MAPPING QUANTITATIVE TRAIT LOCI ASSOCIATED WITH RESISTANCE TO PREHARVEST SPROUTING IN WHEAT

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ABSTRACT

Preharvest sprouting (PHS) is the precocious germination of the grains in the spike following physiological maturity. In wheat, the main problem associated with PHS is reduction in end-product quality. White wheats are the most susceptible class of wheat to PHS whereas the red wheats have high levels of resistance due to the pleiotropic effect of the red color genes with dormancy. However, recent studies reported that several sources of resistance to PHS are available in white wheat germplasm. Our objective was to map quantitative trait loci (QTL) associated with PHS resistance in a recombinant inbred population of 94 lines from a cross between Grandin*5/ND614-A, an elite hard white spring wheat susceptible to PHS and NY6432-18/Clark’s Cream 40-1, a soft white winter wheat selected for its high level of PHS resistance. Multiple interval mapping analysis revealed seven QTL for PHS based on the combined data across eight environments over three years (2005-2007). The QTL on chromosomes 2D, 5A and 7A had LOD score \( \geq 2.5 \) and were not associated with QTL for plant height and heading date. These three QTL jointly explained 39.4\% of the phenotypic variation for PHS. Other QTL were found on chromosomes 1B, 4B, 6A and 6D that explained 2.1\% to 14.2\% of the phenotypic variation. A significant QTL x QTL interaction was found between the chromosomal regions in 1B and 2D that explained 5.5\% of the phenotypic variation. The 4B and 6A QTL coincided with chromosomal regions associated with plant height. The 4B QTL explained only 5.2\% phenotypic variation for PHS, but 58\% of phenotypic variation for plant height. Conversely, the QTL on chromosome 6A explained...
the largest phenotypic variation for PHS (14.2%) of any individual QTL, but accounted for only 5% of the phenotypic variation for plant height. Overall, the results indicated the complexity of the genetic architecture of PHS and a strong genotype x environment interaction and trait correlations. Some of the QTL found in this study may aid in marker-assisted breeding for improvement of PHS resistance in wheat.
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CHAPTER I

REVIEW OF LITERATURE

To fully understand how preharvest sprouting operates on cereal grains such as wheat, it is important to explore the major seed components (embryo, endosperm and seed coat) and the physiological processes and mechanisms involved during seed formation and germination. This chapter describes the morphology, developmental phases and the nutrient and water influx during seed formation and growth. This chapter also focuses on the action and interaction of hormones, particularly abscisic acid (ABA) and gibberellins (GAs) and other regulatory and external factors involved in seed germination. Finally, gene/QTL mapping studies conducted on preharvest sprouting with emphasis on wheat are discussed.

Seed Structures

*Embryo*

The embryo is the part of the seed which gives rise to a plant. Different tissue structures are formed during embryo development in cereals. These include the embryo axis (radicle), scutellum and suspensor. Based on morphological criteria, the development of the embryo in cereals can be divided into several stages. The initial stage of embryo development is the proliferation of the terminal cells leading to the formation of the globular-shaped embryo which then elongates to a club-like shape showing radial symmetry (proembryo stage). This is followed by the coleoptile stage wherein the shape of the developing embryo becomes bilaterally-symmetrical through differentiation of the
scutellum and coleoptile. This stage also indicates the establishment of the embryo axis. This is followed by the leaf stage (third stage) where shoot and root meristems become visible. Seed storage products, such as oil bodies, are accumulated. Finally, at the maturation stage, the embryonic cells stop dividing. The embryo is fully developed and desiccates to become quiescent. Some cereal grains also develop embryo-imposed dormancy at seed maturity.

The length of time to complete embryogenesis varies among different species. For instance, under normal growing conditions a typical wheat embryo completes development to the final three leaf stage in more than 20 days after fertilization (Smart and O’Brien, 1983), whereas other cereals like a rice embryo requires only 12 days to reach an equivalent stage (Hong et al., 1995).

**Endosperm**

In cereals, the fully developed endosperm constitutes the major portion of the grain which consists mainly of four major cell types: the starchy endosperm, the aleurone layer, transfer cells and the cells of the embryo-surrounding region (ESR). The endosperm is basically the source for human food, animal feed and industrial raw materials. The grain filling period normally takes place 25-60 days after pollination (DAP). For example, in spring wheat, grain filling duration is between 25-30 DAP. During this period, the endosperm grows fast and is filled with starch granules and prolamin storage proteins. The cells of the starchy endosperm desiccate and die towards the end of the seed maturation period. In contrast, the cells of the aleurone layer are desiccation tolerant, and are alive in the mature, dry grain. These cells remain quiescent
until upon imbibition. Imbibition cause the embryo to produce the hormone gibberellin (GA), which diffuses into the endosperm. This hormonal activity induces the expression of glucanases, amylases and proteases that break down cell walls and starchy endosperm (Sun and Gubler, 2004). The mobilization of the degraded endosperm as a nutrient source contributes to embryo growth after germination. The degradation of the cereal endosperm has been studied intensively, especially the synthesis and secretion of amylases and proteases from the aleurone layer and regulation of their synthesis by gibberellins and abscisic acid (Gubler et al., 2005).

**Seedcoat**

Seedcoat development commences after fertilization. The inner and outer integuments of the ovule progress into seedcoat layers, hence, seedcoat is of maternal origin. In cereals, there appear to be no detailed studies on the development of the seedcoat by itself (Gong and Bewley, 2006). However, it is generally known that the seedcoat plays an important role in embryo nutrition during seed development. All sugars, amino acids, and mineral ions transported to the developing seeds are unloaded in the seedcoat (where phloem ends) and move apoplastically to the embryo and endosperm (Patrick and Offler, 2001). Several roles of the seedcoat in seed dormancy and germination have been demonstrated such as: the seedcoat (1) interferes with water uptake; (2) acts as a mechanical restraint to radicle protrusion; (3) interferes with gas exchange, particularly oxygen and carbon dioxide; (4) prevents inhibitor (hormone) leakage from the embryo; (5) supplies inhibitors to the embryo; and (6) is involved in light filtration (Bewley and Black, 1994). Studies have demonstrated that phenolic
compounds, particularly flavonoids, contribute to the above-mentioned germination-inhibiting effects of the seedcoat (Debeaujon et al., 2007).

**Seed Development**

Seed development pertains specifically to the growth and maturation of the seed including desiccation and acquisition of dormancy. It can be divided into three stages (Bewley and Black, 1994). In the first stage (Phase I), the embryonic organs and tissues are formed within the embryo, endosperm and surrounding seed as a result of extensive cell division. This phase is also known as a period of morphogenesis during which the body plan of the embryo is established (reviewed by Goldberg et al., 1994). The completion of this stage is manifested by a large increase of seed weight. After this, little cell division occurs. The second stage (Phase II) is concerned with the maturation of the seed, which includes essentially all of the events that occur after cell division has ceased within the embryo. In this stage, majority of the reserves (oil, starch and protein) within the embryo and storage tissues are synthesized. The synthesis comes primarily from sucrose and some amino acids and mineral nutrients supplied by the parent plant. Thus, the supply of carbon and nitrogen plays a major role in determining the rate and duration of seed growth and the final weight of a seed (Bewley et al., 2006). As reserves are deposited, water is replaced from the seed by these reserves; hence, the dry weight of the seed increases, and its proportional water content declines. During the third stage (Phase III), accumulation of storage reserves slows and stops at physiological maturity. Water loss continues after maturation until the seed reaches a harvestable moisture level (about 7-15%). Some respiration may continue during the early part of this stage but there is
probably no significant loss of dry matter. The disconnection of the seed from the parent plant (shriveling of the funiculus) follows and the seed becomes desiccation tolerant and quiescent, i.e. with undetectable metabolic activity. In this stage, some seeds may also have acquired dormancy during their development.

**Role of ABA and GA during Seed Development**

While the seed is accumulating its major storage reserves, changes also occur in its hormone content such as ABA and GAs. These regulatory hormones have been implicated to play a major role in seed development (Benech-Arnold et al., 2006; Feurtado and Kermode, 2007).

ABA has been isolated from immature seeds of many species and has been studied intensively (Steinbach et al., 1995; Kawakami et al., 1997). ABA is involved in the control of several processes that take place during the development of the seed including storage reserve deposition and acquisition of desiccation tolerance (reviewed by Nambara and Marion-Poll, 2003). ABA content of seeds is extremely low or undetectable early in development (until about 15 DAP) but as development proceeds, ABA content increases to reach a maximum of about one-third to one-half from seed initiation to maturity. ABA levels generally decline at seed maturity, with little carry-over into the dry seed. ABA can be detected in various parts of the seed, but its concentration is much higher in the embryo than elsewhere. It may result from both the synthesis in the embryo and translocation from the mother plant through the vascular supply in the seed coat. The content can be artificially lowered either genetically or by chemicals that interfere with any of the steps of its biosynthesis. For example, the chemical, fluridone, inhibits the
formation of the carotenoids that are precursors of ABA (reviewed by Bewley and Black, 1994). During the course of development, seeds change in their sensitivity to ABA. ABA sensitivity is very high during the early stages of seed development. In sorghum, for example, a very low concentration of between 0.5 and 1 µM ABA at its early stage of development is enough to allow the expression of genes that are associated with seed development. Sensitivity to ABA gradually decreases throughout seed development and considerably higher concentrations (5-50 µM ABA) are usually required to maintain the embryo in a developmental mode (Steinbach et al., 1995). Desiccation greatly reduces embryo responsiveness to ABA. Embryo sensitivity to ABA during seed development can also be modulated by the environment experienced by the mother plant (i.e. water or nutrient availability) (Steinbach et al., 1995).

GAs are involved in seed/embryo development as well as fruit growth and development (e.g. Hays et al., 2002; Koornneef et al., 2002; Singh et al., 2002). GA levels are usually high during embryo development, which is the first peak of its accumulation. When seeds approach maturity, most active GAs are deactivated (Yamaguchi et al., 2007). The second peak commences at the onset of germination just prior to radicle protrusion. A good correlation between the content of intermediate precursors of bioactive GAs, GA₉ and GA₂₀, and seed growth has been observed (Hedden and Kamiya, 1997). The concentration peaks for these two GAs coincide with the active growth phase of the seeds. Similarly, GA₁, a major component at the early stages of embryo development has been shown to play a role in embryo growth (Hays et al., 2002). GAs may be distributed unequally in the seed. It may be that GAs are transported around
the seed and may be subjected to differential metabolism at various sites during development.

**Nutrient and Water Import**

Patrick and Offler (2001) illustrated how nutrient and water are transported during the development of wheat seed. This initiates via a single crease vein running through the length of the seed in the maternal seedcoat and nucellus. Beneath the crease vein, the nucellus extends into the endosperm cavity that forms between the maternal and enclosed embryonic and endosperm tissues prior to seed fill. A pigment strand separates the apoplasm of the maternal seedcoat and nucellus from the aleurone layer and starchy endosperm tissues and extends between the crease vein and nucellar projection. This pigment strand regulates the exchange of nutrients from maternal to filial tissues through the endosperm cavity.

**Seed Germination**

Seed germination follows seed development when the germination conditions required by the matured seed are met. Germination is strictly defined as those events that start with the uptake of water by the quiescent dry seed (imbibition) and the emergence of the embryonic axis (the radicle) through its surrounding structures (Bewley and Black, 1994; Bewley, 1997). Generally, germination is complete when the radicle can be seen visibly penetrating the seedcoat. Protrusion of the radicle is a turgor-driven process that requires yielding of cell walls of the embryonic root axis located between the root cap and the base of the hypocotyl (Consgrove, 1997). Subsequent events including the
mobilization of reserves are associated with growth of the seedling. In most cases, all of the cellular and metabolic events are similar between the imbibed dormant seeds and non-dormant seeds before completion of germination. That being said, a dormant seed may experience all of the metabolic steps required to complete germination, however, it is not known why the radicle fails to elongate.

Germination, generally, consists of three major phases. Imbibition (Phase I) starts with the uptake of water into the cells of dry seeds. This results in temporary structural changes in the membranes, which lead to an immediate and rapid leakage of solutes and low molecular weight metabolites into the surrounding imbibition solution. This is followed by a plateau phase (Phase II) when the seed has swollen to maximum size (can also be referred to as germination in a strict sense). The third phase (Phase III) occurs only after germination is completed which culminates in the formation of young seedling (Bewley, 1997). Dormant seed, thus, can not enter phase III since they do not complete germination. Seed dormancy is normally acquired during seed maturation and maintained to seed maturity. This is exhibited by the failure of an intact viable seed to complete germination under favorable conditions. Dormancy is affected by several environmental factors, such as light, temperature, water and the duration of seed storage (after ripening) (Bewley, 1997; Koornneef et al., 2002).

**Influences of ABA and GA on Seed Germination**

Abscisic acid (ABA) is a strong germination inhibitor (Koornneef et al., 2002). It is generally known to be the primary moderator of seed dormancy. Gubler et al. (2005) reviewed the role of ABA metabolism in dormancy release. The authors reported that
after-ripening, stratification and other dormancy releasing mechanisms that promote germination cause a rapid decline in the ABA content of imbibed dormant seeds.

GAs appear not to be involved in the control of dormancy per se but rather important in the promotion and maintenance of germination, that is, they act after the ABA-mediated inhibition of germination has been overcome (reviewed by Bewley, 1997). GA can often reverse the inhibitory effect of ABA. Studies have also revealed that changes in GA and ABA concentrations might be causally linked (Jacobsen et al., 2002; Ogawa et al., 2003). It appears that the accumulation of active GA in the embryos occur only after the level of ABA had decreased. There is strong evidence that GA plays a role in germination by promoting the growth potential of the embryo and by mediating the weakening of tissues that surround the embryo to overcome the mechanical restraint conferred by the seed-covering layers (Bewley, 1997; Koornneef et al., 2002). GA₃ (gibberellic acid), GA₄ and GA₇ have been found to have a very wide spectrum of stimulatory action as shown in numerous plant species (Feurtado and Kermode, 2007). However, their action depends on other external factors, including temperature, oxygen and light, and their interaction among themselves.

In maize, White and Rivin (2000) and White et al. (2000) showed that the GA/ABA ratio, and not the absolute hormone amounts, appears to control vivipary. This led to the conclusion that GA may directly antagonize ABA signaling during maize kernel development.
Regulators of ABA and GA

Some growth regulators have been recorded as stimulators or inhibitors of seed germination by regulating the effect of ABA or GA. These are ethylene, cytokinin, indole acetic acid (IAA) and brassinosteroids (reviewed by Bewley and Black, 1994; Kucera et al., 2005).

Several studies of mutants in Arabidopsis indicated that ethylene may suppress seed dormancy by inhibiting ABA action (Beaudoin et al., 2000; Ghassemian et al., 2000). Beaudoin et al. (2000) suggested that ethylene suppresses seed dormancy by inhibiting ABA action. Also, ethylene influences the sensitivity of the seed to ABA. A key conclusion is that ethylene can promote germination by directly interfering with ABA signaling. However, a study on the exogenous application of ethylene showed that ethylene alone is not sufficient to release seed dormancy (Kepczynski and Kepczynska, 1997; Matilla, 2000). Cytokinin, on the other hand, appears to contribute to the promotion of release from dormancy and subsequent germination by enhancing ethylene biosynthesis (Matilla, 2000). Auxin (IAA) may also play a role in regulating ABA. A relationship between IAA, dormancy and preharvest sprouting of wheat has been reported (Ramaih et al., 2003). IAA and its precursor tryptophan and indole-acetaldehyde inhibited germination of excised embryos from a dormant wheat cultivar, but not from a non-dormant one. In addition, embryos from a dormant cultivar gradually lose sensitivity to IAA throughout maturation period. From these results, Ramaih et al. (2003) suggested that IAA (Auxin) might complement the action of ABA by regulating dormancy but not germination.
DELLA proteins are negative regulators of GA signaling. The first DELLA genes cloned were *Arabidopsis GAI* and *RGA*. *GAI* was originally identified by Koornneef et al. (1985) as a gain-of-function mutation (*gai-1*) causing a GA-insensitive dwarf phenotype similar to the ‘green revolution’ genes of wheat (shown to inhibit stem elongation) (Peng et al., 1999). However, it is not known whether these DELLA proteins function similarly in seed germination (Steber, 2007). Research involving *Arabidopsis* brassinosteroid (BR) mutants *de-etiolated2* (*det2*) and *brassinosteroid insensitive1* (*bri1*) showed that brassinosteroids are not absolutely required for germination (Steber and McCourt, 2001). However, brassinosteroids interact with GA (in the presence of light) in regulating elongation of shoots and photomorphogenesis of seedling. Kucera et al. (2005) noted a complex interaction between GA and BR in regulating seed germination. It is clear that GA and BR act in parallel to promote cell elongation and germination and to counteract the inhibitory action of ABA.

**External Factors Affecting Germination**

Temperature affects germination of seeds itself or by affecting dormancy and viability (Bennett, 2004). The temperature range over which germination is possible is species specific. This is a highly important factor because it has a strong influence on all biochemical reactions as well as regulating flux of oxygen reaching the embryo through the seedcoat.

Light can promote or inhibit germination (reviewed by Bewley and Black, 1994). Some species are incapable of germinating in darkness, or do so only at a low frequency. The photoreceptor responsible for most types of light-sensitivity is phytochrome which
exists in two photoreversible forms, the re-absorbing Pr (inactive form) and the far-red-absorbing Pfr (active form). Pfr is the form that promotes germination. Seeds of species that can germinate in the dark also require Pfr. But in these cases, the Pfr requirement for germination has taken place during seed development and the mature seed relies on this earlier exposure. A mechanism postulated for Pfr to enhance embryo growth potential and to weaken tissues enclosing the radicle tip is through the promotion of active gibberellin (GA$_1$). There is also evidence that sensitivity to GA is provoked by Pfr. Furthermore, in lettuce, there is some evidence that abscisic acid (ABA) content is reduced by Pfr.

The amount of oxygen is also an important factor that may influence germination. Oil storing seeds (lettuce, sunflower, radish, turnip, cabbage, flax, and soybean) usually require more oxygen for completion of germination than starchy seeds (rice, wheat, maize, sorghum, and pea) (Corbineau and Côme, 1995).

Smoke is another factor that may influence germination. A study that first demonstrated that smoke from burning plants stimulates germination was done on dormant seeds of *Audouinia capitata* (de Lange and Boucher, 1990). It was suggested that nitrogen oxides and butenolides produced during this process have very potent germination-enhancing action on the seed of this species.

**Preharvest Sprouting**

Preharvest sprouting (PHS) or precocious germination of seeds is a result of heavy selection against dormancy in order to attain uniform germination for plants grown as crops and permits the utilization of crops for industrial purposes (e.g. malting). This
selection pressure combined with rainy or damp conditions prevailing during the late stages of maturation may lead to germination of the seed on the mother plant. It is important to note that preharvest sprouting is distinct from vivipary, which is the germination of developing seed while still on the mother plant prior to seed maturation. Although dormancy is established very early during development in cereals, PHS is still predominantly a feature of cereal crops. The mechanism of seedcoat (testa plus pericarp plus glumes)-imposed dormancy prevents precocious germination, the duration of which depends on the genotype and on the growing environment experienced by the mother plant during seed maturation process. Nevertheless, sprouting usually occurs on those susceptible cultivars whose coat-imposed dormancy is terminated before harvest maturity (Benech-Arnold et al., 2006). Embryos are capable of germination even from early stages (i.e. 15-20 DAP, days after pollination) when water is imbibed (Paulsen and Auld, 2004). Thus, sprouting can occur if damp conditions prevail during maturation, sometimes even regardless of the level of coat-imposed dormancy.

**Mechanisms involved in PHS**

The rate of water uptake by kernels is one factor that contributes to preharvest sprouting. A high rate of water uptake or, conversely, the possibility of long exposure to moistened conditions, might add to sprouting susceptibility. The morphology of the inflorescence and characteristics of the seedcoat influence the absorption of moisture. In wheat and barley, imbibition is increased by features associated with awns and is affected by waxiness, pubescence, and angle of the inflorescence (King and Wettstein-Knowles, 2000; Benech-Arnold, 2002). Also implicated in controlling the rate of water absorption
are grain hardness, colour, restriction by the seedcoat, thickness of the testa, grain size and surface-to-volume ratio of the grain (Paulsen and Auld, 2004).

The content of and sensitivity to ABA in the embryo are also the primary cause for preharvest sprouting susceptibility (Bewley, 1997; Gubler, 2005). Support for the hypothesis that the absence of, or insensitivity to, ABA during seed development results in the isolation of viviparous mutants that are deficient in ABA content or responsiveness. These include maize *viviparous* (*VP*), tomato *sitiens* (*sit*) and *Arabidopsis ABA-deficient* (*aba*) and *ABA-insensitive* (*abi*) mutants (reviewed by Koornneef et al., 2002). Sensitivity to ABA during development can also be modulated by the environment experienced by the mother plant (i.e. temperature, water or nutrient availability) (Steinbach et al., 1995; Garello and LePage-Degivry, 1999). Although, it should be noted that endogenous ABA, which is required continuously, and exogenous ABA, which is applied only temporarily, may have different modes of preventing sprouting or germination (LePage-Degivry and Garello, 1992).

Temperature during seed development is one of the main factors that appear to be responsible for year-to-year variation in grain dormancy of a particular genotype. Positive relationships between temperature experienced during development and extent of release from dormancy have been established for some cereals (i.e. the higher the temperature during grain filling, the higher the level of dormancy). A study conducted about the effect of drought and high temperature in wheat supports this finding (Biddulph et al., 2005). Other studies have shown that low temperatures can affect ABA content and sensitivity, that in turn influence the degree of dormancy during development and in the mature grain (Walker-Simmons, 1988; Garello and LePage-Degivry, 1999). Exceptions are for seeds
that show deep dormancy and consequently do not germinate at any temperature (it is also a common feature for some cereals that seed dormancy is expressed at certain temperatures and not others). For example, in winter wheat and barley, dormancy is not expressed at low temperatures (i.e. 10°C or lower) while in summer cereals like sorghum, at high temperature, dormancy is not expressed (Paulsen and Auld, 2004). This lack of expression of dormancy at, for example, high temperatures in grains from summer cereals, implies that in years when damp conditions are combined with high air temperatures around harvest time, both resistant (high dormancy) and susceptible (low dormancy) cultivars might be expected to sprout.

**Genes and QTL involved in PHS resistance in wheat**

Preharvest sprouting genes

Wheat grain dormancy is affected both by the pleiotropic effect of *R* (*Red grain color*) genes conferring red pericarp color and by genes such as *PHS*, which has a major effect on the embryo. Therefore, wheat PHS is regulated both by coat-imposed and embryonic pathways controlled by separate genetic systems (Flintham, 2000; Himi, et al., 2002; Mares, et al., 2002). Dominant alleles of *R* genes (1 each on chromosomes 3A, 3B, and 3D) that promote the biosynthesis of red phlobaphenes have a pleiotropic effect on dormancy. Recently, the wheat *R* gene was cloned and shown to encode a MYB-type transcription factor (Himi and Noda, 2005). The red kernel wheats tend to have higher levels of PHS resistance than the white wheats although some studies have shown that sources of resistance to PHS can be acquired in white wheat germplasm (Anderson et al., 1993; Mares et. al., 2005).
Comparative genetics and mapping have been used to identify candidate loci that play an important role in preharvest sprouting in wheat (Flintham et al., 2002). Orthologues of \textit{Vp1} in maize, for example, have been identified in several cereal crops like wheat and rice (Bailey et al., 1999). Also, some studies suggested a relationship between the maize \textit{Viviparous1 (Vp1)} gene and seed dormancy (Gale et al., 2002; Li et al., 2004). In another use of comparative mapping, it was suggested that the \textit{PHS} gene in wheat is orthologous with the barley seed dormancy QTL, \textit{Seed Dormancy4 (SD-4)} (Kato et al, 2001).

Preharvest sprouting QTL

QTL mapping of PHS resistance has been extensively done in wheat throughout the major wheat producing regions in the world. Anderson et al. (1993), was the first to report QTL associated with PHS resistance in wheat. They found eight regions related to PHS resistance in white winter wheat populations that were obtained from the crosses: NY6432-18 (NY18) x ‘Clark’s Cream’ (CC) and NY18 x NY6432-10. This study was among the few of the QTL mapping studies conducted using North American germplasm. Recently, Fofana et al. (2008) cited 9 new QTL clusters for PHS resistance identified in two doubled haploid (DH) populations involving the Canadian bread wheat cultivar ‘AC Domain’. Seven QTL located on chromosomes 3A, 3B, 3D and 5D were identified in one of the DH populations from the cross AC Domain x ‘White-RL4137’. The QTL on homoeologous group 3 chromosomes were found to overlap with three seed coat color QTL (Fofana et al., 2007 as cited by Fofana et al., 2008). In another mapping population
also involving AC Domain, 13 PHS QTL were reported on chromosomes 3A, 3D, 4A, 4B and 7D (Rasul, 2007 as cited by Fofana et al., 2008).

Australia is one of the most vulnerable regions in the world to PHS in wheat due to the occurrence of summer rainfall that coincide with grain harvest (Ogbonnaya et al., 2007). In the early 1980’s, several studies were conducted to find sources of PHS resistance, especially in white wheat germplasm, which are the most susceptible to PHS. In 2001, Mares and Mrva reported QTL associated with variation in grain dormancy in Australian wheat. Using a doubled haploid population derived from ‘Cranbrook’ x ‘Halberd’, they identified chromosome arms 2AL, 2DL and 4AL carrying QTL for grain dormancy. Mares et al. (2005) found a QTL on chromosome 4A using a doubled haploid population derived from three bread wheat genotypes (white- and red-grained) of diverse origin. Two significant QTL for grain dormancy located on chromosomes 4AL and 5BL were reported using ‘AUS1408’, a current major source of PHS resistance in Australian breeding programs (Tan et al., 2006). Recently, Ogbonnaya et. al (2008) identified two QTL on chromosome 4A using recombinant inbred lines derived from a cross between ‘CN19055’ (white-grained, PHS-resistant) with locally adapted Australian cultivar ‘Annuello’ (white-grained, PHS-susceptible).

Using germplasm from India, Roy et al. (1999), identified a microsatellite and a STS marker on chromosomes 6B and 7D, respectively, from RILs derived from a cross between a PHS-tolerant genotype with red kernels, ‘SPR8198’ and a PHS-susceptible genotype with white kernels, ‘HD2329’. A follow up study using a population derived from the same cross was conducted and revealed a major QTL on chromosome 3A (Kulwal et al., 2005). In Japan, a recombinant inbred population from a cross between
Zenkoujikomugi (Zen) and Chinese Spring (CS) evaluated under controlled environments using growth chambers was used to map two QTL for seed dormancy on chromosome 3A (Osa et al., 2003). A follow up study using the same population was evaluated under field conditions that confirmed the QTL on chromosome 3A (Mori et al., 2005). Additional QTL on the group 4 chromosomes were likewise identified in this study.

In Europe, Flintham et al. (2002), identified QTL regions on chromosomes 1B, 4B and 7A that were associated with PHS resistance using RILs from ‘RL4137’ x ‘Timgalen’. Groos et al. (2002) detected four QTL, one each in group 3 homoeologous chromosomes and the short arm of chromosome 5A using a cross between ‘Renan’, a red kernel variety resistant to PHS and ‘Recital’, a white-grained variety highly susceptible to PHS.

QTL mapping studies have also been conducted using germplasm that are close relatives of bread wheat. Zanetti et al. (2000), reported six QTL with major effects on PHS resistance located on chromosomes 2A, 3B, 5AS, 5AL, 6A and 7B using RILs from a wheat x spelt cross. Imtiaz et al. (2008) identified nine QTL with main effects located on chromosomes 3DL and 4AL and 18 additive x additive interactions for germination index, sprouting index and visible sprouted seeds from synthetic backcross-derived wheat lines constructed from Aegilops tauschii and Triticum turgidum ssp. durum var. Altar84.

These QTL for PHS in wheat that have been identified at different genetic loci, in part, reflect the different sources of resistance available from a wide array of germplasm. This implies that many loci are involved in PHS resistance. However, there were reports where similar locations for the QTL were identified over different crosses. For example, QTL on homoeologous group 3 and 4A chromosomes were identified in more than one
study using different germplasm sources. Furthermore, in all these QTL mapping studies, genotype x environment interactions were frequently observed.
CHAPTER II

INTRODUCTION

Preharvest sprouting (PHS) is the precocious germination of the grains in the spike following physiological maturity resulting in reduction of end-product quality in wheat. Sprouted wheat will have smaller bread volume and compact structure due to its loss of thickening power brought about by starch breakdown (Mansour, 1993). Another negative effect of PHS is lower yield due to harvest losses and decrease of grain volume weight. In worst cases, sprouted wheat cannot be processed for human consumption and ends up as animal feed.

Among the classes of wheat, the white wheats are the most susceptible to PHS while the red kernel wheats have higher levels of PHS resistance. This is mainly due to pleiotropic effect of the red color \( R \) genes located on chromosomes 3A, 3B, and 3D (Sears, 1944). Flintham (2000), demonstrated that the \( R \) genes have a direct effect on dormancy. However, other studies have shown that several sources of resistance to PHS are available in white wheat germplasm (Anderson et al., 1993; Mares, et. al., 2005), suggesting that there are several genes for dormancy other than those associated with kernel color.

Several mechanisms have been proposed that contribute to increased levels of preharvest sprouting resistance. These include slower water uptake (King, 1984), germination inhibitive substances in the bracts (Derera and Bhatt, 1980), sensitivity to abscisic acid (Walker-Simmons, 1987), reduced alpha-amylase level in the grain (Bhatt et al., 1976) and ear morphology (Zanetti, et. al., 2000). King and Richards (1984) have
shown, for example, that the grain of awnless wheat cultivars absorbed and imbibed less water than awned lines, thereby, reducing their susceptibility to PHS.

Resistance to PHS in white wheat has relatively low heritability, is expressed as a quantitative character and is affected by environment and genotype x environment interaction (G X E) (Hagemann and Ciha, 1987; Anderson et al., 1993); therefore, lines should be evaluated in multiple environments with replication to account for variation caused by the genotype x environment interaction and reduce error.

Considerable efforts have been made to find DNA markers associated with PHS in wheat. To date, 20 out of the 21 chromosomes of wheat (except 1D) have been shown to carry quantitative trait loci (QTL)/genes for PHS resistance (Anderson et al., 1993; Roy, et. al, 1999; Zanetti, et. al, 2000; Mares and Mrva., 2001; Flintham et. al., 2002; Groos, et. al, 2002; Kulwal, et. al., 2004; Mori et. al., 2005; Chen, et. al, 2008). Most of these studies revealed QTL on homoeologous group 3 chromosomes and 4A. A QTL mapping study conducted by Anderson et. al (1993) was one of the few studies that used wheat germplasm of US origin for PHS resistance. In their study, they used RILs from two populations derived from NY6432-18 x Clark’s Cream, and NY6432-18 x NY6432-10. NY6432-18 is moderately resistant to PHS whereas Clark’s Cream is highly resistant. Anderson et al. (1993) found eight RFLP loci associated with resistance to PHS from these populations. These were located on chromosome 1AS, homoeologous group 2 chromosomes, 3BL, 4AL, 5DL and 6BL. Although several genomic regions for PHS resistance were identified, the authors speculated that additional QTL could be identified with i) more complete genome coverage with markers; and ii) use of a cross involving Clark’s Cream and a susceptible line instead of a moderately resistant line. Therefore,
the objectives of this study were to validate QTL for preharvest sprouting identified in a previous study conducted by Anderson et al. (1993) and identify new PCR-based DNA markers for resistance to preharvest sprouting.
MATERIALS AND METHODS

Plant Materials and Experimental Design

A highly resistant line, NY6432-18/Clark’s Cream RIL 40, was identified from the Anderson et al. (1993) study. A population of BC₁F₈ recombinant inbred lines (RILs) developed from the cross (Grandin*5/ND614-A) *2 / (NY6432-18/Clark’s Cream 40-1) was used for this study. Grandin*5/ND614-A is a hard white spring wheat with good adaptation to the Upper Midwest and with excellent bread-making quality, but is very susceptible to preharvest sprouting. NY6432-18/Clark’s Cream 40-1 is a soft white winter wheat selection from the cross of two winter wheat lines with very high resistance to preharvest sprouting (Anderson et al., 1993). The RILs were developed by single seed descent. Out of the 150 BC₁F₈, only 94 RILs with spring growth habit were retained in the population. Also, very early and very late maturing (± 5 d from the mean heading date) RILs were removed from the population to obtain similar maturity among the lines. The parent Grandin*5/ND614-A was evaluated as a susceptible check with four other hard red spring wheat cultivars - ‘Granite’, ‘Briggs’, ‘Granger’ and ‘Ulen’. ‘Granite’ and ‘Briggs’ were used as highly resistant checks whereas ‘Granger’, and ‘Ulen’ were used as moderately susceptible checks. In addition, these hard red spring wheats with distinct phenotypes also served as checks to determine the correct planting plan in the field.

The RILs and the checks were evaluated in three years at three locations in Minnesota (St. Paul in 2005 and 2007; Morris in 2006 and 2007; Crookston in 2005, 2006 and 2007) and in Watertown, South Dakota in 2006 for a total of eight environments (location*year combinations). Two other environments were also included, but Fusarium head blight infection in Morris 2005 and drought in St. Paul 2006.
compelled us to exclude them from our analysis. Entries were evaluated in plots consisting of a single 1-m row with two replications laid out in a randomized complete block design (RCBD). A few RILs were not evaluated in Crookston in 2005 and some lines had only one replication in St. Paul in 2005 due to lack of seeds. The four hard red spring wheat checks were included only in the 2006 and 2007 experiments.

Trait evaluation

PHS evaluation was carried out as described by Anderson et al. (1993) with some modifications. Briefly, intact spikes (10 spikes per plot cut approx. 20 cm from the base of the spike) from each plot were harvested at physiological maturity, which was approximated by the loss of green color from the peduncle and glumes and/or the first appearance of a dark layer of cells along the crease (pigment strand) of the wheat kernel (Hanft and Wych, 1982). Sampling of all entries was done within 3 to 7 d depending on the location. Harvested spikes were air dried for 5 d under ambient temperature and humidity and then stored at –20°C to preserve dormancy prior to evaluation. Sprouting experiments were carried out by first cutting the peduncles (3 to 4 cm from the base of the spike) and inserting them in pre-made racks consisting of galvanized wire mesh (20 x 20 mm holes) framed with wood or plastic pipes measuring 1 m x 1 m x 1 m. These were placed in a dew chamber with controlled temperature set at 20°C and 100% humidity. The racks were rotated clockwise (one quarter turn) daily to improve uniformity of temperature and wetting of the spikes. After 7 d in the dew chamber, individual spikes were scored on a scale of 0 – 9 following the rating system of Anderson et al. (1993) that takes into account both the number of visually sprouted kernels and the rate at which they
germinate by observing the length of the coleoptile. Two runs (five spikes per run) were carried out to accommodate all experiments within a year because of limited space on the racks. Mean sprout scores for individual plots were calculated by first taking the average score of five spikes for each run then averaging the scores of the two runs. Spikes with evidence of fungal infection were discarded.

Plots were also evaluated for presence/absence of awns, plant height and heading date. The presence/absence of awns was recorded by visual inspection of the spikes. Plant height was recorded as the length of the stem from the soil to the tip of the spike excluding awns, measured between maturity and harvest. Heading date was taken on each of the plots and was recorded as days from planting until 50% of the heads were completely emerged from the flag leaf.

The average temperature during the period of sensitivity to sprouting (25-30 days post anthesis (DPA)) which coincides with the grain filling period of wheat were also recorded. Average temperature was calculated from the mean temperature for each day from heading date to physiological maturity using the formula \((\text{max. } ^\circ\text{C} + \text{min. } ^\circ\text{C})/2\).

Genotyping and linkage map construction

Young leaves from 20 plants for each RIL and parent lines were sampled for DNA extraction. The protocol for DNA isolation has been described (Liu et. al, 2006). Parental DNAs were screened for polymorphism with genomic and EST-derived Simple Sequence Repeat (SSR) and Single Nucleotide Polymorphism (SNP) markers (Chao et al., 2007; Chao et al., 2008). PCR amplification using fluorescent labeled M13-tailed primers followed the method of Schuelke (2000) as described by Chao et al. (2007). The
details for PCR amplification conditions were described by Somers et al. (2004). The
PCR products were assayed using the ABI 3130x1 Genetic Analyzer platform (Applied
Biosystems, Foster City, CA) and GeneMapper 3.7 (Applied Biosystems) was used for
fragment analysis and allele calling. Polymorphic SNP markers were selected based on
their co-location in chromosomal regions that contained putative QTL
(http://wheat.pw.usda.gov/SNP). SNP genotyping based on the template-directed dye-
terminator incorporation assay with fluorescence polarization detection (FP-TDI) was
carried out as described by Chao et al. (2008). Linkage maps were constructed using
MAPMAKER/EXP v3.0b (Lincoln et al., 1993). Markers less than 1 cM apart from their
nearest neighbor were excluded from the map except in groups where significant QTL
based on analysis across environments were found. Segregation distortion was tested with
chi-square tests in Microsoft Excel.

Statistical Analysis of Phenotypic data

A standardized sprout score calculated from PHS line means was used for
subsequent analysis of variance (ANOVA) and QTL analyses, as described by Anderson
et al. (1993). Briefly, the raw scores were converted to a percentage of the mean of all
RILs (excluding checks) harvested from a single experiment on the same day. The
standardization was necessary to account for the variation due to sampling dates.
ANOVA and estimates of heritabilities (plot and entry mean basis) across environments
and for each individual environment were computed using PROC GLM of SAS version
9.1. For these analyses, all effects were considered random. For the combined analysis,
each location and year combination was considered distinct environments as opposed to
separating the effects of years and locations. Heritability on an entry mean basis and 90% confidence intervals were calculated (Knapp et al., 1985). Correlation coefficients for preharvest sprouting means of RILs were tested among the eight environments. Also, simple product-moment phenotypic correlations ($r_P$) and genetic correlations ($r_A$) of standardized preharvest sprouting line means with presence/absence of awns, plant height, heading date and average temperature were calculated according to Bernardo (2002). The approximate standard error and 95% CI for $r_A$ was calculated (Falconer and Mackay, 1996; Lynch and Walsh, 1998), and an $r_A$ estimate was declared significant at $P = 0.05$ if the approximate 95% CI did not include zero.

Detection and Estimation of QTL for preharvest sprouting and other traits

All QTL analyses were based on the standardized PHS line means using Windows QTL Cartographer v2.5 (Wang et al., 2007). Initially, single marker analysis was done to analyze the effect of each marker independently. This is important to serve as a basis for comparing the results of subsequent QTL mapping analyses. The analysis fits the data to the simple linear regression model $y = b_0 + b_1 x + e$ where $y$ is the mean preharvest sprouting score, $b_0$ is the population mean, $b_1$ is the additive effect of the locus, $x$ is the designated genotypic code at the locus being tested for the line considered (1 and -1 for Grandin*5/ND614 and NY18/CC40-1, respectively) and $e$ is the residual error term. The test would indicate whether the marker is linked to a QTL by determining if $b_1$ is significantly different from zero at a specified p-value. Also, single marker analysis was performed to locate the marker that is associated with awnedness.
Composite interval mapping (CIM) analysis was conducted using forward and backward step-wise regression with a probability into and out of the model at $P = 0.1$. A 5-cM window size for selecting cofactors was used for the genome scans. The LOD threshold value of 2.5 (default value) was used to declare the presence of a QTL. Setting the LOD score value to default for declaring a QTL minimizes the risk of type II error (i.e. missing a QTL). The 1,000 permutations performed for all CIM analysis using WinQTLCart v2.5 calculated LOD scores of 3.1 to 4.2 at the 5% level for a Type I error (Doerge and Churchill, 1996), therefore, the LOD score (2.5) used in this study increases the risk of Type I errors. CIM analysis for PHS was performed for each of the eight environments and the combined data across environments. CIM analysis was also conducted for heading date and plant height from the combined data across environments.

Multiple interval mapping (MIM) was implemented only for PHS data across environments. QTL output from CIM analysis (with a LOD threshold value of 2.4 and minimum 5-cM between QTLs) provided initial models for MIM. The MIM models were developed in an iterative, stepwise fashion as described by Zeng et al. (1999). Epistatic effects between significant main QTL declared by the MIM analysis were also included. The final MIM model was chosen based on Bayesian Information Criterion (BIC) which favors models with higher likelihoods but included a penalty for each additional parameter added to the model to help prevent overfitting the model (Piepho and Gauch, 2001).

Epistatic interactions between all pairs of loci (295) were tested by pairwise analysis of variance in R as described by Wu et al., 2007.
Phenotypic effects of major QTL alleles were estimated using the closest linked marker for each main QTL. The approximate PHS scores were calculated by multiplying the preharvest sprout line means (standardized scores) by the average raw score of 3.6 (average sprout score across environments). Multiple comparisons between different QTL allele groups were carried out using Fisher’s least significant difference (LSD) at $P=0.05$. 
RESULTS

Genetic Linkage Map

Out of 1,009 SSRs screened for polymorphism, 467 (46%) were polymorphic between the parents, Grandin*5/ND614-A and NY18/CC40-1. The final map consisted of 287 loci from 269 SSRs and 4 SNPs. Twenty-five linkage groups were formed spanning a genetic distance of 2,056 cM giving an average interval distance of 7.1 cM (Appendix I). The linkage groups covered all the wheat chromosomes, although chromosomes 1A, 4D and 6A contained fewer than 10 markers each.

Fifty one markers deviated significantly ($P<0.05$) from the expected ratio of 3:1 for this BC$_1$F$_8$ population. These were mostly located on chromosomes 2D, 3B, 4B, 5D and 7D. We did not found segregation distortion on chromosome 5A which is the primary chromosomal region that affects vernalization. All the markers with skewed segregation had more than the expected number of Grandin*5/ND614-A alleles. All markers within 12.2 cM of putative QTL identified in this study segregated in the expected ratio except for markers for the QTL in 4B and 6A that had skewed segregation.

Phenotype analysis

The approximated PHS scores of the RILs ranged from 0.5 to 5.6 (Fig. 1). Except for a few highly resistant lines, the population showed approximately normal distribution with a moderate range of phenotypes comparable to the pattern observed in individual environments, suggesting that the PHS trait is quantitatively inherited. We cannot determine if there were transgressive segregants among the RILs on the resistant side because the resistant parent used in this population had a winter growth habit. There was
no indication of transgressive segregants on the susceptible side based on the LSD 
\((P=0.05)\). The most susceptible RIL had a PHS mean of 5.6 whereas the recurrent parent 
had a mean of 5.1.

The analysis of variance across environments showed highly significant variation 
among RILs for PHS and genotype x environment interaction \((P<0.001)\) (Table 1). The 
heritability on an entry mean and plot basis was 0.90 and 0.45, respectively. The 
heritability on a per plot basis is equivalent to the proportion of phenotypic variance 
explained by the RILs. This indicates that 55\% of the phenotypic variance was accounted 
for by RILs x environment interaction (20\% ) and experimental error (35\%).

The approximate mean preharvest sprouting score in the individual environments 
ranged from 2.0 to 5.5 (Table 2). The variation among RILs was significant in all 
individual environments (data not shown). The variance of RILs in both St. Paul 2005 
and 2007 were the largest among the environments and were two to three times larger 
than the error variance. This reflects the wider range of PHS scores in these two 
environments owing to a few extreme phenotypes on both the susceptible and resistant 
side. In all other environments, the variance of RILs was about equal to the error 
variance. Among individual environments, the heritability on a per plot basis ranged from 
0.50 to 0.76 while the heritability on an entry mean basis ranged from 0.67 to 0.86 (Table 
2). The moderate to high heritability estimates in the combined analysis and in individual 
environments indicate a strong genetic influence on this trait. This suggests that the 
methods used to assess PHS were effective; however, testing in multiple environments is 
necessary.
The estimates of correlation coefficients for preharvest sprouting scores of RILs among the eight environments were all positive and highly significant at $P<0.001$, ranging from 0.39 to 0.80 (Table 3). The highest correlations were found between Crookston 2005 and Watertown 2006, and Morris 2006 and Watertown 2006 ($r = 0.80$, $P<0.001$), whereas the lowest was found between St. Paul 2005 and Crookston 2006 ($r = 0.39$, $P<0.001$).

QTL analyses for preharvest sprouting

Single marker analysis from the mean across eight environments revealed 50 significant ($P<0.05$) markers associated with PHS resistance (data not shown). At least one significant marker was found in each of the wheat chromosomes except for 1D, 4D, 5D and 7D. The presence of this many markers significantly associated with preharvest sprouting suggests that many QTL contribute to variation in this trait although it cannot be disregarded that some of these associations may also be random.

A composite interval mapping (CIM) analysis of PHS in individual environments showed 21 different chromosome regions significantly associated (LOD $\geq 2.5$) with PHS resistance in at least one environment (Table 4). Surprisingly, none of these QTL regions were significantly associated with PHS in more than two environments. Thirteen regions were significant in two environments whereas the rest were significant in only one environment. On average, four significant regions (ranging from 3 to 7) were found in each individual environment. Five regions, having the nearest markers $gwm358b$ on chromosome 2D ($R^2 = 7.0$, LOD = 2.5), $barc20$ in 4B ($R^2 = 10.0$, LOD = 3.8), $gwm186a$
in 5A ($R^2 = 21.0$, LOD = 4.1), barc4 in 6A ($R^2 = 13.0$, LOD = 2.6) and gwm130a in 7A ($R^2 = 11.0$, LOD = 4.2), were found significant across environments (Table 4, Figure 2).

A multiple interval mapping (MIM) model based on scanning through composite interval mapping (CIM) results was performed to accurately detect the location and magnitude of QTL for PHS in the population. From the data across environments, seven QTL from different linkage groups were included in a model that explained 76.9% of the phenotypic variance (Table 5). The QTL with the nearest marker barc4 on chromosome 6A accounted for 14.2% phenotypic variation whereas markers gwm358b on chromosome 2D, gwm186a on chromosome 5A, and marker gwm130a on chromosome 7A accounted for 14.0%, 11.8% and 13.6% phenotypic variation, respectively (Table 5). The remaining four QTL accounted for 2.1 to 10.5% of the phenotypic variation. The QTL on chromosome 1B accounted for the lowest $R^2$ value (2.1%) but was retained in the model due to a significant interaction it contributed to the total phenotypic variation. This QTL x QTL interaction was detected between the QTL on chromosome 1B with marker wmc134 and the QTL on chromosome 2D that contributed 5.5% of the phenotypic variation.

Multiple QTL effects

The effects of combining at least two PHS QTL that were not co-located with heading date or plant height and/or had a LOD threshold value greater than or equal to 2.5 were calculated. The QTL on chromosomes 5A (LOD = 4.1) and 7A (LOD = 4.2), which had the highest LOD scores exceeding the LOD threshold value at the 5% level for a Type I error based on 1,000 permutations (LOD = 3.3) was first taken into account.
These two QTL jointly explained 25.4% of the phenotypic variation. Forty five RILs did not have either of the favorable 5A and 7A QTL alleles from NY18/CC40-1 and had a mean PHS score of 4.0 (Table 6). The group of RILs with only the favorable allele from chromosome 5A and the group having only the favorable allele from chromosome 7A had significantly ($P < 0.05$) lower mean PHS scores of 3.3 and 3.2, respectively, than the group without both the favorable alleles. These groups, however, did not differ significantly from each other. When the two QTL were present, the mean PHS score was further reduced to 1.5. Overall, the 5A QTL improved PHS resistance by 20.0%, the 7A QTL reduced PHS score by 22.5%, whereas the combination of the two QTL provided a 62.5% reduction in PHS score.

The effect of combining the QTL on chromosome 2D with the QTL in 5A and 7A was also taken into account. The QTL on chromosome 2D was one of the QTL with LOD $\geq 2.5$ in the study that was not co-located with QTL for heading date or plant height. Furthermore, the favorable allele for this QTL was derived from the susceptible parent, Grandin*5/ND614-A, thus could make it easier to be incorporated in the breeding program. The total phenotypic variation explained by combining the three QTL was 39.4%, which constitute 51% of the total phenotypic variation for PHS resistance in this population. The results showed a very high PHS score (4.7) for the RILs without favorable allele from any of the three QTL (Table 7). Adding a single favorable QTL provided a significant reduction in PHS score whereas adding two or more favorable alleles provided even significantly greater resistance to PHS. For example, the combined effect of the three favorable QTL alleles reduced the PHS score from 4.7 to 1.5 which was equivalent to a 68.1% reduction in PHS score; significantly ($P \leq 0.05$) lower than
having only one or even two favorable QTL alleles present. Similar patterns were observed when the QTL on chromosome 6A, which also has LOD ≥ 2.5 and had the largest $R^2$ but was co-located with QTL for plant height, was combined with the 5A and 7A QTL. However, there were no recombinants found to have all the three favorable alleles from NY18/CC40-1 (data not shown). This was likely due to the skewed segregation towards the Grandin*5/ND614-A allele of the nearest marker for the 6A QTL.

QTL analyses for other traits

Single marker analysis identified marker *cfa2149* located on chromosome 5A linked to awnedness.

QTL were identified for plant height and heading date across seven and four environments, respectively. Four significant QTL linked to plant height were identified on chromosomes 2B, 4B, 4D and 6A (Table 8). Two of these QTL for plant height (4B and 6A) were co-located with QTL for PHS in this study. The 4B QTL with the closest marker *barc20* explained 58% of the phenotypic variation for plant height but it explained only a small portion of the phenotypic variation for PHS (5.2%). Conversely, the QTL on chromosome 6A with the closest marker *barc3* accounted for only 5% of phenotypic variation for plant height but explained the largest phenotypic variation for PHS (14.2%). The QTL on chromosome 2B and 4D each explained 6% of the phenotypic variation for plant height. We also found two QTL associated with heading date and located them on chromosomes 4A and 7D with the closest markers *dupw4* and *gwm130b*, respectively. The QTL on chromosome 4A accounted for 24% of the phenotypic
variation whereas the QTL on 7D accounted for 15%. None of the QTL for heading date was associated with PHS QTL. All QTL identified for both plant height and heading date had LOD score value greater than 4.0 and exceeded the 5% significance level based on 1000 permutations.

Correlation of preharvest sprouting with other traits and environmental factors

The phenotypic and genetic correlations of three traits: presence or absence of awns, plant height and heading date as well as correlation of average temperature (taken at 25-30 DPA) with preharvest sprouting were analyzed. All three traits had low to moderate and negative correlation with preharvest sprouting (Table 9). The phenotypic correlation of PHS with plant height \( r_p = -0.42, P \leq 0.001 \) and heading date \( r_p = -0.21, P \leq 0.05 \) was significant. Also, a slightly stronger genetic correlations of preharvest sprouting with plant height \( r_A = -0.46, P \leq 0.05 \) and heading date \( r_A = -0.29, P \leq 0.05 \) than the phenotypic correlations was found. No significant correlation was found between preharvest sprouting resistance and presence or absence of awns \( r_p = -0.03 \) and average temperature during grain fill \( r_p = 0.14 \).
DISCUSSION

Across environments, seven QTL for preharvest sprouting resistance were found that accounted for about 77% of the phenotypic variance. The QTL on chromosomes 5A and 7A were among the three QTL that exceeded the LOD threshold value at the 5% level of probability for a Type I error and that were not associated with QTL for other traits we studied. The five other QTL were found on chromosomes 1B, 2D, 4B, 6A and 6D.

We imposed selection on the population by retaining those lines with spring growth habit. Therefore, this may affect segregation of markers associated with genes/QTL that affect growth habit such as vernalization. Vernalization genes are found on homoeologous group 5 chromosomes. The primary vernalization gene, Vrn-1A, located on chromosome 5A, was segregating in the population we studied. However, no segregation distortion was found on this chromosome. We found skewed segregation on several regions on the chromosome such as on 5D and 7D. This may likely be due to distorter loci observed on these chromosomal regions. Faris et al. (1998) found similar distorted segregation ratios of genetic markers on these chromosomes. Furthermore, they found that these distorter loci affect gametophyte competition in male gametes.

One of the parents used in our study, NY18/CC40-1, is a highly resistant RIL from a previous PHS QTL mapping experiment (Anderson et al., 1993). This, therefore, may allow us to validate some of the QTL found in that previous experiment. Our results showed agreement with that of the previous study that found a QTL on one of the homoeologous group 2 chromosomes from a population derived from NY6432-18 x Clark’s Cream. This QTL was most likely located on the D genome based on the
molecular mapping of wheat homoeologous group 2 chromosomes (Nelson et al., 1995). The QTL we found on 2D \( (R^2 = 14.0\%) \) mapped on the same region as the previous study, though, this was derived from the Grandin*5/ND614-A parent in the present study. The previous study also found a QTL near RFLP marker cdo795 from the same population. The location of this marker was not determined in the previous study but current data showed that it was located on chromosome 4B (http://wheat.pw.usda.gov). The QTL we found on chromosome 4B \( (R^2 = 5.2\%) \) was likely in the same region as the one found in the previous study. In our study, we did not find any QTL on chromosome 1A corresponding to the one identified in the NY6432-18/Clark’s Cream population.

Several QTL on chromosomes 3BL, 4AL, 5DL and 6BL were identified from a second population derived from NY6432-18 x NY6432-10 (Anderson et al., 1993). However, none of the QTL identified our study were coincident with those QTL. This was because NY18/CC 40 contained the Clark’s Cream alleles for all the RFLP markers nearest the QTL identified from that population. The parent we used in our study, NY18/CC 40-1, which is a single plant selection from the NY18/CC 40 line was not genotyped for these RFLP markers. It may be possible that there may have been recombination in the region of the QTL that contained NY6432-18 fragment in NY18/CC40-1. In this case, those QTL identified in the NY6432-18/NY6432-10 population could not be validated in our study.

For the new QTL we discovered, it is probable that their effects were masked in the earlier study since their experiment involved a cross between a line, NY6432-18 (NY18), moderately resistant to PHS and a highly resistant line, Clark’s Cream (CC). It could also be that the regions we found associated with resistance to PHS were not
covered by the RFLP clones used in the previous QTL mapping experiment. The chromosomal regions on chromosomes 5A and 7A, although screened with 10-15 RFLP markers in the previous study, contained less than five markers that were found polymorphic for each. Compared with the current study, we mapped more than three times the number of markers for these QTL regions than the previous study. Another factor that may have caused the difference in our QTL mapping results from that of the previous study was the small population size both studies used in the QTL mapping experiment. Beavis et al. (1991) found that QTL detection was hindered by the limited size of mapping populations even if the QTL were segregating in the population.

A preharvest sprouting QTL in the short arm of chromosome 5A has been found in a previous study from a cross between red (‘Renan’) x white (‘Recital’) wheat lines (Groos et al., 2002). Interestingly, this QTL for resistance to PHS was due to the allele from the white wheat line ‘Recital’. Flintham et al. (2002) reported association of a region in the short arm of chromosome 7A with PHS resistance from the cross RL4137 × Timgalen. This population involved a cross between red and white grained wheat lines, but they restricted the analysis to only the red-grained subset of 79 RILs. Nonetheless, these QTL were located within the same region found in this study.

We also found a significant QTL x QTL interaction between the chromosomal regions of 1B and 2D that accounted for 5.5% of the phenotypic variation. The QTL on chromosome 1B could be the same as one discovered in a double haploid population from the cross Cayuga x Caledonia (Sorrells, [http://maswheat.ucdavis.edu/protocols/PHS/index.htm](http://maswheat.ucdavis.edu/protocols/PHS/index.htm)) that explained 32% of the
phenotypic variation over eight environments. Cayuga is a backcross-derived line having PHS resistance from Clark’s Cream (CC).

The two other PHS QTL on chromosomes 4B and 6A coincided with chromosomal regions associated with plant height. The 4B QTL explained only a minor proportion of the phenotypic variation for PHS (5.2%) but explained a very large phenotypic variation for plant height (58%). One of the dwarfing (Rht) genes, RhtB1, is located on chromosome 4B. The RILs in the present study segregated for this gene; therefore, it is likely that the QTL on chromosome 4B is the same as the RhtB1 gene. Conversely, the QTL on chromosome 6A explained the largest phenotypic variation for PHS (14.2%) but accounted for only a minor phenotypic variation for plant height (5%).

A highly significant negative phenotypic correlation ($r_P = -0.42, P \leq 0.001$) and moderate genetic correlation ($r_A = -0.46, P \leq 0.05$) between PHS and plant height were found, suggesting pleiotropy and/or linkage of the two traits. The negative correlation between the two traits suggests that selection for resistance to PHS may be impeded by simultaneously selecting for shorter plant height. No studies have been accomplished that suggest the same findings in wheat, but a study on the different cultivars and breeding lines in triticale suggested a negative correlation between sprouting resistance and plant height (Herrmann, 2007).

We found a significant correlation between preharvest sprouting and heading date ($r_P = -0.21; r_A = 0.29$). However, none of the PHS QTL coincided with either of the two QTL for heading date identified in this study. The two QTL for heading date accounted for only a moderate portion of the phenotypic variation (39%) for this trait. Grandin*5/ND614-A is a relatively early line whereas NY18/CC40-1 is late maturing.
We may have expected a large phenotypic variation in the RILs for this trait, but the moderate phenotypic variation observed for heading date could be due to the effective culling of very late and very early maturing RILs during development of this population.

We identified a region on chromosome 5A associated with awnedness. This was likely the awn inhibitor, \textit{B1}, located on the long arm of chromosome 5A based on aneuploid analysis (Sears, 1954). We found no correlation between preharvest sprouting resistance and presence or absence of awns. Likewise, none of the QTL for PHS coincided with the region on chromosome 5A associated with awnedness. Previous studies found a significant effect of the presence of awns on preharvest sprouting. In the study conducted by King and Richards (1984), cultivars and near-isogenic lines with awns absorbed up to 30\% more water and water penetrated more rapidly to the grain in ears of awned lines than in awnless lines. Consequently, sprouting in the ear was enhanced by at least 40\%. This finding disagrees with the results from our study. In our case, the presence or absence of awns may not have had an effect due to the length of time that the spikes were exposed to 100\% humidity in the dew chamber. It could be that our method of PHS screening allowed for similar absorption of water by the grains regardless of the presence or absence of awns.

We also found no correlation between preharvest sprouting resistance and average temperature during grain fill (taken 25-30 DPA). This contradicts with the results from previous studies that had been conducted to show that higher temperature increased dormancy in non-dormant wheat lines (Nyachiro et al., 2002; Auld and Paulsen, 2003; Biddulph et al., 2005). PHS score was not significantly correlated with the average temperature even when the raw score for PHS was used for the calculations (data not
shown). This may be in part due to almost similar rainfall average received by these locations during the growing season (data not shown). Mares (1993) found that rainfall in combination with temperature had significant effect on variation in response of wheat lines to PHS. The average temperature, though, affected the overall PHS score means in individual environments. The experiments in both years in St. Paul had low within environment average PHS score particularly due to high temperature in these environments during the RILs sensitivity period (about 25-30 DPA) to sprouting. For example, in St. Paul 2007 the average temperature during the sensitivity period of the lines was 23.0°C while the average temperature in Crookston in the same year was 21.5°C. The wheat lines most sensitive to temperature are the moderately resistant to moderately susceptible phenotypes (Biddulph et al., 2005; Biddulph et al., 2008). Our data showed that the majority of the RILs fall into this category. This could be the reason for the low PHS score average in the experiments in St. Paul.

Preharvest sprouting resistance genes are reportedly located throughout the genome with the exception of chromosome 1D (Flintham et al., 2002). In some cases QTL are cultivar dependent whereas in others they are contingent on the method of evaluation. The environmental conditions and the effect of the genetic background also play major roles in the expression of individual QTL (Tanksley and Hewitt, 1988; Beavis et al., 1991). Therefore, we would not expect complete agreement among different studies, even when the same resistance source is used.

As has been true with other QTL studies, it could be that the remaining phenotypic variance not explained by our QTL model suggests that there were other chromosomal regions not detected that may contribute to variation in PHS in this
population. Furthermore, there were probably more QTL with minor effects that were not accounted for by our QTL mapping model. The single marker analysis we conducted showed 50 markers from 18 chromosomal regions that were significantly associated with PHS. Furthermore, 21 QTL for preharvest sprouting resistance were found in individual environments based on our CIM analysis, suggesting that many QTL with small effects affect this trait.

It is also likely that additional epistatic interactions remain undetected. All possible (4,033) two-way interactions between pairs of markers used in this study were analyzed. The results showed a higher number of significant interactions than expected from this total number of tests for interactions at all levels of significance (3% were significant at the 1% level of probability, 0.7% at the 0.1% level and 0.2% at the .01% level). Similar results were obtained when tests were limited only to markers (based on single marker analysis) with significant additive effects on the trait. Furthermore, a number of significant epistatic interactions were found among markers that did not have significant main effects. Many of the pairwise tests are not independent; therefore, the expectation that a fraction (α) of tests are significant with $P$-value < α does not necessary hold precisely. However, the results show considerable evidence for two-locus epistatic interactions. Anderson et al. (1993) also found significant epistatic interaction between markers associated with PHS from the two populations they studied.

Marker assisted breeding could prove very useful when breeding for a trait such as preharvest sprouting resistance that is very difficult to phenotype and is highly influenced by environment, provided that the true QTL can be accurately identified. The number of loci conditioning a trait influences the potential efficiency of marker assisted
breeding. Bernardo (2001) used computer simulation to determine the usefulness of marker-assisted selections, assuming that all of the QTL affecting a trait were known. He found that it is more advantageous to make selections on the basis of known QTL if there are only a few loci controlling the trait. With large number of QTL controlling the trait, estimates of QTL effects become imprecise and selection based on genotypes become less and less useful. Therefore, marker-assisted selection is most effective when a few QTL, each with moderate to large effects on target traits and consistent effects across breeding populations, can be identified. In our study, at least three of the seven QTL (2D, 5A and 7A) that had LOD $\geq 2.5$ and did not coincide with other traits can be useful for marker assisted-selection. We showed that the effect of combining the favorable alleles from two of these QTL (5A and 7A) provided for 62.5% better PHS resistance than without any favorable QTL. Furthermore, combining the three QTL provided even higher PHS resistance (68.1%) as compared with no favorable alleles present.

Recent simulation studies conducted by Bernardo and Yu (2007) indicated that genomewide selection was more effective than marker-assisted selection when a complex trait controlled by many QTL (20 or more) with small effects and low heritability is considered. Genomewide selection focuses purely on prediction of performance and avoids QTL mapping (Bernardo, 2008). However, the usefulness of this method has yet to be proven empirically.

The success of marker assisted breeding, on the other hand, can be greatly affected by inaccuracies in QTL mapping. We caution that our QTL mapping estimates were likely overestimated because of the moderate population size used. Studies have found that QTL mapping in small populations leads to the overestimation of the
proportion of phenotypic variance explained by QTL, also known as Beavis effect (Beavis, 1998; Melchinger et al., 1998). However, theoretical studies have found that the power to detect QTL is not only a function of the size of the mapping population, but is also a function of the heritability of the trait (Lande and Thompson, 1990). With a very high heritability of 0.90 (0.84-0.90) in our current study, it is possible that the QTL effects we reported were only slightly overestimated. Greater power to detect QTL and more precise estimates of QTL effect for PHS could be further gained by increasing population size, improving methods for screening PHS, increasing the number of environments for phenotyping and increasing the number of markers used.

Overall, the results indicated the complexity of the genetic architecture for PHS and strong genotype x environment interaction and correlated traits. Phenotypic selection can be challenging for this trait. Some of the QTL found in this study may aid in marker-assisted breeding for improvement of PHS resistance in wheat.
Figure 1. Histogram of preharvest sprout score for 94 recombinant inbred lines from the cross (Grandin*5/ND614-A)*2//NY18/CC40-1 averaged across all environments (mean of 8 environments with 2 replications each). Sprout scores were approximated by multiplying the standardized scores by the average raw score of 3.6. Higher sprout scores indicate greater sprouting.
Figure 2. Approximate location of significant QTL for PHS (■) and plant height (فاق) (LOD ≥ 2.5) based on composite interval mapping (CIM) analysis from the (Grandin*5/ND614-A)*2/NY18/CC40-1 recombinant inbred population. The QTL for PHS were identified based on the mean across eight environments whereas the QTL for plant height were identified based on the mean across seven environments.
Table 1. Analysis of variance for preharvest sprouting from recombinant inbred lines from the cross (Grandin*5/ND614-A)*2//NY18/CC40-1 across eight environments.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Mean Square</th>
<th>F-Value</th>
<th>Pr &gt; F</th>
<th>Prop. of phenotypic variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environment</td>
<td>7</td>
<td>0.05</td>
<td>0.02</td>
<td>1.0000</td>
<td></td>
</tr>
<tr>
<td>Replications</td>
<td>8</td>
<td>1.88</td>
<td>30.72</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>RILs</td>
<td>93</td>
<td>1.32</td>
<td>10.13</td>
<td>&lt;0.0001</td>
<td>0.45††</td>
</tr>
<tr>
<td>RILs x Environment</td>
<td>644</td>
<td>0.13</td>
<td>2.15</td>
<td>&lt;0.0001</td>
<td>0.20</td>
</tr>
<tr>
<td>Error</td>
<td>704</td>
<td>0.06</td>
<td></td>
<td></td>
<td>0.35</td>
</tr>
<tr>
<td>Total</td>
<td>1456</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heritability (entry mean basis) ‡ 0.90 (0.84-0.90)

† Heritability on per plot basis.
‡ Heritability on an entry mean basis was based on RILs evaluated over all replications and environments used in this study. Numbers in parenthesis are 90% confidence intervals (Knapp et al., 1985).

Table 2. Mean preharvest sprouting scores, variance of RILs and heritability estimates for preharvest sprouting from RILs from the cross (Grandin*5/ND614-A)*2//NY18/CC40-1 in individual environments.

<table>
<thead>
<tr>
<th>Environment</th>
<th>Mean†</th>
<th>Variance of RILs</th>
<th>Error</th>
<th>Heritability (plot basis)</th>
<th>Heritability (entry mean basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Paul 2005</td>
<td>2.4</td>
<td>0.29</td>
<td>0.09</td>
<td>0.76</td>
<td>0.86</td>
</tr>
<tr>
<td>Crookston 2005</td>
<td>5.5</td>
<td>0.06</td>
<td>0.03</td>
<td>0.67</td>
<td>0.78</td>
</tr>
<tr>
<td>Morris 2006</td>
<td>4.6</td>
<td>0.07</td>
<td>0.06</td>
<td>0.54</td>
<td>0.70</td>
</tr>
<tr>
<td>Crookston 2006</td>
<td>3.4</td>
<td>0.06</td>
<td>0.05</td>
<td>0.56</td>
<td>0.72</td>
</tr>
<tr>
<td>Watertown 2006</td>
<td>3.8</td>
<td>0.06</td>
<td>0.05</td>
<td>0.56</td>
<td>0.72</td>
</tr>
<tr>
<td>St. Paul 2007</td>
<td>2.0</td>
<td>0.22</td>
<td>0.10</td>
<td>0.69</td>
<td>0.82</td>
</tr>
<tr>
<td>Morris 2007</td>
<td>2.6</td>
<td>0.10</td>
<td>0.08</td>
<td>0.56</td>
<td>0.71</td>
</tr>
<tr>
<td>Crookston 2007</td>
<td>4.0</td>
<td>0.03</td>
<td>0.03</td>
<td>0.50</td>
<td>0.67</td>
</tr>
</tbody>
</table>

† Mean PHS scores were approximated by averaging the product of PHS line means (standardized scores) by the average raw score for each environment. The rating scale for preharvest sprouting score was from 0 (resistant) to 9 (susceptible).
Table 3. Correlation coefficients for preharvest sprouting means of RILs from the cross (Grandin*5/ND614-A)*2//NY18/CC40-1 tested in eight environments.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Crookston 2005</td>
<td>0.63***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morris 2006</td>
<td>0.62***</td>
<td>0.76***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crookston 2006</td>
<td>0.39**</td>
<td>0.60***</td>
<td>0.59***</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watertown 2006</td>
<td>0.62***</td>
<td>0.80***</td>
<td>0.80***</td>
<td>0.64***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>St. Paul 2007</td>
<td>0.63***</td>
<td>0.56***</td>
<td>0.55***</td>
<td>0.43***</td>
<td>0.53***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morris 2007</td>
<td>0.65***</td>
<td>0.70***</td>
<td>0.70***</td>
<td>0.68***</td>
<td>0.73***</td>
<td>0.54***</td>
<td></td>
</tr>
<tr>
<td>Crookston 2007</td>
<td>0.56***</td>
<td>0.69***</td>
<td>0.73***</td>
<td>0.55***</td>
<td>0.67***</td>
<td>0.52***</td>
<td>0.64***</td>
</tr>
</tbody>
</table>

* significant at $P<0.001$; ** significant at $P<0.0001$
Table 4. $R^2$ and LOD scores from composite interval mapping (CIM) analysis for DNA markers associated with preharvest sprouting resistance from RILs from the cross (Grandin*5/ND614-A)*2//NY18/CC40-1 in individual environments and across environments (mean of eight environments).

<table>
<thead>
<tr>
<th>Marker</th>
<th>Location</th>
<th>Stp05</th>
<th>Crk05</th>
<th>Crk06</th>
<th>Mor06</th>
<th>Wat06</th>
<th>Stp07</th>
<th>Crk07</th>
<th>Mor07</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>gwp94024</td>
<td>1A</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>13.0 (2.8)*</td>
<td>5.0 (1.5)</td>
<td>2.0 (0.7)</td>
<td>11.0 (3.0)*</td>
<td>2.0 (0.7)</td>
<td>3.0 (0.5)</td>
<td>3.0 (0.9)</td>
</tr>
<tr>
<td>barc8</td>
<td>1B</td>
<td>0.0 (0.0)</td>
<td>7.0 (2.0)</td>
<td>5.0 (1.6)</td>
<td>13.0 (3.2)*</td>
<td>2.0 (0.5)</td>
<td>2.0 (0.7)</td>
<td>9.0 (2.6)*</td>
<td>3.0 (1.1)</td>
<td></td>
</tr>
<tr>
<td>wmc134</td>
<td>1B</td>
<td>0.0 (0.1)</td>
<td>3.0 (0.5)</td>
<td>6.0 (1.1)</td>
<td>20.0 (4.0)*</td>
<td>9.0 (2.4)</td>
<td>0.0 (0.1)</td>
<td>1.0 (0.2)</td>
<td>2.0 (0.4)</td>
<td>8.0 (1.7)</td>
</tr>
<tr>
<td>barc340c</td>
<td>1D</td>
<td>1.0 (0.2)</td>
<td>15.0 (4.3)*</td>
<td>2.0 (0.2)</td>
<td>9.0 (1.8)</td>
<td>8.0 (2.8)*</td>
<td>1.0 (0.3)</td>
<td>1.0 (0.2)</td>
<td>1.0 (0.2)</td>
<td>3.0 (0.6)</td>
</tr>
<tr>
<td>cfd20</td>
<td>2B</td>
<td>2.0 (0.4)</td>
<td>4.0 (0.6)</td>
<td>28.0 (4.3)*</td>
<td>3.0 (0.6)</td>
<td>6.0 (1.0)</td>
<td>1.0 (0.2)</td>
<td>19.0 (1.5)</td>
<td>10.0 (1.8)</td>
<td>7.0 (0.8)</td>
</tr>
<tr>
<td>wmc344</td>
<td>2B</td>
<td>0.0 (0.2)</td>
<td>0.0 (0.1)</td>
<td>14.0 (4.3)*</td>
<td>0.0 (0.1)</td>
<td>1.0 (0.5)</td>
<td>0.0 (0.2)</td>
<td>1.0 (0.1)</td>
<td>3.0 (1.1)</td>
<td>2.0 (0.6)</td>
</tr>
<tr>
<td>gwm358b</td>
<td>2D</td>
<td>10.0 (3.3)*</td>
<td>1.0 (0.5)</td>
<td>2.0 (0.7)</td>
<td>1.0 (0.4)</td>
<td>2.0 (0.6)</td>
<td>3.0 (0.9)</td>
<td>0.0 (0.0)</td>
<td>7.0 (2.6)*</td>
<td>7.0 (2.5)*</td>
</tr>
<tr>
<td>barc270</td>
<td>2D</td>
<td>6.0 (2.5)*</td>
<td>3.0 (0.9)</td>
<td>2.0 (0.8)</td>
<td>1.0 (0.2)</td>
<td>2.0 (0.7)</td>
<td>1.0 (0.4)</td>
<td>2.0 (0.5)</td>
<td>5.0 (1.8)</td>
<td>3.0 (1.4)</td>
</tr>
<tr>
<td>cfd41</td>
<td>3A</td>
<td>8.0 (1.7)</td>
<td>4.0 (0.8)</td>
<td>1.0 (0.1)</td>
<td>9.0 (1.5)</td>
<td>3.0 (0.6)</td>
<td>4.0 (1.0)</td>
<td>14.0 (3.6)*</td>
<td>16.0 (4.3)*</td>
<td>5.0 (1.0)</td>
</tr>
<tr>
<td>wmc48</td>
<td>4A</td>
<td>1.0 (0.6)</td>
<td>12.0 (2.6)*</td>
<td>4.0 (1.4)</td>
<td>3.0 (0.7)</td>
<td>0.0 (0.1)</td>
<td>2.0 (0.7)</td>
<td>13.0 (2.8)*</td>
<td>1.0 (0.5)</td>
<td>3.0 (1.2)</td>
</tr>
<tr>
<td>dupw4</td>
<td>4A</td>
<td>0.0 (0.0)</td>
<td>1.0 (0.3)</td>
<td>3.0 (0.8)</td>
<td>1.0 (0.2)</td>
<td>1.0 (0.3)</td>
<td>15.0 (3.5)*</td>
<td>2.0 (0.5)</td>
<td>0.0 (0.0)</td>
<td>2.0 (0.6)</td>
</tr>
<tr>
<td>barc20</td>
<td>4B</td>
<td>13.0 (5.0)*</td>
<td>2.0 (0.8)</td>
<td>1.0 (0.5)</td>
<td>1.0 (0.3)</td>
<td>2.0 (1.1)</td>
<td>0.0 (0.1)</td>
<td>2.0 (0.8)</td>
<td>8.0 (2.9)*</td>
<td>10.0 (3.8)*</td>
</tr>
<tr>
<td>gwm165b</td>
<td>4D</td>
<td>11.0 (4.0)*</td>
<td>0.0 (0.1)</td>
<td>0.0 (0.2)</td>
<td>3.0 (1.1)</td>
<td>0.0 (0.0)</td>
<td>1.0 (0.2)</td>
<td>3.0 (0.6)</td>
<td>0.0 (0.1)</td>
<td>1.0 (0.4)</td>
</tr>
<tr>
<td>gwm186a</td>
<td>5A</td>
<td>28.0 (7.8)*</td>
<td>0.0 (0.2)</td>
<td>1.0 (0.2)</td>
<td>6.0 (1.5)</td>
<td>1.0 (0.2)</td>
<td>1.0 (0.4)</td>
<td>6.0 (1.2)</td>
<td>9.0 (1.2)</td>
<td>21.0 (4.1)*</td>
</tr>
<tr>
<td>barc143</td>
<td>5D</td>
<td>4.0 (0.7)</td>
<td>1.0 (0.1)</td>
<td>13.0 (2.7)*</td>
<td>14.0 (1.3)</td>
<td>1.0 (0.2)</td>
<td>3.0 (1.3)</td>
<td>1.0 (0.0)</td>
<td>3.0 (0.5)</td>
<td>2.0 (0.3)</td>
</tr>
<tr>
<td>barc4</td>
<td>6A</td>
<td>5.0 (1.6)</td>
<td>7.0 (0.4)</td>
<td>14.0 (1.2)</td>
<td>1.0 (0.1)</td>
<td>12.0 (2.5)*</td>
<td>10.0 (2.5)*</td>
<td>11.0 (1.0)</td>
<td>13.0 (1.8)</td>
<td>13.0 (2.6)*</td>
</tr>
<tr>
<td>gwm469c</td>
<td>6D</td>
<td>2.0 (0.9)</td>
<td>2.0 (0.7)</td>
<td>0.0 (0.1)</td>
<td>7.0 (1.8)</td>
<td>12.0 (3.8)*</td>
<td>11.0 (3.4)*</td>
<td>3.0 (0.8)</td>
<td>1.0 (0.2)</td>
<td>7.0 (2.2)</td>
</tr>
<tr>
<td>barc49</td>
<td>7A</td>
<td>1.0 (0.4)</td>
<td>1.0 (0.6)</td>
<td>0.0 (0.1)</td>
<td>8.0 (2.5)*</td>
<td>14.0 (4.8)*</td>
<td>0.0 (0.0)</td>
<td>1.0 (0.5)</td>
<td>2.0 (0.6)</td>
<td>3.0 (1.4)</td>
</tr>
<tr>
<td>gwm130a</td>
<td>7A</td>
<td>0.0 (0.1)</td>
<td>4.0 (1.4)</td>
<td>5.0 (1.8)</td>
<td>16.0 (4.5)*</td>
<td>19.0 (6.5)*</td>
<td>5.0 (2.0)</td>
<td>7.0 (1.9)</td>
<td>1.0 (0.4)</td>
<td>11.0 (4.2)*</td>
</tr>
<tr>
<td>barc278</td>
<td>7B</td>
<td>1.0 (0.5)</td>
<td>14.0 (2.6)*</td>
<td>4.0 (0.8)</td>
<td>1.0 (0.4)</td>
<td>2.0 (0.7)</td>
<td>0.0 (0.1)</td>
<td>8.0 (2.2)</td>
<td>21.0 (5.6)*</td>
<td>3.0 (1.0)</td>
</tr>
<tr>
<td>cfd175</td>
<td>7B</td>
<td>2.0 (0.9)</td>
<td>4.0 (1.6)</td>
<td>2.0 (0.6)</td>
<td>3.0 (1.0)</td>
<td>9.0 (2.6)*</td>
<td>1.0 (0.3)</td>
<td>14.0 (3.6)*</td>
<td>0.0 (0.2)</td>
<td>2.0 (0.8)</td>
</tr>
</tbody>
</table>

* LOD score ≥ 2.5. LOD scores are in parenthesis
Table 5. Multiple interval mapping (MIM) results for DNA markers associated with preharvest sprouting resistance from RILs from the cross (Grandin*5/ND614-A)*2//NY18/CC40-1 across eight environments.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Location</th>
<th>LOD(^a)</th>
<th>(R^2)(^b) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wmc134</td>
<td>1B</td>
<td>1.7</td>
<td>2.1</td>
</tr>
<tr>
<td>gwm358b</td>
<td>2D</td>
<td>2.5</td>
<td>14.0</td>
</tr>
<tr>
<td>barc20</td>
<td>4B</td>
<td>3.8</td>
<td>5.2</td>
</tr>
<tr>
<td>gwm186a</td>
<td>5A</td>
<td>4.1</td>
<td>11.8</td>
</tr>
<tr>
<td>barc4</td>
<td>6A</td>
<td>2.5</td>
<td>14.2</td>
</tr>
<tr>
<td>gwm469c</td>
<td>6D</td>
<td>2.1</td>
<td>10.5</td>
</tr>
<tr>
<td>gwm130a</td>
<td>7A</td>
<td>4.2</td>
<td>13.6</td>
</tr>
<tr>
<td>wmc134 x gwm358b</td>
<td></td>
<td></td>
<td>5.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>76.9</td>
</tr>
</tbody>
</table>

\(^a\) LOD was based on composite interval mapping.

\(^b\) \(R^2\) were estimated by multiple interval mapping; proportion of phenotypic variance explained by the QTL.

Table 6. QTL effects on preharvest sprouting from RILs from the cross (Grandin*5/ND614-A)*2//NY18/CC40-1 for QTL on chromosomes 5A and 7A.

<table>
<thead>
<tr>
<th>PHS QTL</th>
<th>Allele Type(^\dagger)</th>
<th>Number of RILs</th>
<th>PHS Score(^\ddagger) (standard dev)</th>
<th>QTL Effect(^§) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>gwm186a</td>
<td>gwm130a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- , -</td>
<td>-</td>
<td>43</td>
<td>4.0 (1.0)a</td>
<td></td>
</tr>
<tr>
<td>5A , -</td>
<td>+</td>
<td>18</td>
<td>3.2 (0.7)b</td>
<td>20.0</td>
</tr>
<tr>
<td>- , 7A</td>
<td>-</td>
<td>15</td>
<td>3.1 (0.6)b</td>
<td>22.5</td>
</tr>
<tr>
<td>5A . 7A</td>
<td>+</td>
<td>4</td>
<td>1.5 (1.1)c</td>
<td>62.5</td>
</tr>
</tbody>
</table>

\(^\dagger\) (-) Represents the Grandin*5/ND614-A allele; (+) represents the NY18/CC40-1 allele.

\(^\ddagger\) Mean PHS scores were approximated by multiplying the standardized scores by the average raw score of 3.6; means with the same letter are not statistically significant at \(P<0.05\).

\(^§\) QTL effects were calculated as the difference between the mean PHS score of the RILs with marker allele(s) from NY18/CC40-1 from the mean PHS score of the RILs without both the resistance alleles (- -) divided by the mean PHS score of the RILs with genotype (- -), expressed as percentage.
Table 7. QTL effects on preharvest sprouting from RILs from the cross (Grandin*5/ND614-A)*2//NY18/CC40-1 for QTL on chromosomes 2D, 5A and 7A.

<table>
<thead>
<tr>
<th>PHS QTL Allele Type‡</th>
<th>Number of RILs</th>
<th>PHS Score† (standard dev)</th>
<th>QTL Effect§ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>gwm358b</td>
<td>10</td>
<td>4.7 (0.7)a</td>
<td></td>
</tr>
<tr>
<td>2D,<em>,</em>,_</td>
<td>33</td>
<td>3.8 (0.9)b</td>
<td>19.1</td>
</tr>
<tr>
<td><em>,5A,</em>,_</td>
<td>6</td>
<td>3.5 (0.3)bc</td>
<td>25.5</td>
</tr>
<tr>
<td>_,7A</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2D,5A,<em>,</em></td>
<td>12</td>
<td>3.0 (0.7)c</td>
<td>36.2</td>
</tr>
<tr>
<td>2D,_,7A</td>
<td>14</td>
<td>3.1 (0.6)c</td>
<td>34.0</td>
</tr>
<tr>
<td>_,5A, 7A</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2D,5A,7A</td>
<td>4</td>
<td>1.5 (1.1)d</td>
<td>68.1</td>
</tr>
</tbody>
</table>

‡ (-) Represents the Grandin*5/ND614-A allele; (+) represents the NY18/CC40-1 allele.
† Mean PHS scores were approximated by multiplying the standardized scores by the average raw score of 3.6; means with the same letter are not statistically significant at P<0.05
§ QTL effects were calculated as the difference between the mean PHS score of the RILs with marker allele(s) from NY18/CC40-1 from the mean PHS score of the RILs without both the resistance alleles (--), divided by the mean PHS score of the RILs with genotype (--), expressed as percentage.
¶ Recombinants of these types were not found in the population.

Table 8. Composite interval mapping (CIM) analysis for DNA markers associated with plant height and heading date from RILs from the cross (Grandin*5/ND614-A)*2//NY18/CC40-1.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Marker</th>
<th>Location</th>
<th>LOD</th>
<th>R²(%)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
<td>gwm257</td>
<td>2B</td>
<td>4.4</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>barc20</td>
<td>4B</td>
<td>29.0</td>
<td>58.0</td>
</tr>
<tr>
<td></td>
<td>gwm194</td>
<td>4D</td>
<td>5.5</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>barc3</td>
<td>6A</td>
<td>3.8</td>
<td>5.0</td>
</tr>
<tr>
<td>Heading date</td>
<td>dupw4</td>
<td>4A</td>
<td>4.7</td>
<td>24.0</td>
</tr>
<tr>
<td></td>
<td>gwm130b</td>
<td>7D</td>
<td>4.1</td>
<td>15.0</td>
</tr>
</tbody>
</table>

a based on data from seven environments.
b based on data from four environments.
† Proportion of phenotypic variance explained by the QTL.
Table 9. Estimates of phenotypic correlation ($r_P$) and genetic correlation ($r_A$) coefficients of preharvest sprouting with presence/absence of awns, plant height, heading date from RILs from the cross (Grandin*5/ND614-A)*2//NY18/CC40-1 and average temperature.

<table>
<thead>
<tr>
<th>Estimate</th>
<th>Awns (Presence or absence)§</th>
<th>Plant height†</th>
<th>Heading date‡</th>
<th>Average temperature‡¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_P$</td>
<td>-0.03$^{ns}$</td>
<td>-0.42$^{***}$</td>
<td>-0.21$^*$</td>
<td>0.14$^{ns}$</td>
</tr>
<tr>
<td>$r_A$</td>
<td>-0.02$^{ns}$</td>
<td>-0.46$^*$</td>
<td>-0.29$^*$</td>
<td>-</td>
</tr>
</tbody>
</table>

$^{ns}$ Not significant  
* Significant at $P<0.05$  
$^{***}$ Significant at $P<0.001$

§ Correlation was calculated from average of eight environments.  
† Correlation was calculated from average of seven environments.  
‡ Correlation was calculated from average of four environments.  
¶ average temperature during the sensitivity period of wheat to sprouting (25-30 days post anthesis).
LITERATURE CITED


Appendix I. Genetic linkage map from 94 BC1F_8-derived recombinant inbred lines from the cross (Grandin*5/ND614-A)*2//NY18/CC40-1 using MAPMAKER/EXP v3.0b. The chromosomes were drawn using the DrwChr function of Windows QTL Cartographer v.2.5. The linkage groups were based mainly on the consensus map for bread wheat (Somers et al., 2004).
Appendix I continued…
Appendix II. Approximate PHS scores of RILs from the cross (Grandin*5/ND614-A)*2//NY18/CC40-1 arrange from most resistant to most susceptible based on mean of eight environments.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Mean of 8 envts</th>
<th>Environment</th>
<th>Presence/absence of favorable QTL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stp05 Crk05 Crk06 Wat06 Mor06 Stp07 Crk06 Mor07 1B 2D 4B 5A 6A 6D 7A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-53</td>
<td>0.5 0.2 1.1 0.7 0.4 0.5 0.0 1.6 0.3 / / X / X / /</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-71</td>
<td>0.7 0.2 1.1 0.3 1.1 2.3 0.0 1.2 0.3 - / / - / / X /</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-11</td>
<td>0.9 0.5 0.0 0.7 0.0 0.9 1.2 2.0 0.5 X / X X - / X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-54</td>
<td>1.0 0.0 1.7 1.3 0.4 1.4 0.2 2.8 1.0 / / X / X / /</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-58</td>
<td>1.0 0.0 2.8 1.7 0.8 1.4 0.4 2.0 0.3 / / X / X / /</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-19</td>
<td>2.0 1.0 - 1.7 2.6 2.3 1.6 2.4 0.8 / / / X / X / X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-99</td>
<td>2.0 0.0 2.8 2.4 1.9 3.2 1.0 4.0 1.3 / / / / / / X X X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-101</td>
<td>2.0 1.7 3.9 0.3 2.6 3.2 0.8 3.2 1.0 X / / X / X / X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-108</td>
<td>2.3 0.2 3.9 2.0 3.0 3.2 1.4 4.0 1.5 / / / / / / X X X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-8</td>
<td>2.4 1.7 3.4 3.7 2.3 3.7 0.4 2.0 2.3 / / / X X X /</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-66</td>
<td>2.5 0.7 2.2 4.0 1.1 2.3 1.6 3.6 3.1 / - / X X X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-45</td>
<td>2.6 1.0 5.0 3.4 3.8 3.2 0.6 2.8 1.8 X / X / / / / X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-76</td>
<td>2.6 0.5 4.5 3.4 3.4 3.7 0.6 4.0 1.8 X / X / X / X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-81</td>
<td>2.7 1.2 4.5 2.0 3.4 3.7 1.4 3.6 1.8 X / X / X X X X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-41</td>
<td>2.7 1.0 3.4 4.0 3.0 4.6 1.0 3.6 1.5 X / X / X / X / X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-111</td>
<td>2.7 2.2 2.8 3.0 3.4 3.2 0.8 3.6 2.0 / - / - X X X /</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-18</td>
<td>2.7 1.9 5.0 2.7 3.4 2.3 1.6 2.8 1.8 X / / / X X X X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-35</td>
<td>2.7 1.9 3.9 2.7 3.0 4.1 0.6 4.0 2.0 X / X X X X X X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-21</td>
<td>2.8 0.7 5.0 4.0 3.0 4.1 0.2 4.0 2.6 X / X X X / / /</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-27</td>
<td>2.8 1.0 - 2.0 3.0 4.6 2.0 3.6 2.0 X / / / X X X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-4</td>
<td>2.8 0.7 5.0 3.7 4.1 4.1 1.4 2.8 1.5 X / / / X - X X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-104</td>
<td>2.8 2.2 4.5 1.7 3.0 4.6 1.2 4.4 1.5 X / / X / - X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-46</td>
<td>2.9 1.4 5.0 3.4 2.3 2.3 2.4 3.6 1.8 / / / X / X / X</td>
<td></td>
<td></td>
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<tr>
<td>Entry</td>
<td>Mean of 8 envts</td>
<td>Environment</td>
<td>Presence/absence of favorable QTL</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------</td>
<td>-------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td></td>
<td>Stp05</td>
<td>Crk05</td>
<td>Crk06</td>
</tr>
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<td>Bulk 8-52</td>
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<td>Bulk 8-1</td>
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<td>2.4</td>
<td>4.5</td>
</tr>
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<td>Bulk 8-86</td>
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<td>0.7</td>
<td>5.0</td>
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<td>Bulk 8-92</td>
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<tr>
<td>Bulk 8-24</td>
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<td>2.6</td>
<td>5.0</td>
</tr>
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<td>Bulk 8-38</td>
<td>3.0</td>
<td>1.0</td>
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<td>6.7</td>
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<td>Bulk 8-17</td>
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<td>3.6</td>
<td>-</td>
</tr>
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<td>3.6</td>
<td>6.2</td>
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<td>3.7</td>
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<td>1.2</td>
<td>6.2</td>
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<td>-</td>
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<td>4.5</td>
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<td>1.4</td>
<td>6.7</td>
</tr>
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</table>
## Appendix II continued…

<table>
<thead>
<tr>
<th>Entry</th>
<th>Mean of 8 envts</th>
<th>Environment</th>
<th>Presence/absence of favorable QTL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stp05 Crk05 Crk06 Wat06 Mor06 Stp07 Crk06 Mor07 1B 2D 4B 5A 6A 6D 7A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-10</td>
<td>3.6 2.4 5.6 3.4 3.0 4.1 3.0 3.6 2.6 /</td>
<td>/ / X X X / X</td>
<td></td>
</tr>
<tr>
<td>Bulk 8-33</td>
<td>3.6 2.4 5.6 3.4 3.8 3.7 2.8 4.0 2.3 /</td>
<td>/ X / X X X X</td>
<td></td>
</tr>
<tr>
<td>Bulk 8-39</td>
<td>3.7 1.9 6.2 4.0 4.1 5.0 2.2 3.6 2.6 X</td>
<td>/ X - X / X</td>
<td></td>
</tr>
<tr>
<td>Bulk 8-55</td>
<td>3.7 3.4 4.5 2.7 3.8 5.0 2.0 4.8 2.6 /</td>
<td>/ / X X X /</td>
<td></td>
</tr>
<tr>
<td>Bulk 8-115</td>
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<td>/ X X X / X</td>
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<td>Bulk 8-40</td>
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<td>/ X X X X X X</td>
<td></td>
</tr>
<tr>
<td>Bulk 8-44</td>
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<td>/ / X X / X</td>
<td></td>
</tr>
<tr>
<td>Bulk 8-2</td>
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<td>/ X X - - X</td>
<td></td>
</tr>
<tr>
<td>Bulk 8-20</td>
<td>3.9 1.7 5.6 4.4 4.1 5.5 2.8 3.2 2.8 X X - / X X X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk 8-118</td>
<td>4.0 2.6 6.2 3.7 4.9 4.6 1.0 5.3 3.6 X</td>
<td>/ X X X - X</td>
<td></td>
</tr>
<tr>
<td>Bulk 8-51</td>
<td>4.0 4.1 6.2 2.4 3.4 5.5 2.2 4.8 2.6 /</td>
<td>/ X X X X X X</td>
<td></td>
</tr>
<tr>
<td>Bulk 8-78</td>
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<td>/ X X X X X X</td>
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Appendix III. $R^2$ from composite interval mapping (CIM) analysis for DNA markers associated with plant height and heading date from RILs from the cross (Grandin*5/ND614-A)*2//NY18/CC40-1 in individual environments and across environments.

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* LOD scores ≥ 2.5