IDENTIFICATION OF GENETIC VARIATION FOR PREHARVEST SPROUTING RESISTANCE AND ALPHA-AMYLASE ACTIVITY IN WHEAT TO REDUCE THE EFFECTS OF SPROUTING DAMAGE

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Plant Breeding, Genetics and Biotechnology - Crop and Soil Sciences—Doctor of Philosophy

2018

ABSTRACT

IDENTIFICATION OF GENETIC VARIATION FOR PREHARVEST SPROUTING RESISTANCE AND ALPHA-AMYLASE ACTIVITY IN WHEAT TO REDUCE THE EFFECTS OF SPROUTING DAMAGE

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Wheat quality, marketability, and profitability are threatened by the risks of sprouting damage. Development of wheat varieties with improved sprouting resistance will mitigate the risk of sprouting and increase the production of high quality wheat; however, breeding efforts for improved sprouting resistance are limited by time- and labor-intensive phenotyping and by the lack of major-effect loci associated with sprouting resistance which are independent of grain color. The wheat growing regions of the Pacific Northwest and the Great Lakes are particularly vulnerable to sprouting related quality issues because the high-value soft white winter wheat varieties grown in those areas are susceptible to sprouting damage induced by frequently rainy conditions between physiological and harvest maturity. Sprouting damage is the result of precocious germination prior to grain harvest (preharvest sprouting, PHS) and the enzymatic degradation of the starchy endosperm by elevated alpha-amylase activity (αAmy). Sprouting damage is affected by genetic factors such as gibberellin sensitivity, grain color and maturity, by environmental factors such as temperature and moisture, and by the interaction of genotype and environment.

The objectives of this dissertation were to: 1) develop standardized phenotyping protocol which produce consistent, reliable results across environments and can be practically implemented to improve phenotypic selection in breeding programs; 2) associate sprout resistance phenotypes with specific loci that might be leveraged to improve sprouting resistance; and 3) utilize sprouting resistance alleles from the wild-wheat relative *Aegilops tauschii* to reduce sprouting damage in wheat. Preharvest sprouting rating criteria and a modified enzyme assay were developed to phenotype elite wheat varieties, breeding lines, and experimental wheat populations in replicated field trials. Genome wide association analysis (GWAS) was used to identify significant quantitative trait loci (QTL), and genome-wide regression models were used to predict genomic estimated breeding values (GEBVs) for sprouting resistance. Standardized phenotyping methods were validated for the characterization of PHS and αAmy and were used for phenotyping in all succeeding studies. Additionally, significant sprout resistance loci were identified by GWAS, and genetic values for improved PHS resistance were successfully predicted by GS. Finally, advantageous genetic loci derived from *Ae. tauschii* were identified which are significantly associated with improved PHS

resistance. To improve genetic gain for sprouting resistance in wheat, the methods described herein will enable more accurate selection of superior genotypes through improved phenotyping methods, genomic selection, and pyramiding of significant QTL using marker assisted selection.

To my supportive and loving parents for encouraging me on this adventure
To my brilliant and beautiful sister for her inspiring example
To my partner in life for being patient and supportive

...and Mr. Jasper Cat for being himself

ACKNOWLEDGEMENTS

I would first like to thank my advisor, Dr. Eric L Olson. Surprisingly, I initially interviewed for my position in the Michigan State University Wheat Breeding program while attending a KACTA Crops Team competition at Kansas State University. As a new professor, Dr. Olson generously provided me with the opportunity to pursue an advanced degree in his laboratory. I strived to be a valuable member of the Wheat Breeding program and hope my achievements over that last several years met and exceeded Dr. Olson's expectations.

I also acknowledge the members of my graduate committee who helped guide me on this journey. I feel very fortunate that my committee included Dr. James Kelly, Dr. Mitch McGrath, and Dr. Dechun Wang who are successful breeders in their respective crops and Dr. Perry Ng who is a respected cereal chemist.
Each of these mentors benefited me uniquely: Dr. Kelly instilled an appreciation for the contributions and discoveries of researchers who provided the foundation that current work is built upon; Dr. McGrath encouraged me to question the borders of knowledge to discover new perspectives; Dr. Wang emphasized thoughtful and deliberate interrogation of data to yield valuable results; and, Dr. Ng taught me to consider the value chain beyond the breeder or the grower when looking for research targets.

The members of the MSU Wheat Breeding program also deserve my great appreciation for their contributions. Lee Siler and Matt Graham were essential to the success of the field components of my experiments. Through his attention to detail and vast experience, Lee Siler taught me how to successfully design field experiments, and for that knowledge I will be forever grateful. My fellow graduate students and the post-docs were also essential to my growth as a scientist. Dr. Beth Brisco McCann, Dr. Amber Hoffstetter, Jeff Kovach, Kyle McCarthy, and Dr. Andrew Wiersma were not only coworkers, but also generous sources of collaboration.

I would also like to thank those who lent their time and talents to the reading and editing of this dissertation, especially Roger and Rosanne Brown, Andrew Wiersma, and Eric Olson.

Finally, I would like to thank a few individuals who impacted me personally. Kyle McCarthy was immeasurably helpful to my programming capabilities. No one was as patient with me or as willing to find the bugs in my coding, and for that I am forever thankful. Amber Hoffstetter was a short-term office mate, but a long-term friend. Amber was always willing to lend a helping hand and we enjoyed many long days running the combine at harvest with Lee. And most of all, I need to thank Andrew Wiersma. Andrew was not just my office mate for four years—he was my example. Andrew guided me through the first years of graduate school and I am gratefully indebted to him for his friendship, mentorship, and shared experiences during my time at MSU.

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KEY TO ABBREVIATIONS

Abbreviations

αAmy	alpha-amylase activity
ANOVA	analysis of variance
BLAST	basic local alignment search tool
FD	flowering date
FDR	false discovery rate
GA	gibberellic acid
GC	grain color
gDNA	genomic DNA
GI	germination index
GR	germination resistance
GS	genomic selection
GWAS	genome wide association mapping study
H^2	broad sense heritability
HD	heading date
HFN	Hagberg falling number
IBL	inbred backcross line
Index	index of sprouting
LMA	late maturity alpha-amylase
MAF	minor allele frequency
PG	percent germination
PHS	preharvest sprouting
PS	percent sprouting
QS	quantitative sprouting
QTL	quantitative trait locus
r	Pearson's correlation coefficient
r^2	coefficient of determination

SNP	single nucleotide polymorphism
SVREC	Saginaw Valley Research and Extension Center
UAN	Urea ammonium nitrate (28-0-0)

CHAPTER 1: Review of literature and rationale

Summary of the problem

The quality and marketability of the most widely grown cereal crop, wheat, is threatened by the detrimental impacts of sprouting damage. Wheat breeders need effective phenotyping strategies and a better understanding of the underlying genetic control to make continual progress in variety improvement for this trait.

Impact in Michigan

Soft winter wheat is Michigan's third largest crop, grown on approximately half a million acres by 8,000 farmers producing 38 million bushels each year (Nagelkirk and Black 2012; NASS 2015). Michigan wheat is used by millers, including Chelsea Milling, King Milling, Knappen Milling, Mennel Milling, and Star of the West; processors, including Kellogg Company, Kraft Foods, General Mills, Jiffy, Mondelez, and Post; distillers, including American Fifth Spirits, Red Cedar Spirits, and Grand Traverse Distillery; and more than 160 breweries (Michigan Wheat Program 2017). With such a significant acreage and high value to stakeholders, maintaining the profitability of wheat is critical to the Michigan agricultural economy. However, the entire wheat value chain in Michigan, a $3.9 billion industry (Peterson et al. 2006), is at risk of losses to sprouting damage resulting in reduced grain quality. The integration of sprouting resistance genes into elite soft winter wheat varieties and the adoption of these varieties by Michigan wheat farmers will help reduce sprouting-related losses in the future and support the production of this high value commodity.

Broader impacts

In the United States, soft white winter wheat is produced in the Pacific Northwest while both soft red and white winter wheat market classes are produced in Great Lakes Region. These growing regions frequently experience precipitation events or cool weather near the time of harvest which can result in significant sprouting related losses. Global yield and quality losses due to sprouting damage have a financial impact estimated at $1 billion annually (Bewley et al. 2006). Wheat growers in the state of Washington experienced millions of dollars in sprout-related losses in 2013 (Steber et al. 2014) and were severely impacted again in 2016.

More farmland is dedicated to the production of wheat than any other single food crop (United Nations Food and Agriculture Organization Statistics Division (FAOSTAT) 2014). The majority of wheat is produced for human consumption and makes up about 20% of total caloric intake. Wheat varieties are developed for market classes with unique end uses. Hard red and hard white wheats are primarily used in bread flour while the soft red and soft white wheats are used in pastry flour and ready-to-eat cereal products. Wheat growers work to produce a high quality product to meet these demands, but sprout damage threatens wheat quality and profitability. The economic impact of sprouting damage is most severe in soft white winter wheat which can be sold for profitable premiums if quality standards are met, but also carries increased risks compared to the generally more sprout-resistant red wheat market classes.

Causes of sprouting damage

Sprouting damage in wheat and other grains is caused by two processes: (1) visual preharvest sprouting (PHS); and (2) enzymatic preharvest sprouting as measured by elevated alpha-amylase activity (αAmy) after physiological maturity. Typically, these features are observed together; however, they can occur independently. High αAmy may be caused by activation of enzyme systems in early seed germination prior to visible symptoms, or by the undesirable synthesis of αAmy during the middle stages of grain development. PHS and αAmy can be triggered by genetics or environmental conditions, but the predominant factor in sprouting damage is rainy conditions during the period between physiological maturity and harvest maturity (Gale 1989; Mares and Mrva 2014).

Description of sprouting related traits

PHS and seed dormancy

Preharvest sprouting is moisture induced vivipary, or precocious germination of grain while in the intact spike (Figure 1.1). PHS is primarily controlled by seed dormancy, abscisic acid (ABA) sensitivity, and giberellic acid (GA) sensitivity. During normal grain maturation, the seed enters a dormancy period which prevents premature germination and is interrupted once ideal conditions for growth occur. Seed dormancy may be due to coat-imposed dormancy or embryo dormancy. Seed dormancy in wheat appears to be primarily coat-imposed as shown through genetic and mutational studies (Noll et al. 1982; Warner et al. 2000; Mares and Mrva 2014) although embryo dormancy does play an important role in the expression of dormant phenotype (Noll et al. 1982). PHS is a deviation from this natural dormancy process. Sufficient seed dormancy is desired to prevent PHS, but poor stand establishment can occur if seed dormancy is too great.

Abscisic acid (ABA) is important in the initiation and maintenance of seed dormancy. The ABA content of seeds is very low at the start of embryogenesis, peaks during mid–to–late seed development, and tapers to low levels during maturation. The peak during embryogenesis is controlled by the maternal genotype and is important in suppressing precocious germination (Taiz and Zeiger 2006). ABA can be produced by the seed coat or pericarp and functions in seed coat-imposed dormancy. ABA can also be produced by the embryo and functions in embryo-imposed dormancy. Embryonic ABA sensitivity decreases in sprouting susceptible varieties compared to resistant varieties as the developing grain enters the desiccation stage. A study using excised embryos and whole grain demonstrated that this decrease in embryonic sensitivity is likely not due to a decreased capability of ABA uptake (Walker-Simmons 1987).

Gibberellins (GAs) also function in seed development and germination. The ratio of ABA to GA is very important in maintaining seed dormancy as demonstrated by Koornneef et al. (Koornneef et al. 1982). Mutants with GA-deficiency failed to germinate unless treated with exogenous GA. Mutants with ABA-deficiency germinated under all conditions (no dormancy). Revertants with both GA- and ABA-deficiency germinated due to the absence of dormancy. Therefore, it appears that the relative—not the absolute—levels of ABA and GA determine dormancy and germination.

Alpha-amylase and Hagberg falling number

Alpha-amylase is the enzyme responsible for starch hydrolyzation from large, intact starch molecules into simple sugars to provide energy for the developing seedling. Gibberellins are thought to be influential in alpha-amylase regulation and have long been known to be important in development, dormancy, and germination of seeds. Alpha-amylase is synthesized in the pericarp shortly after anthesis during normal grain development. The levels of alpha-amylase typically decrease rapidly as the grain matures and no additional alpha-amylase synthesis occurs unless moisture induces germination. At the onset of germination GA is synthesized by the embryo and released into the endosperm. Once there, GA diffuses to the aleurone layer and triggers the transcriptional upregulation of alpha-amylase.

The alpha-amylase activity of grain is referred by various terms depending on the stage of grain development. If the right conditions are present, moisture induced synthesis of alpha-amylase enzymes can occur after physiological maturity leading to elevated alpha-amylase activity in the grain (αAmy). The breakdown of the starchy endosperm into simple sugars by αAmy negatively affects flour quality and results in sprouting damage. In some material, a genetic defect is present which results in increased alpha-amylase levels prior to maturity, which is referred to as late maturity alpha-amylase (LMA). In LMA-affected grain, a new period of undesirable alpha-amylase synthesis occurs during the later stages of grain maturation prior to physiological maturity and remains at harvest maturity (Mares and Mrva 2008; Barrero et al. 2013; Mares and Mrva 2014).

Hagberg Falling Number (HFN) (Hagberg 1961) is the metric used by grain elevators and grain buyers to evaluate the level of αAmy or LMA caused sprouting damage (Bewley et al. 2006). The HFN test measures the rheological properties of the flour which is affected by starch hydrolyzation, and thus HFN indirectly measures alpha-amylase activity. In brief, the HFN test consists of mixing flour (7 g, 14% moisture) with water (25 mL) in a test tube (21 mm x 220 mm), heating while stirring in a water bath (100°C) for 1 minute, and then allowing a viscometer-stirrer to drop by its own weight through the flour slurry and recording the total time in seconds required for the viscometer-stirrer to drop a given distance (70 mm) (ICC 107/1; AACCI 56-81B; ISO 3093). There is a strong curvilinear relationship between HFN and alpha-amylase activity (Hagberg 1961; Barnes and Blakeney 1974; Cornaggia et al. 2016). High falling numbers (>300 s) indicate good gelatinization of starch and low αAmy/LMA while low falling numbers (<200 s) indicate hydrolyzation of starch and high αAmy/LMA. A low HFN indicates sprouted grain and can result in dockages and outright rejection of grain.

Genetics of PHS and αAmy

Association of reduced sprouting with red grain color

Previous studies of PHS have found sprouting resistance QTLs co-localizing with grain color loci *R-A1*, *R-B1*, and *R-D1* on chromosome arms 3AL, 3BL, and 3DL respectively (Groos et al. 2002; Fofana et al. 2009). However, grain color loci are not the only source of sprouting resistance. Grain dormancy and ABA sensitivity could not be completely eliminated using induced mutations of *R-D1* in 'Chinese Spring' indicating that other loci are contributing to sprouting resistance in this genotype (Warner et al. 2000). Interestingly, dos Santos et al. (2010) found no significant correlation between grain color and PHS resistance.

The relationship of PHS resistance and red grain color is not fully understood but one hypothesis proposes a role of phenolic compounds in seed dormancy. The seed coat, pericarp, and hulls of grain accumulate multiple phenolic compounds. A type of phenols, Flavan-4-ols, are a major precursor responsible for proanthocyanidins and anthrocyanins and the red color in wheat. Germination assays using extracts of various phenolic compounds have found germination-inhibitory effects and that phenolic compounds may affect dormancy or ABA sensitivity through seed coat-imposed seed dormancy (Warner et al. 2000; Mares et al. 2009; Rodríguez et al. 2015).

GA-sensitivity and other associations with sprouting

Plant hormones such as GA have long been known to be important in development, dormancy, and germination of seeds. Several reduced height *Rht* alleles influenced by GA sensitivity and insensitivity have already been implicated in increased Hagberg falling number and decreased α-amylase activity (Mrva et al. 2008; Mrva et al. 2009; Gooding et al. 2012). These include the GA-insensitive alleles *Rht-B1* (4B) and *Rht-D1* (4D) and the GA-sensitive *Rht8c* (2DS). These dwarfing genes were a central part of the Green Revolution and nearly all cultivated wheat varieties possess semi-dwarf genotypes.

In developing embryos, *Vp1* serves to promote maturation and dormancy, and to suppress germination. In maize, *Viviparous1* (*Vp1*) is a transcription factor and is an important regulator of embryo development. Orthologues of maize *Vp1* have been mapped to the long arm of group 3 chromosomes: *Vp-A1*, *Vp-B1*, and *Vp-D1* on chromosomes 3AL, 3BL, and 3DL respectively in wheat (Bailey et al. 1999; Yang et al. 2007). Allelic variation in *Vp1* at any of these loci may result in variation of PHS resistance (Bailey et al. 1999; McKibbin et al. 2002; Yang et al. 2007). The structure and expression of the three *Vp-1* homoeologes in wheat are described by McKibbin et al. (2002). McKibbin et al. (2002) discovered that the *Vp-1* homoeologes are frequently mis-spliced in common wheat and ancestral wheat species compromising dormancy promotion and germination suppression.

Variation in sprouting resistance among wheat cultivars

Extensive variation exists among wheat populations fixed for grain color, *Rht*, and *Vp1* loci. Some of this variation has been explained by the identification of the sprout-related genes *Phs1* on chromosome 4AL (Torada et al. 2008) and *TaMFT*/*TaPHS1* on chromosome 3AS (Liu et al. 2008; Liu et al. 2013) or by QTL (comparative map of known QTL associated with sprouting resistance prepared by Martinez et al. (2018)). However, our current understanding of the underlying genetic control does not account for all of the genetic or genetic x environment variation which has been described.

Prior to the studies described in this dissertation (Chapters 2 and 3), a majority of the wheat varieties cultivated in Michigan had not been evaluated for PHS or αAmy under controlled conditions. Identification of strong PHS resistance or reduced αAmy QTL in varieties or elite breeding lines allows rapid advancements to be made in wheat improvement. This sprouting resistance would avoid the drag associated with introgressions from wild or unimproved accessions. Effective phenotyping of PHS resistance and αAmy also provides breeders with the tools to makes informed selections based on direct observation or through training of genome wide prediction models.

Role of domestication in increased sprouting damage

Evolution and domestication history of wheat

Wheat is an allopolyploid with a unique evolutionary history of interspecific hybridization. Modern hexaploid wheat *Triticum aestivum* (2n = 6x = 42, AABBDD) arose through two separate hybridization events (Figure 1.2). The first occurred five hundred thousand years ago between the wild A genome species, *T. urartu* (A^uA^u) and a B genome species (BB) related to modern S genome species (e.g. *Ae. speltoides*), resulting in tetraploid emmer wheat (*T. dicoccoides* or *T. turgidum* subsp. *diccocoides*) (2n = 4x = 28, AABB). This tetraploid species then hybridized with the diploid *Aegilops tauschii* Coss (2n = 2x = 14, DD) ~8,000 years ago (McFadden and Sears 1946; Marcussen et al. 2014). *Ae. tauschii* provided the genes for soft grain endosperm and broad environmental adaptation allowing wheat to expand into the geographical range it is cultivated in today. Very few hybridization events led to the formation of hexaploid wheat resulting in a genetic bottleneck that reduced genetic diversity in the D genome (Wang et al. 2013). Selection pressures during the domestication process likely contributed to increased sprouting damage as lines with uniform and rapid germination were favored (Gao and Ayele 2014; Mares and Mrva 2014; Rodríguez et al. 2015).

Wheat wild relatives as a source of novel alleles

The vast genetic variation represented in D genome species today is not represented in hexaploid wheat. This allows for the potential of novel, superior alleles in the D-genome of *Ae. tauschii* to be exploited in hexaploid wheat. Dormancy is an important component of sprouting resistance (Mares and Mrva 2014). The wild relatives of wheat express strong seed dormancy which inhibits germination until optimum environmental conditions are present (Gatford et al. 2002; Chen et al. 2008). Given that sources of sprouting resistance in soft white wheat breeding germplasm are limited, and strong dormancy exists among the wild relatives of wheat, sprouting resistance from the *Ae. tauschii* D genome could be useful in the development of sprout-resistant soft wheat varieties.

Synthetic hexaploid wheat (SHW) populations have been investigated in various studies for sprouting resistant traits. Researchers found the SHW line to contribute reduced sprouting resistance in mapping populations (Lohwasser et al. 2005), but other studies found the SHW line to be a source of sprouting resistance (Xiu-Jin et al. 1997; Yang et al. 2014). LMA and *Rht-B1* alleles were evaluated in over 250 SHW genotypes (developed from 27 durum cultivars and 140 *Ae. tauschii* accessions) and over 300 derived synthetics of the CIMMYT SHW collection by Mrva et al. (Mrva et al. 2009). Wide variation in LMA levels was found in both the SHW and derived lines and LMA rating appeared to be influenced by plant height (Mrva et al. 2009). An association mapping study by Emebiri, et al. (2010) in 91 SHW lines of the CIMMYT SHW collection did not identify any QTL contributing to LMA within the D genome. However, this study did find significant pedigree and within-pedigree effects. Families with diverse *Ae. tauschii* parents and a shared durum parent varied significantly for LMA phenotypes. These results suggest that unidentified D genome QTL underlie the phenotypic variation for LMA. The sum of these SHW studies indicate that variation in sprouting resistance exists in D genome progenitors and can be donated to hexaploid progeny.

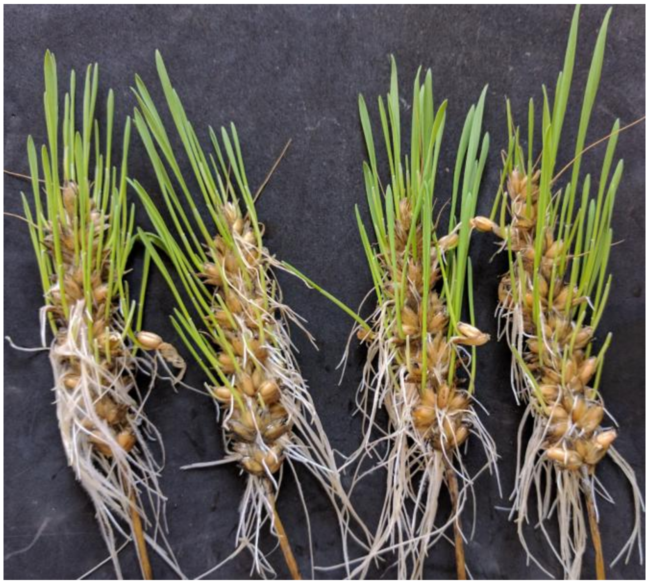

Figure 1.1. Signs of PHS in severely sprout damaged wheat spikes.

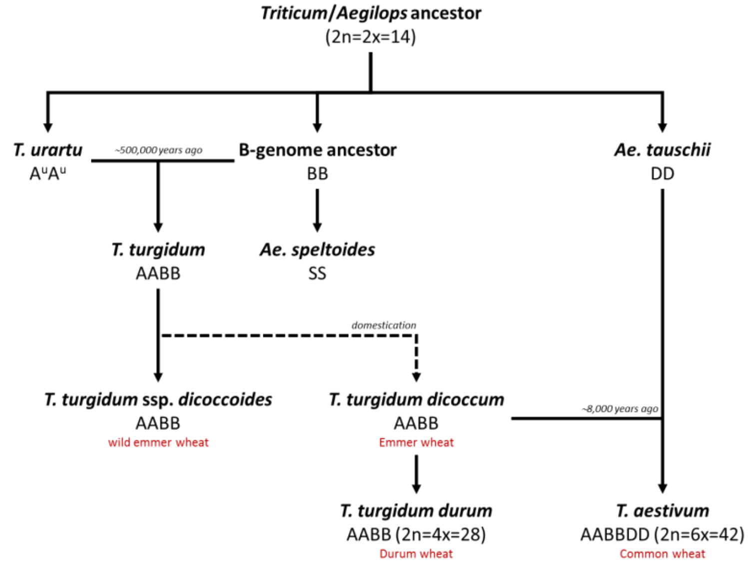

Figure 1.2. Evolution and domestication history of wheat.

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CHAPTER 2: Preharvest sprouting and α-amylase activity in soft winter wheat

Previously published in Journal of Cereal Science 79 (2018) 311-318
DOI: 10.1016/j.jcs.2017.11.016

Abstract

The wheat value chain is vulnerable to reduced grain quality caused by preharvest sprouting. The Great Lakes region and the Pacific Northwest are particularly vulnerable to sprouting related quality issues because precipitation events near or at the time of harvest are common and are the leading cause of sprout related losses. Both visual preharvest sprouting (PHS) and alpha-amylase activity (αAmy) influence grain quality. In this study, standardized methods for phenotying PHS and αAmy have been implemented in soft winter wheat varieties and advanced breeding lines. The methods utilized here for rating visual PHS and αAmy using a scaled down Ceralpha method produce consistent and reliable results across years and can be implemented in screening large wheat breeding populations. The populations phenotyped for PHS and αAmy were also genotyped for grain color (*R*), photoperiod (*Ppd*), and reduced height (*Rht*) genes to determine their influence on PHS and αAmy. Phenotypic variation for PHS and αAmy is present in both soft red and soft white wheat market classes. Soft red winter wheat genotypes were identified that have PHS susceptibility and moderate levels of αAmy. Conversely, genotypes of soft white winter wheat were identified that have PHS resistance and low levels of αAmy. As variation exists for PHS and αAmy in soft wheat germplasm, progress can be made using phenotypic selection for these traits in breeding programs. Development of varieties with improved sprouting resistance will mitigate the risk of sprouting and increase the production of high quality wheat.

Chapter 3: Genomic analyses for preharvest sprouting and alpha-amylase in a diverse population of soft winter wheat

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Abstract

Sprout damage threatens the quality and profitability of wheat production through preharvest sprouting (PHS) and alpha-amylase activity (αAmy). Grain color is known to be associated with sprouting resistance. However, wide variation in sprouting resistance is observed within market classes. Few major-effect quantitative trait loci (QTL) independent of grain color have been identified for PHS resistance or reduced αAmy which hampers varietal improvement within certain wheat market classes. The primary objectives of this study were to 1) characterize a population of soft winter wheat for PHS and αAmy; 2) identify QTL associated with PHS resistance or reduced αAmy; 3) to determine the potential of genomic selection methods for improving PHS and αAmy traits. A genome wide association study (GWAS) was performed to identify QTL significantly associated with PHS resistance or low αAmy. Genomic selection (GS) using genome wide regression models BRR, BayesA, and BayesB was conducted to evaluate the potential of line selection by predicted genetic values. GWAS did not yield any significant QTL for sprouting resistance. However, GS models were effective at predicting lines with reduced PHS. The findings of this study indicate GS can be used to improve PHS and αAmy trait in populations with wide trait variation and in the absence of major-effect QTL.

CHAPTER 4: Identification of D genome variation for preharvest sprouting resistance and reduced alpha-amylase activity in hexaploid wheat

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Abstract

Sprouting of wheat prior to harvest results in poor grain quality, reduced marketability, and loss of profit for growers. Preharvest sprouting (PHS) is induced by rainy conditions between physiological and harvest maturity and is expressed through precocious germination of wheat seeds and enzymatic degradation of the starchy endosperm by alpha-amylase (αAmy). Lack of genome-wide genetic variation, particularly in the D genome, has hampered resistance breeding strategies. The primary objective was to identify grain color-independent QTLs which contribute to preharvest sprouting resistance for utilization in white wheat germplasm development. A D-genome mapping population of inbred backcross lines (IBLs) was developed by direct crossing of five *Aegilops tauschii* genotypes to the hexaploid wheat line, 'KS05HW14.' Phenotypic variation for PHS and αAmy activity within the IBLs was characterized, genome-wide association analysis was used to identify SNPs associated with PHS and αAmy phenotypes, and whole genome regression analysis was used to predict genomic estimated breeding values of IBLs. Putative QTL derived from *Ae. tauschii* were identified for quantitative sprout and a visual sprout index on 2DS (*QPHS.msu-2D.1*) and 3DL (*QPHS.msu-3D.1*). A putative QTL was also identified for αAmy on 5DL (*QAMY.msu-5D.1*) and is associated with the KS05HW14 haplotype. Prediction accuracies of whole genome regression analysis ranged from 0.109 to 0.804 depending on the trait and indicate that genomic selection strategies can be used to improve some sprouting resistance traits. This study demonstrates the value of novel genetic variation from a wild relative for increasing seed dormancy and improved sprouting resistance in wheat. The IBLs containing favorable QTL alleles identified in this study will be used to improve sprouting resistance in wheat breeding programs.