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# A new genetic linkage map of barley (*Hordeum vulgare* L.) facilitates genetic dissection of height and spike length and angle

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ABSTRACT

Plant height and spike length and angle are important agronomic traits in the production of barley (*Hordeum vulgare* L.) due to strong correlations with lodging and disease. The objective of this study was to use QTL analysis to identify genetic regions associated with each trait in a recombinant inbred line (RIL) mapping population derived from a cross of Falcon by Azhul. Falcon is a spring six-row hulless feed barley with long spikes displaying obtuse angles, while Azhul is a spring dwarf, six-row hulless food barley with short spikes displaying acute angles. The population was genotyped using SNP, DArT and SSR markers and quantitative trait loci (QTL) were detected on chromosomes 2H (102.8 cM, spike length), 3H (89.2 cM, plant height and 38.2, spike angle and length), 4H (19.0 cM, spike length), and 5H (106.7 cM, spike angle). In conclusion, we developed a barley genetic map, which incorporated SNP, DArT and SSR markers, for detection of height and spike length and angle QTL. Three spike angle, one spike length and one plant height QTL were novel and by using comparative genomics we identified possible candidate genes involved in gibberellic acid signaling and auxin- and ethylene-responsive pathways. This knowledge can be used to generate suitable markers for barley breeding improvement.

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## 1. Introduction

Barley (*Hordeum vulgare* L.) is the fourth most abundant cereal in world. The crop is adapted to various environments and is used as feed for livestock, malted beverages and food products. US barley production is currently around 320 million bushels per year with an estimated value of $760 million (National Barley Grower Association).

A key trait in developing new barley varieties with improved agronomics, including yield, is plant height. Plant height directly relates to lodging thus reducing this trait would allowed agricultural practices focused on increased yield, such as dense planting and increased use of fertilizer. Genetic studies have been done to identify key alleles involve in this important trait. Chutimanitsakun et al. (2011) and Dahleen et al. (2012) identified QTL affecting plant height on chromosome 1H at 131 cM, 2H at 65, 81 and 156 cM, 3H at 51 and 120 cM, 4H at 188 cM and 6H at 100 cM. Extensive research in model species, including barley, has discovered that most of the genes involved in height were also involved in the gibberellic acid (GA) biosynthetic and signal transduction pathways (Sakamoto et al., 2004; Peng et al., 1999; Helliwell et al., 2001; Gottwald et al., 2004; Spielmeyer et al., 2004). The *sdw1/denso* (semidwarf) gene encodes for GA-20 oxidase and it is one of the most studied genes in barley (Franckowiak and Pecio, 1992; Barua et al., 1993; Sasaki et al., 2002; Spielmeyer et al., 2002). It has been mapped to chromosome 3H (Barua et al., 1993; Laurie et al., 1993)

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and in a recent study it has been sequenced and applied as a diagnostic marker for selection of semidwarf gene in barley (Jia et al., 2009).

Spike length is an additional component affecting yield. Chutimanitsakun et al. (2011) study identified a major QTL on barley chromosome 2H at 156 cM influencing spike length, grain number, plant height and grain yield. In the same study, on 1H at 157 cM, 3H at 20 cM, 5H at 138 cM and 6H at 95 cM affected only spike length. Work by Xue et al. (2010) identified QTL in the same region on chromosome 2H and 4H affecting both spike length and yield. Overall, this provides strong evidence of an underlying genetic mechanism on chromosome 2H and 4H, however, suitable genetic markers for breeding application and underlying mechanisms of this trait were not provided.

Studies have suggested that spike angle can reduce fusarium head blight (FHB), which is a major disease significantly impacting barley production. For example, a FHB outbreak in 1993 caused yield losses of 70 million tons in the USA (McMullen et al., 1997). FHB also leads to contamination of barley with toxins (deoxynivalenol or DON), which can be a significant problem for barley producers and millers. A study showed that spike angle QTL partially overlapped with QTL for low fusarium head blight (FHB) severity and deoxynivalenol (DON) (Ma et al., 2000) while another study suggested that architecture of spike is associated with FHB resistance (Yoshida et al., 2005). Since spike length and angle require careful measurements at maturity, and are linked to important traits such as FHB and yield, markers tightly linked to these regions would be beneficial in order to sequence genes participating in these traits.

Over the last ten years, barley breeders have benefited from the availability of various genetic markers, including RFLP, AFLP, microsatellite, DArT, SNP, and STS (Kleinhofs et al., 1993; Rostoks et al., 2005; Wenzl et al., 2006; Hearnden et al., 2007; Marcel et al., 2007; Stein et al., 2007; Varshney et al., 2007; Potokina et al., 2008; Sato et al., 2009; Szűcs et al., 2009). These markers facilitated numerous mapping studies which led to marker assisted breeding applications. Recently, barley marker resources were greatly expanded due to the development of Illumina GoldenGate SNP assays by the Barley Coordinated Agricultural Project. This alone provided a resource to interrogate several thousand alleles in tandem and led to the construction of a high density consensus map defined by 2943 SNP loci (Close et al., 2009). The same study showed a orthologous relationship between barley and rice chromosomes, which can lead to gene discovery for various traits linked to specific QTL regions, such as beta glucan content in barley grain (Islamovic et al., 2013).

The scope of this study was to use barley mapping population for detection of height and spike length and angle QTL based on SNP loci, use comparative genomic studies to identify candidate genes responsible for these traits, and present suitable markers for barley breeding improvement.

## 2. Materials and methods

### 2.1. Plant material

The Falcon/Azhul mapping population developed by Islamovic et al. (2013) to explore the genetics of beta glucan and amylose grain content was used in this study. Falcon (Helm et al., 1996) is a six-row hulless feed barley (Reg. No. CV-253, PI591612) released by the Field Crop Development Centre of Alberta Agriculture, Food and Rural Development, Lacombe, AB, Canada, and is well adapted to Idaho, with long spikes displaying obtuse angles. Azhul is a dwarf, six-row hulless food barley derived from mutation breeding and release by USDA ARS and the Arizona Agricultural Experiment Station (Dr. R. Thomas Ramage) with short spikes displaying acute angles. The population was developed via single seed descent in the greenhouse to the $F_6$ generation. Spikes of individual plants were not isolated until the $F_5$–$F_7$ stages, so outcrossing between sibs could have possibly occurred up to the $F_4$ generation. $F_8$ seed harvested from individual $F_7$ plants was bulk increased to produce $F_{7:9}$ seed for each FA RIL.

'Baronesse' and 'CDC Alamo' were used as controls in field experiments. Baronesse is a two-row hulled barley under U.S. Plant Variety Protection (PVP9300211) by Peterson Seed Company, Inc., with intermediate spike lengths displaying obtuse angels. CDC Alamo is a two-row hulless feed barley developed by the University of Saskatchewan, with long spikes displaying right angles.

### 2.2. Field experiments

Parents, RILs, and checks were planted in Baton Rouge, LA; College Station, TX; and Leeston, Irwell, New Zealand in the fall of 2008 and in Aberdeen and Tetonia, ID in the spring of 2009. A completely randomized block design was used at each location, with parental and check lines represented by four plots randomized within each block, and RILs represented by a single plot. Plots consisted of a single 1.2 meter row with 0.4 m spacing between rows. A wheat border consisting of four rows was planted around the entire experiment to minimize the edge effect.

Prior to ripening, four plants evenly spaced within each row were measured (cm) for plant height using a standard meter stick (5 m). Measurements were taken from the crown of each plant to the tip of each spike, awns not included. At maturity, spike angles from three spikes were visually assessed by estimating the degree of deviation on a 0–10 scale with 0 = 0° and 10 = 180° from the main tiller. Once the angles were collected, spikes were harvested and images of each spike were captured with a standard ruler (cm) at a fixed distance of 27.9 cm using a Cannon Rebel digital camera ($3072 \times 2304$ pixels). Spike lengths excluding awns were measured in cm from each of the images using the ruler as a reference point. All measurements were recorded into a continuous data set so that plant height and spike length and angle were captured from the replicates at each location for ANOVA and marker/trait association.

### 2.3. Genotyping

Primary and secondary leaves were harvested from each parent and $F_7$ RIL plant used to derive the FA population. Harvested leaves from each line were placed into a 2.0-ml microcentrifuge tube, and ground into a fine powder using a sterilized test tube pestle and liquid nitrogen. DNA was extracted using a CTAB protocol as previously described in Islamovic et al. (2013).

Barley microsatellites, diversity arrays technology (DArT), and single nucleotide polymorphism (SNP) markers were used to genotype each FA parent and RIL. Barley microsatellites from previously published work (Varshney et al., 2007) were screened for polymorphism between Falcon and Azhul using the ABI 3730XL genetic analyzer and previously described protocols for polymerase chain reaction and fragment discrimination (Chao et al., 2007). Alleles for each of the polymorphic markers were subsequently interrogated using the same protocols.

DArT genotyping was provided by Diversity Arrays Technology Pty Ltd (DArT P/L) (http://www.diversityarrays.com/index.html). DNA from each parental line and RIL was hybridized to the PstI(BstNI) v1.7 array which contains 2500 representations from a wide range of barley cultivars and accessions (Wenzl et al., 2006). Dominant genotypes were called using DArTsoft v.7 (Wenzl et al., 2006) and a binary data matrix was provided with various measures of quality and polymorphism information content for each marker.

SNP alleles were assayed via the Barley Oligo Pooled Assay 1 (BOPA1), which contains 1536 SNPs developed as part of the

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