ASSOCIATION STUDIES ON PRE-GERMINATION FLOODING TOLERANCE AND CELL WALL COMPONENTS RELATED TO PLANT ARCHITECTURE IN DRY BEAN

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Katelynn Ann Walter

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Plant Sciences

May 2018

Fargo, North Dakota

North Dakota State University
Graduate School

Title

Association Studies on Pre-Germination Flooding Tolerance and Cell Wall Components Related to Plant Architecture in Dry Bean

By

Katelynn Ann Walter

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dr. Juan M. Osorno
Chair

Dr. Phillip E. McClean

Dr. Elias M. Elias

Dr. Amitava Chatterjee

Approved:

May 17, 2018
Date

Dr. Harlene Hatterman-Valenti
Department Chair

ABSTRACT

Dry bean breeding programs have made significant advances in combating both abiotic and biotic stresses as well as improving plant architectural traits via selective breeding. Flooding can cause complete crop loss in dry bean. On the other hand, breeding for an upright architecture in dry bean has been a breeding target in several programs. However, the stem cell wall components underlying this change have yet to be studied. This research focused on analyzing the cell wall components that might be involved in dry bean architecture as well as pre-germination flooding tolerance in dry bean. For the plant architecture study, two significant genomic regions were identified on Pv07 and Pv08 associated with lignin accumulation in dry bean stems. For the pre-germination flooding study, one unpigmented seed coat genotype (Verano) and three pigmented seed coat genotypes (Indeterminate Jamaica Red, Durango, and Midnight) had germination rates similar to that of the tolerant check.

ACKNOWLEDGEMENTS

I would first like to thank Dr. Juan Osorno for his continued guidance, support, and encouragement throughout my graduate studies. I am grateful for the experiences I have had during my time working with Dr. Osorno and the NDSU dry bean breeding program. I would also like to thank my committee members: Drs. Phil McClean, Elias Elias, and Amitava Chatterjee for their helpful suggestions and guidance during my research. A special thanks to Dr. Ali Soltani for his assistance in several aspects of this research. His kindness and patience while teaching was extremely helpful during this research. Furthermore, I would like to thank Dr. Atena Olazad Abbasabadi for her generous help and guidance with the association mapping work in my projects. I would also like to thank Drs. Marisol Berti and Hui Li for their assistance. Additionally, I would like to thank Dr. Jawahar Jyoti for his help on the statistical analyses.

I am appreciative of all the individuals in the Dry Bean Breeding Program at NDSU for their knowledge and help, especially Jody VanderWal, Mike Kloberdanz, Stephan Schroder, Edgar Escobar, and Federico Velasquez. I must also thank my fellow graduate students who helped make my time at NDSU an enjoyable and memorable experience: Carlos, Courtney, Erin, Gaby, James, Lucy, Luz, and Morgan.

Lastly, I would like to express my profound gratitude to my parents for their love, support, and encouragement throughout my education.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

LIST OF APPENDIX FIGURES

GENERAL LITERATURE REVIEW OF COMMON BEAN

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important legume for human consumption worldwide (Broughton et al., 2003; Bitocchi et al., 2017). Common bean is a dominant crop in the Americas and one of the most ancient crops of the New World (Broughton et al., 2003). Beans are a staple crop in many areas throughout the world and most common bean production takes place in Latin America and Africa, accounting for over 30% of production worldwide. Dry beans are a major source of protein and micronutrients making them valuable in the human diet (Broughton et al., 2003). Aside from the human health benefits dry bean offers, they can also improve cropping systems by adding nitrogen to the soil via symbiotic nitrogen fixing with *Rhizobium* bacteria.

Dry bean breeding began in the United States in the early 1900s (Kelly, 2010). Since then, several traits have been improved leading to cultivars better adapted to certain regions of the United States. Major advancements in disease resistance, seed yield, and plant architecture have been achieved with selective breeding. The advancements in plant architecture have changed the growth habit of many dry bean cultivars from a prostrate type to a more upright architecture; however, the stem strength components underlying this change in architecture have not yet been studied. Abiotic stress tolerance has also been a major focus of several breeding programs with advancements made in drought tolerance and nutrient efficiencies. However, flooding stress tolerance has been largely overlooked even though major dry bean production regions have been affected by excess water in recent years (Knodel et al., 2016).

This research focuses on two main topics/areas: stem strength in relation to plant architecture and flooding tolerance at germination stages. Chapter I studies cell wall components to determine how they account for stem strength and plant architecture in dry bean. Chapter II

examines flooding tolerance to better understand the tolerance mechanisms Middle American dry bean genotypes might be using.

Economic Importance

Dry bean is an economically important crop and common bean production is more than twice that of the second leading grain legume, chickpea (*Cicer arietinum*) (Gepts et al., 2008). From 2006-2008, 28 million hectares (ha) of dry beans were harvested worldwide (Akibode and Maredia, 2011). The United States is the sixth leading producer of dry bean in the world following Brazil, India, China, Burma, and Mexico (Food and Agriculture Organization of the United Nations (FOA), 2017). However, it is difficult to distinguish the top producer of *Phaseolus vulgaris* because the FAO includes species from the *Vigna* genus in the production numbers which can be misleading (Osorno and McClean, 2014).

In 2017, over 1.38 million ha were used for dry bean production in the United States (USDA-NASS, 2018). Pinto, black, and navy are the leading market classes produced in the United States accounting for 50, 20, and 16% of total production, respectively in 2016. North Dakota and Michigan are the two leading producers of dry bean in the nation accounting for 38 and 14% of total production, respectively (USDA-ERS, 2017). Other important states include Nebraska (11%), Minnesota (10%), Idaho (7%), California (4%), Washington (4%), and Colorado (3%). North Dakota, the leading producer of dry bean in the United States, also leads the nation in the production of pinto and navy market classes (USDA-ERS, 2017). Dry beans are an excellent source of protein, vitamins, fiber, and minerals, yet only about 14% of the population in the United States eat dry edible beans on any given day (USDA-ERS, 2017). Dry bean consumption is much higher in other areas of the world such as Uganda and Brazil, where consumption is around 22 and 16kg person^{-1} year^{-1}, respectively (FAOSTAT, 2018).

Origin and Domestication of Common Bean

Phaseolus vulgaris belongs to the family Fabaceae (Leguminosae) (Kelly, 2010). Members of this family have a symbiotic relationship with *Rhizobium* bacteria to fix atmospheric nitrogen. This family also produces pods and has protein-rich seeds. There are four domesticated species within the *Phaseolus* genus other than *P. vulgaris*. These species are*, P. coccineus* (scarlet runner bean), *P. dumosus* (syn. *P. polyanthus*) (year bean), *P. acutifolius* (tepary bean), *and P. lunatus* (lima bean), which are listed in order of genetic similarity to common bean (Kelly, 2010). There are a total of nine clades within the *Phaseolus* genus; however, the five domesticated species come from only two of the clades (*P. vulgaris* and *P. lunatus*) (Delgado-Salinas et al., 1999). Common, scarlet runner, year, and tepary bean all belong to the *P. vulgaris* clade and lima bean belongs to the *P. lunatus* clade.

Present day common bean evolved from its wild ancestor ~165,000 years ago (Schmutz et al., 2014). Dry bean originated in Central America and two independent domestication events took place leading to two genetically distinct gene pools, the Middle American and Andean (Mamidi et al., 2013). There are multiple races within each gene pool. Races Chile, Nueva Granada, and Peru for the Andean gene pool and races Durango, Jalisco, Mesoamerica (Singh et al., 1991), and more recently race Guatemala (Tobar Piñón, 2017) for the Middle American gene pool. The Middle American gene pool consists of small (<25 g 100 seed weight^{-1}) and medium (25-40 g 100 seed weight^{-1}) size seeded individuals, whereas the Andean gene pool consists of individuals with much larger seeds (<40 g 100 seed weight^{-1}). For the Middle American gene pool, black and navy market classes belong to race Mesoamerica and pinto, pink, small red, and great northern market classes belong to race Durango. For the Andean gene pool, large-seeded

kidney market classes belong to race Nueva Granada and cranberry market classes belong to both race Nueva Granada and race Chile.

Both the cultivated and wild forms of dry bean belong to the same species (Gepts and Debouck, 1991). Some of the traits associated with dry bean domestication include a larger seed size, non-shattering pods, increased seed coat permeability, and an upright, bush architecture (Gepts and Debouck, 1991). During domestication, genetic diversity was greatly reduced which is commonly associated with the founder effect (Bitocchi et al., 2013). The reduction in genetic diversity was more pronounced for the Mesoamerican domestication event than for the Andean, and wild common bean could have adaptive traits that are not present in the domesticated forms due to domestication and the bottleneck created (Acosta-Gallegos et al., 2007). Wild common bean could be a source of useful alleles that are not present in domesticated forms and breeders could utilize this variation to introgress traits into existing varieties.

Dry Bean Improvement

Understanding the genetic diversity of a species is essential for improvement of the species (Acosta-Gallegos et al., 2007). Dry bean has a wide variety of pod and seed types, growth habits, maturity lengths, photoperiod sensitivities, and a range of disease and stress resistances (Kelly, 2010). This genetic variability can be utilized by dry bean breeders to further improve the crop. Major improvements in seed yield, plant architecture, disease resistance, and abiotic stress tolerance have already been made via dry bean breeding efforts (Beaver and Osorno, 2009; Kelly, 2010; Vandermark et al., 2014).

One major advancement that has been made with breeding is the development of upright dry bean varieties (Kelly, 2010). The upright varieties are resistant to lodging and offer a means for growers to utilize direct harvest which is not possible with traditional short bush or prostrate

vine types. Direct harvest is beneficial for growers because it is saves time and money since less personnel and equipment are needed (Gregoire, 2007). Dry bean architecture varies by market class with navy and black being the most erect. On the other hand, pinto and great northern market classes, from race Durango, have been much harder to convert to an upright architecture.

Biotic stresses are another area of dry bean breeding that have been focused on and significant advances have been made thus far. Producing cultivars with increased levels of resistance to biotic stresses is a major objective of many dry bean breeding programs (Beaver and Osorno, 2009). Breeding for resistance to bean common mosaic virus (BCMV) and beet curly top virus (BCTV) was heavily focused on in the beginning of dry bean breeding at the University of Idaho and many resistant varieties exist today (Singh et al., 2007). However, cultivars are much more sensitive to anthracnose (Colletotrichum lindemuthianum), rust (Uromyces appendiculatus (Pers) Unger), and common bacterial blight (Xanthomonas campestris pv. Phaseoli), among others. White mold (caused by *Sclerotinia sclerotiorum* (Lib.) de Bary) is one common bean disease that has proven to be very difficult to breed resistance and there are no current varieties with high levels of resistance (Kelly, 2010; Singh et al., 2007).

Dry bean breeding efforts have also been devoted to minimize the adverse effect of abiotic stresses. One example is breeding dry bean varieties that are more tolerant to low levels of soil micronutrients such as zinc (Moraghan and Grafton, 1999). Drought stress tolerance has also been studied in dry bean and breeding for tolerance to drought has taken place for several years (reviewed in Beebe et al., 2013). Another abiotic stress that severely impacts dry bean in major production areas is excess water. Worldwide, flooding is the most devastating threat of all natural disasters accounting for >57% of crop damage/loss from 2003-2013 (FAO, 2015). Flooding is one of the main reasons for dry bean crop loss in North Dakota and Minnesota in

recent years (Knodel et al., 2016), yet there is very little research focused on flooding tolerance in dry bean.

Dry Bean Genomics and Association Mapping (AM)

Common bean is a diploid species with 11 chromosomes and a genome size of 587Mb (Schmutz et al., 2014). Dry bean diverged from soybean (*Glycine max*) ~19.2 million years ago, but the two species shared a whole genome duplication (WGD) event ~56.5 million years ago. Since the species diverged, soybean underwent another WGD ~10 million years ago. There are many syntenic regions between *P. vulgaris* and *G. max* and 91.2% of the *P. vulgaris* genes were identified in synteny blocks with *G. max*. Studying the synteny between two species can help to gain a better understanding of evolutionary patterns as well as identifying genes and markers linked to specific agronomic traits (Duran et al., 2009).

Molecular markers are useful to plant breeders and are an effective tool for marker assisted selection (MAS) (Eathington et al., 2007). Marker assisted selection can increase the efficiency of breeding programs since the markers are linked to genes of interest (Meziadi et al., 2016). The closer the marker is to the gene, the more efficient the marker is at identifying the gene. Single nucleotide polymorphisms (SNPs) are the most recent markers and have been used in common bean research to improve breeding efforts (Bello et al., 2014).

Genome-wide association studies (GWAS) determine genomic regions associated with a trait of interest. The goal of association mapping (AM) is to find correlations between genetic markers and specific phenotypes within a population (Myles et al., 2009; Rafalski, 2010). Association mapping is more cost effective and faster than traditional linkage mapping (Myles et al., 2009). In contrast to linkage mapping, a population does not have to be developed through controlled crosses for AM. Instead, the natural genetic variation within a population is utilized for AM which saves time and money. For GWAS, a mapping population, phenotypic data, and a

large number of markers are required. The markers are typically SNPs and there must be a sufficient number of markers across the genome that functional alleles will be in linkage disequilibrium (LD) with at least one of the markers. Genetic AM involves a group of individuals, which allows several alleles to be evaluated at each locus at the same time for association within a diverse population as opposed to traditional bi-parental mapping where only two alleles are segregating (Rafalski, 2010).

References

Acosta-Gallegos, J.A., J.D. Kelly, and P. Gepts. 2007. Prebreeding in common bean and use of genetic diversity from wild germplasm. Crop Sci. 47: 1-44.

Akibode, S., and M. Maredia. 2011. Global and regional trends in production, trade, and consumption of food legume crops. Department of Agricultural, Food and Resource Economics, Michigan State Univ., East Lansing, MI. 12-16.

Beaver, J.S., and J.M. Osorno. 2009. Achievements and limitations of contemporary common bean breeding using conventional and molecular approaches. Euphytica 168: 145–175.

Beebe, S.E., I.M. Rao, M.W. Blair, and J.A. Acosta-Gallegos. 2013. Phenotyping common beans for adaptation to drought. Front. Physiol. 4: 35.

Bello, M.H., S.M. Moghaddam, M. Massoudi, P.E. McClean, P.B. Cregan, and P.N. Miklas. 2014. Application of in silico bulked segregant analysis for rapid development of markers linked to bean common mosaic virus resistance in common bean. BMC Genomics 15: 903-926.

Bitocchi, E., E. Bellucci, A. Giardini, D. Rau, M. Rodriguez, E. Biagetti, R. Santilocchi, P. Spagnoletti Zeuli, T. Gioia, G. Logozzo, G. Attene, L. Nanni, and R. Papa. 2013. Molecular analysis of the parallel domestication of the common bean (Phaseolus vulgaris) in Mesoamerica and the Andes. New Phytol. 197: 300–313.

Bitocchi, E., D. Rau, E. Bellucci, M. Rodriguez, M.L. Murgia, T. Gioia, D. Santo, L. Nanni, G. Attene, and R. Papa. 2017. Beans (Phaseolus ssp.) as a model for understanding crop evolution. Front. Plant Sci. 8: 722.

Broughton, W.J., G. Hernández, M. Blair, S. Beebe, P. Gepts, and J. Vanderleyden. 2003. Beans (Phaseolus spp.) – model food legumes. Plant Soil 252: 55–128.

Delgado-Salinas, A., T. Turley, A. Richman, and M. Lavin. 1999. Phylogenetic analysis of the cultivated and wild species of Phaseolus (Fabaceae). Syst. Bot. 24: 438-460.

Duran, C., D. Edwards, and J. Batley. 2009. Genetic Maps and the Use of Synteny. p. 41–55. *In* Humana Press.

Eathington, S.R., T.M. Crosbie, M.D. Edwards, R.S. Reiter, and J.K. Bull. 2007. Molecular Markers in a Commercial Breeding Program. Crop Sci. 47: S-154.

FAO (Food and Agriculture Organiziation of the United Nations) FAOSTAT. 2018. http://www.fao.org/faostat/en/#data/QC. Accessed Feb. 20, 2018.

FAO. 2017. The impact of disasters on agriculture and food security. http://www.fao.org/resilience/resources/resources-detail/en/c/346258/

Gepts, P., F.J.L. Aragão, E. de Barros, M.W. Blair, R. Brondani, W. Broughton, I. Galasso, G. Hernández, J. Kami, P. Lariguet, P. McClean, M. Melotto, P. Miklas, P. Pauls, A. Pedrosa-Harand, T. Porch, F. Sánchez, F. Sparvoli, and K. Yu. 2008. Genomics of Phaseolus Beans, a Major Source of Dietary Protein and Micronutrients in the Tropics. p. 113–143. *In* Genomics of Tropical Crop Plants. Springer New York, New York, NY.

Gepts, P., and D. Debouck. 1991. Origin, domestication, and evolution of the common bean (Phaseolus vulgaris L.). p. 7–53. *In* van Schoonhoven, A., Voysest, O. (eds.), Common Beans: Research for Crop Improvement - Google Books. CAB International, Wallingford, Commonwealth .

Gregoire, T. 2007. Harvest losses, undercutting versus direct cutting. Northarvest bean growers Mag. 13:11-15.

Kelly, J.D. 2010. The Story of Bean Breeding White paper prepared for BeanCAP. PBG Works on the topic of dry bean production and breeding research in the U.S.

Knodel, J.J., P.B. Beauzay, G.J. Endres, D.W. Franzen, H.J. Kandel, S.G. Markell, J.M. Osorno, J.S. Pasche, and R.K. Zollinger. 2016. 2016 Dry Bean Grower Survey of Production, Pest Problems and Pesticide Use in Minnesota and North Dakota E1841.

Mamidi, S., M. Rossi, S.M. Moghaddam, D. Annam, R. Lee, R. Papa, and P.E. McClean. 2013. Demographic factors shaped diversity in the two gene pools of wild common bean Phaseolus vulgaris L. Heredity (Edinb). 110: 267–276.

Meziadi, C., M.M.S. Richard, A. Derquennes, V. Thareau, S. Blanchet, A. Gratias, S. Pflieger, and V. Geffroy. 2016. Development of molecular markers linked to disease resistance genes in common bean based on whole genome sequence. Plant Sci. 242: 351–357.

Moraghan, J.T., and K. Grafton. 1999. Seed-zinc concentration and the zinc-fffficiency trait in navy bean. Soil Sci. Soc. Am. J. 63: 918.

Myles, S., J. Peiffer, P.J. Brown, E.S. Ersoz, Z. Zhang, D.E. Costich, and E.S. Buckler. 2009. Association mapping: critical considerations shift from genotyping to experimental design. Plant Cell 21: 2194–2202.

Osorno, J.M. and P.E. McClean. 2014. Common bean genomics and its applications in breeding programs. *In.* Gupta, S., N. Nadarajan, D.S. Gupta (eds). Legumes in the Omics era. Springer, New York. 185-206.

Rafalski, J.A. 2010. Association genetics in crop improvement. Curr. Opin. Plant Biol. 13: 174–180.

Schmutz, J., P.E. McClean, S. Mamidi, G.A. Wu, S.B. Cannon, J. Grimwood, J. Jenkins, S. Shu, Q. Song, C. Chavarro, M. Torres-Torres, V. Geffroy, S.M. Moghaddam, D. Gao, B. Abernathy, K. Barry, M. Blair, M.A. Brick, M. Chovatia, P. Gepts, D.M. Goodstein, M. Gonzales, U. Hellsten, D.L. Hyten, G. Jia, J.D. Kelly, D. Kudrna, R. Lee, M.M.S. Richard, P.N. Miklas, J.M. Osorno, J. Rodrigues, V. Thareau, C.A. Urrea, M. Wang, Y. Yu, M. Zhang, R.A. Wing, P.B. Cregan, D.S. Rokhsar, and S.A. Jackson. 2014. A reference genome for common bean and genome-wide analysis of dual domestications. Nat. Genet. 46: 707–713.

Singh, S.P., P. Gepts, and D.G. Debouck. 1991. Races of common bean (Phaseolus vulgaris, Fabaceae). Econ. Bot. 45: 379–396.

Singh, S.P., H. Terán, M. Lema, D.M. Webster, C.A. Strausbaugh, P.N. Miklas, H.F. Schwartz, and M.A. Brick. 2007. Seventy-five Years of Breeding Dry Bean of the Western USA. Crop Sci. 47: 981-989.

Tobar Piñón, M.G. 2017. Genetic diversity of the Guatemalan climbing bean collections. Available from Dissertations & Theses @ North Dakota State University. Retrieved from https://ezproxy.lib.ndsu.nodak.edu/login?url=https://search-proquest-com.ezproxy.lib.ndsu.nodak.edu/docview/2014462119?accountid=6766

USDA-NASS. 2018. National Agriculture Statistics Service. Available at www.nass.usda.gov/ (verified 20 Feb. 2018). USDA, Washington, DC.

USDA-ERS. 2017. Economic Research Service. Available at http://www.ers.usda.gov/ (verified 11 Apr. 2017). USDA, Washington, DC.

Vandermark, G.J., M.A. Brick, J.M. Osorno, J.D. Kelly, and C.A. Urrea. 2014. Edible Grain Legumes. *In* S. Smith, B. Diers, J. Specht, and B. Carver. eds. Yield gains in major U.S. field crops. ASA, CSSA, SSSA. Madison, WI. 87-124.

CHAPTER I. COMMON BEAN PLANT ARCHITECTURE AND ITS RELATION TO STEM STRENGTH AND CELL WALL COMPONENTS

Introduction

During crop domestication, traits such as determinate growth type, early flowering time and maturity, seed size, and non-shattering pods were selected for (Kelly, 2001). One of the major differences between wild and cultivated forms of common bean is the growth habit (Smartt, 1976). The modern cultivated form of dry bean has an erect growth type whereas the wild form is an indeterminate climber and much more branched (Kelly, 2000). Wild *Phaseolus* types generally have long internodes with unorderly branching (Gentry, 1969; Kelly, 2000). In contrast, cultivated *Phaseolus* has two growth types, determinate and indeterminate. The variation in growth habit can be accounted for by internode length and node number (Kelly, 2000). The internode length is responsible for controlling if the plant has a climbing habit or not and node number corresponds to the stage when the apical bud goes from vegetative to reproductive growth.

Today, common bean can be classified into four main habits which are summarized in Table 1.1: Type I-bush determinate, Type II-bush indeterminate, Type III-prostrate indeterminate, and Type IV-climbing indeterminate (Singh, 1982). Dry bean genotypes from the Durango race are mostly Type III which typically have higher seed yield than genotypes with Types I or II growth habits. Although Type III dry beans have the greatest yield potential, they are also more susceptible to diseases such as white mold (caused by *Sclerotinia sclerotiorum* (Lib.) de Bary) (Kelly and Adams, 1987). Type III dry beans have a dense canopy and tend to lodge; therefore, the pods and stems of Type III plants are on the ground which is one of the reasons why they are more susceptible to diseases that thrive in humid conditions. Aside from Type II dry beans being more stable in terms of yield potential, they also offer agronomic

benefits to growers in comparison to their Type III counterparts. With a more upright architecture, Type II plants can be planted in narrower row spacings, which in turn increases seed yield in dry bean (Eckert, 2009; Grafton et al., 1988).

Upright architecture in dry bean allows for more efficient harvest practices as well as decreased disease pressure for certain pathogens which thrive in humid environments. Developing cultivars with upright architecture has been challenging for pinto and great northern market classes (race Durango) whereas breeders have had much more success with navy and black market classes (race Mesoamerica) since the upright architecture is commonly found within the Mesoamerican race (Brick and Grafton, 1999). The cell wall components underlying this stem architecture are not well understood. Determining which cell wall components might be involved in the shift from Type III to Type II architecture can aid in breeding efforts to continue to convert common bean varieties to a more upright architecture.

Table 1.1. Characterization of dry bean growth habits (Adapted from Kelly, 2001).

Growth Habit	Terminal bud	Growth Type	Stem Strength	Climbing Ability
Type I	Reproductive	Determinate	Strong, upright	Absent/weak climber
Type II	Vegetative	Indeterminate	Strong, upright	Absent/weak climber
Type III	Vegetative	Indeterminate	Weak, open/ prostrate	Weak/facultative climber
Type IV	Vegetative	Indeterminate	Very weak	Strong climber

Dry Bean Harvest Practices

There are two different methods for harvesting dry bean; conventional harvesting which consists of pulling out the plants and putting them in windrows, and direct harvesting where the plants are straight cut with combines having sickle bars cutting plants at the stem base (Eckert, 2010). Direct harvest is utilized for some market classes more than others. For the most part, in North Dakota direct harvest has mostly been utilized only for navy beans since most cultivars have an upright architecture (Thomas et al., 2016). More recently, pinto beans have been

harvested with this method and in 2012 at least 70% of pinto beans grown in North Dakota were direct harvested. According to the 2017 North Harvest bean growers survey, >80% of the growers surveyed indicated they direct harvested some of their dry bean fields in 2016 and 54.4% of respondents indicated they direct harvested all their dry beans in North Dakota and Minnesota (Knodel et al., 2017).

In most cases, direct harvest saves time and money since it only requires one pass through the field compared to the conventional method; however, direct harvest can result in seed yield and quality losses. One of the major downsides and risks of the direct harvest system is a high harvest loss (Thomas et al., 2016). Eckert (2009) found seed yield was on average 830 kg ha^{-1} less when direct harvest is utilized instead of conventional harvest. In a survey from the North Dakota State University extension service, all the respondents who indicated they utilized the direct harvest method in North Dakota and Minnesota also indicated yield loss in 2016 with 33% having 1-5% yield losses and 66% with losses of 6-20% (Knodel et al., 2017).

Many factors can contribute to harvest losses with the direct harvest system including the harvest speed and combine set up. The main contributors to high harvest losses are pods that are <5 cm from the soil surface at the time of harvest and a high sickle height of the combine header (Thomas et al., 2016). The pod height is highly dependent upon the variety being grown as well as environmental factors during the growing season whereas the height of the header is dependent upon the levelness of the field and model of the header. Seed quality is also an important factor to consider and the dry bean seeds can be damaged with direct harvest more so than with the conventional harvest system. For example, Eckert (2009) found that when seeds had a high moisture content, there was no significant difference in split seeds between conventional and direct harvest. On the other hand, if the seed moisture content was low at

harvest, the percentage of split seeds was significantly higher for the direct harvest method compared to conventional.

Breeding for Upright, Type II Architecture

The first reports of specific breeding efforts for modified growth habits was in Michigan in the 1940s (Kelly, 2001). Down and Anderson (1956) used X-ray mutagenesis to develop a determinate bush bean that was more upright than traditional navy beans at the time which had many vines and were more prostrate. The goal of Down and Anderson's work was to increase seed yield, quality, and biotic stress resistance associated with the prostrate growth habit. 'Sanilac' was the first navy bean cultivar successfully converted to possess the determinate growth habit in 1957 (Down and Anderson et al., 1956).

Sanilac revolutionized the way navy beans were produced in Michigan since the upright architecture helped combat white mold which was the most serious dry bean disease at that time. However, the change in growth type also resulted in the cultivar being very susceptible to low zinc (Zn) levels in the soil. None of the previous Type III navy beans were sensitive to soil Zn deficiency which made it clear that there was an association between the change in plant architecture and Zn inefficiency. Breeders wanted to exploit the valuable plant architecture of Sanilac and in doing so, unknowingly transferred the Zn inefficiency to many determinate bush navy beans in different countries (Kelly, 2001) and many progeny developed from Sanilac are very sensitive to soil Zn deficiency (Moraghan and Grafton, 1999). One explanation for the Zn inefficiency displayed in the upright varieties is that determinate shoot growth results in determinate root growth which could limit the plant's Zn mining abilities (Kelly, 2001).

Many navy and black cultivars possess the Type II growth habit, but other market classes such as pinto and great northern have been more challenging to convert to Type II (Osorno et al., 2010). For race Mesoamerica beans, upright varieties were available in the U.S.; however, this

was not the case for market classes belonging to race Durango. Breeding for upright pinto bean varieties became increasingly important in the 1980s to develop cultivars with resistance to fungal diseases such as white mold (Park, 1993). The major challenge of converting Durango market classes to Type II growth habit was combining the architectural traits from the Mesoamerican race with the seed size of the Durango race. Interracial recurrent selection was chosen as the method to accomplish this task and Sierra was the first pinto cultivar released with an upright architecture (Kelly et al., 1990) and since then, several others have been released such as Lariat and Stampede (Osorno et al., 2010), Santa Fe (Kelly et al., 2010), La Paz (ADM, PVP#200500219), and Long's Peak (Brick et al., 2015). Great northern cultivars with upright architecture have also been released such as Matterhorn (Kelly and Copeland, 1998), Coyne (Urrea et al., 2009), Powderhorn (Kelly et al., 2014), and Draco (ProVita, PVP#201400414).

Improved architectural types exist for pinto, great northern, small red, and pink market classes (Kelly, 2010). Larger seeded kidney market classes exhibit the determinate, bush type architecture, and they have been more difficult to convert to upright types. One reason the kidney beans have not been converted to an upright architecture is because a uniform seed size is lost with the upright architecture which is important for large seeded kidney beans.

Although there are current pinto and great northern varieties available with the upright, Type II growth habit, determining which factors contribute to this growth habit can increase the efficiency of breeding these varieties. Studying the cell wall components that might be involved in this change in dry bean architecture and furthermore determining the genomic regions associated with those components can lead us to genetic markers which can be used in marker assisted selection (MAS).

Cell Wall Components and Biosynthesis

Plant cell walls function to surround and protect plant cells and are essential for plant survival (Caffall and Mohnen, 2009). Cell wall structure can be modified in response to changing environmental conditions as well as the developmental stage of the plant. Plant cell walls are mainly composed of cellulose, hemicellulose, pectin, protein and lignin (Zhong and Ye, 2007). Cell wall composition varies depending on plant species and genotype, but cellulose, hemicellulose, and lignin are usually in a 4:3:3 ratio (Chen, 2014). The cell walls define cell shape, regulate cell growth, provide support to plants, and act as barriers to biotic and abiotic stresses.

Cellulose is the primary component of cell walls and is the most load-bearing macromolecule in the cell wall (Caffall and Mohnen, 2009). Cellulose fibers aggregate into bundles to form microfibers which are interconnected into a matrix of polysaccharides to make up a fiberglass-like structure (Cosgrove, 2005). The polysaccharides can be separated into two classes, pectins and hemicelluloses. Pectins are soluble in aqueous buffers and dilute acidic solutions whereas hemicelluloses are soluble only in strong alkali solutions. Hemicelluloses along with cellulose form an extremely strong network. Pectins on the other hand are more important for cell wall porosity and thickness.

Lignin is another cell wall component important for cell structure. Lignin content varies depending on the species and genotype but is usually ~27-32% in woody plants and ~14-25% in herbaceous plants (Chen et al., 1996). Lignin is a phenylpropane polymer found in the cell wall structures of plants (Bilbro et al., 1991). Lignin is involved in several plant functions that are necessary for plant survival including water, nutrient, and metabolite transport. Lignin is also involved in bonding cells to form a rigid structure that can be very resistant to lodging (Goering

and Van Soest, 1970). Altering lignin content can decrease lodging without impacting plant morphology and development (Vanholme et al., 2012). Lodging resistance can be achieved by reducing plant height but a reduction in plant height can lead to yield loss and therefore is not desirable (Berry et al., 2004). On the other hand, increasing stem strength by targeting lignin composition has shown to decrease lodging without the negative impacts dwarfing has (Wei et al., 2017).

Forage quality parameters typically measured include acid detergent fiber (ADF), neutral detergent fiber (NDF), and protein (Stokes and Prostko, 1998). Neutral detergent fiber measures the amount of total fiber in the forage and therefore consists of hemicellulose, cellulose, and lignin. Acid detergent fiber is a measure of the amount of cellulose and lignin content in the plant. The amount of lignin, cellulose, and hemicellulose can therefore be determined with measurements for ADF, NDF, and lignin. There are two main approaches for analyzing forages and feeds: Near infrared (NIR) spectroscopy and wet chemistry (de Ondarza and Ward, 2013). Wet chemistry methods are more accurate; however, they are time consuming and expensive. For example, wet chemistry analyses for ADF, NDF, and lignin cost ~$23 per sample whereas NIR for the same analyses cost ~$1 per sample. NIR is widely used for predicting physical and chemical properties including lignin, cellulose, and hemicellulose in a quick manner (Li et al., 2015). However, for NIR to be utilized, a calibration model against wet chemistry data must be developed. For many forages and feed crops, calibrated NIR equations are commonly available. Forage measurements are not common in crops such as dry bean because bean straw is rarely used as forage. Therefore, an accurate calibration for NIR is not available and wet chemistry analyses were required for this experiment.

A large-scale study on common bean architectural traits was previously performed. In this study, Soltani et al. (2016) screened a Durango Diversity Panel (DDP) for plant architectural traits. This study consisted of screening 122 genotypes with different growth habits grown at three environments with 16 traits measured. Stem strength was measured by taking stem segments from the lower stem (~10 cm above the soil level) and the amount of force needed to cut the stem was measured. Soltani et al. (2016) determined dry bean stem strength is negatively correlated to lodging (r = -0.41) and when the stem diameter is greater than 5.6 mm, lodging is significantly reduced. The objective of the current research is to measure different cell wall components (cellulose, hemicellulose, and lignin) to determine if there are differences in the accumulation of these components between Type II and Type III dry bean genotypes from the DDP. A Genome-Wide Association Study (GWAS) will also be utilized to determine which genomic regions could be underlying the upright architectural trait.

Materials and Methods

A total of 180 dry bean genotypes from the DDP were grown in Prosper, ND and Othello, WA in 2015 with two replications per location (Soltani et al., 2016). The panel consists of 140 genotypes with Type III growth habit and 40 genotypes with Type II. The DDP is comprised of pinto (n=94), great northern (n=41), small red (n=24), pink (n=20), and black mottled (n=1) market classes. Stem samples were randomly collected from 3 plants per experimental unit. The samples were collected from the base of the stem (~10 cm in length) during harvest when the plants were mature. The stem samples were analyzed for stem strength (Newtons; N) using a universal testing machine (Test Resources, Shakopee, MN; model#312).

Preliminary Experiment

The goal of the preliminary experiment was to calibrate an equation for NIR as well as to determine which locations/cell wall components could be analyzed for the entire DDP. Due to

the time and cost of analyses, a preliminary experiment using a small subset of genotypes (n=30) was performed to evaluate if differences in cell wall component accumulation exist between Type II and Type III dry bean genotypes. The subset of genotypes was selected based on the stem strength of each genotype. This preliminary experiment was performed at the NDSU Animal Nutrition Laboratory using wet chemistry analyses. The calibration equation was optimized by the NDSU Forage and Biomass Crop Production laboratory (Drs. M. Berti and H. Li).

Plant Material. The results from the stem sheer test were used to determine which genotypes were selected for the preliminary experiment. Based on the results obtained from Soltani et al. (2016), the 15 genotypes with the highest stem strength across both locations (strong stem genotypes) and the 15 with the lowest stem strength (weak stem genotypes) were selected as extremes (Table 1.2). All weak stem genotypes selected possess Type III growth habit except for CDC Pintium which is Type II. For the strong stem genotypes, Sierra and BelMiNeb RR-2 have Type III growth habits whereas all other genotypes are Type II. The three stem samples from each experimental unit were ground and bulked together.

Lab experiments. Stem segments were ground to a fine powder using a Udy Cyclone sample mill (Seedburo, Chicago, IL). Both replications from Prosper and Othello were analyzed for ADF, NDF, and lignin, for a total of 120 samples. Wet chemistry analyses were made at the NDSU animal nutrition laboratory following standard methods (Horwitz and Latimer, 2010) and samples were run twice to improve accuracy.

Table 1.2. Extreme genotypes used in preliminary study selected based on stem strength (unpublished data).

Genotypes	Release Year	Stem Strength (Newtons)	Growth Habit	Market Class
---------- Strong Stem ----------				
Nodak	1984	290.72	III	Pinto
UI 126	1983	327.04	III	Pinto
Ivory	1983	329.67	III	Great Northern
ABCP-15	2004	332.42	III	Pinto
Pindak	1981	334.93	III	Pinto
Buckskin	1996	336.05	III	Pinto
NW-410	1981	339.05	III	Pinto
Common Pinto†	-‡	345.29	III	Pinto
Emerson	1971	363.62	III	Great Northern
Belmineb RMR-3	1996	369.83	III	Great Northern
Montrose	1999	372.00	III	Pinto
UI-59	1932	372.16	III	Great Northern
CDC Pintium	1999	399.08	II	Pinto
AC Early Rose	2004	404.10	III	Pink
CDC Rosalee	1999	404.66	III	Pink
---------- Weak Stem ----------				
NE-1-09-20†	-	984.93	II	Great Northern
Sinaloa	2011	1006.91	II	Pinto
USPT CBB-3	2001	1011.61	II	Pinto
Sierra	1990	1013.57	III	Pinto
PR0401-259	2012	1031.98	II	Pink
Matterhorn	1998	1036.24	II	Great Northern
Monterrey	2012	1055.40	II	Pinto
Belmineb-1	1993	1065.49	II	Great Northern
Long's peak	2011	1083.05	II	Pinto
Stampede	2007	1094.45	II	Pinto
Belmineb RR-2	1993	1136.22	III	Great Northern
P12606†	-	1174.26	II	Pinto
El Dorado	2012	1176.88	II	Pinto
SR9-4†	-	1223.88	II	Small Red
PT11-13†	-	1300.84	II	Pinto

† Breeding line or landrace.

‡ Release date could not be obtained.

Statistical Analyses. In the lab experiments, dry weight, NDF, ADF, and lignin were measured. Hemicellulose is calculated by subtracting NDF from ADF and cellulose is calculated by subtracting lignin from ADF (Van-Soest and Wine, 1967). Hemicellulose, cellulose, and lignin were reported as a percent content based on dry weight. Least square (LS) means were

calculated for each genotype at each location using the PROC MIXED procedure in SAS version 9.3 (SAS Institute, Cary, NC). Analysis of variance (ANOVA) on cellulose, hemicellulose, and lignin was conducted to determine differences among genotypes. The ANOVAs were completed for each location as an RCBD using PROC MIXED procedure in SAS software 9.3. Replications were considered random and genotypes were fixed effects.

Analysis of DDP Grown in Prosper

Plant Material. In the preliminary study, the genotype effect was significant for cellulose and lignin at both locations and genotype was significant for hemicellulose at the Prosper location only. Due to time and cost of analyses, only one location could be selected for further analyses with the entire DDP panel. Prosper was selected as the location since more variation was observed for all traits at the Prosper location compared to the Othello location. At the Prosper location, the ranges for hemicellulose, cellulose, and lignin were 13.8-19.1%, 32.3-46.9%, and 12.9-20.5%, respectively (Figure 1.1). For the Othello location, the ranges for hemicellulose, cellulose, and lignin were 13.4-18.3%, 31.5-45.6%, and 12.1-16.6%, respectively (Figure 1.1). Therefore, the stem segments that came from the Prosper location were selected for further analyses using the full panel of 180 Durango genotypes.

Experimental Design. All experimental units (n=380, 190 genotypes and 2 replications) were analyzed for cellulose, hemicellulose, and lignin using NIR in the NDSU Forage and Biomass Crop Production laboratory in collaboration with Drs. M. Berti and H. Li. Since currently there is not a standard NIR calibration for dry bean, 150 samples were selected for wet chemistry analyses for the same traits to develop a reliable NIR calibration for dry bean stems. The 150 samples were selected based on the variability in the values obtained from NIR.

Statistical Analyses. Hemicellulose and cellulose were calculated from ADF and NDF. The wet chemistry data were plotted against the NIR data for all traits to determine if NIR could be used for the analyses (a correlation coefficient >0.70 was determined to be suitable). For the traits NIR could be used for, least squares (LS) means were calculated for each genotype using the PROC MIXED procedure in SAS version 9.3 (SAS Institute, Cary, NC). Analysis of variance (ANOVA) was conducted to determine differences among genotypes. The ANOVA was completed as an alpha lattice using PROC MIXED procedure in SAS software 9.3 with replications being random and genotypes fixed. Pearson's phenotypic correlations between lignin and all traits measured in Soltani et al. (2015) were performed using the psych and corrplot packages in R (R Development Core Team, 2011).

Genome-Wide Association Study. GWAS was performed using the GAPIT package in R (Lipka et al., 2012). A total of 780,531 SNP markers were obtained from 6x sequencing provided by the NDSU dry bean genomics lab. After filtering for MAF <5%, ~552K makers remained and were utilized for the analyses (Soltani et al., unpublished). Four models (Naïve, EMMA, PC, and EMMA+PC) were tested for each trait analyzed. PC controls for population structure, EMMA accounts for kinship, EMMA+PC controls for both population structure and kinship, and Naïve does not account for neither kinship nor population structure. The best model was selected based on which one had the lowest mean square deviation (MSD) (Mamidi et al., 2011). Significant SNPs ($P < 0.01$) were selected from the best model. Principal component analysis was performed by the NDSU dry bean genomics lab and three principal components were selected to account for ~27% of the variation.

Potential candidate genes were searched on the second version on the bean genome annotation (Schmutz et al., 2014) which is available at:

https://legumeinfo.org/genomes/jbrowse/?data=phavu.G19833.gnm2. A 100Kb window around the most significant SNP was searched for potential candidate genes. The phenotypic variation explained by the most significant markers was calculated using the likelihood-ratio-based R^2 (Sun et al., 2010) using the genABLE package in R (Aulchenko et al., 2007). The R^2 value was also calculated for each trait using the top 0.001% of SNPs with the lowest p-value.

Results

Preliminary Experiment

The preliminary experiment was performed to determine if cell wall component accumulation differs for weak and strong stemmed genotypes. The genotypes selected as extremes for stem strength (Table 1.2) were analyzed for hemicellulose, cellulose, and lignin using wet chemistry analyses. The genotype effect was significant ($P<0.05$) for hemicellulose and highly significant ($P<0.01$) for cellulose and lignin at the Prosper location. For the Othello location, cellulose was highly significant ($P<0.01$) and lignin was also significant ($P<0.05$) (Table 1.3).

Table 1.3. Mean squares for hemicellulose, cellulose, and lignin for both locations studied (PR=Prosper, ND; OT=Othello, WA).

		Hemicellulose		Cellulose		Lignin	
	df	PR	OT	PR	OT	PR	OT
Replication	1	0.74	4.26	0.72	15.77	0.14	1.05
Genotype	29	4.58*	1.51	33.98**	18.86**	7.44**	1.41*

*,**Significant at 0.05 and 0.01 levels, respectively

The mean for hemicellulose at Prosper was 15.9% with a range of 13.7-19.1% (Figure 1.1). At Othello, hemicellulose ranged from 13.4-18.3% with a mean of 15.6% (Figure 1.1). For cellulose, the values ranged from 32.3-46.9% with a mean of 38.0% at Prosper and from 31.5-45.6% with a mean of 40.6% at Othello (Figure 1.1). There was more variation for lignin at Prosper (ranging from 12.9-20.5%) compared to at Othello (12.1-16.6%) (Figure 1.1). The

correlation coefficients between growth type and hemicellulose, cellulose, and lignin were all negative except for lignin at the Othello location (Table 1.4). For all traits, the correlation coefficient is lower at the Prosper location (Table 1.4) indicating there is more of a correlation between growth type and all cell wall components analyzed at the Prosper location compared to Othello.

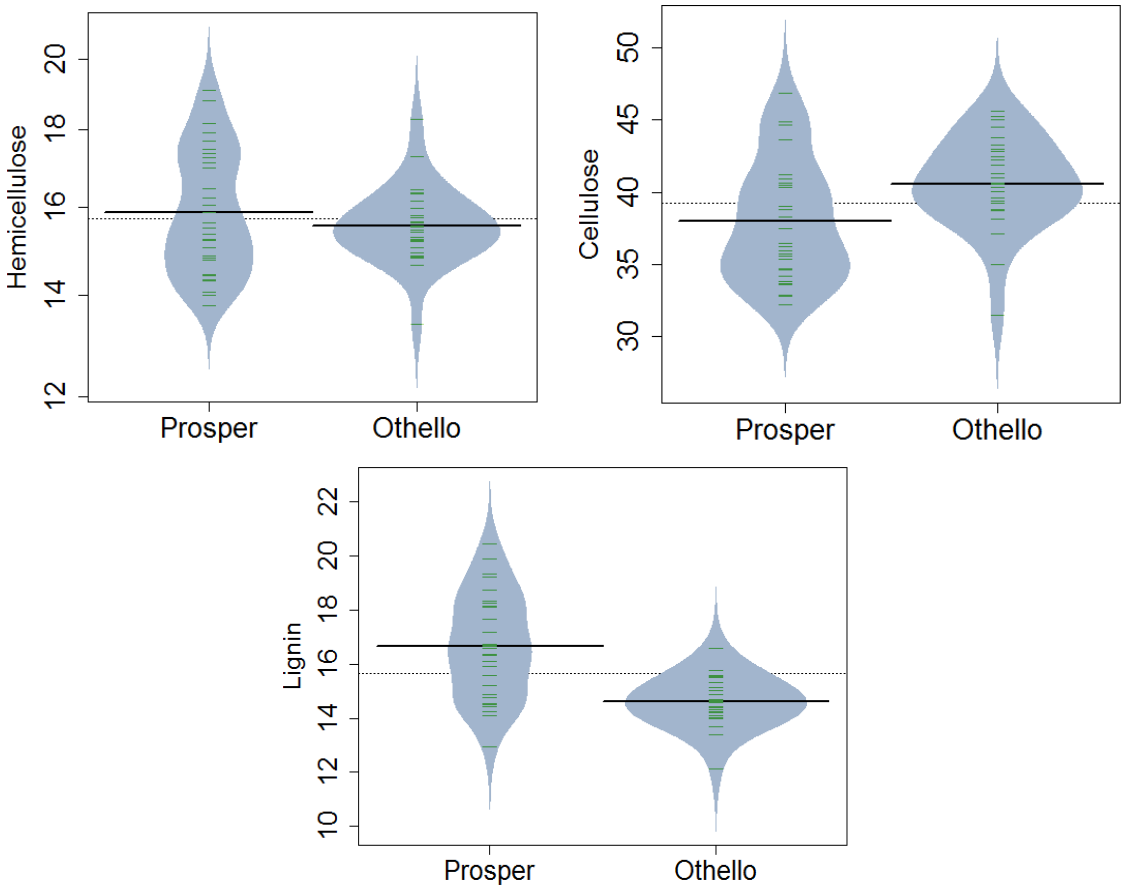

Figure 1.1. Distribution of hemicellulose, cellulose, and lignin for Prosper, ND and Othello, WA locations in the preliminary experiment. Green lines depict individual observations, the blue area shows the distribution, the black solid lines represent the location means, and the black dashed lines represent the trait mean.

Table 1.4. Correlation coefficients between trait and growth type from wet chemistry data for preliminary study.

Location	Hemicellulose	Cellulose	Lignin
Prosper	-0.22*	-0.47*	-0.48*
Othello	-0.13*	-0.21*	0.06

* Significant at 0.05 level

Prosper Location Analyses

From the preliminary experiment results, all genotypes (n=191) grown in the Prosper, ND location were selected for further analyses. This selection to only analyze one location was based on the correlation coefficients and that more variation was detected for Prosper compared to Othello. For each trait, the correlation coefficients between stem strength and growth type were stronger for the Prosper location than Othello (Table 1.4) and the range of individual values was wider for all traits at the Prosper location (Figure 1.1). All samples (191 genotypes, 382 samples) were analyzed for lignin, cellulose, and hemicellulose using NIR. However, only a subset of 150 samples that were selected based on the variability of the sample were analyzed for the traits using wet chemistry due to time and cost of the analyses. The NIR data and wet chemistry data were compared to determine if the correlation was strong enough to use the NIR data as a good proxy for wet chemistry analyses (R^2 >0.70). Of the three traits, lignin was the only trait with a correlation coefficient greater than 0.70 (R^2=0.77) and therefore lignin was the only trait analyzed for all genotypes (Figure 1.2).

Lignin was highly significant (P<0.01) for the genotypes analyzed (Table 1.5). The genotype means ranged from 12.9 (Pindak) to 23.5 (Medicine Hat) with an average of 17.4±1.8 (Figure 1.3, Table A1). Among the top 10% of genotypes with the highest values for lignin, 74% (14/19) have the Type II growth habit. Among the bottom 10% of the genotypes with the lowest values for lignin, 84% (16/19) have the Type III growth habit (Table A1).

Table 1.5. Analysis of variance for lignin evaluated for all genotypes in the DDP grown in Prosper.

Source of Variation	df	SS	MS	F-Value	P>F
Replication	1	9.29	9.29	1.70	0.2136
Block (rep) – error (a)	18	60.3	3.35	3.55	<0.0001
Genotype	189	861	4.56	4.83	<0.0001
Error (b)	166	156	0.94	-	-

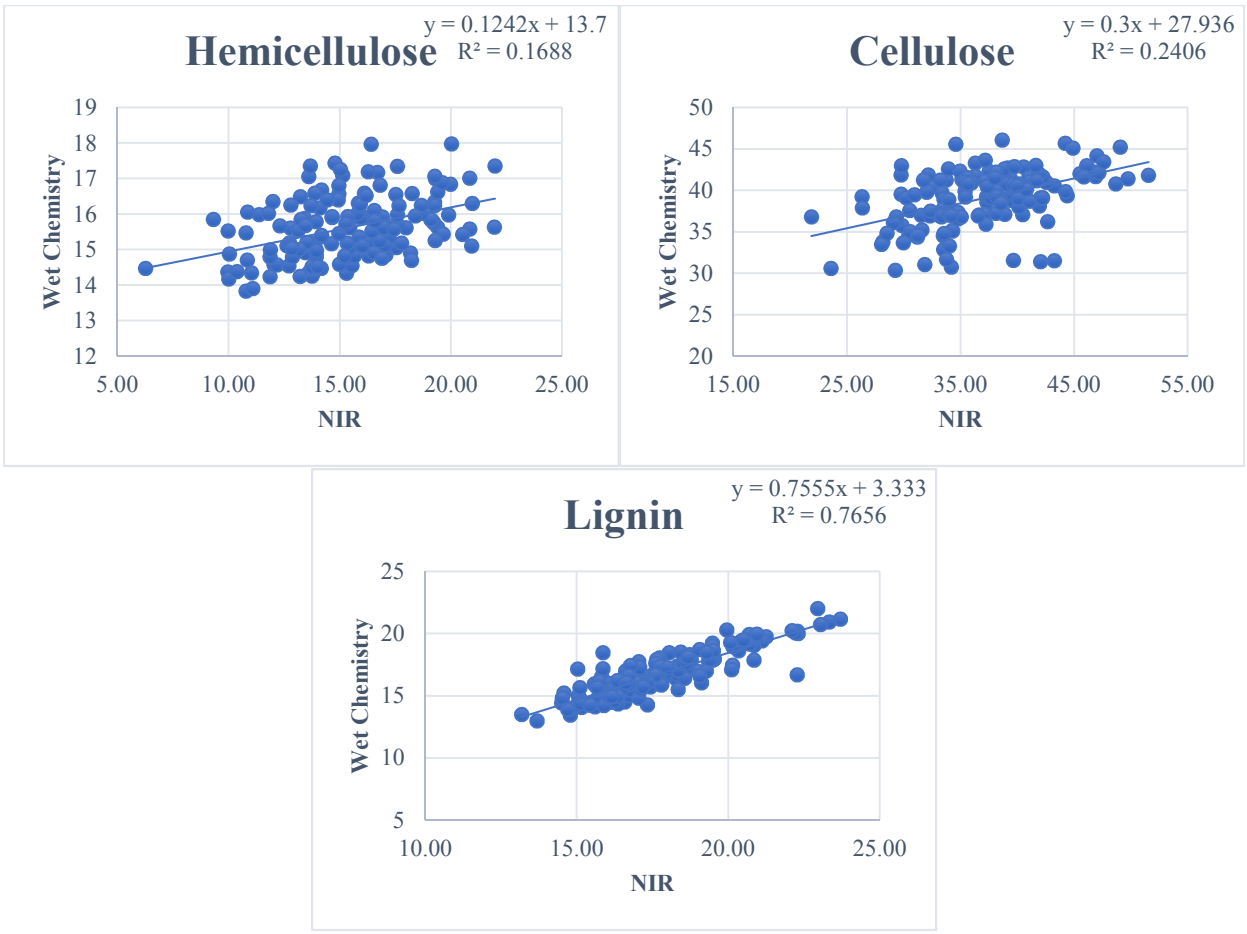

Figure 1.2. Correlation between NIR and wet chemistry values for lignin, cellulose, and hemicellulose.

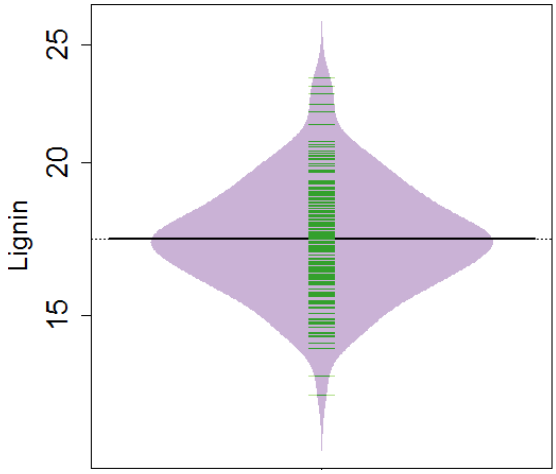

Figure 1.3. Distribution of lignin values for genotypes grown in Prosper. Green lines depict individual observations, the purple area shows the distribution, and the black line depicts the mean.

Correlation coefficients were measured for 14 traits, 12 traits were previously analyzed in Soltani et al. (2016) and lignin and growth habit were also included in this analysis (Figure A1). Significant negative correlations were found between lignin and several traits previously examined in Soltani et al. (2016; Table 1.5). A negative but weak correlation was found between lignin accumulation and lodging (r = -0.47; Table 1.6). Furthermore, a negative but weak correlation was also found between lignin and growth habit (r = -0.39), porosity, plant length, seed yield, and plot yield (r = -0.43; Table 1.5).

Table 1.6. Significant correlation coefficients between lignin and traits examined in Soltani et al. (2016).

Trait	Pearson's Correlation Coefficient
Lodging	-0.47
Growth Habit	-0.39
Porosity	-0.43
Plant Length	-0.43
Seed Yield	-0.43
Plot Yield	-0.43
Dry Matter	-0.33
Seed Weight	-0.12

Association Mapping

After filtering for MAF >5%, a total of 551,825 SNP markers were utilized for GWAS. Genomic regions associated with lignin accumulation were determined using GWAS. Four models were tested, and PC+EMMA was selected as the best model based on the lowest MSD (Table 1.7). Two significant regions were identified, one at the proximal end of Pv07 and one at the distal end of Pv08 (Figure 1.4; Table 1.8). The region identified on Pv08 is 2kb downstream gene model *Phvul.008G279600,* which encodes a cellulose synthase like EI enzyme. The region on Pv07 is 8.2Kb upstream gene model *Phvul.007G009300,* is a homolog of an *Arabidopsis* pectin lyase-like superfamily protein and 736bp downstream of gene model *Phvul.007G009500*

which encodes an alpha/beta-Hydrolase superfamily protein thought to be involved in cutin biosynthesis (Chen, 2014).

Table 1.7. Mean square deviations for each model tested in GWAS for lignin study.

Model	MSD
EMMA+3PC	2.67E-04
EMMA	6.40E-04
PC	3.29E-03
Naïve	6.32E-03

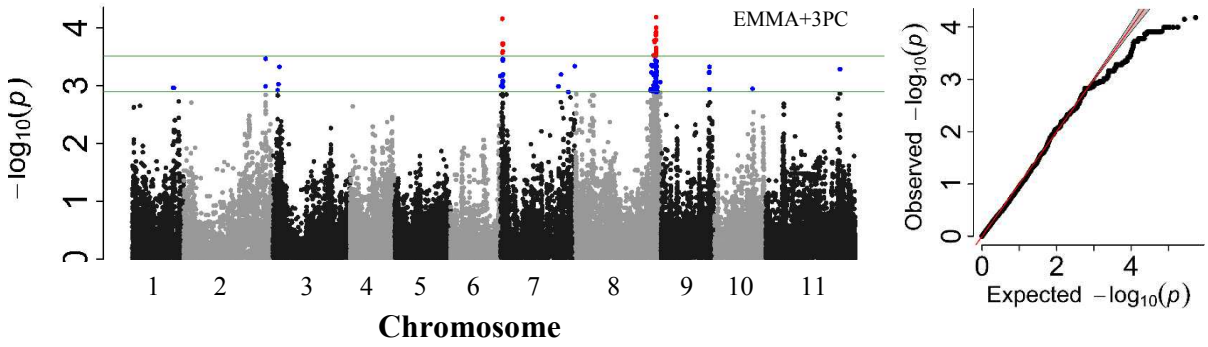

Figure 1.4. Manhattan and quantile-quantile plots for lignin content at Prosper location.

Table 1.8. Major loci associated with lignin accumulation in DDP grown in Prosper, ND. R^2 values are calculated individually for each significant SNP as well as combined for most significant 0.001% of SNPs.

Chromosome	Position	-log10 (P-value)	R^2
7	685,369	4.15	18.3
8	62,096,599	4.00	20.0
7 & 8			33.8†

† combined R^2 value calculated from most significant 0.001% of SNPs.

Discussion

Upright plant architecture is favorable over a more prostrate growth type for dry bean since upright varieties are usually less susceptible to diseases that thrive in humid environments as well as allowing for more efficient harvest practices. Through dry bean breeding, cultivars with the upright, Type II architecture have been developed; however, the structural components

underlying this change have not been explored. This research analyzed different cell wall components to better understand the structural changes associated with dry bean growth types.

The range of individual values for each trait was larger for the Prosper location compared to Othello. Furthermore, lignin was the trait with the best correlation between the NIR and wet chemistry data. From this data, the Prosper location was selected and lignin was analyzed for all 191 lines in the DDP. The genotype effect was significant for lignin indicating there is variation within the panel.

In several plant species such as wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.), lignification in secondary cell walls results in stronger lodging resistance (Zheng et al., 2017). Wang et al. (2014) determined lignin content can be used to evaluate lodging resistance in buckwheat (*Fagopyrum esculentum* L.). Flint-Garcia et al. (2003) performed QTL analysis for stalk strength in four different maize populations and discovered candidate genes involved in lignin synthesis consistent across all populations. Furthermore, lignin accumulation was shown to be significantly correlated with mechanical strength and lodging resistance in wheat (Peng et al., 2014). In the current study, lignin was negatively correlated with growth habit (r = -0.39) and lodging (r = -0.47). The negative correlation between lignin and growth habit further supports the hypothesis that dry bean genotypes with a type II growth habit may contain more lignin than genotypes with a type III growth habit. During the conversion of type III dry bean architecture to type II via selective breeding, genes for lignin accumulation could have indirectly been selected for.

Based on the results from this experiment, lignin accumulation seems to be controlled by multiple loci. Three genes found within the GWAS peaks with the highest R^2 values are possible candidates for lignin accumulation in dry bean. A cellulose synthase-like E1 enzyme was

identified on Pv08. The cellulose synthase-like (*Csl*) gene family is believed to encode enzymes that synthesize non-cellulosic wall polysaccharides (Sommerville et al., 2004). Furthermore, transcriptional regulation of lignin biosynthesis is under the control of the same transcriptional network regulating cellulose biosynthesis (Zhong and Ye, 2009). *Phvul.007G009300* encodes for a pectin lyase-like superfamily protein and the GWAS peak identified on Pv07 is 8.2Kb upstream from this gene. The same region on Pv07 is also 736bp downstream an alpha/beta-hydrolase superfamily protein thought to be involved in cutin biosynthesis (Pineau et al., 2017). Both cutin and pectin are cell wall components along with lignin, cellulose, and hemicellulose. Pectin is generally found between the cellulose microfilaments and cutin is primarily found coating the cell wall surface to aid in water loss (Chen, 2014).

Other studies have analyzed agronomic traits in dry bean diversity panels using GWAS. Moghaddam (2016) performed GWAS for several agronomic traits using a Middle-American diversity panel of dry bean. In this study, a highly significant peak was identified on Pv07 associated with lodging as well as canopy height. Although a significant negative correlation between lignin accumulation and lodging was identified in the current study, the genomic region identified in this study was found at the proximal end of Pv07 (~685Kb) whereas the region identified by Moghaddam (2016) was at the distal end of Pv07 (~46Mb). On the other hand, the plants analyzed by Soltani et al. (2016) were further analyzed for stem strength, and GWAS was performed for all parameters analyzed (unpublished data). In the current study, a significant correlation was not found between lignin accumulation and stem diameter. However, a significant region on Pv07 was identified for stem diameter (Soltani, unpublished data) which is near the same region as the peak identified in this study for lignin content.

Although dry bean is not commonly used as a forage crop and therefore fiber analyses are not typically performed for dry bean stems, the dietary fiber content of dry bean stems has been studied. Dietary fiber consists of the portions of plant foods that humans are unable to digest and include lignin, hemicellulose, and cellulose (Kay, 1982). Dietary fiber content in the MDP of dry bean was analyzed by Moghaddam et al. (2018). Moghaddam et al. (2018) identified a significant region on Pv08 (50.9Mb) associated with total dietary fiber and candidate genes were identified that are part of dietary fiber component synthesis pathways. This region identified on Pv08/50.9Mb is ~11Mb downstream the peak associated with lignin in the current study. Many of the candidate genes for insoluble dietary fiber were related to cellulose synthesis and therefore could also be important in dry bean plant architecture. Furthermore, the authors concluded levels of insoluble dietary fiber was higher for cultivars released since 1997 suggesting the improvement in plant architecture through breeding efforts could have indirectly increased levels of insoluble dietary fiber. Although many of the cultivars released since 1997 have Type II growth habit, the insoluble dietary fiber content did not differ between Type II and Type III cultivars (Moghaddam et al., 2018).

Lignin accumulation in dry bean was analyzed and three potential candidate genes were identified in this study. These results provide a basis for future studies to begin to identify actual genes controlling dry bean plant architecture. Furthermore, adding phenotypic data and analyzing more locations could further improve the accuracy of these results. With further validation, MAS could potentially be utilized with markers near candidate genes associated with lignin content. While lignin was found to be highly variable among dry bean genotypes grown in both Prosper, ND and Othello, WA, the other cell wall components (cellulose and hemicellulose) could still play a large role in the shift from Type III to Type II growth habit in dry bean and

should be further studied using wet chemistry analyses or NIR if a suitable reference equation is generated.

References

Aulchenko, Y.S., S. Ripke, A. Isaacs, and C.M. van Duijn. 2007. GenABEL: an R library for genome-wide association analysis. *Bioinformatics* 23: 1294-1296.

Berry, P.M., M. Sterling, J.H. Spink, C.J. Baker, R. Sylvester-Bradley, S.J. Mooney, A.R. Tams, and A.R. Ennos. 2004. Understanding and reducing lodging in cereals. Adv. Agron. 84: 217-271.

Bilbro, J.D., D.J. Undersander, D.W. Fryrear, and C.M. Lester. A survey of lignin, cellulose, and acid detergent fiber ash contents of several plants and implications for wind erosion control. J. of soil and water conservation. 46:314-316.

Brick, M.A., and K.F. Grafton. 1999. Improvement of Medium-Seeded Race Durango Cultivars. p. 223–253. *In* Sing, S.P. (eds) Common bean improvement in the twenty-first century. Develoments in plant breeding. 7. Springer, Dordrecht.

Brick, M.A., J.B. Ogg, H.F. Schwartz, J.J. Johnson, F. Judson, and M.A. Pastor-Corrales. 2015. Registration of 'Long's Peak' pinto bean. J. of plant reg. 9:279-282.

Caffall, K.H., and D. Mohnen. The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. Carbohydr. Res. 344: 1879–1900.

Chen, H. Chemical composition and structure of natural lignocellulose. p. 25-71. *In* Biotechnology of lignocellulose: Theory and practice. Springer, Houten.

Chen, H.Q., Y. Gong, and Z. Fang. 1996. The situation and aspect of application for macromolecule (l)-cellulose, lignin, and starch. Yunnan Chem. Technol. 11: 41-46.

Cosgrove, D.J. 2005. Growth of the Plant Cell Wall. Nature. 6: 850–861.

de Ondarza, M. and R. Ward. 2013. Accurate analysis: NIRS versus wet chemistry. Feeding. 129.

Down, E.E. and A.L. Anderson. 1956. Agronomic Use of an X-ray-Induced Mutant. Science 124: 223–224.

Eckert, F.R.. 2009. Dry bean yield loss of commercial cultivars under conditions of direct harvesting. MS Thesis, North Dakota State Unviersity. Fargo, ND.

Flint-Garcia, S., C. Jampatong, L.L. Darrah, and M.D. McMullen. 2003. Quantitative trait locus analysis of stalk strength in four maize populations. Crop Sci. 43:13-23.

Gepts, P., and D. Debouck. 1991. Origin, domestication, and evolution of the common bean (Phaseolus vulgaris L.). p. 7–53. *In* van Schoonhoven, A., Voysest, O. (eds.), Common Beans: Research for Crop Improvement - Google Books. CAB International, Wallingford, Commonwealth .

Goering, H.K., and P.J. Van Soest. 1970. Forage Analyses. Agriculture Handbook. 379. Agric. Res. Serv. U.S. Dept. of Agriculture.

Horwitz, W. and Latimer, G.W. 2010. Official methods of analysis of AOAC International. 18th ed. Gaithersburg, MD.:AOAC International.

Kay, R.M. 1982. Dietary fiber. J. of lipid research. 23: 221-242.

Kelly, J.D. and L.O. Copeland. 1998. Matterhorn: A new great northern bean for Michigan. Michigan State University Extension Bulletin. E-2669.

Kelly, J.D. 2001. Remaking bean plant architecture for efficient production. Adv. Agron. 71: 109–143.

Kelly, J.D., and M.W. Adams. 1987. Phenotypic recurrent selection in ideotype breeding of pinto beans. Euphytica 36: 69–80.

Kelly, J.D., M.W. Adams, A.W. Saettler, G.L. Hosfield, G. V. Varner, M.A. Uebersax, and J. Taylor. 1990. Registration of "Sierra" pinto bean. Crop Sci. 30: 745–746.

Kelly, J.D., G.V. Varner, and B. Long. 2010. Registration of "Santa Fe" pinto bean. J. Plant Regist. 4:12-16.

Kelly, J.D., G.V. Varner, K.A. Cichy, and E.M. Wright. 2014. Registration of "Powderhorn" great northern bean. J. Plant Regist. 8:1-4.

Knodel, J.J., P.B. Beauzay, G.J. Endres, D.W. Franzen, H.J. Kandel, S.G. Markell, J.M. Osorno, J.S. Pasche, and R.K. Zollinger. 2017. 2017 Dry Bean Grower Survey of Production, Pest Problems and Pesticide Use in Minnesota and North Dakota E1841.

Li, X., C. Sun, B. Zhou, and Y. He. 2015. Determination of hemicellulose, cellulose, and lignin in moso bamboo by near infrared spectroscopy. Sci. Rep. 5:1-11.

Lipka, A.E., F. Tian, Q. Wang, J. Peiffer, M. Li, P.J. Bradbury, M.A. Gore, E.S. Buckler, and Z. Zhang. 2012. GAPIT: genome association and prediction integrated tool. Bioinformatics 28: 2397–2399.

Moghaddam, S.M., M.A. Brick, D. Echeverria, H.J.Thompson, L.A. Brick, R. Lee, S. Mamidi, and P.E. McClean. 2018. Genetic architecture of dietary fiber and oligosaccharide content in a Middle American Panel of edible dry bean. The Plant Genome. 11:1-11.

Moghaddam, S.M., S. Mamidi, J.M. Osorno, R. Lee, M. Brick, J. Kelly, P. Miklas, C. Urrea, Q. Song, P. Cregan, J. Grimwood, J. Schmutz, and P.E. McClean. 2016. Genome-wide association study identifies candidate loci underlying agronomic traits in a Middle American diversity panel of common bean. The Plant Genome 9:1-21.

Mamidi, S., S. Chikara, R.J. Goos, D.L. Hyten, and D. Annam. 2011. Genome-Wide Association Analysis Identifies Candidate Genes Associated with Iron Deficiency Chlorosis in Soybean. Plant Genome 4: 154-164.

Moraghan, J.T., and K. Grafton. 1999. Seed-Zinc Concentration and the Zinc-Efficiency Trait in Navy Bean. Soil Sci. Soc. Am. J. 63: 918-922.

Osorno, J.M., K.F. Grafton, G.A. Rojas-Cifuentes, R. Gelin, and A.J. Vander Wal. 2010. Registration of "Lariat" and "Stampede" pinto beans. J. Plant Regist. 4: 5-11.

Park, S., A.L. Szumlanski, F. Gu, F. Guo, and E. Nielsen. 2011. pa Nat. Cell Biol. 8:973-980.

Peng, D. X. Chen, Y. Yin, K. Lu, W. Yang, Y. Tang, and Z. Wang. 2014. Lodging resistance of winter wheat (*Triticum aestivum* L.): Lignin accumulation and its related enzymes activities due to the application of paclobutazol or gibberellin acid. Field Crops R. 157:1-7.

Pineau, E., L. Xu, H. Renault, A. Trolet, N. Navrot, P. Ullmann, B. Legeret, G. Verdier, F. Beisson, and F. Pinot. 2017. Arabidopsis thaliana EPOXIDE HYDROLASE1 (AtEH1) is a cytosolic epoxide hydrolase involved in the synthesis of polyhydrolated cutin monomers. New Phytol. 215: 173-186.

R Develepment Core Team (2011). *R: A Language and Environment for Statistical Computing.* Vienna: R Foundation for Statistical Computing.

SAS Institute Inc. 2011. Base SAS® 9.3 Procedures Guide. Cary, NC: SAS Institute Inc.

Singh, S. 1982. A key for identification of different growth habits of Phaseolus vulgaris L. Ann. Rep. Bean Improv. Coop. 25:92–95.

Soltani, A., M. Bello, E. Mndolwa, S. Schroder, S.M. Moghaddam, J.M. Osorno, P.N. Miklas, and P.E. McClean. 2016. Targeted Analysis of Dry Bean Growth Habit: Interrelationship among Architectural, Phenological, and Yield Components. Crop Sci. 56: 3005-3015.

Somerville, C., S. Bauer, G. Brininstool, M. Facette, T. Hamann, J. Milne, E. Osborne, A. Paredez, S. Persson, T. Raab, S. Vorwerk, and H. Youngs. 2004. Toward a Systems Approach to Understanding Plant Cell Walls. Science. 306:2206-2211.

Stokes, S.R. and E.P. Prostko. 1998. Understanding forage quality analysis. Texas agricultural extension service. L-5198, College Station, TX.

Sun, G., C. Zhu, M.H. Kramer, S.S. Yang, W. Song, H.P. Piepho. 2010. Variation explained in miced-model assocaition mapping. Heredity. 105:333-340.

Test Resources. 2018. Universal testing machine. Shakopee, MN.

Thomas, J.A., J.A. Smith, and R.G. Wilson. 2016. Direct Harvest of Dry Edible Beans. Univ. Nebraska Ext. 309: 1-19. Available at http://extensionpublications.unl.edu/assets/pdf/ec309.pdf (verified 22 November 2017).

Urrea, C.A., J.R. Steadman, M.A. Pastor-Corrales, D.T. Lindgren, and J.P. Venegas. 2009. Registration of great northern common bean cultivar "Coyne" with enhanced disease resistance to common bacterial blight and bean rust. J. Plant Regist. 3: 219.

Vanholme, R., K. Morreel, C. Darrah, P. Oyarce, J.H. Grabber, J. Ralph, and W. Boerjan. 2012. Metabolic engineering of novel lignin in biomass crops. New Phytol. 196: 978-1000.

Van-Soest, P.J., and Wine, R.H. 1967. Use of detergents in the analysis of fibrous feeds IV. Determination of plant cell-wall constituents. J. Assoc. Off. Anal. Chem. 50: 50-55.

Wang, C., R. Ruan, X. Yuan, D. Hu, H. Yang, Y. Li, and Z. Yi. 2014. Relationship between lignin metabolism and lodging resistance of culm in buckwheat. J. Ag. Sci. 6:29-36.

Wei, L., H. Jian, K. Lu, N. Yin, J. Wang, X. Duan, W. Li, L. Liu, X. Xu, R. Wang, A.H. Paterson, and J. Li. 2017. Genetic and transcriptomic analyses of lignin- and lodging-related traits in *Brassica napus*. Theoretical and Applied Genetics. 9:1961-1973.

Zheng, M., J. Chen, Y. Shi, Y. Li, Y. Yin, D. Yang, Y. Luo, D. Pang, X. Xu, W. Li, J. Ni, Y. Wang, Z. Wang, and Y. Li. (2017). Manipulation of lignin metabolism by plant densities and its relationship with lodging resistance in wheat. Sci. Rep. 7:1-12.

Zhong, R. and Z. Ye. 2007. Regulation of Cell Wall Biosynthesis. Current Opinions in Plant Biology. 10:564-572.

CHAPTER II. PRE-GERMINATION FLOODING TOLERANCE OF MIDDLE-AMERICAN DRY BEAN GENOTYPES

Introduction

There are numerous abiotic and biotic stresses affecting dry bean production. Flooding is one such abiotic stress that negatively affects dry bean production. Flooding incidents are becoming more common worldwide resulting in lower crop yields (Bailey-Serres et al., 2011). Crop damage due to excess water accounted for over $3 billion in the United States in 2011 (Bailey-Serres et al., 2012). Worldwide, flooding has the largest impact on cropland among all natural disasters, accounting for an average of $7.8 billion in crop loss from 2003 to 2013 (FAO, 2015).

Dry bean is an extremely sensitive crop to flooding and is the most sensitive crop among all grown crops in North Dakota. Furthermore, excess water was the leading production issue for dry bean growers in North Dakota and Minnesota from 2013-2015 (Knodel el al., 2014, 2015, 2016). With the increasing demand of crop production to meet the needs of the world's growing population, developing flooding tolerant germplasm is essential. Flooding tolerance is extremely difficult to breed for due to the complexity of the trait (Zhou, 2010). Economically, the best way to reduce damage caused by flooding is to introduce tolerance into current varieties.

Flooding Stress

Flooding occurs when soil is saturated with water, leading to oxygen deficiency (hypoxia/anoxia) in the soil profile (Rajashekar and Baek, 2014; Voesenek and Bailey-Serres, 2013) which is the main cause of crop damage due to flooding (Ransom, 2011a). Hypoxia or anoxia can affect several plant processes both short and long term (Rajashekar and Baek, 2014). Oxygen is necessary for several plant functions including nutrient uptake and transport, cell

division, and plant growth (Ransom, 2011a). Germination also requires oxygen and several factors control the amount of oxygen necessary for germination to occur. These factors include moisture content of the seed, temperature, seed vigor, and the energy substrate utilized for respiration (starch, protein, and lipids) (Cardwell, 1984).

There are two main causes for decreased germination rates in seeds subjected to flooding prior to germination (Ismail et al., 2012). The seeds are either injured resulting in failure to germinate or they germinate normally but the seedlings are unable to emerge. If seeds are able to germinate under flooding conditions, the impacts of flooding at the germination stage can affect subsequent growth stages. Germination of most food crops, excluding rice (*Oryza sativa* L.), does not take place without oxygen (Setter and Waters, 2003). Seed germination is very sensitive to oxygen deficiency and the oxygen requirement for seeds varies by species (Cardwell, 1984). Dry bean is particularly sensitive to oxygen deficiency at germination stages. Dry bean seeds subjected to hypoxic conditions during the whole germination process fail to germinate, indicating that oxygen is essential for dry bean germination (Rajashekar and Baek, 2014).

Metabolism under Anoxia

For an organism to survive, it must use energy and continue to synthesize ATP (Catalanotti et al., 2013). Aerobic respiration is utilized by many eukaryotic organisms to produce ATP via oxidative phosphorylation where oxygen serves as the terminal electron acceptor. Cells must produce ATP and recycle NADPH, NADH, and $FADH_2$ to maintain viability. There are two methods of conserving energy when eukaryotes are in an oxygen-limiting environment: fermentation and anaerobic respiration (Atteia et al., 2013).

One of the limiting factors for seed germination under hypoxic conditions is the limitation of energy supply. A shift from aerobic to anaerobic metabolism is one of the main

adjustments for seed germination under oxygen deficient conditions. Under aerobic metabolism, for every mole of glucose, 34-36 ATP are produced. Whereas with anaerobic metabolism, as few as 2 ATP per mole of glucose can be produced (Bailey-Serres et al., 2012). Under hypoxic stress, the oxygen concentration limits oxidative phosphorylation and under anoxic stress, there is no oxygen available for oxidative phosphorylation and a shift to anaerobic respiration is necessary (Borisjuk and Rolletschek, 2009). In plant cells, tolerance to anoxia involves the adaptation to this energy crisis (Gibbs and Greenway, 2003; Bailey-Serres et al., 2011). Although the ATP production is much lower in anaerobic conditions compared to aerobic, as long as carbohydrate substrates are present, cells are able to survive with anaerobic ATP production (Bailey-Serres et al., 2011).

Physiological Mechanisms of Pre-Germination Flooding Tolerance

Carbohydrate metabolism is severely inhibited when oxygen is limiting (Miro and Ismail, 2013). The ability to maintain carbohydrate catabolism and sustain energy supply via anaerobic respiration are traits associated with waterlogging tolerant rice genotypes. Many factors including species, genotype, organ, and cell type can influence the efficiency of converting soluble carbohydrates and starch to energy during anoxia (Bailey-Serres et al., 2012).

Plants could utilize various tolerance mechanisms to survive flooding during germination stages. Tolerance mechanisms include decreasing metabolic rate, maintaining enzyme activity, and increasing antioxidant enzyme scavenging during hypoxic conditions. Since rice is known to tolerate flooding conditions and even germinate under hypoxic conditions, the majority of research on flooding tolerance at germination stages has been performed in rice and much less attention has been given to other crops. Cereal crops are generally more tolerant than legumes to flooding stress, with oats (*Avena sativa* L.) and wheat (*Triticum aestivum* L.) being the most

tolerant cereals (Ransom, 2011b). Among legumes, soybean and faba bean (*Vicia faba* L.) are more tolerant than peas (*Pisum sativum* L.) and common bean. Few studies have examined common bean tolerance to flooding with a limited number of genotypes (Nelson et al., 1983; Pociecha, 2012) and common bean has only recently been studied for flooding tolerance at a larger scale (Soltani et al., 2017). Nelson et al. (1983) determined flooding tolerance in dry bean is most likely heritable and Pociecha (2012) analyzed antioxidant enzymes in dry bean plants subjected to flooding and determined dry bean growth was significantly reduced when flooding occurred during vegetative stages.

Crawford (1977) found that tolerance to anoxia during germination is correlated to metabolic rate and species able to lower their metabolic rate during anoxic conditions are more tolerant to the stress. In several types of seeds, very low rates of carbohydrate catabolism can occur. One mechanism to survive anoxic conditions is to decrease the energy cost for maintenance, which prolongs the carbohydrate reserves (Greenway and Gibbs, 2003). Energy requirements for maintenance would thus be higher in anoxia intolerant plants than they would be for anoxia tolerant plants.

During anoxia, carbohydrate metabolism is strongly inhibited (Miro and Ismail, 2013). This is in part because most of the enzymes involved in breaking down starch into simple sugars to be used in glycolysis have reduced activity without oxygen. In rice genotypes that are tolerant to hypoxia during germination, several enzymes remain active even under hypoxic conditions (Ismail et al., 2009). Sucrose synthase, α-amylase, and aldolase are three enzymes which were active in pre-germination tolerant rice genotypes but inhibited in sensitive genotypes.

Anoxia can also change metabolic activity via altering the antioxidant system (Chugh et al., 2011). Several abiotic stresses, including flooding, promote the generation and accumulation

of reactive oxygen species (ROS) which can damage proteins, lipids, and nucleic acids. Antioxidant scavengers can help counteract the accumulation of ROS caused by stress (Gill and Tuteja, 2010). Plants have both enzymatic and non-enzymatic defense systems to manage ROS. Antioxidant enzymes can scavenge ROS, inhibit their formation, and repair the damage they cause (Gill and Tuteja, 2010). Superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) are three common antioxidant enzymes found in plants. Genotypes tolerant to flooding at germination stages could have higher activities of these antioxidant enzymes.

Germination Processes and Seed Coat Influence on Germination

Germination begins when a viable seed takes up water (imbibition) and is terminated once the radicle emerges (Bewley and Black, 1978; Nonogaki et al., 2008). Until a seed becomes a photosynthesizing, autotrophic plant, it must depend on food reserves within the seed (Bewley and Black, 1978). Germination requires the aerobic release of energy from storage materials in the cotyledons of dry bean (Cardwell, 1984). The oxygen that reaches the embryo is dependent upon the external concentration of oxygen, the solubility of oxygen in water, the seed coat characteristics, and the affinity of enzymes in the seed for oxygen. During germination, all seeds undergo a period of anoxia following imbibition, however, if the period of anoxia is prolonged by flooding, seed death can occur.

Functions of the seed coat consist of maintaining the integrity of seed parts, protecting the embryo from mechanical damage, regulating gas exchange between the environment and embryo, and regulating imbibition (Dubbern de Souza and Marcos-Filho, 2001). The outermost layer of the seed coat is the waxy cuticle which can vary in thickness and is the main barrier to imbibition (Cardwell, 1984; Dubbern de Souza and Marcos-Filho, 2001; Nokogaki et al., 2007).

The color of the seed coat is known to be associated with water absorption. In many leguminous species, pigmented seed coats often have slower imbibition rates (Dubbern de Souza and Marcos-Filho, 2001). In lima bean, a pigmented seed coat offers more protection against seed damage to the embryo during germination than an unpigmented, white seed coat (Kannenberg and Allard, 1964). Studies in soybean have also shown that seed coat pigmentation plays a role in germination vigor. Pigmented soybean varieties with a black or brown seed coat are more tolerant to pre-germination flooding than yellow soybean varieties (Hou and Thseng, 1992; Sayama et al., 2009). Seeds with white testae generally have higher levels of solute leakage and have faster imbibition rates than seeds with colored testae which has been observed in common bean (Powell et al., 1986a), pea (Powell and Matthews, 1978), and soybean (Powell et al., 1986b). In pea and soybean, rapid imbibition causes damage resulting in cell death in cotyledons due to physical disruption of membranes (Powell and Matthews, 1978; Powell et al., 1986b).

In dry bean, market classes with pigmented seed coats (e.g. black and small red) showed the highest germination rates under pre-germination flooding whereas unpigmented market classes (navy and great northern) showed the lowest rates (Soltani et al., 2017). In this study, there was an 84% reduction in germination rate of navy beans when they were subjected to 24 hr of flooding prior to germination whereas there was only a 63% reduction for the black market class. GWAS was also performed for this study and the authors identified four significant genomic regions (one on Pv01, two on Pv02, and one on Pv03) associated with germination rate under pre-germination flooding conditions. Soltani et al. (2018) also performed a study analyzing an Andean diversity panel (ADP) for flooding tolerance. In this study, three significant regions on Pv04, Pv06, and Pv08 were identified for germination rate under pre-germination flooding

conditions. The genomic regions identified in the MDP were different from those identified in the ADP indicating the different gene pools might utilize different mechanisms to tolerate pre-germination flooding stress.

Soltani et al. (2017) screened a Mesoamerican Diversity Panel (MDP) consisting of 296 dry bean genotypes. In this study, seeds were subjected to pre-germination flooding stress for 24 hours directly after planting, then plots were drained to field capacity and maintained for 15 days until germination rates (percentage of germinated seeds from 10 seeds planted) were evaluated. Based on germination rate percentages from Soltani et al. (2017), unpigmented (navy and great northern) market classes had an average germination rate of 9.8% whereas pigmented (black, small red, pink, and pinto) market classes had an average germination rate of 16.5%.

From the results of the previous study (Soltani et al., 2017), the main objective of the current research is to further examine the differences in pigmented and unpigmented seed coat genotypes to determine pre-germination flooding tolerant genotypes and genomic regions associated with the tolerance. This research uses the same genotypes as Soltani et al. (2017); however, this study examines pigmented and unpigmented seed coat genotypes separately to find more variation within each of the market classes. The specific objectives are to:

1. Determine the optimal water stress levels (number of hours submerged) that would allow efficient screening of both pigmented and unpigmented genotypes
2. Identify pre-germination flooding tolerant genotypes from various market classes, and
3. Determine genomic regions associated with pre-germination flooding tolerance using Genome-Wide Associate Studies (GWAS).

Materials and Methods

The results from the previous study (Soltani et al., 2017) suggested that dry bean genotypes with pigmented seed coats are more tolerant to pre-germination flooding than

unpigmented seed coat genotypes. Soltani et al. (2017) determined black and small red seeded genotypes had the highest germination rate (22.5% and 16.8% respectively) under flooded conditions and navy and great northern genotypes had the lowest (7.2% and 12.3%, respectively). From the previous study, it is clear there are differences in pre-germination flooding tolerance between market classes; however, to find variation within each market class, it is unfair to screen unpigmented and pigmented seed coat genotypes under the same conditions. In fact, the study from Soltani et al. (2017) mentioned that many of the unpigmented genotypes had a germination rate of 0%, which suggests that the stress level was too harsh to be able to recognize different tolerance levels among unpigmented genotypes. Therefore, for this study, genotypes with unpigmented seed coats (navy and great northern market classes) were analyzed separately from genotypes with pigmented seed coats (black, small red, pink, and pinto market classes) in an attempt to find more variation within each market class.

Preliminary Study

Genetic Material. The most tolerant (pigmented seed coat) and sensitive (unpigmented seed coat) pre-germination flooding genotypes were selected from the results of the previous study (Table 2.1; Soltani et al., 2017). The selection of tolerant and sensitive genotypes was performed to recognize phenotypic variation within each group of pigmented and unpigmented seed coat genotypes. By selecting the most tolerant and sensitive genotypes, the aim was to determine the optimal duration of flooding where variation is found within each group of pigmented and unpigmented seed coat genotypes.

To determine the threshold at which the tolerant genotypes can withstand, the 11 most tolerant pigmented genotypes were selected. To determine the length of flooding the least tolerant (unpigmented) genotypes could withstand, the 11 most sensitive unpigmented genotypes

were selected. 'Royalty', a cream colored dry bean, was used as a tolerant check since it has shown to be very tolerant to flooding at both germination and vegetative stages (Soltani et al., 2017). The genotype's tolerance levels were based on Equation 1.

$$flooding\ index = \frac{flooding\ value - nonflooded\ value}{nonflooded\ value} \quad \text{(Eq.1)}$$

Table 2.1. Most tolerant pigmented seed coat genotypes and most sensitive unpigmented seed coat genotypes selected for threshold study based on germination rate flooding effect.

Genotype	Flooding Index†	Market Class
---------- Pigmented Seed Coat ----------		
Indeterminate Jamaica Red	-0.19	Red Mottled
Rojo Chiquito	-0.21	Small Red
Shania	-0.24	Black
PR-0443-151	-0.25	Black
Blackmagic	-0.27	Black
I9365-5	-0.27	Pink
I9365-25	-0.30	Pink
CDC-Jet	-0.33	Black
CDC-Nighthawk	-0.33	Black
F07-014-22-2	-0.34	Small Red
Inta Precoz	-0.36	Small Red
---------- Unpigmented Seed Coat ----------		
Crestwood	-0.86	Navy
BelNeb RR 1	-0.91	Great Northern
Lightning	-0.93	Navy
CDC Crocus	-0.96	Great Northern
GN9-4	-0.96	Great Northern
GN9-1	-0.96	Great Northern
BelMiNeb RMR-4	-0.98	Navy
BelMiNeb-1	-1.00	Great Northern
BelMiNeb-2	-1.00	Great Northern
HY-4181	-1.00	Navy
OAC Laser	-1.00	Navy

† Flooding index based on results from Soltani et al. (2017), calculated by subtracting the germination rate in the control condition from the germination rate in the flooded condition and then dividing that value by the germination rate in the control condition.

Experimental design. The genotypes were planted in the NDSU greenhouse facilities using an autoclaved play sand (Nurserymen's Preferred® Play Sand, Mendota Heights, MN) in

24-well trays. Ten seeds per genotype were planted into a single well of the tray (Figure 2.1a). The genotypes were arranged in a randomized complete block design (RCBD) with five treatments and three replications. For the unpigmented genotypes, the treatments were 0 (non-flooded), 3, 6, 12, and 24 hours of flooding. For the pigmented genotypes, the treatments were 0, 4, 5, 6, and 7 days of flooding. The number of hours/days for each experiment were chosen based on previous research analyzing a small subset of genotypes at many more different time points (data not shown).

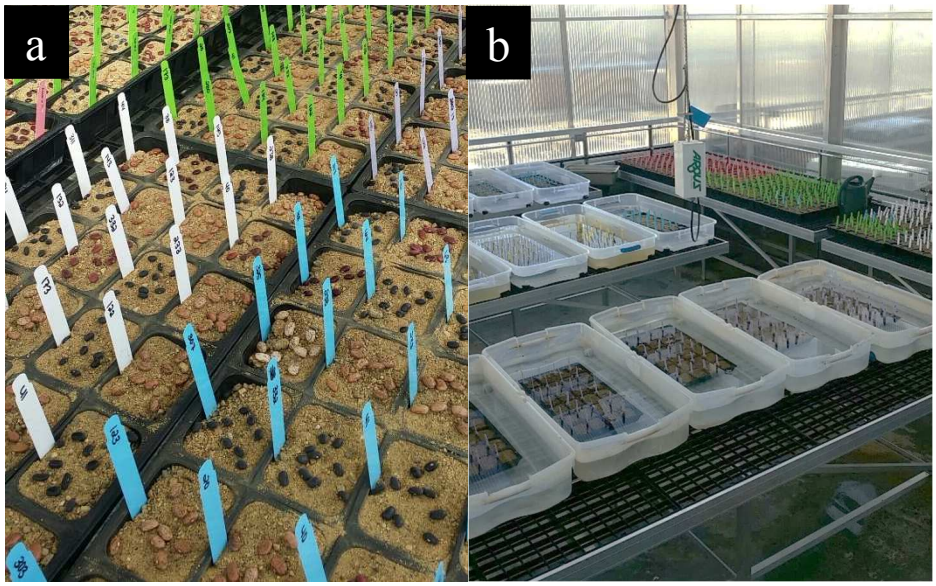

Figure 2.1. Protocol followed for pre-germination flooding experiments. A) Ten seeds per experimental unit planted into play sand. B) Pre-germination flooding procedure, trays submerged 3cm above sand.

Immediately after planting, the flooded main plots were subjected to their respective treatment by completely submerging the trays under water (3 cm above sand surface) (Figure 2.1b). The trays were taken out of the flooding treatment and allowed to drain until the sand reached field capacity, then they were watered to field capacity for the remainder of the experiment. For the non-flooded treatment, the trays were watered to field capacity for the

entirety of the experiment. The greenhouse was maintained within the range of 19℃ to 26℃ with an average of 22.5℃ during the day and 19.7℃ during the night.

Data collection and Statistical Analyses. The following variables were measured: germination rate (GR; percentage of germinated seeds out of the ten total seeds planted per well), root weight (RW; g), shoot weight (SW; g), and total weight (TW; g). For both the unpigmented and pigmented genotype studies, the plants were analyzed for GR 14 d after planting. The plants were harvested and dried for 48 hrs after germination rates were recorded so dry weights could be analyzed. Dry weights were analyzed as a single value for each well regardless of the number of plants that germinated in that well. This approach was taken since many plants were grown in a very small area and therefore competition would have played a large role in the results if they were to be reported as an average for each well. Although competition most likely was present in this experiment, growing the plants in larger pots would have been unfeasible due to the magnitude of the study and the limited greenhouse space.

Least square (LS) means and analysis of variance (ANOVA) on GR, RW, SW, and TW were conducted to determine differences among genotypes. The ANOVAs were completed as a RCBD using PROC MIXED procedure in SAS® software version 9.4 with replications as random effects and genotypes and treatments as fixed (SAS Institute, Cary, NC). Mean separation was performed in SAS (9.4) at α=0.05 level of significance for traits shown to be significant in ANOVAs.

Greenhouse Screening for Pre-Germination Flooding Tolerance

Plant Material. A Middle-American Diversity Panel (MDP) (Moghaddam et al., 2016) was screened for pre-germination flooding tolerance. The MDP was separated into pigmented seed genotypes (n=187) and unpigmented seed genotypes (n=82). The subset of pigmented seed genotypes consists of 46 black, 23 pink, 32 small red, and 86 pinto genotypes. For the subset of

unpigmented seed genotypes, there are 46 navy and 36 great northern genotypes. 'Royalty' was used as a tolerant check in both pigmented and unpigmented trials.

Experimental Design. The procedures for planting and data collection were the same for this study as they were for the preliminary experiment. The genotypes were arranged in a RCBD with split-plot arrangement. The main plots were the treatments (non-flooded and flooded) and the sub-plots were the genotypes. Plant height (PH; cm) was also measured in the greenhouse screening whereas it was not measured in either of the preliminary studies. The PH measurement was added since visible differences were observed between treatments in the preliminary study. Based on the results from the preliminary study, the pigmented genotypes were subjected to 4 days of pre-germination flooding stress and the unpigmented genotypes were placed under 3 hours of pre-germination flooding stress.

Data Collection and Statistical Analyses. Germination rates were recorded 14 days after planting for both the pigmented and unpigmented seed coat genotype experiments and plants were harvested and dried using the same methods previously mentioned. LS-means and ANOVAs on GR, RW, SW, TW, and PH were conducted to determine differences among genotypes. The ANOVAs were completed as a RCBD using PROC MIXED procedure in SAS software version 9.4 (SAS Institute, Cary, NC). Replications were considered random and treatments and genotypes were considered fixed effects. Mean separation was performed in SAS (9.4) at α=0.05 level of significance for traits shown to be significant in ANOVAs. Frequency distribution graphs (beanplots) were produced using the *beanplot* package in R (Kampstra, 2008). For all traits analyzed, a flooding index (Equation 1) was calculated for each genotype. For each trait, flooding indices were calculated for all genotypes analyzed. The flooding index is calculated using equation 1.

Association Mapping

GWAS was performed using the GAPIT package in R (Lipka et al., 2012). There was a total of 211,765 SNP markers that were obtained from genotype-by-sequencing in the NDSU dry bean genomics lab (Moghaddam et al., 2016). After filtering for MAF <5%, 125,745 SNP markers remained that were used in this analysis. Analyses were run for each trait in both flooded and non-flooded conditions for both the unpigmented and pigmented seed coat genotype sets by separate. In addition, a pooled GWAS analysis between pigmented and unpigmented seed coat genotypes was performed for each trait only in the non-flooded condition. The pooled GWAS could be performed for the non-flooded condition since the conditions were similar in both the unpigmented and pigmented seed coat experiments. The goal of the combined GWAS was to determine genomic regions associated with dry bean germination under non-flooded (normal) conditions. Since both pigmented and unpigmented experiment were run in different conditions, standardization of data was done in order to be able to compare between the different experiments. Therefore, a Z-score (Clark-Carter, 2005) was calculated for each genotype and the values for the unpigmented and pigmented sets were combined for the non-flooded condition only. To calculate the Z-score, the sample value was subtracted from the population mean and that value is divided by the standard deviation.

Four models (Naïve, EMMA, PC, and EMMA+PC) were tested for each trait analyzed. PC controls for population structure, EMMA accounts for kinship, EMMA+PC controls for both population structure and kinship, and Naïve does not account for kinship nor population structure. Principle component analysis (PCA) was used to evaluate population structure and based on the matrix, four principle components were used to account for ~40% of the variation. For each trait, the best model was selected based on which one had the lowest mean square deviation (MSD, Mamidi et al., 2011). Manhattan plots were generated using the mhplot()

function from the gap R package (Zhao, 2007). Significant SNPs ($P<0.01$) were selected from the best model.

Potential candidate genes were searched on the second version on the bean genome annotation (Schmutz et al., 2014) using a 100kb window around the most significant SNP. The annotation data is available at: https://legumeinfo.org/genomes/jbrowse/?data=phavu.G19833.gnm2. The phenotypic variation explained by the most significant markers was calculated using the likelihood-ratio-based R^2 (Sun et al., 2010) using the genABLE package in R (Aulchenko et al., 2007).

Results

A preliminary experiment was performed to determine the optimal length of pre-germination flooding for the unpigmented and pigmented seed coat genotypes. The results from the preliminary experiment were then used to develop the protocol for the greenhouse screening experiments. The results presented below are separated into two sections: the preliminary experiment results followed by the greenhouse screening results.

The aim of the preliminary experiment was to determine the threshold levels of pre-germination flooding for the unpigmented and pigmented seed coat genotypes. Germination rate was the trait used to determine the threshold levels since it was the main trait of interest. However, dry weights were also measured in the preliminary experiments to determine if there are significant genotype-by-treatment interactions and would therefore be a useful measurement in the greenhouse screening with a larger number of genotypes.

Preliminary Study

For the unpigmented seed coat genotypes, significant treatment effects were detected for GR (Table A2). The GR for the non-flooded treatment was significantly higher than that of all flooding treatments (Figure 2.2). For the non-flooded treatment, GR ranged from 79-93% with a

mean of 88% which was significantly greater than all other treatment means. The treatment means for GR were 18%, 14%, 15%, and 15% for the 3, 6, 12, and 24 h treatments, respectively. None of the flooding treatment means were significantly different from one another (Figure 2.2). From these results, 3 hours of pre-germination flooding was determined a suitable threshold for the unpigmented seed coat genotypes to be screened at.

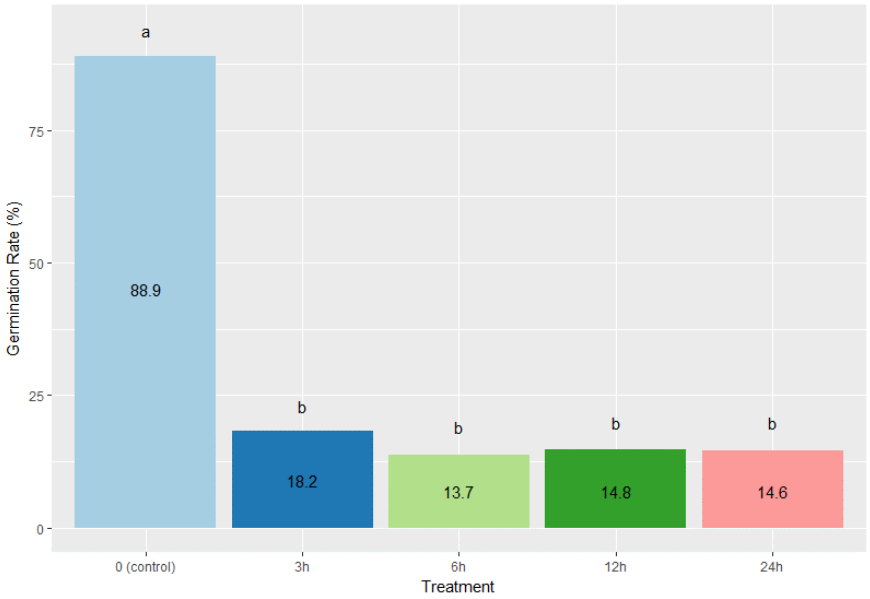

Figure 2.2. Germination rate averages for all treatment levels in unpigmented genotype preliminary study (n=12).

For the pigmented seed coat genotypes, treatment was shown to be significant for GR (Table A3). The GR for the non-flooded treatment ranged from 73-96% with a mean of 82% which was significantly greater than all other treatment means. The flooded treatment means were 35%, 38%, 39%, and 37% for the 4, 5, 6, and 7d treatments, respectively which were not statistically different (Figure 2.3). For the pigmented seed coat genotypes, 4 days was determined as the threshold for the greenhouse screening.

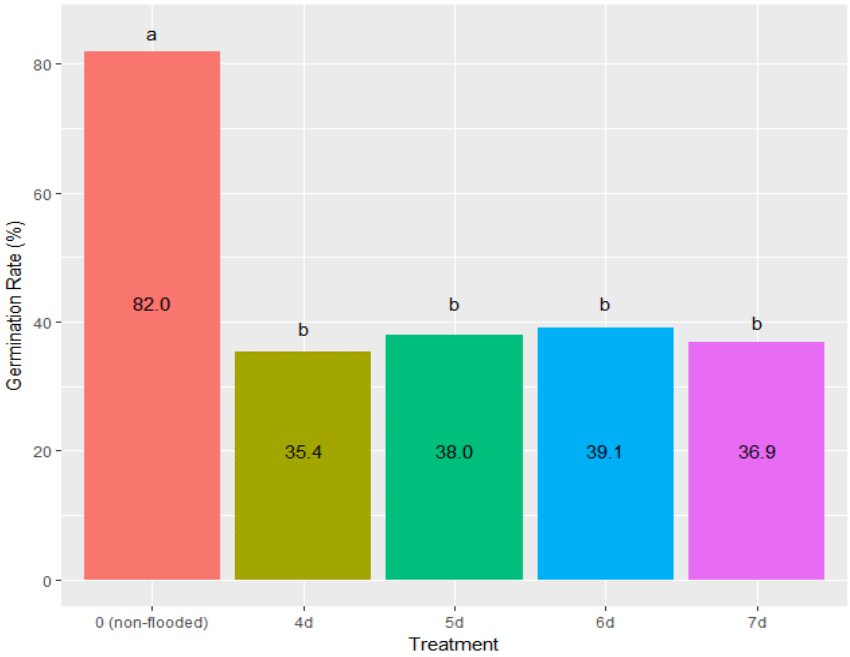

Figure 2.3. Germination rate averages for all treatment levels in pigmented seed genotype preliminary study.

Unpigmented Seed Coat Genotypes Greenhouse Screening

A total of 83 unpigmented seed coat genotypes were screened in the greenhouse for pre-germination flooding tolerance after 3 hours of flooding. The same traits that were analyzed in the preliminary study were analyzed for this experiment plus plant height (PH; cm) was added for this experiment due to differences that were observed during the preliminary experiment.

Significant differences between treatments ($P<0.01$) were found for germination rate (GR) in this study (Table 2.2). For the non-flooded treatment, GR ranged from 23-100% with a mean of 89% whereas for the flooded treatment, GR ranged from 0-93% with a mean of 14% (Table 2.3; Figure 2.4). Genotype-by-treatment interactions were also significant ($P<0.01$) for GR (Table 2.2). All genotypes, except for Matterhorn (23%), had a GR >50% in the non-flooded condition (Figure 2.4). On the other hand, only Verano (63%) and Royalty (93%) had GRs >50% in the flooded treatment (Table 2.4).

Table 2.2. Mean squares for germination rate of unpigmented seed coat genotypes in greenhouse screening.

Source of Variation	df	MS
Replication	2	1.28
Treatment	1	6865**
Error (a)	2	15.10
Genotype	82	5.84**
Genotype x Treatment	82	5.76**
Error (b)	321	1.50

*,**Significant at 0.05 and 0.01 levels, respectively

Royalty and Verano were the only two genotypes where the GR in the flooded condition was not significantly lower than that in the non-flooded condition (Table 2.4). Of the top 20% of genotypes for GR in the unpigmented seed coat study, 14 were from the great northern market class whereas only 3 were from the navy market class (Table 2.4). Furthermore, of the 83 genotypes analyzed, 21 of them (25%) had a GR of 0% in the flooded treatment (Table A4). All the genotypes with a GR of 0 belonged to the navy market class and accounted for 47% of all navy genotypes analyzed.

Table 2.3. Treatment means for germination rate (GR) total weight (TW), shoot weight (SW), and plant height (PH) for unpigmented seed coat genotypes in greenhouse screening.

	Mean ± sd				
Treatment	GR	TW	SW	RW	PH
	-------%-------	----------g----------			-----cm-----
Non-Flooded	89.1 ± 11.2 a	1.77 ± 0.51 a	1.41 ± 0.65a	0.486 ± 0.19 a	15.46 ± 3.01 a
Flooded	13.8 ± 16.3 b	1.00 ± 0.55 b	0.57 ± 0.37 b	0.479 ± 0.22 a	10.35 ± 2.24 b

Means in a column followed by the same letter are not significantly different ($\alpha<0.05$).

Table 2.4. Germination rates in non-flooded and flooded conditions of top and bottom 20% of genotypes† from unpigmented seed coat genotype study.

	Germination Rate (%)		
Genotype	Flooded	Non-Flooded	Market Class
---------- Top 20% ----------			
Royalty	93 a	100 a	Cream
Verano	63 def	60 efg	Navy
UI 59	50 fgh	83 abcd	Great Northern
Belneb RR 1	43 ghi	87 abc	Great Northern
Morales	37 hij	93 abc	Navy
AC resolute	33 hijk	83 abcd	Great Northern
Sawtooth	33 hijk	93 abc	Great Northern
Starlight	33 hijk	85 abcd	Great Northern
Emerson	33 hijk	93 abc	Great Northern
NE1 09 20	33 hijk	93 abc	Great Northern
Hyden	33 hijk	97 ab	Navy
BelMineb RR 2	30 ijkl	87 abc	Great Northern
Belmineb 2	30 ijkl	93 abc	Great Northern
NE1 09 13	30 ijkl	97 ab	Great Northern
UI 123	30 ijkl	90 abc	Great Northern
Belneb 2	27 ijklm	90 abc	Great Northern
Beryl	27 ijklm	87 abc	Great Northern
Sapphire	27 ijklm	90 abc	Great Northern
---------- Bottom 20% ----------			
USWA 50	0 o	100 a	Navy
Crestwood	0 o	100 a	Navy
Envoy	0 o	100 a	Navy
N05324	0 o	100 a	Navy
Michelite	0 o	97 ab	Navy
Mchale	0 o	97 ab	Navy
Newport	0 o	97 ab	Navy
Voyager	0 o	93 abc	Navy
Seabiskit	0 o	93 abc	Navy
OAC gryphon	0 o	93 abc	Navy
Albion	0 o	93 abc	Navy
Arthur	0 o	90 abc	Navy
Lightning	0 o	90 abc	Navy
Navigator	0 o	87 abc	Navy
NW 395	0 o	87 abc	Navy
Reliant	0 o	87 abc	Navy
Belmineb RMR 7	0 o	80 abcde	Navy
Avanti	0 o	77 bcde	Navy

† Top and bottom percentages based on germination rates in flooded conditions.
Means followed by same letter are not significantly different ($\alpha<0.05$) across column or row.

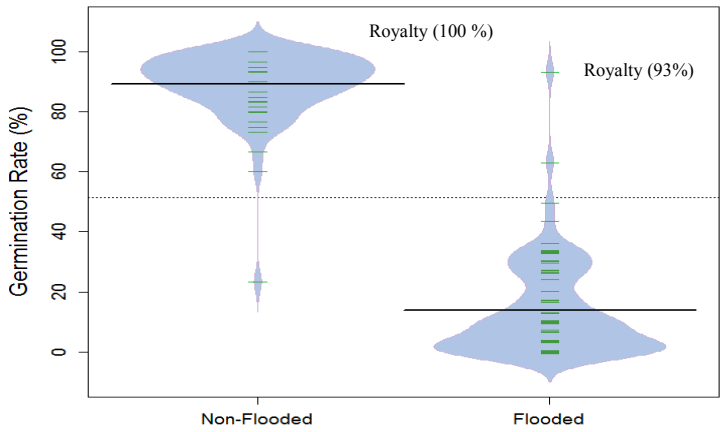

Figure 2.4. Distribution of germination rates for non-flooded and flooded treatments in unpigmented seed greenhouse screening. Green lines depict individual observations, the blue area shows the distribution, the black solid lines indicate treatment means, and the black dashed line indicates the trait mean.

The treatment effects were also significant (P<0.05) for TW, SW, and PH (Table 2.5). The means for TW, SW, and PH were all significantly higher in the non-flooded treatment compared to the flooded treatment (Table 2.3; Figure 2.5). Genotype-by-treatment interactions were significant (P<0.05) for SW, and RW (Table 2.5). Under flooded conditions, Royalty had the highest SW and RW of all genotypes analyzed. Furthermore, Verano was also in the top 10 genotypes for both these traits (Table A4).

Table 2.5. Mean squares for total weight (TW), shoot weight (SW), root weight (RW), and plant height (PH) for unpigmented seed coat genotypes in greenhouse screening.

Source of Variation	df	TW	SW	RW	PH
Replication	2	0.27	0.36*	0.064	9.02
Treatment	1	46.74*	69.90*	0.765	2122**
Error (a)	2	1.95	0.76	0.066	26.06
Genotype	82	0.79**	0.90**	0.122**	23.87**
Genotype x Treatment	59	0.59	0.23**	0.074**	8.72
Error (b)	223	0.05	0.11	0.032	6.72

*,**Significant at 0.05 and 0.01 levels, respectively

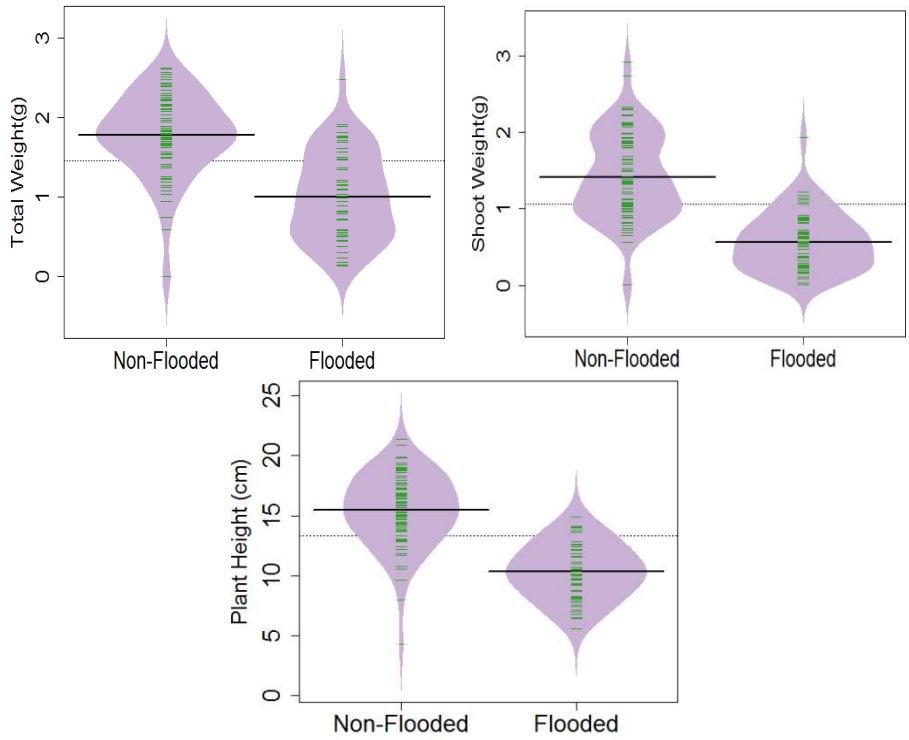

Figure 2.5. Distributions of total weight, shoot weight, and plant height for non-flooded and flooded treatments in unpigmented seed coat greenhouse screening. Green lines depict individual observations, the blue area shows the distribution, the black solid lines indicate treatment means, and the black dashed line indicates the mean across both treatments.

Flooding indices were calculated for each genotype. The flooding index indicates the severity of the flooding effect for each genotype. Results showed that the navy market class was more affected by the flooding stress for every trait in comparison to the great northern market class (Figure 2.6). Verano (0.05) and Royalty (-0.06) had the highest flooding indices for GR (Table 2.6). Several varieties in the top 10% of genotypes analyzed had positive flooding indices indicating the value for the trait in the flooded condition was higher than the value in the non flooded condition for that genotype. This trend was primarily observed for RW (Table 2.6).

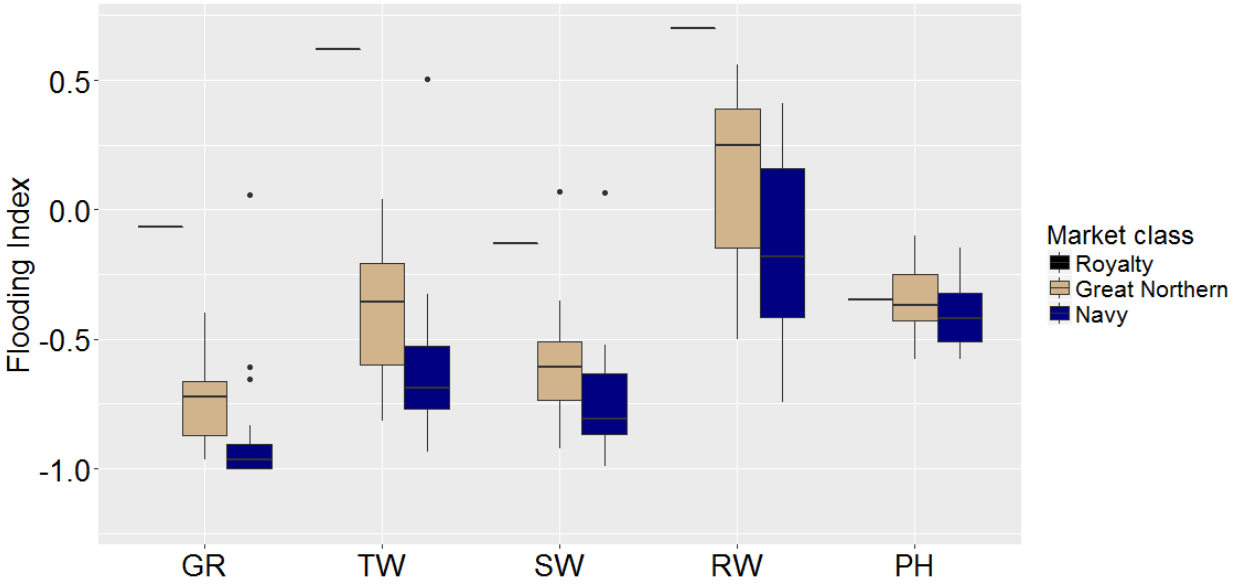

Figure 2.6. Flooding indices of great northern and navy genotypes analyzed in greenhouse screening for germination rate (GR), shoot weight (SW), total weight (TW), root weight (RW), and plant height (PH).

Table 2.6. Flooding indices† for germination rate (GR), total weight (TW), shoot weight (SW), root weight (RW), and plant height (PH) for top and bottom 10% of genotypes‡ in unpigmented greenhouse screening.

Genotype	GR	TW	SW	RW	PH	Market Class
---------- Top 10% ----------						
Verano	0.05	0.50	-0.65	0.86	-0.14	Navy
Royalty	-0.06	0.62	-0.62	0.16	-0.34	Cream
UI 59	-0.39	0.04	-0.81	0.17	-0.21	Great Northern
Belneb RR 1	-0.50	-0.13	-0.99	-0.25	-0.25	Great Northern
Matterhorn	-0.57	-0.52	-0.98	0.70	1.95	Great Northern
AC Resolute	-0.60	-0.15	-0.80	0.30	-0.19	Great Northern
Morales	-0.60	-0.37	-0.78	-0.16	-0.52	Navy
Starlight	-0.60	0.98	-0.71	0.86	-0.31	Great Northern
Sawtooth	-0.64	-0.12	-0.86	-0.33	-0.20	Great Northern
---------- Bottom 10% ----------						
Avalanche	-0.95	. §	-0.64	.	.	Navy
OAC Laser	-0.96	-0.73	-0.92	-0.05	-0.51	Navy
C 20	-0.96	-0.91	-0.99	-0.76	-0.45	Navy
NE1 09 22	-0.96	-0.61	-0.79	-0.20	-0.45	Great Northern
Huron	-0.96	-0.93	-0.99	-0.86	.	Navy
HY 4181	-0.96	-0.60	-0.94	-0.57	-0.32	Navy
T9903	-0.96	-0.74	-0.93	-0.53	-0.49	Navy
Laker	-0.96	-0.69	-0.59	-0.60	-0.39	Navy
Belmineb 1	-0.96	-0.62	.	-0.19	-0.57	Great Northern

† Flooding indices are calculated by subtracting the trait value in the control condition from the trait value in the flooded condition and then dividing that value by the trait value in the control condition.
‡ Top and bottom percentages based on germination rates in flooded conditions; genotypes with 0% germination in flooded condition were excluded.
§ Missing data point.

Pigmented Seed Coat Genotypes Greenhouse Screening

A total of 190 MDP genotypes were analyzed in this experiment. Of the 190 genotypes, 188 had pigmented seed coats (black, small red, pinto, and pink market classes), Royalty (cream) was included as a tolerant check, and Verano (navy) was also included due to its high tolerance in the unpigmented seed coat genotype study. The same traits that were analyzed in the unpigmented seed coat genotype experiment were also analyzed in this study.

Table 2.7. Mean squares for germination rate of pigmented seed coat genotypes in greenhouse screening.

Source of Variation	df	Mean Square
Replication	2	0.21
Treatment	1	11637*
Error (a)	2	14.59
Genotype	189	8.39**
Genotype x Treatment	186	5.45**
Error (b)	321	1.50

*,**Significant at 0.05 and 0.01 levels, respectively

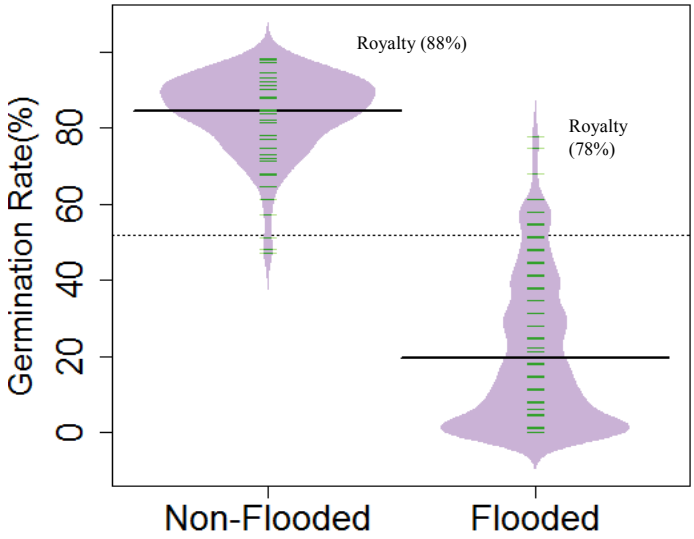

Figure 2.7. Distribution of germination rates for non-flooded and flooded treatments in pigmented seed coat genotypes screening. Green lines depict individual observations, the blue area shows the distribution, the black solid lines depict the treatment mean, and the black dashed lines depict the trait mean.

Significant differences (P<0.05) between treatments were found for germination rate (GR) in this study (Table 2.7). For the non-flooded treatment, GR ranged from 47-100% with a mean of 85% whereas for the flooded treatment, GR ranged from 0-78% with a mean of 20% (Figure 2.7). Genotype and genotype-by-treatment effects were also significant (P<0.01) (Table 2.7). Indeterminate Jamaican Red (68%), Rojo Chiquito (61.3%), DOR364 (61.3%), TARS09 RR004 (58%), Durango (58%), Inta Precoz (58%), Blackjack (58%), Xan 176 (58%), and A55

(58%) had the highest GRs under the flooded condition and were not significantly different from Royalty, which had the highest GR (78%) in the flooded condition (Table 2.8).

Furthermore, Royalty, Midnight, Indeterminate Jamaican Red, and Durango all had GR values in the flooded condition that were not significantly lower than the values for that genotype in the non-flooded condition (Table 2.8). Of the top 10% of genotypes in the flooded condition for GR, eight were from the black market class, six from the small red market class, and two from both pinto and pink market classes (Table 2.8). There were 29 genotypes (15.3%) with a GR of 0% in the flooded condition (Table A5).

Table 2.8. Germination rates of top and bottom 10% of genotypes† from pigmented seed coat genotype study.

	Germination Rate (%)		
Genotype	Flooded	Non-Flooded	Market Class
---------- Top 10% ----------			
Royalty	78 abcde	88 abc	Cream
Midnight	75 bcdef	88 abc	Black
Indeterminate Jamaican Red	68 bcdefghi	88 abc	Red mottled
Rojo chiquito	61 defghij	91 ab	Small red
DOR 364	61 defghij	98 ab	Small red
TARS09 RR004	58 efghi	88 abc	Small red
Durango	58 efghi	81 abcde	Pinto
INTA Precoz	58 efghi	95 ab	Small red
Blackjack	58 efghi	85 abc	Black
Xan 176	58 efghi	91 ab	Black
A55	58 efghi	93 ab	Black
AC Early Rose	55 fghijk	85 abc	Pink
Sonora	55 fghijk	95 ab	Pinto
PR 0443 151	55 fghijk	98 ab	Black
Black hawk	55 fghijk	98 ab	Black
AC Black Diamond	51 ghijkl	85 abc	Black
CDC nighthawk	51 ghijkl	85 abc	Black
PR 0401-259	48 hijklm	91 ab	Pink
---------- Bottom 10% ----------			
A801	0 t	77 abcdef	Carioca
92bg 7	0 t	78 ebdac	Black
AC Scarlet	0 t	78 ebdac	Small red
I9365 31	0 t	81 ebdac	Black
UI 906	0 t	85 bac	Black
Loreto	0 t	85 bac	Black
S08418	0 t	85 abc	Pink
Harold	0 t	85 abc	Pink
Victor	0 t	88 abc	Pink
USPT-WM-1	0 t	88 abc	Pinto
IBC 301 204	0 t	91 ba	Small red
PR-0340-3-3-1	0 t	91 ba	Small red
CENTA pupil	0 t	91 ab	Small red
Big bend	0 t	91 ab	Small red
SDIP-1	0 t	93 ab	Pinto
Amadeus 77	0 t	95 ab	Small red
Othello	0 t	95 ab	Pinto
UI 239	0 t	98 ab	Small red
UCD 9634	0 t	98 ab	Pink

† Top and bottom percentages based on germination rates in flooded conditions.
Means followed by same letter are not significantly different ($\alpha<0.05$) across column or row.

The treatment significantly (P<0.01) affected TW, SW, RW, and PH for pigmented seed coat genotypes (Table 2.9). For all traits, treatment means were significantly higher for the non-flooded treatment compared to the flooded (Figure 2.8; Table 2.10). Furthermore, the genotype-by-treatment interactions were significant (P<0.05) for all traits (Table 2.9).

Table 2.9. Mean squares for total weight (TW), shoot weight (SW), root weight (RW), and plant height (PH) for pigmented seed coat genotypes in greenhouse screening.

Source of Variation	df	TW	SW	RW	PH
Replication	2	0.373	0.098	0.110	24*
Treatment	1	573**	365**	24.850**	9932**
Error (a)	2	0.740	0.391	0.053	67
Genotype	189	0.667**	0.317**	0.101**	14.2**
Genotype x Treatment	150	0.576**	0.257*	0.095**	8.97**
Error (b)	552	0.210	0.109	0.044	6.33

*,**Significant at 0.05 and 0.01 levels, respectively

Table 2.10. Treatment means for total weight (TW), shoot weight (SW), root weight (RW), and plant height (PH) for pigmented seed coat genotypes in greenhouse screening.

Treatment	Mean + sd				
	GR	TW	SW	RW	PH
	--------%--------	----------g----------			-------cm-------
Non-Flooded	84.5 ± 9.5 a	2.30 ± 0.51 a	1.69 ± 0.27 a	0.61 ± 0.32 a	17.63 ± 2.20 a
Flooded	19.4 ± 19.1 b	0.57 ± 0.34 b	0.43 ± 0.20 b	0.26 ± 0.17 b	9.26 ± 2.80 b

Means in a column followed by the same letter are not significantly different (α<0.05).

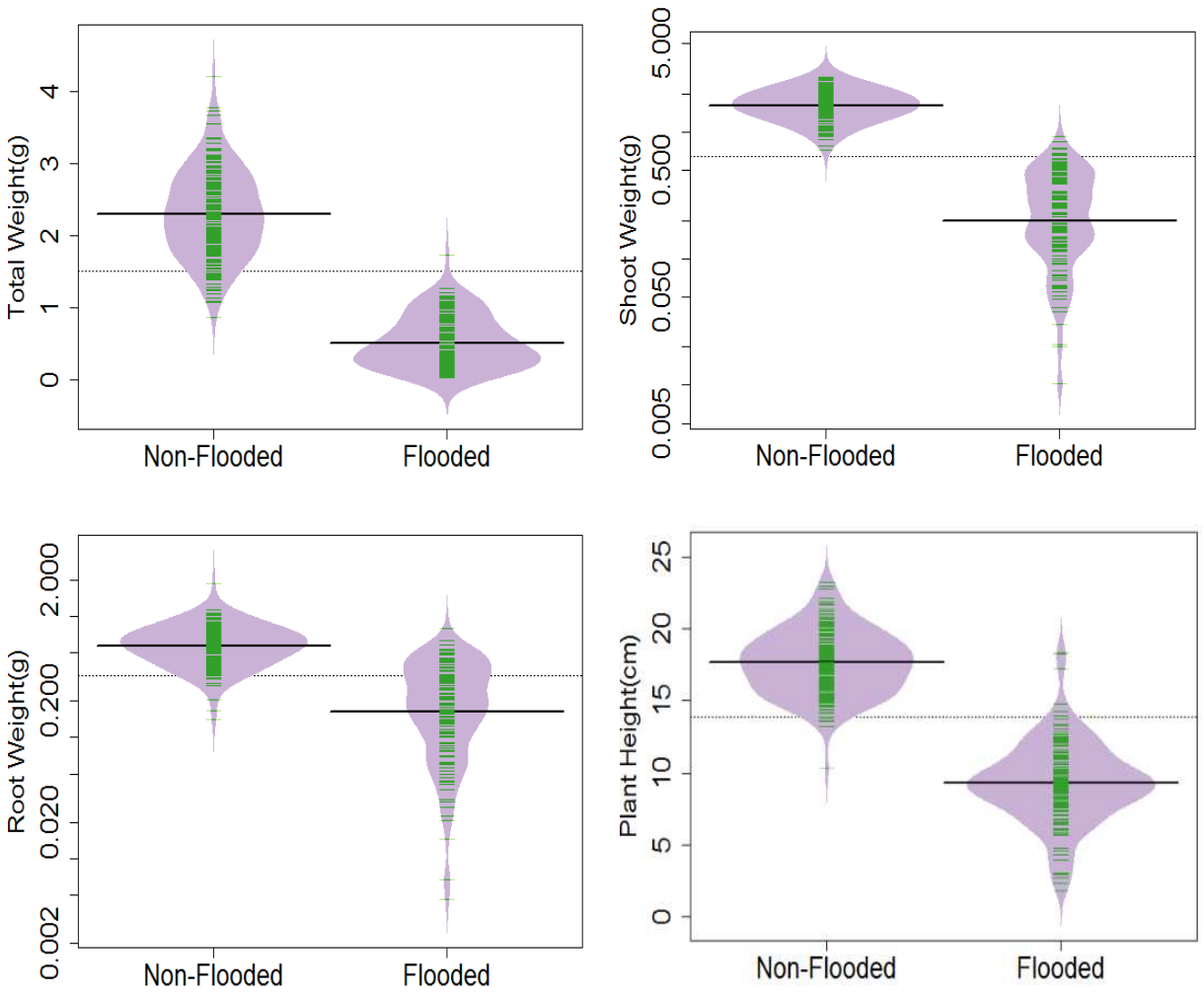

Figure 2.8. Distributions of total weight, shoot weight, root weight, and plant height for treatments in pigmented seed coat genotypes screening. Green lines depict individual observations, the blue area shows the distribution, the black solid lines depict the treatment mean, and the black dashed lines depict the mean across both treatments.

Flooding indices were calculated for each genotype in the pigmented seed coat greenhouse screening. Results show that market classes respond similarily for all traits analyzed (Figure 2.9). With the exception of RW for Eclipse and PH for B05055, all flooding indices were negative for the top and bottom 10% of genotypes in the pigmented seed coat study (Table 2.11). Eclipse had a RW of 0.17g in the non-flooded condition and 0.41g in the flooded condition and B05055 had a PH of 14.5cm in the non-flooded condition and 17.2cm in the flooded condition

leading to positive flooding indices. Royalty, Indeterminate Jamaica Red, Rojo Chiquito, CDC Nighthawk, and Inta Precoz were all in the top 10% of genotypes analyzed in this study (Table 2.11). These genotypes were also chosen as five of the most tolerant pigmented genotypes from Soltani et al. (2017) and were included in the preliminary experiment.

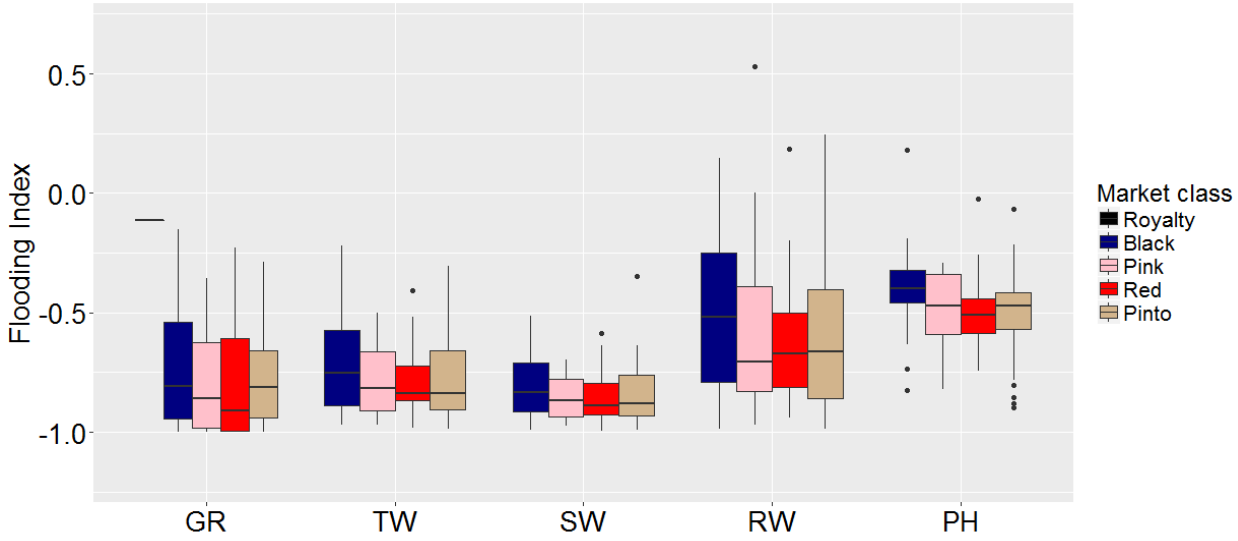

Figure 2.9. Flooding indices of black, pink, red, and pinto genotypes analyzed in greenhouse screening for germination rate (GR), root weight (RW), shoot weight (SW), total weight (TW), and plant height (PH).

Abnormal morphology was observed within the pigmented seed coat genotype study whereas it was not noted in the unpigmented seed coat genotype experiment. The most prominent abnormalities observed were deformed primary leaves, hypogeal germination, and seedlings without primary leaves (Figure A2). Common bean usually exhibits epigeal germination, however, hypogeal germination was noted several times in this experiment. Out of the 119 genotypes that had germination values after 4 days of flooding, 19 (16%) displayed 100% epigeal germination, 95 (80%) displayed between 6 and 89% hypogeal germination, and 5 (4%) displayed 100% hypogeal germination.

Table 2.11. Flooding indices† for germination rate (GR), total weight (TW), shoot weight (SW), root weight (RW), and plant height (PH) for top and bottom 10% of genotypes‡ in pigmented greenhouse screening.

Genotype	GR	TW	RW	SW	PH	Market Class
---------- Top 10% ----------						
Royalty	-0.11	.	.	.	.	Cream
Midnight	-0.15	-0.47	-0.12	-0.93	-0.28	Black
Indeterminate Jamaica Red	-0.23	.	.	.	.	Small red
Durango	-0.29	-0.65	-0.42	-1.66	-0.43	Pinto
Blackjack	-0.32	-0.43	0.05	-0.76	-0.33	Black
Rojo Chiquito	-0.33	-0.52	-0.20	-1.03	-0.45	Small red
TARS09 RR004	-0.34	-0.55	-0.38	-0.90	-0.43	Small red
AC Early Rose	-0.35	-0.62	-0.39	-1.30	-0.59	Pink
Xan 176	-0.37	-0.44	0.05	-0.86	-0.43	Black
DOR 364	-0.37	-0.41	0.19	-0.86	-0.51	Small red
A55	-0.38	-0.48	-0.13	-0.93	-0.38	Black
TARS09 RR007	-0.39	.	.	.	.	Small red
INTA Precoz	-0.39	-0.70	-0.57	-1.24	-0.41	Small red
AC Black Diamond	-0.39	-0.52	-0.22	-0.83	-0.43	Black
CDC Nighthawk	-0.39	-0.48	-0.28	-0.65	-0.22	Black
Eclipse	-0.42	-0.22	1.47	-0.50	-0.37	Black
Sonora	-0.42	-0.59	-0.29	-1.57	-0.51	Pinto
Chase	-0.44	-0.65	-0.38	-1.10	-0.37	Pinto
---------- Bottom 10% ----------						
B05055	-0.98	-0.81	-0.53	-0.72	0.18	Black
Raven	-0.98	-0.93	-0.87	-1.07	-0.63	Black
Gloria	-0.98	-0.97	-0.97	-1.34	-0.82	Pink
Max	-0.98	-0.98	-0.99	-1.63	-0.77	Pinto
Jaguar	-0.98	-0.86	-0.38	-1.11	-0.83	Black
Desert Rose	-0.98	-0.91	-0.84	-1.97	-0.34	Pink
UI 37	-0.98	-0.98	-0.94	-1.63	-0.74	Small red
Quincy	-0.98	-0.96	-0.93	-2.24	-0.80	Pinto
6R 42	-0.98	-0.82	-0.71	-1.64	-0.34	Pink
F04-2801-4-6-6	-0.99	-0.97	-0.99	-1.30	-0.57	Black
Phantom	-0.99	.	.	.	.	Black
Nodak	-0.99	-0.99	-0.98	-1.97	-0.78	Pinto
NW 590	-0.99	-0.97	-0.97	-1.54	-0.47	Pinto
Fiesta	-0.99	-0.97	-0.96	-1.89	-0.90	Pinto
F07-449-9-3	-0.99	-0.86	-0.80	-1.51	-0.02	Small red
JM 126	-0.99	-0.96	-0.96	-1.99	-0.85	Pinto
Orca	-0.99	-0.96	-0.92	-1.49	.	Black
Merlot	-0.99	-0.95	-0.86	-2.55	.	Small red

† Flooding indices are calculated by subtracting the trait value in the control condition from the trait value in the flooded condition and then dividing that value by the trait value in the control condition.

‡Top and bottom percentages based on germination rates in flooded conditions; genotypes with 0% germination in flooded condition were excluded.

Association Mapping

GWAS was performed for all five traits in both the flooded and non-flooded treatments for both the unpigmented and pigmented seed coat genotype sets. For the non-flooded treatment, a combined GWAS between pigmented and unpigmented seed coat groups (entire MDP) was analyzed. A total of 23 and 17 significant genomic regions were identified across all traits for the non-flooded and flooded treatments, respectively. For the combined analysis, a total of 12 significant regions were identified across all traits. The results are presented for each trait below.

Germination Rate. Under non-flooded conditions, three significant regions were associated with GR for the unpigmented seed coat genotypes and two regions for the pigmented seed coat genotypes (Table 2.12; Figure 2.10). The region on Pv08/61Mb was identified for both the unpigmented and pigmented seed coat genotypes; however, it was not identified in the combined analysis. In the pigmented seed coat genotype analysis, this peak (Pv08/61.4Mb) explained 7% of the variation and lies within *Phvul.008G275300* which encodes a WRKY family transcription factor. WRKY family transcription factors are involved in several plant functions including hormone signaling which is important for normal germination (Bakshi and Oelmuller, 2014). Additionally, a region on Pv10 was associated with GR under non-flooded conditions for the unpigmented seed coat genotypes as well as for the entire MDP (combined analysis). Under flooded conditions, one and four significant regions were associated with GR for unpigmented and pigmented seed coat genotypes, respectively. For the unpigmented seed coat genotypes, the significant region was identified near Pv06/25.3Mb. This peak explained 11% of the variation and is 2.7Kb upstream *Phvul.006G148300* which is predicted to encode a peroxisomal membrane protein (PMP22) which are thought to be involved in the metabolism of ROS.

Table 2.12. Significant loci (P<0.01) associated with germination rate under each condition. R^2 values are represented for each significant locus as well as combined for each trait and condition using the most significant 0.01% of markers.

Condition	Seed Coat	Chr.	Position	-log10 (p-value)	R^2
Non-Flooded	Unpigmented	Pv08	61,878,220	3.7	8.49
		Pv10	42,083,778	3.4	7.63
		Pv11	236,163	3.5	4.67
					16.45†
	Pigmented	Pv06	27,539,930	3.7	8.61
		Pv08	61,427,849	3.5	6.80
					33.09†
	Combined	Pv06	1,822,850	4.2	0.97
		Pv06	27,408,071	4.4	0.51
		Pv08	49,541,597	3.6	0.10
		Pv10	42,320,475	4.2	0.10
					1.05†
Flooded	Unpigmented	Pv06	25,339,288	5.0	10.81
					27.82†
	Pigmented	Pv03	22,309,648	3.9	6.60
		Pv03	45,759,683	4.5	5.90
		Pv04	16,936,146	4.5	7.13
		Pv04	45,806,133	4.0	5.78
					1.41†

† Combined R^2 value. Calculated from most significant 0.01% of markers.

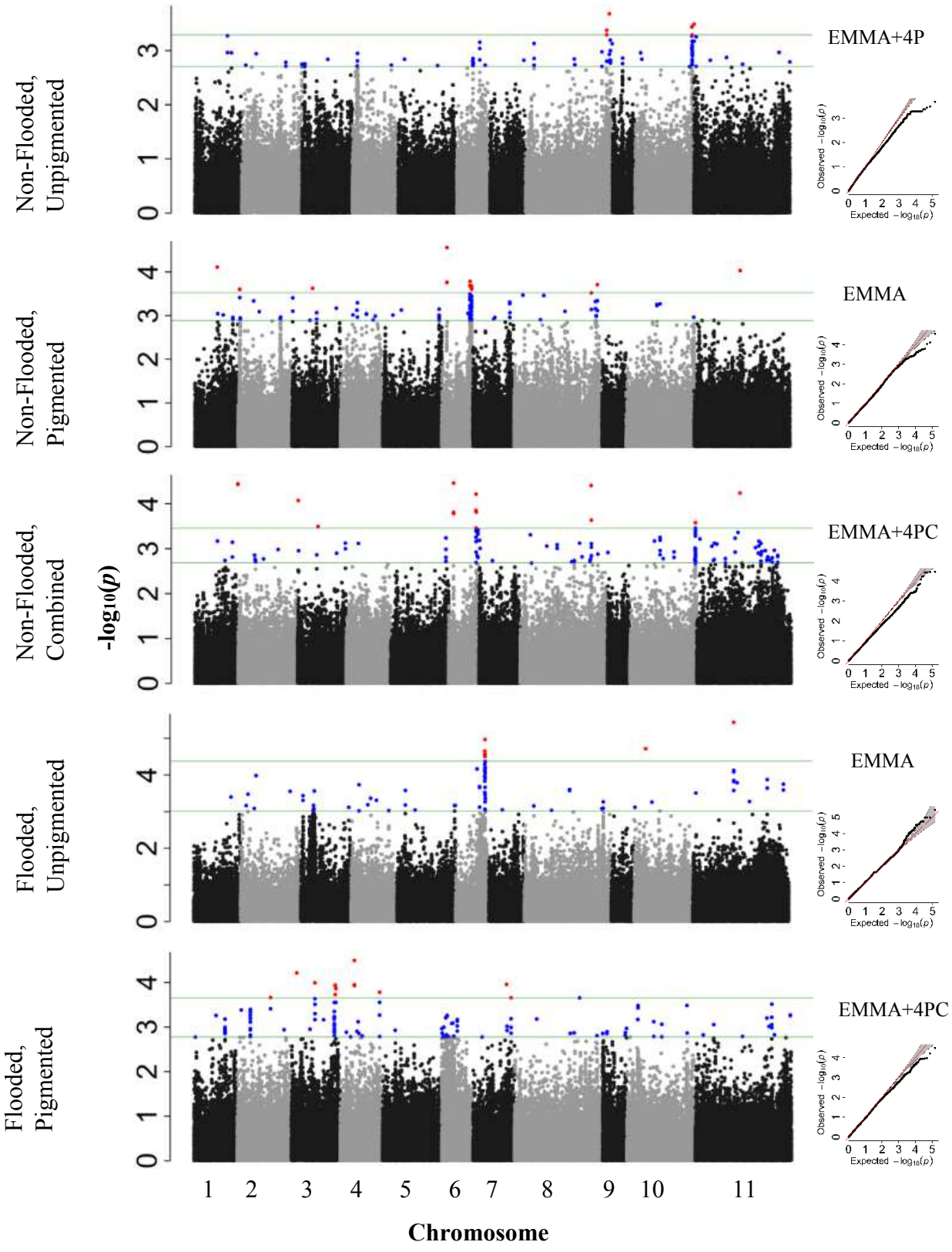

Figure 2.10. Manhattan and quantile-quantile plots for germination rate in all conditions analyzed.

Total Weight. Under non-flooded conditions, one significant region (Pv08/62.4Mb) was associated with TW for the unpigmented seed coat genotypes and two (Pv05/38.7Mb and Pv11/4.61Kb) for the pigmented seed coat genotypes (Table 2.13; Figure 2.11). The region on Pv05 identified for the pigmented seed coat genotypes was also associated with TW in the combined analysis. Furthermore, in the combined analysis, a significant peak that explained 39% of the variation was identified on Pv11/4.6Kb. Under flooded conditions, two significant peaks were identified in the unpigmented seed coat genotype analysis and one in the pigmented seed coat genotype analysis.

Table 2.13. Significant loci (P<0.01) associated with total weight under each condition. R^2 values are represented for each significant locus as well as combined for each trait and condition using the most significant 0.01% of markers.

Condition	Seed Coat	Chr.	Position	-log10 (p-value)	R^2
Non-Flooded	Unpigmented	Pv08	62,470,470	3.7	19.5
					-†
	Pigmented	Pv05	38,738,519	4.6	37.91
		Pv11	4,617,534	5.1	39.12
					-†
	Combined	Pv05	38,738,519	3.9	7.46
		Pv07	30,449,293	3.3	5.18
					35.12‡
Flooded	Unpigmented	Pv06	25,273,076	3.5	8.66
		Pv08	1,929,695	3.5	7.75
					13.99‡
	Pigmented	Pv02	29,860,923	3.9	8.56
					16.15‡

† Combined R^2 cannot be calculated for traits where Naïve model was selected.
‡ Combined R^2 value. Calculated from most significant 0.01% of markers.

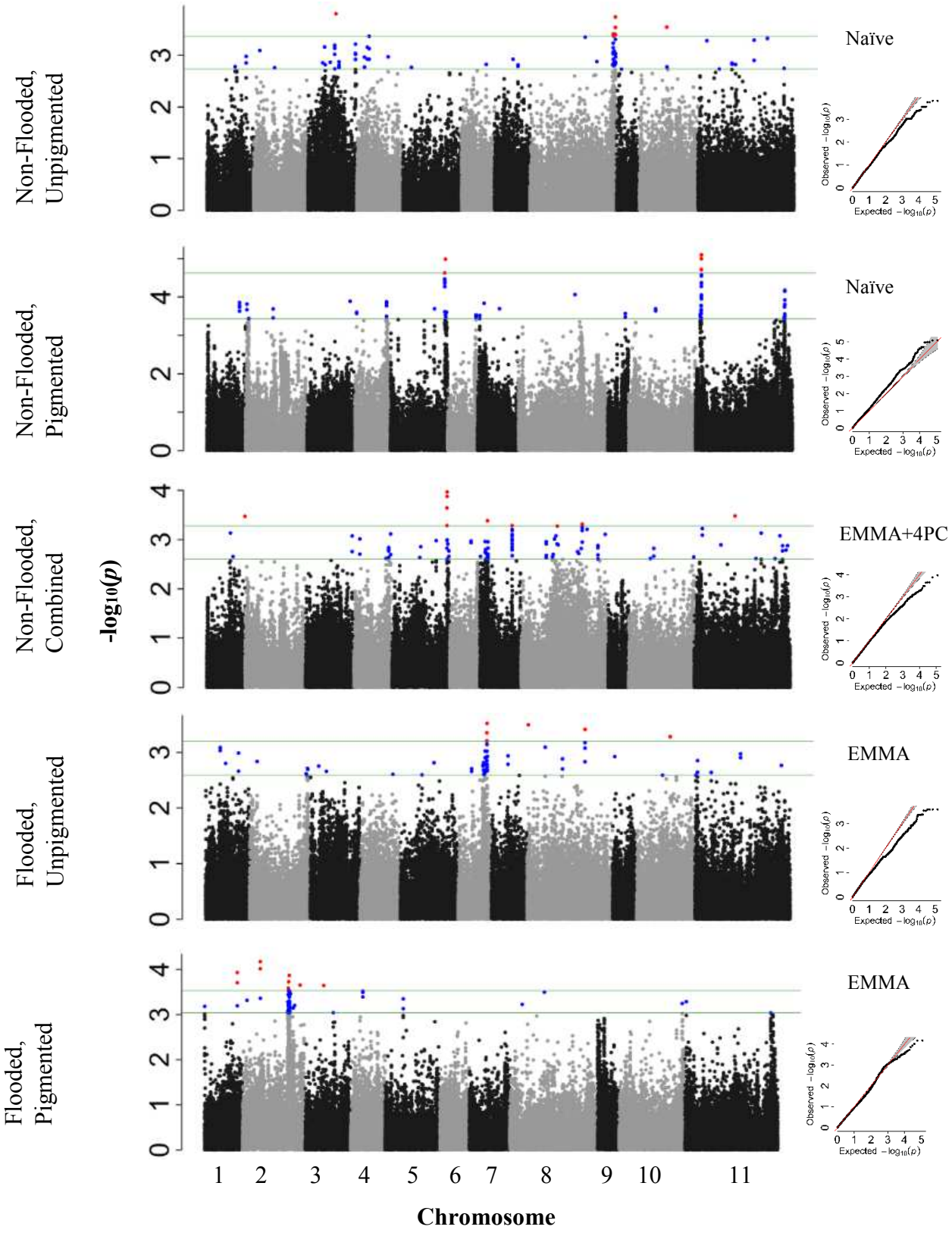

Figure 2.11. Manhattan and Q-Q plots for total weight in all conditions analyzed.

Shoot Weight. Three significant regions were associated with SW for the unpigmented seed coat genotypes and three for the pigmented (Table 2.14; Figure 2.12). A region at the proximal end of Pv11 was identified in both the unpigmented (Pv11/6.74Mb) and pigmented (Pv11/1.67Mb) seed coat genotype analyses. Furthermore, a region on Pv08/26.4Mb was associated with SW in the combined analysis but was not identified for either the unpigmented or pigmented seed coat genotypes. There were three regions associated with shoot weight under flooded conditions, two were identified for the unpigmented seed coat genotypes and one for the pigmented seed coat genotypes.

Table 2.14. Significant loci (P<0.01) associated with shoot weight under each condition. R^2 values are represented for each significant locus as well as combined for each trait and condition using the most significant 0.01% of markers.

Condition	Seed Coat	Chr.	Position	-log10 (p-value)	R^2
Non-Flooded	Unpigmented	Pv02	29,646,465	3.7	7.49
		Pv10	44,068,757	4.0	8.21
		Pv11	6,749,815	4.1	9.66
					25.94†
	Pigmented	Pv03	12,307,279	4.0	25.48
		Pv07	6,315,951	3.9	25.11
		Pv11	1,673,010	3.9	24.99
					-‡
	Combined	Pv08	26,413,869	4.4	8.03
					16.15†
Flooded	Unpigmented	Pv08	41,382,139	3.3	8.14
		Pv09	25,363,499	4.2	6.96
					21.71†
	Pigmented	Pv09	9,264,676	3.9	7.00
					21.12†

† Combined R^2 value. Calculated from most significant 0.01% of markers.

‡Combined R^2 cannot be calculated for traits where Naïve model was selected.

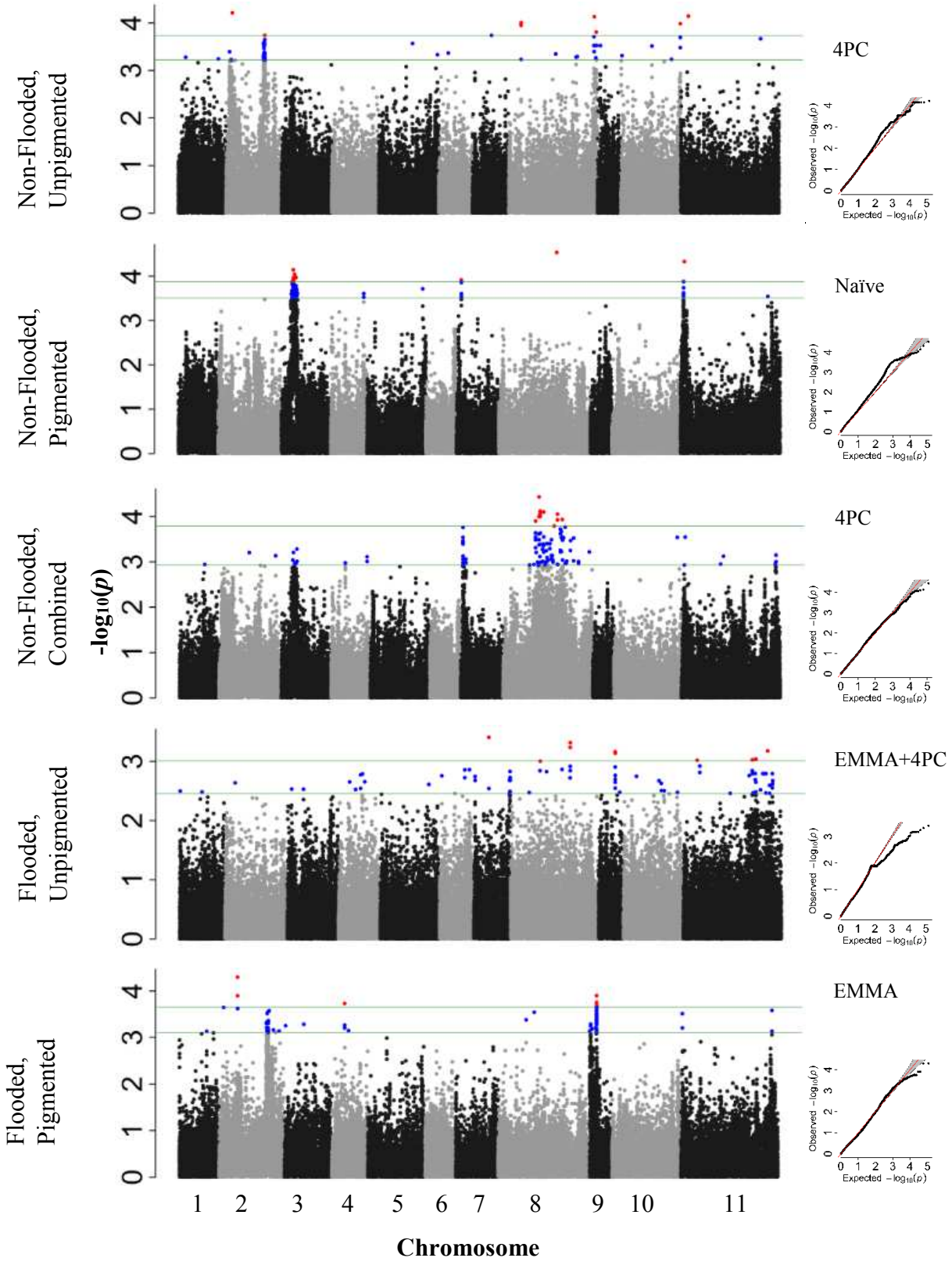

Figure 2.12. Manhattan and Q-Q plots for shoot weight in all conditions analyzed.

Root Weight. Two genomic regions were associated with root weight under non-flooded conditions and two for flooded conditions for both pigmented and unpigmented seed coat genotypes (Table 2.15; Figure 2.13). Neither of these regions were in the same locations for unpigmented and pigmented seed coat genotypes. However, a region on Pv05/39.4Mb was identified under non-flooded conditions for the pigmented seed coat genotypes and was also identified in the combined analysis. Under flooded conditions, a region was identified on Pv01/26.5Mb for unpigmented seed coat genotypes and a region on Pv02/30.4Mb for pigmented seed coat genotypes.

Table 2.15. Significant loci (P<0.01) associated with root weight under each condition. R^2 values are represented for each significant locus as well as combined for each trait and condition using the most significant 0.01% of markers.

Condition	Seed Coat	Chr.	Position	-log10 (p-value)	R^2
Non-Flooded	Unpigmented	Pv02	41,796,655	3.5	6.93
		Pv08	2,422,957	4.3	9.36
					12.64†
	Pigmented	Pv05	39,421,292	4.0	7.74
		Pv11	4,617,654	4.3	11.38
					28.34†
	Combined	Pv04	44,645,363	3.8	8.97
		Pv05	39,421,292	4.0	6.16
		Pv07	30,449,293	3.8	6.49
					33.74†
Flooded	Unpigmented	Pv01	26,451,197	3.4	6.83
					23.21†
	Pigmented	Pv02	30,435,031	5.3	19.2
					-‡

† Combined R^2 value. Calculated from most significant 0.01% of markers.
‡ R^2 cannot be calculated for traits where Naïve model was selected.

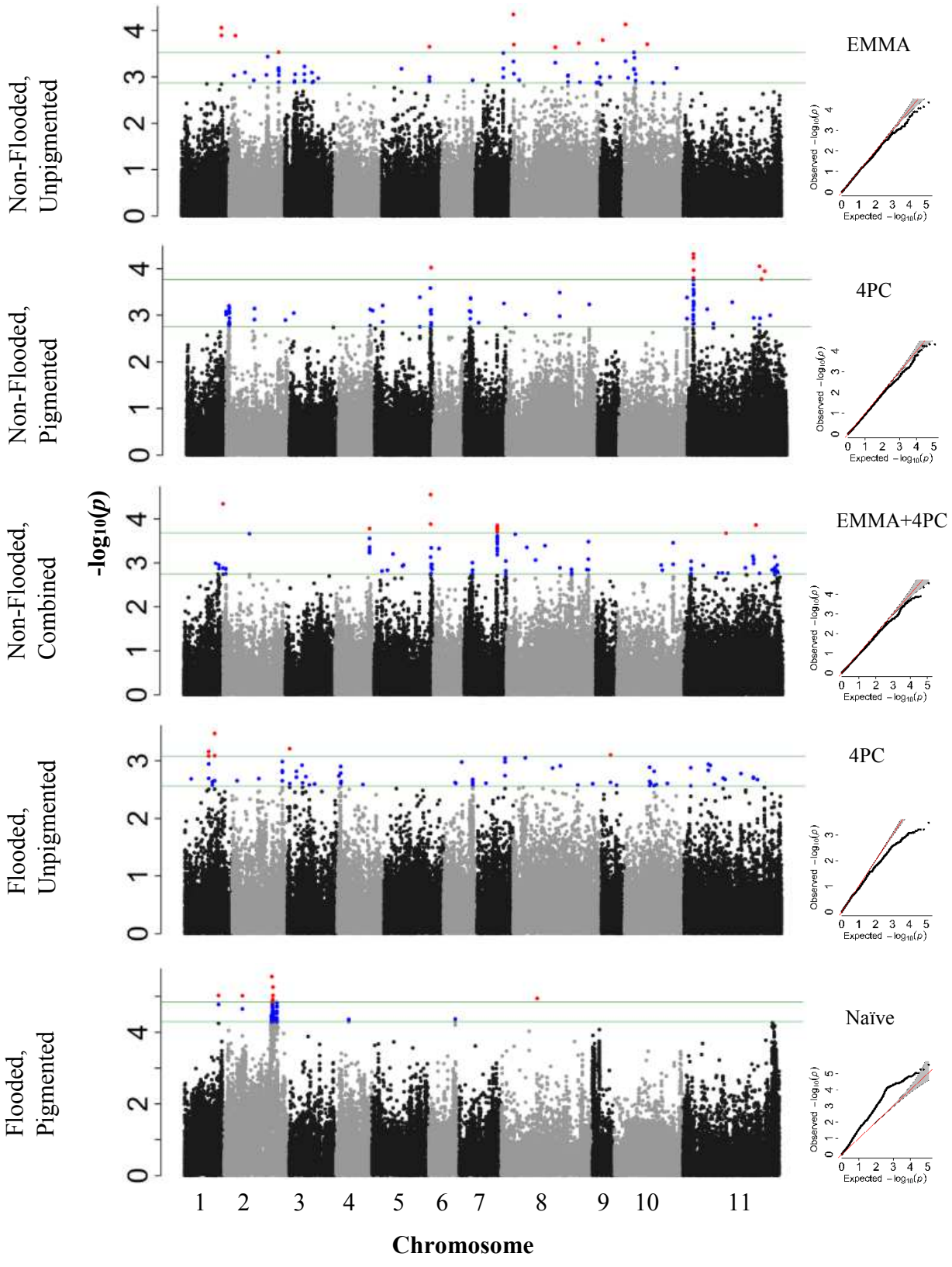

Figure 2.13. Manhattan and Q-Q plots for root weight in all conditions analyzed.

Plant Height. For PH, three significant regions were identified for the unpigmented seed coat genotypes under non-flooded conditions and two regions were identified for pigmented seed coat genotypes (Table 2.16; Figure 2.14). Additionally, two other significant regions were associated with plant height for the combined analysis. A total of four significant regions were associated with plant height under flooded conditions, two were identified for the unpigmented seed coat genotypes and two different regions were detected for the pigmented seed coat genotypes. Of these four peaks identified, three were located on Pv04 but all in different locations.

Table 2.16. Significant loci (P<0.01) associated with plant height under each condition. R^2 values are represented for each significant locus as well as combined for each trait and condition using the most significant 0.01% of markers.

Condition	Seed Coat	Chr.	Position	-log10 (p-value)	R^2
Non-Flooded	Unpigmented	Pv02	13,073,088	3.2	6.20
		Pv09	35,882,614	3.5	6.17
		Pv10	27,208,394	3.4	5.52
					31.2†
	Pigmented	Pv05	2,031,224	3.6	6.09
		Pv07	7,003,126	3.7	6.56
					5.32†
	Combined	Pv01	24,481,298	4.3	7.86
		Pv02	39,493,566	4.0	6.51
					40.22†
Flooded	Unpigmented	Pv04	9,841,853	3.7	1.21
		Pv07	1,160,911	3.7	0.51
					7.78†
	Pigmented	Pv04	28,048,289	4.1	9.80
		Pv04	39,129,024	4.7	12.90
					5.90†

† Combined R^2 value. Calculated from most significant 0.01% of markers.

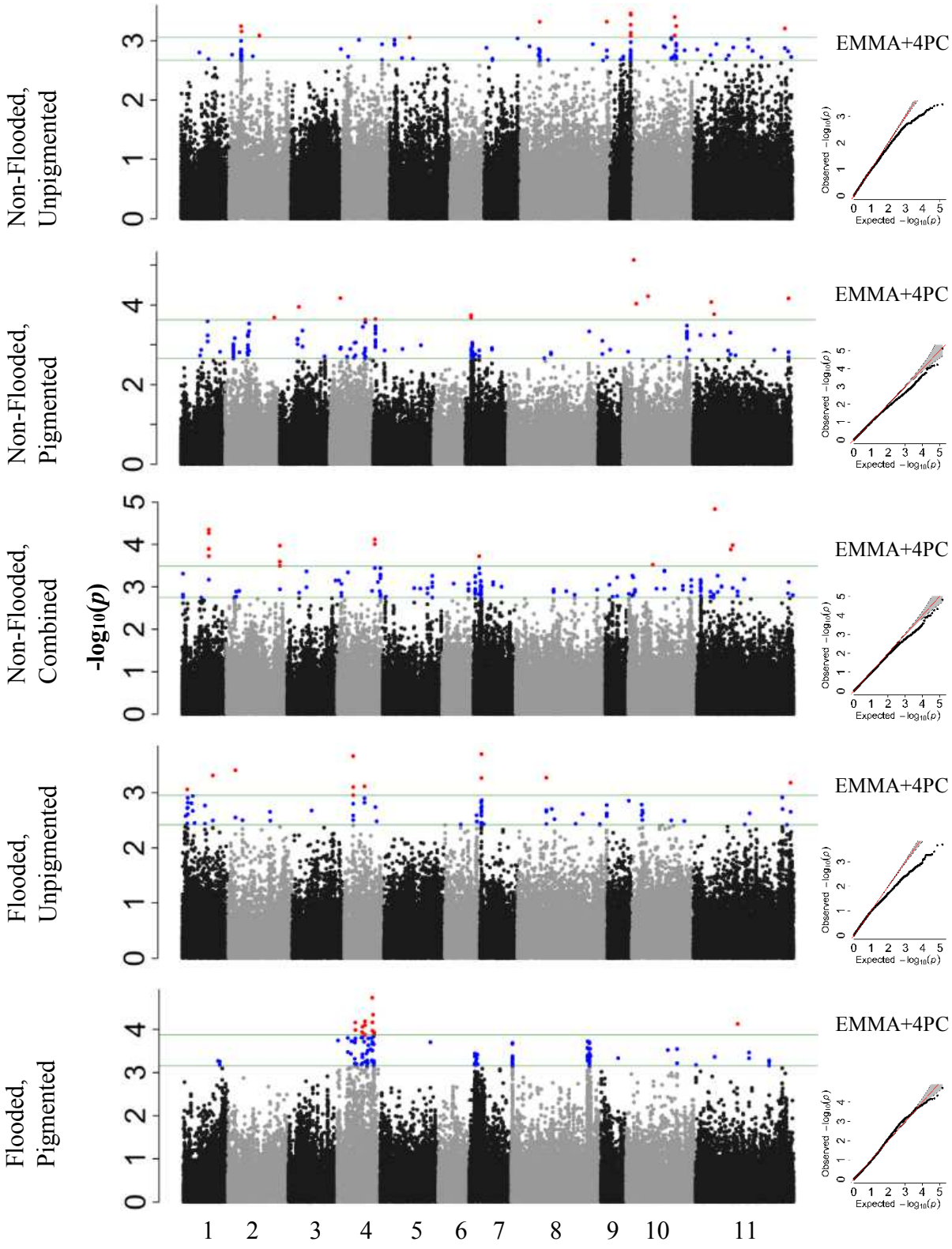

Figure 2.14. Manhattan and Q-Q plots for plant height in all conditions analyzed.

Discussion

Flooding is a major crop production issue worldwide and common bean is a very susceptible species to flooding, especially at germination stages. In a recent study focusing on pre-germination flooding in dry bean, Soltani et al. (2017) concluded dry bean genotypes with a pigmented seed coat are more tolerant than those with a white/unpigmented seed coat. To better understand why the pigmented seed coat genotypes are more tolerant than the unpigmented, this study further analyzed the same genotypes Soltani et al. (2017) examined. However, in contrast to the previous study, this study analyzed pigmented and unpigmented seed coat genotypes separately, under different pre-germination flooding pressures to get a better idea of the tolerance within each group. By separating the panel into groups of unpigmented and pigmented seed coat genotypes, more variation within each group and market class could be identified.

The preliminary study indicated three hours and four days are the threshold levels for the unpigmented and pigmented seed coat groups, respectively. For both the unpigmented and pigmented seed coat genotypes, any amount of flooding resulted in a significant decrease in GR from the non-flooded treatment yet none of the treatment levels were significantly different from one another (Figure 2.2 and 2.3). These results are consistent with a study performed with soybean where Wuebker et al. (2001) concluded that any amount of flooding significantly lowered germination percentage. The pigmented seed coat genotypes were flooded for ≥4 days and required ~3 additional days for the trays to drain to field conditions; therefore, the duration of flooding significantly reduced the GR in all flooded treatments compared to the non-flooded treatment. For the unpigmented seed coat genotypes, although the genotypes were not subjected to long durations of flooding, the trays still required ~3d to reduce water content in the sand to normal levels (field capacity), which could be the cause of significantly lower GRs even after 3h of pre-germination flooding stress.

Both the navy and great northern market classes consist of genotypes with unpigmented seed coats and are generally considered sensitive to pre-germination flooding. However, in this study, genotypes from the navy market class were more sensitive to the flooding stress than those of the great northern market class (Figure 2.6). These results are consistent with the results presented in Soltani et al. (2017) and could indicate seed size may play an important role in pre-germination flooding tolerance in dry bean since navy genotypes usually have a 100 seed weight of ~22 g and great northern of ~38 g. The navy market class belongs to race Mesoamerica whereas the great northern market class belongs to race Durango; however, Soltani et al. (2017) reported the Durango and Mesoamerican races were not significantly different in terms of pre-germination flooding tolerance.

In spite of the navy bean genotypes being the most susceptible market class, Verano (navy) was the most tolerant genotype among the unpigmented genotypes evaluated, having a significantly higher GR under flooded conditions than all other unpigmented seed coat genotypes analyzed. DOR 364 (small red) was one of the more tolerant genotypes among the pigmented genotypes and DOR 364 (Rosas et al., 2004) is in Verano's pedigree (Beaver et al., 2008), suggesting that favorable alleles for pre-germination flooding could have been passed from DOR 364 to Verano. Furthermore, WBB-20-1 is a white-seeded breeding line also used in Verano's pedigree. WBB-20-1 is resistant to common bacterial blight and derived from a cross with *P. coccineus* (Zapata et al., 2004). Velasquez et al. (2017) reported *P. coccineus* wild accessions to be the most tolerant group among all tested at seedling stages for waterlogging. In this study, Velasquez et al. (2017) screened *P. vulgaris, P. coccineus*, *P. acutifolius*, and *P. dumosus* for six traits under waterlogging stress at seedling stages. Of the top 12 accessions for each trait, 95% were *P. coccineus* genotypes.

Abnormal morphology was observed for many pigmented seed coat genotypes under flooded conditions (Figure A2). The current study only analyzed early growth stages under pre-germination flooding stress. Although the plants did germinate, the abnormalities noted might affect later growth stages of the plants. More research studying latter growth stages and seed yield after pre-germination flooding must be conducted to confirm this hypothesis.

Based on GWAS results, pre-germination tolerance is a very complex trait controlled by multiple loci in dry bean. For GR in the unpigmented seed coat genotypes under flooded conditions, a significant region Pv06 was identified which is 2.7Kb upstream a gene model (*Phvul.006G148300*) predicted to encode for a peroxisomal membrane protein (PMP22). Reactive oxygen species production is increased under oxidative stress that occurs during flooding. Peroxisomal membrane proteins are suggested to be involved in enzymatic antioxidant defense systems (Murphy et al., 2003); therefore, tolerant navy and great northern genotypes might have increased ROS scavenging systems compared to their sensitive counterparts. In order to confirm this hypothesis, enzymatic activity of enzymes related to ROS (SOD, CAT,GPx) could be analyzed in tolerant genotypes and compared to that of sensitive genotypes.

Under non-flooding conditions, a significant peak was identified for GR on Pv08 in the unpigmented seed coat genotype analysis. This region is within *Phvul.008G275300* which is suggested to code for a WRKY family transcription factor. WRKY family transcription factors are one of the largest families of plant transcriptional regulators (Bakshi and Oelmuller, 2014) and are known to be involved in many plant processes, including germination (Rushton et al., 2011). Germination is regulated by two key hormones: abscisic acid (ABA) and gibberellic acid (GA). Germination is initiated by GA and ABA represses it and is responsible for maintaining

seed dormancy. WRKY transcription factors are known to act as ABA-inducible repressors of seed germination in many flowering plants (reviewed in Rushton et al., 2011).

Soltani et al. (2017) performed GWAS on the entire MDP panel for both pre-germination flooding and non-flooded conditions and identified four and five peaks, respectively. None of the genomic regions Soltani et al. (2017) found to be associated with pre-germination flooding tolerance were also identified in this study which could be due to the differing durations of flooding between the current (4 days and 3 hours for pigmented and unpigmented seed coat genotypes, respectively) and previous study (24 hours). However, Soltani et al. (2017) also analyzed dry weights and found a significant region on Pv11/1.5Mb for RW, SW, and TW under non-flooded condition. In the current study, a region from Pv11/1.6Mb to Pv11/4.6Mb was associated with RW, SW, and TW for pigmented genotypes under non-flooded conditions. Soltani et al. (2017) analyzed dry weights for plants at the seedling stage whereas the plants were analyzed at germination stages in the current study indicating a major QTL for root and shoot growth at both germination and seedling stages could be located at the proximal end of Pv11.

With the rise in flooding occurrences worldwide, it is essential to produce cultivars adapted to flooding stress. Pre-germination flooding tolerance was analyzed for Middle-American dry bean genotypes in this study. Tolerant genotypes and genomic regions associated with the tolerance were identified. Pre-germination tolerant pigmented genotypes were identified by Soltani et al. (2017); however, this is the first report of pre-germination tolerant genotypes with an unpigmented seed coat. The tolerant varieties identified (such as Royalty, Verano, Indeterminate Jamaica Red, Durango, and Midnight) could be utilized in dry bean breeding programs as parents to pass favorable alleles for pre-germination flooding tolerance to develop more varieties adapted to this stress. Several significant loci were identified related to pre-

germination flooding tolerance and with further research, these regions can be confirmed, markers for pre-germination flooding tolerance can be developed and utilized for MAS, and genes can be identified which could play a key role in this tolerance.

References

Atteia, A., R. van Lis, A.G.M. Tielens, and W.F. Martin. 2013. Anaerobic energy metabolism in unicellular photosynthetic eukaryotes. Biochim. Biophys. Acta - Bioenerg. 1827: 210–223.

Aulchenko, Y.S., S. Ripke, A. Isaacs, and C.M. van Duijn. 2007. GenABEL: an R library for genome-wide association analysis. *Bioinformatics* 23: 1294-1296.

Bailey-Serres, J., T. Fukao, D.J. Gibbs, M.J. Holdsworth, S.C. Lee, F. Licausi, P. Perata, L.A.C.J. Voesenek, and J.T. Van Dongen. 2011. Making sense of low oxygen sensing. Trends Plant Sci. 17: 129–138.

Bailey-Serres, J., S.C. Lee, and E. Brinton. 2012. Waterproofing crops: effective flooding survival strategies. Plant Physiol. 160: 1698–709.

Bakshi, M. and R. Oelmuller. 2014. WRKY transcription factors: Jack of many trades in plants. Plant Signal Behav. 9:1-18.

Beaver, J.S., T.G. Porch, and M. Zapata. 2008. Registration of 'Verano' white bean. J. Plant Reg. 2:187-189.

Bewley, J.D. and M. Black. 1978. Physiology and biochemistry of seeds in relation to germination. Vol. 2. Viability, dormancy, and environmental control. Springer-Verlag, New York.

Borisjuk, L., and H. Rolletschek. 2009. The oxygen status of the developing seed. New Phytol. 182: 17–30.

Catalanotti, C., W. Yang, M.C. Posewitz, and A.R. Grossman. 2013. Fermentation metabolism and its evolution in algae. Frotiers in Plant Sci. 4:150.

Cardwell, V.B. 1984. Seed germination and crop production. p. 53–92. *In* Tesar, M.B. (ed.), Physiological basis of crop growth and development . ASA-CSSA. Madison, WI.

Chugh, V., N. Kaur, and A.K. Gupta. 2011. Role of antioxidant and anaerobic metabolism enzymes in providing tolerance to maize (*Zea mays* L.) seedlings against waterlogging. Indian J. Biochem. Biophys. 48: 346–352.

Clark-Carter D. 2005. Standard deviation. *In* Everitt B.S. and Howell D., (eds.) Encyclopedia of Statistics in Behavioral Science. 4. Hoboken, NJ: John Wiley and Sons, Ltd.

Crawford, R.M.M. 1977. Tolerance of anoxia and ethanol metabolism in germinating seeds. New Phytol. 79: 511–517.

Dubbern de Souza, F.H. and J. Marcos-Filho. 2001. The seed coat as a modulator of seed-environment relationships in Fabaceae. Rev. Bras. Botânica 24: 365–375.

FAO. 2015. Food and agriculture organization of the United Nations. FAOSTAT statistical database. [online]. Available at http://faostat.fao.org/site/609/default. (verified on 5 Jan 2018).

Gibbs, J., and H. Greenway. 2003. Review: Mechanisms of anoxia tolerance in plants. I. Growth, survival and anaerobic catabolism. Funct. Plant Biol. 30: 1.

Gill, S.S., and N. Tuteja. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48: 909–930.

Greenway, H., and J. Gibbs. 2003. Mechanisms of anoxia tolerance in plants. II. Energy requirements for maintenance and energy distribution to essential processes. Funct. Plant Biol. 30: 999–1036.

Hou, F. and F. Thseng. 1992. Studies on the Screening Technique for Pre-germination Flooding Tolerance in Soybean. Japanese J. Crop Sci. 61: 447–453.

Ismail, A.M., E.S. Ella, G. V Vergara, and D.J. Mackill. 2009. Mechanisms associated with tolerance to flooding during germination and early seedling growth in rice (*Oryza sativa*). Ann. Bot. 103: 197–209.

Ismail, A.M., D.E. Johnson, E.S. Ella, G.V. Vergara, and A.M. Baltazar. 2012. Adaptation to flooding during emergence and seedling growth in rice and weeds, and implications for crop establisment. AoB Plants. plso19. 1-18.

Kampstra, P. 2008. Beanplot: A boxplot alternative for visual comparison of distributions. J. Statistical Software. 28: 1-9.

Kannenberg, L.W., and R.W. Allard. 1964. An association between pigment and lignin formation in the seed coat of the lima bean. Crop Sci. 4: 621.

Knodel, J.J., P.B. Beauzay, G.J. Endres, D.W. Franzen, H.J. Kandel, S.G. Markell, J.M. Osorno, J.S. Pasche, and R.K. Zollinger. 2015. 2015 Dry Bean Grower Survey of Production, Pest Problems and Pesticide Use in Minnesota and North Dakota (E1802).

Knodel, J.J., P.B. Beauzay, G.J. Endres, D.W. Franzen, H.J. Kandel, S.G. Markell, J.M. Osorno, J.S. Pasche, and R.K. Zollinger. 2016. 2016 Dry Bean Grower Survey of Production, Pest Problems and Pesticide Use in Minnesota and North Dakota (E1841).

Knodel, J.J., P.B. Beauzay, D.W. Franzen, H.J. Kandel, S.G. Markell, J.M. Osorno, J.S. Pasche, and R.K. Zollinger. 2014. 2014 dry bean grower survey of production, pest problems, and pesticide use in Minnesota and North Dakota (E1750).

Lipka, A.E., F. Tian, Q. Wang, J. Peiffer, M. Li, P.J. Bradbury, M.A. Gore, E.S. Buckler, and Z. Zhang. 2012. GAPIT: genome association and prediction integrated tool. Bioinformatics 28: 2397–2399.

Mamidi, S., S. Chikara, R.J. Goos, D.L. Hyten, and D. Annam. 2011. Genome-Wide Association Analysis Identifies Candidate Genes Associated with Iron Deficiency Chlorosis in Soybean. Plant Genome 4: 154-164.

Miro, B., and A.M. Ismail. 2013. Tolerance of anaerobic conditions caused by flooding during germination and early growth in rice (*Oryza sativa* L.). Front. Plant Sci. 4: 1-18.

Moghaddam, S.M., S. Mamidi, J.M. Osorno, R. Lee, M. Brick, J. Kelly, P. Miklas, C. Urrea, Q. Song, P. Cregan, J. Grimwood, J. Schmutz, and P.E. McClean. 2016. Genome-wide association study identifies candidate loci underlying agronomic traits in a Middle American diversity panel of common bean. The Plant Genome 9:1-21.

Murphy, M.A., B.A Phillipson., A. Baker, R.T. Mullen. 2003. Characterization of the targeting signal of the Arabidopsis 22-kD integral peroxisomal membrane protein. Plant Phys. 133:813-828.

Nelson, R.B, D.W. Davis, J.P. Palta, and D.R. Laing. 1983. Measurment of soil waterlogging tolerance in *Phaseolus vulgaris* L.: a comparison of screening techniques. Sci. Hortic. 20:303-313.

Nonogaki, H., Nonogaki, and Hiro. 2008. Seed Germination and Reserve Mobilization. *In* Encyclopedia of Life Sciences. John Wiley & Sons, Ltd, Chichester.

Pociecha, E. 2012. Different physiological reactions at vegetatative and gernatative stage of development of field bean plants exposed to flooding and undergoing recovery. J. Agron. Crop Sci. 199:195-199.

Powell, A.A, M. Oliveira, and S. Matthews. 1986a. The role of imbibition damage in determining the vigour of white and coloured seed lots of dwarf french beans (*Phaseolus vulgaris*). J. of Exp. Botany. 37:716-722.

Powell, A.A., M. Oliveira, and S. Matthews. 1986b. Seed vigour of dwarf french beans (*Phaseolus vulgaris*) in relation to the color of the testa. J. Ag. Sci. 106: 419-425.

Powell, A.A. and S. Mathews. 1978. The damaging effect of water on dry pea embryos during imbibition. J. of Experimental Botany. 29: 1215-1229.

Rajashekar, C.B., and K.H. Baek. 2014. Hydrogen peroxide alleviates hypoxia during imbibition and germination of bean seeds. Am. J. Plant Sci. 5: 3572–3584.

Ransom, J. 2011a. "Impacts of Flooding/Waterlogging on Crop Development" Crop and pest report. 03 July 2013. 17 January 2018.

Ransom, J. 2011b. Flooding/waterlogging will affect crop development-Ag news from NDSU. Available at https://www.ag.ndsu.edu/news/newsrelease/2011/may-30-2011/flooding-waterloggin-will-affect-crop-development/ (verified 6 March 2018).

Rosas, J.C., J.S. Beaver, S.E. Beebe. 2004. Names of common bean varieties released in Central America and the Caribbean. Bean Improvement Cooperative. Annual Report. 47: 329-330.

Rushton, D.L., P. Tripathi, R.C. Rabara, J. Lin, P. Ringler, A.K. Boken, T.J. Langum, L. Smidt, D.D. Boomsma, N.J. Emme, X. Chen, J.J. Finer, Q.J. Shen, and P.J. Rushton. 2011. WRKY transcription factors: key components in abscisic acid signalling. Plant Biotch. J. 10:2-11.

SAS Institute Inc. 2011. Base SAS® 9.3 Procedures Guide. Cary, NC: SAS Institute Inc.

Schmutz, J., P.E. McClean, S. Mamidi, G.A. Wu, S.B. Cannon, J. Grimwood, J. Jenkins, S. Shu, Q. Song, C. Chavarro, M. Torres-Torres, V. Geffroy, S.M. Moghaddam, D. Gao, B. Abernathy, K. Barry, M. Blair, M.A. Brick, M. Chovatia, P. Gepts, D.M. Goodstein, M. Gonzales, U. Hellsten, D.L. Hyten, G. Jia, J.D. Kelly, D. Kudrna, R. Lee, M.M.S. Richard, P.N. Miklas, J.M. Osorno, J. Rodrigues, V. Thareau, C.A. Urrea, M. Wang, Y. Yu, M. Zhang, R.A. Wing, P.B. Cregan, D.S. Rokhsar, and S.A. Jackson. 2014. A reference genome for common bean and genome-wide analysis of dual domestications. Nat. Genet. 46: 707–713.

Sayama, T., T. Nakazaki, G. Ishikawa, K. Yagasaki, N. Yamada, N. Hirota, K. Hirata, T. Yoshikawa, H. Saito, M. Teraishi, Y. Okumoto, T. Tsukiyama, and T. Tanisaka. 2009. QTL analysis of seed-flooding tolerance in soybean (*Glycine max* [L.] Merr.). Plant Sci. 176: 514–521.

Setter, T.L., and I. Waters. 2003. Review of prospects for germplasm improvement for waterlogging tolerance in wheat, barley and oats. Plant Soil 253: 1–34.

Soltani, A., S. MafiMoghaddam, K. Walter, D. Restrepo-Montoya, S. Mamidi, S. Schroder, R. Lee, P.E. McClean, and J.M. Osorno. 2017. Genetic architecture of flooding tolerance in the dry bean middle-american diversity panel. Front. Plant Sci. 8: 1183:1-15.

Soltani, A., S. MafiMoghaddam, A. OladzadAbbasabadi, K. Walter, P.J. Kearns, J. Vasquez Guzman, S, Mamidi, R. Lee, A. Shade, J.L. Jacobs, M.I. Chilivers, D.B. Lowry, P.E. McClean, and J.M. Osorno. 2018. Toward developing waterproof dry beans: genetic architecture of flooding tolerance in the Andean diversity panel. Under Review.

Sun, G., C. Zhu, M.H. Kramer, S.S. Yang, W. Song, H.P. Piepho. 2010. Variation explained in miced-model assocaition mapping. Heredity. 105:333-340.

Velasquez, F, K. Walter, A. Soltani, and J.M. Osorno. 2017. Waterlogging tolerance in wild accessions of common bean (*Phaseouls vulgaris* L.) and related species. Bean Improvement Cooperative Meeting, East Lansing, MI.

Voesenek, L.A. and J. Bailey-Serres. 2013. Flooding tolerance: 02 sensing and survival strategies. Curr. Opinions in Plant Bio. 16:647-653.

Wuebker, E.F., R.E. Mullen, and K. Koehler. 2001. Flooding and temperature effects on soybean germination. Crop Sci. 41:1857-1861.

Zapata, M., G. Freytag, and R. Wilkinson. 2004. Release of five common bean germplasm lines resistant to common bacterial blight W-BB-11, W-BB-20-1, W-BB-35, W-BB-52, and W-BB-56. J. Agric. Univ. Puerto Rico. 88: 91-95.

Zhao, J. H. 2007. gap:Genetic analysis package. J. Stat. Softw. 23. doi10.18637/jss.v.i08.

Zhou, M. 2010. Improvement of Plant Waterlogging Tolerance. p. 267–285. *In* Waterlogging Signalling and Tolerance in Plants. Springer Berlin Heidelberg, Berlin, Heidelberg.

APPENDIX

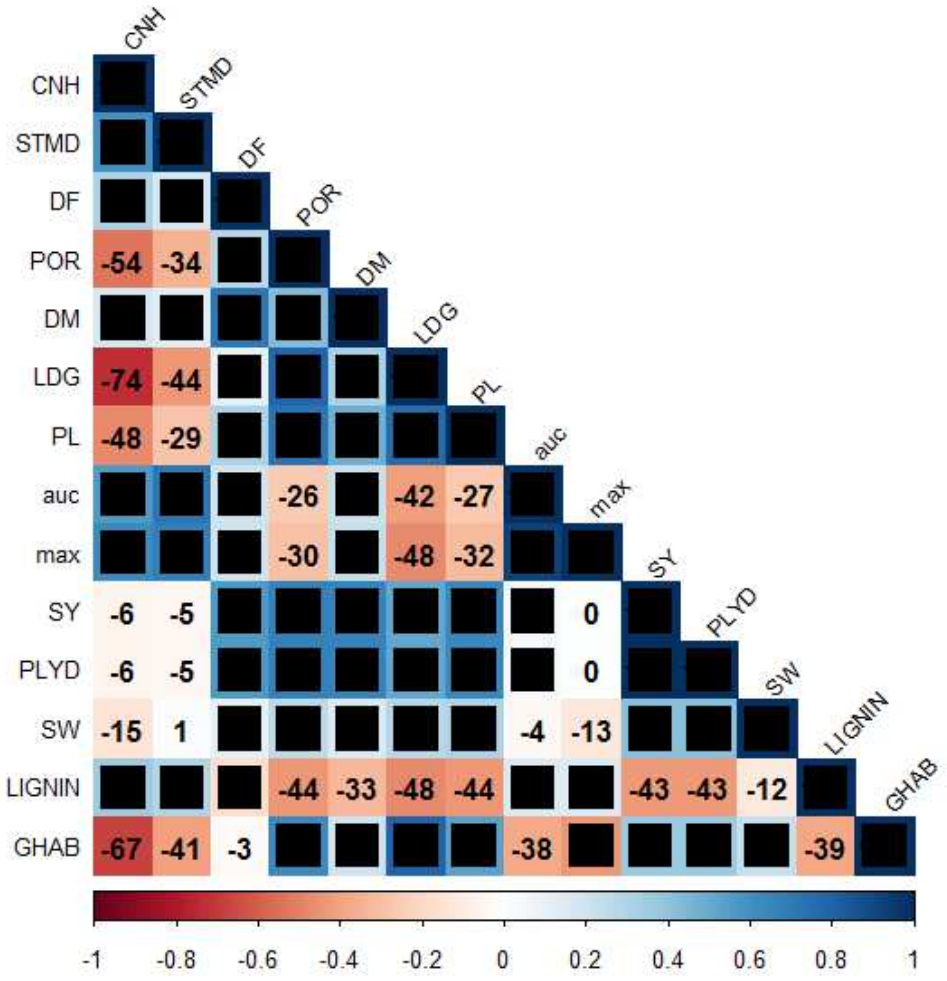

Figure A1. Correlation heatmaps among traits studied in Soltani et al. (2016) and lignin. Values represent Pearson's correlation coefficient x 100. CNH, canopy height; STMD, stem diameter; DF, days to flowering; POR, porosity; DM, days to maturity; LDG, lodging; PL, plant length; auc, area under curve; max, force needed to cut stem; SY, seed yield; PLYD, plant yield; SW, seed weight; LIGNIN, lignin accumulation; GHAB, growth habit.

Table A1. Least square means for lignin from DDP genotypes grown in Prosper, ND.

Genotype	Lignin	Growth Habit
Medicine Hat	23.47 a	II
USPT_CBB_5	23.10 ab	III
AC Redbond	22.81 abc	II
Santa Cruz	22.33 abcd	II
Aries	22.03 abcd	II
Powderhorn	21.50 abcde	II
92US 1006	20.82 abcdef	II
USPT WM 1	20.72 abcdefg	II
3138	20.62 abcdefgh	III
CDC Pintium	20.46 abcdefgh	II
Ouray	20.37 abcdefghi	II
Remington	20.30 abcdefghi	II
GN9 4	20.23 abcdefghij	II
NE1_09_22	20.17 abcdefghijk	II
BelMiNeb RMR 7	20.17 abcdefghijk	III
P08339	20.12 abcdefghijk	II
USWA 13	19.98 abcdefghijk	III
AC Early Rose	19.91 abcdefghijk	III
Lapaz	19.88 abcdefghijkl	II
ND041062 1	19.73 bcdefghijklm	III
Claret	19.68 bcdefghijklm	II
F07_449_9_3	19.68 bcdefghijklm	II
Ember	19.67 cdefghijklm	III
Santa Fe	19.64 cdefghijklm	III
BelMiNeb 1	19.32 cdefghijklm	II
Yolano	19.30 cdefghijklmn	III
Marquis	19.29 cdefghijklmn	III
Gemini	19.27 cdefghijklmn	III
Buster	19.25 defghijklmn	III
PT11 13	19.23 defghijklmn	II
BelMiNeb RMR 4	19.10 defghijklmn	II
Orion	19.07 defghijklmn	III
CDC Crocus	19.04 defghijklmn	III
Lariat	18.95 defghijklmno	III
UCD 9623	18.93 defghijklmno	III
INTA Precoz	18.92 defghijklmno	II
SR7 3	18.89 defghijklmno	NA
ND 307	18.85 efghijklmno	III
Pink Floyd	18.79 efghijklmno	III
Matterhorn	18.75 efghijklmno	II
UI 537	18.71 efghijklmno	III
AC Resolute	18.67 efghijklmno	III
NE2 09 4	18.59 efghijklmno	II

Means followed by same letter are not significantly different ($\alpha<0.05$).

Table A1. Least square means for lignin from DDP genotypes grown in Prosper, ND (continued).

Genotype	Lignin	Growth Habit
Rosetta	18.58 efghijklmno	II
Galeena	18.56 efghijklmnop	III
UI 111	18.56 efghijklmnop	III
PK9 7	18.55 efghijklmnop	III
BelDakMi rr 5	18.55 efghijklmnop	III
Frontier	18.54 efghijklmnop	III
Big Bend	18.48 efghijklmnop	III
Sonora	18.43 fghijklmnop	II
Monterrey	18.35 fghijklmnop	II
PT9 22	18.28 fghijklmnop	II
Longspeak	18.27 fghijklmnop	II
AC Scarlet	18.22 fghijklmnopq	III
Sinaloa	18.14 fghijklmnopq	II
Emerson	18.11 fghijklmnopq	III
USPT CBB 3	18.11 fghijklmnopq	II
NE1_09_19	18.09 fghijklmnopq	III
Desert Rose	18.08 fghijklmnopq	III
CDC Nordic	18.01 fghijklmnopq	NA
Aztec	17.97 fghijklmnopq	III
Mariah	17.97 fghijklmnopq	III
Focus	17.96 fghijklmnopq	III
Fiesta	17.88 fghijklmnopq	III
UCD 9634	17.86 fghijklmnopqr	III
TARS09 RR023	17.82 fghijklmnopqrs	III
BelMiNeb 2	17.79 fghijklmnopqrs	III
Win Mor	17.78 fghijklmnopqrs	III
PT9 17	17.76 fghijklmnopqrs	III
Vision	17.75 ghijklmnopqrs	III
ABCP_15	17.66 ghijklmnopqrs	III
USPT CBB 1	17.65 ghijklmnopqrst	III
USRM 20	17.63 ghijklmnopqrst	III
Sedona	17.56 ghijklmnopqrstu	III
Kimberly	17.56 ghijklmnopqrstu	III
Garnet	17.56 ghijklmnopqrstu	III
USWA 12	17.55 ghijklmnopqrstu	III
ND060197	17.53 ghijklmnopqrstu	III
R11801	17.52 ghijklmnopqrstu	III
Lebaron	17.50 ghijklmnopqrstu	III
Orca	17.50 ghijklmnopqrstu	III
I06 2575 17	17.48 ghijklmnopqrstu	III
Coyne	17.45 ghijklmnopqrstu	III
Jackpot	17.44 ghijklmnopqrstu	III

Means followed by same letter are not significantly different ($\alpha<0.05$).

Table A1. Least square means for lignin from DDP genotypes grown in Prosper, ND (continued).

Genotype	Lignin	Growth Habit
NE2 09 8	17.40 ghijklmnopqrstu	NA
Poncho	17.40 ghijklmnopqrstu	III
ICB 12	17.39 ghijklmnopqrstu	III
Kodiak	17.37 ghijklmnopqrstuv	III
Apache	17.36 ghijklmnopqrstuv	III
USWA 61	17.36 ghijklmnopqrstuv	II
Windbreaker	17.35 ghijklmnopqrstuv	III
Victor	17.35 ghijklmnopqrstuv	III
Grand Mesa	17.34 ghijklmnopqrstuv	III
US 1140	17.34 ghijklmnopqrstuv	III
AC Earlired	17.32 ghijklmnopqrstuv	III
GN9 1	17.26 ghijklmnopqrstuv	III
GTS 900	17.26 ghijklmnopqrstuv	III
Merlot	17.22 ghijklmnopqrstuv	II
Flint	17.21 ghijklmnopqrstuv	III
Coulee	17.20 ghijklmnopqrstuvw	III
Agassiz	17.20 ghijklmnopqrstuvw	II
Sapphire	17.19 ghijklmnopqrstuvw	III
Stampede	17.19 hijklmnopqrstuvw	II
CDCWM 2	17.18 hijklmnopqrstuvw	III
Hungerford	17.10 hijklmnopqrstuvw	III
PT9 5 6	17.08 hijklmnopqrstuvw	II
GN Star	17.06 hijklmnopqrstuvw	III
PK 915	17.04 hijklmnopqrstuvw	III
Gala	17.03 hijklmnopqrstuvw	III
UI 196	17.03 hijklmnopqrstuvw	III
CDC Camino	17.02 hijklmnopqrstuvw	II
NE2 09 10	17.01 hijklmnopqrstuvwx	III
AC Island	16.97 ijklmnopqrstuvwx	III
UI 425	16.94 ijklmnopqrstuvwx	III
TARS VCI 4b	16.93 ijklmnopqrstuvwx	III
Topaz	16.93 ijklmnopqrstuvwx	III
UI_114	16.93 ijklmnopqrstuvwx	III
Common Pinto	16.72 jklmnopqrstuvwx	III
BelMiNeb RR 2	16.71 jklmnopqrstuvwx	III
Red Ryder	16.71 jklmnopqrstuvwx	III
Maverick	16.71 jklmnopqrstuvwx	III
Roza	16.68 jklmnopqrstuvwx	III
NE1 09 20	16.67 jklmnopqrstuvwx	II
Beryl R	16.61 jklmnopqrstuvwx	III
UI_3	16.60 klmnopqrstuvwx	III
P12 606	16.58 klmnopqrstuvwx	II

Means followed by same letter are not significantly different ($\alpha<0.05$).

Table A1. Least square means for lignin from DDP genotypes grown in Prosper, ND (continued).

Genotype	Lignin	Growth Habit
IP08 2	16.55 klmnopqrstuvwx	III
USPT ANT 1	16.53 lmnopqrstuvwx	III
NE2 09 3	16.52 lmnopqrstuvwx	II
AC OLE	16.51 lmnopqrstuvwx	NA
AC Polaris	16.45 lmnopqrstuvwx	III
Sawtooth	16.42 lmnopqrstuvwx	III
GN Harris	16.42 lmnopqrstuvwx	III
Rio Rojo	16.38 lmnopqrstuvwx	NA
Sierra	16.37 lmnopqrstuvwx	III
NW 590	16.35 lmnopqrstuvwx	III
El Dorado	16.34 lmnopqrstuvwx	II
UI 228	16.31 lmnopqrstuvwx	III
Holberg	16.29 lmnopqrstuvwx	III
Shoshone	16.20 mnopqrstuvwx	III
UI 239	16.19 mnopqrstuvwx	III
Bill Z	16.19 mnopqrstuvwx	III
Beryl	16.16 mnopqrstuvwx	III
ABC Weihing	16.13 mnopqrstuvwx	II
Fargo	16.11 mnopqrstuvwx	III
BelMiNeb RMR 3	16.09 nopqrstuvwx	III
Max	16.05 nopqrstuvwx	III
Common Red Mexican	16.01 nopqrstuvwx	III
CDC Pinnacle	16.00 nopqrstuvwx	III
6R 42	15.96 nopqrstuvwx	III
NE 63	15.96 nopqrstuvwxy	III
SDIP 1	15.94 nopqrstuvwxy	NA
UI 123	15.92 nopqrstuvwxy	III
Nodak	15.90 nopqrstuvwxy	III
Croissant	15.89 nopqrstuvwxy	III
F07 014 22 2	15.89 nopqrstuvwxy	II
Starlight	15.89 nopqrstuvwxy	III
Viva	15.76 nopqrstuvwxy	III
Sequoia	15.67 nopqrstuvwxy	III
AC Pintoba	15.65 nopqrstuvwxy	NA
Hatton	15.60 opqrstuvwxy	III
Montrose	15.57 opqrstuvwxy	III
Durango	15.55 opqrstuvwxy	III
Burke	15.55 opqrstuvwxy	III
Gloria	15.41 opqrstuvwxy	III
ABCP 8	15.38 opqrstuvwxy	III
Arapaho	15.35 opqrstuvwxy	III
Baja	15.35 opqrstuvwxy	III

Means followed by same letter are not significantly different ($\alpha<0.05$).

Table A1. Least square means for lignin from DDP genotypes grown in Prosper, ND (continued).

Genotype	Lignin	Growth Habit
JM 24	15.33 pqrstuvwxy	III
Harold	15.24 pqrstuvwxy	III
PR 0340 3 3 1	15.24 pqrstuvwxy	II
SR9 4	15.21 pqrstuvwxy	II
Chase	15.05 pqrstuvwxy	III
BelNeb RR 1	14.92 pqrstuvwxy	III
Ivory	14.87 qrstuvwxy	III
Gypsyrose	14.83 qrstuvwxy	III
URS 117	14.81 qrstuvwxy	III
PT7 2	14.80 rstuvwxy	III
Buckskin	14.77 rstuvwxy	III
Othello	14.68 stuvwxy	III
UI 126	14.52 tuvwxy	III
NW 410	14.51 tuvwxy	III
JM 126	14.45 uvwxy	III
PR0401 259	14.42 uvwxy	II
UI 59	14.25 vwxy	III
Quincy	14.24 vwxy	III
CRC Rosalee	14.09 wxy	III
UI 37	13.39 xy	III
Pindak	12.92 y	III

Means followed by same letter are not significantly different ($\alpha<0.05$).

Table A2. Analysis of variance for germination rate of unpigmented seed genotypes in preliminary study.

Source of Variation	df	Mean Square
Replication	2	73
Treatment	4	39076**
Error (a)	8	231
Genotype	11	41679**
Genotype x Treatment	44	17207*
Error (b)	110	188

*,**Significant at 0.05 and 0.01 levels, respectively

Table A3. Analysis of variance for germination rate of pigmented seed genotypes in preliminary study.

Source of Variation	df	Mean Square
Replication	2	234
Treatment	4	14422**
Error (a)	8	390
Genotype	11	1876**
Genotype x Treatment	44	326
Error (b)	110	267

*,**Significant at 0.05 and 0.01 levels, respectively

Table A4. Least square means for all traits in both flooded and non-flooded condition for all genotypes in unpigmented seed coat screening.

	Germination Rate		Total Weight		Shoot Weight		Root Weight		Plant Height		
Genotype	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Market Class
	----------%----------		---------- g ----------						--------- cm ---------		
Verano	60	63	1.12	1.69	1.07	0.36	0.30	0.55	11.73	9.98	Navy
Royalty	100	93	1.53	2.47	2.22	0.84	0.46	0.54	20.90	13.64	Cream
Ui 59	83	50	1.83	1.91	2.10	0.38	0.68	0.79	14.79	11.59	Great Northern
Belneb rr 1	87	43	2.10	1.81	1.99	0.01	0.80	0.59	18.87	14.02	Great Northern
Matterhorn	23	10	1.50	0.71	0.77	0.01	0.19	0.32	4.27	12.62	Great Northern
Ac resolute	83	33	1.74	1.47	1.97	0.38	0.46	0.60	17.54	14.12	Great Northern
Morales	93	37	1.74	1.08	1.65	0.36	0.47	0.39	19.42	9.28	Navy
Starlight	85	33	0.75	1.48	1.35	0.38	0.32	0.60	18.82	12.81	Great Northern
Sawtooth	93	33	1.96	1.71	2.91	0.38	0.82	0.54	17.18	13.65	Great Northern
Emerson	93	33	2.10	1.76	2.74	0.38	0.61	0.71	18.93	14.91	Great Northern
Ne1 09 20	93	33	1.67	1.68	1.92	0.38	0.58	0.83	19.04	10.72	Great Northern
BelMineb rr 2	87	30	2.20	1.75	1.52	.†	0.71	0.87	17.21	10.93	Great Northern
Hyden	97	33	1.89	1.19	1.50	0.36	0.40	0.65	19.84	9.73	Navy
Ui 123	90	30	1.79	1.56	1.94	0.38	0.58	0.65	16.47	10.14	Great Northern
Belmineb 2	93	30	2.48	1.50	1.80	.	0.56	0.60	16.03	7.92	Great Northern
Ne1 09 13	97	30	1.94	1.34	2.11	0.84	0.76	0.53	17.76	10.00	Great Northern
Beryl	87	27	1.49	1.01	1.32	0.38	0.51	0.44	15.24	9.62	Great Northern
Abc weihing	87	27	2.22	1.36	2.09	0.38	0.51	0.54	16.78	10.59	Great Northern
Sapphire	90	27	1.99	1.61	1.68	0.38	0.72	0.89	16.14	11.09	Great Northern
Belneb 2	90	27	1.72	1.34	2.12	0.84	0.59	0.50	17.68	13.96	Great Northern
Coyne	90	27	2.40	1.89	2.33	0.38	0.58	0.67	21.38	12.56	Great Northern
Uswa 13	81	23	0.59	1.37	0.65	0.36	0.21	0.67	19.96	10.51	Great Northern
Cdc nordic	100	27	1.99	1.15	2.12	0.32	0.83	0.53	18.84	11.66	Great Northern
CDC Crocus	90	20	2.16	1.04	2.31	0.32	0.73	0.47	18.61	12.14	Great Northern
Ne1 09 19	100	20	2.08	0.98	1.98	0.38	0.63	0.37	18.86	12.52	Great Northern
Jm 24	87	17	1.86	1.36	2.09	0.36	0.57	0.84	19.24	11.10	Great Northern
Gn9 4	90	17	2.62	0.72	1.64	0.36	0.58	0.21	15.70	10.04	Great Northern
Beryl r	97	17	1.57	1.09	1.61	0.38	0.53	0.59	16.88	10.08	Great Northern
Orion	100	17	2.60	0.98	2.30	0.08	0.78	0.35	18.96	9.34	Great Northern
Neptune	100	17	1.82	0.51	1.36	0.03	0.58	0.25	13.36	7.58	Navy
Ac compass	87	13	1.23	0.82	0.96	0.08	0.24	0.48	13.85	8.17	Navy

† Missing data point

Table A4. Least square means for all traits in both flooded and non-flooded condition for all genotypes in unpigmented seed coat screening (continued).

	Germination Rate		Total Weight		Shoot Weight		Root Weight		Plant Height		
Genotype	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Market Class
	----------%----------		---------- g ----------						--------- cm ---------		
Marquis	93	13	2.51	1.74	1.85	0.38	0.55	0.65	14.48	11.73	Great Northern
Gn star	77	10	2.25	0.88	1.88	0.38	0.71	0.47	16.60	12.60	Great Northern
T9905	77	10	1.82	1.14	1.27	0.08	0.58	0.58	10.77	7.73	Navy
Ac polaris	83	10	1.79	1.21	1.87	0.38	0.52	0.56	16.15	12.69	Great Northern
Morden 003	83	10	1.26	0.44	1.13	0.36	0.33	0.25	12.17	7.98	Navy
BelMiNeb RMR 4	90	10	2.42	0.81	2.21	0.03	0.98	0.34	17.97	12.20	Navy
Nautica	93	10	1.72	0.58	1.05	0.08	0.41	0.39	15.72	7.40	Navy
Belmineb rmr 3	95	10	1.99	1.01	1.35	0.38	0.58	0.35	18.68	10.62	Great Northern
Swan valley	97	10	2.34	0.45	1.22	0.84	0.55	0.29	13.02	5.52	Navy
Gemini	97	10	2.25	0.95	1.92	0.08	0.47	0.31	18.17	12.42	Great Northern
Oac rex	100	10	1.79	0.58	1.35	0.26	0.68	0.32	14.92	6.40	Navy
Ne1 09 9	100	10	2.15	0.45	1.83	0.36	0.59	0.19	17.26	10.39	Great Northern
Ensign	100	10	1.41	0.30	1.32	0.38	0.47	0.12	16.39	8.23	Navy
Belmineb 5	87	7	2.27	0.55	1.58	. †	0.63	0.17	15.36	13.80	Great Northern
Ivory	90	7	2.57	0.58	2.07	0.84	0.82	0.42	17.35	9.80	Great Northern
Gn9 1	93	7	2.42	0.44	1.89	0.36	0.89	0.21	18.74	11.10	Great Northern
Cdc whitecap	95	7	1.03	0.57	0.68	0.32	0.20	0.24	14.18	10.93	Navy
Midland	100	7	1.18	0.14	0.91	0.03	0.33	0.10	13.07	6.47	Navy
Seahawk	73	3	1.84	0.57	0.78	0.01	0.30	0.34	11.83	8.73	Navy
Belmineb rmr 8	75	3	0.94	0.23	0.88	0.01	0.32	0.07	14.81	10.00	Navy
Medalist	77	3	2.23	.	0.91	0.38	0.19	.	10.59	.	Navy
Vista	80	3	1.79	0.18	1.03	0.36	0.40	0.09	11.86	8.23	Navy
Avalanche	80	3	1.62	.	1.02	0.36	0.34	.	13.71	.	Navy
Oac laser	83	3	1.39	0.37	1.05	0.08	0.27	0.26	13.78	6.73	Navy
C 20	90	3	1.56	0.13	0.99	0.01	0.52	0.12	12.94	7.00	Navy
Ne1 09 22	93	3	2.29	0.88	1.80	0.36	0.64	0.51	16.82	9.13	Great Northern
Huron	93	3	2.21	0.14	1.00	0.01	0.35	0.05	14.47	.	Navy
Hy 4181	97	3	1.36	0.53	1.49	0.08	0.65	0.28	15.14	10.23	Navy

† Missing data point

Table A4. Least square means for all traits in both flooded and non-flooded condition for all genotypes in unpigmented seed coat screening (continued).

	Germination Rate		Total Weight		Shoot Weight		Root Weight		Plant Height		
Genotype	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Market Class
	----------%----------		---------- g ----------						--------- cm ---------		
T9903	97	3	1.94	0.49	1.32	0.08	0.44	0.20	18.33	9.30	Navy
Laker	100	3	2.54	0.78	1.34	0.55	0.63	0.25	14.42	8.77	Navy
Belmineb 1	100	3	1.54	0.58	1.52	. †	0.39	0.32	16.67	7.03	Great Northern
Newport	97	0	1.22	.	1.33	0.01	0.65	.	15.89	.	Navy
Michelite	97	0	1.85	.	1.36	0.84	0.57	.	13.31	.	Navy
Crestwood	100	0	1.40	.	1.20	0.36	0.53	.	15.66	.	Navy
Mchale	97	0	1.64	.	1.13	0.03	0.51	.	14.72	.	Navy
Lightning	90	0	1.56	.	1.06	0.08	0.48	.	12.80	.	Navy
Seabiskit	93	0	1.57	.	1.08	0.38	0.48	.	13.00	.	Navy
Envoy	100	0	1.80	.	1.39	0.36	0.48	.	13.97	.	Navy
Albion	93	0	1.73	.	0.96	0.08	0.45	.	15.84	.	Navy
Uswa 50	100	0	2.38	.	1.43	0.36	0.44	.	17.21	.	Navy
Nw 395	87	0	1.14	.	1.11	0.36	0.39	.	14.29	.	Navy
Belmineb rmr 7	80	0	1.08	.	0.89	0.03	0.36	.	15.08	.	Navy
Sanilac	77	0	1.66	.	0.82	0.03	0.34	.	10.74	.	Navy
Oac gryphon	93	0	1.69	.	1.04	0.08	0.34	.	16.59	.	Navy
N05324	100	0	1.82	.	0.96	0.01	0.34	.	14.36	.	Navy
Reliant	87	0	1.67	.	0.96	0.36	0.32	.	15.07	.	Navy
Arthur	90	0	2.03	.	0.81	0.36	0.30	.	12.44	.	Navy
Navigator	87	0	1.75	.	0.89	0.38	0.29	.	11.68	.	Navy
Voyager	93	0	2.14	.	1.08	0.38	0.27	.	14.77	.	Navy
mackinac	73	0	1.99	.	0.72	0.01	0.24	.	9.65	.	Navy
Norstar	67	0	1.66	.	0.56	0.36	0.22	.	7.98	.	Navy
Avanti	77	0	1.84	.	0.74	0.08	0.21	.	11.68	.	Navy

† Missing data point

Table A5. Least square means for all traits in both flooded and non-flooded condition for all genotypes in pigmented seed coat screening.

	Germination Rate		Total Weight		Shoot Weight		Root Weight		Plant Height		
Genotype	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Market Class
	--------- % ---------		---------- g ----------						--------- cm ---------		
Royalty	88	78	2.19	.†	1.84	.	0.35	.	18.72	.	Cream
Midnight	88	75	2.11	1.12	1.60	0.67	0.51	0.45	14.58	10.53	Black
Indeterminate Jamaican Red	88	68	2.72	.	1.93	.	0.79	.	17.00	.	Small red
Durango	81	58	3.06	1.09	2.32	0.65	0.75	0.43	16.46	9.31	Pinto
Blackjack	85	58	1.73	0.99	1.23	0.47	0.50	0.52	13.71	9.27	Black
Rojo chiquito	91	61	2.24	1.07	1.56	0.53	0.67	0.54	14.71	8.03	Small red
Tars09 RR004	88	58	2.12	0.95	1.41	0.51	0.71	0.44	16.16	9.14	Small red
Ac early rose	85	55	2.48	0.94	1.86	0.56	0.62	0.38	20.29	8.28	Pink
Xan 176	91	58	1.90	1.07	1.44	0.58	0.46	0.48	16.03	9.19	Black
Dor 364	98	61	1.92	1.14	1.47	0.61	0.45	0.53	17.43	8.61	Small red
A 55	93	58	2.09	1.08	1.48	0.56	0.60	0.53	14.67	9.14	Black
Tars09 RR007	78	48	1.79	.	1.23	.	0.55	.	19.22	.	Small red
Inta precoz	95	58	2.39	0.72	1.65	0.41	0.74	0.32	15.93	9.43	Small red
Ac black diamond	85	51	1.73	0.82	1.38	0.55	0.35	0.27	16.30	9.24	Black
Cdc nighthawk	85	51	1.66	0.86	1.16	0.51	0.49	0.35	18.88	14.76	Black
Eclipse	71	41	1.13	0.88	0.96	0.47	0.17	0.41	17.67	11.22	Black
Sonora	95	55	2.96	1.20	2.32	0.75	0.63	0.45	19.64	9.69	Pinto
chase	68	38	1.99	0.70	1.49	0.39	0.50	0.31	20.48	12.86	Pinto
Pr 0443 151	98	55	1.51	0.94	1.07	0.44	0.44	0.50	15.39	8.70	Black
Black hawk	98	55	2.61	1.07	1.81	0.62	0.80	0.45	18.66	12.65	Black
Windbreaker	81	45	2.77	1.11	2.08	0.68	0.69	0.44	17.53	9.99	Pinto
Verano	90	48	2.28	.	1.59	.	0.69	.	20.54	.	Navy
Pr 0401 259	91	48	2.08	1.04	1.70	0.45	0.38	0.59	17.16	12.14	Pink
Puebla 152	68	35	2.26	1.05	1.60	0.42	0.66	0.50	15.91	9.27	Black
Sierra	95	48	1.82	1.27	1.29	0.84	0.53	0.42	15.28	10.76	Pinto
Harrowhawk	97	48	2.19	0.68	1.48	0.33	0.71	0.35	19.02	9.92	Black
92us 1006	85	41	1.83	0.62	1.45	0.40	0.38	0.32	19.33	7.67	Pinto
Ne2 09 8	78	38	2.42	1.16	1.82	0.66	0.60	0.49	19.08	11.14	Pinto
Dehoro	95	45	2.83	0.78	2.13	0.43	0.70	0.35	15.45	9.47	Small red
Apache	95	45	3.07	1.02	2.26	0.57	0.81	0.45	18.50	8.87	Pinto

† Missing data point

Table A5. Least square means for all traits in both flooded and non-flooded condition for all genotypes in pigmented seed coat screening (continued).

	Germination Rate		Total Weight		Shoot Weight		Root Weight		Plant Height		
Genotype	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Market Class
	--------- % ---------		---------- g ----------						--------- cm ---------		
Black velvet	95	45	1.95	0.89	1.37	0.41	0.58	0.49	13.23	9.64	Black
CDC Pintium	88	41	2.61	0.76	2.15	0.47	0.46	0.29	18.19	12.67	Pinto
i9365 25	90	41	1.47	0.64	1.07	0.29	0.41	0.35	16.19	8.55	Pink
Tars09 rr023	61	28	1.67	0.56	1.28	0.26	0.39	0.21	15.39	6.50	Pinto
Agassiz	61	28	2.65	1.01	1.98	0.43	0.67	0.58	19.28	10.67	Pinto
Nd 307	95	41	3.66	1.72	2.62	0.93	1.04	0.79	22.13	10.20	Pinto
Montrose	72	31	2.50	0.50	1.87	0.34	0.63	0.17	20.25	8.90	Pinto
Ac redbond	88	38	2.29	0.67	1.66	0.31	0.64	0.35	14.87	6.49	Small red
Fargo	88	38	2.70	1.16	1.92	0.53	0.78	0.63	17.65	10.97	Pinto
Pink floyd	88	38	2.37	0.99	1.84	0.45	0.53	0.53	15.74	11.11	Pink
i9365 5	88	38	1.73	0.59	1.32	0.29	0.41	0.29	13.77	8.02	Pink
Blackmagic	98	41	2.61	0.84	2.03	0.48	0.58	0.36	16.52	9.76	Black
Cdc jet	98	41	2.31	0.70	1.83	0.31	0.51	0.39	16.11	8.88	Black
Rog 312	91	38	3.01	.†	2.06	.	0.95	.	15.46	.	Pink
Pt9 17	91	38	3.17	1.11	2.19	0.53	0.98	0.58	15.28	9.69	Pinto
Baja	85	35	2.40	0.80	1.80	0.52	0.60	0.32	16.84	7.74	Pinto
Ne2 09 4	78	31	2.41	0.93	1.77	0.48	0.64	0.46	19.12	12.84	Pinto
Cornell 49 242	81	31	2.00	.	1.46	.	0.54	.	15.23	.	Black
Bandit	91	35	1.19	0.50	0.92	0.26	0.27	0.23	15.17	9.11	Black
Gts 900	85	31	2.40	0.56	1.76	0.33	0.64	0.23	17.60	9.54	Pinto
Red ryder	88	31	2.88	0.54	2.13	0.33	0.75	0.21	19.01	9.93	Small red
icb 12	81	28	1.65	0.55	1.24	0.29	0.41	0.25	15.48	10.24	Pinto
Burke	81	28	2.33	0.91	1.82	0.42	0.51	0.49	17.57	9.08	Pinto
Bat 477	91	31	2.12	0.63	1.67	0.31	0.45	0.32	19.97	10.08	Black
Hatton	91	31	3.21	0.32	2.49	0.17	0.72	0.15	18.31	8.64	Pinto
Sequoia	91	31	3.54	0.68	2.58	0.30	0.96	0.37	21.21	12.30	Pinto
Ne2 09 3	84	28	3.56	0.70	2.65	0.40	0.91	0.30	19.44	11.03	Pinto
Black knight	95	31	1.97	0.67	1.40	0.26	0.57	0.40	15.76	9.62	Black
icb 10	95	31	1.94	0.63	1.55	0.40	0.39	0.23	16.39	10.53	Black
Nd021717	98	31	1.58	0.43	1.25	0.27	0.34	0.16	16.25	11.41	Black
Medicine hat	91	28	2.15	0.78	1.50	0.49	0.66	0.30	17.10	10.00	Pinto
Abcp 17	81	25	2.74	1.14	2.07	0.74	0.67	0.40	21.69	12.52	Pinto

† Missing data point

Table A5. Least square means for all traits in both flooded and non-flooded condition for all genotypes in pigmented seed coat screening (continued).

	Germination Rate		Total Weight		Shoot Weight		Root Weight		Plant Height		
Genotype	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Market Class
	--------- % ---------		---------- g ----------						--------- cm ---------		
Kodiak	71	21	2.25	0.94	1.64	0.50	0.60	0.44	18.64	9.96	Pinto
Ac earlired	95	28	2.57	0.34	1.78	0.13	0.79	0.22	14.94	6.51	Small red
Aztec	95	28	2.14	0.80	1.59	0.55	0.55	0.25	21.04	11.60	Pinto
Poncho	85	25	2.68	0.58	2.12	0.23	0.56	0.35	16.78	8.86	Pinto
Abcp 8	85	25	2.15	0.72	1.78	0.42	0.37	0.30	18.08	11.49	Pinto
Beldakmi rr 5	88	25	2.78	0.45	2.15	0.27	0.63	0.19	22.90	11.91	Pinto
Aifi wuriti	91	25	2.00	0.40	1.53	0.21	0.46	0.19	14.11	8.86	Black
Gala	91	25	2.28	0.55	1.76	0.33	0.52	0.21	19.99	7.55	Pinto
Buster	91	25	2.30	0.83	1.70	0.54	0.60	0.29	13.55	9.50	Pinto
ui 537	68	18	1.99	0.51	1.56	0.31	0.44	0.21	16.31	9.69	Pink
Frontier	81	21	3.03	0.48	2.12	0.40	0.91	0.08	15.03	8.64	Pinto
CDC Camino	95	25	3.55	0.35	2.48	0.19	1.07	0.16	17.20	6.44	Pinto
Yolano	85	21	2.47	0.43	1.59	0.28	0.88	0.16	13.91	8.33	Pink
F07 014 22 2	71	18	2.38	0.63	1.45	0.33	0.94	0.31	17.97	13.31	Small red
Nd060197	47	11	1.77	0.35	1.38	0.17	0.39	0.19	17.17	9.59	Pinto
P07863	75	18	2.10	0.34	1.49	0.18	0.61	0.16	15.56	6.73	Pinto
Comman red mexican	91	21	2.19	0.36	1.55	0.19	0.64	0.17	18.80	6.04	Small red
Tars vci 4b	78	18	1.86	0.40	1.45	0.23	0.41	0.17	15.83	7.54	Pinto
Domino	98	22	1.40	0.22	1.04	0.14	0.36	0.08	16.00	8.41	Black
Uspt ant 1	81	18	2.23	0.60	1.63	0.32	0.6	0.29	15.90	12.06	Pinto
Grand mesa	82	18	2.68	0.38	1.96	0.19	0.72	0.19	17.99	9.13	Pinto
Ui 126	91	18	3.09	0.45	2.38	0.26	0.71	0.18	18.25	7.32	Pinto
Ui 228	91	18	2.26	0.60	1.62	0.26	0.65	0.33	17.29	8.37	Small red
icb 3	91	18	1.82	0.34	1.43	0.23	0.39	0.11	17.82	11.86	Black
Pk9 15	91	18	2.78	0.58	2.20	0.30	0.58	0.29	20.46	13.96	Pink
Nw 410	95	18	3.74	0.35	2.61	0.17	1.13	0.18	17.06	6.76	Pinto
F04 2801 4 5 1	78	15	1.72	0.36	1.30	0.17	0.42	0.19	18.53	10.61	Black
Uspt cbb 1	78	15	2.64	0.26	1.88	0.14	0.76	0.12	18.25	9.36	Pinto
Ucd 96114	78	15	1.23	0.22	0.92	0.13	0.31	0.09	16.55	7.65	Black
Win mor	81	15	3.02	0.40	2.42	0.34	0.60	0.06	19.53	18.24	Pinto
Uswa 61	84	15	1.60	0.30	1.17	0.15	0.42	0.16	19.90	9.60	Pink
Abcp 15	85	15	2.42	0.33	1.85	0.14	0.57	0.19	21.32	13.05	Pinto

† Missing data point

Table A5. Least square means for all traits in both flooded and non-flooded condition for all genotypes in pigmented seed coat screening (continued).

	Germination Rate		Total Weight		Shoot Weight		Root Weight		Plant Height		
Genotype	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Market Class
	--------- % ---------		---------- g ----------						--------- cm ---------		
Usrm 20	85	15	3.36	0.30	2.57	0.17	0.79	0.13	19.90	5.69	Small red
Uspt cbb 3	68	11	1.66	0.61	1.33	0.12	0.32	0.40	17.43	8.12	Pinto
Shiny crow	92	15	2.77	0.27	1.91	0.15	0.87	0.12	15.49	7.81	Black
Sedona	95	15	2.97	0.13	2.43	0.07	0.54	0.05	20.66	6.58	Pink
i06 2575 17	75	11	1.66	0.43	1.22	0.25	0.43	0.18	17.06	8.14	Pinto
La paz	81	11	2.67	0.29	1.89	0.18	0.78	0.11	19.85	13.35	Pinto
Pt7 2	85	11	2.41	0.35	1.72	0.21	0.69	0.13	16.81	7.86	Pinto
Lariat	88	11	2.61	0.25	1.77	0.17	0.84	0.07	22.80	13.76	Pinto
Cdc rosalee	88	11	2.00	0.35	1.49	0.21	0.50	0.13	16.16	6.69	Pink
Bill z	91	11	2.47	0.23	1.73	0.12	0.73	0.11	18.14	6.72	Pinto
Ui 911	91	11	1.81	0.16	1.50	0.10	0.30	0.06	15.35	11.06	Black
Cdc expresso	93	11	1.64	0.37	1.30	0.22	0.33	0.14	16.21	9.67	Black
Dpc 4	95	11	1.84	0.20	1.40	0.12	0.44	0.07	17.41	4.59	Black
Common pinto	95	11	2.89	0.44	2.18	0.19	0.72	0.26	18.20	7.85	Pinto
Kimberly	75	8	2.49	0.22	1.96	0.15	0.52	0.07	18.21	14.27	Pinto
Roza	75	8	1.84	0.27	1.31	0.13	0.53	0.14	20.30	8.40	Pink
Nd041062 1	75	8	2.10	0.26	1.50	0.15	0.60	0.11	19.48	10.42	Pinto
Ouray	81	8	3.10	0.19	2.12	0.12	0.98	0.08	14.95	11.07	Pinto
Ac harblack	81	8	2.02	0.18	1.63	0.12	0.39	0.06	18.10	13.03	Black
Uspt cbb 5	48	5	1.54	0.34	1.16	0.10	0.38	0.23	18.81	8.77	Pinto
Buckskin	85	8	2.91	0.28	2.15	0.15	0.76	0.13	16.99	10.65	Pinto
Shania	85	8	1.90	0.14	1.36	0.12	0.54	0.03	15.23	8.94	Black
Jackpot	85	8	2.73	0.39	2.11	0.16	0.62	0.23	19.23	6.02	Pinto
Ui 3	88	8	2.27	0.38	1.67	0.16	0.59	0.22	14.86	5.74	Small red
Sr9 4	88	8	2.46	0.23	1.62	0.15	0.83	0.07	18.24	10.10	Small red
Nd040494 4	88	8	3.28	0.22	2.31	0.16	0.97	0.06	16.40	6.10	Pinto
Nd021574	88	8	1.67	0.25	1.24	0.11	0.43	0.14	10.31	8.37	Black
Flint	91	8	2.84	0.28	2.05	0.12	0.79	0.16	17.77	10.55	Pinto
Shoshone	92	8	3.78	0.17	2.72	0.11	1.06	0.06	22.11	12.42	Pinto
ip08 2	73	6	1.41	0.05	0.94	0.02	0.47	0.04	15.68	1.85	Pinto
Tars09 rr029	68	5	1.25	0.16	0.98	0.07	0.27	0.09	18.12	7.92	Small red
Focus	75	5	2.84	0.21	2.12	0.17	0.71	0.05	18.59	7.94	Pinto
F04 2801 4 1 2	81	5	1.75	0.10	1.41	0.10	0.34	.†	16.98	8.99	Black

† Missing data point

Table A5. Least square means for all traits in both flooded and non-flooded condition for all genotypes in pigmented seed coat screening (continued).

	Germination Rate		Total Weight		Shoot Weight		Root Weight		Plant Height		
Genotype	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Market Class
	--------- % ---------		---------- g ----------						--------- cm ---------		
Zorro	81	5	1.63	0.18	1.23	0.09	0.40	0.09	14.90	9.47	Black
Garnet	81	5	1.75	0.27	1.24	0.07	0.51	0.19	14.07	5.92	Small red
Ui 196	81	5	2.94	0.23	1.91	0.08	1.02	0.11	17.90	10.22	Pinto
T 39	85	5	1.54	0.14	1.02	0.06	0.52	0.08	16.71	11.60	Black
Ne2 09 1	88	5	2.80	0.03	2.17	0.02	0.63	0.01	18.73	8.95	Pinto
Cdc pinnacle	88	5	4.20	0.10	2.33	0.05	1.88	0.04	18.04	7.40	Pinto
Mariah	88	5	2.73	0.23	2.11	0.10	0.61	0.12	20.16	8.22	Pinto
Pk9 7	91	5	2.86	0.13	2.21	0.09	0.65	0.04	19.52	7.04	Pink
Ember	91	5	2.71	0.36	1.68	0.17	1.03	0.19	15.91	7.92	Small red
Maverick	51	1	1.38	0.11	1.07	0.06	0.31	0.05	20.89	12.24	Pinto
Sr7 3	68	1	1.78	0.10	1.31	0.05	0.46	0.06	14.25	4.75	Small red
Viva	71	1	1.43	0.13	1.07	0.06	0.36	0.06	17.38	7.10	Pink
Condor	75	1	1.08	0.30	0.88	0.14	0.20	0.15	17.01	10.60	Black
B05055	75	1	1.06	0.21	0.78	0.07	0.28	0.13	14.58	17.20	Black
Raven	78	1	1.42	0.10	1.11	0.05	0.31	0.04	16.60	6.10	Black
Gloria	81	1	2.07	0.07	1.37	0.04	0.69	0.02	16.92	3.00	Pink
Max	81	1	2.22	0.05	1.67	0.04	0.55	0.01	17.10	3.94	Pinto
Jaguar	85	1	1.46	0.21	1.12	0.01	0.34	0.21	16.96	2.95	Black
Desert rose	85	1	2.73	0.25	2.11	0.14	0.62	0.10	17.44	11.5	Pink
Ui 37	85	1	2.36	0.04	1.64	0.01	0.72	0.04	15.43	3.95	Small red
Quincy	85	1	3.12	0.14	2.31	0.07	0.81	0.06	21.84	4.30	Pinto
6r 42	85	1	2.83	0.52	1.87	0.23	0.96	0.28	18.69	12.4	Pink
F04 2801 4 6 6	88	1	1.82	0.05	1.34	0.04	0.48	0.01	18.68	7.94	Black
Phantom	88	1	2.17	.†	1.52	.	0.65	.	18.57	.	Black
Nodak	88	1	2.91	0.04	2.00	0.03	0.91	0.02	18.26	3.95	Pinto
Nw 590	88	1	2.33	0.06	1.59	0.05	0.73	0.02	17.37	9.25	Pinto
Fiesta	88	1	2.57	0.07	1.93	0.04	0.64	0.03	22.90	2.34	Pinto
F07 449 9 3	91	1	2.28	0.31	1.70	0.19	0.58	0.12	18.81	18.34	Small red
Jm 126	91	1	2.75	0.10	2.05	0.06	0.70	0.03	18.36	2.70	Pinto
Orca	95	1	1.95	0.08	1.53	0.04	0.42	0.04	16.99	.	Black
Merlot	97	1	3.34	0.15	2.61	0.06	0.74	0.10	19.68	.	Small red
Fisher	75	0	2.61	.	1.64	.	0.97	.	19.52	.	Pinto
Croissant	57	0	2.05	.	1.52	.	0.53	.	18.61	.	Pinto

† Missing data point

Table A5. Least square means for all traits in both flooded and non-flooded condition for all genotypes in pigmented seed coat screening (continued).

	Germination Rate		Total Weight		Shoot Weight		Root Weight		Plant Height		
Genotype	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Non-Flooded	Flooded	Market Class
	%		g						cm		
Pr 0340 3 3 1	91	0	2.38	.†	1.82	.	0.56	.	18.58	.	Small red
Amadeus 77	95	0	1.98	.	1.28	.	0.70	.	14.68	.	Small red
ibc 301 204	91	0	1.44	.	1.11	.	0.34	.	17.79	.	Small red
Centa pupil	91	0	2.30	.	1.67	.	0.63	.	15.89	.	Small red
Santa fe	75	0	2.94	.	2.20	.	0.74	.	19.85	.	Pinto
S08418	85	0	2.50	.	1.88	.	0.62	.	20.19	.	Pink
Loreto	85	0	1.46	.	1.10	.	0.36	.	15.05	.	Black
Medalist	68	0	0.86	.	0.72	.	0.14	.	17.39	.	Navy
Sdpi 1	93	0	3.19	.	2.40	.	0.79	.	.	.	Pinto
Ui 239	98	0	2.06	.	1.51	.	0.55	.	14.69	.	Small red
Ui 906	85	0	1.74	.	1.28	.	0.45	.	15.35	.	Black
Ac scarlet	78	0	2.06	.	1.56	.	0.50	.	18.42	.	Small red
I9365 31	81	0	1.91	.	1.47	.	0.44	.	.	.	Black
92bg 7	78	0	1.28	.	0.94	.	0.34	.	19.27	.	Black
Pindak	75	0	2.57	.	2.00	.	0.57	.	18.81	.	Pinto
Holberg	75	0	2.01	.	1.49	.	0.51	.	18.97	.	Pinto
Othello	95	0	2.54	.	1.85	.	0.68	.	21.67	.	Pinto
Uspt wm 1	88	0	3.08	.	2.26	.	0.82	.	20.01	.	Pinto
Victor	88	0	2.28	.	1.54	.	0.74	.	20.75	.	Pink
Harold	85	0	2.59	.	1.89	.	0.70	.	14.42	.	Pink
A801	77	0	1.33	.	1.05	.	0.28	.	20.33	.	carioca
Ndz06249	75	0	1.82	.	1.45	.	0.37	.	23.21	.	Small red
cdcwm 2	65	0	1.80	.	1.45	.	0.34	.	17.82	.	Pinto
Vision	71	0	2.17	.	1.54	.	0.63	.	17.45	.	Pinto
Big bend	91	0	2.94	.	2.04	.	0.90	.	15.29	.	Small red
UCD 9634	98	0	3.12	.	2.14	.	0.98	.	19.71	.	Pink

† Missing data point

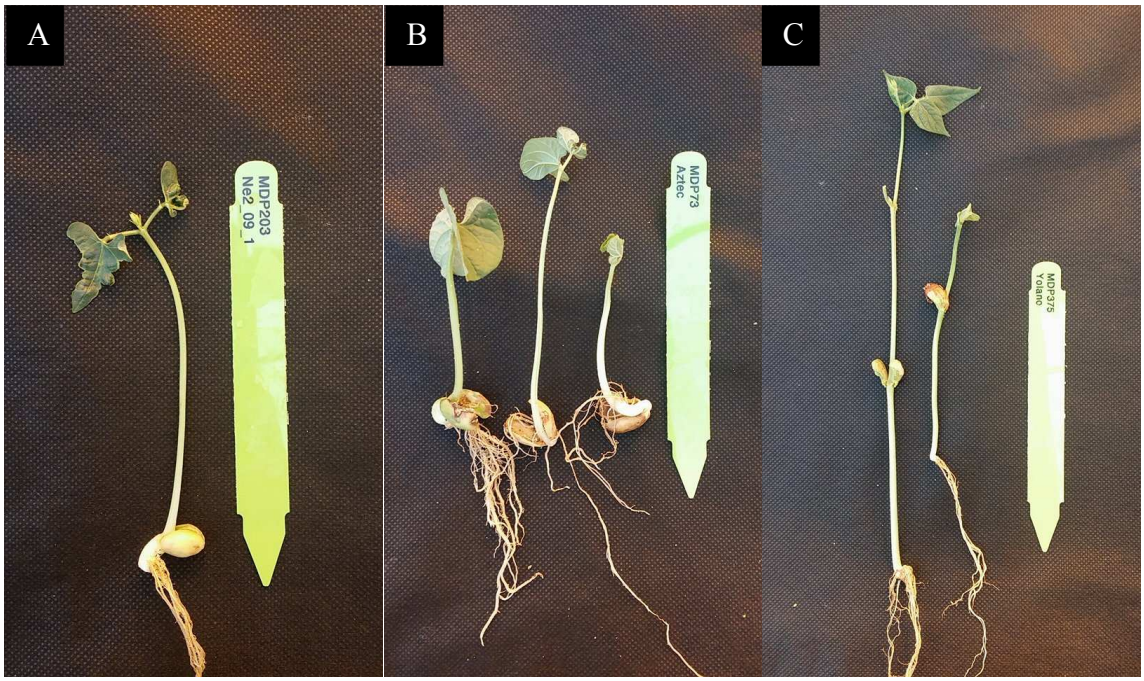

Figure A2. Abnormal morphology observed in pigmented seed coat genotype greenhouse screening. (A) Deformed primary leaves, (B) Hypogeal germination, and (C) Seedlings missing primary leaves.