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A major photoperiod-sensitivity gene tagged with RFLP and isozyme markers in rice

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Summary. Photoperiod-sensitive rice (*Oryza sativa* L.) cultivars are widely grown in rainfed lowland areas with unfavorable water regimes. A molecular marker for the trait would be useful in genetic and physiological studies and in developing improved photoperiod-sensitive cultivars. Previous genetic studies identified a major gene for photoperiod sensitivity on chromosome 6. We have tested an isozyme marker and several RFLP probes mapping to chromosome 6 in an attempt to identify marker(s) tightly linked to photoperiod sensitivity in tropical rice cultivars. We report here that the isozyme gene *Pgi-2* is linked (23.2 ± 4.7 cM) to the photoperiod-sensitivity gene in the cultivar GEB-24. Although association of duration with *Pgi-2* alleles can be used to detect segregation of the photoperiod sensitivity gene in crosses, it will probably not be useful as a marker in selection because of its loose linkage. In contrast, a gene for photoperiod sensitivity in the cultivar Puang Rai 2 was found to be closely linked to the rice genomic clone RG64. Among 15 F_3 lines homozygous for photoperiod insensitivity, no recombinants were detected with RG64. This clone is thus an excellent probe to follow segregation of the major photoperiod-sensitivity gene in rice crosses.

Key words: Molecular markers – Restriction fragment length polymorphism – *Oryza sativa* L. – Isozymes – Rainfed lowland

Introduction

Developing early-maturing cultivars by eliminating or reducing sensitivity to photoperiod has been a common objective of rice breeders over several decades. Recent attention to less favorable environments has stimulated interest among rice breeders in developing improved photoperiod-sensitive cultivars (Mohanty et al. 1984; Miah et al. 1986; Nwe and Mackill 1986). Additional interest in photoperiod sensitivity (PS) stems from recent developments in hybrid seed production using a male-sterility gene that is sensitive to photoperiod (Tong et al. 1990). Measurement of PS under field or greenhouse conditions is time-consuming and subject to error; therefore, a linked marker gene would be useful in selecting for the trait.

A major PS gene (*Se-1*) has been located on chromosome 6, close to the blast resistance gene *Pi-z* (Yokoo et al. 1980). These genes appear to be tightly linked (Kinoshita 1986). This linkage is supported by a study using near-isogenic lines (Oosumi et al. 1989). The latter study also showed that *Pgi-2* is tightly linked to these genes in the order *Se-1–Pgi-2–Pi-z*. Poonyarit et al. (1989) detected linkage between *Pgi-2* and photoperiod sensitivity in three indica cultivars, but several recombinants were obtained in the relatively small populations they analyzed. In the present study, we measured linkage between PS and *Pgi-2* and also searched for an RFLP probe(s) more tightly linked to the major PS gene.

Materials and methods

Recombination with the locus Pgi-2

The source of PS in this study was the cultivar GEB24, a selection from a traditional local cultivar from Tamil Nadu, India. Experiments were conducted at the International Rice Research Institute (IRRI), Los Baños, Philippines (14°N latitude). GEB24 was crossed with IR4563-52-1-3-6 and IR13146-179, two breeding lines developed at IRRI. The former cross was designated 'IR38697' and the latter 'IR38699'. Segregation was detected as differences in heading date under the appropriate

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planting date. Photoperiod-sensitive plants flowered late (in October) under early seeding (May–June). Plants heterozygous for PS (i.e., those that produced segregating progeny) were allowed to self-pollinate up to the F_5 generation. Homozygous F_6 lines, which were derived from the same F_5 plant, were bulk-harvested to constitute near-isogenic lines. A photoperiod-sensitive and insensitive line were selected for two crosses with the following designations:

	Sensitive	Insensitive
PS/Isozyme cross 1	IR38697-6-1-6-3	IR38697-6-1-6-1
PS/Isozyme cross 2	IR38699-53-3-3-2	IR38699-53-3-3-1

(The numbers after the cross number, such '-6-1-6-3', refer to the row numbers for each generation, F_3 through F_6. Thus, IR38697-6-1-6-3 and IR38697-6-1-6-1 are identical until the F_5 generation, where different plants were selected and given different F_6 row numbers.)

Crosses were made between the sensitive and insensitive line of each pair. Single F_1 plants of each cross were harvested to generate two F_2 populations. The F_2 populations were seeded on 10 May 1987 and transplanted to a screenhouse concrete bed. Heading of individual plants was determined as the day when 50% of the panicles were fully emerged. For chi-square analysis, plants that flowered before October were considered photoperiod-insensitive.

Horizontal starch-gel electrophoresis was conducted following the method of Glaszmann et al. (1988) for determining the isozyme variants of *Pgi-2*. The photoperiod-sensitive parents derived *Pgi-2* allele 1 from GEB24, while the insensitive parents possessed allele 2. Chi-square tests were performed to determine goodness of fit to expected genetic ratio as well as independence of *Pgi-2* and photoperiod sensitivity. The maximum likelihood method (Allard 1956) was used to estimate linkage between the two loci.

Recombination with RFLP loci

This study used F_3 lines from an F_2 population studied by Poonyarit et al. (1989). They measured days to heading of F_2 progeny from a cross between a photoperiod-sensitive cultivar, Puang Rai 2, and IR26760-27-1-3-2-1, a breeding line here designated IR26760-27, which has weak PS. Puang Rai 2 has two dominant alleles for PS, designated *Se-1* and *Se-3*, while IR26760-27 has a recessive allele for photoperiod insensitivity at one locus (*se-1*) and a dominant allele for PS at the other (*Se-3*). The F_2 population of the cross Puang Rai 2 × IR26760-27 showed a clear 3:1 segregation for the genotypes *Se-1* – (sensitive): *se-1se-1* (insensitive).

Total genomic DNA was extracted from the two parents as described by McCouch et al. (1988). DNA was digested with five restriction enzymes (*Eco*RI, *Eco*RV, *Pst*1, *Hin*dIII and *Xba*I) and run overnight in agarose (1%) gels. DNA was transferred to nylon filters (Hybond N^+, Amersham), and probed with rice genomic clones previously mapped to chromosome 6 (McCouch et al. 1988). Out of 12 clones used, two (RG213 and RG64) showed clear polymorphism on the two parents.

F_3 seed from the earliest (insensitive) and latest (sensitive) F_2 plants were planted in the greenhouse at Cornell University. At least four seeds from each F_3 line were seeded in each pot. Leaf tissue from all plants within a pot were bulked for DNA extraction. Twenty homozygous-insensitive and six sensitive F_3 lines were used (Table 2). Because the trait was scored on F_2 plants, heterozygotes could not be distinguished from homozygous-sensitive plants. The insensitive lines would therefore be more useful for measuring linkage, and a larger number was used. Filters prepared from the F_3 plants were probed with the two polymorphic clones mapping to chromosome 6.

Results and discussion

Previous research has indicated that photoperiod-sensitive rice cultivars flower in October or later when planted before June, and that photoperiod-insensitive cultivars do not have a duration to flowering longer than 130 days in the tropics (Vergara et al. 1976; Nanda and Coffman 1979; personal observation). Therefore, in both crosses scored for *Pgi-2* alleles and PS, F_2 plants that flowered in October were considered photoperiod-sensitive. All of these flowered more than 130 days after seeding. There were 9-day and 14-day gaps in flowering between plants classified as sensitive and insensitive in crosses 1 and 2, respectively. Both crosses showed discrete distribution of flowering; however, a bimodal distribution was clear for cross 2 but not for cross 1 (Fig. 1). In cross 1, the insensitive plants had a wider distribution for days to heading. This has been observed in other sensitive × insensitive crosses where the insensitive parent is early (Nwe and Mackill 1986; Poonyarit et al. 1989). The insensitive parent in cross 1 was about 3 weeks earlier in flowering than that in cross 2 (Fig. 1). In the former, PS showed a good fit to a 9:7 ratio (sensitive:insensitive) (Table 1). This would indicate that, in addition to a dominant gene conferring sensitivity, there is a recessive gene which inhibits sensitivity. Such a recessive inhibitor was also observed by Chang et al. (1969), and other workers have reported a reduced number of sensitive plants in crosses involving early insensitive-parents (Nwe and Mackill 1986; Poonyarit et al. 1989). But alternative ratios could be possible in this cross, particularly considering that the 'insensitive' class had a range of flowering dates (Fig. 1). In cross 2

Table 1. Segregation for photoperiod-sensitivity and the isozyme phosphoglucoisomerase (*Pgi*-2) allele in two rice crosses

Cross	Sensitivity class	No. of segregants for each *Pgi-2* allele[a]				χ^2
		1/1	1/2	2/2	Total	
Cross 1	Sensitive	17	56	6	79	
	Insensitive	7	32	29	68	
	Total	24	88	35	147	
	Chi-square test:					
	Sensitivity (9:7)					0.28
	Pgi-2 locus (1:2:1)					7.37*
	Independence					24.85**
Cross 2	Sensitive	21	43	8	72	
	Insensitive	3	8	16	27	
	Total	24	51	24	99	
	Chi-square test:					
	Sensitivity (3:1)					0.17
	Pgi-2 locus (1:2:1)					0.09
	Independence					21.68**

[a] *Pgi-2* alleles are designated 1 for *Pgi-2*^{1} and 2 for *Pgi-2*^{2}
*,** Significant at the 5% and 1% level of probability

the population showed a good fit to a 3:1 (sensitive: insensitive) ratio (Table 1) implicating a major dominant gene conferring PS.

The isozyme assay was performed at seedling and tillering stages for each cross. The results were the same for both stages. The chi-square value for cross 1 was significant at the 5% level of probability; the number of homozygotes for allele 1 was less, and the number of heterozygotes more, than expected for a 1:2:1 ratio (Table 1). The results showed a good fit to the expected 1:2:1 ratio in cross 2. In both crosses, the chi-square for independence of *Pgi-2* and PS was highly significant, indicating a strong association between allele 1 (from GEB24) and PS. Linkage between PS and *Pgi-2* in cross 2 was calculated as 23.2 ± 4.7 cM by the maximum likelihood method.

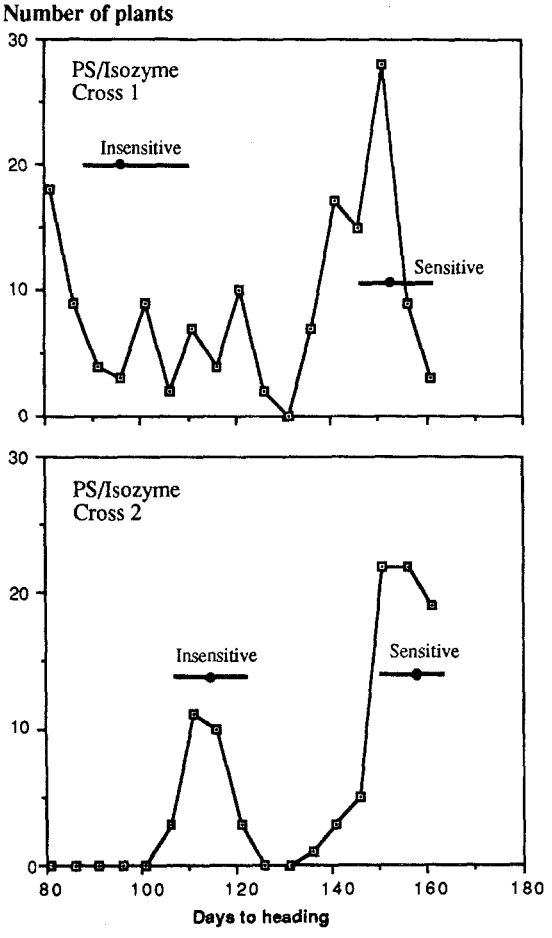

Fig. 1. Distribution of days to heading in F_2 plants from two crosses between photoperiod-sensitive and insensitive near-isogenic lines. Ranges of parents are shown by *horizontal bars* with means indicated by *circles*

The RFLP survey on the parents of PR2 × IR26760-27 revealed two polymorphic probes, RG64 and RG213, on the region of chromosome 6 near *Se-1*. RG213 showed some association with PS, but linkage was not very tight. Eleven of the photoperiod-insensitive F_3 lines were homozygous for the marker of the insensitive parent, and four were heterozygous (i.e., 13 cM). RG64 alleles, however, were directly associated with the PS phenotype (Fig. 2, Table 2). Of the 15 insensitive families that gave a good autoradiogram signal, none had the low-molecular-weight band of the sensitive parent PR2. (There is a slight indication of such a band in lane 5 which could theoretically be due to only one plant with this allele.) Of the six sensitive F_3 families used, three were homozygous for the low molecular weight band of PR2 and three were heterozygous. The PS genotype of the sensitive F_3 could not be ascertained from this study, because only F_2 data were available. As PS is partially to completely dominant (Poonyarit et al. 1989), the three RFLP heterozygotes could also be heterozygous for PS. The recombination between PS and RG64 could only be determined from the insensitive F_3. As no recombinants were observed, the genes must be closely linked. Because only 15 plants were assayed, there is a 95% chance that the map distance between the two loci is less than 12 cM. If the very weak band in lane 5 was interpreted as a recombinant, the map distance would be 3 cM.

The present study indicates that the major PS gene in GEB24 and Puang Rai 2 (and, by implication, Soc Nau) is loosely linked to the isozyme gene *Pgi-2*, and closely linked to RG64. This contradicts a previous study, which showed that *Pgi-2* is tightly linked to the major-PS-locus *Se-1* (Oosumi et al. 1989). A possible explanation is that the gene in GEB24 and PR2 is different from *Se-1*. A new

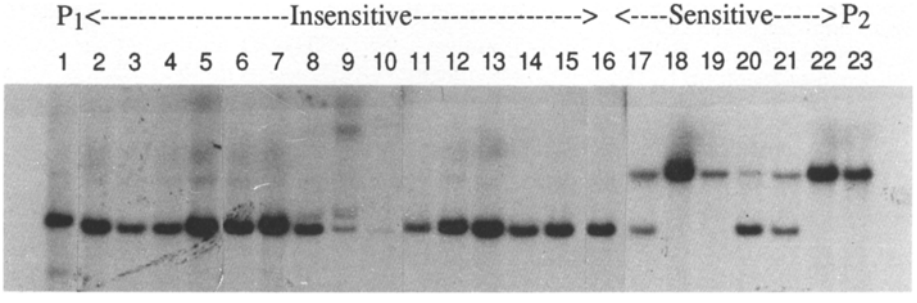

Fig. 2. Autoradiogram showing hybridization of rice clone RG64 to *Xba1* restriction fragments of DNA from the parents and F_3 progeny that are insensitive and sensitive to photoperiod from the cross Puang Rai 2 × IR26760. (Five lanes with uncut or degraded DNA, or with too low a DNA concentration to detect bands are not shown)

Table 2. Photoperiod class, days to flowering and flowering date (for seeding date of May 5) of F_2 plants of the cross Puang Rai 2 × IR26760-27 from which F_3 lines used in the RFLP analysis shown in Fig. 2 were derived. F_2 data are from unpublished results of M. Poonyarit (1989)

Line no.	Class[a]	Days to flowering	Flowering date	Lane (Fig. 2)
IR26760-27 (parent)	I	115	30 Jul	1
F_3, no. 168	I	119	3 Aug	2
F_3, no. 125	I	122	6 Aug	3
F_3, no. 410	I	122	6 Aug	4
F_3, no. 263	I	123	7 Aug	5
F_3, no. 54	I	124	8 Aug	6
F_3, no. 77	I	124	8 Aug	7
F_3, no. 331	I	124	8 Aug	8
F_3, no. 165	I	126	10 Aug	9
F_3, no. 174	I	126	10 Aug	10
F_3, no. 418	I	126	10 Aug	11
F_3, no. 111	I	127	11 Aug	12
F_3, no. 195	I	128	12 Aug	13
F_3, no. 412	I	129	13 Aug	14
F_3, no. 98	I	130	14 Aug	15
F_3, no. 338	I	133	17 Aug	16
F_3, no. 320	S	186	9 Oct	17
F_3, no. 290	S	190	13 Oct	18
F_3, no. 313	S	192	15 Oct	19
F_3, no. 13	S	205	28 Oct	20
F_3, no. 184	S	206	29 Oct	21
F_3, no. 335	S	208	31 Oct	22
Puang Rai 2 (parent)	S	225	17 Nov	23

[a] I photoperiod insensitive; S photoperiod sensitive

set of crosses would have to be made to determine if this is correct. Some results, however, indicate that the PS gene linked to RG64 is the major-PS-gene *Se-1*. The linkage between *Se-1* and the blast resistance gene *Pi-z* is well documented (Yokoo and Fujimaki 1971; Yokoo et al. 1980). Yu et al. (1991) found a tight linkage between RG64 and a blast-resistance gene designated *Pi-2*. Furthermore, Brar et al. (1991) observed 11.6% recombination between RG64 and *Pgi-2*, confirming that the gene tightly linked to RG64 was not tightly linked to *Pgi-2*.

The linkage of RG64 to PS has practical implications in rice improvement. It will certainly be useful in genetic studies of PS, as it may serve as an accurate assay of the PS genotype in segregating populations. This is often difficult to do because of the partial to complete dominance of the trait and its dependence on the date of seeding. In a practical breeding program it would be expensive to use RG64 in selection for PS plants in large populations. In tropical locations, such as Los Baños, Philippines, rice breeders can usually select photoperiod-sensitive plants in the field with proper choice of seeding dates. But developing improved photoperiod-sensitive cultivars is more difficult and time-consuming than developing insensitive cultivars. Most breeding programs in rainfed lowland areas have therefore concentrated on insensitive cultivars, even though photoperiod sensitivity would often be an advantage.

Because highly productive photoperiod-insensitive cultivars are easier to develop than are sensitive cultivars, it would be advantageous to be able to convert insensitive cultivars with demonstrated yield performance into photoperiod-sensitive cultivars. It has been shown that RFLP markers can reduce the number of backcrosses needed to recover the recurrent parent genotype (Tanksley et al. 1989). With a more saturated RFLP map, flanking markers for PS could be identified. These could be used to select for recombination between the PS gene and nearby markers, allowing transfer of PS without the accompanying donor genes. The photoperiod-sensitive cultivars developed from such a conversion program would be more suitable for rice-growing environments with unpredictable water regimes than the original insensitive parent.

References

Allard RW (1956) Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24:235–278

Brar DS, de los Reyes BG, Panaud O, Sanchez A, Khush GS (1991) Genetic mapping in rice using isozyme and RFLP markers. In: Rice genetics II. Proc 2nd Int Rice Genet Symp, 14–18 May 1990, Int Rice Res Inst, Los Baños, Philippines, pp 137–145

Chang TT, Li CC, Vergara BS (1969) Component analysis from seeding to heading in rice by the basic vegetative phase and photoperiod sensitive phase. Euphytica 18:79–91

Glaszmann JC, de los Reyes BG, Khush GS (1988) Electrophoretic variation of isozymes in plumules of rice (*Oryza sativa* L.) – A key to identification of 76 alleles at 24 loci. IRRI Res. Paper Series No. 134. Int Rice Res Inst, Los Baños, Philippines

Kinoshita T (1986) Standardization of gene symbols and linkage maps in rice. In: Rice genetics. Proc Int Rice Genet Symp, 27–31 May 1985, Int Rice Res Inst, Los Baños, Philippines, pp 215–228

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

Miah NM, Miah MAA, Dewan SBA (1986) Integrated breeding program for medium-deep, stagnant-water, rainfed rice in Bangladesh. In: Progress in rainfed lowland rice. Proc Int Rice Res Conf, 15–20 Oct 1984, Bhubaneswar, India, Int Rice Res Inst, Los Baños, Philippines, pp 217–222

Mohanty HK, Roy A, Das SR (1984) Varietal improvement in lowland rice. Oryza 21:72–87

Nanda JS, Coffman WR (1979) Alternative plant types and other agronomic characteristics for rainfed lowland rice. In: Rainfed lowland rice: Selected papers from the 1978 International Rice Research Conference. 17–21 Apr 1978, Los Baños, Philippines. Int Rice Res Inst, Los Baños, Philippines, pp 123–133

Nwe KT, Mackill DJ (1986) Photoperiod sensitivity in three rice crosses. Euphytica 35:751–760

Oosumi T, Miyazaki A, Uchimiya H, Kikuchi F, Yokoo M (1989) Analysis of glucose phosphate isomerase in near-isogenic

lines, and cultivars of rice (*Oryza sativa* L.). Bot Mag Tokyo 102:283–289

Poonyarit M, Mackill DJ, Vergara BS (1989) Genetics of photoperiod sensitivity and critical daylength in rice. Crop Sci 29:647–652

Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding: new tools for an old science. Bio/Technology 7:257–264

Tong Z, Wang T, Xu Y (1990) Evidence for involvement of phytochrome regulation in male sterility of a mutant of *Oryza sativa* L. Photochem Photobiol 52:161–164

Vergara BS, Jackson B, De Datta SK (1976) Deep water rice and its response to deep water stress. In: Climate and rice. Proc Symp, 24–27 Sep 1974. Int Rice Res Inst, Los Baños, Philippines, pp 301–319

Yokoo M, Fujimaki H (1971) Tight linkage of blast-resistance with late maturity observed in different indica varieties of rice. Jpn J Breed 21:35–39

Yokoo M, Kikuchi F, Nakane A, Fujimaki H (1980) Genetic analysis of heading date by aid of close linkage with blast resistance in rice. Bull Nat Inst Agric Sci Ser D, 31:95–126

Yu ZH, Mackill DJ, Bonman JM, Tanksley SD (1991) Tagging genes for blast resistance in rice via linkage to RFLP markers. Theor Appl Genet 81:471–476