGAGGTCTTGGAGTGTGGTGTGCAGCAGATCTGCATGC
ACG

No good hits in ESTs, or nucleotides

TCTC'''a-2 (556/413)

GATGAGTCCTGAGTAACTCCTATCCAGTTTTTTTGCGTGTAAGTTGTTGTGCTTCACCTATTC
ATGGGTGTGGAAAGTGTTTTTTGTCAAGATGCGTAGCTTGTTCAATTTGGCTCAATATAGCT
AGAATTTGCAGGATGAAACCTGACAAAATCTGGCCGAAGCTGAACATTCATGCCAGTTATA
TTAGTGAAACATGCCAATGTTCATGGGTTGCGACATGCCACGGGTTCAGTTCTGTGGTATGT
GCATGTTCCCTCATTCAGAGCTGCAAAGATGCAGTCTAGTTGTATTTTGCATGGAAGAAGTC
TGGCGTCATTGTATATTGAGCCTACCAGTGATCGCCGATAGAGATTGAGCTGTTTTTATTGA
GGATCTGAAAATGTATAGACCAGCATCTTCAACTGCAAGGGTGTTAGAAAAACAAGAAAA
CAAGACGATTCAGGAAGCTACTAGGCTAAGAGTGGTGGAGAGTAACAGAAAAAGTAATGA
AGTTGCACACATTCTAGTTTAGTAGAGGTCTTGGAGTGCGGTGTGCAGCAGATCTGCATGC
ACG

Same as TCTC'''a-1

TCTC’’’a-4 (551/413)

GATGAGTCCTGAGTAACTCCTATCCAGTTTTTTTGCGTGTAAGTTGTTGTGCTTCACCTATTC
ATGGGTGTGGAAAGTGTTTTTTGTCAAGATGCGTAGCTTGTTCAATTTGGCTCAATATAGCT
AGAATTTGCAGGATGAAACCTGACAAAATCTGGCCGAAGCTGAACATTCATGCCAGTTATA
TTAGTGAAACATGCCAATGTTCATGGGTTGCGACATGCCACGGGTTCAGTTCTGTGGTATGT
GCATGTTCCCTCATTCAGAGCTGCAAAGATGCAGTCTAGTTGTATTTCGCATGGAAGAAAT
CTGGCGTCATTGTATATTGAGCCTACCGGTGATCGCCGATAGAGATTGAGCTGTTTTTATTG
AGGATCTGAAAATGTATAGACCAGCATCTTCAACTGCAAGGGTGTTAGAAAAACAAGAAA
ACAAGACGATTCAGGAAGCTACTAGGCTAAGAGTGGTGGAGAGTAACAGAAAAAGTAATG
AAGTTGCACACATTCTAGTTTAGTAGAGGTCTTGGAGTGTGGTGTGCAGCAGATCTGCAT

Same as TCTC’’’a-1, just a little shorter

TCTC’’’a-5 (Vector sequence missing before second restriction site) **(561/413)**

GATGAGTCCTGAGTAACTCCTATCCAGTTTTTTTGCGTGTAAGTTGTTGTGCTTCACCTATTC
ATGGGTGTGGAAAGTGTTTTTTGTCAAGATGCGTAGCTTGTTCAATTTGGCTCAATATAGCT
AGAATTTGCAGGATGAAACCTGACAAAATCTGGCCGAAGCTGAACATTCATGCCAGTTAAA
TTAGTGAAACATGCCAATGTTCATGGGTTGCGACATGCCACGGGTTCAGTTCTGTGGTATGT
GCATGTTCCCTCATTCAGAGCTGCAAAGATGCAGTCTAGTTGTATTTTGCATGGAAGAAATC
TGGCGTCATTGTATATTGAGCCTACCGGTGATCGCCGATAGAGATTGAGCTGTTTTTATTGA
GGATCTGAAAATGTATAGACCAGCATCTTCAACTGCAAGGGTGTTAGAAAAACAAGAAAA
CAAGACGATTCAGGAAGCTACTAGGCTAAGAGTGGTGGAGAGTAACAGAAAAAGTAATGA
AGTTGCACACATTCTAGTTTAGTAGAGGTCTTGGAGTGTGGTGTGCAGCAGATCTGCATGT
ACGCAGTC

Same as TCTC’’’a-1, but longer

TCTC’’’a-6 (556/413)

GATGAGTCCTGAGTAACTCCTATCAAGTTTTTTTGCGTGTAAGTTGTTGTGCTTCACCTACTC
ATGGGTGTGGAAAGTGTTTTTTGTCAAGATGCGTAGCTTGTTCAATTTGGCTCAATATAGCT
AGAATTTGCAGGATGAAACCTGACAAAATCTGGCCGAAGCTGAACATTCATGCCAGTTATA
TTAGTGAAACATGCCAATGTTCATGGGTTGTGACATGCCACGGGTTCAGTTCTGTGGTATGT

GCATGTTCCCTCATTCAGAGCTGCAAAGATGCAGTCTAGTTGTATTTTGCATGGAAGAAATC
TGGCGTCATTGTATATTGAGCCTACCGGTGATCGCCGATAGAGATTGAGCTGTCTTTATTGA
GGATCTGAAAATGTATAGACCAGCATCTTCAACTGCAAGGGTGTTAGAAAAACAAGAAAA
CAAGACGATTCAGGAAGCTACTAGGCTAAGAGTGGTGGAGAGTAACAGAAAAAGTAATGA
AGTTGCACACATTCTAGTTTAGTAGAGGTCTTGGAGTGTGGTGTGCAGCAGATCTGCATGC
ACG

Same as TCTC'''a-1

TCTC'''b-1 (559/413)

CTGCGTACATGCAGATCTGCTGCACACCACACTCCAAGACCTCTACTAAACTAGAATGTGT
GCAACTTCATTACTTTTTCTGTTACTCTCCACCACTCTTAGCCTAGTAGCTTCCTGAATCGTC
TTGTTTTCTTGTTTTTCTAACACCCTTGCAGTTGAAGATGCTGGTCTATACATTTTCAGATCC
TCAATAAAAACAGCTCAATCTCTATCGGCGATCACCGGTAGGCTCAATATACAATGACGCC
AGATTTCTTCCATGCAAAATACAACTAGACTGCATCTTTGCAGCTCCGAATGAGGGAACAT
GCACATACCACAGAACTGAACCCGTGGCATGTCGCAACCCATGAACATTGGCATGTTTCAC
TAATATAACTGGCATGAATGTTCAGCTTCGGCCAGATTTTGTCAGGTTTCATCCTGCAAATT
CTAGCTATATTGAGCCAAATTGAACAAGCTACGCATCTTGACAAAAAACACTTTCCACACC
CATGAATAGGTGAAGCACAACAACTTACACGCAAAAAAACTGGATAGGAGTTACTCAGGA
CTCATC

No good hits in ESTs, or nucleotides

TCTC'''b-2 (575/413)

GATGAGTCCTGAGTAACTCCTATCCAGTTTTTTTGCGTGTAAGTTGTTGTGCTTCACCTATTC
ATGGGTGTGGAAAGTGTTTTTTGTCAAGATGCGTAGCTTGTTCAATTTGGCTCAATATAGCT
AGAATTTGCAGGATGAAACCTGACAAAATCTGGCCGAAGCTGAACATTCGTGCCAGTTATA
TTAGTGAAACATGCCAATGTTCATGGGTTGCGACATGCCACGGGTTCAGTTCTGTGGTATGT
GCATGTTCCCTCATTCAGAGCTGCAAAGATGCAGTCTAGTTGTATTTTGCATGGAAGAAATC
TGGCGTCATTGTATATTGAGCCTACCGGTGATCGCCGATAGAGATTGAGCTGTTTTTATTGA
GGATCTGAAAATGTATAGACCAGCATCTTCAACTGCAAGGGTGTTAGAAAAACAAGAAAA
CAAGACGATTCAGGAAGCTACTAGGCTAAGAGTGGTGGAGAGTAACAGAAAAAGTAATGA

AGTTGCACACATTCTAGTTTAGTAGAGGTCTTGGAGTGTGGTGTGCAGCAGATCTGCATGT
ACGCAGTCGATCTGCATGTACG

Same as TCTC’’’a-1

TCTC’’’b-3 (Vector sequence missing after first restriction site) **(561/413)**

GACTGCGTACATGCAGATCTGCTGCACACCACACTCCAAGACCTCTACTAAACTAGAATGT
GTGCAACTTCATTACTTTTTCTGTTACTCTCCACCACTCTTAGCCTAGTAGCTTCCTGAATCG
CCTTGTTTTCTTGTTTTTCTAACACCCTTGCAGTTGAAGATGCTGGTCTATACATTTTCAGAT
CCTCAATAAAAACAGCTCAATCTCTATCGGCGATCACCGGTAGGCTCAATATACAATGACG
CCAGATTTCTTTCATGCAAAATACAACTAGACTGCATCTTTGCAGCTCTGAATGAGGGAAC
ATGCACATACCACAGAACTGAACCCGTGGCATGTCGCAGCCCATGAACATTGGCATGTTTC
ACTAATATAACTGGCATGAATGTTCAGCTTCGGCCAGATTTTGTCAGGTTTCA
TCCTGCAAATTCTAGCTATATTGAGCCAAATTGAACAAGCTACGCATCTTGACAAAAAACA
CTTTCCACACCCATGAATAGGTGAAGCACAACAACTTACACGCAAAAAAACTGGATAGGA
GTTACTCAGGACTCATC

Same as TCTC’’’b-1

TCTCa’’’’-2 (510/413)

GCACGTGCACGTGACTGCGTACATGCAGATCCGATCGGTGGTATTGAATTTGGGGTGTTGA
TTTTGCAGGTCAATTGTTGATTGGGATATAGTACATGGATTTGTTCAGTTGCATTTTTTATAC
ATGGAAAAGTCTGCTTCCCGGTTGTGAAGCTATTCATTGCGGAGGGGTGGAGCCCGCTCGC
GATTTGTACACGTTAGTTGTTTTTTTGTCTGTCTTGTTCTTCAGGAAGTTGTGTCTTGTTACT
GTATTAGATCTGCATCTTTCTGTAAGCTTAGTAATATATACATACTATTTTGAGGATGGATT
GGTGCACCCTCTATTTGTATTGCATTGCCGCGGTACCTTGTCTGCATGACTGTTTTGGGCAT
AATTATATTGTGATTACATACACCATCTAGAATTTCTTGCACCGGAAACAAAGGGTTTTCCG
CAGCTAAATTACTAGTGTGTCAGGGGAACCTGAGATCGTGTCTGAGGGAGTTGTGGAGAGT
TACTCAGGACTCATC

Hordeum vulgare **cDNA, no good hits in nucleotides**

TCTCa’’’’-3 (Vector sequence missing after first restriction site) **(498/413)**

GACTGCGTACATGCAGATCCGATCGGTGGTATTGAATTTGGGGTGTTGATTTTGCAGGTCA
ATTGTTGATTGGGATATAGTACATGGATTTGTTCAGTTGCATTTTTTATACATGGAAAAGTC
TGCTTCCCGGTTGTGAAGCTATTCATTGCGGAGGGGTGGAGCCCGCTCGCGATTTGTACATG
TTAGTTGTTTTTTTGTCTGTCTTGTTCTTCAGGAAGTTGTGTCTTGTTACTGTATTAGATCTGC
ATCTTTCTGTAAGCTTAGTAATATATACATACTATTTTGAGGATGGATTGGTGCACCCTCTA
TTTGTATTGCATTGCCGCGGTACCTTGTCTGCATGACTGTTTTGGGCATAATTATATTGTGAT
TACATACACCATCTAGAATTTCTTGCACCGGAAACAAAGGGTTTTCCGCAGCTAAATTACT
AGTGTGTCAGGGGAACCTGAGATTGTGTCTGAGGGAGTTGTGGAGAGTTACTCAGGACTCA
TC

Like TCTCa’’’’-2, but a little shorter

TCTCa’’’’-4 (Vector sequence missing after first restriction site) **(498/413)**

GACTGCGTACATGCAGATCCGATCGGTGGTAATGAATTTGGGGTGTTGATTTTGCAGGTCA
ATTGTTGATTGGGATATAGTACATGGATTTGTTCAGTTGCATTTTTTATACGTGGAAAAGTC
TGCTTCCCGGTTGTGAAGCTATTCATTGCGGAGGGGTGGAGCCCGCTCGCGATTTGTACATG
TTAGTTGTTTTTTTGTCTGTCTTGTTCTTCAGGAAGTTGTGTCTTGTTACTGTATTAGATCTGC
ATCTTTCTGTAAGCTTAGTAATATATACATACTATTTTGAGGATGGATTGGTGCACCCTCTA
TTTGTATTGCATTGCCGCGGTACCTTGTCTGCATGACTGTTTTGGGCATAATTATATTGTGAT
TACATACACCATCTAGAATTTCTTGCACCGGAAACAAAGGGTTTTCCGCAGCTAAATTACT
AGTGTGTCAGGGGGACCTGAGATTGTGTCTGAGGGAGTTGTGGAGAGTTACTCAGGACTCA
TC

Same as TCTCa’’’’-3

TCTCa’’’’-5 (484/413)

GATGAGTCCTGAGTAACTCCTAGCTGAGACAAGCGGTTGTGTAACCTAGACATTTTGAGCA
TATGCTCGCTGACAGAACTGTTTTCCTCCATCTTACAATTGTAGAATTTGTCGGAGACTTCA
TATCTCTCGACCCGGGCATGAGCTTGGAAAACCATTTCCAGCTCTTGGAACATCTCATATGC
TCCGTGTTGCACAAAATGCTTTGGAGCCCCGATTCTAAGCTATAAAGCATGTCACACTGAA
CCAGGGAGTAATCATCACTACGCCACTGCCAGGCGTTCATAACGTCTTGAGTTGCTGGGAA
AATGGGTGCGTCACCTAGCGGTGCTTCATGGACATATGCTTTCTTGGCAGCTATGAGGATA

ATCCTCAAGTTACGGACCCAATCCTTATAGTTGCTACCATCGTCTTTCAGCTTGGTTTTCTCT
AGGAACGCATTGAAATTGAGGGCAACATTAGCATGGGCCATTGGATCTGCATG

Durum etiolated seedling cDNA, *Triticum* (many species)

TCTCb’’’’-1 (447/413)

GATGAGTCCTGAGTAACTCTAGAATACTTATGGAACTCTAACAATCCTACCATAATAGGAA
GTCCTGGGAAATTTGTATATTGATCTGCATGTACGCAGGACTGCGTACATGCAGATCGAAC
CTCAACGGAAATATGGCGCGAGGTGCCACGCACTTGTCTGATAGGTTGCGCGAGTAATTCG
GCCATCAAGGCGGCCCTGAATGGAAAAACCTTCAACACGAAAAAGTTTTGTCTCGTCGAGC
CGATTGTTTTTCATATAAAATTTGTCTCAATTCGAGATCGTATGCAACCTGGAGAGGCAAAT
CAAGATCAGGCTGTGTTTTCAGTCGGGAAATCCGAGTGAAAAGTTTACCATCCGGGTGAAA
ACCTACCAATCGAGTAAAAGTTCGAGTGAAAACATATCGATCGAGTAAAAGATTGGCTTCC
GAGTTACTCAGGACTCATC

***Triticum aestivum* cDNA, *Hordeum vulgare* putative proteins**

Table B.1 Sequences and annealing temperatures for designed primers

	Forward Primer	Reverse Primer	An. Temp
tccc2	TGC TCC GTG TCT CTC AAA AC	TGT TAT GAA TGC TTG GCA GG	55
tccc3	CCT GGA ATG TTG GAC TGG ACC	TGT CAA CGC ACT ACT GAT ACG G	55
tccc4	CAA CCA GGG CAC CTT CTT C	ACA GTC ACG GCG TAT CTC G	60
cgta2	GCC ACT AGA CAA CTG TGT AG	TCA TTT TGA TGG CAA TCC	50
cgta3	CCG GCA AAA GTC CTT CTA G	AAA TGA CAT AAA TGG TGG	50
ctca1	ATT CAC AGG GGT GCC AAA G	ACA TCA CAA CCG CCA CTC AC	60
tcta2	ATC CCA GAA GAA GTT GTG	TAA GAC TAA CGG GGT CCA TTA	55
cccc3	CGT CCA CTT GAT GGG ATG AC	AAT GAA GCA ACC AAC CGT TC	60
		AAA TAC GGA CAA CTG ACC CC	60

Appendix C - EST Primer Sequences

All oligos were synthesized by IDT, Coralville, IA.

Primers from ESTs in Rice Chromosome 12 Group

	Forward	Reverse	An. Temp
BF146083	CTT CCT GTC TTC CCC TCT TTC	TTC CTC TGG TGA ACA ATG TGA C	60
BF202632	ACA TTG CCA GAA GTC GTT ACC	ATC CCA AAG TGA TGA CAG GTC	60
BE405614	ACA CAT CTT GTA GGA GTT TCG C	ACC ACT TCA GAG GCA ACA GTA G	60
BF484701	AAG TGA GAG AAT CGT TGC TGG	ATC TTT ACC CCT TAC ACC ATC C	60
BF292081	CGA TTT GTT TGG CGA CAG G	GCA GGA TGT TGC TGA ACT CG	60
BE495282	AGG CAA TCA GTG CTA CTT TGG	CTC CTG TTT CTG CTG CTA TCA C	60

Primers from ESTs with Disease Resistance Sequence Hits

	Forward	Reverse	An. Temp
BE498930	TGG TTC CTG GTT GAG GTT TC	CCA ATC CTT GAG TGC TCT GAG	60
BE500856	GTG TCG CTC ATT GGC TAC TG	GAG GAT GTT GGT GGA CTT GAC	60

Primers from other ESTs

	Forward	Reverse	An. Temp
BG607386	GCC TTC TCC TCC TAC TAC TAT TAC C	ATC TTG CTG TGT CTG TCT TTC C	60
BG263391	ATG CCA TCA AAC ATC GCC	GCT CAA GAA ACT TAC GAG ACT GG	60
BG312607	TTG AAC AAA GGA CAC CTA CAG C	CCA TTG ACC TGA ACA GTG GC	60
BG314248	TGA TTG GTG AGG ATG TCG G	TTG GTT GTA GGC AAG GAG AAG	55
BI479113	AAA TCG GCA AGC AAG GAG G	TGA TGG CGA AAT GGA CGA C	60
BE403373a	AAG AAC AGC AAG GTT GGG TC	TGG GTT GGA TGT GAC AAA TC	60
BE403471	GGA ACC AGT GGA AGG AGA GG	CGT GCC AGA CAC AGG ACT TG	60
BE405630	ACG GAC TGG AAA GAT AGA TGC	GAG GAA AGG AAC GAT GAA GG	55
BQ170480	TGC TTC CTG GAG TAG AAA CAT C	AAC AGA ATG ACG CTA TGG GAG	55
BF145493	CGA CAA GGA GTG CTT TTG C	TTG AGG TAA ATG GCA TCG G	50
BE496036	GGA GAT AAG GAG AAG AAG AAA CGG	CGG AAA GAG AAG AGA GAA AGC G	60
BG274989	TCA CTC ACC TCA CCA TTC CAC	GCT GAC CAT CAT CAA TCG C	50
BE494514	CCA ATG AGT CCT GCT GAA GG	CCT CTT GAA GTT GCC AAA TGT C	55
BQ166799	ATT TCC TTT GAC AGC AGC G	GTA GAA TGC CAT CAC TTG CG	55
CD454535	TGG TTT GGA GCA GCA ACT G	GAT TAT GTT CTG GAT TGG AGG C	60
BE499458	CCC AAT CGG AAG CAA AAC	TCA AGA ATA GGA AGC AAG CG	55
BF200555	TGG TGG AAA GGT TAG TAT GGC	CCA GCA ACA ATA GAT GGA CAA C	55
BF202010	AGG ATG CTG GTT GTT GTG G	CGC TGT AGC CCT TCT TCA G	60
BE403373b	AAG TCA CCT GTG CGA GTA AAT G	CCC AAC CTT GCT GTT CTT AGT ATC	60
BF496903	GGT TCA TTG GTC TCT TCC TCT C	ATT CAC CTG GCA ACT GCT G	60
BM134523	CCA GCC TTC CTT ACT GTG AAA G	ATC TCT CAT CGT CGG TAG CG	60
BI479830	GAA GAT GTC AGT CTC GTC ACC TC	TTC ACA CTC ACC ACT CCA GG	60