Dormancy Genes From Weedy Rice Respond Divergently to Seed Development Environments

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ABSTRACT

Genes interacting with seed developmental environments control primary dormancy. To understand how a multigenic system evolved to adapt to the changing environments in weedy rice, we evaluated genetic components of three dormancy QTL in a synchronized nondormant genetic background. Two genetically identical populations segregating for *qSD1*, *qSD7-1*, and *qSD12* were grown under greenhouse and natural conditions differing in temperature, relative humidity, and light intensity during seed development. Low temperatures tended to enhance dormancy in both conditions. However, genotypes responded to the environments divergently so that two populations displayed similar distributions for germination. Additive and/or dominance effects of the three loci explained ~90% of genetic variances and their epistases accounted for the remainder in each environment. The *qSD1* and *qSD7-1* main effects were increased, while the *qSD12* additive effect was decreased by relatively low temperatures. Both gene main and epistatic effects were involved in $G \times E$ interactions, which in magnitude were greater than environmental main effect. The divergent responses of dormancy genes observed in this simple multigenic system presumably have selective advantages in natural populations adapted to changing environments and hence represent a genetic mechanism stabilizing the dormancy level of weedy rice ripened in different seasons or temperature regimes.

GENES interact with each other and with environments to regulate phenotypic variation for many adaptive traits in natural populations. Environmental factors during seed development strongly influence the level of primary dormancy, and plant species or genotypes differ in their response to the environment (BEWLEY and BLACK 1982). Relatively low temperatures often enhance dormancy, which is characterized by delayed or reduced rate of germination in cereal crops (REINER and LOCH 1975; GOLDBACH and MICHAEL 1976; HAYAS and HIDAKA 1979; REDDY *et al.* 1985). Genotypic difference in response to the environment affects the genetic composition of a weedy or wild population in subsequent seasons (SAWHNEY and NAYLOR 1979). Historical data for the response to local environments can be used to forecast dormancy levels of cultivars to assist in malting and breeding (REINER and LOCH 1975). Despite the broad impact on weed and crop sciences, little is known about how individual dormancy genes respond to seed development environments to regulate adaptive variation at a population level.

An early genetic model for seed dormancy proposed three Mendelian factors (JOHNSON 1935), which demonstrated the importance of a multigenic system in con-

trolling this adaptive trait. Subsequent research using classical genetic approaches added nonallelic interaction to the model (JANA *et al.* 1979, 1988; FENNIMORE *et al.* 1999; GU *et al.* 2003) and emphasized environment and genotype-by-environment ($G \times E$) interaction effects (CHANG and YEN 1969; UPADHYAY and PAULSEN 1988; PATERSON and SORRELLS 1990). Recently, the Mendelian factors associated with seed dormancy were resolved as quantitative trait loci (QTL) in major cereal crops to seek dormancy genes that impart resistance to preharvest sprouting (PHS) in breeding (ANDERSON *et al.* 1993; ULLRICH *et al.* 1993; LIN *et al.* 1998; LIJAVETZKY *et al.* 2000) and in wild and weedy species to determine evolutionary and genetic mechanisms underlying the adaptive trait and germination (CAI and MORISHIMA 2000; ALONSO-BLANCO *et al.* 2003; GU *et al.* 2004; ZHANG *et al.* 2005). Dormancy QTL epistases were often detected in the QTL analyses (ANDERSON *et al.* 1993; OBERTHUR *et al.* 1995; ALONSO-BLANCO *et al.* 2003; GU *et al.* 2004; KULWAL *et al.* 2004). However, the interlocus interactions have rarely been examined in relation to their gene additive and dominance components. Without information about the magnitude of component effects, it is difficult to understand how a multigenic system regulates the adaptive variation under changing environments and to predict the expression of selected dormancy genes in successive generations of breeding populations.

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FIGURE 1.—Graphic genotype for plant (no. 60) selected from an SS18-2-derived BC₄F₂ population. This plant is heterozygous only for genomic regions encompassing seed dormancy loci *qSD1*, *qSD7-1*, and *qSD12*. Open and solid bars denote the EM93-1- or SS18-2-derived chromosomes or chromosomal segments, respectively, which were determined by rice microsatellite (RM) markers at tick mark positions on the framework linkage map (Gu *et al.* 2004). Circles depict QTL from SS18-2 detected in previous research (Gu *et al.* 2004, 2005b).

Epistasis imparts an important genetic basis for the evolution of adaptation in plants (ALLARD 1996), but it often complicates a QTL analysis for adaptive traits and interpretation of the mapping results (WADE 2001). Different analytic systems, such as F-infinite and F₂-metric models, can be used to estimate QTL epistases, and statistically each system has its own advantages (KEARSEY and POONI 1996). The F₂-metric, or COCKERHAM's (1954) model, is considered to be more appropriate than others for modeling epistasis in a primary segregation (*e.g.*, F₂) population when the genes are in linkage equilibrium (KAO and ZENG 2002). In such a diploid population, the genetic variance can be partitioned into $3^n - 1$ (n is the number of loci) independent component variances using Cockerham's orthogonal contrast scales, which correspond to n additive, n dominance, and $3^n - 2n - 1$ epistatic effects. Cockerham's model has been used to determine the epistatic components between two QTL for maize domestication-related traits (DOEBLEY *et al.* 1995). Extension of Cockerham's model from two to three loci is statistically straightforward (COCKERHAM 1954). However, an increase in loci also adds to the difficulty of interpretation of estimates based on data from a limited experimental population, especially when environmental factors affect gene expression (WADE 2001). Fortunately, the difficulty should be mitigated by introduction of target genes into the same genotypic background (DOEBLEY *et al.* 1995).

Previous research detected interactions of some dormancy QTL (*e.g.*, *qSD12*) with environments (*i.e.*, time of afterripening and year) in the weedy rice-derived primary segregation [backcross (BC)₁] population (Gu *et al.* 2004, 2005a). Some other dormancy QTL such as *qSD1* and *qSD7-1* varied significantly in effect with generations of backcrossing or populations (Gu *et al.*

2005b). In this research, we introduced the *qSD1*, *qSD7-1*, and *qSD12* dormancy alleles into a nondormant genetic background and evaluated their component genetic effects in two distinct environments during seed development. On the basis of the experimental results, we discussed: (1) to what extent epistases may contribute to the adaptive variation in a multigenic system, (2) some characteristics of dormancy $G \times E$ interactions, and (3) how dormancy genes evolved to adapt to changing environments, with emphasis on temperatures during seed development in weedy rice.

MATERIALS AND METHODS

Development of the segregation population: The donor of the dormancy genes at *qSD1*, *qSD7-1*, and *qSD12* is SS18-2, an accession of wildlike weedy rice that originated from Thailand (SUH *et al.* 1997). The recipient parent used in the repeated backcross to transfer these dormancy genes is EM93-1, a nondormant, extremely early maturation breeding line. From the SS18-2-derived BC₄F₂ (132) population (Gu *et al.* 2005b), a plant (no. 60), which was heterozygous for *qSD1*, *qSD7-1*, and *qSD12* regions, was self-pollinated to develop the trigenic segregation population. The remaining chromosomal (chr) regions, including other dormancy QTL, in the selected plant are identical to EM93-1 (Figure 1), which was determined by the markers distributed on the framework genetic map (Gu *et al.* 2004). It is known that the above three dormancy loci do not link with the QTL for seed shattering (Gu *et al.* 2005a,b), which facilitates the management of segregation populations during harvesting and reduces the possible influence of shattering on dormancy evaluation. In addition, correlation between markers on these three SS18-2-derived segments and flowering time was not detected in our experiment to isolate these three QTL as single Mendelian factors using a BC₄F₂ genotype similar to plant no. 60 (Gu *et al.* 2006). Therefore, the highly synchronized genetic background and the absence of discernable segregation for other life-history traits, which

FIGURE 2.—Distributions of daily temperatures in the greenhouse and natural environments during the period from the beginning of flowering to the end of harvest in the populations. The arrows depict the flowering and harvesting periods for individual plants in each environment.

may influence germination due to tight linkage (CAI and MORISHIMA 2000; TAKEUCHI *et al.* 2003), allow us to focus on dormancy genes underlying the three QTL.

Plant cultivation and environmental conditions: Seeds from plant no. 60 were completely afterripened and germinated, and seedlings cultured using methods previously described (GU *et al.* 2004). Five weeks after germination, approximately one-half of the tillers from each plant were split; split tillers were transplanted into a new pot, and the remaining tillers were left in the original pot. The pots (28-cm diameter $\times$ 25-cm height) were filled with a mixture of clay soil and SUNSHINE medium (Sun Gro Horticulture Canada, Seba Beach, AB). A total of 234 plants were duplicated using the split-tiller technique. The two genetically identical populations were maintained in a greenhouse before flowering. Day/night temperatures were set at 29°/21°. Day length was the same as that under the local condition (Fargo, North Dakota at 46.89° north latitude and 96.79° west longitude) with supplementary light applied before 10 AM and after 2 PM.

Flowering date was recorded daily by tagging the first panicle emerging from the leaf sheath. The original set of plants from which tillers were taken flowered from July 5 to 18, and this population was maintained in the greenhouse during seed development. The split-tiller-derived population flowered from July 15 to 30 and was moved outdoors (~ 5 m away from the greenhouse) on July 17. Climatic data were automatically recorded using a HOBO Micro Station equipped with a photosynthetically active radiation (PAR) Smart Sensor (Onset, Pocasset, MA). The sensors were mounted at the plant canopy level. Mean temperature, relative humidity (RH), and PAR during the period from the onset of flowering to the end of harvest were $26.3^\circ \pm 1.8^\circ$, $70.0 \pm 5.6\%$, and $198.8 \pm 43.0 \mu\text{m}^{-2} \text{sec}^{-1}$, respectively in the greenhouse and $22.0^\circ \pm 2.8^\circ$, $73.3 \pm 8.2\%$, and $440.3 \pm 126.9 \mu\text{m}^{-2} \text{sec}^{-1}$, respectively, under natural conditions. Daily mean temperatures during the period are shown in Figure 2, as the data analysis below revealed their correlations with germination in each environment.

Seeds were harvested at 40 days after flowering, cleaned by removal of empty and immature spikelets, and air dried in the greenhouse for 3 days to $\sim 12\%$ moisture content. Dried seeds were stored at -20° to maintain dormancy.

Phenotypic and genotypic identifications: Weak or moderate dormancy usually reduces germination rate, while strong dormancy delays germination. Thus, the degree of dormancy for a plant was evaluated with percentage of germination of

seed samples afterripened at room temperature (24° – 25°) for 10, 30, and 50 days to better display genotypic difference in a segregation population (GU *et al.* 2003). About 50 seeds were placed in 9-cm petri dishes lined with a Whatman no. 1 filter paper and wetted with 10 ml deionized water. Three replications for each afterripening treatment were incubated at 30° and 100% RH in the dark for 7 days. Germination was evaluated visually by protrusion of the radicle or coleoptile from the hull by ≥ 3 mm.

The population of 234 plants was genotyped with rice microsatellite (RM) markers on the three SS18-2-derived segments (Figure 1). Genomic DNA was prepared from young leaves. DNA was extracted, the markers were amplified by polymerase chain reaction (PCR), and the PCR products were displayed using the same methods as previously described (GU *et al.* 2004). The genotyping data show that each of the 27 trigenic genotypes was replicated no less than three times in each environment (see Figure 5). Intermarker distances were adjusted with MAPMAKER/EXP 3.0 (LINCOLN *et al.* 1992).

Data analysis and genetic parameter estimation: Germination percentage (y) for each sample was transformed by $\sin^{-1}(y)^{-0.5}$, and the transformed y was averaged over three replications for further analysis. Linear correlation analysis was used to estimate the influence of climatic factors on dormancy in each environment. For this analysis, germination data from plants flowering on the same day regardless their genotypes were averaged, and the 40-day period from flowering to harvest for each plant was divided into four 10-day intervals to calculate mean temperature, RH, and PAR. Then, the mean germination was correlated with the mean temperature, RH, and PAR, respectively, to determine major climatic factors affecting dormancy and to estimate the most sensitive period of seed development in response to the environmental cues under greenhouse and natural conditions. In addition, a pairwise comparison between two genetically identical plants was used to determine mean environmental effects.

One-way ANOVA was used to confirm the three QTL and their peak positions. This analysis was based on the linear model in which a phenotypic value was partitioned into the mean, genotypic, and residual (including random error and those unexplained by the other genetic effects) components. The contribution (R^2) of each QTL was calculated as the proportion of the component type III sum of squares (SS) to the corrected total SS. The ANOVA was performed using the SAS procedure GLM (SAS INSTITUTE 1999).

COCKERHAM's (1954) model was extended to three loci to partition the genetic effect and variance of these QTL into the additive, dominance, and epistatic components. Germination data from the two environments were analyzed separately on the basis of a reduced multiple linear model,

$$\begin{aligned}
 y_{ijkl} &= G_{ijk} + \varepsilon_{ijkl} \\
 &= \mu + \sum_m^3 a_m x_m + \sum_m^3 d_m z_m + \sum_{m < n}^3 i_{mn} w_{mn} + \varepsilon_{ijkl} \\
 \sum_{m < n}^3 i_{mn} w_{mn} &= \sum_{m < n}^3 i_{aman} w_{aman} + \sum_{m < n}^3 i_{amd n} w_{amd n} \\
 &\quad + \sum_{m < n}^3 i_{dman} w_{dman} + \sum_{m < n}^3 i_{dmd n} w_{dmd n}, \quad (1)
 \end{aligned}$$

where y_{ijkl} is the phenotypic value of the l th plant ($l = 1$ to N , where N is the number of plants evaluated for germination); G_{ijk} is the genetic effect of the genotype for loci i ($qSD1$), j ($qSD7-1$), and k ($qSD12$), with i , j , and $k = 0, 1$, or 2 indicating the number of SS18-2-derived dormancy alleles at a QTL; μ is the mean of the model; x_m 's are the variables for additive (linear) components

of the loci i , j , and k , respectively, with x coded as -1 , 0 , and 1 when i , j , or $k = 0$, 1 , and 2 , respectively; z_m 's are the variables for dominance (quadratic) components of the loci i , j , and k , respectively, with z coded as $\frac{1}{2}$ and $-\frac{1}{2}$ when i , j , or $k = 1$ and 0 or 2 , respectively; w_{mn} 's are the variables for all 12 possible digenic epistases, including additive $\times$ additive (w_{aman}), additive $\times$ dominance (w_{amd_n}), dominance $\times$ additive (w_{dman}), and dominance $\times$ dominance (w_{dmd_n}), of the three QTL, with each component epistasis coded with the product of codes for the corresponding additive or dominance variables; a_m 's, d_m 's, and i_m 's are the partial regression coefficients for the corresponding variables and are also the estimates for corresponding additive, dominant, and epistatic effects, respectively; and ε_{ijkt} is the residual including random error and trigenic epistatic effects, if any. Trigenic epistases are ignored in the model because of a relatively small sample size for some trigenic genotypes in the population.

One of the advantages of Cockerham's model is that genetic variance can be partitioned into independent components, and there is no genetic covariance between components because of the property of orthogonal scales (KAO and ZENG 2002). The twenty-six orthogonal contrast scales for an F_2 population segregating for loci A (a), B (b), and C (c) were developed (Table 1) to estimate individual genetic variances (δ_i^2)

$$\delta_i^2 = (\sum P_{ijk} G_{ijk} W_{ijkt})^2 / \sum P_{ijk} W_{ijkt}^2 \quad (2)$$

(COCKERHAM 1954), where P_{ijk} and G_{ijk} are the genotypic frequency and genotypic value, respectively, for a trigenic genotype in the population, and W_{ijkt} is the t th orthogonal contrast scale for the genotype (Table 1). The component genetic variances were further used to estimate broad- (H_B^2) and narrow- (H_N^2) sense heritabilities in the population under each environment. H_B^2 and H_N^2 were calculated as the proportions of summations of δ_i^2 for all significant components and for both component additive and additive $\times$ additive variables, respectively, to the phenotypic variance. The additive $\times$ additive components were included in the H_N^2 estimation, because this fraction of additive epistasis can be passed across generations in a diploid, random-mating system and contributes to the response to selection (KEARSEY and POONI 1996).

Germination data from the greenhouse and natural conditions were also combined together to estimate each component $G \times E$ effect. This analysis was based on the joint model,

$$y_{ijkl} = \mu' + \sum_{m=1}^3 a_m x_m + \sum_{m=1}^3 d_m z_m + \sum_{m<n}^3 i_{mn} w_{mn} + b_e v_e + \sum_{m=1}^3 I_{am \times e} x_{am \times e} + \sum_{m=1}^3 I_{dm \times e} z_{dm \times e} + \sum_{m<n}^3 I_{mn \times e} w_{mn \times e} + \varepsilon_{ijkl}, \quad (3)$$

where y_{ijkl} is the phenotypic value of the l th plant grown in the s th environment; μ' is the mean of the joint model; v_e is the environment variable and it is coded as 1 and -1 for greenhouse and natural conditions, respectively; $x_{am \times e}$, $z_{dm \times e}$, and $w_{mn \times e}$ are the variables for interactions of v_e with individual additive, dominance, and digenic epistasis components, respectively; the component $G \times E$ variables are coded as the product of the code for v_e and the code for individual genetic component variables; b_e and I 's are the partial regression coefficients for the environment and interaction variables, respectively; and the remaining variables and parameters are defined as those in model (1). The above regression analyses were implemented by the SAS procedure REG with a stepwise selection set at a significant level of 5% (SAS INSTITUTE 1999).

RESULTS

Environmental influence and the population response:

Mean germination averaged over the plants flowering on the same day significantly correlated with mean temperature in both environments and with mean RH and PAR in the natural environment (Table 2). The largest environmental influence occurred during the period from 11 to 30 days after flowering, when relatively low temperature or low PAR and high RH reduced germination, as indicated by the signs of the correlation coefficients. Temperature also correlated with RH and PAR (Table 2), suggesting that these three climatic factors did not independently influence dormancy, especially in the natural environment. Relatively, plants appeared most sensitive to temperature because the greenhouse plants responded only to variation in mean temperature from 25.8° to 26.9° (Table 2).

Mean temperature in the greenhouse (26.3°) was ~4° higher than that (22.0°) under natural conditions during seed development. Pairwise comparison between split-tiller-derived identical plants detected relatively small (<5%) differences in mean germination at 10–50 days of after ripening (DAR) (Figure 3). The largest (4.9%) and smallest (2.5%) differences occurred at 10 and 50 DAR, respectively, when the plants grown in the greenhouse had higher mean germination than those under the natural conditions. However, at 30 DAR the plants under the natural condition displayed a little (3%) higher mean germination than the greenhouse plants. This set of estimates suggests that afterripening tends to diminish the effect of seed development environment on germination and may interact with the environmental effect as dormancy is gradually released.

The two genetically identical populations displayed similar segregation patterns for germination at 10, 30, and 50 DAR (Figure 3), although they experienced distinctly different temperatures during seed development (Figure 2). Germination of seeds from the split-tiller-derived identical plants grown under greenhouse and natural conditions was correlated, with $r = 0.61$ – 0.82 (Figure 3). The correlation suggests that a common reason (mainly the identical genotypes) accounted for only part (~37–68%) of the above phenotypic similarities between two environments.

Component genetic variances and effects of the three dormancy QTL: One-way ANOVA detected $qSD1$, $qSD7-1$, and $qSD12$ from each of the two populations (Table 3), and they are nearest to the markers RM220, RM5672, and RM270, respectively (Figure 1). These codominant markers were used to represent the respective dormancy loci in the following analyses. The three QTL contributed (R^2) a relatively small, moderate, and large amount, respectively to total variances in the synchronized genetic background (Table 3). Individual QTL contributions differed between greenhouse and natural conditions and the differences varied

TABLE 1
Twenty-six orthogonal contrast scales (W 's) for an F_2 population segregating for three genes in linkage equilibrium

Genotype	G_{ijk}	P_{ijk}	W_1	W_2	W_3	W_4	W_5	W_6	W_7	W_8	W_9	W_{10}	W_{11}	W_{12}	W_{13}	W_{14}	W_{15}	W_{16}	W_{17}	W_{18}	W_{19}	W_{20}	W_{21}	W_{22}	W_{23}	W_{24}	W_{25}	W_{26}
AABBCC	G222	$\frac{1}{64}$	1	$-\frac{1}{2}$	1	$-\frac{1}{2}$	1	$-\frac{1}{2}$	1	1	1	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	1	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$
AABBCc	G221	$\frac{1}{32}$	1	$-\frac{1}{2}$	1	$-\frac{1}{2}$	0	$-\frac{1}{2}$	1	0	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$
AABBcc	G220	$\frac{1}{64}$	1	$-\frac{1}{2}$	1	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	1	-1	-1	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$
AABbCC	G212	$\frac{1}{32}$	1	$-\frac{1}{2}$	0	$-\frac{1}{2}$	1	$-\frac{1}{2}$	0	1	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	0	$-\frac{1}{2}$
AABbCc	G211	$\frac{1}{16}$	1	$-\frac{1}{2}$	0	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	0	0	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	0	0	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	0	$-\frac{1}{2}$	$-\frac{1}{2}$	0	0	0	$-\frac{1}{2}$
AABbcc	G210	$\frac{1}{32}$	1	$-\frac{1}{2}$	0	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	0	-1	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$
AAbbCC	G202	$\frac{1}{64}$	1	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	1	$-\frac{1}{2}$	-1	1	-1	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	0	0	0	$-\frac{1}{2}$
AAbbCc	G201	$\frac{1}{32}$	1	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	0	$-\frac{1}{2}$	-1	0	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$
AAbbcc	G200	$\frac{1}{64}$	1	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	-1	-1	1	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	1	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$
AaBBCC	G122	$\frac{1}{32}$	0	$-\frac{1}{2}$	1	$-\frac{1}{2}$	1	$-\frac{1}{2}$	0	0	1	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	0	0	0	0	0	0	$-\frac{1}{2}$
AaBBCc	G121	$\frac{1}{16}$	0	$-\frac{1}{2}$	1	$-\frac{1}{2}$	0	$-\frac{1}{2}$	0	0	0	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	0	0	0	0	0	0	$-\frac{1}{2}$
AaBbCC	G120	$\frac{1}{32}$	0	$-\frac{1}{2}$	1	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	0	0	-1	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	0	0	0	0	0	0	$-\frac{1}{2}$
AaBbCc	G112	$\frac{1}{16}$	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	1	$-\frac{1}{2}$	0	0	0	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	0	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	0	0	0	0	0	0	$-\frac{1}{2}$
AaBbCC	G111	$\frac{1}{8}$	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	0	0	0	0	0	0	0	0	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	0	0	0	0	0	0	$-\frac{1}{2}$
AaBbcc	G110	$\frac{1}{16}$	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	0	0	0	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	0	0	0	0	0	0	$-\frac{1}{2}$
AabbCC	G102	$\frac{1}{32}$	0	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	1	$-\frac{1}{2}$	0	0	-1	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	0	0	0	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$
AabbCc	G101	$\frac{1}{16}$	0	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	0	$-\frac{1}{2}$	0	0	0	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	0	0	0	0	0	0	$-\frac{1}{2}$
Aabbcc	G100	$\frac{1}{32}$	0	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	0	0	1	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	0	0	0	0	0	0	$-\frac{1}{2}$
aaBBCC	G022	$\frac{1}{64}$	-1	$-\frac{1}{2}$	1	$-\frac{1}{2}$	1	$-\frac{1}{2}$	-1	-1	1	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$
aaBBCc	G021	$\frac{1}{32}$	-1	$-\frac{1}{2}$	1	$-\frac{1}{2}$	0	$-\frac{1}{2}$	-1	0	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$
aaBBcc	G020	$\frac{1}{64}$	-1	$-\frac{1}{2}$	1	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	-1	1	-1	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	1	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$
aaBbCC	G012	$\frac{1}{32}$	-1	$-\frac{1}{2}$	0	$-\frac{1}{2}$	1	$-\frac{1}{2}$	0	-1	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$
aaBbCc	G011	$\frac{1}{16}$	-1	$-\frac{1}{2}$	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	0	-1	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$
aaBbcc	G010	$\frac{1}{32}$	-1	$-\frac{1}{2}$	0	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	0	0	0	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$
aabbCC	G002	$\frac{1}{64}$	-1	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	1	$-\frac{1}{2}$	1	-1	-1	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	1	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$
aabbCc	G001	$\frac{1}{32}$	-1	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	0	$-\frac{1}{2}$	1	0	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$
aabbcc	G000	$\frac{1}{64}$	-1	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	1	1	1	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	-1	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$

G_{ijk} and P_{ijk} are the genotypic values and expected genotypic frequencies of genotypes for loci A , B , and C ; the subscript distinguishes the 27 genotypes, with i (or j , or k) = 2, 1, and 0 indicating that there are two dormancy alleles, one dormancy and one nondormancy allele, and two nondormancy alleles, respectively at the locus A (or B , or C). W 's are adopted from the eight orthogonal scales of Kao and ZENG (2002). W_1 and W_2 , W_3 and W_4 , and W_5 and W_6 are additive and dominance orthogonal contrasts for A , B , and C loci, respectively.

TABLE 2

Correlation between mean germination and mean temperature, relative humidity (RH), and photosynthetic active radiation (PAR) during the period from 11 to 30 days after flowering for the segregation populations grown in natural and greenhouse environments

Environment	Climatic factors (range)	Correlation coefficient at DAR ^a		
		10	30	50
Natural	Temperature ($\sim 21.3^{\circ}$ – 24.4°)	0.547*	0.766**	0.793**
	RH (~ 64.6 – 78.0%)	–0.522*	–0.707**	–0.712**
	PAR (~ 417 – $481 \mu\text{M m}^{-2} \text{sec}^{-1}$)	0.483 ^{ns}	0.734**	0.761**
	($r_{t,rh} = -0.387^{**}$; $r_{t,par} = 0.403^{**}$; $r_{rh,par} = -0.124^{ns}$) ^b			
Greenhouse	Temperature ($\sim 25.8^{\circ}$ – 26.9°)	0.502*	0.549*	0.772**
	RH (~ 70.6 – 72.2%)	–0.210 ^{ns}	–0.244 ^{ns}	–0.408 ^{ns}
	PAR (~ 196 – $205 \mu\text{M m}^{-2} \text{sec}^{-1}$)	0.108 ^{ns}	0.160 ^{ns}	0.083 ^{ns}
	($r_{t,rh} = -0.4724^{**}$; $r_{t,par} = 0.469^{**}$; $r_{rh,par} = -0.717^{**}$)			

^a Germination after the stated days of afterripening (DAR) was averaged among plants that flowered on the same day. The superscripts denote nonsignificance (ns) or significance at the $P < 0.05$ (*) or < 0.01 (**) probability levels.

^b Correlation coefficients between temperature and RH ($r_{t,rh}$), temperature and PAR ($r_{t,par}$), and RH and PAR ($r_{rh,par}$) for the period from the onset of flowering to the end of harvest for the population.

with QTL. For example, at 10 DAR both *qSD1* and *qSD7-1* contributed about two times more, while *qSD12* contributed approximately two times less to the phenotypic variances under natural than under greenhouse conditions. The distinct differences between the populations segregating for the same set of three dormancy QTL demonstrate that the underlying genes responded divergently to seed developmental environments.

The population of 234 plants consisted of all 27 genotypes for *qSD1* (*A/a*), *qSD7-1* (*B/b*), and *qSD12* (*C/c*), with the upper- and lowercase letters standing for dormancy and nondormancy alleles at each locus, respectively (refer to Figure 5). The observed allelic frequencies are $p_A = 0.5171$ and $p_a = 0.4829$, $p_B = 0.4957$ and $p_b = 0.5043$, and $p_C = 0.4701$ and $p_c = 0.5299$.

Ten of the 27 genotypes had sample sizes of less than five (refer to Figure 5), which is too small for the chi-square approximation for a trigenic segregation ratio. However, the observed digenic genotypic frequencies fit the expectations for two unlinked loci, *i.e.*, (0.25:0.5:0.25) (0.25:0.5:0.25), with $\chi^2 = 8.2$ ($P = 0.41$) for *qSD1* and *qSD7-1*, $\chi^2 = 10.7$ ($P = 0.22$) for *qSD1* and *qSD12*, and $\chi^2 = 4.8$ ($P = 0.78$) for *qSD7-1* and *qSD12*.

The analysis based on Equation 1 in MATERIALS AND METHODS detected additive effects for *qSD1* (a_1), *qSD7-1* (a_2), and *qSD12* (a_3), dominance effects for *qSD7-1* (d_2) and *qSD12* (d_3), and some epistases involving all aforementioned main effects and the *qSD1* dominance (d_1) effect (Table 4). The a_1 – a_3 and d_2 components reduced germination at 10–50 DAR under both conditions,

FIGURE 3.—Frequency distribution of the plants in each of the genetically identical segregation populations for percentage germination. Germination was evaluated at 10 (solid lines with squares), 30 (dotted lines with triangles), and 50 (solid lines with circles) days of afterripening (DAR). Solid and open symbols depict data for germination of seeds developed in greenhouse and natural environments, respectively. *N* represents the number of plants in each population evaluated for germination. Population mean and standard deviation that were calculated on the basis of arcsin-transformed percentage of germination are provided to relate the angle-transformed to the percentage scale. Asterisks indicate that the differences in both transformed and percentage scales and correlations (r) between split-tiller-derived identical plants grown in the greenhouse and natural environments were significant at the 0.01 (**) or < 0.0001 (***) levels.

TABLE 3

Contribution of dormancy QTL on germination evaluated at 10, 30, and 50 days of afterripening (DAR) in the genetically identical populations of rice grown in greenhouse and natural environments

QTL ^a	Environment	10 DAR		30 DAR		50 DAR	
		R^2 (%) ^b	Prob. ^b	R^2 (%)	Prob.	R^2 (%)	Prob.
<i>qSD1</i> (RM220)	Greenhouse	2.3	0.0639	4.2	0.0069	10.6	<0.0001
	Natural	5.6	0.0015	9.2	<0.0001	13.1	<0.0001
<i>qSD7-1</i> (RM5672)	Greenhouse	8.1	<0.0001	5.6	0.0003	5.1	0.0005
	Natural	20.2	<0.0001	7.6	<0.0001	8.9	<0.0001
<i>qSD12</i> (RM270)	Greenhouse	51.3	<0.0001	61.2	<0.0001	38.3	<0.0001
	Natural	28.4	<0.0001	36.9	<0.0001	29.4	<0.0001

^a Marker in parentheses is nearest to the QTL.

^b Proportion of the phenotypic variance explained by the QTL (R^2) and the F -test probability (Prob.) calculated by one-way ANOVA.

whereas d_3 increased germination and was significant only at 50 DAR. Absolute values of a_1 and a_2 or d_2 were higher, while a_3 -values were lower under natural than under greenhouse conditions. The a_1 – a_3 components totally accounted for a vast majority (80–90%) of the genetic variance, with the a_3 ($a_3 = -0.178$ to -0.376 equivalent to 3.5–13.5% germination) contributing most (42–78%) at the three DAR and in the two environments (Table 4). In contrast, d_2 and d_3 totally explained a relatively small amount (2–12%) of the genetic variances. It is clear that together these gene additive and dominant effects accounted for a major part of the above divergent responses of the three-locus system to the environments.

Seven sets of digenic epistasis, including additive $\times$ additive, additive $\times$ dominance, dominance $\times$ additive, and dominance $\times$ dominance, totally accounted for 1–7% of genetic variances (Table 4). Different from the above additive effects on reducing germination, the epistatic effects increased or decreased germination, which varied depending on environment and DAR. For example, four of the five epistases at 10 DAR were present under the greenhouse condition and increased germination, whereas the other one was present under the natural condition and reduced germination; the $d_1 \times d_2$ and $d_1 \times d_3$ epistases occurred only at 10 DAR, and the $a_2 \times a_3$ and $d_2 \times a_3$ occurred only at 30 and 50 DAR. Dissection of the epistases, such as the four types of *qSD12*-involved epistases, revealed a variety of interaction patterns (Figure 4, A–D). For example, when *qSD12* was homozygous for nondormancy (cc) and dormancy (CC) alleles, the *qSD1* additive effect reduced (*i.e.*, $AA < aa$) and increased (*i.e.*, $AA > aa$) germination, respectively (Figure 4A); similarly, when *qSD12* was cc and CC , the dormancy allele at *qSD7-1* was completely dominant (*i.e.*, $Bb = BB$) and overdominant (*i.e.*, $Bb < BB$) over the nondormancy allele, respectively (Figure 4B). With respect to diversity and their presence or absence, epistases also made an important contribution to the regulation of genetic variation with growth environment and DAR.

Genic effects estimated on the basis of Equation 1 together accounted for 94–97% of the total genetic variances in germination at different DAR and environments (Table 4). According to these estimates based on the reduced model, broad-sense heritabilities were greater under greenhouse ($H_B^2 = 0.64$ – 0.78) than under natural ($H_B^2 = 0.64$ – 0.66) conditions, and the largest heritability was obtained at 30 DAR in the greenhouse environment (Table 4). Narrow-sense heritabilities (H_N^2) ranged from 0.59 to 0.75 in the greenhouse environment and from 0.55 to 0.57 in the natural environment. Nonadditive effects, which include dominance effects and the effects of epistases excluding the additive $\times$ additive interactions, explained up to ~5 and 8% of the phenotypic variances, respectively, in greenhouse and natural environments. The above single-plant-based estimates suggest that the heritability for dormancy with the three-locus system is relatively high, and a majority (~83%) of genetic variation could be fixed by selection even under the natural condition.

Genotype-by-environment interactions: Most of the 27 trigenic genotypes varied in germination between greenhouse and natural environments (Figure 5). Some genotypes (*e.g.*, *aaBbcc*, *AaBbcc*, and *AABBCC*) had lower, while others (*e.g.*, *AabbCc*, *AabbCC*, and *AaBBCC*) had higher germination in the natural than in the greenhouse environment. The divergent genotypic responses on average contributed to phenotypic similarity between the populations under different environments (Figure 3) and suggest the presence of genotype-by-environmental interactions.

The analysis based on Equation 3 in MATERIALS AND METHODS corroborated the additive and dominance effects and some of the epistatic effects (Table 4) and yielded additional information about epistatic, environmental, and $G \times E$ interaction effects (Table 5). The $a_1 \times a_2$ epistasis absent in model (1) was significant at 50 DAR in the combined analysis; whereas, the $a_1 \times d_3$ epistasis in model (1) at 10 DAR was not significant

TABLE 4

Component genetic effects of the three dormancy loci on germination at 10, 30, and 50 days of afterripening (DAR) and their variances (σ_t^2) based on the genetically identical populations of rice grown in greenhouse and natural environments

Parameter ^a	Greenhouse				Natural			
	Estimate	Error	Prob.	σ_t^2 (%) ^b	Estimate	Error	Prob.	σ_t^2 (%)
Germination at 10 DAR								
<i>u</i>	0.395	0.011	<0.0001	NA ^c	0.353	0.010	<0.0001	NA
<i>a</i> ₁	-0.036	0.015	0.0207	1.1	-0.072	0.013	<0.0001	6.8
<i>a</i> ₂	-0.118	0.016	<0.0001	11.0	-0.148	0.014	<0.0001	33.5
<i>d</i> ₂	-0.084	0.022	0.0002	3.2	-0.140	0.019	<0.0001	11.7
<i>a</i> ₃	-0.298	0.017	<0.0001	73.1	-0.178	0.014	<0.0001	42.2
<i>i</i> _{<i>a</i>₁ <i>a</i>₃}	0.078	0.021	0.0003	3.3	— ^f	—	—	—
<i>i</i> _{<i>a</i>₁ <i>d</i>₃}	—	—	—	—	-0.056	0.027	0.0375	1.0
<i>i</i> _{<i>d</i>₁ <i>d</i>₂}	0.114	0.045	0.0129	1.4	—	—	—	—
<i>i</i> _{<i>d</i>₁ <i>d</i>₃}	0.113	0.045	0.0132	1.6	—	—	—	—
<i>i</i> _{<i>a</i>₂ <i>d</i>₃}	0.067	0.033	0.0423	0.5	—	—	—	—
H_B^{2c}				69.0				66.0
H_N^{2d}				64.0				57.2
Germination at 30 DAR								
<i>u</i>	0.924	0.011	<0.0001	NA	0.959	0.012	<0.0001	NA
<i>a</i> ₁	-0.080	0.015	<0.0001	3.3	-0.101	0.016	<0.0001	10.3
<i>a</i> ₂	-0.115	0.016	<0.0001	8.6	-0.113	0.017	<0.0001	13.8
<i>d</i> ₂	-0.095	0.022	<0.0001	2.5	-0.105	0.024	<0.0001	7.3
<i>a</i> ₃	-0.376	0.016	<0.0001	78.4	-0.244	0.017	<0.0001	56.4
<i>i</i> _{<i>a</i>₁ <i>a</i>₃}	0.063	0.021	0.0035	1.2	—	—	—	—
<i>i</i> _{<i>a</i>₂ <i>a</i>₃}	-0.069	0.023	0.0038	1.6	-0.066	0.025	0.0085	1.6
<i>i</i> _{<i>d</i>₂ <i>a</i>₃}	-0.115	0.033	0.0006	1.5	-0.114	0.035	0.0012	4.2
H_B^2				78.1				63.7
H_N^2				74.9				55.8
Germination at 50 DAR								
<i>u</i>	1.152	0.010	<0.0001	NA	1.113	0.010	<0.0001	NA
<i>a</i> ₁	-0.101	0.013	<0.0001	13.3	-0.103	0.014	<0.0001	15.3
<i>a</i> ₂	-0.079	0.014	<0.0001	9.7	-0.105	0.015	<0.0001	17.0
<i>d</i> ₂	-0.068	0.019	0.0006	2.6	-0.065	0.021	0.0021	3.8
<i>a</i> ₃	-0.214	0.014	<0.0001	59.6	-0.177	0.015	<0.0001	48.8
<i>d</i> ₃	0.043	0.019	0.028	2.3	0.062	0.021	0.0032	3.7
<i>i</i> _{<i>a</i>₂ <i>a</i>₃}	-0.061	0.020	0.0032	3.3	-0.059	0.022	0.0077	2.4
<i>i</i> _{<i>a</i>₂ <i>d</i>₃}	—	—	—	—	0.075	0.031	0.0161	2.6
<i>i</i> _{<i>d</i>₂ <i>a</i>₃}	-0.101	0.028	0.0004	3.0	-0.071	0.030	0.0213	2.4
H_B^2				64.3				63.7
H_N^2				58.9				55.4

^a *u* and *a*'s, *d*'s, and *i*'s represent the mean and additive, dominance, and epistatic effects, respectively, in model (1); only significant parameters are shown.

^b Proportion of the component genetic variance, which was estimated on the basis of Equation 2, to the total genetic variance.

^c Broad-sense heritability estimated as the proportion of the summation of genetic variances for all significant component effects to the phenotypic variance.

^d Narrow-sense heritability estimated as the proportion of the summation of genetic variances for significant additive and additive $\times$ additive component effects to the phenotypic variance.

^e Not applicable.

^f Not significant in the environment.

and the $d_1 \times d_2$ and $d_1 \times d_3$ epistases in model (1) were shifted to $G \times E$ interactions in this analysis. Of the five gene main effects, only the *qSD12* additive effect (*a*₃) was also involved in the $G \times E$ interaction. The environmental effects ($b_e = 0.022$, -0.018 , and

0.018 at 10, 30, and 50 DAR, respectively) were minor, as they were much smaller than any component gene and $G \times E$ interaction effect in absolute value (Table 5). The three sets of $G \times E$ interactions displayed divergent effects on germination and were

FIGURE 4.—Digenic epistases involving $qSD12$ detected in the two genetically identical populations grown in different environments. Dormancy (nondormancy) alleles at $qSD1$, $qSD7-1$, and $qSD12$ are represented by A (a), B (b), and C (c), respectively. Solid and open symbols depict data for germination of seeds developed in the greenhouse and natural environments, respectively. Dotted, solid, and dashed lines indicate germination evaluated after 10, 30, and 50 days of afterripening, respectively.

maintained for different DAR in the population. Specifically, the $a_3 \times E$ interaction ($I_{a_3 \times e}$) reduced germination and the effect lasted for ~ 30 days; whereas, the $d_1 \times d_2$ (or d_3) $\times E$ interactions ($I_{d_1 d_2 \times e}$ or $I_{d_1 d_3 \times e}$) increased germination and the effects lasted for ~ 10 days.

DISCUSSION

Contribution of epistatic effects to dormancy in a multigenic system: The synchronized genetic background improved estimation for genic effects of dormancy QTL. All three loci had significant main effects

FIGURE 5.—Mean germination at 10 (A), 30 (B), and 50 (C) days of afterripening (DAR) for 27 trigenic genotypes of the three loci segregating in the rice population grown in greenhouse and natural environments during seed development. A (a), B (b), and C (c) denote the dormancy (nondormancy) alleles at $qSD1$, $qSD7-1$, and $qSD12$, respectively; the number of the plants is in the parentheses after each genotype.

TABLE 5
Joint analysis based on model (3) for the data from the two environments in Table 4

Parameter ^a	10 DAR ^b			30 DAR			50 DAR		
	Estimate	Error	Prob.	Estimate	Error	Prob.	Estimate	Error	Prob.
u'	0.376	0.007	<0.0001	0.939	0.008	<0.0001	1.130	0.007	<0.0001
a_1	-0.052	0.010	<0.0001	-0.090	0.011	<0.0001	-0.102	0.010	<0.0001
a_2	-0.132	0.011	<0.0001	-0.114	0.012	<0.0001	-0.092	0.011	<0.0001
d_2	-0.109	0.015	<0.0001	-0.103	0.016	<0.0001	-0.067	0.014	<0.0001
a_3	-0.235	0.011	<0.0001	-0.308	0.012	<0.0001	-0.192	0.011	<0.0001
d_3	— ^c	—	—	—	—	—	0.057	0.014	<0.0001
$i_{a_1 a_2}$	—	—	—	—	—	—	-0.030	0.014	0.0282
$i_{a_1 a_3}$	0.055	0.014	0.0001	0.044	0.016	0.0049	—	—	—
$i_{a_2 a_3}$	—	—	—	-0.066	0.017	0.0001	-0.061	0.015	<0.0001
$i_{a_2 d_3}$	—	—	—	—	—	—	0.069	0.021	0.0015
$i_{d_2 a_3}$	—	—	—	-0.112	0.024	<0.0001	-0.079	0.021	0.0002
b_e	0.022	0.008	0.0037	-0.018	0.008	0.0273	0.018	0.007	0.0120
$I_{a_3 \times e}$	-0.056	0.011	<0.0001	-0.062	0.012	<0.0001	—	—	—
$I_{d_1 d_2 \times e}$	0.084	0.030	0.0056	—	—	—	—	—	—
$I_{d_1 d_3 \times e}$	0.064	0.030	0.0326	—	—	—	—	—	—
Model R^{2d}	0.64			0.68			0.57		

^a u' is mean of the model, b_e is environmental effect with the greenhouse and natural environments coded with 1 and -1, respectively; I s denote interactions between environment and gene component effects; the remaining parameters are similar to those in Table 4; only significant parameters in model (3) are shown.

^b Days of afterripening (DAR) prior to germination.

^c Not significant.

^d Proportion of the phenotypic variance accounted for by parameters retained in the model.

and were also involved in digenic epistases through additive and/or dominance effects (Table 4). The locus *qSD1* was suggested only by interactions between its flanking marker RM259 (Figure 1) and other dormancy loci in the primary segregation (BC_1) population (Gu *et al.* 2004). In this three-locus system, the *qSD1* additive effect (a_1) contributed ~0.9% (or 1.1%), while the *qSD1*-involved digenic epistases together contributed 4.9% (or 6.3%) to the phenotypic (or genotypic) variance in germination at 10 DAR under the greenhouse condition (Table 4). The relatively higher proportion of component epistatic variance partly explains why researchers could detect more epistatic-effect (E) than main-effect (M)-QTL in a complex genetic background (KULWAL *et al.* 2004) and suggests that an E-QTL for an adaptive trait is not necessarily only a regulatory locus (WADE 2001). Synchronizing the genetic background appears crucial to clarifying the nature of E-QTL and how they regulate or are regulated by an interacting gene system. For example, the simulation of a hypothesized three-locus interacting system suggests that the genetic background at two regulatory loci alters dominance performances of the third locus in its effects on fitness from neutral, additive, dominant, and over- or underdominant (WADE 2001). The dormancy genes in the present research differ from the hypothesized loci, because both *qSD1* and *qSD12* are basically additive and *qSD7-1* is nearly completely dominant on the basis of their main effects (Table 4). However, the phenomena

simulated by WADE (2001) also occurred in our three-locus system where the effect of a dormancy allele could be enhanced, offset, or inverted by a change in non-allelic combinations at the other loci (Figure 4). Gene frequency varies in weedy populations in agroecosystems because of human disturbance. Epistases make it difficult to determine which genotype(s) is a favorable nonallelic combination(s) in a population to adapt best to a particular environment. A similar difficulty also exists in breeding activities where dormancy genes are employed to improve resistance to preharvest sprouting.

Digenic epistases together contributed up to 5% (or 8%) to phenotypic (or genetic) variances in the three-locus system (Table 4). Exclusion of trigenic epistases in model (1) due to limitation of sample size for some genotypes must have led to an underestimation of the epistatic contribution. This is because: (1) this model detected 94–97% or missed 3–6% of the total genetic variances, (2) higher-order epistases were detected in the BC_1 population (Figures 7 and 8 in Gu *et al.* 2004), and (3) our simulation based on the same data and the full model, which is modified by addition of all trigenic epistases to Equation 1 (refer to Table 1), suggests the presence of several trigenic epistatic effects, such as $a_1 \times a_2 \times a_3$ ($i_{a_1 a_2 a_3} = -0.104$, $R^2 = 1.5\%$, $P = 0.0006$), $a_1 \times a_2 \times d_3$ ($i_{a_1 a_2 d_3} = 0.040$, $R^2 = 0.6\%$, $P = 0.0127$), and $d_1 \times d_2 \times d_3$ ($i_{d_1 d_2 d_3} = -0.218$, $R^2 = 0.7\%$, $P = 0.0172$) under greenhouse and/or natural conditions. Most likely, the trigenic epistatic components are responsible

TABLE 6

Expectations of genotypic means (G_{ijk}) and their genetic components based on model (1) for genotypes selected to represent three types of responses to the greenhouse (GH) and natural (NT) environments

Component ^a	<i>AAbbCc</i> ^b			<i>AaBbCc</i>			<i>AabbCC</i>		
	GH	NT	GH-NT	GH	NT	GH-NT	GH	NT	GH-NT
u	0.395	0.353	0.042	0.395	0.353	0.042	0.395	0.353	0.042
a_1x_1	-0.036	-0.071	0.036	NA	NA	NA	NA	NA	NA
a_2x_2	0.118	0.148	-0.030	NA	NA	NA	0.118	0.148	-0.030
d_2z_2	0.042	0.070	-0.028	-0.042	-0.070	0.028	0.042	0.070	-0.028
a_3x_3	NA ^c	NA	NA	NA	NA	NA	-0.298	-0.178	-0.120
$i_{a_1d_3}w_{a_1d_3}$	— ^c	-0.028	0.028	NA	NA	NA	NA	NA	NA
$i_{a_2d_3}w_{a_2d_3}$	-0.034	—	-0.034	NA	NA	NA	0.034	—	0.034
$i_{d_1d_2}w_{d_1d_2}$	0.028	—	0.028	0.028	—	0.028	-0.028	—	-0.028
$i_{d_1d_3}w_{d_1d_3}$	-0.028	—	-0.028	0.028	—	0.028	-0.028	—	-0.028
G_{ijk}	0.486	0.472	0.014	0.410	0.283	0.127	0.234	0.394	-0.159

^a See MATERIALS AND METHODS for definitions of the variable and parameter in each component.

^b A/a , B/b , and C/c donate the dormancy/nondormancy alleles at $qSD1$, $qSD7-1$, and $qSD12$, respectively; GH-NT is the difference between greenhouse and natural conditions in arcsin-transformed germination.

^c This component is not applicable (NA) or not significant (—) for the genotype under the environment.

mainly for the missing genetic variances estimated on the basis of model (1).

Partitioning genotypic means with model (1) revealed that the relatively small proportion of epistatic variation played an important role in regulating genotypic responses to seed development environments (Figure 5). For example, the *AAbbCc* genotypic means at 10 DAR were similar in both environments with the difference being 0.014, but their genetic components differed, including the four sets of digenic epistases (Table 6). The $a_1 \times d_3$ epistasis was present in the natural, but absent under the greenhouse conditions, while the remaining three epistases were present in the greenhouse, but absent under the natural conditions. The presence or absence of epistatic effect(s) partly counteracted background (u) or gene main effects in each environment to contribute to the phenotypic similarity across the two environments (Figure 5A). Similarly, the *AaBbCc* and *AabbCC* genotypic means were 0.127 higher and 0.159 lower in the greenhouse than in the natural environments, respectively, partly because both the $d_1 \times d_2$ and $d_1 \times d_3$ epistatic effects increased germination in the genotype *AaBbCc*, but inhibited germination in the genotype *AabbCC* in the greenhouse environment (Table 6). These two epistatic effects together contributed 44% to the *AaBbCc* and 36% to the *AabbCC* genotypic differences between the two environments. Divergent genotypic responses to germination environments were reported for an Arabidopsis recombinant inbred line population, which led to the hypothesis that there may be different sets of genes controlling germination timing in alternative environments or that the same genes increase germination in one but decrease germination in the other environment (DONOHUE *et al.* 2005). Genetic mechanisms governing the acquiring

and release of seed dormancy are likely different. Our observations demonstrate that even a simple multigenic system is capable of regulating genotypic responses through adjusting gene component effects to adapt to changing environments.

Implications of component $G \times E$ interactions for dormancy: Although interactions between genotypes and seed development environments were frequently reported in classical genetic analysis for seed dormancy and preharvest sprouting (UPADHYAY and PAULSEN 1988; PATERSON and SORRELLS 1990), usually a QTL analysis could detect the involvement only of loci with a relatively larger effect in a significant $G \times E$ interaction (OBERTHUR *et al.* 1995; LIJAVETZKY *et al.* 2000; KULWAL *et al.* 2004; GU *et al.* 2005a). In the present research, the additive effect of the major locus $qSD12$ (a_3) was involved in a significant $G \times E$ interaction; the other two QTL with a moderate or relatively small main effect also interacted with the environment through epistases, which were confirmed by the joint model (Table 5). It seems that no dormancy gene in a multigenic system can be completely independent of the seed development environment in the phenotypic effect on germination. However, detection of $G \times E$ s with relatively small effects appears to be dependant on the analytical method employed. For example, although the magnitude of a_1 was small at 10 DAR, and the component d_3 was not significant at 30 DAR according to model (1) (Table 4), we detected significant $G \times E$ interactions for $a_1 \times E$ at 10 DAR ($I_{a_1 \times e} = 0.020$, $T = 2.01$, $P = 0.045$) and $d_3 \times E$ ($I_{d_3 \times e} = -0.028$, $T = -2.52$, $P = 0.012$) at 30 DAR, using F -infinite metrics (KEARSEY and POONI 1996) on the basis of model (3) (data not shown).

A population segregating solely for three loci may represent a statistically manageable multigenic system

to examine component $G \times E$ interactions for a complex trait like dormancy. The information from the present research provides several additional insights. First, a $G \times E$ effect may increase or reduce the phenotypic effect (Table 5), which varies depending on individual genotypes (Table 6). Therefore, genetic-by-seed development environment interactions contribute directly to germination flexibility (DEKKER *et al.* 1996) or phenotypic plasticity regardless of its active or passive responses in adaptation (VAN KLEUNEN and FISCHER 2005). Second, the dominance $\times$ dominance epistases are more frequently involved in the interactions as compared with other component epistatic effects; this implies that some $G \times E$ interactions cannot be determined in a homogenous segregation population, such as the often-used recombinant inbred lines in a QTL analysis. Finally, the $G \times E$ interactions for seed dormancy can be detected during the early to midstages of afterripening, when the magnitude of a $G \times E$ interaction can be greater than the environmental main effect (b_e) (Table 5). Information from this research provides the basis to examine more complex genetic systems in environments with broader variation.

Adaptive significance of dormancy gene divergent responses to seed development environments: Rice plants are more sensitive to temperature than to RH and light intensity in acquiring primary seed dormancy, with relatively low and high temperatures during seed development tending to enhance and reduce dormancy, respectively (Table 2). This tendency has been described for cultivars or pure lines that are different in seed dormancy (REINER and LOCH 1975; GOLDBACH and MICHAEL 1976; HAYAS and HIDAKA 1979; SAWHNEY and NAYLOR 1979; REDDY *et al.* 1985). Apart from the general tendency, we observed that some genotypes (*e.g.*, *AaBbCC* and *AAbbCc*) were relatively constant in degree of dormancy in different temperature regimes, and some others (*e.g.*, *AabbCC* and *AaBBCC*) had weaker dormancy under relatively low temperature conditions (Figure 5). Divergently genotypic responses to seed development environment were also reported for germination timing in an *Arabidopsis* (*Arabidopsis thaliana*) recombinant inbred line population grown under different photoperiods (MUNIR *et al.* 2001; DONOHUE *et al.* 2005). Thus, a heterogeneous population may have an adaptive advantage over pure lines with respect to maintaining relative stability of seed dormancy in changing environments. Weeds often experience changing maturation environments due to human disturbance, such as change in cropping systems; dormancy as a major adaptive trait promotes survival of weed seeds in disturbed environments. Therefore, similar divergent responses of dormancy genotypes must also occur in weedy populations.

Differential regulation of underlying genes is behind the divergent genotypic responses. The *qSD1* and *qSD7-1* loci and the *qSD12* locus were up- and downregulated,

respectively, by the low-temperature regime under natural conditions, with respect to their gene main effects in this highly synchronized nondormant genetic background (Tables 3 and 4). All loci in the simple multigenic system interacted with each other and with environments (Table 5), but there was no one environmental condition best suited to promote full expression of all the naturally occurring dormancy genes at a population level. The gene regulatory system seems geared to maintain dormancy homeostasis in natural populations under varying environmental conditions. The question is if homeostasis for dormancy genes has a selective advantage under natural conditions.

The gene donor SS18-2 originated from Thailand (SUH *et al.* 1997), and its dormancy genes are presumably derived from wild rice (*Oryza rufipogon*) in the tropical region, as suggested by the QTL clusters or haplotypes for wild-like adaptive traits (GU *et al.* 2005a,b). Weedy rice accompanies cultivated rice year-round in multiple cropping systems in tropical regions (WATANABE *et al.* 2000). Dormancy genes, such as that underlying *qSD12*, with phenotype enhanced by a relatively high temperature would be important for weed seeds ripened under seasonal humid, hot conditions to prevent immediate germination after maturation or shattering. Conversely, genes such as those at *qSD1* and *qSD7-1*, with phenotype enhanced by low temperatures are important for weed seeds ripened under seasonal cool conditions. This set of dormancy genes was simultaneously introduced from the weedy rice SS18-2 by six generations of phenotypic selection alone for low germination extremes (GU *et al.* 2005b). The cointroduction suggests that this set of dormancy genes is a favorable epistatic combination (ALLARD 1996) under high selection pressure. It is reasonable to believe that weedy and wild rice distributed in tropical and likely other areas have selected such a dichotomous genetic mechanism for dormancy to persist in a range of ecosystems by distributing germination over time.

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