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Quantitative Trait Loci Associated with Waterlogging Tolerance in a Soft Red Winter Wheat Mapping Population

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QUANTITATIVE TRAIT LOCI ASSOCIATED WITH WATERLOGGING TOLERANCE IN A SOFT RED WINTER WHEAT MAPPING POPULATION

Quantitative Trait Loci Associated with Waterlogging Tolerance in a Soft Red Winter Wheat Mapping Population

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Abstract

Waterlogging is caused when water stays superficially in the soil for an extended period of time, creating an anaerobic environment which decreases plant growth and grain yield at maturity. Despite the impact of waterlogging on wheat production both globally and in the southeastern U.S., very little is known about the genetic control of waterlogging tolerance in wheat. The objective of this study was to determine the amount of genetic variation for vegetative stage waterlogging tolerance present within a wheat recombinant inbred line (RIL) population and to identify quantitative trait loci (QTL) associated with tolerance and productivity. Experiments were carried out in both the greenhouse and the field using a RIL population derived from the wheat cultivars 'USG3209' and 'Jaypee'. The effect of vegetative stage waterlogging was determined by quantifying fresh shoot biomass, fresh root biomass, dry shoot biomass, dry root biomass, root length, chlorophyll content, tiller number, elongation, and plant height pre and post-treatment under stressed and non-stressed conditions.

In both the greenhouse and the field experiments, biomass traits, chlorophyll content ere significantly reduced by waterlogging stress with percent reduction ranging from 10 to 54%. Significant genetic variation was detected for biomass traits, chlorophyll content, tiller number, elongation, and plant height post-treatment. Root traits showed high sensitivity to waterlogging and significant genotype by treatment interaction. Shoot biomass and root biomass were highly correlated, indicating the possibility of indirect selection for root biomass. The QTL analysis revealed 53 total QTLs, with 34 detected under waterlogging stress. These QTLs clustered into 19 regions distributed throughout the wheat genome. QTL on chromosomes 2A, 2B, 2D, 5A and 5B were found to localize with known genes regulating plant height and flowering time. Other QTL regions located on chromosomes 1B, 2A and 6B appear to be novel for biomass production

specific to waterlogging stress and can be used for marker-assisted selection to more efficiently select for waterlogging tolerant lines.

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Dedication

To

God, my parents, sister and

Art

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Chapter I
Introduction and Literature Review

Waterlogging reduces wheat yield and biomass

Waterlogging is caused when water from either rain or irrigation stays superficially in the soil or subsoil for an extended period of time (Lee et al. 2007a). Globally, some 10% of arable soil is subjected to waterlogging with yield losses ranging from 15% to 80% (Shabala 2011). Waterlogging is a common problem in the United States, affecting approximately 16% of cultivated land, which is second only to drought in terms of impact on crop yield (Paux et al. 2008; Setter and Waters 2003; Shabala 2011). Every year 10 to 15 million ha cultivated with wheat are subjected to waterlogging globally (Boru et al. 2001; Lee et al. 2007a), corresponding to 15% to 20% of the 70 million ha of global wheat producing land (Lee et al. 2007a). In Arkansas, where moderate waterlogging during stand establishment occurs on a yearly basis, yield losses up to 30% have been observed (Mason 2011).

Waterlogging stress inhibits leaf and root growth, promoting seed dormancy, leaf dormancy, and leaf chlorosis (Lee et al. 2007a).Waterlogging stress can also be expressed in a decrease in the number of tillers, number of kernels, and reduction of root/shoot weight. Cumulatively, these effects are reflected in a loss of yield at maturity. In a study by Collaku and Harrison (2002) the average yield of 15 genotypes was reduced by 44% under waterlogging stress due to a decrease in the number of tillers by 41% and kernels per tiller by 20%. If wheat plants are subject to waterlogging for as little as six days, yield can be reduced by 47% in sodic soils (Sharma and Swarup 1988).

A reduction in the root/shoot weight, inhibition of the apical root and the emergence of adventitious roots are characteristic symptoms of waterlogging stress. The inhibition of roots limits the plants ability to uptake nutrients and to have normal development, resulting in detrimental effects on the biomass produced by the plant. Two wheat cultivars, ‘Gamenya’ and

'Kite' showed a shoot weight decrease of 63% and 82%, respectively under waterlogging conditions in comparison to well-drained controls (Thomson et al. 1992). Malik et al. (2001) showed the root mass of three week old wheat seedlings to be reduced 2.7 fold after fourteen days of waterlogging. In the same experiment, shoot growth was reduced by approximately 6% to 27% and root growth reduced by 15% to 74%. It was also shown that plants subjected to waterlogging developed 1.5 times more adventitious roots per stem, but produced fewer tillers per plant, reducing tiller number by 24% to 62%.

Wheat is most vulnerable to waterlogging at the early phenological stages. Cannell et al. (1980) found that all wheat plants between the phenological stages of germination and emergence subjected to waterlogging for sixteen days died. Shorter periods of waterlogging, such as 6 days, decreased the wheat population to 12% in clay soils and 38% in sandy loam soils, with yield reductions of 82% observed (Cannell et al. 1980). Meyer and Barrs (1988) found wheat to be susceptible to waterlogging at the phenological stages of stem elongation and flag leaf emergence when compared to control plants. In this study, waterlogging significantly impacted the growth rate of the stem and flag leaves. Wheat plants subjected to waterlogging for 44 days at 93 days after sowing and 58 days at 64 days after sowing had grain yield reductions of 20 and 24%, respectively (Dickin and Wright 2008). Evidence also suggests that the plant can overcome waterlogging stress more easily when it occurs in later stages (Meyer and Barrs 1988).

Effect of waterlogging on the soil environment

Waterlogging reduces the diffusion rate of dissolved oxygen transported through the pore space of the soil around the roots (Christianson et al. 2010; Lee et al. 2007b). The proportion of gases that are diffused in water is 10,000 times lower than the gases that diffuse in air. When water covers the soil, the internal movement of atmospheric oxygen is restricted an average of 320,000

times (Armstrong 1979; Barrett-Lennard 2003; Colmer and Greenway 2011; Lee et al. 2007b). The oxygen reduction rate in waterlogged soil depends on the microorganisms and plant roots present and their metabolic activity. The-air filled porosity becomes low when it is 10% or less and it can affect the normal activity of the plants and microorganisms (Grable 1966; Setter and Waters 2003). The amount of oxygen drops dramatically in waterlogged soils with high temperatures and with a significant quantity of organic matter (Colmer and Greenway 2011; Drew 1992).

Waterlogging periods promote the reduction of oxygen in the soil layers (Setter and Waters 2003), exposing plants to an anoxic environment. Anoxic conditions are generated when microbes and plants consume all the oxygen present in the soil and there is no exchange of gases between the atmosphere and the soil to replace the oxygen used by them (Bailey-Serres and Voesenek 2008; Malik et al. 2001). An anoxic environment in waterlogged soil hampers plant growth by changing the soil's physical, chemical and biological properties (Malik et al. 2001). Quantification of the intensity of waterlogging is related to the chemical process of oxido-reduction of chemical elements in the soil (Setter and Waters 2003). Acid soil as a result of waterlogging hampers the adequate development of wheat by promoting deficiencies of essential nutrients such as Ca, Mg, Mo and decreasing the amount of P available to the plant (Carver and Ownby 1995). Waterlogged soil generally contains greater amounts of manganous and ferrous ions, which can be toxic for the plant and affect the uptake of some nutrients, as well as smaller proportions of nitrates and sulfates (Malik et al. 2001; Ponnamperuma et al. 1984). Although wheat and barley plants did not present toxicity by Fe and Mn in anaerobic conditions, they presented deficiencies in P, K, N, Cu, Zn, and Mn (Steffens et al. 2005). Submerged plants may

also contain a significant amount of some secondary products that can be toxic to the plant such as ethylene, carboxylic acid, and carbon dioxide (Boru et al. 2001; Ponnamperuma et al. 1984).

Plant adaptation to waterlogging

The effect of waterlogging in wheat plants is observed first in the roots, in which the seminal root dies and the adventitious roots increase in length (Malik et al. 2002). However, plants have developed many tactics to decrease the negative effect of waterlogging and submergence. One of them is seed dormancy, so the plant would have a benevolent environment during growth and reproductive stages (Voesenek et al. 2004). Other species acquire tolerance tactics that help them to survive and to avoid stress due to waterlogging. Two classic responses to flooding and waterlogging include the "Low Oxygen Escape Syndrome" (LOES) and "Low Oxygen Quiescence Strategy" (LOQS) (Bailey-Serres and Voesenek 2008).

LOES is a mechanism in which the plant improves internal oxygen levels. Plants which use the LOES mechanism increase the growth rate of shoots (Voesenek and Blom 1989), petioles and leaves (Bailey-Serres and Voesenek 2008) and create anatomical and morphological structures to transport gases within the plant and interchange gases with the environment through radial oxygen loss (ROL) (Colmer 2003). These structures include adventitious roots and aerenchyma tissue (Bailey-Serres and Voesenek 2008; Colmer and Voesenek 2009; Justin and Armstrong 1987; Rocha et al. 2010).

Aerenchyma is an aerated cortical tissue formed in the roots and shoots of wetland and some dry land species (Evans 2004). Aerenchyma develops as a consequence of common development of the cells or as a response to abiotic stresses such as waterlogging, high temperatures, deficient nutrition, and drought (Evans 2004). Two types of aerenchyma have been described, known as a lysogenic and schizogenic (Armstrong et al. 1991; Evans 2004).

Lysogenic aerenchyma can be found in crop species including rice, barley, wheat, and maize (Drew et al. 2000; Evans 2004). Schizogeneous aerenchyma occurs when gas space is formed due to cell separation (Armstrong et al. 1991; Evans 2004). This type of tissue is present principally in wetland species such as rumex. However, some species like *Saggitaria lancifolia* known as a duck potato (Paradis et al. 2012) can present both classes of aerenchyma (Evans 2004). Some waterlogging-tolerant wheat genotypes have been shown to form aerenchyma in their adventitious roots (Malik et al. 2001). Aerenchyma tissue has been shown to develop after 24 hours of submergence, and become well-developed at 120 hours post submergence (Jiang et al. 2010).

Under the LOES mechanism, the number of chloroplasts and thickness of leaves are reduced in order to help the diffusion of gases such as CO_2 and O_2 (Bailey-Serres and Voesenek 2008; Voesenek et al. 2004). Plants which are completely under shallow water may utilize the LOES mechanism simply for survival, although they are not always successful. LOES plays an important role during survival because the submerged shoots continue to grow, allowing the leaves to reach the surface and for oxygen exchange. However, if all the energy reserves of the plants are used during growth without reaching the surface, the plant dies (Bailey-Serres and Voesenek 2008). Setter and Laureles (1996) showed that rice genotypes with reduced seedling elongation during fourteen days of complete submergence had a higher rate of survival. The correlation between the number of plants which survived and elongation was negative ($r= -0.81$).

LOQS is a mechanism used by plants to both conserve ATP and increase the efficiency of enzymes involved in the production of ATP in an environment with an oxygen deficit. Additionally, LOQS activates the production of molecules that help to neutralize adverse cellular modifications associated with submergence (Colmer and Voesenek 2009). LOQS is

distinguished by avoidance of elongation of underwater organs and then by preserving energy and carbohydrates. The plants modify their metabolism as a consequence of the depletion of oxygen in the environment (Bailey-Serres and Voesenek 2008). Plants change from an aerobic metabolic pathway to an anaerobic metabolic pathway to avoid energy losses and cell destruction due to a reduction in respiration (Geigenberger 2003; Mommer et al. 2004; Perata and Alpi 1993). The LOQS mechanism has been observed in lowland rice in which the tolerant submerged cultivar has the locus *Sub1*, which encodes the ERF gene *Sub1A* (Fukao et al. 2006). The *Sub1A* gene restricts leaf and internode prolongation, chlorophyll degradation, carbohydrate losses and promotes the activities of dehydrogenase and pyruvate decarboxylase enzymes (Fukao et al. 2006).

Plant adaptations to elemental toxicity and deficiency

High concentrations of Al, Mn, and Fe have been found in shoot tissue of wheat grown under waterlogging conditions in Western Australia's acidic soils and the survival of some wheat plants can be due to tolerance to toxic elements in those genotypes (Khabaz-Saberi et al. 2010). High amounts of aluminum can promote toxicity in the plants, affecting DNA synthesis, mitosis, membrane function and structure, cell wall rigification, and low cell elongation rates (Tice et al. 1992). There are several theories about the mechanisms used by plants for protection from Al toxicity. First is the capacity of the plant either to eliminate the toxins generated by Al or tolerate Al (Taylor 1991; Tice et al. 1992). Second is the ability to prevent the entrance of Al to the symplasm (exclusion) by immobilization of Al or production of chelating ligands (Delhaize 1993; Taylor 1991). The third mechanism is to defend cell wall and membrane function and structure from lesions through variations in the apoplastic pathway (Tice et al. 1992). Wheat has been shown to use an exclusion mechanism to avoid toxicity from Al rather than internal

tolerance mechanisms (Carver and Ownby 1995). For example, Al tolerant wheat genotypes excreted 5 to 10 times more malic acid from roots than susceptible wheat plants (Delhaize 1993). Malic acid is an organic acid which chelates aluminum and protects the root apex from high levels of Al^{3+} (Ma et al. 2001).

Manganese is an essential element for photosystem II. Acid soil can contain high Mn levels that can be toxic to the plants. Wheat plants with Mn toxicity have shown a reduction in net photosynthesis and chlorophyll content (Ohki 1985). Internal tissue tolerance to Mn is the most common mechanism used by plants to survive in an environment with excess Mn (González and Lynch 1999). Plants tolerant to Mn are able to maintain low concentrations of Mn^{2+} in the cytoplasm by accumulating Mn^{2+} into the vacuole. In addition, plants use active transport to mobilize Mn^{2+} from cytoplasm out of the cell or into the vacuole, while Na^{2+} is up taken by the root through facilitate diffusion (Quiquampoix et al. 1993). Additionally, Mn^{2+} tolerance implies intra and extracellular chelation, translocation (Kumar et al. 1995), storage of the excess Mn^{2+} in the epidermis (Memon et al. 1981), and the formation of glandular trichomes (Blamey et al. 1986), a hair derived from the epidermis which secretes secondary metabolites (Levin 1973). Mn excess in wheat promotes the reduction of vegetative dry biomass by 54%, due to a reduction in both tiller number and plant height, and a reduction in root dry biomass by 25% (Ohki 1984). In another study, Mn tolerant wheat cultivars were found to have higher photosynthetic rates, respiration, chlorophyll a and chlorophyll b levels compared with a susceptible wheat cultivar (Macfie and Taylor 1992).

Flooded soils can contain high concentrations of Fe^{2+} due to oxido-reduction reactions in the soil (Gotoh and Patrick 1974). Iron toxicity tolerance mechanisms have been classified into three strategies: exclusion/avoidance, inclusion/avoidance and inclusion/tolerance (Becker and

Asch 2005). Exclusion/avoidance is the mechanism by which the plant does not uptake Fe^{2+} in order to prevent Fe^{2+} injuries in the shoot tissue. Inclusion/avoidance implies uptake of Fe^{2+} by the root and then immobilization of iron in the leaf apoplast. Inclusion tolerance is when the plant is resistant to high amounts of Fe^{2+} in the leaf cells through enzymatic detoxification (Becker and Asch 2005). Khabaz-Saberi et al. (2010) evaluated the effects of different Fe concentrations in waterlogged acid soils on wheat cultivars, including a tolerant wheat genotype, 'Siete Cerros', and an intolerant wheat genotype, 'BH1146'. They found that the Siete Cerros cultivar used the exclusion mechanism to overcome Fe^{2+} stress.

Use of physiology to understand waterlogging

Waterlogging stress hampers the growth and development of the plant. A major reason for the reduced growth rate under waterlogging conditions is due to the negative effect of submergence on photosynthesis. Photosynthesis efficiency can be measured indirectly through the quantification of chlorophyll. Chlorophyll content in wheat is negatively affected by waterlogging stress. The soil plant analysis development (SPAD) value has been used as a measure of wheat chlorophyll levels in several studies (Malik et al. 2001; Tan et al. 2008; Thomson et al. 1992). A SPAD meter is an efficient method to calculate an accurate and instantaneous measure of leaf chlorophyll content. A SPAD meter is able to quantify the amount of red and infra-red light which pass on the leaf via a photo-diode detector (Markwell et al. 1995). Chlorophyll absorbs red light (650 nm), but does not absorb infrared light (940 nm), which allows the SPAD meter to calculate chlorophyll levels in the plant (Hoel and Solhaug 1998). According to Uddling et al. (2007), a high correlation (r^2=0.9) is observed between SPAD values and chlorophyll in wheat.

Following fifteen days of waterlogging a significant reduction in the SPAD value of flag leaves of waterlogged wheat cultivars compared with control wheat cultivars was observed, with the waterlogging-tolerant cultivar 'Yangmai9' having a SPAD value 10.1% less than the control, while the susceptible cultivar 'Yumai 34' had a SPAD value16.2% less than the control (Tan et al. 2008). In another study, the chlorophyll level of the youngest leaf in wheat cultivars was severely reduced when waterlogging was applied both at the soil surface and at 100 mm under the soil surface in relation to the control plants (Malik et al. 2001).

Genetic variation for waterlogging tolerance in wheat

Several studies under waterlogging conditions have been carried out in wheat at different phenological stages in order to identify genetic diversity that can be exploited for genetic improvement through breeding. Gardner and Flood (1993) evaluated fourteen genotypes in drained and undrained areas, with genotypes classified by different apex developmental times. They found that earlier types were more susceptible to undrained areas than long season wheat. Another study evaluated two winter wheat genotypes, 'Bayles' and 'Savannah' in a waterlogged environment over a 17 day period. Savannah was more resistant to waterlogging than Bayles due to the formation of aerenchyma tissue in the roots (Huang et al. 1994b). Wheat growth response and recovery was studied in six wheat genotypes (Bayles, FL302, BR34, Coker-9766, Gore and Savannah) under a hypoxic environment. Bayles and FL302 were found to be very susceptible to hypoxia. However, Gore and Savannah were found to be tolerant to hypoxia resulting from the presence of a large amount of aerenchyma (Huang et al. 1994a). Davies and Hillman (1988) compared the tolerance levels of different hexaploid and tetraploid wheat species. The hexaploid wheat species *Triticum macha* was more flooding tolerant than the species *Triticum aestivum*, and *Triticum spelta*. In the population of tetraploid wheat, *Triticum dicoccum* (Bordeaux) was

more susceptible to flooding than *Triticum dicoccum* (Pontus). Tolerant species showed more vegetative growth, higher inflorescence number and higher grain weight (Davies and Hillman 1988).

Wheat cultivars 'Gamenya' and 'Kite' and a triticale cultivar 'Muir' grown in a stagnant solution presented genetic variation in reaction to oxygen deprivation resulting from the formation of differential amounts of aerenchyma and nodal root elongation rates (Thomson et al. 1992). Collaku and Harrison (2005) evaluated genetic variance components to waterlogging tolerance in 80 families. The families were collected from the F_2 generation of four soft red winter wheat populations. They found that kernel weight, chlorophyll and tiller number had high heritability estimates (Collaku and Harrison 2005). Cai et al. (1996) estimated the heritability of waterlogging tolerance to be 71.5% in 10 wheat varieties based on the number of green leaves per stem during 25 days of waterlogging. They concluded that early generation is a good time to select plants with high waterlogging tolerance. Additionally, nine wheat genotypes were evaluated in waterlogging conditions, including the Arkansas cultivar 'Jaypee', which was classified into the highest group for tillering under waterlogging conditions. Jaypee presented a smaller reduction in tiller number compared to most genotypes (Collaku and Harrison 2002).

Wheat genetics and mapping

Wheat is allopolyploid, with seven basic chromosomes (n=7) (Ganal and Röder 2007) and different levels of polyploidy depending on the species (Feldman and Levy 2005; Levy and Feldman 2004). Polyploidy often occurs during the process of adaptation and specialization (Levin 1983). Polyploidy can be generated between different individuals of a single species, called autopolyploids or can be generated by hybridization of different species, known as allopollyploids (Ramsey and Schemske 1998). Levy and Feldman (2004) stated that

allopolyploidy in wheat varies from diploid to hexaploid. For example, *Triticum turgidum* ssp., or durum wheat, is a tetraploid wheat formed by AA and BB genomes (2n=4x=28) and is commonly used for pasta products. Common bread wheat, *Triticum aestivum* ssp. is a hexaploid wheat, with three sets of chromosomes (2n=6x=42) and the AABBDD genomes.

Common wheat arose from the hybridization of *T. turgidum* L. (2n=28, AABB), a tetraploid wheat, and *Aegilops tauschii* (Coss.), a diploid grass with the genome DD (Pestsova et al. 2000). According to Levy and Feldman (2004) and Feldman and Levy (2005), the AA genome of wheat is homologous to the AA genome of *T. urartu* (2n=2x=14) and the DD genome is highly related to the DD genome of *Ae. tauschi* (2n=2x=14). The donor of the B genome to durum and bread wheat is not well known. However, *Ae. speltoides* (2n=2x=14) is the most closely related species as a progenitor of the B genome (Levy and Feldman 2004). Genetic studies in wheat are very complex due to the size and structure of the genome (Langridge et al. 2001). Wheat genome size has been estimated at approximately 5000 Mb, 13000 Mb, and 17300 Mb for diploid, tetraploid and hexaploid wheat, respectively (Qi et al. 2004), making it much larger than both the human (3200 Mb) (Morton 1991) and rice genomes (450 Mb) (Qi et al. 2004).

A major objective for plant breeders and geneticists is to map quantitative trait loci (QTL). QTL are areas of the genome identified by the presence of a molecular marker allele that are associated with important agronomical traits (Röder et al. 1998). Molecular markers can be described as signs or flags which help to find target genes (Collard et al. 2005). Molecular markers originate as a result of diverse DNA mutations such as insertions, deletions and errors at the moment of replication (Collard et al. 2005; Paterson and Wing 1993). Molecular markers

allow plant breeders to be more efficient in selection, by combining the process of conventional phenotypic selection with marker assisted selection for QTL (Gupta et al. 1999).

Several molecular markers are currently available to perform genetic studies in wheat, including fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR) and single nucleotide polymorphism (SNPs) Landjeva et al. (2007). Over the last ten years, SSR markers have been the most popular choice for genetic studies in wheat (Ganal and Röder 2007). SSRs are a repeat sequence of two to four nucleotides randomly distributed along the eukaryotic genome (Pestsova et al. 2000). SSR markers are stable, co-dominant, and have a high rate of polymorphism in wheat, a species with relatively low levels of polymorphism (Ganal and Röder 2007). SSRs have been used in wheat to perform studies of genetic diversity (Dreisigacker et al. 2004; Gupta et al. 2003; Manifesto et al. 2001), and construction of genetic linkage maps (Gupta et al. 2002; Pestsova et al. 2000; Röder et al. 1998; Varshney et al. 2005). Unlike SSRs, SNP markers can identify variation in the genome that arises as a result of the modification of a single nucleotide (Zhang et al. 2003), so there is the potential for many more SNP markers within a genome (Landjeva et al. 2007). Likewise, the polymorphism of a single nucleotide sequence can be reflected in the modification of the phenotype in an organism (Yanagisawa et al. 2003). SNPs are currently being used in plant breeding programs, for example in the selection of wheat genotypes that contain grain hardness genes (Huang and Roder 2005).

QTL and genes regulating waterlogging tolerance in wheat and other crops

A QTL is a region within a genome that is able to promote alterations in a quantitative trait (Doerge 2002). Despite the economic importance of waterlogging stress, there is little information about the genetic control or QTLs associated with waterlogging tolerance in wheat.

A quantitative analysis made in F_3 populations from crosses between three tolerant wheat genotypes (Ducula, PR1/Sara, and Vee/Myna) and two susceptible wheat genotypes (Seri-82 and Kite/Glen), indicated that tolerance to waterlogging is controlled by four tolerance genes with additive effect (Boru et al. 2001). Additionally, five QTLs located on chromosomes 2B, 3B, 5A and 7S were associated with seedling survival when waterlogging was applied at germination in a winter spelta (*Triticum spelta* L.) by winter wheat population, Forno/Oberkulmer. Those QTLs explained 40.6% of the total phenotypic variation for plant survival. In the same population, ten QTLs detected on chromosomes 2A, 2B, 2D, 3A, 4B, 5A, 5B, 6A and 7S were related to seedling growth index following waterlogging and explained 35.5% of the phenotypic variance (Burgos et al. 2001).

Unlike wheat, our understanding of waterlogging and flooding tolerance in other plant species including rice, barley, maize, and soybean is much more defined. In rice, QTLs related to increased submergence tolerance were mapped on the chromosomes 1, 2, 3, 7, 10, 11. These QTLs promoted plant survival by reducing shoot elongation and maintaining chlorophyll levels during long periods of flooding (Toojinda et al. 2003). Two QTLs were linked to early stem elongation under flooding, with a major QTL on chromosome 12 accounting for 50% of the genetic variation (Nemoto et al. 2004). Three major QTLs associated with internode elongation during submergence were found in deep-water rice (Hattori et al. 2007). However, QTLs associated with a lower rate of internode elongation have also been associated with flooding tolerance in deep-water rice (Kawano et al. 2008).

QTLs associated with germination stage waterlogging tolerance have also been identified in rice. In a BC_2F_2 population from the cross IR64/Khaiyan/IR65, four QTLs were detected during germination on chromosomes 1, 2, 11, and 12, explaining from 12% to 29% of the

phenotypic variance (Angaji 2008). All positive alleles for the four QTLs were inherited from the tolerant parent Khaiyan. Abdolhamid et al. (2010) identified five QTLs for germination in a BC_2F_2 population derived from the cross Khao Hlan On/IR64/IR64. QTLs were located on chromosomes 1, 3, 7, and 9, accounting for 17% to 33.5% of the phenotype variation.

Zheng et al. (2003) identified eighteen QTLs associated with seminal root length, lateral root number, lateral root length and adventitious root number under both flooding and upland conditions in rice. In the same study, two genes associated with cell wall expansion, *OsEXP2* and *4-b-D-glucanase EGase,* were found to co-localize with a QTL for seminal root length and lateral root length under upland conditions (Zheng et al. 2003). A similar study identified a QTL associated with seminal root length under waterlogging located on the chromosome 1 between the genes *RM315* and *OsEXP2* (Zheng et al. 2006).

A number of QTLs associated with waterlogging tolerance have been identified in barley. Four QTLs were identified in a double haploid population for leaf chlorosis, including two major QTLs explaining 24% and 17% of the phenotypic variation and two minor QTLs explaining 8% and 7% of the phenotypic variance (Zhou 2011). Li et al. (2008) identified twenty QTLs associated with waterlogging tolerance in two barley double haploid populations. Traits measured included leaf chlorosis, vegetative biomass and plant survival. A QTL on chromosome 4H contributed by the tolerant parent, 'TX9435', was associated with decreased leaf chlorosis and increased plant biomass under waterlogging conditions.

In maize, leaf injury and dry matter production were linked to a QTL on chromosome 1 which explained 10% of the variance for those traits under flooded conditions (Mano et al. 2006). Evaluation of a F_2 population from the cross B64/Na4 under flooding conditions revealed three QTLs associated with formation of adventitious roots, located on chromosomes 3, 7 and 8.

The positive alleles for these QTLs, which increased the number of adventitious roots, were inherited from the Na4 parent (Mano et al. 2005). Similarly, three QTLs for adventitious root formation under waterlogging conditions were identified on chromosomes 3, 7 and 8 in a population of 317 seedlings derived from a cross between 'MI29' and the progenitor of common maize, teosinte *Zea nicaraguensis* (Mano et al. 2009). Interestingly, the alleles inherited from *Zea nicaraguensis* enhanced adventitious root formation for all QTLs. Qiu et al. (2007) found thirty four QTL in maize under control and waterlogging conditions associated with root and shoot dry weight, total dry weight, waterlogging tolerance coefficient and plant height (Qiu et al. 2007).

VanToai et al. (2001) identified a QTL related to waterlogging tolerance in two soybean recombinant inbred line (RIL) populations derived from the crosses Archer/Minsoy and Archer/Noir I. The positive allele from Archer was responsible for increasing plant growth by 11% to 18% and increasing grain yield by 47% to 180%. Cornelious et al. (2005) evaluated a population of 103 RILs derived from A5403/Archer and identified a QTL associated with 10% of the phenotypic variation for leaf chlorosis and plant death under waterlogging conditions. This QTL was located near to the marker Satt385 on the linkage group Al with the positive allele inherited from Archer. In the same study, a QTL close to the marker Satt269 on the linkage group F was identified in a population of 67 P9641/Archer RILs. Again, the favorable allele was inherited from Archer and explained 16% of the phenotypic variation (Cornelious et al. 2005).

QTL associated with elemental toxicity in wheat and other crops

Tolerance to elemental toxicity is an important mechanism contributing to overall tolerance to waterlogging conditions. Wheat genotypes tolerant to iron toxicity were found to be more tolerant to waterlogging conditions (Khabaz-Saberi et al. 2012) and it is therefore hypothesized

that the identification of QTL associated with elemental toxicity tolerance would lead to improved performance under waterlogging. In rice, twenty-four QTLs associated with morphological and physiological traits related to iron toxicity tolerance were identified, including leaf bronzing index, root and shoot dry weight, shoot water content, relative variation of root and shoot, chlorophyll content, stomatal resistance and shoot iron concentration (Dufey et al. 2009). A similar study in rice identified four QTLs under Fe^{2+} stress, explaining from 20.5% to 38.8% of the phenotypic variance for leaf bronzing index, root dry weight, tiller number and stem dry weight (Wan et al. 2003). In wheat, the *Nax1* and *Nax2* genes related with Na^+ exclusion were transferred from tetraploid wheat to hexaploid wheat. Hexaploid wheat plants which contained either one or two of these genes were more resistant to saline and waterlogging conditions by inhibiting the storage of Na^+ in leaves (James et al. 2011).

Approach of the current study

Despite the impact of waterlogging on wheat production both globally and in the southeastern U.S., very little is known about the genetic control of waterlogging tolerance in wheat. The *main objective* of this study was to identify quantitative trait loci (QTL) associated with waterlogging tolerance and productivity in wheat which can be utilized for marker assisted breeding. To accomplish this, the following specific objectives were carried out:

Objective 1: Determine the amount of genetic variation for biomass productivity and tolerance to vegetative stage waterlogging stress within a soft red winter wheat mapping population. For this objective, waterlogging tolerance will be defined as maintaining biomass traits under waterlogging stress compared to control conditions, as determined by a flooding tolerance index and the presence of significant genotype by treatment interaction. Productivity is defined as the amount of biomass produced under waterlogging or control conditions and the

presence of significant genetic variation. A recombinant inbred line (RIL) population derived from a cross between the wheat cultivars ‘USG3209’ and ‘Jaypee’ (U/J) that segregates in its response to waterlogging, will be evaluated for agronomic and physiological traits in both waterlogging and non-waterlogging environments. The *working hypothesis* for this objective is that important agronomic and physiological traits will show significant genetic variation and significant genotype by treatment interaction for traits which are important to vegetative stage waterlogging tolerance.

Objective 2: **Identify quantitative trait loci (QTL) associated with waterlogging tolerance and productivity.** This objective will be accomplished by combining the phenotypic data collected in objective 1 with the genotypic data and a genetic map for the U/J RIL population. The *working hypothesis* for this objective is that there is significant genetic variation for waterlogging tolerance in wheat and this variation is controlled by QTL.

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Chapter II
Phenotypic and Genetic Analysis of Waterlogging Tolerance in Wheat

Abstract

Soil waterlogging is a primary constraint to wheat production both globally and in the United States. The goal of this study was to determine the amount of genetic variation for waterlogging tolerance present within a wheat recombinant inbred line (RIL) population, and to identify quantitative trait loci (QTL) associated with tolerance and productivity. A RIL population derived from a cross between the cultivars 'USG3209' and 'Jaypee' was evaluated under control and waterlogging conditions in both greenhouse and field conditions. Traits measured included shoot and root biomass, tiller number, plant height pre and post- treatment, elongation, and chlorophyll content (SPAD). Waterlogging resulted in a reduction of 8-58% for biomass traits, tiller number, root length, plant height post-treatment, elongation evaluated in the parents and 10 to 54% in the RIL population, with the largest reduction observed for root length. A significant genotype by treatment effect was observed for root traits, indicating that root traits have a differential response to waterlogging and can be targeted to improve waterlogging tolerance. The QTL analysis revealed 53 total QTLs, with 34 detected under waterlogging stress explaining 6 to 32% of the total phenotypic variation. These QTLs clustered into 19 regions distributed throughout the wheat genome. QTL on chromosomes 2A, 2B, 2D, 5A and 5B were found to localize with known genes regulating plant height and flowering time. Other QTL regions located on chromosomes 1B, 2A and 6B appear to be novel for biomass production specific to waterlogging stress and can be used for marker-assisted selection to more efficiently select for waterlogging tolerant lines.

Introduction

Waterlogging occurs in 10% of worldwide soils, causing yield reductions from 15% to 80% (Shabala 2011) . In the United States, it is the second most important abiotic stress after drought in terms of impact on crop yield (Paux et al. 2008; Setter and Waters 2003; Shabala 2011). In wheat, periods of waterlogging cause a reduction of root and vegetative biomass (Huang et al. 1994a; Lee et al. 2007a; Malik et al. 2001) decreased photosynthesis (Huang et al. 1994b), and leaf chlorosis (Huang et al. 1994b; Lee et al. 2007a), resulting in lower yield components and total yield at maturity (Collaku and Harrison 2002). Waterlogging hampers the performance of plants by decreasing the dispersion of oxygen through the pore space of the soil around the roots (Christianson et al. 2010; Lee et al. 2007b) as the diffusion of atmospheric oxygen is restricted an average of 320,000 times in submerged soils (Armstrong 1979; Barrett-Lennard 2003; Colmer and Greenway 2011; Lee et al. 2007b). Oxygen levels are reduced to the point that plants are subjected to an anoxic environment (Setter and Waters 2003) which alters aerobic metabolism and results in an energy crisis, with catastrophic consequences on plant growth and development (Colmer and Greenway 2011).

Plants have developed physiological strategies to reduce the harmful effects of submergence and soil waterlogging. Two classical responses include the "Low Oxygen Escape Syndrome" (LOES) and "Low Oxygen Quiescence Strategy" (LOQS) (Bailey-Serres and Voesenek, 2008). In LOES, plants maintain internal oxygen levels by utilizing escape mechanisms such as increased shoot, petiole and leaf growth and by creating anatomical and morphological structures to transport gases (Bailey-Serres and Voesenek 2008; Voesenek and Blom 1989). These structures include aerenchyma tissue (Bailey-Serres and Voesenek 2008; Colmer and Voesenek 2009; Justin and Armstrong 1987; Rocha et al. 2010), radial oxygen loss

(ROL) (Colmer 2003), and adventitious roots (Bailey-Serres and Voesenek 2008). Species which utilize LOQS adjust their metabolism as a consequence of oxygen depletion, utilizing fewer adenosine triphosphate (ATP) or by producing ATP in the absence of oxygen (Bailey-Serres and Voesenek 2008; Colmer and Voesenek 2009). ATP are conserved by inhibiting the elongation of underwater organs which conserves carbohydrates and thus energy (Bailey-Serres and Voesenek 2008).

Previous studies have been successful at identifying genetic variation for waterlogging tolerance in wheat. Gardner and Flood (1993) found that early flowering genotypes were more susceptible to soil waterlogging than later flowering genotypes. Other studies have shown that waterlogging tolerance involves the formation of aerenchyma tissue in the roots (Huang et al. 1994a; Huang et al. 1994b; Thomson et al. 1992) and fast coleoptile growth (Burgos et al. 2001). Collaku and Harrison (2002) evaluated nine soft red winter wheat genotypes under waterlogging starting at the three to four leaf stage and identified tolerant genotypes, including the Arkansas cultivar "Jaypee" which showed a moderate reduction in tiller number and yield following five weeks of continuous flooding.

Previous studies have shown waterlogging tolerance to be quantitatively inherited. A quantitative analysis of F_3 populations derived from crosses between three tolerant (Ducula, PR1/Sara, and Vee/Myna) and two susceptible (Seri-82 and Kite/Glen) wheat genotypes estimated waterlogging tolerance to be controlled by four genes with additive effect (Boru et al. 2001). In a study utilizing a winter spelta (*Triticum spelta* L.) by winter wheat population, five quantitative trait loci (QTLs), located on chromosomes 2B, 3B, 5A, and 7S were identified for seedling survival when waterlogging was applied at germination (Burgos et al. 2001). Those QTLs explained 40.6% of the total phenotypic variation for plant survival. In the same study,

QTLs detected on chromosomes 2A, 2B, 2D, 3A, 4B, 5A, 5B, 6A and 7S were related to seedling growth index following waterlogging and explained 35.5% of the phenotypic variance. With the exception of the study by Burgos et al. (2001) very little is known about the genetic control of waterlogging tolerance in wheat, despite the impact of waterlogging on wheat production in the United States and globally. This gap in knowledge becomes much more apparent when compared to the amount of information that has been reported for other important crop species including rice (Toojinda et al. 2003), barley (Li et al. 2008; Zhou 2011), soybean(Cornelious et al. 2005; VanToai et al. 2001) and maize (Mano et al. 2006; Mano et al. 2005; Qiu et al. 2007). Therefore, the goal of this study was to determine the impact of vegetative stage waterlogging on a wheat recombinant inbred line population and to identify QTL associated with both waterlogging tolerance and plant productivity under waterlogging stress.

Materials and methods

Plant material

A population of 130 $F_{11:12}$ recombinant inbred lines (RILs) derived from the soft red winter wheat cultivars "USG3209" and "Jaypee" was used for this study (referred to herein as U/J RILs). Jaypee was developed at the University of Arkansas (Bacon et al. 1998) and has previously been shown to be moderately tolerant to vegetative stage waterlogging (Collaku and Harrison 2002). USG3209 and the U/J RILs were developed by the Virginia Polytechnic Institute (VPI) and their development has previously been reported (Hall et al. 2010).

Experimental design in the greenhouse

Greenhouse waterlogging experiments were conducted in Fayetteville, Arkansas in the spring 2012 and repeated in the fall 2012. Parental cultivars and RILs were sown in the greenhouse in a sandy loam soil (56.9% sand, 37% silt, and 6.1% clay) at five seeds per 1.65 liter tree pot (10 cm wide x 24 cm tall). Pots were placed in 99-liter plastic tubs at 28 pots per tub. The greenhouse was maintained at an average temperature of 20°C/18°C day/night cycle with 16-hour days. Pots and tubs were randomized in a split plot with complete blocks design and two replications per treatment. After planting, each pot received 5 grams of Osmocote 20-20-20 slow release fertilizer (The Scotts Company, LLC) to promote establishment and growth. Following establishment, plants were thinned to 3 plants per pot based on plant uniformity. At 16 days after sowing, pots were fertilized with the recommended rate of Peters 20-20-20 nutrient mix. Waterlogging was applied for 28 days at 24 days after sowing by filling the plastic tubs containing the pots with deionized water to slightly above the soil level. Additional water was added daily to maintain waterlogging above the soil level. Control plants were maintained in plastic tubs with no waterlogging treatment to maintain normal growth conditions. After 28 days of waterlogging the plastic tubs were drained and the plants were harvested for trait measurement. Four independent soil samples from both the waterlogging and control treatments each were analyzed to determine the amount of extractable soil nutrients such as P, K, Mg , Ca , S, Na, using the procedure described by Mehlich (1984).

Trait measurement in the greenhouse

Plant height was measured pre-treatment from the soil level to the node of the tallest tiller. Chlorophyll was measured weekly using a SPAD meter (Spectrum Technologies Inc.) on the healthiest leaf of each of the three plants per pot starting during the first week of waterlogging.

Following the waterlogging treatment, whole plants including roots and shoots were removed from pots, excess soil was removed and roots were washed clean with tap water and dried with paper towels. The number of tillers per plant was manually counted. Root length was measured as the distance from the tip of the longest root to the crown of the plant. Plant height post-treatment was measured as the distance from the crown to the node of the tallest tiller. Root and shoots of harvested plant were weighed for fresh-weight, oven dried at 65˚C for 72 hours or until tissue reached a constant weight, and re-weighed for dry-weight.

Field experimental design

The field experiment was carried out in Stuttgart, Arkansas during the 2012-2013 growing season. Stuttgart soils are characterized by a silt loam surface layer and a clayey subsoil with low permeability (NRCS 2013) and are prone to periodic waterlogging. Parental lines and RILs were drill seeded on November 7, 2012 in plots that consisted of two 1.5 meter rows at 115 seed m^{-2}. Rows and lines were arranged in a randomized complete block design with two treatments (waterlogged and non-waterlogged) with two replications per treatment. Plots were fertilized with 120 units of nitrogen in a split application prior to starting the waterlogging treatment. Field waterlogging was imposed by establishing 0.30 meter high levees surrounding the experimental field and applying water to saturate the soil twice weekly for the duration of the treatment. The waterlogging treatment was started on March 20, 2013 at Feekes growth stage 4/5 and continued until April 17, 2013 at Feekes growth stage 5/6.

Trait measurement in the field

Chlorophyll was measured using a SPAD meter one day prior to waterlogging and one day post-waterlogging as the average of five healthy leaves in each plot. Following waterlogging fresh biomass was calculated by harvesting and weighing a 0.10 m^2 sample of vegetative biomass.

Dry biomass was calculated after the samples were oven dried at 65˚C for 72 hours or until tissue reached a constant weight.

Statistical analysis

Data was analyzed using PROC MIXED in SAS 9.3 (SAS Institute Inc. 2011, Cary, NC). For the greenhouse experiment a split plot with randomized complete blocks design was used for all traits with the exception of chlorophyll which was analyzed as a split split plot design. Homogeneous error between the experimental runs (spring and fall) allowed for data to be analyzed in a combined analysis. In the field experiment shoot biomass was evaluated using as a randomized complete blocks design. Chlorophyll pre-treatment and post treatment were analyzed using a paired T-test. For the U/J RILs, treatments were considered fixed allowing the detection of significant differences between means from both treatments. For the parental cultivars analysis, both genotype and treatment were considered fixed to detect significant differences between means. Broad sense heritability (H^2) was estimated for each treatment individually (control and waterlogging) from variance components with all factors (genotype, run, replications (run) and genotype x run) considered random and using the following formula:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{e} + \frac{\sigma_{error}^2}{re}}$$

where σ_g^2, σ_{ge}^2 and σ_{error}^2 represent genotype variance, genotype by environmental variance and error variance, respectively, and e and r the number of environments or in the case of this study runs (n=2) and replications (n=2). Pearson's correlation was done using PROC CORR and principal component analysis was carried out using SAS code developed by Crossa et al (unpublished).

QTL mapping

A genetic map previously developed using 225 SSR markers, 363 DART markers and 3 SNP markers was used in this study. Detail information on genetic map construction can be found at Hall et al. (2010). Genotypic means were determined across treatments (waterlogged and control) for the greenhouse and field experiments separately and used for detecting QTL using WinQTL Cartographer v2.5 (Wang, et al., 2006). The association between genetic markers and phenotypic traits was first analyzed using single marker analysis. QTL positions were then determined using composite interval mapping (CIM). For CIM, background cofactors with a 10 cM window were identified using forward (P = 0.05) and backward regression (P = 0.05). The LOD threshold for each trait was calculated using a 1000 permutation test at a significance level of P = 0.05. The nomenclature described in the catalog for gene symbols for wheat (http://wheat.pw.usda.gov/ggpages/wgc/98/) (USDA 1998) was used to designate QTLs. QTL images were redrawn using MapChart (Voorrips 2002).

Results

Phenotypic analysis in the greenhouse

For the parental lines, greenhouse waterlogging resulted in a reduction of 6-58% for the traits measured with the largest reduction observed for root length (Table 1). The parents showed a similar response to waterlogging in terms of flooding tolerance index. Jaypee had significantly higher means than USG3209 under waterlogging for most of the traits measured, including 13-25% higher root and shoot biomass. A significant difference was also observed for final plant height, with Jaypee being the taller cultivar following waterlogging stress. Similar results were observed in the U/J RILs, with reductions of 10 to 54% due to waterlogging stress and the largest reduction also observed for root length (Table 2). A significant treatment effect was detected

for fresh shoot biomass and root length while significant genetic variation was observed for all traits with the exception of root length (Table 2). Significant genotype by treatment interaction was detected for the root traits, including fresh root biomass, dry root biomass and root length. Chlorophyll was significantly reduced in both the parents and the U/J RILs due to waterlogging stress. While the parents had comparable levels of chlorophyll in both treatments across weeks (Figure 1), significant genetic variation was observed in the U/J RILs (Table 3). A significant treatment by week interaction was also observed for chlorophyll in both the parents and U/J RILs, indicating that leaf chlorosis occurred during the time that the plants were under waterlogging stress.

Phenotypic distribution of the progeny was near to normal for most of the traits (Figure 2). However, there was evidence for skewedness toward small fresh root biomass, dry root biomass and plants height post-treatments under waterlogging (means 2.2, 0.24, 14.31 and medias 2.03, 0.18, 13.83 respectively), suggesting that for those traits a greater proportion of the lines were more susceptible to waterlogging stress. Although parents had similar means for most of the traits, means of the RILs showed a wide range of variation and fell outside the range of the parents, indicating the presence of transgressive segregation in both directions for most traits.

Broad sense heritability estimates (H^2) were moderate to high for both the control and waterlogging treatments (Table 4). Under waterlogging, heritability ranged from 22% to 83%, with the highest heritability observed for plant height post-treatment and the lowest for fresh root biomass. In the control treatment, heritability ranged from 11% to 88% with the highest heritability observed for plant height post-treatment and the lowest for fresh root biomass. Most traits showed similar heritability across the two treatments, with root length being the notable exception. Root length had higher heritability (H^2=0.47) under waterlogging compared to

control (H^2=0.11), due to higher genotypic variance and lower environmental variance present under waterlogging.

Phenotypic analysis in the field

Waterlogging was applied in the field for 28 days beginning at Feekes growth stage 4. For both the parental lines (Table 1) and U/J RILs (Table 2), a significant treatment effect was observed for fresh biomass with waterlogging stress reducing mean shoot biomass compared to control conditions.. USG3209 was slightly more tolerant to field waterlogging with a reduction in fresh shoot biomass of 22% and dry shoot biomass of 27%, compared to 31% and 35% for Jaypee, although these differences were not significant. Similar to the greenhouse, a significant reduction in chlorophyll was observed for both the parents and U/J RILs as a result of waterlogging (Figure 3). Overall, mean chlorophyll was reduced by 15% in Jaypee, 12% in USG3209, and 13% in the U/J RILs.

Correlations

Trait correlations under waterlogging stress are presented in Table 5. Agronomic and physiological traits showed moderate to high correlation in the greenhouse. Fresh root biomass had a high positive correlation with fresh shoot biomass (r=0.77) and with tiller number (r=0.62). A moderate and positive correlation was observed between root length and fresh shoot biomass (r=0.41) as well as between elongation and fresh shoot biomass (r=0.43). SPAD was moderately correlated with fresh biomass under both greenhouse (r=0.38) and field waterlogging conditions (r=0.39). Comparing across field and greenhouse experiments, both dry and fresh shoot biomass in the greenhouse exhibited a low but significant correlation (r=0.13) with the measurement in the field.

Principal component analysis bi-plot (PCA) using the greenhouse waterlogging means explained 64% of the experimental variation using the first two dimensions (Figure 4). Associations between traits were similar to those observed for the Pearson's correlation analysis, with a strong association between root and shoot biomass and between plant height traits and elongation. SPAD showed a positive association with both root and shoot biomass although very little of the total variance for SPAD could be explained by the PCA. Overall, the PCA explained a large percent of the variance for root and shoot biomass, plant height traits, and tiller number, and a smaller percent of the variance for root length and SPAD.

QTL mapping

Composite interval mapping (CIM) detected a total of 51 QTLs at the 1000 permutation threshold and two putative QTLs, under both control and waterlogging conditions in the greenhouse and field (Table 6). These 53 QTLs clustered into 19 regions throughout the wheat genome, located on chromosomes 1A, 1B, 1D, 2A, 2B, 2D, 3B, 4B, 4D, 5A, 5B, 6A, 6B, and 6D (Figure 5). Of the 53 total QTLs, 19 QTLs were identified under control conditions and 34 QTLs under waterlogging conditions. Both parents donated favorable alleles with 27 from Jaypee and 26 from USG3209. The LOD value for QTLs ranged from 2.5 to 13.8 with individual QTLs explaining from 6% to 32% of the phenotypic variation.

QTL for shoot biomass traits

Fifteen QTLs for shoot biomass traits were identified and located on chromosomes 1A, 1B, 2A, 2B, 2D, 3B, 5B, 6A, and 6D. Seven QTLs were detected under waterlogging and six QTLs under control conditions in the greenhouse. QTLs for shoot biomass in the greenhouse had moderate to high genetic effects, accounting for 6 to 33% of the phenotypic variance. Two QTLs were

identified for shoot biomass under field conditions, on chromosomes 2D and 3B, explaining 6% and 7% of the phenotypic variance, respectively.

QTL for root traits

Ten QTLs were identified for root traits, located on chromosomes 1B, 2A, 2B, 4D, 5A, 6B, and 6D. Eight of the 10 QTLs were detected under waterlogging conditions. Four QTL associated with root biomass were identified specific for waterlogging conditions and accounted for 12-23% of the phenotypic variance. Three of the five QTLs for root length were identified under waterlogging and explained 8 to 11% of the root length variance, whereas two QTLs were observed under control conditions, explaining 10 to 12% of the phenotypic variance.

QTL for plant height and elongation

Only one QTL was detected each for the traits plant height pre-treatment, plant height post-treatment and elongation that was above the threshold LOD value. These three QTL, *QElon.ua-5A, QHgtpost.ua-5A, QHgtpre.ua-5A*, co-localized on chromosome 5A, and were inherited from USG3209, explaining 19 to 28 % of the phenotypic variance for those traits.

QTL for chlorophyll

Chlorophyll QTLs were distributed throughout the wheat genome. Nine QTLs were detected under waterlogging stress, located on chromosomes 1B, 1D, 2A, 5A, 5B, and 6B and accounting for 10 to 22% of the phenotypic variance. In addition, eight QTLs were detected under control conditions in the greenhouse, located on the chromosomes 1B, 1D, 2B, 3B, 5B, and 6B, explain 8 to 25% of the phenotypic variance. Thirteen of the seventeen QTLs for chlorophyll were inherited from Jaypee.

QTL for flooding tolerance index

Two QTLs were detected for the flooding tolerance indices. *QSffti.ua-1B* was identified for fresh shoot FTI on the chromosomes 1B, explaining 14% of the phenotypic variance and contributed by USG3209. A second FTI QTL, *QRlengthfti.ua-2B*, was detected for root length on the chromosome 2B, explaining 10% of the phenotypic variance and inherited from Jaypee.

Discussion

Phenotypic variation under waterlogging

Jaypee and USG3209 showed contrasting levels of productivity for root and shoot biomass traits when grown under waterlogging and control conditions in the greenhouse, with Jaypee having higher means for all traits measured. Despite differences in their levels of productivity, the two parents had similar levels of waterlogging tolerance as determined by a lack of genotype x treatment interaction (data not shown) and similar values for FTI. Jaypee had a larger FTI for shoot biomass and elongation while USG3209 had a larger FTI for root biomass. It is therefore possible that these two genotypes may partition energy differently under waterlogging stress, with Jaypee utilizing energy to maintain vegetative growth and USG3209 to maintain root development. Those are two mechanisms that are associated with "Low Oxygen Escape Syndrome," an adaptive mechanism used by plants under flooding (Bailey-Serres and Voesenek, 2008). Less variation was observed between the parents in the field experiment, with no significant differences detected for either dry or fresh shoot biomass or chlorophyll content. Both parents also showed similar levels of biomass reduction as a results of waterlogging, with USG3209 slightly more tolerant compared to Jaypee but not significantly different. It is likely that differences in the growth stage at the time of treatment and differences in soil type would lead to differences in response to waterlogging. In the greenhouse experiment, a significant

reduction in the soil concentrations of sulfur, sodium, and iron was detected under waterlogging compared to control conditions (Data not shown). However, the concentrations of the elements in the soil were under optimum levels for wheat in both environments thus the wheat plants were not under toxicity or nutrient deficiency stresses.

The negative effect of waterlogging in the RIL population was reflected in a reduction of the means for all of the traits measured with exception of tiller number. These findings are in agreement with previous studies showing the negative effects of waterlogging on vegetative and root biomass, root growth and shoot growth (Huang et al. 1994b; Malik et al. 2001; Meyer and Barrs 1988; Thomson et al. 1992). Chlorophyll decline observed during waterlogging in both the greenhouse and field experiments was also in agreement with previous studies showing waterlogging stress to accelerate leaf senescence (Malik et al. 2001). A low but significant correlation (r=0.13) was observed between biomass in the greenhouse and biomass in the field and between chlorophyll (r=0.18). These low correlations show the need to screen material under waterlogging conditions in the target environment when at all possible.

The continuous and approximately normal distribution of the progeny for most of the agronomic and physiological traits suggests polygenic variation in agreement with previous studies showing waterlogging to be quantitatively inherited (Boru et al. 2001; Collaku and Harrison 2005; Li et al. 2008). High heritability was observed for plant height post-treatment and elongation under both the stressed and non-stressed treatments, indicating these traits to be simply inherited and under strong genetic control. This result was in agreement with the QTL analysis, which detected only one QTL on chromosome 5A for plant height and elongation. Other traits such as root biomass and root length were lowly heritable with a large portion of the phenotypic variance explained by environment and were found to be controlled by a larger

number of small effect QTLs. Root traits exhibited a high sensitivity to waterlogging stress and significant genotype by treatment interaction, indicating that root growth and development of some genotypes is different between control and waterlogged environments. Given this low heritability and difficulty in measuring these traits, particularly root biomass, marker assistance selection (MAS) could help to make selection more efficient (Burgos et al. 2001; Hospital et al. 1997). In addition, a strong correlation was observed between root biomass and shoot biomass traits in agreement with the correlations for those traits described previously by Bai et al. (2013). Given the high correlation between these traits and the difficulty in quantifying root traits, especially under field conditions (Bai et al. 2013), selecting plants for shoot biomass may allow for indirect selection of greater root biomass under waterlogging.

QTL analysis

Significant phenotypic variation made it possible to map QTL for the quantitative traits measured in this study. QTL analysis detected 53 total QTLs under both waterlogging and control conditions, with QTL contributed by both parents. This finding was in agreement with the distribution of the phenotypic data for the RIL population which showed transgressive segregation in both directions for most traits, suggesting that both parents contributed positive alleles (Qiu et al. 2007).

For biomass traits under waterlogging, chromosomes 1B and 6B contained the largest effect QTL. *QSfbio.ua-1B* explained 27% of the phenotypic variation for shoot fresh biomass, with an additive effect of 0.98 grams. *QRfbio.ua-6B.1* explained 23% of the phenotypic variance for root fresh biomass, with an additive effect of 0.27 grams. Other notable QTLs under waterlogging were detected for root length (*QRlength.ua-2A*), tiller number (*QTiller.ua-5B*), and shoot biomass (*QSfbio.ua-6D*), with R^2 values ranging from 6% to 8%. QTLs for elongation

(*QElon.ua-5A*), height post-treatment (*QHgtpost.ua-5A*), chlorophyll (*QSpad1.ua-5A*) under waterlogging stress were co-localized on the chromosomes 5A and QTLs for tiller number (*QTiller.ua-5B*) and chlorophyll (*QSpadpost.ua-5B*) on chromosome 5B. Those QTLs explained 8% to 21% of the phenotypic variance, suggesting that chromosomes 5A and 5B have a strong influence on waterlogging tolerance. Findings from this study are parallel to results from Poysa (1984) in which the chromosomes 5A,5B, and 5D contributed to increased flooding tolerance in wheat.

The major QTL under control conditions for shoot biomass, *QSdbio.ua-3B.1*, was localized on chromosome 3B, explaining 33% of the variance. QTL mapping did not detect any QTL for plant height post treatment and elongation under control conditions. These traits had very high 1000 permutation LOD threshold as a result of their high heritability and qualitative inheritance.

QTL clustering and pleiotropy

In quantitative genetics, correlation between traits is produced by pleiotropic effects or by genes which are very close and linked to the traits of interest (Qiu et al. 2007). Thus, the position of the QTLs associated with the correlated traits would be in similar regions in the genome (Qiu et al. 2007). In this experiment, shoot biomass was correlated with root biomass, tiller numbers, root length, and elongation. Plant height pre-treatment was positive correlated with plant height post-treatment. Those results were in agreement with the co-localization of QTLs for correlated traits in similar genomic regions. QTLs for root biomass and shoot biomass co-localized on chromosomes 1B (*QRdbio.ua-1BI, QRfbio.ua-1B, QSfbio.ua-1B*) and on 4D (*QRfbio.ia-4D, QSfbio.ua-4D.1, QSdbio.ua*-4D). This was also observed on chromosome 1B for root biomass and tiller number (*QRdbio.ua-1B, QTiller.ua-1B.*3) and on 5A for elongation and plant height

post treatment (*QElon.ua-5A*, *QHgtpost.ua-5A*). Although QTLs for several traits co-localized in the same genome regions, the favorable alleles at some of these QTLs came from different parents and therefore showed a negative pleiotropic effect. This was common for QTL for SPAD and biomass related traits on chromosomes 2A, 5A, 5B, and 6D and shoot biomass and root length on chromosomes 2A and 6D. Negative pleitropy between traits can make the process of selection, in this case for waterlogging tolerance, more difficult due to the fact that a specific allele can be favorable for one trait but unfavorable for another trait (Mason et al. 2010).

Comparison to known QTLs

QTLs associated with seedling survival under waterlogging have previously been identified on chromosomes 2B, 3B, and 5A in a winter spelta (*Triticum spelta* L.) by winter wheat population (Burgos et al. 2001). In addition, Liang et al. (2010) found a QTL associated with accumulation of dry biomass on chromosome 3B. These results are comparable with the findings in both the greenhouse and field experiments in which QTLs associated with root length (*QRlength.ua-2B*), root length FTI (*QRlengthfti.ua-2B*), and shoot biomass (*QSdbio.ua-2B*) under waterlogging were detected on chromosome 2B. Similarly, *QSfbio.ua-3B.1* for shoot fresh biomass in the field was located on the chromosome 3B. QTLs on the chromosome 5A in the greenhouse associated with plant height post-treatment (*QHgtpost.ua-5A*), elongation (*QElon.ua-5A*), and chlorophyll (*QSpad1.ua-5A*) were expressed under waterlogging.

Height regulating genes in wheat have been mapped on chromosomes 5A (*Rht12*) (Sutka and Kovács 1987) and 2A (*Rht7*) *(Worland et al. 1980).* Several QTLs were located on chromosomes 2A and 5A in this study, including QTLs for height and elongation on chromosome 5A. These results suggest that the presence of dwarfing genes may be implicated in the tolerance of wheat to waterlogging, possibly by faster production of biomass early in the

season prior to soil waterlogging. Likewise, the vernalization gene *Vrn-A1* is located on chromosome 5A and has been also associated with variation in plant height (Griffiths et al. 2012; Kato et al. 1999). Co-localization of QTLs for root and shoot biomass were identified on chromosome 4D in this study, close to the marker barc048. Similar results were found by Bai et al. (2013), in which they suggested that the dwarfing *gene Rht-D1* could be associated with observed differences in biomass. A QTL for shoot dry biomass was detected on the chromosome 2B in a similar position to a QTL reported by An et al. (2006). Previous studies have identified QTLs for chlorophyll on the chromosomes 2A (Diab et al. 2008), 5B (Peleg et al. 2009), 6B (Czyczyło-Mysza et al. 2013). In this study chlorophyll QTL detected by SPAD were mapped on chromosomes 2A, 5B, and 6B in agreement with previous reports.

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Table 1. Trait means, flooding tolerance indices, and paired T-test for parental lines under control and waterlogging conditions

Trait	Control			Waterlogging			Flooding tolerance Index	
	Jaypee	USG 3209	p-value	Jaypee	USG 3209	p-value	Jaypee	USG 3209
Fresh shoot biomass (Gh) (g)	15.23	13.65	0.1200	10.94	8.45	0.0009***	0.72	0.62
Fresh root biomass (Gh) (g)	3.72	2.44	0.0070**	2.32	1.95	0.0226*	0.62	0.80
Dry shoot biomass (Gh) (g)	1.89	1.50	0.0029**	1.36	1.03	0.0008***	0.72	0.69
Dry root biomass (Gh) (g)	0.30	0.19	0.0128*	0.19	0.15	0.0127*	0.63	0.79
Tiller greenhouse (Gh)	5.83	5.54	0.4600	5.32	5.21	0.6450	0.91	0.94
Plant height pre-treatment (Gh) (cm)	7.56	7.97	0.2510	8.0	7.0	0.0100*	1.01	0.88
Root length (Gh) (cm)	36.65	31.48	0.0040**	15.6	15.4	0.7400	0.43	0.49
Plant height post- treatment (Gh) (cm)	19.50	17.23	0.0030**	17.11	15.02	0.0080**	0.88	0.87
Elongation (Gh) (cm)	11.94	10.19	0.0040**	9.13	8.04	0.1312	0.76	0.79
Fresh shoot biomass (F) (g)	245	242	0.6329	168	188	0.5464	0.68	0.78
Dry shoot biomass (F) (g)	59	62	0.8577	38	45	0.1053	0.64	0.72

Gh = Greenhouse
F = Field
* Significant at P = 0.05
** Significant at P = 0.01
*** Significant at P = 0.001

Table 2. Trait means and analysis of variance for the U/J RIL population under control and waterlogging conditions

	RIL Population Means			Analysis of variance (p-value)		
Trait	**Control**	**Water**	**FTI**	**Treatment (T)**	**Genotype (G)**	**GxT**
Fresh shoot biomass (Gh)	13.17	8.89	0.67	0.0401*	0.0001***	0.2180
Fresh root biomass (Gh)	2.95	2.22	0.75	0.2010	0.0050**	0.018*
Dry shoot biomass (Gh)	1.53	1.08	0.70	0.0181*	0.0001**	0.3627
Dry root biomass (Gh)	0.24	0.18	0.75	0.0576	0.0223*	0.0118*
Tiller (Gh)	5.90	5.34	0.90	0.2297	0.0001***	0.7200
Height pre-treatment (Gh)	6.49	6.48	1.00	0.6462	0.0001***	0.6151
Root length (Gh)	34.0	15.46	0.45	0.0001***	0.5725	0.0024**
Height post-treatment Gh)	15.78	14.31	0.90	0.0749	0.0001***	0.1291
Elongation (Gh)	9.34	7.83	0.83	0.0737	0.0001***	0.0661
Fresh shoot biomass (F)	209.8	142.4	0.68	0.0384*	0.0001*	0.9297
Dry shoot biomass (F)	50.08	34.7	0.69	0.0428*	0.0002***	0.8682

* Significant at P = 0.05
** Significant at P = 0.01
*** Significant at P = 0.001
Water = Waterlogging
Gh = Greenhouse
F = Field
G = Genotype
T = Treatment
FTI= Flooding tolerance index = Waterlogging/control

Table 3. Analysis of variance for chlorophyll in the parental lines and the U/J RIL population

Source	Parental lines		RIL population	
	Mean Square	**Pr > F**	**Mean Square**	**Pr > F**
Run	81	0.1011	1155	0.3076
Treatment	1168	0.0162*	16367	0.0217*
Container (run*block*treatment)	13	0.0003**	-	-
Block(run)	10	0.8302	627	0.5466
Error A	49		843	
Genotype	8	0.4467	32	0.0001***
Treatment*genotype	4	0.5826	10	0.0668
Error B	12		10	
Week	325	0.0001***	5446	0.0001***
Week*genotype	6	0.3937	6	0.1648
Week*treatment	125	0.0001***	1737	0.0001***
Week*treatment*genotype	4	0.5734	5	0.9999
Residual	6	.	5	

* Significant at P = 0.05
** Significant at P = 0.01
*** Significant at P = 0.001
Run = 2
Container = 20
Blocks = 4
Weeks = 4

Table 4. Percent variance and broad sense trait heritability under control and waterlogging for the U/J RIL population in the greenhouse

	Waterlogging				Control			
Trait	σ^2_g	σ^2_e	σ^2_{ge}	H^2	σ^2_g	σ^2_e	σ^2_{ge}	H^2
Fresh shoot biomass	14	61	7	42	11	60	1	41
Fresh root biomass	7	82	6	22	11	57	5	38
Dry shoot biomass	10	72	11	30	13	52	1	50
Dry root biomass	6	65	3	26	8	52	7	32
Tiller	7	71	7	24	3	42	2	23
Plant height pre-treatment	30	26	5	77	33	26	4	79
Root length	9	38	2	47	3	74	13	11
Plant height post-treatment	34	19	4	83	60	30	2	88
Elongation	27	36	10	66	44	48	1	78
SPAD	8	43	0	44	13	83	2	37

Table 5. Trait correlations in the U/J RIL population under waterlogging conditions in the greenhouse and field

	Fresh shoot bio (GH)	Fresh root bio (GH)	Dry root bio (GH)	Dry shoot bio (GH)	Tiller no. (GH)	Hgt Pre-trt (Gh)	Root length (GH)	Hgt post-trt (GH)	Elon-gate (GH)	SPAD (GH)	Fresh shoot bio (F)	Dry shoot bio (F)	SPAD (F)
Fresh shoot bio (GH)		0.77*	0.49*	0.84*	0.49*	0.32*	0.41*	0.48*	0.43*	0.38*	0.13*	0.05	0.06
Fresh root bio (GH)			0.76*	0.82*	0.62*	0.13*	0.28*	0.20*	0.19*	0.18*	0.10	0.03	0.01
Dry root bio (GH)				0.72*	0.64*	-0.09*	0.08*	-0.07*	-0.03	-0.12	0.02	0.02	-0.03
Dry shoot bio (GH)					0.68*	0.14	0.16	0.26	0.25	0.15	0.13*	0.03	0.04
Tiller no. (GH)						-0.20	0.04	-0.22	-0.17	-0.01	-0.02	-0.03	-0.05
Hgt pre-trt (GH)							0.34*	0.66*	0.28*	0.3*	0.08	0.10	0.13*
Root length (GH)								0.35*	0.26*	0.43*	0.05	0.01	0.02
Hgt post-trt (GH)									0.91*	0.35*	0.17*	0.09	0.20
Elongate (GH)										0.27*	0.15*	0.05	0.15
SPAD (GH)											0.01	0.02	0.18*
Fresh shoot bio (F)												0.78*	0.39*
Dry shoot bio (F)													0.31*

*Significant at P =0.05

GH = Greenhouse Hgt pre-trt = Height pre-treatment Hgt post-trt= Height post- treatment

F = Field Elongate= Elongation Bio = Biomass

Table 6. QTLs associated with agronomic and physiological traits under waterlogging and control conditions in the U/J RIL population

QTL	Chrom	Pos (cM)	Marker	LOD	R^2	Add	Allele
Elongation							
QElon.ua-5A(WGH)	5A	28	gwm415	7.8	0.19	0.70	U
Height post-treatment							
QHgtpost.ua-5A(WGH)	5A	25	gwm415	10.9	0.21	1.36	U
Height pre-treatment							
QHgtpre.ua-5A(WGH)	5A	25	wpt1712	13.8	0.28	0.61	U
Root dry biomass							
QRdbio.ua-1B(WGH)	1B	184	gwm259	3.7	0.18	0.02	U
Root fresh biomass							
QRfbio.ua-1B(WGH)	1B	211	wmc728v	6.6	0.22	0.29	U
QRfbio.ua-4D(WGH)	4D	0	cfd054	5.3	0.12	0.22	U
QRfbio.ua-6B.1(WGH)	6B	16	wpt4164	4.2	0.23	0.30	U
QRfbio.ua-6B.2(WGH)	6B	88	wpt7576	6.2	0.15	0.27	J
Root length							
QRlength.ua-2A(WGH)	2A	179	barc1015	4.4	0.08	0.50	J
QRlength.ua-2B(WGH)	2B	18	barc55p	4.9	0.11	0.68	J
QRlength.ua-5A.1(CGH)	5A	16	wpt0302	3.3	0.12	1.65	U
QRlength.ua-5A.2(CGH)	5A	28	gwm415	3.4	0.10	1.39	U
QRlength.ua-6D(WGH)	6D	0	barc273	3.8	0.08	0.52	J
Root length FTI							
QRlengthfti.ua-2B(WGH)	2B	122	gwm047	3.6	0.10	0.03	J
Shoot dry biomass							
QSdbio.ua-1A(WGH)	1A	19	cla2153v	4.9	0.10	0.09	U
QSdbio-2A.1(CGH)	2A	0	cfd36v	6.9	0.23	0.14	J
QSdbio.ua-2A.2(WGH)	2A	186	barc1015	5.2	0.11	0.10	U
QSdbio.ua-2B(WGH)	2B	165	gwm501b	3.2	0.12	0.11	J
QSdbio.ua-3B.1(CGH)	3B	70	gwm533	3.4	0.33	0.19	J
QSdbio.ua-4D(WGH)	4D	7	barc048	5.3	0.14	0.12	U
QSdbio.ua-6A(CGH)	6A	4	wpt9131	5.0	0.14	0.12	J
QSdbio.ua-6D(WGH)	6D	0	barc273	3.6	0.06	0.07	U
Shoot fresh biomass							
QSfbio.ua-1B(WGH)	1B	204	wmc728v	6.7	0.27	0.98	U
QSfbio.ua-2D(WF) [a]	2D	5	cfd053	2.5	0.06	11.58	U
QSfbio.ua-3B(WF)[a]	3B	111	cfd053	2.6	0.07	11.73	J
QSfbio.ua-4D.1(WGH)	4D	0	cfd054	3.2	0.08	0.57	U

Table 6. continued

QTL	Chrom	Pos (cM)	Marker	LOD	R^2	Add	Allele
QSfbio.ua-4D.2(CGH)	4D	18	gwm194	5.0	0.14	0.65	U
QSfbio.ua-5B(CGH)	5B	49	wpt6971	5.0	0.18	0.90	J
QSfbio.ua-6A(CGH)	6A	4	wpt9131	5.3	0.17	0.72	J
Shoot fresh FTI							
QSffti.ua-1B(WGH)	1B	198	gwm259	3.2	0.14	0.05	U
Spad week 1							
QSpad1.ua-3B(CGH)	3B	100	gwm340	4.2	0.25	0.63	J
QSpad1.ua-5A(WGH)	5A	29	gwm415	3.3	0.10	0.45	J
QSpad1.ua-5B(WGH)	5B	158	wpt0935	4.8	0.13	0.89	J
QSpad1.ua-6B.1(WGH)	6B	7	wpt9356	9.0	0.22	0.81	U
QSpad1.ua-6B.2(WGH)	6B	87	wpt7576	5.8	0.14	0.53	J
Spad week 2							
QSpad2.ua-1D(WGH)	1D	13	wpt2206	4.3	0.10	0.58	J
QSpad2.ua-2B(CGH)	2B	37	wmc261N	3.7	0.09	0.61	J
Spad week 3							
QSpad3.ua-1B(CGH)	1B	34	scm09	3.5	0.08	0.39	U
QSpad3.ua-1D.1(WGH)	1D	12	wpt2206	6.2	0.20	0.63	J
QSpad3.ua-1D.2(CGH)	1D	26	wpt2206	3.0	0.24	1.08	J
QSpad3.ua-2B(CGH)	2B	145	gwm388	4.5	0.20	0.84	J
Spad week 4							
QSpad4.ua-1B(WGH)	1B	1	ba128p2	3.1	0.14	0.61	U
QSpad4.ua-1D(CGH)	1D	13	wpt2206	3.2	0.11	1.09	J
QSpad4.ua-2A(WGH)	2A	172	wpt8826	5.4	0.18	0.88	J
QSpad4.ua-5B(CGH)	5B	182	wpt8094	3.8	0.23	1.16	J
QSpad4.ua-6B(CGH)	6B	0	wpt9660	5.1	0.14	0.90	U
Spad post-treatment							
QSpadpost.ua-5B(WF)	5B	37	wpt3616	3.2	0.11	0.99	J
Tiller							
QTiller.ua-1B.1(CGH)	1B	3	ba128p2	4.0	0.14	0.35	J
QTiller.ua-1B.2(WGH)	1B	47	gwm273f	4.5	0.09	0.35	J
QTiller.ua-1B.3(WGH)	1B	156	bar188p2	5.7	0.32	0.65	U
QTiller.ua-4B(CGH)	4B	2	wpt1046	4.9	0.14	0.34	U
QTiller.ua-4D(CGH)	4D	18	gwm194	4.0	0.10	0.33	U
QTiller.ua-5B(WGH)	5B	50	wpt7238	3.8	0.08	0.39	U

WGH = Waterlogging greenhouse;
CGH = control greenhouse; F = Waterlogging field; Chrom = Chromosome Pos = Position
Add = Additive effect ; U = USG3209; J = Jaypee
[a] = Putative QTLs under 1000 permutation threshold

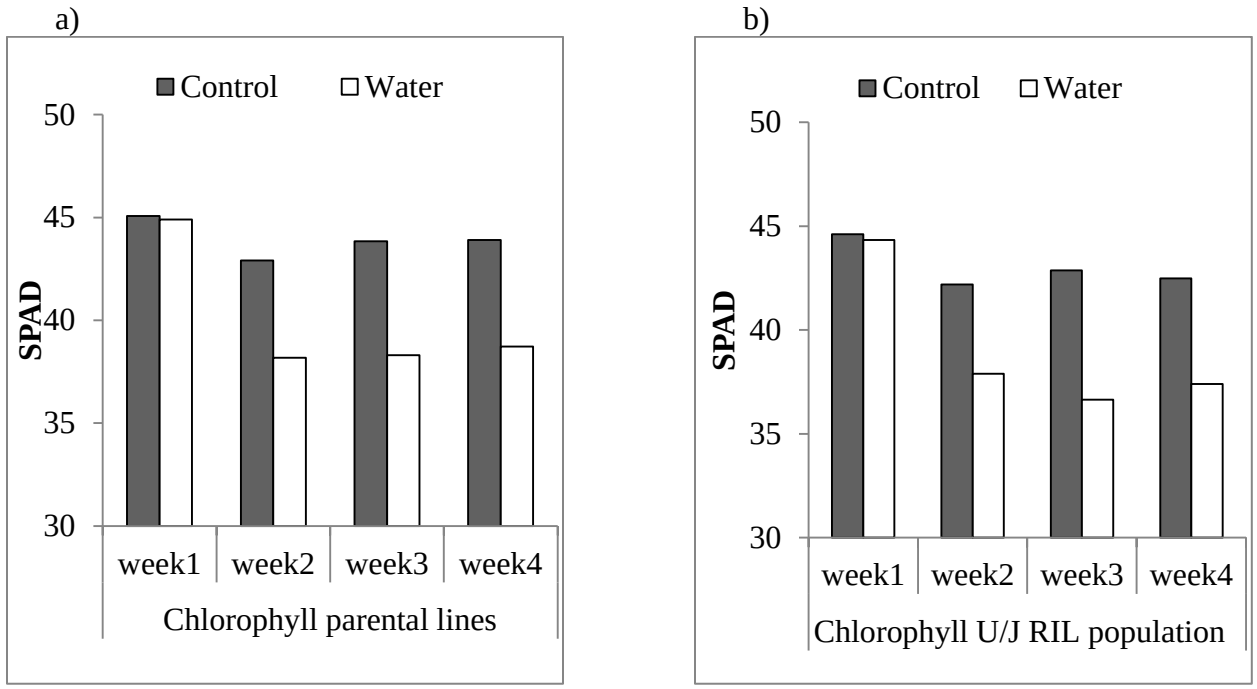


Figure 1. a) Chlorophyll content means for the parental lines in the greenhouse. b) Chlorophyll means for the U/J RIL population in the greenhouse. LSD to compare weeks for same treatment in the parents = 5.82 and U/J RIL 0.28. LSD to compare means different treatments in the parents = 3.39 and U/J RIL 2.87

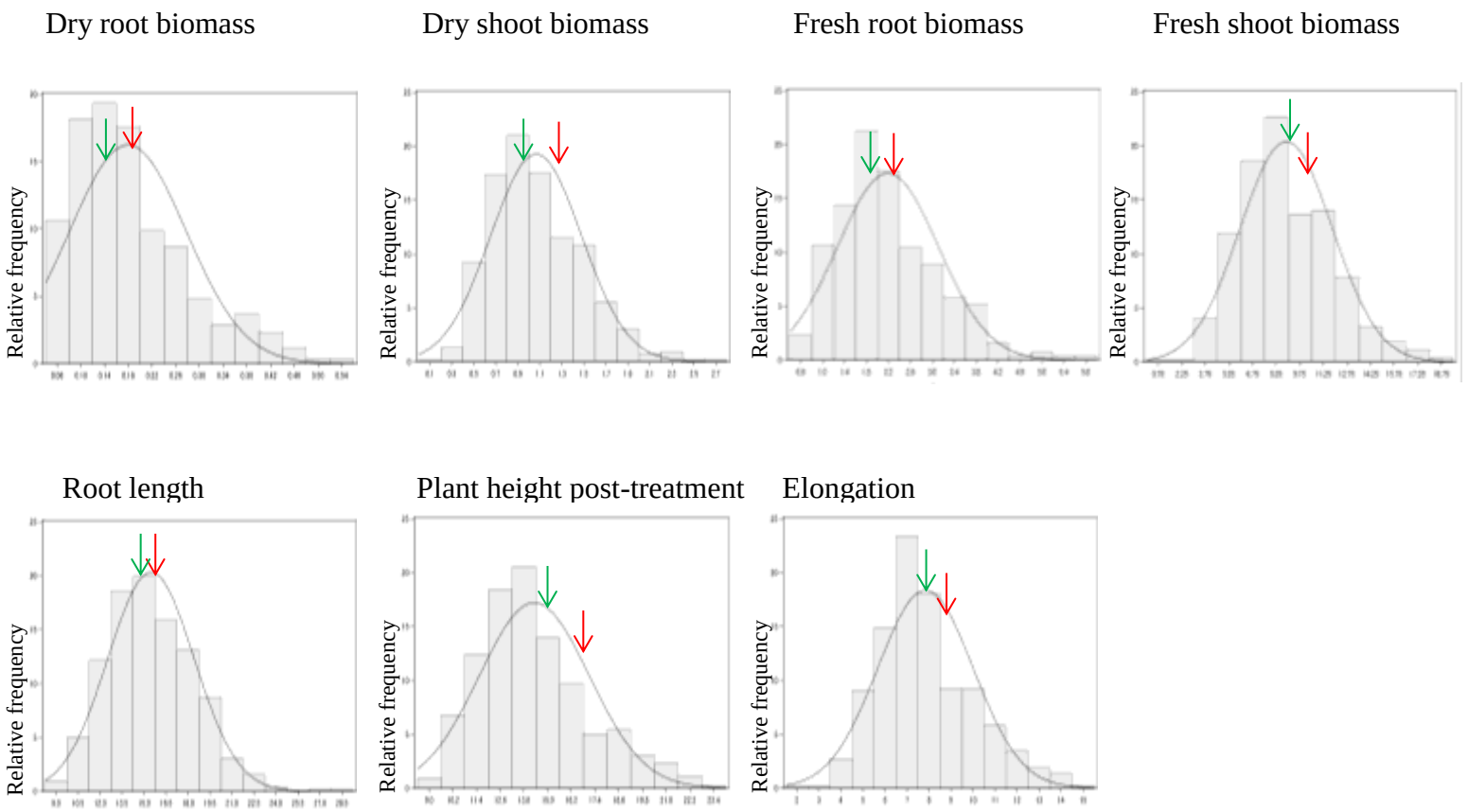


Figure 2. Frequency distribution of agronomic traits under waterlogging conditions for the U/J RIL population in the greenhouse

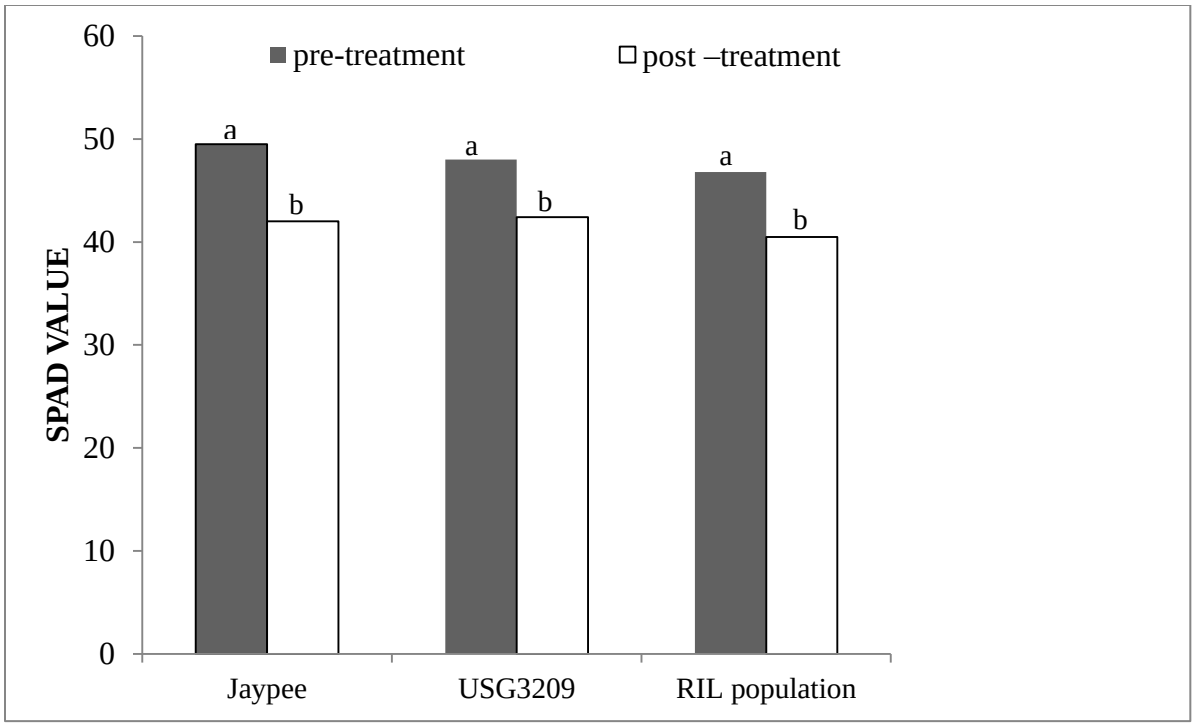


Figure 3. Chlorophyll content means for parental lines and U/J RIL population under waterlogging conditions in the field. Means followed by different letter are significantly different at α=0.05 within pairs of columns

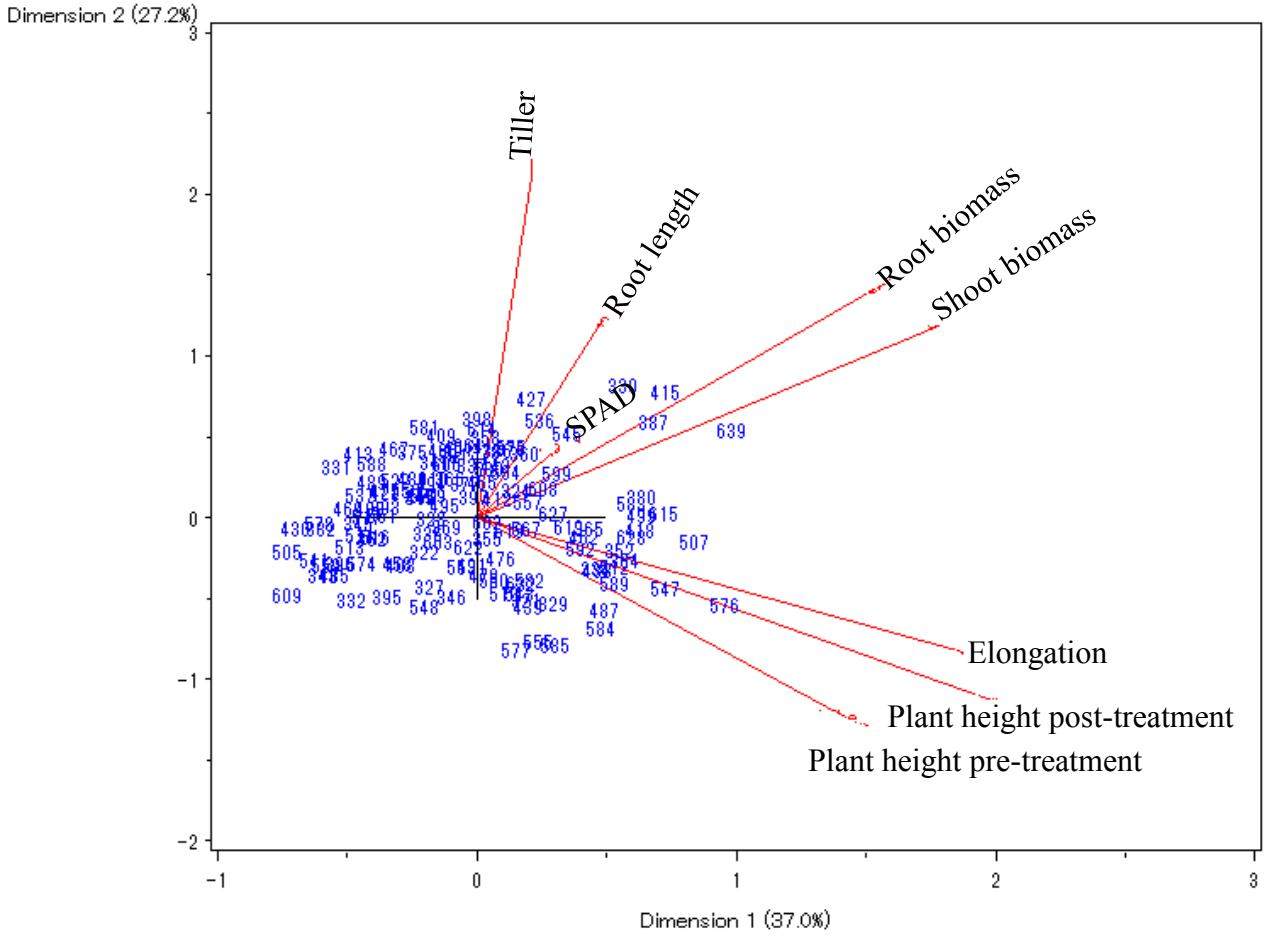
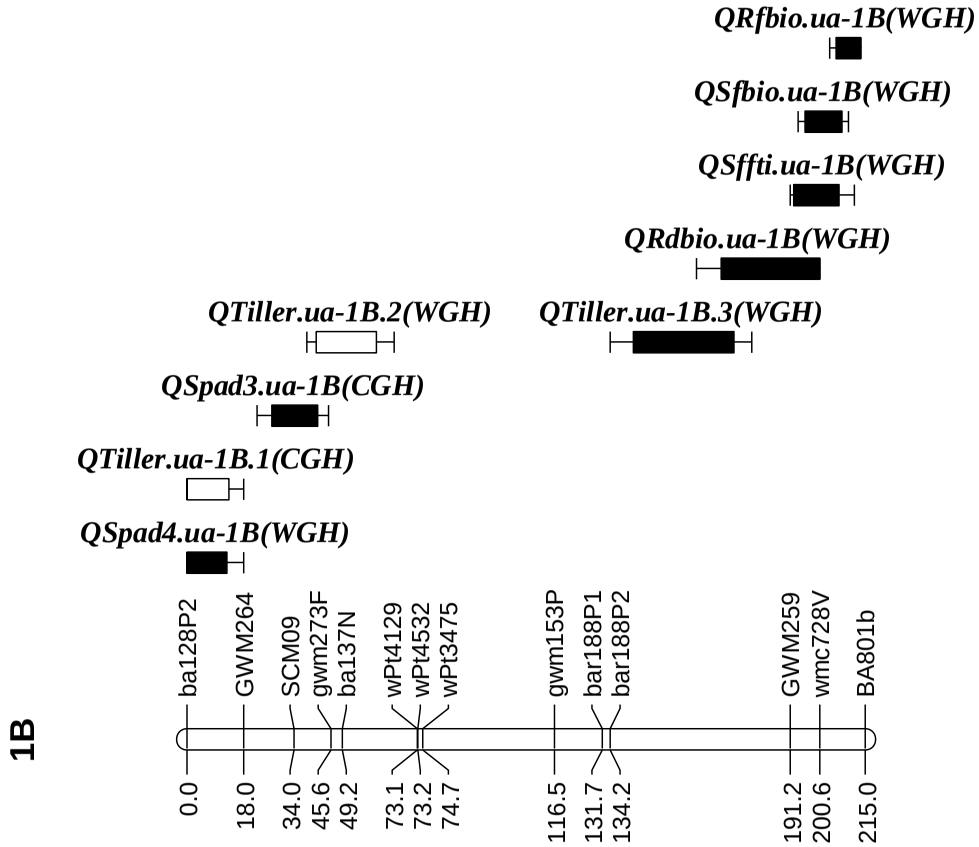


Figure 4. Principal component analysis bi-plot for agronomic and physiological traits in the U/J RIL population under waterlogging greenhouse conditions.


QRfbio.ua-1B(WGH)
QSfbio.ua-1B(WGH)
QSffti.ua-1B(WGH)
QRdbio.ua-1B(WGH)
QTiller.ua-1B.2(WGH)
QTiller.ua-1B.3(WGH)
QSpad3.ua-1B(CGH)
QTiller.ua-1B.1(CGH)
QSpad4.ua-1B(WGH)
1B
ba128P2
GWM264
SCM09
gwm273F
ba137N
wPt4129
wPt4532
wPt3475
gwm153P
bar188P1
bar188P2
GWM259
wmc728V
BA801b
0.0
18.0
34.0
45.6
49.2
73.1
73.2
74.7
116.5
131.7
134.2
191.2
200.6
215.0

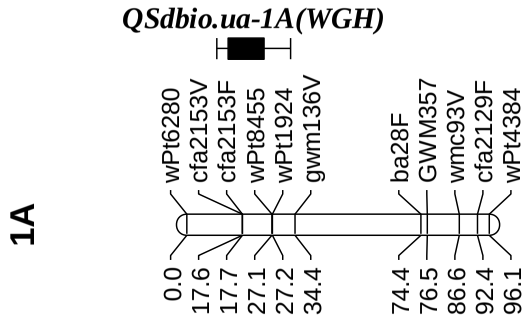
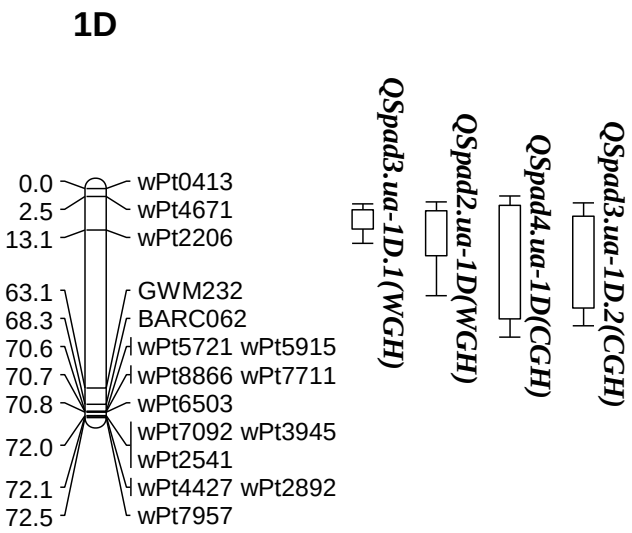

QSdbio.ua-1A(WGH)
1A
wPt6280
cfa2153V
cfa2153F
wPt8455
wPt1924
gwm136V
ba28F
GWM357
wmc93V
cfa2129F
wPt4384
0.0
17.6
17.7
27.1
27.2
34.4
74.4
76.5
86.6
92.4
96.1

Figure 5 continued



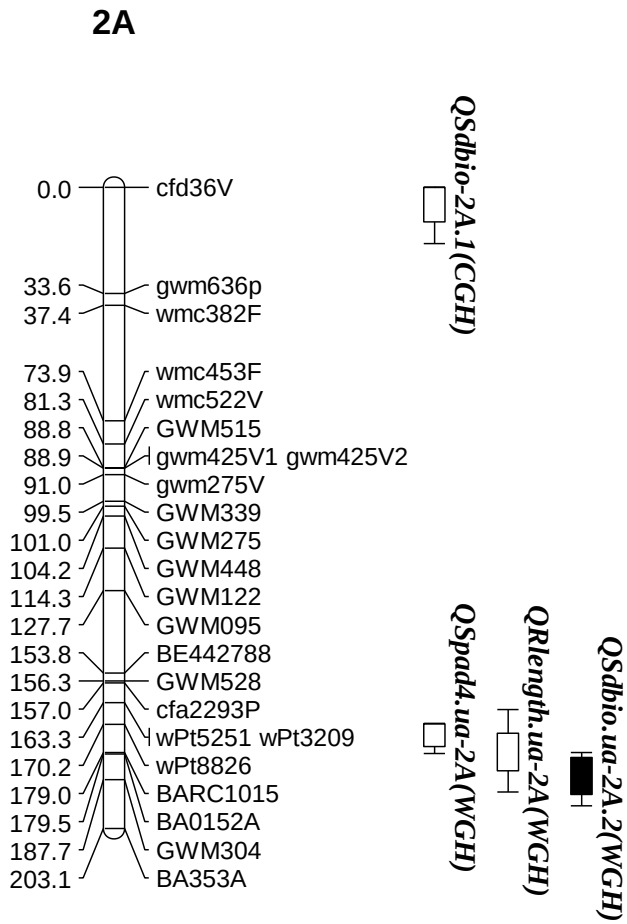


Figure 5 continued

Figure 5 continued

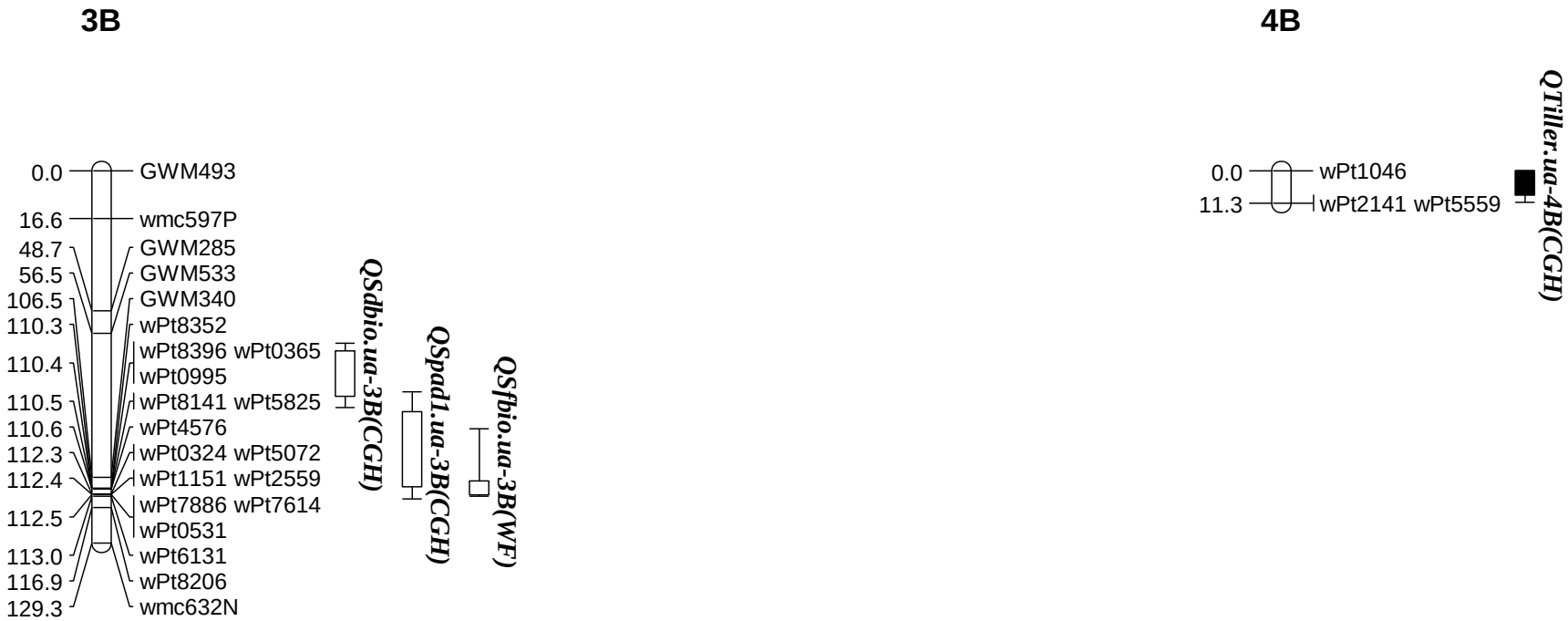
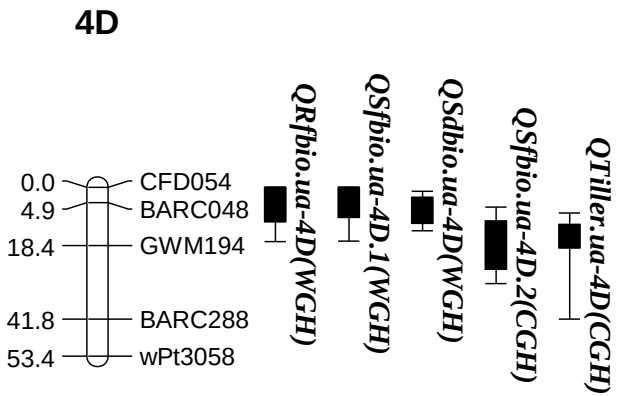


Figure 5 continued



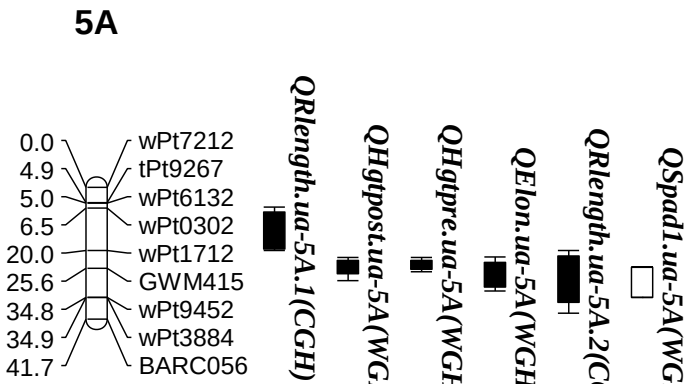
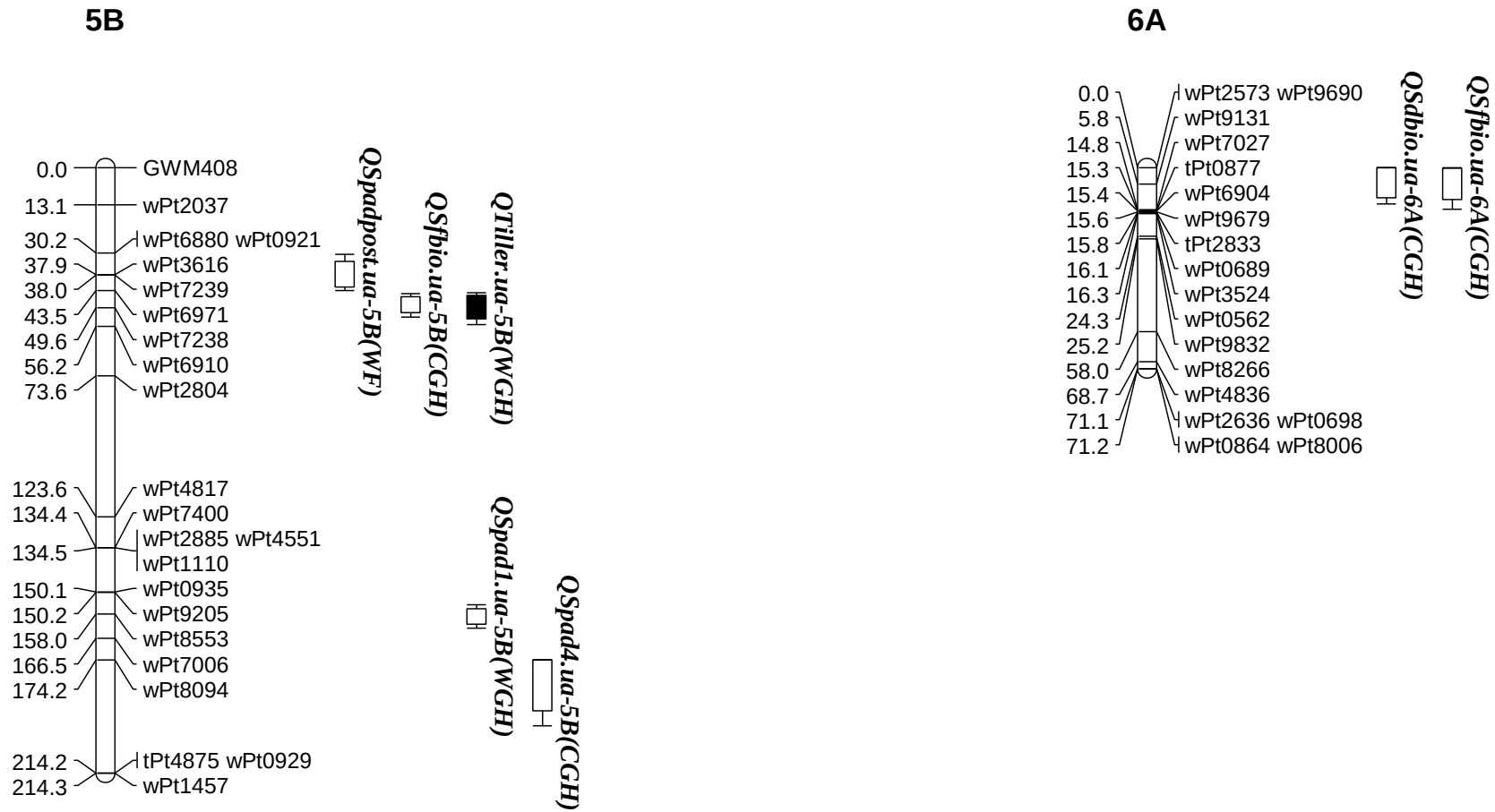


Figure 5 continued



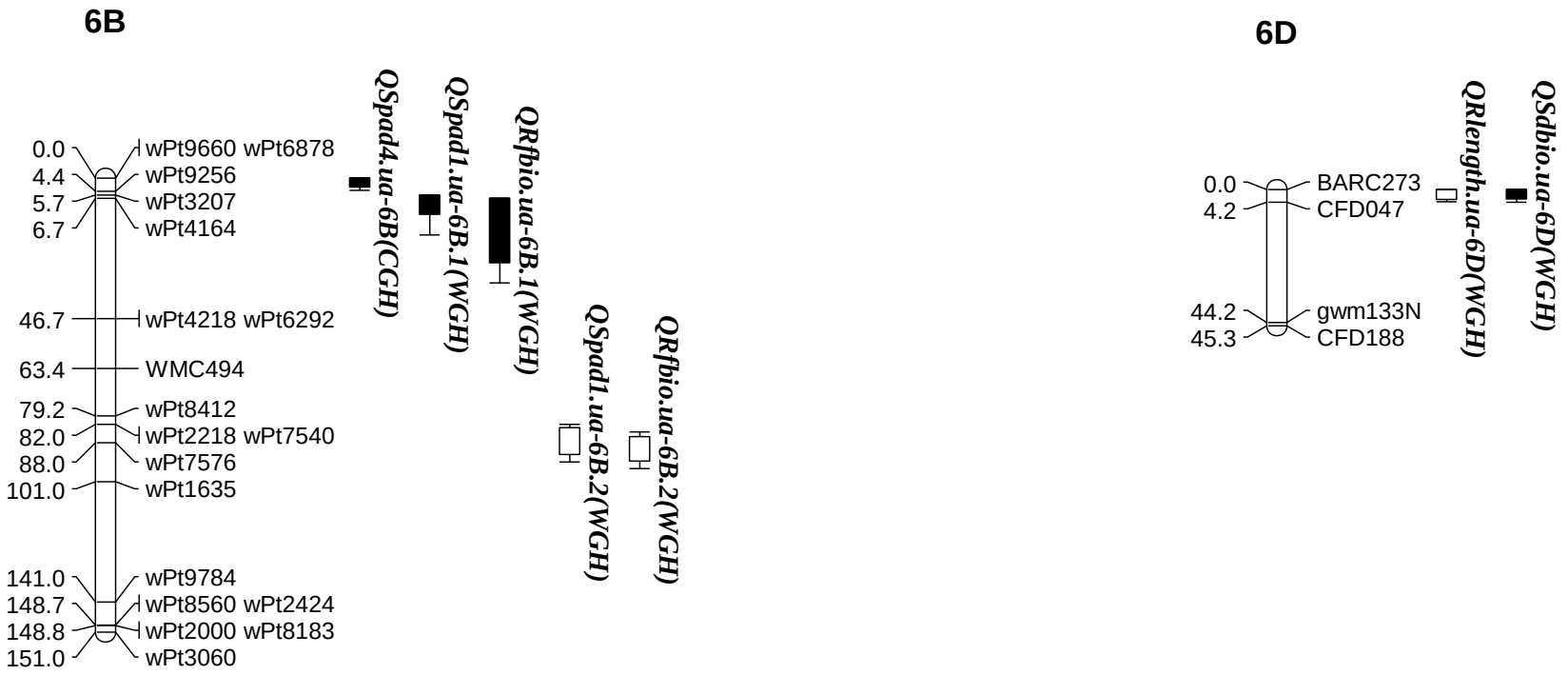


Figure 5. QTLs associated with agronomic and physiological traits under waterlogging and control conditions in the U/J RIL population . Characteristics of QTLs are summarized in table 6. Abbreviations: Sfbio (Shoot fresh biomass), Sdbio (Shoot dryh biomass), Rfbio (Root fresh biomass), Rdbio (Root dry biomass), Rlength (Root length), Hgtpre (Plant height pre-treatment), Hgtpost (Plant height post-treatment), Spad1 (Chlorophyll week1), Spad2 (Chlorophyll week2), Spad3 (Chlorophyll week3), Spad4 (Chlorophyll week4), Tiller (Tiller numbers), Spadpre (Chlorophyll pretreatment), Spadpost (Chlorophyll post-treatment), Sffti (Shoot fresh flooding tolerance index), Rlengthfti (Root length flooding tolerance index).QTLs with empty bars designating QTL from Jaypee and dark bars representing QTL from USG3209.

Chapter III
Overall Conclusions

Overall Conclusions

Waterlogging is an abiotic stress with catastrophic effects on wheat growth and development. The results presented here confirmed this, as vegetative stage waterlogging significantly reduced biomass and growth traits. Interestingly, the number of tillers was not affected by waterlogging stress in either the parents or U/J RILs, indicating a possible tolerance mechanism present in this soft red winter wheat germplasm. While genetic variation was detected for most traits, only root related traits showed genotype by treatment interaction, indicating that it would be possible to select plants that have different root responses depending on environment and thus select for improved waterlogging tolerance. The high correlation between root and shoot biomass indicated the possibility of indirect selection which could prove extremely useful given the difficulty in phenotyping roots in wheat. The data shows the likelihood of wheat using the "Low oxygen escape syndrome" to survive in a waterlogging environment, although future work is necessary to better understand this mechanism. Genetic variation within the U/J RIL population allowed for identification of QTLs underlying tolerance to waterlogging conditions. A total of 53 QTLs were detected, including 34 specific to the waterlogging treatment and these were located on 14 of the 21chromosomes. The 53 QTLs were clustered in 19 genomics regions. Chromosome 5A could have an important role conferring waterlogging tolerance in wheat due to the presence of loci that affect plant height and flowering time. A positive pleiotropic association between QTLs for plant height, elongation, and root length was observed on chromosome 5A and selection for these traits and QTL would help to select taller plants with greater tolerance to waterlogging. QTLs for root biomass on chromosomes 1B, 4D, and 6B were observed only in the waterlogging treatment and are likely adaptive QTL.

Root biomass traits were most sensitive to waterlogging stress, had relatively low heritability, genotype by treatment interaction and are the most difficult to phenotype. Therefore, molecular marked assisted selection could be used to select plant with higher root biomass that would help to improve productivity in a waterlogged environment.