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Identification of QTLs for Seed Germination Capability after Various Storage Periods Using Two RIL Populations in Rice

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Seed germination capability of rice is one of the important traits in the production and storage of seeds. Quantitative trait loci (QTL) associated with seed germination capability in various storage periods was identified using two sets of recombinant inbred lines (RILs) which derived from crosses between Milyang 23 and Tong 88-7 (MT-RILs) and between Dasanbyeo and TR22183 (DT-RILs). A total of five and three main additive effects (QTLs) associated with seed germination capability were identified in MT-RILs and DT-RILs, respectively. Among them, six QTLs were identified repeatedly in various seed storage periods designated as *qMT-SGC5.1*, *qMT-SGC7.2*, and *qMT-SGC9.1* on chromosomes 5, 7, and 9 in MT-RILs, and *qDT-SGC2.1*, *qDT-SGC3.1*, and *qDT-SGC9.1* on chromosomes 2, 3, and 9 in DT-RILs, respectively. The QTL on chromosome 9 was identified in both RIL populations under all three storage periods, explaining up to 40% of the phenotypic variation. Eight and eighteen pairs additive × additive epistatic effect (epistatic QTL) were identified in MT-RILs and DT-RILs, respectively. In addition, several near isogenic lines (NILs) were developed to confirm six repeatable QTL effects using controlled deterioration test (CDT). The identified QTLs will be further studied to elucidate the mechanisms controlling seed germination capability, which have important implications for long-term seed storage.

INTRODUCTION

Seed germination capability during storage can be defined as the maximum time period that pure seeds retain germination viability when stored under ideal ambient conditions. It varies among the species due to the occurrence of natural variability and is usually regarded to be related with seed longevity or seed storability traits (Bentsink et al., 2000; Ellis and Roberts, 1981; Singh and Ram, 1986). Seed germination capability is an important index for determining the regeneration cycle of germplasm conserved in genebanks (Miura et al., 2002). Without proper storage facilities, rapid seed deterioration in humid tropical climates can be a serious problem for rice production (Siddique et al., 1988).

Although storage conditions are important for seed germination capability, genetic factors also largely affect seed germination capability (Bewley and Black, 1994; Clerkx et al., 2004; Miura et al., 2002). To date, some quantitative genetic studies have been conducted to detect QTLs for analysis of seed germination capability (Miura et al., 2002; Sasaki et al., 2005; Xue et al., 2008; Zeng et al., 2006). Among these QTL analyses, the QTL on chromosome 9 was consistently identified in three independent studies (Miura et al., 2002; Sasaki et al., 2005; Xue et al., 2008), and subsequently, the effect of this QTL was confirmed with chromosome segment substitution lines (CSSLs) by Shigemune et al. (2008).

The genetics and molecular basis of seed germination capability have not yet been clearly elucidated, mainly due to the difficulty in accurate phenotyping. Since it takes a long period of time for seeds to age naturally, an alternative method for evaluating seed germination capability was suggested based on the fact that moisture content and storage temperatures are the most important factors affecting the germination capability of stored seeds (Ellis et al., 1982; Roberts, 1972). The combination of temperature, humidity, time, and controlled deterioration test (CDT) are considered a reliable method to measure seed germination capability and seed longevity (Padma and Reddy, 2000; Powell and Matthews, 1984; Xue et al., 2008; Zeng et al., 2002; 2006). This treatment is presumed to mimic natural aging (Delouche and Baskin, 1973), while allowing considerably accelerates the seed deteriorations, which is convenient for quickly assessing the seed germination capability. Therefore, seed companies largely rely on this treatment as a prognosis for seed germination capability in stored seeds (Rajjou and Debeaujon, 2008). The procedure in rice is simple: seeds are placed in a chamber controlled at a temperature of 40°C and 95% relative humidity (RH%) for 10-14 days, and then germina-

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tion rate is scored.

In the present study, we conducted QTL analysis for seed germination capability in various seed storage periods under natural conditions using two RIL populations, both derived from the combination of *indica* and *japonica* varieties (Milyang 23 × Tong 88-7 and Dasanbyeo × TR22183). We also attempted to confirm the effects of the QTLs using two sets of NILs that separately developed from each RIL population through backcrossing and marker-assisted selection (MAS) using a CDT.

MATERIALS AND METHODS

Plant materials

Two sets of RILs were used. One set of RILs (F_6, MT-RILs) consisted of 166 lines derived from the cross between Milyang 23 and Tong 88-7 by single seed descent (SSD) (Jiang et al., 2010). The other set of RILs (F_8, DT-RILs) consisted of 166 lines derived from the cross between Dasanbyeo and TR22183 by SSD (Cho et al., 2007). Milyang 23 and Dasanbyeo are Korean *tongil*-type rice varieties derived from an *indica* × *japonica* cross and similar to *indica* in its genetic make-up (Chung and Heu, 1991; Kown et al., 2001), whereas Tong 88-7 and TR22183 are temperate *japonica* varieties, both originating from northeast China.

To confirm QTL effects of seed germination capability, three RILs harboring one or more target QTL regions were separately selected in each RIL population for developing NILs. These RILs were backcrossed with their *tongil*-type parents (Milyang 23 or Dasanbyeo) twice to produce two sets of BC_2F_1 populations. A total of 123 markers for MT-NILs (developed from MT-RILs) and 132 markers for DT-NILs (developed from DT-RILs) were used to background and foreground selection. After a marker selection, several BC_2F_1 plants which contained *japonica* segments harboring target QTLs (alleles from Tong 88-7 or TR22183), and a small portion of other segments originating from *japonica* varieties, were selected and backcrossed again to produce BC_3F_1 populations. In this generation, 24 markers for MT-NILs and 25 markers for DT-NILs were used in selecting NILs. Finally, 10-18 BC_3F_1 plants of each introgression type were selected and the seed germination capabilities were evaluated using the BC_3F_2 seeds with three replications per plant.

Seed production and field experiments

Two sets of RILs were seeded on plastic-tunnel seed beds, and the seedlings were transplanted at a planting density of 30 × 15 cm in the experiment field of Seoul National University, Suwon, Korea (127°36′E, 37° 51′N). Fertilizer was applied at the rate of 110-80-80 kg N-P-K ha^{-1}. Field management and chemical input for disease and pest control followed the conventional methods of the experimental farm. The seeds were harvested 45 days after the heading date, from the beginning of September to the beginning of October, and they were dried in a well-ventilated glass house to a constant weight. Well-dried seeds were put in envelopes and then stored under natural room conditions at temperatures from > 10°C in January to < 35°C in August and relative humidity (RH%) from 50% in February to 85% in July (monthly average data from year 2004 to 2006).

Evaluation of seed germination capability

Bulked seeds from five plants of each line were harvested in 2003, 2004, and 2005 and stored for 3, 2, and 1 years, respectively. The seeds harvested in 2006 served as a control. Seed germination capability was evaluated in March 2007. Seed germination tests for each storage period were replicated three times with 100 seeds per replication. Seeds were placed on a whatman filter paper moistened with distilled water in a 6-cm Petri-dish and incubated at 30°C and 100% relative humidity in the dark for 7 days. Germination was evaluated visually by protrusion of the radicle from the hull by 2 mm. Seed germination capability (%) was generated by SGR/NSGR × 100, where the SGR was the germination rate of stored seeds, and the NSGR was the germination rate of the non-stored seeds. The same formula applied to all storage periods. Seed germination capability was transformed by $\sin^{-1}(x)^{-0.5}$ for statistical analysis (Gu et al., 2004).

For evaluating seed germination capability on the developed NILs, the CDT was used. Seeds of NILs and their parents were harvested in autumn in 2008 and well-dried in a greenhouse. To break seed dormancy, seeds were kept in an oven at 30°C for 1 day, followed by 45°C for 3 days, then followed by 30°C for 1 day. For prompt aging, the seeds were put in a Constant Temperature and Humidity Chamber (Hanbaek Sci. Co., Ltd., Korea) at 40°C and > 90% RH for 2 weeks. After germination test, the seed germination capability was calculated by SGR/NSGR × 100, where the SGR was the seed germination rate for CDT, and the NSGR was the germination rate of the non-CDT seeds.

Data analysis

Following classical quantitative genetics theory (Falconer and Mackay, 1996), the phenotypic value of a RIL (y_{ijk}) is described by the genetic model:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$
(i = 1-66; j and k = 1, 2, 3),

Where y_{ijk} is the k^{th} observation for the j^{th} storage period of the i^{th} RIL; μ is the mean of germination capability over all lines and all storage periods, α_i and β_j are the main genotype, storage period effects, respectively; $(\alpha\beta)_{ij}$ is the genotype × storage period interaction, and ε_{ijk} is the error term, including random error and residual effect. ANOVA for each RIL population over storage periods was performed using the SAS PROC MIXED procedure (SAS Institute, 1999). Variance components for estimating broad sense heritability (h^2) were estimated by a model, wherein all factors were considered randomly using the restricted maximum likelihood (REML) option of the SAS PROC VARCOMP procedure (SAS Institute, 1999). The h^2 of seed germination capability over storage periods was estimated as:

$$h^2 = \delta_\alpha^2 / (\delta_\alpha^2 + \delta_{\alpha\beta}^2 / j + \delta_\varepsilon^2 / jk)$$

where δ_α^2, $\delta_{\alpha\beta}^2$, and δ_ε^2 are the genotype, genotype × storage period, and residual variance components, respectively; j is the storage period; and k is the replicates (Hill et al., 1998).

PCR amplification of markers and linkage map construction

The DNA of each RIL was extracted from leaves collected at the maximum tillering stage, according to the method of Jiang et al. (2008). The new genetic map in MT-RILs was constructed. A total of 363 SSR and 525 STS markers were tested for polymorphisms, which found 196 of SSR (54.0%) and 321 of STS (61.1%) polymorphic markers between Milyang 23 and Tong 88-7. An integrated genetic linkage map consisting of 119 SSR and 97 STS markers was constructed, which covered a total length of 1,666.2 cM, with an average distance of 7.64 cM between adjacent markers. Molecular markers for the DT-RILs were essentially the same as those described earlier (Cho et al.,

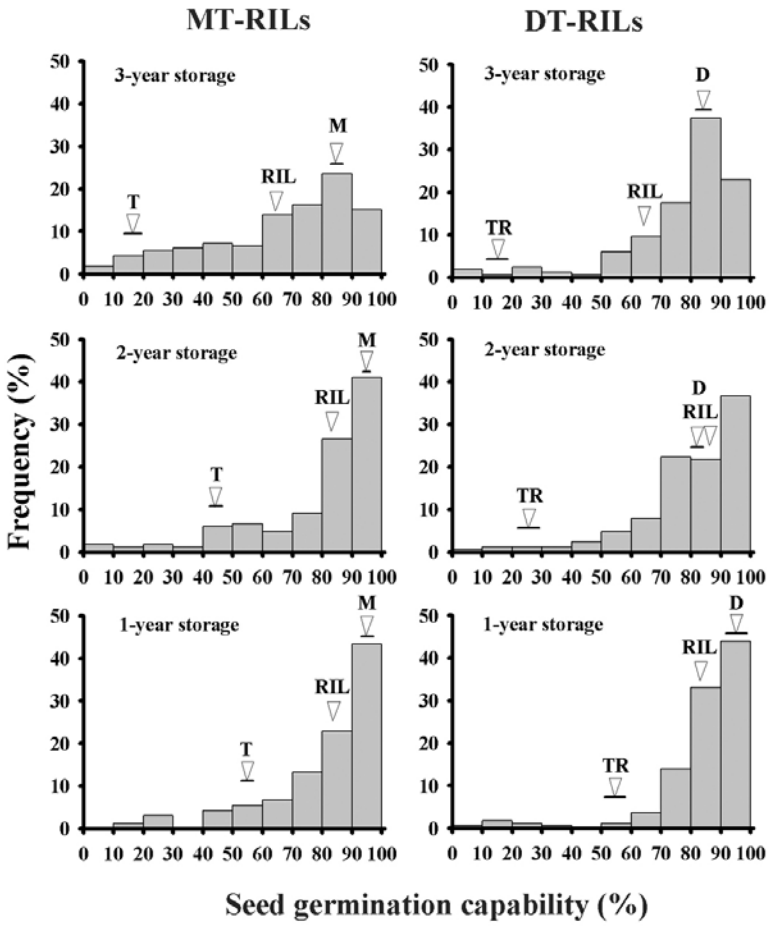

Fig. 1. Frequency distributions of seed germination capability in the two RIL populations. Arrowheads showed mean values for parents and RILs. M, T, D, and TR on arrowheads indicated Milyang 23, Tong 88-7, Dasanbyeo, and TR22183, respectively. Bars under arrowheads indicated standard deviation (10 replications) for seed germination capability in respective parents.

2007). This map contained 216 markers consisting of 113 SSR and 103 STS markers. The total map length was 1409.0 cM, with average distance of 6.5 cM between adjacent markers. The molecular linkage map was constructed using "Mapmaker/EXP 3.0" software, and the Kosambi function was used for calculating map distances. A log of the odds (LOD) score of 3.0 was used as the threshold for declaring linkage (Kosambi, 1944; Lincoln et al., 1992).

QTL analysis

The chromosomal locations of main additive effects (QTLs) and additive × additive epistatic effects (epistatic QTLs) were performed separately in each storage period and RIL population by composite interval mapping using the mixed linear model approach and QTLMapper 1.0 (Wang et al., 1999). The value of likelihood ratio (LR) corresponding to $P \leq 0.005$ (equivalent to LOD = 2.79 for $df = 3$) was used in this QTL analysis. To determine the empirical significance threshold for declaring a QTL, 10,000 permutations were performed to calculate the thresholds of LOD for seed germination capability of each storage period at $P = 0.05$ using the software Qgene 3.06 for Macintosh (Kim et al., 2004; Nelson, 1997). The threshold of LOD score ($P = 0.05$) by permutation test was ranged from 2.96 to 3.02 in various storage periods of both populations. For epistatic QTLs, the LR value corresponding to $P \leq 0.001$ was used as the threshold for claiming the presence of putative epistatic QTLs. The description of epistatic QTLs was followed by Yang et al. (2010). The proportion of total phenotypic variance explained (PVE) was estimated by the sum of phenotypic effects (R^2) of each QTL or epistatic QTL for each storage period. Nomenclature for the QTL was modified from that described by McCouch et al. (1997).

RESULTS

Variation of seed germination capability

The seed germination capability of the two *indica* varieties, Milyang 23 and Dasanbyeo, maintained high seed germination capability (above 80%) in all storage periods. However, the two *japonica* varieties, Tong 88-7 and TR22183, was reduced as the storage period increased, extremely reduced at the 3-year storage period (below 20%) (Fig. 1). The means of seed germination capability were evidently reduced in 3-year storage period than the other two storage periods in both RIL populations, indicating that germination capability was largely affected by storage period (Fig. 1). The h^2 computed across storage periods were 87.2% in the MT-RILs and 82.5% in the DT-RILs, showing high amounts of genetic effect in seed germination capability.

Main additive effect (QTL)

In the MT-RILs, a total of five QTLs for all seed storage periods were identified on chromosomes 1, 5, 7 (2 regions), and 9 (Table 1 and Fig. 2). Two QTLs, *qMT-SGC7.2* (S07080-RM234) and *qMT-SGC9.1* (S09040-S09045), were consistently identified in all seed storage periods. One QTL, *qMT-SGC5.1* (S05095-

Table 1. Additive main effect (QTL) characteristics for seed germination capability in two RIL populations

Storage period	QTLs	Chromosome	Flanking markers	LOD	A[a]	R^2 (%)	PVE (%)[b]
MT-RILs							
1-year	*qMT-SGC7.1*	7	RM214-S07055	4.80	6.4	11.6	45.7
	qMT-SGC7.2	7	S07080-RM234	8.21	7.9	17.5	
	qMT-SGC9.1	9	S09040-S09045	3.90	5.8	9.5	
2-year	*qMT-SGC5.1*	5	S05095-RM480	3.15	5.9	7.9	32.4
	qMT-SGC7.2	7	S07080-RM234	8.68	8.4	16.0	
	qMT-SGC9.1	9	S09040-S09045	3.17	5.9	8.5	
3-year	*qMT-SGC1.1*	1	S01027-RM1	6.19	-8.4	10.7	47.5
	qMT-SGC5.1	5	S05095-RM480	5.96	9.1	12.5	
	qMT-SGC7.2	7	S07080-RM234	6.76	9.1	12.5	
	qMT-SGC9.1	9	S09040-S09045	5.97	8.9	11.8	
DT-RILs							
1-year	*qDT-SGC3.1*	3	S03048-RM251	4.56	4.2	10.5	36.6
	qDT-SGC9.1	9	S09040-S09049	9.16	6.6	26.1	
2-year	*qDT-SGC2.1*	2	S02054-S02057	9.86	6.2	12.2	63.6
	qDT-SGC3.1	3	S03048-RM251	7.25	6.0	11.3	
	qDT-SGC9.1	9	S09040-S09049	22.32	11.3	40.1	
3-year	*qDT-SGC2.1*	2	S02054-S02057	7.50	6.0	12.4	48.4
	qDT-SGC3.1	3	S03048-RM251	5.79	6.7	15.6	
	qDT-SGC9.1	9	S09040-S09049	10.29	7.7	20.5	

[a]QTL effects of the maternal parents (Milyang 23 or Dasanbyeo); positive (+) means maternal genotype increasing seed germination capability and negative (-) means maternal genotype decreasing seed germination capability.
[b]Total percentage of phenotypic variance explained (PVE) by all QTLs for each storage period

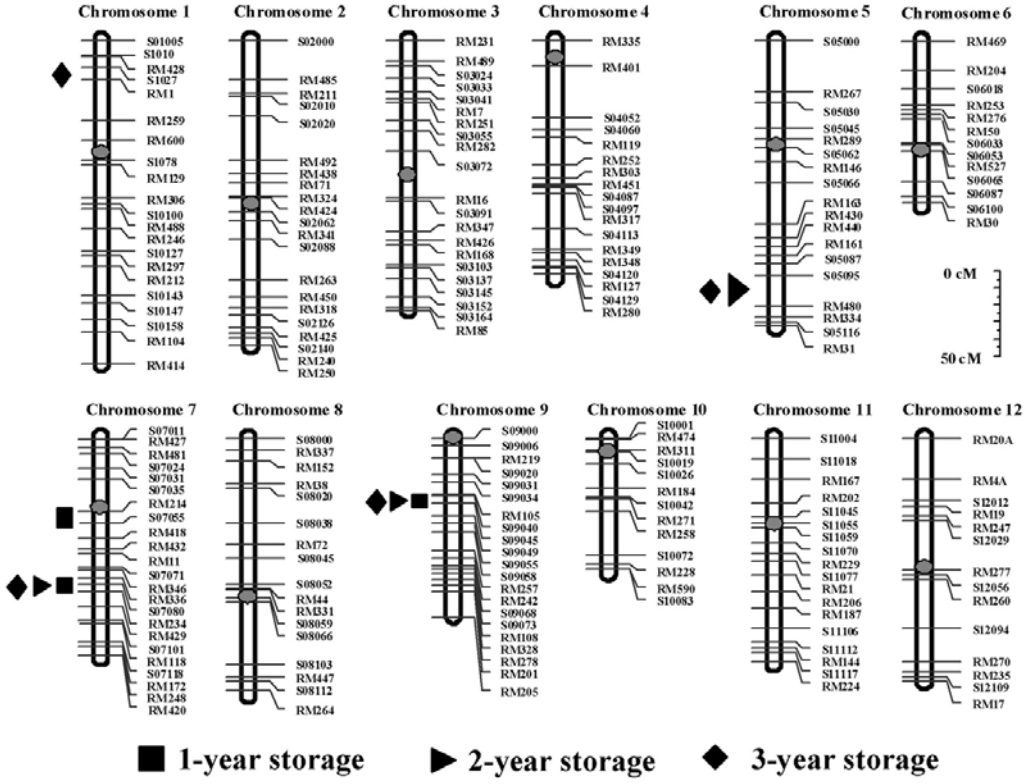

Fig. 2. Milyang 23/Tong 88-7 linkage map showing the genetic location of QTLs for seed germination capability in various storage periods

RM480), was identified in 2-year and 3-year seed storage periods, whereas both *qMT-SGC1.1* and *qMT-SGC7.1* were identified only in one seed storage period (3-year and 1-year storage period, respectively). The phenotypic variance explained by individual QTL ranged from 8.5% to 17.5%. Cumulative effects of these QTLs in each storage period ranged from 32.4% (2-year storage) to 47.5% (3-year storage) of the PVE. Milyang 23 alleles at four loci (*qMT-SGC5.1*, *qMT-SGC7.1*, *qMT-SGC7.2*,

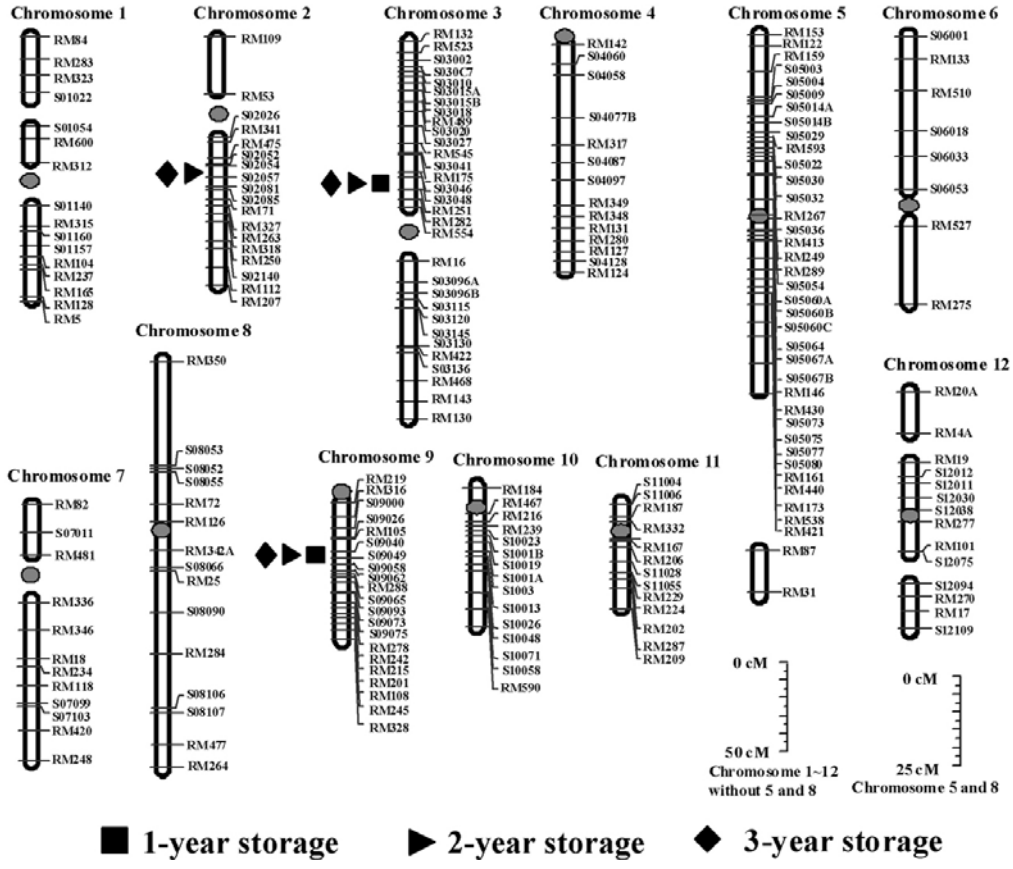

Fig. 3. Dasanbyeo/TR22183 linkage map showing the genetic location of QTLs for seed germination capability in various storage periods

and *qMT-SGC9.1*) seemed to increase seed germination capability, but the allele at *qMT-SGC1.1* had the opposite effect.

In the DT-RILs, a total of three QTLs, *qDT-SGC2.1*, *qDT-SGC3.1* and *qDT-SGC9.1*, were identified on chromosomes 2, 3, and 9, respectively (Table 1 and Fig. 3). The two QTLs, *qDT-SGC3.1* and *qDT-SGC9.1*, were consistently identified in all seed storage periods, and *qDT-SGC2.1* was identified in 2-year and 3-year seed storage periods. The phenotypic variance explained by individual QTL ranged from 10.5% to 40.1%. Collectively, the PVE by these QTLs ranged from 32.4% (2-year storage) to 47.5% (3-year storage). The QTL, *qDT-SGC9.1,* had the largest phenotypic variance in all seed storage periods, accounting for 26.1% (1-year storage), 40.1% (2-year storage), and 20.5% (3-year storage) of the phenotypic effect. The PVE in each storage period ranged from 36.6% (1-year storage) to 63.6% (2-year storage). Dasanbyeo alleles increased seed germination capability in all QTLs.

Additive × additive epistatic effect (epistatic QTL)

In MT-RILs, a total of eight epistatic QTL pairs were identified; three pairs in 1-year storage period, three pairs in 2-year storage period, and two pairs in 3-year storage period (Table 2). Among these loci, the phenotypic variance contributed by each epistatic QTL pair ranged from 3.5% to 9.7% and the PVE in the three storage periods ranged from 9.9% to 23.7%. These epistatic QTL pairs were partitioned into six interactions between two loci with only epistatic effects (NN) and two interactions between a QTL with additive effect and a locus without significant additive effect (AN). In DT-RILs, six epistatic QTL pairs were separately identified in the three storage periods (Table 2). The phenotypic variance explained by each epistatic QTL pair ranged from 1.4% to 7.5% and the PVE in the three storage periods ranged from 11.9% to 34.2%. These epistatic QTL pairs were partitioned into fourteen NN interactions, two AN interactions, and three interactions between two QTLs with additive effects (AA). In the AA types, the interactions between two QTLs, *qDT-SGC3.*1 and *qDT-SGC9.1,* were identified in two storage periods with an increased epistatic effect originated from the parental digenic combination, and an epistatic QTL pair between *qDT-SGC2.1* and *qDT-SGC9.1* was identified in 2-year storage period with an increased epistatic effect arose from different parents.

QTL validation for seed germination capability in NILs

Six NILs developed from the MT-RILs were evaluated for seed germination capability by CDT (Fig. 4). The MT_NIL1 and MT_NIL2 did not contain Tong 88-7 segments in QTL regions on chromosome 5, 7, and 9. As expected, the averages of seed germination capability in the MT_NIL1 and MT_NIL2 were 81.2% and 79.9%, respectively, which was similar to that of Milyang 23 (81.3%). The MT_NIL3 and MT_NIL4 contained Tong 88-7 allele at the *qMT-SGC7.2* and *qMT-SGC5.1* on chromosome 7 and 5, respectively. In the MT_NIL3 and MT_NIL4, seed germination capability was significantly reduced comparing to that of Milyang 23, suggesting that the severe reduction of germination capability in these NILs was caused by the effect of the Tong 88-7 alleles at *qMT-SGC7.2* and *qMT-SGC5.1.*The MT_NIL5 and MT_NIL6 contained Tong 88-7 allele at *qMT-SGC9.1* on chromosome 9, and their seed germination capability was reduced the most severely among six MT-

Table 2. Additive × additive epistatic interactions (epistatic QTLs) for seed germination capability identified in two RIL populations

Storage period	Chr/In[a]	Flanking markers	Chr/In	Flanking markers	LOD	Interaction[b]	AA[c]	R^2 (aa) (%)	PVE[d]
MT-RILs									
1-year	4/1	RM335-S04052	4/5	RM252-RM303	3.36	NN	-4.9	6.2	23.7
	4/9	S04097-RM317	6/6	RM50-S06033	3.79	NN	5.2	6.9	
	9/8	S09040-S09045	7/18	RM118-S07101	7.12	AN	-6.5	10.6	
2-year	1/8	S01078-RM129	2/1	S02000-RM485	4.27	NN	7.3	9.7	21.5
	4/4	RM119-RM252	8/6	S08038-RM72	3.29	NN	-6.0	6.7	
	7/15	S07080-RM234	9/5	S09031-S09034	9.74	AN	-5.3	5.2	
3-year	2/15	RM450-RM318	7/19	RM118-S07118	3.46	NN	-5.7	4.5	9.9
	5/10	RM430-RM440	8/10	RM44-RM331	2.97	NN	6.3	5.5	
DT-RILs									
1-year	2/13	RM263-RM318	3/1	RM132-RM523	6.77	NN	4.7	6.9	34.2
	3/16	S03048-RM251	9/6	S09040-S09049	5.78	AA	4.5	6.4	
	6/3	RM510-S06018	9/1	RM219-RM316	5.33	NN	-4.1	5.4	
	8/13	S08107-RM477	9/13	S09073-S09075	5.12	NN	4.0	5.0	
	8/14	RM477-RM264	12/12	RM270-RM17	5.66	NN	-4.0	5.0	
	10/1	RM184-RM467	10/6	S1001B-S10019	3.67	NN	-4.2	5.5	
2-year	2/6	S02054-S02057	9/6	S09040-S09049	4.41	AA	-4.3	2.1	11.9
	3/16	S03048-RM251	7/9	S07099-S07103	10.50	AN	-4.3	2.1	
	3/25	S03145-S03130	5/5	S05004-S05009	3.70	NN	-3.2	1.2	
	4/2	S04060-S04058	5/16	RM413-RM249	3.85	NN	3.8	1.7	
	5/8	S05014B-S05029	9/17	RM215-RM201	2.89	NN	4.0	1.8	
	7/7	RM234-RM118	8/9	RM25-S08090	4.68	NN	-5.2	3.1	
3-year	1/5	S01054-RM600	5/37	RM87-RM31	3.20	NN	4.5	4.1	32.8
	2/4	RM341-RM475	5/34	RM173-RM538	5.47	NN	-5.9	7.2	
	3/1	RM132-RM523	3/6	S03015A-S03015B	3.72	NN	4.3	3.8	
	3/16	S03048-RM251	9/6	S09040-S09049	12.70	AA	5.5	6.3	
	4/2	S04060-S04058	4/12	RM127-S04128	4.17	NN	4.3	3.8	
	4/7	S04097-RM349	5/17	RM249-RM289	5.01	NN	-6.0	7.5	

[a]Chr/In and Chr/In represent the chromosome number interval of the tested points in the analysis.
[b]Types of epistatic interactions. *AA* interactions between two QTL with additive effects, *NA* (*AN*) interactions between a QTL with additive effects and a locus without significant additive effects, *NN* interaction between two loci with epistatic effects only
[c]AA means the epistatic effects. Positive (+) means that parental digenic genotypes increasing seed germination capability, and negative (-) means that recombinant alleles from two parents increasing seed germination capability.
[d]Total percentage of phenotypic variance explained (PVE) by all epistatic QTLs for each storage period

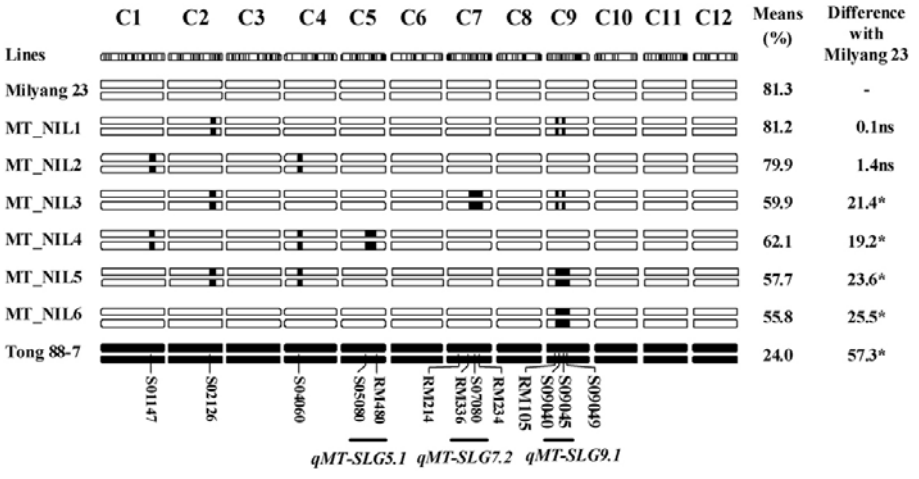

Fig. 4. Genomic composition of NILs derived from backcrossing Milyang 23 to MT-RILs. Black bar: Tong 88-7 homozygote segment; white bar: Milyang 23 homozygote segment. *Seed germination capability of NIL significantly different with Milyang 23 at $P < 0.0001$ based on the *t*-test. ns: not significant.

NILs.

The same strategy was applied to DT-NIL for confirmation of three QTLs identified from the DT-RIL population. Among four DT-NILs, the DT_NIL2, DT_NIL3, and DT_NIL4 contained the TR22183 allele of *qDT-SGC2.1*, *qDT-SGC3.1*, and *qDT-SGC9.1* on chromosome 2, 3, and 9, respectively (Fig. 5). Seed

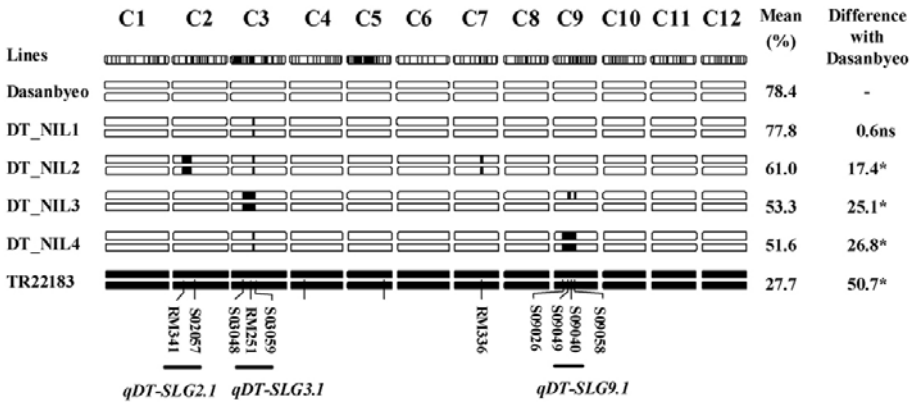

Fig. 5. Genomic composition of NILs derived from backcrossing Dasanbyeo to DT-RILs. Black bar: TR22183 homozygote segment; white bar: Dasanbyeo homozygote segment; gray bar: heterozygote segment; broken line: chromosome regions for the identified QTLs. *Seed germination capability of NIL significantly different with Dasanbyeo at $P < 0.0001$ based on the *t*-test. ns: not significant.

germination capability of the DT_NIL2, DT_NIL3, and DT_NIL4 was 61.0%, 53.3%, and 51.6%, respectively, which was significantly lower than that of recurrent parent Dasanbyeo.

These results collectively demonstrate that the QTLs identified in two sets of RILs reliably affected the seed germination capability of certain genotypes having different genetic backgrounds.

DISCUSSION

We were able to identify five and three QTLs associated with seed germination capability in MT-RILs and DT-RILs in all storage periods, respectively (Table 1, Figs. 2 and 3). Among the QTLs identified in both RIL populations, one pair of QTLs was located at the same chromosome region on chromosome 9: *qMT-SGC9.1* (S09040-S09045) for the MT-RILs and *qDT-SGC9.1* (S09040-S09049) for the DT-RILs. This chromosomal region originated from *indica* varieties, was also identified for seed longevity or seed storability in the previous studies (Miura et al., 2002; Sasaki et al., 2005; Xue et al., 2008), suggesting that this QTL might be widespread among *indica* rice varieties. In the MT-RILs, one QTL, *qMT-SGC7.2*, on chromosome 7 was detected in all storage periods and possibly corresponds with *RC7* detected by Sasaki et al. (2005). A novel QTL, *qMT-SGC5.1*, was repeatedly identified in 2-year and 3-year storage periods, implying that it was likely expressed in the severely deteriorated seeds caused by long-term seed storage. The *qMT-SGC7.1* (RM214-S07055) on chromosome 7 is an interesting QTL because it was only detected in the 1-year storage period. Since 1-year storage period may not be regarded as long-term storage, *qMT-SGC7.1* may represent QTL for seed dormancy rather than seed germination capability. It is difficult to clearly distinguish the phenotypes of seed dormancy from seed germination capability. Unexpectedly, the allele originating from Milyang 23 in the locus of *qMT-SGC1.1* decreased seed germination capability, whereas all other alleles from Milyang 23 in the other loci of the QTLs increased seed germination capability. It was revealed that seed germination capability exhibited different expression patterns between different rice varieties, which was consistent with the findings of previous studies (Kameswara and Jackson, 1996; 1997; Zeng et al., 2006). In DT-RILs, we identified two novel QTLs (*qDT-SGC2.1* and *qDT-SGC3.1*) for seed germination capability, which increased the seed germination capability in Dasanbyeo alleles.

Developing NILs is an important fundamental step for QTL cloning and molecular marker-assisted selection. When a QTL is 'Mendelized', the candidate gene can be identified by high-resolution linkage mapping (Yamamoto et al., 2009). In rice, among the identified several QTLs for seed germination capability on seven chromosomes, the QTL on chromosome 9 was identified consistently in various mapping populations (Miura et al., 2002; Sasaki et al., 2005; Shigemune et al., 2008; Xue et al., 2008). In the intermediate stage for cloning these QTLs, we developed NILs corresponding to QTLs, including *qMT-SGC5.1*, *qMT-SGC7.2*, *qMT-SGC9.1* from MT-RILs and *qDT-SGC2.1*, *qDT-SGC3.1*, *qDT-SGC9.1* from DT-RILs, by using molecular marker-assisted selection through foreground selection and background selection. After developing NILs using the CDT method, we confirmed that each genomic region on target QTLs controlled the seed germination capability (Figs. 4 and 5). This should allow the introgression of favorable alleles from high-germination capability varieties into high-yielding varieties, such as *japonica* hybrid rice, using marker-assisted backcrossing.

Since we are also interested in the improvement of seed germination capability under the proper storage condition compared to that under the natural condition, the phenotype performance of seed germination capability was calculated by dividing SGR with NSGR, instead of using primary seed germination rates even though this secondary data process may enlarge errors and the results may not be interpreted directly. In addition, we detected high correlations between seed germination capability calculated with SGR/NSGR and primary seed germination rates in each seed storage period of both RIL populations ($r > 0.993$ in MT-RILs and $r > 0.986$ in DT-RILs). Moreover, the high h^2 for seed germination capability in both RIL populations (86.9% in MT-RILs and 81.7% in DT-RILs) suggests that natural seed storage method can evaluate seed germination capability and reduce environmental errors. The *indica* alleles of six confirmed QTLs from the two RIL populations showed that *indica* alleles employed increasing seed germination capability. Although these QTLs are the most important determinants of the seed germination capability, epistatic interactions explained relatively high phenotypic variance in some storage periods, indicating that epistatic interactions were an important component on the genetic variance for seed germination capability. A series of NILs for a target trait provided the opportunity to analyze epistatic interactions among identified genes by combining individual NILs (Doi et al., 2008; Rahman et al., 2009; Yano et al., 2001). In addition, sometimes the effects of QTLs are also subjected to environmental changes, which can lead to dramatic differences in the phenotypic effects of the QTLs. The results demonstrate that for the marker-assisted breeding programs for improving seed germination capability, attention should be paid to the direct effects of the minor QTLs, epistatic QTLs in addition to the most important

process of pyramiding reliable QTLs (especially major QTLs).

The molecular identification of several reliable QTLs in the two sets of RILs, reported herein, may help to elucidate the poorly understood germination capability under different seed storage conditions. We expect that our results will provide the genetic information on map-based cloning and MAS that will be vital to the advancement of breeding strategies and breeding resources for increased seed germination capability.

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REFERENCES

Bentsink, L., Alonso-Blanco, C., Vreugdenhil, D., Tesnier, K., Groot, S.P.C., and Koornneef, M. (2000). Genetic analysis of seed-soluble oligosaccharides in relation to seed storability of Arabidopsis. Plant Physiol. *124*, 1595-1604.

Bewley, J.D., and Black, M. (1994). Seeds: physiology of development and germination. (New York, NY, USA: Plenum Press).

Cho, Y.I., Jiang, W., Chin, J.H., Piao, Z., Cho, Y.G., McCouch, S.R., and Koh, H.J., (2007). Identification of QTLs associated with physiological nitrogen use efficiency in rice. Mol. Cells *23*, 72-79.

Chung, G.S., and Heu, M.H. (1991). Improvement of *tongil*-type rice cultivars from *indica*/*japonica* hybridization in Korea. In Biotechnology in Agriculture and Forestry 14-Rice, Y.P.S. Bajaj, ed. (Springer, Berlin Heidelberg, New York), pp. 105-112.

Clerkx, E.J.M., Blankestijn-De Vries, H., Ruys, G.J., Groot, S.P.C., and Koornneef, M. (2004). Genetic differences in seed longevity of various Arabidopsis mutants. Physiol. Plant *121*, 448-461.

Doi, K., Yasui, H., and Yoshimura, A. (2008). Genetic variation in rice. Curr. Opin. Plant Biol. *11*, 144-148.

Delouche, J.C., and Baskin, C.C. (1973). Accelerated aging techniques for predicting the relative storability of seed lots. Seed Sci. Technol. *1*, 427-452.

Ellis, R.H., Roberts, and E.H. (1981). The quantification of aging and survival in orthodox seeds. Seed Sci. Technol. *9*, 373-409.

Ellis, R.H., Osei-Bonsu, K., and Roberts, E.H. (1982). The influence of genotype, temperature and moisture on seed longevity in chickpea, cowpea and soybean. Ann. Bot. *50*, 69-82.

Falconer, D.S., and Mackay, T.F.C. (1996). Introduction to quantitative genetics. (Harlow, Essex, UK: Longmans Green).

Gu, X.Y., Kianian, S.F., and Foley, M.E. (2004). Multiple loci and epistases control genetic variation for seed dormancy in weedy rice (*Oryza sativa*). Genetics *166*, 1503-1516.

Hill, J., Becker, H.C., and Tigerstedt, P.M.A. (1998). Quantitative and ecological aspects of plant breeding. (London, UK: Chapman & Hall).

Jiang, W., Chu, S.H., Piao, R.H., Chin, J.H., Jin, Y.M., Lee, J., Qiao, Y., Han, L., Piao, Z., and Koh, H.J. (2008). Fine mapping and candidate gene analysis of *hwh1* and *hwh2*, a set of complementary genes controlling hybrid breakdown in rice. Theor. Appl. Genet. *116*, 1117-1127.

Jiang, W., Lee, J., Chu, S.H., Ham, T.H., Woo, M.O., Cho, Y.I., Chin, J.H., Han, L.Z., Xuan, Y., Yuan, D., et al. (2010). Genotype × environment interactions for chilling tolerance of rice recombinant inbred lines under different low temperature environments. Field Crops Res. *117*, 226-236.

Kameswara, N., and Jackson, M.T. (1996). Seed longevity of environment and storage longevity of *japonica* rices (*Oryza sativa* L.). Seed Sci. Res. *6*, 17-21.

Kameswara, N., and Jackson, M.T. (1997). Variation in seed longevity of rice cultivars belonging to different isozyme groups. Gene Res. Crop Evol. *44*, 159-164.

Kim, K.M., Kown, Y.S., Lee, J.J., Eun, M.Y., and Shon, J.K. (2004). QTL mapping and molecular marker analysis for the resistance of rice to ozone. Mol. Cells *17*, 151-155.

Kosambi, D.D. (1944). The estimation of map distances from recombination values. Ann. Eugn. *12*, 172-175.

Kwon, Y.S., Kim, K.M., Eun, M.Y., and Sohn, J.K. (2001). Quantitative trait loci mapping associated with plant regeneration ability from seed derived calli in rice (*Oryza sativa* L.). Mol. Cells *11*, 64-67.

Lincoln, S., Daly, M., and Lander, E.S. (1992). Constructing genetic maps with MAPMAKER/EXP 3.0. Whitehead Institute Technical Report, 2nd eds. (Massachusetts, USA: Whitehead Institute, Cambridge).

McCouch, S.R., Cho, Y.G., Yano, M., Paul, E., and Kinoshita, T. (1997). Report on QTL nomenclature. Rice Genet. Newslett. *14*, 11-13.

Miura, K., Lin, S.Y., Yano, M., and Nagamine, T. (2002). Mapping quantitative trait loci controlling seed longevity in rice (*Oryza sativa* L.). Theor. Appl. Genet. *104*, 981-986.

Nelson, J.C. (1997). QGENE, software for marker-based genome analysis and breeding. Mol. Breed. *3*, 239-245.

Padma, V., and Reddy, B.M. (2000). Evaluation of rice genotypes for dormancy duration and seed storability under natural and accelerated ageing. Seed Res. *28*, 158-165.

Powell, A., and Matthews, S. (1984). Application of the controlled deterioration vigour test to detect seed lots of Brussels sprouts with low potential for storage under commercial conditions. Seed Sci. Technol. *12*, 649-657.

Rahman, M.L., Jiang, W., Chu, S.H., Qiao, Y., Ham, T.H., Woo, M.O., Lee, J.H., Khanam, M.S., Chin, J.H., Jeung, J.U., et al. (2009). High-resolution mapping of two rice brown planthopper resistance genes, *Bph20(t)* and *Bph21(t)*, originating from *Oryza minuta*. Theor. Appl. Genet. *119*, 1237-1246.

Rajjou, L., and Debeaujon, I. (2008). Seed longevity: survival and maintenance of high germination ability of dry seeds. C.R. Biol. *331*, 796-805.

Roberts, E.H. (1972). Loss of viability and crop yields. In Viability of Seeds, E.H. Roberts, ed. (London, UK: Chapman and Hall), pp. 307-359.

SAS Institute Inc. (1999). SAS/Stat User's Guide, Version 8.2. SAS institute, Inc., Cary, NC, USA.

Sasaki, K., Fukuta, Y., and Sato, T. (2005). Mapping of quantitative trait loci controlling seed longevity of rice (*Oryza sativa* L.) after various periods of seed storage. Plant Breed. *124*, 361-366.

Shigemune, A., Miura, K., Sasahara, H., Goto, A., and Yoshida, T. (2008). Role of maternal tissues in *qLG-9* control of seed longevity in rice (*Oryza sativa* L.). Breed. Sci. *58*, 1-5.

Siddique, S.B., Seshu, D.V., and Pardee, W.D. (1988). Rice cultivar variability in tolerance for accelerated aging of seed. IRRI Res. Pap. Ser. *131*, 2-7.

Singh, R.K., and Ram, H.H. (1986). Inheritance study of soybean seed storability using an accelerated aging test. Field Crops Res. *13*, 89-98.

Wang, D.L., Zhu, J., Li, Z.K., and Paterson, A.H. (1999). Mapping QTLs with epistatic effects and QTL × environment interactions by mixed linear model approaches. Theor. Appl. Genet. *99*, 1255-1264.

Xue, Y., Zhang, S.Q., Yao, Q.H., Peng, R.H., Xiong, A.S., Li, X., Zhu, W.M., Zhu, Y.Y., and Zha, D.S. (2008). Identification of quantitative trait loci for seed storability in rice. Euphytica *164*, 739-744.

Yamamoto, T., Yonemaru, J., and Yano, M. (2009). Towards the understanding of complex traits in rice: substantially of superficially? DNA Res. *16*, 141-154.

Yang, X., Guo, Y., Yan, J., Zhang, J., Song, T., Rocheford, T., and Li, J.S. (2010). Major and minor QTL and epistasis contribute to fatty acid composition and oil content in high-oil maize. Theor. Appl. Genet. *120*, 665-678.

Yano, M., Kojima, S., Takahashi, Y., Lin, H.X., and Sasaki, T. (2001). Genetic control of flowering time in rice, a short-day plant. Plant Physiol. *127*, 1425-1429.

Zeng, D.L., Qian, Q., Yasukumi, K., Teng, S., and Hirosi, F. (2002). Study on storability and morphological index in rice (*Oryza sativa* L.) under artificial aging. Acta Agron. Sin. *28*, 551-554.

Zeng, D.L., Guo, L.B., Xu, Y.B., Yasukumi, K., Zhu, L.H., and Qian, Q. (2006). QTL analysis of seed storability in rice. Plant Breed. *125*, 57-60.