Linkage Map of SSR Markers and QTLs Detection for Heading Date of Iranian Rice Cultivars

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ABSTRACT

The construction of molecular maps and identification of genomic regions controlling quantitative traits have great significance for plant breeders. In this study, a genetic analysis of quantitative trait loci (QTLs) affecting the heading date of rice was performed using an F2 population of a cross between two Iranian landrace cultivars, Domsephid and Gerdeh, comprising 192 plants. An approximately normal distribution was observed for the heading date in the F2 population. A genetic linkage map with 88 informative microsatellite markers (SSR) was constructed, which covered 1367.9 cM of the rice genome with an average distance of 18 cM between markers. Single marker analysis (SMA) and interval mapping (IM) procedures were used to detect the QTLs controlling heading date and QTLs identified were further confirmed using composite interval mapping (CIM). Six significant QTLs (LOD ≥ 3.0) were identified for the heading date, of which three major QTLs mapped on chromosomes 6 (hd6), 7 (hd7) and 8 (hd8) had particularly high LOD scores and explained 23.5%, 19.8% and 20.5% of the total phenotypic variance, respectively. Three other minor QTLs detected for the heading date, located on chromosomes 1 (hd1), 3 (hd3) and 11 (hd11), accounted for 6.6%, 11.7% and 6.6% of the phenotypic variation, respectively. The additive effect of a single QTL ranged from 1.67 to 3.91 days. In the QTL hd6, alleles from Domsephid were responsible for reducing the heading date, while in the other five QTLs, alleles from Gerdeh caused a decrease in the heading date. The QTLs hd1, hd3 and hd8 showed over dominance effects for increasing the heading date, whereas the other three QTLs had partial to incomplete dominance effects for increasing (hd7 and hd11) and reducing (hd6) the heading date.

Keywords: Heading date, Linkage map, Microsatellite markers, Rice, Quantitative trait loci.

INTRODUCTION

Most agronomic traits in rice have continuous phenotypic variation and are controlled by quantitative trait loci (QTLs). The identification and mapping of QTLs for such traits has great significance for rice breeders. Since the introduction of molecular markers and the development of high-density molecular linkage maps in rice (Causse et al., 1994; Chen et al., 1997; Temnykh et al., 2000; McCouch et al., 2002), many quantitative traits like the heading date has been studied and QTLs affecting this important trait have also been identified (Li et al., 1995; Lu et al., 1997; Lin et al., 1998; Maheswaran et al., 2000; Zhou et al., 2001; Brondani et al., 2002; Yu et al., 2002; Hitatalmani et al., 2002).

Xiao et al. (1996), using recombinant inbred lines, mapped a major QTL for heading date on chromosome 8 and two minor QTLs on chromosomes 3 and 11, while Yano et al. (1997) using an F2 population, detected two major QTLs on chromosomes 6 (Hd1) and 7 (Hd2) and other three minor QTLs on chromosomes 6, 7 and 8 (Hd3, Hd4 and Hd5, respectively). Lin et al. (2000), using near isogenic lines, revealed that Hd1, Hd2 and Hd3 are involved in photoperiod sensitivity. Additional analysis using a large segregating
population derived from advanced backcross progeny constructed a high-resolution linkage map of *Hd3* on the short arm of chromosome 6 and detected two tightly linked genes controlling the heading date in the *Hd3* region (Monna et al., 2002). He et al. (2001) identified a major QTL on chromosome 8 which explained 35% of the phenotypic variation, and a minor QTL on chromosome 12. Yamamoto et al. (2001) mapped four QTLs on chromosomes 3, 4, 6 and 8, of which two QTLs identified on chromosomes 6 and 8 had large effects on heading date. Septiningsih et al. (2003) detected the QTLs controlling days to heading using an advanced backcross population and found six QTLs with small effects on chromosomes 2 (two QTLs), 7 (two QTLs), 11 and 12.

The high-coincidence of QTLs identified for heading date on chromosomes 3, 6, 7 and 8 for rice varieties belonging to the Japonica and Indica subspecies aroused our interest in verifying as to whether the QTLs detected for Iranian rice varieties show similar tendencies. While many researchers have identified the QTLs in rice varieties apart from Rabiei et al. (2004), who found QTLs controlling grain size and shape, there is no other report on QTL mapping in Iranian rice cultivars.

In this study, a genetic analysis of QTLs governing the heading date of rice was carried out using an *F₂* population derived from a cross between two Iranian landrace cultivars. Genetic analysis then followed to determine the number, chromosomal location, gene action and magnitude of effects of QTLs controlling heading date.

**MATERIALS AND METHODS**

**Plant Materials and Field Experiments**

Two Iranian landrace rice cultivars, Domsephid as the female parent and Gerdeh as the male parent, were crossed in 2001. Domsephid is a tall aromatic cultivar with long grains, low yield and a late heading date cultivated in Guilan Province of Iran, while Gerdeh is a semidwarf cultivar with short grains, relatively high yielding and with an early heading date cultivated in Azarbaijan Province of Iran. Both cultivars are basically landraces that have been cultivated by farmers over years and now carefully maintained along with other landraces for their genetic purity at the Rice Research Institute of Iran (RRII) at Rasht, Iran.

From this cross, approximately 40 *F₁* hybrid plants were obtained. *F₁* selfed seeds were collected from those *F₁* plants and grown as an *F₂* population. One hundred and ninety two *F₂* plants, 20 replications of *F₁* plants and the two parents, were raised in an experimental field with a spacing of 25×25 cm at the RRII. The heading date was measured as the days from sowing to emergence of the first panicle for each *F₂* plant.

**Construction of SSR Map**

Genomic DNA was extracted from young and fresh leaves of *F₂* plants and the two parents, as described by Dellaporta *et al.* (1983) with minor modifications. Based on the published rice SSR maps (Chen *et al.*, 1997; Temnykh *et al.*, 2000; McCouch *et al.*, 2002), 88 informative SSR markers distributed throughout 12 chromosomes of rice were selected and scored on *F₂* plants. SSR analysis was performed as described by Chen *et al.* (1997) and silver staining according to Panaud *et al.* (1996), with slight modifications. The software Mapmaker/EXP ver. 3.0 (Lander *et al.*, 1987; Lincoln *et al.*, 1993a) was used to construct the linkage map with a minimum threshold LOD score of 5.0 and a maximum recombination fraction of 0.3. The Kosambi map function (Kosambi, 1944) was employed to convert the recombination frequencies to marker distances in centiMorgan (cM), and the order of the markers was established using three-point analysis.
QTL Identification

Segregation ratios at the marker loci for the three genotypic classes (homozygous Domsephid, heterozygous Domsephid/Gerdeh, and homozygous Gerdeh) were compared with the expected ratios 1:2:1, based on Chi-square tests, and the skewedness of the marker loci were determined. The analysis of quantitative trait loci (QTLs) was performed using three analytical methods. Firstly, single marker analysis and interval mapping (Lander and Botstein, 1989) were used to identify the genomic regions affecting the heading date using Mapmaker/QTL ver. 1.1b (Lincoln et al., 1993b). Since the single marker analysis and the interval mapping is generally affected by two or more linked QTLs located on a chromosome, the composite interval mapping (Zeng, 1993 and 1994) was then used to confirm the identified QTLs using QTL Cartographer ver. 1.3 (Basten et al., 2001).

RESULTS AND DISCUSSION

Phenotypic Variation

The phenotypic distribution of heading dates in 192 F₂ plants is shown in Figure 1. An approximately normal distribution was observed for the heading dates. Some F₂ progenies showed extreme values, with values more than the higher parent (Domsephid) and less than the lower parent (Gerdeh), indicating a transgressive segregation for the heading date. The phenotypic value of the heading date was significantly different (P<0.01) between the two parents. The heading date for the female parent, Domsephid, was 99.19 ± 2.25 days, which was 18.7 days more than the male parent, Gerdeh. The mean value of heading dates in F₁ and F₂ progenies was closer to the higher parent Domsephid (95.45 ± 1.68 and 93.52 ± 7.27 for F₁ and F₂ plants, respectively).

Construction of Linkage Map

The linkage map consisting of 88 SSR markers was constructed, which covered 1367.9 cM of the 12 rice chromosomes with an average distance of 18.0 cM between marker loci. The chi-square tests of the observed frequencies of each marker locus in 192 F₂ plants indicated that deviations from the expected ratio of 1:2:1 were significant.
(P<0.05) for 12 of 88 (13.64%) mapped markers. These skewed marker loci corresponded to four linked chromosomal regions on three different chromosomes (Figure 2). Comparison of the resulting linkage map and the maps of the Chen et al. (1997), Temnykh et al. (2000) and McCouch et al. (2002) showed that all markers were located in the expected order on the chromosomes.

QTL Mapping

Six QTLs (hd1, hd3, hd6, hd7, hd8 and hd11) were mapped for their heading date on chromosomes 1, 3, 6, 7, 8 and 11 (Table 1 and Figure 2). Amongst these, three QTLs located on chromosomes 6 (hd6), 7 (hd7) and 8 (hd8) had particularly high LOD scores and explained 23.5%, 19.8% and 20.5% of the total phenotypic variation, re-
respectively. The additive effect of a single QTL ranged from 1.67 to 3.91 days. Domsephid alleles at \textit{hd6} (chromosome 6) caused a decrease in heading date, whereas in the other five putative QTLs, alleles from Gerdeh were responsible for a reduced heading date. There were QTLs (\textit{hd1}, \textit{hd3} and \textit{hd8}) that showed overdominance effects (with \(d/a\) ratios of 1.12, 1.21 and 1.10, respectively) for an increased heading date, while the other three QTLs showed partial to incomplete dominance effects for increased (\textit{hd7} and \textit{hd11}) and reduced (\textit{hd6}) heading date.

### Gene Action and Effect of QTLs on Heading Date

For successful QTL mapping studies, large differences between parents for traits studied are the essential requirements. Likewise in this study, the female parent Domsephid and the male parent Gerdeh differed significantly in terms of heading date. The percentage of phenotypic variation explained by each QTL ranged from 6.6\% to 23.5\%, with an average of 14.78\%. Among these six QTLs, three of them accounted for a phenotypic variation of more than 19.8\% (Table 1). This showed that the heading date might be governed by two or three major QTLs that explained the large proportion of phenotypic variation and several minor QTLs each explaining a small proportion of phenotypic variance. Similar findings have been reported by several researchers who had also identified two or three major loci similar to \textit{hd6}, \textit{hd7} and/or \textit{hd8} that control heading date. Yano \textit{et al.} (1997) had detected two major QTLs for heading date on chromosomes 6 (linked to R1679 marker locus) and 7 (linked to C728 marker locus), and three other minor QTLs on chromosomes 6, 7 and 8. Among the QTLs that identified relatively large genetic effects in this study, \textit{hd6} within the interval of RM121-RM527 should be the same locus as with the earlier studies (Yano \textit{et al.}, 1997; Lin \textit{et al.}, 1998; Maheswaran \textit{et al.}, 2000; Yamamoto \textit{et al.}, 2001). Further, the major locus mapped on chromosome 7 in this study (\textit{hd7}) between RM234-RM420 interval could be the same locus reported in previous studies (Yano \textit{et al.}, 1997; Lin \textit{et al.}, 1998). Xiao \textit{et al.} (1996) had detected a major QTL controlling the days to heading on chromosome 8 (linked to RZ562) that accounted for 51\% of total phenotypic variance. Moreover, Lu \textit{et al.} (1997), He \textit{et al.}

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**Table 1.** QTLs affecting heading date (HD) in the 192 F2 plants derived from a cross between two Iranian rice cultivars Domsephid and Gerdeh.

| QTL*   | Interval   | Pos. (cM) | LOD* | \(R^2\) | \(a^e\) | \(d^f\) | \(d/|a|\)^g |
|--------|------------|-----------|------|---------|--------|--------|-----------|
| \textit{hd1} | RM473A-RM543 | 10        | 3.10 | 6.6     | -2.08  | 2.33   | 1.12      |
| \textit{hd3} | RM545-RM7 | 8         | 3.48 | 11.7    | -1.82  | 2.21   | 1.21      |
| \textit{hd6} | RM121-RM527 | 12       | 5.76 | 23.5    | 2.71   | -1.06  | -0.39     |
| \textit{hd7} | RM234-RM420 | 4         | 4.85 | 19.8    | -1.67  | 1.12   | 0.67      |
| \textit{hd8} | RM38-RM25 | 10        | 5.13 | 20.5    | -3.91  | 4.32   | 1.10      |
| \textit{hd11} | RM332-RM167 | 7        | 3.14 | 6.6     | -2.45  | 1.76   | 0.72      |

*a: QTLs are named by trait abbreviation plus chromosomal numbers.*

*b: QTL positions are measured as distance from left marker per interval.*

*c: Log10-likelihood.*

*d: Percentage phenotypic variance explained.*

*e: Additive gene effect of putative QTL.*

*f: Dominance gene effect of putative QTL.*

*g: Degree of dominance.*
(2001) and Yamamoto et al. (2001) had also found a QTL for days to heading on chromosome 8 which explained a large proportion of the phenotypic variation. The QTL mapped on chromosome 8 (interval RM38-RM25) in this study (hd8) is located in approximately the same region as a QTL for days to heading reported previously. Li et al. (1995) and Hittalmani et al. (2002) had identified a major QTL for the heading date on chromosome 3 (near to RG348). However, in this study, a minor QTL was detected on chromosome 3 (hd3) within the interval of RM545-RM7, which should be the same locus as that of the earlier mapped as major QTL. Two new minor QTLs i.e. hd1 (interval RM473A-RM543) and hd11 (interval RM332-RM167) that were mapped for the heading date in this study, may be loci unique to the Iranian parents studied. Nevertheless, the QTL hd11 detected in this study can be the same locus as compared with the minor QTL identified by Xiao et al. (1996) and Septiningsih et al. (2003).

The degree of dominance, which is presented as the ratio of dominance to additive effects (d/a), for three QTLs detected for heading date was overdominance towards an increased heading date and three QTLs were partial to incomplete dominance towards an increased heading date, except for hd6 which exhibited a negative partial dominance for a reduced heading date (Table 1). These high levels of dominance can be related to the heterosis as observed in the F1 hybrid. Several researchers such as Li et al. (1995) and Yu et al. (2002) have also reported partial dominance or overdominance effects for the QTLs controlling heading date.

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نفشه لپتازی نشانگرهای QTL و شناسایی های کنتورل کننده تاربخ گلددهی در ارقام برنج ایرانی

ب. ریعی

چکیده

تهیه نفشه‌های مولکولی و شناسایی نواحی زنومی کنترل کندنده صفات کمی (QTLs) اهمیت زیادی برای به‌نواختن گیاهی‌های دارد. در این مطالعه، تجزیه‌زنبستی QTL‌های کنترل کننده تاربخ گلددهی با استفاده از ۱۹۲ گیاه F۲ حاصل از تلاقی دو رقم برنج ایرانی (دلم سفید و گرده) انجام شد. توزیع فوتیبی تاربخ گلددهی در جمعیت مورد مطالعه تقریباً نرمال بود. نفشه بیوستگی‌زنبستی QTL شناسایی شدند. در مهره‌های ماهواره (SSR) حاصل از جمعیت F۲ سالی از کل زنوم برنج را پیوستگی تک نیست و برابر با ۸۹ نسیمی و مانند تک نیست. در مجموع، برای تایید (LOD≥۳.۰) QTL از مکان‌های فاصله‌ای مارک (CIM) استفاده شد. در مجموع، شش گلددهی شناسایی گردد که در کنار اثر بوده و با QTL LOD نسبتاً بالا در مرکز کروموزم‌های ۶ و ۸ QTL (hd8) و (hd7) از مکان‌های شدن و به ترتیب ۲۰/۵ و ۲۰/۵ نتیجه QTL کنترلی از گلددهی QTL (hd8) را کنترل کردند. سه QTL (hd8) و (hd7) و (hd6) در اثرات فوق بودند. برآورد درجه غلیبی QTL (hd11) به نشان داد که اثرات فوق غلیبی زن جزء گلدهی در برنج از QTL (hd11) و (hd7) را برآورد کردند. اثر افزایشی (hd6) و کاهش (hd11) اثرات QTL (hd7) و (hd11) نشان دادند.