Quantitative Trait Loci for Rice Phyllochron in Lemont × IR36 Cross

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Abstract: In rice, genetic variation in "phyllochron" (the time interval between the appearance of successive leaves) affects many aspects of shoot system development. The objective of our study was to identify quantitative trait loci (QTLs) that control phyllochron in a Lemont (japonica) × IR36 (indica) F₂ population. Composite interval mapping detected four phyllochron QTLs located on chromosomes 1, 2, 9 and 11. Individually, these QTLs accounted for 8-14% of the phenotypic variation, indicating that phyllochron is controlled by multiple QTLs rather than by a major gene system. At these QTLs, the presence of a Lemont allele increased phyllochron. The only exception was for the QTL on chromosome 9, where the Lemont allele decreased phyllochron. Based on the synteny between rice and maize genomes, the ortholog of the maize terminal ear 1 gene was considered a candidate gene for the QTL on chromosome 1.

Key words: Leaf, Phyllochron, QTL, Rice.

In grasses, development of sequentially growing leaves and tiller buds are highly coordinated, where the time interval of successive leaf emergence, referred to as the "phyllochron", sets the pace of the whole shoot system development (Mitchell, 1953; Friend, 1965; Klepper et al., 1982; Hay and Kirby, 1991). In rice, this issue was pioneered by T.Katayama who studied rice shoot development since the 1920s. His studies were compiled in a monograph (Katayama, 1951), where he pointed out that (i) the first leaf of the tiller emerges three phyllochrons after emergence of the tiller's subtending leaf, and (ii) the unfolding of successive leaves on the tiller is closely synchronized with those on the main stem. He postulated a generalized scheme of rice tiller system development based on these rules. His studies formed the basis for many modern agronomic studies, and the phyllochron concept has seen widespread acceptance among rice scholars in Japan (see Nemoto et al., 1995 for a review). Genetic variation in rice phyllochron (Goto and Hoshikawa, 1989) has attracted the attention of researchers. For example, Japanese rice breeders are aware that the long phyllochron in some U.S. rice cultivars (e.g., Lemont), when crossed with Japanese cultivars, is associated with such characters as reduced tiller number and thick roots, which would be beneficial for direct seeding rice culture (H. Araki, personal communication).

In the last decade, DNA marker technology has facilitated the identification of genomic regions that control quantitative traits (quantitative trait loci, QTLs). Such information would be useful in understanding the regulatory mechanism of the traits. Very recently, we first reported QTLs for grass phyllochron (Miyamoto et al., 2004) using a rice recombinant inbred population. The objective of this study was to examine consistency of phyllochron QTLs across genetic backgrounds. In this study, we used the cross between Lemont and IR36. QTL information of the long phyllochron in Lemont would be particularly useful for improving the plant habit of Japanese rice cultivars.

Materials and Methods

1. Plant material

We conducted a preliminary survey of the genotypic variation in phyllochron among elite rice cultivars from Japan (Nipponbare, Koshihikari and Akihikari), Philippines (IR8, IR20, IR24, IR26, IR36, IR72 and IR65598-112-2), China (Nanjin11, Guang-lu-ai 4, Quickiao 2 and Taichung Native 1), Korea (Milyang 23, Milyang 54, Suwon 258 and Suwon 264), and the U.S.A. (Lemont, Blue Belle, Bluebonnet 50, L202, L203 and M202) at the University of Tokyo. Among these cultivars, Lemont (japonica, Mackill, 1995) and IR36 (indica) represented long and short extremes in phyllochron, respectively (data not shown). In 1998, IR36 was crossed as the male parent to Lemont. From this cross, an F₂ population of 190 plants was generated.

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2. Phenotypic evaluation

Phenotypic evaluation of the 190 F₂ plants, along with their parents (30 plants each) for phyllochron was conducted in a greenhouse at the University of Tokyo during summer 2000 (June-July). The parental and F₂ seeds were pre-germinated at 30°C for 3 days and sown in plastic pots (10 cm diameter, 12 cm height) filled with 0.5 L clay soil containing 0.1 g each of N, P₂O₅ and K₂O. The pots were watered regularly and the water maintained at approximately 3 cm above the soil level throughout the growth period. Some F₂ plants started reproductive development as early as eight phyllochrons after sowing (i.e., when the 8th leaf had fully appeared and the 9th leaf had begun to appear). This asynchronous reproductive development poses a problem because reproductive development increases phyllochron dramatically in rice (Nemoto et al., 1995) and therefore, it may cause a discrepancy in evaluating the phyllochron. For consistency, and to eliminate the effect of reproductive development on phyllochron, we only used the first seven phyllochrons for phenotypic evaluation.

3. DNA analysis

The DNA of the parents and 190 F₂ plants was extracted from 3 g of fresh leaf tissue using the benzyl chloride method (Zhu et al., 1993). For restriction fragment length polymorphism (RFLP) analysis, DNA was digested with six restriction enzymes: BamHI, BglII, DraI, EcoRI, EcoRV and HindIII. Southern blotting, hybridization and detection using the ECL direct nucleic acid labeling and detection kit (Amersham Pharmacia Biotech, U.K.) were conducted as described by Kurata et al. (1994). The 192 RFLP landmarkers set (Rice Genome Research Program, RGP http://rgp.dna.affrc.go.jp) derived from the published RFLP linkage map (Kurata et al., 1994) were surveyed on the parents, and selected probes were mapped. Some simple sequence repeat (SSR) markers (Chen et al., 1997) and cleaved amplified polymorphic sequence (CAPS) markers (RGP, http://rgp.dna.affrc.go.jp) were also mapped. Polymerase chain reaction (PCR) amplifications were conducted as described on the RGP homepage (http://rgp.dna.affrc.go.jp) and by Chen et al. (1997). The amplified products of the mapping population were separated on 3% agarose gels in 1 X TAE buffer. The patterns were visualized using ethidium bromide.

4. Data analysis

A linkage map was constructed using MAPMAKER/EXP ver. 3.0 (Lander et al., 1987). Composite interval mapping was performed using Windows QTL Cartographer 2.0 (Wang et al., 2001-2003). LOD threshold was determined by permutation test and resultant value was 3.6.

Results and Discussion

1. Linkage map and marker segregation

Approximately 70% of the probes detected RFLPs, and monomorphic regions on chromosomes 1, 2, 7, 8, 10 and 11 were observed. For these regions, we tested SSR and CAPS markers. Finally, one CAPS, 11 SSR and 77 RFLP markers comprised a map of 12 linkage groups that spanned 1791 cM with an average marker distance of 23.3 cM. Of the 89 markers, 23 (26%) that were mainly on the long arm of chromosomes 1, 3 and 12, showed significant deviations (P < 0.05) from the expected segregation ratios based on the Chi-square test. Among the 23 markers, 22 showed an excess of the IR36 homozygote. Only one marker (R2289 on chromosome 5) showed an excess of the Lemont homozygote.

2. Trait variation

In the 190 F₂ plants and two parents, the 8th leaf began to appear at 21-28 day after sowing (DAS). From these dates, the mean values of the first seven phyllochrons were calculated for each plant. The phyllochron for Lemont and IR36 averaged 3.96 and 3.10 days per leaf, respectively (Fig. 1). The frequency distribution of phyllochron of the F₂ population was approximately normal and within the range of the two parental values. The broad-sense heritability was high (0.83) (Fig. 1), which was similar to that observed in barley, which had heritability values ranging from 0.71 to 0.91 (Dofing, 1999).

3. QTLs for phyllochron

The analysis with QTL Cartographer revealed four phyllochron QTLs located on chromosomes 1, 2, 9 and 11 with LOD scores higher than 3.6 (Fig.2).
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1). Of the four QTLs, the QTL on chromosome 11 had relatively large effects, explaining 14% of the total phenotypic variation. Recently, we identified QTLs for phyllochron in IR36 × Genjah Wangkal (a japonica cultivar from Indonesia) cross (Miyamoto et al., 2004), and only this QTL on chromosome 11 is common to the two crosses. At the QTLs on chromosomes 1, 2 and 11, the presence of a Lemont allele increased phyllochron, while the Lemont allele decreased phyllochron at the QTL on chromosome 9 (Table 1). The ratio of the dominance effect to the additive effect was relatively high (0.86) for the QTL on the chromosome 9, but much lower for the other QTLs.

Based on segregation data, Yamamoto (1991) supposed that a single major gene might be responsible for the long phyllochron of Lemont rice. However, our results indicate that phyllochron is controlled by a polygenic system rather than by major genes. This discrepancy could be due to the fact that he used an intra-subspecific cross, which generally gives a less complex mode of inheritance (Zhuang et al., 2002). Some of our phyllochron QTLs might directly control the leaf initiation rate at the shoot apical meristem since the leaf emergence rate (phyllochron) is synchronized with the leaf initiation rate (plastochron) at the shoot apical meristem in rice (Nemoto et al., 1995). In maize, a gene having such a function was recently cloned, terminal ear 1. The terminal ear 1 regulates the timing and position of leaf initiation at the shoot apical meristem, presumably

Table 1. Location, peak LOD, additive effects, dominance effects, dominance effect / |additive effect| ratio, and percent of the phenotypic variation explained (R^2) for QTLs detected for phyllochron in F2 population from the cross Lemont × IR36.

| Chromosome | Interval       | LOD  | Add  | Dom  | Dom|Add| | R^2         |
|------------|----------------|------|------|------|----|---||             |
| 1          | R3203-R117     | 5.42 | 0.08 | -0.02| -0.27| 0.10|
| 2          | C1221-C1445    | 5.13 | 0.07 | -0.02| -0.25| 0.08|
| 9          | C711-R1687     | 5.04 | -0.06| 0.05 | 0.86| 0.09|
| 11         | RM20B-R3202    | 7.94 | 0.09 | 0.01 | 0.08| 0.14|

Add; additive effect of the Lemont allele, Dom; dominance effect, R^2; percentage of variance explained.
by acting as a suppressor of leaf initiation (Veit et al., 1998). Interestingly, one of the phyllochron QTLs detected in this study (the QTL between markers M3203 and R117 on chromosome 1) is positioned on the genomic region that is syntenous to the terminal ear 1 region on maize chromosome 3 (Devos and Gale, 1997). The current rice genomic sequence data also revealed that a putative coding sequence that shows a high homology with this gene actually exists near the marker R3203 (RGP, http://rgp.dna.affrc.go.jp). A fine mapping of this QTL would be worth determining to test that the rice ortholog of this gene may control phyllochron as a QTL.

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