Genetic and Physiological Characterization of Two Clusters of Quantitative Trait Loci Associated With Seed Dormancy and Plant Height in Rice

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ABSTRACT

Seed dormancy and plant height have been well-studied in plant genetics, but their relatedness and shared regulatory mechanisms in natural variants remain unclear. The introgression of chromosomal segments from weedy into cultivated rice (Oryza sativa) prompted the detection of two clusters (qSD1-2/qPH1 and qSD7-2/qPH7) of quantitative trait loci both associated with seed dormancy and plant height. Together, these two clusters accounted for >96% of the variances for plant height and ~71% of the variances for germination rate in an isogenic background across two environments. On the initial introgression segments, qSD1-2/qPH1 was dissected genetically from OsVp1 for vivipary and qSD7-2/qPH7 separated from Sdr4 for seed dormancy. The narrowed qSD1-2/qPH1 region encompasses the semidwarf1 (sd1) locus for gibberellin (GA) biosynthesis. The qSD1-2/qPH1 allele from the cultivar reduced germination and stem elongation and the mutant effects were recovered by exogenous GA, suggesting that sd1 is a candidate gene of the cluster. In contrast, the effect-reducing allele at qSD7-2/qPH7 was derived from the weedy line; this allele was GA-insensitive and blocked GA responses of qSD1-2/qPH1, including the transcription of a GA-inducible α-amylase gene in imbibed endosperm, suggesting that qSD7-2/qPH7 may work downstream from qSD1-2/qPH1 in GA signaling. Thus, this research established the seed dormancy-plant height association that is likely mediated by GA biosynthesis and signaling pathways in natural populations. The detected association contributed to weed mimicry for the plant stature in the agro-ecosystem dominated by semidwarf cultivars and revealed the potential benefit of semidwarf genes in resistance to preharvest sprouting.

KEYWORDS

seed dormancy, plant height, quantitative trait locus, gibberellin, signaling, weedy rice

Seed dormancy and plant height contribute to adaptation by regulating the timing of germination and plant architecture, respectively. Both traits have been intensively selected during domestication or breeding, resulted in the divergence between cereal crops and their wild relatives. For example, domestication tended to reduce seed dormancy to synchronize germination, which also caused the problem of preharvest sprouting in production (Harlan et al. 1973; Bewley and Black 1994); crop breeding has been in favor of semidwarf varieties to prevent lodging since the “Green Revolution” commenced in the middle of the last century (Khush 2001). Despite years of detached research on seed dormancy and plant height, little attention has been paid to their relatedness and shared regulatory mechanisms. Thus, it remains unclear whether and how the selection for one of the two traits impacted the other.

Many seed dormancy and plant height genes or quantitative trait loci (QTL) were identified, but none of them is known to influence both of the two traits. In wheat (Triticum aestivum), the Reduced height1 genes encode DELLA proteins, which act as master regulators of gibberellin (GA) signaling, and the gain-of-function mutants Rht-B1b and Rht-D1b produce truncated DELLA proteins resulting in reduced GA response and plant height (Peng et al. 1999). In rice (Oryza sativa), the Semi-dwarf1 gene (Sd1 or GA20ox2) encodes GA20-OXIDASE2 catalyzing the second-to-last step of the GA
biosynthesis, and the sd1 loss-of-function mutants produce defective enzymes resulting in reduced GA and plant height (Ashikari et al. 2002; Monna et al. 2002; Spielmeyer et al. 2002). These and other genes for GA biosynthesis or signaling were not reported to have an effect on germination. On the other hand, the seed dormancy QTL DOG1 in Arabidopsis, and Sdr4 and qSD7-1 in rice were map-based cloned. DOG1 and Sdr4 encode predicted proteins with unknown molecular functions (Bentsink et al. 2006; Sugimoto et al. 2010). The qSD7-1 underlying gene encodes a basic helix-loop-helix family transcription factor that enhanced the biosynthesis of the dormancy-inducing hormone abscisic acid in developing seeds (Gu et al. 2011). None of the dormancy genes was reported for an effect on plant height. Thus, characterization of additional QTL for seed dormancy may help clarify the relationship between the two adaptive traits.

Weedy rice, the Oryza genus plants adapted to rice-growing areas, retained some wild traits (e.g., strong seed dormancy) and also developed crop mimics for agronomic traits such as plant height (Oka 1988; Delouché et al. 2007). The previous research identified several QTL for seed dormancy (qSD) in a primary segregating population (BC1F1) developed from the cross between a weedy and a cultivated line of rice (Gu et al. 2004). In the subsequent research to transfer individual QTL alleles from the weedy into the cultivated rice, qSD1-2 and qSD7-2 were also associated with plant height, or co-located with plant height QTL in different advanced generations. Interestingly, the isolated qSD1-2 region encompasses the loci GA20ox2 and OsVp1 (ortholog of the Maize Viviparous1 gene) (McCarty et al. 1991) and qSD7-2 was colocated with the Sdr4 seed dormancy QTL (Lin et al. 1998; Gu et al. 2004). Therefore, the objectives of this research were: (1) to estimate genetic component effects of the qSD1-2 and qSD7-2 regions on both seed dormancy and plant height in an isogenic background; (2) to determine allelic relations between qSD1-2 and GA20ox2 or OsVp1, and between qSD7-2 and Sdr4; and (3) to characterize the QTL clustered in the qSD1-2 and qSD7-2 regions for GA responses, including germination, seedling elongation, and transcription of a GA-induced α-amylase gene in the endosperm of imbibed seeds.

**MATERIALS AND METHODS**

**Plant materials and breeding scheme**

The qSD7-2 QTL was identified from the BC1F1 (EM93-1/EM93-1/SS18-2) population and mapped on the long arm of chromosome 7 (Gu et al. 2004), whereas the qSD1-2 was detected in two BC2F1 plant-derived BC3F2 populations and located on the long arm of chromosome 1 (Ye et al. 2010). SS18-2 is a weedy (O. sativa) and EM93-1 is a cultivated line of rice, and both lines are semidwarf in plant height. The parental and hybrid F1 (EM93-1/SS18-2) lines and their recombinants for each of the two QTL regions (Figure 1A) were used to infer an inheritance pattern and to map QTL for plant height.

Recurrent backcrossing, combined with marker-assisted foreground and background selection, started with a BC1F1 plant and continued for four (BC2F1—BC5F1) generations to transfer only the qSD1-2- and qSD7-2-containing segments from SS18-2 into the EM93-1 background (Figure 1). The BC5F1 heterozygote for these two segments (e.g., plant 144 in Figure 1B) was advanced to the BC6F2 and higher generations to select a digenic system of all nine digenic genotypes, nearly isogenic lines (NILs) for the four homozygotes, and recombinants for each of the two QTL regions.

**Marker genotyping and genotypic selection**

Rice microsatellite (RM) markers on the framework linkage map of weedy rice (Gu et al. 2004) were used to genotype advanced backcross populations for QTL mapping. Markers flanking the qSD1-2 and qSD7-2 peak positions were used to genotype the BC2F2 and their progeny lines to select for the digenic system, NILs, and recombinants. The digenic system consisted of all nine digenic genotypes for the two
QTL and the selected genotypes were similar in frequency to minimize biased estimates of genetic component effects. The NILs were progeny lines of the four homoygotes from the digenic system and were designated 1-1-7-7-, 1*1-7-7-, 1-1-7-7*, and 1*1-7-7*, with the superscripts indicating germination/height-promoting (+) or -reducing (−) alleles at qSD1-2 on chromosome 1 or qSD7-2 on chromosome 7. The recombinants were identified from about 500 segregating for one of the two initial introgression segments. Marker genotyping was conducted on 3- to 4-wk-old seedlings using the previously described methods (Gu et al. 2004).

On the initial introgression segments from SS18-2, the qSD1-2 interval encompasses the loci sdi (Os1g666100) and OsVp1 (Os1g68370), and the qSD7-2 interval covers the locus Sdr4 (Os07g39700), according to the reference genome sequence of japonica rice ([IRGSP 2005 Calendar Database release 35 at http://www.gramene.org]). Thus, more than 20 RM markers physically aligned to the two QTL regions on the reference genome (McCouch et al. 2004) were screened for polymorphism between EM93-1 and SS18-2. Polymorphic markers were used to delimit the SS18-2 derived segments retained in individual recombinants. In addition, the marker sdi(k), which targets the 383-bp functional deletion within sdi (Monna et al. 2002), was used to genotype the parental and recombinant plants to infer allelic differentiation at the GA synthesis gene. Based on the marker genotypes, six recombinants that were heterozygous for different sub-segments of the qSD1-2 or qSD7-2-containing regions were selected for progeny testing. About 90 seedlings from each of the progeny lines were genotyped with a marker on the heterozygous region and grown in the greenhouse for phenotypic assessment.

Genetic component effects of the two QTL-containing regions on seed dormancy and plant height were evaluated in a greenhouse and a field experiment. Fully after-ripened BC1F2 seeds were germinated in a 30°C incubator for 5 d, and sprouting seeds transferred to 200-cell Plug Trays filled with rice nutrient solution (Yoshida et al. 1976) for 4 wk to synchronize seedling size. For the greenhouse experiment, ~25 seedlings for each of the nine genotypes were transplanted into pots (one plant/pot), which were filled with a mixture of clay soil and potting medium (Sunshine Mix #1; SUNGRO Horticulture Ltd., Canada) and placed in watertight containers (60 cm x 120 cm). The day/night temperatures in the greenhouse were set at 29/21°C, and photoperiods were natural for the summer or 12 hr for the winter season. Plants were tagged for flowering date when the first panicle in a plant emerged from the leaf sheath. Seeds were harvested at 40 d after flowering and air-dried in the greenhouse for 3 d before storage in a -20°C freezer to maintain the dormant status. For the field experiment, ~25 seedlings for each of the nine genotypes were transplanted in individual plots, at a density of 16 cm by 30 cm, on the Rice Research Farm of Southeast Missouri State University in Malden, MO. Seed harvesting and storage were conducted using the aforescribed methods.

Phenotypic assessment for seed dormancy and plant height

Seed dormancy was quantified by germination rate under controlled conditions. Before germination, seed samples from individual plants were left at the room temperature (~24°C, i.e., after-ripening) for varying lengths (0–30 d depending on experiments) to maximize the variance in a population or to estimate the after-ripening period required for a genotype to achieve 50% germination. A germination experiment consisted of three replications for all plants from a segregating population or a progeny line. A replicate of approximately 30 seeds distributed in 9-cm Petri dishes, each lined with a filter paper and wetted with 8 mL of deionized water, was germinated at 30°C, 100% relative humidity and in dark for 7 d. Germinated seeds (protrusion of the radicle from the hull by ≥3 mm) were counted daily from 2 to 7 d. Germination percentage (x) for a sample was transformed by sin⁻¹(x⁻¹) to improve the normality of the frequency distribution.

Plant height was measured as the length of the main stem from the soil surface to the top of the panicle at maturation and recorded for single plants cultivated using the aforescribed methods, with the exception of the parental, F1 and BC1F1 generations. Five plants from each of the parental and F1 generations and 156 BC1F1 plants were duplicated four times by a splitting-tiller technique at the seedling stage; the genetically identical duplicates were grown in large pots (28 cm diameter and 25 cm height) placed in the same greenhouse (Gu et al. 2004). For these generations, plant height data recorded for the four tiller-derived plants were averaged for genetic and QTL analyses.

QTL mapping and genetic effect estimation

Linkage maps constructed based on the BC1F1, BC1F2, and BC1F3 populations (Gu et al. 2004, Ye et al. 2010) were used to scan for plant height QTL. The map construction and QTL mapping were conducted using the backcross F1 model for the BC1F1 data, or using the intercross F2 model for the BC1F2 and BC1F3 data. The composite interval mapping program of Windows QTL Cartographer v2.5 (Wang et al. 2006) was used to generate likelihood ratio distributions along all (BC1F1) or part (BC1F2 and BC1F3) of the 12 chromosomes to infer QTL segregating in the populations. Parameters set to run the program included Kosambi’s map function, 1-cM walking speed, and 1000 random permutations at a type-I error rate of 5%.

Genetic component effects of the narrowed qSD1-2 and qSD7-2 regions on seed dormancy and plant height were estimated using the additive (a)-dominance (d)-epistasis (I) model for two loci,

\[ y_ijk = \mu + a_1 x_1 + a_2 x_2 + a_1 a_2 + \ldots + I_{a_1 a_2} + I_{a_1 d} + I_{a_2 d} + I_{d_1 d_2} + e_{ijk} (\text{Model 1}) \]

where, \( y_{ijk} \) is the phenotypic value for the \( k \)th plant (\( k = 1 \), the population size) of a digenic genotype for qSD1-2 (i) and qSD7-2 (j), with \( i \) and \( j \) = 0, 1, 2 representing the copy number of the SS18-2-derived allele; \( \mu \) is the model mean; \( x_1 \) and \( x_2 \) are variables for additive (linear) components of loci \( i \) and \( j \), respectively, with \( x \) coded as -1, 0, and 1, when \( i \) or \( j \) = 0, 1, and 2, respectively; \( z_1 \) and \( z_2 \) are the variables for the dominance (quadratic) components of loci \( i \) and \( j \), respectively, with \( z \) coded as 1 and 0 when \( i \) or \( j \) = 1 and 0 or 2, respectively; \( w_{a_1 a_2} \), \( w_{a_1 d} \), \( w_{a_2 d} \), \( w_{d_1 d_2} \) are the variables for additive-additive \((w_{a_1 a_2})\), additive-dominance \((w_{a_1 d})\), and dominance-dominance \((w_{d_1 d_2})\) types of epistasis between the two loci, with each type coded as the product of codes for the corresponding additive or dominance variables; \( a_1, a_2, d_1, d_2, I_{a_1 a_2}, I_{a_1 d}, I_{a_2 d}, I_{d_1 d_2}, \) and \( e_{ijk} \) is the residual including random error and environmental effects. Regression analysis for the greenhouse and field data sets was implemented separately using the SAS REG procedure, with a stepwise selection at a significance level of 5% (SAS Institute 1999).
Data from the six progeny lines were analyzed by linear correlation to infer if the recombinants differentiate at qSD1-2, or qSD7-2, or known gene (sd1, OsVp1, or Sdr4). Marker genotypes like EM93-1, heterozygous, and like SS18-2 in a progeny line were coded as 0, 1, and 2, respectively, to correlate with the trait values for germination and plant height. A significant marker-trait correlation indicates that the recombinants retained a copy of functional dormancy/plant height gene on the marked heterozygous region. A nonsignificant correlation suggests that the recombinant is fixed for the QTL/gene or that the QTL underlying gene is located outside the marked heterozygous region. For the progeny lines with a significant marker-trait correlation, the data were also used to estimate additive and dominance effects of the QTL/gene using the model,

\[ y_{ik} = \mu + ax + dz + e_{ik} \] (Model 2)

Model (2) was modified from model (1) by removing variables related to the second locus.

GA induced seedling growth, germination, and gene expression experiments

The four NILs were used to evaluate genotypic responses to exogenous GA (GA3; Aceros Organic, NJ) for germination, seedling growth, and the expression of the GA-inducible α-amylase gene Amy1A to infer relations between the seed dormancy/plant height QTL and GA synthesis or signal transduction. To evaluate the germination response, dried seeds without an after-ripening treatment were soaked in each of the 0 (control), 0.1, 1.0, 10.0, and 100.0 μM GA3 solutions and incubated under the aforesaided conditions for 7 d. The sample size was about 40 seeds, and the germination experiment was replicated three times for 10 plants from each of the four NILs.

Two experiments were conducted to evaluate GA effects on seedling growth. In the initial experiment, 10 seedlings (2-d-old) from an NIL were grown in a 9-cm Petri dish, which was lined with two layers of filter paper and irrigated with one of the 0 (control), 0.1, 1.0, 10.0, and 100.0 μM GA3 solutions, in a growth chamber set for 30° and 16-hr light. Seedling length was measured after 7 d. In the second experiment, 18 seedlings at 5 d of age were planted in nutrient solutions (Yoshida et al. 1976) with different GA3 concentrations in the greenhouse. The length of the second foliage leaf sheath was measured every 3 d during the following 15-d period.

The expression of Amy1A in the aleuron layer was induced by GA from the embryo to synthesize α-AMYLASE1A required to hydrolyze starchy endosperm in imbibed seeds of rice (Kaneko et al. 2002). Quantitative real-time polymerase chain reaction (PCR) assays were used to compare genotypic differences in the transcription level of Amy1A. Three samples of 50 seeds from an RIL were incubated in 9-cm Petri dish, which was lined with two layers of filter paper and irrigated with one of the 0 (control), 0.1, 1.0, 10.0, and 100.0 μM GA3 solutions and incubated under the aforesaided conditions for 7 d. The sample size was about 40 seeds, and the germination experiment was replicated three times for 10 plants from each of the four NILs.

Genetic effects of the isolated QTL clusters qSD1-2/qPH1 and qSD7-2/qPH7

The two chromosomal segments introduced from SS18-2 cover the qSD1-2/qPH1 and qSD7-2/qPH7 peak-containing intervals, respectively. As expected, plant height correlated positively with germination rate in the digenic system consisting of the nine genotypes for the two segments (Figure 4). The linear correlation

\[ y_{ik} = \mu + ax + dz + e_{ik} \] (Model 2)

Model (2) was modified from model (1) by removing variables related to the second locus.

RESULTS

Inheritance and QTL for plant height in the weed-cultivar cross

The data from the parental, F1, and BC1F1 generations revealed a complex inheritance pattern for plant height in the cross. Hybrid F1s (92 cm) were taller than the parents EM93-1 (73 cm) and SS18-2 (81 cm); the BC1F1s (60–126 cm) varied beyond the parental and F1 limits and displayed a bimodal distribution (Figure 2). A genome-wide scan detected only one QTL (qPH1) for plant height on chromosome 1, which accounted for 65% (R2) of the phenotypic variance in the BC1F1 population (Figure 3A). The major effect of qPH1 could explain the bimodal distribution, but not the transgressive segregation. There must be undetectable QTL in the primary segregating population.

The BC1F2 was similar to the BC1F1 population for the bimodal distribution (Figure 2). QTL analysis confirmed qPH1 (R2 = 50%) and detected no additional loci associated with plant height in the BC1F2 population (Figure 3B). The qSD1-2 seed dormancy locus was detected in this BC1F2 population and colocated with qPH1 in the RM315 to RM3602 region of ~2.3 Mb in physical length (Ye et al. 2010).

The BC1F3 was similar to the BC1F2 population for the range of variation in plant height (Figure 2). Two plant height QTL, qPH1 and qPH7, were detected in the BC1F3 population (Figure 3C). The locus qPH7 was located on chromosome 7 and accounted for less (R2 = 18%) phenotypic variance than qPH1 (R2 = 27%). The qPH1 allele from EM93-1 and the qPH7 allele from SS18-2 reduced plant height. The distribution of height-reducing alleles across the two parents explained the transgressive segregation observed in the BC1F1, BC1F2, and BC1F3 populations. The qSD7-2 seed dormancy QTL was detected in all three populations and colocated with qPH7 in the RM346 to RM234 interval of ~6.3 Mb detected in BC1F3 population (Gu et al. 2004, Ye et al. 2010).

The four NILs were used to compare genotypic differences in the transcription level of Amy1A. Three samples of 50 seeds from an RIL were incubated in 9-cm Petri dish, which was lined with two layers of filter paper and irrigated with one of the 0 (control), 0.1, 1.0, 10.0, and 100.0 μM GA3 solutions and incubated under the aforesaided conditions for 7 d. The sample size was about 40 seeds, and the germination experiment was replicated three times for 10 plants from each of the four NILs.

Two experiments were conducted to evaluate GA effects on seedling growth. In the initial experiment, 10 seedlings (2-d-old) from an NIL were grown in a 9-cm Petri dish, which was lined with two layers of filter paper and irrigated with one of the 0 (control), 0.1, 1.0, 10.0, and 100.0 μM GA3 solutions, in a growth chamber set for 30° and 16-hr light. Seedling length was measured after 7 d. In the second experiment, 18 seedlings at 5 d of age were planted in nutrient solutions (Yoshida et al. 1976) with different GA3 concentrations in the greenhouse. The length of the second foliage leaf sheath was measured every 3 d during the following 15-d period.

The expression of Amy1A in the aleuron layer was induced by GA from the embryo to synthesize α-AMYLASE1A required to hydrolyze starchy endosperm in imbibed seeds of rice (Kaneko et al. 2002). Quantitative real-time polymerase chain reaction (PCR) assays were used to compare genotypic differences in the transcription level of Amy1A. Three samples of 50 seeds from an RIL were incubated in the 0 (control) or 10 μM GA3 solution for 36 hr. Total RNA was extracted from the endosperm tissue cut from the imbibed seeds using TRI Reagent (Sigma-Aldrich). Total RNA (4 μg) was reverse-transcribed into cDNA using SuperScript III First-Strand Synthesis Kit (Invitrogen). PCR primers for Amy1A (F: TCCCTTTCCGTCCTCATCGTCC and R: CGCCTCGGCTGGCCTTAC) and the control gene Actin (F: AGGAATGGAAGCTGGTGAT and R: CAGGAGGACGCGATAACA) were designed based on the coding sequences Os02g52710.1 and Os03g50885.1, respectively, available in the Gramene database (release 35 at http://www.gramene.org/Oryza_sativa). Real-time reactions were performed using the SYBER Green PCR Master Mix (Applied Biosystems Co). Primer validation and differential expression analysis were conducted using the method described in Chao (2008).
explained 48 and 57% of the phenotypic variances, respectively, in the greenhouse (r = 0.69) and field (r = 0.75) experiments. Genetic analysis demonstrated that both qSD1-2/qPH1 and qSD7-2/qPH7 had significant effects on both seed dormancy and plant height in the isogenic background (Table 1).

Genetic component effects of the digenic system partitioned using Model 1 varied with the traits and environments (Table 1). For plant height, the phenotypic variation was attributed to additive (a1 and a2) and some dominance (d1 and d2) and epistatic (Iaa1a2 and Idd1d2) effects of qPH1 and qPH7 in the greenhouse, or to a1, a2, d1, and Iaa1a2 in the field environment; qPH1 had a greater main effect than qPH7. For seed dormancy, the phenotypic variation was attributed to additive effects (a1 and a2) of qSD1-2 and qSD7-2 in the greenhouse, or to a1, a2, and their epistasis (Iaa1a2) in the field environment; qSD7-2 had a greater additive effect than qSD1-2. Heritability was higher for plant height (0.96–0.98) than for seed dormancy (~0.71) across the two environments (Table 1).

The four NILs showed three phenotypes for plant height (Figure 5A): dwarf (1’1’7’’7’’), semidwarf (1’1’7’’7’’ and 1’1’7’’7’’), and tall (1’1’7’’7’’). The two semidwarf genotypes were also similar in the greenhouse or to 1’1’1’1’7’’7’’1’ for the dwarf, semidwarf, and tall genotypes to reach 50% germination, respectively, under the controlled conditions (Figure 5B). Therefore, the four digenic homozygotes displayed only three types of trait (plant height/seed dormancy) combinations: dwarf/strong dormancy, semidwarf/moderate dormancy, and tall/weak dormancy.

A partial high-resolution map for the qSD1-2/qPH1 region located sd1 and OsVp1 on different marker intervals (Figure 6A). Three recombinants, which were heterozygous for sd1 (Rec.1-1), both sd1 and OsVp1 (Rec.1-2), and OsVp1 (Rec.1-3), respectively, were selected for progeny testing. Marker-trait correlations were significant only in the Rec.1-1 and -2 progeny lines, with r = 0.53–0.58 for germination and 0.92–0.98 for plant height. The absence of marker-trait association in the progeny line of Rec.1-3 suggests that OsVp1 has no effect on germination in this background. The similar strength of correlation in the other two progeny lines suggests that qSD1-2/qPH1 locates on the sd1-containing interval of <400 Kb between RM11988 and RM11987. Genetic analysis of the progeny data using Model 2 confirmed that the narrowed qSD1-2/qPH1 cluster had an additive effect on germination and both additive and dominance effects on plant height, with the effect-increasing allele from SS18-2 (Supporting Information, Table S1).

Marker genotyping with sd1(k) demonstrated that the two parental lines differentiated at the site of the 383-bp functional deletion, with EM93-1 and SS18-2 carrying the mutant and wild-type alleles, respectively (Figure 6B). Both Rec.1-1 and -2 were heterozygous for sd1(k) and their progeny lines segregated for plant height (61-101 cm), whereas Rec.1-3 was an SS18-2-like homozygote at sd1(k) and its progeny line was true-breeding for the tall phenotype (100 cm) (Table S1). The consistence between marker genotypes and segregation patterns suggest that sd1 is the underlying gene of qPH1.

A partial high-resolution map for the qSD7-2/qPH7 region was developed with 10 polymorphic markers, including two flanking Sdr4 (Figure 7). Three recombinants (Rec.7-1 to -3) were selected for progeny testing. Marker-trait correlations were not significant in the line from Rec.7-1, which was heterozygous for an Sdr4-containing segment, suggesting that there is no functional differentiation at Sdr4 between the two parental lines. The correlations were significant in the two lines from Rec.7-2 and -3, which were heterozygous for an overlapping segment between RM21773 and RM5495, with r = 0.61–0.67 for germination and 0.87 for plant height, indicating that qSD7-2/qPH7 locates.
on the RM21773 to RM5945 interval of <700 Kb. Genetic analysis of the progeny data using Model 2 confirmed that the narrowed qSD7-2/qPH7 consisted of only additive effects, with the SS18-2-derived allele reducing germination and plant height (Table S1).

### Genotypic differences in GA response

The GA treatments generally promoted seedling elongation, germination, and the expression of Amy1A in the four NILs, but the promoting effects varied with the genotypes. For example, GA application completely recovered inhibiting effects on seedling height/leaf sheath length and germination for the qSD1-2/qPH1 (1+1+7+7+ = 1+1+7+7+), but not for the qSD7-2/qPH7 (1+1+7−7− < 1+1+7−7+) single mutant (Figure 8A–C). These data indicate that the natural variants of qSD1-2/qPH1 and qSD7-2/qPH7 belong to GA-sensitive and -insensitive mutants, respectively, and that qSD1-2/qPH1 is likely involved in GA biosynthesis. The effect-reducing allele of qSD7-2/qPH7 blocked the GA responses of the qSD1-2/qPH1 wild type (i.e., 1+1+7−7− < 1+1+7−7+), including Amy1A’s transcription (Figure 8D), suggesting that qSD7-2/qPH7 may work downstream of qSD1-2/qPH1 to regulate plant height and seed dormancy (refer to Figure 9).

It was noticed that the genotypic differences of qSD1-2/qPH1 in GA sensitivity varied between seedling height and seed dormancy. qPH1’s mutant effect on seedling elongation was compensated by ~1 μM GA (1−1−7+7+ = 1+1+7−7+ and 1−1−7−7− = 1+1+7−7−), whereas qSD1-2’s mutant effect on germination was offset by ~10 μM GA only when qSD7-2 fixed for the effect-promoting allele (1−1−7+7+ = 1+1+7−7+; Figure 8A–C). The difference in GA sensitivity indicates that cell elongation in the vegetative tissue is more sensitive to GA than germination, which is determined by the velocity of dormancy release during seed imbibition. In addition, the application of GA at 10 μM or higher concentration could not compensate for the inhibiting effect of the 1−1−7−7− genotype on germination (1−1−7−7− < 1+1+7−7− < 1+1+7−7− = 1+1+7−7+ in Figure 8C), suggesting that the QTL underlying genes may be also involved in the regulation of dormancy development.

### Table 1 Summary of genetic component effects of qSD1-2/qPH1 and qSD7-2/qPH7 on plant height and germination in greenhouse and field experiments

| Parameter | Effectb | Prob. | R² (%)c | Effect | Prob. | R² (%) |
|-----------|---------|-------|---------|--------|-------|--------|
| **Greenhouse experiment** | | | | | | |
| μ (intercept) | 74.1 | <0.0001 | N/A | 0.76 | <0.0001 | N/A |
| a1 | 16.4 | <0.0001 | 82.2 | 0.14 | <0.0001 | 30.2 |
| d1 | 3.2 | <0.0001 | 1.3 | Not significant | | |
| a2 | −5.7 | <0.0001 | 11.6 | −0.16 | <0.0001 | 40.8 |
| d2 | 1.3 | 0.0045 | 0.2 | Not significant | | |
| la1a2 | −1.9 | <0.0001 | 0.8 | Not significant | | |
| ld1a2 | −1.6 | 0.003 | 0.2 | Not significant | | |
| Total (model R²) | | | 96.2 | | | 71.0 |
| **Field experiment** | | | | | | |
| μ | 75.9 | <0.0001 | N/A | 0.91 | <0.0001 | N/A |
| a1 | 13.1 | <0.0001 | 81.4 | 0.13 | <0.0001 | 31.1 |
| d1 | 1.9 | <0.0001 | 0.4 | Not significant | | |
| a2 | −5.8 | <0.0001 | 14.4 | −0.15 | <0.0001 | 34.6 |
| ld1a2 | −2.3 | <0.0001 | 1.9 | −0.07 | <0.0001 | 5.7 |
| Total (model R²) | | | 98.0 | | | 71.3 |

a Additive (a), dominance (d), and epistatic (l) effects were estimated based on Model 1, with the subscript 1 or 2 representing the qSD1-2/qPH1 or qSD7-2/qPH7 cluster.

b A positive (negative) value indicates that the parental line SS18-2 (EM93-1) contributed an effect-increasing allele to the cluster. The effect on germination is arcsine-transformed.

c R², proportion of the variance explained by the component or model. N/A, not applicable.
Plant height (et al. Nagano et al. 2002) and the dwarf phenotype, which is caused by an accumulation of the effect-reducing alleles in natural variants. For example, the elongation. The observed association appeared to help scatter the QTL at which the alleles reducing germination also reduced stem height. The association is underlain by two clusters of markers on the partial map of chromosome 7 were aligned against the Nipponbare genome sequence (IRGSP 2005) and used to select recombinants (Rec. 7-1 to -3) to dissect the initial introgression segment from SS18-2 (black bar in the top panel) in the EM93-1 background (empty bars). Marker targeting the 383-bp functional deletion within the sd1 semidwarf gene (Monna et al. 2002); N, the number of plants in a recombinant-derived progeny line; \( r_g \) and \( r_h \), marker-trait correlation coefficients for germination \( (r_g) \) or plant height \( (r_h) \), with a significant value \( (** for P < 0.0001 and ns for P = 0.05 or greater) indicating that the SS18-2-derived allele at locus or loci on the marked heterozygous region increased germination or plant height. Vertical lines delimit qSD1-2/qPH1, based on the progeny testing. (B) Gel image showing the polymorphism at sd1(k) among the parental and recombinant genotypes.

**DISCUSSION**

**Consequences of the association between seed dormancy and plant height**

This research established the association between seed dormancy and plant height in rice. The association is underlain by two clusters of QTL at which the alleles reducing germination also reduced stem elongation. The observed association appeared to help scatter the effect-reducing alleles in natural variants. For example, the sd1 height-reducing allele was reported in two wild rice \( (O. rufigriza) \) accessions (Nagano et al. 2005), and the qSD7-2/qPH7 mutant allele is present in the weedy rice accession SS18-2. This scattering distribution was likely because a dwarf phenotype, which is caused by an accumulation of the height-reducing alleles from different loci, has no selective advantage in natural populations. Of the 10 validated seed dormancy QTL differentiated between EM93-1 and SS18-2 (Ye et al. 2010), two (or 20%) were colocalized with the QTL for plant height. This finding suggests that there is a selection for/against seed dormancy would have about 20% chance to retain/eliminate a height-reducing allele. Therefore, the seed dormancy-plant height association represents a balancing mechanism that helps maintain the genetic diversity for these two adaptive traits in natural populations.

One more beneficial effect of the “Green Revolution” gene sd1 that has not been described would be the association with an increase in seed dormancy, due to a tight linkage or pleiotropy. The increased effect lengthened the after-ripening time by ~5 d to reach 50% germination (Figure 5). This effect must have contributed to the resistance of semidwarf cultivars to preharvest sprouting in rice production in the past decades. The qSD7-2/qPH7 allele isolated from SS18-2 is similar to sd1 and could be used as an alternative gene to breed semidwarf varieties with an enhanced resistance to preharvest sprouting.

The reduction in plant height for major crops after the “Green Revolution” imposed a selection force on accompanying weeds. Morphological mimicry is an evolutionary mechanism regulating weed adaptation to agro-ecosystems to avoid eradication by agricultural practices (Barrett 1983; Smith 1988). This hybridization experiment identified genetic bases underlying the weed mimicry for semidwarf plant height. A semidwarf genotype could be defined as nonallelic combinations of at least two loci, with one fixed for an effect-increasing and the other fixed for an effect-reducing allele (Figure 5A). The genetic diversity for the associated traits, or only for plant height,
maintained in wild and weed populations could contribute to the morphological mimicry. The semidwarf weed line SS18-2 is such an example, as the qPH1 allele from the parent offsets the effect of the qPH7 wild-type allele on plant height in the weed genotype. For conspecific weeds, the introgression of a semidwarf gene from accompanying cultivars by outcrossing, or "gene flow," could also contribute to the mimicry. The development of two morphologically identical semidwarf NILs (Figure 5) would be a simulation of the introgression. Recent research detected an sd1-like haplotype in three of 58 weedy rice accessions, which was presumably derived from the gene flow from the local semidwarf cultivars (Reagon et al. 2011).

**Shared mechanisms regulating the associated traits**

This research isolated GA-sensitive (qSD1-2/qPH1) and GA-insensitive (qSD7-2/qPH7) natural mutants and provided a set of isogenic materials to test a model for shared mechanisms regulating both seed dormancy and plant height (Figure 9). In addition to seedling and plant height, qSD1-2/qPH1 was also associated with the duration of seed dormancy, the functional 383-bp deletion mutation in OsGA20ox2, the change in expression of the GA-inducible gene Amy1A, and GA sensitivity for germination (Figure 8). This series of associations strongly suggest that OsGA20ox2 is a qSD1-2/qPH1-underlying gene, which controls plant height and likely also affects seed dormancy and germination by regulating GA biosynthesis in developing and/or imbibed seeds. The GA level was reduced in stems of the OsGA20ox2 mutants, which accounted for the semidwarf phenotype (Ashikari et al. 2002; Kaneko et al. 2003). However, differences between the OsGA20ox2 wild-type and mutant genotypes for the GA level in developing and imbibed seeds and for the degree of seed dormancy were not determined, which could have contributed to the uncertainty about the role of GA in regulating germination in rice (Steber 2007). The plant height QTL ph1 was mapped onto the qSD1-2/qPH1 region of <400 kb and proposed to be the locus Os01g65900 encoding a predicted chitin-inducible GA-responsive protein (Kovi et al. 2011). Research is being conducted to characterize the regulatory role of OsGA20ox2 in dormancy development and release and to determine if Os01g65900 also contributes an effect to qPH1, or qSD1-2, or both.

The genetic analysis based of the double mutants provided evidence that qSD7-2/qPH7 works downstream of qSD1-2/qPH1 or the GA signaling pathway (Figure 9). In the narrowed qSD7-2/qPH7 region of <700 kb, there is no gene or predicted paralog with a known function for GA biosynthesis or signaling. It is possible that qSD7-2/qPH7 is a new gene controlling the associated traits through GA signaling. Research is also being conducted to clone and characterize qSD7-2/qPH7.

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