

7-2015

Association Mapping for Elemental Concentration Resulting from Vegetative Stage Soil Waterlogging Stress in Wheat (*Triticum aestivum* L.)

Maria Nelly Arguello-Blanco
University of Arkansas, Fayetteville

Follow this and additional works at: <http://scholarworks.uark.edu/etd>

 Part of the [Agronomy and Crop Sciences Commons](#), and the [Plant Breeding and Genetics Commons](#)

Recommended Citation

Arguello-Blanco, Maria Nelly, "Association Mapping for Elemental Concentration Resulting from Vegetative Stage Soil Waterlogging Stress in Wheat (*Triticum aestivum* L.)" (2015). *Theses and Dissertations*. 1239.
<http://scholarworks.uark.edu/etd/1239>

This Thesis is brought to you for free and open access by ScholarWorks@UARK. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of ScholarWorks@UARK. For more information, please contact scholar@uark.edu, ccmiddle@uark.edu.

Association mapping for elemental concentration resulting from vegetative stage soil
waterlogging stress in wheat (*Triticum aestivum* L.)

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Crop, Soil & Environmental Sciences

By

Maria Nelly Arguello-Blanco
Universidad Nacional de Colombia
Agronomical Engineer, 2010

July 2015
University of Arkansas

This thesis is approved for recommendation to the Graduate Council.

Dr. R. Esten Mason
Thesis Director

Dr. Edward Gbur
Committee Member

Dr. Trenton Roberts
Committee Member

Dr. Ainong Shi
Committee Member

Overall Abstract

Soil waterlogging (WL) affects wheat production worldwide, including the southern of the U.S. Little is known about the genetics of tolerance mechanisms to WL, particularly of potentially toxic elements such as aluminum, iron and sodium. The objectives of this study were to use association mapping (AM) to identify SNP associated with the EC in the vegetative tissue of a set of 240 diverse soft winter wheat genotypes (AM panel) grown under field WL and to determine the impact of WL on wheat yield (GY) and yield components in a set 28 adapted cultivars (YT)

YT study showed reductions in GY of 42%, resulting of the combined effect of decreases in total biomass, kernel-weight per spike and spikes m^{-2} . Strong negative correlations were observed between GY and accumulation of aluminum, iron and sodium, indicating likely elemental toxicity. Cultivars 'Jamestown' and 'USG3555' were found to be the most tolerant to WL, as these prevented large reductions in spikes m^{-2} , kernel weight per spike, kernel number per spike, thousand kernel weight and thus total grain yield.

The AM panel was evaluated over two growing seasons in both Stuttgart Arkansas (AR13 and AR14) and St. Joseph Louisiana (LA13 and LA14) under WL. Elemental accumulation (EC) measured using ICP-AES found significant genotypic variation for Al in both AR13 and LA14, for Fe in AR13 and for Na in AR13 and AR14, with the percent of phenotypic variation explained by genotype ranging from 0 to 60%. Overall, EC were higher in the 2012-2013 than in 2013-2014 growing season. AM for aluminum, sodium and iron identified nine chromosomes that had marker-trait associations that were consistently detected at the same SNP or SNPs in LD (<30 cM) across multiple data-sets. Overall, detected individual SNPs explained a small percent of the phenotypic variance, ranging from $R^2 = 0.03$ to 0.07, indicating variation in EC to be

quantitatively inherited within this AM panel. Chromosomes 2B and 5A were found to contain the majority of SNP with Fe, Na and Al concentration. This is the first study evaluating genetic diversity for the EC in wheat under WL.

Acknowledgements

I would like to express my deepest gratitude to my advisor, Dr. Esten Mason, for giving me the chance to join his wheat breeding and genetics team, for his guidance, patience, and providing me with an excellent working atmosphere and encouragement for finishing this research.

I would like to thank Dr. Edward Gbur, Dr. Trent Roberts and Dr. Ainong Shi for serving on my Master's research committee for their guidance, advice and assisting me in the data analysis process.

I would also like to thank all staff in the Department of Crop, Soil and Environmental Sciences at the University of Arkansas for all their assistance during the time spent here, and to the University of Arkansas Agriculture Research and Extension Center for allowing me to use their facilities where this research was conducted.

Finally, I would like to express how thankful I am for all the help, patience and encouragement of the wheat breeding and genetics laboratory crew. Thanks for such great opportunity to work with you all, everything is awesome when you are part of a team living a dream.

I would finish here by acknowledging my friends for always taking care of Jhon while I was away working in the wheat fields, their love and care for him is incredibly helpful.

Dedication

To

Jhon Fajardo y Cenaida Arguello

Table of content

CHAPTER I.....	1
INTRODUCTION AND LITERATURE REVIEW	1
Introduction.....	2
Literature review	4
Wheat background	4
Wheat breeding for yield and stress tolerance	5
Wheat production is affected by waterlogging	6
Plant adaptation to waterlogging	8
Linkage and association mapping.....	11
Quantitative trait loci for waterlogging tolerance.....	13
Quantitative trait loci associated with elemental toxicity tolerance	15
CHAPTER II.....	24
ELEMENTAL TOXICITY: IMPACT ON GRAIN YIELD AND ASSOCIATION MAPPING FOR ALUMINUM, SODIUM AND IRON CONCENTRATION	24
Introduction.....	26
Material and Methods	29
Plant material and experimental design for association mapping.....	29
Plant material and experimental design for yield trial	30
Elemental accumulation analysis.....	31
Statistical analysis of phenotypic data	31
Genome wide association analysis (GWAS)	32
Results.....	33
Correlations of yield and elemental accumulation under waterlogging	33
Elemental accumulation in the AM panel.....	34
Population structure, kinship and linkage disequilibrium in the AM panel	35
Genome-wide association analysis (GWAS) for elemental accumulation	35
Discussion.....	37
GY reduction associated with accumulation of elements	37
Population structure of the AM panel.....	39
Genome-wide association analysis for Al, Fe and Na	40

References Chapter II	51
CHAPTER III	57
SOIL WATERLOGGING: IMPACT ON GRAIN YIELD AND YIELD COMPONENTS IN SOFT RED WINTER WHEAT GERMPLASM	57
Abstract	58
Introduction	59
Material and Methods	61
Plant material and field experiments	61
Trait measurement	62
Statistical analysis	62
Results	63
Impact of WL on yield and yield components	63
Analysis of variance	63
Trait correlations	64
Differences between cultivars	64
Discussion	65
Impact of WL stress on wheat yield	65
Cultivar performance	66
References Chapter III	72
CHAPTER IV	74
OVERALL CONCLUSIONS	74
Conclusions Chapter II	75
Conclusions Chapter III	76

List of tables

Tables Chapter II

Table 1 Descriptive statistics for yield and elemental concentrations and percentage of variance attributed to genetic, treatment, genotype x treatment and other effects in 28 wheat genotypes evaluated under waterlogging and non-waterlogging conditions in Stuttgart, AR 2012-2013. ... 42

Table 2 Descriptive statistics for elemental concentrations and percentage of variance attributed to genetic and other effects in 240 wheat genotypes evaluated under waterlogging conditions across four site-years..... 43

Table 3 Summary of marker-trait associations for aluminum, iron and sodium concentrations in 240 wheat genotypes using 5227 markers. 44

Tables Chapter III

Table 1. Descriptive statistics for yield and yield components and percentage of variance attributed to genetic, treatment, genotype x treatment and other effects in 28 wheat genotypes evaluated under waterlogging and non-waterlogging conditions in Stuttgart, AR 2012-2013. ... 68

Table 2 Descriptive statistics for yield and yield components and percentage of variance attributed to genetic, treatment, genotype x treatment and other effects in 28 wheat genotypes evaluated under waterlogging and non-waterlogging conditions in Stuttgart, AR..... 69

Table 3 Phenotypic correlations (r) of yield and yield components with total grain yield in 28 wheat genotypes..... 70

Table 4 Five most tolerant and susceptible cultivars based on percent reduction of yield and yield components under waterlogged versus non-waterlogged conditions. 71

Figures Chapter II

Fig. 1 Waterlogging treatment in Stuttgart, AR 2014	46
Fig. 2 Correlogram of P phosphorus, K potassium, Ca calcium, Mg magnesium, S sulfur, Na sodium, Fe iron, Mn manganese, Zn zinc, Cu copper, B boron, Al aluminum and GY grain yield in a wheat under waterlogging stress in Stuttgart, AR. Diameter of the circle increases with the correlation value.....	47
Fig. 3 Principal component analysis for population structure in 240 wheat genotypes using 5227 single nucleotide polymorphism markers	48
Fig. 4 Heat map of the kindship relationship in 240 wheat genotypes using 5227 SNP markers	49

CHAPTER I
INTRODUCTION AND LITERATURE REVIEW

Introduction

Wheat is the most important staple food for humans, supplying more than 20% of total consumed calories (Peng et al. 2011; Shepherd et al. 2010) and cultivated on 17% of all crop land worldwide (Peng et al. 2011). As a result of its widespread cultivation, wheat production faces many constraints, including both biotic and abiotic factors (Spiertz 2012). With global climate change predicted to result in increased winter precipitation and waterlogging of cereals, improving the tolerance of wheat to soil waterlogging is a growing priority area for breeders in affected regions. Waterlogging is described as the saturation of the pore spaces in the soil and results in an energy crisis within the plant due to low ATP (Adenosine triphosphate) production resulting from a decrease in root respiration and an increase in trace element availability resulting in toxicity (Colmer and Voesenek 2009). In combination, these two abiotic factors can decrease wheat yield by as much as 50%, resulting from poor establishment and root development, decreased biomass and plant senescence. In Arkansas more than US\$20 million in economic losses were reported in 2011 as a result of soil waterlogging and flooding (Watkin et al. 2011). Adaptive traits known to be involved in tolerance to waterlogged soils include the development of root aerenchyma, vigorous stem elongation and resistance to element toxicity. Collectively, these adaptive traits contribute to a stay-green phenotype and more biomass for yield production.

Despite the impact of waterlogging on wheat yield, very little is known about the genetic control of tolerance mechanisms and in particular tolerance to ion accumulation and elemental toxicity during waterlogging. Therefore, the objectives of this research are as follows:

Objective 1. Determine the impact of vegetative stage waterlogging on wheat yield and yield components in adapted soft red winter wheat.

Objective 2. Determine the genetic diversity for elemental toxicity tolerance in adapted soft red winter wheat and its impact on wheat yield and tolerance to waterlogged soils.

Objective 3. Identify quantitative trait loci (QTL) associated with elemental toxicity tolerance using an association mapping approach.

To accomplish these three objectives, two studies were carried out. The first study evaluated 28 adapted soft red winter wheat (SRWW) cultivars and breeding lines under waterlogged and non-waterlogged conditions in two locations. In this study, the impact of waterlogging on wheat yield and yield components and the relationship between elemental accumulation and wheat yield were investigated. The results of this study provide a better understanding of the impact of soil waterlogging on yield, tolerance mechanisms and recommendations to producers on the tolerance levels of currently grown SRWW cultivars. The second study characterized elemental accumulation under waterlogging in a panel of 240 soft winter wheat lines and identify single nucleotide polymorphism markers associated with variation in elemental accumulation using an association mapping approach. The results of this study provide molecular markers to wheat breeders that can be used for marker assisted selection to improve adaptation to environments prone to waterlogging.

Literature review

Wheat background

Wheat is the most important staple food for humans, supplying more than 20% of total consumed calories (Peng et al. 2011; Shepherd et al. 2010). Wheat-derived products provide essential amino acids, minerals, vitamins, beneficial phytochemicals and dietary fiber components to the human diet (Shewry 2009). A serving of 100 grams of commercially prepared whole-wheat bread provides 247 Kcal, including 12.95 grams of protein, 3.35 grams of total lipids, 41.29 grams of carbohydrates, 6.6 grams of total dietary fiber and 5.57 grams of total sugars (USDA 2011).

Wheat is cultivated on 17% of all crop land worldwide (Peng et al. 2011). Although optimal conditions are required for wheat to perform at its highest potential, it is a broadly adapted crop in terms of latitude, temperature, soil moisture and precipitation (Mergoum et al. 2009). Wheat is primarily grown in China, the European Union, India and the United States. In the United States, this field crop is third in planted area, as well as in gross return for farmers, after corn and soybean. Since 1992, the United States has contributed 10% to total wheat production worldwide (USDA 2012b). During 2011-2012, wheat was grown on 22.01 million hectares throughout the country with total production of 54.413 million metric tons (USDA 2012a).

Wheat belongs to the Poaceae family and Pooideae sub-family of grasses, with a center of origin in the Levant region of the Near East (Mergoum et al. 2009). Wheat is an allopolyploid species, with hexaploid bread wheat (*Triticum aestivum*, $2n=6x=42$, genomes AuAuBBDD) and tetraploid pasta wheat (*T. durum*, $2n=4x=28$, genomes AuAuBB) being the predominantly cultivated forms. Hybridization of wild diploid wheat (*T. urartu*, genome AuAu) and goat grass

1 (*Aegilops speltoides*, genome SS), a close ancestor of the BB genome, generated wild emmer wheat (*T. dicoccoides*, AuAuBB) (Dubcovsky and Dvorak 2007). Through selection, a cultivated emmer (*T. dicoccum*, genomes AABB) was created and its hybridization with *A. tauschii* (genome DD) produced *T. spelta* (genomes AuAuBBDD). Subsequent natural mutation resulted in the evolution of free threshing ears in both emmer and spelt, resulting in *T. durum* and *T. aestivum*, respectively (Peng et al. 2011).

The economic importance of wheat has led to many cytogenetic and genetic studies. However, due to the complexity and genome size (15961 Megabases for bread wheat; 11660 Megabases for durum), genomic resources for wheat still lag behind other species such as rice *Oryza sativa*, maize *Zea mays* and soybean *Glycine max*. Nevertheless, a more comprehensive understanding of the wheat genome is being developed using new techniques and technologies that are quickly advancing (Paux et al. 2012). Recent sequencing of the diploid ancestors of the A genome (Ling et al. 2013) and D genome (Jia et al. 2013) will aid in future gene discovery and genetic improvement.

Wheat breeding for yield and stress tolerance

With increasing population, the demand for wheat and wheat-derived products also increases. Therefore, wheat supply needs to be augmented in order to overcome the deficit (Paux et al. 2012; Sajjad et al. 2012). In addition, cultivated land area is limited, hence wheat breeding for increased yield, particularly in stressed environments is a current and future challenge to breeders (Araus et al. 2008; Mergoum et al. 2009; Sajjad et al. 2012).

Increasing yield has always been the main objective in wheat breeding programs. This can be achieved through both conventional breeding, which considers grain yield as the targeted trait and analytical breeding, which takes in consideration the heritability and genotype-by-environment interaction of traits such as grain yield, yield components, and quality traits (Araus et al. 2008; Mergoum et al. 2009). Such traits are considered to be quantitatively inherited, are controlled by many genes known as quantitative trait loci (QTL) and are significantly influenced by the environment (Collard et al. 2005).

The creation of new wheat varieties through conventional schemes considers performance evaluation of a genotype in different environments and subsequent selection of stable genotypes containing as many desirable characters as possible (Araus et al. 2008; Mergoum et al. 2009). Due to the complexity of many important agronomic traits, other approaches including molecular biology and physiological trait breeding are often used within a breeding program to complement the overall wheat improvement goal (Reynolds and Trethowan 2007). Breeding for tolerance to cold, drought, heat, pre-harvest sprouting, salinity and waterlogging (WL) have been associated with improved genotype performance and can therefore be incorporated in breeding programs to improved overall yield potential (Mir et al. 2012).

Wheat production is affected by waterlogging

In general, crop production has many constraints, including both biotic and abiotic factors (Spiertz 2012). WL affects up to 10% of global land area, (Ruttan 2005) including 10% to 25% of the worldwide area planted to wheat (Powell et al. 2012), and has become an increasing

problem throughout the United States. In Arkansas more than US\$20 million in economic losses were reported in 2011 (Watkin et al. 2011).

Waterlogging affects the physiology and metabolism of the plant, resulting in reduced growth and accelerated development, ultimately lowering yield and yield components. Wheat has shown a 68% root growth reduction, up to 50% reduction in tiller number and a 37% shoot growth reduction, all negatively influencing the number of spike-bearing tillers, number of ears, grain weight and yield (Dickin and Wright 2008; Robertson et al. 2009; Yavas et al. 2012). In soft red winter wheat, yield losses of 44% have been reported, due mainly from a reduction in kernel number and tiller number (Collaku and Harrison 2002). Araki et al. (2012) investigated the effect of WL after heading and found root growth to be intensely affected and that recovery of root biomass and development of new roots is difficult after the stress has ceased. Khabaz-Saberi et al. (2006) reported as much as 52% reduction in shoot dry weight while Ali et al. (2012) reported a reduction of 1.5% in grain yield, 0.7% of spikes m^{-2} and 1.3% for grain weight for each day of WL treatment. In the same study, Ali et al. (2012) showed that ten days of WL are sufficient to have a significant effect on yield and yield components.

Robertson et al. (2009) investigated the effect of a 14-day WL treatment and observed a 50% reduction in tiller number due to less production of secondary tillers and fewer spike-bearing tillers, resulting in 71% lower shoot dry weight. Shao et al. (2013) investigated the effect of WL on photosynthetic and yield traits in winter wheat at different growth stages. The authors reported a significant decrease in root length, root mass and root/shoot ratio when the stress was applied at both the tillering and booting stages. Measurements of transpiration rate, stomatal conductance and net photosynthetic rate (P_N) were also affected after 4 days of WL. Overall, P_N was decreased by 13.6% when WL stress was applied at the tillering stage and by 12.2% for

booting stage stress, with the observed decrease in photosynthesis resulting from stomatal closure. In the same study, a grain yield decrease of 7.1 to 11.2% resulting from fewer spikes ha⁻¹, impacted by a delay in flowering time and maturity. The study concluded that reduction in photosynthesis and thus yield could be explained by less leaf turgor and stomatal conductance due to decreased hydraulic conductivity, leading to a CO₂ (carbon dioxide) deficit and a shortage of assimilate accumulation. Other studies have also shown negative effects on crop establishment, yield and yield components by Li et al. (2011), Yaduvanshi et al. (2012) and Yavas et al. (2012).

Few studies have been conducted to evaluate the genetic variation for waterlogging and tolerance to elemental toxicity. Research by Collaku and Harrison (2002), showed that there is significant genetic variation among wheat genotypes for waterlogging tolerance in terms of yield performance in both commercial cultivars and breeding lines. Similarly, studies have reported wheat tolerance to elemental toxicity by Khabaz-Saberi et al. (2012) under waterlogging stress.

Plant adaptation to waterlogging

Waterlogging is described as the saturation of the pore spaces in the soil (Colmer and Voesenek 2009), which occurs as a result of excessive rainfall followed by both inadequate drainage and reduced percolation through the soil profile. Waterlogging causes hypoxia and anoxia, which is a reduction and depletion of oxygen, respectively, and consequently influences gas exchange, mineral nutrition and micro elemental toxicity in plants (Sairam 2008). A WL-tolerant genotype would survive or maintain its yield under non-drained conditions compared to well-drained conditions. Studies have reported various plant WL tolerance mechanisms, including

morphological and metabolic adaptations, as well as elemental toxicity mechanisms (Hossain et al. 2012; Hossain and Uddin 2011)

The development of roots is affected by waterlogged soils and root dry mass is reduced under WL conditions in several crop species including corn (Abiko et al. 2012), barley (Pang et al. 2004) and wheat (Malik et al. 2001). Morphological adaptations include the ability to persist and maintain growth of seminal roots, the formation of adventitious roots, the formation of aerenchyma tissue (Hague et al. 2012) and root porosity, and the ability to reduce radial oxygen loss (ROL) (Hossain and Uddin 2011). Aerenchyma is a tissue containing air-filled cavities that provide a low resistance internal pathway for the exchange of O₂ between root and shoots, therefore allowing aerobic respiration and maintenance of growth under hypoxia and anoxia stresses (Hossain and Uddin 2011). Ultimately, waterlogging tolerant plants develop suberin or lignin on epidermis, which act as barriers to radial oxygen loss (Pearson et al. 2011)

Metabolic adaptations, including glycolysis, ethanolic fermentation and lactic fermentation, provide tolerant genotypes the ability to supply its demanded energy through anaerobic respiration. These pathways are activated because of the increased activity of fermentative enzymes such as aldolase, enolase, ADH (Alcohol dehydrogenase) and PDC (Pyruvate dehydrogenase complex) (Albrecht et al. 2004). In wheat for example, higher ethanol production is due to higher activity of ADH and PDC enzymes under low oxygen supply (Hossain and Uddin 2011). Anaerobic respiration requires the availability of glucose and fructose provided by high sucrose synthase (SS) activity and greater sugar reserves (Sairam et al. 2009), which has been seen in hypoxia tolerant wheat genotypes (Hossain and Uddin 2011).

Several soil chemical and biochemical reactions occur when oxygen is depleted, which affects plant metabolism and consequently growth and development. Modifications in a waterlogged soil include: higher concentration of ethylene, accumulation of ethanol and acetaldehyde, dissociation of fatty acids and phenols, and changes in element solubility and availability (Abiko et al. 2012; Shabala 2011). These changes, mainly due to changes in pH and redox potential, disturb soil properties and propitiate inadequate nutritional status (Setter et al. 2009). This may result in micronutrient toxicities, such as manganese (Mn), iron (Fe) (Shabala 2011), sulfur S (Colmer and Voesenek 2009) and Al (Khabaz-Saberi et al. 2012) in addition to possible N, P, K, Mg, Cu, Zn, Mn deficiencies (Setter et al. 2009; Steffens et al. 2005)

Plants can tolerate WL by enhancing elemental toxicity tolerance. Khabaz-Saberi et al. (2012) indicated that wheat genotypes tolerant to elemental toxicity, including Mn, Fe and Al, had improved early root growth and shoot growth with maturity much less affected by WL than susceptible genotypes. Both external and internal mechanisms have been suggested to be associated with elemental toxicity tolerance. External mechanisms include: exudation of phosphate and organic acids, immobilization of elements at the cell wall, efflux across the plasma membrane, element exclusion through alteration of rhizosphere pH and selective permeability of the plasma membrane (Hossain et al. 2012). Meanwhile, chelation of elements in the cytosol, compartmentation in the vacuole, elevated enzyme activity and element-binding proteins have been suggested as internal mechanisms associated with elemental toxicity tolerance for aluminum (Reynolds et al., 2001) and other elements (Hossain et al. 2012; Millaleo et al. 2010).

Linkage and association mapping

The majority of traits associated with yield performance and abiotic stress tolerance are governed by quantitative trait loci (QTL). A QTL is described as a region within the genome that contains segregating genes associated with a given trait, with each gene causing a small effect on the phenotype (Semagn et al. 2010). Genetic mapping is a strategy widely used to explain the phenotypic variation within a population and bi-parental linkage mapping has traditionally been used for achieving this goal (Collard et al. 2005; Paux et al. 2012).

By analyzing the recombination frequencies and the mean genetic value for alleles at a locus, linkage mapping provides information about markers linked to a QTL, allowing for a better understanding of the genetic control of phenotypic variation for a trait (Semagn et al. 2010). For waterlogging tolerance, a good example of a large effect QTL would be the *Sub1A* (ethylene-response gene) in rice which was mapped to chromosome 9 and explained as much as 69% of the phenotypic variation for tolerance to flooding stress (Xu et al. 2006).

Although linkage mapping remains a fundamental tool for genetic studies, it has some limitations, including low mapping resolution (QTLs located in 10-20 cM intervals), time and resource consuming and a limitation to the number of segregating alleles present within the population. Consequently, association mapping, also known as linkage disequilibrium or LD mapping is growing in popularity as an alternative to bi-parent QTL analysis. LD mapping takes advantage of natural variation and multiple historical recombination events (Al-Maskri et al. 2012; Hall et al. 2010; Paux et al. 2012), resulting in higher QTL resolution, faster population development and screening time, and the ability to use previously collected phenotype data for the association analysis (Sajjad et al. 2012).

In wheat, several QTL for both complex and simply inherited traits have been detected from bi-parental segregating populations. However, QTLs detected for qualitative traits have been more useful to plant breeders as these provide suitable molecular markers to screen large segregating progenies, the ability to discard undesirable genotypes at early generations and assisting in marker-assisted backcrossing programs. For quantitative traits, markers are scarcely used due to lack of precision and reproducibility in different genetic backgrounds, (Buerstmayr et al. 2009; Gupta et al. 2010; Paux et al. 2012).

Likewise, association mapping studies have been published on wheat. Sajjad et al. (2012) reviewed some association mapping studies in wheat, addressing various agronomical and quality traits, utilizing SSRs, SNPs and DArT markers. Main results encompass significant associations with kernel width (chromosome 2D), protein content (chromosomes 1B, 1D), plant height and grain per spike (chromosome 4A), grain yield (chromosomes 1B, 3B and 6B), yield components, leaf rust resistance (chromosome 1B), as well as drought tolerance and earliness (chromosome 4B)

Similar studies have found associations with aluminum tolerance in triticale (Niedziela et al. 2012) yield and yield stability in barley (Kraakman et al. 2004) and drought tolerance in maize (Lu et al. 2012). Studies on elemental toxicity (Al, Fe, Mn) tolerance under WL stress have demonstrated genetic variation of wheat genotypes (Khabaz-Saberi et al. 2012). However, LD methodologies; which would dissect the genetic control of WL tolerance given by ion-toxicity tolerance remain unknown. Moreover, knowledge on markers associated with tolerance mechanisms to elemental toxicity would provide valuable information for genetic improvement in wheat by assisting in the screening and selection of tolerant parental lines for future crosses and development of WL tolerant wheat varieties.

Quantitative trait loci for waterlogging tolerance

QTL studies contribute to a better understanding of the genetic basis of crop performance under environmental constraints such as WL. Various studies have been conducted to analyze the genetic control of tolerance to WL in different crop species for a number of traits (Hattori et al. 2009; Li et al. 2008; Mano et al. 2005; Pearson et al. 2011; Reyna et al. 2003; Setter and Waters 2003; Yeboah et al. 2008; Zhou 2011).

Mano et al. (2005) mapped QTL for adventitious root formation in an F₂ population derived from a cross between the maize progenitor teosinte and the maize inbred line B64 subjected to a two-week WL treatment during seedling stage. A single QTL was reported for adventitious root formation on chromosome 8, provided by the teosinte parent, suggesting a genetic resource to introducing waterlogging tolerance into commercial adapted maize lines. Qiu et al. (2007) performed a QTL analysis on 288 F_{2:3} lines and reported a major QTL on chromosome 9 associated with both root and shoot dry weight that co-localized with the sucrose synthase 1 gene known to be responsive to anaerobic conditions. QTLs associated with the same traits have also been reported by Zhang et al. (2013), including a QTL located on chromosome 9.

In barley (*Hordeum vulgare* L.), QTL have been mapped for leaf chlorosis, plant survival and plant biomass under waterlogging and many of these QTL have been shown to be conserved across populations and studies (Li et al. 2008; Zhou 2011). Zhou (2011) reported four QTLs 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. From this study, four molecular markers were identified suitable for incorporation into screening routines for waterlogging tolerance.

The most successful genetic studies of flooding tolerance come from rice where QTL studies have subsequently led to the map based cloning of several agronomical important genes. For example, the *Sub1* locus was originally identified as a QTL on chromosome 9 that provided WL tolerance by reducing energy consumption during stress and providing the ability to withstand flash floods (Xu and Mackill 1996). This QTL was eventually isolated through map-based cloning and shown to be an ERF gene (Xu et al. 2006). QTLs regulating internode elongation in deep water rice were also mapped, including *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*) (Hattori et al. 2008) and isolated through map-based cloning. Both *SK1* and *SK2* are ethylene response factors that are highly expressed under WL conditions.

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 A1 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).

In wheat, Boru et al. (2001) determined WL tolerance to be quantitatively inherited and controlled by a minimum of 4 genes however this paper does not show genetic mapping information. Yu and Chen (2013) reported multiple QTL distributed throughout the wheat genome, including 17 alleles present in synthetic wheat.

The genetic mechanisms of WL tolerance in wheat remain relatively unknown, especially when compared to reports in other crop species. Knowledge on the genetics of tolerance to this important constraint would provide tools to enhance yield performance under waterlogging stress, including molecular tools to detect important agronomic and physiological traits correlated with yield performance.

Quantitative trait loci associated with elemental toxicity tolerance

Chemical and biochemical reactions that occur during WL stresses disturb soil properties, create inadequate nutritional status and expose crops to inadequate element concentrations in the soil solution (Khabaz-Saberi and Rengel 2010). Khabaz-Saberi et al. (2012) demonstrated that genotypes tolerant to Al, Mn and Fe perform better while growing under WL stress. Cai et al. (2008) mapped three QTLs for aluminum resistance in wheat, located on chromosomes 2A, 3BL and 4DL. The 4DL QTL was found to co-segregate with a molecular marker located within the promoter of an aluminum-activated malate transporter gene (ALMT1) making it a suitable marker for marker-assisted selection for aluminum tolerance. Ma et al. (2005) had previously reported the same QTL explaining as much as 50% of the phenotype variation for root growth and root tolerance index.

Aluminum, manganese and iron tolerance under WL were studied by Khabaz-Saberi and Rengel (2010) who suggested that wheat genotypes carrying ion toxicity tolerance would also exhibit WL tolerance. However, there are currently no reports on genetic mapping of QTL for elemental concentration and ion toxicity tolerance in wheat growing under WL. With climate change and given the extension of the cultivated land area prone to WL constraints, knowledge

on the genetic control of elemental concentrations and ion toxicity for cereals, including wheat becomes an important goal of current wheat improvement efforts. These tools would provide new screening and selection tools for breeders that would make development and selection of tolerant genotypes more efficient

References Chapter I

- Abiko T, Kotula L, Shiono K, Malik AI, Colmer TD, Nakazono M (2012) Enhanced formation of aerenchyma and induction of a barrier to radial oxygen loss in adventitious roots of *Zea nicaraguensis* contribute to its waterlogging tolerance as compared with maize (*Zea mays* ssp *mays*). *Plant Cell and Environment* 35
- Al-Maskri AY, Sajjad M, Khan SH (2012) Association Mapping: A Step Forward to Discovering New Alleles for Crop Improvement. *International Journal of Agriculture and Biology* 14:153-160
- Albrecht G, Mustroph A, Fox TC (2004) Sugar and fructan accumulation during metabolic adjustment between respiration and fermentation under low oxygen conditions in wheat roots. *Physiologia Plantarum* 120:93-105
- Ali R, Mohammad-Eghbal G, Saeid J-H, Mokhtar G, Mohsen S (2012) Impacts of waterlogging on shoot apex development and recovery effects of nitrogen on grain yield of wheat. *European Journal of Experimental Biology*, 2012 2:8
- Araki H, Hossain MA, Takahashi T (2012) Waterlogging and Hypoxia have Permanent Effects on Wheat Root Growth and Respiration. *Journal of Agronomy and Crop Science* 198
- Araus JL, Slafer GA, Royo C, Dolores Serret M (2008) Breeding for Yield Potential and Stress Adaptation in Cereals. *Critical Reviews in Plant Sciences* 27
- Boru G, van Ginkel M, Kronstad WE, Boersma L (2001) Expression and inheritance of tolerance to waterlogging stress in wheat. *Euphytica* 117:91-98
- Buerstmayr H, Ban T, Anderson JA (2009) QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. *Plant Breeding* 128
- Cai SB, Bai GH, Zhang DD (2008) Quantitative trait loci for aluminum resistance in Chinese wheat landrace FSW. *Theoretical and Applied Genetics* 117:49-56
- Collaku A, Harrison SA (2002) Losses in Wheat Due to Waterlogging Approved for publication by the Director of the LAES as manuscript no. 01-09-220. *Crop Sci* 42:444-450

Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica* 142:169-196

Colmer TD, Voesenek L (2009) Flooding tolerance: suites of plant traits in variable environments. *Funct Plant Biol* 36:665-681

Cornelius B, Chen P, Chen Y, De Leon N, Shannon JG, Wang D (2005) Identification of QTLs underlying waterlogging tolerance in soybean. *Molecular Breeding* 16:103-112

Dickin E, Wright D (2008) The effects of winter waterlogging and summer drought on the growth and yield of winter wheat (*Triticum aestivum* L.). *European Journal of Agronomy* 28:234-244

Dubcovsky J, Dvorak J (2007) Genome plasticity a key factor in the success of polyploid wheat under domestication. *Science* 316:1862-1866

Gupta PK, Langridge P, Mir RR (2010) Marker-assisted wheat breeding: present status and future possibilities. *Molecular Breeding* 26:145-161

Hague ME, Oyanagi A, Kawaguchi K (2012) Aerenchyma Formation in the Seminal Roots of Japanese Wheat Cultivars in Relation to Growth under Waterlogged Conditions. *Plant Production Science* 15

Hall D, Tegstrom C, Ingvarsson PK (2010) Using association mapping to dissect the genetic basis of complex traits in plants. *Briefings in Functional Genomics* 9

Hattori Y, Nagai K, Furukawa S, Song XJ, Kawano R, Sakakibara H, Wu JZ, Matsumoto T, Yoshimura A, Kitano H, Matsuoka M, Mori H, Ashikari M (2009) The ethylene response factors SNORKEL1 and SNORKEL2 allow rice to adapt to deep water. *Nature* 460:1026-U1116

Hattori Y, Nagai K, Mori H, Kitano H, Matsuoka M, Ashikari M (2008) Mapping of three QTLs that regulate internode elongation in deepwater rice. *Breed Sci* 58:39-46

Hossain MA, Piyatida P, da Silva JAT, Fujita M (2012) Molecular Mechanism of Heavy Metal Toxicity and Tolerance in Plants: Central Role of Glutathione in Detoxification of Reactive Oxygen Species and Methylglyoxal and in Heavy Metal Chelation. *Journal of Botany* 2012

Hossain MA, Uddin SN (2011) Mechanisms of waterlogging tolerance in wheat: Morphological and metabolic adaptations under hypoxia or anoxia. *Australian Journal of Crop Science* 5

Jia JZ, Zhao SC, Kong XY, Li YR, Zhao GY, He WM, Appels R, Pfeifer M, Tao Y, Zhang XY, Jing RL, Zhang C, Ma YZ, Gao LF, Gao C, Spannagl M, Mayer KFX, Li D, Pan SK, Zheng FY, Hu Q, Xia XC, Li JW, Liang QS, Chen J, Wicker T, Gou CY, Kuang HH, He GY, Luo YD, Keller B, Xia QJ, Lu P, Wang JY, Zou HF, Zhang RZ, Xu JY, Gao JL, Middleton C, Quan ZW, Liu GM, Wang J, Yang HM, Liu X, He ZH, Mao L, Wang J, *Int Wheat Genome Sequencing C* (2013) *Aegilops tauschii* draft genome sequence reveals a gene repertoire for wheat adaptation. *Nature* 496:91-95

Khabaz-Saberi H, Barker SJ, Rengel Z (2012) Tolerance to ion toxicities enhances wheat (*Triticum aestivum* L.) grain yield in waterlogged acidic soils. *Plant and Soil* 354

Khabaz-Saberi H, Rengel Z (2010) Aluminum, manganese, and iron tolerance improves performance of wheat genotypes in waterlogged acidic soils. *J Plant Nutr Soil Sci* 173:461-468

Khabaz-Saberi H, Setter TL, Waters I (2006) Waterlogging induces high to toxic concentrations of iron, aluminum, and manganese in wheat varieties on acidic soil. *Journal of Plant Nutrition* 29:899-911

Kraakman ATW, Niks RE, Van den Berg PMMM, Stam P, Van Eeuwijk FA (2004) Linkage Disequilibrium Mapping of Yield and Yield Stability in Modern Spring Barley Cultivars. *Genetics* 168:435-446

Li CY, Jiang D, Wollenweber B, Li Y, Dai TB, Cao WX (2011) Waterlogging pretreatment during vegetative growth improves tolerance to waterlogging after anthesis in wheat. *Plant Sci* 180:672-678

Li H, Vaillancourt R, Mendham N, Zhou M (2008) Comparative mapping of quantitative trait loci associated with waterlogging tolerance in barley (*Hordeum vulgare* L.). *BMC Genomics* 9:Article No.: 401

Ling HQ, Zhao SC, Liu DC, Wang JY, Sun H, Zhang C, Fan HJ, Li D, Dong LL, Tao Y, Gao C, Wu HL, Li YW, Cui Y, Guo XS, Zheng SS, Wang B, Yu K, Liang QS, Yang WL, Lou XY, Chen J, Feng MJ, Jian JB, Zhang XF, Luo GB, Jiang Y, Liu JJ, Wang ZB, Sha YH, Zhang BR, Wu HJ, Tang DZ, Shen QH, Xue PY, Zou SH, Wang XJ, Liu X, Wang FM, Yang YP, An XL, Dong ZY, Zhang KP, Zhang XQ, Luo MC, Dvorak J, Tong YP, Wang J, Yang HM, Li ZS, Wang DW, Zhang AM, Wang J (2013) Draft genome of the wheat A-genome progenitor *Triticum urartu*. *Nature* 496:87-90

Lu Y, Xu J, Yuan Z, Hao Z, Xie C, Li X, Shah T, Lan H, Zhang S, Rong T, Xu Y (2012) Comparative LD mapping using single SNPs and haplotypes identifies QTL for plant height and biomass as secondary traits of drought tolerance in maize. *Molecular Breeding* 30

Ma HX, Bai GH, Carver BF, Zhou LL (2005) Molecular mapping of a quantitative trait locus for aluminum tolerance in wheat cultivar Atlas 66. *Theoretical and Applied Genetics* 112:51-57

Malik A, Colmer TD, Lambers H, Schortemeyer M (2001) Changes in physiological and morphological traits of roots and shoots of wheat in response to different depths of waterlogging. *Australian Journal of Plant Physiology* 28

Mano Y, Muraki M, Fujimori M, Takamizo T, Kindiger B (2005) Identification of QTL controlling adventitious root formation during flooding conditions in teosinte (*Zea mays* ssp *huehuetenangensis*) seedlings. *Euphytica* 142:33-42

Mergoum M, Singh PK, Anderson JA, Singh RP, Xu SS, Ransom JK (2009) Cereals: Spring Wheat Breeding. In: Carena M (ed) *Handbook of Plant Breeding*. Springer

Millaleo R, Reyes- Diaz M, Ivanov AG, Mora ML, Alberdi M (2010) MANGANESE AS ESSENTIAL AND TOXIC ELEMENT FOR PLANTS: TRANSPORT, ACCUMULATION AND RESISTANCE MECHANISMS. *Journal of soil science and plant nutrition* 10:470-481

Mir RR, Zaman-Allah M, Sreenivasulu N, Trethowan R, Varshney RK (2012) Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. *Theoretical and Applied Genetics* 125

Niedziela A, Bednarek PT, Cichy H, Budzianowski G, Kilian A, Aniol A (2012) Aluminum tolerance association mapping in triticale. *Bmc Genomics* 13

Pang JY, Zhou MX, Mendham N, Shabala S (2004) Growth and physiological responses of six barley genotypes to waterlogging and subsequent recovery. *Australian Journal of Agricultural Research* 55

Paux E, Sourdille P, Mackay I, Feuillet C (2012) Sequence-based marker development in wheat: Advances and applications to breeding. *Biotechnology Advances* 30:1071-1088

Pearson A, Cogan NOI, Baillie RC, Hand ML, Bandaranayake CK, Erb S, Wang J, Kearney GA, Gendall AR, Smith KF, Forster JW (2011) Identification of QTLs for morphological traits

influencing waterlogging tolerance in perennial ryegrass (*Lolium perenne* L.). *Theoretical and Applied Genetics* 122

Peng J, Sun D, Nevo E (2011) Domestication evolution, genetics and genomics in wheat. *Molecular Breeding* 28:281-301

Powell N, Ji X, Ravash R, Edlington J, Dolferus R (2012) Yield stability for cereals in a changing climate. *Funct Plant Biol* 39:539-552

Qiu FZ, Zheng YL, Zhang ZL, Xu SZ (2007) Mapping of QTL associated with waterlogging tolerance during the seedling stage in maize. *Annals of Botany* 99:1067-1081

Reyna N, Cornelious B, Shannon JG, Sneller CH (2003) Evaluation of a QTL for waterlogging tolerance in southern soybean germplasm. *Crop Science* 43:2077-2082

Reynolds MP, Trethowan RM (2007) Physiological interventions in breeding for adaptation to abiotic stress

Robertson D, Zhang H, Palta JA, Colmer T, Turner NC (2009) Waterlogging affects the growth, development of tillers, and yield of wheat through a severe, but transient, N deficiency. *Crop & Pasture Science* 60

Ruttan V (2005) Scientific and Technical Constraints on Agricultural Production: Prospects for the Future. www.amphilsoc.org, p 15

Sairam RK, Dharmar K, Chinnusamy V, Meena RC (2009) Waterlogging-induced increase in sugar mobilization, fermentation, and related gene expression in the roots of mung bean (*Vigna radiata*). *Journal of Plant Physiology* 166:602-616

Sairam RK, Kumutha, D., Ezhilmathi, K., Deshmukh, P.S. And G.C. Srivastava (2008) Physiology and biochemistry of waterlogging tolerance in plants. *Biologia Plantarum* 52 (3):13

Sajjad M, Khan S, Kazi A (2012) The low down on association mapping in hexaploid wheat (*Triticum aestivum* L.). *J Crop Sci Biotechnol. The Korean Society of Crop Science*, pp 147-158

Semagn K, Bjornstad A, Xu Y (2010) The genetic dissection of quantitative traits in crops. *Electronic Journal of Biotechnology* 13

Setter TL, Waters I (2003) Review of prospects for germplasm improvement for waterlogging tolerance in wheat, barley and oats. *Plant and Soil* 253:1-34

Setter TL, Waters I, Sharma SK, Singh KN, Kulshreshtha N, Yaduvanshi NPS, Ram PC, Singh BN, Rane J, McDonald G, Khabaz-Saberi H, Biddulph TB, Wilson R, Barclay I, McLean R, Cakir M (2009) Review of wheat improvement for waterlogging tolerance in Australia and India: the importance of anaerobiosis and element toxicities associated with different soils. *Annals of Botany* 103

Shabala S (2011) Physiological and cellular aspects of phytotoxicity tolerance in plants: the role of membrane transporters and implications for crop breeding for waterlogging tolerance. *New Phytologist* 190

Shao GC, Lan JJ, Yu SE, Liu N, Guo RQ, She DL (2013) Photosynthesis and growth of winter wheat in response to waterlogging at different growth stages. *Photosynthetica* 51:429-437

Shepherd LVT, Alexander CA, Sungurtas JA, McNicol JW, Stewart D, Davies HV (2010) Metabolomic analysis of the potato tuber life cycle. *Metabolomics* 6

Shewry PR (2009) Wheat. *Journal of Experimental Botany* 60:1537-1553

Spiertz H (2012) Avenues to meet food security. The role of agronomy on solving complexity in food production and resource use. *European Journal of Agronomy* 43:1-8

Steffens D, Huetsch BW, Eschholz T, Losak T, Schubert S (2005) Water logging may inhibit plant growth primarily by nutrient deficiency rather than nutrient toxicity. *Plant Soil and Environment* 51:545-552

USDA (2011) Composition of Foods Raw, Processed, Prepared. In: 24 (ed) USDA National Nutrient Database for Standard Reference. United States Department of Agriculture, <http://www.ars.usda.gov/SP2UserFiles/Place/12354500/Data/SR24/nutrlist/sr24w307.pdf>, p 100

USDA (2012a) Wheat: Planted acreage, harvested acreage, production, yield and farm price. In: USDA (ed) Economic Reserach Service, October 2012 edn, p 1

USDA USDoA (2012b) **Wheat Overview**. Economic Research Service, <http://www.ers.usda.gov/topics/crops/wheat/background.aspx>

- VanToai TT, St. Martin SK, Chase K, Boru G, Schnipke V, Schmitthenner A, Lark KG (2001) Identification of a QTL Associated with Tolerance of Soybean to Soil Waterlogging. *Crop Sci* 41:1247-1252
- Watkin B, Wailes EJ, Miller W (2011) Arkansas Economic Losses Due to Flooding of Crops - 2011. University of Arkansas, United States Department of Agriculture and County Governments Cooperating.
- Xu K, Xu X, Fukao T, Canlas P, Maghirang-Rodriguez R, Heuer S, Ismail AM, Bailey-Serres J, Ronald PC, Mackill DJ (2006) Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice. *Nature* 442:705-708
- Xu KN, Mackill DJ (1996) A major locus for submergence tolerance mapped on rice chromosome 9. *Molecular Breeding* 2:219-224
- Yaduvanshi NPS, Setter TL, Sharma SK, Singh KN, Kulshreshtha N (2012) Influence of waterlogging on yield of wheat (*Triticum aestivum*), redox potentials, and concentrations of microelements in different soils in India and Australia. *Soil Research* 50:489-499
- Yavas I, Unay A, Aydin M (2012) The Waterlogging Tolerance of Wheat Varieties in Western of Turkey. *Scientific World Journal*
- Yeboah MA, Chen XH, Liang GH, Gu MH, Xu CW (2008) Inheritance of waterlogging tolerance in cucumber (*Cucumis sativus* L.). *Euphytica* 162:145-154
- Yu M, Chen G-Y (2013) Conditional QTL mapping for waterlogging tolerance in two RILs populations of wheat. *SpringerPlus* 2:245
- Zhang X, Tang B, Yu F, Li L, Wang M, Xue Y, Zhang Z, Yan J, Yue B, Zheng Y, Qiu F (2013) Identification of Major QTL for Waterlogging Tolerance Using Genome-Wide Association and Linkage Mapping of Maize Seedlings. *Plant Mol Biol Rep* 31:594-606
- Zhou M (2011) Accurate phenotyping reveals better QTL for waterlogging tolerance in barley. *Plant Breeding* 130:203-20

CHAPTER II
ELEMENTAL TOXICITY: IMPACT ON GRAIN YIELD AND ASSOCIATION
MAPPING FOR ALUMINUM, SODIUM AND IRON CONCENTRATION

Abstract

Soil waterlogging affects 25% of the worldwide area planted to wheat and continues to be an important problem for crop production in the United States, including in Arkansas, where more than US\$20 million in economics losses were reported in 2011. Enhanced elemental toxicity tolerance is a desirable trait that has been reported to confer increased tolerance to soil waterlogging. This study evaluated the impact of elemental accumulation as a result of waterlogging stress at the vegetative stage on wheat yield production in 28 adapted soft-red winter wheat breeding lines and performed association mapping for elemental concentration (EC) in a panel of 240 wheat genotypes. Overall, WL reduced mean GY by 42% in the 28 genotypes with the fold change for ECs ranging from 0.8 to 12. Pearson correlations between grain yield and Al ($r = -0.76$), Na ($r = -0.41$) and Fe ($r = -0.72$) were strongly negative under WL, indicating that elemental toxicity likely contributed to reductions in grain yield. In the panel of 240 genotypes, significant genotypic variation for elemental accumulation was shown for Al, Fe and Na, dependent on environment, with the percent of phenotypic variation explained by genotype ranging from 0 to 60%. Overall, elemental concentrations were higher in the 2012-2013 than in 2013-2014 growing season. Association mapping for aluminum, sodium and iron using 5,227 single nucleotide polymorphism markers identified nine chromosomes, including 1B, 2B, 5A, 3A, 3B, 5A, 5B, 6A and 7A which had marker-trait associations that were consistently detected at the same SNP or SNPs in LD (<30 cM) across multiple data-sets. Overall, detected individual SNPs explained a small percent of the phenotypic variance, ranging from $R^2 = 0.03$ to 0.07 , indicating variation in tissue elemental accumulation to be quantitatively inherited within this panel of lines. Chromosomes 2B and 5A were found to contain the majority of markers associated with Fe, Na and Al concentration in this study. In agreement with the high phenotypic correlation between Al and Fe concentrations ($r = 0.89$), IWA8118 on chromosome

5B was found to be associated with both Al and Fe accumulation, suggesting there could be a similar tolerance mechanism. This study represents the first study aiming to evaluate genetic diversity for the elemental accumulation in a diverse panel of wheat under WL stress.

Introduction

Wheat is cultivated on 17% of all crop land worldwide and supplies more than 20% of total consumed calories (Peng et al. 2011; Shepherd et al. 2010). Soil waterlogging (WL), the saturation of the pore spaces in the soil (Colmer and Voesenek 2009), and affects up to 25% of the worldwide area planted to wheat (Powell et al. 2012). It has become an increasing crop production constraint throughout the United States, including Arkansas where more than US\$20 million in economics losses were reported in 2011 (Watkin et al. 2011).

Waterlogging affects the physiology and metabolism of the plant, resulting in reduced growth and accelerated development, ultimately lowering yield and yield components. Wheat has shown a 68% root growth reduction, up to 50% reduction in tiller number and a 37% shoot growth reduction (Robertson et al. 2009), all negatively influencing the number of spike-bearing tillers, number of ears, grain weight and yield (Dickin and Wright 2008). In soft red winter wheat (SRWW), yield losses of 44% have been reported, due mainly from a reduction in kernel number and tiller number (Collaku and Harrison 2002).

Waterlogging causes hypoxia and anoxia, influencing mineral nutrition and micro elemental toxicity in plants resulting from chemical and biochemical reactions which occur while oxygen is depleted (Sairam et al. 2008). These changes, due mainly to changes in soil pH and redox potential, disturb the soil properties and propitiate inadequate nutritional status (Setter et al. 2009). This can result in micronutrient toxicities, including for manganese (Mn), iron (Fe) (Shabala 2011), sulfur (S) (Colmer and Voesenek 2009) and aluminum (Al) (Khabaz-Saberi et al.

2012), in addition to possible deficiencies in nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), copper (Cu), zinc (Z) and Mn (D. Steffens 2005; Setter et al. 2009).

Enhanced elemental toxicity tolerance has been suggested as an adaptive trait that confers waterlogging tolerance. Khabaz-Saberi et al. (2012) found that wheat genotypes tolerant to toxic concentrations of Mn, Fe and Al had improved early root growth and shoot growth with maturity much less affected by WL than susceptible genotypes. Both external and internal mechanisms have been suggested to be associated with elemental toxicity tolerance. External mechanisms include: exudation of phosphate and organic acids, immobilization of elements at the cell wall, efflux across the plasma membrane, element exclusion through alteration of rhizosphere pH and selective permeability of the plasma membrane (Hossain et al. 2012). Meanwhile, chelation of elements in the cytosol, compartmentalization in the vacuole, elevated enzyme activity and element-binding proteins have been suggested as internal mechanisms associated with tolerance for Al (Reynolds et al. 2001), Mn (Millaleo et al. 2010) and heavy metals (Hossain et al. 2012)

Quantitative trait loci (QTL) studies contribute to a better understanding of the genetic basis of crop performance under environmental constraints such as WL. Various studies have been conducted to analyze the genetic control of tolerance to WL in different crop species for a number of traits including rice (Hattori et al. 2009), barley (Li et al. 2008); Zhou (2011), maize (Mano et al. 2005), ryegrass (Pearson et al. 2011) and small grains including oats and wheat (Ballesteros et al. 2015; Setter and Waters 2003). Ballesteros et al. (2015) studied a recombinant inbred line population and reported one QTL for chlorophyll content in chromosome 1B, two in 1D and one in 3B that explained 32%, 20%, 24%, and 25% of the phenotypic variance respectively. They also reported QTL with a large effect for root fresh biomass on 1B, shoot dry biomass on 3B and shoot fresh biomass on 5B. Boru et al. (2001) determined WL tolerance in

wheat to be quantitatively inherited and controlled by a minimum of four genes. Yu and Chen (2013) reported multiple QTL distributed throughout the wheat genome, including 17 alleles present in synthetic wheat. Cai et al. (2008) mapped three QTLs for aluminum resistance in wheat, located on chromosomes 2A, 3BL and 4DL. The 4DL QTL was found to co-segregate with a molecular marker located within the promoter of an aluminum-activated malate transporter gene (ALMT1) making it a suitable marker for marker-assisted selection for aluminum tolerance. Ma et al. (2005) had previously reported the same QTL explaining as much as 50% of the phenotype variation for root growth and root tolerance index. With the exception of these studies, the genetic mechanisms of waterlogging and elemental toxicity tolerance in wheat remain relatively unknown, especially when compared to reports in other crop species.

Association mapping (AM) has grown in popularity as an alternative and complementary approach to bi-parent QTL analysis. AM takes advantage of historical recombination events and natural variation (Al-Maskri et al. 2012; Hall et al. 2010; Paux et al. 2012), resulting in higher QTL resolution, faster population development and screening time, and the ability to use previously collected phenotype data for the association analysis (Sajjad et al. 2012). AM studies have been conducted for a number of traits in wheat, including agronomic and quality traits, utilizing simple sequence repeats (SSRs), single nucleotide polymorphisms SNPs and diversity array technologies (DArT) markers. Recent advances in high-throughput genotyping platforms such as the Illumina® 9K array and 90K arrays and genotype-by-sequencing (GBS), whole genome selection for traits highly desirable in multi environments is now possible (Ballesteros et al. 2015; Niedziela et al. 2012; Sajjad et al. 2012).

Khabaz-Saberi and Rengel (2010) suggested that wheat genotypes carrying ion toxicity tolerance would also exhibit WL tolerance, but there are currently no reports on genetic mapping

of QTL for elemental concentration and ion toxicity tolerance in wheat subjected to WL stress. With current climate change prediction models and given the extent of cultivated land area prone to WL, knowledge on the genetic control of elemental concentrations and ion toxicity for cereals, including wheat, becomes an important goal of current wheat improvement efforts. Therefore, this research aimed to perform an association mapping study on elemental accumulation in the vegetative tissue of a diverse set of wheat genotypes grown under field waterlogging conditions. Results of this study will provide new screening methods and tools for selection of WL tolerant genotypes by plant breeders.

Material and Methods

Plant material and experimental design for association mapping

A panel of 240 soft winter wheat lines was used for this study (referred to herein as AM panel), comprised of both public and private cultivars, advanced breeding lines and other germplasm representative of the genetic diversity in soft winter wheat adapted to the eastern and southern United States (Supplementary Table 1). Field experiments were carried out at the Rice Research and Extension Center in Stuttgart Arkansas during the 2012-2013 and 2013-2014 growing seasons (AR13 and AR14) and the Northeast Louisiana Research Station in Saint Joseph, LA (LA13 and LA14) during the 2012-2013 and 2013-2014 growing seasons. Stuttgart soils are characterized by a silt loam surface layer and a clay subsoil with low permeability (NRCS 2013) and are prone to periodic waterlogging. In Louisiana, the waterlogged experiment was carried out in a sharkey clay which are poorly drained soils that have a clayey surface layer and are prone to waterlogging (NRCS 2001). Plots at both locations consisted of two 1.5 meter rows at 115 seed m⁻² arranged in a randomized complete block design with two replications. In AR13

and AR14, all plots were fertilized with 170 kg N ha⁻¹ as urea in a split application, with 60% applied prior to the WL treatment and 40% applied post WL treatment. In LA13 and LA14, all plots were fertilized with 225 kg N ha⁻¹ as urea in a split application, with 50% applied prior to the WL treatment and 50% applied post WL treatment. At both sites, field WL was imposed by establishing 0.30 m high levees surrounding the experimental field and applying water to saturate the soil twice weekly for the duration of the treatment (Fig 1). In the AR13, the WL treatment was started on March 20, 2013 at Feekes stage 4 and continued until April 17, 2013 at Feekes stage 5. In AR14, the treatment was started on April 1 at Feekes stage 4 and continued until April 14 at Feekes stage 5. In LA13 no treatment was applied due to excessive rainfall. In LA14, the WL treatment was started on Feb 24 and continued until March 19.

Plant material and experimental design for yield trial

In addition to the AM panel, a yield trial was conducted in 2012-2013 using a set of 28 adapted soft red winter wheat cultivars and advanced breeding lines, including entries from both public and private breeding programs (Supplementary Table 1). The field experiment was carried out at the Rice Research and Extension Center in Stuttgart, Arkansas. Plots were drill seeded on at a rate of 118 kg of seed hectare⁻¹ in seven row plots measuring 1.25 meters wide x 6 meters long in a split plot with randomized complete blocks design with 4 replications and two treatments (waterlogged and non-waterlogged). Crop maintenance and WL treatment conditions were the same as those for the AM Panel. Grain yield (GY) in g m⁻² was determined on whole plots by combine harvesting, drying and weighing the grain, and adjusted to 13% moisture.

Elemental accumulation analysis

For both the AM Panel and yield trial at all site-years, vegetative biomass was harvested from a 0.10 m² section of plot one day after the WL treatment was terminated or at Feekes stage 5. Samples were decontaminated of soil and dust particles using deionized water, dried at 65°C for 72 hours or until tissue reached a constant weight. Tissue was then ground and homogenized to a 0.5-1.0-mm particle size. A 0.25 g sample of ground and homogenized tissue was digested with concentrated HNO₃, heat (125°C during one hour for digestion and 80°C for sample drying after digestion) and 30% H₂O₂ according to the Organic Matter Destruction-Wet Ashing protocol by Campbell and Plank (1992). Total phosphorus (P), potassium K, calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), aluminum (Al), boron (B), copper (Cu), and zinc (Zn) content were determined in shoots using inductively coupled plasma atomic emission spectroscopy (ICP-AES) according to Donohue and Aho (1992). Soil samples were also taken both pre and post waterlogging and analyzed using ICP-AES to determine pH, soil organic matter and plant-available elements including P, K, Ca, Mg, Na, Fe, Mn, Zn, Cu, B and S accumulation (Supplementary table 3)

Statistical analysis of phenotypic data

Phenotypic traits were analyzed using PROC MIXED in SAS 9.3 (SAS Institute Inc. 2011, Cary, NC). For the yield trial, an analysis of variance (ANOVA) was performed using Type 3 sum of squares with the treatment (WL vs. non-WL) as a fixed effect and all other components (genotype, rep and genotype x treatment interaction (GxT)) treated as random in order to determine significant differences between treatment means. For the AM panel, least square means (LSMeans) were calculated for each line and trait with genotype considered a fixed effect

and location, year, replication and interactions considered random. In addition, best linear unbiased predictors (BLUPs) for each trait and line were estimated with all effects including genotype considered random. Variance components were estimated using Type 3 sum of squares with all effects, including genotypes considered random and were used for determining the percent of total variation explained by each component. The Shapiro-Wilk test for normality determined that the elemental concentrations were normally distributed ($P \leq 0.0001$). Pearson's correlation using PROC CORR in SAS was used to determine associations between GY and measured elemental concentrations.

Genome wide association analysis (GWAS)

The AM panel was genotyped for single nucleotide polymorphism (SNP) markers using either the 9K (Cavanagh et al. 2013) or 90K (Wang et al. 2014) Illumina Infinium iSelect array (Illumina Inc. San Diego, CA), in collaboration with the USDA-ARS Regional Genotyping Centers in Fargo North Dakota and Raleigh North Carolina. Genome locations were obtained from consensus maps published by Cavanagh et al. (2013) and Wang et al. (2014). Allele specific KASP assays for major genes for vernalization (*Vrn-A1* and *Vrn-B1*) and photoperiod (*Ppd-A1*, *Ppd-B1* and *Ppd-D1*) were also evaluated (Diaz et al. 2012; Guedira et al. 2014; Nishida et al. 2013). Polymorphic SNP markers were used for assessment of population structure using principal component analysis implemented in TASSEL (Bradbury et al. 2007) and for calculating a kinship matrix using the VanRaden method (VanRaden 2008) implemented in the Genomic Association and Prediction Integrated Tool – GAPIT R package (Lipka et al. 2012). The same SNP markers were utilized for the estimation of linkage disequilibrium patterns using JMP Genomics 6.0 (SAS Institute Inc. Cary, NC).

A total of 5287 SNP markers with minor allele frequency (MAF) greater than 0.04 and less than 10% missing data were used to conduct GWAS on elemental concentrations (EC) determined by ICP for aluminum, iron and sodium under WL stress. The elements Al, Na and Fe were selected for analysis in the AM panel based on their negative correlation with GY. A compressed mixed linear model (CMLM) implemented in GAPIT was used for GWAS (Lipka et al. 2012; Zhang et al. 2010). This model was selected by its ability to account for population structure (fixed effect) and genetic relatedness (random effect), as well as the computational performance as it uses the EMMA (efficient mixed model association) algorithm, which reduces computational time. In this model, principal components were included as fixed effects, and kinship relationships as a variance-covariance structure of the random effect of individuals. Marker trait association of 5287 SNP markers and measured elements was performed using both LSMeans and BLUPs (best linear unbiased predictor), however association results were highly similar and therefore only associations obtained by using LSMeans for each element are reported.

Results

Correlations of yield and elemental accumulation under waterlogging

For the yield trial consisting of 28 SRWW genotypes, a significant WL treatment effect ($P \leq 0.05$) was observed for GY and all elemental concentrations (EC) with the exception of Mg and S (Table 1). Overall, WL reduced mean GY by 42% with the fold change for elements ranging from 0.8 to 12. The elements Al, Na and Fe showed the greatest increase under WL compared to control, with fold-changes of 12, 5 and 5 respectively. The percent of total variance explained by the WL treatment ranged from 4 to 91%, with greater than 50% explained for GY, Zn, K, B, Mn,

Fe, Na and Al. A significant GxT was observed for GY, Zn, Cu, K, Mg, S and Ca which contributed to non-significant genotype variation within the 28 genotypes evaluated. Pearson correlations between GY and ECs under WL ranged from $r = -0.76$ to 0.55 , with negative correlations observed for all elements with the exception of Ca, Mn, P, and Cu (Fig. 2). Al ($r = -0.76$), Na ($r = -0.41$) and Fe ($r = -0.72$) were all found to be strongly negatively correlated with GY under WL.

Elemental accumulation in the AM panel

A total of 240 wheat genotypes were screened for tissue Al, Fe and Na EC under WL stress in four site-years in Arkansas and Louisiana (AR13, AR14, LA13 and LA14). Overall, EC was higher in 2013 compared to 2014 for both locations and all elements, with higher mean concentrations observed in the Arkansas sites. Mean Al, Fe and Na concentrations were highest in the AR13 site-year, with mean ECs of 392 ppm, 534, ppm and 1496 ppm, respectively. Fe concentration reached maximum of 2,329 ppm in AR13 in genotype 'NC08-23925'. Plank and Donohue (2000) reported sufficient Fe levels at seedling to tillering stage and flag leaf maturity to be between 30-200 ppm and as such the levels observed here may indicate potentially toxicity. No reports were found for Na and Al to establish a sufficient or toxic amount in tissue at the vegetative stage (Supplementary table 4).

Significant genotypic variation was observed for Al in both AR13 and LA14, for Fe in AR13 and for Na in AR13 and AR14, with the percent of phenotypic variation explained by genotype ranging from 0 to 60%.

Population structure, kinship and linkage disequilibrium in the AM panel

Principal component analysis, performed using a total of 5227 SNP markers, found the first three principal components (PCs) to explain 16% of the genotypic variance in the AM panel. No apparent population stratification was extracted from first two PCs, however, plots of PC2 (5.6%) and PC3 (4.3%) separated the population into two clusters (Fig. 3 and Fig. 4), and thus both were included in the mixed model of marker-trait association. Determination of linkage disequilibrium (LD) showed different patterns of LD decay, increasing with genetic distance, and varied based on linkage group and genome (Fig. 5). Markers mapped on chromosomes of the A genome exhibited high LD values with $r^2 > 0.50$ at a genetic distance of up to 80 cM. Markers mapped on chromosomes of the B genome exhibited $r^2 > 0.50$ in distances up to 70 cM, with the exception of chromosome 7B, which showed an $r^2 = 0.90$ at 100 cM. The D genome, likewise showed different patterns of LD decay, for example on chromosomes 1D, 2D and 6D which showed $r^2 \geq 0.50$ at a genetic distance of up to 120 cM. Chromosomes 3D and 5D showed more rapid LD decay (10 cM) and chromosome 4D very low LD ($r^2 \leq 0.20$ at 25 cM).

Genome-wide association analysis (GWAS) for elemental accumulation

Trait means for each line in the four site-years and in a combined analysis were used for GWAS. Three models were used for identifying marker trait associations (MTA), including; 1) a general linear mixed model; 2) mixed model using the kinship matrix (K), and; 3) mixed model using PCs and K (P+K). Only markers which were significant at $P=0.01$ using the P+K mixed model with the first three PCs are reported herein. A total of 92 marker trait association (MTA) were detected for EC of the three elements, including 5 for Al, 43 with iron and 44 with sodium (Table 3 and Supplementary Table 2). Of these association, 9 chromosomes, including 1B, 2B, 5A, 3A,

3B, 5A, 5B, 6A and 7A had MTA that were consistently detected at the same SNP or SNPs in LD (<30 cM) across multiple site-years or in most cases a single site-year and the combined analysis. Overall, detected individual SNPs explained a small percent of the phenotypic variance, ranging from $R^2 = 0.03$ to 0.07.

Genome-wide association analysis for Aluminum

MTA for Al tissue concentration were located on chromosomes 2B and 5A with $-\log(P)$ ranging from 3.1 to 4.3 and the variance explained by the markers ranging from 5% to 7% (Table 3). Markers IWA5261 and IWA3942 in LD on chromosome 2B were significant in AR14 and LA14, respectively. IWA3942 explained 7% of the phenotypic variance for AL EC in LA14 with an average allele effect of 58 ppm, the largest effect SNP observed for Al. On chromosome 5A, IWA8118 was associated with Al EC in AR13 and the MEAN, explaining 5% of the phenotypic variance with an allele effect of 17 ppm.

Genome-wide association analysis for iron

Marker-trait associations for Fe tissue concentration were located on chromosomes 3B, 5A, 5B and 7A, including 4 MTA for AR13, 5 for AR14 and two for LA14 (Table 3). Genome regions on 5A and 5B were detected in both AR13 and AR14. SNPs IWA2446 and IWA8118 on chromosome 5A were in LD and located at 111 cM with allele effects ranging from 9-17 ppm. On chromosome 5B, three SNPs (IWA1342, IWA2610 and IWA4631) located from 54-82 cM, were associated with Fe concentration in AR14, AR13 and LA14, respectively, with allele effects ranging from 17-23 ppm.

Genome-wide association analysis for Sodium

Marker-trait associations for Na were located on chromosomes 1B, 2B, 3A, 5A, 6A and 7A, with the phenotypic variance explained ranging from 3 to 6%. Genome regions on chromosomes 1B, 2B, 5A, and 6A had MTA which were detected in more than one site-year. On chromosome 1B, SNPs IWA6063 and IWA7560 were associated with Na concentration in AR13 and LA14, respectively, with allele effects of 17 and 19 ppm. IWA905 and IWA6554 in LD on 2B showed significant MTA for LA13 and LA14, with allele effects of 19 and 25 ppm. Chromosome position 188 cM on 5A showed a significant MTA for Na in LA13, AR13 and LA14, with allele effects ranging from 13 to 18 ppm. Similarly, IWA416 was significant in AR13 and LA14, with allele effects of 18 and 19 ppm, respectively. A second SNP, IWA7349, in LD with IWA416 was also found to be significantly associated with Na in AR13.

Discussion

GY reduction associated with accumulation of elements

The WL stress response in plants is influenced by genetics in addition to the timing, intensity and duration of the hypoxia or anoxia, as well as other environmental conditions such as temperature and soil type (Fageria et al. 2011; Setter and Waters 2003). Redox potential changes due to anaerobiosis influence Fe and other element availability which are potentially toxic. In evaluating a set of 28 wheat cultivars, a 42% reduction in GY was observed, in agreement with previous studies reporting losses of 32 to 44% (Collaku and Harrison 2002; Robertson et al. 2009). Based on the observed negative correlation between GY and accumulation of micro-elements under WL (Fig. 2), particularly for aluminum, iron, sodium and magnesium, and the increase in tissue concentration of these elements (Table 1) reductions in grain-yield observed in

this study can, at least in part, be attributed to potential elemental toxicity. Enhanced tolerance to increased aluminum and iron concentrations have previously been suggested as a mechanism for WL tolerance in acidic soils by Khabaz-Saberi et al. (2006) and similar findings were reported by Setter et al. (2009) for environments in India and Australia. Therefore, it is important to not only continue improving wheat for GY, but also emphasize the importance of stacking genetic diversity for other traits that confer adaptability to stressed environments, including WL and resulting elemental toxicity.

Evaluation of the panel of 240 wheat genotypes for EA of Na, Fe and Al found significant genetic variation which was highly determined by environment (Table 2). Setter et al. (2009) also reported that WL response does not always relate to responses in other environments, in agreement with the results presented here, where genotype explained a large proportion of the phenotypic variation for EC within individual locations, mainly in the 2013 site-years, but to a lesser extent in 2014 (Table 2). EC data in the AM panel showed a large range within the genotypes, up to levels reported to be toxic to wheat productivity (Plank and Donohue 2000). These results suggest the presence of mechanism of tolerance and/or exclusion for Al, Fe and Na within this diverse panel as can be seen in the wide range between lowest and highest values for within this population. Elemental toxicity tolerance and exclusion mechanisms have been documented for Fe and other metals (Hossain and Uddin 2011). However, this is the first study screening for Al, Na and Fe accumulation under WL of diverse wheat genotypes using inductively coupled plasma multi-element detection, which establishes a starting point for the characterization of the genetic diversity for this important adaptive trait that confers WL tolerance in wheat.

Population structure of the AM panel

Overall, the AM panel showed low population structure, indicating diversity (Fig. 3), consistent with findings in other association studies (Benson et al. 2012) and likely due in part to both the majority of genotypes being SRWW and cooperation among wheat breeding programs which enables exchange of genetic material. These findings are not unexpected as LD patterns differ between populations due to population size, effective recombination rate, mutation and gene flow (Yu et al. 2014).

This AM panel showed overall LD decay in a distance of 30 cM (Fig 4). There are gaps in the marker coverage in the genetic map used for this study, particularly in the D genome that showed rapid LD. Also, an inbreeding species such as wheat could have slow LD decay due to reduced effective recombination rates (Caldwell et al. 2006)

Determining LD patterns is crucial to AM studies and is influenced by factors such as population structure, genetic linkage, genetic drift and cultivar selection (Würschum et al. 2013). Assessing LD of marker pairs and observed LD decay at a distance of 10 cM found high r^2 values at long chromosome distances, although the number of paired markers that were in complete LD was very low and this was consistent across all wheat chromosomes. Previous studies have reported similar results investigating genetic diversity in spring wheat with LD decay at approximately 2 cM in the A and B genomes and ~7 cM in the D genome (Edae et al. 2014). In this study, genome D showed low r^2 measurements and rapid LD decay, likely due to the low marker coverage. Sukumaran et al. (2015) reported genome D to have the highest LD and LD decay at 5 cM ($r^2 = 0.02$), and similar values of LD in genome were observed on this study. Edae et al. (2014) also reported LD decay in genome D below $r^2 = 0.20$ at 6.8 cM, which

is in agreement to results here reported, where most of the chromosomes of genome D had low LD values at distances greater than 5 cM.

Genome-wide association analysis for Al, Fe and Na

Genetic variation within the AM panel allowed detection of MTA between concentrations of Al, Fe and Na with SNP markers (Table 3). A total of 92 MTA were detected for EC including MTA on 1B, 2B, 5A, 3A, 3B, 5A, 5B, 6A and 7A that were detected across data-sets. The genetic effects of detected MTA were generally small, with R^2 values ranging from 0.03 to 0.07%, indicating a quantitative inheritance of EC tolerance within the AM panel. A region on chromosome 5A at 111 cM significant for Al and Fe is in a similar region as was reported by Sukumaran et al. (2015) associated with grain yield (5A at 115 cM), thousand-kernel weight and grain number m^{-2} (5A at 98 cM). Likewise, MTA detected on chromosome 1B at 72 cM for Na is 2 cM from that reported for peduncle length by Sukumaran et al. (2015). This is also in agreement with Ballesteros et al. (2015) who reported adaptive QTL in chromosome 1B for shoot biomass, root biomass and tiller number.

Chromosomes 2B and 5A were found to contain the majority of markers associated with Fe, Na and Al concentration in this study. In agreement with the high phenotypic correlation between Al and Fe concentrations ($r = 0,89$, data not shown), IWA8118 on chromosome 5B was found to be associated with both Al and Fe accumulation, suggesting there could be a similar tolerance mechanism which has previously been suggested for heavy metal toxicity in plants (Hossain et al. 2012). A similar pleiotropic effect has been observed for Al and Fe in rice using ICP analysis in two recombinant inbred lines populations (Zhang et al. 2014).

In conclusion, this study reports important wheat genome regions associated with the accumulation of Al, Fe and Na under WL and represents the first study aiming to evaluate genetic diversity for the elemental accumulation in a diverse panel of wheat under WL stress.

Table 1 Descriptive statistics for yield and elemental concentrations and percentage of variance attributed to genetic, treatment, genotype x treatment and other effects in 28 wheat genotypes evaluated under waterlogging and non-waterlogging conditions in Stuttgart, AR 2012-2013.

Source	Trait												
	GY (g m ⁻²)	Zn (ppm)	Cu (ppm)	K (%)	Mg (%)	S (%)	Ca (%)	P (%)	B (ppm)	Mn (ppm)	Fe (ppm)	Na (ppm)	Al (ppm)
<u>Descriptives</u>													
Control	451	41	21	4.27	0.12	0.25	0.26	0.27	2.22	133	89	292	25
Waterlogging	263	29	15	3.33	0.11	0.27	0.32	0.34	3.75	257	474	1571	307
Standard error	41	2	1	0.08	0.004	0.01	0.01	0.02	0.15	12	59	56	52
Fold change	1	1	1	0.78	0.94	1.09	1.20	1.25	1.69	2	5	5	12
<u>Variance component of each effect divided by the total of all variance components</u>													
Treatment	59*	61**	35**	68*****	4	9	23*	39*	59*****	80*****	72**	91*****	66**
Rep (Treatment)	24*****	9*****	5**	1*	16*****	15*****	7*****	25*****	2*	4*****	9*****	1***	14*****
Genotype	0	1	0	0	1	1	0	0	0	0	0	0	0
GenotypexTreatment	4**	5*	9*	11*****	17**	11*	32*****	4	4	1	0	0	0
Residual	14	25	51	21	62	64	38	32	34	15	19	7	20

* Significant at P = 0.05, ** Significant at P = 0.01, *** Significant at P = 0.001, **** Significant at P = 0.0001

Table 2 Descriptive statistics for elemental concentrations and percentage of variance attributed to genetic and other effects in 240 wheat genotypes evaluated under waterlogging conditions across four site-years.

Source	Aluminum (ppm)				Iron (ppm)				Sodium (ppm)			
	AR13	AR14	LA13	LA14 ^a	AR13	AR14	LA13	LA14 ^a	AR13	AR14	LA13	LA14 ^a
<u>Descriptives</u>												
Mean	392	66	199	33	534	168	376	94	1496	1182	196	104
Minimum	23	4	75	6	87	51	157	38	480	352	78	45
Maximum	2223	382	553	145	2329	1038	1010	306	3347	2996	605	308
Standard error	298	54	68	20	290	79	110	44	439	419	77	38
<u>Analysis of variance</u>												
Genotype	12***	0	11*		28*****	7	8		60*****	25*****	8	
Replication	41*****	0	0		33*****	15*****	2**		2***	2	15*****	
Residual	47	100	89		39	78	90		39	73	77	

* Significance at P = 0.05

** Significance at P = 0.01

*** Significance at P = 0.001

**** Significance at P = 0.0001

^a One replication was analyzed in this environment

Table 3 Summary of marker-trait associations for aluminum, iron and sodium concentrations in 240 wheat genotypes using 5227 markers.

Trait (Chr)	Env	Marker Name	SNP	Position	-Log (P) ^a	Maf	Mean Major Allele	Mean minor allele	Allele Effect ^b	R ²
Al (2B)	AR14	IWA5261	A/G	196	3.1	0.38	58	75	22	0.05
	LA14	IWA3942	A/C	198	4.3	0.05	32	75	58	0.07
Al (5A)	AR13	IWA8118	T/G	111	3.1	0.44	358	433	17	0.05
	MEAN	IWA8118	T/G	111	3.2	0.44	159	183	13	0.05
Fe (3B)	AR14	IWA2712	T/C	110	2.6	0.12	161	195	18	0.04
	MEAN	IWA6002	A/C	218	2.9	0.04	276	779	65	0.05
Fe (5A)	MEAN	IWA2144	T/C	20	2.6	0.20	272	310	12	0.04
	AR13	IWA2144	T/C	20	2.9	0.20	514	639	20	0.04
	MEAN	IWA2145	T/C	20	2.6	0.20	272	310	12	0.04
	AR13	IWA2145	T/C	20	2.9	0.20	514	639	20	0.04
Fe (5A)	AR14	IWA2446	T/C	111	3.0	0.20	159	191	17	0.05
	MEAN	IWA8118	T/G	111	2.4	0.44	266	294	9	0.04
	AR13	IWA8118	T/G	111	2.6	0.44	494	585	16	0.04
Fe (5B)	AR13	IWA2610	A/G	54	2.7	0.38	566	483	17	0.04
	AR14	IWA1342	T/C	61	2.9	0.07	162	209	23	0.05
	LA14	IWA4631	T/C	82	2.7	0.24	87	112	22	0.04
Fe (5B)	AR14	IWA7123	T/C	122	2.9	0.26	158	186	15	0.05
	MEAN	IWA721	A/C	121	2.7	0.07	275	331	17	0.04
Fe (7A)	AR14	IWA4620	T/C	207	3.3	0.44	155	176	12	0.05
	MEAN	IWA614	A/G	178	2.7	0.10	274	336	19	0.04
Fe (7A)	LA14	IWA3655	A/G	107	2.8	0.41	100	83	21	0.04
	MEAN	IWA3987	T/C	101	2.7	0.31	269	299	10	0.04
Na (1B)	AR13	IWA6063	T/C	72	2.9	0.21	1544	1321	17	0.04
	LA14	IWA7560	A/C	99	3.3	0.13	101	125	19	0.05

Table 3 (Cont.)

Na (2B)	MEAN	IWA4189	A/G	218	3	0.38	728	768	5	0.04
	LA14	IWA4189	A/G	218	2.9	0.38	99	113	12	0.04
Na (2B)	LA13	IWA905	A/G	164	3.6	0.15	203	163	25	0.06
	LA14	IWA6554	T/C	164	2.8	0.15	101	124	19	0.04
Na (3A)	MEAN	IWA5212	T/G	210	3.2	0.21	730	806	9	0.04
	AR14	IWA5212	T/G	210	3.4	0.21	1149	1317	13	0.05
Na (5A)	LA13	IWA2	T/G	188	2.5	0.47	208	184	13	0.04
	AR13	IWA454	T/C	188	3.7	0.18	1455	1685	14	0.05
	LA14	IWA454	T/C	188	2.7	0.18	100	122	18	0.04
Na (5A)	MEAN	IWA7303	T/C	17	2.6	0.13	734	801	8	0.03
	LA14	IWA7303	T/C	17	2.7	0.13	101	123	18	0.04
Na (6A)	AR13	IWA416	T/G	106	2.6	0.34	1576	1338	18	0.03
	LA14	IWA416	T/G	106	2.7	0.34	110	93	19	0.04
	AR13	IWA7349	A/C	106	2.8	0.34	1398	1678	17	0.04
Na (7A)	MEAN	IWA7306	A/G	10	2.6	0.13	734	801	8	0.03
	LA14	IWA7306	A/G	10	2.7	0.13	101	123	18	0.04
Na (um)	MEAN	IWA1170	A/G	97	2.5	0.13	734	798	8	0.03
	LA14	IWA1170	A/G	97	3.1	0.13	101	127	20	0.05

^a-Log (*P*) Negative logarithm of the p-value,

^bAllele effect absolute value of allele effect relative to major allele,

Maf Minor allele frequency, *AR13* Arkansas 2013, *AR14* Arkansas 2014, *LA13* Louisiana 2013, *LA14* Louisiana 2014, *MEAN* overall least square mean across all site-years



Fig. 1 Waterlogging treatment in Stuttgart, AR 2014
Photograph: Mason, 2014

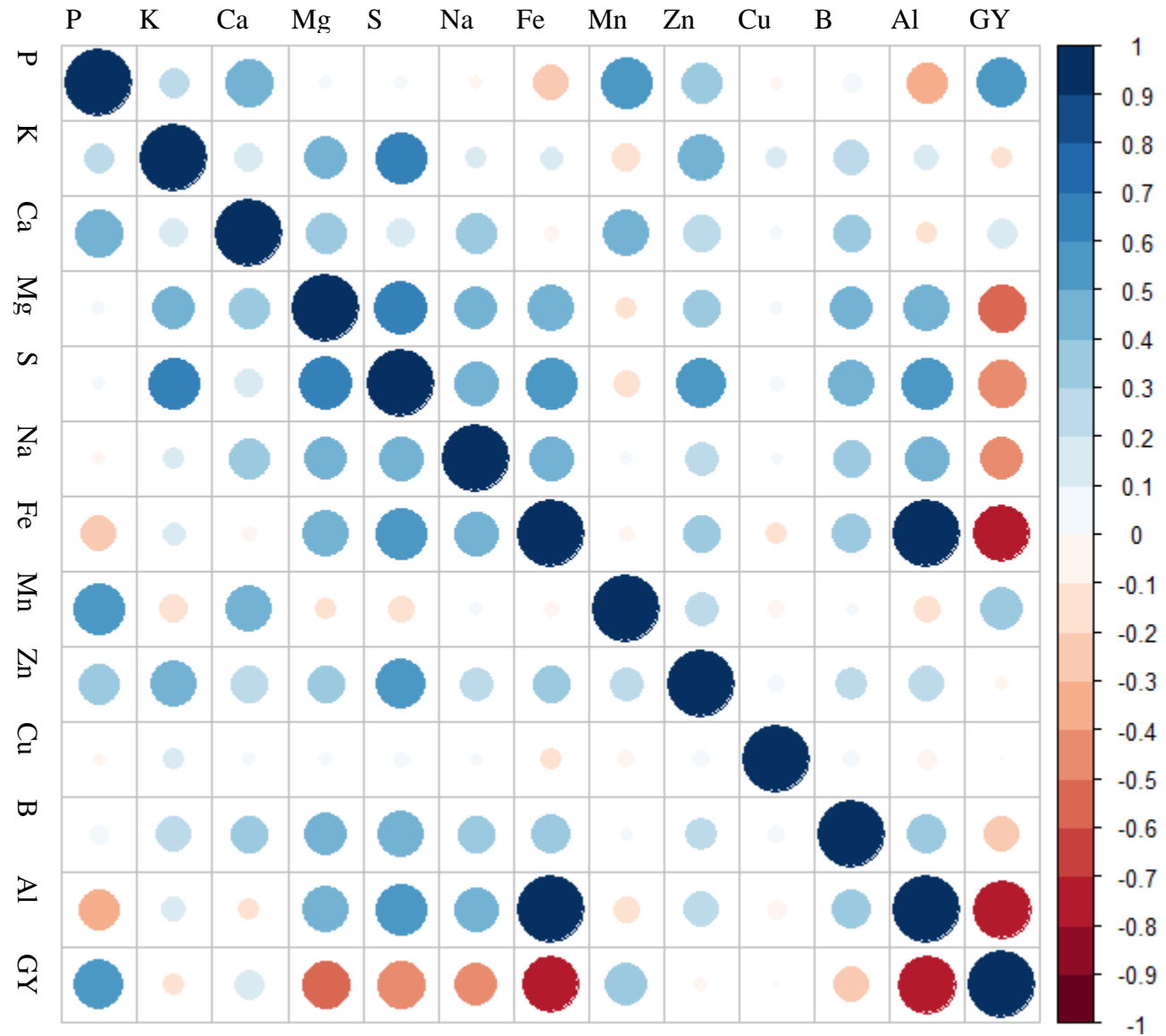


Fig. 2 Correlogram of *P* phosphorus, *K* potassium, *Ca* calcium, *Mg* magnesium, *S* sulfur, *Na* sodium, *Fe* iron, *Mn* manganese, *Zn* zinc, *Cu* copper, *B* boron, *Al* aluminum and *GY* grain yield in a wheat under waterlogging stress in Stuttgart, AR. Diameter of the circle increases with the correlation value

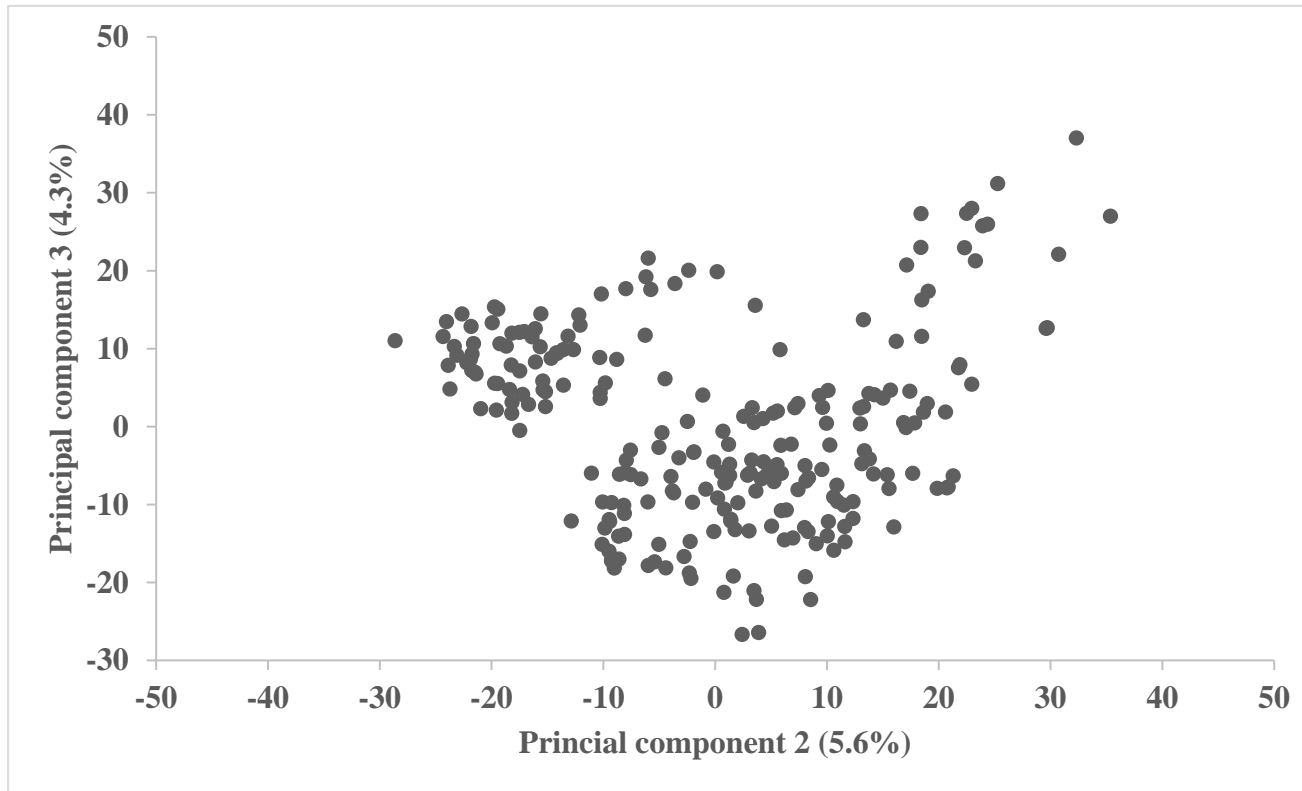


Fig. 3 Principal component analysis for population structure in 240 wheat genotypes using 5227 single nucleotide polymorphism markers

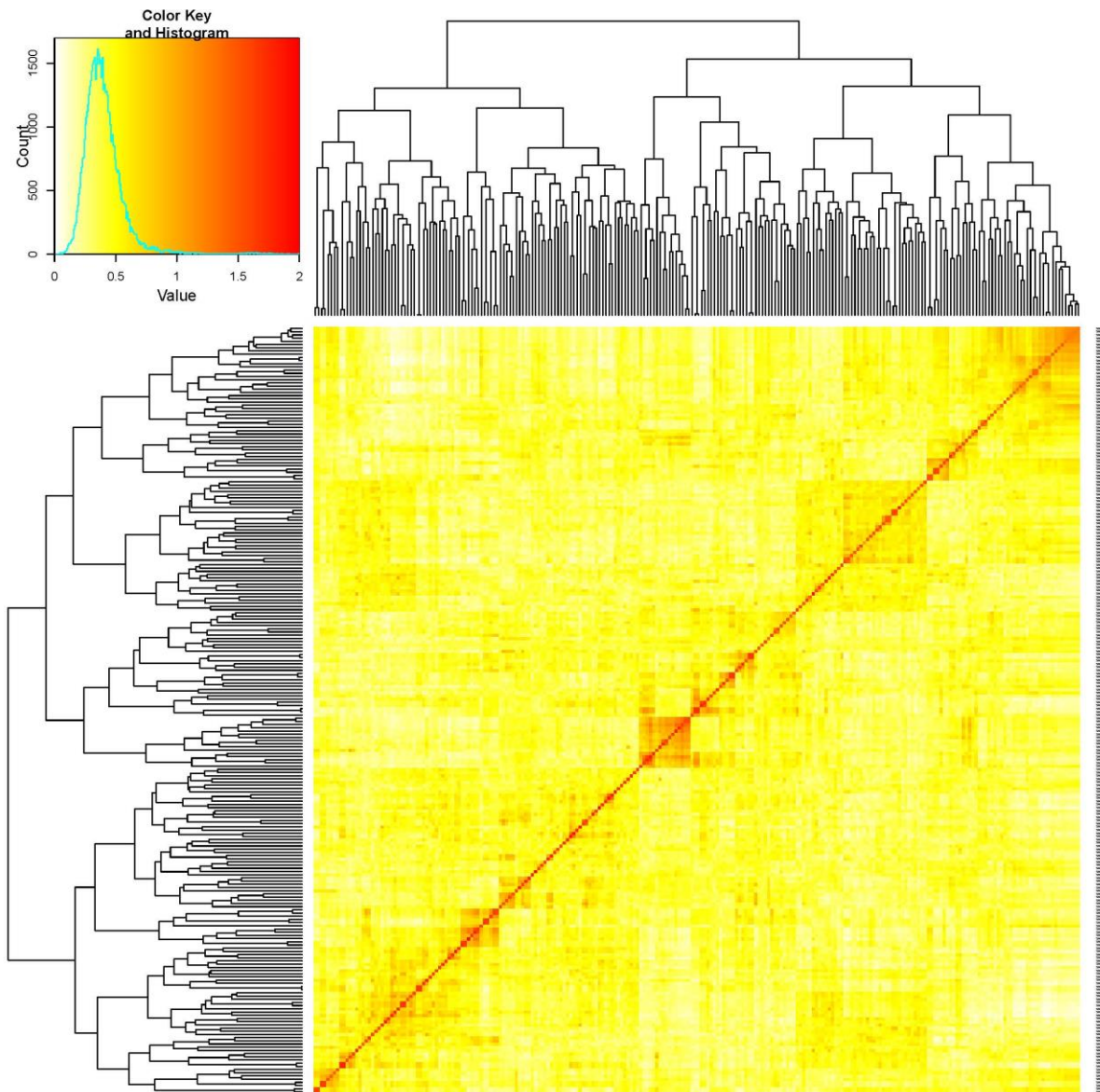


Fig. 4 Heat map of the kindship relationship in 240 wheat genotypes using 5227 SNP markers

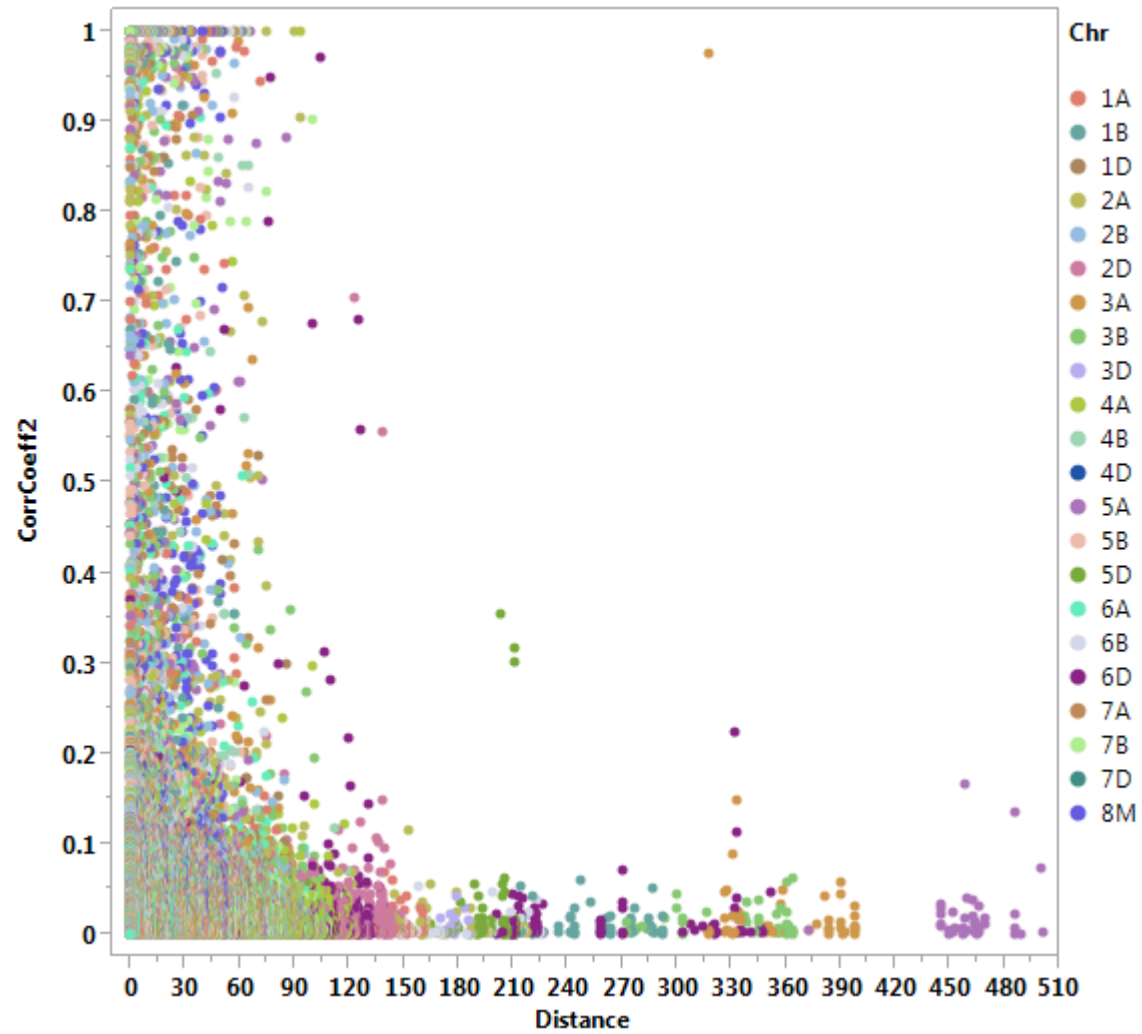


Fig. 5 Linkage disequilibrium decay (r^2 -decay) plot for all chromosomes in 240 wheat genotypes. 8M refers to unmapped markers

References Chapter II

- Al-Maskri AY, Sajjad M, Khan SH (2012) Association Mapping: A Step Forward to Discovering New Alleles for Crop Improvement. *International Journal of Agriculture and Biology* 14:153-160
- Ballesteros D, Mason RE, Addison C, Andrea Acuña M, Nelly Arguello M, Subramanian N, Miller R, Sater H, Gbur E, Miller D, Griffey C, Barnett M, Tucker D (2015) Tolerance of wheat to vegetative stage soil waterlogging is conditioned by both constitutive and adaptive QTL. *Euphytica* 201:329-343
- Benson J, Brown-Guedira G, Murphy JP, Sneller C (2012) Population structure, linkage disequilibrium, and genetic diversity in soft winter wheat enriched for Fusarium head blight resistance. *The Plant Genome* 5:10
- Boru G, van Ginkel M, Kronstad WE, Boersma L (2001) Expression and inheritance of tolerance to waterlogging stress in wheat. *Euphytica* 117:91-98
- Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics* 23:2633-2635
- Cai SB, Bai GH, Zhang DD (2008) Quantitative trait loci for aluminum resistance in Chinese wheat landrace FSW. *Theoretical and Applied Genetics* 117:49-56
- Caldwell KS, Russell J, Langridge P, Powell W (2006) Extreme Population-Dependent Linkage Disequilibrium Detected in an Inbreeding Plant Species, *Hordeum vulgare*. *Genetics* 172:557-567
- Campbell CR, Plank CO (1992) Organic Matter Destruction-Wet Ashing. In: Plank CO (ed) *Plant Analysis Reference Procedures for the Southern Region of the United States*, #368 edn. The University of Georgia, Crop & Soil Science Department, p 68
- Cavanagh CR, Chao S, Wang S, Huang BE, Stephen S, Kiani S, Forrest K, Saintenac C, Brown-Guedira GL, Akhunova A, See D, Bai G, Pumphrey M, Tomar L, Wong D, Kong S, Reynolds M, da Silva ML, Bockelman H, Talbert L, Anderson JA, Dreisigacker S, Baenziger S, Carter A, Korzun V, Morrell PL, Dubcovsky J, Morell MK, Sorrells ME, Hayden MJ, Akhunov E (2013) Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. *Proceedings of the National Academy of Sciences of the United States of America* 110:8057-8062

Collaku A, Harrison SA (2002) Losses in wheat due to waterlogging. *Crop Science* 42:444-450

Colmer TD, Voeselek LACJ (2009) Flooding tolerance: suites of plant traits in variable environments. *Funct Plant Biol* 36:665-681

D. Steffens BWH, T. Eschholz, T. Lošák, S. Schubert, Justus Liebig (2005) Water logging may inhibit plant growth primarily by nutrient deficiency rather than nutrient toxicity. *Plant Soil Enviro*:8

Diaz A, Zikhali M, Turner AS, Isaac P, Laurie DA (2012) Copy Number Variation Affecting the Photoperiod-B1 and Vernalization-A1 Genes Is Associated with Altered Flowering Time in Wheat (*Triticum aestivum*). *Plos One* 7

Dickin E, Wright D (2008) The effects of winter waterlogging and summer drought on the growth and yield of winter wheat (*Triticum aestivum* L.). *European Journal of Agronomy* 28:234-244

Donohue SJ, Aho DW (1992) Determination of P, K, Ca, Mg, Mn, Fe, Al, B, Cu, and Zn in Plant Tissue by Inductively Coupled Plasma (ICP) Emission Spectroscopy. In: Plank CO (ed) *Plant Analysis Reference Procedures for the Southern Region of the United States*. The University of Georgia, Crop & Soil Science Department

Edae E, Byrne P, Haley S, Lopes M, Reynolds M (2014) Genome-wide association mapping of yield and yield components of spring wheat under contrasting moisture regimes. *Theoretical and Applied Genetics* 127:791-807

Fageria NK, Carvalho GD, Santos AB, Ferreira EPB, Knupp AM (2011) Chemistry of Lowland Rice Soils and Nutrient Availability. *Communications in Soil Science and Plant Analysis* 42:1913-1933

Guedira M, Maloney P, Xiong M, Petersen S, Murphy JP, Marshall D, Johnson J, Harrison S, Brown-Guedira G (2014) Vernalization Duration Requirement in Soft Winter Wheat is Associated with Variation at the VRN-B1 Locus. *Crop Science* 54:1960-1971

Hall D, Tegstrom C, Ingvarsson PK (2010) Using association mapping to dissect the genetic basis of complex traits in plants. *Briefings in Functional Genomics* 9

Hattori Y, Nagai K, Furukawa S, Song XJ, Kawano R, Sakakibara H, Wu JZ, Matsumoto T, Yoshimura A, Kitano H, Matsuoka M, Mori H, Ashikari M (2009) The ethylene response factors SNORKEL1 and SNORKEL2 allow rice to adapt to deep water. *Nature* 460:1026-U1116

Hossain MA, Piyatida P, da Silva JAT, Fujita M (2012) Molecular Mechanism of Heavy Metal Toxicity and Tolerance in Plants: Central Role of Glutathione in Detoxification of Reactive Oxygen Species and Methylglyoxal and in Heavy Metal Chelation. *Journal of Botany* 2012

Hossain MA, Uddin SN (2011) Mechanisms of waterlogging tolerance in wheat: Morphological and metabolic adaptations under hypoxia or anoxia. *Australian Journal of Crop Science* 5

Khabaz-Saberi H, Barker SJ, Rengel Z (2012) Tolerance to ion toxicities enhances wheat (*Triticum aestivum* L.) grain yield in waterlogged acidic soils. *Plant and Soil* 354

Khabaz-Saberi H, Rengel Z (2010) Aluminum, manganese, and iron tolerance improves performance of wheat genotypes in waterlogged acidic soils. *J Plant Nutr Soil Sci* 173:461-468

Khabaz-Saberi H, Setter TL, Waters I (2006) Waterlogging induces high to toxic concentrations of iron, aluminum, and manganese in wheat varieties on acidic soil. *Journal of Plant Nutrition* 29:899-911

Li H, Vaillancourt R, Mendham N, Zhou M (2008) Comparative mapping of quantitative trait loci associated with waterlogging tolerance in barley (*Hordeum vulgare* L.). *BMC Genomics* 9:Article No.: 401

Lipka AE, Tian F, Wang Q, Peiffer J, Li M, Bradbury PJ, Gore MA, Buckler ES, Zhang Z (2012) GAPIT: genome association and prediction integrated tool. *Bioinformatics* 28:2397-2399

Ma HX, Bai GH, Carver BF, Zhou LL (2005) Molecular mapping of a quantitative trait locus for aluminum tolerance in wheat cultivar Atlas 66. *Theoretical and Applied Genetics* 112:51-57

Mano Y, Omori F, Muraki M, Takamizo T (2005) QTL mapping of adventitious root formation under flooding conditions in tropical maize (*Zea mays* L.) seedlings. *Breed Sci* 55:343-347

Millaleo R, Reyes- Diaz M, Ivanov AG, Mora ML, Alberdi M (2010) MANGANESE AS ESSENTIAL AND TOXIC ELEMENT FOR PLANTS: TRANSPORT, ACCUMULATION AND RESISTANCE MECHANISMS. *Journal of soil science and plant nutrition* 10:470-481

- Niedziela A, Bednarek P, Cichy H, Budzianowski G, Kilian A, Anioł A (2012) Aluminum tolerance association mapping in triticale. *BMC Genomics* 13:1-16
- Nishida H, Yoshida T, Kawakami K, Fujita M, Long B, Akashi Y, Laurie DA, Kato K (2013) Structural variation in the 5' upstream region of photoperiod-insensitive alleles Ppd-A1a and Ppd-B1a identified in hexaploid wheat (*Triticum aestivum* L.), and their effect on heading time. *Molecular Breeding* 31:27-37
- Paux E, Sourdille P, Mackay I, Feuillet C (2012) Sequence-based marker development in wheat: Advances and applications to breeding. *Biotechnology Advances* 30:1071-1088
- Pearson A, Cogan NOI, Baillie RC, Hand ML, Bandaranayake CK, Erb S, Wang J, Kearney GA, Gendall AR, Smith KF, Forster JW (2011) Identification of QTLs for morphological traits influencing waterlogging tolerance in perennial ryegrass (*Lolium perenne* L.). *Theoretical and Applied Genetics* 122
- Peng JH, Sun D, Nevo E (2011) Domestication evolution, genetics and genomics in wheat. *Molecular Breeding* 28
- Plank CO, Donohue SJ (2000) Small Grain: Barley, Oats, Rye, Wheat. In: Plank CO (ed) Reference sufficiency ranges for plant analysis in the Southern Region of the United States -Field Crops-. North Carolina Department of Agriculture and Consumer Services Agronomic Division
- Powell N, Ji X, Ravash R, Edlington J, Dolferus R (2012) Yield stability for cereals in a changing climate. *Funct Plant Biol* 39:539-552
- Reynolds MP, Ortiz-Monasterio JI, McNab A (2001) Application of Physiology in Wheat Breeding. *CIMMYT*, p 240
- Robertson D, Zhang H, Palta JA, Colmer T, Turner NC (2009) Waterlogging affects the growth, development of tillers, and yield of wheat through a severe, but transient, N deficiency. *Crop & Pasture Science* 60
- Sairam RK, Kumutha D, Ezhilmathi K, Deshmukh PS, Srivastava GC (2008) Physiology and biochemistry of waterlogging tolerance in plants. *Biologia Plantarum* 52:401-412
- Sajjad M, Khan S, Kazi A (2012) The low down on association mapping in hexaploid wheat (*Triticum aestivum* L.). *J Crop Sci Biotechnol*. The Korean Society of Crop Science, pp 147-158

Setter TL, Waters I (2003) Review of prospects for germplasm improvement for waterlogging tolerance in wheat, barley and oats. *Plant and Soil* 253:1-34

Setter TL, Waters I, Sharma SK, Singh KN, Kulshreshtha N, Yaduvanshi NPS, Ram PC, Singh BN, Rane J, McDonald G, Khabaz-Saberi H, Biddulph TB, Wilson R, Barclay I, McLean R, Cakir M (2009) Review of wheat improvement for waterlogging tolerance in Australia and India: the importance of anaerobiosis and element toxicities associated with different soils. *Ann Bot* 103:221-235

Shabala S (2011) Physiological and cellular aspects of phytotoxicity tolerance in plants: the role of membrane transporters and implications for crop breeding for waterlogging tolerance. *New Phytologist* 190

Shepherd LVT, Alexander CA, Sungurtas JA, McNicol JW, Stewart D, Davies HV (2010) Metabolomic analysis of the potato tuber life cycle. *Metabolomics* 6

Sukumaran S, Dreisigacker S, Lopes M, Chavez P, Reynolds M (2015) Genome-wide association study for grain yield and related traits in an elite spring wheat population grown in temperate irrigated environments. *Theoretical and Applied Genetics* 128:353-363

VanRaden PM (2008) Efficient methods to compute genomic predictions. *Journal of dairy science* 91:4414-4423

Wang S, Wong D, Forrest K, Allen A, Chao S, Huang BE, Maccaferri M, Salvi S, Milner SG, Cattivelli L, Mastrangelo AM, Whan A, Stephen S, Barker G, Wieseke R, Plieske J, International Wheat Genome Sequencing C, Lillemo M, Mather D, Appels R, Dolferus R, Brown-Guedira G, Korol A, Akhunova AR, Feuillet C, Salse J, Morgante M, Pozniak C, Luo M-C, Dvorak J, Morell M, Dubcovsky J, Ganal M, Tuberosa R, Lawley C, Mikoulitch I, Cavanagh C, Edwards KJ, Hayden M, Akhunov E (2014) Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. *Plant Biotechnology Journal* 12:787-796

Watkin B, Wailes EJ, Miller W (2011) Arkansas Economic Losses Due to Flooding of Crops - 2011. University of Arkansas, United States Department of Agriculture and County Governments Cooperating.

Würschum T, Langer SM, Longin CFH, Korzun V, Akhunov E (2013) Population structure, genetic diversity and linkage disequilibrium in elite winter wheat assessed with SNP and SSR markers. *Theoretical and Applied Genetics* 126: 1477-1486.:9

Yu H, Deng Z, Xiang C, Tian J (2014) Analysis of Diversity and Linkage Disequilibrium Mapping of Agronomic Traits on B-Genome of Wheat. *Journal of Genomics* 2:20-30

Zhang M, Pinson SM, Tarpley L, Huang X-Y, Lahner B, Yakubova E, Baxter I, Guerinot M, Salt D (2014) Mapping and validation of quantitative trait loci associated with concentrations of 16 elements in unmilled rice grain. *Theoretical and Applied Genetics* 127:137-165

Zhang Z, Ersoz E, Lai C-Q, Todhunter RJ, Tiwari HK, Gore MA, Bradbury PJ, Yu J, Arnett DK, Ordovas JM, Buckler ES (2010) Mixed linear model approach adapted for genome-wide association studies. *Nat Genet* 42:355-360

Zhou M (2011) Accurate phenotyping reveals better QTL for waterlogging tolerance in barley. *Plant Breeding* 130:203-208

CHAPTER III
SOIL WATERLOGGING: IMPACT ON GRAIN YIELD AND YIELD COMPONENTS
IN SOFT RED WINTER WHEAT GERMPLASM

Abstract

Wheat is grown on 17% of all crop land worldwide and because of its widespread cultivation faces many production constraints, such as soil waterlogging (WL), which has been reported to reduce grain yield by as much as 50%. With global climate change predicted to result in increased winter precipitation, improving the tolerance of wheat to soil WL is a priority area for breeders in regions prone to WL such as the southern U.S. In this study, 28 soft red winter wheat breeding lines and cultivars were evaluated over two growing seasons (YR1 and YR2) from 2012-2014. Experiments were planted in a randomized complete block design with four replications and two treatments, WL and non-WL. In YR1 a 28 day WL treatment reduced grain yield by 42%, compared to 13% in YR2 where a 14 day WL treatment was applied. GY reduction in YR1 resulted from the combined effect of reductions in total biomass, kernel weight per spike and spikes m^{-2} . In YR2, the largest reductions were observed for kernel weight per spike, thousand-kernel weight and kernel-number per spike. In YR1 a genotype by treatment effect (GxT) was observed for all traits with the exception of thousand kernel weight, indicating differences in genotype performance under WL and non-WL conditions. In YR2, lower GxT was observed, with more of the phenotypic variation explained by genetic variation between cultivars. All yield components were positively correlated with grain yield, regardless of treatment, with spikes m^{-2} the most important for yield production and total biomass highly correlated with grain yield under WL conditions. Overall, the cultivars 'Jamestown' and 'USG3555' were found to be tolerant to waterlogging across both growing seasons, preventing large reductions in spikes m^{-2} , kernel weight per spike, kernel number per spike and thousand kernel weight which contributed to a lower reduction total grain yield.

Introduction

Soil waterlogging (WL) affects up to 10% of global land area, including 25% of the worldwide area planted to wheat (Powell et al. 2012). WL has continued to become an increasingly important problem for crop production in the United States including in Arkansas, where more than US\$20 million in economics losses were reported in 2011 (Watkin et al. 2011). During WL stress, saturation of the air pore spaces in the soil results in hypoxia, or low oxygen availability, or anoxia, the complete absence of oxygen for plants (Colmer and Voesenek 2009). Low oxygen availability disturbs plant physiology and metabolism, resulting in reduced growth, delayed development and lower total wheat yield and yield components (Dickin and Wright 2008). Reduction in grain yield production is physiologically explained by a decreased number of molecules of adenosine triphosphate (ATP) produced in a hypoxic environment, which triggers lactic acid and alcohol fermentation, subjecting the plants to less water and less nutrients available for growth and development compared to aerobic conditions (Colmer and Greenway 2011).

The severity of yield reduction for wheat exposed to soil WL depends greatly on the intensity and duration of the WL stress, as well as the developmental stage when the stress is imposed. Under 14 days of waterlogging at the tillering stage, wheat showed a 50% reduction in tiller number resulting in 71% lower shoot dry weight (Robertson et al. 2009). A reduction of 50% of tillers per unit area following 44 days of WL was observed by Dickin and Wright (2008) who suggested that maintaining 450-550 m⁻² spike-bearing tillers should be addressed to maintain yield under WL conditions in Australia. Collaku and Harrison (2002) reported that five weeks of uninterrupted waterlogging at the tillering stage resulted in a 44% reduction in grain yield in soft red winter wheat (SRWW), resulting mainly from a reduction in kernel number and tiller number. Araki et al. (2012) investigated the effect of 8 days of WL after heading and found root

growth to be largely affected and that recovery of root biomass and development of new roots was difficult even after the stress had ceased. Khabaz-Saberi et al. (2006) reported up to a 52% reduction in shoot dry weight while Ali et al. (2012) reported a reduction of 1.5% in grain yield, 0.7% for spikes m^{-2} and 1.3% for grain weight for each day of WL stress applied at the four-to-five leaf stage. In the same study, the authors showed that ten days of WL are sufficient to have a significant effect on yield and yield components. Shao et al. (2013) investigated the effect of WL on photosynthetic and yield traits in winter wheat at different growth stages (tillering, jointing-booting, onset of flowering and milky stage) and reported a significant decrease in root length, root mass and root/shoot ratio when the stress was applied at both the tillering and booting stages. Measurements of transpiration rate, stomatal conductance and net photosynthetic rate (PN) were also affected after 4 days of WL. Overall, PN decreased by 13.6% when WL stress was applied at the tillering stage and by 12.2% for booting stage stress, with the observed decrease in photosynthesis resulting from stomatal closure. In the same study, a grain yield decrease of 7.1 to 11.2% was observed, resulting from fewer spikes ha^{-1} and also impacted by a delay in flowering time and maturity. The study concluded that reduction in photosynthesis and thus yield could be explained by less leaf turgor and stomatal conductance due to decreased hydraulic conductivity, leading to a CO_2 deficit and a shortage of assimilate accumulation.

Despite the economic impacts of soil WL on wheat production, previous reports on the tolerant germplasm in SRWW are limited to a single study in which Collaku and Harrison (2002) evaluated 15 SRWW cultivars under 5 weeks of continuous stress. This lack of information is an obstacle toward determining genetic sources of tolerance traits that can be integrated for developing wheat cultivars with improved WL tolerance. Therefore, the focus of this study was

to evaluate the performance of 28 SRWW cultivars and breeding lines adapted to the southeastern U.S. subjected to WL stress during the vegetative stage.

Material and Methods

Plant material and field experiments

A set of 28 adapted SRWW cultivars and advanced breeding lines was used for this study, including entries from both public and private breeding programs. Field waterlogging, experiments were carried out at the Rice Research and Extension Center in Stuttgart Arkansas in the 2012-2013 and 2013-2014 growing seasons. Stuttgart soils are characterized by a silt loam surface layer and a clay subsoil with low permeability (NRCS 2013) and are prone to periodic WL. Plots were drill seeded at a rate of 118 kg of seed hectare⁻¹ in seven row plots measuring 1.25 meters wide x 6 meters long. The experimental design was a split plot with randomized complete blocks with 4 replications and two treatments (WL and non-WL). All plots were fertilized with 170 kg of nitrogen as urea in a split application, with 60% applied prior to the WL treatment and 40% applied post WL treatment.

Field WL was imposed by establishing 0.30 m high levees surrounding the experimental field and applying water to saturate the soil twice weekly for the duration of the treatment (Fig 1). In the 2012-2013 season (YR1), the WL treatment was started on March 20, 2013 at Feekes stage 4 and continued until April 17, 2013 at Feekes stage 5. In 2013-2014 season (YR2), the treatment was started on April 1 at Feekes stage 4 and continued until April 14 at Feekes stage 5.

Trait measurement

Yield components including spike density (Sm²), kernel number per spike (KNS), kernel weight per spike (KWS), total biomass (TB), and harvest index (HI) were estimated by harvesting 50 spike-bearing culms from each plot at maturity prior to whole plot harvest. The Seedburo 801 (Seedburo Equipment Company, Des Plaines, IL) was used to count and calculate the weight of 1000 kernels (TKW). Plant height (PH) was measured at maturity from the soil surface to the top of each plot, excluding awns. Days-to-heading (HD) was measured in Julian days when 50% spike emergence for each plot. Grain yield (GY) in g m⁻² was determined on whole plots by combine harvesting, drying and weighing the grain with final GY adjusted to 13% moisture. Test weight (TW) was determined on a volume basis in kg hl⁻¹ using a GAC2500AGRI DICKEY-John Grain Moisture Tester (Churchill Industries, Minneapolis, MN).

Statistical analysis

Data were analyzed using procedures in SAS 9.3 (SAS Institute Inc. 2011, Cary, NC). To determine the impact of the WL treatment, an analysis of variance was performed using Type 3 sum of squares with WL treatment (WL vs non-WL) as a fixed effect and genotype, rep(treatment) and genotype by treatment interaction (GxT) as random effects. Variance components were estimated using Type 3 sum of squares with all effects, including treatment treated as random and were used to determine the percentage of total phenotypic variation explained by individual variance components. Pearson's correlation using PROC CORR were used to determine associations among the measured phenotypic traits. This correlation analysis was conducted individually for the WL and non-WL treatments.

Results

Impact of WL on yield and yield components

In 2012-2013 (YR1), a significant WL treatment effect ($p \leq 0.05$) was observed for PH, GY, KWS, TKW, KNS, TB, SB and HI. Mean GY was reduced by 42%, with genotypes showing a range in reduction from 15 to 60%. TB, KWS, Sm² and TB were the traits most affected while TW and HI were the least affected by WL. Likewise, in the 2013-2014 season (YR2), a significant treatment effect ($p \leq 0.05$) was observed for PH, TW, KWS, TKW, KNS and HI. Although not significant ($p \leq 0.05$), a 13% GY reduction was observed in YR2 with genotypes showing a range in reduction of 1 to 47%. The most affected traits in YR2 were KWS, TKW and KNS, while the least impacted were Sm², SB and TB. Overall a greater GY reduction was observed with the 28 days of WL in YR1 than the 14 days of WL in YR2. Similarly, there was a greater reduction in YR1 for yield components including KWS, KNS, TB and Sm² than in YR

Analysis of variance

In YR1, treatment accounted for the largest portion of phenotypic variance for most traits, ranging from 9% for TW up to 59% for GY, with genotypic variation observed only for TKW (Table 1). Significant genotype by treatment (GxT) interaction was observed for all traits, indicating that genotypes performed differently under WL versus non-WL conditions and this contributed to the low level of observed genotypic variation. This was in contrast to YR2, where significant genotype variation was observed for all traits, with genotype explaining from 11 to 44% of the phenotypic variance (Table 2). While a large portion of the phenotypic variance was still explained by the WL treatment, a lower level of GxT, significant for only KWS and TKW, explaining only 5% of the variation for both.

Trait correlations

All measured yield components were positively correlated with total GY, although the magnitude of the correlations differed between treatments. Spike density (Sm2) was the most strongly correlated with GY regardless of treatment, with $r = 0.88$ and $r = 0.74$ under WL and control treatments, respectively. Total biomass ($r = 0.79$) and straw biomass ($r = 0.63$) were more strongly correlated to GY under WL stress compared to control conditions ($r = 0.49$ and $r = 0.09$, respectively), while plant height showed a strong correlation under control ($r = 0.70$) with a low but significant correlation ($r = 0.19$) observed under WL conditions.

Differences between cultivars

Overall there was a wide range in cultivar performance for the majority of phenotypic traits measured (Table 1 and Table 2). However, differences in cultivar performance and WL tolerance based on percent reduction under WL versus non-WL conditions was highly dependent on year (Table 4). For example, cultivar 'Baldwin' had the highest GY in YR1 under waterlogging and was the most WL-tolerant with only a 15% GY reduction. 'Baldwin' was the least impacted in YR1 because of its ability to maintain KNS (5% reduction) and Sm2 (14% reduction). In contrast, 'Baldwin' showed a 40% GY reduction in YR2, including 39% KWS reduction and 22% TB reduction. In contrast, Cultivar 'AR01163-3-1', had the highest GY under control conditions in YR1 but was also the most susceptible and lowest yielding under WL, with a 60% GY reduction, 23% KNS reduction, 53% Sm2 reduction and 70% TB reduction.

Cultivar ‘Pioneer 26R20’ had high GY performance in both WL and non-WL in YR1, however in response to WL stress, it reduced GY by 38%, with KWS and TB being the most reduced yield components at (29% and 35% respectively). Likewise, ‘Terral TV8861’ had the highest GY under control conditions in YR1 and YR2, but had a 53% GY reduction in YR1 and 23% reduction in YR2 (Table 4). Cultivar ‘AR01167-3-1’ was the 5th most tolerant line with 1% GY reduction showing no reduction in Sm2 and TB.

Cultivar ‘USG3555’ was the most stable genotype, ranking in the top five most WL-tolerant in both YR1 and YR2. ‘USG3555’ maintained yield by preventing large reductions in KWS, KNS and TKW (Table 4). Cultivar ‘Jamestown’ showed similar results, ranking in the top five most WL-tolerant in both YR1 and YR2 by maintaining KWS and KNS in YR1 and Sm2 and TB in YR2.

Discussion

Impact of WL stress on wheat yield

This study evaluated the impact of a 28 (YR1) or 14 (YR2) day field WL treatment on yield and yield components in 28 adapted soft red winter wheat cultivars. Overall, WL stress reduced mean GY by 40%, with a maximum reduction of 60% observed in susceptible cultivars. Yield components including KWS, KNS and TKW were also significantly reduced. These findings are similar to reported findings by Collaku and Harrison (2002), who evaluated 15 wheat cultivars under 5 weeks of continuous WL and reported yield losses up to 44%.

For GY, a significant GxT effect was observed in YR1 but not in YR2, which resulted in increased genotypic variation in YR2. A likely explanation for differences observed between

years is variation in the level of the stress intensity across the two years, with the 28 day WL treatment too drastic to detect differences in the genotype response (Musgrave and Ding 1998). As mentioned before, the soils properties play an important role in WL impact, for example the drainage rate in clay soils (type of soil in this experiment) which could increase the effective duration of the stress and could explain the inability to detect genetic variation in YR1. These results could also simply mean that tolerance for WL is limited in these commercial cultivars and reaffirms the importance on continuing with cultivar development and introgression of traits associated with WL tolerance, such as KWS, TKW, KNS which are highly correlated with yield in both waterlogged and control growing conditions and were greatly reduced in WL conditions (Table 3).

These results reinforce the importance of screening breeding lines under waterlogged soils in the southern of the United States, which continues to be a region prone to waterlogged soils. Furthermore, genotype by WL treatment interaction observed suggests that the evaluation of cultivars under WL regimes across different years and locations is highly relevant if genetic gain for WL tolerance is to be made in modern wheat cultivars.

Cultivar performance

Results of this allow for important observations of genotype performance. Cultivars ‘Terral TV8861’, ‘Pioneer 26R10’, ‘AR01167-3-1’ were found to be among the top yielding cultivars under control conditions in YR1 and YR2, but only high yielding under WL in YR2. Meanwhile, ‘GA-031086-10E29’ and ‘Pioneer 26R22’ were high yielding under WL in YR1 and YR2 but only high yielding in control conditions in YR2. These cultivars maintained their high

performance by combining high Sm2 and TB under WL, which have previously been suggested as traits associated with WL tolerance by Collaku and Harrison (2002). When comparing performance under WL versus control, Pioneer 26R87 was found to have the lowest reduction in GY, Sm2, TB and SB among the cultivars evaluated, in agreement with the high correlation of Sm2 and biomass with GY under WL (Table 3). These findings are in agreement with the study by Zhang et al. (2007) who reported number of ears to be crucial to prevent GY losses and also with Collaku and Harrison (2002) that suggested tiller and kernel number as key traits for WL tolerance traits under WL. Other cultivars including USG3555, Jamestown, and Pioneer 26R22, which were high yielding under WL, were found to have a lower reduction in KNS, TKW, Sm2 and TB.

Taken together, cultivar performance under control and WL conditions and the correlation of yield components with GY suggests that attention should be given to not only cultivars that maintain higher total biomass and tiller number per unit area, but also to KNS, TKW and KWS, which can lead to higher yielding cultivars performing well regardless of WL stress. Overall, these results add important knowledge that can be used by wheat breeders regarding important traits and potential germplasm to be incorporated in future varieties adapted to flood prone areas such as the southeastern U.S. Along with traits reported in this research, future work may target physiological traits such as aerenchyma development and root porosity (Hossain and Uddin 2011), high chlorophyll content and photosynthetic rates (Ballesteros et al. 2015), adventitious root development (Saqib et al. 2005) and tolerance to elemental toxicities (Shabala 2011) and nutrient deficiencies (Yavas et al. 2012).

Table 1. Descriptive statistics for yield and yield components and percentage of variance attributed to genetic, treatment, genotype x treatment and other effects in 28 wheat genotypes evaluated under waterlogging and non-waterlogging conditions in Stuttgart, AR 2012-2013.

Source	HD ^a (days)	PH (cm)	GY (g m ⁻²)	TW (kg hl ⁻¹)	KWS (g)	TKW (g)	KNS	Sm2	TB (g)	SB (g)	HI (%)
Means											
Control	117	84.0	451	73.0	1.03	42.0	25.0	439	844	19961	0.54
Waterlogging	121	79.0	263	70.0	0.74	37.0	20.0	347	505	12304	0.51
Range	112 - 127	61 - 99	25 - 601	68 - 84	0.29 - 1.50	29 - 63	9 - 33	52 - 813	47 - 1359	1054 - 40925	0.36 – 0.95
Reduction (%)	+3	-6	-42	-3	-29	-13	-19	-21	-40	-38	-5
Analysis of variance											
Treatment	-	16*	59*	9.0	57***	32***	37*	15	52*	36*	5.0*
Genotype	-	0.0	0.0	0.0	0.0	4.0**	0.0	0.0	0.0	0.0	4.0
G*T	-	22****	4.0**	28****	12****	13	11**	12**	7.0****	16****	14*
Rep (T)	-	9.0****	24****	6.0***	7.0****	1.0	12****	31****	25****	21****	0.0
Residual	-	53	14	56	24	49	40	41	16	27	77

* Significant at P = 0.05

** Significant at P = 0.01

*** Significant at P = 0.001

**** Significant at P = 0.0001

HD Heading date *PH* Plant height *GY* Grain yield, *TW* Test weight *KWS* Kernel weight spike⁻¹ *TKW* Thousand-kernel weight *KNS* Kernel number per spike *Sm*⁻² Spike m⁻² *TB* Total biomass *SB* Straw Biomass *HI* Harvest index *T* Treatment, *G* Genotype *Rep* Replication

^a Data taken on one replication

Table 2 Descriptive statistics for yield and yield components and percentage of variance attributed to genetic, treatment, genotype x treatment and other effects in 28 wheat genotypes evaluated under waterlogging and non-waterlogging conditions in Stuttgart, AR

Source	HD ^a (days)	PH (cm)	GY (g m ⁻²)	TW (kg hl ⁻¹)	KWS (g)	TKW (g)	KNS	Sm ²	TB (g)	SB (g)	HI %
Means											
Control	117	70.0	306	74.0	0.86	37.0	24	362	953	31874	0.31
Waterlogging	118	63.0	265	71.0	0.63	31.0	20	426	926	33465	0.28
Range	115 - 125	46 - 86	58 - 494	66 - 77	0.34 – 1.35	22 - 50	13 – 38	66 - 878	10 - 1694	150 - 65493	0.20 – 0.49
Reduction (%)	+1	-10	-13	-3	-26	-14	-14	+17	-3	+5	-10
Analysis of variance											
Treatment	4.0	33*	5.0	7.0*	58****	47****	28****	4.0	0.0	0.9	25****
Genotype	44****	12****	14.**	12.*	11**	35****	25****	14**	24****	13**	11*
G*T	0.0	0.0	0.0	1.0	5.0*	5.0****	4.0	0.0	0.0	0.0	5.0
Rep (T)	4.0	15****	6.0**	0.0	0.0	2.0****	1.0	3.0	13****	4.0****	0.0
Residual	48	40	76	81	26	12	43	80	64	83	58

* Significant at P = 0.05

** Significant at P = 0.01

*** Significant at P = 0.001

**** Significant at P = 0.0001

HD Heading date *PH* Plant height *GY* Grain yield, *TW* Test weight *KWS* Kernel weight spike⁻¹ *TKW* Thousand-kernel weight *KNS* Kernel number per spike *Sm²* Spike m⁻² *TB* Total biomass *SB* Straw Biomass *HI* Harvest index *T* Treatment, *G* Genotype *Rep* Replication

Table 3 Phenotypic correlations (r) of yield and yield components with total grain yield in 28 wheat genotypes.

Trait	Control treatment	Waterlogging treatment
Heading date	0.14	0.09
Plant height	0.70****	0.19**
Test weight	0.22***	0.28****
Kernel weight per spike	0.54****	0.39****
Thousand kernel weight	0.25****	0.15*
Kernel number per spike	0.41****	0.35****
Spike m ⁻²	0.74****	0.88****
Total biomass	0.49****	0.79****
Straw biomass	0.09	0.63****
Harvest index	0.04	0.11

* Significant at $P = 0.05$

** Significant at $P = 0.01$

*** Significant at $P = 0.001$

**** Significant at $P = 0.0001$

Table 4 Five most tolerant and susceptible cultivars based on percent reduction of yield and yield components under waterlogged versus non-waterlogged conditions.

Description	Cultivar	% reduction						
		Grain Yield	Kernel weight spike ⁻¹	Kernel number spike ⁻¹	Spikes m ⁻²	Total Biomass	Straw Biomass	Thousand kernel weight
Tolerant YR1	Baldwin	-16	-15	-5	-15	-38	-47	-11
	Terral LA841	-20	-13	-5	-14	-27	-33	-10
	LA02015E201	-23	-18	-11	-6	-28	-35	8
	USG3555	-29	1	-1	-28	-21	-11	+1
	Jamestown	-30	+6	+2	-36	-23	-12	+3
Susceptible YR1	AR01163-3-1	-61	-22	-23	-53	-69	-75	+1
	Pioneer 26R10	-61	-32	-19	-41	-59	-57	-21
	LA0320DE-2	-54	-24	-22	-45	-43	-21	-4
	Terral TV8861	-53	-37	-36	-25	-43	-30	-2
	AGS 2060	51	-44	-38	-27	-58	-63	-9
Tolerant YR2	LA01110D-150	+35	-14	+3	+63	+36	+36	-16
	AGS 2060	+3	-22	-7	+34	+12	+16	-16
	USG3555	+3	-10	+1	+11	3	5	-10
	Jamestown	0	-19	-12	+27	+14	+20	7
	AR01167-3-1	-1	-30	-21	+43	+9	+14	-12
Susceptible YR2	AGS 2035	-47	-36	-19	-17	-35	-28	-21
	AR01179-4-1	-45	-23	0	-28	-39	-15	-22
	Baldwin	-40	-39	-16	-3	-22	15	-26
	Pioneer 26R87	-40	-33	-15	-9	-28	-23	-21
	AGS 2038	-35	-43	-33	-5	-19	-11	-15

References Chapter III

- Ali R, Mohammad-Eghbal G, Saeid J-H, Mokhtar G, Mohsen S (2012) Impacts of waterlogging on shoot apex development and recovery effects of nitrogen on grain yield of wheat. *European Journal of Experimental Biology*, 2012 2:8
- Araki H, Hossain MA, Takahashi T (2012) Waterlogging and Hypoxia have Permanent Effects on Wheat Root Growth and Respiration. *Journal of Agronomy and Crop Science* 198
- Ballesteros D, Mason RE, Addison C, Andrea Acuña M, Nelly Arguello M, Subramanian N, Miller R, Sater H, Gbur E, Miller D, Griffey C, Barnett M, Tucker D (2015) Tolerance of wheat to vegetative stage soil waterlogging is conditioned by both constitutive and adaptive QTL. *Euphytica* 201:329-343
- Collaku A, Harrison SA (2002) Losses in wheat due to waterlogging. *Crop Science* 42:444-450
- Colmer TD, Greenway H (2011) Ion transport in seminal and adventitious roots of cereals during O₂ deficiency. *Journal of Experimental Botany* 62:39-57
- Colmer TD, Voesenek LACJ (2009) Flooding tolerance: suites of plant traits in variable environments. *Funct Plant Biol* 36:665-681
- Dickin E, Wright D (2008) The effects of winter waterlogging and summer drought on the growth and yield of winter wheat (*Triticum aestivum* L.). *European Journal of Agronomy* 28:234-244
- Hossain MA, Uddin SN (2011) Mechanisms of waterlogging tolerance in wheat: Morphological and metabolic adaptations under hypoxia or anoxia. *Australian Journal of Crop Science* 5
- Khabaz-Saberi H, Setter TL, Waters I (2006) Waterlogging induces high to toxic concentrations of iron, aluminum, and manganese in wheat varieties on acidic soil. *Journal of Plant Nutrition* 29:899-911
- Musgrave ME, Ding N (1998) Evaluating wheat cultivars for waterlogging tolerance. *Crop Science* 38:90-97
- Powell N, Ji X, Ravash R, Edlington J, Dolferus R (2012) Yield stability for cereals in a changing climate. *Funct Plant Biol* 39:539-552
- Robertson D, Zhang H, Palta JA, Colmer T, Turner NC (2009) Waterlogging affects the growth, development of tillers, and yield of wheat through a severe, but transient, N deficiency. *Crop & Pasture Science* 60
- Saqib M, Akhtar J, Qureshi RH (2005) Na⁺ exclusion and salt resistance of wheat (*Triticum aestivum*) in saline-waterlogged conditions are improved by the development of adventitious nodal roots and cortical root aerenchyma. *Plant Sci* 169:125-130

Shabala S (2011) Physiological and cellular aspects of phytotoxicity tolerance in plants: the role of membrane transporters and implications for crop breeding for waterlogging tolerance. *New Phytologist* 190

Shao GC, Lan JJ, Yu SE, Liu N, Guo RQ, She DL (2013) Photosynthesis and growth of winter wheat in response to waterlogging at different growth stages. *Photosynthetica* 51:429-437

Watkin B, Wailes EJ, Miller W (2011) Arkansas Economic Losses Due to Flooding of Crops - 2011. University of Arkansas, United States Department of Agriculture and County Governments Cooperating.

Yavas I, Unay A, Aydin M (2012) The waterlogging tolerance of wheat varieties in western of Turkey. *TheScientificWorldJournal* 2012

Zhang HP, Turner NC, Poole ML, Asseng S (2007) High ear number is key to achieving high wheat yields in the high-rainfall zone of south-western Australia. *Australian Journal of Agricultural Research* 58:21-27

CHAPTER IV
OVERALL CONCLUSIONS

Cocnclusion chapter I

Waterlogging impacts wheat yield production worldwide, including the southern of the United States. With current and future precipitation changes due to climate change, it is expected that this soil-type environmental stress will continue to increase its impact and wheat demand will not be sufficiently supplied. Few studies have been conducted that evaluate waterlogging tolerance in current wheat cultivar, which is key for wheat breeders as knowing which traits to select for is crucial in the breeding process.

During waterlogging, the soil properties are affected and plants undergo nutritional unbalances, including nutrient deficiencies such as Nitrogen and Magnesium deficiencies, as well as potential nutrient and elemental toxicity such as Manganese and Iron. Elemental toxicity tolerance is an adaptive trait that has been suggested to confer better waterlogging tolerance in wheat.

Knowledge on the genetic control for waterlogging tolerance and elemental toxicity tolerance is therefore crucial for future wheat breeding efforts.

Conclusions Chapter II

This study suggest that reduction in total grain yield in wheat grown under waterlogging, is partially due to toxicities of Sodium, Aluminum an Iron, as strong negative correlations of these elements with grain yield were found in the yield trial.

Genetic diversity for elemental accumulation in soft winter wheat was found. This diversity is important for current and future wheat breeding schemes, especially for the southern of the United States programs, where soils are constantly prone to waterlogging and potential elemental toxicity could be observed in soft red winter wheat.

Several genetic regions were detected to be involved in the control of elemental accumulation in wheat growing under waterlogging. Chromosomes 2B and 5A were found to contain the majority of markers associated with Aluminum, Iron and Sodium suggesting that there could be a similar tolerance mechanism in wheat for the toxicity tolerance of these elements. This research represents the first study aiming to evaluate genetic diversity for the elemental accumulation in a diverse panel of wheat under waterlogging stress.

Conclusions Chapter III

Grain yield reduction and yield components reduction depends on the duration of the waterlogging stress and the year of evaluation. There was a total grain yield reduction of 42% under 28 days of waterlogging and of 13% under 14 days of waterlogging. Yield reduction in the 28-day waterlogging period resulted of the combined effect of reduction in total biomass, kernel weight per spike and spike m^{-2} . Genetic variance among the adapted soft red winter cultivars was found in year 2 with the shorter period of waterlogging but not with the 28-day waterlogging period. Very low genotype-by-waterlogging was observed in year 1, whereas the opposite was observed for year 2, where low genotype-by-waterlogging effect was found. All yield components were found to be positively correlated with total grain yield regardless of the period of waterlogging and spikes m^{-2} and total biomass accumulation were found to be the most important yield components that need to be enhanced if genetic gain for waterlogging tolerance is to be made.

APPENDIX A. Supplementary Table 1 Germplasm included in yield trial and association mapping studies

Entry #	Genotype name	Entry #	Genotype name	Entry #	Genotype name	Entry #	Genotype
<u>Adapted soft red winter germplasm</u>							
1	AGS 2035	8	GA-031086-10E29	15	Pioneer 26R10	22	Syngenta Magnolia
2	AGS 2038	9	GA-031257-10LE34	16	Pioneer 26R20	23	Syngenta Oakes
3	AGS 2060	10	GA-04570-10E46	17	Pioneer 26R22	24	Syngenta SY Harrison
4	AR01163-3-1	11	Jamestown	18	Pioneer 26R41	25	Terral LA841
5	AR01167-3-1	12	LA01110D-150	19	Pioneer 26R53	26	Terral TV8861
6	AR01179-4-1	13	LA02015E201	20	Pioneer 26R87	27	USG 3120
7	Baldwin	14	LA0320DE-2	21	Syngenta Coker 9553	28	USG3555
<u>Soft winter wheat association mapping diverse panel</u>							
1	McNair_1003	61	McNair_1813	121	SS8641_JJ	181	09283-G1-G1
2	Arthur_CG	62	McNair_701	122	NC-Neuse_PM	182	051336-B-B-1
3	Branson	63	Oasis	123	NC-Cape_Fear	183	081515-G1-G2
4	Coker_747_CG	64	Pat	124	NC-Yadkin	184	011388-8-4-5
5	Coker_9134_CG	65	Pioneer_2568	125	NC06-19896_	185	071694-G5-G5-G1pub
6	Coker_9553	66	Pioneer_25W60	126	NC07-24445	186	001169-7E15
7	Coker_9803_CG	67	Roy	127	NC07-25169	187	01063-1-3-6-2-G2
8	Dominion	68	Sabbe	128	NC07-23880	188	011124-1-42-13
9	FG95195	69	Severn	129	NC08-23089	189	031086-44-4-2
10	Jackson_CG	70	Stacey	130	NC06-20401	190	NC96BGTD1
11	LA97113UC-124	71	VA_96W-247	131	MO_980525	191	NC96BGTD2
12	Massey_CG	72	IL00-8633	132	NC07-22432	192	NC96BGTD3
13	Merl	73	IL00-8641	133	NC08-21273	193	NC96BGTA4
14	MPV_57_CG	74	IL05-4236	134	NC08-23090	194	NC96BGTA5
15	Oakes	75	IL06-13721	135	NC08-23323	195	NC96BGTA6
16	Panola	76	IL06-23571	136	NC08-23324	196	NC97BGTD7
17	Pioneer_2548_CG	77	IL08-24578	137	NC08-23383	197	NC97BGTD8
18	Pioneer_2555_CG	78	IL96-6472	138	AGS_2000_JJ	198	NC97BGTAB9
19	Pioneer_2580_CG	79	INW0304	139	Blueboy_JJ	199	NC97BGTAB10
20	Pioneer_2643_CG	80	P99840C4-8	140	Coker_68-15_PM	200	NC99BGTAG11
21	Pioneer_2684_CG	81	P03528A1-10	141	Coker_916_JJ	201	NC06BGTAG12
22	Pioneer_26R15	82	P07290A1-12	142	Coker_9663_Syn	202	NC09BGTUM15
23	Pioneer_26R24_CG	83	P0570A1-2	143	Coker_9835_PM	203	NC09BGTS16
24	Pioneer_26R31_CG	84	Chesapeake	144	FL_302_JJ	204	NC08-23925
25	Pioneer_26R46_CG	85	McCormick	145	Jamestown_PM	205	USG_3295

Supplementary Table 1. (Cont.)

26	Potomac_CG	86	MD00W16-07-3	146	Pioneer_26R61_JJ	206	ARS05-0074
27	Roane_CG	87	MD99W64-05-11	147	Saluda_PM	207	ARS05-0241
28	Sisson_CG	88	VA01W713	148	Tribute_PM	208	ARS05-0401
29	SS_520	89	KY03C-1237-39	149	USG_3209_PM	209	ARS07-0203
30	SS_5205	90	KY02C-1076-07	150	USG_3555_JJ	210	ARS07-0404
31	Tribute_CG	91	KY03C-1002-02	151	Hunter	211	ARS07-0558
32	VA00W-38	92	KY02C-1121-11	152	Coker_797_JJ	212	ARS07-0815
33	VA01W-21	93	KY02C-2215-02	153	Delta_King_GR9108	213	ARS07-0912
34	VA03W-211	94	KY02C-1043-04	154	Coker_9134_Syn	214	ARS07-1208
35	VA03W-235	95	KY02C-1058-03	155	AGS_2035	215	ARS08-0111
36	Wakefield_CG	96	222-22-5	156	AGS_2010	216	ARS09-776
37	Wakeland_CG	97	AGS_2060	157	AGS_2031	217	MD01W28-08-11
38	Wheeler_CG	98	LA01069D-23-4-4	158	AGS_2485	218	VA05W-139
39	AG_2020	99	LA0110D-150	159	USG_3592__	219	VA05W-151
40	Boone	100	LA01139D-56-1	160	Oglethorpe	220	MO_081652
41	Caldwell	101	LA01164D-94-2-B	161	Baldwin	221	MO_080104
42	Chancellor	102	LA02015E201	162	USG_3120	222	AR00255-16-1
43	Clark	103	Shirley_CG	163	VA_90	223	AR00343-5-1
44	Clemson_201	104	LA02015E58	164	VA_259	224	AR01039-4-1
45	Coker_65-20	105	LA02024E12	165	MO_011126	225	AR01040-4-1
46	Coker_762	106	LA02024E7	166	AGS_2020	226	AR01044-1-1
47	Coker_9152	107	LA03012E-27	167	AGS_2026	227	AR01156-2-1
48	Coker_9375	108	LA03118E117	168	AGS_CL7	228	AR01163-3-1
49	Coker_9766	109	LA03136E71	169	Gore_JJ	229	AR01167-3-1
50	Doublecrop	110	LA03148E12	170	GA001170-7E26	230	AR01179-4-1
51	Elkhart	111	LA03155D-P13	171	GA031238-7E34	231	AR01209-2-1
52	Ernie_CS	112	LA03161D-P1	172	GA00067-8E35	232	AR02061-1-1
53	Flint	113	LA03217D-P2	173	GA001138-8E36	233	LA02015E42
54	GA_1123	114	LA03217E2	174	GA011493-8E18	234	AR910
55	Holley	115	LA04013D-142	175	GA021338-9E15	235	AR97124-4-3
56	Jaypee_CS	116	LA04041D-10	176	GA021245-9E16	236	GA971127
57	Knox_62	117	LA821	177	GA001142-9E23	237	Hazen
58	Kristy	118	LA841	178	991227-6A33	238	Keiser
59	Madison_CS	119	LA95135	179	991371-6E12	239	Nelson
60	Mallard	120	Magnolia	180	071628-G3-G1-G4-G1	240	Rosen

APPENDIX B Supplementary Table 2. Summary of marker-trait associations in soft red winter wheat under waterlogging

Trait (Chr)	Environment	Marker name	SNP	Position	-Log (P)	Maf	Mean major allele	Mean minor allele	Allele difference	R ²
Al (2B)	Ar14	IWA5261	A/G	196	3.1	0.38	58	75	22	0.05
	La14	IWA3942	A/C	198	4.3	0.05	32	75	58	0.07
Al (5A)	Ar13	IWA8118	T/G	111	3.1	0.44	358	433	17	0.05
	All	IWA8118	T/G	111	3.2	0.44	159	183	13	0.05
Fe (3A)	Ar14	IWA2737	A/G	10	4.2	0.08	161	245	34	0.07
	Ar14	IWA4066	T/C	15	2.9	0.43	156	176	11	0.05
Fe (3B)	Ar14	IWA2712	T/C	110	2.6	0.12	161	195	18	0.04
	All	IWA6002	A/C	218	2.9	0.04	276	779	65	0.05
Fe (5A)	All	IWA2144	T/C	20	2.6	0.2	272	310	12	0.04
	Ar13	IWA2144	T/C	20	2.9	0.2	514	639	20	0.04
	All	IWA2145	T/C	20	2.6	0.2	272	310	12	0.04
	Ar13	IWA2145	T/C	20	2.9	0.2	514	639	20	0.04
	Ar14	IWA2446	T/C	111	3	0.2	159	191	17	0.05
	Ar14	IWA7129	T/C	91	2.6	0.12	161	195	18	0.04
	All	IWA8118	T/G	111	2.4	0.44	266	294	9	0.04
	Ar13	IWA8118	T/G	111	2.6	0.44	494	585	16	0.04
Fe (5B)	Ar14	IWA1342	T/C	61	2.9	0.07	162	209	23	0.05
	Ar13	IWA2610	A/G	54	2.7	0.38	566	483	17	0.04
	La14	IWA4631	T/C	82	2.7	0.24	87	112	22	0.04
	All	IWA4708	A/C	77	2.9	0.04	278	279	0	0.05
	Ar14	IWA7123	T/C	122	2.9	0.26	158	186	15	0.05
	All	IWA721	A/C	121	2.7	0.07	275	331	17	0.04
Fe (7A)	Ar14	IWA4620	T/C	207	3.3	0.44	155	176	12	0.05
	All	IWA614	A/G	178	2.7	0.1	274	336	19	0.04
	La14	IWA3655	A/G	107	2.8	0.41	100	83	21	0.04

Supplementary Table 2. (Cont)

	All	IWA3987	T/C	101	2.7	0.31	269	299	10	0.04
Na (1B)	Ar13	IWA6063	T/C	72	2.9	0.21	1544	1321	17	0.04
	La14	IWA7560	A/C	99	3.3	0.13	101	125	19	0.05
Na (2B)	All	IWA4189	A/G	218	3	0.38	728	768	5	0.04
	La14	IWA4189	A/G	218	2.9	0.38	99	113	12	0.04
	La14	IWA6554	T/C	164	2.8	0.15	101	124	19	0.04
	La13	IWA905	A/G	164	3.6	0.15	203	163	25	0.06
Na (3A)	All	IWA5212	T/G	210	3.2	0.21	730	806	9	0.04
	Ar14	IWA5212	T/G	210	3.4	0.21	1149	1317	13	0.05
Na (5A)	La13	IWA2	T/G	188	2.5	0.47	208	184	13	0.04
	Ar13	IWA454	T/C	188	3.7	0.18	1455	1685	14	0.05
	La14	IWA454	T/C	188	2.7	0.18	100	122	18	0.04
	All	IWA7303	T/C	17	2.6	0.13	734	801	8	0.03
	La14	IWA7303	T/C	17	2.7	0.13	101	123	18	0.04
Na (6A)	Ar13	IWA416	T/G	106	2.6	0.34	1576	1338	18	0.03
	La14	IWA416	T/G	106	2.7	0.34	110	93	19	0.04
	Ar13	IWA7349	A/C	106	2.8	0.34	1398	1678	17	0.04
Na (7A)	Ar13	IWA5132	T/C	129	2.7	0.44	1413	1601	12	0.03
	All	IWA7306	A/G	10	2.6	0.13	734	801	8	0.03
	La14	IWA7306	A/G	10	2.7	0.13	101	123	18	0.04
Na (um)	All	IWA1170	A/G	97	2.5	0.13	734	798	8	0.03
	La14	IWA1170	A/G	97	3.1	0.13	101	127	20	0.05

-Log (P) Negative logarithm of the p-value *Maf* Minor allele frequency *Allele difference* Absolute value of allele effect relative to major allele

Ar13 Arkansas 2013 *Ar14* Arkansas 2014 *La13* Louisiana 2013 *La14* Louisiana 2014 *All* Overall least square mean across environments

Supplementary Table 3. Results of waterlogged and non-waterlogged soil analysis

Procedure: Inductively coupled plasma spectrometer SPECTRO ARCOS ICP

Year	Treatment	pH	EC	Phosphorus	Potassium	Calcium	Magnesium	Sulfur	Sodium	Iron	Manganese	Zinc	Copper	Boron
2012 - 2013	Pre-waterlogging	5.51	77	25	160	1193	126	12	56	465	123	2.75	1.00	0.30
	Non-waterlogged	5.49	110	21	131	1253	122	11	65	464	125	2.49	0.98	0.29
	Waterlogged	5.30	132	25	162	1201	131	12	61	462	124	2.75	1.03	0.29
2013-2014	Non-waterlogged	5.22	91	21	98	1106	107	5	42	534	60	0.59	1.33	0.30
	Waterlogged	5.30	142	22	142	1151	124	8	54	495	160	2.74	1.16	0.28

Supplementary Table 4 Low, critical , sufficient and high levels of vegetative stage elemental concentration in wheat *Triticum aestivum* L.

Element	Low	Critical	Sufficient	High
Boron (ppm)	No report	1.00	1.50-4.00	No report
Calcium (%)	0.10 - 0.20	0.15	0.21-1.00	>1.00
Copper (ppm)	3.00 - 5.00	3.00	5.0 - 50.0	51.0-70.0
Iron (ppm)	<10.00	25.00	10.0 -300	301 - 500
Potassium (%)	1.00 - 1.50	2.00	1.51-3.00	3.01-5.00
Magnesium (%)	0.10 - 0.15	0.10	0.16-1.00	>1.00
Manganese (ppm)	10.0 - 15.0	15.00	16.0 -200	201 - 350
Phosphorus (%)	0.11- 0 .20	15.00	0.21-0.50	0.51-0.80
Sulfur (%)	No report	0.10	0.15-0.65	No report
Zinc (ppm)	11.0 - 20.0	15.00	21.0-70.0	1.0 - 150

Reference

Plank and Donohue, 2000

Mill, Benton and Wolf, 1996