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Dissection of a major QTL for photoperiod sensitivity in rice: its association with a gene expressed in an age-dependent manner

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Abstract A proposed major quantitative trait locus (QTL) for photoperiod sensitivity on chromosome 6 in rice was examined by introducing a chromosomal segment from a sensitive line into an insensitive one. The crossing experiments showed that a range of variation in heading date occurred in the later generations and that the region might contain at least a major gene and two additional recessive genes controlling photoperiod sensitivity. Gene mapping experiments showed that the major gene was *Se-1* and that a recessive gene (tentatively named *se-pat*) was loosely linked to it. The responses to photoperiods were examined among the different genotypes under natural and controlled conditions. The two genes acted additively on the degree of photoperiod sensitivity. However, *se-pat* plants showed a response to photoperiods that differed from that of the other sensitive lines; a short-day treatment at the seedling stage delayed heading in the former plants, suggesting that the manner of its expression was age-dependent. A recessive gene similar to *se-pat* seemed to be widely distributed in wild and cultivated rice, suggesting that the gene complex in the region plays a significant role in response to photoperiod.

Key words *Oryza sativa* L. · Photoperiod-sensitivity genes · Quantitative traits · Gene complex · Wild rice

Introduction

The time to flowering varies greatly in rice and depends on the genotype and environment. The inheritance of heading date is of polygenic nature, and hybridization between cultivars frequently produces a continuous variation (Akemine and Kikuchi 1958; Vergara and Chang 1985). Numerous genetic studies have been carried out because of its importance for regional adaptability and adaptive ranges in cultivated rice. The latitudinal cline of photoperiod sensitivity observed in rice suggests that photoperiod sensitivity is a major factor that enables plants to complete their life cycle in a favorable period depending on the temperature and rainfall patterns (Oka and Chang 1960; Oka 1988).

Recently, much interest has been focused on the dissection of quantitative traits into Mendelian loci (Tanksley 1993). Although quantitative traits had been assumed to be controlled by numerous genes with large effects as well as small effects, it has been pointed that there are often specific regions of the genome that are able to account for a large portion of the phenotypic variation observed in nature for many quantitative traits. However, our present information on minor genes (or QTLs) is limited in relation to how variation for quantitative traits is maintained in natural populations under different environments.

Yano et al. (1996) located two major and three minor QTLs (quantitative trait loci) for heading date on a high-resolution map in an Indica-Japonica hybrid of cultivated rice. Of these, a major QTL seemed to be identical to *Se-1*, a gene which Yokoo et al. (1980) suggested plays a role in photoperiod sensitivity in rice. Date of heading is partially determined by the critical daylength (the longest daylength at which floral initiation takes a place). Rice plants with a shorter critical daylength flower later in the summer season in Japan. It has been proposed that a series of alleles at the *Se-1* locus are present among rice cultivars (Yokoo and Kikuchi 1977; Yokoo et al. 1982). However, it is not easy to ascertain whether the major effects associated with the specific regions are due to the action of single genes (orthologous) or clusters of genes. In fact, on chromosome 6, an additional minor QTL was detected

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in an Indica type of rice, and an enhancer for *Se-1* was found in the wild ancestor, *O. rufipogon* (Yano et al. 1996; Sano 1992). This implies that there might be clustered genes on the segment and that the genetic complexity should be studied within chromosome as postulated by McCouch and Doerge (1995). Our intention in the study presented here was to compare the genetic complexity of the region proposed as a major factor for heading date among rice taxa. Our strategy was to examine the complexity by introducing the alien chromosomal segments responsible for heading behavior from various strains into an almost completely insensitive strain. This enabled us to examine phenotypic expression precisely in the same genetic background. We report here the genetic complexity of a major QTL for photoperiod sensitivity of rice showing involvement of a hidden locus that was expressed in an age-dependent manner.

Materials and methods

Plant materials

All the materials used were chosen from collections preserved at the Genetic Stocks Center, National Institute of Genetics, Mishima, Japan. T65*wx* is a near-isogenic line of Taichung 65 (Japonica type of *Oryza sativa*) carrying the *wx* gene (waxy or glutinous) on chromosome 6. Additional material consisted of three Indica types (Patpaku and Pehkuh from Taiwan, Basmati 370 from Pakistan), three strains of of *O. rufipogon* (W1806 from Sri Lanka, W1975 from Indonesia, W1939 from Thailand) and one strain of *O. longistaminata* (W1618 from Madagascar), all of which carry *Wx*. All the strains except for T65*wx* and Pehkuh are strongly sensitive to photoperiod. *O. rufipogon* is assumed to be the wild ancestor of Asian cultivated rice and it is divisible into annual (W1806) and perennial (W1975, W1939) types (Oka 1988). *O. longistaminata* is a distantly related wild relative distributed in Africa which shares the AA genome of the other taxa used in this study.

Backcrossing experiments

Since genic differentiation for photoperiod sensitivity among rice taxa has been reported for chromosome 6 (Morishima et al. 1992), backcrossing experiments were carried out to introduce chromosomal segments containing *Wx* from Patpaku into T65*wx* by using T65*wx* as the recurrent parent. We intended to introduce the segment containing the *Wx* and the *Se-1* loci into an insensitive accession (T65*wx*), if Patpaku had the *Se-1* gene. Patpaku was used as the pollen parent in the initial cross. Since Patpaku shows no heading in summer seasons at Mishima (35°N) due to its strong photoperiod sensitivity, the backcrossed plants were grown in a short-day field (2.6 × 3.5 m) where daylength was experimentally reduced to 10 h from 8 weeks after sowing. It was noticed that in a short-day field backcrossed plants heterozygous for *wx* frequently headed later than the recurrent parent (T65*wx*). Since floral initiation took a place in T65*wx* before 8 weeks after sowing, the heterozygotes with late heading suggested an involvement of gene(s) controlling the degree of photoperiod sensitivity linked to *Wx*. A few heterozygotes for *wx* were then used for further crossings in order to introduce the segment containing *Wx* and the photoperiod-sensitivity gene(s). Heterozygosity at the *wx* locus was examined by staining pollen grains with an iodine solution prior to crossing and was ascertained from selfed seeds. A plant which was shown to be homozygous for *Wx* and late heading was selected from the B_7F_3 generation, and the line was designated T65(*Wx*-pat).

The genic differentiation involved in the introduced segment was examined in the B_8F_2 and later generations of a cross between T65(*Wx*-pat) and the recurrent parent (T65*wx*). With regard to heading date, a range of variation showing transgressiveness was observed in the B_8F_4 generation. True-breeding lines that were different in heading date were found in the B_8F_4 lines under natural daylength. The true-breeding lines were regarded as recombinant inbred lines (RILs) carrying different lengths of alien segments. The RILs were grouped into five types according to their heading dates; Type I headed as early as T65*wx* (late August) and Type V headed later than T65 (*Wx*-pat). As often found in Indica-type rice, the donor parent (Patpaku) carried an additional marker, *c* (anthocyanin coloration), on chromosome 6, which was also used for characterizing the introduced segment.

Similar backcrossings were also carried out using the other six strains (Pehkuh, Basmati 370, W1806, W1975, W1939 and W1618) to compare the genetic constitutions of the regions among wild and cultivated strains. The objective of this experiment was focused on a survey of genes for heading date tightly linked to the *wx* locus because genes controlling heading date had been reported previously to be linked to it (Sano 1992; Yano et al. 1996). F_2 populations from the B_3F_1 and B_4F_1 generations were grown in a paddy field under natural daylength to examine whether or not genes controlling heading date had tightly linked to *wx*. The simply inherited gene detected was further purified by backcrosses.

Cultivation

To examine the genetic segregation for heading date, we allowed seeds to germinate in petri dishes in early May, and 4-week-old seedlings were then transplanted in paddy fields with a spacing of 25 × 20 cm at Mishima (35°N). To look into the degree of photoperiod sensitivity in the different genotypes obtained, we conducted experiments in a short-day field and greenhouse, and in growth chambers. After germination in the dark for 2 days, four pre-germinated seeds were planted in a plastic pot (12-cm diameter). Sets of the pots were placed under natural daylength (NDL), a 10-h photoperiod (short day, SD) or a 18-h photoperiod (long day, LD) until heading. In addition, a set of the pots were subjected to a 10-h photoperiod at the age of 7 weeks (7wkSD) after growth under natural daylength. The plants under LD were reared in a greenhouse where supplementary lighting (about 18 $\mu mol\,m^{-2}\,s^{-1}$ at the plant level) was provided. Since heading was delayed under LD conditions, the temperatures in the greenhouse were kept above 25°C from the middle of September to avoid effects due to low temperatures. Further, sets of the pots were placed in growth chambers after pre-germination, and the plants were subjected to 10-h, 12-h and 14-h photoperiods (about 700 $\mu mol\,m^{-2}\,s^{-1}$) at 30°C until heading.

Heading date of the main stem was individually recorded. To count the number of leaves on the main stem, we marked the leaf number starting with the incomplete leaf of the seedling. In each treatment, four plants per genotype were measured.

Results

Segmentation of an alien segment from Patpaku

T65 (*Wx*-pat) carries an alien segment of chromosome 6 from Patpaku. Based on the segregation pattern in the F_2 of T65*wx* × T65 (*Wx*-pat), it was apparent that T65 (*Wx*-pat) had the *c* allele and an incompletely

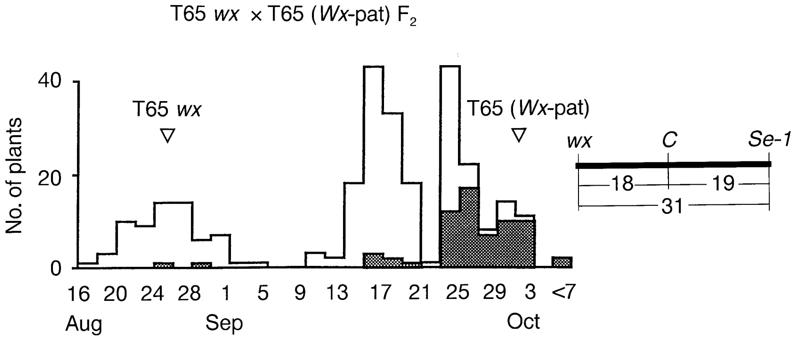

Fig. 1 F_2 segregation for heading date in a cross of T65 *wx* × T65 (*Wx*-pat). Plants with the genotypes of *C*_ and *cc* are shown by *open* and *dotted boxes*, respectively. The gene map is based on the recombination values estimated (n = 283)

dominant gene controlling heading date (Fig. 1). The gene affecting heading date was linked to the *wx* and *C* loci with a recombination value of 0.31 ± 0.03 and 0.19 ± 0.06, respectively, and it was considered to be identical to, or tightly linked to, *Se-1* judging from the linkage intensities reported by Yokoo et al. (1980). It was, however, noticed from the F_2 data (283 plants) that the segregation for *Se-1* deviated significantly from the expected ratio of 1 : 2 : 1 ($\chi^2 = 16.46$, $df = 2$, significant at 1%), although *wx* and *C* showed no such tendency. Infertilities that often cause distorted segregations in rice were not detected in this case. As mentioned below, the significant distortion for *Se-1* was shown to have resulted from the presence of additional genes linked to *Se-1* and present on the introduced segment.

True-breeding lines (or RILs) differing in heading date were obtained from the selfed progeny (B_8F_4 generation), and these were grouped into five types on the basis of their heading dates under natural daylength (Table 1). Type I headed as early as T65*wx*, while the others (Types II–V) headed significantly later. Since the number of days to heading was reduced by short-day treatments in these lines, all the genes responsible for heading date seemed to control the degree of photoperiod sensitivity. Type V headed much later than T65(*Wx*-pat), showing a transgressive segregation. The occurrence of five different true-breeding lines suggested that at least three genes were responsible for the differences. Based on segregations in crosses among them, we determined their genotypes, except for Type V.

se-pat located between *wx* and *C*

Crosses were made among T65*wx* (or T65) and the RILs, except for Type V. All of the F_2 plants from a cross of Type I × T65*wx* headed as early as T65*wx*, indicating that their genotypes are the same for heading date (Table 1). F_2 populations from two crosses (Type I × Type IV and Type II × Type III) showed continuous segregation, while the other four crosses showed monogenic segregation (Table 1). A ratio of 3 : 1 observed in Type II × T65*wx* F_2 indicated that Type II has a recessive gene for heading date, while a ratio of 1 : 2 : 1 in Type III × T65 F_2 indicated that Type III has an incompletely dominant gene for heading date. The former gene was estimated to be linked to the two markers *wx* and *C*, with recombination values of 0.14 ± 0.03 and 0.13 ± 0.04, respectively (Table 2). This gene seemed to be located between *wx* and *C* and was tentatively designated *se-pat*, while the latter gene in Type III was assumed to be *Se-1* from its location (Table 2). No F_2 segregant heading as early as T65*wx* was observed in crosses of Type II × Type IV and Type III × Type IV, showing that Type IV has both genes. The former cross gave a ratio of 1 : 2 : 1, while the latter gave a ratio of 3 : 1 as expected. In the latter cross, F_3 lines fitted a 1 : 2 : 1 ratio of the parental types and segregating line (5 : 11 : 4, $\chi^2 = 0.30$, $df = 2$). Therefore, *Se-1* and *se-pat* were expected to simply segregate in the Type II × Type IV F_2 and in the Type III × Type IV F_2, respectively (Tables 1 and 2). Thus, the two genes seemed to act additively to delay the time of heading in the summer seasons at Mishima.

Photoperiod responses of the RILs

The degree of photoperiod sensitivity was compared among T65*wx* and the four RILs (Types II, III, IV, V) under various photoperiodic regimes. Only T65*wx* headed under an 18-h photoperiod (LD), suggesting that it is almost insensitive to daylength. The four RILs all headed under natural daylength but not under an 18-h photoperiod, showing that they were photoperiod-sensitive (Table 3). When they were subjected to a 10-h photoperiod at the age of 7 weeks (7wkSD), the days to heading was markedly reduced compared to those under natural daylength (NDL). When a 10-h photoperiod was applied from the germination stage (SD), all the lines except for Type II headed earlier than those of 7wkSD, while Type II headed later in SD than in 7wkSD (Table 3).

In growth chambers, the four lines (Types II, III, IV, V) showed no heading until 170 days after sowing

Table 1 Segregation patterns for heading date in the F_2 populations from crosses between the RILs (Types I–IV) and the recurrent parent (T65*wx*) under natural daylength at Mishima (35°N)

Cross	Heading date																										Number of plants observed
	August						September															October					
	20	22	24	26	28	30	1	3	5	7	9	11	13	15	17	19	21	23	25	27	29	1	3	5	7	9	
Type I × T65*wx* F_2	1	27	41	44	20	7																					140
Type II × T65*wx* F_2		2	15	26	24	15	5		4	4	1	2		2	3	3											106
Type II × Type IV F_2										3	6	14	2	4				$\underline{14}$	$\underline{16}$	$\underline{13}^{d}$		5	4	4	6		93
Type III × Type IV F_2																2	17	26	33	24	2	7	12	21	8		152
Type III × Type IV F_3 (E)[a]																	3	15	6	3	1						27
Type III × Type IV F_3 (L)[b]																							3	14	21	7	35
Type III × T65 F_2[c]	4	12	14	3				$\underline{9}$	$\underline{17}$	$\underline{22}$	$\underline{4}$	$\underline{4}$	$\underline{1}$	$\underline{1}$			7	18	11	3	1						131
TypeII × Type III F_2	2			1		3	5	15	20	17	18	15		15	17	21	6	13	15	2			2	2			189
TypeIV × T65*wx* F_2	5	7	4	6	1		3	2	3	2	7	11		14	8	6		2	8	9	1	2	4	3			108
TypeI	6	4	4																								14
Type II											1	3	8	2													14
Type III																	2	4	7	1							14
Type IV																							8	6			14
T65*wx*	6	8																									14

[a] F_3 lines homozygous for early heading from the Type III × Type IV F_2

[b] F_3 lines homozygous for late heading from the Type III × Type IV F_2

[c] T65 (*WxWx*) was used as a parent to examine a linkage intensity between *wx* and a photoperiod-sensitivity gene

[d] Underlined numerals indicate assumed heterozygotes

(DAS) under a 14-h photoperiod (Table 3). Types III, IV and V headed earlier than T65*wx* under a 10-h photoperiod, while the latter two types showed no heading under a 12-h photoperiod, suggesting that they have shorter critical daylengths than Type III. On the other hand, Type II showed no heading under any of the short-day conditions in the growth chambers, even though it headed slightly later (about 10 days) than T65*wx* under natural daylength in a paddy field (NDL). Type II grew vigorously in the growth chambers and continued to produce leaves without floral initiation.

The number of days to heading, which varied greatly in response to photoperiodic regimes (Table 3), was found to be significantly correlated with the number of leaves produced on the main culm in both the field ($r = 0.94, df = 14$) and growth chamber ($r = 0.97, df = 5$) experiments. The linear regression was estimated as $y = 18.6x - 171.6$ in fields and $y = 10.8x - 48.2$ in growth chambers, where x and y stand for the number of leaves produced and the number of days to heading, respectively. This suggests that the time interval of leaf emergence differed depending on environment. Although the pattern of leaf emergence was different in each environmental condition, the delay in the heading resulted from the increased number of leaves produced.

Distribution of *se-pat* in cultivated and wild rice strains

The six cultivated and wild rice accessions (Pehkuh, Basmati 370, W1806, W1975, W1939 and W1618) were examined as to whether or not they carry a gene(s) controlling heading date which is tightly linked to the *wx* locus like *se-pat*. The F_1 hybrids between T65*wx* and the six accessions showed low seed fertilities (9.3–46.8%), and no discrete pattern of segregation was observed in the F_2 populations. The chromosomal segment containing *Wx* from the donors was then introduced into T65*wx* by backcrosses. In the backcross generations, a few heterozygotes for *wx* (*Wx*/*wx*) were arbitrarily chosen for further crossing. Backcrosses were repeated until a simple segregation pattern was observed in the selfed populations. As shown in Table 4, a 3:1 segregation ratio suggested that all the accessions involve a recessive gene linked to the *wx* locus, although the heading date of the recessive segregants varied depending on the donor parents. The recombination values with *wx* also varied from 0.06 to 0.19. Although allelism tests are needed, the present results suggest that *se-pat* might be widely distributed among wild and cultivated rice accessions.

Discussion

Panicle initiation can be triggered by daylength, causing a wide rage of variation in growth duration

Table 2 Recombination values estimated between a photoperiod-sensitivity gene and markers *wx* and *C* (*ns* non-significant)

Cross[a]	Segregation ratio	*df*	χ^2	p of a photosensitivity gene with[b]	
				wx	*C*
Type II × T65*wx* F_2	3 : 1	1	2.83^{ns}	0.14 ± 0.03	0.13 ± 0.04
Type II × Type IV F_2	1 : 2 : 1	2	2.25^{ns}	0.36 ± 0.05	–
Type III × Type IV F_2	3 : 1	1	3.51^{ns}	–	0.09 ± 0.03
Type III × T65 F_2	1 : 2 : 1	2	2.47^{ns}	0.34 ± 0.05	–

[a] Genotypes were *wxwx CC* in T65*wx* and Type III, *WxWxCC* in T65, *WxWx cc* in Type II, and *wxwx cc* in Type IV

[b] Recombination value (p) was estimated by maximum likelihood method. Type II and Type III were estimated to have *se-pat* and *Se-1*, respectively, and Type IV both genes. The gene order was assumed to be *wx-se-pat-C-Se-1* based on the data (see text)

Table 3 Photoperiodic responses of the RILs as measured under different photoperiodic regimes in the field, greenhouse and growth chamber

Treatment[a]	Number of days to heading					
	Type	Type I (= T65*wx*)	Type II	Type III	Type IV	Type V
	Genotype[b]	*se-pat*$^{+}$ *Se-1*$^{+}$	*se-pat* *Se-1*$^{+}$	*se-pat*$^{+}$ *Se-1*	*se-pat* *Se-1*	Unknown
Field and greenhouse:						
NDL	Mean	106.5[d]	123.5	134.3	143.3	168.8
SD	Mean	68.8	137.0	60.8	70.5	66.5
LD	Mean	109.0	NH[e]	NH	NH	NH
7wkSD	Mean	92.5	102.3	91.0	94.3	94.5
Difference	(NDL – 7wkSD)	14.0	21.2	43.0	48.9	74.3
	(NDL – SD)	37.8	– 13.5	73.3	72.8	102.3
	(7wkSD – SD)	23.8	– 34.7	30.3	23.8	28.0
Growth chamber:						
10 h[c]	Mean	87.8	NH	57.4	78.3	63.6
12 h	Mean	91.2	NH	99.0	NH	NH
14 h	Mean	117.0	NH	NH	NH	NH

[a] NDL, Plants were grown under natural daylength; LD, an 18-h photoperiod was applied to plants in a greenhouse; SD, a 10-h photoperiod was applied in a short-day field; 7wkSD, plants were transferred from NDL to SD at the age of 7 weeks

[b] The genotype of T65*wx* was shown as *se-pat*$^{+}$*se-pat*$^{+}$ *Se-1*$^{+}$*Se-1*$^{+}$, and both genes control photoperiod insensitivity, while the opposite alleles (*se-pat* and *Se-1*) control photoperiod sensitivity

[c] 10-h, 12-h and 14-h in growth chambers, 10-h, 12-h and 14-h photoperiods, respectively

[d] LSD0.05 for comparisons between mean values was 3.49 in the field and greenhouse and 4.39 in the growth chamber

[e] NH, shows no heading until 200 days (greenhouse) or 170 days (growth chamber) after sowing

among rice cultivars. Quantitative traits such as heading date are generally determined from the interactions of numerous genes with large and small effects that are influenced by environmental conditions. Both major and minor genes seem to be required for the adjustment of heading date, as expected from the continuous or clinal variations observed among rice cultivars. Information on minor genes, however, is limited.

With respect to major loci for photoperiod sensitivity, it has been proposed that *Se-1* on chromosome 6 plays an important role for determining the duration of growth in rice based on the fact that this gene was frequently detected in experiments conducted both in temperate and tropical regions (Chandraratna 1955; Yokoo et al. 1980). In addition, *Se-3*, *En-Se-1*, and *E-1* have been shown to cause stronger sensitivity when they interact with *Se-1* (Poonyarit et al. 1989; Sano 1992; Okumoto et al. 1996). Furthermore, inhibitors for *Se-1* have also been found to be involved in cultivated rice (Chang et al. 1969; Ohshima et al. 1993). These findings support the assumption that *Se-1* plays a significant role in the regulation of photoperiodic responses in rice. Although a series of alleles at the *Se-1* locus might be a causal factor controlling different degrees of photoperiod sensitivity (Yokoo and Kikuchi 1977; Yokoo et al. 1982), it is impossible to carry out

Table 4 Segregation patterns for heading date under natural daylength at Mishima (35°N) in the hybrid derivatives between six wild and cultivated rice accessions and T65*wx*, showing linkage relations with *wx*

Donor	Generation of backcross[a]	Heading date																		Number of plants observed	χ^2 (3:1)	p (with *wx*)
		August								September												
		16	18	20	22	24	26	28	30	1	3	5	7	9	11	13	15	17	19			
Pehkuh	B_4F_2	7	6	13	9	11	4	12	2		$\underline{1}$	$\underline{6}$	$\underline{2}$	$\underline{8}$	$\underline{8}$	$\underline{1}^b$				90	0.73^{ns}	0.10 ± 0.03
Basmati370	B_4F_2		2	9	10	5	3	1						$\underline{3}$	$\underline{3}$	$\underline{5}$	$\underline{1}$			42	0.29^{ns}	0.06 ± 0.03
W1975	B_7F_2	3	3	2	10	5	3	4		$\underline{4}$	$\underline{2}$	$\underline{6}$								42	0.29^{ns}	0.07 ± 0.03
W1806	B_4F_2	2	9	8	13	10	4	1		$\underline{1}$	$\underline{8}$	$\underline{3}$	$\underline{3}$	$\underline{1}$						63	0.01^{ns}	0.07 ± 0.02
W1939	B_6F_2			1	10	10	13	20	15	12	$\underline{5}$	$\underline{9}$	$\underline{2}$	$\underline{8}$						105	0.26^{ns}	0.19 ± 0.04
W1618	B_6F_2			7	18	39	14	1	1	3	$\underline{1}$	$\underline{5}$	$\underline{5}$	$\underline{3}$	$\underline{1}$					98	1.65^{ns}	0.11 ± 0.03

[a] Chromosome segments carrying *Wx* were introduced into T65*wx* from cultivated and wild rice strains using T65*wx* as the recurrent parent

[b] Underlined numerals indicate assumed recessive homozygotes

complementation (or allelism) tests for dominant alleles by means of conventional analysis. The present results indicate that the position of *Se-1* was correctly estimated, even though recessive modifiers were linked to it (Fig. 1). If T65(*Wx*-pat) is assumed to carry only a major gene (*Se-1*) for photoperiod sensitivity, the present results lead to the conclusion that T65(*Wx*-pat) carried a different allele with a stronger effect at the *Se-1* locus, resulting in instability in the hybrid derivatives. Thus, genetic comparisons among the RILs with the same genetic background were useful in detecting minor genes on the gene complex.

The complex nature of photoperiod sensitivity observed in the present study is supported by the recent QTL analysis of Yano et al. (1996). Two major and three minor QTLs were located on three chromosomes in a cross between Indica ('Kasalath') and Japonica ('Nipponbare') types of rice. Two of these were mapped on chromosome 6. A major QTL (*Hd-1*) seemed to be the same as *Se-1*, and another minor QTL (*Hd-3*) to be closely linked to *wx*. The manner of genic interaction was not clear because it was based only on F_2 data. Although *Hd-3*, carried by 'Kasalath', was estimated to cause late heading under natural daylength, it does not seem to be the same as *se-pat*, because plants carrying the segment *Wx* to *c* introduced from 'Kasalath' headed as early as the recurrent parent of T65*wx* (our unpublished data). The cross T65*wx* × T65(*Wx*-pat) produced transgressive segregants in heading date. Type V headed much later than T65(*Wx*-pat), although the genic analysis is still in progress. The transgressiveness is considered to have resulted from an involvement of an additional recessive gene, which might be located on chromosome 6 of T65*wx*.

The present experiments confirm that a gene complex on chromosome 6 results in a range of variation in heading date by recombining genes on the segment through hybridization. All the genes involved might be related to the degree of photoperiodic response, or its modulation, suggesting that the region plays a more significant role in the time of flowering in rice than expected. Polygenic traits are controlled by the interaction of multiple genes whose effects are essentially interchangeable and small relative to environmental sources of variation. Whether or not the gene complex is the outcome of adaptation is uncertain, however; different combinations of functionally related genes on the segment have the potential to provide a range of heading dates in a hybrid population, allowing adaptation to different localities. Heterogeneity within the segment tends to be maintained in predominantly self-fertilizing species, since gametic disequilibrium is greatly affected by restricted gene flow due to the difference in flowering time as well as inbreeding and linkage (Hedrick et al. 1978).

Since the RILs obtained in this study are regarded as near-isogenic lines with different combinations of genes, our comparisons provide an opportunity to examine their genic effects. Among the estimated four genotypes, the degree of photoperiod sensitivity is assumed to be *se-pat*$^+$ *Se-1*$^+$(T65*wx*) < *se-pat Se-1*$^+$ (Type II) < *se-pat*$^+$*Se-1* (Type III) < *se-pat Se-1* (Type IV) based on data from paddy fields under natural daylength, showing that the two genes, *se-pat* and *Se-1*, act additively. The significant correlation between the number of days to heading and the number of leaves on the main culm suggests that the time interval of leaf emergence (phyllochron) is not changed by different photoperiodic regimes among the genotypes. Because phyllochron is synchronized with the time interval of leaf initiation (plastochron) in rice (Nemoto 1995), plastochron does not seem to be affected by daylengths among the genotypes.

Of special interest are the responses observed in the *se-pat* homozygous plants (Type II). A 10-h photoperiod was effective in the *se-pat* genotype when the short-day treatment started at the age of 7 weeks. However, the heading date was delayed when the treatment started at the germination stage. This shows that a 10-h photoperiod at the early stage of development delayed heading in the *se-pat* genotype, suggesting that the genic effects were age-dependent. The effect of *se-pat* at the juvenile stage seems to be cancelled out by *Se-1* since no such effect was observed in plants with both *se-pat* and *Se-1*. Recently, Yin et al. (1997) reported that short-day treatments (10-h and 12.5-h photoperiods) caused the delay in heading when the treatment was subjected at the seedling stage (about 1 month). The present results also suggest that responses to daylengths change in an unexpected manner during the seedling stage in the *se-pat* genotype. This suggests that caution should be taken in estimating the basic vegetative phase under controlled conditions, although it is assumed in rice that the seedling stage is simply insensitive to photoperiod. The experiments in growth chambers also showed an unexpected phenomenon in that *se-pat* did not head under any of the photoperiods adopted in the present experiments. Although the significance of the gene is obscure at present, the possibility of its wide distribution might imply an important role in the adjustment of floral initiation in response to photoperiods in tropical areas. Detailed investigations of the genic effects of *se-pat* might shed light on the regulatory network controlling photoperiodic responses in rice.

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