Multiple Loci and Epistases Control Genetic Variation for Seed Dormancy in Weedy Rice (*Oryza sativa*)

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ABSTRACT

Weedy rice has much stronger seed dormancy than cultivated rice. A wild-like weedy strain SS18-2 was selected to investigate the genetic architecture underlying seed dormancy, a critical adaptive trait in plants. A framework genetic map covering the rice genome was constructed on the basis of 156 BC_1 [EM93-1 (nondormant breeding line)//EM93-1/SS18-2] individuals. The mapping population was replicated using a split-tiller technique to control and better estimate the environmental variation. Dormancy was determined by germination of seeds after 1, 11, and 21 days of after-ripening (DAR). Six dormancy QTL, designated as *qSD^S-4*, *-6*, *-7-1*, *-7-2*, *-8*, and *-12*, were identified. The locus *qSD^S-7-1* was tightly linked to the red pericarp color gene *Rc*. A QTL × DAR interaction was detected for *qSD^S-12*, the locus with the largest main effect at 1, 11, and 21 DAR (R^2 = 0.14, 0.24, and 0.20, respectively). Two, three, and four orders of epistases were detected with four, six, and six QTL, respectively. The higher-order epistases strongly suggest the presence of genetically complex networks in the regulation of variation for seed dormancy in natural populations and make it critical to select for a favorable combination of alleles at multiple loci in positional cloning of a target dormancy gene.

SEED dormancy, the temporary failure of a viable seed to germinate under favorable conditions, is an adaptive trait that promotes the survival of many plants. Rapid and uniform seed germination has been selected in crops, but a moderate degree of dormancy is desirable for cereals to resist preharvest sprouting (PHS). Preharvest sprouting is germination of seeds on the plant after maturation, but before harvest of the crop, when moist conditions prevail or untimely rains occur. It can cause a substantial loss of yield and reduce grain quality (Ringlund 1993). Dormancy is a genetically complex trait controlled by polygenes with effects modified by the genetic background and environmental factors (Johnson 1935; Anderson *et al.* 1993). A major approach to determine the genetic architecture for seed dormancy is to dissect it into quantitative trait loci (QTL), such as in Arabidopsis (*Arabidopsis thaliana*), barley (*Hordeum vulgare*), sorghum (*Sorghum bicolor*), rice (*Oryza sativa*), and wheat (*Triticum aestivum*; Anderson *et al.* 1993; Oberthur *et al.* 1995; Lin *et al.* 1998; Lijavetzky *et al.* 2000; Mares *et al.* 2002; Alonso-Blanco *et al.* 2003). Quantitative trait loci analysis is also a prerequisite to clone and characterize genes that directly regulate seed dormancy and germination and to facilitate marker-assisted selection for resistance to PHS in breeding programs.

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There are basically two categories of seed dormancy. Coat-imposed dormancy is enforced by seed covering tissues such as the glume and palea (or hull), the pericarp and testa, and in some cases the endosperm. Embryo dormancy is imposed by the factors within the embryo itself (Bewley and Black 1994). Embryo dormancy has been reported for wild oat and wheat (Naylor and Simpson 1961; Flintham 2000) and was suggested in rice (Takahashi 1963); however, most genetic research has focused on coat-imposed dormancy. Coat-imposed dormancy in rice is controlled by the maternal genotype on the basis of research using genetic and somatic approaches (Seshu and Sorrells 1986; Gu *et al.* 2003).

Primary dormancy develops during seed development and maturation. Environmental factors, such as the temperature, humidity, and light, strongly affect the degree of dormancy (Nair *et al.* 1965; Chang and Yen 1969). Heritability for seed dormancy ranges from 0.12 to 0.42 in cultivated rice, 0 to 0.13 in white-grained wheat, and 0.33 to 0.56 in wild oat segregating populations grown in the field (Chang and Yen 1969; Jana and Naylor 1980; Paterson and Sorrells 1990). Genotype-by-environment ($G \times E$) interactions have been reported for seed dormancy in several species (Nair *et al.* 1965; Upadhyay and Paulsen 1988; Paterson and Sorrells 1990). The growth environment greatly affects both the number and the influence of individual QTL in a mapping population (Oberthur *et al.* 1995; Sorrells and Anderson 1996; Van der Schaar *et al.* 1997; Lijavetzky *et al.* 2000; Kato *et al.* 2001; Groos

et al. 2002). To reduce and estimate the confounding effect of environmental factors on dormancy, Seshu and Sorrells (1986) and Koornneef *et al.* (2002) have emphasized the use of controlled growth conditions and permanent mapping populations.

After-ripening is loss of the dormant state over some period of time through exposure of seeds to a set of environmental conditions after maturation and separation from the parent plant (Simpson 1990). Plant species vary in environmental conditions that facilitate after-ripening. For instance, rice and wild oat seeds after-ripen under warm, dry conditions, while Arabidopsis and many other species respond best to cool, moist conditions (Naylor and Simpson 1961; Leopold *et al.* 1988; Koornneef and Karssen 1994). Hypotheses exist, but little information is available on how after-ripening affects the genetic expression of dormancy. Chang and Tagumpay (1973) hypothesized that one or more dormancy alleles in cultivated rice gradually lose their effect during after-ripening, which is based on more individuals in the segregating population shifting from the dormant to nondormant classes during the period of after-ripening. Romagosa *et al.* (1999) used flanking markers to monitor the expression of four dormancy QTL from barley during after-ripening and classified the genotypes into early, intermediate, and late dormancy-release types. Recently, Alonso-Blanco *et al.* (2003) identified seven QTL in Arabidopsis for delay of germination (*DOG*), which was measured as days of after-ripening required to reach 50% germination. Variation in the main effect of the *DOG* QTL with the times of after-ripening suggests that the expression pattern of dormancy genes might be more complicated than originally hypothesized.

Epistasis, the interaction between or among alleles at two or more loci, is critical to advanced quantitative genetic models (Mather and Jinks 1971). Assembly of favorable epistatic combinations is considered as the single most important genetic basis underlying the evolution of adaptiveness in plants (Allard 1996). On the basis of Mendelian approaches, two- and three-locus epistases for the control of dormancy have been postulated for rice, wheat, and wild oat (Johnson 1935; Seshu and Sorrells 1986; Jana *et al.* 1988; Bhatt *et al.* 1993; Fennimore *et al.* 1999; Gu *et al.* 2003). Epistasis between two dormancy QTL was reported in an Arabidopsis, a barley, and a wheat mapping population (Anderson *et al.* 1993; Oberthur *et al.* 1995; Alonso-Blanco *et al.* 2003).

Rice is greatly divergent in the degree of seed dormancy. Some of the most highly dormant genotypes are found among the nondomesticated accessions from wild (*O. rufipogon*) and weedy rice (*O. sativa*; Takahashi 1963; Oka 1988; Suh *et al.* 1997). These nondomesticated genotypes likely harbor major genes or alleles for seed dormancy that might have been eliminated during domestication. As a first step toward cloning dormancy genes, we have characterized some weedy rice strains for the types and levels of seed dormancy and the genetic aspects of coat-imposed dormancy (Gu *et al.* 2003). Here we report construction of a weedy rice genetic map, identification of dormancy QTL, and characterization of the QTL for epistasis and QTL-by-environment (QTL × *E*) interaction.

MATERIALS AND METHODS

Mapping population: A backcross was made to develop the mapping population EM93-1//EM93-1/SS18-2 (BC_1). SS18-2 is a wild-like, indica type of weedy rice originating from Thailand that has a red pericarp/testa color (Suh *et al.* 1997). SS18-2 seeds have mainly hull-imposed dormancy and heritability for this trait ranged from 0.64 to 0.95 in seeds after-ripened for 0 to 60 days (Gu *et al.* 2003). EM93-1 is a nondormant, short growth duration, indica type of breeding line. The cross EM93-1/SS18-2 was selected because there was >90% seed set for the F_1 plants and >70% polymorphism for DNA markers between the parents. Having a sufficient number of seeds is especially important for a precise determination of the dormancy genotypes in a distant cross (Cai and Morishima 2000).

Plant cultivation, population replication, and seed harvest: Germination was synchronized by removing the hull from seeds and drying for 30 days. Upon germination, the seedlings were transferred to rice nutrition solution (Yoshida *et al.* 1976) for 10 days and then transplanted into pots with one plant per pot, filled with a mixture of clay soil and Sunshine greenhouse medium (Sun Gro Horticulture). The population was replicated using a split-tiller technique to increase the accuracy of phenotypic identification. After 38 days when there were 15 (±3.5) tillers per plant, the secondary or tertiary tillers were split from each BC_1 plant and transplanted into three new pots. The greenhouse was divided into three blocks, and the pots with the identical tiller-derived plants were arrayed in different blocks to estimate microenvironmental variation in seed dormancy. Five control parental and F_1 (EM93-1/SS18-2) plants were all cultivated under the same conditions. The greenhouse temperature was set at 29°/21° (day/night). The average temperature and relative humidity were 25.6 (±1.32)° and 60.8 (±11.1)%, respectively. Day length was 14 hr, except from day 40 to 70, when a 10-hr short day-length treatment was applied to synchronize flowering. Flowering date was marked by emergence of the first panicle from the leaf sheath. Panicles were covered with white paper bags at ~10 days after flowering. Bagged panicles were fixed to bamboo poles to prevent shattering during seed development due to brushing or shaking the plant. Seeds were harvested at 40 days after flowering and immature seeds were removed. Seeds were air dried in the greenhouse for 3 days to 12.1 ± 0.6% moisture (estimated by 30 random samples dried at 105° for 3 days) and then stored at −20° to prevent after-ripening.

Seed after-ripening and dormancy evaluation: The degree of seed dormancy for BC_1, F_1, and parental genotypes was determined by percentage of germination of seeds harvested from the BC_1, F_1, and parental plants. The tiller-derived plants from each line were independently evaluated for germination to estimate the block effect. Prior to germination, seeds were after-ripened for 1, 11, and 21 days at 25.7 ± 0.6° and 31.9 ± 2.9% relative humidity. Germination experiments for each level of after-ripening treatment were replicated three times with ~50 seeds per replication. Seeds were placed in 9-cm petri dishes that were lined with a Whatman no. 1 filter paper, wetted with 10-ml deionized water, and incubated at 30° and

TABLE 1

Expectations of ANOVA and analysis of covariance based on the block mean for germination of seeds after-ripened for 1 or 11 (y) and 11 or 21 (y') days from the BC_1 (EM93-1//EM93-1/SS18-2) population

Source of variation[a]	d.f.	ANOVA for y and y'[b]			Analysis of covariance[b]	
		MS	EMS (y)	EMS (y')	MP	EMP (y, y')
Between lines	155	MS_B	$\delta^2_{ey} + 3\delta^2_{gy}$	$\delta^2_{ey'} + 3\delta^2_{gy'}$	MP_B	$cov_e + 3cov_g$
Within lines	312	MS_W	δ^2_{ey}	$\delta^2_{ey'}$	MP_W	cov_e

[a] A line consisted of three tiller-derived plants from the same BC_1 plant and grown in different blocks.
[b] Refer to Kearsey and Pooni (1996).

100% relative humidity in the dark for 7 days. Germination was evaluated visually by protrusion of the radicle from the hull by 3 mm. Germination percentage (x) was transformed by $\sin^{-1}(x)^{-0.5}$ for statistical analysis.

Biometric genetic analysis: The structure of the germination data for the BC_1 population resembles that of a three-factor [genotype (G), DAR, and block] factorial experiment with three replications. Both DAR and block were treated as environmental factors. Thus, the phenotypic variance was partitioned into its components with a partial linear model:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ik} + \rho_k + \varepsilon_{ijkl} \quad (i = 1\text{–}156;\ j,\ k,\ \text{and}\ l = 1,\ 2,\ \text{and}\ 3), \qquad (1)$$

where y_{ijkl} is the lth observation for the kth DAR treatment of the ith BC_1 plant in the jth block; μ is the mean; α_i, β_j, and ρ_k are the main G, DAR, and block effects, respectively; $(\alpha\beta)_{ik}$ is the $G \times$ DAR interaction, and ε_{ijkl} is the error term, including random error and the residual effect that is not explained by the main and interaction effects. ANOVA was based on the mixed model with the genotypic and block effects being random and the DAR effect being fixed. The total phenotypic variance (δ^2_P) was estimated as $\delta^2_\alpha + \delta^2_\beta + \delta^2_\rho + \delta^2_{(\alpha\beta)} + \delta^2_e$, where the δ^2_α, δ^2_β, δ^2_ρ, $\delta^2_{(\alpha\beta)}$, and δ^2_e are the component variances of G, DAR, block, $G \times$ DAR, and error, respectively, calculated on the basis of the expected mean squares (EMS) in Table 2.

Broad sense heritability (h^2_B) in the BC_1 population was defined as the proportion of gross genetic variance to the total phenotypic variance in germination. To estimate h^2_B at different DAR, two-way ANOVAs were performed on the basis of the modified linear model,

$$y_{ikl} = m + \alpha_i + \rho_k + \varepsilon_{ikl} \quad (i = 1\text{–}156;\ k \text{ and } l = 1,\ 2,\ \text{and}\ 3), \qquad (2)$$

where, y_{ikl} is the lth observation of the ith BC_1 genotype in the kth block; m is the mean, α_i and ρ_k are the main genotypic and block effects, respectively; and ε_{ikl} is the error term, including random error and possible interaction between genotype and block. The EMS for each source of variance was dissected on the basis of a random effect to estimate the genetic (δ^2_α), block (δ^2_ρ), and error (δ^2_e) component variances. h^2_B was calculated as $\delta^2_\alpha/\delta^2_P$, or $\delta^2_\alpha/(\delta^2_\alpha + \delta^2_\rho + \delta^2_e)$, where δ^2_P is the phenotypic variance. Coefficient of genetic variation (CVg) at the different DAR was estimated as $100 \times \delta_\alpha/m$.

Evaluation of germination for the individual BC_1 tiller-derived plants provided replications to estimate not only the phenotypic (r_p), but also the genetic (r_g) and environmental (r_e) correlations for degree of dormancy at DAR between 1 or 11 (y) and 11 or 21 (y') days. The statistics required for the correlation analysis were obtained from the EMS and expected mean product (EMP) in the ANOVA and analysis of covariance on the basis of the mean germination of a block (Table 1), as described by Kearsey and Pooni (1996). Correlation coefficients r_p, r_g, and r_e were calculated as $cov_p/\delta_{py}\delta_{py'}$, $cov_g/\delta_{gy}\delta_{gy'}$, and $cov_e/\delta_{ey}\delta_{ey'}$, respectively, where the cov_g and cov_e are the genetic and environmental covariances; the δ^2_{gy} and δ^2_{ey} or $\delta^2_{gy'}$ and $\delta^2_{ey'}$ are the component genetic and environmental variances, respectively, at 1 or 11 (y) or 11 or 21 (y') days; and the cov_p or δ^2_{py} and $\delta^2_{py'}$ are the corresponding phenotypic covariance or variances, which equal $cov_g + cov_e$ or $\delta^2_{gy} + \delta^2_{ey}$ and $\delta^2_{gy'} + \delta^2_{ey'}$, respectively.

Map construction: DNA was extracted from fresh leaves using a chloroform protocol. Rice microsatellite or simple sequence repeat (SSR) markers (Temnykh *et al.* 2001; McCouch *et al.* 2002) were used to construct a framework linkage map. SSR primers were obtained from ResGen (Invitrogen, San Diego) or synthesized by ITD (Integrated DNA Technologies). Polymerase chain reaction (PCR) was performed in a total volume of 15 μl containing 20–40 ng template DNA, 20 μM of each primer, 200 μM of dNTP, 0.2 unit of *Taq* polymerase, and 1.5 μl of 10× buffer with 1.5 mM Mg^{2+} (Promega, Madison, WI). Thirty-five cycles were carried out, with an initial 5-min period at 94° followed by cycles of 1 min at 94°, 1 min at 55°, 2 min at 72°, and a final 7-min period at 72°. Some SSR markers were amplified by adjusting the Mg^{2+} concentration to 2.0 or 3.0 mM or raising the annealing temperature to 61° or 67°. PCR products were separated on a 6% nondenaturing acrylamide gel and visualized with UV light or separated on a 4% denaturing acrylamide gel and detected using silver staining.

The linkage map was generated using MAPMAKER/EXP 3.0 (Lincoln *et al.* 1992). Genetic distance in centimorgans was derived by the Kosambi (1944) mapping function. Markers were grouped at the minimum log-likelihood (LOD) threshold of 4.0, a maximum distance of 40 cM, and placed at LOD of 2.0 or greater. Markers from multilocus primers or those that were different from the reported locus were distinguished with a suffix (A, B, C, or D), with the suffix A given to the first mapped locus.

QTL analysis: MQTL (Beta Version 0.98; Tinker 1996) was employed to identify dormancy QTL and QTL × E interactions. MQTL merges two complementary procedures: simple interval mapping (SIM) and simplified composite interval mapping (sCIM; Tinker and Mather 1995a). Both SIM and sCIM are currently designed for a bigenotype (*i.e.*, two genotypes at a locus) population with phenotypes assayed in multiple environments (Tinker and Mather 1995b). The parameters to run the procedures were 1-cM walking speed, 1000 random permutations to generate a threshold for the presence of QTL or QTL × E interactions, and a genome-wide type I error rate of 5%. The background markers were randomly selected at a genetic distance of ~25 cM for sCIM.

SIM and sCIM multiple-environment models were used to identify QTL and QTL × E (block) interactions associated with germination of seeds after-ripened for 1, 11, and 21 days. These analyses were based upon the mean of a block averaged

over the three germination replications. Blocks were treated as environments because the ANOVAs based on models (1) and (2) revealed a significant block effect. SIM was used to infer the presence of QTL, and sCIM was used to refine the QTL positions and main effects (Tinker and Mather 1995b). The main effect of a QTL in the BC_1 population is defined as the difference between the homozygous and heterozygous genotypes for the locus at the peak position of the test statistics (TS) distribution generated by sCIM. This difference is the gross estimate of the additive and nonadditive effects of the QTL. The proportion of variance that is explained by the main effect of a single QTL to the total variance is estimated using the equation $R^2 = 1 - 1/\exp(\mathrm{TS}/n)$ (Tinker 1996). The product between the number of environments (blocks) and the number of marker-genotyped BC_1 plants is n. The TS is the peak value of a distribution generated by SIM, rather than by the sCIM procedures, because the R^2 calculated from the TS for sCIM may be meaningless in multiple environments (Tinker 1996). A multilocus R^2 is defined as V_M/V_P. V_M is the variance arising from the main effect of a set of QTL that were established by the threshold for DAR, and V_P is the phenotypic variance based on a block mean. These estimates were obtained from MQTL inference files (Tinker 1996).

A SIM single-environment model was used to delimit the 9.1-TS (equivalent to the commonly used 2-LOD computed by MAPMAKER/QTL) supporting genomic regions for the QTL because only with this model can the TS be convertible to a LOD score by the equation 1TS = 0.22 LOD (Tinker and Mather 1995b). These analyses were based on the mean averaged over three blocks at each DAR. A SIM multiple-environment model was also used to identify QTL × DAR interactions. In this analysis, three DAR treatments were treated as different environments because the ANOVA based on model (1) had detected a significant $G \times$ DAR interaction.

Rice QTL nomenclature (McCouch *et al.* 1997) was used with a modification to designate QTL for seed dormancy. An additional superscript S was placed after the trait name to indicate that the source of a dormancy allele was the weedy strain SS18-2. This modification is made to distinguish the weedy rice-derived dormancy QTL on a particular chromosome from those reported for cultivated rice.

Epistasis: Epistasis between/among dormancy QTL is inferred by significant two-, three-, and four-way interactions between/among their nearest markers. ANOVAs for detection of the interactions were based on the generalized linear model,

$$y_{ijk} = \mu + g_i + \varepsilon_{ijk} \quad (i = 1\text{–}2^n;\ j = 1, 2, \ldots, N_i;\ \textstyle\sum N_i = 156;\ k = 1, 2, \text{and } 3), \qquad (3)$$

where y_{ijk} is the mean of the jth BC_1 tiller-derived plant of the ith di-, tri-, or tetragenic genotype in the kth block; μ is the mean; g_i is the genetic effect of the ith genotype; ε_{ikm} is the residual effect, including the genetic effects that are not explained by n ($n = 2$, 3, and 4 for the two-, three-, and four-way interactions, respectively) loci, the block effect, and random error; and N_i is the subtotal number of BC_1 tiller-derived plant lines of the ith genotype. The genetic effect g_i can be further dissected into components by the full model that consists of the main effects of the n loci and all their two-, and/or three-, and/or four-way interactions. The threshold to establish a significant two-, three-, or four-way interaction was adjusted using the sequential Bonferroni procedure (Holm 1979). Contribution of each significant interaction to the phenotypic variance (R^2) was expressed as the proportion of its component sum of squares (SS) to the corrected total SS. All ANOVA or analyses of covariance were implemented using the SAS procedure GLM (SAS Institute 1999).

RESULTS

Genetic map: A weedy rice framework genetic map was constructed on the basis of a primary segregating population of 156 BC_1 individuals. The linkage map consists of 151 SSR markers distributed along 12 chromosomes and a red pericarp/testa color gene *Rc*. The average intermarker distance was 13.5 (±7.6) cM and the total distance was ~1900 cM. *Rc* was recognized because red and white grain color could be clearly distinguished and the segregation (81:75) fit a monogenic ratio of 1:1 (χ^2-test probability = 0.63). This gene was linked to SSR markers on chromosome 7.

The present map has a length similar to that of the rice SSR linkage map based on an intersubspecies cross-derived doubled haploid population (Temnykh *et al.* 2001), for chromosomes 3, 4, 8, 9, and 10 (difference <8 cM); a greater length (10–35 cM) for chromosomes 1, 5, 6, 7, 11, and 12; and a shorter length (22 cM) for chromosome 2. The two SSR linkage maps are identical in the order of markers that were placed at LOD score >2.0, except three pairs of closely linked markers, RM232-RM251 (2.4 cM), RM252-RM241 (7.2 cM), and RM457-RM21 (2.7 cM) on chromosomes 3, 4, and 11, respectively. These linked markers were inverted and were 5–9 cM more distal from one another (Figure 1) than those in the map by Temnykh *et al.* (2001). Sixteen markers that were placed at a lower LOD score (such as RM44, RM164, RM235, RM318, and RM324) or merged from other populations (such as RM52, RM180, RM187, etc.) onto Temnykh *et al.*'s (2001) map are mapped with LOD scores >2.0 in our population. The coverage and resolution of this weedy rice linkage map allow a genome-wide scan for dormancy QTL.

Inheritance and variation for dormancy: Seeds of the parental lines SS18-2 and EM93-1 were extremely dormant and nondormant, respectively, and seeds of the F_1 plants had a degree of dormancy lying between the parents, but closer to the dormant parent SS18-2 (Figure 2). The BC_1 population was distributed over regions delimited by the range of the nondormant parent and the F_1 plants after 1, 11, and 21 DAR, although the segregation patterns varied with the times of after-ripening (Figure 2). Only a small number of BC_1 genotypes had a phenotype similar to the F_1 or EM93-1, and no genotypes in the population were observed between the dormant parent SS18-2 and the F_1's at 11 and 21 DAR. On the basis of the germination data from the parental, F_1, and BC_1 generations, we concluded that seed dormancy is an incompletely dominant and multigenic trait in this cross and that the BC_1 genotypes are much more sensitive to the relatively short periods (10 days) of after-ripening as compared with SS18-2 or the F_1.

The ANOVA based on model (1) detected significant *G*, DAR, and block main effects, and a $G \times$ DAR interaction (Table 2). The DAR, *G*, $G \times$ DAR interaction, and block component variances [*i.e.*, δ^2_β, δ^2_α, $\delta^2_{(\alpha\beta)}$, and δ^2_ρ]

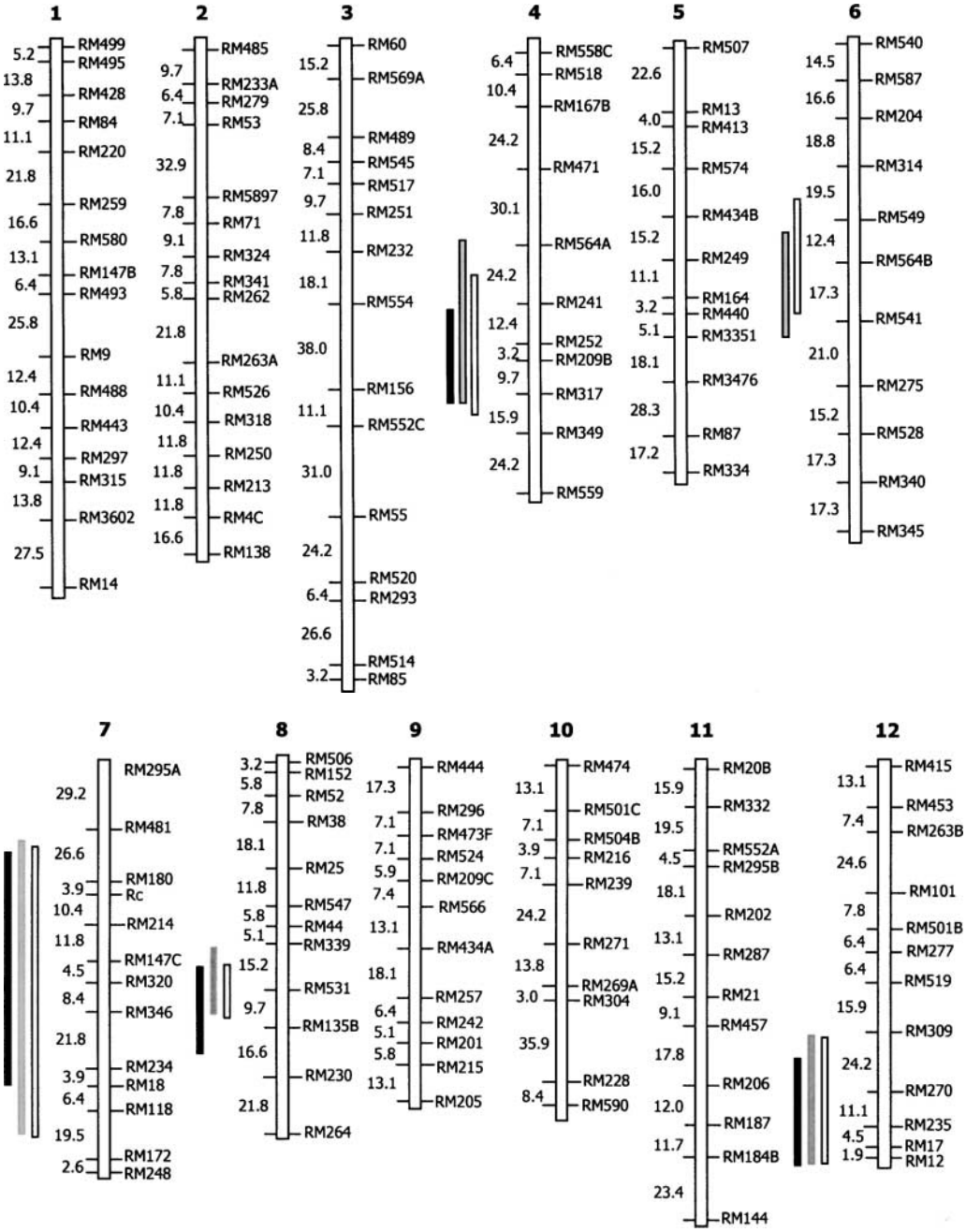

FIGURE 1.—A weedy rice linkage map and the test statistics (TS) support limits for dormancy QTL. The map was constructed on the basis of the BC_1 (EM93-1// EM93-1/SS18-2) population using rice microsatellite (RM) markers. The markers and the intermarker distances in centimorgans are labeled on the right and left of each chromosome, respectively. The solid, shaded, and open vertical bars on the left of chromosomes 4, 6, 7, 8, and 12 depict the 9.1-TS support limit for the QTL detected at 1, 11, and 21 days of after-ripening, respectively. The 9.1-TS reported by MQTL is equivalent to 2 LOD computed by MAPMAKER/QTL.

accounted for 70.2, 19.2, 3.6, and 0.6% of the total variance, respectively. Because of the high DAR variance and the presence of a significant $G \times$ DAR interaction and block effects, we adopted the strategies of QTL analysis as described in MATERIALS AND METHODS.

The ANOVA based on model (2) revealed that the genetic variance contributed most to the total phenotypic variation in germination of seeds after-ripened for 1, 11, and 21 days. In addition, the analysis confirmed the presence of a significant block effect for all three DAR treatments, although its contribution to the total variance was substantially smaller than that to the error variance (Table 3). Broad sense heritability for dormancy in the BC_1 population varied from 0.68 to 0.81 with the smallest and the largest estimate occurring at 1 and 11 days, respectively (Table 3). The standard deviations (SD) of the mapping population for the mean of tiller-derived plant lines at each DAR are in Table 3 to provide a reference to the QTL main and epistatic effects presented in Table 5 and Figures 6–8.

The degrees of seed dormancy between different DAR were positively correlated, not only for the phenotype, but also for its genotypic and environmental components (Table 4). The positive correlations indicate that both genetic and environmental factors had an increasing effect on degree of seed dormancy in the population. However, the genetic correlation (r_g^2 = 0.66–0.76) was more important than environmental correlation

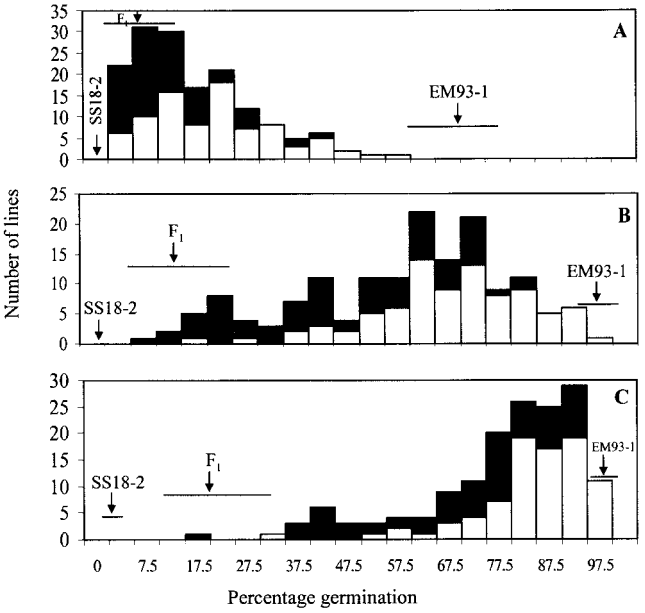

FIGURE 2.—Distribution of tiller-derived plant lines from the BC_1 (EM93-1//EM93-1/SS18-2) population for seed germination evaluated at 1(A), 11 (B), and 21 (C) days of after-ripening. Solid and open parts of a column indicate the contribution of heterozygotes and homozygotes for the EM93-1 allele at RM270, the marker nearest to the largest dormancy QTL *qSD^S-12* in the population, respectively, to the total number of lines in a germination percentage category. Arrows and horizontal lines indicate the mean and range, respectively, of five plants from the parental and F_1 generations.

(r_c^2 = 0.12–0.16). On average, ~70% of the genetic variance in degree of dormancy could be explained by the same genetic bases during the 21 DAR, and the remaining variance likely arises from the DAR-dependent expression of dormancy genes.

Dormancy QTL: Six QTL were identified from the BC_1 population by SIM. Four QTL were located on chromosomes 4, 6, 8, and 12, with the remaining two on chromosome 7 (Figure 3). They are designated as *qSD^S-4*, *qSD^S-6*, *qSD^S-7-1*, *qSD^S-7-2*, *qSD^S-8*, and *qSD^S-12*. Five QTL were detected at each DAR because the TS distribution peaks met the threshold only at 21 days for *qSD^S-6* and at 1 and 11 days for *qSD^S-8*.

The peak positions of TS distributions were refined by the sCIM (Table 5). The loci *qSD^S-7-1* and *qSD^S-7-2* are ~45 cM apart near the markers RM180 and RM346, respectively (Figure 4). The alleles that enhance dormancy at the six QTL are derived from the weedy strain SS18-2 and vary in their main effect (Table 5). The single-locus contribution to the total variance for the five QTL detected at 1, 11, and 21 DAR ranged from 6.6 to 12.9%, 7.3 to 24.3%, and 7.6 to 19.4%, respectively. The QTL *qSD^S-12* had main effects of 0.24 at 11 days and 0.21 at 21 days, which is equivalent to 1 unit of standard deviation for the population at 11 and 21 days (Table 3), and contributed most to the phenotypic variation as depicted in Figure 2. In a multilocus model, the five QTL detected at 1, 11, and 21 DAR accounted for 34, 47, and 47% of the total phenotypic variance, respectively, or 50, 58, and 63% of the genetic variance, respectively. The multilocus estimates are lower than the summation of the five individual estimates, suggesting the existence of additional genetic components contributing to variation in dormancy in this population.

The 9.1-TS support limits for the six QTL are depicted in Figure 1. *qSD^S-6* at 11 and *qSD^S-8* at 21 DAR have intervals of 27 and 16 cM, respectively, although their TS-distribution peaks are lower than the thresholds at these times (Figure 3). The 9.1-TS support limits for *qSD^S-7-1* and *qSD^S-7-2* overlap and cover about two-thirds of chromosome 7 (Figure 1).

QTL × *E* interaction: The QTL × block interaction was not significant (data not shown), which suggests that the six dormancy QTL responded relatively evenly across the blocks to greenhouse conditions. It is reasonable to assume that the genotype × block interaction is almost identical to the error in the linear models (1) and (2).

One QTL × DAR interaction involving *qSD^S-12* was detected by SIM (Figure 5). According to the single-locus model, the interaction accounted for ~22% of the phenotypic variance (V_I/V_P = 0.22) on the basis of

TABLE 2

ANOVA and expected mean square (EMS) for germination data from the BC_1 (EM93-1//EM93-1/SS18-2) population

Source[a]	d.f.	SS	MS	*F*	EMS[b]
Genotype (*G*)	155	160.40	1.0348	81.8*	$\delta_e^2 + 27\delta_\alpha^2$
DAR	2	389.61	194.8037	2524.5*	$\delta_e^2 + 9\delta_{(\alpha\beta)}^2 + 1404\delta_\beta^2$
G × DAR	310	23.92	0.0772	6.1*	$\delta_e^2 + 9\delta_{(\alpha\beta)}^2$
Block	2	3.25	1.6268	128.5*	$\delta_e^2 + 1404\delta_\rho^2$
Error	3742	47.35	0.0127		δ_e^2
Total	4211	624.53			

*Significant at the $P < 0.0001$ level.

[a] Source of variation: *G*, among tiller-derived lines; DAR, days of after-ripening.

[b] δ_α^2, δ_β^2, δ_ρ^2, $\delta_{(\alpha\beta)}^2$, and δ_e^2 are *G*, DAR, block, *G* × DAR, and error variances, respectively.

TABLE 3

Summary of genetic parameters for germination of seeds after-ripened for 1, 11, and 21 days from the BC_1 (EM93-1//EM93-1/SS18-2) population

Days of after-ripening	Mean[a]	Component variances[b]					
		δ^2_α	δ^2_ρ	δ^2_e	δ^2_P[c]	h^2_B[d]	CV_g[e]
1	0.393 ± 0.179	0.0305*	0.0009*	0.0132	0.0446	0.68	44.4
11	0.882 ± 0.240	0.0575*	0.0009*	0.0127	0.0711	0.81	27.0
21	1.126 ± 0.207	0.0436*	0.0020*	0.0122	0.0578	0.75	18.5

* Significant at the $P < 0.0001$ level.

[a] The mean ± SD for tiller-derived lines based on arcsine-transformed percentage of germination.

[b] δ^2_α, δ^2_ρ, and δ^2_e are the genotypic, block, and error variances, respectively, estimated from the ANOVA on the basis of model (2) in MATERIALS AND METHODS.

[c] Phenotypic variance estimated as $\delta^2_\alpha + \delta^2_\rho + \delta^2_e$.

[d] Broad sense heritability estimated as $\delta^2_\alpha/\delta^2_P$.

[e] Coefficient of genetic variation estimated as $100 \times \delta_\alpha$/mean.

the mean germination over the three blocks. During the after-ripening period *qSD^S-12* displayed the smallest and largest effects at 1 and 11 DAR, respectively (Table 5).

Epistasis between/among dormancy QTL: Various epistases are evident by significant two-, three-, and four-way interactions between/among two or more of the markers RM252, RM549, RM180, RM346, RM531, and RM270 nearest the six QTL. Three two-way interactions were detected (Figure 6). The RM252 × RM270 interaction was detected at both 11 and 21 DAR (Figure 6, A and B) and RM180 × RM270 and RM270 × RM549 interactions were detectable at 11 and 21 DAR, respectively (Figure 6, C and D). RM270, which marks *qSD^S-12*, was involved in all the interactions. Thus, the two-locus epistatic model can be generalized that the repressive effect on germination of the dormancy allele at *qSD^S-12* is enhanced by a dormancy allele at *qSD^S-4* or *qSD^S-6* or *qSD^S-7-1*. Each two-way interaction contributed 1.4–2.8% to the phenotypic variance.

Five three-way interactions were detected at different DAR (Figure 7). All six dormancy QTL were involved in at least one of the epistases. The three-locus epistatic effects varied with DAR. The interactions *qSD^S-4* × *qSD^S-7-1* × *qSD^S-12*, *qSD^S-7-2* × *qSD^S-8* × *qSD^S-12* and *qSD^S-6* × *qSD^S-7-2* × *qSD^S-8*, and *qSD^S-7-1* × *qSD^S-7-2* × *qSD^S-8* were detected at only 1, 11, and 21 DAR, respectively (Figure 7, A–D), while the *qSD^S-6* × *qSD^S-7-2* × *qSD^S-12* interaction was detectable at both 11 and 21 DAR (Figure 7, E and F). These interactions each explained 1.7–2.5% of the phenotypic variance. The three-way interactions reveal that the effect of a dormancy allele may increase or be partly or completely offset by the combinations of alleles at the remaining two QTL. For example, at 1 DAR the allelic difference of *qSD^S-12* was almost zero when both *qSD^S-4* and *qSD^S-7-1* are homozygous for nondormancy alleles, and the allelic difference was ~0.22, which was greater than its main effect, 0.14 (Table 5), when the dormancy allele was absent at *qSD^S-4*, but present at *qSD^S-7-1* (Figure 7A).

Six four-way interactions were found at 11 DAR (Figure 8, A–F). The markers RM252, RM531, and RM270, which were linked to *qSD^S-4*, *qSD^S-8*, and *qSD^S-12*, respectively, were involved in six, five, and four of the interactions, respectively. The markers linked to the remaining three QTL were involved in three of the six interactions. Each of the interactions accounted for 1.3–1.9% of the phenotypic variance. The four-locus epistases are more complex than the three-locus ones as the allelic difference for a QTL varies dramatically with the combinations of alleles at the remaining three loci. For example, the dormancy allele at *qSD^S-4* displayed an increased effect on germination while its contrasting alleles for nondormancy exhibited a decreased effect on germina-

TABLE 4

Summary of genetic (r_g), environmental (r_e), and phenotypic (r_p) correlation coefficients for seed germination between after-ripening treatments in the BC_1 (EM93-1//EM93-1/SS18-2) population

After-ripening treatments	r_p	r_g	r_e
1 day *vs.* 11 days	0.71 (21.7*)[a]	0.81 (17.2*)	0.36 (6.7*)
1 day *vs.* 21 days	0.72 (22.8*)	0.83 (18.6*)	0.40 (7.7*)
11 days *vs.* 21 days	0.77 (26.1*)	0.87 (21.7*)	0.35 (6.5*)

* Significance at the $P < 0.01$ level.

[a] The numbers in parentheses are the *t*-test statistics for the corresponding correlation coefficient.

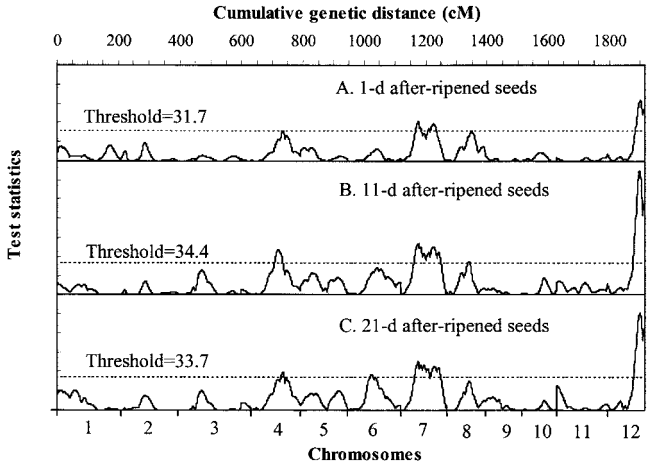

FIGURE 3.—MQTL scan for dormancy QTL from the BC_1 (EM93-1//EM93-1/SS18-2) population. Dormancy was measured by germination of seeds after-ripened for 1 (A), 11 (B), and 21 (C) days, respectively. The test statistics and threshold were generated by SIM for three blocks. The cumulative genetic distance was determined from the top of chromosome 1 to the bottom of chromosome 12.

tion when dormancy alleles were absent at both *qSD^S-7-2* and *qSD^S-12*, but present at *qSD^S-8* simultaneously (Figure 8B).

DISCUSSION

QTL controlling seed dormancy in rice: We identified six QTL controlling seed dormancy from the weedy rice strain SS18-2 (Table 5). Some genomic regions, such as those on chromosomes 1, 2, 3, 5, and 11, may also affect seed dormancy on the basis of the secondary peaks (Figure 3). Markers nearest to these peaks were associated ($P < 0.01$, mean averaged over three blocks) with seed germination in our preliminary one-way ANOVA (data not shown). We did not observe any genotypes with a degree of dormancy lying between the dormant parent and the F_1 when using the nondormant EM93-1 as the recurrent parent (Figure 2). It is possible that these regions may have greater effects in other mating systems, such as a backcross population developed using SS18-2 as the recurrent parent.

QTL for seed dormancy or resistance to PHS in rice have been identified from seven populations. Five populations were derived from cultivated rice, one from wild rice, and one from weedy rice (WAN *et al.* 1997; LIN *et al.* 1998; CAI and MORISHIMA 2000; MIURA *et al.* 2002; DONG *et al.* 2003). These >30 QTL are mapped to all rice chromosomes except chromosome 10. On the basis of similarity of map positions, *qSD^S-7-1* and *qSD^S-7-2* (Figure 4) appear to coincide with the two linked QTL on

TABLE 5

The position and main effects of QTL for seed dormancy detected at 1, 11, and 21 days of after-ripening from the BC_1 (EM93-1//EM93-1/SS18-2) population

	Days of after-ripening								
	1			11			21		
QTL name[a]	Position (cM)[b]	Main effect[c]	R^{2d} (%)	Position (cM)	Main effect	R^2 (%)	Position (cM)	Main effect	R^2 (%)
qSD^S-4	1, RM252	0.08 ± 0.026	6.6	−4, RM252	0.13 ± 0.010	9.6	−1, RM252	0.11 ± 0.025	8.1
qSD^S-6	—[e]	—	—	—	—	—	6, RM549	0.13 ± 0.015	7.6
qSD^S-7-1	−2, RM180	0.09 ± 0.006	8.5	2, RM180	0.12 ± 0.006	10.9	1, RM180	0.11 ± 0.015	10.1
qSD^S-7-2	3, RM346	0.07 ± 0.006	7.9	9, RM346	0.09 ± 0.021	10.2	9, RM346	0.08 ± 0.025	9.3
qSD^S-8	−4, RM135B	0.09 ± 0.012	6.5	−3, RM531	0.09 ± 0.020	7.3	—	—	—
qSD^S-12	1, RM270	0.14 ± 0.006	12.9	1, RM270	0.24 ± 0.031	24.3	2, RM270	0.21 ± 0.035	19.4
		Multilocus estimates:[f]	34			47			47

[a] The letters *q* and *SD* stand for a *Q*TL for the trait *S*eed *D*ormancy; the first number after the trait name indicates the chromosome on which the QTL is located, and the second number indicates more than one QTL on a chromosome; and the superscript *S* indicates the origin of the dormancy allele from the weedy strain SS18-2.

[b] Peak of the test statistic (TS) distribution for the QTL was generated by sCIM and is expressed as the genetic distance in centimorgans to the nearest marker. Absence and presence of a minus sign before cM indicate the peak is located after and before the marker, respectively.

[c] The difference between homozygous and heterozygous genotypes for the QTL in arcsine-transformed percentage of germination; the mean and standard deviation are calculated on the basis of the results generated by MQTL inference files for three blocks.

[d] The proportion of total variance explained by the main effect of a QTL estimated as described in MATERIALS AND METHODS.

[e] Dashes indicate that the peak of TS distribution for the QTL is lower than the threshold generated by SIM.

[f] The multilocus R^2 is estimated only from the QTL established by the threshold.

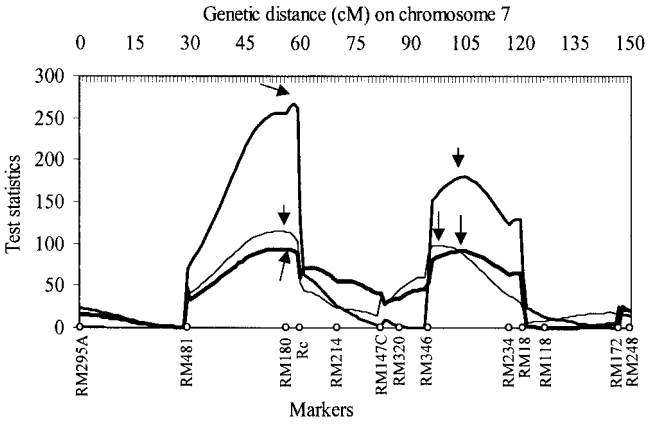

FIGURE 4.—MQTL scan for peaks of test statistics (TS) distributions for two linked dormancy QTL on chromosome 7. The TS was computed by sCIM on the basis of a multiple-environment model for seed germination evaluated at 1 (thin line), 11 (medium line), and 21 (thick line) days of after-ripening. Arrows indicate the peak positions for *qSD*^{S}*-7-1* and *qSD*^{S}*-7-2*.

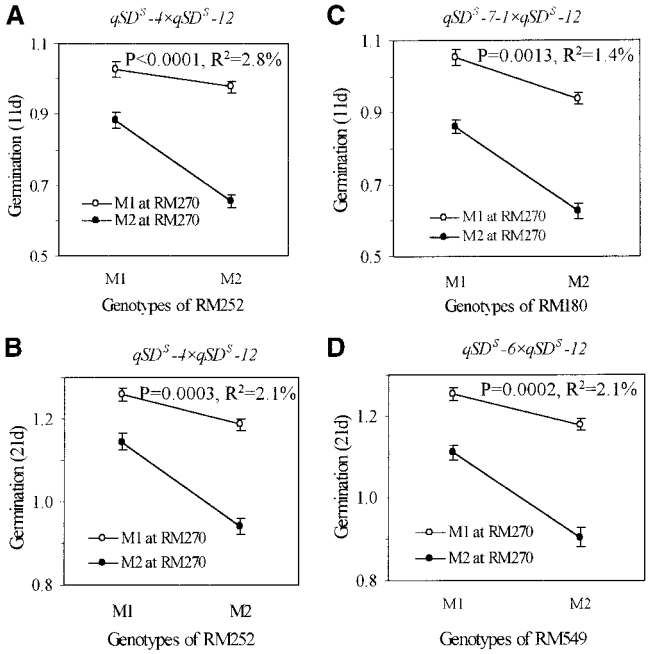

FIGURE 6.—Two-locus epistases of dormancy QTL detected at 11 (A and C) and 21 (B and D) days of after-ripening from the BC_1 (EM93-1//EM93-1/SS18-2) population. The QTL *qSD*^{S}*-4*, *qSD*^{S}*-6*, *qSD*^{S}*-7-1*, and *qSD*^{S}*-12* are represented by their nearest markers RM252, RM549, RM180, and RM270, respectively. The labels M1 and M2 for the lines and horizontal axis represent the marker (M) locus homozygous for EM93-1 alleles and heterozygous, respectively. The solid or open circles and the vertical bars represent the mean and SE for each marker combination, respectively. *P* values are *F*-test probabilities for the effect of the two-way interactions. R^2 is the proportion of component two-way interaction sum of squares (SS) to the corrected total SS based on model (3). The vertical axis is the arcsine transformation of percentage of germination.

chromosome 7 derived from the indica variety Kasalath (LIN *et al.* 1998). *qSD*^{S}*-7-2* is also likely to colocalize with the QTL nearest to markers R1245 and R2677 or *Est-9* identified from the other four cultivated rice populations (WAN *et al.* 1997; LIN *et al.* 1998; MIURA *et al.* 2002; DONG *et al.* 2003), because these markers are closely linked on the integrated genetic map of rice chromosome 7 (http://www.shigen.nig.ac.jp/rice/oryzabase/maps).

Allelic differentiation at the dormancy QTL must have occurred between nondomesticated and domesticated rice during evolution. Dormancy QTL identified from cultivars were detectable immediately after harvest and lost their inhibiting effect on germination after drying or after several days of after-ripening (WAN *et al.* 1997; LIN *et al.* 1998; MIURA *et al.* 2002; DONG *et al.* 2003). Due to sensitivity to drying and short periods of after-ripening, the cultivar-derived alleles usually lack consistency in the trait expression across environments or generations of the population. In contrast, wild (CAI and MORISHIMA 2000) and weedy (Table 5) strain-derived dormancy alleles maintained their effect for a much longer period of after-ripening. Thus, nondomesticated genotypes harbor more wild-type dormancy alleles with a relatively durable effect on germination. These alleles provide candidates for breeding varieties with stronger resistance to preharvesting sprouting.

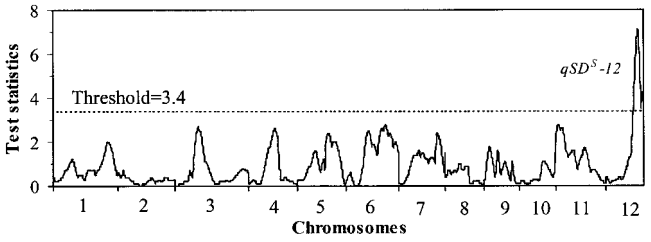

FIGURE 5.—MQTL scan for a QTL × *E* (days of after-ripening) interaction for dormancy from the BC_1 (EM93-1//EM93-1/SS18-2) population.

Orthologous dormancy gene: Red grain color in the pericarp/testa is associated with seed dormancy and resistance to PHS in Arabidopsis and wheat, respectively (GFELLER and SVEJDA 1960; DEBEAUJON *et al.* 2000; FLINTHAM *et al.* 2002). We observed a correlation between seed dormancy and red pericarp color in a segregating population derived from the weedy rice strain LD (GU *et al.* 2003). Red grain color in wheat is controlled by three orthologous genes, *R1–R3*, located on the long arm of homeologous group 3 chromosomes (FLINTHAM and GALE 1996). The three *R* loci colocalize with three QTL for resistance to PHS in a white × red grain bread-wheat cross (GROOS *et al.* 2002). Red pericarp color in rice is thought to be controlled by the genes *Rc* and *Rd* on chromosomes 7 (52 cM from the top) and 1 (65 cM from the top), respectively (KINOSHITA 1984; http://www.gramine.org/perl/mutants). The gene *Rc* is re-

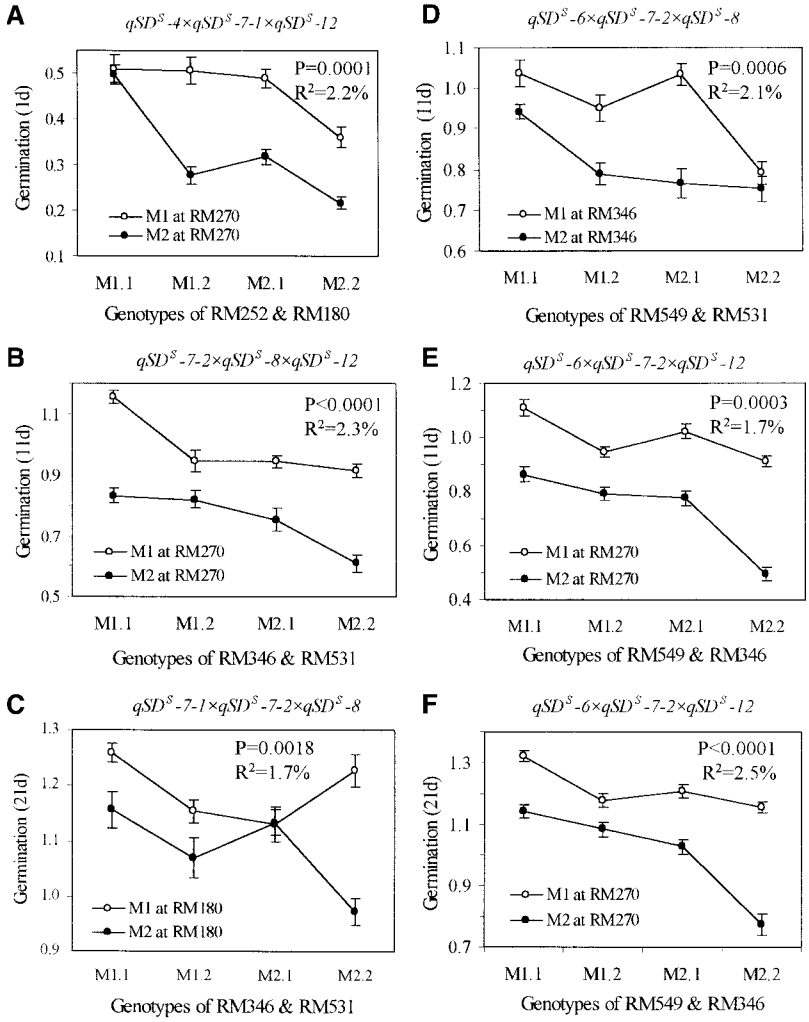

FIGURE 7.—Three-locus epistases of dormancy QTL detected at 1 (A), 11 (B, D, and E), and 21 (C and F) days of after-ripening from the BC_1 (EM93-1//EM93-1/SS18-2) population. The QTL *qSD^S-4*, *qSD^S-6*, *qSD^S-7-1*, *qSD^S-7-2*, *qSD^S-8*, and *qSD^S-12* are represented by their nearest markers RM252, RM549, RM180, RM346, RM531, and RM270, respectively. The labels below the horizontal axis beginning with the letter M and followed by a number-dot-number sequence represent the recombinant genotypes of two markers (M). The numbers 1 and 2 represent each marker locus homozygous for EM93-1 alleles and heterozygous, respectively. The lines for M1 and M2 represent the third marker locus homozygous for EM93-1 alleles and heterozygous, respectively. The solid or open circles and the vertical bars represent the mean and SE for each marker combination, respectively. *P* values are *F*-test probabilities for the effect of the three-way interactions. R^2 is the proportion of component three-way interaction sum of squares (SS) to the corrected total SS based on model (3). The vertical axis is the arcsine transformation of percentage of germination.

sponsible for pigment production, while the gene *Rd* is assumed to be responsible for spreading the *Rc*-controlled pigment (KINOSHITA 1984; http://www.shigen.nig.ac.jp/rice/oryzabase/genes/). In our population, a genetic difference in pericarp/testa color occurred at the *Rc*, rather than at the *Rd*, locus, as indicated by the linkage with markers on chromosome 7. *Rc* is tightly linked to the QTL *qSD^S-7-1* (Figure 4). Apparently, the QTL linked to a red grain color gene is important for dormancy or resistance to PHS in cereals. However, QTL analyses have been unable to determine if the colocalization or linkage are the result of pleiotropic effects of the red grain color genes or of the presence of dormancy genes near *R* or *Rc* loci in wheat (GROOS *et al.* 2002) or rice.

The red grain color gene is thought to be orthologous across the Triticeae, maize, and rice genomes (DEVOS and GALE 1997; GALE *et al.* 2002). The wheat *R* genes were suggested to be orthologous to the rice *Rd* locus (BAILEY *et al.* 1999). However, we did not detect a major effect on dormancy on rice chromosome 1 (Figure 3). In the rice pericarp/testa color system, the gene *Rc* is more important than the assumed gene *Rd*, as the gene *Rd* itself does not produce pigment (KINOSHITA 1984). QTL analysis using other rice populations will be required to evaluate the presence of a dormancy gene linked to the *Rd* locus. Nevertheless, GROOS *et al.* (2002) and our research provide evidence for association between red grain color and seed dormancy in wheat and rice. Comparative research will be also needed to relate the *Rc* locus on rice chromosome 7 to the wheat *R* genes.

Dormancy QTL × *E* interaction: Days of after-ripening is the major postharvest factor affecting the expression of seed dormancy genes (Table 2). Approximately one-quarter to one-third of the genetic variation in degree of dormancy was caused by the differential expression of dormancy genes during the 21 DAR (Table 4). Although most of this variation could be explained by a *qSD^S-12* × DAR interaction (Figure 5), other QTL also contributed to the genetic variation. There are basically three categories of dormancy QTL in Arabidopsis (ALONSO-BLANCO *et al.* 2003) and rice (Table 5) based on the detection of their main effect through the after-ripening period: those QTL with detectable effects throughout the duration of after-ripening and those with either early or late effects. The relatively constant-

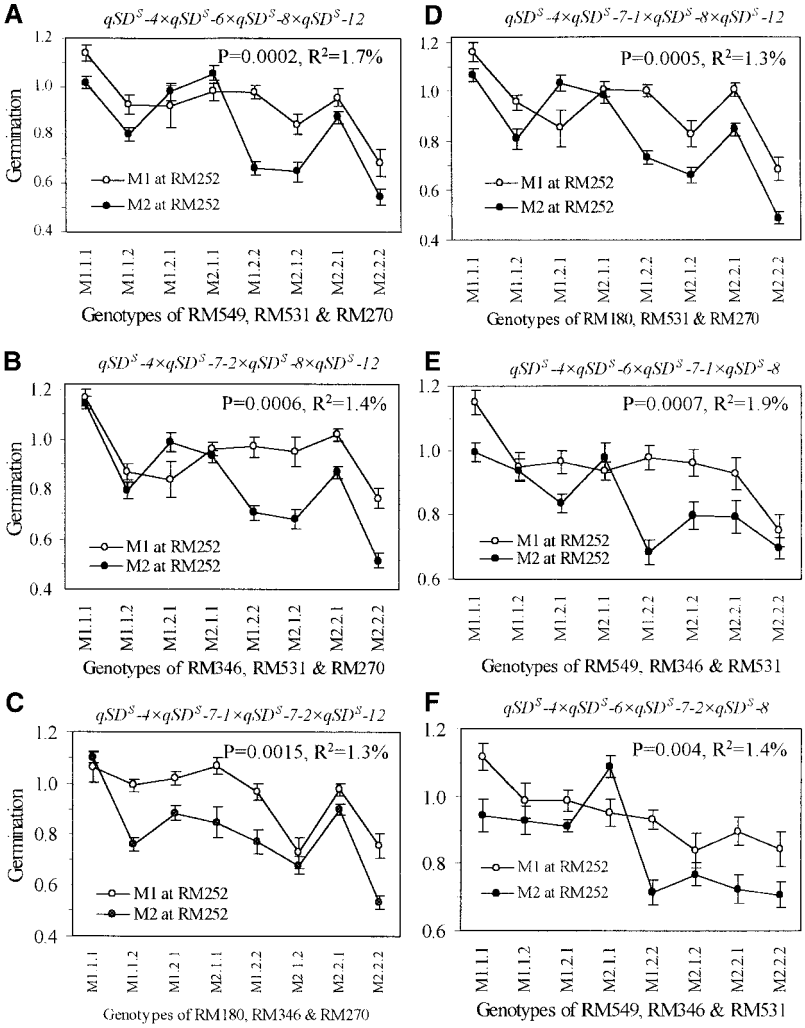

FIGURE 8.—Four-locus epistases of dormancy QTL detected at 11 days of after-ripening from the BC_1 (EM93-1//EM93-1/SS18-2) population. The QTL *qSD^S-4*, *qSD^S-6*, *qSD^S-7-1*, *qSD^S-7-2*, *qSD^S-8*, and *qSD^S-12* are represented by their nearest markers RM252, RM549, RM180, RM346, RM531, and RM270, respectively. The labels below the horizontal axis beginning with the letter M and followed by a number-dot-number-dot-number sequence represent the recombinant genotypes of three markers (M). The numbers 1 and 2 represent each marker locus homozygous for EM93-1 alleles and heterozygous, respectively. The lines for M1 and M2 represent the fourth marker locus homozygous for EM93-1 alleles and heterozygous, respectively. The solid or open circles and the vertical bars represent the mean and SE for each marker combination, respectively. *P* values are *F*-test probabilities for the effect of the three-way interactions. R^2 is the proportion of component four-way interaction sum of squares (SS) to the corrected total SS based on model (3). The vertical axis is the arcsine transformation of percentage of germination.

effect QTL, such as *DOG4*, *qSD^S-4*, and *qSD^S-12*, are detectable immediately with freshly harvested, dried seeds and maintain their inhibiting effect on germination during a relatively long period of after-ripening at room temperature. The early-effect QTL, such as *DOG3*, *DOG6*, and *qSD^S-8*, influence the germination of freshly harvested seeds and are less effective after a few weeks of after-ripening. The late-effect QTL, such as *DOG2*, *DOG5*, and *qSD^S-6*, are detectable after only 1 to a few weeks of after-ripening. This categorization is incomplete unless genetic interactions and possible allelic differentiation are considered. First, we observed that early- or late-effect QTL may interact with other QTL either before or after the time when their main effect is statistically significant. Second, a significant QTL × DAR interaction appeared to favor the QTL with the largest main effect, such as *qSD^S-12* (Figure 5). In addition, it is likely that the status of a dormancy allele or genetic background could affect the expression pattern of an individual QTL. For example, the effect of the dormancy allele marked by R1440 (corresponding to *qSD-7-1*) was detected only in the BC_1F_5 generation, but not in the BC_1F_9 generation in the Kasalath-derived population (LIN *et al.* 1998; MIURA *et al.* 2002). This locus was also not detected in other cultivated rice populations (WAN *et al.* 1997; DONG *et al.* 2003). In any event, understanding genetic mechanisms underlying the response of QTL during after-ripening will be required to provide crop breeders with solutions to PHS and seed physiologists with new insight into the after-ripening phenomenon.

Within a pure line for all the plant species that have been investigated, a range of variation for seed dormancy exists. That is, individuals in a pure-line population vary in percentage of germination (BRADFORD 1996). The within-line variation has been used as the measure of environmental variation in genetic research on seed dormancy (CHANG and YEN 1969; JANA and NAYLOR 1980; PATERSON and SORRELLS 1990; GU *et al.* 2003). Thus, it is particularly important to reduce and/or estimate the environmental variation in a dormancy QTL analysis. We used a split-tiller technique to replicate the mapping population and detected a significant block effect on degree of dormancy, but not for other morphological traits such as flowering time, plant height,

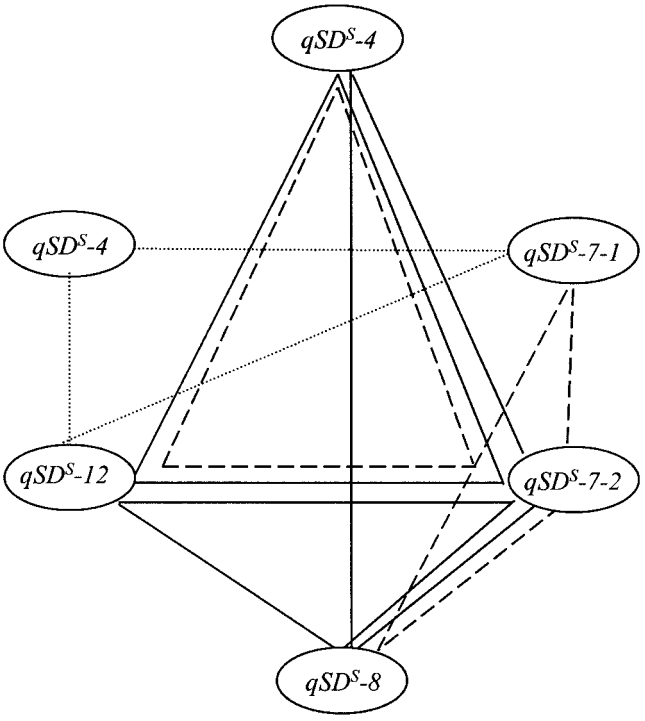

FIGURE 9.—A network consists of six dormancy QTL by three-locus epistases. The dotted, solid, and dashed lines connect QTL involved in the epistases at 1, 11, and 21 days of after-ripening, respectively.

and number of panicles per plant. A block effect in our experiment is similar to the within-line variation in the greenhouse experiment and is likely due to microenvironmental variation. The low heritability of 0–0.56 (CHANG and YEN 1969; JANA and NAYLOR 1980; PATERSON and SORRELLS 1990) implies that microenvironmental variation under field conditions is usually greater than that in the greenhouse (Table 3). Most QTL analyses of seed dormancy used a permanent segregating population such as doubled haploid lines or recombinant inbred lines. A permanent mapping population has many advantages over a temporary segregating population, for example, in replication of seed germination assays. However, germination assessment done with seeds bulked from doubled haploid lines or recombinant inbred lines, as is often the case, will confound environmental variance with other variances. Theoretically, a field experiment where seeds from identical individuals are germinated independently should provide a more accurate determination of a dormancy genotype.

Significance of epistasis: Epistases are involved in the regulation of genetic variation for seed dormancy. Interactions between two dormancy QTL were previously detected using Arabidopsis, barley, and wheat mapping populations (ANDERSON *et al.* 1993; OBERTHUR *et al.* 1995; ALONSO-BLANCO *et al.* 2003). Our research determined two- as well as three- and four-locus epistatic effects on seed dormancy in the weedy rice-derived backcross population (Figures 6–8). A locus without a significant main effect on a quantitative trait may also contribute to the phenotypic variation by interaction with another locus (LI *et al.* 1997). Thus, a two-way ANOVA based on model (3) was applied to 50 markers evenly selected from the whole genome, including the markers nearest to the six QTL (Table 5). Several markers that are independent of the QTL, such as RM259, RM262, RM251, RM3351, and RM304 on chromosomes 1, 2, 3, 5, and 10, were involved in a pairwise interaction with a QTL or with another marker that was not linked with a dormancy QTL (data not shown). Some of these "epistatic" loci may also participate in higher-order interactions, which would increase the complexity of seed dormancy. Our research detected interactions only for genotypes that were homozygous for a nondormancy allele and heterozygous at individual loci. However, it is clear that the commonly used additive and dominance model is inadequate to explain the genetic behavior of seed dormancy in natural populations and for experimental populations developed from a strongly dormant genotype like the weedy rice-strain SS18-2.

Epistasis of dormancy QTL is dependent on the duration of after-ripening. Of the 14 different epistases, 1, 9, and 2 were detected at 1, 11, and 21 DAR, respectively, and the remaining two sets were detected at both 11 and 21 DAR. When dormancy is measured by germination, segregation patterns and estimates of heritability vary with DAR. Classical genetic analyses usually attribute the variations in the pattern or estimate to the change in number of effective dormancy genes with the time of after-ripening (JANA *et al.* 1988; BHATT *et al.* 1993; FENNIMORE *et al.* 1999; GU *et al.* 2003). A majority of the epistases, and especially those involving three or more QTL, were detected at 11 DAR, when heritability was greatest, but the number of dormancy QTL was the same as that at 1 or 21 DAR (Tables 3 and 5). It seems that the change in epistatic effects is more important than the number of loci that have a detectable main effect on germination in regulating the genetic variation for dormancy during a certain period of after-ripening.

All dormancy QTL interact with each other by higher orders of epistases, which strongly suggests the presence of a genetically complex network in the control of seed dormancy. For example, in the network of three-locus epistases, *qSD^S-12* has a direct path to each of the other five QTL, *qSD^S-7-2* acts on three and four loci at 11 and 21 DAR, respectively, and no QTL acts strictly independently (Figure 9). Thus, a mutation at a particular locus could change its main effect and also the expression of one to several other loci. Apparently, the multiple loci and various epistases dramatically increase variation for dormancy, providing abundant raw material for natural selection of this critical adaptive trait, and provide a variety of choices for the artificial selection of domesti-

cated varieties. The question of how the selection for favorable epistases occurred during domestication is also fundamentally important in crop evolution (Harlan *et al.* 1973; Oka 1988; Allard 1996).

The higher-order epistases make it critical to select for a favorable combination of alleles at multiple loci as research moves toward positional cloning of a target dormancy gene. A large allelic difference is key to the success in identifying and verifying the target gene for a quantitative trait (Frary *et al.* 2000). The best allelic recombinant or the ideal genetic background for the target gene varies with the dormancy loci. For example, we might not be able to fine map *qSD^s-12*, the dormancy QTL with the largest effect in this population, from a genetic background where both *qSD^s-4* and *qSD^s-7-1* are homozygous for nondormancy alleles, because almost no allelic difference could be observed (Figure 7A). In contrast, *qSD^s-4* with a main effect of 0.13 at 11 DAR could have an allelic difference equivalent to or greater than 1 unit of the population standard deviation (0.24) with several four-locus recombinants (Figure 8).

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