Journal of Integrative Agriculture 2017, 16(2): 445–453

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RESEARCH ARTICLE

Identification of a major QTL for flag leaf glaucousness using a high-density SNP marker genetic map in hexaploid wheat

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Abstract

Cuticular wax plays an important role in protecting land plant against biotic and abiotic stresses. Cuticular wax production on plant surface is often visualized by a characteristic glaucous appearance. This study identified quantitative trait loci (QTLs) for wheat (*Triticum aestivum* L.) flag leaf glaucousness (FLG) using a high-density genetic linkage map developed from a recombinant inbred line (RIL) population derived from the cross Heyne×Lakin by single-seed descent. The map consisted of 2 068 single nucleotide polymorphism (SNP) markers and 157 simple sequence repeat (SSR) markers on all 21 wheat chromosomes and covered a genetic distance of 2 381.19 cM, with an average marker interval of 1.07 cM. Two additive QTLs for FLG were identified on chromosomes 3AL and 2DS with the increasing FLG allele contributed from Lakin. The major QTL on 3AL, *QFlg.hwwgr-3AL*, explained 17.5–37.8% of the phenotypic variation in different environments. *QFlg.hwwgr-3AL* was located in a 4.4-cM interval on chromosome 3AL that was flanked by two markers IWA1831 and IWA8374. Another QTL for FLG on 2DS, designated as *QFlg.hwwgr-2DS* which was identified only in Yangling in 2014 (YL14), was flanked by IWA1939 and Xgwm261 and accounted for 11.3% of the phenotypic variation for FLG. *QFlg.hwwgr-3AL* and *QFlg.hwwgr-2DS* showed Additive×Environment (AE) interactions, explaining 3.5 and 4.4% of the phenotypic variance, respectively. Our results indicated that different genes/QTLs may contribute different scores of FLG in a cultivar and that the environment may play a role in FLG.

Keywords: wheat, quantitative trait locus (QTL), flag leaf glaucousness, single nucleotide polymorphism, QTL×Environment interactions

1. Introduction

Cuticular wax covers aerial organs of land plants and provides protection against water loss and other environmental stresses, such as drought, pests, pathogens and radiation (Misra and Ghosh 1991; Jenks and Ashworth 1999). Cuticular waxes are complex mixtures of very long-chain fatty acids and their derivatives, such as alkane, aldehyde, alcohol, ketone, and ester, which typically range from

Received 22 January, 2016 Accepted 2 March, 2016
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doi: 10.1016/S2095-3119(16)61339-4

C24 to C32 in length (Kunst and Samuels 2003; Samuels *et al.* 2008). In plants, these components are specifically found as an epicuticular layer that gives the plant surface a glaucous appearance (Jenks and Ashworth 1999). In wheat (*Triticum aestivum* L.), cuticular wax deposits on the surfaces of leaf, stem and spike, and is often visualized by a characteristic glaucous appearance. Genetic studies have demonstrated that the glaucous and non-glaucous phenotypes of wheat are mainly controlled by several loci. Tsunewaki and Ebana (1999) identified glaucousness wax genes *W1* and *W2* and their inhibitor genes *Iw1* and *Iw2*, respectively. *W1* and its inhibitor gene *Iw1* were located on the short arm of chromosome 2B, and *W2* and its inhibitor gene *Iw2* were located on the chromosome arm 2DS (Liu *et al.* 2007; Adamski *et al.* 2013; Wu *et al.* 2013; Zhang *et al.* 2013; Nishijima *et al.* 2014; Lu *et al.* 2015). Böner *et al.* (2002) found three QTLs for wax on chromosome arms 1DL, 2DL and 4AL. Gadaleta *et al.* (2009) identified the *Ws* gene on chromosome arm 1AS as responsible for glaucous spikes. Mason *et al.* (2010) detected a wax QTL, *Qwax.tam-5A*, linked closely to the SSR marker Xwmc150 on the short arm of chromosome 5A. A major QTL for flag leaf glaucousness (*QW.aww-3A*) was detected in a doubled -haploid (DH) population, which accounted for 52% of the variation (Bennett *et al.* 2012). The non-glaucousness locus *Iw3* was mapped at the marker interval Xpsp3000–XWL3096 on chromosome arm 1BS with a genetic distance of 0.13 cM (Wang J *et al.* 2014). In addition, *W3* was necessary for β-diketone synthesis and was localized on chromosome arm 2BS (Zhang *et al.* 2015). Recently, two stable QTLs for leaf wax content were located on chromosomes 1B and 5A (Mondal *et al.* 2015). Taken together, previous studies on wheat wax glaucousness and non-glaucousness suggested that numerous loci influence the glaucous appearance of wheat plant with complex genetic control; furthermore, the extent of deployment of these loci is different in various germplasm.

Genetic map plays a fundamental role in gene or QTL identification. And high-density maps may facilitate identification of closely linked markers to specific traits, which is useful for marker-assisted selection (MAS) in breeding. In previous studies, most QTLs for wheat traits were mapped with low-density maps of SSR or other markers. Single-nucleotide polymorphism (SNP) is the most common polymorphism in plant species, and the availability of high-throughput SNP genotyping platforms makes it possible to develop high-density maps for genetic dissection and MAS of those complex traits (Jannink and Lorenz 2010). Thus, SNP has been increasingly used for developing high density genetic map and QTL mapping in many crops. A few SNP markers are available for agronomic traits. However, progress in SNP discovery and detection in common wheat has been slowed because of the highly repetitive nature of the hexaploid genome. To date, a high-density SNP map has not been used for mapping wax genes in wheat. Cavanagh *et al.* (2013) developed a 9 000-SNP chip and constructed the first high-density wheat consensus SNP map. A total of 7 504 polymorphic loci were mapped in that study. The SNP chip and map developed from that study provide a powerful resource for further mapping of wheat traits of interest and for genomic research and genome-wide association studies in wheat. Using this SNP chip, we analyzed a wheat recombinant inbred line (RIL) population derived from the cross of Heyne×Lakin. We identified 2 070 polymorphic markers between parents. These SNPs provide a great opportunity for construction of a high-density genetic map and for high-resolution mapping of QTLs in common wheat. The objectives of this study were (1) to further characterize additive QTLs for flag leaf glaucousness (FLG) in wheat with the high-density SNP and SSR maps; (2) to find molecular markers closely linked to the QTLs of the glaucousness for MAS.

2. Materials and methods

2.1. Plant materials and phenotypic data collection

A population of 145 F_6 RILs was developed from the cross Heyne×Lakin by single-seed descent. Heyne (PI 612577) and Lakin are both hard white winter wheat, developed cooperatively by the Kansas Agricultural Experiment Station and the United States Department of Agriculture-Agricultural Research Service (USDA-ARS)(Heyne and Niblett 1978; Martin *et al.* 2001). Heyne was resistant to various rust pathogens and *Fusarium graminearum*, whereas Lakin was susceptible to rust but had good yield potential.

Phenotypic data were collected from three field experiments in Yangling and Sanyuan of Shaanxi Province, China, with two crop years in 2014–2015. The three experiments included Yangling in 2014 (YL14), Yangling in 2015 (YL15), and Sanyuan in 2015 (SY15). The RILs along with the parents were measured using a replicates-in-sets design with two replications. Visual flag leaf glaucousness was scored using a 1–6 scale at an average growth stage of anthesis as previously described (Bennett *et al.* 2012). Scores of 1, 2, and 3 occurred when 0, 50, and 100% glaucousness were observed on the abaxial surface of the flag leaf, respectively. Scores of 4, 5, and 6 occurred when glaucousness were visible on 100% of the abaxial and 25, 50, and 100% of adaxial surfaces of the flag leaf, respectively.

2.2. Scanning electron microscopy (SEM) analysis

For epicuticular wax crystal examination, the middle of leaf