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A QTL for early heading in wheat cultivar Suwon 92

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Summary

Heading date is an important trait that determines wheat adaptation to environments. A recombinant inbred line (RIL) population derived from CI 13227 × Suwon 92 was employed to tag the quantitative trait locus (QTL) for early heading in Suwon 92. This population was phenotyped for heading date in 1994, 1995, and 1997, and analyzed with AFLP and SSR markers. Two AFLP markers (*XGCTG.CGCT118* and *XGCTG.CGCT60*) closely associated with heading date were identified. Across years, *XGCTG.CGCT118* and *XGCTG.CGCT60* explained 40.4% and 32.2% of the total phenotypic variances, respectively. Interval analysis revealed a major QTL for heading date, designated *QHd.pser-2DS*, between AFLP marker *XGCTG.CGCT118* and SSR marker *Xgwm261*. Based on the linkage map, *QHd.pser-2DS* was about 41.2 cM proximal to the distal end of chromosome 2DS, and explained 40.5% of the phenotypic variance across three years. The identified markers associated with the early heading QTL have the potential to be used in wheat breeding programs.

Introduction

Understanding the genetic basis of heading date is important for breeding wheat cultivars adapted to diverse environments and to different cropping systems. Three categories of genes influence heading date through their control of photoperiod response (*Ppd*), vernalization response (*Vrn*), and earliness per se (*Eps*). The *Ppd* and *Vrn* genes interact with environments, whereas *Eps* genes control developmental rate independently of the environment (Snape et al., 2001; Bullrich et al., 2002).

An orthologous series of photoperiod response genes located on chromosome 2D (*Ppd-D1*; formerly *Ppd1*), 2B (*Ppd-B1*; formerly *Ppd2*), and 2A (*Ppd-A1*; formerly *Ppd3*) play an important role in determining heading date in response to day length, with dominant alleles controlling early heading (Welsh et al., 1973; Law et al., 1978; Scath & Law, 1983). A previous study demonstrated that *Ppd-B1, Ppd-A1* and *Ppd-D1* shortened the heading time by 3, 5, and 8 days, respectively (Scarth & Law, 1983). Genes on other chromosomes, such as 3D and 4B, also played a minor role in determining day-length response (Miura & Worland, 1994; Halloran & Boydell, 1967). *Ppd-D1* was located on the short arm of chromosome 2D using genetic stocks (Law et al., 1978) and RFLP markers (Worland et al., 1998), whereas *Ppd-B1* was mapped on the short arm of chromosome 2B in three segregating populations employing AFLP and SSR markers. One AFLP marker linked to *Ppd-B1* was converted to a CAPS marker (Mohler et al., 2004).

Vernalization response genes regulate the requirements of exposure to cold temperatures to induce heading and flowering (Bullrich et al., 2002). Four *Vrn* genes, namely *Vrn-A1* on chromosome 5A (formerly *Vrn1*), *Vrn-B1* on chromosome 5B (formerly *Vrn2*), *Vrn-D1* on chromosome 5D (formerly *Vrn3*), and *Vrn-B4* on chromosome 7B, were identified. The *Vrn* genes have been extensively characterized with molecular markers (Dubcovsky et al., 1998; Nelson et al., 1995;

Galiba et al., 1995; Iwaki et al., 2002; Toth et al., 2003). High-density genetic maps are available for *Vrn-A1* and *Vrn-B1* regions, and *Vrn-A1* and *Vrn-B1* were successfully cloned through map-based cloning (Yan et al., 2003, 2004).

Earliness *per se* genes determine the number of vegetative and floral primordia in wheat (Hoogendoorn, 1985). They have received less mapping attention than *Ppd* and *Vrn* genes in wheat. Chromosome 2B, 3A, 3D, 4A, 4D, 5A, and 6B (Flood & Halloran, 1983; Kato et al., 1999; Miura & Worland, 1994; Miura et al., 1999) were reported to harbour *Eps* genes/QTLs. Bullrich et al. (2002) identified a major QTL for heading date on chromosome $1A^m$ of *Triticum monococcum*. Laurie et al. (1995) reported that most of the chromosomes in barley (*Hordeum Vulgare* L.) appeared to contain *Eps* QTLs.

Suwon 92 is a semi-dwarf early maturing Korean wheat cultivar that possesses agronomic features worthy of use in wheat breeding programs (Shaner et al., 1997). Understanding the genetic basis of these traits is helpful for their introgression into commercial cultivars. Here we report the characterization of a QTL for early heading date in Suwon 92 with molecular markers.

Materials and methods

Experimental materials and design

A population of 104 recombinant inbred lines (RILs) was developed by single-seed-descent from a cross between Suwon 92 (early heading parent) and CI 13227 (late heading parent) (Shaner et al., 1997). The 104 RILs and two parents were evaluated for heading date at the Agronomy Centre for Research and Education, Purdue University, West Lafayette, IN, in 1994, 1995, and 1997 using a randomized complete block design with two replications. Each experimental line was planted in three 1 m rows. Natural field conditions in West Lafayette satisfied the vernalization requirements for these RILs. Heading date was recorded as the number of days from 1 January to the date that half of the heads had emerged from flag leaves.

Marker analysis

Genomic DNA was extracted from two-week old wheat seedlings using the CTAB method (Murray & Thomposon, 1980). A bulked segregant analysis (BSA) (Michelmore et al., 1991) was employed to screen informative AFLP primers. Equal amounts of DNA from five early heading RILs were pooled to construct the early heading bulk, while equal amounts of DNA from five late heading RILs were mixed to construct the late heading bulk. The two bulks, together with the two parents, were used to screen informative AFLP primers. Analyses of fluorescence-labeled AFLP and SSR markers were performed using a Li-Cor DNA Analyzer (Li-Cor Inc, Lincoln, NE) (Xu et al., 2005).

Data analysis

Genetic linkage maps were constructed using MAPMAKER 3.0 (Lander et al., 1987) with a LOD threshold of 4.0. Centimorgan (cM) values were calculated based on the Kosambi mapping function (Kosambi, 1944). Single marker analysis and interval analysis were performed using QGENE software (Nelson, 1997) to determine the effects of each marker and the location of the early heading QTL in Suwon 92. Analysis of variance was conducted using SAS software (SAS Institute Inc., Cary, NC) to estimate broad-sense heritability (H^2) as the ratio of genetic variance (V_G) to the total phenotypic variance (V_P).

Results

Suwon 92 headed significantly earlier than CI 13227 in all three years (Figure 1). The difference in heading date between the two parents was 7.4 days, 11.5 days, and 5.3 days in 1994, 1995, and 1997, respectively. A continuous distribution for heading date was observed among the RILs, ranging from 167 days (May 16) to

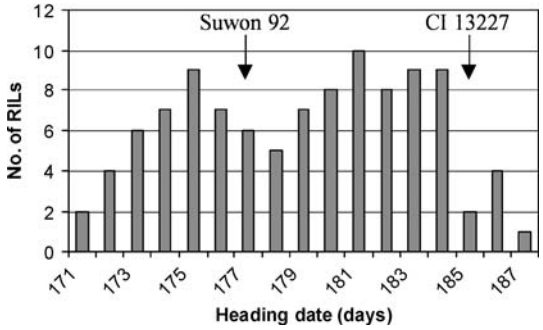

Figure 1. Frequency distribution for heading data across three years for Suwon 92, CI 13227, and RILs derived from CI 13227 × Suwon 92. Heading date was scored from January 1 in each year.

185 days (June 3) in 1994, 166 days (May 15) to 184 days (June 2) in 1995, and 177 days (May 26) to 196 days (June 14) in 1997. Transgressive segregation was observed in all years.

Correlation coefficients were high for heading date between any pairs of years, ranging from 0.70 to 0.89 and the broad-sense heritability (H^2) was high (0.94). Single marker analysis identified two AFLP markers (*XGCTG.CGCT118* and *XGCTG.CGCT60*) closely associated with early heading date. The genetic distance between them is 3.3 cM. Among years, the determination coefficients for markers *XGCTG.CGCT118* and *XGCTG.CGCT60* ranged from 30.1% to 40.3%, and 22.0% to 33.9%, respectively. The additive effects ranged from 2.3 days to 2.9 days for *XGCTG.CGCT118*, and 2.0 days to 2.6 days for *XGCTG.CGCT60* in three years (Table 1). Averaged across years, *XGCTG.CGCT118* explained 40.4% of the phenotypic variance with an additive effect of 2.7 days, whereas *XGCTG.CGCT60* explained 32.2% of the phenotypic variance with an additive effect of 2.4 days. Linkage analysis positioned these two markers on the short arm of chromosome 2D based on the linked SSR markers, *Xgwm261*, *Xbarc95*, *Xgwm455,* and *Xbarc124*, which were previously mapped on chromosome 2DS (Somers et al., 2004; Song et al., 2005).

Interval mapping revealed a major QTL for heading date (Figure 2), designated as *QHd.pser -2DS*, between AFLP marker *XGCTG.CGCT118* and SSR marker *Xgwm261* on the short arm of chromosome 2D, with LOD values ranging from 8.5 to 11.1 in three years (Table 2). This QTL explained 39.8%, 33.5%, and 32.1% of the total phenotypic variance in 1994, 1995, and 1997, respectively. Averaged across years, this QTL resided about 2.6 cM from AFLP marker *XGCTG.CGCT118* and 12.1 cM from SSR marker *Xgwm261* with a LOD value of 11.4, and explained 40.5% of total variance for heading date. Among the SSR markers linked to *QHd.pser-2DS*, *Xbarc124* was previously mapped on the distal end of chromosome 2DS (Somers et al., 2004). Based on the linkage map, *QHd.pser-2DS* was about 41.2 cM proximal to the distal end of chromosome 2DS.

Discussion

Worland et al. (1998) located *Ppd-D1* on chromosome 2DS, 20.9 cM proximal to *Rht8*, and the SSR marker *Xgwm261* was found to be 0.6 cM away from *Rht8* (Korzun et al., 1998). In our study, the QTL *QHd.pser-2DS* for heading date was also identified on chromosome 2DS. This QTL was 12.1 cM proximal to *Xgwm261,* and explained about 40% of the phenotypic variance over three years. It promoted earlier heading by 5–6 days. A previous study also revealed that *Xgwm261* was associated with photoperiod response and explained 5.7% of the phenotypic variance (Sourdille et al., 1999).

Laurie et al. (1995) identified an *Eps* QTL on chromosome 2 (2H) in barley (*Hordeum vulgare* L.). Although it is reasonable to expect an *Eps* QTL on wheat chromosome 2DS, such a QTL should have a relatively small effect on heading date (Snape et al., 2001; Kato et al., 1999) and is unlikely to account substantially for the phenotypic variance. Hence, the coincidence of *QHd.pser-2DS* and *Ppd1* suggests that Suwon 92 may carry a *Ppd1* gene.

The introgression of genes/QTL for early heading into commercial cultivars to shorten the life cycle of

Table 1. Determination coefficients (R^2), LOD values, P values, and allelic substitution effects of AFLP markers associated with heading date in 1994, 1995, and 1997

Year	Marker	R^2 (%)	LOD	*P*	Allele mean		Additive effect
					CI 13227	Suwon 92	
					day		
1994	XGCTG.CGCT118	40.3	11.0	<0.0001	29.3	23.5	2.9
	XGCTG.CGCT60	33.9	8.7	<0.0001	28.7	23.5	2.6
1995	XGCTG.CGCT118	33.9	8.8	<0.0001	27.4	21.6	2.9
	XGCTG.CGCT60	27.4	6.7	<0.0001	26.7	21.6	2.6
1997	XGCTG.CGCT118	30.1	7.6	<0.0001	36.0	31.4	2.3
	XGCTG.CGCT60	22.0	5.2	<0.0001	35.4	31.5	2.0
Mean	XGCTG.CGCT118	40.4	11.0	<0.0001	30.9	25.5	2.7
	XGCTG.CGCT60	32.2	8.2	<0.0001	30.3	25.5	2.4

Table 2. Marker intervals, QTL peak positions to the closest marker (QTL peak), determination coefficients, and LOD values for *QHd.pser-2DS* in Suwon 92

Year	Interval	QTL Peak (cM)	R^2 (%)	LOD
1994	XGCTG.CGCT118/Xgwm261	2.1	39.8	11.1
1995	XGCTG.CGCT118/Xgwm261	2.2	33.5	8.9
1997	XGCTG.CGCT118/Xgwm261	3.6	32.1	8.5
Mean	XGCTG.CGCT118/Xgwm261	2.6	40.5	11.4

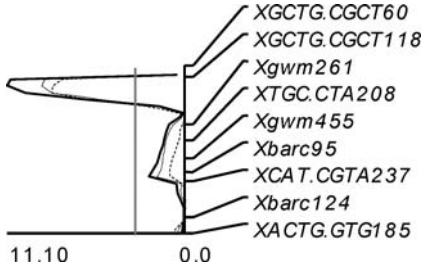

Figure 2. Likelihood plots of a QTL for heading date, *QHd.pser*-2DS. Solid, dot, and slash curves represent results from 1994, 1995, and 1997, respectively. The vertical line represents the threshold LOD value of 3.0. The highest LOD value is given beneath the horizontal line.

wheat is important in most wheat growing areas. In Europe, *Ppd-D1* typically accelerates flowering time by 6–14 days depending on the growing region (Snape et al., 2001; Worland, 1996), with pleiotropic effects on plant height, tiller number, spikelet number, spikelet fertility, grain filling, kernel weight, and harvest index (Worland, 1996; Foulkes et al., 2004). Its influence on yield is therefore complex and varied in different regions. Snape et al. (2001) reported that genotypes with the photoperiod-insensitivity allele (*Ppd-D1*) had significantly higher yields in regions with hot, dry summers, whereas genotypes with the sensitive allele (*ppd-D1*) had higher yields in areas with wetter, cooler summers and a longer vegetative growth period.

The early heading QTL, *QHd.pser-2DS,* should also play an important role in wheat breeding programs, especially in areas where more than one crop is harvested in a year. For example, wheat-maize and wheat-rice are popular double-cropping systems in China, where an early harvest is so imperative that early maturity is one of the top breeding objectives. Thus the manipulation of *QHd.pser-2DS* and other early heading genes/QTL in wheat breeding programs bears agronomic importance. However, PCR-based markers closely linked with early heading genes/QTL are still rare. In this study, the identified QTL, *QHd.pser-2DS*, was 2.6 cM distal to AFLP marker *XGCTG.CGCT118*, and 12.1 cM proximal to SSR marker *Xgwm261*. The successful conversion of the AFLP marker into a STS marker may create a more practical PCR-based marker for marker-assisted selection of QTL *QHd.pser-2DS*. However, in the absence of a STS marker, SSR marker *Xgwm261* is still a useful marker for marker-assisted selection for the QTL. Further fine mapping of this region may identify closer selectable markers to the QTL than *Xgwm261*.

QHd.pser-2DS explained 40% of phenotypic variance in three years, leaving a large portion of the variance unexplained. The absence of other detectable QTLs may be partially attributed to the method used in this study. In order to quickly identify molecular markers linked with early heading, we employed the BSA method that assumes that markers polymorphic between the two bulks are associated with the QTLs encoding early heading. The successful identification of linked markers depends on the selection of plants constituting the bulks. Since plant selection was based on phenotypic data, BSA is most effective when recombination does not occur between the target QTL and the flanking markers for the plants selected. If a plant with recombination between the target QTL and the flanking markers is included in one of the bulks, flanking markers will not be detected using this method. Hence, to detect all the QTLs involved, it is necessary to eventually screen the entire genome.

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