UNRAVELING THE GENETICS OF SEED DORMANCY IN BARLEY USING GENOME-WIDE ASSOCIATION AND BIPARENTAL MAPPING

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Title

Unraveling the Genetics of Seed Dormancy in Barley using Genome-Wide Association and Biparental Mapping

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

DOCTOR OF PHILOSOPHY

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ABSTRACT

Seed dormancy is the delay or inability of viable seeds to germinate under favorable conditions. The differential expression of dormancy levels in barley (*Hordeum vulgare* L.) seeds impacts malt quality. While dormant genotypes are required to avoid the incidence of preharvest-sprouting, genotypes with low dormancy are needed for uniform germination of seeds during malting. The objective of this study was to determine the genetic basis underlying seed dormancy in spring barley using genome-wide association mapping (AM) and linkage mapping. A panel of 3,072 elite U.S. spring barley breeding lines from eight breeding programs participating in the USDA-NIFA Barley Coordinated Agricultural Project and 193 F_1-derived doubled-haploid lines from the cross 'Stander'/ 'Robust' were used to map QTL controlling seed dormancy. The AM panel and the doubled-haploid population were genotyped with SNP markers using the Illumina Golden Gate assay. Four mixed linear models that controlled population structure and kinship were used for the AM analyses, while composite interval mapping was used for the analysis of the biparental population. Our results confirmed the existence of marker-trait associations delineating two QTL regions in the long arm of chromosome 5H (5HL) using the AM panel, and a large effect QTL in the same region using the biparental population. The locations and effects of these marker-trait associations are congruent with previously mapped QTL for seed dormancy and demonstrate the two mapping methods effectively targeted the same genetic regions on the barley genome and provide insights about the genetics of seed dormancy.

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I would like to express my appreciation and gratitude to the Department of Plant Sciences faculty and office staff, in particular to Drs. Ed. Deckard, Ted Helms, and Phillip McClean, and

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Finally but not least, I would like to thank my mother Patricia and family in Colombia and in here for encouraging and supporting me all these years. Undoubtedly, I thank my husband Baird Heilman for his support, understanding, patience and love.

DEDICATION

This dissertation is dedicated to the memory of my mother Patricia and to my auntie Mercedes for whom everything was possible, and who inspired me to set my goals with the confidence that dreams may come true. This is also dedicated to my husband Baird and for those not yet born.

PREFACE

This dissertation includes the result of all my work and understanding about the phenomenon of seed dormancy in barley, which was carried out over the period from January 2009 to June of 2013 in the Department of Plant Sciences at North Dakota State University. Four years ago what started as “baby-sitting” plants in the greenhouse, harvesting spikes at the right maturity level, and counting thousands of seeds, turned into a great journey that helped me to unravel some of the intrinsic genetic and physiological relationships behind this trait, and at the same time help me to overcome some of my difficulties in mathematics. Certainly, I would not have reached to this point if it was not for the valuable help and contributions made by others, specially my advisor Dr. Richard Horsley and Dr. Sujan Mamidi, who believed that something good was about to come from this research. Their constant motivation, support and discussions helped me to address fundamental questions, while opening my mind to new ideas.

The following thesis dissertation contains four chapters. Chapter I includes an introduction followed by the literature review portion, which describes some of the general aspects concerning barley origin and dissemination, the genetics and physiology of seed dormancy, as well as the implications that breeding for malting quality traits has over the selection of barley cultivars with lower levels of seed dormancy. Chapter II gives a broad overview of the genome-wide association tools utilized to identify significant marker-trait associations for seed dormancy using a panel of elite U.S. spring barley breeding lines, as well as a description of the putative gene functions associated with them and their corresponding role in barley physiology. Chapter III gives an insight about the genetic mechanisms underlying seed dormancy in a narrowed genetic base population and provides an idea of what happens inside those breeding programs that breed for malt quality traits. Chapters II and III are written as two

separate papers to be submitted for publication in the near future. Therefore, these chapters include an abstract, introduction, materials and methods, results and discussion, and references section. The references are specific for each chapter. Due to the similarity in genetic and statistical tools used, repetition does occur between chapters. Finally, Chapter V provides a general summary of the results and highlights important findings and conclusions.

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CHAPTER I. INTRODUCTION

Seed dormancy is a physiological phenomenon characterized by a delay in germination of viable seeds, which is modulated by several genetic and environmental factors. The inability of seeds to germinate is an adaptive trait that promotes the survival of the next generations until the right conditions appear (Foley, 2001; Finkelstein et al., 2008) and it's a process that is conditioned by the moisture, temperature, light and oxygen conditions present in the seed bed. However, the causes behind seed dormancy vary depending on the type of inhibition affecting the organism, which could be embryonic, physical, physiological or due to immaturity (Finkelstein et al., 2008). Hydrolytic enzymes that modify the endosperm are secreted by specialized tissues, such as the scutellum and the aleurone in response to plant growth regulators, including abscisic acid (ABA), gibberellic acid (GA), and jasmonate (JA). However, germination can be also be triggered by the exogenous application of other substances, such as hydrogen cyanide (Oracz et al., 2009).

Seed dormancy, seed viability, and germination are key factors affecting malting, which is the process where the endosperm is modified by changing its friability and increasing the enzymatic activity to provide malt with optimal brewhouse performance (Kay, 2005). However, a problem imposed by the differential expression of dormancy on the barley seeds may cause reductions in malt quality associated with either high or low levels of seed dormancy, which makes grains worthless for malting.

In programs that breed for spring malting barley, an important step in determining if a line should be kept for advancement to subsequent generations is to determine its malt quality. In the upper Midwest region of the U.S., the crop is harvested in mid- to late-August and the breeding lines are submitted to the USDA-ARS malt quality laboratory in Madison, WI between

October and November for evaluation. The decision on whether to advance or discard a line needs to be made before March, which makes it hard to determine if poor endosperm modification in a line is due to extended seed dormancy or inherently poor malt quality. Thus, lines with extended seed dormancy are often culled in favor of those with low seed dormancy and desirable levels of endosperm modification. This can lead into the selection of lines that may have acceptable malt quality profiles, but are susceptible to preharvest sprouting (PHS).

Two main approaches were utilized in this study to identify significant marker-trait associations with seed dormancy in barley. One includes the application of association mapping (AM) tools for the analysis of a panel of elite breeding lines representing the eight U.S. spring barley breeding programs, while the second approach includes the utilization of linkage mapping analysis for the study of an F_1-derived doubled-haploid (DH) population that has a narrowed genetic base. My main goal was to gain a better understanding of the genetics underlying seed dormancy and to identify single nucleotide polymorphisms (SNPs) that may be candidates for use in marker-assisted selection (MAS) for each of the breeding programs, with special attention on the Midwest U.S. six-rowed barley germplasm, which has a narrow genetic base.

This review focuses on some physiological aspects of the seed germination, including some remarks about the function of hydrolytic enzymes and their interactions with starch granules during the pre and post-germination processes, as well as the role of ABA, GA and JA hormones and their impact on germination. Briefly, aspects on the domestication, geographical distribution, breeding and genetics of barley crops are critically discussed to give light of two physiological phenomena (dormancy and PHS) that affect the process of starch modification and ultimately malt quality.

Literature review

Introduction

Seed dormancy and PHS are complex traits controlled by several quantitative trait loci (QTL), which creates a large variation in dormancy expression patterns among barley genotypes (Buraas and Skinnes, 1984; Gu et al., 2005). Because of low levels of seed dormancy are preferred by the malting industry, barley breeders have been forced to select for non-dormant genotypes or those with low seed dormancy. The combination of non-dormant genotypes and the occurrence of adverse climatological conditions, such as rainy, damp and cold temperatures may lead to the occurrence of PHS (Lin et al., 2008). Such pre-germinated barley seeds not only exhibit unacceptable levels of germination, but also can imbibe more water during the steeping process, which might induce the formation of mold that can decrease malt quality (Brookes, 1980; and Sole, 1994 cited by Lin et al., 2008). Ultimately, PHS causes financial losses for growers and processors.

Physiological and genetic studies have confirmed the importance of the phytohormones GA, ABA, and JA, and their interactions in seed dormancy and PHS responses in cereals (Barrero et al., 2009). Different methods have been utilized to determine the mode of action of these hormones and their role in the activation/deactivation of genes during the germination process. The discovery of barley mutants exhibiting defects on grain morphology, coupled with the use of biotechnology approaches, which include transgenics, genetic mapping, enzymology, expression analysis and in general all the –onomics approaches have given new insights about the molecular mechanisms controlling seed dormancy and PHS (Green et al., 1997; Jensen et al., 2003; Finkelstein et al., 2008 Barrero et al., 2009).

Different classifications have been proposed for seed dormancy based on the type of inhibition, which could be embryonic, physical, physiological or due to immaturity. There are classifications methods that take into account the controlling structures or substances that are derived from the embryo or the surrounding tissues (Finkelstein et al., 2008). According to Foley (2001) a less complex classification method is one that distinguishes two types of dormancy (primary and secondary) and two categories (seed coat-imposed and embryo imposed). The type refers to the period of time where dormancy is developed, while the category makes reference to the structures or mechanisms that impose a constraint for germination (Foley, 2001).

After removal from the mother plant and imbibition under optimal conditions, mature seeds may show low germinability, which is referred as primary dormancy. On the other hand, secondary dormancy appears in after-ripened seeds as the result of their exposure to prolonged unfavorable conditions (e.g temperature, light, substances) (Foley, 2001; Finkelstein et al., 2008). The mechanisms by which dormancy can be overcome in some species include the use of scarification, after-ripening, stratification, or the exposure to light. Scarification refers to the mechanisms by which the seed coat tissues are removed; these may include chemical or physical methods (e.g. acids/fire). After-ripening refers to a period of dry storage that is needed to overcome seed dormancy, while stratification is associated with the requirement of chilling or moist conditions. In certain plant species, light may induce or reduced the potential for germination depending on the degree of seed dormancy present at the moment of imbibition (e.g. fully dormant, intermediate, non-dormant) (Foley, 2001; Finkelstein et al., 2008). In the particular case of cereals, scientist have observed that blue light mimics the effects of white light, which affects seed germination by repressing jasmonate production. Such repression could be

occurring at the level of lipid hydrolysis, which could affect the production of precursor molecules for jasmonate (i.e., linoleic acid) (Jacobsen et al., 2013)

Benech-Arnold et al. (2006) suggested that dormancy of the barley grain is imposed by the covering structures (i.e., lemma, palea, pericarp and seed coat) based on the observation that embryos can germinate well when isolated from the rest of these structures. These layers form a barrier that delays oxygen diffusion, which they believe may result in increased levels of ABA induced by hypoxia (Benech-Arnold et al., 2006)

To date we have seen that most mechanisms underlying seed dormancy and germination have been correlated with changes in gene expression, hormone accumulation and sensitivity, enzyme activity and environmental factors acting together, which reflects the complexity of this trait.

Germination and mechanisms for dormancy induction and maintenance

Seed development and germination are separated by a quiescent period, which sometimes is incorrectly referred to as dormancy. Quiescent seeds have the capacity to fully germinate; however due to limiting external conditions (i.e., light, oxygen, moisture, termperature) such seeds cannot complete the germination process (Foley, 2001; Sreenivasulu et al., 2008). During seed maturation barley kernels prepare to germinate; however primary dormancy may prevent germination. Primary dormancy is owed to the presence of the covering structures (i.e., glumellae) that imposed a physical barrier for the diffusion of water and oxygen into the embryo (Bradford et al., 2008). Secondary dormancy can be acquired after harvest of non-dormant grains or after the release of primary dormancy of dormant grains if grains are exposed to unfavorable environmental conditions, such high temperatures (30 °C) and high water content, which results in an increased expression of genes related with the catabolism of GA and

increased in ABA content (Hoang et al., 2012). On the other hand, seed dormancy present at harvest is gradually lost during after-ripening (dry storage) as a consequence of the expansion in temperature ranges and the exposure of the seeds to certain environmental conditions that facilitate its germination (Foley, 2001; Bradford et al., 2008). Among scientist, it is widely regarded that the balance between abscisic acid (ABA) and gibberellins (GA) is required for the maintenance and release of seed dormancy. While ABA promotes the induction of seed dormancy, GA is involved in the release and progression towards germination. However, other major controlling elements also include jasmonate (JA) and its intermediates, nitric oxides (NO) and light (Figure 1.1).

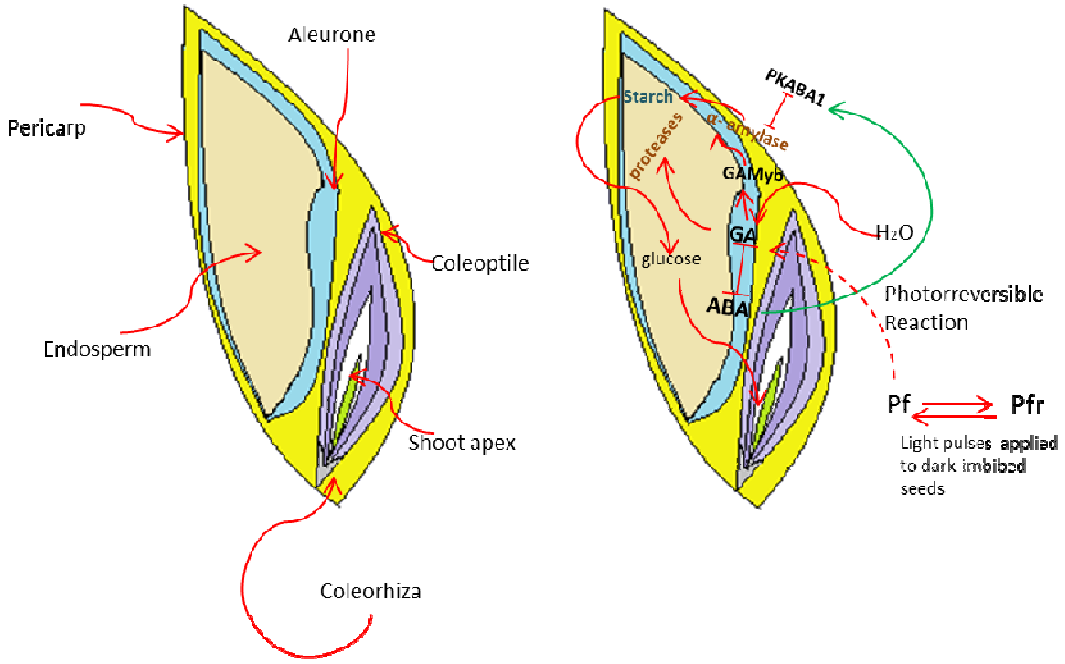

Figure 1.1. Longitudinal section of a barley caryopsis with a brief description of the main seed components and the physiological/genetic mechanism involved in the ABA and GA signal transduction pathways (adapted from http://plantphys.info/plant_biology/seedgerm.shtml accessed: 6 June, 2013; Gómez-Cadenas et al., 2001; Kamiya and García Martinez, 1999).

For the purposes of this review, a brief description of the major dormancy controlling elements will be described in order to integrate the current knowledge on what is seed dormancy and its occurence.

Abscisic acid

The accumulation of ABA is associated with the maintenance of seed dormancy and it has been detected in lower quantities in ABA-deficient mutants, or during the onset of germination due to changes in sensitivity and catabolism of ABA. Genetic studies have shown that ABA is regulated by the genotype of the mother plant, but it seems that the ABA regulated by the embryo is correlated with deepest levels of dormancy expression (Foley, 2001; Finkelstein et al., 2008).

Evaluation of the transcriptome of dormant and after-ripened barley embryos revealed that ABA sensitivity and its catabolism is promoted in after-ripened seeds by the differential regulation of the ABA-8'-hydroxylase, the *LIPID PHOSPHATE PHOSPHATASE* gene family and the *ABI3-INTERACTING PROTEIN2* genes, respectively. Barrero et al. (2009) suggested that the coleorhiza enhances dormancy by acting as a barrier for the root emergence, while after-ripening enhances the up-regulation of genes involved in the jasmonate (JA) and nitric oxide biosynthesis pathways, which seems to counteract ABA levels on the seeds. The concomitant effect of low ABA levels results in the degradation of the coleorhiza, the emergence of the root and germination of the grain (Barrero et al., 2009).

The catabolism of ABA in barley by HvABA-8'-hydroxylases (*HvABA8'OH1*) was observed to occur during after-ripening, while ABA synthesis is mediated by the expression of the gene *HvNCED1* (9-cis-epoxycarotenoid deoxygenase) in response to white light. Gubler et al. (2008) revealed that after-ripening did not have any effect on the expression of biosynthetic

genes for ABA, but it did promote the expression of ABA catabolic genes (i.e., *HvABA8'OH1*) and GA biosynthetic and catabolic genes (i.e., *HvGA3ox2* and *HvGA2ox3*, respectively) following imbibition (Gubler et al., 2008).

ABA has an important role not only in the maintenance of seed dormancy, but also in the protection of seeds against desiccation and the effects of active oxygen species (AOS) (Finkelstein et al., 2008). Late Embryogenesis Abundant (LEA) proteins have been associated with cellular tolerance to dehydration induced by drying, freezing or salinity conditions. Most of the genes encoding LEA proteins have ABA responsive elements (ABRE), as well as temperature responsive elements (LTRE), so its expression is induced by ABA, cold or drought (Hundertmark and Hincha, 2008).

Transcriptomic studies that characterize seed dormancy release in wheat (*Triticum aestivum*) suggested that a decrease in seed sensitivity to the growth regulators abscisic acid (ABA) and indole acetic acid (IAA) during the transition from dormancy to germination seems to be related with the transcriptional repression of a Protein Phosphatase 2c, SNF1-Related Protein Kinase2, ABA Insensitive5, Lipid Phosphate Phosphtase2, Auxin Response Factor, and Related to Ubiquitin1 genes. ABA inhibits seed germination by activating the transcription of genes involved in the catabolism of gibberellin products, while repressing the transcription of genes associated to chromatin assembly and cell wall modification (Jacobsen et al., 2013).

ABA biosynthesis. Abscisic acid is known to occur from a pathway involving carotenoid precursors. According to Milborrow (2001) and Xiong and Zhu (2003) the biosynthesis occur in three main stages, two occurring in the plastids and the final reactions happening in the cytosol: i) initial assembling of small phosphorylated intermediates as precursors; ii) early formation of the uncyclized C_{40} carotenoid phytoene molecule and cleavage of the 9'-cis-neoxanthin; and iii)

formation of an ABA intermediate known as xanthoxal and synthesis of ABA via ABA aldehyde (Milborrow, 2001; Xiong and Zhu, 2003). According to Xiong and Zhu (2003), the molecular and biochemical reactions involved in the production of ABA can be summarized (Figure 1.2) as follow:

1. Epoxidation of zeaxanthin and antheraxanthin to violaxanthin by the zeaxanthin epoxidase (ZEP). This reaction occurs in plastids (Marin et al, 1996 cited by Xiong and Zhu, 2003)
2. Conversion of violaxanthin to an epoxycarotenoid known as 9-*cis*-epoxycarotenoid and from this to a C_{15} intermediate known as xanthoxin by the 9-*cis*-epoxycarotenoid dioxygenase (NCED) (Schwartz et al., 1997 cited by Xiong and Zhu, 2003).
3. Transport of xanthoxin (C_{15}) to the cytosol and its conversion to ABA.
    a. Conversion of xanthoxin to ABA aldehyde by the dehydrogenase/reductase (SDR) (Xiong and Zhu, 2003 and references therein)
    b. Conversion of ABA aldehyde to ABA by the ABA aldehyde oxidase (AAO)/ molybdenum cofactor (MoCo) synthase (Xiong and Zhu, 2003 and references therein).

The active modification of carotenoids by NCED (9-*cis*-epoxycarotenoid dioxygenase) has been detected during the production of new leaves, which may have the concomitant effect of reducing the ABA biosynthesis, thus altering plants resistance to drought and oxidative stress (Cazzonelli, 2001; Du et al., 2010). Lower transcription levels of the NCED genes (i.e., NCED3) associated with a mutation on the locus *atx1* in Arabidopsis has been related with significant

reductions for the synthesis of ABA (Ding et al., 2011; Cazzonelli, 2011). Additionally, Leymarie et al. (2008) identified that the genes *HvNCED1* and *HvNCED2* are involved in ABA-mediated primary and secondary dormancy of the barley caryopses (Leymarie et al., 2008; Graeber et al., 2012).

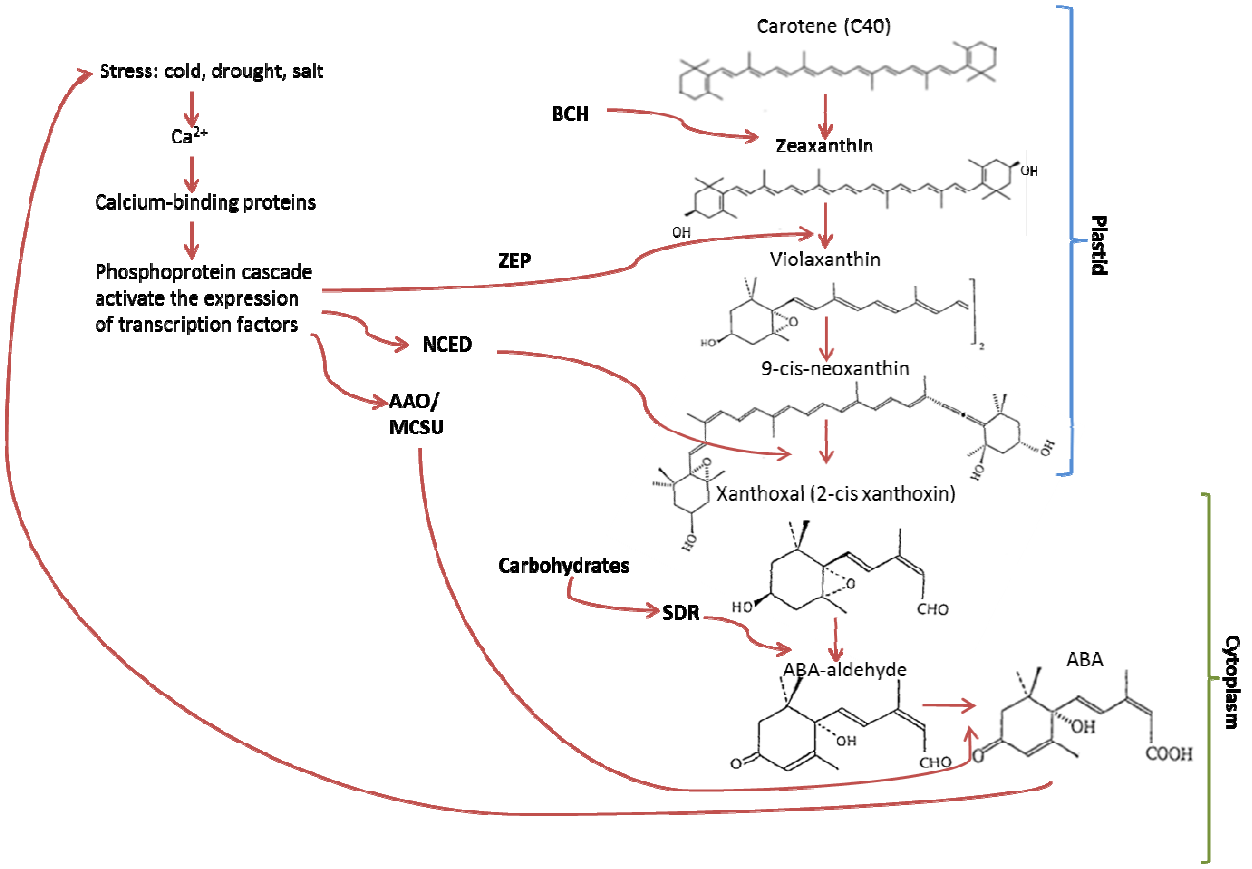

Figure 1.2. ABA biosynthetic pathway derived from the C_{40} epoxicarotenoid precursors and abiotic stresses associated with the expression of transcription factors associated with the degradation of carotenoids for the production of ABA (adapted from Xion and Zhu, 2003; Millborrow, 2001; and Mercadante,1999). BCH=β-carotene hydroxylase; ZEP=zeaxanthin epoxidase; NCED=9-*cis*-epoxycarotenoid dioxygenase; AAO/MCSU=ABA-aldehyde oxidase coupled with the MoCo sulfurase; SDR=alcohol dehydrogenase/reductase.

Gibberellins

Gibberellins belong to a big family of tetracyclic diterpenoid molecules that appear at the onset of germination and are involved in the control of several other physiological processes

including “stem elongation, root growth, leaf expansion, trichome development, flowering and, fruit development” (Israelsson, 2004 and references cited therein). According to Finkelstein et al. (2008) and Israelsson (2004), there are hundreds of tetracyclic diterpens (~136), but only few of these compounds stimulate biological responses in plants (i.e., GA_4 and GA_1). Their role in germination seems to be controversial since the use of GA treatments by itself does not induce the germination of dormant *Arabidopsis* seeds or does not necessarily stimulate germination in all species (Finkelstein et al., 2008). Bradford et al. (2008) identified that sensitivity to GA decreased with hypoxia, which helps to explain why GA may or may not stimulate germination in some species. However, studies by Gubler et al. (2008) in barley revealed that after-ripening promotes the expression of the GA biosynthetic and catabolic genes (*HvGA3ox2* and *HvGA2ox3*, respectively) following imbibition, while inducing the expression of genes for ABA catabolism (i.e., *HvABA8'OH1*). Seo et al. (2006) recognized that *Arabidopsis* ABA-deficient mutants (i.e., *nced6-1, aba2-2* and *aao3-4*), which were imbibed in the dark after irradiation with far red light, showed an enhanced ability to germinate compared to the wild type. These results suggest that ABA is antagonic to GA and is involved in the suppression of GA biosynthesis (Seo et al., 2006). Gibberellins induce the expression of hydrolytic enzymes that help to modify the endosperm and weaken seed coat tissues, which allow the subsequent mobilization of seed storage reserves, which help during the transition from the embryonic to the vegetative development (Finkelstein et al., 2008). There are several factors conditioning the regulation of GA, among them light is a critical factor for the seed germination of some species (Shinomura, 1997 cited by Seo et al., 2006). The effect of red light on the biosynthesis of GA was studied by Toyomasu et al. (1998) using lettuce seeds (*Lactuca sativa* L). Their results suggest that red light promotes the synthesis of GA_1 by inducing the expression of 3β-hydroxylases (i.e., Ls3h1) via

phytochrome regulation. While red light induces the germination of lettuce seeds, far-red light has the reverse effect if applied just after treating the seeds with red light (Toyomasu et al., 1998; Seo et al., 2006). It is also known that stratification (~4°C) induces the production of bioactive GA in *Arabidopsis* seeds. Using a mutant that affected the cold-inducible GA gene AtGA3ox1, Yamauchi et al. (2004) concluded that this gene plays an important role in seed germination and that both red light and GA deficiency act in conjunction to increase the transcript levels of the AtGA3ox1, which suggest this might integrate multiple signals to control seed germination in *Arabidopsis* (Yamauchi et al., 2004; Seo et al., 2006)

The theory of the antagonistic effects exerted by the balance between ABA and GA has been well supported by the observation that a non-germinating phenotype for a GA-deficiency locus can be overcome by an additional mutation in an ABA-deficiency locus (Koornneef et al., 1982 cited by Seo et al., 2006)

GA biosynthesis. Giberellic acid is derived from the complex isoprenoid pathway, where products are involved in the metabolisms of "hormones (i.e., ABA), photosynthetic pigments (i.e., carotenoids and phytol), electron carriers (i.e., ubiquinone and plastoquinone), membrane structural components (i.e., phytosterols), mediators of polysaccharide assembly (i.e., polyprenil phosphates)" among others (McGarvey and Croteau, 1995; Israelsson, 2004).

The base chemical structure of gibberellins is based of small five-carbon molecules (C_5) known as isopentenyl diphosphate (IPP) which join to form a direct precursor known as geranylgeranyl diphosphate (GGDP), which is a 20 carbon molecule (C_{20}). The IPP could be synthesized via the mevalonate-dependent reactions in the cytoplasm or through the mevalonate-independent pathway in the plastids (Figure 1.3; Israelsson, 2004; Milborrow, 2001). According to Israelsson (2004), the synthesis of gibberellins can be divided into three main steps: i)

formation of the *ent*-kaurene intermediates in plastids; i) synthesis of GA_{12} from *ent*-kaurene mediated by cytochrome P-450 monooxygenases present in the membrane of the endoplasmic reticulum; iii) formation and deactivation of C_{19}-GA molecules in the cytoplasm, which provides biologically active GA compounds (Figure 3).

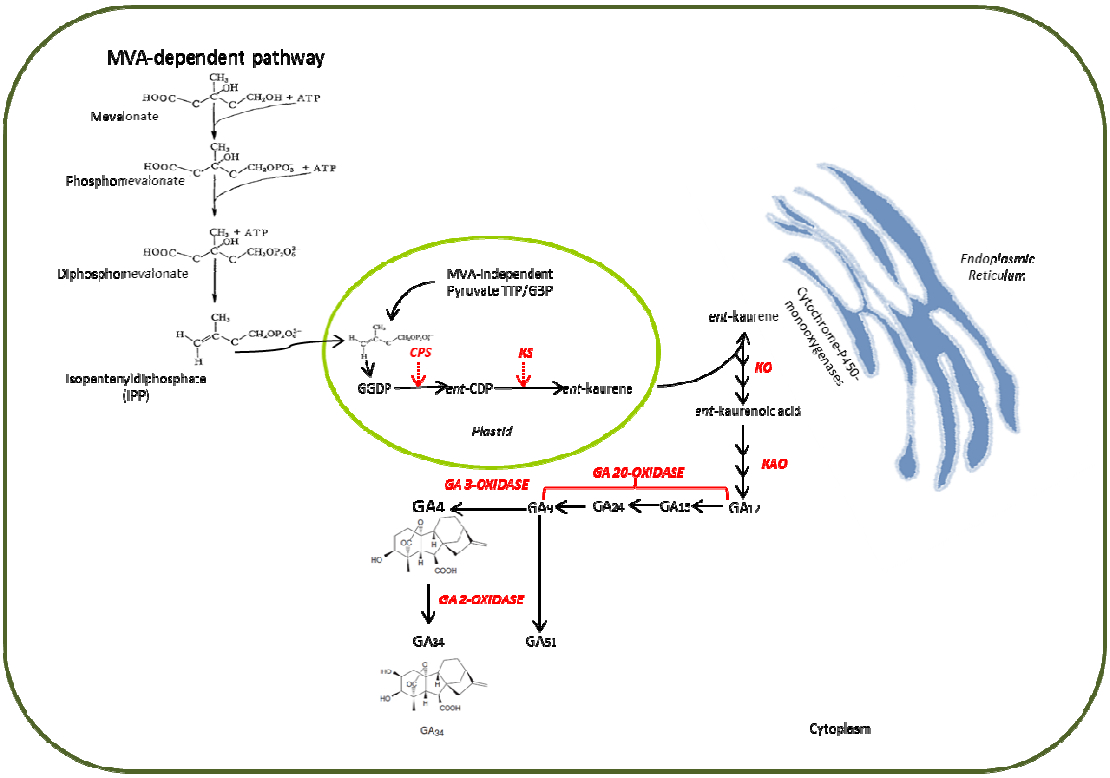

Figure 1.3. The GA biosynthetic scheme featuring the major steps for the biosynthesis of gibberellins in *Arabidopsis* (adapted from Israelsson, 2004; Milborrow, 2001; Yamauchi et al., 2004, 2007). MVA=mevalonate; IPP=Isopentenyldiphosphate; Pyruvate TTP= pyruvate triphosphate; G3P=Glyceraldehyde-3-P; GGDP=geranylgeranyl diphosphate; CPS=*ent*-copalyl diphosphate synthase; *ent*-CDP=*ent*-copalyl diphosphate; KS=*ent*-kaurene synthase; KO=*ent*-kaurene oxidase; KAO=*ent*-kaurenoic acid oxidase.

The conversion of GGDP into *ent*-kaurene in the plastids of *Arabidopsis* is catalyzed by the *ent*-copalyl diphosphate synthase (CPS) and the *ent*-kaurene synthase (KS) (Yamauchi et al., 2004; Israelsson, 2004). The *ent*-kaurene molecule is then transported to the cytoplasm where it is converted into GA_{12} by the action of membrane-bounded enzymes *ent*-kaurene oxidase (KO) and the *ent*-kaurenoic acid oxidase (KAO). While KO is present in the outer membrane of the

chloroplast, KAO is in the membrane of the endoplasmic reticulum (Israelsson, 2004, and references cited therein). Finally, once GA_{12} is synthesized in the cytoplasm there are two metabolic pathways (i.e., the early non-hydroxylation and the early 13-hydroxylation pathways) that lead to the formation of active GA forms. The occurrence of one or the other seems to be species-dependent (Kamiya and García-Martínez, 1999; Israelsson, 2004). Figure 3 only shows the metabolic pathway that corresponds to the early non-hydroxylation mechanism, which has been proposed for *Arabidopsis* (Yamauchi et al., 2004). Kamiya and García-Martínez (1999) highlighted in their review that the early non-hydroxylation pathway is utilized more in *Arabidopsis* and cucumber (*Cucumis sativus*), compared with cowpea (*Vigna unguiculata*), rice (*Oriza sativa*) and lettuce (*Lactuca sativa*), in which the early 13-hydroxylation pathway is more common (Figure 1.4).

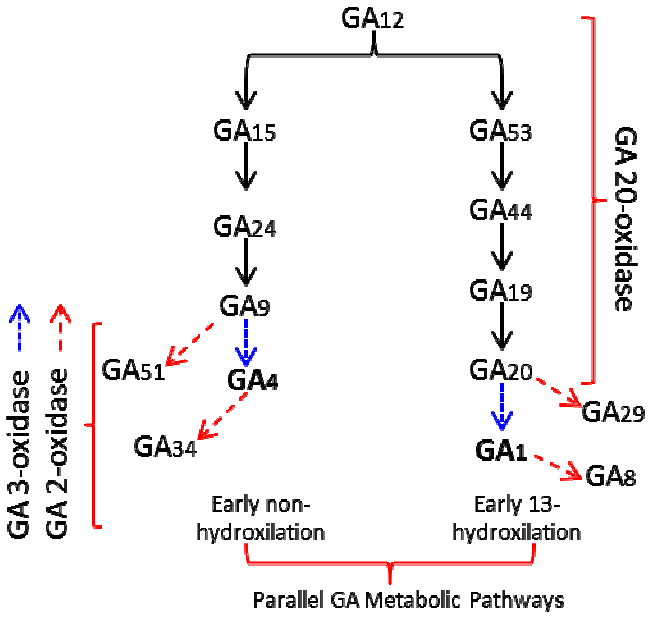

Figure 1.4. Two biosynthetic pathways in plants that lead to the formation of active GA forms (adapted from Israelsson, 2004; Kamiya and García-Martinez, 1999; and Yamauchi et al., 2004).

Jasmonate and other elements controlling seed dormancy

Transcriptomic analyses of dormant and after-ripened barley embryos revealed there are other mechanisms involved in the dormancy maintenance or release which are associated with cell wall modification, JA responses, nitrate and nitrite reduction, mRNA stability, and blue light sensitivity processes (Barrero et al., 2009). Recent studies by Jacobsen et al. (2013) corroborated the results obtained by Barrero et al. (2009) in barley by using wheat caryopses. Their results indicate that blue light, nitric oxide (NO) and JA are important elements controlling seed dormancy. While blue light has an inhibitory effect on dormancy release, methyl-jasmonate (MJ) and NO had the opposite effect by controlling ABA signaling, resulting in lower levels of seed dormancy. Both MJ and NO required each other's presence in order to reduce seed dormancy of wheat grains. Blue light seems to have a negative effect on the production of MJ, which has been associated with dormancy release in wheat kernels, while darkness seems to promote the production of MJ, which has been associated with germination of dormant seeds and defense (Jacobsen et al., 2013).

Endosperm modification

Even though most of the efforts in the enzymology research of germinated barley grains have been directed toward the identification, characterization, and improvement of α-amylases; there are other carbohydrate-hydrolyzing enzymes that have been isolated and characterized that are also involved in the catalysis of starches and their mobilization. Additionally, genes encoding for such enzymes have been mapped to six of the seven barley chromosomes (Fincher and Stone, 1993).

In barley and wheat (*Triticum aestivum* L.), the expression of starch degrading enzymes, especially α-amylases, has been used as an indicator of pre-germination of the grain, as well as a

sign of the hormonal status that is related to the grain maturity (Izydorczyk, 2004; Green et al., 1997).

The degradation of native starch granules is initiated by the α-amylase (1,4-D-glucanohydroxilase), which hydrolyses α-1,4 linkages binding the glucose molecules (Acquistucci et al., 2011). These enzymes have been grouped into a specific glycoside hydrolase family depending upon several aspects, including the genetic information, structural and amino acid constitution, sequence identity, homology, hydrophobic cluster information, as well as physico-chemical properties (Jensen et al., 2003, Delcour and Hoseney, 2010). Enzymology and genetic studies on germinated or malted grains indicate the existence of two main groups of α-amylases, known as α-*Amy*-1 and α-*Amy*-2. These have been classified on the basis of their isoelectric points (pI) differences, as well as other physico-chemical, and activity properties. The members of the low-pI, or α-*Amy*-1 have a pI value approaching 4.6; while the members of the high-pI, or α-*Amy*-2 exhibit pI values that approach 5.9. Both types are categorized as Ca^{2+} metalloproteins, which indicates that their expression and secretion are stimulated by the regulation of Ca^{2+} (Fincher and Stone, 1993). The α-*Amy*-1 enzymes have a remarkable stability at acidic pH, and are also known as “malt” or “germination” α -amylases. On the other hand, the α-*Amy*-2 enzymes, also known as the “green” or “pericarp” enzymes, exhibit a higher specific activity, but a lower affinity for maltodextrins and maltooligosaccharides compared to α-*Amy*-1. Furthermore, α-*Amy*-2 is specifically inhibited by the α -amylase/subtilisin inhibitor (BASI), and exhibits a lower activity towards insoluble blue starch at around 10 mM of Ca^{2+}; while the α-*Amy*-1 has its highest activity at 0.1-1 mM of Ca^{2+}. These features make α-*Amy*-2 less effective in the catalysis of starch grains as opposed to α-*Amy*-1.

Matthews and Jacobson (2001) demonstrated the potential for barley improvement using transformation technologies to produce transgenic barley containing genes encoding for low and hi-pI α-amylases, as well as α-glucosidases. Their results indicated that addition of an extra copy of the low-pI α-amylase gene makes little or no difference compared to the addition of an α-glucosidase gene. This is probably the result of the presence of several endogenous gene copies encoding for α-amylase (three low-pI and six high-pI; Chandler et al., 1984 cited by Jensen et al., 2003; Matthews and Jacobson, 2001), compared to one copy of the gene encoding α-glucosidase (Tibbot and Skadsen, 1996 cited by Jensen et al., 2003; Matthews and Jacobson, 2001). Jensen et al. (2003) suggested that an important impact could be made in the malting, kilning, and mashing processes if improved versions of the α-*Amy*-1 genes were used for the transformation of barley cultivars. This would directly translate into more stable enzymes, which could enhance their activity during the kilning and mashing processes, and thus improve the quality of the end product.

Barley domestication and dissemination

Domestication syndrome, genetic diversity, and geographical distribution

The genus *Hordeum* belongs to the monophyletic tribe Triticeae, which represents an evolutionary successful branch of the Poaceae family that evolved roughly 12 million years ago (Gaut, 2002; Schulte et al., 2009). The members of this tribe show a mode of speciation that includes a variety of polyploidization levels, along with interspecific and intergenetic hybridizations, which have allowed the evolution of different life forms, reproductive and dispersal mechanisms. Approximately 32 species have been assigned to the genus *Hordeum*, of which *H. vulgare* spp *vulgare* is a selfing diploid known as “cultivated barley” and *H. vulgare* spp *spontaneum* as “wild barley” (von Bothmer and Jacobsen, 1985; Salamini et al., 2002).

Fundamental transitions occurred between the cultivated barley and its wild relative during the domestication process. These transitions, also known as the "domestication syndrome", include the reduction in grain shattering due to the development of a non-brittle rachis, an increase in seed weight, appearance of naked seeds, decrease in seed dormancy, and changes from the two-rowed to the six-rowed spike type (Salamini et al., 2002; Pourkheirandish and Komatsuda, 2007; Sang, 2009). In wild barley, there are two lateral spikelets, which are reduced in size and assist in the dispersal of the central spikelet. In contrast, the six-rowed type has developed all three spikelets, which leads to an increase on the number of grains per spike. Such a trait did not change in the two-rowed barley compared to the wild form, and this has been attributed to the gene action exerted by the *Vrs*1 locus. Cultivated barley genotypes having a recessive version of the *vrs*1 locus exhibit a six-rowed spike, while those containing the dominant version (*Vrs*1) have a two-rowed morphology. The results of mutational studies on the *Vrs*1 locus allowed scientist to support the hypothesis that six-rowed barley was originated from a two-rowed type by mutation (Pourkheirandish and Komatsuda, 2007).

Another important adaptive trait that permitted expansion of barley production to different areas around the globe was the development of a spring growth habit. Three genes located on chromosomes 4H, 5H and 7H (respectively) have been associated with the differentiation of growth habits. The *Sgh*1, *Sgh*2 and *Sgh*3 genes are present in the spring growth barley genotypes, while their allelic versions (*Sgh*1, *sgh*2 and *sgh*3) are required for the winter growth habit (Pourkheirandish and Komatsuda, 2007). Winter barley requires vernalization and long days (LD) for determining flowering time while spring-sown genotypes do not require vernalization and have accumulated several mutations that make them LD-insensitive. A reduced response to photoperiod in spring-growth barley genotypes allowed them to accumulate

more biomass as a consequence of an extended vegetative growth. Such characteristics directly translate into higher yield benefits, and major probabilities for the expansion of barley to higher latitudes.

Like many other cereal crop species, barley originated in the Eastern Mediterranean area known as the Fertile Crescent (Rasmusson, 1985). This region includes the current territories of central Israel, Jordan, Syria, Lebanon, Turkey, Iraq and Iran. The expansion of barley around the ancient world began approximately 8,000 years ago, spreading through ancient routes to Greece, North Africa, the Nile and Ethiopia, as well as Iran and India. Approximately 2000-3000 years later, this crop was also found in Northern Germany, Southern Scandinavia, and China (von Bothmer et al., 2003). It is believed that crosses between cultivars and wild species followed by artificial selection resulted in the appearance of the current barley diversity (Sang, 2009).

It seems that the spread of grain crops through the world occurred in response to (1) climate changes, (2) growth in population sizes that forced human migrations into less dense areas, (3) domestication of animals (i.e., cattle) that were used to carry loads of grain, and (4) commercialization of products. According to Ensminger (1994) an unusual warm climate between the 5500 and 3000 B.C. caused the melting of the snow in mountain regions of Europe and Russia, pushing farmers from Macedonia to migrate to the Balkans and Central Europe. However, the establishment of barley and wheat crops only occurred until climate temperatures cooled down.

Recent studies have demonstrated that temperature and precipitation gradients played a major role in the adaption and shaping processes of the population structure of wild barley species in the Fertile Crescent area (Hübner et al., 2009). Changes in temperature and humidity during the development of modern barley crops have also been associated with changes in the

dormancy release patterns and PHS responses. If maturing grain is exposed to rainy and warm temperatures, this leads to an immediate loss of seed viability and quality. When high temperatures occur during grain fill, this may alter the hormonal metabolism of the embryo, which contributes to the disruption of dormancy and triggers the occurrence of PHS (Gualano and Benech-Arnold, 2009). Also, low temperatures and frost conditions play an important role in the activation of molecular mechanisms that turn on plant frost tolerance functions and vernalization responses (Kosová et al., 2011).

The patterns of differentiation and evolution of cultivated barley landraces, as assessed by the use of nuclear loci and morphological traits, indicate that South and East Asian barley types are genetically distinct from those in Europe and North Africa. Such differences might be the result of differential migration of barley from its two domestication centers of origin during the Neolithic age period (Saisho and Purugganan, 2007) and resulted in the development of distinctive morphotypes and growth habits in response to the local environments in which crops were grown.

With the discovery of the new world, barley was introduced most likely during the second voyage of Christopher Columbus into the Americas (Wiebe, 1979). It is believed two main routes allowed for the dissemination of barley into the United States. The first route came through the east coast, while the second route came through the southwest area.

Because of the unbearable climate of the eastern seaboard, barley production was limited until settlements moved into western New York. Interestingly, it was found that the six-rowed cultivars from Europe grew much better than the two-rowed cultivars from England. The barley production then became popular for brewing purposes, which caused its spreading throughout the colonies, suitable or not (Wiebe, 1979). Nevertheless, climate factors, biotic stress, and

farmer's freedom formed the distributional pattern of barley crops grown in the U.S. (Weaver, 1943). There are four main regions devoted to the barley production in the US, and they are: the East, upper Midwest, West, and Southwest. Approximately, 80% of the production comes from the Midwest and West regions, where North Dakota is the largest producer, followed by Idaho and Montana (USDA, 2012 Small Grains 2012 Summary, September 2012 http://usda01.library.cornell.edu/usda/current/SmalGraiSu/SmalGraiSu-09-28-2012.pdf accessed: June 9, 2013). Most of the barley cultivars sown in the upper Midwest (including Minnesota, North Dakota, and South Dakota) are six-rowed malting cultivars, whose ancestry could be traced back to the Manchuria region of China (Rasmusson, 1985; Horsley and Harvey, 2011). Such introductions were adapted to the cold and dry climate conditions present in the area, and had acceptable levels of malt quality. On the other hand, the production of two-rowed barley is circumscribed to the West region, where the states of Idaho and Montana are the largest producers (USDA, 2012 Small Grains 2012 Summary, September 2012 http://usda01.library.cornell.edu/usda/current/SmalGraiSu/SmalGraiSu-09-28-2012.pdf accessed: June 9, 2013). Both states devote between 65-80% of their barley area to the production of malting cultivars, whose ancestry can be traced to introductions coming from central Europe. The East region, comprised by the states of Maryland, Pennsylvania, and Virginia produces on average less than 5% of the total barley area in the U.S. (USDA, 2012 Small Grains 2012 Summary, September 2012 http://usda01.library.cornell.edu/usda/current/SmalGraiSu/SmalGraiSu-09-28-2012.pdf accessed: June 9, 2013). However, the majority of this barley grown in this region are non-malting winter cultivars. Finally, the Southwest region, comprised by the states of Arizona and southern California, generally produces around 1% of the total U.S. total barley production.

Much of the barley grown in this region are non-malting spring barley cultivars that can be grown under irrigated conditions. A big proportion of the barley grown in the U.S. is produced for domestic use, and only a small percentage (around 15%) is exported as feed barley (Horsley and Harvey, 2011).

Breeding barley cultivars for malting quality

The production of new cultivars with improved malt quality and other favorable agronomical attributes starts with the selection of parents adapted to the specific production regions that already have acceptable malt quality. A breeding line is called "Malting-type" only when such line has been inspected and approved by an official private company or a non-profit organization, such as the American Malting Barley Association, Inc. (AMBA). Public and private breeding programs are involved in the development of new malting-cultivars, and the AMBA oversees a malt and brewing evaluation program in which prospective cultivars are evaluated.

Quality traits that receive the most attention of breeders, maltsters and brewers include: (1) grain protein and kernel plumpness, (2) malt extract, (3) enzymatic activity (i.e., α-amylase and diastatic power), (4) wort protein, (5) carbohydrate modification (i.e., β-glucan content and wort viscosity), and (6) protein modification or Kolbach Index (Horsley and Harvey, 2011), among others. One of the reasons why producing malting cultivars is a big challenge for the barley breeders, is that beer brands have their own specific recipes, which include different ratios of barley cultivars mixed into a brewing blend. Such blends provide the characteristic flavor to each brand, and a little change in the composition might compromise the quality, flavor, and consistency of the beer. Such requirements represent a cutoff that can only be overcome by the production of cultivars with similar malting attributes to the already existing malting cultivar, but

that have improved agronomic traits and/or disease resistance (R. Horsley pers. communication, 2012). Another limitation that affects the improvement and development of new malting cultivars in the upper Midwest of U.S. and the eastern Prairie Provinces of Canada is the narrow germplasm base of their respective collections. According to Horsley and Harvey (2011) the current breeding lines produced in these regions can be traced back to only fifteen accessions that were obtained at the end of the nineteen-century. How can traits within a narrow germplasm be improved? Rasmusson and Phillips (1997) suggested that gain from selection in a narrow germplasm could be achieved possibly by *de novo* variation and the occurrence of higher than normal rates of epistasis.

Genetics of seed dormancy and PHS: the genotype by environment interaction

Seed dormancy is a critical adaptative trait present in many species that is imposed during the latter stages of embryo development and prevents the germination prior to the complete maturation of the seed (Gubler et al., 2008). Multiple loci and the environment contribute to the genetic variation present in nature for this trait. In *Arabidopsis*, a large number of mutants in genes such as ABA-insensitive 3 (*ABI3*), Fusca 3 (*FUS3*) and leafy cotyledons (*LEC1* and *LEC 2*), which are non-dormant genotypes, show a deficiency in seed maturation. Additionally, mutants affected in the biosynthesis of GA (non-germinating mutants) and ABA (non-dormant mutants) has shown the importance of ABA in the induction and maintenance of dormancy and the opposing role of GA in the control of germination. Previous studies on *Arabidopsis* suggested that seed dormancy is specifically controlled by the *Delay of germination 1* (*DOG1*) gene family compared to other seed dormancy and germination genes that are usually involved in multiple physiological processes (Bentsink et al., 2006)

In barley, several cultivars are more prone to sprout as a consequence of the stringent selection exerted by breeders (Benech-Arnold et al., 2006; Gubler et al., 2008.) In 2006, Benech-Arnold et al. confirmed that dormancy of barley seeds is a trait typically imposed by the covering structures (lemma and palea, pericarp and seed coat). Their study demonstrated that embryos germinate well during earlier stages of development when they were isolated from the rest of the seed and incubated in water. Thus, limitation of oxygen supply to the embryo was suggested as the mechanism responsible for dormancy of covered seeds due to oxidized phenolic compounds present in the lemma and palea (Benech-Arnold et al., 2006).

Factors affecting the expression of dormancy and its release are determined by the genotype, the stage of the seed development, and environmental conditions present at that moment. Among other environmental factors affecting dormancy and its release pattern, temperature, precipitation, humidity, photoperiod, nitrogen level, global radiation and water deficit have been reported (Gualano and Benech-Arnold, 2009).

Pre-harvest sprouting in barley cultivars is enhanced if maturing seeds are exposed to rain and warm temperatures, which leads to the immediate loss of seed viability. In 2009, Gualano and Benech-Arnold reported on the effect of temperature during grain fill on the dormancy release pattern of five malting cultivars widely used in the Argentina. Their findings suggested that high temperatures during a sensitivity window during grain fill could be altering the hormonal metabolism of the embryo, leading to the disruption of dormancy and the manifestation of PHS. Changes in the thermal environment could be modulating the embryo response to hormones by reduction of the ABA sensitivity and increasing of GA sensitivity.

Alpha amylases effects on dormancy and PHS

Dormancy and PHS often are considered two separate processes governing the development of the embryo in response to internal and external factors. One of the methods that allows for identification of sprout damage in seeds is the presence of α-amylase activity. Alpha-amylases are key enzymes involved in the metabolism of starches, which act in the amylolytic breakdown of the α-1,4 bonds between the amylose and amylopectin sugars. This event is a prerequisite for seed germination and seedling growth in terms of energy production and provision of carbon structures to create new cellular components (Mitsui et al., 1996). Two indirect methods that estimate the levels of α-amylase in the grains are: the falling number (FN) and the stirring number (SN). Both methods provide estimates of the enzyme activity based on the starch pasting properties. The FN method is used more often in wheat (*Triticum* spp.) industry, while the SN method is commonly used in the malting barley industry (Lin et al., 2009).

Two main α-amylase families have been described in cereals and they are named α-*Amy-1* and -*2*, respectively (Derera, 1989). The α-*Amy-2* isozymes, have been detected in the pericarp of immature caryopses, but also have been observed later during stages of germination (Lunn et al., 2001; Mrva and Mares, 2001), while the α-*Amy-1* isozymes, have been detected during the germination process and PHS.

Synthesis of both type of enzymes occurs in the green pericarp tissues where they are more abundant during the early stages of the caryopsis development; however, their activity declines afterwards as the caryopsis reaches maturity (Mares and Gale, 1990; Lunn et al., 2001). This is true for most cultivars where trace amounts of α-amylases remain until maturity, although, high levels of α-amylase activity have also been detected during the later stages of

ripening in triticale and some wheat cultivars (Mrva and Mares, 1996). For this reason, researchers have divided them into two categories, termed “pre-maturity α-amylase” and “late maturity α-amylase”. Apparently, the production of pre-maturity α-amylases is associated with a delayed ripening of the grains caused by environmental factors such as cold temperatures, precipitation, and high humidity. In 1994, Andrzejczuk-Hybel et al. reported that triticale caryopses 9 d after pollination had low levels of α-amylase activity, but later the levels increased during caryopsis development. Their findings suggest that α-amylase activity in the earlier developmental stages may be related to the PHS level exhibited by the triticale cultivars (Andrzejczuk-Hybel et al., 1994) and also contradicts the results previously reported by Lindblom et al., (1989) cited by Andrzejczuk-Hybel et al., (1994) who found significant differences in the α-amylase activity at the end of the maturity stage of triticale cultivars.

In 1997, Pagano et al. reported that PHS susceptibility of sorghum cultivars is related to their α-amylase activity. In the PHS-resistant cultivar low levels of α-amylase were detected during the caryopsis development, while in the susceptible ones high levels were observed during grain maturation. In 2004, Izydorczyk suggested that pre-maturity α-amylase versions might be associated to PHS in barley, however, additional research should be done in order to address this question.

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CHAPTER II. GENOME-WIDE ASSOCIATION MAPPING FOR SEED DORMANCY IN THE SPRING BARLEY CAP LINES

Abstract

Seed dormancy, seed viability, and germination in barley (*Hordeum vulgare* L.) are key factors affecting malt quality. While certain levels of seed dormancy are required to avoid preharvest-sprouting (PHS) in the field, low dormancy is necessary to assure uniform germination of kernels during malting. The objective of this study was to employ genome-wide association mapping (AM) to identify QTL for seed dormancy using elite US spring barley breeding lines from eight breeding programs participating in the USDA- CSREES Barley Coordinated Agricultural Project (Barley CAP). Dormancy tests were performed on 3,072 lines. All 3,072 lines were genotyped using the Illumina GoldenGate assay using two 1,536-SNP arrays Barley Oligo Pool Assays (BOPA 1 and BOPA2), but only 2,965 lines were utilized for GWAS. Phenotype and genotype data were subjected to AM analyses using four linear regression models that controlled for population structure and kinship. A total of 40 AM analyses were performed, including separate analyses for each year within a breeding program and combined across years for each program. Two quantitative trait loci (QTL) regions in the long arm of chromosome 5H were consistently observed across programs and years where QTL have been reported previously. Other QTL specific to each breeding program and year also were identified. Common SNPs that could be used for marker-assisted selection (MAS) across breeding programs were found only on chromosome 5HL. Further studies need to be done to validate the efficacy of these SNPs for MAS in each breeding program, and to determine if the associations of seed dormancy with specific malt quality traits is due to linkage or pleiotropy.

Introduction

Among modern cereals, barley has undergone strong selection by plant breeders against extended seed dormancy in order to promote uniform and quick germination during malting (Oberthur et al., 1995). Seed dormancy is an adaptive trait characterized by the inability of viable seeds to germinate under favorable conditions (Foley, 2001; Li et al., 2004) and is a main factor contributing to PHS tolerance (Mares, 1984; Rodríguez et al., 2001; Chao et al., 2010). It has been well documented that there is large genetic variation underlying seed dormancy and PHS in common wheat (*T. aestivum* L.), rice (*Oryza sativa*) and barley, and that expression is strongly controlled by environmental factors and genotype x environment interactions (Buraas and Skinnes, 1984; Gu et al., 2005a; Lin et al., 2009; Chao et al., 2010).

The ultimate goal in malting is to maximize endosperm modification and increase enzymatic activity, which are imperative to produce fermentable sugars for the production of beer. However, a problem associated with low seed dormancy is the occurrence of PHS, which is characterized by the germination of seeds on the mother plant when maturing grain is exposed to rainy conditions (Gualano and Benech-Arnold, 2009). The occurrence of PHS in barley cultivars with low seed dormancy leads to decreased grain weight and malt quality by promoting hydrolysis of starch molecules and creating the perfect environment for attack by saprophytic pathogens (Li et al., 2004; Gualano and Benech-Arnold, 2009). In severe cases of PHS, the grain can be worthless for malting. On the other hand, dormant genotypes need a prolonged storage time before malting, which increases cost, as well as the probability of seed decay if problems occur during storage. Ultimately, dormancy and PHS cause financial losses for growers and processors.

In breeding programs for spring malting barley, an important step in determining if a line should be kept for additional testing in subsequent generations is to determine its malt quality. In the upper Midwest US, the crop is harvested in mid- to late-August, the breeding lines are submitted to the USDA-ARS malt quality laboratory in October and November for malt quality evaluation, and decisions on whether to advance or discard a line are made before March. It is not unusual to begin malting the lines in early November. A major problem with this scheme is that it can be difficult to determine if poor endosperm modification in a line is due to extended seed dormancy or inherently poor malt quality. Thus, lines with extended seed dormancy often are culled in favor of those with low seed dormancy and desirable levels of endosperm modification. This can lead to lines that may have acceptable malt quality, but are susceptible to PHS.

Traditional genetic studies for seed dormancy in barley have relied on use of biparental mapping, where at least one parent is unadapted. Up to 26 QTL have been mapped across the entire barley genome, with a large effect QTL reported in chromosome 5H proximal to the centromere (5HC) and in the telomeric region in the long arm (5HL; Oberture et al., 1995; Lin et al., 2009; Ullrich et al., 1993; Li et al., 2004a). While this mapping strategy is effective in identifying QTL controlling traits of interest in a population, it may not be effective in identifying QTL conferring differences in one's targeted germplasm. Often, a QTL identified by biparental mapping can span 10 to 30 cM, which results in low mapping resolution due to the restricted number of meiotic events (Zhu et al., 2008; Pasam et al., 2012). An alternative to biparental mapping is genome-wide association mapping (AM), which harnesses the genetic diversity and historical recombination present in natural populations. Association mapping methods allow for increased mapping resolution of polymorphisms to individual nucleotides or

single genes (Zhu et al., 2008; Wang et al., 2012). The use of AM tools promises to increase the speed and efficiency of breeding crops by predicting phenotypes based on the identification of functional polymorphisms associated with marker loci (Blake et al., 2012).

The Barley CAP facilitated the collaboration of scientists from the public and private sectors with the aim of developing and integrating the information of 3,072 SNP markers into a barley consensus linkage map (Close et al., 2009; Blake et al., 2012). This resulted in a platform for the genetic analysis of barley germplasm from across the US. Each of the breeding programs submitted 96 different lines (F_4 or more advanced) each year for a period of four years (2006-2009). The lines were genotyped in the USDA-ARS molecular marker laboratory of Dr. Shiaoman Chao in Fargo, ND and phenotypic data for agronomic and quality traits were collected each year by each breeding program or in collaborative trials (Blake et al., 2012). The barley breeding program at North Dakota State University (NDSU) was responsible for phenotyping seed dormancy in lines with the spring growth habit from eight breeding programs (USDA-ARS, Aberdeen, ID; Busch Agricultural Resources, LLC; NDSU six-rowed; NDSU two-rowed; University of Minnesota; Montana State University; Utah State University; and Washington State University. Both phenotype and genotype data are curated and stored in The Hordeum Toolbox data resource (THT <http://hordeumtoolbox.org/> accessed: 7 May 2013).

In the present study, I applied AM to seed dormancy data collected on 2,965 barley breeding lines submitted by the eight aforementioned spring barley breeding programs. My main goal was to gain a better understanding of the genetics underlying seed dormancy and to identify SNPs for each program that may be candidates for use in MAS.

Materials and methods

Plant materials

A total of 3,072 barley lines submitted by eight of the ten barley breeding programs participating in the Barley CAP were phenotyped for seed dormancy. Breeding programs submitting materials included those from the USDA-ARS-Aberdeen, ID; Busch Agricultural Resources, LLC; Montana State University; NDSU, two-rowed and six-rowed programs; the University of Minnesota; Utah State University; and Washington State University. Each program submitted 96 different lines (F_4 or later generations) each year from 2006-2009, but only those with complete phenotype and genotype data were included in this study (Table 2.1).

Table 2.1. Breeding programs participating in the USDA-CSREES Barley Coordinated Agricultural Project (CAP) from which lines were provided for seed dormancy phenotyping.

Spring breeding program	Number of lines evaluated
USDA-ARS – Aberdeen, ID	369
Bush Agricultural Resources, LLC	377
Montana State University	362
North Dakota State University (two rowed)	379
North Dakota State University (six rowed)	367
University of Minnesota	371
Utah State University	365
Washington State University	375
Total number of CAP lines analyzed	2965†

† Elite breeding lines (F_4 or advanced) submitted from 2006-2009.

To determine seed dormancy, the Barley CAP line entries from each year and two checks were assigned to experimental units using an augmented block design (Federer and Raghavarao, 1975) consisting of 27 blocks. The cultivars Stander (non-dormant) and Robust (dormant/moderately dormant) appeared in each block and each of the CAP entries appeared once in the experiment. Randomizations were performed using the software AGROBASE

Generation II v. 18.18.2 (2010 Agronomix Software, Inc.). Experimental units were 15.24-cm-diameter clay pots (Ceramo, Jackson, Missouri) containing a potting media of Sunshine LC 8 soil mix (Sun Gro Horticulture, Canada). Three seeds from a single entry were sown in each pot. All experiments were conducted in the greenhouse, with a photoperiod of 16 h day/8 h night and temperatures of 20 °C day/18 °C night, respectively. Osmocote Plus (Scotts, Maryville, OH) granular fertilizer was applied at the two-leaf stage, followed by weekly applications of a solution of 20-20-20 Jack's Peat Lite (JR Peters, Inc, Allentown, PA) at the recommended rate. Spikes from the three plants within a pot were harvested in bulk at physiological maturity. Visual indicators of physiological maturity included loss of green color from the glumes and the peduncle (Copeland and Crookston, 1985). Harvested spikes were placed in Ziploc®-type plastic bags and stored at -20°C until germination tests were performed as described by Lin (2007).

Germination test and statistical analyses

The percent of non-dormant seeds was determined using the protocol Barley 3-C of the American Society of Brewing Chemist (1999), with some modifications. The method consists germinating 100 kernels uniformly spread over two sheets of 90-mm Whatman® filter paper in 51-mm Petri dishes previously saturated with 4 mL of distilled water. Petri dishes were sealed with Parafilm M (Pechiney Plastic Packaging Company; Chicago IL) to maintain stable moisture conditions. Samples were incubated in the dark for 72 h at 20 ± 2˚C and relative humidity of 98% in a growth chamber (Percival Scientific; Perry, IA). The percent of non-dormant seeds was determined at 72 h as described by Lin et al. (2009). Data gathered from each test year were analyzed separately using the MIXED procedure of SAS v. 9.3 (SAS Institute, Inc. 2011), where block was considered a random effect and genotypes were a fixed effect.

The heritability of seed dormancy and its associated standard error were estimated on a family-mean basis using a modification of the method described by Holland et al. (2003) for the analysis of random lines in an augmented design,

$$h^2 = \frac{\sigma_G^2}{\sigma_G^2 + {\sigma_e^2}/{r}}$$

where σ_G^2=genetic component of variance, σ_e^2=experimental error variance, and r=number of replicates. Data for both checks were removed prior to the calculations. The variance and covariance parameters were calculated using the COVTEST and ASYCOV options of the MIXED procedure of SAS 9.3 (SAS Institute, Inc. 2011), where blocks were deemed random and genotypes fixed. Heritability estimates were calculated by the multivariate restricted maximum likelihood (REML) option of the MIXED procedure in SAS 9.3 (SAS Institute, Inc. 2011) and as described by Holland et al. (2003) and Holland (2006). Approximate standard errors for heritability were calculated using the delta method of Lynch and Walsh (1998). Matrix computations to estimate the standard errors were calculated using PROC IML (SAS Institute, Inc. 2011). Variance components were estimated using the REML option of the MIXED procedure of SAS v. 9.3 (SAS Institute, Inc. 2011).

Genotype data acquisition

All 3,072 germplasm lines from the eight spring barley participating programs were genotyped with the Illumina (San Diego, CA) GoldenGate assay (Fan et al., 2003) using two 1,536-SNP Barley Oligo Pool Assays (BOPA 1 and BOPA2) previously described in Close et al. (2009) and Blake et al. (2012). Three data sets (annotated alignment, SNP file, and traits) were downloaded from the THT in TASSEL (Bradbury et al., 2007) format using a minor allele

frequency (MAF) of 0.0 and maximum missing data of 100. Original files were formatted for further imputation analyses.

Association mapping analyses

Missing data imputation

To minimize the problems caused by missing genotype data values, imputation analyses were performed. The software package FastPHASE v. 1.3 (Scheet and Stephens, 2006) was used to impute missing genotype data at each of the 3,072 loci using the default parameters and the Expectation-Maximization algorithm was used to estimate the maximum likelihood. Only 2,768 markers having a MAF > 0.05 were considered for analyses herein.

Identification of polymorphic loci

Forty separate AM analyses were performed herein, including 1) analyses for each breeding program combined across the four years (eight analyses), and 2) separately for each breeding program for each of the four years (32 analyses). SNP markers identifying polymorphisms and having a MAF > 0.05 were selected for each of the further steps.

Population structure and kinship

Population structure, defined as the differential relatedness among individuals of different subsets (e.g. breeding programs), was initially inferred by Principal Component Analysis (PCA) using the PRINCOMP procedure of SAS 9.3 (SAS Institute, Inc. 2011). The first principal components (i.e., eigenvectors) that explained at least 25% of the cumulative variation were selected for subsequent analyses. An identical by state pairwise kinship (K) allele sharing matrix (Zhao et al., 2007) was calculated using the DISTANCE procedure and Gower method of SAS 9.3 (SAS Institute, Inc. 2011).

Linkage disequilibrium analyses

Genome-wide LD analysis was performed across breeding programs and years by making pairwise comparisons among SNP markers using the squared allele frequency correlations between pair of loci (pairwise r^2 statistic) as suggested by Hill and Robertson (1968). The squared value of the Pearson's correlation r^2 coefficient was calculated using the CORR procedure of SAS 9.3 (SAS Institute, Inc. 2011). Decay of LD within each chromosome and the whole genome was determined using non-linear regression methods as described by Remington et al. (2001) and implemented by the NLIN procedure of SAS 9.3 (SAS Institute, Inc. 2011). Separate LD analyses for each breeding program, using lines from all four years and for the whole panel were performed. The pattern and distribution of intrachromosomal LD was graphically depicted for each chromosome and the whole genome by plotting significant intra-chromosomal pairwise r^2 values against the genetic distance (cM) between markers. From a total of 3,072 SNP markers, only 2,522 with a MAF > 0.05 were included herein to identify the average LD decay for the genome-wide AM analyses. Cosegregating markers were removed and an r^2 of 0.5 was arbitrarily chosen as a cutoff point beyond which LD was likely due to genetic linkage. This level of LD indicates that the closest marker only captures 50% of the phenotypic variation.

Linear regression models used and identification of marker-trait associations

Four linear regression models comprising both general linear models (GLM) and Mixed Linear Models (MLM) were selected to determine *P*-values associated with tests for marker-trait associations. Descriptions for each of the four models (Naïve, P, K, and P+K) are provided in Table 2.2 and information can be found elsewhere (Pasam et al. 2012; Mamidi et al. 2011; Yu et

al. 2005). All analyses were conducted using the MIXED procedure of SAS 9.3 (SAS Institute, Inc. 2011).

The general linear regression model used herein follows the formula:

$$y = X\alpha + P\beta + Kv + \varepsilon$$

where y is the response vector for phenotypic values for seed dormancy, α is the vector of fixed effects related to SNP marker effects, β is the vector of fixed effects with regard to population structure, v is the vector of random effects for co-ancestry, and ε is a vector of the residual effects. X denotes the genotypes at the marker, P denotes the principal components from the PCA, and K is the Kinship-IBS identity matrix. The variances of the random effects where calculated as follows: Var (v) = $2K\delta_g$ and Var (ε) = $I\delta_R$, where K is the kinship matrix and I is an identity matrix. Diagonal elements in this matrix correspond to the reciprocals of the number of observations for the phenotypic data, while the off-diagonal elements are recorded as zero; δ_g is the genetic variance, and δ_R is the variance of the error term or residuals variance.

Table 2.2. Four statistical models used to identify marker-trait associations.

Linear regression model		Information present in the model
Naïve	$y = X\alpha + \varepsilon$	General Linear Model (GLM) without any correction for population structure. y is related to X, without correction for structure (PCA) or relatedness (K)
P	$y = X\alpha + P\beta + \varepsilon$	GLM with principal components used for correction of population structure and y is related to X. Principal components explaining a minimum of 25% of the cumulative variance were chosen.
K	$y = X\alpha + Kv + \varepsilon$	Mixed Linear Model (MLM) with the K-matrix used as a correction for population structure and y is related to X. Similarity, defined as identity in state, was used as the kinship matrix
P+K	$y = X\alpha + P\beta + Kv + \varepsilon$	MLM with principal components and the K-matrix as corrections for population structure. Principal components explaining 25% of cumulative variance were chosen.

The best linear model for each of the 40 data set combinations was identified using the method suggested by Mamidi et al. (2011), which is based on the estimation of mean square

difference (MSD) values. In this method, all marker *P*-values for each model are ranked from the smallest to the largest values, and MSDs are calculated as follow:

$$MSD = \frac{\sum_{i=1}^{n}\left(p_i - \frac{i}{n}\right)^2}{n}$$

where *i* denotes the rank number, p_i is the probability of the i^{th} ranked *P-value*, and *n* is the total number of markers. The linear model exhibiting the lowest MSD value for each analysis was deemed as the best.

Additionally, the positive false discovery rate (pFDR) for each of the markers with significant marker-trait associations ($P<0.001$) was estimated using the MULTTEST procedure of SAS 9.3 (SAS Institute, Inc. 2011) The estimated pFDR values allowed for correction of multiple marker-trait associations (Storey, 2002). The efficiency of the models was also estimated by comparing their ability to reduce the inflation of false positive associations by plotting the observed *P*-values versus the expected *P*-values (Kang et al. 2008). Uniform distribution of the observed *P*-values exhibiting minimal deviations from the expected *P*-values also served as criteria for model selection. The coefficients of determination (R^2) and the allelic means were calculated for each of the significant markers using the REG and STEPWISE procedures of SAS 9.3 (SAS Institute, Inc. 2011). For the stepwise regression, the defaults in SAS were used (significance level for entry is 0.15 and significance level for staying is 0.15).

Epistasis

Epistatic interactions between significant marker loci were estimated using a MIXED linear regression model with an interaction term using SAS v. 9.3 (SAS Institute, Inc. 2011). The significance threshold used to declare important epistatic interactions was $P<0.001$. Maps containing the network of epistatic interactions between significant loci were built using the software MapChart v. 2.2 (Vorrips, 2002).

Gene annotation

Significant SNP markers found to be associated with seed dormancy were cross referenced with information provided in Table S4 by Close et al. (2009), which included relevant information about BLAST hits to the rice and Arabidopsis genomes, as well as the corresponding Uni-Prot information. Additional information was also gathered from HarvEST v. 1.83 (http://harvest.ucr.edu/ accessed: 15 March 2013), which contains a BLAST server that supplies information for 2,943 mapped SNP unigene sequences that can be used as queries in the database (Close et al., 2009)

Results and discussion

Phenotypic data

The malting process of barley is comprised of three major steps: steeping, germination, and kilning. Since differential expression of dormancy levels of barley seeds can impact germination and malt quality, a collection of 3,072 CAP lines was evaluated to determine the genetic basis of seed dormancy. Of these, only 2,965 lines having both phenotype and genotype data were used for the analyses.

The phenotypic distribution of percent non-dormant seeds for each breeding program showed marked differences from year to year (Appendix Figures A1-A8). Additionally, the individual means varied greatly from one year to another, which is partly the result of the utilization of different elite breeding lines (F_4 or more advanced generations) in each of the four years (Table 2.3). The phenotypic distribution of percent non-dormant seeds for all breeding lines was continuous (KS =0.145; $P<0.01$), with means of individual lines extending beyond the checks. Robust, the dormant check and Stander, the non-dormant check, behaved as expected for seed dormancy (Figure 2.1). The normality tests (Kolmogorov-Smirnov *D* statistic) indicated

that percent non-dormant seed values did not follow a normal distribution in most breeding programs, with the exception of the NDSU two-rowed and six-rowed programs in 2006, NDSU six-rowed in 2007, and Montana State University in 2008. The *P*-values for the analysis of each breeding program across four years (D=0.145; P<0.01) support the results obtained in the individual analyses (Table 2.4).

Based on the histogram distribution plots, it appears that seed dormancy data generally follow a bimodal distribution, which suggest the action of major genes or large QTL effects controlling the trait. Previous studies on barley and other cereal crops, including rice and wheat, led to identical conclusions based on the study of wild and cultivated accessions, half diallel crosses, BC, and F_2 populations (Gu et al., 2003, 2005; Mares et al., 2005; Wan et al., 2006; Andreoli et al., 2006; Takeda and Hori, 2007; Torada et al., 2008).

Heritability estimates ranged between 0.21 ± 0.07 to 0.82 ± 0.06 (Table 2.5), with the lowest values generally observed for the breeding materials submitted in 2007 and highest values for the materials submitted in 2006. Differences in the range of heritability values for dormancy from year to year for a breeding program suggest that selection for seed dormancy has not been a priority in the breeding programs. Traits, such as heading date, which receive high attention because of their importance to adaptation, often have heritability values within narrower ranges (Pasam et al., 2012). It is thought that selection for low seed dormancy in breeding programs has stretched to a level where most commercial cultivars are non-dormant or moderately dormant in order to promote uniform and quick germination of the kernels upon imbibition, which is a main prerequisite for malting (Oberthur et al., 1995; Li et al., 2004).

Table 2.3. Mean, minimum and maximum values for percent non-dormant seeds across four years and eight breeding programs participating in the USDA-CSREES barley CAP project.

Breeding program	2006			2007			2008			2009		
	Range		Mean	Range		Mean	Range		Mean	Range		Mean
	----------%----------			----------%----------			----------%----------			----------%----------		
USDA-ARS – Aberdeen, ID	0	100	56.0 ± 31.2	0	29	4.1 ± 5.9	0	76.7	26.5 ± 25.0	0	100	43.6 ± 24.8
Bush Agricultural Resources, LLC	5	100	78.6 ± 25.8	0	52	11.5 ± 12.5	0	75.0	22.1 ± 20.0	0	100	46.1 ± 19.9
Montana State University	0	100	65.0 ± 31.2	0	27	4.71 ± 5.8	0	71.6	28.9 ± 21.5	0	94	34.4 ± 24.1
North Dakota State University (two row)	0	100	49.3 ± 29.5	0	54	10.3 ± 10.2	0	57.0	13.2 ± 13.4	0	90	40.8 ± 15.2
North Dakota State University (six row)	14	100	62.3 ± 23.2	0	56	18.6 ± 12.7	0	45.0	10.6 ± 11.0	0	92	41.4 ± 23.2
University of Minnesota	3	100	69.0 ± 29.7	0	44	5.7 ± 9.0	0	82.0	24.8 ± 27.5	0	100	35.2 ± 19.8
Utah State University	0	98	27.7 ± 24.1	0	58	4.2 ± 9.8	0	86.2	21.5 ± 22.7	0	90	37.5 ± 21.1
Washington State University	0	100	42.6 ± 33.4	0	33	3.2 ± 6.5	0	70.0	17.4 ± 18.8	0	100	34.4 ± 23.3

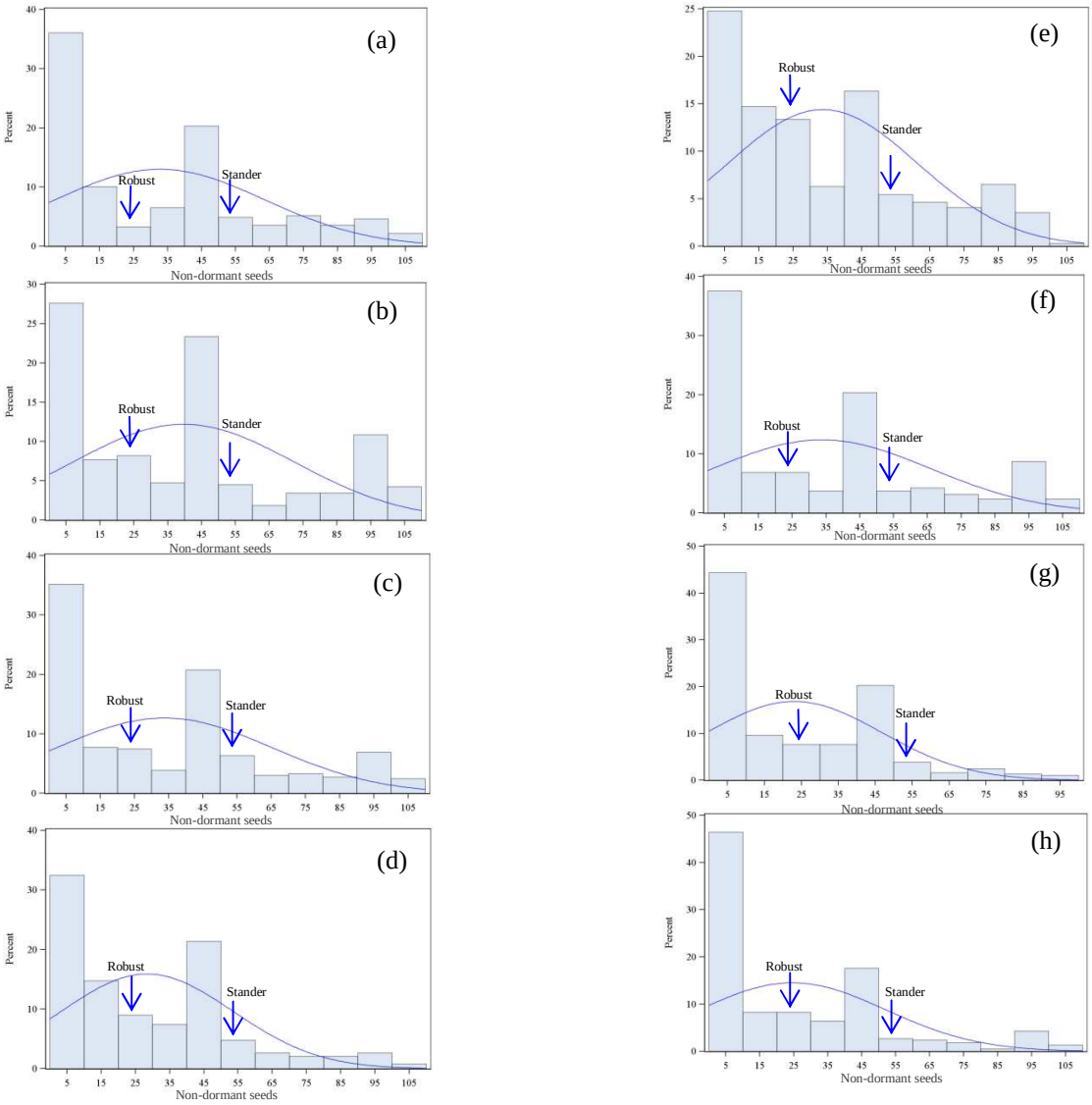

Figure 2.1. Phenotypic distribution of percent non-dormant seeds, averaged across four years, for spring barley lines from the: (a) USDA-ARS Aberdeen, ID, (b) Busch Agricultural Resources, LLC, (c) Montana State University, (d) North Dakota State University (NDSU) two-rowed, (e) NDSU six-rowed, (f) University of Minnesota, (g) Utah State University, (h) and Washington State University barley breeding programs. The arrows indicate the means for Robust (23.6) and Stander (52.9) across four years. The X-axis represents the percentage of germinated seeds, while the Y-axis represents the observed frequency for each of the intervals in X.

Table 2.4. Goodness of fit test for a normal distribution of percent non-dormant seed data for each breeding program in 2006-2009, and across all years and breeding programs.

	2006		2007		2008		2009		2006-2009	
Breeding Program	D†	*P*-value‡	D	*P*-value	D	*P*-value	D	*P*-value	D	*P*-value
USDA-ARS – Aberdeen, ID	0.125	<0.010	0.253	<0.010	0.185	<0.010	0.294	<0.010	0.149	<0.010
Bush Agricultural Resources, LLC	0.231	<0.010	0.189	<0.010	0.148	<0.010	0.302	<0.010	0.114	<0.010
Montana State University	0.158	<0.010	0.246	<0.010	0.107	**0.022**	0.347	<0.010	0.142	<0.010
North Dakota State University (two row)	0.071	**>0.150**	0.157	<0.010	0.179	<0.010	0.330	<0.010	0.152	<0.010
North Dakota State University (six row)	0.090	**0.057**	0.104	**0.014**	0.167	<0.010	0.309	<0.010	0.128	<0.010
University of Minnesota	0.208	<0.010	0.262	<0.010	0.213	<0.010	0.344	<0.010	0.155	<0.010
Utah State University	0.130	<0.010	0.334	<0.010	0.173	<0.010	0.344	<0.010	0.167	<0.010
Washington State University	0.130	<0.010	0.314	<0.010	0.177	<0.010	0.332	<0.010	0.185	<0.010

†Kolmogorov-Smirnov (KS) statistic.

‡Values shown in bold indicate the data followed a normal distribution at $P \leq 0.01$.

The genetic analysis of seed dormancy in weedy rice (*Oryza sativa*) and its association with some adaptive syndrome traits (e.g. shattering, awn length, black hull color, and red pericarp color) revealed that seed dormancy QTL are flanked by one or up to four QTL of these multiple interrelated traits. The biological implication of this QTL organization indicates that reduction in seed dormancy could be the result of indirect selection against multiple interrelated adaptive syndrome traits than the selection against seed dormancy per se (Gu et al., 2004). Continuing with this idea, it seems reasonable to believe that low seed dormancy in barley could be the result of selection for other traits, including heading date, malt extract, and high α-amylase (Li et al., 2004).

Table 2.5. Heritability estimates for percent non-dormant seeds for each of the eight breeding program across four years (2006-2009).

Breeding Program	2006	2007	2008	2009
	----------h^2----------			
USDA-ARS – Aberdeen, ID	0.82 ± 0.06	0.24 ± 0.07	0.70 ± 0.08	0.75 ± 0.08
Bush Agricultural Resources, LLC	0.55 ± 0.14	0.55 ± 0.13	0.44 ± 0.11	0.60 ± 0.12
Montana State University	0.80 ± 0.07	0.21 ± 0.07	0.58 ± 0.10	0.56 ± 0.12
North Dakota State University (two row)	0.78 ± 0.07	0.32 ± 0.13	0.37 ± 0.09	0.45 ± 0.12
North Dakota State University (six row)	0.61 ± 0.12	0.56 ± 0.11	0.38 ± 0.08	0.56 ± 0.14
University of Minnesota	0.79 ± 0.07	0.27 ± 0.11	0.77 ± 0.06	0.64 ± 0.12
Utah State University	0.76 ± 0.06	0.35 ± 0.13	0.53 ± 0.12	0.68 ± 0.11
Washington State University	0.78 ± 0.06	0.27 ± 0.08	0.56 ± 0.11	0.65 ± 0.11

†Heritability estimates based on a family mean basis were calculated using formulas and SAS codes with some modifications as proposed by Holland et al. (2003).

Association mapping analyses

Polymorphic loci

In principle, imputation analysis allows one to estimate missing SNP genotypes using methods that rely on heuristics or expectation-maximization algorithms by comparing each individual and maker locus against the complete data from other individuals. The large amount of information gathered from the use of thousands of markers provides enough information to

impute the missing data with great accuracy (Li et al., 2009; Browning, 2008). From the 3,072 SNPs used to genotype the 2,965 lines, 3.5% of the alleles were missing and needed to be imputed. Among them, a subset of 2,768 markers having a MAF > 0.05 were used for identification of marker-trait associations on a mapping panel that included lines from all programs in all years. These markers were previously used in biparental mapping and other AM studies; therefore, they are considered herein. Information about the total number of polymorphic markers (MAF > 5%) utilized in each of the AM analyses, including the analysis of individual years for each breeding program, as well as the combined analysis across the four years for each breeding program is provided in appendix Table A1. The number of markers found to identify polymorphisms varied within and among breeding programs from year to year as the result of the utilization of different sets of elite breeding lines (~96 lines per year). Some of the highest levels of polymorphism were observed in the lines submitted by the Utah State University (2,608) and USDA-ARS-Aberdeen (2,556) breeding programs, while the lowest number corresponded to the materials submitted by the University of Minnesota (1,853) and NDSU six-rowed (2,055) programs.

Linkage disequilibrium analysis

Linkage disequilibrium, also known as gametic phase disequilibrium, is an estimate of the degree of non-random associations existing between alleles at different loci based on expectations for allele and haplotype frequencies (Zhu et al., 2008). In general, the causes of LD are the same as those for evolution (i.e., mutation, genetic drift, subpopulation structure, and migration). If the effect of selecting for superior genotypes is added, we should expect to see extensive and variable LD across the barley genome. Mapping resolution and the number of candidate genes that can be associated with a phenotype are strongly affected by the extent of LD

(Pasam et al. 2012; Waugh et al., 2009). The genome-wide LD patterns observed for each breeding program were variable (Figures A9 and A10). Linkage disequilibrium decay ranged from rapid in the Utah State University' s program ($r^2 \leq 0.5$ at 2 cM to $r^2 \geq 0.1$ at 7 cM) to extended in the University of Minnesota's program ($r^2 \leq 0.5$ at 4 cM to $r^2 \geq 0.1$ at 44 cM). From a total of 2,522 polymorphic SNP markers used, 908 non-cosegregating loci were identified. These markers covered approximately 1,090 cM of the barley genome, with an average of one marker every 1.2 cM. Results suggest the density of markers used in this study is sufficient for the identification of marker-trait associations for seed dormancy. Additionally, there were only seven intermarker distances >2.5 cM where the chances of finding a QTL were reduced.

Population structure

A model-based approach using PCA and kinship was implemented to determine population structure. The number of eigenvectors per combination of SNP markers that collectively explained at least 25% of the variation is summarized in Table 2.6. In each of the four years, a single principal component sometimes was sufficient to explain up to 35.4, 43.2, 37.7, and 35.3% of the variation for a single breeding program, while in other cases up to seven principal components were necessary to explain a minimum of 25% of the variation (e.g. the NDSU two-rowed program in 2006).

Comparison of models

Four linear models were utilized in this study (Naïve, P, K, and P+K) to detect associations between SNP markers and seed dormancy. In a self-pollinated crop like barley, the level of population structure is expected to be large due to the effect of non-random mating and relatedness, in addition to the pressure exerted by selection of important agronomic traits (Wang et al., 2012; Passam et al., 2012).

Table 2.6. The number of principal components (PC) included in each of the association mapping analyses and the percent of cumulative variance (%) explained by the principal components.

Breeding Program	Individual years								Four years (2006-2009)	
	2006		2007		2008		2009			
	PC	%	PC	%	PC	%	PC	%	PC	%
USDA-ARS-Aberdeen, ID	1	28.7	1	32.0	1	29.6	4	29.3	1	24.6
Bush Agricultural Resources LLC.	1	35.4	1	43.2	1	37.7	1	35.3	1	34.5
Montana State University	3	27.3	2	25.8	3	31.0	2	45.2	4	27.8
North Dakota State University (two-row)	7	26.0	5	28.0	4	30.3	5	27.4	10	25.7
North Dakota State University (six-row)	3	25.2	2	28.6	4	30.5	3	27.7	5	25.4
University of Minnesota	4	28.5	3	30.0	3	26.6	3	28.1	6	27.2
Utah State University	5	28.5	3	28.1	2	28.3	2	30.8	5	25.0
Washington State University	1	26.0	1	25.9	5	26.0	5	27.3	3	25.7

In order to detect significant marker-trait associations and reduce the confounding effect of population structure, I identified those models that performed "best" based on the MSD values (Table 2.7). The lower the MSD value, the better the model (Mamidi et al., 2011). Additionally, I assessed the effectiveness of these four models and their ability to reduce false positive associations by using the method described by Kang et al. (2008). Under the assumption that SNP markers are unlinked and there are only a few true associations, it is expected that the cumulative distribution of the *P*-values should approach a uniform distribution. Thus, a large deviation from the expectation indicates that the model may increase the chances to find spurious associations (Kang et al. 2008; Pasam et al., 2012). Based on this assumption, I identified those models containing kinship (K) or a combination of a structure component, P, and kinship to be significantly better. Eighteen of the P+K models and 16 of the K models generally had lower *P*-values than those in the Naïve or P-only models (appendix Figures A11-A19). Additional information about markers having a convergence with the best linear models is also provided in appendix Table A2.

Marker-trait association analysis and annotation

A concern often expressed with AM is that of false positives. I tried to reduce the number of false positives by using population structure and kinship as covariates in the analyses. A marker-trait association was considered significant if the marker main effect was significant at $P \leq 0.001$ [-log10 (0.001) = 3]. The number of markers significantly associated with seed dormancy based on the analyses of i) individual programs and years, and ii) across all years for each breeding program are summarized in Table 2.7.

In the combined analysis across programs and years, I identified four SNPs significantly associated with seed dormancy (data not shown). Three of the four SNPs mapped to the telomeric region of chromosome 5HL and explained 4.27, 5.14, and 6.05% of the phenotypic variation, respectively. The fourth SNP marker is unmapped, but explained up to 27.9% of the phenotypic variation. An inherent weakness in combined analyses across breeding programs with very different germplasm is that the results may not be meaningful for individual programs. In the AM analyses across years for each program, the number of significant marker-trait associations detected ranged from 20 in the NDSU six-rowed program to 104 in the Busch Agricultural Resources program (Table 2.7; appendix Figures A20-A27). However, in many cases for each breeding program, the chromosome region where significant marker-trait associations were identified in one year was not consistently detected in other years (Appendices Table A3-A10). This may be due, in part, to an insufficient population size (< 96 lines), lack of genetic diversity at specific loci, or both. To determine if specific SNPs may have utility for MAS, interpretation was done on analyses from individual years for each breeding program. Valuable markers for MAS must work across the germplasm base of a breeding program, which includes lines from a wide range of crosses and years. For this purpose, I considered SNP

Table 2.7. Best linear models selected based on the mean square difference (MSD) value and the number of significant makers ($P \leq 0.001$) associated with percent of non-dormant seed.

Breeding Program	Analysis	Linear Model†				Markers
		Naïve	P	K	P+K	
		---------- MSD‡----------				
USDA-ARS-Aberdeen, ID	4 years	0.0054	0.021	0.0414	**0.0018**	65
	2006	0.0427	0.0101	0.0032	**0.0031**	9
	2007	0.1119	**0.0006**	0.0098	0.0013	14
	2008	0.1347	0.0014	**0.0002**	0.0004	7
	2009	0.0175	0.0087	**0.0001**	0.0002	6
Bush Agricultural Resources LLC	4 years	0.0792	**0.0284**	0.0688	0.0285	104
	2006	0.0505	0.0409	0.0157	**0.0099**	52
	2007	0.1272	**0.0014**	0.0088	0.0111	41
	2008	0.1495	0.1862	0.037	**0.016**	8
	2009	0.0782	0.0027	0.0014	**0.0006**	5
Montana State University	4 years	0.0331	0.0701	**0.007**	0.024	61
	2006	0.0225	0.0059	0.0007	**0.0006**	5
	2007	0.0477	0.0643	0.001	**0.0008**	7
	2008	0.025	0.0227	**0.0016**	0.0023	1
	2009	0.0417	0.0337	0.0026	**0.0024**	3
North Dakota State University (two-row)	4 years	0.033	0.0127	0.0212	**0.01**	39
	2006	0.0046	0.0009	**0.0007**	0.001	12
	2007	**0.0009**	0.0032	0.0009	0.0017	29
	2008	0.0049	0.0059	0.0014	**0.0006**	18
	2009	0.0069	**0.0013**	0.1072	0.0039	8
North Dakota State University (six-row)	4 years	0.042	0.0338	**0.0092**	0.0134	20
	2006	0.0242	0.0129	**0.0003**	0.0003	-
	2007	0.0061	0.0166	0.0077	**0.0057**	3
	2008	0.0295	0.0056	**0.0006**	0.0051	13
	2009	0.0126	0.0167	0.0099	**0.0015**	1
University of Minnesota	4 years	0.0528	0.0401	0.0498	**0.0363**	81
	2006	0.0457	0.0088	**0.0004**	0.0009	4
	2007	**0.0009**	0.0012	0.0033	0.0048	13
	2008	0.0403	0.0032	**0.0003**	0.0007	1
	2009	0.0061	0.0121	**0.0016**	0.0019	8
Utah State University	4 years	0.0821	0.0326	0.0214	**0.0172**	57
	2006	0.0074	0.0127	0.0042	**0.0041**	7
	2007	0.0077	0.0005	**0.0003**	0.0005	13
	2008	0.0474	0.0262	**0.0004**	0.0004	6
	2009	0.0147	0.0228	0.004	**0.0029**	12
Washington State University	4 years	0.1079	0.0094	0.0934	**0.0063**	62
	2006	0.1062	0.0039	**0.0002**	0.0004	17
	2007	0.1243	**0.0014**	0.0041	0.1268	34
	2008	0.0177	0.005	**0.0005**	0.0006	13
	2009	0.0051	0.0033	**0.0004**	-	2

†Naïve=Simple model; P=Principal Component Analysis; K=Kinship; P+K= combination of PCA and kinship.

‡Numbers in bold represent the best linear models selected.

markers significantly associated ($P \leq 0.05$) with seed dormancy in two or more years as those that may be used for MAS (appendices Tables A11-A18). Ultimately, the utility of specific SNPs for MAS needs to be validated using completely different materials for each of the breeding programs.

Marker trait associations identified in similar regions across programs. Two large effect QTL on chromosome 5HL were identified in multiple breeding programs and in different years (Table 2.8; Figure 2.2). The QTL named QDrm.BCAP-5H.1 contained two SNP loci (178.43-182.88 cM) and QDrm.BCAP-5H.2 contained 10 SNP loci (189.60-196.85 cM). QDrm.BCAP-5H.1 was detected in breeding lines from the Busch Agricultural Resources, LLC; NDSU two-rowed; University of Minnesota; and Washington State University programs. QDrm.BAP-5H.2 was detected in lines from all eight programs.

Among these 12 SNP loci in the two QTL, five had putative functions associated with seed dormancy. The SNP 12_30360 had significant marker-trait associations in 50% of the breeding programs and is associated with a putative jasmonate O-methyltransferase protein. This enzyme catalyzes the formation of methyl jasmonate from jasmonic acid. Methyl jasmonate is a plant volatile reported to be one of the major elements controlling seed dormancy in cereal grains and other plant species including sunflower (*Helianthus annuus* L.), *Amaranthus* spp., tobacco (*Nicotiana tabacum* L.), oat (*Avena sativa* L.), wheat, rapeseed (*Brassica napus* L.), and flax (*Linum usitatissimum* L.). Methyl jasmonate is involved in regulation of the expression of key biosynthetic and catalytic abscisic acid (ABA) genes that ultimately modulate seed dormancy responses. Additionally, methyl jasmonate is an important cellular regulator mediating the expression of various developmental processes, including flower and fruit development, leaf

abscission, senescence, and seed germination; and it induces plant defense responses (Jacobsen et al., 2013; Seo et al., 2001).

Significant marker-trait associations were found in 75% of the breeding programs using the SNP 12_31123. This SNP is associated with a putative pectinesterase inhibitor domain containing protein. Pectinesterase inhibitors may have an important role in the defense reaction of plants against pathogens and other developmental events including seed germination, microsporogenesis, pollen growth, fruit maturation, and senescence (An et al., 2008). An association between seed dormancy and the SNP 11_10401 was detected in 50% of the breeding programs. This SNP is associated with a putative RCD1 (Radical Induced Cell Death1) protein, which has been identified as a key regulator of stress, hormonal, and developmental responses in *Arabidopsis thaliana*. Mutants for RCD1 showed altered responses to jasmonate, ethylene, and nitric oxide, as well as differential sensitivity to reactive oxygen species (ROS). For this reason it has been suggested that RCD1 plays an important role in the hormone-signaling pathway and in the coordination of ROS responses in plants (Jaspers et al., 2009).

Significant associations between seed dormancy and the SNP 12_10322 were detected in 75% of the breeding programs. This SNP is associated with a putative protein of the plasma membrane encoding another putative ABA induced plasma membrane protein (PM 19) (U35 Uniprot description; Close et al., 2009). These types of proteins are expressed in barley embryos from mid-embryogenesis up to maturity, and their levels decline upon germination. In dormant embryos the PM19 mRNA levels are high and only start to decrease after 72 h upon imbibition. In non-dormant seeds the expression of PM19 mRNA levels can be induced by treatments that prevent the germination of the seeds (e.g. addition of ABA).

Table 2.8. Significant marker-trait associations identified in the long arm of chromosome 5H in two or more years and across breeding programs.

			USDA-ARS – Aberdeen, ID				Bush Agricultural Resources, LLC				Montana State University				North Dakota State University (two rowed)			
Marker	Chr	cM	2006	2007	2008	2009	2006	2007	2008	2009	2006	2007	2008	2009	2006	2007	2008	2009
12_11010	5H	178.43					*			*						***	***	
12_11450	5H	178.43					*			*					*	*	***	
12_31292	5H	189.6					*			*								
11_10401	5H	191.97	**	**	***		***	***		**								
12_30360	5H	191.97	**	***	***		***	***		***	*							
12_31210	5H	191.97	**	***	***		***	***		**								
12_30382	5H	194.64					**		*	*	**		*		***		*	
12_10857	5H	194.84		***	**		*	***		**								
11_20402	5H	195.42	***		**						***		**		***		*	
12_10322	5H	196.12	***		**						***		*		***		*	
12_30958	5H	196.12					***		*		*							
12_31123	5H	196.85	***		**						***		*		***	*	*	

*, **, *** Significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

Table 2.8. Significant marker-trait associations identified in the long arm of chromosome 5H in two or more years and across breeding programs (cont.).

			North Dakota State University (six rowed)				University of Minnesota				Utah State University				Washington State University			
Marker	Chr	cM	2006	2007	2008	2009	2006	2007	2008	2009	2006	2007	2008	2009	2006	2007	2008	2009
12_11010	5H	178.43					**	*	**						***		***	
12_11450	5H	178.43						*	**									
12_31292	5H	189.6			***	*									*		***	
11_10401	5H	191.97					***		*	**					***		***	
12_30360	5H	191.97					***		*	**								
12_31210	5H	191.97					***		*	**								
12_30382	5H	194.64																
12_10857	5H	194.84					***		*	**	***		*	***				
11_20402	5H	195.42													***	*	***	**
12_10322	5H	196.12	*		***						**		**	*	***	*	***	***
12_30958	5H	196.12	*		***						**			*	***		***	**
12_31123	5H	196.85	*		***						**		***	*	***		***	***

*, **, *** Significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

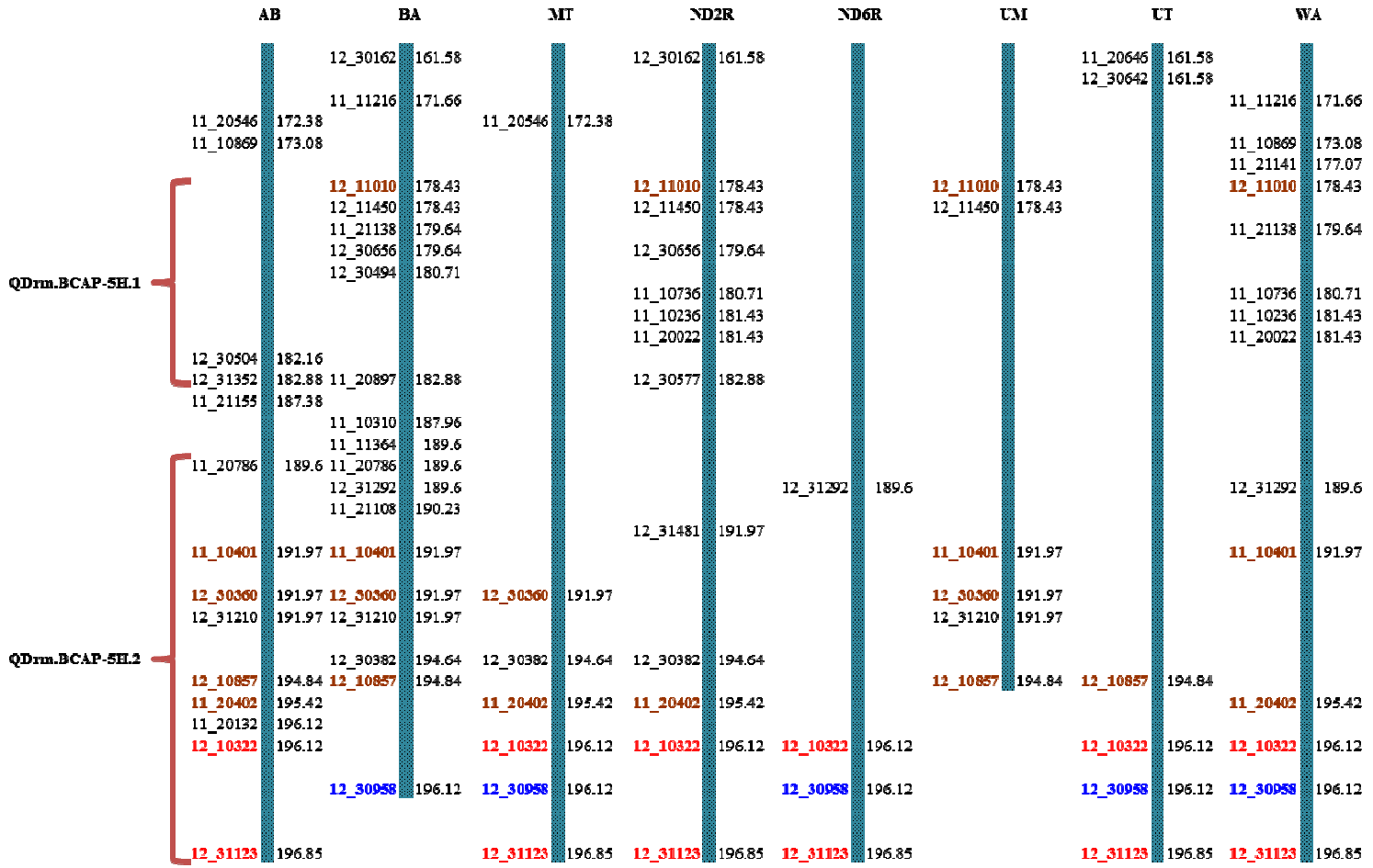

Figure 2.2. Significant marker-trait associations identified across four years and eight breeding programs on the telomeric region of the long arm of chromosome 5HL. AB=USDA-ARS, Aberdeen, ID; BA=Busch Agricultural Resources, LLC; MT=Montana State University; ND 2R=NDSU two-rowed; ND 6R=NDSU six-rowed; UM=University of Minnesota; UT=Utah State University; and WA=Washington State University. Different colors indicate the presence of a specific SNP marker-trait association across different breeding programs and years. Brown, royal blue and red colors for the SNP indicate the presence of a significant marker-trait association in 50%, 63% and 75% of the breeding programs, respectively.

PM19 is part of the Late Embryogenesis Abundant (LEA) proteins, which have also been associated with cellular tolerance to dehydration induced by drying, freezing conditions, or salinity. Most of the genes encoding LEA proteins have ABA responsive elements (ABRE), as well as temperature responsive elements (LTRE), so their expression is induced by ABA, cold, or drought (Hundertmark and Hincha, 2008).

Significant associations between the SNP 11_20402 and seed dormancy were found in 50% of the breeding programs. This SNP is associated with a putative ubiquitin-conjugating enzyme (E2-21 kDa 1), which is part of the post-translational modification machinery that is implicated in the molecular death tagging of proteins (Gao et al., 2013). Once proteins are tagged, they are disposed by the protease complex system, which is responsible of the removal of intracellular polyubiquitinated proteins (Smalle et al., 2003). These proteins have also been implicated in the hypersensitivity response to ABA, and might be implicated in the modulation of seed dormancy during embryo maturation (Smalle et al., 2003; Finkelstein et al., 2008). Liu et al. (2013) suggested that declines in seed sensitivity to ABA and indolacetic acid (IAA) are mediated by transcriptional repression of several family genes, including those related to ubiquitin1 genes (Liu et al., 2013). The remainders of the SNP markers with significant associations with seed dormancy have putative functions implicated with several developmental processes, but with unknown direct relation for seed dormancy (appendices Tables A11-A18).

An interesting hypothesis arose from the observation that certain SNP loci were significantly associated with seed dormancy in some breeding programs, but not others. For the particular case of the University of Minnesota and NDSU six-rowed programs, fewer marker-trait associations were detected on chromosome 5HL than in the other programs. Additionally, some of the loci in the University of Minnesota and NDSU six-rowed programs without

associations were either fixed or approaching fixation (Table 2.8). For example, the genotype BB at locus 12_30382 and AA at locus 11_20402 were highly likely to be found across the four years in these two breeding programs, which led me to conclude that fixation of some alleles and genotypes in this genomic region is likely the result of indirect selection for other traits controlled by loci in this region (Appendices Tables A11-A18).

The distal portion of chromosome 5HL has been identified in multiple studies as an important region harboring QTL that control the expression of multiple malting quality traits, including malt extract, diastatic power, soluble nitrogen, α-amylase activity, wort viscosity, β-glucan, β-glucanase activity, seed dormancy, and PHS (Zhang et al., 2011; Von Korff et al., 2008; Panozzo et al., 2007; Li et al., 2005; Hayes et al., 2003; Collins et al., 2003; Gao et al., 2003; Li et al., 2003; Marquez-Cedillo et al., 2001; Mather et al., 1997; Ullrich et al., 1993; Han et al., 1996). In two crosses where 'Harrington' was the susceptible parent to PHS, Li et al. (2003) identified a large effect QTL for seed dormancy that coincided with a QTL for PHS in chromosome 5HL. The allele from Harrington for increased PHS susceptibility also was associated with increased malt extract, diastatic power, α-amylase activity, and soluble nitrogen. These results suggest that genes controlling dormancy/PHS susceptibility are in repulsion with those for malting quality. However, more research needs to be done to test this hypothesis (Li et al., 2003).

Marker trait associations detected within breeding programs. Apart from comparing the results for marker-trait associations detected across different breeding programs, I aimed to examine those regions within a breeding program where significant ($P \leq 0.05$) marker-trait associations were detected in a minimum of two out of four years. The associations in chromosome 5HL discussed previously will not be discussed in detail in this section.

A total of 32 marker-trait associations were detected in the USDA-ARS-Aberdeen, ID program. Except for chromosome 1H, associations were identified in all chromosomes, with chromosome 5H harboring the most (19 of the 32). The SNP marker 12_31527 was identified in the long arm of chromosome 2H (151.37 cM) in 2006 and 2007 and it is associated with a putative α-amylase/trypsin inhibitor precursor. A QTL with a lesser effect in chromosome 2H associated with α-amylase activity was previously identified by Li et al. (2003) in a biparental mapping study using the cross 'Chebec' x 'Harrington'. The allele contributed by Harrington was associated with higher α-amylase activity levels than those with the allele from Chebec. The rest of the SNP markers identified with significant marker-trait associations in the USDA-ARS-Aberdeen, ID program were associated with energy metabolism (e.g ATP dependent processes, sugar transport, carbohydrate synthesis and catabolism, cell receptors), as well as other proteins involved in the replication of DNA and the ROS pathway.

One hundred and twelve marker-trait associations were detected in the Bush Agricultural Resources LLC program across all seven chromosomes and unlinked markers. The majority of the associations were located in chromosome 5H (71 SNP loci), 4H (20 SNP loci) and 2H (19 SNP loci). The non-chromosome 5HL SNPs were associated with several metabolic pathways. Two marker-trait associations identified were connected to proteins involved in the metabolism of plant regulators. The SNP 11_10793 mapped to the short arm of chromosome 4H (44.94 cM) and it is associated with a putative gibberellin-regulated protein 1 precursor; and SNP 12_30494, mapped to chromosome 5HL (180.71 cM), is associated with a putative ethylene receptor protein. It is well known that gibberellins stimulate seed germination by inducing the production of hydrolytic enzymes that weaken the endosperm and seed coat tissues, as well as prompt the translocation of seed reserves and the expansion of the cell walls that end up in the protrusion of

the radicle and the expansion of the embryo. Additionally, ethylene promotes dormancy breaking and germination via antagonism of the ABA signaling pathway (Finkelstein et al., 2008).

Seventeen marker-trait associations significant were detected in the Montana State University program and they were identified in chromosomes 2H, 4H, 5H and 7H. The majority of the associations were located in chromosome 5H (9 SNP loci) and 4H (6 SNP loci). Most of the marker-trait associations identified in this program are related to carbohydrate assimilation and metabolism, post-translational modification, plant stress responses, and seed dormancy control (e.g. jasmonate and pectinesterase inhibitors).

For the North Dakota State University two-rowed program, 40 marker-trait associations were detected in six of the seven chromosomes and the unlinked group of markers No associations were detected in 3H. The majority of marker-trait associations were identified in chromosomes 5H (16 SNP loci) and 6H (8 SNP loci). The SNP 12_31481 mapped to chromosome 5HL (191.97 cM) and it is associated with a putative *gibberellin 20 oxidase 1*. This locus was previously identified by Li et al. (2004) as part of a major QTL controlling both PHS and seed dormancy in a rice-wheat-barley comparison study. A lesser effect QTL associated with dormancy and previously designated as *SD2*, also mapped to a similar chromosome location in chromosome 5HL (Han et al. 1996; Gao et al. 2003), where other genes controlling malt quality traits also have been identified (Li et al. 2004).

In the North Dakota State University six-rowed program, 34 marker-trait associations were identified in chromosomes 1H, 2H, 5H, 6H and the unlinked group of markers, with the majority of the marker-trait associations located in chromosome 6H (23 SNP loci). Once again, the majority of the marker-trait associations were related with putative proteins involved in a

large number of cell processes. The SNP 12_30856, mapping to chromosome 6H (55.94 cM) corresponds to a putative cryptochrome 1 apoprotein, which is a class of photoreceptor in the blue region of the spectrum that links the control of seed dormancy and germination with light intensity (Yu et al., 2010; Goggin et al., 2008). Gubler et al. (2008) stated that blue light mimics the effects of white light in promoting seed dormancy of freshly harvested cereal grains, which is associated with the regulation of genes for ABA metabolism in embryos.

The University of Minnesota program had the lowest number of marker-trait associations, nine. Most of the associations were found in the long arm of chromosome 5HL and they were previously described. The rest of the marker-trait associations were found on chromosomes 1H, 2H and the unlinked group of markers. None of these SNPs appeared to be linked to any of the processes involved in seed dormancy or germination.

A total of 55 marker-trait associations in the Utah State University program were identified across all chromosomes, including the unlinked group of markers. The majority of the associates were found on chromosome 3H (18 SNP loci), followed by chromosomes 4H and 5H (7 SNP loci each). The SNP 11_10180 maps to chromosome 2HS (21.61 cM) and is in a similar region as a QTL for dormancy identified by Lohwasser et al. (2012). The SNP 11_10180 is associated with a putative auxin-binding protein ABP20 precursor. The auxin-binding protein is of special interest since auxins accumulate in the cotyledons of mature seeds, where they seem to play an important role in embryogenesis and the development of the apical-basal pattern formation (Kucera et al., 2005). The inhibitory effect of L-tryptophan, a precursor of IAA, and other synthetic auxins was confirmed using excised embryos from dormant wheat cultivars, where germination was inhibited unless auxin antagonists were used. Additionally, Ramaih et al. (2003) showed that excised embryos from dormant seeds lose sensitivity to auxins during after-

ripening, which supports the importance of auxins during seed development and their role in the control of seed dormancy and PHS. Two other interesting marker-trait associations were identified in the short and long arms of chromosome 4H (44.94 cM and 76.03 cM, respectively). The SNP on the short arm is associated with a putative gibberellin-regulated protein 1 precursor, while the second is associated with a putative chitin-inducible gibberellin-responsive protein 2. A QTL located in chromosome 4HS was previously identified by Han et al. (1996) and named *SD4*. This QTL was flanked by markers WG622 and BCD402B, which positions it within the region of 13.1-38.41 cM in the barley OPA consensus map (Close et al., 2009). *SD4* was identified only under certain environments and explained only 5% of the phenotypic variation of seed dormancy (Han et al., 1996). Coincidentally, a dominant gibberelic acid (GA_3) insensitive dwarfing gene *Dwf2* has been map to chromosome 4HS near microsatellite marker XhvOle (18.3 cM) using an F_2 population from a cross between '93/B694' (*Dwf2*) and 'Bonus M2' (*dwf2*). The identification of the *Dwf2* gene and other dwarfing genes among homoeologous groups suggest their synteny within the Triticeae tribe (Ivandic et al. 1999). A QTL in the same region of chromosome 4HS has also been identified in other studies and associated with the control of several malt quality traits, including, malt extract percentage, α-amylase activity, diastatic power, β-glucan content and seed dormancy (Gao et al., 2004; Hayes et al., 1993, 1994; Han and Ullrich, 1994 cited by Gao et al., 2004). The second SNP marker in chromosome 4H (12_20143) is associated with a putative *chitin-inducible gibberellin-responsive protein 2*. This protein has been associated with a QTL affecting plant height in rice (Kovi et al. 2011), which in barley corresponds to a genomic region containing GA-insensitive dwarfing genes that seem to be collinear with genes *Rht-D1c* and *Rht-D1b* in wheat (Ivandic et al. 1999).

Finally, 32 marker-trait associations were detected using materials from the Washington State University breeding program in chromosomes 1H, 3H, 5H, 6H, and 7H; and some of the unlinked markers. Most of the significant marker-trait associations were found in chromosome 5H (17 SNPs), towards the telomere (191.97 cM to 196.85), and were discussed in the previous section. However, the SNP 12_31094 on chromosome 5HS was found to be associated with a putative protein enolase 1, which has been observed in higher amounts in germinating barley and rice seeds (Østergaard et al., 2004; Kim et al., 2009). Another interesting association was detected in chromosome 3HS and related to a putative protein cysteine synthase mitochondrial precursor. Cyanide has been identified as a key regulator of seed dormancy in cereals, as well as in other plant species including rice, sunflower, apple (*Malus domestica L.*), and *Arabidopsis thaliana (Oracz et al., 2009; Garcia et al., 2010 and references therein).* Oracz et al. (2009) suggested that the effect of cyanide on dormancy release could be attributed to a response to ROS accumulation. In sunflower it has been observed that dormancy is alleviated by ethylene, in which expression is induced by cyanide through the activation of the transcription factor ethylene response factor1 (ERF1). Other metabolites including cysteine, which is a substrate of the β-cyanoalanine synthase (β-CAS), stimulate seed germination in some plants as well (Maruyama et al., 1998; García et al., 2010). The rest of the significant marker-trait associations are related with proteins unrelated to seed dormancy or germination.

Because many of the QTL for seed dormancy in the present study map to chromosome regions where malt quality QTL have been mapped previously, I wanted to determine if the selection for malt quality traits, such as wort protein, soluble protein/total protein (S/T) and α-amylase, may have indirectly impacted seed dormancy. This hypothesis is supported, in part, by the wide range on heritability values observed for seed dormancy (Table 2.5) and the existence

of fixed alleles at some loci within genomic regions with marker-trait associations (e.g. chromosome 5HL). Correlation analyses between percent non-dormant seeds with wort protein, S/T and α-amylase did not provide conclusive results to support my hypothesis (data not presented). The weak correlations obtained, generally, (r<0.62), may have been due to large amounts of missing phenotype data for the malt quality traits; thus, additional research is needed.

In the present study I have described the application of the genome-wide AM tools using a panel of elite US spring barley breeding lines for the identification of marker-trait associations with percent of non-dormant seeds. Two main QTL regions were identified in the long arm of chromosome 5HL and its locations are corresponding with previously identified QTL in several biparental mapping studies (Ullrich et al., 1993; Han et al., 1996; Li et al., 2003; Lin et al., 2009; Zhang et al., 2011; Lohwasser et al., 2012). The results suggest that in the case of US spring barley, each program needs to conduct their own mapping studies using their own germplasm in order to identify markers than can be used successfully for MAS. Further studies need to be done to validate the different SNPs efficacy for MAS in each breeding program, and to determine if the associations of seed dormancy with specific malt quality traits is due to linkage or pleiotropy.

Candidate markers suggested for further validation and use in MAS

I suggest the following SNP markers be validated to determine their utility for MAS in each breeding program (Table 2.9 and 2.10). Criteria for selecting markers differed based on whether the markers mapped to chromosome 5HL or another region. SNP markers in chromosome 5HL significantly associated (P≤0.05) with seed dormancy in two or more years are included and critically discussed. For the other chromosome regions, significant SNP markers (P≤0.05) that were found to be significant in two out of four years were selected for validation

depended on whether the SNP was associated with a putative function related to seed dormancy/germination, or mapped to a region coinciding with known QTL for seed dormancy (e.g. SD1, SD3, or SD4; Han et al., 1996). Significant SNP's ($P \leq 0.05$) whose function did not appear to be associated with seed dormancy, but that showed up in three or more years were also included herein. Nine candidate SNP markers were identified for the USDA-ARS-Aberdeen program across years, but only eight were detected in the combined analysis with the exception of marker 11_20546. The BB genotype in five SNP loci was found to be associated with nearly 60% more non-dormant seeds ($\bar{X}$=38.8). The remaining four SNP loci showed that the AA genotype was associated with 68% more non-dormant seeds ($\bar{X}$=38.5). Eleven candidate SNP markers were identified for the Bush Agricultural Resources program across years, but only seven markers were detected in the combined analysis. The BB genotype in nine of the markers was associated with nearly 86.4% more non-dormant seeds ($\bar{X}$=42.3). Only two SNP markers having the A allele at both loci were associated with 57% more non-dormant seeds ($\bar{X}$=43.4). Five candidate SNP makers were identified for the Montana State University breeding program, with only one not detected in the combined analysis across years (marker 11_20546). The AA genotype in four out of five SNP markers was associated with 44% more non-dormant seeds than those lines with the BB genotype. Eight candidate SNP markers were identified for the NDSU two-row program, but marker 12_31123 was not detected in the combined analysis across years. The presence of the AA genotype in five out of eight markers was associated with 54% more non-dormant seeds ($\bar{X}$=34.3), while in three other loci (12_11450, 11_10236 and 12_30577) the BB genotype was associated with 36% more non-dormant seed than the AA genotype.

Four SNP markers were identified for the NDSU six-rowed program across years, but none of them were significant in the combined analyses. The means for the percentage of

Table 2.9. Candidate SNP markers identified on chromosome 5HL using breeding lines from eight US spring barley breeding programs from 2006-2009.

Breeding program	Marker	cM	Separate analysis†						Combined analysis‡
			Years	------AA-----		------BB-----			
				%	Mean	%	Mean		
USDA-ARS-Aberdeen, ID	11_20546	172.38	3	75.5	36.43	24.2	30.95	*	ns
	11_10869	173.08	2	55.9	25.2	44.1	41.99	*	***
	12_31352	182.88	2	28.5	22.2	71.5	35.93	*	***
	11_10401	191.97	3	39.5	21.72	58.9	39.21	*	***
	12_31210	191.97	3	33.3	21.77	65.3	37.75	*	***
	12_30360	191.97	3	37.8	21.22	60.8	39.12	*	***
	11_20402	195.42	2	67.1	39.31	32.3	20.60	*	***
	12_10322	196.12	2	64.4	39.40	35.1	21.22	*	***
	12_31123	196.85	2	64.7	39.03	35.1	21.44	*	***
Bush Agricultural Resources LLC	12_30162	161.58	3	78.9	40.35	20.8	31.14	*	ns
	12_30494	180.71	2	14.0	31.64	78.8	42.31	*	ns
	11_20897	182.88	3	13.5	25.54	86.5	41.61	*	ns
	11_11364	189.60	2	7.2	21.18	92.8	40.66	*	***
	11_20786	189.60	3	13.8	20.36	86.2	42.79	*	***
	11_21108	190.23	2	35.0	46.5	49.3	34.0	*	***
	11_10401	191.97	3	17.5	21.29	82.5	43.50	*	***
	12_30360	191.97	3	15.1	19.09	84.9	43.44	*	***
	12_31210	191.97	3	14.0	18.98	86.0	43.05	*	***
	12_30382	194.64	3	13.5	26.00	86.5	42.24	*	ns
	12_10857	194.84	3	6.7	16.78	93.3	40.73	*	***
Montana State University	11_20546	172.38	2	50.4	39.66	49.4	29.66	*	ns
	12_30382	194.64	2	44.5	26.63	54.7	38.44	*	***
	11_20402	195.42	2	42.2	43.01	57.0	25.74	*	***
	12_10322	196.12	2	40.9	42.50	58.3	26.18	*	***
	12_31123	196.85	2	41.7	42.26	57.7	26.16	*	***
North Dakota State University (two-row)	12_11010	178.43	2	6.6	43.9	92.6	27	*	***
	12_11450	178.43	3	93.7	27.20	6.3	44.48	*	***
	11_10736	180.71	2	45.4	34	52.2	22.9	*	***
	11_10236	181.43	3	45.6	23.60	54.1	32.91	*	***
	12_30577	182.88	3	50.7	24.07	47.8	33.22	*	***
	11_20402	195.42	2	72.8	31.2	25.6	19.4	*	***
	12_10322	196.12	2	71.2	31.3	27.4	20.3	*	***
	12_31123	196.85	3	72.0	31.28	27.4	20.34	*	ns

*, *** Significant SNP marker-trait associations at $P \leq 0.05$ and 0.001.

† Analyses of individual years within each breeding program.

‡ Analyses across all years for each breeding program.

Table 2.9. Candidate SNP markers identified on chromosome 5HL using breeding lines from eight US spring barley breeding programs from 2006-2009 (cont.).

Breeding Program	Marker	cM	Separate analysis†						Combined analysis‡
			Years	------AA-----		------BB------			
				%	Mean	%	Mean		
North Dakota State University (six-row)	12_31292	189.60	2	70.4	33.03	27.4	34.79	*	ns
	12_10322	196.12	2	56.6	32.09	41.2	35.22	*	ns
	12_30958	196.12	2	41.2	35.22	57.4	32.10	*	ns
	12_31123	196.85	2	57.7	32.33	41.2	35.22	*	ns
University of Minnesota	12_11010	178.43	3	31.8	39.39	67.4	29.64	*	ns
	11_10401	191.97	3	16.2	13.25	83.6	36.02	*	***
	12_30360	191.97	3	14.6	9.38	85.2	35.78	*	ns
	12_31210	191.97	3	14.6	9.38	85.2	35.78	*	ns
	12_10857	194.84	3	16.1	17.81	83.4	35.90	*	***
Utah State University	12_10857	194.84	3	54.4	17.23	44.5	29.36	*	***
	12_10322	196.12	3	40.3	28.08	58.5	16.71	*	***
	12_31123	196.85	3	41.2	28.08	58.5	16.71	*	***
Washington State University	11_10869	173.08	3	69.7	19.01	29.6	39.37	*	ns
	12_31292	189.60	2	66.6	20.34	32.40	31.60	*	***
	11_10401	191.97	2	64.2	17.56	33.43	36.89	*	***
	11_20402	195.42	4	32.4	39.90	65.2	16.06	*	***
	12_10322	196.12	4	30.5	41.20	67.1	16.65	*	***
	12_30958	196.12	3	60.7	16.79	37.1	36.29	*	***
	12_31123	196.85	3	32.6	40.82	66.6	16.53	*	***

*, *** Significant SNP marker-trait associations at $P \leq 0.05$ and 0.001.
† Analyses of individual years within each breeding program.
‡ Analyses across all years for each breeding program.

non-dormant seeds are not significantly different between genotype groups, and that is the reason why they were not identified as significant in the combined analysis. For example, the presence of the BB genotype in markers 12_31292, 12_10322 and 12_31123 was associated with 37% of the lines having a mean of 35.0% non-dormant seeds, while for the AA genotype was associated with 62% of the lines having a mean of 32.5% non-dormant seeds. Based on these results we cannot suggest the use of these markers for MAS.

Five candidate SNP markers were identified for the University of Minnesota breeding program. Only 11_10401 and 12_10857 were detected in the combined analysis across years.

Overall, the presence of the BB genotype in four out of five SNP loci (11_10401 to 12_10857) was associated with 84.3% more non-dormant seeds ($\bar{X}$=36.0). Three candidate SNP markers were identified for the Utah State University breeding program. All the three loci were identified in the combined analysis across years as well. The AA genotype at loci 12_10322 and 12_31123 was associated with 41% more non-dormant seeds than the BB genotype. The BB genotype at the locus 12_10857 was associated with 45% more non-dormant seeds than the AA. Finally, seven candidate SNP markers were identified for the Washington State University breeding program. Only marker 11_10869 was not detected in the combined analysis across years. The BB genotype at loci 11_10869, 12_31292, 11_10401 and 12_30958 was associated with 33% more non-dormant seeds ($\bar{X}$=36.0). The genotype AA was found to be associated with 32% more non-dormant seeds ($\bar{X}$=40.6), than the BB genotype ($\bar{X}$=16.4) for the SNP loci 11_20402, 12_10322 and 12_31123.

Table 2.10. Candidate SNP markers identified across the genome using breeding lines from eight US spring barley breeding programs from 2006-2009.

				Separate analysis†					
Breeding program	Marker	Chr	cM	Years	--------A--------		--------B--------		
					%	Mean	%	Mean	
USDA-ARS-Aberdeen, ID	12_31527	2H	151.37	2	25.1	35.07	74.1	32.56	*
	12_11154	3H	138.83	2	53.6	30.86	46.1	35.57	*
	11_20675	6H	50.07	2	5.2	49.94	94.6	31.52	*
	11_20211	6H	123.84	2	74.2	33.88	24.5	30.40	*
	11_11012	7H	147.47	3	40.6	33.27	56.6	31.09	*
Bush Agricultural Resources LLC	11_20371	1H	18.05	2	73.4	40.84	26.6	33.50	*
	11_21126	1H	73.94	3	60.8	42.82	38.9	33.61	*
	11_10722	1H	125.27	2	59.5	41.59	40.5	36.81	*
	11_10782	1H	131.89	3	24.4	35.08	75.0	40.85	*
	11_11059	2H	7.14	2	54.1	41.95	45.9	35.66	*
	11_11302	2H	52.47	2	37.0	37.59	63.0	42.01	*
	12_10545	2H	69.13	2	22.4	33.44	77.6	40.82	*
	11_21220	2H	120.02	3	34.3	34.25	65.7	41.57	*

*, *** Significant SNP marker-trait associations at $P \leq 0.05$ and 0.001.
† Analyses of individual years within each breeding program.

Table 2.10. Candidate SNP markers identified across the genome using breeding lines from eight US spring barley breeding programs from 2006-2009 (cont.).

				Separate analysis†					
Breeding program	Marker	Chr	cM	Years	--------A--------		--------B--------		
Bush Agricultural Resources LLC	12_30992	4H	38.63	2	20.2	30.36	79.8	41.28	*
	12_10371	4H	40.36	2	79.2	41.33	20.8	30.42	*
	11_10793	4H	44.94	1	80.3	41.63	19.7	30.40	*
	11_20670	4H	80.79	2	91.2	39.35	8.8	40.71	*
	12_30239	4H	119.84	2	75.3	41.55	24.7	32.62	*
	12_31055	unlinked	0.00	2	38.4	34.64	61.1	42.98	*
	12_31054	unlinked	0.00	2	38.4	34.64	61.1	42.98	*
	12_30351	unlinked	0.00	2	34.2	46.53	65.8	35.58	*
Montana State University	12_30259	2H	54.95	2	50.4	35.83	49.0	34.24	*
	11_10409	4H	3.74	2	13.2	33.35	86.2	34.13	*
	11_11345	4H	5.55	2	72.8	34.75	26.1	28.56	*
	12_30237	4H	61.04	2	69.3	33.07	30.7	33.51	*
North Dakota State University (two-row)	12_30554	4H	96.59	2	27.2	23.48	70.2	30.04	*
	11_20119	4H	99.28	2	28.8	24.95	68.3	30.01	*
	12_30239	4H	119.84	2	38.3	22.49	59.6	31.75	*
	12_10575	6H	45.44	2	71.8	27.67	26.6	30.90	*
	11_20170	7H	161.54	2	77.6	29.20	19.5	25.95	*
	12_31128	unlinked	0.00	2	80.5	28.20	19.0	33.44	*
North Dakota State University (six-row)	11_20371	1H	18.05	2	69.9	33.34	29.8	32.88	*
	12_10166	1H	69.53	2	82.6	34.73	16.1	24.38	*
University of Minnesota	11_20943	2H	149.61	2	3.8	52.47	96.2	32.52	*
	12_31239	unlinked	0.00	2	27.2	38.79	72.2	30.87	*
Utah State University	12_10693	1H	128.14	2	13.9	44.03	86.1	21.90	*
	11_10180	2H	21.61	2	97.4	23.23	2.6	16.61	*
	12_30170	3H	80.89	3	27.9	23.47	71.9	23.87	*
	12_30767	3H	162.15	3	32.1	21.98	67.0	22.55	*
	11_10793	4H	44.94	2	89.2	23.06	8.7	28.03	*
	12_20143	4H	76.03	2	50.6	26.63	48.6	16.88	*
	11_20725	6H	105.60	2	24.9	29.43	74.6	21.39	*
	12_30836	7H	4.89	2	78.6	21.88	21.4	31.31	*
	12_30597	unlinked	0.00	2	91.3	23.08	8.7	24.56	*
Washington State University	12_30953	3H	41.00	2	30.8	22.89	67.4	25.19	*
	11_10868	6H	24.36	2	81.9	26.18	17.9	16.30	*
	12_31239	unlinked	0.00	2	38.2	30.38	60.2	18.45	*

*, *** Significant SNP marker-trait associations at $P \leq 0.05$ and 0.001.

† Analyses of individual years within each breeding program.

Epistasis

Epistatic interactions are likely to play an important role in the control of complex traits, in which combinations of certain alleles and the effects of their interactions may result in significant phenotypic differences (Romagosa et al., 1999; Bonnardeaux et al., 2008; Yu et al., 2011). Seed dormancy has been a difficult trait to dissect because of its polygenic nature and the effect of environmental and gene-gene interactions. Oberthur et al. (1995) identified two regions on chromosome 5HL, one near RFLP marker PRS 128 (80.61 cM; Close et al., 2009) that strongly and consistently affected seed dormancy in a doubled-haploid population from the cross Steptoe/Morex; and a second one that had a lesser effect on seed dormancy close to the telomere of chromosome 5HL near marker ABG390 (269.63 cM; Close et al., 2009). Two other QTL regions were also identified in chromosome 7HL near the *Amy2* locus (126.28 cM; Close et al., 2009) and in chromosome 4HS near marker BCD402B (38.41 cM; Close et al., 2009). However, these loci had a minor influence on seed dormancy and appeared only under specific growing/environmental conditions. The results from the study of Oberthur et al. (1995) suggest that the allelic state of the gene near locus PRS128 (5HL) is epistatic to the genes near the ABG390 (5HL) and *Amy2* (7HL) loci; while the expression of the gene near marker BCD402B (4HS) appears to depend moderately upon the allelic state of the gene near ABG390 (5HL). Further studies from Han et al. (1996) using reciprocal crosses between doubled-haploid lines from the cross of Steptoe (dormant parent) and Morex (non-dormant parent) were used to verify interactions between loci present in chromosome 5HL that were previously detected by Oberthur et al. (1995). The two regions were named *SD1* and *SD2*. The *SD1* corresponds to the QTL region at 80.61 cM near marker PRS128, while SD2 correspond to QTL region located at 269.63 cM near marker ABG390. Han et al. (1996) suggested that seed dormancy could be modulated

not only by gene-gene interactions among dormancy loci, but also might be the result from the interaction between nuclear genes and cytoplasmic factors. However, this last statement could not be verified in this study because of the composition of the mapping populations used.

Several QTL with large and small effects have been identified across the length of the barley genome, specifically on chromosomes 2H, 3H, 4H, 5H, 6H, and 7H (Oberthur et al., 1995; Han et al., 1996; Larson et al., 1996; Romagosa et al. 1999; Ullrich et al., 2008; Bonnardeaux et al., 2008; Lohwasser et al., 2013). Many of these studies have confirmed the existence of epistatic interactions between two QTL (*SD1* and *SD2*) located in chromosome 5HL. Romagosa et al. (1999) stated that *SD1*, which is close to the *aleurone* gene in 5HL is "the most important QTL in determining the time of dormancy release" and that "*SD1* is epistatic to *SD2* (telomere of 5HL) at early after ripening". Bonnardeaux et al. (2008) confirmed these results and determined that the epistatic interactions between them had a negative effect on germination, reducing it by 7.6%. Additional epistatic interactions were also documented to occur between lesser effect QTL located in chromosomes 2H, 3H, and 4H. A QTL located in the long arm of chromosome 2H showed additive x additive interactions with a locus in the long arm of chromosome 3H. Another interaction was found between a QTL located in the short arm of chromosome 4H and two QTL located in the long arm of the same chromosome (Bonnardeaux et al. 2008).

All of the aforementioned studies suggest that epistatic interactions are an important genetic factor controlling seed dormancy maintenance and release, and therefore I wanted to test for gene-gene interactions using the Barley CAP materials. The information gathered from significant markers identified in the analyses of four years combined within each breeding program was used to estimate epistatic interactions between SNP loci. The significance threshold

used to declare important epistatic interactions was $P<0.001$ (Table 2.11). Intra and inter-chromosomal loci interactions were detected across the whole barley genome (Figures 3-4; appendix Table A19) with marked differences being observed for each breeding program.

From the analysis of the USDA-ARS, Aberdeen breeding program, I detected 21 significant pairwise interactions between loci in chromosomes 2H, 3H, 5H, 6H, and the unlinked group of markers (Figure 2.3; Table 2.11). The most significant epistatic interactions ($-Log_{10}(P)= 8.53$) were detected between loci 11_20402 (195.40 cM) and 11_10901 (158.40 cM) located in the long arm of chromosome 5H, followed by the epistatic interactions between the loci 11_20402 (195.40 cM) in chromosome 5HL and 11_10325 (54.95 cM) in chromosome 2HS. Interactions between QTL regions in chromosome 5HL has been previously described by Han et al. (1996) and confirmed by Bonnardeaux et al. (2008). The loci 11_20402 coincides with the position of the QTL SD2, which has been identified to have a major effect on seed dormancy and is partly epistatic to SD1, which is located in the same chromosome. Other interactions between loci at chromosomes 2HL and 3HS, and between 3HS and 5HL were detected. Similar interactions were reported by Bonnardeaux et al. (2008); however, the genetic positions used in this study differed from those reported by Bonnardeaux et al. (2008), which could be the result of the use of a high density map herein (~2,522 SNP markers, 908 non-cosegregating markers) compared to the map built by Bonnardeaux et al. (~128 SSRs).

The results of the analysis for the Bush Agricultural Resources breeding program revealed five significant pairwise interactions between loci located in chromosomes 1H, 2H, 4H, 7H, and the unlinked group of markers (Figure 2.3 and Table 2.11). Significant interactions between the SNP loci 11_10756 (48.5 cM) in chromosome 4HS and 11_21079 (83.44 cM) located in chromosome coincide with the positions of the QTL SD4 and SD3, respectively.

Oberthur et al. (1995) identified SD3 and SD4 only under specific environments, with each of them explaining about 5% of the phenotypic variability in seed dormancy.

The analyses of the Montana State University breeding program revealed the presence of 12 significant pairwise epistatic interactions between loci located in chromosomes 3H, 5H, and the unlinked group of markers (Figure 2.4; Table 2.11). Interestingly, significant interactions were detected between loci from the distal portion of chromosome 5HL and the unlinked group of markers. Several studies have reported important epistatic effects between the two SD QTL regions in chromosome 5H (Oberthur et al., 1995; Han et al., 1996; Larson et al., 1996; Romagosa et al. 1999; Bonnardeaux et al. 2008), which suggests that some of the unlinked group of markers may be in chromosome 5H. Additional support comes from the observation the same epistatic interactions occur in nearly two-thirds of the breeding programs (Table 2.11).

Six and two significant epistatic interactions were identified for the NDSU two-rowed and six-rowed breeding programs, respectively (Figure 2.4; Table 2.11). Pairwise interactions between loci located in chromosomes 3H, 4H, 5H, and the unlinked group of markers was detected for the NDSU two-rowed breeding program. From these interactions, those occurring between loci in chromosomes 3HL and 5HS, and 3HL and 5HL have been previously described by Bonnardeaux et al. (2008). The results for the NDSU six-rowed breeding program revealed the interaction between loci in chromosome 5HS with 5HL and between 5HS and the unlinked group of markers.

Twenty-seven significant intra- and inter-chromosomal epistatic interactions were identified across the whole genome in the Utah State University breeding program (Figure 2.4; Table 2.11). The most significant epistatic interaction was observed between loci 11_11436 (155.85 cM) and 11_20755 (15.93cM) located in chromosomes 3HL and 7HS, respectively.

However, the most interesting interactions identified were those involving loci on chromosomes 4HL and 7HL, and 5HL and 7HL (appendix Table A19), since they contain putative loci associated with dormancy (e.g. hormonal regulation and malting traits) and because dormancy QTL have been highlighted in these chromosomal regions in other studies (Oberthur et al., 1995; Han et al., 1996; Larson et al., 1996; Romagosa et al., 1999; Li et al., 2003; Li et al., 2004; Bonnardeaux et al., 2008; Lohwasser et al. 2013).

Only one significant pairwise epistatic interaction was detected in the Washington State University breeding program. This included SNP locus 12_31123 (196.9 cM) in chromosome 5HL and 11_10150 (unlinked). Both markers have been identified in the marker-trait association analysis and their relevance to seed dormancy was previously discussed. Finally, the results from the analysis of across all breeding programs and years revealed only three significant epistatic interactions between four loci located in the telomeric region of 5HL (Figure 2.3) and marker 11_10150 (unlinked).

Summary

Overall the analysis of 2,965 barley CAP lines using genome-wide AM tools allowed me to identify two main QTL regions in the long arm of chromosome 5H that have been previously detected thorough biparental QTL mapping. The consistent identification of these QTL regions in different studies and the large variability explained in seed dormancy using different populations are indicative of their importance in the regulation and maintenance of the physiological and metabolic processes involved in seed dormancy.

Several lesser effect QTL/marker-trait associations for seed dormancy were identified independently in some breeding programs, including those on chromosomes 2H, 4H, 6H and 7H using the combined analysis across years for each breeding program.

Table 2.11. Significant epistatic interactions for seed dormancy detected in the spring barley CAP lines from eight US spring barley breeding programs from 2006-2009.

Epistatic Interaction	USDA-ARS-Aberdeen	Bush Agricultural Resources LLC	Montana State University	NDSU two-rowed	NDSU six-rowed	University of Minnesota	Utah State University	Washington State University
1HS-2HS							***	
1HS-3HL							***	
1HS-5HL							***	
1HS-6HS							***	
1HL-2HS		***						
1HL-4HS		***						
1HL-6HS							***	
1HL-UG		***						
2HS-3HL							***	
2HS-5HL	***							
2HS-6HS							***	
2HS-6HL	***							
2HS-3HL								
2HS-7HS							***	
2HL-3HS	***							
2HL-3HL	***						***	
2HL-UG	***							

*** Significant SNP marker-trait associations at $P \leq 0.001$ identified from the analyses across all years for each breeding program.

Table 2.11. Significant epistatic interactions for seed dormancy detected in the spring barley CAP lines from the eight US spring barley breeding programs from 2006-2009 (cont.).

Epistatic Interaction	USDA-ARS-Aberdeen	Bush Agricultural Resources LLC	Montana State University	NDSU two-rowed	NDSU six-rowed	University of Minnesota	Utah State University	Washington State University
3HS-UG			***					
3HS-5HL	***		***					
3HL-5HS				***				
3HL-5HL	***			***				
3HL-UG	***							
4HL-UG				***				
4HL-6HS							***	
4HL-7HL							***	
5HS-UG				***	***			
5HL-UG	***	***	***	***				***
5HL-7HL							***	
6HS-7HS							***	
6HL-5HL	***							
7HS-3HL							***	
7HL-4HS		***						

*** Significant SNP marker-trait associations at $P \leq 0.001$ identified from the analyses across all years for each breeding program.

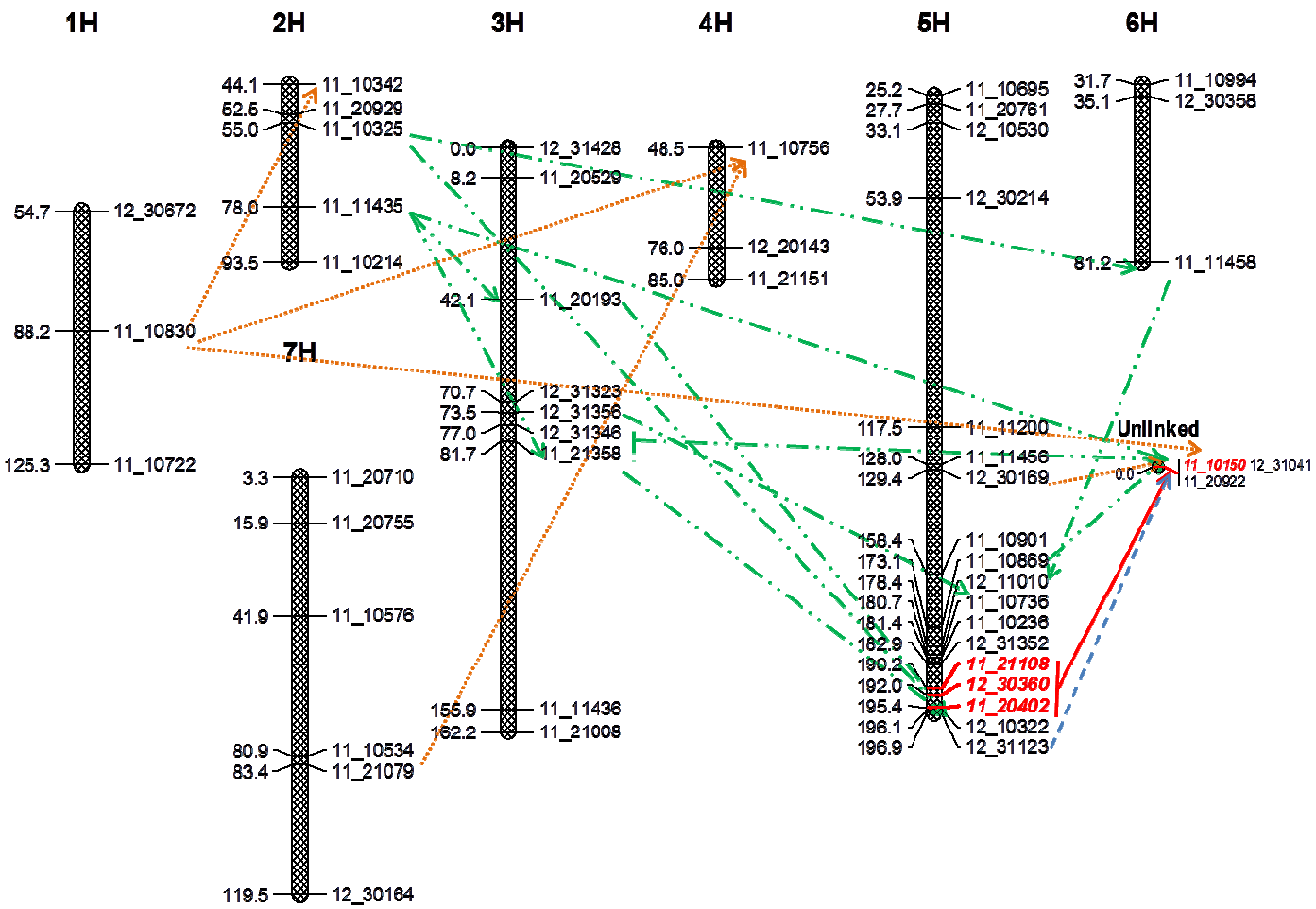

Figure 2.3. Gene-gene interactions network for all SNP loci associated with dormancy for the analysis of the whole panel (WBCAP, red arrows), USDA-ARS-Aberdeen, ID, four years (AB, green dotted lines), Bush Ag. Resources four years (BA, orange dotted lines), Washington (WA, gray dotted line). Chromosome positions for the significant SNP markers were based on the Infinium Assay Barley iSelect 9K SNPchip. Different lines and colors represent interactions for each of the analysis previously described.

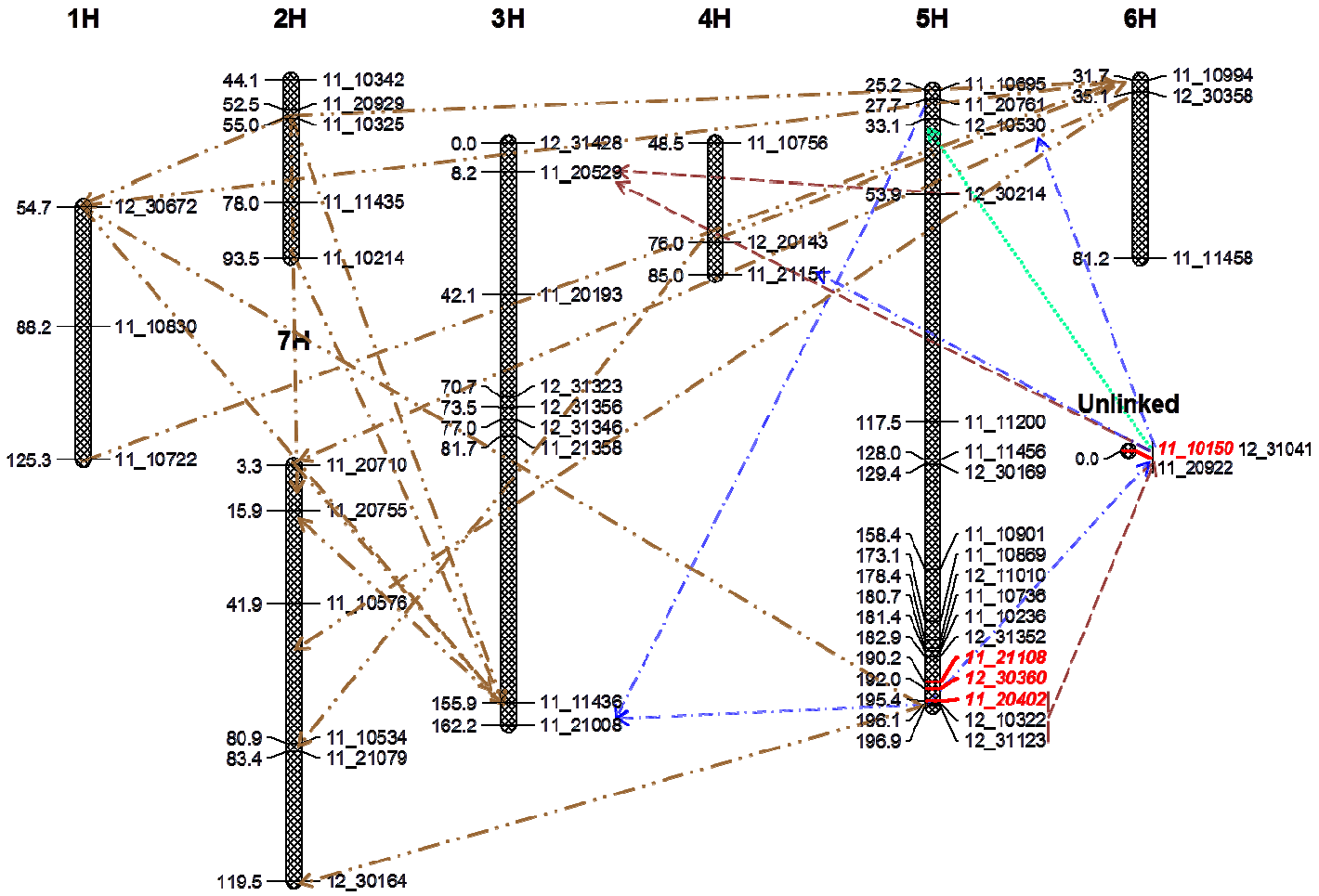

Figure 2.4. Gene-gene interactions network for all SNP loci associated with dormancy for the analysis of Montana four years (MT, red scarlet dotted lines); North Dakota 2-R four years (ND2R, blue dotted lines); North Dakota 6-R four years (ND6R, aqua dotted line), Utah four years (UT, brown dotted lines). Chromosome positions for the significant SNP markers were based on the Infinium Assay Barley iSelect 9K SNPchip. Different lines and colors represent interactions for each of the analysis previously described.

Putative functions are described and some pairwise epistatic interactions were discussed (appendices Table A20-A27). Most of these QTL regions have been documented before giving credence to the results obtained in the present study (Oberthur et al., 1995; Han et al., 1996; Larson et al., 1996; Romagosa et al. 1999; Ullrich et al., 2008; Bonnardeaux et al., 2008; Lohwasser et al., 2013). Even though a QTL relative to SD1 (76.6cM-83.2 cM) was not detected across years within any breeding program, the QTL QDrm.BCAP-5H.2, which is effectively SD2 or QDrm.StMo-5H.2 (Figure 2.5) in the agronomic QTL consensus map from Rostoks et al. (2005), was consistently identified across years and breeding programs.

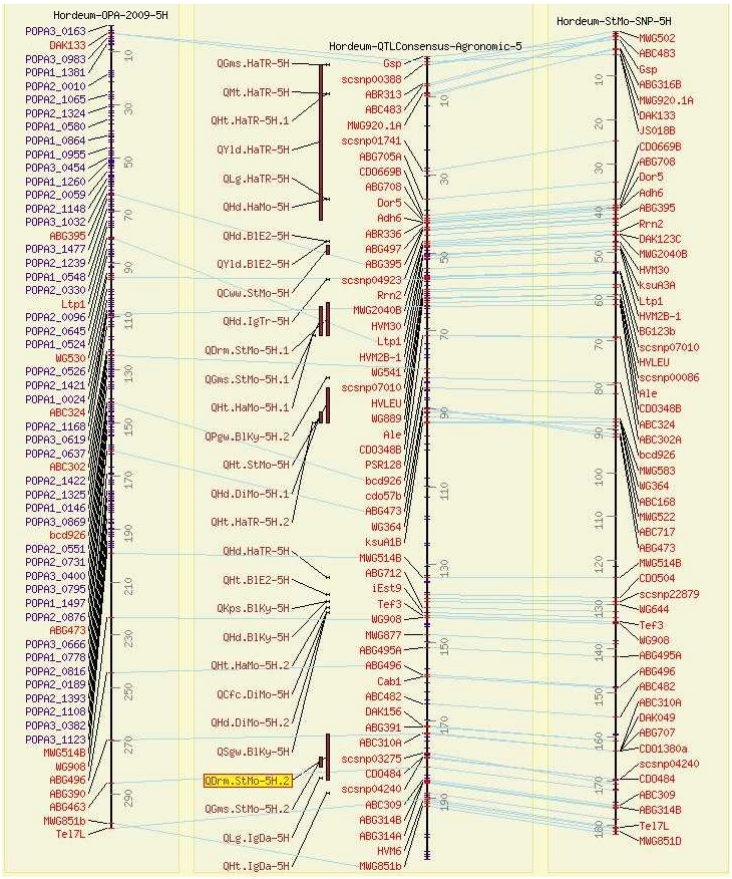

Figure 2.5. Position of the QDrm.BCAP-5H.2 (SD2 or QDrm.StMo-5H.2) in the Barley OPA Consensus map (Close et al., 2009), agronomic QTL consensus map, and the Steptoe/Morex SNP map (Rostoks et al., 2005).

This QTL was also found to be most important one in the study by Bonnardeaux et al. (2008). My results do not support the hypothesis of Romagosa et al. (1999) about that SD1 is the most important QTL in seed dormancy release based on the results for the Steptoe/Morex population; however, I cannot disproved them either. It seems plausible that epistatic interactions between these two QTL on 5HL, and between these two and other genomic regions are responsible for most of the dormancy release responses occurring at early-after-ripening. Chromosome 5HL also harbors other QTL implicated in the regulation of several malting quality and agronomic traits including: diastatic power, free α-amino acid, α-amylase, heading date and test weight (Mather et al., 1997; Marquez-Cedillo et al., 2001; Panozzo et al., 2007), which complicates the ability of breeding for seed dormancy and PHS tolerance since changes in dormancy could cause changes in malt quality (Li et al. 2003; Bonnardeaux et al., 2008). My results highlight the importance of two major QTL in chromosome 5HL near the telomere involved in the regulation of seed dormancy, as well as other minor ones located in chromosomes 2H, 4H, 6H and 7H. The observation of numerous epistatic interactions between loci in chromosome 5HL and other chromosomal regions is indicative of its importance for the control of this trait. Additionally, the observation of positive correlations between α-amylase, wort protein, and Kolbach Index with seed dormancy in some of the years and two breeding programs (e.g. University of Minnesota and Washington State University) suggest that further studies should include the validation of the SNP markers identified herein, and the assessment of correlations between malting/agronomic traits and seed dormancy in order to determine if the associations are due to linkage or pleiotropy (Li et al., 2003; Lohwasser et al., 2013). Finally, it has yet to be determined if the implementation of a MAS breeding strategy combining the selection of the appropriate dormancy levels with the desired malt quality attributes would highly

benefit the selection of undesirable genotypes before submitting samples to malt quality assessments that are costly, labor intensive and time consuming.

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CHAPTER III. UNRAVELING THE GENETICS OF SEED DORMANCY IN SIX-ROWED BARLEY USING A DOUBLE HAPLOID POPULATION DERIVED FROM A NARROW CROSS

Abstract

The problem imposed by differential expression of dormancy levels on barley (*Hordeum vulgare* L.) seed is a key factor affecting malt quality. Dormant genotypes need a prolonged storage time before malting, which increases the probability of seed decay if problems with storage conditions appear. In contrast, low dormant genotypes are more prone to pre-harvest sprouting (PHS), which affects seed viability and makes grains worthless for malting. An F_1-derived doubled-haploid (DH) population with 193 individuals was developed by intercrossing two closely related six-rowed malting cultivars, Stander and Robust, which fit the requirements and preferences of the two major brewing companies in the U.S., Anheuser-Bush InBev (ABI) and MillerCoors Brewing Company (MillerCoors). The population was used to determine the genetics of seed dormancy in a narrow genetic germplasm base. The progeny and parents of this population were grown in three greenhouse experiments using a simple partial lattice design. Spikes were harvested at physiological maturity and their grains were used to determine the germination percentage after 72 h. Eighty-eight SNP markers and 191 individuals were used to build a linkage map covering 206.7 cM, which represents six of the seven barley chromosomes, except chromosome 7H. A single QTL was detected towards the telomere of chromosome 5H that accounts for 69.2% (LOD=48.87) of the phenotypic variation observed for seed dormancy, where the allele coming from 'Stander' increased the overall phenotypic mean 17%.

Introduction

Barley (*Hordeum vulgare* L.) has been one of the most important crops since ancient times based on archeological evidence and the study of genes involved in the domestication process (Pourkheirandish and Komatsuda, 2007). Barley's high adaptation to a wide range of environments, including the equatorial to boreal zones, has allowed its dissemination and expansion around the globe begining 8,000 years ago (von Bothmer et al., 2003; Pedraza-Garcia, 2011). The introduction of barley cultivars into the new world most likely occurred during the second voyage of Christopher Columbus; however, the first documented evidence about barley crops being successfully grown in the U.S. territories of Martha's Vineyard and Virginia date back to 1630 (Wiebe, 1979). Due to the unbearable climate of the eastern seaboard, barley production was limited until settlements penetrated into western New York. The crop's popularity for brewing purposes caused its production to spread through all the colonies and by mid-1800 most growers incorporated the use of six-rowed barley cultivars. By 1873 the University of Wisconsin introduced the cultivar Manchuria, which became very popular amongst farmers. Selected Manchuria seeds were distributed to Wisconsin farmers and after several cycles of selection seed was send to state farms in Minnesota and North Dakota, starting the wide-spread use of Manchuria barley in the Midwest (Wiebe 1979; Weaver, 1943).

U.S. barley production from 2003 to 2012 has averaged about 4.8 million tones per year, with an average annual value of $785 million as a raw commodity (USDA\NASS <http://www.nass.usda.gov/> accessed: 2 May 2013). Barley domestic use is mostly devoted to the production of alcoholic beverages including beer (53.5%) and whiskey (1.7%), and is used in lesser amount for livestock feed and food purposes (~41.6%). The three states producing the most barley in the country are North Dakota (68.1 m bu) followed by Idaho (48.7 m bu) and

Montana (37.5 m bu) (Source: USDA\NASS http://quickstats.nass.usda.gov/, verified 10 June 2013 and cited by the American Malting Barley Association: 'Economic Significance of Barley' <http://ambainc.org/media/AMBA_PDFs/NBIC/2013_Economics.pdf> accessed: May 30th, 2013).

Since barley is the raw material required for the production of beer, the selection of parents with optimal quality traits is critical for the development of new cultivars that follow industry quality guidelines (Horsley et al., 1995; Rasmusson and Phillips, 1997). Most of these parents are closely related elite cultivars bred for low seed dormancy and adaptation to specific production areas, which has the concomitant effect of reducing the genetic diversity within the crop (Horsley and Harvey, 2010; Mikel et al., 2008). Additionally, the malt quality specifications from the two major brewing companies in the U.S., ABI and MillerCoors has been quite different since the year 2000 (Lewis, 2012). MillerCoors use a blend of six and two-rowed cultivars that have low dormancy (>98% non-dormant seeds) and moderate protein modification/enzymatic levels (Kay, 2005). On the other hand, ABI prefers the use of barley cultivars that have low dormancy as well, but that exhibit higher levels of protein modification, enzymatic activity, and higher soluble protein levels (Hertrich, 2005). These factors, coupled with premiums paid to growers for producing the same malting cultivars each year, has augmented the incidence of PHS and diseases in the Upper Midwest (Rasmusson and Phillips, 1997; Schwarz el al., 2004).

Among modern cereals, barley has undergone strong selection by plant breeders against extended seed dormancy in order to promote uniform and quick germination during malting (Oberthur, 1995). In malting, the ultimate goal is to maximize endosperm modification of the kernel by changing its friability and increasing the enzymatic activity. However, a problem

imposed by differential expression of seed dormancy leads to reductions in grain and malt quality, affecting the production of fermentable sugars that are needed for the production of beer.

Seed dormancy is an adaptive trait characterized by the inability of viable seeds to germinate under favorable conditions (Foley, 2001) and is a main factor contributing to PHS tolerance (Mares, 1984; Rodríguez et al., 2001; Chao et al., 2010). It has been well documented that there is a large amount of genetic variation underlying both traits in common wheat (*T. aestivum* L.), rice (*Oryza sativa*) and barley, where expression is strongly controlled by environmental factors and their genotype x environment interactions (Buraas and Skinnes, 1984; Gu et al., 2005; Lin et al., 2009; Chao et al., 2010).

Traditional genetic studies of seed dormancy in barley have relied on the use of biparental mapping in which at least one parent is unadapted. While this strategy is effective in identifying quantitative trait loci (QTL) controlling traits of interest, it may not be as effective in identifying QTL conferring differences in one's targeted germplasm. Burass and Skinnes (1984) suggested that several recessive genes, with no cytoplasmic effects, control seed dormancy in barley; however, neither genes nor gene locations were identified in the study. Additionally, no associations with other agronomic traits and dormancy were identified (Burass and Skinnes, 1984). In later studies, up to 26 QTL were identified for seed dormancy across the entire barley genome, with a large effect QTL reported in chromosome 5H proximal to the centromere (5HC) and in the telomeric region in the long arm (5HL; Oberture et al., 1995; Lin et al., 2009; Ullrich et al., 1993; Li et al., 2004).

For the purpose of this study, I used an F_1-derived doubled-haploid (DH) population derived from the cross between two closely related spring six-rowed malting cultivars (Stander and Robust) for biparental mapping for seed dormancy . Both parents differ greatly in

agronomic, malting quality, and seed dormancy performance, but also exhibit low levels of genetic variability, which is the result of the selection and intermating of a small number of founder lines belonging to an elite gene pool. Robust and Stander were developed by the University of Minnesota and released by the Minnesota Agricultural Experimental Station in 1983 and 1993, respectively. The cross 'Manker' x 'Morex' gave origin to 'Robust', which is present in the pedigree of 'Stander' four times [Robust*2/3/'Cree'/'Bonanza'//'Manker'/4/'Robust'/'Bumper'] (Rasmusson et al., 1993; Rasmusson and Phillips, 1997; Pedraza-Garcia, 2011; Lewis, 2011). The close kinship of both cultivars results in most of the shared genomic regions being monomorphic, which represents a challenge for the identification of functional polymorphisms associated with traits of interest (Lin, 2007; Pedraza-Garcia, 2011).

Previous studies conducted by Lin (2007) and Pedraza-Garcia (2011) using the Robust x Stander DH population found that less than 10% of the SSR and DArT markers identified polymorphism between 'Stander' and 'Robust', which suggests the presence of few genomic regions that account for most of the phenotypic differences between the two parents. To overcome some of the limitations associated with the identification of functional polymorphisms in this genetically narrowed cross, I utilized a 9,000-SNP iSelect Illumina platform to genotype the DH population. This chip was developed by Martin Ganal (IPK, Gaterslaben, Germany) and Robbie Waugh (James Hutton Institute, Dundee, Scotland) in a collaborative effort to genotype all barley present in the USDA-ARS National Small Grain Collection, and to make the data available for the barley scientific community (Triticeae Coordinated Agricultural Project http://www.triticeaecap.org accessed: January 11, 2012). The premise of mapping in a very narrow population is that even though are very few regions with polymorphisms, those areas

containing them are more likely to be in regions harboring genes important for Midwest U.S. six-rowed malting barley.

The objective of this study was to determine the location and effect of QTL controlling seed dormancy in the genetically narrow F_1-derived DH population from the cross Stander x Robust. Information gathered in this study will provide clues on the genetics of seed dormancy in barley with special attention on Midwest US six-rowed barley germplasm, which has a narrow genetic base.

Materials and methods

Plant materials and genotyping approach

One-hundred and ninety-six DH lines from the cross 'Stander x Robust' were generated by The New Zealand Institute for Plant and Food Research Limited (Lincoln, New Zealand) using the *Hordeum bulbosum* (bulbous barley grass) method (Houben et al., 2011). A subset of 54 lines from this population was used in previous studies to identify QTL controlling malting quality and agronomic traits (Lin, 2009; Pedraza-Garcia, 2011; Lewis, 2012). An additional set of 142 DH lines was received from our collaborators in New Zealand and increased in Fall 2010 greenhouse for the purpose of this study. From the initial population, one line was lost during the process of seed increase and two others were discarded due to heterozygosity, which was unexpected. A total of 193 lines, two parents and one check (Tradition) were assigned to experimental units using a simple 14 x 14 partial lattice. The experiment was repeated over three consecutive greenhouse seasons. Randomizations were performed using the software AGROBASE Generation II v. 18.18.2 (Mulitze, 1990; 2010 Agronomix Software, Inc.). Experimental units were 15.24-cm-diameter clay pots (Ceramo, Jackson, Missouri) containing a potting media of Sunshine LC 8 soil mix (Sun Gro Horticulture, Canada) and each pot contained

three seeds from a single entry. All experiments were conducted under greenhouse conditions at a photoperiod of 16 h day/8 h night and temperatures of 20 °C day/18 °C night, respectively. Granular fertilizer Osmocote Plus (Scotts, Maryville, OH) was applied at the two-leaf stage, followed by weekly applications of a solution of 20-20-20 Jack's Peat Lite (JR Peters, Inc, Allentown, PA) at recommended rate. Spikes were harvested at physiological maturity (PM), described as the point at which 95 % maximum kernel dry weight is attained. Visual indicators of PM included loss of green color from the glumes and the peduncle (Copeland and Crookston, 1985). Harvested spikes were placed in Ziploc-type plastic bags and stored at -20°C until germination test (GT) were performed.

Additionally, the entire biparental population, Robust and Stander were sown in fall 2011 greenhouse to obtain leaf tissue samples for DNA extraction. One seed per genotype was sown in 21-cm-tall Ray Leach UV stabilized cone-tainers (Stuewe & Sons, Inc., Tangent, OR) filled with a potting media of Sunshine LC 8 soil mix (Sun Gro Horticulture, Canada) and placed in plastic trays that held up to 98 cone-tainers. The methods for collection of leaf tissue and DNA extraction are the same as those described by Bodo Slotta et al. (2008) in the laboratory of Dr. Shiaoman Chao at USDA-ARS in Fargo, ND. The population was genotyped utilizing a customized 9,000-Infinium iSelect HD Custum BeadChip panel and the Infinium HD assay protocol developed by Illumina, Inc. (San Diego, CA). This assay interrogates the genome through a two-step process that includes the hybridization of 50-mer probes to the loci of interest, followed by an enzymatic single base extension reaction that incorporates a fluorescent labeled (i.e., Cy5= red or Cy3=green) nucleotide. The Illumina iScan imaging system was used to detect specific alleles at a locus based on signal intensity and color, which is the basis for genotype calling. A cutoff threshold 'GenCall' of 0.15, plus the use of a clustering algorithm

and normalization of the data was possible though the implementation of the software GenomeStudio™ v. 1.0 (Illumina, Inc., San Diego, CA). Heterozygous genotypes were rare (1.04%) as expected from the process of production of DH lines by the *H. bulbosum* method. These lines were discarded from the analysis of data.

Germination test and statistical analysis

The percent of non-dormant seeds was determined using the protocol Barley 3-C of the American Society of Brewing Chemist (1999), with some modifications. The method consists germinating 100 kernels uniformly spread over two sheets of 90 mm Whatman® filter paper in 51 mm Petri dishes previously saturated with 4 mL of distilled water. Petri dishes were sealed with Parafilm M (Pechiney Plastic Packaging Company; Chicago IL) to maintain stable moisture conditions. Samples were incubated in the dark for 72 h at 20 ± 2˚C and relative humidity of 98% in a growth chamber (Percival Scientific; Perry, IA). The percent of non-dormant seeds was determined at 72 h as described by Lin et al. (2009). Data gathered from each test year were analyzed separately using the MIXED procedure of SAS v. 9.3 (SAS Institute, Inc. 2011), where block was considered a random effect and genotypes were a fixed effect.

Using SAS v. 9.3 (SAS Institute, Inc. 2011), germination data from each greenhouse run were considered an environment and they were analyzed separately using the PROC LATTICE statement to calculate the individual error mean squares (EMS) and the intrablock EMS. Homogeneity of variances among experiments was determined by the ratio of the largest to the smallest intrablock EMS, or in other words, if the EMS did not differ by more than a factor of 10 then variances were considered homogeneous. Based on this premise a combined ANOVA across experiments was done using the MIXED procedure, with experiments deemed random and genotypes fixed. *F*-tests were considered significant at $P<0.05$.

Heritability estimates for the combined environments were calculated for seed dormancy from the components of variance using the following equation:

$$h^2 = \frac{\sigma_G^2}{\sigma_G^2 + {\sigma_{GE}^2}/{e} + {\sigma_e^2}/{er}}$$

were σ_G^2=genetic component of variance, σ_{GE}^2=variance due to genotype*environment interaction, σ_e^2=experimental error variance, e=number of environments, and r=number of replicates. The heritability of seed dormancy and its associated standard error were estimated using the method described by Holland et al. (2003) for the analysis of random lines in an incomplete block design, in this case a simple 14 x 14 lattice design conducted at three environments. Both parental lines and check (Tradition) were removed from the data set to assess the variance and covariance components, as well as the heritability on a plant and family basis, respectively. The variance and covariance parameters were calculated using the COVTEST and ASYCOV options of the MIXED procedure **(**SAS Institute, Inc. 2011**),** with environments and genotypes deemed random. The heritability estimates were calculated using the multivariate restricted maximum likelihood (REML) method implemented using PROC MIXED **(**SAS Institute, Inc. 2011**)** and as described by Holland et al. (2003) and Holland (2006). Approximate standard errors for heritability were estimated using the delta method (Lynch and Walsh, 1998). Matrix computations to estimate the standard errors were calculated using PROC IML **(**SAS Institute, Inc. 2011**).**

Genotypic and phenotypic coefficients of variation were calculated according to Singh and Chaudhary (1977) as follow:

$$GCV = \frac{\sqrt{\sigma_G^2}}{\bar{X}} x100$$

$$PCV = \frac{\sqrt{\sigma_P^2}}{\bar{X}} x100$$

where, GCV=genotypic coefficient of variation, PCV=phenotypic coefficient of variation, σ^2_G=genotypic variance, σ^2_P=phenotypic variance, and $\bar{X}$ = general mean**.**

Genetic data analysis and mapping approach

The 9,000-Infinium iSelect HD Custum BeadChip was used for genotyping the lines and parents in the study. Cosegregating markers were manually removed and the number of linkage groups was determined using the software MapDisto v. 1.7.5 (Lorieux 2012; http://mapdisto.free.fr) with a minimal LOD score of 3.0 and maximum recombination of 0.30. The order of the markers in each linkage group was determined using the Order, Ripple, and Check inversions commands. The Seriation II algorithm and SARF (Sum of Adjacent Recombination Frequencies) criteria were chosen to determine the best linkage order of each sequence (Buetow and Chakravarti, 1987; Lorieux 2012). Additionally, the stability and robustness of each sequence was validated with 1,000 bootstrappings, and the Kosambi function was used to convert recombination fractions into centiMorgans (cM) (Kosambi 1944). Segregation ratios of individual markers were assessed statistically at an individual marker locus for deviations from the expected Mendelian ratio (1:1) by a X^2-test. If the marker deviated from the expected 1:1 ratio, then the equation proposed by Zhang et al. (2010) was used to explain the effect of marker distortion over the estimation of QTL detection power as follow:

$$k = \frac{\sigma_{SD}^2}{\sigma^2} = \frac{p\,(1-p)}{f(1-f)} = \frac{1-(1-2p)^2}{1-(1-2f)^2}$$

were *k* denotes the ratio of the variance under distortion to the variance of no distortion, *p* and 1-*p* are the frequencies of two QTL types, and *f* and 1-*f* are the frequencies of two genotypes that segregate according to the Mendelian ration 1:1. So, in the case of distortion, *k* will be smaller than 1, and therefore the QTL detection power will be reduced.

QTL analysis

QTL analyses were performed using the phenotypic adjusted means across all experiments utilizing the software QGene v. 4.3.10 (Nelson, 1997). The population distribution for seed dormancy was plotted and tested for normality using the Kolmogorov-Smirnov test. Data were considered normally distributed if *P* value >0.05. Single Marker Regression (SMA) Analysis was initially done to identify chromosomal regions associated with dormancy using the statistics –log p(*F*), R^2 and additive effect. Markers were considered significantly associated at a *P* value < 0.001. Next, simple interval mapping (SIM) was done using the step-wise interval analysis every 2 cM, and permutation tests with 1,000 iterations were done to determine the LOD scores for the $\alpha_{0.01}$ and $\alpha_{0.05}$ experiment-wide errors needed to declare significant marker-trait associations. Composite interval mapping (CIM) was conducted as well using the default parameters for cofactor selection suggested in QGene v. 4.3.10 (Nelson, 1997), in which markers outside an interval containing a QTL are selected as cofactors. This method allowed the positioning and estimation of the magnitude of the QTL. Again, to determine the LOD score for the experiment-wide error rate, a permutation test with 10,000 iterations was used. Graphic representation of the linkage groups and QTL was obtained using the software MapChart v 2.2 (Voorrips, 2002) and map locations for the identified QTL were estimated based on the published consensus maps by Close et al., (2009)

Results and discussion

Phenotypic data

Robust, the dormant parent, and Stander, the non-dormant, parent behaved as expected for seed dormancy. Robust had germination percentages ranging from 0 to 31%, while Stander had relatively higher levels of germination ranging from 32 to 74%. The phenotypic distribution for the mapping population was continuous and showed a bimodal distribution with population means intermediate to both parents, and ranges extending beyond the parents. The minimum value observed for germination was 4.1% and the maximum 74.5% (Figure 3.1).

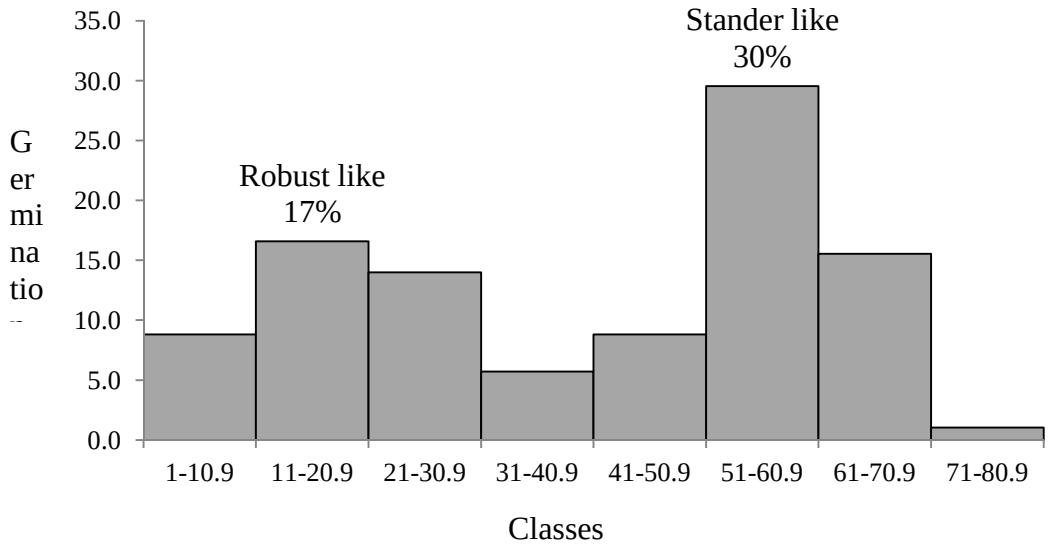

Figure 3.1. Phenotypic distribution of the Stander/Robust DH population means estimated based on the LSD (*P*=0.05) for percent of non-dormant seeds across environments.

Approximately 17% of the progeny behaved similar to Robust, while 30% behaved similar to Stander. Our results corroborate those of Lin (2007) who conducted a similar study with a smaller number of lines from the Robust x Stander DH population. Lin (2007) reported mean germination values across environments for Robust, Stander and the DH population to be 23.3%, 65%, and 43.7%, which are similar to the values reported herein. Mean germination

percentage values for the parents and progeny are presented for the individual analysis of environments, as well as the means across experiments (Table 3.1). The relative efficiency of the three greenhouse experiments analyzed, as a partial simple Lattice design compared to RCBD was 107.13%, 121.70% and 100.86%, corresponding to the 2011 spring greenhouse (11sgh), 2011 fall greenhouse (11fgh), and 2012 spring greenhouse (12sgh) seasons, respectively. Intrablock error mean squares from the individual environment analysis of variance (ANOVAs) were homogeneous among experiments, as determined by the ratio of the largest to the smallest intrablock EMS (Table 3.2). Thus, a combined analysis across environments was performed based on the premise of homogeneity of variances (Table 3.1).

Table 3.1. Mean percent non-dormant seeds measured at 72 h on caryopses harvested at physiological maturity from parents and the Stander/Robust DH progeny, based on the individual and combined analysis of the three experiments.

Season	Robust	Stander	Population			
	----------Mean----------		Mean	LSD	SD	Range
11sgh‡	5.2 a†	58.2b	31.6	32.5	22.4	0-76.6
11fgh‡	13.2a	61.2b	46.7	30.9	22.5	1.1-91.3
12sgh‡	30.5a	48.5a	42.2	31.8	21.1	2.5-87.5
Combined	15.2a	54.3b	40.1	17.8	20.0	1.1-75.0

† The 2011 spring greenhouse (11sgh), 2011 fall greenhouse (11fgh), 2012 spring greenhouse (12sgh).
‡ Means for parents between columns followed by the same letter are not significantly different ($P \leq 0.05$) as determined by an *F*-test.

Table 3.2. Results from the analysis of variance for seed dormancy coming from three greenhouse season experiments.

Source of Variation	DF	11sgh	11fgh	12sgh
		----------MS----------		
Replications	1	1657	16316	54757
Blocks(rep) (Adj.)	26	530	785	337
Genotypes (Unadj.)	195	1039	1104	889
Intra Block Error	169	253	224	260
Total	391	667	742	718

† 11sgh=the 2011 spring greenhouse, 11fgh=2011 fall greenhouse, and 12sgh=2012 spring greenhouse.

The combined analysis revealed a non-significant genotype-by-environment interaction, and a highly significant (P<0.01) genotype main effect**.** Most of the variance observed for seed dormancy was associated with the genotype main effect (47%), and the variance associated with the environment main effect was negligible as expected for the given greenhouse conditions (Table 3.4). The estimates of heritability based on individual environment analyses were high and consistent, ranging from 0.71±0.04 to 0.77±0.03, while the estimate of heritability for the combined analysis was comparatively higher at 0.90±0.01 (Table 3.4).

Table 3.3. Results from the combined analysis of seed dormancy from three greenhouse seasons.

Sources of Variation	Degrees of freedom	MS
Environment	2	22585
Rep(environment)	3	24243
Block(environment*rep)	78	550**
Genotype	195	2129**
Genotype*environment	390	225
Error	507	246

*, **Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 3.4. Estimate of variance components, heritability plus the standard error, and genotypic (GCV) and phenotypic coefficients of variation (PCV) for seed dormancy.

Covariance parameter	11sgh	11fgh	12sgh	Combined
Environment	-	-	-	0
Rep(environment)	2.72	76.05	267.95	115.37
Block(rep*environment)	51.31	87.16	24.34	50.61
Genotype	358.27	380.61	301.78	358.57
Genotypes*environment	-	-	-	0
Error	251.24	226.62	252.51	234.50
Heritability	0.74±0.04	0.77±0.03	0.71±0.04	0.9±0.01
CV	50.10	32.23	37.65	39.14
GCV	59.84	41.77	41.15	47.27
PCV	69.50	47.54	49.02	49.77

The estimates for the phenotypic and genotypic coefficients of variation for the individual analyses of environments were very close to each other (Table 3.4), ranging from 41.2% to

59.8% for the GCV and 47.5% to 69.5% for the PCV. The GCV and PCV values were higher in the 2011 spring greenhouse compared to the other two seasons. The estimates of GCV and PCV based on the combined analysis were very close as well; suggesting that phenotypic selection based on the germination test is reliable for selection of genotypes with desired levels of seed dormancy.

Creation of a genetic linkage map and QTL analysis

From a total of 6,715 SNP markers used to screen the mapping population, only 6.6% (445 SNPs) were found to identify polymorphisms between Stander and Robust. The lack of polymorphism detected between them can be explained as the result of their close kinship, which results in most shared genomic regions being monomorphic and only few being responsible for most of the phenotypic differences observed between them (Pedraza-Garcia, 2011). The few polymorphic regions identified may harbor good candidate genes for MAS for seed dormancy and other important quality and agronomic traits.

After the removal of cosegregating markers, a total of 88 SNPs were used to build the final map (Figure 3.2). Six out of seven barley chromosomes are represented herein, with three linkage groups representing chromosome 5H (5H-1, 5H-2, and 5H-3). A representation for chromosome 7H was not found in either this study or in Pedraza-García (2011). This could be the result of most chromatin regions in chromosome 7H being identical between Stander and Robust. As reported by Pedraza-García (2011), there are linkage gaps in chromosome 5H, which are represented herein by two small linkage groups of 4.1 and 5.2 cM and a larger one of 80.6 cM distance (Figure 3.2). Linkage group 5H-1 corresponds to a small section of the long arm of chromosome 5H between the 159cM to 163.29 cM region on the barley consensus map by Close et al. (2009). The linkage group 5H-2 contains markers near the telomere of the short arm of 5H

corresponding to the region 5.38 cM to 10.58 cM (Close et al., 2009). Finally, linkage group 5H-3 corresponds to the region near the telomere on chromosome 5HL. Some of the SNP markers mapping within this linkage group have not been mapped before (e.g. SCRI_RS_141226), but most of the BOPA SNPs had (Close et al., 2009; Muñoz-Amatriaín et al. (2011), which allowed me to determine that 5H-3 comprise a region between distances173 cM (1_0869; Close et al., 2009) to 194 cM (12_20775; Close et al., 2009) and extending beyond that in both directions (Figure 3.2). Again, a hypothesis that explains why we obtained several linkage groups representing chromosome 5H is the existence of complete homology in certain chromosomal regions between both parents spanned by few polymorphic regions. It is not surprising that much of their genome would be fixed since they were produced by intermating a small number of founder lines belonging to an elite gene pool followed by selection of parents that follow strict malt quality guidelines.

Interestingly, segregation distortion was observed on a group of 10 SNP makers clustered in chromosome 5H-3 and on those markers belonging to chromosomes 1H and 2H, respectively. We detected that segregation distortion for those SNP markers located on 5H-3 was toward the maternal allele (Stander), while the distortion on chromosomes1H and 2H was towards the paternal allele (Robust) (appendix Table A28). Pedraza-Garcia, (2011) documented the existence of deviations from the expected 1:1 ration for about 12 SNP markers that detected polymorphism between Stander and Robust.

This phenomenon of segregation distortion has been reported for other DH populations including Morex/'Barke', 'Oregon Wolfe', Steptoe/Morex and 'Haruna Nijo'/OHU602. Most of the segregation distortion was observed in the Morex/Barke population towards the pericentric regions of chromosomes 1H, 2H, 5H, 7H and the long arm of 7H (Close et al., 2009). Sayed et

al. (2002) studied this phenomenon comparing 71 DH lines from the cross 'Tadmor'/'WI2291' with the segregation of 92 F_2 lines coming from the same cross by using simple sequence repeat (SSR) markers. Their results showed that loci deviated 44% more in the DH than in the F_2 population (16%). Even though, the production of DH lines allows reaching homozygosity faster than using other conventional breeding methods, including pedigree and backcrossing, it seems to be less effective than recombinant inbred lines (RILs) for mapping purposes (R. Brueggemann, personnel communication, 2013).

Segregation distortion has been documented to occur in DH populations produced using the anther culture and *Hordeum bulbosum* (H.b.) methods. While the anther culture method targets male gametes, the H.b. method targets female gametes. The former uses the F_1 as the male parent, from which microspores are taken for the regeneration of entire barley plants by *in vitro* tissue culture; while the latter involves the interspecific hybridization of F_1 individuals (used as females) with *H. bulbosum,* followed by embryo rescue, regeneration, uniparental chromosome elimination (*H. bulbosum*), and chromosome doubling of the *H. vulgare* chromatin by colchicine (Cistué et al. 2011; Houben et al. 2011).

Cistué et al. (2011) reported that DH populations derived by male gametes having segregation distortion is the result of differential performance of the parents in *in vitro* tissue culture, while with the H.b. method segregation distortion is more likely the result of allelic variation. The comparative mapping study by Cistué et al. (2011) using two subpopulations of the 'Oregon Wolfe' barley derived from the utilization of both methods revealed there was a greater amount of segregation distortion in the anther culture derived subpopulation than in the H.b.-derived subpopulation, which could affect the power of QTL estimation. However, the impact of segregation distortion on QTL analysis will depend on the degree of dominance of the

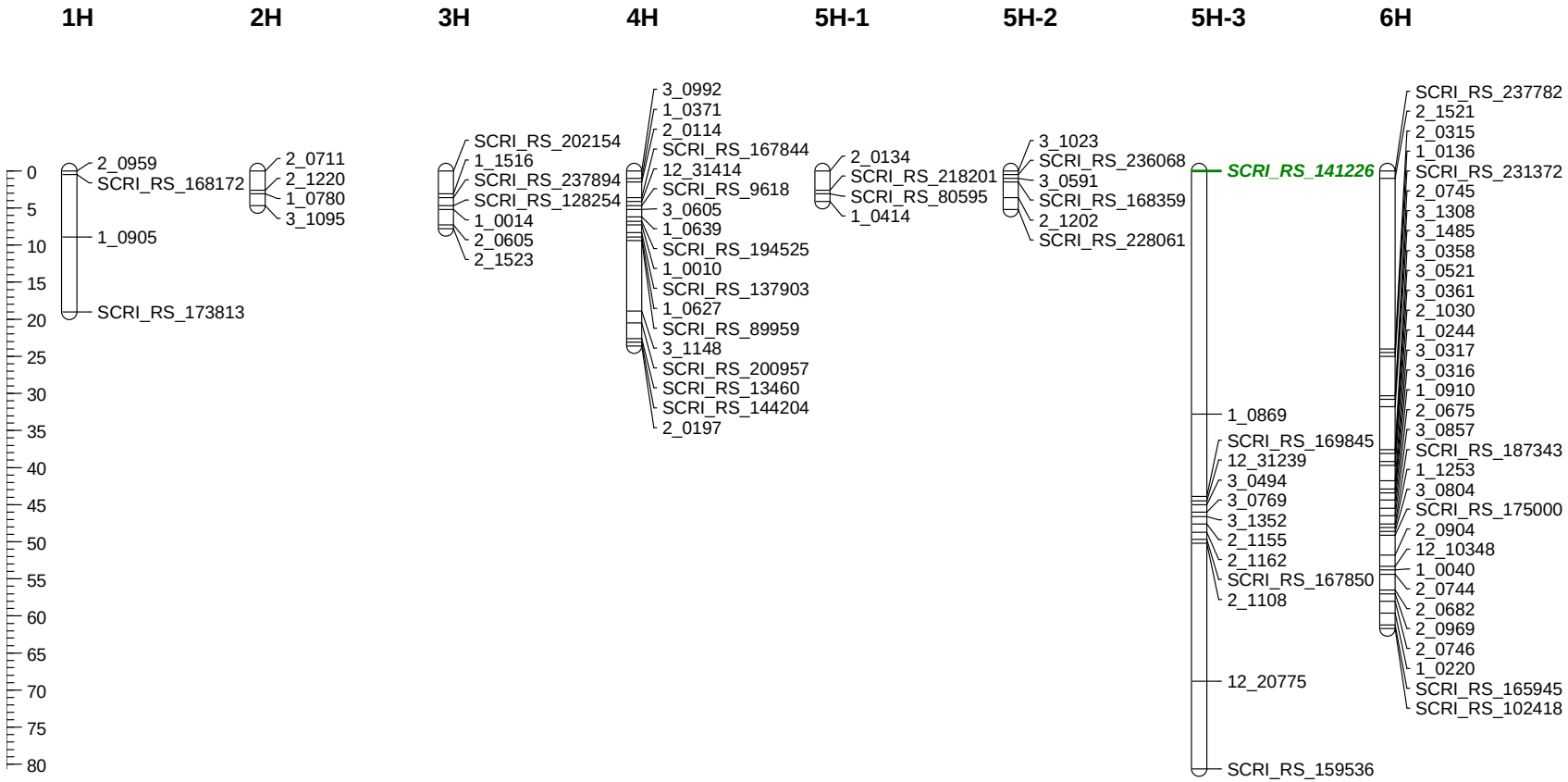

Figure 3.2. Linkage map for the Stander/Robust population built with 88 SNP non-cosegregating markers from the 9K iSelect chip.

QTL, the level of linkage existing between distorted markers and the QTL, as well as the population size (Zhang et al., 2010).Recent simulation studies by Zhang et al. (2010) documented the effect of segregation distortion on QTL mapping detection using an F_2 population. Their results indicate that the effect of distortion decreases rapidly if there is not a tight linkage relationship between the distorted markers with the QTL, and in some cases the higher genetic variance resulted from the distortion may benefit the detection of linked QTL (Zhang et al., 2010). For the particular case of DH populations only the additive effects can be estimated due to the existence of only two distinct genotypic classes (e.g. AA or BB at a single locus) compared to an F_2 population for which three distinct genotype classes are detected (e.g. AA, AB, and BB), making easier the estimation of both additive and dominance effects.

The effect of marker distortion on the estimation of QTL detection power was initially assessed using the equation proposed by Zhang et al. (2010) [eq.4] and through the implementation of 10,000 permutations using the CIM function on QGene v. 4.3.10 (Nelson, 1997). Even though, we performed single marker regression, SIM and CIM analyses, only the results for the CIM will be presented and discussed herein, since the final outcome for the three analyses was nearly identical. Marker cofactors were selected using the default parameters of QGene v. 4.3.10 (Nelson, 1997). The R^2, the threshold of the odds (LOD), and the additive effects were determined for each of the four QTL analyses corresponding to the separate analysis of each environment and the combined analysis across environments. Ten thousand permutations were used to determine the LOD scores for the $\alpha_{0.01}$=3.39 and $\alpha_{0.05}$=2.38 experiment-wide error needed to declare significant marker-trait associations. One QTL for seed dormancy ($\alpha_{0.01}$=3.39) was identified in chromosome 5H-3. Markers SCRI_RS_141226 and 1_0869 flanked the QTL, and it spanned 32.8 cM (Figure 3.3; appendix Table A29). I named the

QTL QDrm.SdRo-5H.2 had an LOD=48.87 and accounts for 69.2 % of the phenotypic variation observed in seed dormancy for the 'Stander/Robust' population (Figure 3.3; appendix Table A29).

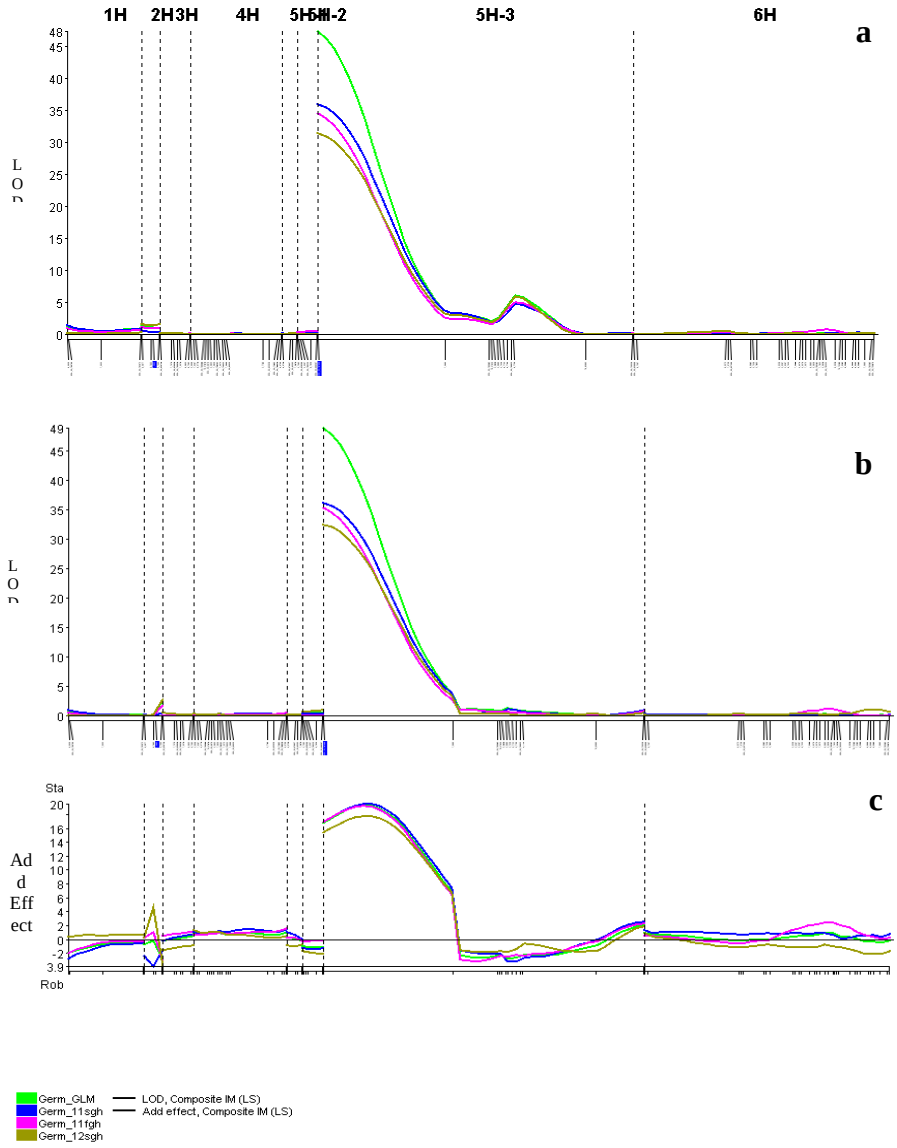

Figure 3.3. Genome-wide distribution of LOD values (Y-axis) for percent of non-dormant seeds using composite interval mapping. Ten thousand iterations were carried out to determine the LOD scores for the $\alpha_{0.01}$ and $\alpha_{0.05}$ ($\alpha_{0.01}$=3.39 and $\alpha_{0.05}$=2.38) needed to declare significant marker-trait associations. a) Distribution of LOD values previous to cofactor selection; b) Distribution of LOD values after cofactor selection; c) Additive effect.

The alleles from ‘Stander’ were found in about 17% of the non-seed dormant phenotypes, suggesting there may have been negative selection against ‘Robust’ alleles, which are associated with significantly higher levels of seed dormancy. The position of QDrm.SdRo-5H.2 corresponds with the position of QDrm.BCAP-5H.2 (Close et al., 2009), which is effectively QTL SD2 (Han et al., 1996; Bonnardeaux et al., 2008; Lohwasser et al., 2013).

The segregation distortion of some of the markers located in 5H-3 (Figure 3.4) did not affect the estimation of QTL detection power based on a *k* value higher than 1 (*k*=1.2) (Zhang et al., 2010).

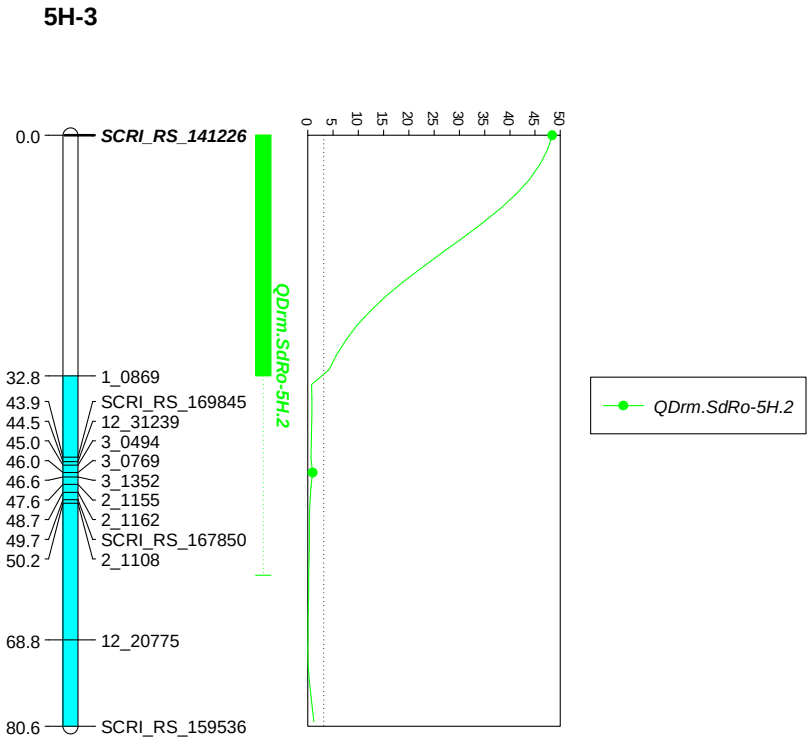

Figure 3.4. Position of QDrm.SdRo-5H.2 on linkage group 5H-3 and distribution of LOD values associated with the percent of non-dormant seeds. The blue shadowed area on the chromosome corresponds to region where markers showed significant segregation distortion (P<0.0001) from the Mendelian 1:1 ration. The green solid box represents the interval where the QTL was detected.

For several years the effect of distorted markers in QTL analysis was unknown, and for that reason markers were discarded as a preventive solution. However, recent studies have shown that distorted markers can be safely used for the purpose of QTL mapping with low or no detrimental effect on the QTL detection power (Xu et al., 2009; Zhang et al., 2010). This phenomenon occurs as the result of gametic selection, zygotic selection, or both (Xu et al., 2009). The SNP markers that showed significant segregation distortion on chromosome 5HL are located in a region that also harbors other QTL implicated in the regulation of several malting quality and agronomic traits including: diastatic power, free α-amino acid, α-amylase, heading date ant test weight (Mather et al., 1997; Marquez-Cedillo et al., 2001; Panozzo et al., 2007), which complicates the ability to breed for seed dormancy and PHS tolerance since direct changes in dormancy could cause concomitant changes in malting attributes (Li et al. 2003; Bonnardeaux et al., 2008). It is not surprising that this cluster of markers show significant segregation distortion since this particular genomic region has been the target of strict selection for malt quality.

The repeatable expression and importance of this QTL for seed dormancy in both association mapping and biparental mapping analyses is supported by the results of Bonnardeaux et al. (2008) based on the analysis of the 'Stirling'/'Harrington' DH population. They confirmed that the QTL 5Hqb or SD2, which is located on the telomere of chromosome 5H had the largest additive effect and accounted for most of the variability observed for seed dormancy in barley. Even though, most biparental studies in seed dormancy have confirmed the existence of two QTL regions on chromosome 5HL, SD1 located near the centromere and SD2 on the telomere region, only one was detected herein and it corresponds to QTL SD2. Romagosa et al. (1999) proposed that SD1 was the most important QTL in seed dormancy release, based on the high

variability that this one explained (~50% of the phenotypic variance) across different environments (Oberthur et al., 1995; Han et al., 1996). The QTL SD1 was never detected in this study and should not be expected to be for different reasons, including: i) use of a narrow based population, which make it hard to find polymorphism due to most shared genomic regions being monomorphic between both parents; ii) the fact that different markers have been used across different mapping studies makes difficult to make valid comparisons for QTL positions based on different crosses; iii) marker order and map distances vary among crosses depending on the saturation of the maps; and iv) the statistical methods and thresholds used to declare significance vary among studies (Clancy et al., 2003). Another possibility is that the SD1 region in Robust and Stander has been genotypically fixed. Even though most biparental mapping studies have relied on the use of populations derived from parents distantly related for the identification of significant QTL, I believe that identifying these regions using a narrow germplasm based population will better represent the case of barley breeding programs that breed for malting barley adapted to specific growing areas. Additionally, identification of specific genes conferring actual phenotypic differences in Robust and Stander should be easier.

Gene annotation for the SCRI_RS_141226

Pedraza-García (2011) identified a DArT marker bPb-9660 that explains 81% of the phenotypic variation observed for percent of non-dormant seeds using a subpopulation of the 'Stander/Robust' cross. This marker mapped to a similar region on chromosome 5HL comparable to that identified by SCRI_RS_141226. According to Pedraza-García this locus is under selection pressure, and "only certain allele combinations increase the percentage of the non-dormant seed" phenotype. I wanted to determine if bPb-9660 and SCRI_RS_141226 are indeed detecting the same locus. To test this hypothesis, I compared the nucleotide sequence

from the DArT marker bPb-9660 with the complete codon sequence (GenBank accession #: AK363215.1) from which marker SCRI_RS_141226 was obtained (Matsumoto et al., 2011). To do so, I utilized the software Clutal Omega v. 1.1.0 (EMBL-EBI < http://www.ebi.ac.uk/Tools/msa/clustalo/> accessed: 3 June 3 2013). The results from the alignment indicate there is a similarity of only 53.5%, which makes it hard to determine if both markers map exactly to the same region. The alignment between the sequences is not shown herein due to proprietary rights held by Triticarte® (Yarralumla, Australia) over all DArT technology.

On the other hand, the results from searching for translated nucleotide databases using the complete translated nucleotide query sequence for AK363215.1 (Figure 3.5) indicates its association with a large family of protein kinases (PKs; E-value: 9.21e-52; http://blast.ncbi.nlm.nih.gov/ accessed: 3 June 2013). Protein kinases (PKs) have been proposed as candidate proteins involved in the signaling pathway of the phytohormone abscisic acid (ABA) based on the observation of increased mRNA levels for a serine/threonine kinase known as PKABA1, which accumulates on wheat and barley developing embryos as ABA levels rise (Anderberg and Walker-Simmons, 1992; Gómez-Cadenas et al., 1999). ABA is well known for its important role in seed dormancy induction and maintenance, as well as in mediating plant responses to environmental, biotic, and abiotic stresses, including drought, salinity, and cold (Gómez-Cadenas et al., 1999; Finkelstein et al., 2008). Gómez-Cadenas et al. (1999) using particle bombardment transformation techniques to introduce two types of genetic constructs in the barley genome to determine the role of protein kinases in the signaling transduction pathway mediating ABA expression. Their results indicate that PKABA1 acts as an antagonist of GA-inducible genes by mimicking ABA. The constitutive expression of the PKABA1 construct

resulted in the suppression of the expression both low and high PI α-amylase genes, as well as other protease genes that are induced by GA.

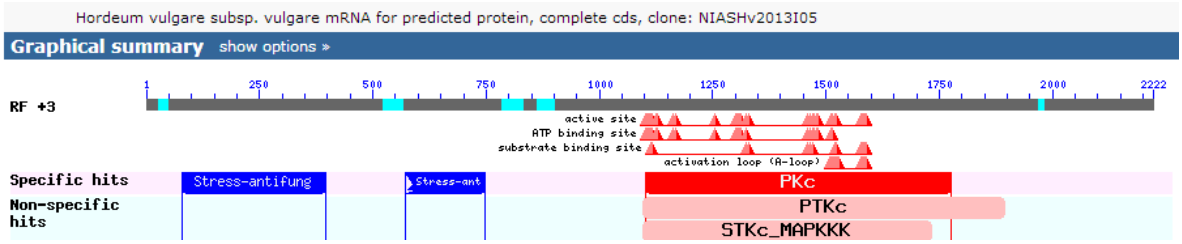

Figure 3.5. Results from the TBLASTX search for the sequence AK363215.1 from which marker SCRI_RS_141226 was obtained.

Based on the results obtained from the analysis of the sequence AK363215.1 and its predicted protein product (protein ID: BAJ94419), we identified that marker SCRI_RS_141226 is associated with a putative protein kinase having 69% identity with a cysteine-rich receptor like protein kinase 10-like from *Brachypodium distachyon* (L.) P.Beauv. (Gene Bank accession #: XP_003563975.1); and it is also 53.7% similar to PKABA1 from *Secale cereale* L. (Gene Bank accession #:DQ295068.1) and 55.0% similar to PK4 on wheat (Gene Bank accession #:AF519805.1). Tanaka et al., (2012) identified stress-inducible receptor-like kinases encoded by the genes *ARCK1* and *CRK36*, to be involved in the control of ABA and stress signaling transduction responses in Arabidopsis. The identification of protein kinases induced by ABA involved in the regulation of ABA provide a basis for the study of the role of these type of proteins in seed dormancy and stress responses (Anderberg and Walker-Simmons, 1992; Gómez-Cadenas et al., 1999; Tanaka et al., 2012).

Summary

The AA genotype (A allele=A) was detected in 105 individuals, while the BB genotype (B allele=G) was detected in about 86 individuals of the 'Stander/Robust' DH population for marker SCRI_RS_141226. The QTL on chromosome 5HL associated with marker SCRI_RS_141226

accounts for 69.2% (LOD=48.87) of the phenotypic variation observed for seed dormancy, were the A allele coming from 'Stander' increased the overall phenotypic mean by 17% for the non-dormant phenotype. Further studies should include the identification of markers on the interval between markers SCRI_RS_141226 and 1_0869 in order to saturate the region with informative markers that could be used for MAS of seed dormancy. Additionally, we suggest the validation of marker SCRI_RS_141226 and those markers that were found to be in segregation distortion to determine the relationships between malt quality traits and dormancy. The validation of such markers should be done using other narrow germplasm base populations (e.g. Barley CAP breeding lines from the University of Minnesota and the NDSU six-rowed program) in order to determine their utility for MAS.

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CHAPTER IV. SUMMARY AND CONCLUSIONS

Historically, most U.S spring barley breeding programs have bred cultivars with similar malting and brewing attributes in order to satisfy the preferences and demands of the malting and brewing industries. This makes the development of new cultivars a very difficult task, since many quality traits need to be considered and the decision to determine which lines to advance for further testing must to be made within a narrow period of time after the crop is harvested. A major problem associated with this scheme is that it can be difficult to determine if poor endosperm modification in a line during malting is due to extended seed dormancy or inherently poor malt quality. Thus, lines with extended seed dormancy are often culled in favor of those with low seed dormancy and desirable levels of endosperm modification. This can lead to lines that may have acceptable malt quality, but are susceptible to pre-harvest sprouting (PHS).

Since differential expression of dormancy levels in barley seeds impacts malt quality, there is a need to identify genomic regions that account for most of the phenotypic variation in order to design an optimal breeding strategy for the selection of cultivars with acceptable malt quality and seed dormancy. Marker-assisted selection (MAS) has been proposed as a means of identifying markers linked to important traits that follow a quantitative inheritance; however, its utility will depend on how reliable marker-trait associations are for predicting the phenotype (e.g. seed dormancy) based on the genotype. Validation studies and the development of strong predictive methodologies are imperative for the development of molecular applications that take advantage of the genotyping instead of phenotyping; thus, benefiting the selection of barley cultivars with low to intermediate dormancy levels and desirable malting attributes.

The objective of this study was to unravel the genetic basis underlying seed dormancy in spring barley using genome-wide association mapping (AM) and linkage mapping tools for the

analysis of: i) a panel of 3,072 elite U.S. spring barley breeding lines from eight breeding programs participating in the USDA-NIFA Barley Coordinated Agricultural Project and ii) a population of 193 F_1-derived doubled-haploid lines from the cross 'Stander' x 'Robust', respectively. All these with the aim to:

1. Identify marker-trait associations for seed dormancy that are specific to each breeding program that are candidates for use in MAS.
2. Identify polymorphic regions between Stander and Robust that can lead to the identification of marker-trait associations for use in MAS, with special attention on Midwest U.S. six-rowed barley germplasm, which has a narrow genetic base.
3. Propose a set of SNP markers for further validation studies to determine their utility for MAS.
4. Identify possible correlations between malt quality traits and seed dormancy
5. To identify pairwise epistatic interaction among SNP markers

Some important results and conclusions drawn from this research are:

- Two main QTL regions were consistently detected across breeding programs and years in the long arm of chromosome 5H using 2,965 barley CAP lines and genome-wide AM. This is indicative of the importance of this chromosome region in the regulation and maintenance of the physiological processes associated with seed dormancy in U. S. spring barley germplasm.
- Smaller effect QTL were identified independently in some breeding programs in chromosomes 2H, 4H, 6H and 7H using combined analyses across years for each breeding program.

- Even though a QTL relative to *SD1* (76.6cM-83.2 cM) was not detected across years within any breeding program or in the combined analysis across years within a program, a QTL which is likely *SD2* and named in here as QDrm.BCAP-5H.2 was detected in most breeding programs and years. Bonnardeaux et al. (2008) stated that *SD2* was the most important QTL region accounting for most of the variability observed for seed dormancy in their study.
- The annotation analyses for the genome-wide AM revealed that most of the genes identified near the telomere in chromosome 5HL are associated with the metabolism of hormones, as well as other mechanisms associated with defense, stress responses to dehydration and ROS, and hormone sensitivity mediated responses (i.e., jasmonate, pectinesterases, gibberellins, protease complex, radical induced cell death proteins, LEA proteins).
- The observation of positive correlations between α-amylase, wort protein, and Kolbach Index with seed dormancy in some of the years and two breeding programs (e.g. University of Minnesota and Washington State University) suggest that further studies should include the validation of the SNP markers identified herein, and the assessment of correlations between malting/agronomic traits and seed dormancy in order to determine if the associations are due to linkage or pleiotropy.
- The observation of numerous epistatic interactions between loci in 5HL and other chromosomal regions is indicative of the importance chromosome 5HL region for the control of this trait.
- The combined analysis on the 'Stander/Robust' DH population revealed a non-significant genotype-by-environment interaction for seed dormancy, and a highly significant

(P<0.01) genotype main effect (47%). The heritability estimate based on this analysis was very high **(**0.90±0.01) compared to the separate analysis of each environment. The estimated values for the genotypic and phenotypic coefficients of variation (GCV and PCV, respectively) were very close (GCV=47.27; PCV=49.77), which suggests that phenotypic selection based on the use of germination test is reliable for the identification of genotypes with desired levels of seed dormancy in barley.

- From a total of 6,715 SNP markers used to screen the 'Stander x Robust' population, only 6.6% (445 SNPs) were found to identify polymorphisms between the parents. The lack of polymorphisms can be explained by the close relationship of the two parents, which results in most shared genomic regions being monomorphic and only a few regions likely accounting for most observed of the phenotypic differences.
- An 88-marker linkage map covering 206.7 cM was developed and used to identify a single QTL for seed dormancy in the long arm of chromosome 5H that accounted for nearly 69.2% of the phenotypic variation.

  The SNP marker SCRI_RS_141226 within the QTL region is proposed for use in MAS. The A allele coming from Stander (non-dormant parent) increased the overall phenotypic mean of the non-dormant phenotype by 17%.
- The annotation analysis of the full coding sequence for marker SCRI_RS_141226 indicates it is associated with a putative protein kinase. Protein kinases (PKs) have been proposed as candidate proteins involved in the signaling pathway of the phytohormone abscisic acid (ABA), which is implicated in the seed dormancy maintenance and the activation of plant defense mechanisms.

- A cluster of SNP markers that showed significant segregation distortion on chromosome 5HL, approximately 32.8 cM from marker SCRI_RS_141226 was identified. Results suggest that the segregation distortion of those markers did not affect the estimation of QTL detection power based on a *k* value higher than 1 (*k*=1.2). This cluster of markers is located in a region that also harbors other QTL implicated in the regulation of several malt quality and agronomic traits, including: diastatic power, free α-amino acid, α-amylase, heading date, and test weight. The fact that QTL for these traits and seed dormancy reside in a similar region complicates the ability of breeding for seed dormancy and PHS tolerance because direct changes in dormancy could cause concomitant changes in malt quality. It is not surprising that this cluster of markers show significant segregation distortion since this particular genomic region has been the target of strict selection for malt quality.

APPENDIX

Table A1. Polymorphic markers identified for each of the forty AM analyses.

Breeding Program†	2006	2007	2008	2009	Combined 4 years
AB	2334	2359	2370	2330	2556
BA	2320	2162	2266	2314	2428
MT	1544	1689	2204	1441	2302
ND2R	2278	2197	2050	1855	2481
ND6R	1335	1287	1736	1474	2055
UM	1295	1539	1304	1186	1853
UT	2502	2195	2144	2115	2608
WA	2343	2341	2067	1979	2532

† AB=USDA-ARS-Aberdeen, ID; BA= Bush Agricultural Resources LLC..; MT= Montana State University; ND2R= North Dakota State University (2-Rowed); ND6R= North Dakota State University (6-Rowed); UM=University of Minnesota; UT= Utah State; WA=Washington State University.

Table A2. SNP markers having a convergence with the best linear models.

		Number of SNP Markers			
Breeding Program	Analysis	Naïve†	P	K	P+K
USDA-ARS-Aberdeen, ID	4 years	2436	2446	2468	2468
	2006	2257	2257	2257	2257
	2007	2359	2344	2354	2345
	2008	2362	2362	2355	2360
	2009	2330	2330	2329	2329
Bush Agricultural Resources LLC	4 years	2325	2320	2303	2297
	2006	2319	2319	2312	2319
	2007	2159	2159	2153	2134
	2008	2266	5207	2227	3993
	2009	2314	2314	2312	2314
Montana State University	4 years	2164	2167	2302	2302
	2006	1544	1544	1541	1544
	2007	1689	1689	1686	1688
	2008	2201	2202	2202	2202
	2009	1441	1441	1415	1441
North Dakota State University (two-row)	4 years	2468	2461	2465	2429
	2006	2277	2278	2278	2275
	2007	2197	2197	2197	2195
	2008	2050	2050	2050	2050
	2009	1855	1855	16	1768
North Dakota State University (six-row)	4 years	2029	2035	2046	2046
	2006	1335	1335	1335	1335
	2007	1283	1283	1283	1279
	2008	1731	1732	1732	1732
	2009	1460	1460	1456	1439
University of Minnesota	4 years	1809	1825	1852	1840
	2006	1295	1295	1295	1295
	2007	1539	1539	1420	1365
	2008	1304	1304	1304	1304
	2009	1102	1102	1102	1102
Utah State University	4 years	2537	2540	2607	2607
	2006	2502	2479	2502	1806
	2007	2161	2161	2153	2109
	2008	2130	2130	2130	2130
	2009	2115	2114	2112	2110
Washington State University	4 years	2515	2492	2476	2434
	2006	2343	2343	2341	2343
	2007	2341	2341	2011	78
	2008	2067	2067	1419	2067
	2009	1979	1979	1965	-

†Simple model=Naïve; P=principal component analysis (PCA); K=kinship; P+K=PCA+Kinship

Table A3. Significant marker-trait associations ($P \leq 0.001$) for percent of non-dormant seed identified for the USDA-ARS-Aberdeen, ID breeding program across years.

					Allele A		Allele B		Heterozygote			In
Year	Chr	cM	Marker	-log10(p)	Count	Mean	Count	Mean	Count	Mean	R^2	model
2006	5H	0.00	11_10593	5.44	15	81.60	78	51.12			0.13	
		173.08	11_10869	4.25	56	41.96	37	77.34			0.31	x
		179.06	11_10254	4.37	42	74.40	51	40.91			0.29	x
		179.64	11_21138	3.94	39	75.81	53	41.97	1	30.00	0.29	
		179.64	12_30656	3.94	39	75.81	53	41.97	1	30.00	0.29	
		194.64	12_30382	3.55	41	38.87	51	70.35	1	30.00	0.21	
		195.42	11_20402	4.93	51	71.19	41	37.82	1	30.00	0.28	x
		196.12	12_10322	3.96	49	71.16	43	39.41	1	30.00	0.26	
		196.85	12_31123	3.35	51	69.56	42	39.61			0.23	
2007	1H	36.95	11_21072	4.45	1	29.00	92	3.85			0.20	x
	2H	54.95	11_21096	3.58	91	3.76	2	20.50			0.17	x
		150.67	11_21436	3.81	52	3.49	39	4.92	2	5.00	0.01	x
	3H	43.23	11_20647	4.45	1	29.00	92	3.85			0.20	
	4H	0.00	11_10846	3.56	86	3.36	6	15.00	1	4.00	0.14	
		65.05	12_30620	3.65	88	3.54	4	17.00	1	4.00	0.11	
		65.05	11_11224	3.65	88	3.54	4	17.00	1	4.00	0.11	x
		65.05	11_11229	3.56	6	15.00	86	3.36	1	4.00	0.20	x
		65.80	12_30455	3.65	4	17.00	88	3.54	1	4.00	0.17	
	5H	191.97	12_31210	3.33	38	2.18	54	5.56	1	0.00	0.06	
		191.97	12_30360	3.30	40	2.15	52	5.72	1	0.00	0.07	x
		194.84	12_10857	5.66	25	3.24	68	4.45			0.01	
	6H	3.11	12_31233	4.24	76	3.70	16	4.56	1	29.00	0.06	
	Unlinked	0.00	12_31128	3.53	48	3.78	45	4.49			0.00	
2008	2H	113.48	11_11118	3.17	53	15.85	33	42.33	1	68.00	0.30	x
	4H	3.74	11_21228	3.35	5	2.21	81	27.48	1	68.00	0.08	x
		12.02	12_31458	3.86	19	12.82	66	29.12	2	69.50	0.13	
	5H	182.88	12_31352	4.39	31	6.25	56	37.70			0.37	x
		191.97	12_31210	4.21	26	5.72	60	35.93	1	0.00	0.25	
		191.97	12_30360	6.60	29	5.42	57	37.68	1	0.00	0.30	
		191.97	11_10401	7.14	31	5.58	55	38.76	1	0.00	0.33	x
2009	1H	126.48	11_11481	3.16	6	70.55	90	41.75			0.08	x
	3H	8.23	11_21190	3.91	49	50.39	43	38.40	4	15.17	0.11	x
	4H	77.31	11_11004	3.53	81	38.28	12	79.25	3	43.11	0.15	
		77.31	11_21353	3.02	82	38.37	14	73.90			0.26	x
		77.31	12_31231	3.02	82	38.37	14	73.90			0.26	
		77.31	12_30136	3.02	82	38.37	14	73.90			0.26	

Table A4. Significant marker-trait associations ($P \leq 0.001$) for percent of non-dormant seed identified for the Bush Agricultural Resources LLC breeding program across years.

					Allele A		Allele B		Heterozygote			In
Year	Chr	cM	Marker	-log10(p)	Count	Mean	Count	Mean	Count	Mean	R^2	model
2006	2H	0.00	11_10017	4.10	13	50.64	82	82.58			0.18	x
		27.29	11_20394	4.18	9	38.16	86	82.40			0.24	x
		63.53	11_10909	4.03	41	73.92	54	81.46			0.02	
		71.12	12_10719	3.55	60	72.02	35	88.81			0.10	
		78.03	12_30696	3.65	42	65.04	53	88.64			0.20	
		78.03	11_10196	3.65	42	65.04	53	88.64			0.20	
		133.94	11_20715	3.45	41	67.45	54	86.37			0.13	
	5H	2.09	11_20894	3.46	76	75.05	19	90.83			0.06	
		6.40	11_20206	5.32	62	86.01	33	63.55			0.17	x
		47.39	11_11432	3.10	88	81.08	7	42.11			0.15	
		47.39	12_30105	3.95	6	32.97	89	81.26			0.20	
		51.00	11_20129	3.08	37	76.05	58	79.58			0.00	
		51.00	11_20958	3.08	37	76.05	58	79.58			0.00	
		51.30	12_30728	3.08	37	76.05	58	79.58			0.00	
		51.30	12_30575	3.08	37	76.05	58	79.58			0.00	
		51.60	11_10661	3.08	58	79.58	37	76.05			0.00	
		65.49	11_20713	3.67	78	84.45	17	49.56			0.26	
		69.90	12_30080	4.50	51	71.00	44	86.57			0.09	
		87.35	11_21445	3.40	5	34.00	90	80.66			0.16	
		143.27	11_20551	3.68	26	54.49	69	87.15			0.31	
		175.90	11_10778	3.94	70	84.91	25	59.45			0.18	
		176.62	11_21012	3.94	25	59.45	70	84.91			0.18	
		176.62	11_10600	4.17	65	87.21	30	58.70			0.26	
		177.07	11_21141	3.00	23	64.52	72	82.58			0.09	x
		177.65	11_20536	3.86	72	84.37	23	58.91			0.17	
		179.06	11_10254	4.02	63	86.64	32	61.60			0.20	
		179.64	12_30656	3.63	61	87.65	34	61.27			0.23	
		179.64	11_21138	4.02	62	87.72	33	60.34			0.25	
		181.43	11_20022	3.87	15	46.89	80	84.08			0.27	
		189.60	11_20786	4.58	17	45.78	78	85.27			0.33	
		190.23	11_21108	3.02	51	88.92	43	66.46	1	36.96	0.21	
		191.97	12_31210	6.32	17	45.01	78	85.44			0.35	
		191.97	11_10401	7.54	20	46.73	75	86.60			0.38	
		191.97	12_30360	7.86	19	45.19	76	86.46			0.40	
		195.42	11_20402	7.94	76	86.46	19	45.18			0.40	x
		196.12	11_20132	6.19	17	45.00	78	85.44			0.35	x
		196.12	12_10322	7.41	75	86.60	20	46.72			0.38	
		196.12	12_30958	7.41	20	46.72	75	86.60			0.38	
		196.85	12_31123	7.41	75	86.60	20	46.72			0.38	
	6H	34.40	11_10427	4.99	18	63.09	77	81.74			0.08	x
		35.07	12_30358	3.91	13	64.14	82	80.44			0.05	
	7H	140.21	11_10454	3.42	27	61.54	68	84.83			0.16	x
		144.45	11_20452	3.06	45	81.85	50	74.93			0.02	
		144.45	12_30593	3.06	50	74.93	45	81.85			0.02	
		144.45	12_31166	4.27	67	83.68	28	65.10			0.10	
		159.27	11_21086	3.29	2	12.50	93	79.62			0.14	
		166.56	11_20365	3.19	34	78.90	61	77.82			0.00	
		166.56	11_10174	3.19	34	78.90	61	77.82			0.00	
	Unlinked	0.00	12_30685	3.58	12	66.12	83	79.95			0.03	
		0.00	12_31521	3.10	7	42.11	88	81.08			0.15	
		0.00	12_20985	3.87	4	25.50	91	80.52			0.18	
		0.00	12_31240	4.39	8	31.11	87	82.54			0.30	x

Table A4. Significant marker-trait associations ($P \leq 0.001$) for percent of non-dormant seed identified for the Bush Agricultural Resources LLC breeding program across years (cont.)

					Allele A		Allele B		Heterozygote			In
Year	Chr	cM	Marker	-log10(p)	Count	Mean	Count	Mean	Count	Mean	R^2	model
2007	1H	127.38	12_31387	4.42	29	21.26	63	6.98			0.29	
		128.14	11_11038	5.14	28	21.95	64	6.91			0.31	x
	2H	119.05	11_10780	3.03	7	29.14	85	10.03			0.17	
	3H	141.54	11_20851	3.22	59	7.44	32	17.73	1	50.00	0.23	
		142.32	12_30137	3.22	59	7.44	32	17.73	1	50.00	0.23	
		144.64	11_10631	3.28	60	7.57	31	17.82	1	50.00	0.23	
		155.09	11_20155	3.12	36	17.10	55	7.11	1	50.00	0.08	
		155.85	12_30921	3.52	18	7.78	73	11.87	1	50.00	0.05	x
	4H	0.00	11_10379	3.39	27	20.98	65	7.54			0.24	
		40.36	12_20240	3.18	86	10.13	6	30.83			0.17	
		47.60	11_11405	4.02	23	9.09	69	12.28			0.01	
		51.30	12_11063	3.48	21	9.14	71	12.18			0.01	
		54.25	11_11114	3.39	27	20.98	65	7.54			0.24	
		61.04	12_30054	3.33	22	9.55	70	12.09			0.01	
		65.05	12_31515	4.47	6	33.77	86	9.93			0.23	
		65.05	12_30620	4.47	86	9.93	6	33.77			0.23	
		65.05	11_11229	4.47	6	33.77	86	9.93			0.23	x
		65.05	11_20906	4.47	86	9.93	6	33.77			0.23	
		65.05	11_11224	4.47	86	9.93	6	33.77			0.23	
		65.05	11_20924	3.61	23	22.15	69	7.93			0.25	
		65.05	11_10639	3.61	69	7.93	23	22.15			0.25	
		65.05	11_11431	3.61	23	22.15	69	7.93			0.25	
		65.05	11_10052	3.61	69	7.93	23	22.15			0.25	
		65.80	12_30455	4.47	6	33.77	86	9.93			0.23	
		67.46	11_10606	4.47	6	33.77	86	9.93			0.23	
	5H	94.43	12_21497	3.13	78	9.14	14	24.54			0.20	
		94.43	12_11106	3.13	14	24.54	78	9.14			0.20	
		94.43	12_10930	3.13	14	24.54	78	9.14			0.20	
		95.08	11_10578	3.13	14	24.54	78	9.14			0.20	x
		180.71	12_30494	3.00	9	11.89	73	12.31	10	5.10	0.02	
		182.88	12_31352	5.66	31	9.52	61	12.48			0.01	
		182.88	11_20897	3.06	9	4.56	83	12.24			0.03	
		189.60	11_20786	3.29	10	4.80	82	12.30			0.04	x
		189.60	11_11364	3.97	11	4.36	81	12.45			0.04	x
		191.97	11_10401	3.35	15	6.47	77	12.46			0.03	x
		191.97	12_31210	4.21	10	3.30	82	12.48			0.05	
		191.97	12_30360	4.21	10	3.30	82	12.48			0.05	
		194.84	12_10857	4.21	10	3.30	82	12.48			0.05	
	7H	91.79	12_30026	3.37	20	8.25	69	12.82	3	2.42	0.01	
	Unlinked	0.00	12_30597	3.48	71	12.18	21	9.14			0.01	
		0.00	12_31414	4.02	69	12.28	23	9.09			0.01	
2008	4H	78.77	11_10523	3.54	54	17.20	39	29.25	2	0.50	0.04	x
		85.04	12_10670	8.86	94	21.23	1	75.00			0.08	
	6H	51.41	12_30569	6.19	35	8.49	60	29.56			0.26	x
		54.60	12_31007	8.86	94	21.23	1	75.00			0.08	
		58.55	12_10803	8.86	94	21.23	1	75.00			0.08	
		60.23	12_30804	8.86	1	75.00	94	21.23			0.08	x
		83.89	11_11147	5.49	6	27.06	89	21.44			0.00	
	Unlinked	0.00	12_30655	3.03	41	8.73	54	31.72			0.33	x
2009	4H	62.83	11_20453	3.35	64	52.33	31	33.23			0.21	x
		62.83	11_21296	3.35	31	33.23	64	52.33			0.21	
	5H	182.88	11_20897	3.27	13	26.20	82	49.25			0.16	x
		191.97	12_30360	3.15	12	25.39	83	49.09			0.16	x
	Unlinked	0.00	12_20295	3.07	93	45.95	1	100.00	1	6.00	0.00	

Table A5. Significant marker-trait associations ($P \leq 0.001$) for percent of non-dormant seed identified for the Montana State University breeding program across years.

					Allele A		Allele B		Heterozygote			In
Year	Chr	cM	Marker	-log10(p)	Count	Mean	Count	Mean	Count	Mean	R^2	model
2006	5H	190.23	11_21108	4.83	19	92.79	66	51.45	11	95.36	0.01	
		195.42	11_20402	11.84	41	92.56	54	43.72	1	52.00	0.57	x
		196.12	12_10322	11.84	41	92.56	54	43.72	1	52.00	0.57	
		196.85	12_31123	10.57	42	91.60	54	43.72			0.58	x
	Unlinked	0.00	12_31267	3.28	39	44.72	56	78.79	1	52.00	0.25	
2007	2H	31.72	11_20864	3.67	35	7.60	56	2.67	1	18.00	0.11	x
		57.12	11_20444	3.29	8	16.88	83	3.45	1	12.00	0.31	
	3H	59.89	11_10653	3.60	17	10.29	74	3.33	1	12.00	0.16	x
		168.40	11_20057	4.08	83	3.38	9	17.00			0.49	x
	4H	0.74	12_30764	4.37	9	16.56	83	3.43			0.45	x
		3.74	11_21056	5.59	7	18.43	84	3.44	1	16.00	0.31	
	7H	161.54	11_20170	3.84	87	3.94	5	18.20			0.31	x
2008	7H	28.27	12_30329	3.29	39	22.56	42	34.74			0.08	x
2009	6H	22.35	12_30843	3.17	88	33.05	5	57.20			0.05	
		22.35	11_10023	3.17	88	33.05	5	57.20			0.05	x
	Unlinked	0.00	12_30050	3.17	88	33.05	5	57.20			0.05	

Table A6. Significant marker-trait associations ($P \leq 0.001$) for percent of non-dormant seed identified for the North Dakota State University (two-rowed) breeding program across years.

					Allele A		Allele B		Heterozygote			In
Year	Chr	cM	Marker	-log10(p)	Count	Mean	Count	Mean	Count	Mean	R^2	model
2006	1H	4.51	12_10636	3.62	6	76.00	88	47.70			0.06	x
		93.95	11_10433	3.20	86	52.59	8	16.38			0.12	x
	4H	26.19	11_20302	3.40	90	48.07	4	81.75			0.05	x
		119.84	12_30239	4.23	35	32.66	58	59.68	1	49.00	0.18	x
	5H	190.23	11_21108	3.25	12	81.75	82	44.78			0.18	x
		194.64	12_30382	3.93	25	28.40	35	50.94	34	63.54	0.22	x
		195.42	11_20402	3.78	71	56.38	23	28.26			0.17	
		196.12	12_10322	3.54	68	57.02	26	29.85			0.17	
		196.85	12_31123	3.54	68	57.02	26	29.85			0.17	
	Unlinked	0.00	12_11368	3.05	5	20.00	88	50.63	1	98.00	0.08	
		0.00	12_30793	3.46	6	85.50	88	47.05			0.10	
		0.00	12_31267	3.90	45	36.51	49	61.44			0.18	
2007	1H	57.77	12_10198	3.80	4	28.59	91	9.49			0.14	
	2H	54.95	11_21388	3.21	93	9.78	2	34.18			0.12	
		105.77	11_10630	3.21	24	16.31	71	8.26			0.12	
		122.21	12_30152	3.09	43	9.46	51	10.27	1	47.37	0.02	
		149.36	11_21299	4.16	91	9.56	3	34.46	1	4.00	0.05	
		149.61	11_20943	8.40	92	9.48	2	50.68	1	4.00	0.08	
		150.67	11_21436	9.26	93	9.42	2	50.68			0.34	
	4H	28.40	11_21374	4.89	90	9.26	5	28.87			0.19	
		36.37	11_20411	5.70	4	32.84	91	9.30			0.22	
		96.59	12_30554	4.46	22	8.80	72	10.14	1	54.00	0.03	
	5H	107.59	11_10024	8.93	32	10.59	58	7.94	5	35.62	0.05	
		108.18	11_21321	4.98	51	9.92	39	8.22	5	30.22	0.04	
		108.18	12_10844	4.33	40	6.50	52	12.07	3	30.06	0.16	x
		109.56	11_21168	9.33	44	10.66	46	7.19	5	35.62	0.04	x
		110.26	12_30705	5.56	53	9.94	39	8.65	3	37.79	0.04	x
		113.11	11_10477	4.54	34	10.90	60	9.22	1	54.00	0.00	
		122.38	11_20629	4.30	4	29.84	91	9.43			0.16	
		123.08	11_20637	9.26	2	50.68	93	9.42			0.34	x
		151.36	12_30062	3.67	5	26.27	90	9.40			0.14	
		151.36	12_30183	5.45	91	9.32	4	32.34			0.21	
		178.43	12_11010	5.65	6	18.50	88	9.24	1	54.00	0.00	x
		179.64	12_30656	5.03	7	15.57	87	9.36	1	54.00	0.00	
		181.43	11_20189	6.21	26	14.19	68	8.16	1	54.00	0.02	
		181.43	11_10236	3.19	52	7.12	43	14.13			0.12	x
	6H	38.42	12_30521	4.41	25	10.32	69	9.65	1	54.00	0.01	
		67.70	11_21469	5.02	76	9.04	18	13.16	1	54.00	0.11	
	7H	161.54	11_20170	3.04	77	8.50	13	19.26	5	14.55	0.09	
	Unlinked	0.00	12_30827	3.04	13	19.26	77	8.50	5	14.55	0.05	
		0.00	12_31128	4.30	91	9.43	4	29.84			0.16	

Table A6. Significant marker-trait associations ($P \leq 0.001$) for percent of non-dormant seed identified for the North Dakota State University (two-rowed) breeding program across years (cont.)

					Allele A		Allele B		Heterozygote			In
Year	Chr	cM	Marker	-log10(p)	Count	Mean	Count	Mean	Count	Mean	R^2	model
2008	1H	131.15	11_20383	3.48	14	27.36	81	10.80			0.20	x
		137.83	11_20840	3.76	9	27.78	85	11.59	1	23.00	0.09	
		138.92	11_20509	3.66	8	31.13	86	11.47	1	23.00	0.13	
	4H	48.50	11_10261	3.11	3	3.33	90	12.68	2	53.50	0.15	
		48.50	11_10942	3.53	4	2.75	89	12.81	2	53.50	0.15	x
		50.40	12_30605	3.11	90	12.68	3	3.33	2	53.50	0.10	
		51.30	11_20496	3.11	90	12.68	3	3.33	2	53.50	0.10	
		53.50	12_30427	3.11	3	3.33	90	12.68	2	53.50	0.15	
	5H	178.43	12_11010	4.18	7	36.00	87	11.07	1	43.00	0.13	
		178.43	12_11450	3.33	88	11.43	7	36.00			0.23	
		182.88	11_20897	3.04	66	8.82	26	23.66	3	20.33	0.22	
		182.88	12_30769	3.92	24	25.40	71	9.13			0.28	x
		187.96	11_10310	4.20	81	10.82	14	27.25			0.19	x
		189.60	11_21052	4.47	79	10.70	16	25.78			0.18	
	6H	31.73	11_10994	3.07	91	12.97	4	19.50			0.01	
	7H	61.32	11_10346	3.89	7	35.60	88	11.46			0.23	x
		62.88	11_10721	3.06	9	29.02	86	11.59			0.15	
	Unlinked	0.00	12_10257	4.47	79	10.70	16	25.78			0.18	
2009	5H	94.43	12_11106	3.62	2	1.00	90	42.11	3	29.33	0.02	x
		94.43	12_10930	3.62	2	1.00	90	42.11	3	29.33	0.02	
		95.08	11_10578	3.15	5	18.00	90	42.11			0.13	x
	6H	0.00	11_11329	3.27	87	42.66	7	21.09	1	20.00	0.15	
		67.70	11_20468	3.27	87	42.66	7	21.09	1	20.00	0.15	
		67.70	11_20636	3.27	87	42.66	7	21.09	1	20.00	0.15	
		69.38	12_31289	3.27	7	21.09	87	42.66	1	20.00	0.08	
		70.04	11_20673	4.28	89	42.44	6	17.00			0.17	x

Table A7. Significant marker-trait associations ($P \leq 0.001$) for percent of non-dormant seed identified for the North Dakota State University (six-rowed) breeding program across years.

					Allele A		Allele B		Heterozygote			In
Year	Chr	cM	Marker	-log10(p)	Count	Mean	Count	Mean	Count	Mean	R^2	model
2007	1H	121.12	12_21172	3.98	17	33.00	77	15.37			0.29	x
		128.14	11_20133	3.55	18	32.11	76	15.35			0.27	
		128.14	12_30649	3.98	77	15.37	17	33.00			0.29	
2008	5H	135.72	12_30883	3.19	9	27.24	81	8.76			0.26	
		142.20	11_11532	3.19	81	8.76	9	27.24			0.26	
		142.20	12_31366	3.19	9	27.24	81	8.76			0.26	
		142.20	11_10845	3.19	81	8.76	9	27.24			0.26	
		142.20	11_21289	3.19	81	8.76	9	27.24			0.26	
		143.92	11_20375	3.19	81	8.76	9	27.24			0.26	
		143.92	12_30556	3.19	9	27.24	81	8.76			0.26	
		143.92	11_10819	3.19	9	27.24	81	8.76			0.26	x
		187.38	11_21155	3.02	27	7.44	63	11.96			0.04	
		196.12	12_30958	4.06	26	16.71	63	7.86	1	25.00	0.10	
		196.12	12_10322	4.06	63	7.86	26	16.71	1	25.00	0.15	x
		196.85	12_31123	4.25	64	8.13	26	16.71			0.13	
	Unlinked	0.00	12_30502	4.06	26	16.71	63	7.86	1	25.00	0.10	
2009	3H	162.15	12_30767	3.06	49	40.64	36	40.74	2	71.50	0.01	

Table A8. Significant marker-trait associations ($P \leq 0.001$) for percent of non-dormant seed identified for the University of Minnesota breeding program across years.

					Allele A		Allele B		Heterozygote			In
Year	Chr	cM	Marker	-log10(p)	Count	Mean	Count	Mean	Count	Mean	R^2	model
2006	5H	191.97	12_31210	3.58	4	8.75	91	70.65	1	50.00	0.11	
		191.97	12_30360	3.58	4	8.75	91	70.65	1	50.00	0.11	
		191.97	11_10401	3.09	7	23.57	88	71.58	1	50.00	0.13	x
		194.84	12_10857	3.33	10	42.50	85	71.05	1	50.00	0.06	
2007	5H	25.23	11_10695	3.41	2	27.50	90	5.25			0.13	
	6H	105.60	11_20036	3.62	89	5.11	3	24.00			0.14	
		110.32	11_20355	3.62	3	24.00	89	5.11			0.14	
		110.32	12_30734	3.62	3	24.00	89	5.11			0.14	x
	7H	36.77	12_30242	4.48	1	44.00	89	5.42	2	0.50	0.11	
		86.44	12_30199	5.11	1	44.00	91	5.31			0.20	
		87.97	12_10089	5.11	1	44.00	91	5.31			0.20	
		91.79	12_30996	5.11	1	44.00	91	5.31			0.20	
		99.67	12_30806	5.11	91	5.31	1	44.00			0.20	x
		166.56	12_10378	4.35	89	5.26	2	7.50	1	44.00	0.15	
		166.56	12_30826	4.35	89	5.26	2	7.50	1	44.00	0.15	
		166.56	11_20365	5.11	91	5.31	1	44.00			0.20	
		166.56	11_10174	5.11	91	5.31	1	44.00			0.20	
2008	7H	133.79	11_10861	4.36	5	61.00	90	22.76			0.10	x
2009	4H	39.76	11_20012	3.01	68	35.72	20	29.53			0.02	
		40.96	12_30328	3.43	10	21.70	78	35.93			0.05	
		42.45	11_10048	3.43	78	35.93	10	21.70			0.05	x
	7H	148.25	11_10896	3.20	78	37.64	10	8.40			0.21	x
		161.54	11_20170	3.04	78	37.64	9	5.33	1	36.00	0.16	x
		166.56	12_10378	3.04	78	37.64	9	5.33	1	36.00	0.16	
		166.56	12_30826	3.04	78	37.64	9	5.33	1	36.00	0.16	
	Unlinked	0.00	12_30827	3.04	9	5.33	78	37.64	1	36.00	0.21	

Table A9. Significant marker-trait associations ($P \leq 0.001$) for percent of non-dormant seed identified for the Utah State University breeding program across years.

					Allele A		Allele B		Heterozygote			In
Year	Chr	cM	Marker	-log10(p)	Count	Mean	Count	Mean	Count	Mean	R^2	model
2006	4H	26.19	11_20109	3.15	22	26.97	71	25.96	3	71.00	0.02	
		40.36	12_10063	3.46	8	58.47	88	24.79			0.15	x
		55.63	12_31462	3.35	77	29.73	19	18.96			0.03	x
		55.63	12_30995	3.35	77	29.73	19	18.96			0.03	
	5H	187.96	11_10310	4.14	73	21.39	22	48.41	1	23.00	0.18	x
		189.60	12_31292	4.28	92	25.52	4	75.43			0.17	x
		194.84	12_10857	5.20	66	22.27	30	39.33			0.11	
2007	1H	136.31	11_20594	4.23	39	1.61	48	4.75	3	29.52	0.13	x
	4H	116.85	11_21130	3.77	5	23.80	85	3.06			0.24	x
		120.58	12_31422	3.02	6	19.83	84	3.10			0.18	
	6H	71.87	12_31101	3.48	75	2.09	13	13.27	2	25.00	0.26	x
		72.54	12_30940	5.77	27	4.51	62	3.34	1	50.00	0.01	x
		72.54	11_20488	6.08	22	2.50	67	4.09	1	50.00	0.04	
		72.54	11_10469	5.78	56	2.46	33	5.80	1	50.00	0.10	x
		76.55	12_30573	6.08	67	4.09	22	2.50	1	50.00	0.02	
		77.89	11_21224	6.07	35	1.63	54	5.04	1	50.00	0.09	
	7H	46.19	11_21528	3.60	4	24.25	86	3.28			0.20	x
	Unlinked	0.00	12_30939	5.68	65	3.44	24	4.41	1	50.00	0.05	x
		0.00	12_30908	5.68	65	3.44	24	4.41	1	50.00	0.05	
		0.00	12_31200	3.77	85	3.06	5	23.80			0.24	
2008	1H	135.56	12_11496	4.21	25	5.12	58	28.58			0.23	
	3H	80.89	11_20115	3.57	24	4.50	59	28.44			0.23	x
		80.89	12_30170	3.57	24	4.50	59	28.44			0.23	
		81.66	12_31262	3.57	24	4.50	59	28.44			0.23	
		162.15	12_30767	3.01	20	12.43	61	22.51	2	82.08	0.12	
	5H	196.85	12_31123	3.42	19	28.35	64	19.49			0.03	x
2009	2H	9.28	11_20563	4.12	10	10.60	84	41.03	2	22.83	0.12	x
	3H	120.59	11_11330	3.29	43	31.90	53	42.01			0.06	
		120.59	12_31220	3.38	53	42.43	43	31.39			0.07	
		123.68	11_21405	3.57	37	28.85	59	42.89			0.11	
		123.68	11_10918	3.39	60	42.94	36	28.39			0.11	x
		173.17	12_20345	3.04	89	35.93	6	59.17	1	45.66	0.05	x
	4H	50.40	11_20289	3.30	49	42.90	43	28.24	4	70.42	0.01	
	5H	161.58	11_20646	3.40	8	40.88	87	36.57	1	90.00	0.00	x
		161.58	12_30642	3.40	87	36.57	8	40.88	1	90.00	0.04	x
		182.88	12_31352	4.77	19	19.88	74	41.71	3	44.67	0.16	
		191.97	12_30360	5.37	31	20.90	63	45.53	2	41.00	0.27	x
		194.84	12_10857	5.73	49	29.07	45	46.49	2	41.00	0.15	x

Table A10. Significant marker-trait associations ($P \leq 0.001$) for percent of non-dormant seed identified for the Washington State University breeding program across years.

					Allele A		Allele B		Heterozygote			In
Year	Chr	cM	Marker	-log10(p)	Count	Mean	Count	Mean	Count	Mean	R^2	model
2006	3H	37.17	11_10672	3.24	57	43.16	35	41.17	2	79.00	0.00	x
	4H	66.00	12_30904	3.15	21	69.52	72	34.72	1	99.00	0.13	
		66.00	12_30755	3.15	21	69.52	72	34.72	1	99.00	0.13	
		66.00	11_10010	3.15	21	69.52	72	34.72	1	99.00	0.13	
		66.00	12_31385	3.15	72	34.72	21	69.52	1	99.00	0.22	x
		66.00	12_30905	3.15	72	34.72	21	69.52	1	99.00	0.22	
	5H	178.43	12_11010	3.16	10	85.67	82	37.15	2	78.00	0.11	
		178.43	12_11450	3.39	84	38.12	10	85.67			0.19	x
		179.06	11_10254	3.43	22	78.51	71	31.97	1	62.00	0.30	
		179.64	11_21138	4.95	20	84.37	73	31.64	1	62.00	0.36	
		179.64	12_30656	4.95	20	84.37	73	31.64	1	62.00	0.36	
		180.71	11_10736	3.15	15	83.11	77	34.50	2	78.00	0.17	
		191.97	11_10401	4.13	65	31.59	26	68.21	3	77.33	0.26	x
		195.42	11_20402	5.52	32	71.96	60	26.97	2	69.00	0.29	
		196.12	12_30958	4.01	58	29.34	34	65.27	2	69.00	0.26	
		196.12	12_10322	6.63	26	77.01	66	29.07	2	69.00	0.30	
		196.85	12_31123	6.92	28	76.44	66	29.07			0.42	x
2007	1H	69.53	12_10166	4.03	85	2.31	10	8.80	1	22.00	0.17	
		69.53	12_30298	6.43	4	24.75	92	2.25			0.48	x
		72.43	12_11267	5.49	4	24.75	88	2.25	4	2.25	0.25	
		75.45	11_20121	4.28	88	2.26	8	13.38			0.22	x
		77.29	11_20657	4.28	88	2.26	8	13.38			0.22	
	2H	17.85	11_20107	3.40	88	1.67	7	19.57	1	22.00	0.56	x
		69.13	12_10545	4.03	8	13.63	87	1.94	1	28.00	0.10	
		71.12	12_31020	3.43	8	17.75	87	1.56	1	28.00	0.24	
		71.56	12_10717	3.43	87	1.56	8	17.75	1	28.00	0.62	
		73.04	11_20528	3.43	87	1.56	8	17.75	1	28.00	0.62	
		133.94	12_30106	3.50	10	18.00	86	1.47			0.60	
		133.94	12_30396	10.27	10	20.00	86	1.23			0.78	x
		139.65	11_10625	3.31	85	1.25	11	18.18			0.69	
		147.12	12_10181	3.31	11	18.18	85	1.25			0.69	
	4H	26.19	11_21418	4.38	10	11.30	85	1.88	1	33.00	0.06	x
		26.19	11_20109	4.39	86	1.86	9	12.56	1	33.00	0.44	x
		111.68	11_11299	4.83	4	24.00	91	2.13	1	16.00	0.25	
	5H	26.28	11_20873	3.10	59	3.03	34	2.44	3	14.67	0.02	
		27.00	11_10974	3.27	33	2.52	59	3.03	4	11.00	0.03	
		94.43	12_10930	3.04	6	20.50	90	2.03			0.47	
		100.28	11_10771	3.04	6	20.50	90	2.03			0.47	x
		108.18	11_21321	3.31	85	1.25	11	18.18			0.69	
		108.18	12_30852	3.31	85	1.25	11	18.18			0.69	
		110.26	12_10507	3.31	11	18.18	85	1.25			0.69	
		110.26	12_30705	3.31	85	1.25	11	18.18			0.69	
		149.64	11_20791	6.80	91	2.68	4	8.50	1	28.00	0.16	
		149.64	11_21297	6.80	91	2.68	4	8.50	1	28.00	0.16	
		151.36	12_10333	6.61	92	2.96	4	8.50			0.03	
		151.36	11_20100	3.10	91	2.53	4	12.00	1	28.00	0.23	
		161.58	12_30162	4.53	83	1.81	12	10.25	1	33.00	0.37	
	7H	112.46	12_10241	4.86	87	1.47	9	19.78			0.67	
		166.56	12_10378	3.31	11	18.18	85	1.25			0.69	
		166.56	12_30826	3.31	11	18.18	85	1.25			0.69	
	Unlinked	0.00	12_30602	6.61	4	8.50	92	2.96			0.03	

Table A10. Significant marker-trait associations ($P \leq 0.001$) for percent of non-dormant seed identified for the Washington State University breeding program across years (cont.)

					Allele A		Allele B		Heterozygote			In
Year	Chr	cM	Marker	-log10(p)	Count	Mean	Count	Mean	model	Mean	R^2	model
2008	1H	0.00	11_10501	4.28	44	28.52	45	6.54			0.35	x
	2H	41.66	12_30432	3.14	75	12.88	13	42.76	1	27.00	0.27	x
	3H	57.12	11_20444	3.66	6	54.53	83	14.72			0.29	x
	5H	178.43	12_11010	3.60	35	30.03	53	9.23	1	9.00	0.28	
		179.64	11_21138	3.60	35	30.03	53	9.23	1	9.00	0.28	
		181.43	11_10236	4.00	44	6.12	45	28.44			0.36	x
		189.60	12_31292	4.85	52	8.52	37	29.89			0.32	x
		191.97	11_10401	3.62	51	8.69	36	29.06	2	30.00	0.27	
		195.42	11_20402	4.41	33	30.91	54	8.69	2	30.00	0.24	
		196.12	12_10322	4.35	32	31.03	54	8.69	3	29.00	0.20	x
		196.12	12_30958	3.15	51	9.02	36	28.59	2	30.00	0.25	
		196.85	12_31123	4.48	33	30.39	54	8.69	2	38.50	0.20	
	7H	86.44	12_31199	3.21	68	13.08	21	31.42			0.17	x
2009	5H	196.12	12_10322	3.03	26	48.22	70	28.11			0.16	
		196.85	12_31123	3.50	27	48.80	69	27.59				

Table A11. SNP markers significantly associated ($P \leq 0.05$) with percent of non-dormant seeds for the USDA-ARS-Aberdeen, ID breeding program across years, and located on chromosome 5HL.

		2006							2007						
			AA		BB		AB			AA		BB		AB	
Marker	cM	*P*value	%	Mean	%	Mean	%	Mean	*P*value	%	Mean	%	Mean	%	Mean
11_20546	172.38	0.004	74.2	63.5	25.8	34.6			1.000	82.8	4.6	17.2	1.6		
11_10869	173.08	0.000	60.2	42.0	39.8	77.3			0.807	64.5	4.6	35.5	3.3		
12_11010	178.43	0.003	72.0	50.0	28.0	71.7			0.490	32.3	4.2	67.7	4.1		
12_11450	178.43	0.003	72.0	50.0	28.0	71.7			0.490	68	4.10	32	4.17		
12_30504	182.16	0.300	15.1	37.3	82.8	60.0	2.2	36.5	0.045	20.4	1.3	79.6	4.9		
12_31352	182.88	0.032	35.5	51.6	64.5	58.5			0.311	38.7	5.6	61.3	3.2		
11_21155	187.38	0.741	3.2	79.0	96.8	55.3			0.010	8.6	2.5	91.4	4.3		
11_20786	189.6	0.002	31.2	44.5	66.7	62.0	2.2	36.5	0.333	24.7	3.7	75.3	4.3		
12_31292	189.6	0.281	48.4	57.5	48.4	55.1	3.2	48.0	0.265	57	5.75	42	2.01	1	0.0
11_10401	191.97	0.002	34.4	44.6	63.4	62.2	2.2	57.0	0.008	46.2	3.0	51.6	5.2	2.2	2.5
12_31210	191.97	0.001	32.3	44.0	65.6	61.9	2.2	57.0	0.000	40.9	2.2	58.1	5.6	1.1	0.0
12_30360	191.97	0.001	33.3	43.7	64.5	62.4	2.2	57.0	0.000	43.0	2.2	55.9	5.7	1.1	0.0
12_30382	194.64	0.000	44.1	38.9	54.8	70.3	1.1	30.0	0.852	33	2.0	66.0	5.20	1.0	4.0
12_10857	194.84	0.138	31.2	51.9	67.7	58.2	1.1	43.0	0.000	26.9	3.2	73.1	4.4	0.0	
11_20402	195.42	0.000	54.8	71.2	44.1	37.8	1.1	30.0	0.149	67.7	5.8	31.2	0.5	1.1	4.0
11_20132	196.12	0.030	12.9	42.4	84.9	58.6	2.2	36.5	0.764	15.1	0.4	83.9	4.9	1.1	0.0
12_10322	196.12	0.000	52.7	71.2	46.2	39.4	1.1	30.0	0.699	62.4	5.4	36.6	1.9	1.1	4.0
12_30958	196.12	0.110	16.1	45.7	81.7	58.6	2.2	36.5	0.942	26	2.83	73	4.64	1	0.0
12_31123	196.85	0.000	54.8	69.6	45.2	39.6			0.405	62.4	5.4	37.6	2.0	0.0	

Table A11. SNP markers significantly associated ($P \leq 0.05$) with percent of non-dormant seeds for the USDA-ARS-Aberdeen, ID breeding program across years, and located on chromosome 5HL (cont.)

	2008							2009						
		AA		BB		AB			AA		BB		AB	
Marker	pvalue	%	Mean	%	Mean	%	Mean	pvalue	%	Mean	%	Mean	%	Mean
11_20546	0.009	92.0	23.9	8.0	55.8			0.044	53.1	53.7	45.8	31.8	1.0	45.7
11_10869	0.921	41.4	20.1	58.6	31.0			0.018	57.3	34.1	42.7	56.3		
12_11010	0.133	54.0	34.9	46.0	16.6			0.808	41.7	54.5	58.3	35.7		
12_11450	0.133	46	16.56	54	34.95			0.808	58.3	35.7	41.7	54.5		
12_30504	0.010	5.7	9.2	93.1	27.9	1.1	0.0	0.757	17.7	37.9	79.2	45.1	3.1	37.1
12_31352	0.000	35.6	6.2	64.4	37.7			0.502	4.2	25.3	95.8	44.3		
11_21155	0.027	8.0	2.9	92.0	28.6			0.779	1.0	45.7	99.0	43.5		
11_20786	0.003	23.0	5.5	77.0	32.7			0.502	4.2	25.3	95.8	44.3		
12_31292	0.844	43	15.62	56	35.24	1	0.0	0.853	36.5	35.2	61.5	48.4	2.1	45.7
11_10401	0.000	35.6	5.6	63.2	38.8	1.1	0.0	0.516	41.7	33.7	57.3	50.7	1.0	45.7
12_31210	0.000	29.9	5.7	69.0	35.9	1.1	0.0	0.727	30.2	35.1	68.8	47.6	1.0	20.0
12_30360	0.000	33.3	5.4	65.5	37.7	1.1	0.0	0.516	41.7	33.7	57.3	50.7	1.0	45.7
12_30382	0.142	8	13.51	92	27.63			0.525	31.3	34.1	66.7	48.3	2.1	32.8
12_10857	0.001	26.4	3.8	72.4	35.2	1.1	0.0	0.923	31.3	36.4	67.7	47.2	1.0	20.0
11_20402	0.006	89.7	28.2	10.3	11.4			0.144	56.3	52.0	43.8	32.6		
11_20132	0.048	4.6	14.6	95.4	27.1			.		.	100.0	43.6		
12_10322	0.003	86.2	29.0	13.8	10.9			0.144	56.3	52.0	43.8	32.6		
12_30958	0.003	14	10.91	86	28.98			0.811	32.3	35.1	66.7	47.6	1.0	45.7
12_31123	0.003	86.2	29.0	13.8	10.9			0.742	55.2	52.2	43.8	33.2	1.0	20.0

Table A12. SNP markers significantly associated ($P \leq 0.05$) with percent of non-dormant seeds for the Bush Agricultural Resources LLC breeding program across years, and located on chromosome 5HL.

		2006							2007						
			AA		BB		AB			AA		BB		AB	
Marker	cM	pvalue	%	Mean	%	Mean	%	Mean	pvalue	%	Mean	%	Mean	%	Mean
12_30162	161.58	0.006	78.9	80.2	21.1	70.7			0.039	66.3	7.9	32.6	18.0	1.1	34.6
11_11216	171.66	0.006	84.2	83.3	15.8	51.3			0.842	96.7	11.7	3.3	5.8		0.0
12_11010	178.43	0.039	52.6	84.7	47.4	71.0			0.106	56.5	7.4	43.5	16.8		
12_11450	178.43	0.039	48.4	69.9	51.6	86.0			0.106	43.5	16.8	56.5	7.4		
11_21138	179.64	0.000	65.3	87.7	34.7	60.3			0.245	81.5	11.2	18.5	12.6		
12_30656	179.64	0.000	64.2	87.6	35.8	61.3			0.192	80.4	11.4	19.6	11.9		
12_30494	180.71	0.174	12.6	67.8	82.1	80.0	5.3	75.0	0.001	9.8	11.9	79.3	12.3	10.9	5.1
11_20897	182.88	0.037	12.6	60.8	87.4	80.7			0.001	9.8	4.6	90.2	12.2		
11_10310	187.96	0.034	11.6	61.9	88.4	80.3			0.002	15.2	6.9	84.8	12.3		0.0
11_11364	189.6	0.203	3.2	65.7	96.8	78.6			0.000	12.0	4.4	88.0	12.5		0.0
11_20786	189.6	0.000	17.9	45.8	82.1	85.3			0.001	10.9	4.8	89.1	12.3		
12_31292	189.6	0.013	43.2	68.8	56.8	85.3			0.567	44.6	15.3	55.4	8.5		
11_21108	190.23	0.001	53.7	88.9	45.3	66.5	1.1	37.0	0.870	37.0	7.1	39.1	15.7	23.9	11.3
11_10401	191.97	0.000	21.1	46.7	78.9	86.6			0.000	16.3	6.5	83.7	12.5		0.0
12_30360	191.97	0.000	20.0	45.2	80.0	86.5			0.000	10.9	3.3	89.1	12.5		
12_31210	191.97	0.000	17.9	45.0	82.1	85.4			0.000	10.9	3.3	89.1	12.5		
12_30382	194.64	0.001	24.2	57.1	75.8	84.9			0.611	5.4	13.4	94.6	11.4		
12_10857	194.84	0.039	2.1	49.5	97.9	78.8			0.000	10.9	3.3	89.1	12.5		
11_20402	195.42	0.000	80.0	86.5	20.0	45.2			.	100.0	11.5	0.0			
12_30958	196.12	0.000	21.1	46.7	78.9	86.6			0.842	4.3	14.5	95.7	11.3		
12_10322	196.12	0.000	78.9	86.6	21.1	46.7			0.842	95.7	11.3	4.3	14.5		
12_31123	196.85	0.000	78.9	86.6	21.1	46.7			0.842	95.7	11.3	4.3	14.5		

Table A12. SNP markers significantly associated ($P \leq 0.05$) with percent of non-dormant seeds for the Bush Agricultural Resources LLC breeding program across years, and located on chromosome 5HL (cont.)

	2008							2009						
		AA		BB		AB			AA		BB		AB	
Marker	pvalue	%	Mean	%	Mean	%	Mean	pvalue	%	Mean	%	Mean	%	Mean
12_30162	.	83.2	24.4	16.8	9.1			0.011	87.4	48.9	12.6	26.8		
11_11216	0.014	81.1	24.8	18.9	9.1			0.665	87.4	45.7	11.6	48.8	1.1	46.0
12_11010	0.700	64.2	26.7	35.8	13.0			0.029	65.3	50.7	34.7	37.4		
12_11450	0.619	36.8	12.8	63.2	27.1			0.029	34.7	37.4	65.3	50.7		
11_21138	0.171	67.4	27.1	32.6	10.9			0.027	74.7	49.2	25.3	36.9		
12_30656	0.171	67.4	27.1	32.6	10.9			0.035	72.6	49.3	27.4	37.6		
12_30494	0.032	18.9	4.1	74.7	28.0	6.3	1.3	0.093	14.7	42.8	78.9	48.9	6.3	18.6
11_20897	0.709	17.9	10.6	82.1	24.2			0.001	13.7	26.2	86.3	49.3		
11_10310	0.612	10.5	8.4	89.5	23.4			0.169	11.6	30.9	88.4	48.1		
11_11364	0.197	8.4	1.5	91.6	23.7			0.005	5.3	13.2	94.7	47.9		
11_20786	0.036	16.8	3.3	83.2	25.6			0.079	9.5	27.6	90.5	48.0		
12_31292	0.545	30.5	11.2	69.5	26.5			0.039	35.8	34.9	64.2	52.3		
11_21108	0.672	29.5	39.2	54.7	11.5	15.8	25.0	0.355	20.0	50.8	57.9	42.5	22.1	51.3
11_10401	.	16.8	2.5	83.2	25.7			0.006	15.8	29.4	84.2	49.2		
12_30360	.	16.8	2.5	83.2	25.7			0.001	12.6	25.4	87.4	49.1		
12_31210	.	16.8	2.5	83.2	25.7			0.003	10.5	25.1	89.5	48.6		
12_30382	0.022	12.6	3.4	87.4	24.5			0.045	11.6	30.1	88.4	48.2		
12_10857	.	8.4	1.1	91.6	23.7			0.005	5.3	13.2	94.7	47.9		
11_20402	.	89.5	24.0	10.5	2.9			0.072	92.6	47.1	7.4	34.1		
12_30958	0.018	10.5	2.9	89.5	24.0			0.446	12.6	38.9	87.4	47.1		
12_10322	.	89.5	24.0	10.5	2.9			0.446	87.4	47.1	12.6	38.9		
12_31123	.	89.5	24.0	10.5	2.9			0.446	87.4	47.1	12.6	38.9		

Table A13. SNP markers significantly associated ($P \leq 0.05$) with percent of non-dormant seeds for Montana State University breeding program across years, and located on chromosome 5HL.

		2006							2007						
			AA		BB		AB			AA		BB		AB	
Marker	cM	pvalue	%	Mean	%	Mean	%	Mean	pvalue	%	Mean	%	Mean	%	Mean
11_20546	172.38	0.048	27.1	86.0	72.9	56.7			0.340	66.3	5.5	32.6	3.3	1.1	1.0
12_11450	178.43	0.146	76.0	56.8	22.9	90.4	1.0	72.0	0.487	33.7	2.9	66.3	5.6		
12_11010	178.43	0.146	22.9	90.4	76.0	56.8	1.0	72.0	0.713	66.3	5.6	32.6	3.0	1.1	1.0
12_31292	189.6	0.021	17.7	44.8	82.3	68.9	0.0		0.469	23.9	6.3	76.1	4.2		
11_10401	191.97	0.025	16.7	44.6	83.3	68.7	0.0		0.921	21.7	6.6	78.3	4.2		
12_30360	191.97	0.025	16.7	44.6	83.3	68.7			0.921	21.7	6.6	78.3	4.2		
12_31210	191.97	0.139	1.0	98.0	99.0	64.3	0.0		0.921	21.7	6.6	78.3	4.2		
12_30382	194.64	0.001	40.6	44.8	58.3	78.8	1.0	52.0	0.151	38.0	6.1	59.8	3.9	2.2	3.0
12_10857	194.84	.			100.0	64.7			0.719	14.1	2.6	85.9	5.1		
11_20402	195.42	0.000	42.7	92.6	56.3	43.7	1.0	52.0	0.369	40.2	5.0	57.6	4.5	2.2	5.0
12_10322	196.12	0.000	42.7	92.6	56.3	43.7	1.0	52.0	0.369	40.2	5.0	57.6	4.5	2.2	5.0
12_30958	196.12	0.025	16.7	44.6	83.3	68.7			0.316	42.4	4.4	54.3	5.0	3.3	3.7
12_31123	196.85	0.000	43.8	91.6	56.3	43.7			0.510	42.4	5.0	55.4	4.4	2.2	6.5

Table A13. SNP markers significantly associated ($P \leq 0.05$) with percent of non-dormant seeds for Montana State University breeding program across years, and located on chromosome 5HL (cont.)

	2008							2009						
		AA		BB		AB			AA		BB		AB	
Marker	pvalue	%	Mean	%	Mean	%	Mean	pvalue	%	Mean	%	Mean	%	Mean
11_20546	0.792	54.3	31.9	45.7	25.3			0.005	53.8	35.3	46.2	33.3		
12_11450	0.265	64.2	28.7	35.8	29.3			0.921	53.8	30.1	46.2	39.3		
12_11010	0.265	35.8	29.3	64.2	28.7			0.921	46.2	39.3	53.8	30.1		
12_31292	0.742	33.3	27.0	66.7	29.8			0.359	1.1	54.0	98.9	34.1		
11_10401	0.157	25.9	21.3	74.1	31.5			.			100.0	34.3		
12_30360	0.157	25.9	21.3	74.1	31.5			.			100.0	34.3		
12_31210	0.296	23.5	22.8	76.5	30.7			.			100.0	34.3		
12_30382	0.046	45.7	26.1	54.3	31.2			0.783	53.8	29.6	46.2	39.9		
12_10857	0.510	12.3	25.6	87.7	29.3			.			100.0	34.3		
11_20402	0.004	39.5	34.6	60.5	25.2			0.783	46.2	39.9	53.8	29.6		
12_10322	0.020	34.6	32.5	65.4	26.9			0.783	46.2	39.9	53.8	29.6		
12_30958	0.568	30.9	25.7	69.1	30.3			.			100.0	34.3		
12_31123	0.020	34.6	32.5	65.4	26.9			0.783	46.2	39.9	53.8	29.6		

Table A14. SNP markers significantly associated ($P \leq 0.05$) with percent of non-dormant seeds for the North Dakota State University (two-rowed) breeding program across years, and located on chromosome 5HL.

		2006							2007						
			AA		BB		AB			AA		BB		AB	
Marker	cM	pvalue	%	Mean	%	Mean	%	Mean	pvalue	%	Mean	%	Mean	%	Mean
12_30162	161.58	0.692	37.23	45.86	60.64	52.29	2.13	34.00	0.036	30.53	7.03	66.32	12.14	3.16	3.00
12_11010	178.43	0.051	8.51	76.88	90.43	46.83	1.06	58.00	0.000	6.32	18.50	92.63	9.24	1.05	54.00
12_11450	178.43	0.013	91.49	46.96	8.51	76.88			0.017	94.74	9.71	5.26	20.80		
12_30656	179.64	0.181	13.83	64.00	85.11	47.04	1.06	58.00	0.000	7.37	15.57	91.58	9.36	1.05	54.00
11_10736	180.71	0.053	57.45	58.64	41.49	37.10	1.06	40.00	0.002	40.00	14.63	57.89	7.32	2.11	9.50
11_10236	181.43	0.042	39.36	37.22	60.64	57.48			0.001	54.74	7.12	45.26	14.13		
11_20022	181.43	0.088	4.3	28.5	95.7	50.4			0.490	5.3	7.2	94.7	10.5		
12_30577	182.88	0.006	47.9	38.0	52.1	60.0			0.008	51.6	7.4	47.4	13.6	1.1	2.0
12_31292	189.6	0.185	42.6	54.0	57.4	46.2			0.025	35.8	11.8	62.1	8.9	2.1	27.0
11_10401	191.97	0.149	2.1	29.0	97.9	49.9			0.435	5.3	6.8	93.7	10.6	1.1	0.0
12_31210	191.97	0.633	1.1	36.0	98.9	49.6			0.435	5.3	6.8	93.7	10.6	1.1	0.0
12_30360	191.97	1.000	4.3	44.0	95.7	49.7			0.504	11.6	8.7	87.4	10.6	1.1	0.0
12_31481	191.97	0.013	37.2	61.5	62.8	42.4			0.007	25.3	13.6	72.6	8.7	2.1	27.0
12_30382	194.64	0.000	26.6	28.4	37.2	50.9	36.2	63.5	0.090	22.1	6.6	37.9	10.0	40.0	12.6
12_10857	194.84	0.545	3.2	41.3	96.8	49.8			0.602	8.4	10.1	90.5	10.4	1.1	0.0
11_20402	195.42	0.000	75.5	56.4	24.5	28.3			0.086	69.5	11.8	26.3	6.7	4.2	7.6
12_10322	196.12	0.000	72.3	57.0	27.7	29.8			0.107	70.5	11.7	26.3	6.7	3.2	8.8
12_30958	196.12	0.682	6.4	41.7	93.6	50.0			0.608	8.4	7.0	89.5	10.5	2.1	13.1
12_31123	196.85	0.000	72.3	57.0	27.7	29.8			0.039	73.7	11.6	26.3	6.7		

Table A14. SNP markers significantly associated ($P \leq 0.05$) with percent of non-dormant seeds for North Dakota State University (two-rowed) breeding program across years, and located on chromosome 5HL (cont.)

	2008							2009						
		AA		BB		AB			AA		BB		AB	
Marker	pvalue	%	Mean	%	Mean	%	Mean	pvalue	%	Mean	%	Mean	%	Mean
12_30162	0.150	48.42	12.08	47.37	13.23	4.21	26.75	0.039	49.47	37.66	47.37	43.84	3.16	45.66
12_11010	0.000	7.37	36.00	91.58	11.07	1.05	43.00	0.368	4.21	44.25	95.79	40.69		
12_11450	0.000	92.63	11.43	7.37	36.00			0.368	95.79	40.69	4.21	44.25		
12_30656	0.010	11.58	26.22	87.37	11.16	1.05	43.00	0.347	11.58	41.70	86.32	41.11	2.11	25.00
11_10736	0.026	41.05	19.26	55.79	8.41	3.16	20.33	0.240	43.16	43.35	53.68	38.60	3.16	44.55
11_10236	0.032	57.89	9.05	41.05	19.26	1.05	9.00	0.842	30.53	41.01	69.47	40.76		
11_20022	0.031	2.1	15.5	95.8	13.4	2.1	4.0	0.018	27.4	35.2	72.6	42.9		
12_30577	0.042	60.0	9.9	37.9	18.8	2.1	9.0	0.787	43.2	41.0	53.7	40.4	3.2	46.4
12_31292	0.678	28.4	10.7	69.5	14.2	2.1	16.3	0.540	16.8	45.1	82.1	39.9	1.1	45.7
11_10401	0.240	1.1	1.0	98.9	13.4			0.807	1.1	45.7	98.9	40.8		
12_31210	.	.	.	100.0	13.2			0.951	1.1	45.7	97.9	40.7	1.1	45.7
12_30360	.	.	.	100.0	13.2			0.951	1.1	45.7	97.9	40.7	1.1	45.7
12_31481	0.827	21.1	12.6	76.8	13.7	2.1	1.5	0.358	15.8	45.1	81.1	39.8	3.2	45.7
12_30382	0.047	9.5	4.4	65.3	14.8	25.3	12.6	0.341	43.2	38.9	41.1	41.5	15.8	44.4
12_10857	0.240	1.1	1.0	98.9	13.4			0.325	27.4	37.4	69.5	41.9	3.2	46.4
11_20402	0.017	89.5	14.3	10.5	4.0			0.457	56.8	42.3	41.1	38.5	2.1	45.7
12_10322	0.026	87.4	14.3	12.6	6.0			0.448	54.7	42.2	43.2	38.9	2.1	45.7
12_30958	0.071	3.2	10.7	96.8	13.3			0.303	29.5	38.0	67.4	41.8	3.2	46.4
12_31123	0.026	87.4	14.3	12.6	6.0			0.448	54.7	42.2	43.2	38.9	2.1	45.7

Table A15. SNP markers significantly associated ($P \leq 0.05$) with percent of non-dormant seeds for North Dakota State University (six-rowed) breeding program across years, and located on chromosome 5HL.

		2006							2007						
			AA		BB		AB			AA		BB		AB	
Marker	cM	pvalue	%	Mean	%	Mean	%	Mean	pvalue	%	Mean	%	Mean	%	Mean
12_11450	178.43	0.921	59.4	65.1	40.6	60.3			0.951	53.2	16.3	43.6	21.3	3.2	19.0
12_11010	178.43	0.921	40.6	60.3	59.4	65.1			0.870	43.6	21.3	52.1	16.0	4.3	22.0
12_31292	189.6	0.792	82.3	62.3	17.7	67.1			0.266	50.0	16.9	43.6	19.5	6.4	25.2
11_10401	191.97	0.524	51.0	65.9	49.0	60.2			0.420	39.4	16.8	57.4	19.1	3.2	31.3
12_31210	191.97	0.320	10.4	62.0	89.6	63.2			.	.	.	100.0	18.6		
12_30360	191.97	0.320	10.4	62.0	89.6	63.2			.	.	.	100.0	18.6		
12_30382	194.64	.	.	.	100.0	63.1			.	.	.	100.0	18.6		
12_10857	194.84	0.320	10.4	62.0	89.6	63.2			.	.	.	100.0	18.6		
11_20402	195.42	.	100.0	63.1	.	.			.	100.0	18.6	.	.		
12_10322	196.12	0.027	42.7	59.9	57.3	65.5			0.458	56.4	18.4	37.2	17.6	6.4	25.2
12_30958	196.12	0.027	57.3	65.5	42.7	59.9			0.449	37.2	17.6	59.6	18.5	3.2	31.3
12_31123	196.85	0.027	42.7	59.9	57.3	65.5			0.951	59.6	19.1	37.2	17.6	3.2	19.0

Table A15. SNP markers significantly associated ($P \leq 0.05$) with percent of non-dormant seeds for North Dakota State University (six-rowed) breeding program across years, and located on chromosome 5HL (cont.)

	2008							2009						
		AA		BB		AB			AA		BB		AB	
Marker	pvalue	%	Mean	%	Mean	%	Mean	pvalue	%	Mean	%	Mean	%	Mean
12_11450	0.407	54.4	12.9	45.6	7.9			0.046	54.0	43.5	44.8	39.9	1.1	0.0
12_11010	0.407	45.6	7.9	54.4	12.9			0.046	44.8	39.9	54.0	43.5	1.1	0.0
12_31292	0.001	67.8	11.7	31.1	7.6	1.1	25.0	0.042	81.6	41.2	17.2	45.0	1.1	0.0
11_10401	1.000	23.3	8.3	76.7	11.3			1.000	51.7	42.8	48.3	39.9		
12_31210	0.208	6.7	2.7	93.3	11.2			1.000	14.9	48.2	85.1	40.2		
12_30360	0.208	6.7	2.7	93.3	11.2			1.000	14.9	48.2	85.1	40.2		
12_30382	0.199	3.3	2.0	95.6	10.7	1.1	25.0	.	.	.	100.0	41.4		
12_10857	0.208	6.7	2.7	93.3	11.2			1.000	14.9	48.2	85.1	40.2		
11_20402	.	100.0	10.6	.	.			.	100.0	41.4	.	.		
12_10322	0.000	70.0	7.9	28.9	16.7	1.1	25.0	0.288	57.5	42.2	41.4	41.1	1.1	12.0
12_30958	0.000	28.9	16.7	70.0	7.9	1.1	25.0	0.288	41.4	41.1	57.5	42.2	1.1	12.0
12_31123	0.000	71.1	8.1	28.9	16.7			0.288	57.5	42.2	41.4	41.1	1.1	12.0

Table A16. SNP markers significantly associated ($P \leq 0.05$) with percent of non-dormant seeds for the University of Minnesota breeding program across years, and located on chromosome 5HL.

		2006							2007						
			AA		BB		AB			AA		BB		AB	
Marker	cM	pvalue	%	Mean	%	Mean	%	Mean	pvalue	%	Mean	%	Mean	%	Mean
12_11010	178.43	0.009	21.9	72.7	77.1	67.1	1.0	19.0	0.018	30.4	9.7	68.5	4.0	1.1	2.0
12_11450	178.43	0.149	78.1	66.5	21.9	72.7			0.018	68.5	4.0	30.4	9.7	1.1	2.0
12_31292	189.6	0.007	39.6	81.3	60.4	59.1			0.573	31.5	7.1	67.4	5.1	1.1	2.0
11_10401	191.97	0.001	7.3	23.6	91.7	71.6	1.0	50.0	0.077	20.7	2.5	79.3	6.6		
12_30360	191.97	0.000	4.2	8.8	94.8	70.6	1.0	50.0	0.055	17.4	1.8	82.6	6.6		
12_31210	191.97	0.000	4.2	8.8	94.8	70.6	1.0	50.0	0.055	17.4	1.8	82.6	6.6		
12_30382	194.64	0.025	6.3	32.2	93.8	70.2			0.640	6.5	7.4	93.5	5.6		
12_10857	194.84	0.000	10.4	42.5	88.5	71.0	1.0	50.0	0.140	17.4	1.8	81.5	6.6	1.1	2.0
11_20402	195.42	0.193	93.8	68.0	6.3	65.0			0.640	93.5	5.6	6.5	7.4		
12_10322	196.12	0.150	90.6	68.7	9.4	59.4			0.672	90.2	5.6	9.8	6.9		
12_30958	196.12	0.150	9.4	59.4	90.6	68.7			1.000	3.3	6.0	96.7	5.7		
12_31123	196.85	0.484	96.9	68.5	3.1	48.3			0.672	90.2	5.6	9.8	6.9		

Table A16. SNP markers significantly associated ($P \leq 0.05$) with percent of non-dormant seeds for the University of Minnesota breeding program across years, and located on chromosome 5HL (cont.)

	2008							2009						
		AA		BB		AB			AA		BB		AB	
Marker	pvalue	%	Mean	%	Mean	%	Mean	pvalue	%	Mean	%	Mean	%	Mean
12_11010	0.004	43.2	38.4	56.8	14.4			0.932	31.8	36.8	67.0	32.9	1.1	45.7
12_11450	0.004	56.8	14.4	43.2	38.4			0.932	67.0	32.9	31.8	36.8	1.1	45.7
12_31292	0.053	22.1	33.0	77.9	22.4			0.085	28.4	37.9	71.6	32.9		
11_10401	0.046	10.5	5.6	89.5	27.0			0.004	26.1	21.3	73.9	38.9		
12_30360	0.046	10.5	5.6	89.5	27.0			0.004	26.1	21.3	73.9	38.9		
12_31210	0.046	10.5	5.6	89.5	27.0			0.004	26.1	21.3	73.9	38.9		
12_30382	0.154	5.3	0.6	94.7	26.1			.			100.0	34.3		
12_10857	0.046	10.5	5.6	89.5	27.0			0.004	26.1	21.3	73.9	38.9		
11_20402	.	100.0	24.8	.	.			.	100.0	34.3	.	.		
12_10322	.	100.0	24.8	.	.			0.534	90.9	34.4	9.1	33.8		
12_30958	.			100.0	24.8			0.534	9.1	33.8	90.9	34.4		
12_31123	.	100.0	24.8	.	.			0.534	90.9	34.4	9.1	33.8		

Table A17. SNP markers significantly associated ($P \leq 0.05$) with percent of non-dormant seeds for the Utah State University breeding program across years, and located on chromosome 5HL.

		2006							2007						
			AA		BB		AB			AA		BB		AB	
Marker	cM	pvalue	%	Mean	%	Mean	%	Mean	pvalue	%	Mean	%	Mean	%	Mean
11_20646	161.58	0.807	18.8	33.6	81.3	26.2			0.001	12.2	6.4	87.8	3.9		
12_30642	161.58	.	76.0	25.6	24.0	33.9			0.001	87.8	3.9	12.2	6.4		
12_11450	178.43	0.710	93.8	26.4	6.3	45.2			0.099	94.4	4.4	5.6	1.6		
12_11010	178.43	0.500	56.3	26.3	43.8	29.3			0.199	54.4	3.8	43.3	4.0	2.2	18.5
12_31292	189.6	0.000	95.8	25.5	4.2	75.4			0.216	94.4	3.8	5.6	10.4		
11_10401	191.97	0.138	66.7	21.4	25.0	41.9	8.3	34.5	0.757	85.6	3.9	12.2	7.1	2.2	0.8
12_31210	191.97	0.470	22.9	23.0	77.1	29.0			0.820	30.0	4.9	68.9	4.0	1.1	0.0
12_30360	191.97	0.196	59.4	23.1	40.6	34.1			0.757	85.6	3.9	12.2	7.1	2.2	0.8
12_30382	194.64	0.673	59.4	28.9	40.6	25.7			0.417	24.4	3.1	75.6	4.6		
12_10857	194.84	0.000	68.8	22.3	31.3	39.3			0.686	54.4	4.3	44.4	4.2	1.1	2.0
12_10322	196.12	0.004	33.3	36.3	66.7	23.2			0.436	17.8	8.9	78.9	3.3	3.3	0.5
12_30958	196.12	0.002	36.5	21.8	63.5	30.9			0.836	55.6	3.2	41.1	5.8	3.3	1.2
11_20402	195.42	0.278	22.9	37.9	61.5	26.0	15.6	18.8	0.420	17.8	8.9	80.0	3.3	2.2	0.8
12_31123	196.85	0.004	33.3	36.3	66.7	23.2			0.382	20.0	8.0	78.9	3.3	1.1	0.0

Table A17. SNP markers significantly associated ($P \leq 0.05$) with percent of non-dormant seeds for the Utah State University breeding program across years, and located on chromosome 5HL (cont.)

	2008							2009						
		AA		BB		AB			AA		BB		AB	
Marker	pvalue	%	Mean	%	Mean	%	Mean	pvalue	%	Mean	%	Mean	%	Mean
11_20646	0.328	20.5	29.1	78.3	19.9	1.2	0.0	0.000	8.3	40.9	90.6	36.6	1.0	90.0
12_30642	0.328	78.3	19.9	20.5	29.1	1.2	0.0	0.000	90.6	36.6	8.3	40.9	1.0	90.0
12_11450	0.012	65.1	29.3	34.9	7.0			0.844	72.9	38.5	24.0	36.9	3.1	18.6
12_11010	0.449	81.9	19.4	18.1	31.2			0.780	31.3	34.1	67.7	38.9	1.0	45.7
12_31292	0.238	63.9	24.7	36.1	16.0			0.951	72.9	36.2	26.0	42.2	1.0	10.0
11_10401	0.155	54.2	20.3	44.6	22.4	1.2	44.4	0.087	24.0	28.5	76.0	40.3		
12_31210	0.921	9.6	21.8	90.4	21.5			0.092	22.9	27.7	77.1	40.4		
12_30360	0.133	51.8	19.3	44.6	23.7	3.6	25.8	0.000	32.3	20.9	65.6	45.5	2.1	41.0
12_30382	0.300	34.9	20.4	65.1	22.1			0.018	11.5	20.8	88.5	39.6		
12_10857	0.014	43.4	13.3	55.4	27.5	1.2	44.4	0.000	51.0	29.1	46.9	46.5	2.1	41.0
12_10322	0.002	21.7	27.5	77.1	19.5	1.2	44.4	0.018	88.5	39.6	11.5	20.8		
12_30958	0.765	20.5	28.7	79.5	19.7			0.018	11.5	20.8	88.5	39.6		
11_20402	0.002	21.7	27.5	77.1	19.5	1.2	44.4	0.064	87.5	39.6	11.5	20.8	1.0	45.7
12_31123	0.000	22.9	28.3	77.1	19.5			0.018	88.5	39.6	11.5	20.8		

Table A18. SNP markers significantly associated ($P \leq 0.05$) with percent of non-dormant seeds for the Washington State University breeding program across years, and located on chromosome 5HL.

		2006							2007						
			AA		BB		AB			AA		BB		AB	
Marker	cM	pvalue	%	Mean	%	Mean	%	Mean	pvalue	%	Mean	%	Mean	%	Mean
11_11216	171.66	0.002	29.8	71.3	70.2	31.2			0.887	34.4	7.2	62.5	1.1	3.1	0.3
11_10869	173.08	0.004	85.1	36.4	13.8	84.2	1.1	52.0	0.011	79.2	2.4	18.8	4.9	2.1	17.0
11_21141	177.07	0.017	63.8	31.0	35.1	64.7	1.1	62.0	0.942	74.0	1.4	25.0	8.5	1.0	1.0
12_11010	178.43	0.001	10.6	85.7	87.2	37.1	2.1	78.0	0.749	11.5	2.2	87.5	3.3	1.0	1.0
12_11450	178.43	0.000	89.4	38.1	10.6	85.7			0.567	91.7	3.2	8.3	2.6		
11_21138	179.64	0.000	21.3	84.4	77.7	31.6	1.1	62.0	0.980	21.9	9.7	77.1	1.4	1.0	1.0
11_10736	180.71	0.001	16.0	83.1	81.9	34.5	2.1	78.0	0.090	17.7	12.8	80.2	1.1	2.1	0.5
11_10236	181.43	0.017	67.0	31.6	33.0	66.7			0.556	78.1	1.1	21.9	10.7		
11_20022	181.43	0.027	72.3	34.2	25.5	65.6	2.1	78.0	0.504	67.7	1.1	32.3	7.6		
12_31292	189.6	0.041	73.4	39.7	25.5	52.5	1.1	62.0	0.414	67.7	3.7	29.2	2.3	3.1	0.3
11_10401	191.97	0.000	69.1	31.6	27.7	68.2	3.2	77.3	0.089	63.5	1.1	33.3	7.4	3.1	0.3
12_31210	191.97	0.423	21.3	35.4	76.6	44.2	2.1	85.0	0.551	11.5	3.3	87.5	3.2	1.0	0.0
12_30360	191.97	0.001	62.8	29.7	33.0	66.8	4.3	59.0	0.089	63.5	1.1	33.3	7.4	3.1	0.3
12_30382	194.64	0.289	23.4	29.7	75.5	46.9	1.1	76.0	0.337	13.5	1.2	85.4	3.5	1.0	0.0
12_10857	194.84	0.238	8.5	26.5	91.5	44.7			0.104	4.2	2.8	95.8	3.2		
11_20402	195.42	0.000	34.0	72.0	63.8	27.0	2.1	69.0	0.036	31.3	8.5	64.6	0.7	4.2	1.0
12_10322	196.12	0.000	27.7	77.0	70.2	29.1	2.1	69.0	0.036	31.3	8.5	64.6	0.7	4.2	1.0
12_30958	196.12	0.000	61.7	29.3	36.2	65.3	2.1	69.0	0.083	58.3	0.7	37.5	7.3	4.2	1.0
12_31123	196.85	0.000	29.8	76.4	70.2	29.1			0.057	35.4	7.6	63.5	0.8	1.0	0.0

Table A18. SNP markers significantly associated ($P \leq 0.05$) with percent of non-dormant seeds for the Washington State University breeding program across years, and located on chromosome 5HL (cont.)

	2008							2009						
		AA		BB		AB			AA		BB		AB	
Marker	pvalue	%	Mean	%	Mean	%	Mean	pvalue	%	Mean	%	Mean	%	Mean
11_11216	0.034	46.1	25.7	52.8	9.5	1.1	50.0	0.087	46.9	40.2	53.1	27.7		
11_10869	0.001	53.9	7.3	46.1	29.2			0.484	60.4	29.9	39.6	39.2		
11_21141	0.004	58.4	10.1	41.6	27.7			0.921	69.8	32.2	30.2	36.6		
12_11010	0.000	39.3	30.0	59.6	9.2	1.1	9.0	0.914	29.2	37.6	69.8	31.7	1.0	45.7
12_11450	.	59.6	9.2	39.3	30.0	1.1	9.0	0.795	70.8	31.2	28.1	39.0	1.0	45.7
11_21138	0.000	39.3	30.0	59.6	9.2	1.1	9.0	0.914	29.2	37.6	69.8	31.7	1.0	45.7
11_10736	0.002	52.8	27.7	46.1	5.8	1.1	9.0	.	43.8	41.2	56.3	27.6		
11_10236	0.000	49.4	6.1	50.6	28.4			.	54.2	27.8	45.8	40.4		
11_20022	0.003	44.9	5.9	55.1	26.8			.	49.0	26.9	51.0	40.0		
12_31292	0.000	58.4	8.5	41.6	29.9			0.082	66.7	29.5	33.3	41.7		
11_10401	0.000	57.3	8.7	40.4	29.1	2.2	30.0	0.089	66.7	28.9	32.3	42.9	1.0	45.7
12_31210	0.029	12.4	15.0	85.4	17.2	2.2	38.5	0.618	19.8	34.1	80.2	33.4		
12_30360	.	56.2	8.9	41.6	28.3	2.2	30.0	0.089	66.7	28.9	32.3	42.9	1.0	45.7
12_30382	.	15.7	12.4	83.1	17.9	1.1	50.0	0.286	27.1	31.9	72.9	34.2		
12_10857	.	.	.	100.0	17.4			0.486	2.1	22.8	97.9	33.8		
11_20402	0.000	37.1	30.9	60.7	8.7	2.2	30.0	0.003	27.1	48.2	71.9	27.9	1.0	45.7
12_10322	0.000	36.0	31.0	60.7	8.7	3.4	29.0	0.001	27.1	48.2	72.9	28.1		
12_30958	0.001	57.3	9.0	40.4	28.6	2.2	30.0	0.008	65.6	28.1	34.4	44.0		
12_31123	0.000	37.1	30.4	60.7	8.7	2.2	38.5	0.000	28.1	48.8	71.9	27.6		

Table A19. Epistatic interactions among SNP markers significantly associated with dormancy using the results from the analysis of four years combined for each breeding program.

Breeding Program	Marker	Chr	cM	Marker	Chr	cM	P-value	-Log10(P)
AB	11_11435	2H	78.03	11_20193	3H	42.06	3.5223E-06	5.45317737
	11_11435	2H	78.03	11_10325	2H	54.95	0.00041022	3.38698156
	11_11435	2H	78.03	11_21358	3H	81.66	0.00065059	3.18669037
	11_10325	2H	54.95	11_11458	6H	81.17	0.00072198	3.1414754
	12_31356	3H	73.53	12_11010	5H	178.4	0.00082652	3.08274499
	12_31428	3H	0	12_31323	3H	70.71	0.00091815	3.03708599
	11_20402	5H	195.4	11_10901	5H	158.4	2.9332E-09	8.53266002
	11_20402	5H	195.4	11_10325	2H	54.95	2.8657E-07	6.5427633
	11_10901	5H	158.4	12_11010	5H	178.4	3.2662E-06	5.48596011
	11_20402	5H	195.4	11_21358	3H	81.66	1.4975E-05	4.82463139
	11_10869	5H	173.1	11_10736	5H	180.7	2.5883E-05	4.58698954
	11_10901	5H	158.4	11_10736	5H	180.7	6.4919E-05	4.18762668
	11_20402	5H	195.4	11_20193	3H	42.06	0.00036619	3.43628909
	11_10869	5H	173.1	11_10236	5H	181.4	0.0004571	3.33998432
	11_10869	5H	173.1	12_11010	5H	178.4	0.00072531	3.13947838
	12_31352	5H	182.9	12_11010	5H	178.4	0.00075225	3.1236379
	11_11458	6H	81.17	12_11010	5H	178.4	0.00047397	3.32425354
	11_10150	unlinked	0	12_31346	3H	76.98	9.9365E-05	4.00276445
	11_10150	unlinked	0	11_11435	2H	78.03	0.00018494	3.73297096
	11_10150	unlinked	0	11_21358	3H	81.66	0.00029036	3.53706852
	11_10150	unlinked	0	11_10869	5H	173.1	0.00058299	3.23433691
BA	11_10830	1H	88.23	11_10756	4H	48.5	0.000519	3.28483658
	11_10342	2H	44.13	11_10830	1H	88.23	0.00028386	3.54690288
	11_21079	7H	83.44	11_10756	4H	48.5	0.00013709	3.86300489
	11_10150	unlinked	0	11_10830	1H	88.23	3.2777E-05	4.48443687
	11_10150	unlinked	0	12_30169	5H	129.4	0.00085547	3.06779716
MT	12_31123	5H	196.9	12_31041	unlinked	0	1.5897E-07	6.79869312
	12_10322	5H	196.1	12_31041	unlinked	0	3.0984E-07	6.50885697
	11_20402	5H	195.4	12_31041	unlinked	0	8.8592E-07	6.05260561
	11_11456	5H	128	12_31123	5H	196.9	1.3374E-06	5.87374965
	11_20402	5H	195.4	11_11456	5H	128	2.0777E-06	5.68241796
	11_11456	5H	128	12_10322	5H	196.1	2.5968E-06	5.58555335
	12_30214	5H	53.9	11_20529	3H	8.23	0.00014986	3.82432307
	12_30214	5H	53.9	12_31123	5H	196.9	0.00038073	3.41938021
	11_10150	unlinked	0	12_31123	5H	196.9	5.7236E-14	13.242327
	11_10150	unlinked	0	12_10322	5H	196.1	3.3202E-13	12.4788325
	11_10150	unlinked	0	11_20402	5H	195.4	1.7725E-12	11.7514136
	11_10150	unlinked	0	11_20529	3H	8.23	0.00047033	3.32759703
ND2R	11_20402	5H	195.4	11_21008	3H	162.2	0.00019098	3.719001
	11_20761	5H	27.72	11_21008	3H	162.2	0.00038615	3.41324123
	11_20402	5H	195.4	11_20761	5H	27.72	0.00042614	3.37044798
	11_20922	unlinked	0	11_21151	4H	85.04	1.016E-11	10.993121
	11_10150	unlinked	0	11_20402	5H	195.4	7.7216E-06	5.11229384
	11_20922	unlinked	0	11_20402	5H	195.4	7.5246E-05	4.12351497
ND6R	11_11200	5H	117.5	11_10695	5H	25.23	2.0922E-05	4.67940134
	11_10150	unlinked	0	12_10530	5H	33.09	2.7858E-05	4.55505575

† AB=USDA-ARS-Aberdeen, ID; BA= Bush Agricultural Resources LLC..; MT= Montana State University; ND2R= North Dakota State University (2-Rowed); ND6R= North Dakota State University (6-Rowed); UM=University of Minnesota; UT= Utah State; WA=Washington State University.

‡ Marker 1and 2 indicate interacting markers and their chromosomal positions.

Table A19. Epistatic interactions among SNP markers significantly associated with dormancy using the results from the analysis of four years combined for each breeding program (cont.)

Breeding Program †	Marker 1‡	Chr	cM	Marker 2‡	Chr	cM	*P*-value	-Log10(P)
UT	11_10722	1H	125.3	11_10994	6H	31.73	1.8273E-08	7.74
	11_10722	1H	125.3	12_30358	6H	35.07	5.9396E-06	5.23
	12_30672	1H	54.73	11_11436	3H	155.9	9.5119E-06	5.02
	12_30672	1H	54.73	11_10994	6H	31.73	4.5083E-05	4.35
	11_20929	2H	52.47	11_20710	7H	3.34	3.1412E-06	5.50
	11_20929	2H	52.47	11_11436	3H	155.9	9.3265E-05	4.03
	11_20929	2H	52.47	12_30672	1H	54.73	0.00016505	3.78
	11_10214	2H	93.5	11_11436	3H	155.9	0.00017086	3.77
	11_20929	2H	52.47	11_20755	7H	15.93	0.0002414	3.62
	11_20929	2H	52.47	11_10994	6H	31.73	0.00029699	3.53
	11_11436	3H	155.9	11_20755	7H	15.93	2.4712E-09	8.61
	12_20143	4H	76.03	12_30358	6H	35.07	5.7663E-07	6.24
	12_20143	4H	76.03	11_10534	7H	80.94	1.4864E-05	4.83
	12_20143	4H	76.03	11_10994	6H	31.73	5.9477E-05	4.23
	12_10322	5H	196.1	12_30164	7H	119.5	0.00018604	3.73
	12_10322	5H	196.1	12_30672	1H	54.73	0.00060233	3.22
	12_30358	6H	35.07	12_30672	1H	54.73	1.4026E-08	7.85
	12_30358	6H	35.07	11_20929	2H	52.47	5.0706E-05	4.29
	12_30358	6H	35.07	11_10576	7H	41.85	0.00013843	3.86
	12_30358	6H	35.07	11_20710	7H	3.34	0.00026467	3.58
	11_20710	7H	3.34	11_11436	3H	155.9	3.766E-08	7.42
	11_20710	7H	3.34	11_10994	6H	31.73	7.638E-07	6.12
	11_10534	7H	80.94	11_10994	6H	31.73	8.8547E-05	4.05
	11_10534	7H	80.94	11_20755	7H	15.93	0.00030217	3.52
	11_10534	7H	80.94	12_30358	6H	35.07	0.00037656	3.42
	11_10534	7H	80.94	11_20710	7H	3.34	0.00047422	3.32
	12_30164	7H	119.5	12_30360	5H	192	0.00059707	3.22
WA	11_10150	unlinked	0	12_31123	5H	196.9	1.2555E-08	7.90

† AB=USDA-ARS-Aberdeen, ID; BA= Bush Agricultural Resources LLC..; MT= Montana State University; ND2R= North Dakota State University (2-Rowed); ND6R= North Dakota State University (6-Rowed); UM=University of Minnesota; UT= Utah State; WA=Washington State University.

‡ Marker 1and 2 indicate interacting markers and their chromosomal positions.

Table A20. SNP annotation summary for marker-trait associations identified on chromosome 1H based on the analysis of four years combined by breeding program.

cM	Marker	U35 Rice(v5) Description
0	11_20149	protein soluble inorganic pyrophosphatase, putative, expressed
0	11_10501	protein metallothionein-like protein type 2, putative, expressed
0.95	11_10460	protein keratin-associated protein 5-4, putative, expressed
11.42	12_30952	protein NAD-dependent epimerase/dehydratase, putative, expressed
36.95	12_31177	protein floral homeotic protein APETALA2, putative, expressed
43.28	11_21134	protein myb-like DNA-binding domain containing protein, expressed
54.73	12_30672	protein peptide transporter PTR2, putative, expressed
62.78	11_10302	protein endo-1,3;1,4-beta-D-glucanase precursor, putative, expressed
75.45	11_20121	protein oxidoreductase, putative, expressed
83.3	12_30072	protein tyrosine aminotransferase, putative, expressed
87.62	12_11144	protein FK506-binding protein 4, putative, expressed
88.23	11_10830	protein bHLH transcription factor, putative, expressed
88.23	12_31160	protein yip1 domain family, member 2, putative, expressed
90.97	11_11189	protein pleckstrin homology domain-containing protein 1, putative, expressed
92.04	11_21446	protein expressed protein
92.04	12_10535	protein ferredoxin-6, chloroplast precursor, putative, expressed
93.95	12_31163	protein isoflavone reductase homolog IRL, putative, expressed
96.92	11_20769	protein GTPase, putative, expressed
97.68	11_11277	protein heat shock 70 kDa protein, mitochondrial precursor, putative
121.12	12_21172	protein OsIAA19 - Auxin-responsive Aux/IAA gene family member
121.77	12_31105	protein expressed protein
125.27	11_10722	protein fasciclin-like arabinogalactan protein 7 precursor, putative, expressed
126.01	11_21140	protein 40S ribosomal protein S27a, putative, expressed
137.83	11_20840	protein endopeptidase Clp, putative, expressed
139.79	11_20772	protein spliceosome RNA helicase BAT1, putative, expressed

Table A21. SNP annotation summary for marker-trait associations identified on chromosome 2H based on the analysis of four years combined by breeding program.

cM	Marker	U35 Rice(v5) Description
0	11_20631	protein glycosyl transferase, group 1 family protein, putative, expressed
0	11_10017	protein DNA-binding protein, putative, expressed
0	11_10996	protein elongation factor 1-delta 1, putative, expressed
10.06	11_21416	protein cyclopropane-fatty-acyl-phospholipid synthase/ oxidoreductase, putative
10.06	12_10502	protein phosphoglycerate mutase-like protein, putative, expressed
15.15	11_20112	protein expressed protein
17.85	11_20107	protein nucleus protein, putative, expressed
31.72	11_20864	protein 2-dehydro-3-deoxyphosphooctonate aldolase, putative, expressed
37.32	11_20173	protein peroxidase precursor, putative, expressed
44.13	11_10342	protein nucleolar protein NOP5, putative, expressed
50.49	11_21005	protein novel plant SNARE 11, putative, expressed
51.75	11_10234	protein hydrophobic protein LTI6A, putative, expressed
51.75	12_30691	protein ubiquitin-protein ligase, putative, expressed
51.75	12_30604	protein h/ACA ribonucleoprotein complex subunit 4, putative, expressed
52.47	11_20929	protein transmembrane emp24 domain-containing protein 10 precursor, putative
53.53	12_31474	protein cyclin delta-2, putative, expressed
53.53	11_11522	protein fasciclin-like arabinogalactan protein 8 precursor, putative, expressed
54.95	11_10325	protein phospho-2-dehydro-3-deoxyheptonate aldolase 1, chloroplast precursor, putative
54.95	11_10733	protein copper-transporting ATPase PAA1, putative, expressed
54.95	11_21096	protein glucan endo-1,3-beta-glucosidase 4 precursor, putative, expressed
54.95	12_30259	protein hydrolase, putative, expressed
55.67	12_11272	protein strictosidine synthase 1 precursor, putative, expressed
58.24	11_20500	protein insulin-degrading enzyme, putative, expressed
58.24	11_10602	protein chlorophyll a-b binding protein of LHCII type III, chloroplast precursor, putative
58.24	11_11133	protein polygalacturonase inhibitor 1 precursor, putative, expressed
58.24	12_10485	protein ribosome recycling factor, chloroplast precursor, putative, expressed
58.24	12_30634	protein myosin-5C, putative, expressed
58.9	11_20417	protein autophagy-related protein 8 precursor, putative, expressed
58.9	11_11354	protein ATP-dependent RNA helicase dbp4, putative, expressed
58.9	11_10012	protein 60S ribosomal protein L38, putative, expressed
58.9	11_20039	protein pre-rRNA processing protein ESF1, putative, expressed
58.9	11_21286	protein uncharacterized Cys-rich domain, putative, expressed
58.9	11_11046	protein cytochrome b6-f complex iron-sulfur subunit, chloroplast precursor, putative
58.9	11_10070	protein expressed protein
58.9	11_20458	protein succinate dehydrogenase subunit 3, putative, expressed
58.9	12_10099	protein ATP synthase gamma chain, chloroplast precursor, putative, expressed
58.9	12_31175	protein casein kinase II subunit beta-4, putative, expressed
58.9	12_30828	protein 60S ribosomal protein L38, putative, expressed

Table A21. SNP annotation summary for marker-trait associations identified on chromosome 2H based on the analysis of four years combined by breeding program (cont.)

cM	Marker	U35 Rice(v5) Description
58.9	12_30582	protein rho-GTPase-activating protein 8, putative, expressed
58.9	12_30179	protein protein binding protein, putative, expressed
58.9	11_20160	protein mitochondrial protein, putative, expressed
59.21	11_10624	protein xylanase inhibitor protein 1 precursor, putative, expressed
59.21	12_10474	protein UL36 very large tegument protein, putative, expressed
59.21	12_30853	unknown
59.9	11_11178	protein prolyl-tRNA synthetase, putative, expressed
59.9	11_10317	protein ubiquitin carboxyl-terminal hydrolase 7, putative, expressed
59.9	11_20032	protein oxygen-evolving enhancer protein 2, chloroplast precursor, putative, expressed
59.9	11_20251	protein peptidyl-prolyl isomerase, putative, expressed
59.9	11_10358	protein chloroplastic quinone-oxidoreductase, putative, expressed
59.9	11_20669	protein aldo-keto reductase/ oxidoreductase, putative, expressed
59.9	12_30561	protein vegetative cell wall protein gp1 precursor, putative, expressed
59.9	12_30514	protein glycine-rich protein, putative, expressed
60.68	11_11384	protein expressed protein
62.82	12_10035	protein cysteine proteinase 1 precursor, putative, expressed
63.53	11_20438	protein extensin precursor, putative, expressed
63.53	11_21399	protein protein phosphatase type 2A regulator/ signal transducer, putative, expressed
63.53	11_20532	protein expressed protein
63.53	11_10191	protein 2-cys peroxiredoxin BAS1, chloroplast precursor, putative, expressed
63.53	11_10685	protein expressed protein
63.53	11_20585	protein 2-oxoglutarate dehydrogenase E1 component, mitochondrial precursor, putative
63.53	11_20390	protein 1,4-alpha-glucan branching enzyme IIB, chloroplast precursor, putative, expressed
63.53	11_20887	protein alkaline/neutral invertase, putative, expressed
63.53	12_11504	protein protein kinase KIPK, putative, expressed
63.53	12_11324	protein CPL3, putative, expressed
63.53	12_30323	protein AHK5, putative, expressed
63.53	12_30275	protein expressed protein
63.53	12_30265	protein carbonic anhydrase precursor, putative, expressed
65.67	11_21094	protein calmodulin binding protein, putative, expressed
66.12	11_11072	protein zeaxanthin epoxidase, chloroplast precursor, putative, expressed
67.54	12_11121	protein cryptochrome 1 apoprotein, putative, expressed
71.12	11_20833	protein brain protein 44-like protein, putative, expressed
71.12	11_10407	protein endochitinase A precursor, putative, expressed
71.12	12_31021	protein endochitinase A precursor, putative, expressed
71.12	12_31020	protein endochitinase A precursor, putative, expressed
71.56	12_10717	protein glycoside transferase, six-hairpin, subgroup, putative, expressed
75.89	12_30178	protein ammonium transporter 1, member 2, putative, expressed

Table A21. SNP annotation summary for marker-trait associations identified on chromosome 2H based on the analysis of four years combined by breeding program (cont.)

cM	Marker	U35 Rice(v5) Description
78.03	11_10818	protein expressed protein
78.03	11_10196	protein beta-mannosidase 4, putative, expressed
78.03	11_11435	protein NB-ARC domain containing protein, expressed
78.03	12_31398	protein NB-ARC domain containing protein, expressed
78.03	12_30696	protein zinc finger, C3HC4 type family protein, expressed
79.19	12_31445	protein expressed protein
79.19	12_20489	protein phenylalanine ammonia-lyase, putative, expressed
81.33	12_10859	protein flavonol sulfotransferase-like, putative, expressed
85.92	11_20340	protein vacuolar processing enzyme, beta-isozyme precursor, putative, expressed
86.63	11_10213	protein endothelial differentiation-related factor 1, putative, expressed
86.63	12_30900	protein DNA binding protein, putative, expressed
88.74	11_21037	protein deoxyribonuclease ycfH, putative, expressed
90.1	11_21351	protein mechanosensitive ion channel family protein, expressed
93.5	11_10214	protein HMG1/2-like protein, putative, expressed
96.82	11_10138	protein phospholipid hydroperoxide glutathione peroxidase, putative, expressed
113.92	12_21396	protein SET domain containing protein, expressed
115.08	11_10429	protein calcium-dependent protein kinase, isoform AK1, putative, expressed
116.49	11_10707	protein expressed protein
116.49	12_31095	protein lipid binding protein, putative
120.02	11_21220	protein expressed protein
121.5	11_10092	protein nonspecific lipid-transfer protein 4 precursor, putative, expressed
126.03	11_20480	protein beta-fructofuranosidase, insoluble isoenzyme 7 precursor, putative, expressed
126.03	11_21440	protein expressed protein
131.77	11_20895	protein sulfate transporter 3.3, putative, expressed
133.22	11_11227	protein vacuolar cation/proton exchanger 3, putative, expressed
133.94	12_30396	protein cysteine proteinase RD21a precursor, putative, expressed
133.94	12_30106	protein transposon protein, putative, unclassified, expressed
137.51	11_21274	protein expressed protein
149.36	11_21299	protein ubiquinone biosynthesis protein COQ4, putative, expressed

Table A22. SNP annotation summary for marker-trait associations identified on chromosome 3H based on the analysis of four years combined by breeding program .

cM	Marker	U35 Rice(v5) Description
0	11_20952	protein gamma-secretase subunit APH-1B, putative, expressed
0	12_31428	protein expressed protein
0	11_11411	unknown
8.23	11_20529	protein fructose-bisphosphate aldolase, chloroplast precursor, putative, expressed
8.23	12_30297	protein glycosyltransferase, putative, expressed
16.33	11_20172	protein eukaryotic translation initiation factor 3 subunit 10, putative, expressed
22.68	11_20982	protein nonspecific lipid-transfer protein 1 precursor, putative, expressed
24.99	11_10559	protein metalloendopeptidase, putative, expressed
32.83	11_20607	protein IWS1 C-terminus family protein, expressed
32.83	12_30571	protein zinc finger in N-recognin family protein, putative, expressed
37.17	11_10672	protein ubiquitin family protein, expressed
39.45	11_20410	protein NADP-dependent oxidoreductase P1, putative, expressed
39.45	11_10825	protein cysteine synthase, mitochondrial precursor, putative, expressed
39.45	11_10081	protein 40S ribosomal protein S5, putative, expressed
39.45	11_10710	protein sphingosine-1-phosphate lyase, putative, expressed
41	12_30953	protein cysteine synthase, mitochondrial precursor, putative, expressed
42.06	11_20193	protein senescence-associated-like protein, putative, expressed
42.06	12_10114	protein senescence-associated-like protein, putative, expressed
42.47	11_21145	protein CENP-E like kinetochore protein, putative, expressed
43.23	11_20647	protein integral membrane protein like, putative, expressed
44.76	11_21259	protein NADPH quinone oxidoreductase 1, putative, expressed
47.09	11_20356	protein mitochondrial-processing peptidase alpha subunit, mitochondrial precursor
51.73	11_10380	protein expressed protein
51.73	11_11313	protein ATP binding protein, putative, expressed
51.73	12_30680	protein heme-binding protein 2, putative, expressed
54.4	11_11099	protein argonaute-like protein, putative, expressed
54.4	12_21475	protein expressed protein
54.4	12_20574	protein monoglyceride lipase, putative, expressed
55.57	12_30809	protein ATP binding protein, putative, expressed
70.71	11_20877	protein ATP binding protein, putative, expressed
70.71	12_31323	protein oligosaccharyl transferase STT3 subunit, putative, expressed
72.26	11_20694	protein early nodulin-like protein 3 precursor, putative, expressed
73.53	11_10350	protein ras-related protein Rab7, putative, expressed
73.53	12_31356	protein 50S ribosomal protein L13, chloroplast precursor, putative, expressed
74.15	11_20521	protein expressed protein
74.78	12_30399	protein ATP synthase epsilon chain, mitochondrial, putative, expressed
76.98	12_31346	protein DNA polymerase eta, putative, expressed
78.53	12_11454	protein alpha-1,4-glucan-protein synthase, putative, expressed
80.89	11_20115	protein expressed protein

Table A22. SNP annotation summary for marker-trait associations identified on chromosome 3H based on the analysis of four years combined by breeding program (cont.)

cM	Marker	U35 Rice(v5) Description
80.89	12_30170	protein expressed protein
81.66	11_21358	protein plus-3 domain containing protein, expressed
87.24	11_21348	protein calmodulin, putative, expressed
87.24	11_10444	protein expressed protein
87.24	12_31299	protein apurinic endonuclease-redox protein, putative, expressed
88.82	11_21294	protein lipid-transfer protein, putative, expressed
89.31	12_31018	protein transcription factor GAMYB, putative, expressed
93.43	11_10747	protein ATP binding protein, putative, expressed
107.63	11_20009	protein homeodomain protein JUBEL2, putative, expressed
109.14	11_21513	protein laccase, putative
114	11_10753	protein protein GPR108 precursor, putative, expressed
117.1	11_10584	protein vignain precursor, putative, expressed
141.54	11_21427	protein NB-ARC domain containing protein, expressed
147.43	12_11297	protein leucoanthocyanidin dioxygenase, putative, expressed
155.85	11_11436	protein ran GTPase binding protein, putative, expressed
160.08	11_10935	protein expressed protein
162.15	11_21008	protein cell division protein ftsY, putative, expressed
167.77	11_10893	protein membrane protein, putative, expressed
168.4	11_10694	protein ubiquitin-fold modifier 1 precursor, putative, expressed
168.4	12_10014	protein 60S ribosomal protein L38, putative, expressed

Table A23. SNP annotation summary for marker-trait associations identified on chromosome 4H based on the analysis of four years combined by breeding program.

cM	Marker	U35 Rice(v5) Description
0	11_10379	protein 30S ribosomal protein S1, chloroplast precursor, putative, expressed
26.19	11_20109	protein DNA-directed RNA polymerase I subunit 12, putative, expressed
26.19	11_20680	protein expressed protein
26.19	11_20302	protein DELLA protein SLR1, putative, expressed
26.19	11_21418	protein 14-3-3-like protein S94, putative, expressed
26.19	11_20606	protein phytanoyl-CoA dioxygenase, putative, expressed
28.4	11_10031	protein phosphoglucomutase, cytoplasmic 2, putative, expressed
36.37	11_21389	protein monoglyceride lipase, putative, expressed
37.12	12_31524	protein peptidase, M50 family, putative, expressed
38.63	12_30992	protein sugar transporter family protein, putative, expressed
40.36	11_20180	protein expressed protein
40.36	11_20114	protein serine hydroxymethyltransferase, mitochondrial precursor, putative, expressed
40.36	12_10371	protein endo-1,4-beta-glucanase Cel1, putative, expressed
40.36	12_10063	protein serine hydroxymethyltransferase, mitochondrial precursor, putative, expressed
44.94	11_10793	protein gibberellin-regulated protein 1 precursor, putative, expressed
46.41	11_21490	protein YKL151C, putative, expressed
47.6	11_11405	protein selenium-binding protein-like, putative, expressed
48.5	11_21073	protein DNA-directed RNA polymerases I and III 14 kDa polypeptide, putative, expressed
48.5	11_20853	protein digalactosyldiacylglycerol synthase 1, putative, expressed
48.5	11_10756	protein protein kinase domain containing protein, expressed
48.5	11_10577	protein protein transport protein Sec24-like CEF, putative, expressed
48.5	12_31382	protein non-imprinted in Prader-Willi/Angelman syndrome region protein 1, putative
48.5	12_30331	protein transcriptional corepressor SEUSS, putative, expressed
49.5	12_30777	protein structural constituent of ribosome, putative, expressed
50.4	11_20289	protein 26S protease regulatory subunit 4, putative, expressed
50.4	12_11190	protein cupin family protein, expressed
51.3	11_11332	protein NAC domain transcription factor, putative, expressed
51.3	12_11063	protein gamma-interferon-inducible lysosomal thiol reductase precursor, putative
61.04	12_30054	protein phosphoenolpyruvate carboxykinase, putative, expressed
76.03	12_20143	protein chitin-inducible gibberellin-responsive protein 2, putative, expressed
78.77	12_31148	protein phosphoserine aminotransferase, chloroplast precursor, putative, expressed
85.04	11_21151	protein ufm1-conjugating enzyme 1, putative, expressed
90.29	12_30138	protein homeodomain-leucine zipper transcription factor TaHDZipI-1, putative, expressed
96.59	11_20838	protein 24-methylenesterol C-methyltransferase 2, putative, expressed
97.06	11_21243	protein glutathione S-transferase, putative, expressed

Table A23. SNP annotation summary for marker-trait associations identified on chromosome 4H based on the analysis of four years combined by breeding program (cont.)

cM	Marker	U35 Rice(v5) Description
101.62	11_20515	protein NADH-ubiquinone oxidoreductase B18 subunit, putative, expressed
101.62	11_20454	protein glyceraldehyde-3-phosphate dehydrogenase B, chloroplast precursor, putative
103.11	11_21111	protein GMFP5, putative, expressed
103.11	11_10334	protein CIPK-like protein 1, putative, expressed
106.03	11_20974	protein 50S ribosomal protein L11, putative, expressed
116.85	11_21130	protein major pollen allergen Ory s 1 precursor, putative, expressed
119.84	12_30239	protein ZAC, putative, expressed

Table A24. SNP annotation summary for marker-trait associations identified on chromosome 5H based on the analysis of four years combined by breeding program.

cM	Marker	U35 Rice(v5) Description
0	11_10405	protein salt tolerance protein, putative, expressed
0	11_10593	protein 50S ribosomal protein L18, chloroplast precursor, putative, expressed
2.81	12_30543	protein ATP binding protein, putative, expressed
18.72	11_20010	protein GTPase family protein, putative, expressed
25.23	11_10695	protein inositolphosphorylceramide-B C-26 hydroxylase, putative, expressed
27	11_10974	protein cysteine synthase, putative, expressed
27.72	11_20761	protein glutathione S-transferase IV, putative, expressed
33.09	12_10530	protein serine/threonine-protein phosphatase BSL2, putative, expressed
48.83	11_11198	protein serine/threonine-protein kinase SAPK9, putative, expressed
48.83	11_21401	protein disease resistance protein, putative, expressed
50.27	11_20841	protein UNC93 homolog A, putative, expressed
50.27	11_21447	protein nucleotide pyrophosphatase/phosphodiesterase, putative, expressed
50.27	12_30729	protein PAPA-1-like conserved region family protein, expressed
51.3	12_30728	protein fiber protein Fb19, putative, expressed
51.6	11_11506	protein surfactant protein B containing protein, expressed
53.9	12_30214	protein indole-3-acetate beta-glucosyltransferase, putative, expressed
57.36	11_20239	protein asparagine synthetase, putative, expressed
57.98	11_21148	protein sucrose responsive element binding protein, putative, expressed
57.98	11_20105	protein monoglyceride lipase, putative
57.98	12_10079	protein 60S ribosomal protein L15, putative, expressed
59.4	12_11512	protein 4-nitrophenylphosphatase, putative, expressed
59.4	12_10034	protein 60S ribosomal protein L17, putative, expressed
60.74	12_31033	protein alcohol dehydrogenase 2, putative, expressed
62.15	11_20265	protein expressed protein
63.31	11_21344	protein serine/threonine-protein kinase 16, putative, expressed
90.84	12_31427	protein NOL1/NOP2/sun family protein, expressed
100.28	12_30533	protein 3-hydroxy-3-methylglutaryl-coenzyme A reductase 3, putative, expressed
108.01	11_20549	protein glutathione S-transferase, putative, expressed
108.18	12_10844	protein expressed protein
108.18	12_30854	protein dehydration-responsive element-binding protein 1B, putative, expressed
110.26	11_20805	protein NC domain containing protein, expressed
117.47	11_11200	protein phospholipase D delta, putative, expressed
123.08	11_20637	protein transmembrane 9 superfamily protein member 4, putative, expressed
127.96	11_11456	protein glutamyl-tRNA, putative, expressed
129.41	12_30169	protein nuclear transcription factor Y subunit A-10, putative, expressed
132.48	12_11472	protein expressed protein
149.1	12_30580	protein 25.3 kDa vesicle transport protein, putative, expressed
151.36	11_20100	protein hydroxyacid oxidase 1, putative, expressed
151.36	11_21360	protein 40S ribosomal protein S18, putative, expressed
151.36	12_31050	protein dehydrin Rab16C, putative, expressed
153.51	12_10016	protein 40S ribosomal protein S15, putative, expressed

Table A24. SNP annotation summary for marker-trait associations identified on chromosome 5H based on the analysis of four years combined by breeding program (cont.)

cM	Marker	U35 Rice(v5) Description
158.37	11_10901	protein lipid binding protein, putative
159.79	11_10536	protein carboxyl-terminal peptidase, putative, expressed
161.58	12_30162	protein acid phosphatase, putative, expressed
173.08	11_10869	protein expressed protein
177.65	11_20536	protein cytidine/deoxycytidylate deaminase family protein, putative, expressed
177.65	12_21009	protein polygalacturonase, putative, expressed
178.43	12_20816	protein electron transfer flavoprotein alpha-subunit, mitochondrial precursor, putative
178.43	12_11010	protein actin-3, putative, expressed
178.43	12_11450	protein ferredoxin-3, chloroplast precursor, putative, expressed
179.06	11_10254	protein CESA3 - cellulose synthase, expressed
179.64	11_21138	protein transport protein particle subunit trs31, putative, expressed
179.64	12_30656	protein expressed protein
180.71	11_10736	protein phytosulfokine receptor precursor, putative, expressed
181.43	11_20022	protein protein kinase Pti1, putative, expressed
181.43	11_10236	protein mitochondrial prohibitin complex protein 2, putative, expressed
182.16	12_30504	protein RNA-binding protein Luc7-like 2, putative, expressed
182.88	12_31352	protein regulatory protein, putative, expressed
182.88	12_30577	protein mitochondrial carrier C12B10.09, putative, expressed
187.96	11_10310	protein transcription factor BTF3, putative, expressed
189.6	11_20786	protein 26S proteasome non-ATPase regulatory subunit 12, putative, expressed
189.6	12_31292	protein nucleotide binding protein, putative, expressed
189.6	11_11364	protein pantoate--beta-alanine ligase, putative, expressed
190.23	11_21108	protein derlin-2, putative, expressed
191.97	12_31210	protein DANA2, putative, expressed
191.97	11_10401	protein RCD1, putative, expressed
191.97	12_30360	protein jasmonate O-methyltransferase, putative, expressed
194.64	12_30382	protein OsMKK3 - putative MAPKK based on amino acid sequence homology, expressed
194.84	12_10857	protein RNA polymerase sigma factor rpoD1, putative, expressed
195.42	11_20402	protein ubiquitin-conjugating enzyme E2-21 kDa 1, putative, expressed
196.12	12_30958	protein expressed protein
196.12	12_10322	protein plasma membrane associated protein, putative, expressed
196.85	12_31123	protein pectinesterase inhibitor domain containing protein, expressed

Table A25. SNP annotation summary for marker-trait associations identified on chromosome 6H based on the analysis of four years combined by breeding program.

cM	Marker	U35 Rice(v5) Description
0	11_10496	protein allene oxide cyclase 4, chloroplast precursor, putative, expressed
30.06	12_31485	protein rhodanese like protein, putative, expressed
31.73	11_10994	protein peptidyl-prolyl cis-trans isomerase, putative, expressed
35.07	12_30358	protein GDSL-like Lipase/Acylhydrolase family protein, expressed
42.36	11_10494	protein expressed protein
42.36	12_11455	protein tetratricopeptide repeat protein KIAA0103, putative, expressed
48.74	11_10461	protein AP-4 complex subunit sigma-1, putative, expressed
52.75	11_10003	protein tubulin beta-3 chain, putative, expressed
58.01	11_11067	protein inner envelope membrane protein, chloroplast precursor, putative
60.23	12_30804	protein zinc finger, C3HC4 type family protein, expressed
64.36	11_10455	protein monodehydroascorbate reductase, cytoplasmic isoform 2, putative, expressed
65.03	11_11261	protein catalytic/ hydrolase, putative, expressed
65.03	11_10040	protein omega-6 fatty acid desaturase, endoplasmic reticulum isozyme 2, putative
72.54	12_31111	protein expressed protein
81.17	11_11458	protein PDE317, putative, expressed
85.16	11_10815	protein expressed protein
90.15	11_10202	protein phosphate carrier protein, mitochondrial precursor, putative, expressed
91.79	12_31235	protein expressed protein
93.66	11_20728	protein subtilisin-like protease precursor, putative, expressed
94.73	11_10595	protein dnaJ protein, putative, expressed
112.32	11_20558	protein electron transporter/ thiol-disulfide exchange intermediate, putative, expressed
112.32	11_20733	protein mRNA decapping enzyme 2, putative, expressed
112.32	11_11534	protein PAP-specific phosphatase, putative, expressed
119.02	11_10107	protein MTN3, putative, expressed
121.22	11_11187	protein ABC-1, putative, expressed
129.38	12_30627	protein zinc finger C-x8-C-x5-C-x3-H type family protein, expressed

Table A26. SNP annotation summary for marker-trait associations identified on chromosome 7H based on the analysis of four years combined by breeding program.

cM	Marker	U35 Rice(v5) Description
0.62	11_11132	protein expressed protein
3.34	11_20710	unknown
4.12	11_11179	protein MTA/SAH nucleosidase, putative, expressed
6.78	12_11433	protein serine/threonine-protein kinase MAK, putative, expressed
9.84	11_20307	protein ATOZI1, putative, expressed
15.93	11_20755	protein expressed protein
19.11	12_30723	protein spastin, putative, expressed
25.7	11_20495	protein ATP-dependent Clp protease proteolytic subunit 2, putative, expressed
25.93	12_30530	protein GDP-mannose 4,6 dehydratase 2, putative, expressed
39.04	12_10218	protein alpha-L-fucosidase 2 precursor, putative, expressed
41.85	11_10576	protein caffeoyl-CoA O-methyltransferase 1, putative, expressed
58.57	12_10959	protein expressed protein
61.32	12_30880	protein sucrose synthase 1, putative, expressed
61.32	12_30879	protein sucrose synthase 1, putative, expressed
64.8	12_10605	protein gibberellin receptor GID1L2, putative, expressed
68.46	11_11098	protein nuclear migration protein nudC, putative, expressed
68.46	12_10267	protein annexin-like protein RJ4, putative, expressed
73.75	12_30496	protein ribonucleoside-diphosphate reductase small chain, putative, expressed
74.52	11_10983	protein transmembrane 9 superfamily protein member 2 precursor, putative, expressed
76.08	12_30344	protein multiple stress-responsive zinc-finger protein ISAP1, putative, expressed
76.17	12_10655	protein lipid transfer protein, putative, expressed
76.17	12_30595	protein signal recognition particle receptor beta subunit, putative, expressed
77.85	11_10924	protein protein ariadne-1, putative, expressed
77.85	11_20879	protein NAC domain-containing protein 68, putative, expressed
77.85	12_10698	protein glycyl-tRNA synthetase 1, mitochondrial precursor, putative, expressed
77.85	12_30794	protein protein ariadne-1, putative, expressed
77.85	12_30760	protein VIP2 protein, putative, expressed
77.85	11_10256	protein nuclear transport factor 2, putative, expressed
79.6	11_20460	protein APOBEC1 complementation factor, putative, expressed
79.6	12_11146	protein topoisomerase-like protein, putative, expressed
79.6	12_10581	protein fructokinase-2, putative, expressed
79.6	12_30589	protein expressed protein
80.94	11_10534	protein far upstream element-binding protein 1, putative, expressed
83.44	11_21079	protein peptide methionine sulfoxide reductase msrB, putative, expressed
83.44	12_10369	protein expressed protein
83.44	12_30213	protein vacuolar protein sorting protein 72, putative, expressed
84.07	12_30645	protein nicalin precursor, putative, expressed
84.92	12_11499	protein histone-like transcription factor and archaeal histone family protein, expressed
86.44	11_20896	protein OsPP2Ac-1 - Phosphatase 2A isoform 1 belonging to family 1, expressed

Table A26. SNP annotation summary for marker-trait associations identified on chromosome 7H based on the analysis of four years combined by breeding program (cont.)

cM	Marker	U35 Rice(v5) Description
86.44	12_31199	protein xyloglucan endotransglucosylase/hydrolase protein 32 precursor
86.44	12_31137	protein expressed protein
86.44	12_30199	protein cyclopropane-fatty-acyl-phospholipid synthase, putative
86.44	11_21330	protein FK506 binding protein, putative, expressed
87.21	11_20771	protein expressed protein
87.97	12_10089	protein elongation factor 1-gamma 3, putative, expressed
91.79	12_30026	protein expressed protein
107.11	12_31261	protein expressed protein
114.78	12_30362	protein DNA polymerase alpha subunit B, putative, expressed
119.54	12_30164	protein nuclear transcription factor Y subunit B-3, putative, expressed
140.21	12_31241	unknown
144.45	11_11440	protein transcriptional corepressor SEUSS, putative, expressed
144.45	11_10843	protein expressed protein
144.45	11_21363	protein structural constituent of ribosome, putative, expressed
144.45	11_20452	protein proteasome subunit beta type 3, putative, expressed
144.45	12_30593	protein phosphopantothenate--cysteine ligase, putative, expressed
149.8	11_20962	protein expressed protein

Table A27. SNP annotation summary for marker-trait associations identified on the unlinked group of markers based on the analysis of four years combined by breeding program.

Marker	U35 Rice(v5) Description
11_20922	protein DNA-directed RNA polymerases II 24 kDa polypeptide, putative, expressed
11_20131	protein 40S ribosomal protein SA, putative, expressed
11_20639	protein expressed protein
12_11408	protein peptidyl-prolyl isomerase, putative, expressed
12_10915	protein pathogenesis-related 10 protein PR10-1, putative
12_21157	protein polyubiquitin 2, putative, expressed
12_10752	protein transcription initiation factor IIB, putative, expressed
12_20632	protein profilin A, putative, expressed
12_10313	protein coatomer subunit delta, putative, expressed
12_20323	protein 60S acidic ribosomal protein P2B, putative, expressed
12_31414	protein expressed protein
12_31267	protein expressed protein
12_31239	protein cysteine-type peptidase, putative, expressed
12_31230	protein ATP binding protein, putative, expressed
12_31229	protein hypothetical protein
12_31200	protein expressed protein
12_31041	protein water stress-inducible protein Rab21, putative, expressed
12_30982	protein prolamin PPROL 17 precursor, putative, expressed
12_30939	protein OsPDIL2-3 - Oryza sativa protein disulfide isomerase, expressed
12_30908	protein glutamine synthetase root isozyme 3, putative, expressed
12_30845	protein dehydration-responsive element-binding protein 1D, putative, expressed
12_30822	protein alpha-glucosidase precursor, putative, expressed
12_30646	protein signal recognition particle 9 kDa protein, putative, expressed
12_30603	protein serine/threonine-protein kinase Cx32, chloroplast precursor, putative, expressed
12_30597	protein dehydrogenase/reductase SDR family member 7 precursor, putative, expressed
12_30503	protein cytochrome P450 51, putative, expressed
12_30502	protein expressed protein
12_30224	protein cytokinin-O-glucosyltransferase 2, putative
11_21213	protein retrotransposon protein, putative, unclassified, expressed
11_10150	protein ubiquitin-conjugating enzyme E2 I, putative, expressed

Table A28. SNP markers detecting polymorphisms between Stander and Robust were used to build a linkage genetic map with a minimal LOD score of 3.0 and maximum recombination fraction of 0.30. Chi-square tests were used to determined segregation distortion from the 1:1 Mendelian ration for all the loci.

Marker Name	Chr	cM	Allele		$\chi^2_{1:1}$	
			A	B		
2_0959	1H	0.00	39	152	66.85	*****
SCRI_RS_168172	1H	0.50	40	151	64.51	*****
1_0905	1H	8.90	56	135	32.68	*****
SCRI_RS_173813	1H	19.00	61	130	24.93	*****
2_0711	2H	0.00	77	114	7.17	**
2_1220	2H	2.60	76	115	7.96	**
1_0780	2H	3.10	75	116	8.80	**
3_1095	2H	4.70	76	115	7.96	**
SCRI_RS_202154	3H	0.00	94	97	0.05	ns
1_1516	3H	3.10	94	97	0.05	ns
SCRI_RS_237894	3H	3.60	95	96	0.01	ns
SCRI_RS_128254	3H	4.70	95	96	0.01	ns
1_0014	3H	5.20	94	97	0.05	ns
2_0605	3H	7.30	92	99	0.26	ns
2_1523	3H	7.80	93	98	0.13	ns
3_0992	4H	0.00	86	105	1.89	ns
1_0371	4H	1.00	86	105	1.89	ns
2_0114	4H	1.50	87	104	1.51	ns
SCRI_RS_167844	4H	3.60	85	106	2.31	ns
12_31414	4H	4.10	86	105	1.89	ns
SCRI_RS_9618	4H	4.70	87	104	1.51	ns
3_0605	4H	5.20	88	103	1.18	ns
1_0639	4H	6.20	86	105	1.89	ns
SCRI_RS_194525	4H	6.80	85	106	2.31	ns
1_0010	4H	7.30	86	105	1.89	ns
SCRI_RS_137903	4H	8.30	86	105	1.89	ns
1_0627	4H	8.90	87	104	1.51	ns
SCRI_RS_89959	4H	9.40	86	105	1.89	ns
3_1148	4H	18.90	92	99	0.26	ns
SCRI_RS_200957	4H	20.50	91	100	0.42	ns
SCRI_RS_13460	4H	22.60	89	102	0.88	ns
SCRI_RS_144204	4H	23.10	88	103	1.18	ns
2_0197	4H	23.60	87	104	1.51	ns

ns,***, *****non-significant and significant SNP marker-trait associations at $P \leq 0.001$ and 0.00001.

Table A28. SNP markers detecting polymorphisms between Stander and Robust were used to build a linkage genetic map with a minimal LOD score of 3.0 and maximum recombination fraction of 0.30. Chi-square tests were used to determined segregation distortion from the 1:1 Mendelian ration for all the loci (cont.)

Marker Name	Chr	cM	Allele		$\chi^2_{1:1}$	
			A	B		
2_0134	5H-1	0.00	93	98	0.13	ns
SCRI_RS_218201	5H-1	2.60	90	101	0.63	ns
SCRI_RS_80595	5H-1	3.10	89	102	0.88	ns
1_0414	5H-1	4.10	91	100	0.42	ns
3_1023	5H-2	0.00	90	101	0.63	ns
SCRI_RS_236068	5H-2	0.50	89	102	0.88	ns
3_0591	5H-2	1.00	88	103	1.18	ns
SCRI_RS_168359	5H-2	1.50	89	102	0.88	ns
2_1202	5H-2	3.60	91	100	0.42	ns
SCRI_RS_228061	5H-2	5.20	94	97	0.05	ns
SCRI_RS_141226	5H-3	0.00	105	86	1.89	ns
1_0869	5H-3	32.80	140	51	41.47	*****
SCRI_RS_169845	5H-3	43.90	159	32	84.45	*****
12_31239	5H-3	44.50	160	31	87.13	*****
3_0494	5H-3	45.00	159	32	84.45	*****
3_0769	5H-3	46.00	157	34	79.21	*****
3_1352	5H-3	46.60	158	33	81.81	*****
2_1155	5H-3	47.60	160	31	87.13	*****
2_1162	5H-3	48.70	160	31	87.13	*****
SCRI_RS_167850	5H-3	49.70	158	33	81.81	*****
2_1108	5H-3	50.20	157	34	79.21	*****
12_20775	5H-3	68.80	165	26	101.16	*****
SCRI_RS_159536	5H-3	80.60	143	48	47.25	*****
SCRI_RS_237782	6H	0.00	103	88	1.18	ns
2_1521	6H	1.00	103	88	1.18	ns
2_0315	6H	24.00	102	89	0.88	ns
1_0136	6H	24.50	101	90	0.63	ns
SCRI_RS_231372	6H	25.00	100	91	0.42	ns
2_0745	6H	30.30	100	91	0.42	ns
3_1308	6H	30.80	99	92	0.26	ns
3_1485	6H	31.80	99	92	0.26	ns
3_0358	6H	37.60	104	87	1.51	ns
3_0521	6H	38.10	103	88	1.18	ns
3_0361	6H	39.20	101	90	0.63	ns
2_1030	6H	39.70	100	91	0.42	ns
1_0244	6H	41.80	98	93	0.13	ns
3_0317	6H	42.90	98	93	0.13	ns
3_0316	6H	43.40	97	94	0.05	ns

ns,***, *****non-significant and significant SNP marker-trait associations at $P \leq 0.001$ and 0.00001.

Table A28. SNP markers detecting polymorphisms between Stander and Robust were used to build a linkage genetic map with a minimal LOD score of 3.0 and maximum recombination fraction of 0.30. Chi-square tests were used to determined segregation distortion from the 1:1 Mendelian ration for all the loci (cont.)

Marker Name	Chr	cM	Allele			
			A	B	$\chi^2_{1:1}$	
1_0910	6H	44.40	97	94	0.05	ns
2_0675	6H	45.50	95	96	0.01	ns
3_0857	6H	46.50	93	98	0.13	ns
SCRI_RS_187343	6H	47.60	91	100	0.42	ns
1_1253	6H	48.10	90	101	0.63	ns
3_0804	6H	48.60	89	102	0.88	ns
SCRI_RS_175000	6H	49.10	90	101	0.63	ns
2_0904	6H	51.80	89	102	0.88	ns
12_10348	6H	53.30	92	99	0.26	ns
1_0040	6H	53.80	93	98	0.13	ns
2_0744	6H	54.40	94	97	0.05	ns
2_0682	6H	56.50	94	97	0.05	ns
2_0969	6H	57.00	95	96	0.01	ns
2_0746	6H	58.00	95	96	0.01	ns
1_0220	6H	59.60	94	97	0.05	ns
SCRI_RS_165945	6H	61.20	97	94	0.05	ns
SCRI_RS_102418	6H	61.70	96	95	0.01	ns

ns,***, *****non-significant and significant SNP marker-trait associations at $P \leq 0.001$ and 0.00001.

Table A29. Statistics from the composite interval mapping analysis based on the separate means for each environment and overall means from the Stander x Robust DH population.

			11FGH			11SGH			12SGH			Combined		
Chr	Marker	cM Interval	Additive	LOD	R^2	Additive	LOD	R^2	Additive	LOD	R^2	Additive	LOD	R^2
1H	2_0959	0	-1.956	0.485	0.012	-2.779	1.025	0.024	0.324	0.014	0	-1.989	0.855	0.02
1H		2	-1.456	0.267	0.006	-2.13	0.596	0.014	0.55	0.04	0.001	-1.558	0.519	0.012
1H		4	-1.122	0.16	0.004	-1.788	0.425	0.01	0.576	0.045	0.001	-1.324	0.38	0.009
1H		6	-0.757	0.078	0.002	-1.38	0.27	0.006	0.57	0.047	0.001	-1.041	0.251	0.006
1H		8	-0.417	0.027	0.001	-0.976	0.151	0.004	0.539	0.047	0.001	-0.757	0.148	0.004
1H		10	-0.309	0.015	0	-0.813	0.106	0.003	0.559	0.051	0.001	-0.657	0.113	0.003
1H		12	-0.351	0.018	0	-0.794	0.096	0.002	0.618	0.06	0.001	-0.673	0.112	0.003
1H		14	-0.38	0.021	0.001	-0.738	0.082	0.002	0.655	0.066	0.002	-0.66	0.107	0.003
1H		16	-0.393	0.023	0.001	-0.653	0.066	0.002	0.664	0.07	0.002	-0.62	0.097	0.002
1H		18	-0.39	0.024	0.001	-0.55	0.05	0.001	0.646	0.071	0.002	-0.559	0.085	0.002
2H	2_0711	0	0.221	0.001	0	-2.424	0.146	0.004	0.241	0.001	0	-0.797	0.026	0.001
2H		2	1.049	0.006	0	-3.892	0.081	0.002	4.629	0.117	0.003	-0.218	0	0
2H		4	-3.102	1.74	0.041	-1.591	0.484	0.012	-3.763	2.709	0.063	-2.873	2.51	0.059
3H	SCRI_RS_202154	0	0.517	0.053	0.001	-0.134	0.004	0	-1.598	0.54	0.013	-0.264	0.023	0.001
3H		2	0.721	0.099	0.002	0.276	0.015	0	-1.296	0.344	0.008	0.029	0	0
3H		4	0.842	0.138	0.003	0.493	0.049	0.001	-1.051	0.23	0.006	0.18	0.011	0
3H		6	1.121	0.241	0.006	0.726	0.105	0.003	-0.817	0.137	0.003	0.428	0.06	0.001
4H	3_0992	0	0.635	0.078	0.002	1.072	0.232	0.006	1.282	0.341	0.008	0.908	0.272	0.007
4H		2	0.627	0.075	0.002	0.953	0.182	0.004	0.908	0.169	0.004	0.724	0.171	0.004
4H		4	0.729	0.103	0.002	1.006	0.205	0.005	1.027	0.219	0.005	0.815	0.219	0.005
4H		6	0.848	0.139	0.003	1.123	0.255	0.006	1.085	0.244	0.006	0.917	0.277	0.007
4H		8	0.756	0.111	0.003	0.996	0.2	0.005	0.558	0.064	0.002	0.669	0.147	0.004

Table A29. Statistics from the composite interval mapping analysis based on the separate means for each environment and overall means from the Stander x Robust DH population (cont.)

			11FGH			11SGH			12SGH			Combined		
Chr	Marker	cM Interval	Additive	LOD	R^2	Additive	LOD	R^2	Additive	LOD	R^2	Additive	LOD	R^2
4H		10	0.826	0.13	0.003	1.369	0.372	0.009	0.621	0.078	0.002	0.863	0.241	0.006
4H		12	0.922	0.152	0.004	1.404	0.369	0.009	0.562	0.06	0.001	0.864	0.227	0.005
4H		14	0.991	0.172	0.004	1.39	0.353	0.008	0.477	0.042	0.001	0.831	0.206	0.005
4H		16	1.024	0.187	0.004	1.324	0.325	0.008	0.373	0.026	0.001	0.768	0.178	0.004
4H		18	1.019	0.195	0.005	1.215	0.289	0.007	0.264	0.014	0	0.682	0.148	0.004
4H		20	1.188	0.269	0.006	1.013	0.203	0.005	0.16	0.005	0	0.625	0.126	0.003
4H		22	1.541	0.451	0.011	1.5	0.445	0.011	0.431	0.037	0.001	1.051	0.355	0.009
5H-1	2_0134	0	0.213	0.009	0	0.966	0.191	0.005	-0.948	0.189	0.005	0.31	0.032	0.001
5H-1		2	0.241	0.011	0	0.664	0.088	0.002	-1.049	0.227	0.005	0.119	0.005	0
5H-1		4	-0.259	0.013	0	-0.126	0.003	0	-0.812	0.138	0.003	-0.265	0.023	0.001
5H-2	3_1023	0	-0.318	0.019	0	-1.26	0.319	0.008	-1.678	0.583	0.014	-1.019	0.341	0.008
5H-2		2	-0.183	0.006	0	-1.366	0.368	0.009	-1.968	0.787	0.019	-1.152	0.427	0.01
5H-2		4	-0.138	0.004	0	-1.35	0.362	0.009	-2.096	0.902	0.022	-1.196	0.464	0.011
5H-3	SCRI_RS_141226	0	16.925	35.367	0.574	16.864	36.136	0.582	15.399	32.494	0.543	16.793	48.87	0.692
5H-3		2	17.609	34.554	0.565	17.596	35.62	0.576	16.063	32.025	0.538	17.495	47.765	0.684
5H-3		4	18.22	33.412	0.553	18.266	34.775	0.568	16.669	31.27	0.529	18.13	46.14	0.671
5H-3		6	18.722	31.906	0.537	18.836	33.548	0.555	17.183	30.185	0.517	18.661	43.951	0.653
5H-3		8	19.067	30.022	0.515	19.261	31.909	0.537	17.564	28.739	0.5	19.042	41.195	0.63
5H-3		10	19.21	27.774	0.488	19.493	29.855	0.513	17.767	26.927	0.478	19.225	37.92	0.599
5H-3		12	19.103	25.212	0.455	19.483	27.423	0.484	17.75	24.777	0.45	19.165	34.228	0.562
5H-3		14	18.71	22.419	0.418	19.194	24.692	0.449	17.476	22.352	0.417	18.823	30.265	0.518
5H-3		16	18.015	19.504	0.375	18.602	21.772	0.408	16.926	19.745	0.379	18.181	26.2	0.468
5H-3		18	17.022	16.589	0.33	17.709	18.79	0.364	16.102	17.07	0.337	17.241	22.202	0.415

Table A29. Statistics from the composite interval mapping analysis based on the separate means for each environment and overall means from the Stander x Robust DH population (cont.)

			11FGH			11SGH			12SGH			Combined		
Chr	Marker	cM Interval	Additive	LOD	R^2	Additive	LOD	R^2	Additive	LOD	R^2	Additive	LOD	R^2
5H-3		20	15.765	13.788	0.283	16.544	15.874	0.318	15.031	14.441	0.294	16.036	18.421	0.359
5H-3		22	14.303	11.197	0.237	15.161	13.133	0.271	13.762	11.958	0.25	14.62	14.969	0.303
5H-3		24	12.71	8.884	0.193	13.632	10.648	0.226	12.36	9.698	0.209	13.066	11.917	0.25
5H-3		26	11.064	6.884	0.153	12.032	8.466	0.185	10.895	7.708	0.17	11.452	9.297	0.201
5H-3		28	9.438	5.205	0.118	10.435	6.604	0.147	9.435	6.005	0.135	9.85	7.105	0.157
5H-3		30	7.889	3.833	0.088	8.9	5.053	0.115	8.032	4.585	0.105	8.317	5.314	0.12
5H-3		32	6.459	2.742	0.064	7.47	3.791	0.087	6.727	3.429	0.079	6.896	3.882	0.089
5H-3		34	-2.897	0.974	0.023	-1.619	0.314	0.008	-1.575	0.305	0.007	-2.279	1.024	0.024
5H-3		36	-3.094	0.974	0.023	-1.851	0.36	0.009	-1.747	0.329	0.008	-2.497	1.078	0.026
5H-3		38	-3.167	0.931	0.022	-2.038	0.398	0.01	-1.864	0.341	0.008	-2.629	1.09	0.026
5H-3		40	-3.068	0.838	0.02	-2.134	0.419	0.01	-1.89	0.337	0.008	-2.629	1.046	0.025
5H-3		42	-2.794	0.706	0.017	-2.112	0.418	0.01	-1.81	0.315	0.008	-2.48	0.947	0.023
5H-3		44	-2.442	0.569	0.014	-2.083	0.43	0.01	-1.658	0.279	0.007	-2.266	0.834	0.02
5H-3	3_0769	46	-2.506	0.61	0.015	-3.219	1.052	0.025	-1.868	0.361	0.009	-2.736	1.243	0.03
5H-3		48	-2.217	0.404	0.01	-3.245	0.904	0.022	-1.377	0.165	0.004	-2.657	0.99	0.024
5H-3		50	-2.346	0.459	0.011	-2.603	0.589	0.014	-0.665	0.039	0.001	-2.307	0.757	0.018
5H-3		52	-2.139	0.343	0.008	-2.523	0.498	0.012	-0.695	0.039	0.001	-2.234	0.638	0.015
5H-3		54	-2.134	0.305	0.007	-2.481	0.429	0.01	-0.943	0.063	0.002	-2.315	0.611	0.015
5H-3		56	-2.037	0.255	0.006	-2.323	0.346	0.008	-1.203	0.095	0.002	-2.314	0.561	0.013
5H-3		58	-1.837	0.198	0.005	-2.042	0.255	0.006	-1.447	0.131	0.003	-2.21	0.489	0.012
5H-3		60	-1.547	0.141	0.003	-1.657	0.168	0.004	-1.637	0.168	0.004	-2.005	0.402	0.01
5H-3		62	-1.204	0.089	0.002	-1.22	0.095	0.002	-1.751	0.201	0.005	-1.724	0.311	0.007
5H-3		64	-0.856	0.049	0.001	-0.788	0.043	0.001	-1.782	0.227	0.005	-1.411	0.226	0.005

Table A29. Statistics from the composite interval mapping analysis based on the separate means for each environment and overall means from the Stander x Robust DH population (cont.)

			11FGH			11SGH			12SGH			Combined		
Chr	Marker	cM Interval	Additive	LOD	R^2	Additive	LOD	R^2	Additive	LOD	R^2	Additive	LOD	R^2
5H-3		66	-0.541	0.022	0.001	-0.407	0.013	0	-1.746	0.244	0.006	-1.104	0.155	0.004
5H-3		68	-0.279	0.007	0	-0.097	0.001	0	-1.663	0.254	0.006	-0.831	0.101	0.002
5H-3		70	0.202	0.004	0	0.413	0.016	0	-1.19	0.134	0.003	-0.341	0.017	0
5H-3		72	0.868	0.066	0.002	1.096	0.11	0.003	-0.397	0.015	0	0.35	0.018	0
5H-3		74	1.461	0.198	0.005	1.694	0.277	0.007	0.38	0.014	0	0.994	0.155	0.004
5H-3		76	1.909	0.372	0.009	2.136	0.485	0.012	1.037	0.117	0.003	1.506	0.393	0.009
5H-3		78	2.19	0.558	0.013	2.404	0.701	0.017	1.52	0.286	0.007	1.855	0.681	0.016
5H-3		80	2.326	0.734	0.018	2.522	0.899	0.021	1.832	0.484	0.012	2.054	0.974	0.023
6H	SCRI_RS_237782	0	0.529	0.055	0.001	1.266	0.326	0.008	0.909	0.172	0.004	0.958	0.305	0.007
6H		2	0.452	0.038	0.001	0.919	0.164	0.004	0.4	0.032	0.001	0.692	0.152	0.004
6H		4	0.383	0.025	0.001	0.965	0.167	0.004	0.272	0.014	0	0.653	0.125	0.003
6H		6	0.296	0.014	0	1	0.168	0.004	0.119	0.002	0	0.597	0.097	0.002
6H		8	0.192	0.006	0	1.022	0.166	0.004	-0.057	0.001	0	0.523	0.071	0.002
6H		10	0.075	0.001	0	1.027	0.162	0.004	-0.247	0.01	0	0.431	0.046	0.001
6H		12	-0.05	0	0	1.013	0.155	0.004	-0.443	0.03	0.001	0.327	0.026	0.001
6H		14	-0.175	0.004	0	0.98	0.145	0.003	-0.633	0.062	0.001	0.215	0.011	0
6H		16	-0.294	0.013	0	0.929	0.134	0.003	-0.806	0.103	0.002	0.103	0.003	0
6H		18	-0.399	0.025	0.001	0.866	0.121	0.003	-0.953	0.151	0.004	-0.004	0	0
6H		20	-0.488	0.039	0.001	0.794	0.108	0.003	-1.07	0.202	0.005	-0.1	0.003	0
6H		22	-0.558	0.055	0.001	0.718	0.096	0.002	-1.155	0.254	0.006	-0.183	0.01	0
6H	2_0315	24	-0.609	0.072	0.002	0.643	0.083	0.002	-1.211	0.304	0.007	-0.251	0.021	0
6H		26	-0.27	0.014	0	0.723	0.103	0.002	-0.969	0.189	0.005	-0.052	0.001	0

Table A29. Statistics from the composite interval mapping analysis based on the separate means for each environment and overall means from the Stander x Robust DH population (cont.)

			11FGH			11SGH			12SGH			Combined		
Chr	Marker	cM Interval	Additive	LOD	R^2	Additive	LOD	R^2	Additive	LOD	R^2	Additive	LOD	R^2
6H		28	-0.244	0.011	0	0.813	0.127	0.003	-0.892	0.157	0.004	-0.026	0	0
6H		30	-0.204	0.008	0	0.851	0.146	0.004	-0.762	0.12	0.003	0.002	0	0
6H		32	0.357	0.025	0.001	0.738	0.11	0.003	-0.878	0.16	0.004	0.062	0.001	0
6H		34	0.828	0.127	0.003	0.818	0.128	0.003	-1.043	0.215	0.005	0.189	0.011	0
6H		36	1.272	0.301	0.007	0.853	0.141	0.003	-1.154	0.265	0.006	0.312	0.031	0.001
6H		38	1.591	0.495	0.012	0.949	0.182	0.004	-1.164	0.282	0.007	0.437	0.063	0.002
6H		40	2.001	0.784	0.019	0.818	0.135	0.003	-1.211	0.305	0.007	0.511	0.086	0.002
6H		42	2.119	0.885	0.021	0.878	0.156	0.004	-1.138	0.27	0.006	0.636	0.134	0.003
6H		44	2.368	1.103	0.026	0.935	0.177	0.004	-0.814	0.137	0.003	0.892	0.263	0.006
6H		46	2.432	1.164	0.028	0.677	0.093	0.002	-0.978	0.199	0.005	0.827	0.226	0.005
6H		48	1.963	0.757	0.018	0.118	0.003	0	-1.222	0.311	0.007	0.397	0.052	0.001
6H		50	1.696	0.552	0.013	0.257	0.013	0	-1.38	0.389	0.009	0.312	0.032	0.001
6H		52	1.073	0.224	0.005	0.427	0.037	0.001	-1.714	0.611	0.015	0.019	0	0
6H		54	0.573	0.064	0.002	0.475	0.046	0.001	-2.11	0.94	0.022	-0.297	0.029	0.001
6H		56	0.415	0.033	0.001	0.593	0.071	0.002	-2.127	0.942	0.022	-0.329	0.035	0.001
6H	2_0746	58	0.095	0.002	0	0.347	0.025	0.001	-2.124	0.956	0.023	-0.525	0.092	0.002
6H		60	0.243	0.011	0	0.699	0.099	0.002	-1.726	0.623	0.015	-0.216	0.015	0

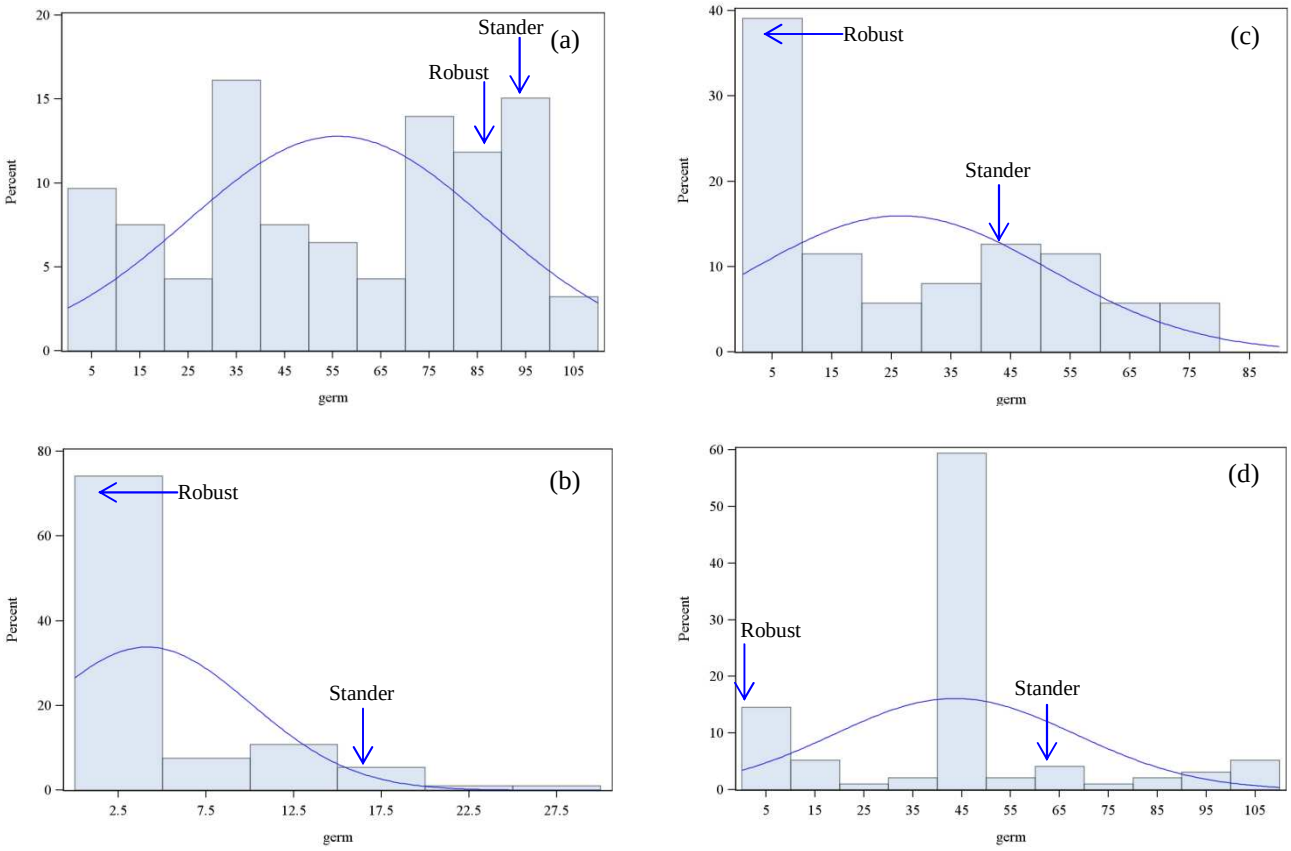

Figure A1. Phenotypic distribution of seed dormancy for the breeding materials from USDA-ARS-Aberdeen, ID (AB). (a) 2006, Robust mean=89; Stander=91; (b) 2007, Robust mean=1.96; Stander=16.85; (c) 2008, Robust mean=3.44; Stander=42.51; (d) 2009, Robust mean=0; Stander=61.28. The X-axis represents the percentage of germinated seeds, while the Y-axis represents the observed frequency for each of the intervals in X.

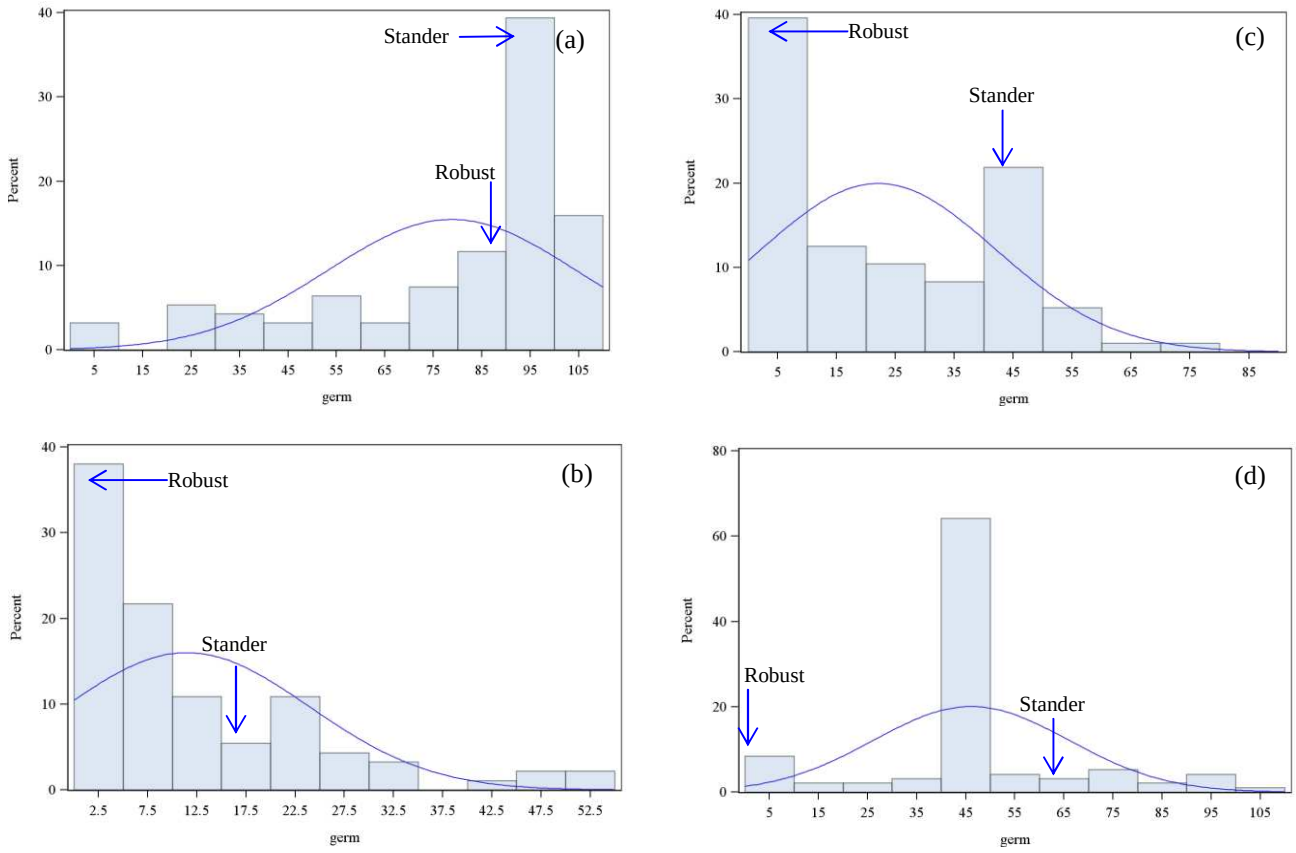

Figure A2. Phenotypic distribution of seed dormancy for the breeding materials from Bush Agricultural Resources (BA). (a) 2006, Robust mean=89; Stander=91; (b) 2007, Robust mean=1.96; Stander=16.85; (c) 2008, Robust mean=3.44; Stander=42.51; (d) 2009, Robust mean=0; Stander=61.28. The X-axis represents the percentage of germinated seeds, while the Y-axis represents the observed frequency for each of the intervals in X.

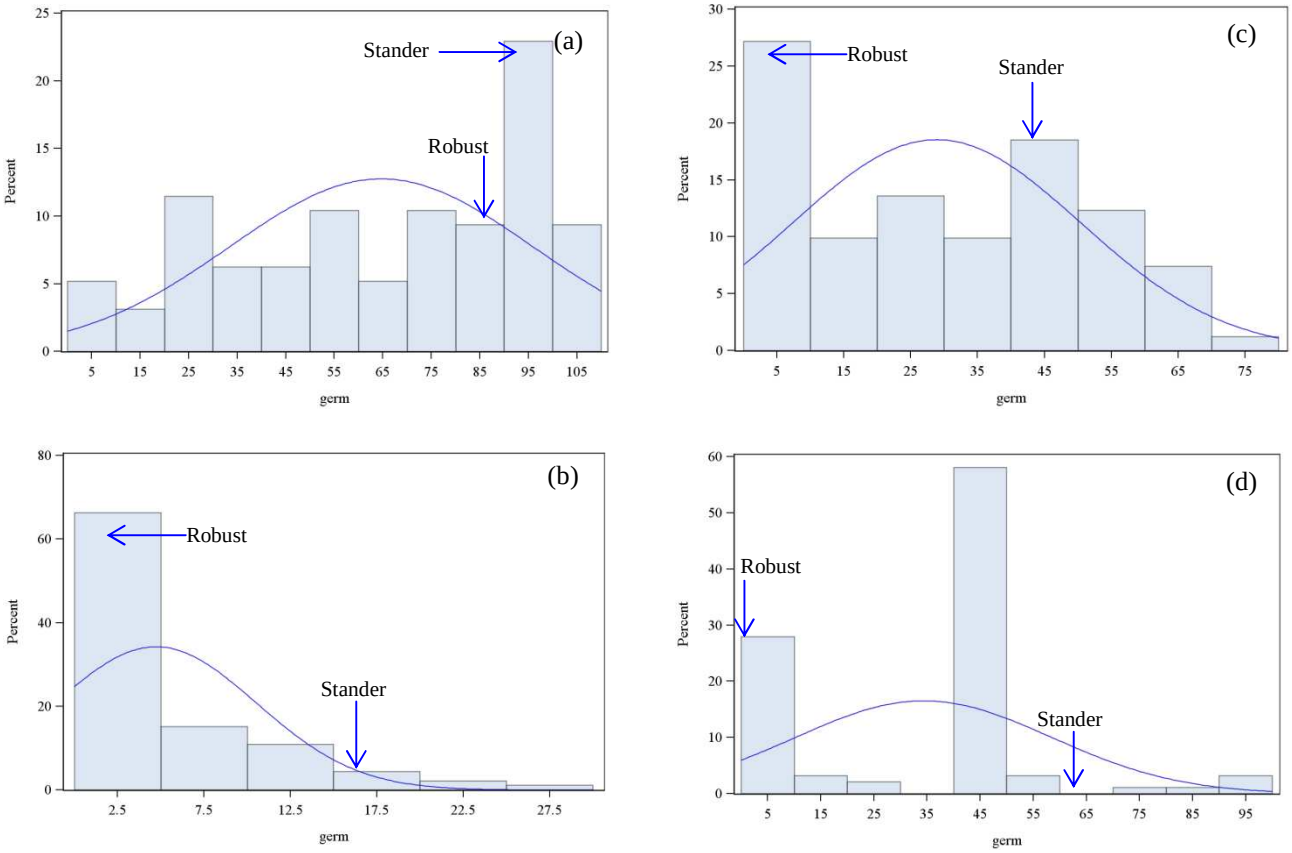

Figure A3. Phenotypic distribution of seed dormancy for the breeding materials from Montana State University (MT). (a) 2006, Robust mean=89; Stander=91; (b) 2007, Robust mean=1.96; Stander=16.85; (c) 2008, Robust mean=3.44; Stander=42.51; (d) 2009, Robust mean=0; Stander=61.28. The X-axis represents the percentage of germinated seeds, while the Y-axis represents the observed frequency for each of the intervals in X.

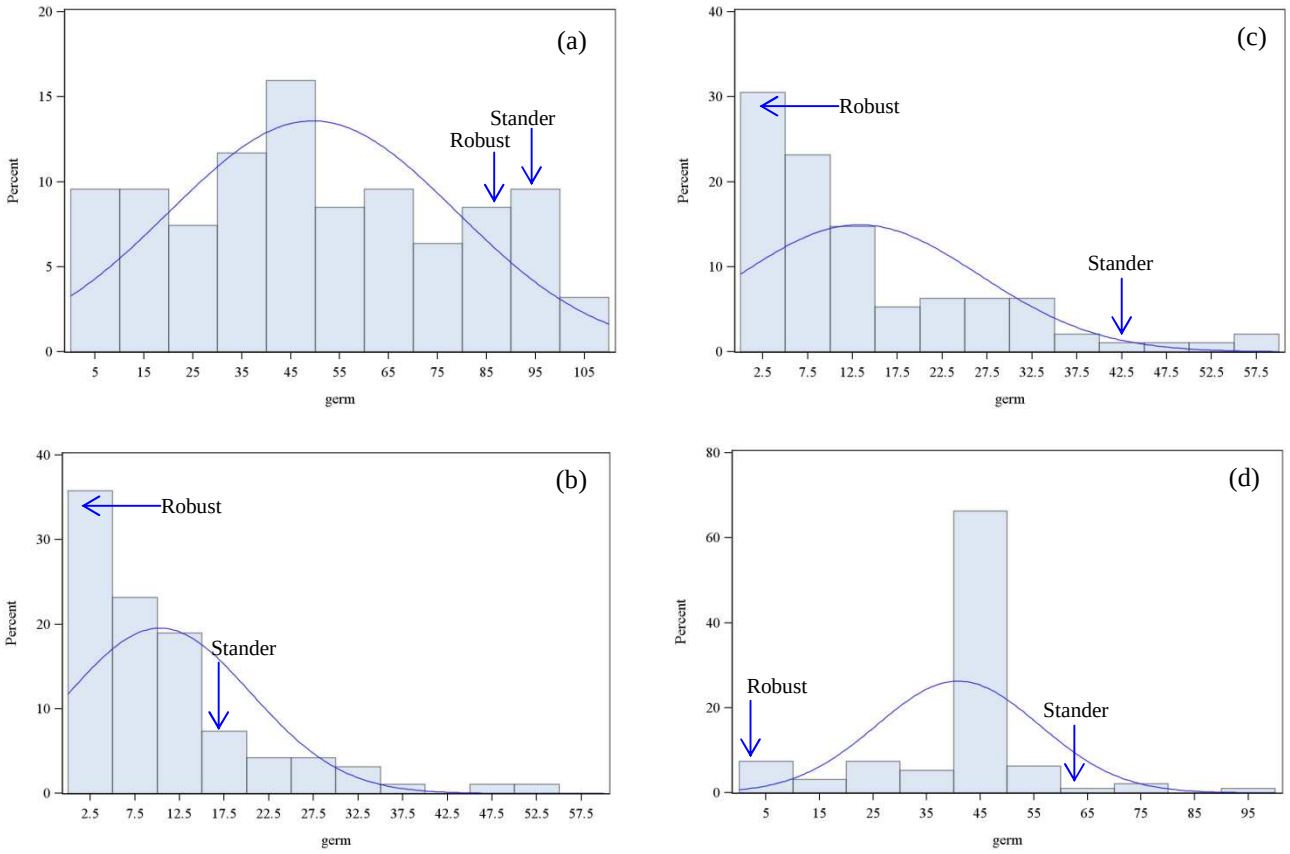

Figure A4. Phenotypic distribution of seed dormancy for the breeding materials from North Dakota State University-two rowed (ND2R). (a) 2006, Robust mean=89; Stander=91; (b) 2007, Robust mean=1.96; Stander=16.85; (c) 2008, Robust mean=3.44; Stander=42.51; (d) 2009, Robust mean=0; Stander=61.28. The X-axis represents the percentage of germinated seeds, while the Y-axis represents the observed frequency for each of the intervals in X.

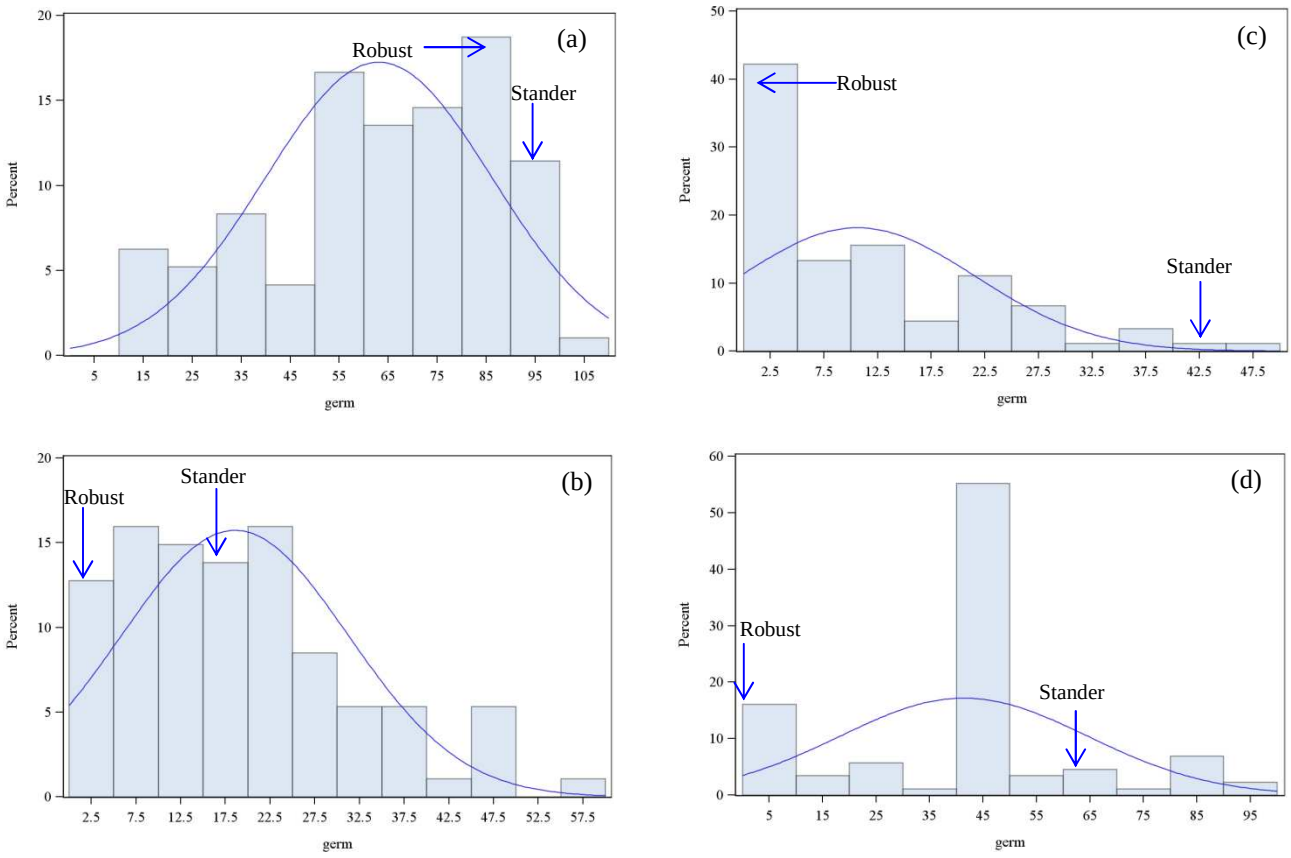

Figure A5. Phenotypic distribution of seed dormancy for the breeding materials from North Dakota State University-six-rowed (ND6R). (a) 2006, Robust mean=89; Stander=91; (b) 2007, Robust mean=1.96; Stander=16.85; (c) 2008, Robust mean=3.44; Stander=42.51; (d) 2009, Robust mean=0; Stander=61.28. The X-axis represents the percentage of germinated seeds, while the Y-axis represents the observed frequency for each of the intervals in X.

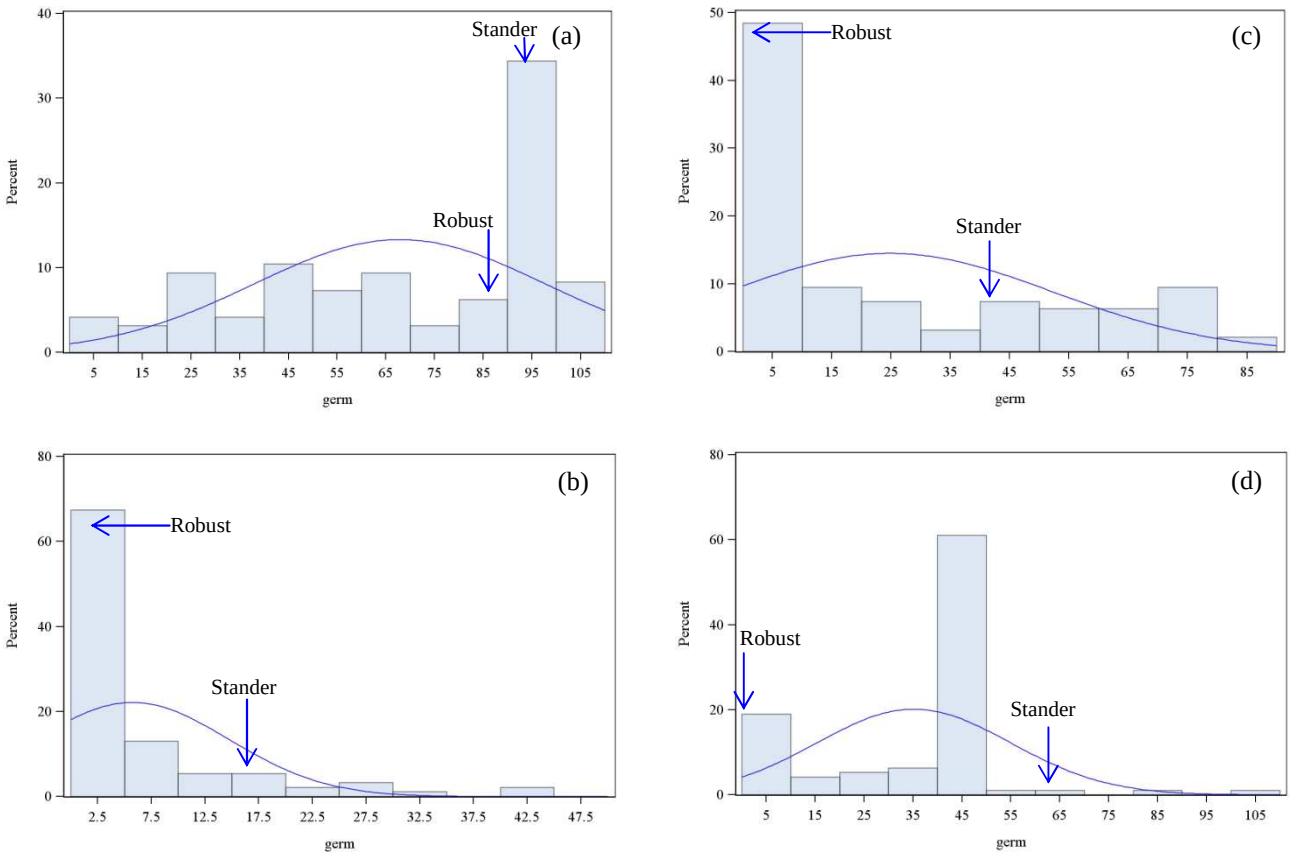

Figure A6. Phenotypic distribution of seed dormancy for the breeding materials from University of Minnesota (UM). (a) 2006, Robust mean=89; Stander=91; (b) 2007, Robust mean=1.96; Stander=16.85; (c) 2008, Robust mean=3.44; Stander=42.51; (d) 2009, Robust mean=0; Stander=61.28. The X-axis represents the percentage of germinated seeds, while the Y-axis represents the observed frequency for each of the intervals in X.

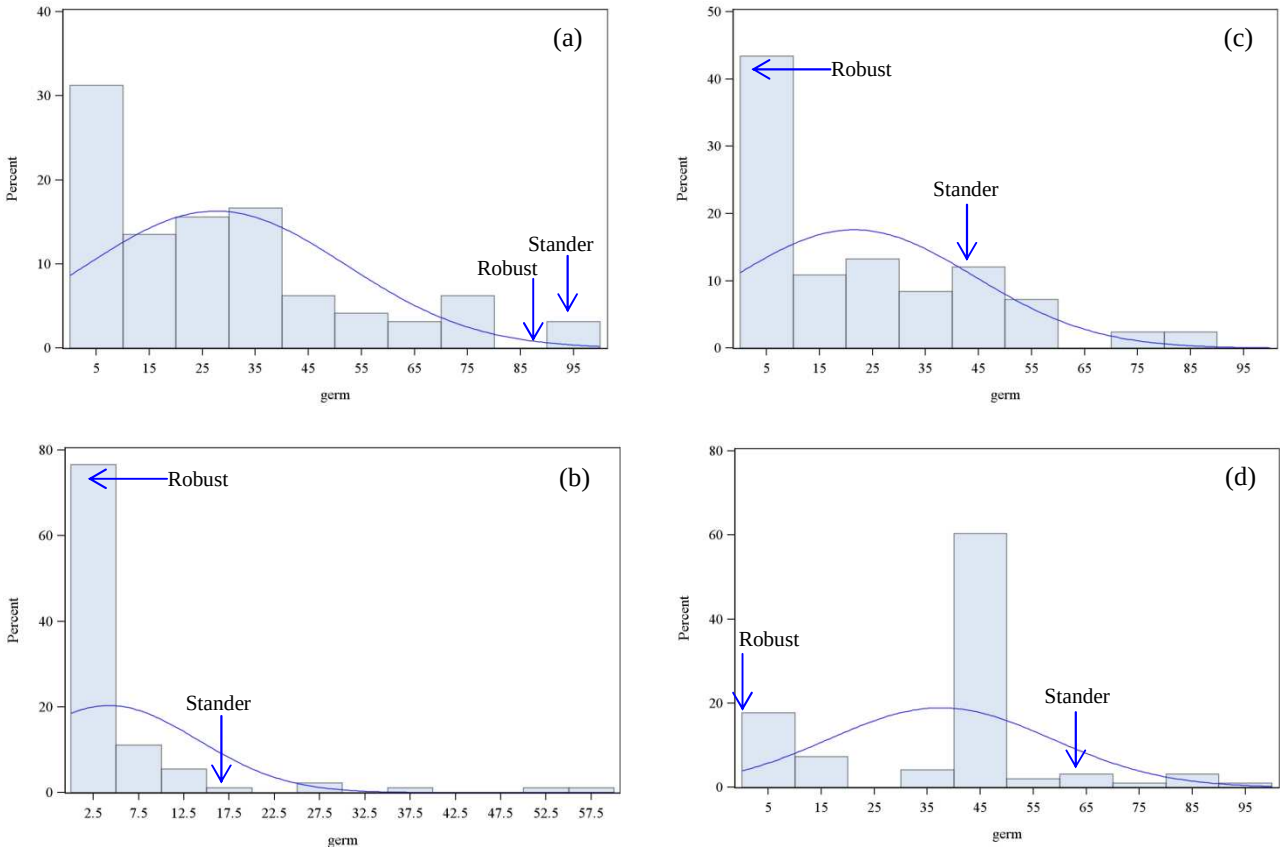

Figure A7. Phenotypic distribution of seed dormancy for the breeding materials from Utah State University (UT). (a) 2006, Robust mean=89; Stander=91; (b) 2007, Robust mean=1.96; Stander=16.85; (c) 2008, Robust mean=3.44; Stander=42.51; (d) 2009, Robust mean=0; Stander=61.28. The X-axis represents the percentage of germinated seeds, while the Y-axis represents the observed frequency for each of the intervals in X.

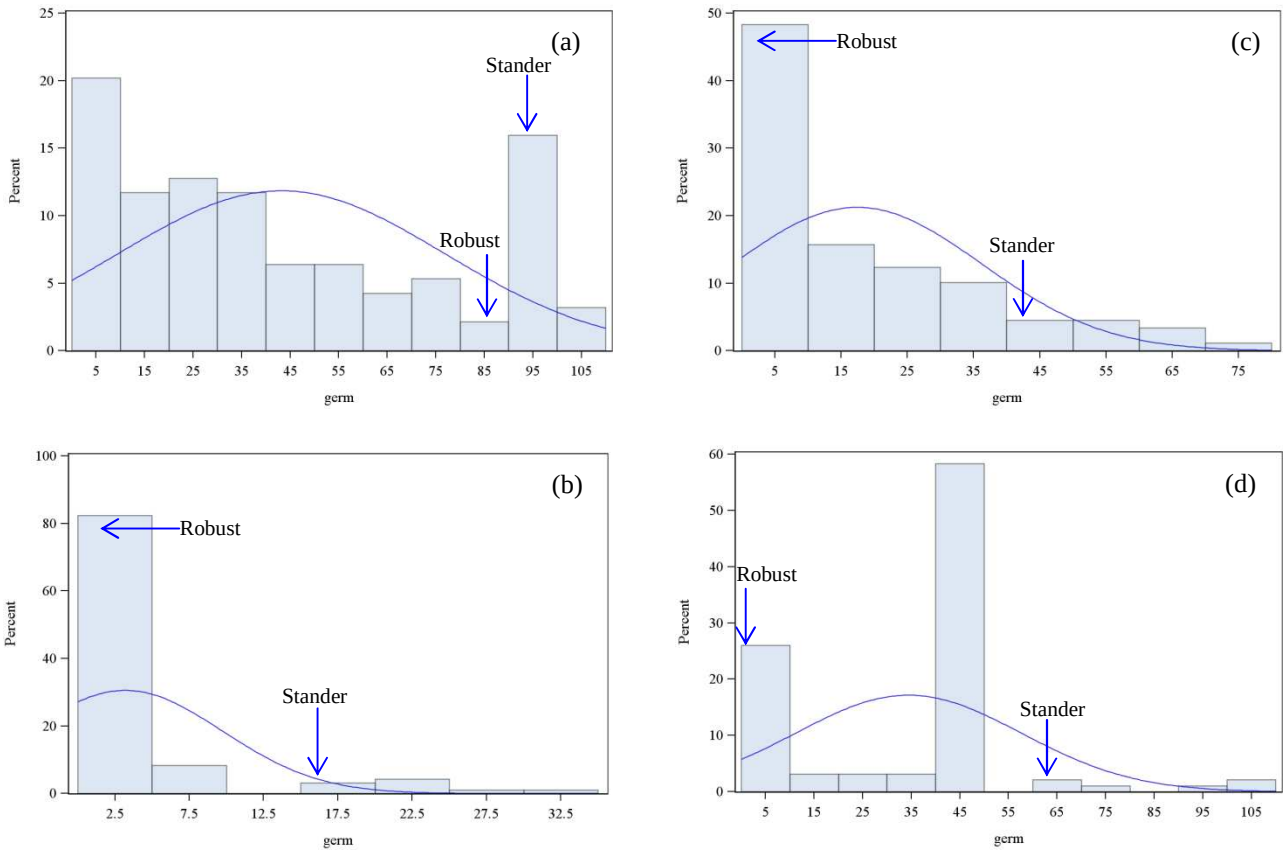

Figure A8. Phenotypic distribution of seed dormancy for the breeding materials from Washington State University (WA). (a) 2006, Robust mean=89; Stander=91; (b) 2007, Robust mean=1.96; Stander=16.85; (c) 2008, Robust mean=3.44; Stander=42.51; (d) 2009, Robust mean=0; Stander=61.28. The X-axis represents the percentage of germinated seeds, while the Y-axis represents the observed frequency for each of the intervals in X.

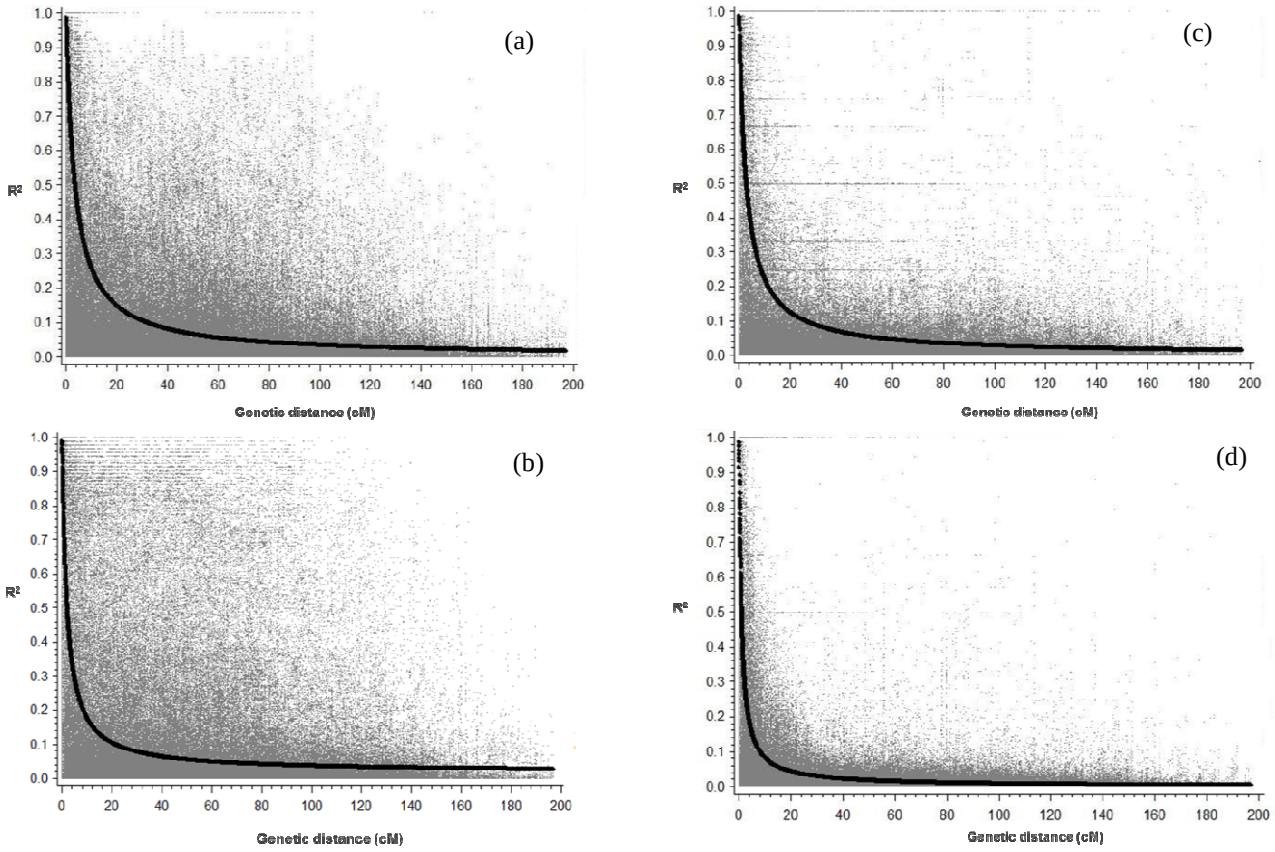

Figure A9. Genome-wide LD decay scatterplots for the analysis of each breeding program (four years): a) USDA-ARS-Aberdeen, ID (AB); b) Bush Ag. (BA); c) Montana State University (MT); d) North Dakota State two-row (ND2R). LD measured as R^2 between pairs of polymorphic loci (MAF>0.05) was plotted against the genetic distance (cM).

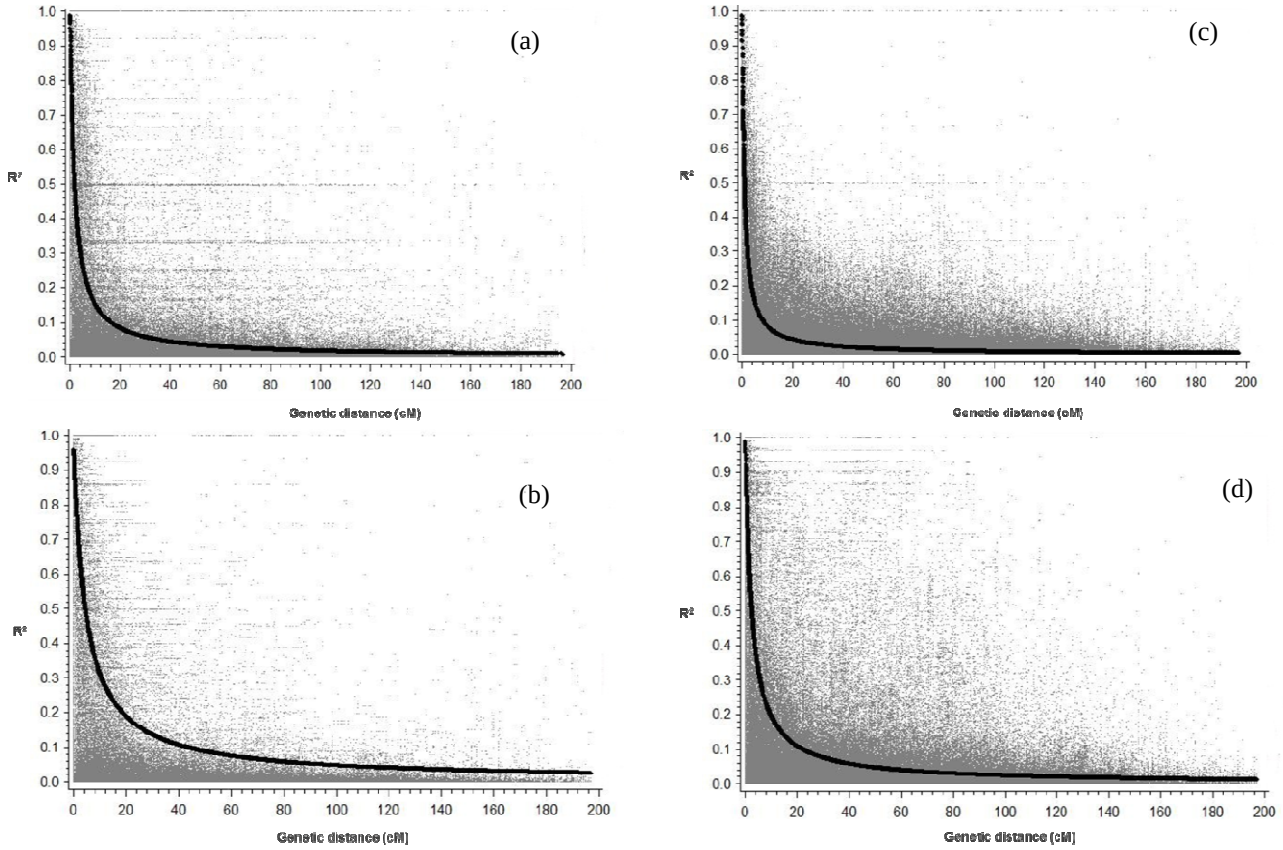

Figure A10. Genome-wide LD decay scatterplots for the analysis of each breeding program (four years): a) North Dakota State University six-row (ND6R); b) University of Minnesota (UM); c) Utah State (UT); d) Washington State (WA). LD measured as R^2 between pairs of polymorphic loci (MAF>0.05) was plotted against the genetic distance (cM).

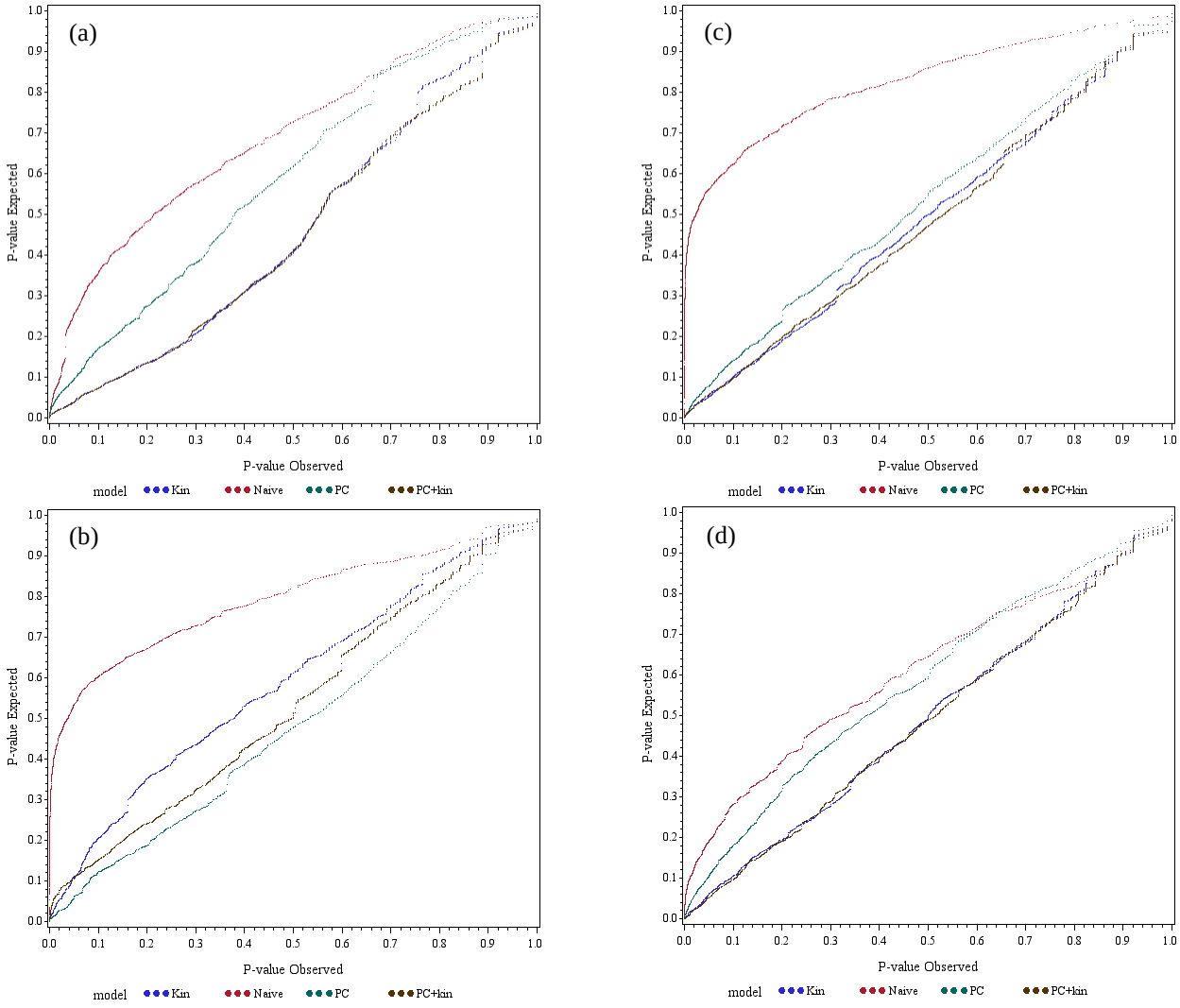

Figure A11. Comparison of four linear models for individual AM analysis of materials submitted by USDA-ARS-Aberdeen, ID (Aberdeen) across four years. Cumulative distribution of *P*-values was computed as follow: (a) 2006= 2,334 SNPs and 95 lines; (b) 2007: 2,359 and 96 lines; (c) 2008: 2,370 and 96 lines; (d) 2009= 2,330 and 96 lines.

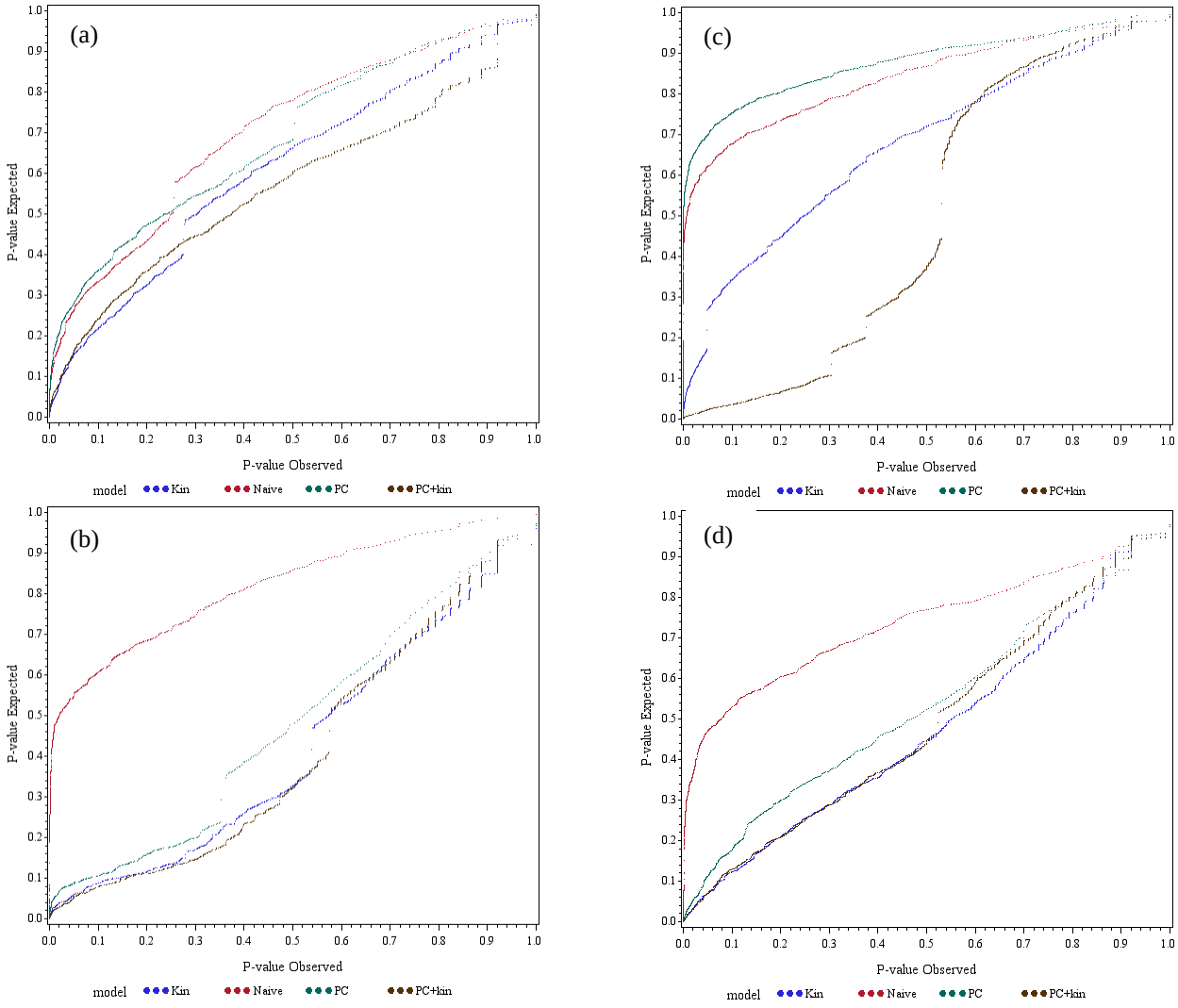

Figure A12. Comparison of four linear models for individual AM analysis of materials submitted by Bush Agricultural Resources LLC., across four years. Cumulative distribution of *P*-values was computed as follow: (a) 2006= 2,320 SNPs and 96 lines; (b) 2007: 2,162 and 96 lines; (c) 2008: 2,266 and 95 lines; (d) 2009= 2,314 and 96 lines.

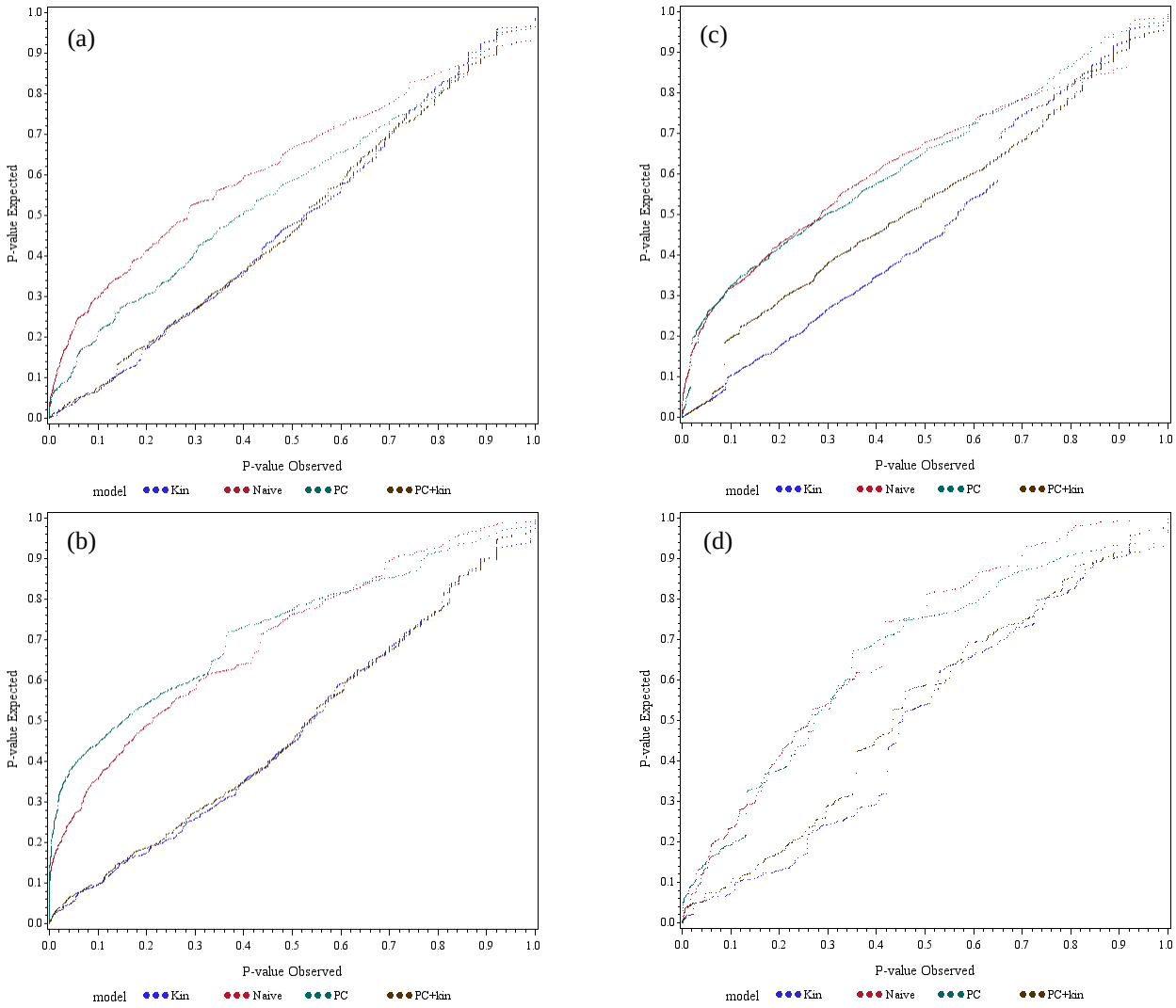

Figure A13. Comparison of four linear models for individual AM analysis of materials submitted by Montana State University across four years. Cumulative distribution of *P*-values was computed as follow: (a) 2006= 1,544 SNPs and 96 lines; (b) 2007: 1,689 and 96 lines; (c) 2008: 2,204 and 96 lines; (d) 2009= 1,441 and 96 lines.

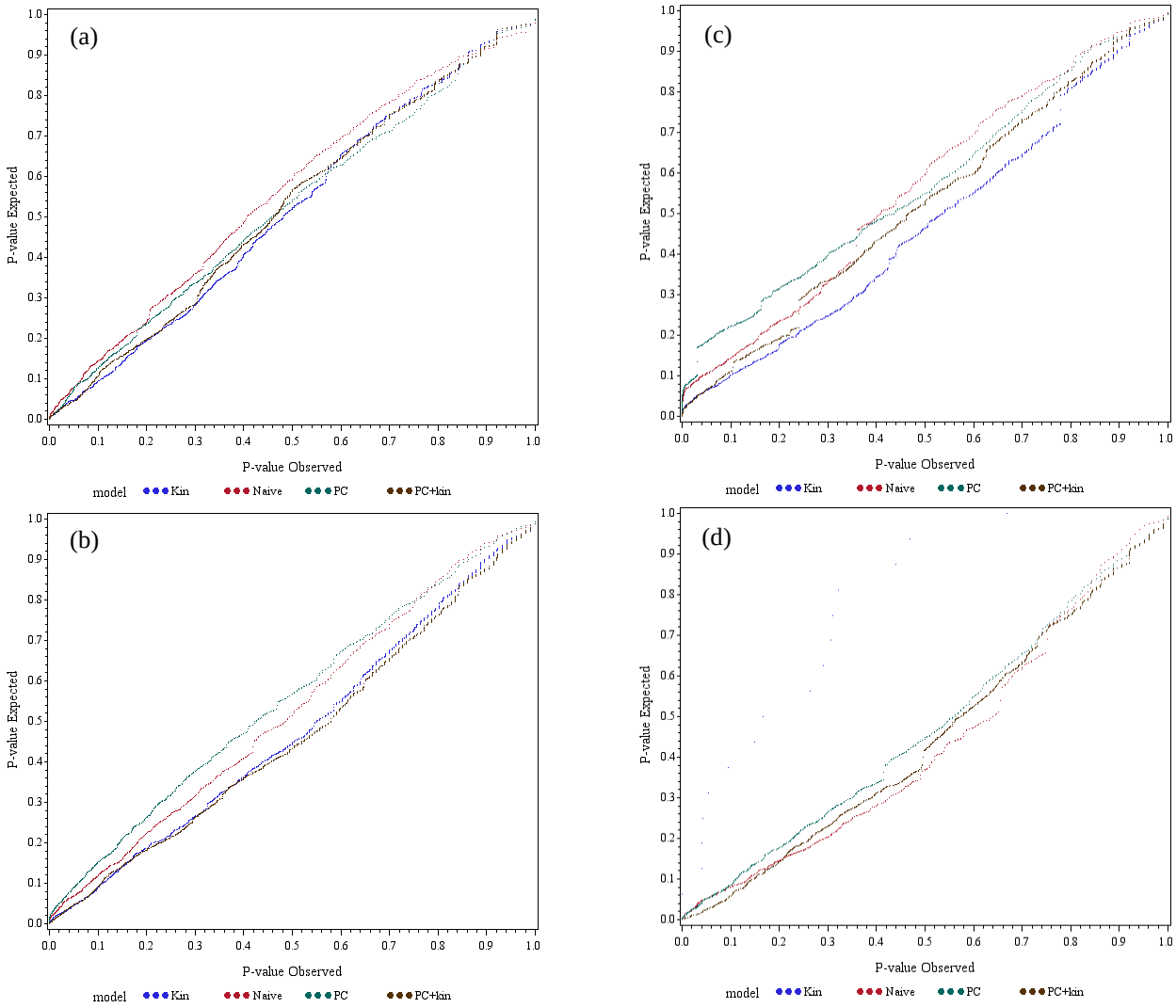

Figure A14. Comparison of four linear models for individual AM analysis of materials submitted by North Dakota State University (two-row) across four years. Cumulative distribution of *P*-values was computed as follow: (a) 2006= 2,278 SNPs and 96 lines; (b) 2007: 2,197 and 96 lines; (c) 2008: 2,050 and 96 lines; (d) 2009= 1,855 and 96 lines.

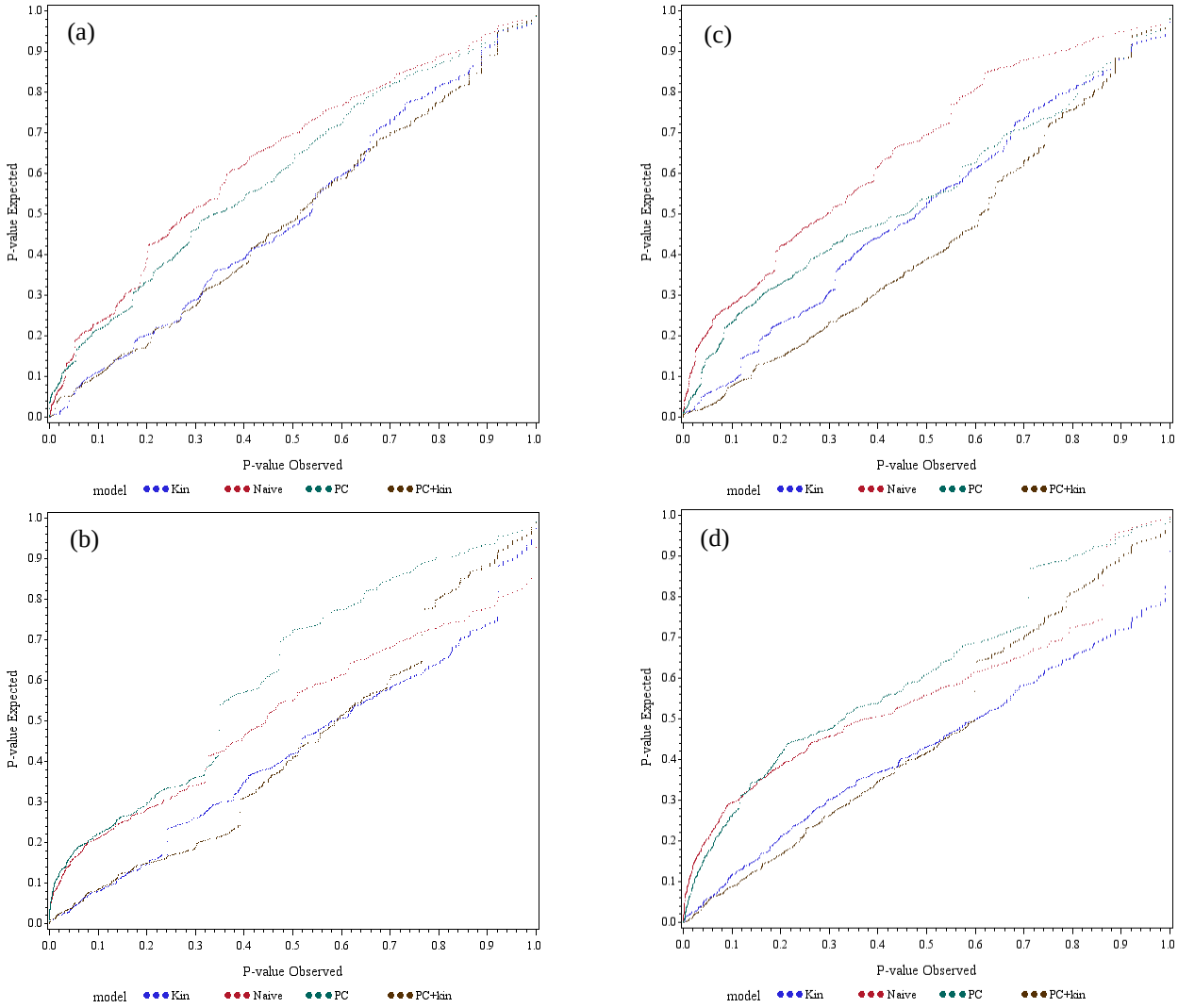

Figure A15. Comparison of four linear models for individual AM analysis of materials submitted by North Dakota State University (six-row) across four years. Cumulative distribution of *P*-values was computed as follow: (a) 2006= 1,335 SNPs and 96 lines; (b) 2007: 1,287 and 96 lines; (c) 2008: 1,736 and 96 lines; (d) 2009= 1,474 and 96 lines.

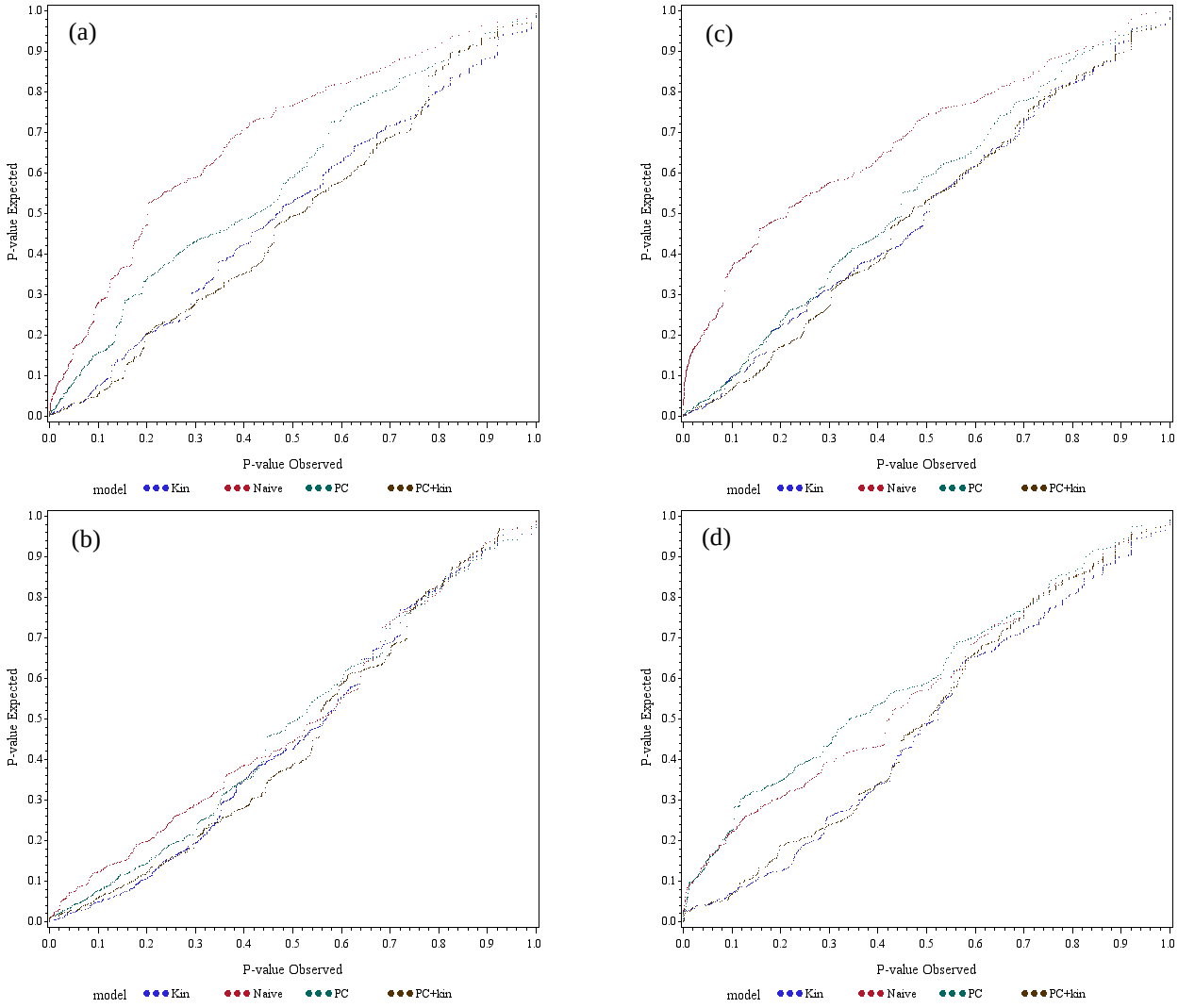

Figure A16. Comparison of four linear models for individual AM analysis of materials submitted by University of Minnesota across four years. Cumulative distribution of *P*-values was computed as follow: (a) 2006= 1,295 SNPs and 96 lines; (b) 2007: 1,539 and 96 lines; (c) 2008: 1,304 and 96 lines; (d) 2009= 1,186 and 96 lines.

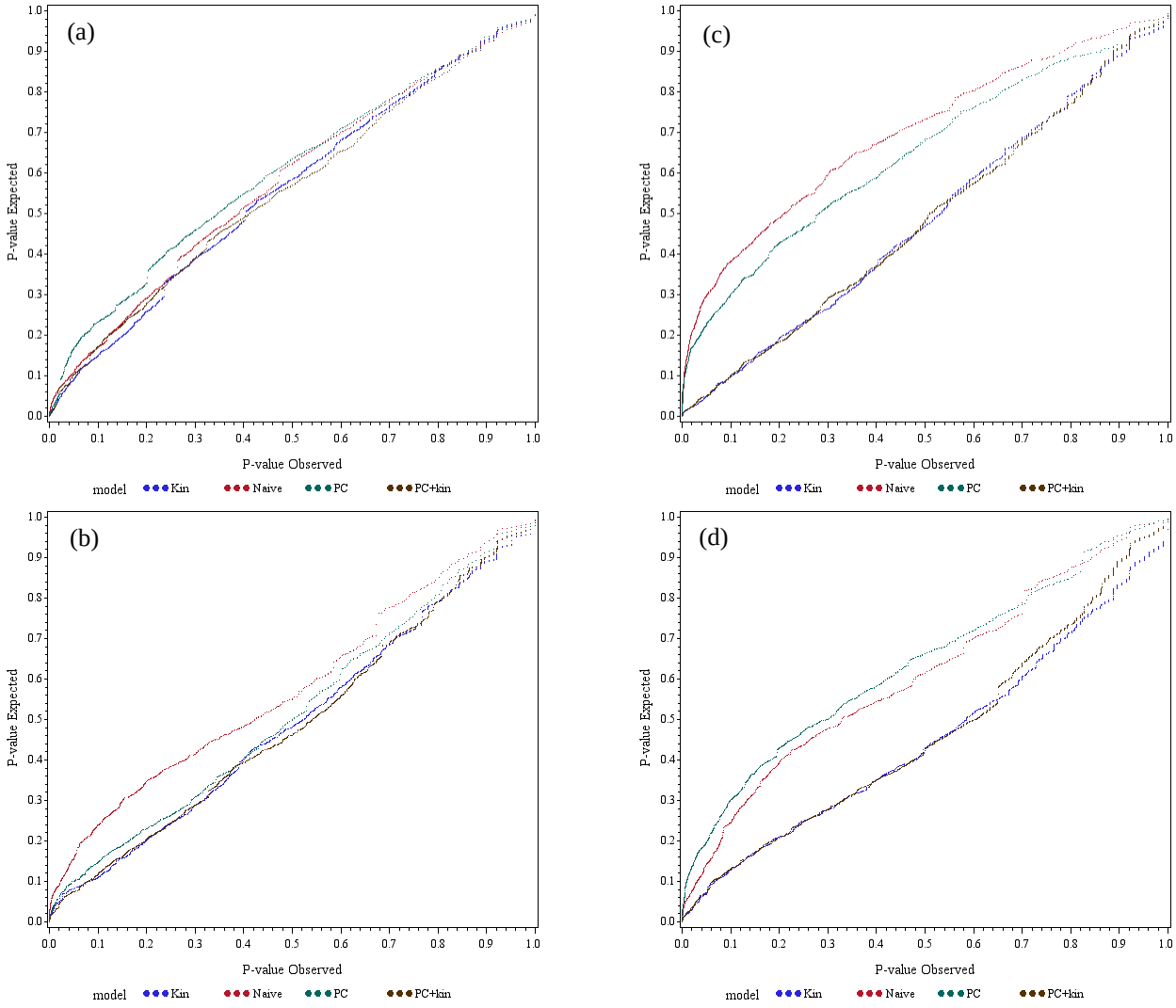

Figure A17. Comparison of four linear models for individual AM analysis of materials submitted by Utah State University across four years. Cumulative distribution of *P*-values was computed as follow: (a) 2006= 2,502 SNPs and 96 lines; (b) 2007: 2,195 and 96 lines; (c) 2008: 2,144 and 96 lines; (d) 2009= 2,115 and 96 lines.

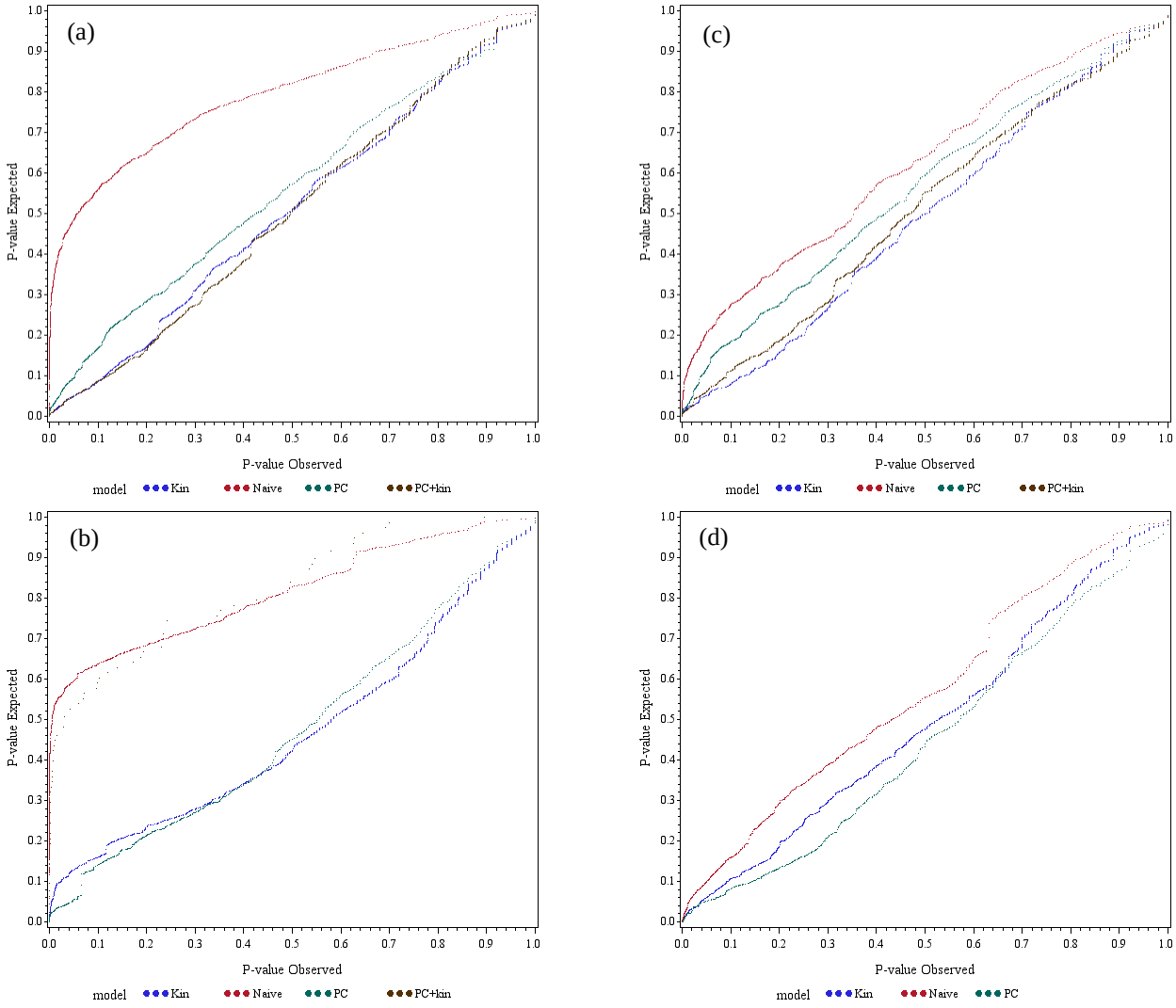

Figure A18. Comparison of four linear models for individual AM analysis of materials submitted by Washington State University across four years. Cumulative distribution of *P*-values was computed as follow: (a) 2006= 2,343 SNPs and 96 lines; (b) 2007: 2,341 and 96 lines; (c) 2008: 2,067 and 96 lines; (d) 2009= 1,979 and 96 lines.

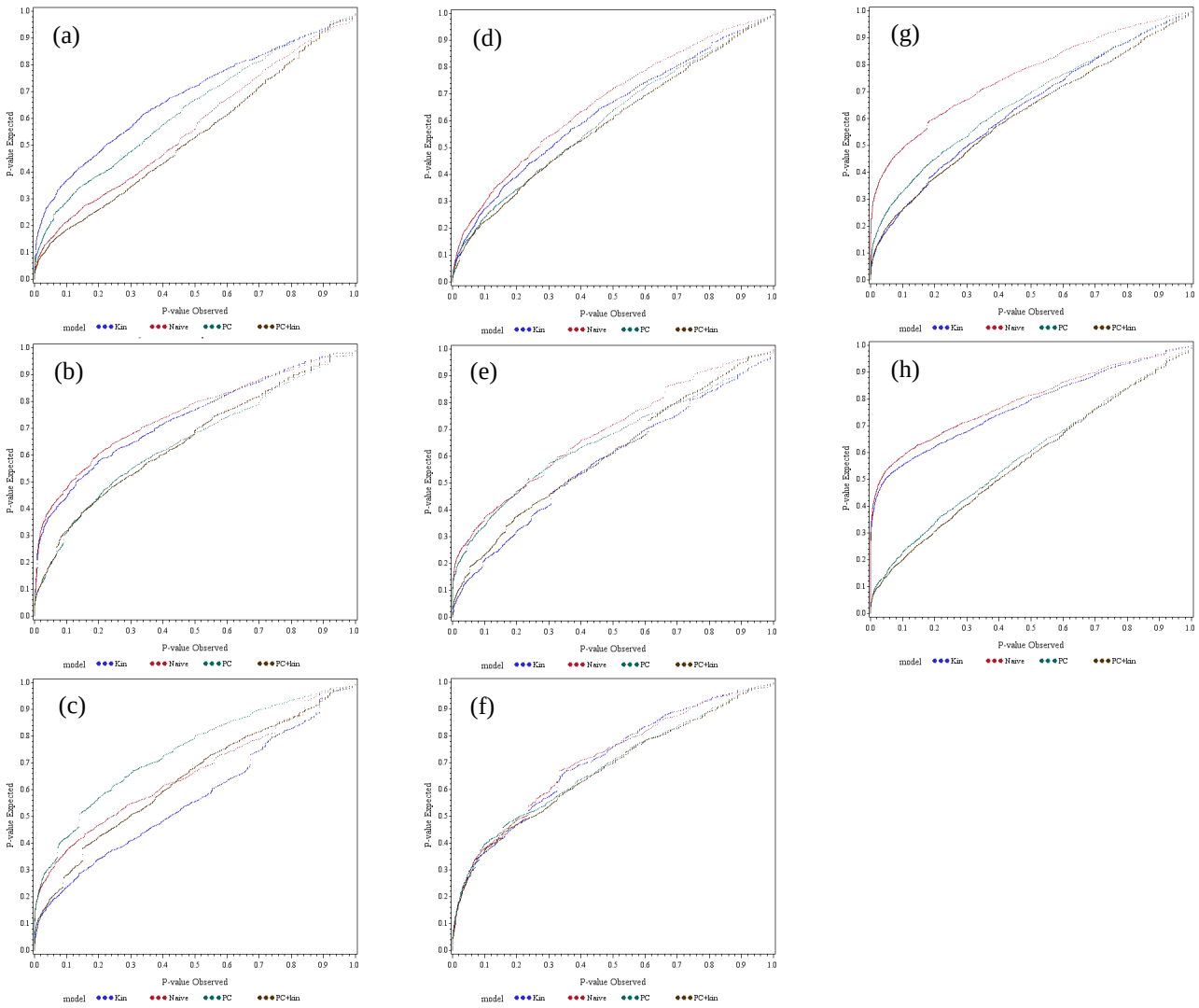

Figure A19. Comparison of four linear models from the combined analysis across four years for individual breeding programs. The cumulative distribution of *P*-values was computed for each individual breeding program and four years as follow: (a) AB= 2,556 SNP markers and 369 barley CAP lines; (b) BA= 2,428 SNP markers and 377 lines; (c) MT= 2,302 SNP markers and 362 lines; (d) ND2R= 2,481 SNP markers and 379 lines; (e) ND6R= 2,055 SNP markers and 367 lines; (f) UM= 1,853 SNP markers and 371 lines; (g) UT= 2,608 SNP markers and 365 lines; (h) WA= 2,532 SNP markers and 375 lines.

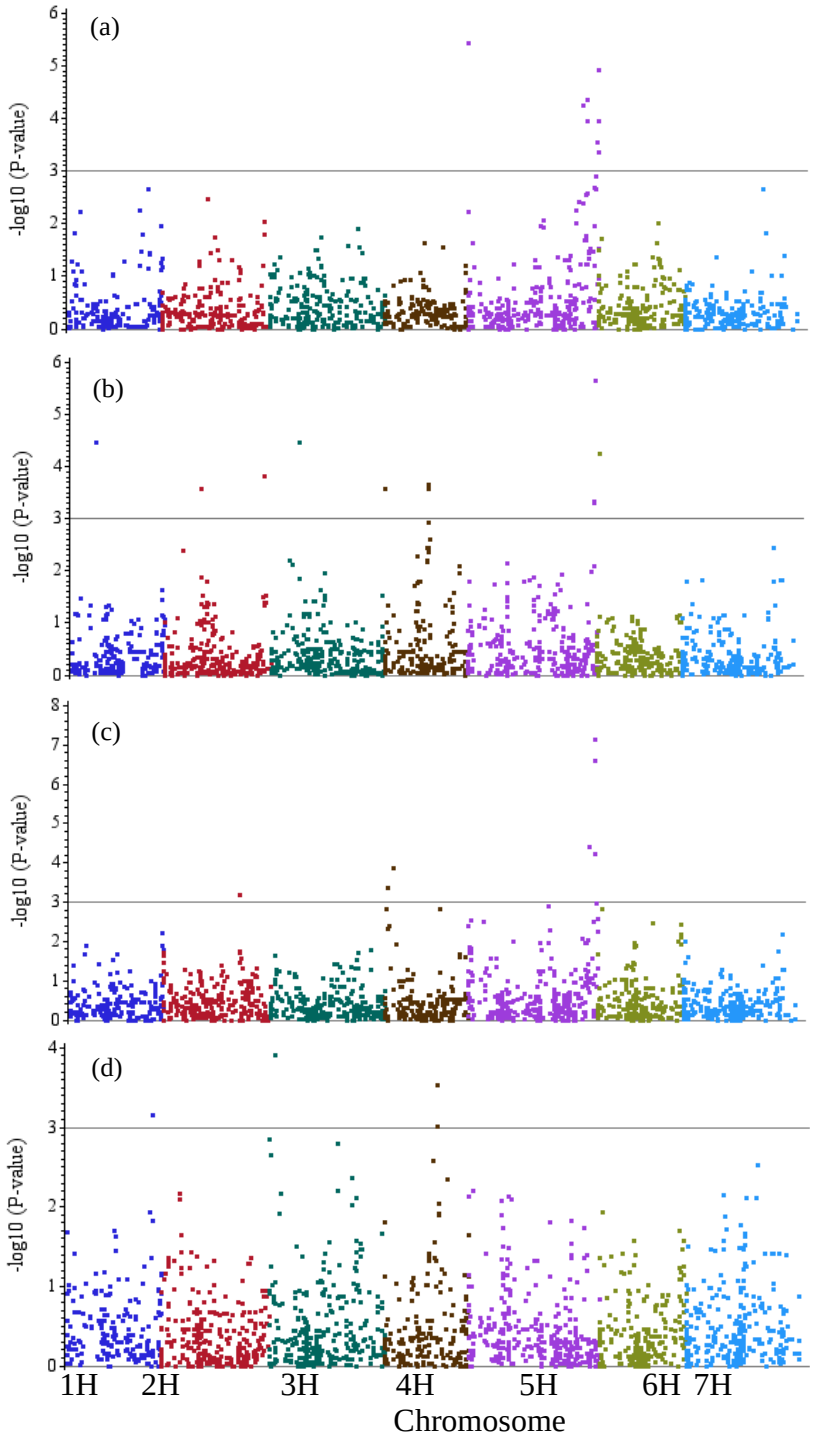

Figure A20. Genome-wide association results for seed dormancy on each of the four subpopulations submitted by USDA-ARS-Aberdeen, ID (AB). a) 2006, model=PK; b) 2007, model=P; c) 2008, model=K; d) 2009, model=K.

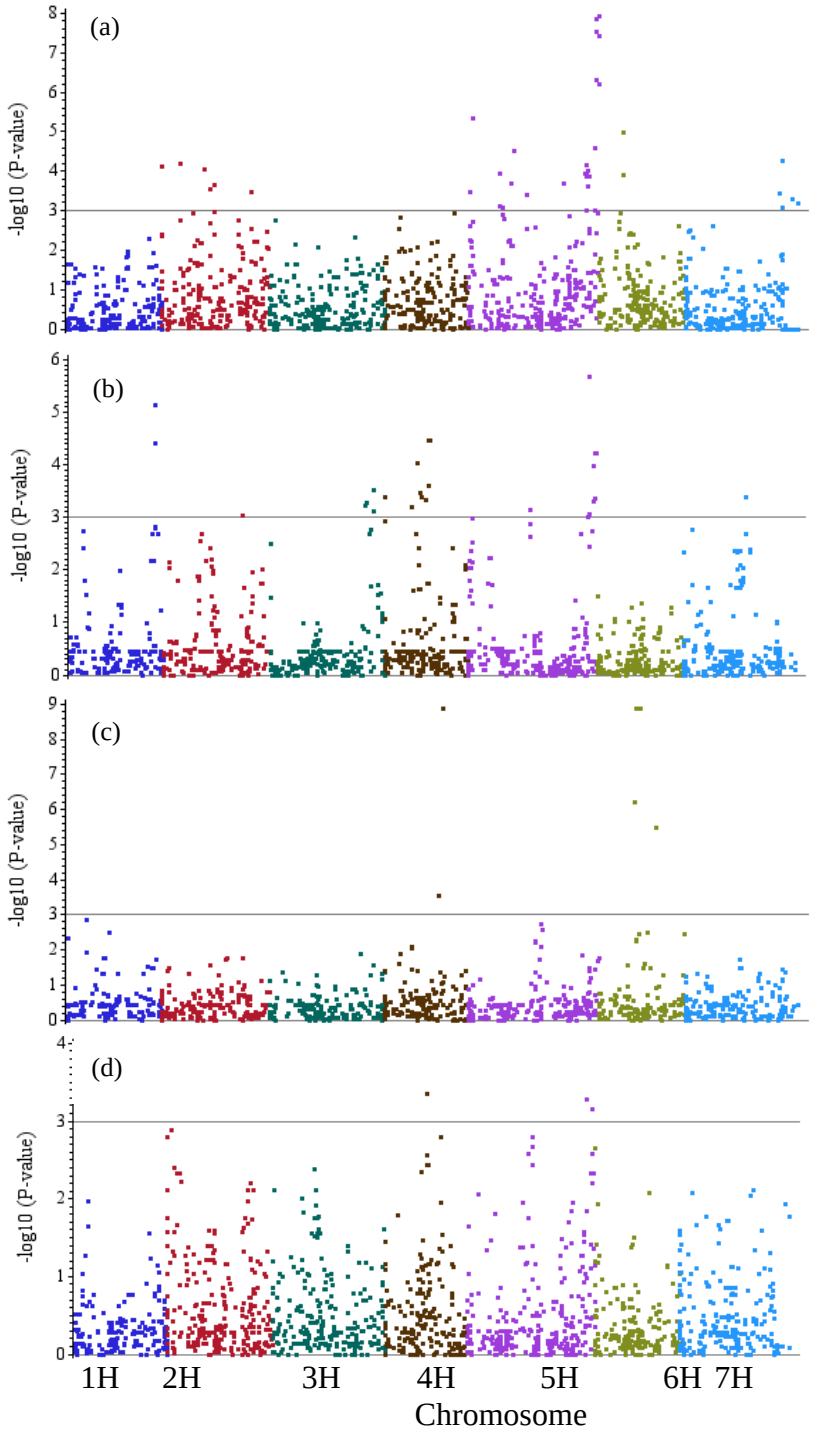

Figure A21. Genome-wide association results for seed dormancy on each of the four subpopulations submitted by Bush Agricultural Resources LLC (BA). a) 2006, model=PK; b) 2007, model=P; c) 2008, model=PK; d) 2009, model=PK.

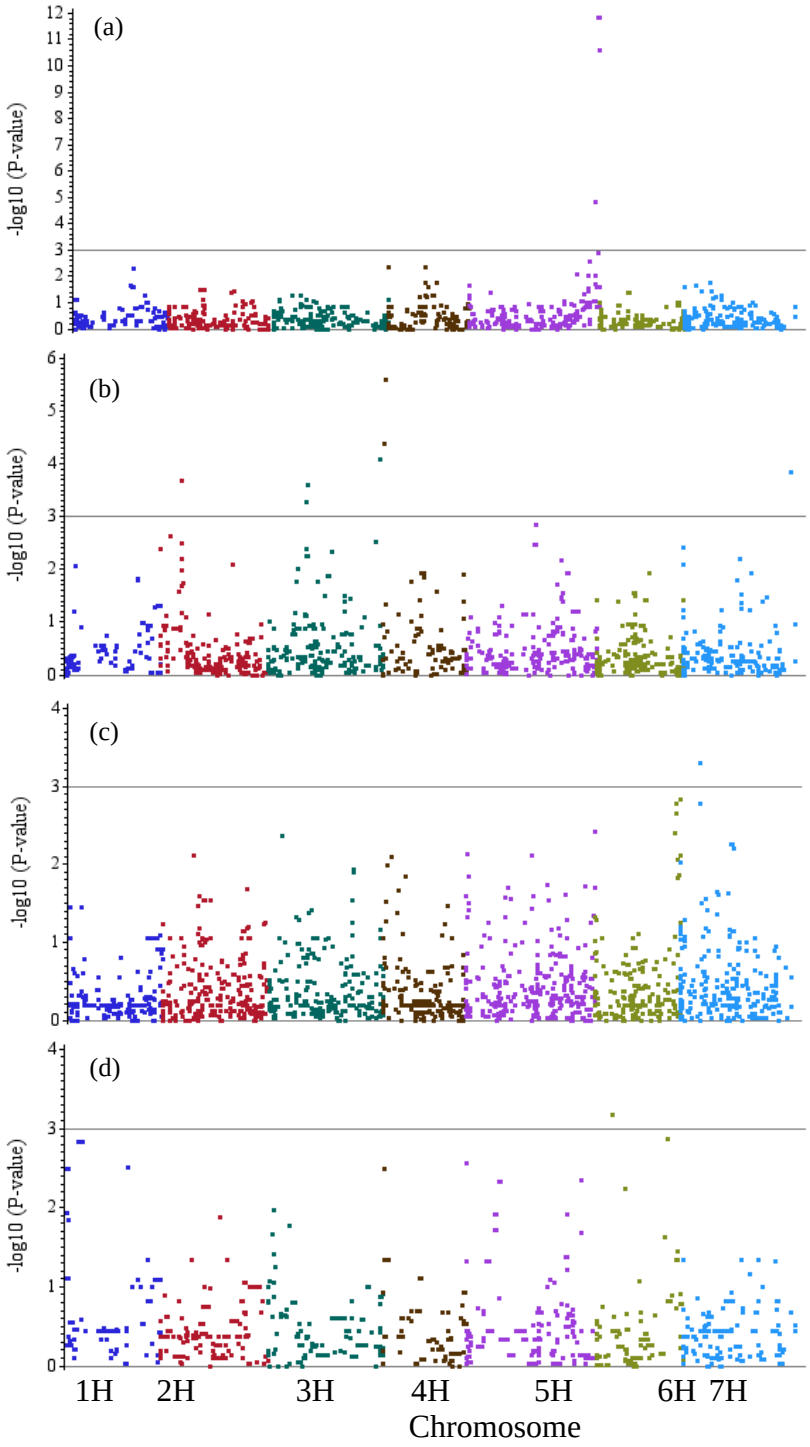

Figure A22. Genome-wide association results for seed dormancy on each of the four subpopulations submitted by Montana State University (MT). a) 2006, model=PK; b) 2007, model=PK; c) 2008, model=K; d) 2009, model=PK.

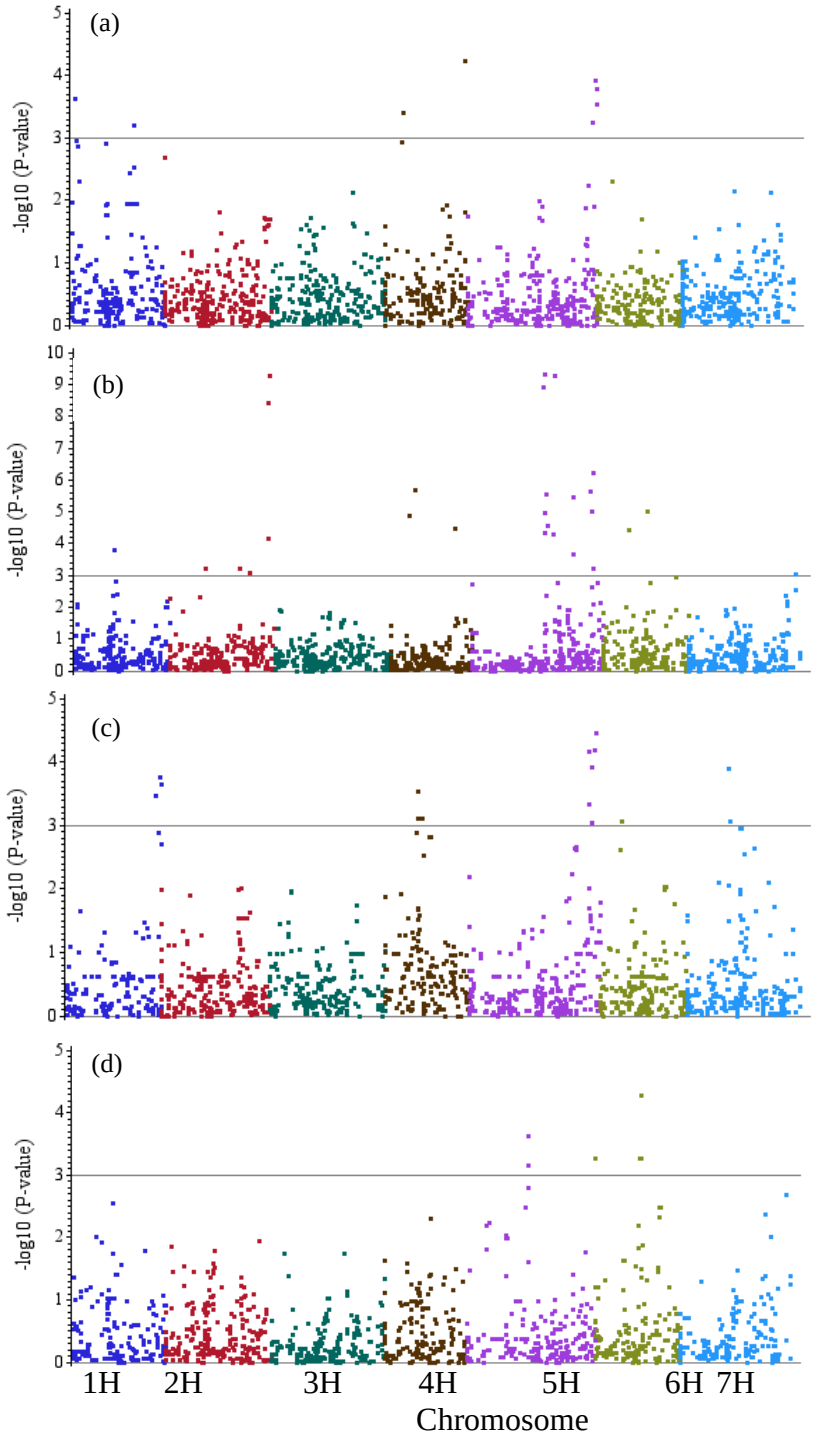

Figure A23. Genome-wide association results for seed dormancy on each of the four subpopulations submitted by North Dakota State University two-rowed (ND2R). a) 2006, model=K; b) 2007, model=Naïve; c) 2008, model=PK; d) 2009, model=P.

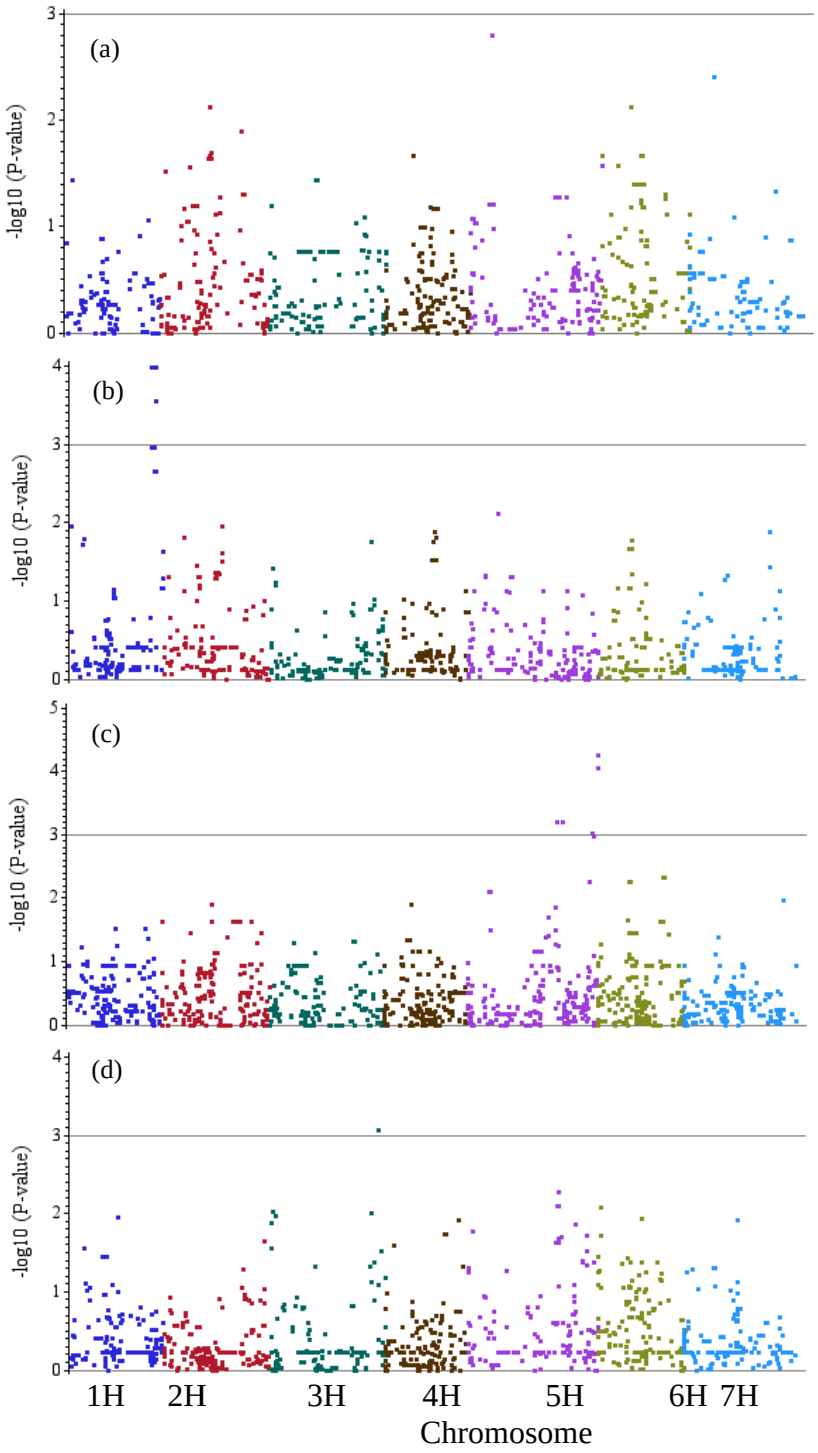

Figure A24. Genome-wide association results for seed dormancy on each of the four subpopulations submitted by North Dakota State University six-rowed (ND6R). a) 2006, model=K; b) 2007, model=PK; c) 2008, model=K; d) 2009, model=PK.

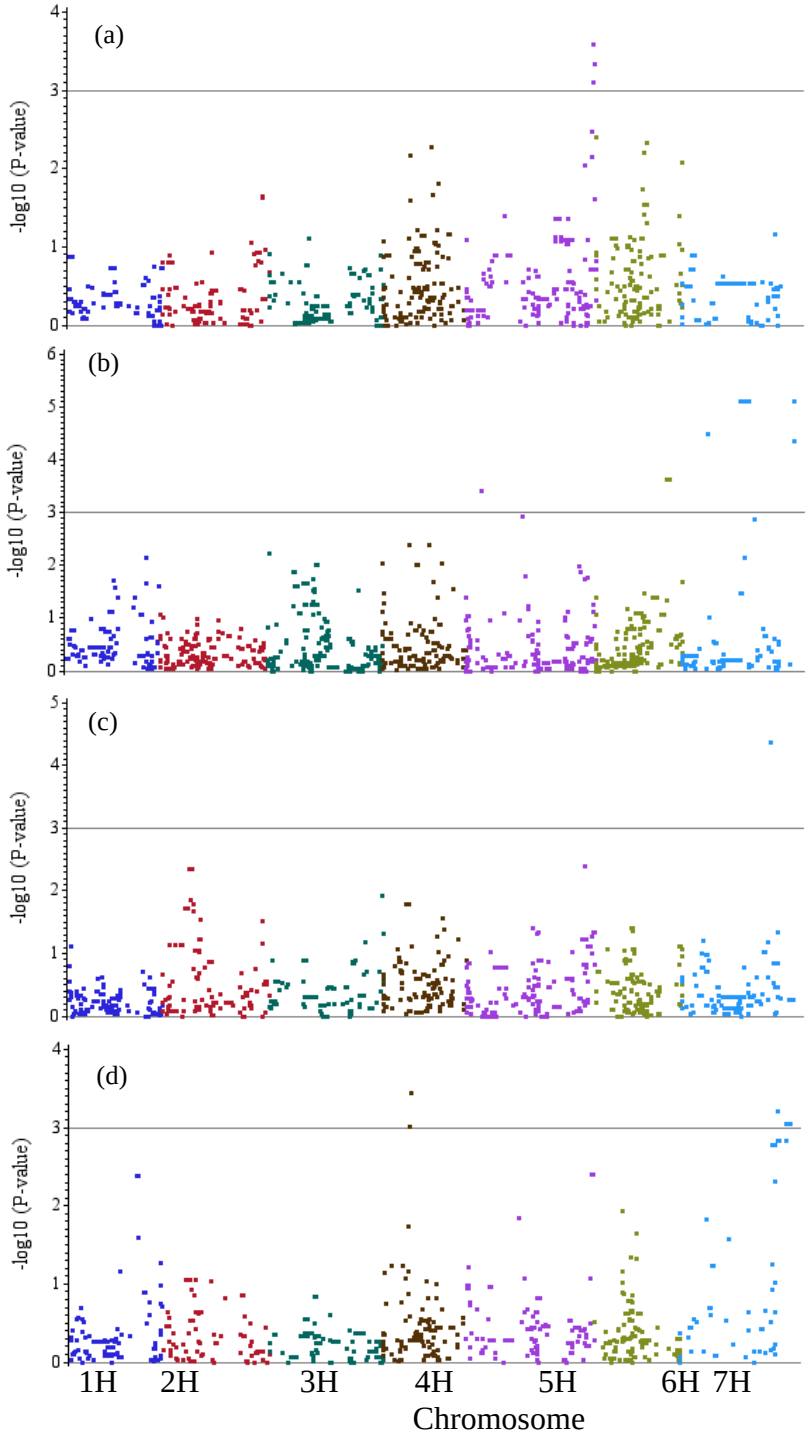

Figure A25. Genome-wide association results for seed dormancy on each of the four subpopulations submitted by University of Minnesota (UM). a) 2006, model=K; b) 2007, model=Naïve; c) 2008, model=K; d) 2009, model=K.

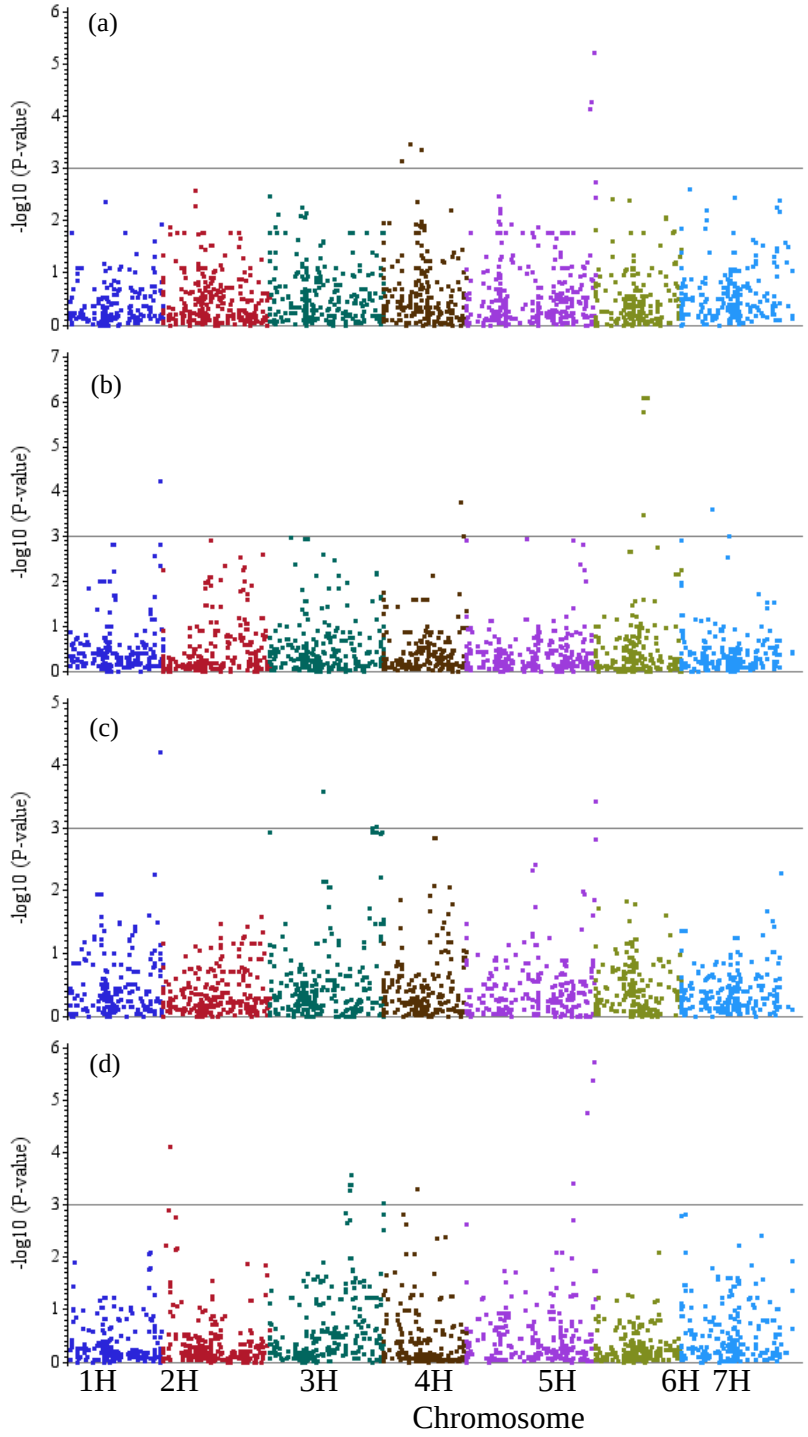

Figure A26. Genome-wide association results for seed dormancy on each of the four subpopulations submitted by Utah State University (UT). a) 2006, model=PK; b) 2007, model=K; c) 2008, model=K; d) 2009, model=PK.

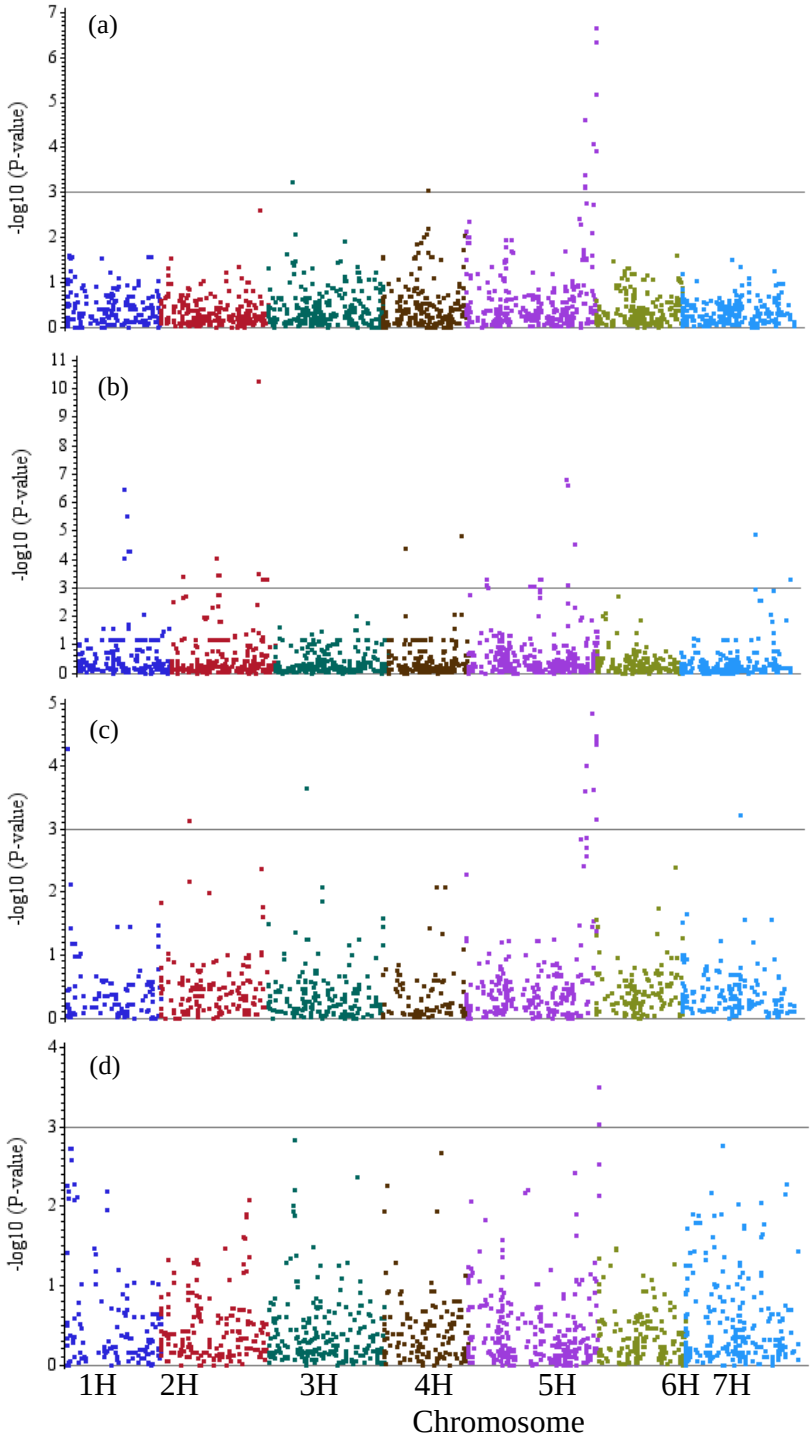

Figure A27. Genome-wide association results for seed dormancy on each of the four subpopulations submitted by Washington State University (WA). a) 2006, model=PK; b) 2007, model=P; c) 2008, model=K; d) 2009, model=K.