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ORIGINAL PAPER

# Epistatic interactions of three loci regulate flowering time under short and long daylengths in a backcross population of rice

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**Abstract** The short-day plant rice varies greatly in photoperiod sensitivity (PS) for flowering. The hybrid $F_1$ from a cross between the day-neutral pure line EM93-1 and the weedy rice accession SS18-2 had stronger PS than SS18-2. Some $BC_1$ (EM93-1/$F_1$) segregates were even more sensitive to photoperiod than the $F_1$, as indicated by later flowering or no flowering after 250 days under a 14-h long daylength. A genome-wide scan identified the quantitative trait loci $Se_{7.1}$, $Se_{7.2}$ and $Se_8$ for PS from the $BC_1$ population, with all alleles that inhibit flowering derived from SS18-2. These three loci regulate the time of flowering under long daylength through their main effects, and di- and trigenic epistases. Under a 10-h short daylength, the regulation is through $Se_{7.1}$ and $Se_8$ main effects and digenic epistases involving all three loci. The short daylength not only nullified the main effect of $Se_{7.2}$, but also changed its epistatic effects from inhibiting flowering under long daylength to promoting flowering. The epistases indicate that genes underlying the three PS loci work in the same pathway for the control of flowering. Many non-flowered $BC_1$s were the trigenic heterozygote; this suggests that the three PS loci are also involved in genetic control of critical daylength.

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## Introduction

Flowering time is an adaptive trait of biological and agricultural importance as it determines seasonal, latitudinal and altitudinal adaptations of wild and domesticated plants. Photoperiod sensitive plants may flower only under, or have their flowering accelerated by, a daylength longer (i.e., long-day plant) or shorter (i.e., short-day plant) than a particular threshold, called critical daylength (Garner and Allard 1920). Genetic diversity in floral response to photoperiod exists in long- and short-day plant species. Arabidopsis (*Arabidopsis thaliana* L.) and rice (*Oryza sativa* L.) are model long- and short-day plants, respectively, for research on genetic pathways of floral control (Yano et al. 2001; Boss et al. 2004; Jack 2004). Recently, map-based cloning of quantitative trait loci (QTLs) for heading date revealed that underlying genes from rice share a high degree of similarity with those from Arabidopsis, although they appeared to play different roles in promoting or inhibiting flowering under long or short daylengths (Yano et al. 2000; Takahashi et al. 2001; Kojima et al. 2002; Doi et al. 2004). To understand photoperiod control of flowering, a wide range of allelic variation from cultivated and wild rice should be examined (Yano et al. 2001).

The genetic variation in flowering time in rice is largely determined by the vegetative growth phase, the period from germination to panicle initiation. This is

because the length of the reproductive growth phase (from panicle initiation to flowering) is relatively constant among rice cultivars (it is about 35 days in a normal condition) (Vergara and Chang 1985). The vegetative phase can be further divided into the basic vegetative phase and the successive photoperiod sensitivity phase (Vergara and Chang 1985). Chang et al. (1969) devised a split-tiller technique, combined with controlled short and long daylengths, to simultaneously determine the genetic bases for these two physiological phases in the generations of a cross. They proposed that a few early flowering (*Ef*) and photoperiod sensitivity (*Se*) genes control flowering time under short (10 h) and long (16 h) daylengths, respectively, and that the *Se* genes may be epistatic to *Ef* genes in expression for earliness under a short daylength. QTL analysis based on a permanent segregation population under controlled long and short daylengths improved the detection of putative *Ef* and *Se* genes, with respect to the number and relative position on chromosomes. Maheswaran et al. (2000) detected 15 QTLs associated with flowering time under 10- (13 loci) or 14-h (9 loci) daylengths, or photoperiod response (4 loci) from a recombinant inbred line population. These 15 putative loci distribute over all 12 rice chromosomes, and 10 may be allelic to previously identified genes/QTLs (Yokoo and Fujimaki 1971; Mackill et al. 1993; Li et al. 1995; Xiao et al. 1996; Yano et al. 1997). It remains a challenge to assign the multiple loci to genetic pathway(s) for floral control and to use this multi-locus information to explain a variety of inheritance patterns of flowering time across generations. For example, only five QTLs (*Hd1* to *Hd5*) for heading date were detected from the $F_2$ Nipponbare/Kasalath population, with an additional eight heading date QTLs identified from different advanced backcross populations (Yano et al. 1997, 2001).

Naturally occurring variants in the photoperiod response of flowering, from qualitative (obligate) to quantitative (facultative) to day-neutral, are present in rice germplasm collections. Of the 457 cultivars or breeding lines from different countries, about 31% and <2% have qualitative (critical) and day-neutral responses, respectively, and the remaining display quantitative responses (Vergara and Chang 1985). Cultivars with a critical daylength are limited to low latitude tropical areas and could not be found in subtropical and temperate regions (Yu and Yao 1967); day-neutral cultivars are essential for adaptation to regions of rice cultivation at high latitudes (Okumoto et al. 1996). However, both obligate short-day and day-neutral genotypes could be obtained from segregation populations derived from crosses between facultative short-day cultivars, such as the middle-season varieties ''Nanjing 11'' and ''Wujinxiangxuan'' (Gu et al. 2004a). EM93-1 is such a day-neutral genotype isolated as the trigenic mutant for a set of three Mendelian factors for photoperiod sensitivity from the ''Wujinxiangxuan''/''Nanjing 11'' cross, where the mutant alleles at two loci and one locus were derived from the female and male parents, respectively (Gu et al. 2004a). In this research, EM93-1 was chosen as the parent for the cross and backcross (BC) with an accession of wild-like weedy rice to relate the above putative genes to QTLs. As expected, we identified three major QTLs for photoperiod sensitivity from the $BC_1$ population. This set of three QTLs was further evaluated for gene component effects on flowering time under both long and short daylengths and was related to critical response to photoperiod.

## Materials and methods

### Mapping population

Two pure lines, EM93-1 and SS18-2, were selected as parents to generate a backcross population ($BC_1$), i.e., EM93-1//EM93-1/SS18-2. EM93-1 is photoperiodically insensitive or day-neutral with an extremely short duration of about 60 days (d) to flowering under both long and short daylengths (Gu et al. 2004a). SS18-2 is an accession of weedy rice from Thailand (Suh et al. 1997) and is sensitive to photoperiod. The $F_1$ EM93-1/SS18-2 plants were also strongly sensitive to photoperiod and flowered later than the SS18-2 plants under the natural long daylength during the summer season. Therefore, a 10-h short daylength was used to synchronize flowering to produce the $BC_1$ hybrid seeds.

### Plant cultivation and long and short daylength treatment

Seeds were germinated in the incubator set at 30°C in late June when natural daylength begins to decrease. Germinated seeds were cultured with rice nutrition solution (Yoshida et al. 1976) for 10 days. More than 200 $BC_1$ and five parental and $F_1$ seedlings were transplanted into pots (27 cm diameter × 24 cm height), with one plant per pot. The pots were filled with a mixture of clay soil and SUNSHINE medium (Sun Gro® Horticulture Canada Ltd., Seba Beach, AB, Canada) and placed in a greenhouse. The spit-tiller technique (Chang et al. 1969) was applied to a subpopulation of 156 $BC_1$ plants and the parental and $F_1$ plants at 38 days after germination to generate genet-

ically identical plants for replication. Four tillers were gently separated from the base of each plant and immediately transplanted into new pots that were randomly arranged in four blocks, respectively, in the adjacent greenhouse. The tiller-derived plants were treated with a short daylength (SD) of 10 h from 40 to 70 days by covering with black sheets from 6:00 p.m. to 8:00 a.m. the following morning. The plants in the original pots, together with the additional 48 intact $BC_1$ plants, were maintained under a long daylength (LD) of 14 h. Different replications were used in the daylength treatment, because genetic variation in flowering time in the population segregating for photoperiod sensitivity is much smaller under SD than under LD conditions (Chang et al. 1969; Poonyarit et al. 1989; Maheswaran et al. 2000; Gu et al. 2004a). Day/night temperatures were set at 29/21°C and supplementary light was used before 10:00 a.m. and after 4:00 p.m. in both greenhouses throughout the experiment.

## Phenotypic identification

The flowering date was recorded daily by tagging the first panicle emerging from the leaf sheath of a plant. All plants under SD flowered and days to flowering (DTF) for the four tiller-derived plants were averaged to estimate the phenotypic value of individual $BC_1$s. A majority (185) of $BC_1$ plants flowered before 190 days, one plant flowered at 214 days and the remaining 18 plants could not flower after 250 days under LD. The LD experiment was ended at 250 days because dissecting the meristems from the non-flowered $BC_1$s did not find visible young panicle at the time. The $BC_1$s that flowered under SD but not flowered under LD represent a category of segregates having a critical daylength (Vergara and Chang 1985; Poonyarit et al. 1989; Gu et al. 2004a). To distinguish these non-flowered from the flowered $BC_1$s under LD, DTF for the 18 non-flowered $BC_1$s were represented by the experiment period of 250 days for data analysis.

## QTL mapping and effect analysis

The framework linkage map, which consists of 153 markers relatively evenly distributed over the whole rice genome (Gu et al. 2004b), was used to scan for QTLs associated flowering time and PS in a preliminary analysis. QTL mapping was conducted by the MQTL simple interval mapping (SIM) procedure (Tinker and Mather 1995). The threshold of test statistic (TS) for a significant QTL was generated by 1,000 permutations at a type-I error rate of 0.05. The TS values exported from SIM for a single environment are convertible to the commonly used LOD score computed by MAPMAKER/QTL (Lincoln et al. 1993), with 1 TS = 0.22 LOD (Tinker and Mather 1995). After the preliminary analysis, additional rice microsatellite (RM) markers (McCouch et al. 2002) were screened for specific chromosome (chr) regions, including the *Hd1* and *Hd3* QTL regions on chr 6 (Yano et al. 1997). Polymorphic markers were added to the linkage map constructed based on the whole population of 204 $BC_1$ plants using methods as previously described (Gu et al. 2004b). The new map was used to refine QTLs for flowering time under SD and LD, respectively based on the subpopulation and whole populations. The 95% confidence intervals (CI) in centiMorgen (cM) of detected QTLs were estimated using the method developed by Darvasi and Soller (1997). CI = $530/(NR^2)$, where the constant 530 is derived from the simulation of a $BC_1$ population (Darvasi and Soller 1997), $N$ is the population size and $R^2$ is the proportion of phenotypic variance explained by the QTL that was estimated based on the MQTL single locus model.

Two-way ANOVA was used to identify additional chr regions epistatically interacting with each of the three QTLs detected by the above SIM. This analysis was conducted between each of the markers located outside the three QTL regions and the fixed marker, or the marker nearest a QTL peak position. The linear model for the analyses and the sequential Bonferroni test procedure were same as previously described (Gu et al. 2004b). ANOVAs were performed using the SAS GLM procedure (SAS Institute 1999).

Multiple linear regression analysis was used to estimate individual main and epistatic effects of the three QTLs detected in the $BC_1$ population using the model:

$$y_{ij} = \mu + m_1x_1 + m_2x_2 + m_3x_3 + i_{12}z_{12} + i_{13}z_{13} + i_{23}z_{23} + i_{123}w_{123} + \varepsilon_{ij}, \quad (1)$$

where $y_{ij}$ is the phenotypic value of the $j$th $BC_1$ plant for the $i$th trigenic genotype, with $i = 1$ to 8 and $j = 1$ to $N_i$ ($N_i$ is the number of plants in the $i$th genotypic category, and $\Sigma N_i$ equals to 156 or 204, i.e., the sub- or whole population size); $\mu$ is the mean of the model; $x_1$ to $x_3$ are the variables for main effects of the loci 1 to 3, respectively, with $x$ coded as 0.5 and –0.5 when the locus has one (i.e., heterozygous) and 0 (i.e., homozygous for the mutant allele) wild-type allele, respectively; $z_{12}$, $z_{13}$ and $z_{23}$ are the variables for digenic epistases between loci 1 and 2, 1 and 3, and 2 and 3, respectively, with $z_{12}$, $z_{13}$ and $z_{23}$ coded with the

products of codes for $x_1$ and $x_2$, $x_1$ and $x_3$, $x_2$ and $x_3$, respectively; $z_{123}$ is the variable for the trigenic epistasis among the three loci coded with the product of the variables $x_1$, $x_2$ and $x_3$; $m_1$ to $m_3$ are the partial regression coefficients for $x_1$ to $x_3$ that estimate the main effects of loci 1 to 3, respectively; $i_{12}$, $i_{13}$, $i_{23}$ and $i_{123}$ are partial regression coefficients that estimate corresponding di- or trigenic epistatic effects, respectively; and $\varepsilon_{ij}$ is the residual including the random error and genetic effect that are unexplained by these three loci. The regression analysis was performed by the SAS procedure REG with a stepwise selection set at a significant level of 5% (SAS Institute 1999).

Component genetic variances $\delta_t^2$ were estimated based on Cockerham's model (1954):

$$\delta_t^2 = \frac{\left(\sum P_{ijk} G_{ijk} W_{ijkt}\right)^2}{\sum P_{ijk} W_{ijkt}^2},$$

where $P_{ijk}$ and $G_{ijk}$ are the genotypic frequency and genotypic value, respectively, for a trigenic (i.e., loci $i$, $j$ and $k$) genotype in the population; $W_{ijkt}$ is the $t$th orthogonal contrast scale for the genotype (Table 1), which is adopted from Kao and Zeng (2002). The component genetic variances were further used to estimate their contribution ($R^2$) to the total phenotypic variance ($s^2$) where $R_t^2 = \delta_t^2/s^2$.

## Results

### Inheritance of flowering time in the cross

The day-neutral genotype EM93-1 flowered after about 65 and 60 days under SD and LD, respectively (Fig. 1). The previous research using seed-derived EM93-1 plants did not detect an effect of the 10-h SD treatment on flowering time of the genotype (Gu et al. 2004a). Thus, recovery from transplanting tillers mainly accounted for the 5-day delay in flowering under SD observed in this experiment. The weedy rice accession SS18-2 flowered about 30 and 70 days later than EM93-1 under SD and LD, respectively (Fig. 1), indicating that both parents differ not only in photoperiod sensitivity (PS) but also in basic vegetative growth phase. The $F_1$ plants were more sensitive to the photoperiod than the late-flowering parent SS18-2, as they flowered about two weeks earlier under SD and approximately 30 days later under LD than SS18-2 (Fig. 1). The elongation of daylength changed the overall dominance of flowering time from partially dominant to overdominant.

The subpopulation of 156 $BC_1$ plants varied in DTF from 64 to 104 days under SD and from 58 to >250 days under LD (Fig. 1). Transgression segregation over the late-flowering parent SS18-2 occurred under LD, which is evidenced by many $BC_1$ plants flowered later than the SS18-2 or $F_1$ plants (Fig. 1). The earliest-flowering $BC_1$ segregates under LD also flowered earliest under SD, while the $BC_1$s that did not flower or flowered later than the $F_1$ plants under LD were $F_1$-like rather than the latest-flowered individuals under SD (Fig. 1). The difference in DTF of genetically identical plants between LD and SD varied from 0 to >173 days in the $BC_1$ subpopulation. Correlation of the difference with DTF under SD was relatively low ($r = 0.4744$, $r^2 = 22.5\%$), and the correlation with DTF under LD was very high ($r = 0.9933$, $r^2 = 98.7\%$). The high correlation indicates that the phenotypic variation in flowering time under LD was almost completely caused by genotypic differences in PS in this population.

**Table 1** Seven orthogonal contrast scales ($W_1$–$W_7$) for partitioning genetic variance in the $BC_1$ (*aabbcc* × *AaBbCc*) population

| Genotype | *AaBbCc* | *AaBbcc* | *AabbCc* | *Aabbcc* | *aaBbCc* | *aaBbcc* | *aabbCc* | *aabbcc* |
|---|---|---|---|---|---|---|---|---|
| $G_{ijk}$ | $G_{111}$ | $G_{110}$ | $G_{101}$ | $G_{100}$ | $G_{011}$ | $G_{010}$ | $G_{001}$ | $G_{000}$ |
| $P_{ijk}$ | 1/8 | 1/8 | 1/8 | 1/8 | 1/8 | 1/8 | 1/8 | 1/8 |
| $W_1$ | 1/2 | 1/2 | 1/2 | 1/2 | –1/2 | –1/2 | –1/2 | –1/2 |
| $W_2$ | 1/2 | 1/2 | –1/2 | –1/2 | 1/2 | 1/2 | –1/2 | –1/2 |
| $W_3$ | 1/2 | –1/2 | 1/2 | –1/2 | 1/2 | –1/2 | 1/2 | –1/2 |
| $W_4$ | 1/4 | 1/4 | –1/4 | –1/4 | –1/4 | –1/4 | 1/4 | 1/4 |
| $W_5$ | 1/4 | –1/4 | 1/4 | –1/4 | –1/4 | 1/4 | –1/4 | 1/4 |
| $W_6$ | 1/4 | –1/4 | –1/4 | 1/4 | 1/4 | –1/4 | –1/4 | 1/4 |
| $W_7$ | 1/8 | –1/8 | –1/8 | 1/8 | –1/8 | 1/8 | 1/8 | –1/8 |

$G_{ijk}$ and $P_{ijk}$ denote the genotypic values and expected genotypic frequencies of the genotypes for unlinked loci *A*, *B* and *C*. The subscribes $i$ ($j$ or $k$) = 0 or 1, with 0 or 1 indicating that there are two recessive alleles or one dominant and one recessive alleles at the loci *A* (*B* or *C*). $W_1$ ($W_2$ and $W_3$), $W_4$ ($W_5$ and $W_6$) and $W_7$ are orthogonal contrast scales for the marginal effect of *A* (*B* and *C*), digenic epistasis between loci *A* and *B* (*A* and *C*, and *B* and *C*) and trigenic epistasis, respectively

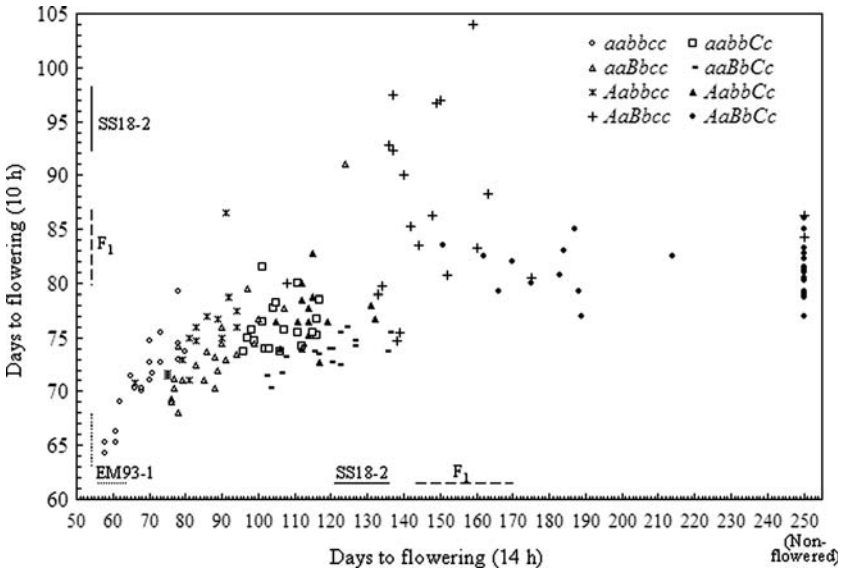


**Fig. 1** Distribution of the $BC_1$ (EM93-1//EM93-1/SS18-2) subpopulation and range of the parental (*solid* or *dotted lines*) and $F_1$ (*dashed line*) plants for flowering time (*DTF*) under 10- and 14-h daylengths. The *symbols* depict the eight genotypes for the markers RM214, RM25 and RM172 that are nearest to the $Se_{7.1}$ (*A/a*), $Se_8$ (*B/b*) and $Se_{7.2}$ (*C/c*) QTLs, respectively

## QTL mapping

The MQTL SIM mapping identified three and two QTLs associated with DTF under LD and SD, respectively (Fig. 2a). Two (one in the middle of chr 7 and the other on chr 8) of the three QTLs detected under LD are mapped at the same positions as the two QTLs detected under SD (Fig. 2b, c); these two loci differed greatly in main effect between the LD (about 54 days) and SD (7.3 and 4.6 days, respectively) conditions (Table 2). The other QTL detected only under LD is located near the end of the long arm of chr 7 (Fig. 2b). Because their main effects varied greatly with photoperiod (Table 2), we defined the three

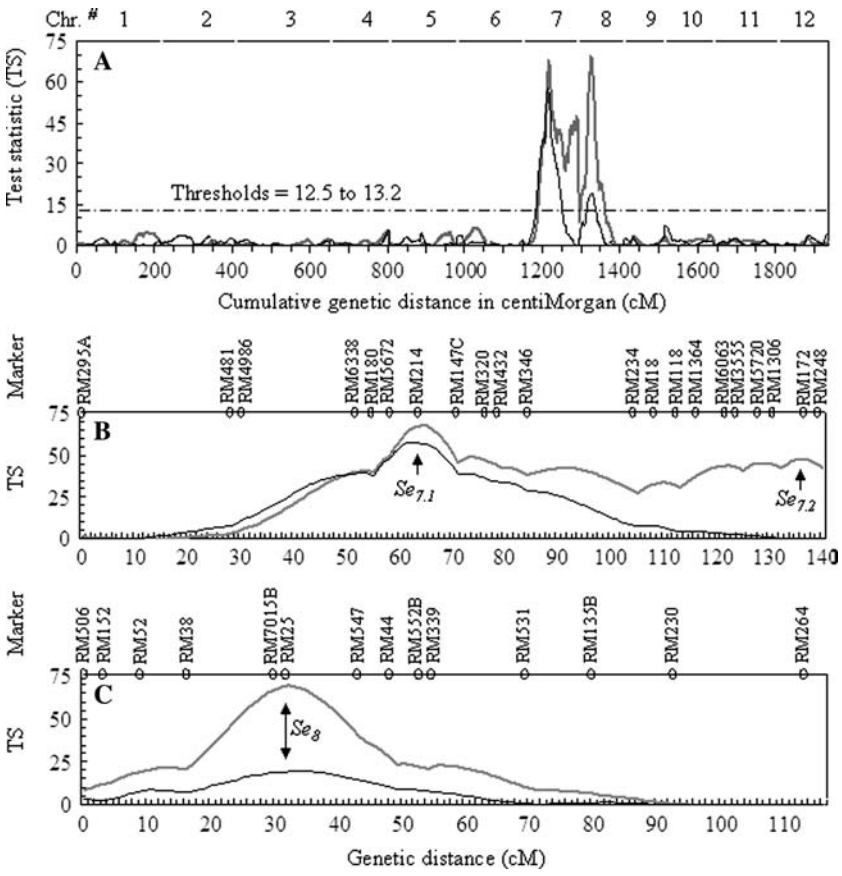


**Fig. 2** Scan for QTLs for flowering time under 14- (*heavy gray line*) and 10- (*fine black line*) daylengths. **a** Genome-wide scan by the MQTL simple interval mapping procedure. **b**, **c** Enlargement of test statistic (*TS*) distributions on chromosomes 7 and 8, respectively. One TS is equivalent to 0.22 LOD that is computed by MAPMAKER/QTL (Tinker and Mather 1995). The genetic map developed using rice microsatellite (*RM*) markers is at the top of each distribution; *arrows* depict the peak positions for QTLs $Se_{7.1}$, $Se_{7.2}$ and $Se_8$

**Table 2** QTLs for days to flowering (DTF) detected under 10- and 14-h daylengths from the $BC_1$ population by simple interval mapping

| QTL (nearest marker)[a] | DTF (14 h) | | DTF (10 h) | |
|---|---|---|---|---|
| | Effect (days)[b] | $R^2$ | Effect (days) | $R^2$ |
| $Se_{7.1}$ (RM214) | 53.6 | 0.28 | 7.3 | 0.31 |
| $Se_{7.2}$ (RM172) | 53.0 | 0.22 | –[c] | – |
| $Se_8$ (RM25) | 54.2 | 0.29 | 4.6 | 0.12 |
| Full model | | 0.68 | | 0.39 |

[a] See Fig. 2 for QTL position

[b] QTL main effect was estimated as the difference between heterozygous and EM93-1-like homozygous genotypes at the peak position; the proportion ($R^2$) of variance explained by the locus or by the full model to the total variance was computed by MQTL inference files

[c] This locus was not detectable under the 10-h daylength

QTLs as photoperiod sensitivity (*Se*) loci and designated them as $Se_{7.1}$, $Se_{7.2}$ and $Se_8$, respectively.

The two-way ANOVA did not detect markers outside the above three QTL regions significantly associated with flowering time, including those located in the interval between RM204 and RM314 on chr 6 (the RM204–RM314 interval corresponds to the secondary peak located between 1,000 and 1,050 cM in Fig. 2a; the markers are not shown in the figure), which is supposed to be identical to the QTL *Hd3* (Yano et al. 1997).

The $Se_{7.1}$ peak is co-located with the marker RM214 (the 95% confidence interval or CI = 9.2 cM) and the $Se_{7.2}$ (CI = 11.8 cM) and $Se_8$ (CI = 8.9 cM) peaks are about 1 cM proximal to the nearest markers RM172 and RM25, respectively, based on the population of 204 $BC_1$ plants (Fig. 2b, c). All the alleles from the parent SS18-2 delayed flowering under LD or enhancing PS, as indicated by positive values of their main effects (Table 2); the main effects of $Se_{7.1}$, $Se_8$, and $Se_{7.2}$ accounted for 22–29% of the phenotypic variances in DTF under LD, respectively, based on the MQTL single locus model. The $Se_{7.1}$ (31%) contributed more than $Se_8$ (12%) to the phenotypic variance in DTF under SD; the alleles delaying flowering under LD also delayed flowering under SD (Table 2).

## Epistatic effects of the three QTLs

Genetic distance between $Se_{7.1}$ and $Se_{7.2}$ peaks is 73 cM, and the recombination fraction between their nearest markers RM214 and RM172 is 49% (100/204) in the $BC_1$ population (Fig. 2b), indicating that alleles at these two QTLs were nearly independent of each other in segregation during meiosis. The observed frequencies of eight genotypes for $Se_{7.1}$ (*A*/*a*), $Se_8$ (*B*/*b*) and $Se_{7.2}$ (*C*/*c*) were 25 (*aabbcc*), 23 (A*abbcc*), 28 (*aaBbcc*), 28 (*aabbCc*), 25 (*AaBbcc*), 24 (*aaBbCc*), 20 (A*abbCc*) and 31 (*AaBbCc*), respectively, which fit the expected equal frequencies of a testcross for three genes ($\chi^2 = 3.22$, $P = 0.86$).

Multiple linear regression analysis based on the model (1) detected that all component genetic effects of the three loci on DTF under LD were significant (Table 3). These components together accounted for 76% of phenotypic variance in the flowering time greater than the estimate (68%) based on the MQTL multi-locus model (Table 2). The main effects of the three loci on DTF ranged from 40 to 51 days, and accounted for 16–25% of the phenotypic variance. Of the three sets of digenic epistases, the $Se_{7.1} \times Se_8$ interaction had the largest effect ($i_{12} = 70$ days) and contributed most (12%) to the phenotypic variance. All the digenic epistases displayed similar interaction patterns, that is, the allelic difference of a QTL was enlarged in the presence of the wild type allele at the other QTL (Fig. 3a, and data not shown). The trigenic epistasis

**Table 3** Component genetic effects of the three loci on flowering time (DTF) detected in the $BC_1$ population under 10- and 14-h daylengths

| Parameter[a] | Estimate (days) | Error (days) | *t* value | Probability | $R^{2b}$ |
|---|---|---|---|---|---|
| DTF under 14 h (0.86) | | | | | |
| $u$ | 119.8 | 1.36 | 88.20 | <0.0001 | N/A |
| $m_1$ ($Se_{7.1}$) | 46.4 | 2.72 | 17.08 | <0.0001 | 0.211 |
| $m_2$ ($Se_8$) | 50.9 | 2.72 | 18.75 | <0.0001 | 0.254 |
| $m_3$ ($Se_{7.2}$) | 40.4 | 2.72 | 14.89 | <0.0001 | 0.160 |
| $i_{12}$ ($Se_{7.1} \times Se_8$) | 69.6 | 5.43 | 12.81 | <0.0001 | 0.119 |
| $i_{13}$ ($Se_{7.1} \times Se_{7.2}$) | 15.5 | 5.43 | 2.86 | 0.0047 | 0.006 |
| $i_{23}$ ($Se_8 \times Se_{7.2}$) | 12.2 | 5.43 | 2.25 | 0.0255 | 0.004 |
| $i_{123}$ ($Se_{7.1} \times Se_8 \times Se_{7.2}$) | 37.8 | 10.9 | 3.48 | 0.0006 | 0.009 |
| DTF under 10 h (0.56) | | | | | |
| $u$ | 76.9 | 0.34 | 227.3 | <0.0001 | N/A |
| $m_1$ ($Se_{7.1}$) | 6.5 | 0.68 | 9.57 | <0.0001 | 0.262 |
| $m_2$ ($Se_8$) | 3.8 | 0.68 | 5.68 | <0.0001 | 0.094 |
| $m_3$ ($Se_{7.2}$) | –[c] | – | – | – | – |
| $i_{12}$ ($Se_{7.1} \times Se_8$) | 7.7 | 1.35 | 5.68 | <0.0001 | 0.093 |
| $i_{13}$ ($Se_{7.1} \times Se_{7.2}$) | –4.2 | 1.35 | –3.14 | 0.0020 | 0.027 |
| $i_{23}$ ($Se_8 \times Se_{7.2}$) | –5.9 | 1.35 | –4.35 | <0.0001 | 0.055 |
| $i_{123}$ ($Se_{7.1} \times Se_8 \times Se_{7.2}$) | –[c] | – | – | – | – |

[a] $u$ is the mean of model (1); $m$'s are the main effects of the locus indicated in parenthesis; $i$'s are the effects of epistases between/among the indicated loci

[b] $R^2$ indicates the contribution of each genetic component to the phenotypic variance; N/A indicates this parameter is not applicable to the model mean; the figure in the parenthesis is the adjusted $R^2$ of the linear model

[c] Dashes indicate that the main ($m_3$) and trigenic epistatic ($i_{123}$) effects were non-significant under the short-day condition

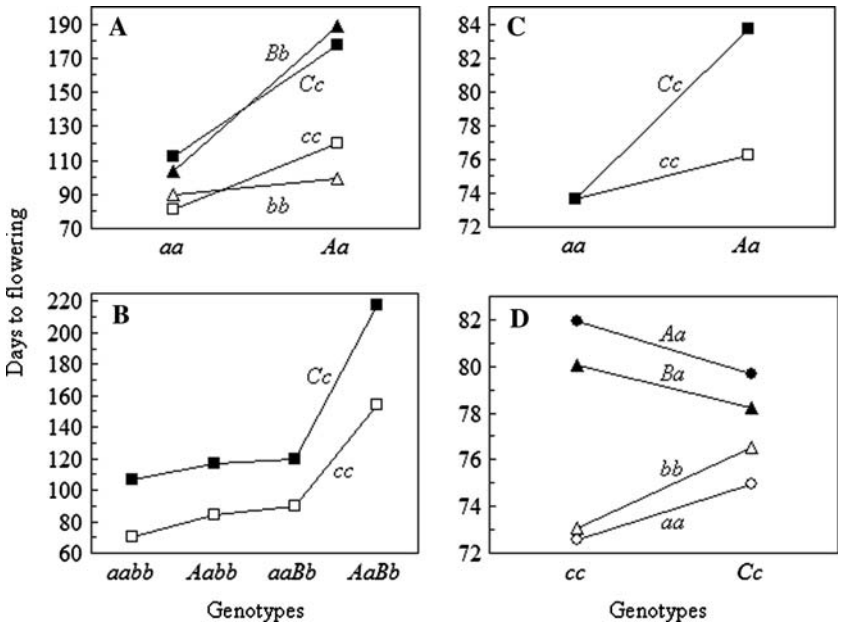


**Fig. 3** Di- and trigenic epistases for flowering time (*DTF*) detected in the $BC_1$ population under 14- (**a**, **b**) and 10- (**c**, **d**) h daylength. *Italic* upper/lower cases of *A/a*, *B/b* and *C/c* denote photoperiod sensitive/insensitive alleles at $Se_{7.1}$, $Se_8$ and $Se_{7.2}$, respectively, which are represented by their nearest markers RM214 (*circle*), RM25 (*triangle*) and RM172 (*square*), respectively. *Solid* and *open symbols* donated heterozygous and EM93-1-type homozygous genotypes, respectively for the locus

contributed a little (<1%) to the total variance, which is similar to the other two sets of digenic epistases, but it ($i_{123}$ = 38 days) exhibited a larger effect on DTF than the digenic interactions (12 and 16 days). This three-order epistasis showed that the allelic differences of $Se_{7.2}$ varied with genotypes for the other two loci from 29 to 64 days; the largest difference occurred in the presence of the wild type alleles at both $Se_{7.1}$ and $Se_8$ (*AaBb*), and the smallest difference occurred in the presence of the mutant allele at $Se_{7.1}$ combining with the wild type allele at $Se_8$ (*aaBb*) (Fig. 3b).

The analysis based on the model (1) only detected $Se_{7.1}$ and $Se_8$ main effects and three sets of digenic epistases on DTF under SD (Table 3). Compared with $Se_8$, $Se_{7.1}$ had a greater (6.5 vs. 3.8 days) main effect on delaying flowering and contributed more (26 vs. 9%) to the phenotypic variance. The $Se_{7.1} \times Se_8$ epistasis delayed flowering ($i_{12}$ = 8 days); the epistatic effect was greater than the individual main effects and contributed about 9% to the phenotypic variance (Table 3). There was a difference in the $Se_{7.1} \times Se_{7.2}$ interaction pattern between LD and SD; the $Se_{7.2}$ wild type allele had delaying and null effects on flowering under LD and SD, respectively, when $Se_{7.1}$ was homozygous for mutant alleles (*aa*) (Fig. 3a, c).

Although the main effect of $Se_{7.2}$ was not detectable, its interactions with $Se_{7.1}$ and $Se_8$ promoted flowering ($i_{13}$ = –4 days and $i_{23}$ = –6 days) by enhancing the effects of the mutant alleles under SD (Table 3). Both $Se_{7.1} \times Se_{7.2}$ and $Se_{7.2} \times Se_8$ epistatic interactions displayed similar patterns; the allelic differences for $Se_{7.1}$ and $Se_8$ were greater in the absence (*cc*) than in the presence (*Cc*) of a wild type allele at $Se_{7.2}$ (Fig. 3d).

## Discussion

Flowering of the hybrid $F_1$ later than the late-flowering parent is often observed in crosses between rice ecotypes, where both parents are weakly or moderately sensitive to photoperiod (Li et al. 1980). Classic genetic analysis explains the above phenomenon using complementation between/among major PS genes from different parents (Poonyarit et al. 1989; Gu et al. 2004a). However, this phenomenon has been rarely reported for a cross such as the EM93-1/SS18-2, because EM93-1 is a day-neutral, extremely early flowering genotype. The three major PS QTLs identified from the $BC_1$ population are insufficient to explain the elongated growth duration of the $F_1$ generation using a complementary model. This is because: (1) both SS18-2 (*AABBCC*) and the $F_1$ (*AaBbCc*) have wild type allele at each of the three loci, but the $F_1$ flowered later than SS18-2, and (2) both the $BC_1$ trigenic heterozygotes (*AaBbCc*) and the $F_1$ are all heterozygous for the three QTLs, but the $BC_1$ heterozygotes flowered even later than the $F_1$ or displayed obligate floral response to the 14-h daylength (Fig. 1). Such an across-generation inheritance pattern has been rarely reported for crosses developed for QTL analyses of flowering time in rice, as phenotypic data for the $F_1$ generation are not presented in the publications. To explain the across-generation inheritance pattern, we propose that the weedy rice SS18-2 contains flowering promoting genes to inhibit expression of PS genes at the $Se_{7.1}$, $Se_{7.2}$ and $Se_8$ loci, and the promoting effect of these putative genes on flowering was inhibited in the EM93-1 genetic background under LD. We failed to detect the putative

QTLs by the MQTL analysis and ANOVAs based on the BC population. However, such putative genes could be identified using different mate systems such as $F_2$ and advanced BC populations (Yano et al. 2001).

The genes underlying the $Se_{7.1}$, $Se_{7.2}$ and $Se_8$ loci most likely originate from wild rice (*O. rufipogon*) in the tropical area and they may represent primitive forms of PS alleles at these loci. The gene donor SS18-2 was collected from Thailand, where it could evolve directly from the co-existing wild rice or originate from natural hybridization between wild and cultivated rice (Suh et al. 1997). Wild rice perennial and primitive forms have stronger PS than the annual form (Oka and Chang 1960). Several accessions of annual wild rice, such as "P16" (Xiong et al. 1999), "W1944" (Cai and Morishima 2002) and "IRGC 105491" (Thomson et al. 2003), have been identified for QTLs associated with flowering time. None of these accessions had QTLs in all of the $Se_{7.1}$, $Se_{7.2}$ and $Se_8$ regions, suggesting that mutations occurred at the three PS loci in annual populations of wild rice. The presence of all three PS alleles implies that SS18-2 may derive from primitive wild rice. Therefore, the question is how the weedy rice SS18-2 has maintained all the three major genes during its evolution. One major reason is the lack of selection pressure due to the relatively short daylength (<13 h) in the low-latitude tropics in summer. The other possible reason could be the presence of flowering promoting genes that partly offset the effect of the three PS genes in SS18-2, as hypothesized above. For example, we did not detect a QTL for flowering time in the *Se-1* or *Hd1* region on chr 6 from the $BC_1$ population (Fig. 2a). The *Se-1* and *Hd1* loci are allelic (Yano et al. 2000). *Se-1* is a major, dominant PS gene distributed in a range of rice cultivars (Yokoo and Fujimaki 1971; Poonyarit et al. 1989; Mackill et al. 1993; Yano et al. 2000). The absence of *Se-1* in the EM93-1 (day-neutral) genetic background indicates that SS18-2 carries the mutant or flowering promoting alleles at this major locus.

The PS genes identified from SS18-2 in the EM93-1 background distribute separately in cultivars. The parent EM93-1 is homozygous for the mutant alleles at the $Se_{7.1}$, $Se_{7.2}$ and $Se_8$ loci. This day-neutral genotype was isolated as the trigenic mutant for a set of three Mendelian factors for PS from a complementary test; wild-type alleles at two loci and one locus were derived from the middle-season varieties "Nanjing 11" and "Wujinxiangxuan," respectively (Gu et al. 2004a). It is likely that the genes underlying the three PS QTLs from SS18-2 include all or some of the three Mendelian factors identified by Gu et al. (2004a). Allelic variation for the $Se_{7.1}$, $Se_{7.2}$ and $Se_8$ regions has been reported for other varieties of rice. Based on the approximate map position, $Se_{7.1}$ is likely allelic to the flowering time (or heading date) QTLs *dth7*, *Hd4*, *E1*, *Dth7a* and *QDTH7-1* (Xiao et al. 1996; Yano et al. 1997; Ichitani et al. 1998; Maheswaran et al. 2000; Hittalmani et al. 2003; Fujino and Sekiguchi 2005). Similarly, $Se_{7.2}$ may be allelic to *Hd2*, *Dtf7b* and *qDTH7-2* (Yano et al. 1997; Maheswaran et al. 2000; Bres-Patry et al. 2001; Fujino and Sekiguchi 2005), and $Se_8$ is probably allelic to *QHd8a*, *dth8*, *Hd5*, *DTf8a* and *QHd8* (Li et al. 1995; Xiao et al. 1996; Yano et al. 1997; Maheswaran et al. 2000; Mei et al. 2005). Most of the parental lines for the above mapping populations are elite varieties cultivated from tropical to temperate areas. Similar to that at the *Se-1* locus (Yokoo and Fujimaki 1971; Poonyarit et al. 1989; Mackill et al. 1993; Yano et al. 2000), genetic differentiations at the $Se_{7.1}$, $Se_{7.2}$ and $Se_8$ loci must have played an important role in the expansion of Asian cultivated rice from the tropics to high latitudinal temperate regions.

Based on epistatic effects, many Arabidopsis mutants for flowering have been assigned to different genetic pathways (Koornneef et al. 1998). The epistatic interactions (Table 3) strongly suggest that genes underlying $Se_{7.1}$, $Se_{7.2}$ and $Se_8$ work in the same genetic pathway to regulate floral initiation. We proposed a working model to estimate floral responses of the major PS genes to photoperiod (Fig. 4). These genes

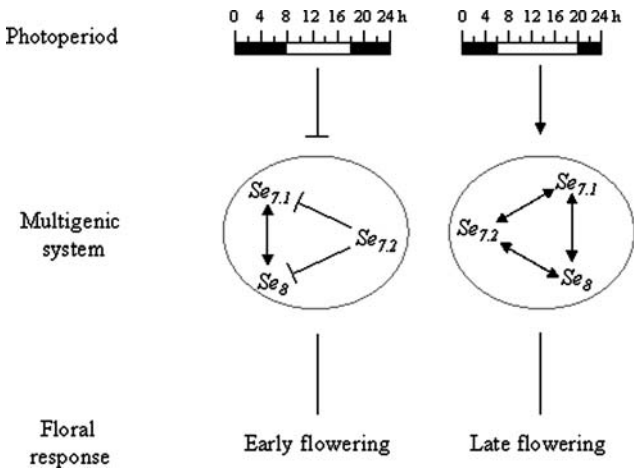


**Fig. 4** A working model for the three-locus system regulating flowering time of the rice plants under the short (10-h) and long (14-h) photoperiods. The black and empty bars depict dark and light periods, respectively used during the photoperiod sensitive phase of the short-day plants. The long photoperiod activated effects of the system on repressing flowering, where the photoperiod sensitive genes at $Se_{7.1}$, $Se_{7.2}$ and $Se_8$ interacted synergistically with each other. The short photoperiod dramatically lowered the repressing effect of the system, where the main effect of Se7.2 was nullified but Se7.2 interacted with the other two loci to promote flowering

work as a system to delay floral initiation, and the delaying effect is greatly promoted by LD and inhibited by SD. The $Se_{7.2}$ allele appears to be a pivotal gene in the putative pathway. Its main effect was activated by LD and completely nullified by SD, suggesting that it may sense circadian rhythm in the short-day plant. In addition, $Se_{7.2}$ inhibited the expression of $Se_{7.1}$ and $Se_8$ under SD through epistasis, indicating that it is an important regulatory gene in the pathway. The three major PS QTLs differ from *Hd1*, the major locus coding for a homolog of the Arabidopsis *CONSTANS* gene (Yano et al. 2000), because the *Hd1* QTL promoted flowering under SD and inhibited flowering under LD through its main effect (Yano et al. 2000). However, *Hd1* may be part of the genetic pathway, as suggested by its interaction with a QTL mapped in the $Se_{7.2}$ region (Yano et al. 1997; Lin et al. 2000). Our previous research led to a hypothesis that floral response to critical photoperiod results from the synergistic action among a set of major PS genes rather than single genes (Gu et al. 2004a). Many of the trigenic heterozygotes (*AaBbCc*) $BC_1$ plants that flowered under the 10-h daylength did not flower after 250 days under the 14-h daylength condition (Fig. 1), indicating they are obligate short-day genotype. The presence of the obligate short-day genotype suggests that the above putative pathway also may be involved in the genetic control of critical daylength. We are transferring the PS alleles at the $Se_{7.1}$, $Se_{7.2}$ and $Se_8$ loci from the non-flowered segregates into the EM93-1 genetic background to further examine the hypothesis and their component additive, dominance and epistatic effects to improve the model (1).

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