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Title: FR-H3: A New QTL to Assist in the Development of Fall-Sown Barley with Superior Low Temperature Tolerance

Abstract approved:

Patrick M. Hayes

Fall-sown barley will be increasingly important in the era of climate change due to higher yield potential and efficient use of water resources. Resistance/tolerance to biotic and abiotic stresses will be critical. Low temperature is an abiotic stress of great importance. Resistance to barley stripe rust (incited by *Puccinia striifomis* f. sp. *hordei)* and scald (incited by *Rhynchosporium secalis*) will be important in higher rainfall areas. Simultaneous gene discovery and breeding will accelerate the development of agronomically relevant germplasm. The role of *FR-H1* and *FR-H2* in low temperature tolerance (LTT) has been well documented. However the question still remains: is LTT due only to *FR-H1* and *FR-H2* or are there other, undiscovered, determinants of this critical trait? We developed two doubled haploid mapping

populations using two lines from the University of Nebraska (NE) with superior cold tolerance and one line from Oregon State University (OR) with good malting quality and disease resistance: NB3437f/OR71 (facultative x facultative) and NB713/OR71 (winter x facultative). Both were genotyped with a custom 384 oligonucleotide pool assay (OPA). QTL analyses were performed for LTT, vernalization sensitivity (VS), and resistance to barley stripe rust and scald. Disease resistance QTL were identified with favorable alleles from both NE and OR germplasm. The role of *VRN-H2* in VS was confirmed and a novel alternative winter allele at *VRN-H3* was discovered in the Nebraska germplasm. *FR-H2* was identified as a determinant of LTT and a new QTL, *FR-H3*, was discovered on chromosome 1H that accounted for up to 48% of the phenotypic variation in field survival at St. Paul, Minnesota, USA. The discovery of *FR-H3* is a significant advancement in barley LTT genetics and will assist in developing the next generation of fall-sown varieties.

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FR-H3: A New QTL to Assist in the Development of Fall-Sown Barley with Superior Low Temperature Tolerance

by
Scott P. Fisk

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature bellow authorizes release of my thesis to any reader upon request.

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CONTRIBUTION OF AUTHORS

Dr. Patrick M. Hayes initiated the project and advised at every phase as it was carried out. Dr. Alfonso Cuesta-Marcos designed the custom OPA, advised in the genetic mapping, QTL analysis and all other statistical aspects as well as edits. Ann Corey conducted much of the data collection. Tanya Filichkin conducted the in-house genotyping and DNA preparation. Dr. Luis Cistue produced the doubled haploids. Dr. Kevin P. Smith conducted the low temperature tolerance work at SPMN. Dr. Stephen Baenziger provided the NE germplasm. Dr. Zoltan Bedo and Dr. Ildikó Karsai carried out the controlled environment studies at MRI. Dr. Robbie Waugh and Joanne Russel conducted the SNP genotyping.

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FR-H3: A New QTL to Assist in the Development of Fall-Sown Barley with Superior Low Temperature Tolerance

General Introduction

Barley is one of the founder crops of Old World agriculture (Badr et al. 2000). Evidence shows that barley was domesticated approximately 10,000 years ago in the Fertile Crescent from its wild relative, *Hordeum spontaneum*. Barley is believed to have been originally used as human food, eaten roasted and raw as well as in breads, soups, and porridges. Barley then evolved into an animal feed, malting, brewing and distillation grain (Ullrich 2011). Today it is utilized in all of these ways.

H. spontaneum is naturally a winter annual plant. Through domestication, spring growth habit was selected and spread around the world. Now the majority of cultivated barley is of spring growth habit. Therefore most of the selection done for malt quality has also been done in this spring germplasm base and the ability of barley to withstand winter conditions was not historically of primary interest. Winter barley has potential which has only recently been exploited. Growing a winter crop as opposed to spring has advantages in terms of disease, water usage and yield. In areas where fusarium head blight (FHB) is an issue the early maturity of the winter crop may lower the risk and it is also possible to grow the crop in areas where FHB is not an issue. In regions which receive ample winter precipitation dryland winter barley

yields are generally greater than that of spring barley and in irrigated areas fewer irrigation applications are required than with a spring crop.

One of the three main components of growth habit in barley is vernalization sensitivity (VS). VS is described as the requirement of a period of low temperature in order to transition from a vegetative to reproductive state (Karsai et al. 2005). The response to vernalization is controlled by three genes: *VRN-H1*, *VRN-H2*, and *VRN-H3*.

There are two different epistatic models proposed controlling VS. In *Triticeae* Yan et al. (2004) proposed that a dominant *Vrn2* allele is a repressor which inhibits the expression of the recessive *vrn1* allele. Vernalization down regulates *Vrn2* allowing for the expression of *vrn1*. Recessive *vrn2* alleles (deletion), regardless of the state of *VRN1* alleles will cause for the lack of VS. If *Vrn2* and *Vrn1* are both dominant alleles they will show minimal VS. *VRN3* was not included in the model because most cultivated barley is monomorphic for the recessive allele at this locus. Trevaskis et al. (2007) proposed a model where the *VRN-H2* allele represses VRN-H3 and blocks long day promotion of flowering before winter. *VRN-H2* is not expressed in the short days of the winter. After a prolonged exposure to the cold; *VRN-H1* is up-regulated, promoting inflorescence initiation and represses *VRN-H2*, allowing the long-day induction of *VRN-H3*, further enhancing the activity of *VRN-H1*.

Day length can also have an effect on the growth and development of barley similar to VS. Photoperiod sensitive plants require the day length to reach a critical threshold to become reproductive. The two main determinants of photoperiod

sensitivity (PS) are *PPD-H1* and *PPD-H2*. The recessive allele at *ppd-H1* causes barley to be insensitive to long photoperiod conditions (Turner et al. 2005). This is an advantage for spring barley as it increases the growing period allowing for the crop to be more productive (Jones et al. 2008). The candidate gene for *PPD-H1* is *HvPRR7* with the variation at this locus being due to changes in the amino acid sequence in the CCT domain (Turner et al. 2005). The allelic variation at *PPD-H2* (candidate gene *HvFT3*) is caused by a complete or partial deletion of the gene (Faure et al. 2007; Kikuchi et al. 2009). Genotypes which have the deletion are sensitive to short photoperiods thus staying vegetative during short days.

There are three growth habit types (winter, spring and facultative) all with variations of low temperature tolerance (LTT), VS and PS. Winter types are low temperature tolerant, vernalization sensitive and PS varies. Spring types are not low temperature tolerant, vernalization insensitive and short PS is irrelevant since they are grown under long day conditions. Facultative types are low temperature tolerant, vernalization insensitive and most often photoperiod sensitive (von Zitzewitz et al. 2011). This growth habit is attributed to a deletion at *VRN-H2*. The length of deletion causes a variation in flowering time with a complete deletion leading to the loss of VS (Szucs et al. 2007).

Vegetative LTT is an induced response requiring exposure of low, nonfreezing temperatures in order for the plant to reach its maximum level of tolerance. During this acclimation period hundreds of cold responsive genes are up and down

regulated (Fowler and Thomashow 2002). Once barley transitions to a reproductive state its LTT is reduced. PS (short day length sensitivity) and VS has the ability to help keep barley vegetative through the cold (short day) winter months.

Facultative types, being vernalization insensitive and low temperature tolerant, have many advantages to winter growth habit. Facultative varieties can be planted under spring or fall-sown conditions. This could serve as a safety net for farmers in case of serious winter injury they could replant the same variety in the spring. From a breeding perspective it could provide acceleration of generation advancement. Using the method of single seed decent under controlled conditions, vernalization sensitive germplasm must be subjected to a six week period of low temperatures between generations, unnecessary for facultative germplasm. It also makes it possible for offseason increases in areas where a vernalization requirement may not be met (von Zitzewitz et al. 2010). PS is a more reliable environmental cue than VS, especially in the ever-changing climate conditions, as vernalization requirements may be met well before the coldest part of the winter while sensitivity to short days would keep the plant vegetative throughout the winter months.

It is important to understand the driving force behind barley winter hardiness in order to target genes in the development of new barley varieties, increasing the speed and precision of selecting material with the traits of interest. There are two main determinants of LTT in the *Triticeae, FR-1* and *FR-2* (Francia et al. 2004; Skinner et al. 2005; Galiba et al. 2009). In barley they are termed *FR-H1* and *FR-H2* located

approximately 30 cM apart on chromosome 5H. *FR-H1* cosegregates with *VRN-H1* (candidate gene *HvBM5A*). It is not known whether the effect of *FR-H1* is pleiotropic or if it is tightly linked with *VRN-H1* (Francia et al. 2007). There is evidence for the latter as a low temperature quantitative trait locus (QTL) was mapped to the *FR-H1* region in the Dicktoo x Morex population which does not segregate for *VRN-H1* (Pan et al. 1994). However in support of the pleiotropic effect it has been found that vernalization saturation corresponds with maximum LTT (Limin et al. 2007). There are two physically linked clusters of more than 11 C-repeat Binding Factor (CBF) genes located at *FR-H2* (Francia et al. 2004; Skinner et al. 2005; Galiba et al. 2009). One or more of these genes are the candidate(s) for *FR-H2*. Evidence has been shown that variation in LTT due to this locus may be caused by variations in gene copy number (Stockinger et al. 2007) and/or the presence/absence of specific CBF genes (Francia et al. 2007).

Biparental QTL mapping has been used extensively to discover the genes involved with VS and winter hardiness (Hayes et al. 1993; Pan et al. 1994; Laurie et al. 1995; Karsai et al. 1997; Francia et al. 2004; Szűcs et al. 2006; Szűcs et al. 2007). The first QTL reported at the *FR* loci in barley, *FR-H1*, was in the Dicktoo x Morex population (Hayes et al. 1993). Recently a genome wide association mapping (GWA) study was performed in which marker/trait associations were identified at *FR-H1* and *FR-H2* confirming their importance (von Zitzewitz et al. 2011). The use of GWA mapping has some advantages to biparental QTL mapping as it allows for the use of

lines with a wider genetic base and lines already within a breeding program without the development of populations specifically for a QTL study.

Mapping of disease resistance genes has also been done using biparental mapping populations including the two most problematic diseases in Oregon's Willamette Valley, scald and barley stripe rust (BSR) (Chen et al. 1994; Hayes et al. 1996; Toojinda et al. 2000; Jensen et al. 2002; Castro et al. 2003; Vales et al. 2005; Cheong et al. 2006; Rossi et al. 2006; Li and Zhou 2011). Scald (incited by *Rhynchosporium secalis*) is a major foliar disease in barley. It is found in most production areas but most severe in areas where it is cool and moist. The pathogen can survive on seed, volunteer barley plants, alternate grass hosts and debris. The wind alone will not spread the conidia of *R. secalis* as it also needs splashing rain (Steffenson 1988). If the conditions are conducive scald can be a serious issue. Upon infection lesions are formed on the leaves, reducing the functional green leaf area on the plant thus making it less productive, having the greatest effect on kernel weight. There have been a number of QTL identified for scald resistance (Abbott et al. 1992; Garvin et al. 1997; Jensen et al. 2002; Cheong et al. 2006; Zhan et al. 2008; Li and Zhou 2011).

BSR (incited by *Puccinia striiformis* f. sp. *hordei*) was first reported in the United States in 1991 (Marshall and Sutton 1995). It is now one of the most prominent and detrimental diseases in the Western United States. Depending upon severity it is possible for this disease to completely devastate a crop. The optimal

conditions for infection are low temperatures in the evening (11-15°C) and free moisture (Stubbs 1985; Roelfs et al. 1992). Genetic resistance to BSR is necessary in regions where the disease is severe. Fungicide can be effective but expensive. Major effect genes and QTL have been identified for resistance (Toojinda et al. 2000; Castro et al. 2003; Yan and Chen 2008). Both BSR and scald have the ability to readily change and generate new virulence types. The pyramiding of resistance genes has the ability to provide higher levels of resistance as well as a more durable and stable resistance to both scald and BSR (Brown et al. 1996; Richardson et al. 2006).

One of the most important traits in barley, malting quality, is a composite trait with a more complex inheritance. With a market premium, malting quality is valuable and economically significant. Above all other grains barley possesses a perfect combination of starch, enzymes, flavors and aromas for this use. Szucs et al. (2009) summarized 154 malting quality QTL in barley and placed them on the Oregon Wolfe Barley map. These QTL are distributed throughout the entire genome. The quality traits of the greatest interest are grain protein, kernel plumpness, malt extract, enzymatic activity (alpha-amylase and diastatic power), wort protein, carbohydrate modification (beta-glucan and wort viscosity) and protein modification. The complexity of this trait makes successful breeding an arduous task, therefore the parents used in developing barley for malting are generally of acceptable quality already (Horsely and Harvey 2011).

The growing amount of genomic data gathered needs to be utilized in order to accelerate and advance the breeding process. There is an abundant amount of these resources available, including an extensive single nucleotide polymorphism (SNP) database as well as genotyping platforms. Close et al. (2009) developed a barley consensus map including all available SNP data, located in the barley HarvEST database (http://harvest.ucr.edu; verified 15 November 2011). These genomic tools make it possible to design custom genotyping platforms that can be used for biparental QTL mapping and GWA mapping, as well as facilitate in the use of marker assisted selection and genome wide selection schemes.

In this study we used two doubled haploid populations (N=111 and N=124) derived from crosses between an Oregon winter malting line and two low temperature tolerant Nebraska winter feed lines for biparental QTL mapping. The populations were thoroughly phenotyped for LTT, VS (flowering time and final leaf number), BSR, and scald severity. Both were genotyped with 384 SNP markers using a custom oligonucleotide pool assay (OPA) which was designed using the barley consensus map to maximize polymorphism, sufficiently cover the genome, and focus on specific areas of interest. The two populations were half-sibs so the two were also combined to form one large population (N=225) for analysis, in order to give a better estimate of effects. The principal research goal was to gain further understanding of LTT, VS and disease resistance in facultative/winter germplasm with the practical goal

of developing winter malting barley varieties with superior LTT, disease resistance and quality.

FR-H3: A New QTL to Assist in the Development of Fall-Sown Barley with Superior Low Temperature Tolerance

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ABSTRACT

Fall-sown barley will be increasingly important in the era of climate change due to higher yield potential and efficient use of water resources. Resistance/tolerance to biotic and abiotic stresses will be critical. Low temperature is an abiotic stress of great importance. Resistance to barley stripe rust (incited by *Puccinia striifomis* f. sp. *hordei)* and scald (incited by *Rhynchosporium secalis*) will be important in higher rainfall areas. Simultaneous gene discovery and breeding will accelerate the development of agronomically relevant germplasm. We developed two doubled haploid mapping populations using two lines from the University of Nebraska (NE) with superior cold tolerance and one line from Oregon State University (OR) with good malting quality and disease resistance: NB3437f/OR71 (facultative x facultative) and NB713/OR71 (winter x facultative). Both were genotyped with a custom 384 oligonucleotide pool assay (OPA). QTL analyses were performed for low temperature tolerance (LTT), vernalization sensitivity (VS), and resistance to barley stripe rust and scald. Disease resistance QTL were identified with favorable alleles from both NE and OR germplasm. The role of *VRN-H2* in VS was confirmed and a novel alternative winter allele at *VRN-H3* was discovered in the Nebraska germplasm. *FR-H2* was identified as a determinant of LTT and a new QTL, *FR-H3*, was discovered on chromosome 1H that accounted for up to 48% of the phenotypic variation in field survival at St. Paul, Minnesota, USA. The discovery of *FR-*

H3 is a significant advancement in barley LTT genetics and will assist in developing the next generation of fall-sown varieties.

INTRODUCTION

Winter malting barley is a relatively new crop to the world and certainly to the U.S. Only two varieties (Charles and Endeavor) have been approved by the American Malting Barley Association (AMBA). Both are two-rows, and on average 49% of the malting barley planted in the U.S. over the past 5 years have been six-rows. Both varieties lack sufficient low temperature tolerance (LTT) for extensive production in target environments (Obert et al. 2009). Selecting for improved LTT in a crop that has undergone intensive selection for the past ~10,000 years within a spring growth habit framework is a challenge and a necessity in a time of climate change. Fall-sown barley is higher yielding than spring-sown barley in regions that receive ample winter precipitation. Fall-sown barley conserves scarce water resources in regions where irrigation is required. The challenge is to ensure that the crop survives LT stresses. LTT alone is not a sufficient attribute for winter malting barley. Malting quality is a composite trait consisting of many component traits, each of which shows complex inheritance (Marquez-Cedillo et al. 2000). Resistance to diseases prevalent in target areas is essential, and quantitative resistance is preferred due to a higher probability of durability (Niks and Rubiales 2002).

LTT is the critical component of “winter-hardiness”. Two other associated traits are photoperiod sensitivity (PS) and vernalization sensitivity (VS) (Hayes et al. 1993). Maximum vegetative stage LTT requires cold acclimation, a process in which many cold responsive genes are up and down regulated (Fowler and Thomashow

2002). LTT is reduced with the vegetative to reproductive transition (Galiba et al. 2009). The associations of PS and VS with LTT are due to their involvement in the timing of this transition (Fowler et al. 2001; von Zitzewitz et al. 2005). PS delays the vegetative to reproductive transition under short days and promotes under long days. VS delays the transition until a sufficient number of "cold units" are achieved. VS can be satisfied, in target northern hemisphere environments, as early as December but there is still considerable risk of LT injury after VS is satisfied. PS, in contrast, will maintain plants in a vegetative stage until the risk of LT injury is past. At 45° N, for example, photoperiod reaches 10h light/24h on February 9.

PS, VS, and LTT define the three growth habits in barley (winter, facultative, and spring). As defined by von Zitzewitz et al. (2011), winter types have varying degrees of LTT, they have VS, and PS varies. Facultative types have varying degrees of LTT, they do not have VS, and for maximum LTT they should have short PS. Spring types do not have LTT, they are not VS, and if they have short PS it is usually not of agronomic importance since they are grown under long photoperiod conditions. Long photoperiod insensitivity is beneficial for spring types, increasing the growing period. A key point is that facultative and winter types show comparable variation in LTT: VS is not a prerequisite for maximum LTT. Despite the data in support of this assertion, it is still a widely-held perception that varieties with the best LTT also have VS. VS is determined by the epistatic interactions between alleles at three loci: *VRN-H1* (5H), *VRN-H2* (4H), and *VRN-H3* (7H). Winter genotypes are *Vrn-H2_/vrn-H1vrn-H1/vrn-*

H3vrn-H3 and all other allele combinations do not have VS (Szucs et al. 2007). Facultative types have a winter allele at the *VRN-H1* locus and a complete deletion of the *VRN-H2* locus (Karsai et al. 2005; von Zitzewitz et al. 2005; Szűcs et al. 2007). The determinant of *VRN-H1* is *HvBM5A*, a MADS-box floral meristem identity gene which promotes flowering after a period of cold temperatures (von Zitzewitz et al. 2005). The size of deletions in the critical region of the first intron of *VRN-H1* cause phenotypic variation in the degree of VS (Fu et al. 2005; Von Zitzewitz et al. 2005; Szucs et al. 2007). The determinant of *VRN-H2* is a zinc finger-CCT domain transcription factor which encodes a flowering repressor that is down-regulated by short days and the expression of *VRN-H1* (von Zitzewitz et al. 2005). In barley there are three *ZCCT* genes at this complex locus (*ZCCT-Ha, ZCCT-Hb*, and *ZCCT-Hc*) (Dubcovsky et al. 2005; Karsai et al. 2005). *ZCCT-Ha* is the candidate for *VRN-H2* (Dubcovsky et al. 2005). *VRN-H3* is an orthologue of the *Arabidopsis Flowering Locus T* (*FT*) gene (Trevaskis et al. 2007; Hemming et al. 2008; Kikuchi et al. 2009). *HvFT1* is the candidate gene for *VRN-H3*. Mutations in the first intron of *HvFT1* have been proposed to differentiate between dominant and recessive *VRN-H3* alleles (Yan et al. 2006). SNPs have also been reported in the promoter to better differentiate between dominant and recessive alleles (Cuesta-Marcos et al. 2010). Dominant alleles are associated with a lack of VS and may also lead to very early flowering (Yan et al. 2006). The expression of *HvFT1* is dependent upon day length: long days induce expression, which promotes flowering (Turner et al. 2005). The two main

determinants of photoperiod sensitivity are *PPD-H1* (candidate gene *HvPRR7*) and *PPD-H2* (candidate gene *HvFT3*). The variation at the *PPD-H1* locus is due to changes in the amino acid sequence in the CCT domain with the recessive allele conferring insensitivity to long photoperiod conditions (Turner et al. 2005). Variation at *PPD-H2* is caused by a complete or partial deletion of the gene leading to sensitivity to short photoperiods (Faure et al. 2007, Kikuchi et al. 2009).

There are two principal LTT QTL reported in the *Triticeae*. The first LTT QTL reported in barley was in the 'Dicktoo' X 'Morex' population (Hayes et al. 1993) and corresponds to what was subsequently designated as *Frost Resistance-1* (*FR-H1*) (Galiba et al. 1995; Francia et al. 2004). *Frost Resistance-2* (*FR-H2*) was discovered subsequently (Francia et al. 2004; Skinner et al. 2005; Galiba et al. 2009). In barley, *FR-H1* and *FR-H2* are approximately 30 cM apart on chromosome 5H. *FR-H1* cosegregates with *VRN-H1*, however it has not yet been shown if this is due pleiotropic effects of *VRN-H1* or to the effects of a tightly linked gene (or genes) (Francia et al. 2007). Underlying *FR-H2* are two physically linked clusters of more than 11 *C-repeat binding factor* (*CBF*) genes (Francia et al. 2004; Skinner et al. 2005; Galiba et al. 2009). A polymorphism in *HvCBF4* accounted for 31% of the variation in LTT based on controlled freeze tests (Francia et al. 2004). Phenotypic variation in LTT associated with *FR-H2* may be due to copy number variation in one or more CBF genes (Stockinger et. al. 2007).

Genome-wide association mapping (GW-AM) validated the importance of *FR-H1* and *FR-H2* (von Zitzewitz et al. 2011) and confirmed that maximum LTT is achieved with specific alleles at each of the two loci. The question remains: is LTT due only to *FR-H1* and *FR-H2* or are there other undiscovered determinants of this critical trait?

Developing winter malting barley adapted to target regions in the U.S. will also require resistance to biotic stresses. Barley stripe rust (BSR) (incited by *Puccinia striiformis* f. sp. *hordei)* is a disease of worldwide importance (Dubin and Stubbs 1986; Vales et al. 2005), and it is especially problematic in the Pacific Northwest (Chen and Line 2003). Many genes and QTLs conferring resistance to this disease have been mapped (Chen et al. 1994; Hayes et al. 1996; Toojinda et al. 2000; Castro et al. 2003; Vales et al. 2005; Rossi et al. 2006). Scald (incited by *Rhynchosporium secalis*) is also a major disease of barley. Although yield losses may not be as devastating as those caused by BSR, scald can cause significant decreases in grain quantity and quality (Brown et al. 1996). Many scald resistance genes and QTLs have also been reported (Abbott et al. 1992; Garvin et al. 1997; Jensen et al. 2002; Cheong et al. 2006; Li and Zhou 2011). Fungicides can be used to control BSR and scald. However it is much more effective, especially in sustainable cropping systems, to effectively deploy disease resistance genes (Li and Zhou 2011).

In order to advance our understanding of LTT genetics and to further the improvement of winter six-row malting barley, we developed two doubled haploid

(DH) populations from crosses between germplasm originating in the Pacific Northwest of the US (Oregon) and the Upper Midwest (Nebraska). The Oregon parent (OR71) has moderate LTT, is resistant to BSR, and was a candidate for release as a malting variety. The Nebraska parents (NB3437f and NB713) have high LTT, are susceptible to BSR, and lack malting quality. The DH populations were phenotyped for LTT, VS, BSR resistance, and scald resistance. QTL analyses were performed using each of the two populations, and the pooled data of the two half-sib populations, when appropriate.

MATERIALS AND METHODS

Germplasm

NB3437f (NB92711/P-954) and NB713 (P-954/Pennco) were chosen as donors of LTT based on the long-term record of Nebraska germplasm in the Uniform Barley Winterhardiness Nursery, conducted by the USDA ARS in cooperation with State Agricultural Experiment Stations since 2003. The “f” designation in NB3437f indicated facultative growth habit. In the seed lot we received from the University of Nebraska, we found that < 1% of plants grown without vernalization flowered under long day conditions. A report on this finding and subsequent characterization of the germplasm at the genotype and phenotype levels is presented in Supplemental Figure 1. OR71 (Stab 47/Kab 51-7) is an advanced selection from the Oregon State University breeding program. At the time this research was initiated, it was a candidate for release as a facultative malting barley variety. Subsequently, it was not advanced in the AMBA qualification process.

The two doubled haploid mapping populations, consisting of 111 and 124 lines, were derived via anther from the F1 of NB3437f/OR71 and NB714/OR71 by Dr. Luis Cistue, Aula Dei Experiment Station, Zaragoza, Spain as described in Cistue et al. (2003). OR71 and NB3437f are facultative (*VRN-H2* deletions) and we therefore expected the DH progeny to be facultative. NB713 was VS. Therefore, we expected the DH progeny of NB713/OR71 to segregate for VS.

Phenotyping

The two DH populations and parents were phenotyped for final leaf number (FLN) and flowering time (FT) under greenhouse conditions in vernalized and unvernalized treatments. Each DH line and parent was replicated twice within each treatment. The vernalized treatment consisted of planting seed directly into moist soil and maintaining the experiment in a growth chamber, without light, at 4°C for six weeks. Seedlings were then transferred to a greenhouse maintained at 18±2°C day and night. Supplemental light was used to provide a photoperiod of 16 h light/8 h dark. After one week of acclimation, seedlings were transplanted to 1.65 liter pots filled with Sunshine Professional Growing Mix. For the unvernalized treatment, seed was sown directly into 13.5 cm pots under the same greenhouse conditions. FLN was assessed as the total number of leaves on the main stem of each plant. FT was recorded as the number of days from planting to the appearance of awns 1-2 cm out of the boot (for unvernalized plants) and as the number of days from transfer to the greenhouse (for vernalized plants). The vernalized and unvernalized experiments were terminated 100 days after planting/transfer. Plants that did not flower were assigned an FT value of 150.

For disease resistance phenotyping and measurement of FT under field conditions (FTF), the two populations were grown in field trials at the Oregon State University Hyslop Farm near Corvallis, OR (COR). The NB3437f/OR71 population was sown in the fall of 2008 and the NB713/OR71 population in the fall of 2009. Each DH

line, and the parents, were planted in two-row plots, 1 m in length. BSR and scald severity were assessed visually using a scale of 0-100% based on the percent of plot canopy affected by each of the two diseases. FTF was recorded as the number of days after the January 1 when 50% of the heads in a plot were 50% emerged from the boot.

LTT was assessed in field experiments and in a controlled freeze test. The field experiments were conducted at St. Paul, MN (SPMN), Lincoln, NE (LNE), Fort Collins, CO (FCCO), and Fairfield, MT (FMT). LNE, FCCO, and FMT trials were planted in fall 2009. The SPMN trial was planted in fall 2010. Each DH line and parent was grown in single row plots using two replications. LTT was rated as the percent of plants in each plot surviving the winter. The controlled freeze tests were carried out at the Agricultural Research Institute of the Hungarian Academy of Sciences in Martonvásár, Hungary (MRI) as described by Skinner et al. (2006).

Genotyping

Genomic DNA was extracted from a single plant of each DH line and each parent using ~100mg of leaf tissue collected from greenhouse grown plants using the Qiagen DNeasy Plant Maxi Kit (Qiagen, Valencia, CA, USA). A custom 384 oligonucleotide pool assay (OPA) was designed based on polymorphisms identified between parents using Barley OPA 1 and Barley OPA 2 (Close et al. 2009). SNPs represented in the custom OPA (Supplemental Table 1) were selected to maximize

polymorphism, provide complete genome coverage, and to focus on specific genome regions/genes. Genotyping was performed using an Illumina BeadXpress at the James Hutton Institute (Invergowrie, Scotland). For quality assurance purposes, two markers were developed and assayed “in-house” based on two of the SNPs targeted in the custom 384-OPA (2_0653, and 3_0162). These markers were generated by identifying a restriction site polymorphism in the sequence flanking each of the SNPs, based on the OPA SNP sequence data obtained from the barley HarvEST database (http://harvest.ucr.edu; verified 9 November 2011).

Linkage Mapping

JoinMap 4 (Van Ooijen 2006) was used for construction of three linkage maps: NB3437f/OR71 (220 SNPs), NB713/OR71 (217 SNPs) and a combined map of the two populations created *de novo* using only the 157 markers polymorphic in both of the populations. A minimum LOD score of 4 was used in the formation of linkage groups. The maximum likelihood mapping algorithm was used to determine marker order in each linkage group. Recombination frequencies were converted to centiMorgans using Haldane’s mapping function as implemented in Joinmap 4.

QTL Analysis

QTL analyses were performed for 11 traits using each of the DH populations and the combined population using Composite Interval Mapping (CIM) as

implemented in Windows QTL Cartographer 2.5 (Wang et al. 2005). Up to seven cofactors were chosen using the CIM standard model with forward selection and backward elimination, 0.1 probabilities into and out of the model, a walk speed of 1 cM, and a scan window of 10 cM. Experiment-wise significance (α = 0.05) likelihood ratio test (LR) thresholds for QTL identification were determined with 1,000 permutations, and expressed as LOD (LOD = 0.217 LR). Multiple Interval Mapping (MIM) was used to evaluate epistatic interactions between QTL.

Allele effects for markers that were not assigned a position in the linkage map were estimated using PROC MIXED with the Maximum Likelihood (ML) method in SAS v9.1 (SAS Institute Inc., Cary, NC, USA). The cofactors and thresholds determined using QTL cartographer were used in calculating the significance of these unmapped markers. The proportion of variance explained by a marker at a position (R^2) was calculated using SAS PROC GLM as $R^2 = (MS_1 - MS_2)/MS_3$ where MS_1 is the mean square error including only the cofactors, MS_2 is the mean square error including the cofactors and the marker being tested and MS_3 is the total mean square for the trait. These unmapped markers are addressed in detail in the Results and Discussion sections. Broad-sense heritability values were estimated with SAS PROC VARCOMP using the following formula: $H^2 = \sigma_G^2/(\sigma_G^2 + \frac{\sigma_e^2}{r})$ where σ_G^2 represents the genetic variance, σ_e^2 the residual variance, and r the number of replicates per genotype.

RESULTS

Linkage Maps

Linkage maps are shown in Supplemental Figures 2, 3 and 4. Orders and distances of markers in the two population linkage maps, and in the combined map, are as expected for chromosomes 1H, 2H, 3H, 4H, and 6H. The chromosome 7H maps are as expected, except for three markers in the short arm that showed segregation distortion and do not map as expected with the rest of the linkage group in NB713/OR71. Markers in chromosome 5H did not map as expected in several regions. Based on the parental genotyping with BOPAs 1 and 2, we expected a ~24 cM region of monomorphism in the vicinity of *VRN-H1*. However, we found six discrete linkage groups in NB3437f/OR71 and two linkage groups in NB713/OR71 (Figure 1). As is apparent in this Figure, linkage distances on 5H are also much greater in each of the two populations than in the consensus map. Most markers adjacent to gaps, or areas of apparently high recombination, also show segregation distortion.

Low-Temperature Tolerance

Phenotypic assessment of LTT is a challenge. No DH line or parent survived at FMT. Nearly all lines in the NB713/OR71 population survived at FCCO. There was differential survival in the remaining tests. Phenotypic frequency distributions were diverse (Figure 2, Supplemental Figure 5), ranging from nearly bi-modal (NB713/OR71 - SPMN, considering the 100% surviving lines vs. “others”) to nearly

normal (both populations, MRI). In the MRI and SPMN trials, LTT patterns for the parents fit expectations: the Nebraska germplasm had superior LTT. In the LNE trials, the Oregon and Nebraska parents had similar levels of survival and there were positive and negative phenotypic transgressive segregants. In FCCO, OR71 was superior to NB3437f and the same as NB713. Overall, the two populations (one facultative and the other segregating for VS) had similar means, ranges, and distributions. Within the NB713/OR71 population there were no patterns of LTT survival for VS vs. facultative progeny (Supplemental Figure 6).

Based on prior reports, the most likely region for LTT QTL in barley is at *FR-H1* and/or *FR-H2* (reviewed by von Zitzewitz et al. 2011). However, due to the 5H linkage map issues addressed in the prior section, it is not possible at the current level of map resolution to unequivocally state that one or both of these loci are determinants of LTT in this germplasm. As shown in Figure 1, using the MRI data, there is a marker/trait association coincident with *FR-H2* in NB713/OR71 but it does not reach the significance threshold. In this population there is a significant effect at SNP 3_0850, with NB713 contributing the favorable allele based on an adjusted single locus test (Table 1). This marker maps to the *FR-H2* region in the consensus map but was highly distorted and did not coalesce with the 5H markers in the NB713/OR71 population. In NB3437f/OR71 there are significant effects for markers that map to the *FR-H2* region in the consensus map.

The largest effect QTL, which we designate as *FR-H3*, was detected in the SPMN and MRI data on the short arm of chromosome 1H. The best estimates of the position of this QTL are based on the individual populations because of the higher density of markers whereas the best estimates of effects are based on the combined population due to number of individuals. Considering each of the populations and data sets separately, the 2-LOD confidence intervals overlap for both populations at MRI and one of the populations at SPMN. However, the confidence intervals for the two populations at SPMN are contiguous. Therefore we believe that all peaks represent the same QTL. The maximum LOD in the combined population is 22.9 for the SPMN data and 8.6 for the MRI data (Figure 3). The Nebraska parents contributed the favorable allele at this QTL in the combined population and in each of the two populations. Additional QTL information is shown in Table 2. Most other QTL were environment and/or population-specific. Considering all environments and populations, QTL were detected on all chromosomes except 2H and 6H. Assuming QTL with overlapping confidence intervals (as well as the SPMN QTL) to represent the effects of the same gene or genes, a total of 8 distinct QTL were detected and at all but one of these environments (LNE) the Nebraska parent contributed the favorable allele.

Vernalization Sensitivity

Assessment of the two DH populations under greenhouse conditions allowed for assessment of VS (the unvernalized treatment) and flowering time per se (the vernalized treatment). We used two measures of the phenotype, FLN and FT. The latter is simpler to measure but the former is reported to be more accurate (Cuesta-Marcos et al. 2008, Baga et al. 2009, Sasani et al. 2009). The populations behaved as expected: NB3437f/OR71 did not segregate for VS and NB713/OR71 did segregate for VS. All DH lines, and the parents, in the facultative x facultative population (NB3437f/OR71) flowered within 95 days of planting in the unvernalized treatment and had a maximum FLN value of 17. The phenotypic frequency distributions for the vernalized and unvernalized treatments are very similar, with flowering times, on average, 10 days earlier in the vernalized treatment (Supplemental Figure 7). FLN values ranged from 8 to 15 in the vernalized treatment and from 10 to 17 in the unvernalized treatment (Figure 4). FLN values were the same for the two parents with vernalization and differed by two leaves in the unvernalized treatment. As with FT, there were positive and negative phenotypic transgressive segregants with both treatments. Comparison of flowering time in the field is confounded by the year effect, since the two populations were tested in different years. Overall, both populations showed a range of flowering times, spanning nearly one month. There were positive and negative phenotypic transgressive segregants in both populations, and OR71 was later than either of NB3437f or NB713.

VS in NB713/OR71 was determined by *VRN-H2* (Supplemental Figure 8 and Table 1). Expressed as QTL effects for FT, segregation of alleles at this locus had a LOD of 119 and an R^2 of 0.96. NB713 contributed the larger value (e.g. later flowering, higher FLN) allele. The corresponding values for FLN were LOD 28, and R^2 of 0.56. For FLN, there was also a QTL on chromosome 7H, at the position of *VRN-H3*, with a LOD of 6.1 and R^2 of 0.07, where OR71 contributed the larger value allele. The *VRN-H3* effect was also significant for FT in NB713/OR71 (unvernalized) with a LOD of 3.3 and it was highly significant for both FT and FLN in the unvernalized treatment for NB3437f/OR71. In this population, OR71 also contributed the later flowering and higher FLN alleles. With vernalization the primary determinant of FT per se, and FLN, in both populations was *VRN-H3*. The later flowering, and higher FLN allele, was contributed by OR71. Additional QTL were detected on chromosomes 3H, 4H, 5H, and 6H. The 4H QTL is coincident with *VRN-H2*.

Under field conditions, *VRN-H3* had the largest effect on FT, accounting for a five day additive effect, with OR71 contributing the later flowering allele. The magnitude and sign of this *VRN-H3* effect reflects the results obtained under greenhouse conditions. *PPD-H2*, on chromosome 1H, had a significant effect, but only in NB3437f/OR71. OR71 contributed the later flowering allele. Other lesser effect QTL were detected on chromosomes 3H, 6H, and 7H (short arm, not coincident with *VRN-H3*).

Disease Severity

Both populations displayed abundant phenotypic variation for resistance to BSR and scald (Figure 5). BSR disease severities ranged from 0 – 90% and 0 – 100% in NB3437f/OR71 and NB713/OR71, respectively. NB3437f and NB713 had BSR disease severities of 75% and 95% respectively, while OR71 had no disease symptoms. There were nearly equal numbers of DH lines with severities <30% and >70% in the NB713/OR71 population. The phenotypic distribution was not as discrete in NB3437f/OR71. The Nebraska parents, in the case of scald, were more resistant than OR71, with severities of 0 for NB3437f and 5% for NB713. Severities for OR71 were 22% and 10% in years 1 and 2. Scald severities ranged from 0 - 100% in NB3437f/OR71 and from 0 - 50% in NB713/OR71.

The principal QTL for BSR resistance, with OR71 contributing the favorable allele, was on chromosome 1H. The magnitude of this QTL (LODs of 36, 78, and 76 in NB3437f/OR71, NB713/OR71 and the combined population) indicates the effects of a major gene. QTL with smaller effects, with OR71 contributing the favorable alleles, were observed on chromosomes 1H, 4H, and 7H. All were population specific. The 7H QTL detected in NB3437f/OR71 and the combined population overlapped with the 7H QTL for scald resistance (see below). There was a small (R^2=0.07), but significant, interaction between QTL on 1H and 7H in NB3437f/OR71.

A total of four scald resistance QTL were detected. All were population specific. In NB3437f/OR71 a QTL on 7H suggests the effect of a major gene, with a

LOD of 30.9 and an R^2 of 0.68. NB3437f contributed the resistance allele at this QTL and at a QTL with lesser effect on 4H. In NB713/OR71 two QTL were detected, one on 2H and one on 7H. Both were smaller-effect QTL, accounting for 8% and 13% of the phenotypic variation respectively. In both cases, OR71 contributed the resistance allele.

DISCUSSION

LTT: Challenges and Opportunities

Phenotyping LTT is a challenge due to the difficulty in encountering field environments that provide accurate measures of differential survival. In this project, for example, we had nearly complete survival at FCCO and complete mortality at FMT. The lowest temperatures were quite similar for the field tests: SPMN = -27°C; LNE = -26°C; FCCO = -25°C; and FMT -32°C. The MRI freeze test temperature was -13.5°C. The absolute low temperature is just one of many factors in winter survival: acclimation conditions, snow cover, soil moisture, and plant growth stage are all very important factors. The MRI controlled environment test and the SPMN winter field survival datasets proved to be most informative in assessing LTT. These two data sets provided the greatest differential in survival and also had the highest heritabilities (0.92 and 0.78, respectively). Controlled freeze tests are very expensive ($90 per line) and therefore the principal constraint to using this approach is cost. However, controlled tests cannot reflect the full complexity of field survival. To date, SPMN has been a very informative environment. It provided the best estimates of *FR-H3* in this project and allowed for separation of *FR-H1* and *FR-H2* effects in previous research (von Zitzewitz et al. 2011).

The superior LTT of NE germplasm was recognized but the genetic basis was unknown. Understanding the genetic basis of a complex phenotype is a prerequisite for effective use of molecular breeding tools. Our discovery of *FR-H3* QTL will make it

possible to make efficient use of this unique germplasm. The only previous report of a LTT QTL on chromosome 1H was in the Dicktoo x Morex population. Dicktoo, like NB3437f, is a facultative and it also originated in the Upper Midwest. However, the 1H QTL in Dicktoo x Morex is likely a pleiotropic effect of *PPD-H2* and is distal to *FR-H3*. *FR-H3* had a very large effect in SPMN, accounting for an additive effect of 29% in the combined population. Additional research will be necessary to confirm the precise genome coordinates of this QTL and to proceed with isolating the gene (or genes) responsible.

When we developed these populations we did not expect to find a QTL at *FR-H1*. All parents have a degree of LTT and are monomorphic for all available BOPA 1 and BOPA 2 SNP markers within 5cM of *VRN-H1*/*FR-H1* (n = 27 markers). Even if there were contrasting alleles at *FR-H1* we would not be able to detect them at the current level of marker resolution. We hypothesized that we would detect a QTL at *FR-H2*, based on the presence of marker polymorphisms in the region and the difference in LTT between OR71 and the Nebraska parents. There are indications of a LTT QTL in the region, but the fragmentation of the 5H linkage map precludes definitive characterization. It is curious that linkage map construction was straightforward in all regions of the genome except for the one region, on 5H, known to contain genes determining LTT. Determining the cause(s) of fragmentation is an exciting challenge that will be addressed through localized sequencing, cytogenetic characterization, and high density mapping. Chromosome rearrangements (duplication or inversion)

are possible, but would be expected to lead to recombination "suppression" rather than "expansion". Furthermore, no confirmed inversions or duplications have been detected in the hundreds of molecular marker-based linkage maps constructed over the past 20 years in cultivated x cultivated and cultivated x ancestral barley crosses. Copy number of the CBF genes has been hypothesized to be related to the degree of LTT in barley (Stockinger et al. 2007), and a complex locus of multiple CBF genes underlies *FR-H2*. However, localized gene duplication would not be expected to cause issues in low-density linkage mapping. Heterozygosity in the 5H region in one or both parents is possible, but the genotyping of parents did not reveal heterozygosity (http://triticeaetoolbox.org/; verified 9 November 2011). Since multiple plants were used to make crosses, but not all were genotyped, it is possible that one or more plants used for crossing were heterozygotes. If this were the case, it suggests that retention of heterozygosity at this potentially critical region could be of adaptive significance.

Many minor LTT QTL were also found in addition to *FR-H2* and *FR-H3*, suggesting that this complex trait has major and minor determinants. Detecting, quantifying, and accumulating the essential, but minor-effect, alleles is likely beyond the scope of QTL mapping and marker assisted selection. These strategies will be effective in fixing favorable alleles at FR loci (*FR-H1*, *FRH-2*, and *FR-H3*) and genomic selection should be more effective for accumulating the minor alleles.

Vernalization Sensitivity: Validation and Discovery

The role of *VRN-H2* in determining vernalization sensitivity was confirmed: when grown under unvernalized conditions, all progeny of the facultative x facultative cross (NB3437f x OR71) flowered in a time frame comparable to that of a spring barley grown under the same conditions. The parents and all progeny are *VRN-H2* deletions. In the winter x facultative cross (NB713 x OR71) the parents behaved as expected and the progeny segregated for vernalization sensitivity. Further validation of the role of *VRN-H2* in vernalization sensitivity was the discovery of the facultative line, NB3437f, in the bulk seed of NB3437 received from the University of Nebraska breeding program. NB3437f flowered at the same time as spring barley checks, without vernalization, and carries the *VRN-H2* deletion. All non-flowering plants in the bulk seed lot tested positive for the *Vrn-H2* dominant allele (Supplemental Figure 1). The effect of *VRN-H3* on VS was unexpected. All reports to date, beginning with the original report by Takahashi and Yasuda (1971) state that there is no allelic variation at this locus except in very exotic germplasm. The Nebraska germplasm indeed has exotic ancestry: the variety Wong, released in Jiangsu, China in 1941 (http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1434053; verified 9 November 2011) figures in the pedigrees of both NB713 and NB3437f. The early flowering allele at *VRN-H3* traces to the Nebraska parents in both populations. In the currently accepted vernalization model (Yan et al. 2004; Trevaskis et al. 2007) the recessive allele at *vrn-H3* is necessary for

VS. Very early flowering is the phenotype of the dominant allele at *Vrn-H3*. Since NB713 has VS, its "earlier" allele is most likely an alternative recessive allele. If this alternative earlier flowering allele is triggered only under long days, as is the case of winter alleles (Faure et al. 2007) then the earlier allele would not increase the risk for low temperature damage due to a precocious vegetative to reproductive transition during the short days of winter.

LTT and Vernalization Sensitivity: Uncoupled

Several lines of evidence point to the "uncoupling" of VS and LTT in this germplasm. Overall, LTT values were the same in both populations and in the population segregating for VS there were lines with high survival in both the VS and non-VS categories. However, a QTL in the region of *VRN-H2* was detected in the NB713 x OR71 population under the controlled freeze test conditions of the Martonvasar phytotron. A possible explanation is that under controlled freeze tests the acclimation regime and timing of the freeze occurred at a point in plant development when VS was still effective in delaying the vegetative to reproductive transition and thus ensuring maximum LTT. Finally, the NB3437f variant persisted in the heterogeneous mixture that is NB3437 under the low temperature field selection conditions customary for the University of Nebraska breeding program. Under controlled environment conditions, NB3437f has an LTT value comparable to NB713 (Supplemental Table 2).

Resistance to Barley Stripe Rust and Scald

The very large-effect QTL for BSR resistance is on the short arm of 1H, as is *FR-H3*. The favorable alleles for LTT (NB3437f and NB713) and BSR resistance (OR71) are in repulsion, but are far enough apart (20cM in the combined map) that we recovered progeny with high survival and BSR resistance (Figure 3). This BSR resistance QTL is coincident with a major gene for resistance, *Rps4*, previously known as *Yr4* (von Wettstein-Knowles 1992). Toojinda et al. (2000) reported a BSR resistance QTL in this region accounting for 28-50% of the phenotypic variance, depending on environment. In our populations this QTL accounted for 58-88% of the phenotypic variance depending on population. This is the first report of BSR resistance in a winter barley background. The resistance donors could be either Kold or Strider. Both are winter six-rows released by the Oregon State University breeding program and this resistance has been effective against the spectrum of virulence encountered in the Pacific Northwest of the U.S. ever since the disease was first reported in the region in 1995 (Chen et al. 1995). Future breeding efforts in winter barley should include introgression of additional resistance genes/QTL alleles in order to maximize the likelihood of maintaining durable resistance.

All parents have some level of resistance to scald, although the Nebraska germplasm had higher levels of resistance. Interestingly, OR71 contributed the resistance alleles on 2H and 7HL in progeny derived from NB713 whereas NB3437f contributed the resistance alleles when crossed with OR71 on chromosomes 4H and

7H. This 7H QTL is coincident with *Rrs2* (Schweizer et al. 1995). The *Rrs2* gene seems to be a valuable resistance gene as few isolates have been shown to break its resistance (reviewed by Hanemann et al. 2009). *Rrs15* is a candidate for the QTL found in NB713/OR71 on 7HL. *Rrs15* is a scald resistance gene which originated from wild barley, *H*. *vulgare* ssp. *spontaneum* (Genger et al. 2005). Oregon and Nebraska germplasm has been selected for "field resistance" to scald, as the disease is endemic to both regions. Our scald resistance results are confounded by the year effect, and the severity of this disease can vary substantially depending on the timing and intensity of precipitation and temperature fluctuations. With alleles at a minimum of four loci segregating, and the alleles tracing to all parents, it will be possible to maximize the accumulation of scald resistance alleles in the OrNe germplasm.

Prospects for Disease Resistant, LT Tolerant Fall-Sown Barley

Fall-sown barley offers numerous advantages in many production environments. Likewise, within the fall-sown class, facultative types offer advantages to both breeders and producers. A pre-requisite to realizing these advantages is sufficient LTT, resistance to prevailing diseases, and grain that commands a premium in the marketplace. We have shown, with this research, that gene discovery and breeding can be coupled to generate new knowledge and useful facultative germplasm with good LTT and resistance to barley stripe rust and scald. However, we have not shown that this germplasm produces grain that will command a price

premium. The surest way to add value to barley is through malting quality. A supporting objective of this research was to couple LTT, disease resistance, and malting quality QTL detection. Prior assessment of the parental lines had confirmed that the Oregon germplasm could be expected to contribute BSR resistance, scald tolerance, and malting quality. The Nebraska germplasm was expected to contribute favorable alleles for LTT and scald. Unfortunately, a genetic analysis of malting quality in the full doubled haploid populations was not possible due to the confounding effects of low temperature injury (e.g. SPMN) or barley stripe rust (e.g. COR). A limited number of selected DH lines with LTT and disease resistance have been incorporated into the breeding program and malting quality analyses are in progress based on assessment of these lines in multiple environments. The limited numbers of these lines precludes biparental QTL mapping. However, these lines are included in an association mapping panel (n = 256) that will be assessed for multiple phenotypes, including malting quality, under the auspices of the Triticeae Coordinated Agricultural Project (TCAP) (http://triticeaecap.org/; verified 9 November 2011). The biparental QTL mapping described in this report, together with the results of the genome wide association mapping, are expected to effectively mine a multitude of favorable alleles from the Oregon x Nebraska germplasm for MAS and GS. In this way, we hope to contribute to realizing the potential of fall-sown malting barley. This market class is likely to be of increasing importance in an era of increasingly volatile climate change.

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Figure 1 Linkage maps of chromosome 5H in the NB3437f/OR71 and NB713/OR71 doubled haploid (DH) mapping populations and the 5H quantitative trait locus (QTL) scans for low temperature tolerance (LTT) as measured in a controlled freeze test at the Martonvasar Research Institute aligned with the 5H consensus map. Single nucleotide polymorphism (SNP) marker names are indicated on the left of each linkage group. Distances are given in Haldane cM on the right. The distances on the two DH maps are continuous with distances between unlinked groups (gaps) based on the consensus map

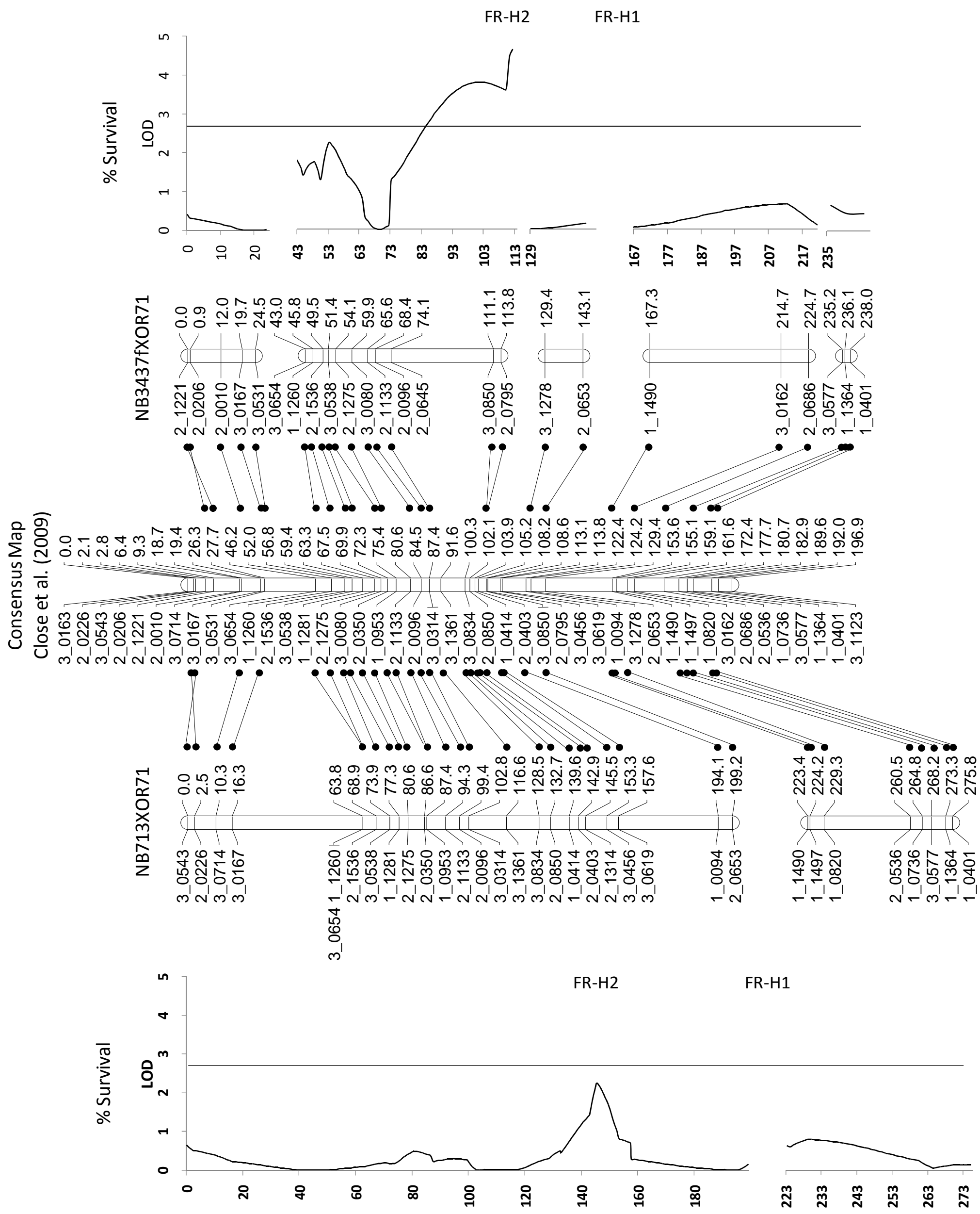

Figure 1

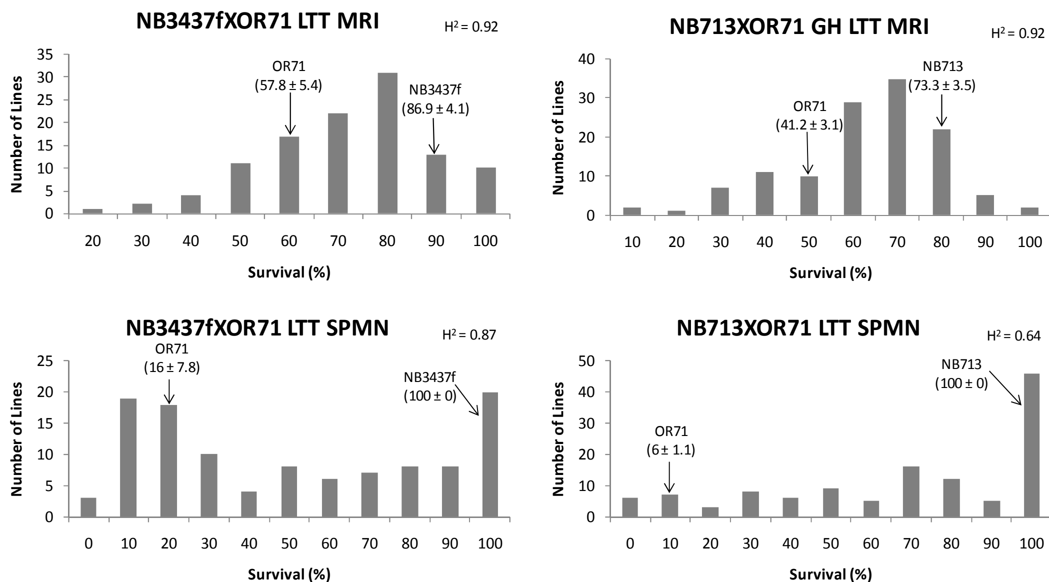

Figure 2 Phenotypic frequency distributions for low temperature tolerance (LTT) evaluated in the NB3437f/OR71 and NB713/OR71 doubled haploid populations in a controlled freeze test at the Martonvasar Research Institute (MRI) and under field conditions in St. Paul, Minnesota (SPMN). Standard deviations are shown for the parents. Broad-sense heritability (H^2) is also noted

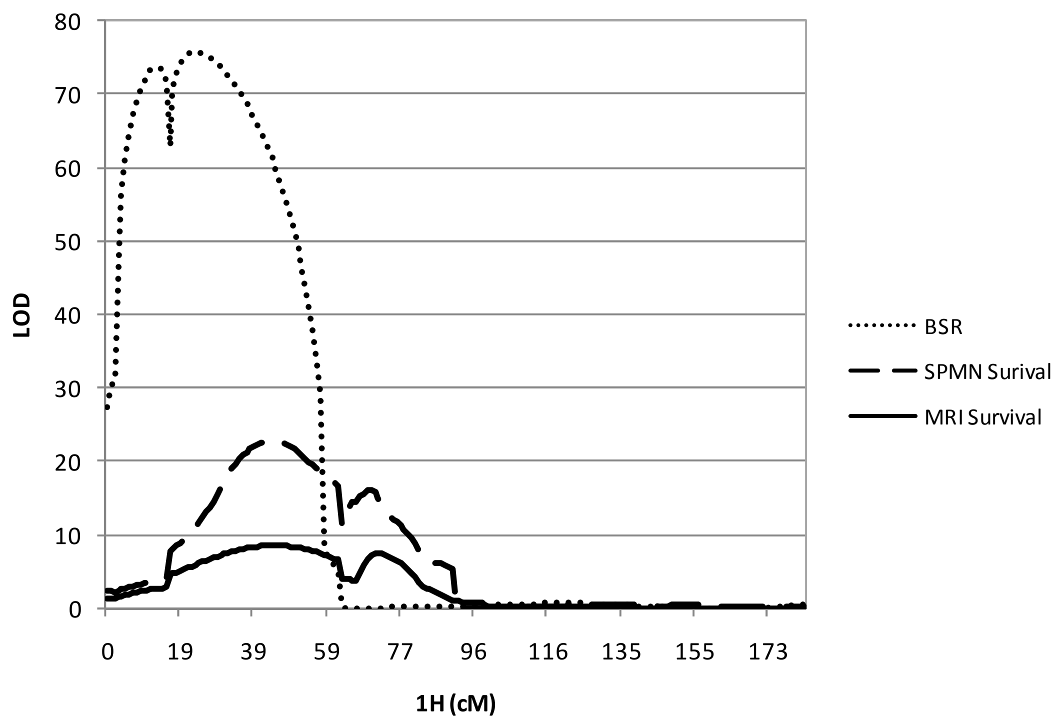

Figure 3 Quantitative trait locus (QTL) scans on chromosome 1H for barley stripe rust (BSR) evaluated under field conditions at Corvallis, OR (COR), low temperature tolerance (LTT) at St. Paul, Minnesota (SPMN) and LTT from the Martonvasar Research Institute (MRI) freeze test. A 20 cM scan window was used for this analysis, which is based on the combined map of two doubled haploid mapping populations: NB3437f/OR71 and NB713/OR71

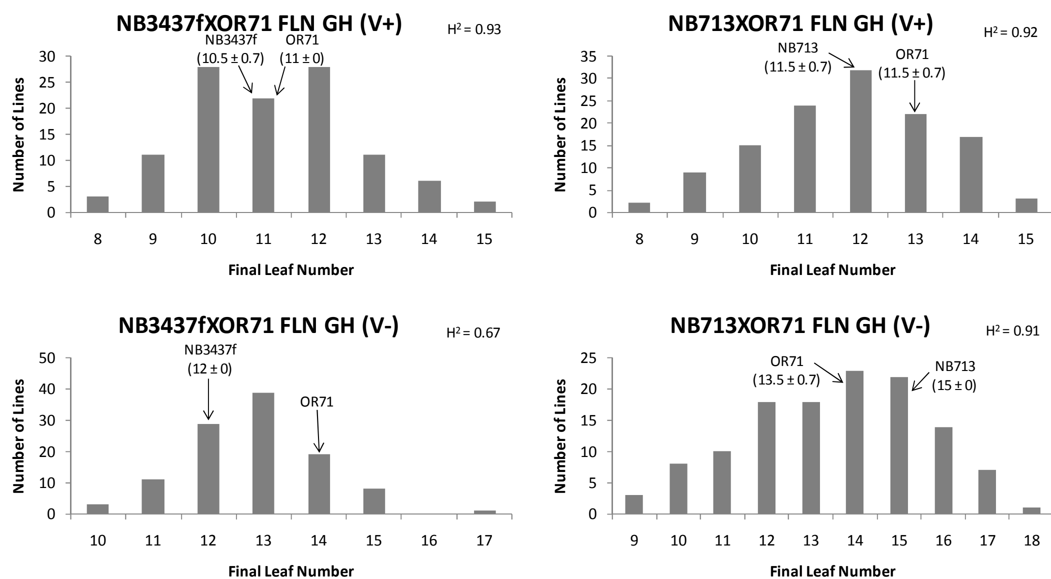

Figure 4 Phenotypic frequency distributions for final leaf number (FLN) evaluated for NB3437f/OR71 and NB713/OR71 doubled haploid populations. FLN were measured with and without vernalization (V+, V-) under greenhouse conditions. Standard deviations are shown for the parents when replicated. Broad-sense heritability (H^2) is also noted

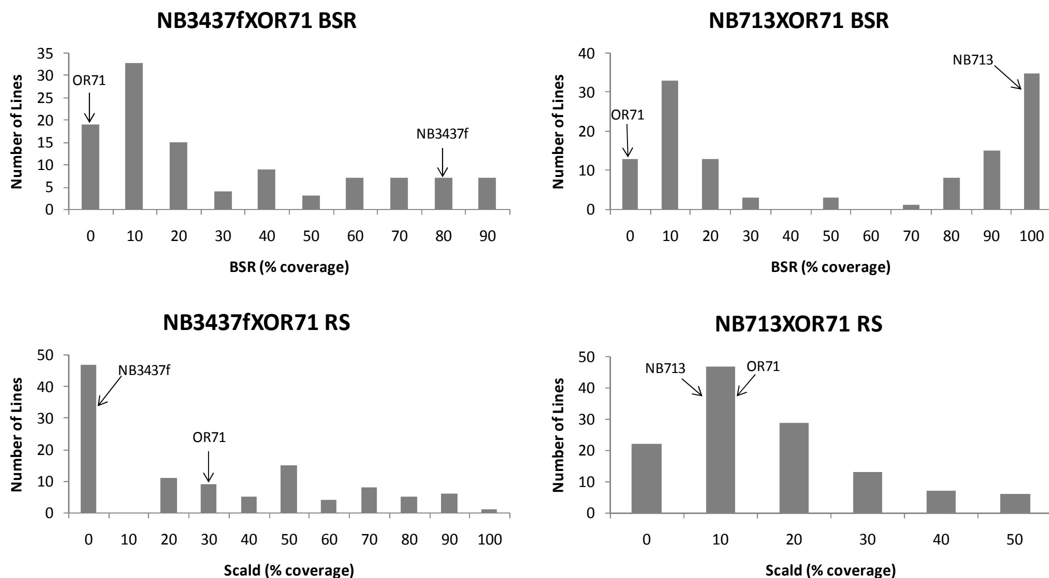

Figure 5 Phenotypic frequency distributions for barley stripe rust (BSR) and scald (RS) evaluated in the NB3437f/OR71 and NB713/OR71 doubled haploid populations. Both populations were evaluated for BSR and RS under field conditions at Corvallis, OR (COR)

Table 1 Summary of QTL detected in the NB3437fXOR71 (A) and NB713XOR71 (B) doubled haploid mapping populations. QTL alignment was based on overlapping 2-LOD confidence intervals. Refer to Supplemental Figures 1 and 2 for detail. Negative additive effect denotes higher value allele contributed by Nebraska parent. All table abbreviations are defined in the footnote

Table 1

Trait	QTL No.	Chromosome		Peak Position		2-LOD CI		Closest Marker**		LOD		R^2		Additive effect		LOD Threshold		MIM R^2	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
LTT-MRI																2.8	2.8	0.4	0.2
	1	1H	-	2.8	-	0-7	-	2_0502	-	3.5	-	0.07	-	-4.7	-				
	2	1H	1H	52.4	75.1	41-60	66-81	1_0938	3_0350	6.0	4.0	0.13	0.09	-6.3	-5.3				
	3	-	4H	-	186.9	-	185-187	-	3_0006	-	3.2	-	0.08	-	-5.7				
	4	5Hb	-	70.1	-	43-70	-	2_0795	-	4.6	-	0.11	-	-6.1	-				
	5	-	7Hb	-	162.9	-	160-166	-	1_0078	-	3.4	-	0.08	-	-4.9				
	6^	-	5H	-	NA	-	NA	-	3_0850	-	4.7	-	0.10	-	-7.2				
LTT-SPMN																2.9	2.9	0.6	0.3
	1*	1H	1H	47.7	42.0	44-52	21-56	3_1467	2_0749	23.2	9.0	0.48	0.27	-26.1	-17.5				
	2	3H	-	0.0	-	0-4	-	3_0192	-	3.3	-	0.04	-	-8.3	-				
LTT-NE																2.6	2.7	0.0	0.1
	1	-	4H	-	55.6	-	47-77	-	3_0864	-	3.9	-	0.16	-	3.9				
LTT-CO																2.7	2.7	0.0	0.1
	1	-	5Ha	-	90.4	-	86-97	-	1_0953	-	3.2	-	0.10	-	-6.0				
FT-F																2.9	2.9	0.8	0.8
	1	1H	-	95.9	-	90-103	-	*PPD-H2*	-	12.8	-	0.18	-	2.7	-				
	2	-	1H	-	67.6	-	48-86	-	3_1467	-	4.9	-	0.05	-	-1.4				
	3	-	1H	-	200.2	-	197-200	-	2_0915	-	3.1	-	0.03	-	1.1				
	4	3H	-	0.0	-	0-7	-	3_0192	-	4.3	-	0.05	-	-1.4	-				
	5	6H	-	80.3	-	70-86	-	3_1253	-	4.0	-	0.05	-	1.4	-				
	6	7H	7Hb	40.5	39.8	35-48	36-43	1_0232	1_0576	35.1	23.4	0.64	0.36	5.2	4.2				

^Marker was not included in map, marker/trait association was performed using SAS Proc Mixed with the same cofactors as QTL analysis
*The two LOD CI do not overlap however they are contiguous and believed to represent the same QTL
**Pilot OPA name
Table abbreviations: CI = Confidence Interval; MIM = Multiple Interval Mapping
Environments and treatments are coded as follows: LTT = low temperature tolerance; FT = flowering time; FLN = final leaf number; BSR = barley stripe rust; RS = scald; MRI = Martonvasar Research Institute; SPMN = St. Paul, Minnesota, USA; LNE = Lincoln, Nebraska, USA; FCCO = Fort Collins, Colorado, USA; F = field; GH = greenhouse; V+ = vernalized; V- = unvernalized

Table 1 Continued

Trait	QTL No.	Chromosome A	Chromosome B	Peak Position A	Peak Position B	2-LOD CI A	2-LOD CI B	Closest Marker** A	Closest Marker** B	LOD A	LOD B	R^2 A	R^2 B	Additive effect A	Additive effect B	LOD Threshold A	LOD Threshold B	MIM R^2 A	MIM R^2 B
FT-GHV+																2.8	2.8	0.6	0.5
	1	-	3H	-	139.1	-	135-143	-	1_0381	-	3.2	-	0.06	-	2.7				
	2	-	4H	-	186.9	-	175-187	-	3_0006	-	4.4	-	0.08	-	-3.2				
	3	-	6H	-	64.3	-	62-69	-	2_0058	-	3.4	-	0.06	-	-2.7				
	4	7H	7Hb	38.5	39.8	33-49	36-47	1_0232	1_0576	16.2	14.0	0.47	0.31	7.3	6.4				
FT-GHV-																2.9	3	0.6	1.0
	1	-	4H	-	177.6	-	174-180	-	2_1210	-	119.0	-	0.96	-	-43.3				
	2	7H	7Hb	39.5	43.7	34-48	43-47	1_0232	1_0576	23.1	3.3	0.56	0.01	6.6	3.7				
FLN-GHV+																2.9	2.8	0.7	0.6
	1	1H	-	61.1	-	52-65	-	2_0956	-	4.1	-	0.05	-	-0.4	-				
	2	-	3H	-	141.0	-	133-153	-	1_1154	-	7.1	-	0.11	-	0.6				
	3	3H	-	191.8	-	183-192	-	1_0283	-	3.9	-	0.05	-	-0.3	-				
	4	-	4H	-	5.5	-	3-7	-	1_0319	-	3.0	-	0.05	-	0.4				
	5	-	4H	-	186.9	-	181-187	-	3_0006	-	11.2	-	0.20	-	-0.7				
	6	5Hb	-	10.4	-	0-11	-	2_1275	-	5.9	-	0.08	-	-0.4	-				
	7	-	6H	-	54.7	-	53-70	-	2_0720	-	4.7	-	0.07	-	-0.4				
	8	7H	7Hb	39.5	49.0	33-49	37-63	1_0232	3_0143	19.5	9.9	0.40	0.18	1.0	0.7				
FLN-GHV-																2.9	2.8	0.8	0.7
	1	3H	-	37.7	-	36-41	-	1_0281	-	3.1	-	0.07	-	0.3	-				
	2	-	4H	-	184.5	-	180-187	-	3_0889	-	28.1	-	0.56	-	-1.6				
	3	6H	-	28.9	-	17-37	-	3_1485	-	4.4	-	0.10	-	0.4	-				
	4	7H	7Hb	39.5	47.0	33-51	36-54	1_0232	3_0143	11.8	6.1	0.37	0.07	0.8	0.6				

^Marker was not included in map, marker/trait association was performed using SAS Proc Mixed with the same cofactors as QTL analysis
*The two LOD CI do not overlap however they are contiguous and believed to represent the same QTL
**Pilot OPA name
Table abbreviations: CI = Confidence Interval; MIM = Multiple Interval Mapping
Environments and treatments are coded as follows: LTT = low temperature tolerance; FT = flowering time; FLN = final leaf number; BSR = barley stripe rust; RS = scald; MRI = Martonvasar Research Institute; SPMN = St. Paul, Minnesota, USA; LNE = Lincoln, Nebraska, USA; FCCO = Fort Collins, Colorado, USA; F = field; GH = greenhouse; V+ = vernalized; V- = unvernalized

Table 1 Continued

Trait	QTL No.	Chromosome A	Chromosome B	Peak Position A	Peak Position B	2-LOD CI A	2-LOD CI B	Closest Marker** A	Closest Marker** B	LOD A	LOD B	R^2 A	R^2 B	Additive effect A	Additive effect B	LOD Threshold A	LOD Threshold B	MIM R^2 A	MIM R^2 B
BSR																2.8	2.9	0.8	0.9
	1	1H	1H	12.5	19.3	12-16	16-22	3_0951	3_0951	35.9	78.2	0.58	0.88	-21.9	-39.9				
	2	1H	-	112.1	-	103-127	-	2_0021	-	4.5	-	0.05	-	-5.4	-				
	3	-	4H	-	3.5	-	0-8	-	1_1345	-	4.4	-	0.02	-	-5.7				
	4	7H	-	0.9	-	0-7	-	1_0894	-	6.5	-	0.12	-	-11.2	-				
	1X4											0.07	-	6.1	-				
RS																2.9	2.9	0.7	0.2
	1	-	2H	-	147.9	-	146-149	-	1_0475	-	3.3	-	0.08	-	-4.0				
	2	4H	-	6.6	-	3-15	-	1_0319	-	3.3	-	0.04	-	6.3	-				
	3	7H	-	0.9	-	0-2	-	1_0894	-	30.7	-	0.68	-	25.8	-				
	4	-	7Hb	-	174.9	-	169-187	-	3_1166	-	6.3	-	0.13	-	-4.7				

^Marker was not included in map, marker/trait association was performed using SAS Proc Mixed with the same cofactors as QTL analysis
*The two LOD CI do not overlap however they are contiguous and believed to represent the same QTL
**Pilot OPA name

Table abbreviations: CI = Confidence Interval; MIM = Multiple Interval Mapping
Environments and treatments are coded as follows: LTT = low temperature tolerance; FT = flowering time; FLN = final leaf number; BSR = barley stripe rust; RS = scald; MRI = Martonvasar Research Institute; SPMN = St. Paul, Minnesota, USA; LNE = Lincoln, Nebraska, USA; FCCO = Fort Collins, Colorado, USA; F = field; GH = greenhouse; V+ = vernalized; V- = unvernalized

Table 2 Summary of QTL detected in the half-sib combined mapping population constructed using 157 markers in common between the NB3437f/OR71 and NB713/OR71 doubled haploid populations. Only QTL which are coincident in both populations are shown. Negative additive effect denotes higher value allele contributed by Nebraska parent. All table abbreviations are defined in the footnote

Trait	Chromosome	Peak Position	2-LOD CI	Closest Marker**	LOD	R^2	Additive effect	LOD Threshold
LTT-MRI	1H	45.2	26-63	1_0764	8.6	0.19	-8.3	2.6
	5Hb	1.0	0-2	2_0795	15.9	0.21	-8.1	
LTT-SPMN	1H	44.2	36-52	1_0764	22.9	0.64	-28.9	2.7
FT-F	7H	75.3	71-81	1_0232	26.0	0.36	5.1	2.7
FT-GHV+	7H	76.3	70-82	1_0232	24.7	0.39	7.0	2.7
FLN-GHV+	7H	75.3	67-82	1_0232	18.8	0.32	0.9	2.7
BSR	1H	23.2	18-30	3_0951	75.6	0.82	-35.0	2.7

**Pilot OPA name
Table abbreviations: CI = Confidence Interval; MIM = Multiple Interval Mapping
Environments and treatments are coded as follows: LTT = low temperature tolerance; FT = flowering time; FLN = final leaf number; BSR = barley stripe rust; MRI = Martonvasar Research Institute; SPMN = St. Paul, Minnesota, USA; F = field; GH = greenhouse; V+ = vernalized

GENERAL CONCLUSIONS

The selection of predominantly spring growth habit in barley has caused for the lack of low temperature tolerance (LTT) in fall-sown barley today. Therefore most selection for malting quality has also occurred in spring types. Thus the malting quality in winter barley has lagged behind that of spring. Some of the genetic diversity available to improve LTT and malting quality in winter barley has been exploited but obviously there is more yet to be discovered and utilized.

This is the first biparental QTL mapping population based on facultative x facultative and winter x facultative germplasm for LTT and vernalization sensitivity (VS). All previous biparental QTL mapping populations assessed were facultative/winter x spring crosses. The cross was between Oregon (moderate LTT) and Nebraska (superior LTT) germplasm in hopes of finding new QTL which may not have been found in a study with such drastic phenotypic and genotypic difference using a cross with a spring type. The Nebraska pedigrees have not been previously studied and the material has exotic ancestry. Wong, a variety developed in China, is in the pedigree of both Nebraska parents.

Using this unique germplasm base it was possible to discover QTL which have been previously reported and characterized, e.g. *PPD-H2*, *FR-H2*, *VRN-H2* and *VRN-H3* as well as the newly discovered QTL, *FR-H3*. Finding *VRN-H3* was unexpected as most cultivated barley is fixed at this locus. The exotic ancestry of the Nebraska material

may be the source for this variation. Both alleles, although the Nebraska allele causes earlier flowering, may fall into the “winter” (late flowering) category with an alternative winter allele at this locus.

PPD-H2 and *VRN-H3* had a significant effect on flowering time but played no role in LTT. Delaying the vegetative to reproductive transition could be a beneficial attribute to avoid the risk of winter injury. If the “early” flowering due to *VRN-H3* only occurs under long day conditions then the transition is most likely happening after the danger of winter injury has passed and may have a neutral effect. A deletion of *PPD-H2* causing short day photoperiod sensitivity, while not beneficial in this case may be in others.

Genome-wide association (GWA) mapping is now being used more frequently for marker/trait associations as it possible to use actual breeding lines without the need to develop a specific population for analysis. However biparental QTL mapping has some advantages to GWA mapping in terms of discovering new QTL. In cases where an allele may exist but in low frequency, GWA mapping may not pick up a significant association (Keurentjes et al. 2011). This is not an issue in biparental QTL mapping. This may or may not have been the case as the parents for the two populations have been included in a GWA mapping study of LTT in which there were no significant marker/trait associations found at the location of *FR-H3* on 1H.

The result of this study is of great significance for the advancement of LTT in barley. The newly discovered *FR-H3* and the superior Nebraska *FR-H2* will serve as

critical targets for future introgression of LTT. Over 200 doubled haploid lines were developed between the two mapping populations and while the progeny may not prove to posses all favorable characteristics, superior LTT, disease resistance and malting quality, they do possess some of these characteristics providing a new source of these key LTT genes for future crosses. Finding undiscovered QTL shows that the search for more LTT QTL should continue as well as the identification of candidate genes and causal polymorphisms.

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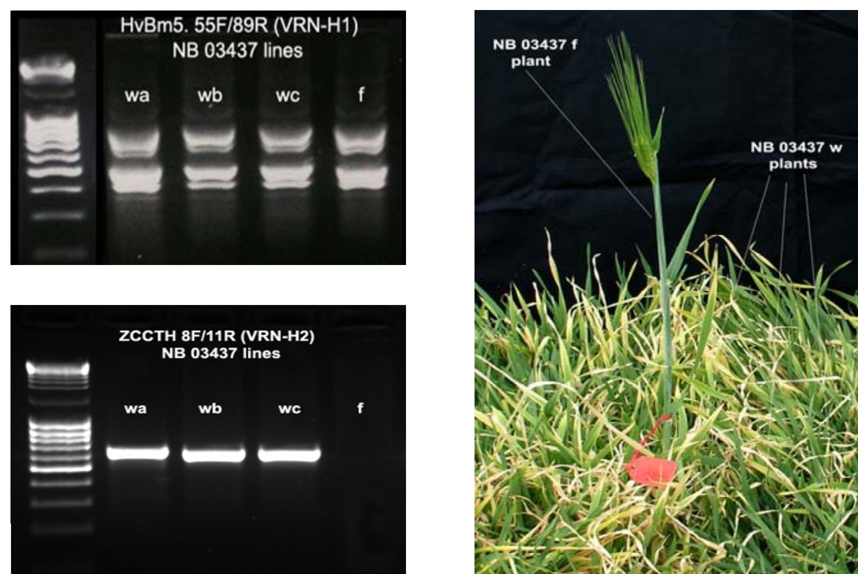
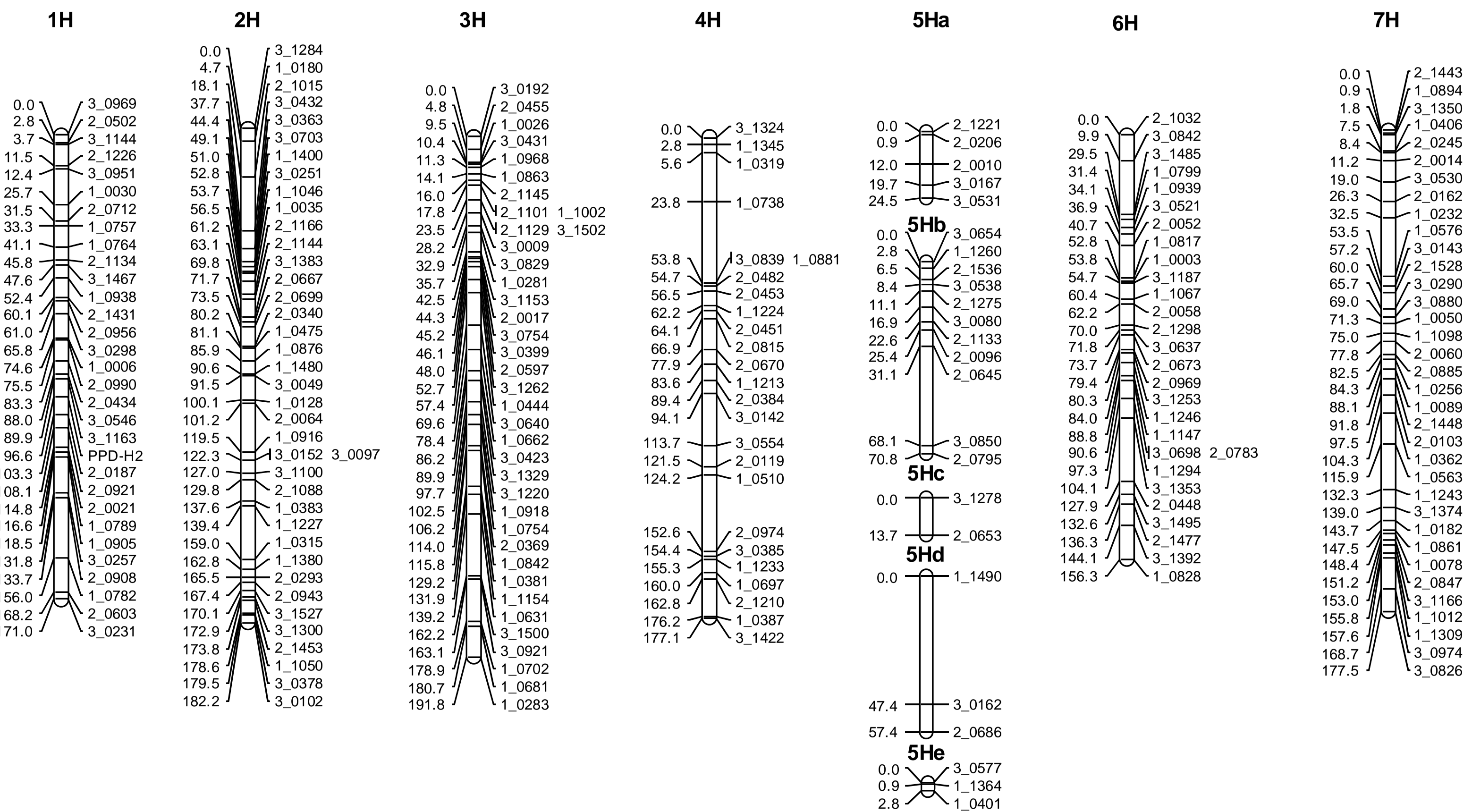
APPENDIX

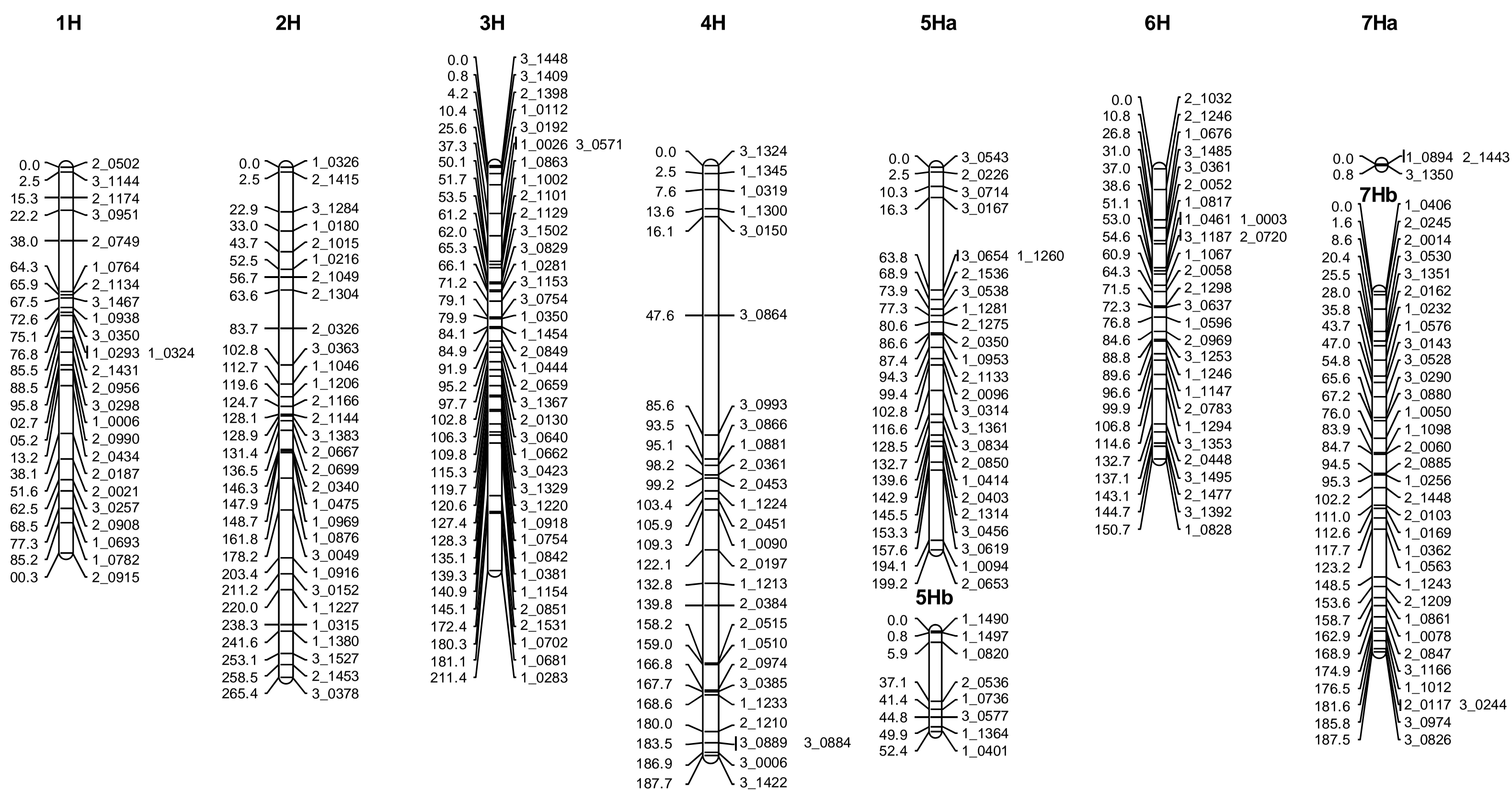


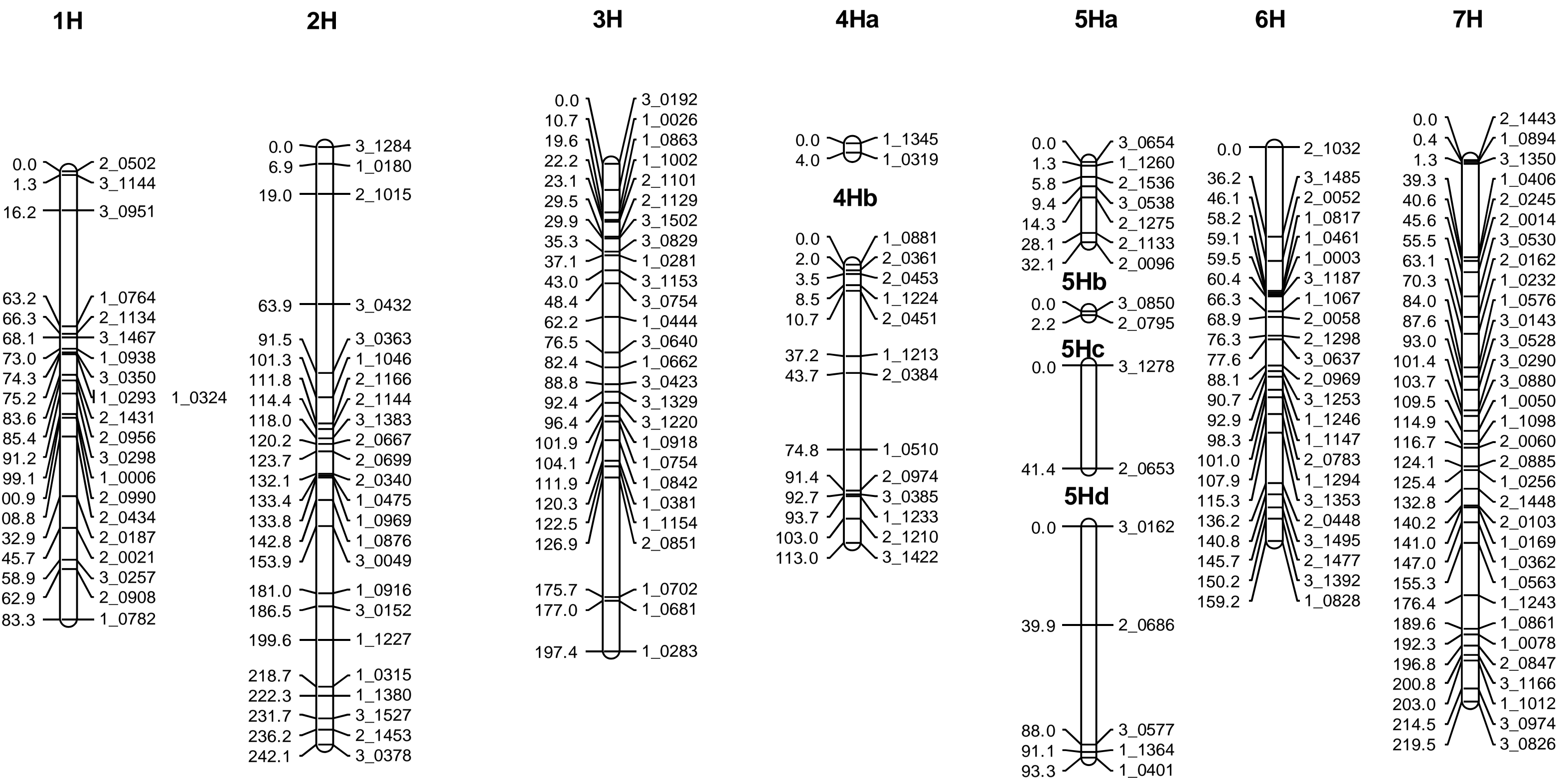
Supplemental Figure 1 Genotype (left panels) and phenotype (right panel) images for one of two NB3437 facultative (f) plants in a population of ~ 200 winter (w) plants. Results were consistent for both 'f' vs. all 'w' plants. All 'f' and 'w' plants had a full-length first intron in *HvBM5 (VRN-H1) (upper left). ZCCT-H* (*VRN-H2*) was present in all 'w' plants and deleted in the two 'f' plants (lower left)

Supplemental Figure 2 NB3437f/OR71 linkage map constructed using a custom 384 oligonucleotide pool assay (OPA). Single nucleotide polymorphism (SNP) marker names are indicated on the right of each linkage group. Distances are given in Haldane cM on the left

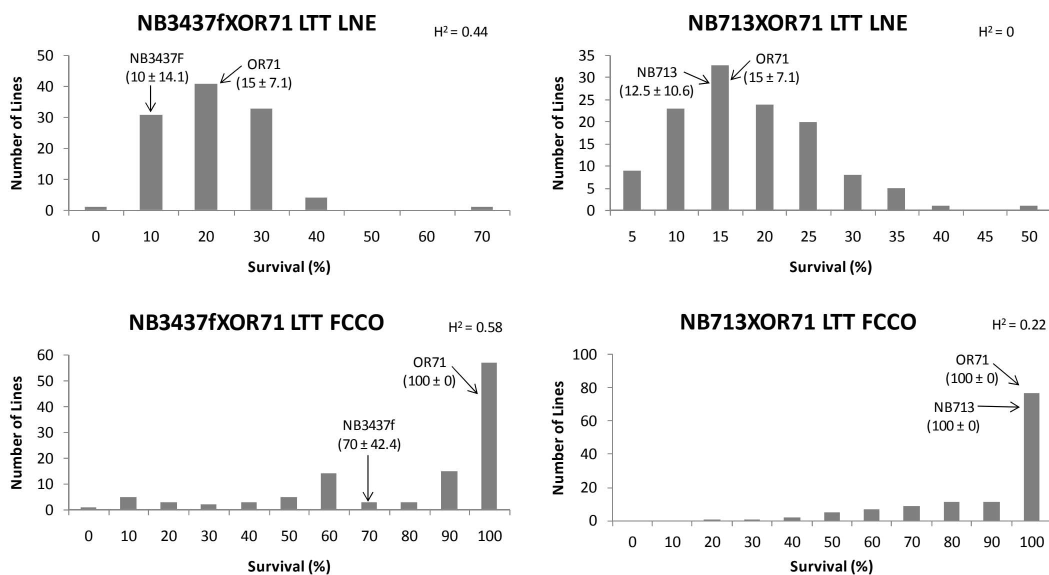


Supplemental Figure 3 NB713/OR71 linkage map constructed using a custom 384 oligonucleotide pool assay (OPA). Single nucleotide polymorphism (SNP) marker names are indicated on the right of each linkage group. Distances are given in Haldane cM on the left

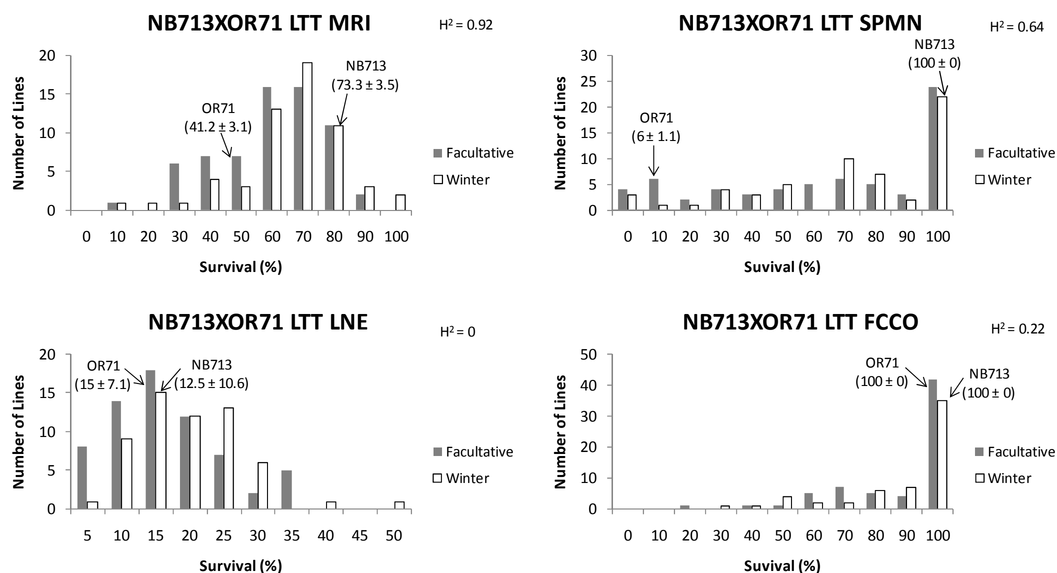




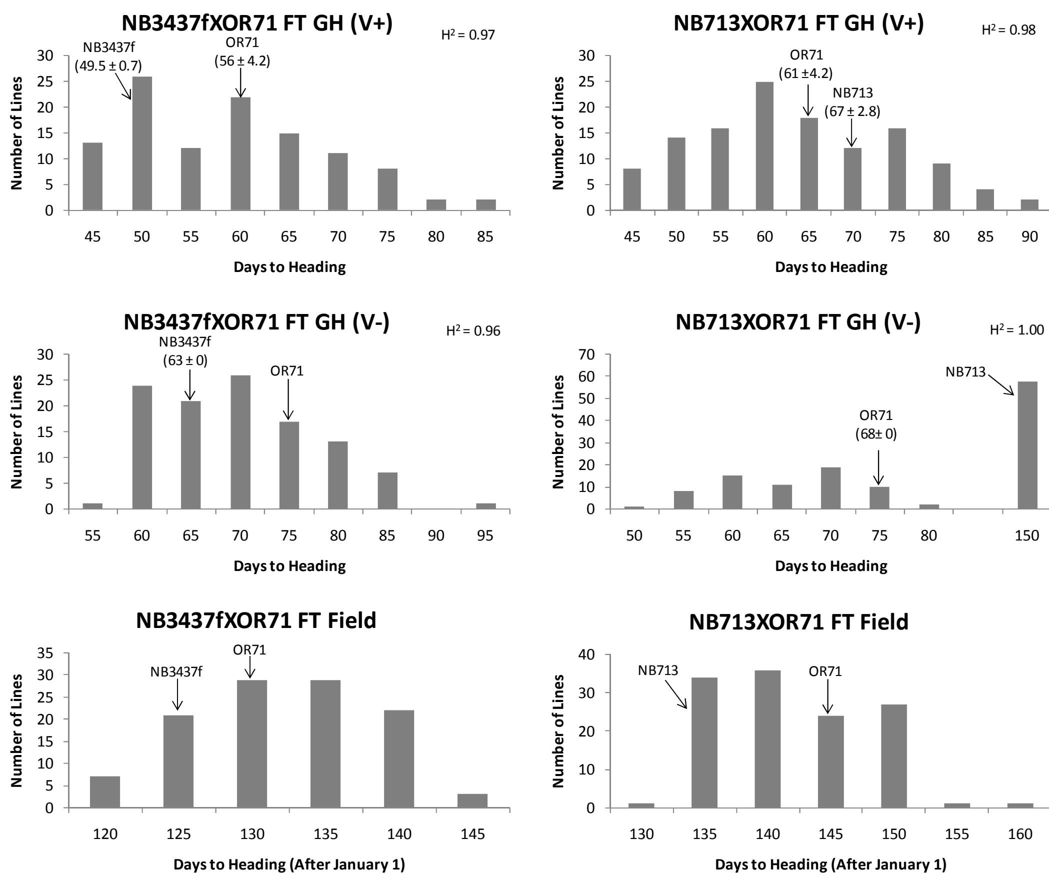
Supplemental Figure 4 Combined linkage map constructed using a custom 384 oligonucleotide pool assay (OPA). The map was created de novo using only single nucleotide polymorphism (SNP) markers segregating for both NB3437f/OR71 and NB713/OR71 doubled haploid populations. SNP marker names are indicated on the right of each linkage group. Distances are given in Haldane cM on the left



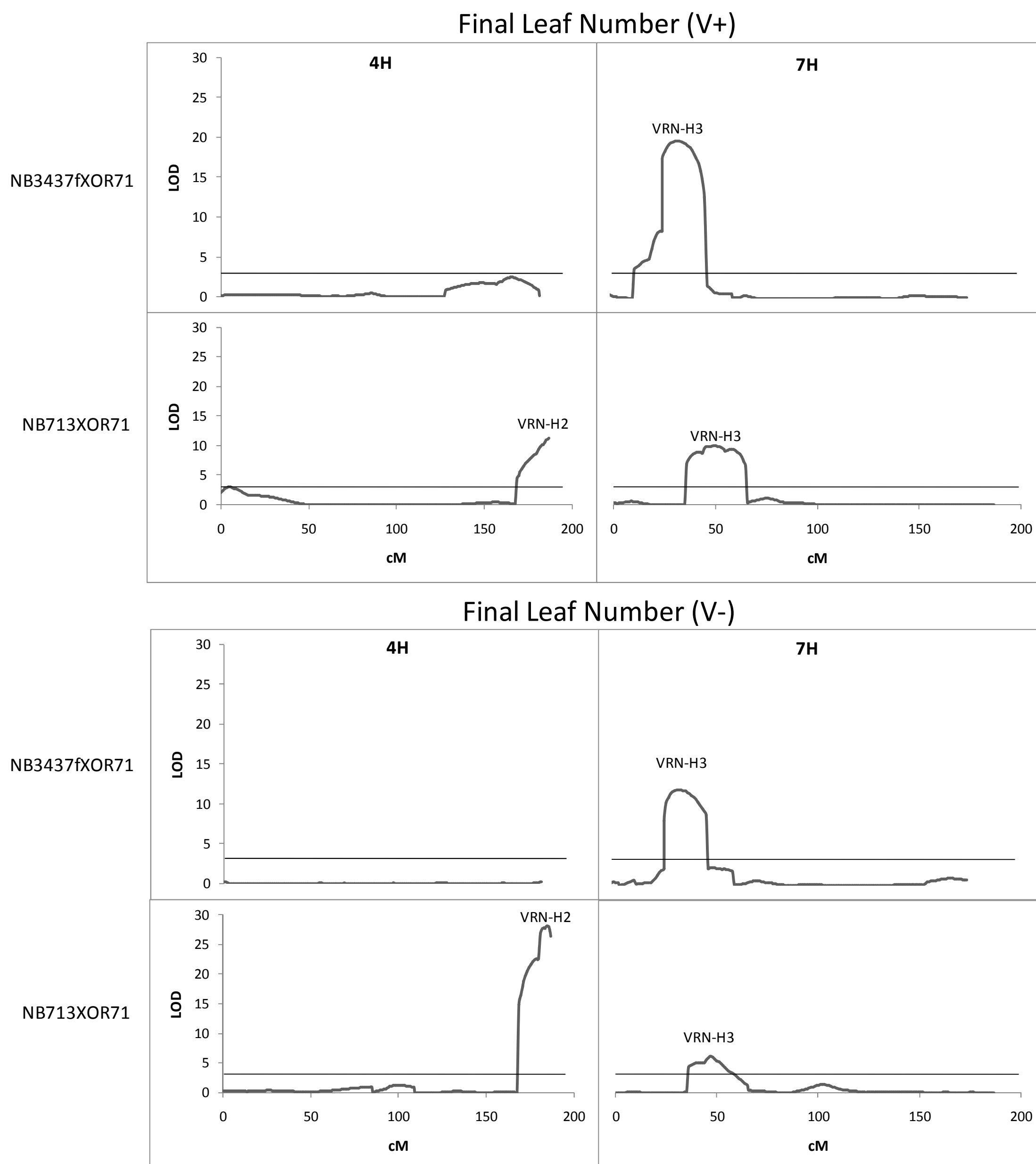
Supplemental Figure 5 Phenotypic frequency distributions for low temperature tolerance (LTT) evaluated in the NB3437f/OR71 and NB713/OR71 doubled haploid populations under field conditions in Lincoln, Nebraska (LNE) and Fort Collins, Colorado (FCCO). Standard deviations are shown for the parents. Broad-sense heritability (H^2) is also noted



Supplemental Figure 6 Phenotypic frequency distributions for low temperature tolerance (LTT) evaluated in the NB713/OR71 doubled haploid population in a controlled freeze test at the Martonvasar Research Institute (MRI) and under field conditions in St. Paul, Minnesota (SPMN), Lincoln, Nebraska (LNE), and Fort Collins, Colorado (FCCO). Standard deviations are shown for the parents. Broad-sense heritability (H^2) is also noted



Supplemental Figure 7 Phenotypic frequency distributions for flowering time (FT) evaluated for NB3437f/OR71 and NB713/OR71 doubled haploid populations. Both populations were evaluated for FT under fall planted field conditions at Corvallis, OR (COR). FT was also measured with and without vernalization (V+, V-) under greenhouse conditions. Standard deviations are shown for the parents when replicated. Broad-sense heritability (H^2) is also noted in replicated trials



Supplemental Figure 8 Quantitative trait locus (QTL) scans for final leaf number (FLN) on chromosome 4H and 7H for NB3437f/OR71 and NB713/OR71 doubled haploid populations evaluated in the greenhouse (GH) under both vernalized (V+) and unvernalized (V-) conditions

Supplemental Table 1 Single nucleotide polymorphism (SNP) markers represented in the custom 384 oligonucleotide pool assay (OPA). Designed at Oregon State University for genotyping the NB3437f/OR71, NB713/OR71 doubled haploid populations and two other BC1 populations used in a concurrent study, at The James Hutton Institute. The custom OPA was designed based on polymorphisms identified between parents using Barley OPA 1 and Barley OPA 2 (http://triticeaecap.org/; verified 15 November 2011). Position is according to the consensus map (Close et al. 2009)

Pilot OPA name	Chromosome	Position (cM)	Locus_Name
3_0969	1H	0	SCRI_bbc15015_01_164
2_0502	1H	1.5	3220-723
3_1144	1H	3.8	U35_16501_168
2_1174	1H	8.3	6949-895
2_1226	1H	8.8	7372-1253
3_0951	1H	11.4	SCRI_bbc05112_01_2
2_0749	1H	17.3	4473-309
1_0030	1H	18.1	10922-503
2_0712	1H	20.8	4226-570
1_0757	1H	23.9	5318-436
3_1276	1H	27.4	U35_19740_954
2_0617	1H	33.6	3751-1136
3_1177	1H	37	U35_17276_547
1_0764	1H	41	5381-1950
2_1134	1H	43.3	6720-641
3_1467	1H	47.5	U35_5390_330
1_0938	1H	50	7833-315
3_0350	1H	52.5	U32_12209_166
1_0293	1H	55.5	2401-1028
1_0324	1H	57	2577-1122
3_0821	1H	59.7	OSU_Aglu3_536
2_0997	1H	60.2	5790-558
3_0304	1H	61.5	U32_10148_338
2_1431	1H	64.9	9638-619
2_0956	1H	66.7	5547-294
3_0298	1H	69.5	AY972619_MX_Nec1_2066
1_0006	1H	73.9	1016-376
2_0990	1H	75.5	5772-1176
2_0657	1H	77.3	4005-530
2_0434	1H	88.2	2881-935
3_0546	1H	92.8	U32_4421_1432
3_1163	1H	94	U35_1704_1053
3_1192	1H	96.9	U35_17735_1334
2_0187	1H	101.5	1498-596
2_0921	1H	105.9	5336-400
2_0625	1H	106.6	3786-2204
2_0021	1H	109.8	10360-563
3_0532	1H	109.8	U32_4190_2397
1_0789	1H	112.5	5690-1045

Supplemental Table 1 Continued

Pilot OPA name	Chromosome	Position (cM)	Locus_Name
3_0014	1H	113.3	ABC02471_2_350
1_0905	1H	114.8	7389-555
1_0729	1H	116.3	5048-1685
3_0257	1H	117.8	ABC16431_1_600
2_0908	1H	121.1	5283-1090
2_1068	1H	126.5	6201-1190
3_1377	1H	126.5	U35_326_272
1_0693	1H	128.1	472-1376
1_0782	1H	131.9	5555-438
2_0603	1H	135.6	3671-59
2_0915	1H	138.3	5316-739
3_0231	1H	138.3	ABC14709_1_359
3_1081	1H	140.5	U35_1042_538
2_1415	2H	2.9	9452-210
1_0326	2H	6.5	2582-767
2_1377	2H	8.6	8930-370
1_0943	2H	18.3	791-1113
3_1284	2H	19.5	U35_20027_279
1_0180	2H	21.6	1865-396
1_0216	2H	26.5	2029-1143
2_1015	2H	27.3	5880-2547
2_1366	2H	28.4	8787-1459
1_0787	2H	31	5652-419
2_1049	2H	32.3	6086-690
2_1304	2H	33.7	816-265
1_0296	2H	39.1	2416-1016
2_0326	2H	39.1	2232-1685
3_0432	2H	41.7	U32_2438_479
3_0363	2H	45.6	U32_1342_283
3_0703	2H	49	U32_7446_193
1_1400	2H	53.5	ABC20402-1-3-298
3_0251	2H	56.3	ABC15603_1_183
1_1046	2H	59.2	ABC01644-1-3-379
1_1206	2H	62.8	ABC08774-1-1-752
1_0035	2H	62.8	1114-892
2_1166	2H	66.8	6911-866
2_1144	2H	69.3	6804-1197
1_0265	2H	70.5	2284-1738
2_0667	2H	72.3	4037-916
3_1383	2H	73.8	U35_3378_1098
3_0178	2H	75.9	ABC11711_1_344
2_0699	2H	78	4164-176
2_0340	2H	85.9	2322-462
1_0475	2H	88.7	3469-1152
1_0969	2H	90.1	8397-432
1_0214	2H	93.5	2020-539
2_1175	2H	96.8	6951-875
1_0876	2H	100.4	682-767

Supplemental Table 1 Continued

Pilot OPA name	Chromosome	Position (cM)	Locus_Name
2_1527	2H	101.8	ConsensusGBS0335-1
3_0049	2H	106.5	ABC06173_1_575
1_1480	2H	108.6	ConsensusGBS0348-2
2_0064	2H	112.9	111-499
1_0128	2H	113.5	1584-263
1_0916	2H	117.9	7576-818
3_0097	2H	121.5	ABC08212_1_434
3_0152	2H	122.2	ABC10730_2_206
3_1100	2H	125.5	U35_1470_1376
2_1088	2H	128.3	6328-736
1_0383	2H	130	285-2932
1_1227	2H	133.2	ABC09559-1-2-143
1_0315	2H	141.3	252-556
1_1380	2H	145	ABC17314-1-1-226
2_0293	2H	147.9	2052-792
2_0943	2H	149.6	5483-787
3_1527	2H	151.4	U35_851_373
3_1300	2H	152.8	U35_20591_995
2_1453	2H	155.3	9910-427
1_1050	2H	156.7	ABC01852-1-1-214
3_0378	2H	158.9	U32_14697_157
3_0102	2H	160.3	ABC08520_1_180
3_1448	3H	2.9	U35_4975_534
3_1409	3H	6.7	U35_3907_2125
2_0529	3H	8.2	3344-1147
2_1398	3H	8.2	918-480
1_0112	3H	10.8	1499-290
2_0172	3H	16.3	1440-1148
3_0192	3H	23.5	ABC11988_1_747
2_0455	3H	28.4	2995-717
3_0284	3H	28.4	ABC22737_1_28
1_0026	3H	32.8	1074-992
3_0571	3H	32.8	U32_4926_1136
3_0431	3H	35.2	U32_2436_2433
1_0672	3H	37.2	4593-2007
1_0968	3H	38.7	8387-187
1_0863	3H	41.7	6634-263
2_1145	3H	42.5	6805-1110
1_1002	3H	44	9018-522
3_0064	3H	46.3	ABC06946_1_359
3_0737	3H	48.6	U32_8184_522
2_1101	3H	48.6	6460-355
3_0467	3H	49.4	U32_3096_1267
2_1129	3H	52.5	6681-314
3_1502	3H	55.6	U35_6656_330
3_0009	3H	58.6	ABC01956_1_787
3_0829	3H	59.9	OSU_Contig2595_at_152
1_0281	3H	64.2	2338-1572

Supplemental Table 1 Continued

Pilot OPA name	Chromosome	Position (cM)	Locus_Name
1_1391	3H	65.5	ABC18717-1-3-215
3_1153	3H	68.3	U35_16856_424
2_0017	3H	69.6	10317-448
3_0754	3H	71.5	U32_8513_567
1_0350	3H	73.5	2677-501
3_0399	3H	74.8	U32_1775_564
2_0597	3H	78.5	3650-311
1_1454	3H	78.5	ConsensusGBS0222-2
2_0849	3H	81.7	4995-889
3_1262	3H	81.7	U35_19389_544
1_1517	3H	83.7	ConsensusGBS0637-1
1_0444	3H	87.2	3266-570
2_0659	3H	91.3	4019-302
3_1367	3H	95.4	U35_3071_1164
2_0130	3H	98.5	1291-936
3_0640	3H	98.5	U32_6288_886
1_0662	3H	104.5	4528-2080
2_0009	3H	107.6	10114-1946
3_0423	3H	111.4	U32_2291_275
3_1329	3H	115.6	U35_22627_571
3_1220	3H	120.6	U35_18257_694
1_0918	3H	123.7	76-1059
1_0754	3H	126.3	5260-462
2_0369	3H	130.8	2481-903
1_0842	3H	131.6	6402-691
1_0381	3H	136.7	2847-485
1_1154	3H	138.8	ABC06897-2-3-312
2_0851	3H	141.5	5008-2402
1_0631	3H	144.6	4270-184
2_1386	3H	145.9	9040-492
2_1531	3H	149.7	ConsensusGBS0431-2
3_1500	3H	152	U35_6520_551
3_0921	3H	155.9	SCRI_abc17007_02_1
1_0702	3H	162.2	4787-1746
2_1008	3H	162.2	5840-659
1_0681	3H	167.8	4643-867
2_0176	3H	170.1	14538-224
1_0283	3H	173.2	2346-318
3_0764	4H	0.7	U32_87_319
3_1324	4H	0.7	U35_21988_580
1_1345	4H	5.6	ABC14522-1-8-350
1_0319	4H	8.3	2533-773
3_1458	4H	12	U35_5192_464
1_1300	4H	12	ABC12449-1-3-227
3_0150	4H	18	ABC10624_1_1026
1_0738	4H	19.5	5149-1645
3_0864	4H	33.4	OSU_HvPhyA_3050
2_1389	4H	36.4	9081-753

Supplemental Table 1 Continued

Pilot OPA name	Chromosome	Position (cM)	Locus_Name
3_0992	4H	38.6	SCRI_olad_101
1_0371	4H	40.4	2800-1016
3_0993	4H	43.8	SCRI_olad_109
2_0939	4H	46.4	5475-1355
3_1382	4H	48.7	U35_3372_1463
3_0866	4H	48.7	OSU_HvPhyB_2292
1_1042	4H	51.3	ABC01247-1-1-92
1_0946	4H	52.8	7942-948
1_0881	4H	54.3	6954-861
3_0839	4H	55.6	OSU_DTDP_237
3_1297	4H	55.6	U35_20464_428
2_0482	4H	59.4	3127-273
2_0361	4H	59.4	2421-520
2_0453	4H	62.8	2977-1925
1_1207	4H	62.8	ABC08788-1-1-329
1_1224	4H	65.1	ABC09432-1-1-160
2_0451	4H	68.2	2955-452
2_1504	4H	69.5	ABC14714-1-1-162
3_0994	4H	72.8	SCRI_olad_112_02
1_0090	4H	76	1375-2534
1_0309	4H	76	2490-1786
2_0815	4H	76	4807-1328
2_0670	4H	80.8	4051-1101
2_0197	4H	81.7	1523-1136
1_1213	4H	86.3	ABC09154-1-1-250
2_0384	4H	91.8	2574-410
3_0142	4H	93.1	ABC10311_1_272
3_0584	4H	96.6	U32_5144_1710
3_0554	4H	96.6	U32_4613_499
2_0119	4H	99.3	1241-1649
2_0515	4H	101.6	3282-555
1_0510	4H	102.4	3652-872
2_0974	4H	106	5692-310
3_0385	4H	107.9	U32_1510_670
1_1233	4H	111.7	ABC09877-1-1-108
1_1066	4H	113.9	ABC02813-1-4-326
1_0697	4H	114.7	4773-1009
2_1210	4H	117.6	7221-766
3_0884	4H	119.1	OSU_VRN_H2_ZCCT_Ha_1430
3_0889	4H	119.1	OSU_VRN_H2_ZCCT_Hb_1580
1_0387	4H	119.8	2878-574
3_0006	4H	119.8	ABC01616_1_514
3_1422	4H	120.6	U35_4290_1313
2_0089	4H	123.3	11707-509
2_0226	5H	2.1	166-154
3_0543	5H	2.8	U32_4362_135
2_0206	5H	6.4	1582-63
2_1221	5H	9.3	7310-996

Supplemental Table 1 Continued

Pilot OPA name	Chromosome	Position (cM)	Locus_Name
2_0010	5H	18.7	10207-1024
3_0714	5H	19.4	U32_7632_376
3_0167	5H	26.3	ABC11259_1_564
3_0531	5H	27.7	U32_4156_2652
3_0410	5H	37.1	U32_2073_1148
3_0707	5H	43.1	U32_7514_744
3_0654	5H	46.2	U32_6548_1115
3_1492	5H	47.4	U35_623_856
1_0923	5H	48.8	7679-530
1_1260	5H	52	ABC10705-1-1-263
2_1318	5H	53.2	8320-955
2_1536	5H	56.8	ConsensusGBS0654-4
3_0111	5H	59.4	ABC08998_1_415
3_0538	5H	59.4	U32_4304_3452
1_1281	5H	63.3	ABC11529-1-1-295
2_0441	5H	63.3	2906-1177
2_1275	5H	67.5	7839-633
3_0080	5H	69.9	ABC07516_1_341
2_0350	5H	72.3	2365-405
1_0953	5H	75.4	8107-154
2_1133	5H	80.6	6714-579
2_0096	5H	84.5	11931-389
2_0645	5H	87.4	3928-513
3_0314	5H	87.4	U32_10259_178
3_1361	5H	91.6	U35_2913_1944
2_0497	5H	95.8	3200-242
3_0834	5H	100.3	OSU_Contig5182_at_132
2_0850	5H	102.1	5004-375
1_0414	5H	103.9	3056-1317
2_0403	5H	105.2	2727-1286
2_1314	5H	108.2	8258-330
3_0850	5H	108.2	OSU_HVCBF4A_875
3_0854	5H	108.2	OSU_HVCBF9_907
3_0855	5H	108.2	OSU_HVCBF9_988
2_0795	5H	108.6	4706-940
2_0805	5H	110.3	4771-380
3_0456	5H	113.1	U32_2783_2471
3_0619	5H	113.8	U32_5830_1337
1_0094	5H	122.4	139-1263
3_1278	5H	124.2	U35_19814_680
2_0653	5H	129.4	3985-824
3_0556	5H	143.9	U32_464_949
3_1234	5H	146	U35_18533_855
2_0731	5H	146	4346-840
3_0400	5H	149.1	U32_1794_707
1_1441	5H	151.4	ConsensusGBS0086-5
1_1490	5H	153.6	ConsensusGBS0397-16
1_1497	5H	155.1	ConsensusGBS0451-1

Supplemental Table 1 Continued

Pilot OPA name	Chromosome	Position (cM)	Locus_Name
1_0820	5H	159.1	6054-1050
3_0162	5H	161.6	ABC11039_1_102
2_0686	5H	172.4	4117-472
1_0869	5H	173.1	6735-754
2_0536	5H	177.7	3362-644
1_0736	5H	180.7	5145-1355
2_0189	5H	181.4	15017-277
3_0577	5H	182.9	U32_5031_1217
1_1364	5H	189.6	ABC16075-2-2-232
1_0401	5H	192	2978-938
1_0857	5H	194.8	6580-293
2_0493	6H	1.3	3178-1276
1_0120	6H	3.1	1553-753
2_1032	6H	9.1	5993-2383
1_0165	6H	17	1769-545
3_0842	6H	17	OSU_HA16L09r_s_at_77
2_1246	6H	22.4	7624-359
3_0843	6H	22.4	OSU_HSP70_429
2_0315	6H	24.4	2188-425
1_0676	6H	28.4	4611-178
3_1485	6H	30.1	U35_5918_746
1_0799	6H	31.7	5771-91
1_0939	6H	33.7	7848-441
3_0358	6H	35.1	U32_13027_353
3_0521	6H	38.4	U32_3978_465
3_0361	6H	40.8	U32_13368_432
2_0052	6H	42.4	10927-876
1_0817	6H	45.4	6018-235
1_0461	6H	48.7	3378-619
1_0003	6H	52.8	1009-1089
2_0720	6H	52.8	4258-1498
3_1187	6H	55.4	U35_17528_729
1_1067	6H	58	ABC02895-1-4-231
2_0058	6H	60.2	11016-603
2_1298	6H	63.3	8048-952
3_0637	6H	64.4	U32_6217_114
2_0673	6H	70	4064-1724
1_0596	6H	70	4077-76
2_0969	6H	75.2	5656-1012
3_1253	6H	77.9	U35_19046_990
1_1246	6H	81.9	ABC10265-sfp25-01
1_1147	6H	83.9	ABC06682-1-1-311
3_0698	6H	86.9	U32_7349_99
2_0783	6H	88.9	4641-266
1_1294	6H	93.7	ABC12239-1-1-316
3_1353	6H	97.4	U35_2762_1119
2_0379	6H	101.4	2562-1191
3_0734	6H	110.3	U32_8114_521

Supplemental Table 1 Continued

Pilot OPA name	Chromosome	Position (cM)	Locus_Name
2_0448	6H	111	2945-515
3_1495	6H	112.3	U35_6278_408
2_1477	6H	117	ABC05412-1-8-275
3_1392	6H	119	U35_3529_1428
1_0828	6H	124.9	617-167
1_0390	6H	128.5	2911-1136
1_0682	7H	0.6	4644-1363
2_1443	7H	0.6	9728-1098
1_0894	7H	1.9	7172-1536
3_1350	7H	3.3	U35_2649_795
3_0836	7H	4.9	OSU_Contig7611_at_147
1_0406	7H	6.8	3011-438
2_0245	7H	12.4	1773-358
1_0841	7H	15	6394-944
2_0014	7H	17.2	10281-245
1_0025	7H	21.1	1073-916
3_0530	7H	25.9	U32_415_260
3_1351	7H	29.8	U35_2705_1795
2_0162	7H	31.8	1404-64
1_0232	7H	34.8	2124-984
1_0576	7H	41.9	398-1244
3_0143	7H	45.7	ABC10328_1_388
2_1528	7H	46.2	ConsensusGBS0356-1
3_0528	7H	49.7	U32_4123_1240
2_0074	7H	54.4	11387-335
3_0290	7H	57.6	ABC25593_eSNP_CNGC_442
3_0880	7H	61.3	OSU_SS1_201
1_0050	7H	63.7	11912-654
1_1098	7H	68.5	ABC04803-1-1-392
2_0060	7H	71.1	1107-392
2_0885	7H	74.5	5-1593
1_0256	7H	78.2	2251-643
1_0534	7H	80.9	3743-1988
3_0998	7H	84.9	SCRI_olad_137_01
1_0089	7H	88	1369-1446
2_1448	7H	98.5	977-1377
2_0103	7H	102.9	12027-128
1_0169	7H	104.8	1800-1101
1_0362	7H	108.7	2745-1082
1_0563	7H	112.5	3900-611
1_1243	7H	122.1	ABC10197-1-1-101
1_0543	7H	122.1	3799-1335
3_1535	7H	125.2	U35_949_361
3_1374	7H	126.7	U35_3205_2012
1_0182	7H	128.4	1894-2132
2_1209	7H	129.9	7216-297
1_0861	7H	133.8	6628-1302
1_0078	7H	136.6	13008-352

Supplemental Table 1 Continued

Pilot OPA name	Chromosome	Position (cM)	Locus_Name
2_0847	7H	140.2	4991-1028
3_1166	7H	144.5	U35_17126_2201
1_1012	7H	147.5	93-413
1_1309	7H	148.3	ABC12986-1-3-308
3_0244	7H	151.4	ABC15259_3_1022
2_0117	7H	157	12368-207
2_0504	7H	157.8	3225-520
3_0974	7H	161.4	SCRI_bbc32814_01_394
3_0826	7H	166.6	OSU_CAT1_200

Supplemental Table 2 Percent survival of NB3437f, NB713, OR71, Dicktoo (winter hardiness check) and Morex (spring check) after freezing at -13.5^{0}C in the Martonvasar Research Institute (MRI) phytotron.

Accession	**Survival (%)**	**Growth habit**
NB3437f	87.8	Facultative
DICKTOO	84.2	Facultative
NB713	77.7	Winter
OR71	72.9	Facultative
MOREX	7.3	Spring