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REVIEW

Genetical Studies on Germination of Seed and Seedling Establishment for Breeding of Improved Rice Varieties Suitable for Direct Seeding Culture

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Abstract

For the introduction of low temperature germinability (LTG), seed longevity and accelerated coleoptile growth derived from foreign varieties to Japanese elite varieties to breed rice varieties for direct seeding culture in Japan, we tried to establish backcross breeding to introduce these traits with marker-assisted selection (MAS). The objectives of this study were to identify quantitative trait loci (QTLs) for LTG and seed longevity using backcross inbred lines derived from a cross between a *japonica* variety Nipponbare and an *indica* variety Kasalath in order to facilitate MAS of these traits. Five putative QTLs controlling LTG were detected on chromosomes 2, 4 and 11. A putative QTL with a large seed longevity effect was detected on chromosome 9. We bred a near isogenic line for coleoptile growth by a backcross between Kitaibuki as the recurrent parent and Arroz da Terra as the donor parent to have more rapid coleoptile growth and higher seedling establishment rate than Kitaibuki. From the above experimental data, we conclude that backcross breeding with MAS is effective to introduce genes for adaptability to direct seeding from foreign varieties in Japanese rice breeding programs.

Discipline: Plant breeding
Additional Key words: *Oryza sativa* L., quantitative trait loci, marker-assisted selection

Introduction

For the establishment of direct seeding culture to reduce rice production costs in Japan, breeding rice varieties exhibiting a high seedling-stand is a high priority. We recognize 3 traits to be necessary for rice varieties with stable seedling establishment; LTG[18], seed longevity[20] and accelerated coleoptile growth[15]. The introduction of these traits from foreign varieties to Japanese elite varieties is necessary to breed varieties for direct seeding culture. However, undesirable traits in foreign varieties, such as, low yielding ability and bad grain appearance, make it difficult. To overcome this difficulty, we tried to establish backcross breeding to introduce these traits with MAS. The objectives of this study were to identify quantitative trait loci (QTLs) for LTG[11] and seed longevity[12] using backcross inbred lines

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(BILs) derived from a cross between *indica* and *japonica* varieties in order to facilitate MAS of these traits[11,12] and to confirm the efficiency of backcross breeding to introduce accelerated coleoptile growth into *japonica* elite varieties from foreign varieties[13].

Re-assessment of rice genetic resources for LTG after removing the effect of seed dormancy

The evaluation of LTG should be carried out taking into consideration the effects of primary and secondary dormancy[3,9,10]. Previously estimated LTG for 140 varieties[6–8,14,19] from high and low latitude regions were re-assessed after removing the effect of seed dormancy. The seeds were stored up to 12 months at 30ºC in a drying machine (Yamato DK63, Tokyo) to remove the effect of seed dormancy. Then they were subjected to a germination test at 15ºC for 14 days. LTG was estimated by Germination Rate Index (GRI)[1]. GRI for 100 seeds was calculated using the following formula:

$$GRI = G_1/T_1+G_2/T_2+ \cdots + G_{n-1} /T_{n-1}+ G_n /T_n$$

where G_1= number germinated at T_1, G_2= number germinated at T_2, G_n= number germinated at T_n minus number germinated at T_{n-1}, T_1= days to first count, T_2= days to second count, T_n= days to final count, T_{n-1}= days to the count immediately preceding the last count.

In the group of varieties previously estimated as high LTG, the frequency of varieties with high LTG was high. Otherwise, the group of varieties previously estimated as low LTG included high and low LTG varieties (Fig. 1). The varieties with high LTG were *indica* varieties originating from India, China and Korea. Their LTG levels were similar to Italica Livorno and Arroz da Terra utilized as parents for improvement of LTG in rice breeding programs and were higher than LTG of *japonica* varieties.

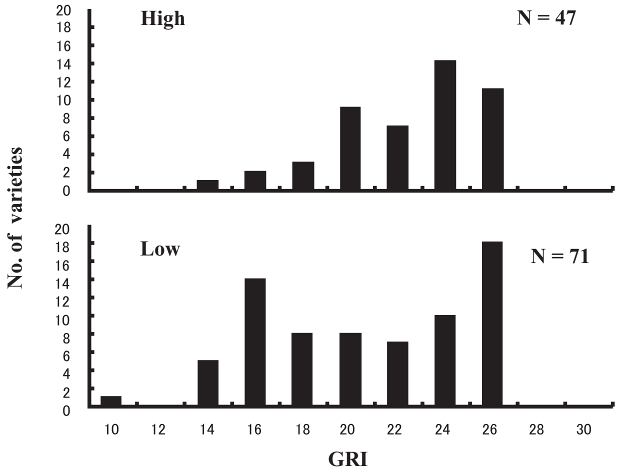

Fig. 1. Frequency distributions of GRI in high and low varietal groups with previously estimated LTG

GRI: Germination rate index during a germination test at 15ºC for 14 days using stored seeds.
N: Population size.

Mapping QTLs controlling LTG

Backcross breeding with MAS is an effective approach to introduce only a higher LTG and not undesirable traits from *indica* varieties into *japonica* elite varieties. QTLs controlling LTG were identified using 98 backcross inbred lines (BILs) derived from a cross between a *japonica* variety Nipponbare and an *indica* variety Kasalath with genotype data of 245 RFLP markers (http://rgp.dna.affrc.go.jp/publicdata/genotypedata BILs/genotypedata.html). Seeds of each BIL were stored at 30ºC in a drying machine for 7 months to exclude the effects of seed dormancy. Then germination was scored

Table 1. Putative QTLs for LTG in rice

QTL	NML[a)]	Chromosome	GLM/SAS Probability	MAPMAKER/QTL			DPE[d)]
				LOD	% Variation[b)]	AE[c)]	
qLTG-2	G1327	2	<0.0001	2.434	11.9	15.8	K
qLTG-4-1	C946	4	0.0039	2.794	14.9	10.7	K
qLTG-4-2	C513	4	0.0054	2.031	10.1	–12.8	N
qLTG-5	R830	5	0.0014	2.345	10.6	–11.6	N
qLTG-11	G1465	11	0.0009	2.672	11.8	14.6	K
				Total[e)]	40.7		

a): Nearest marker locus of putative QTLs.
b): Phenotypic variation explained by each QTL.
c): Additive effects (1/2 weight) of Kasalath allele based on arc sine transformation of germination percentage.
d): Direction of phenotypic effect. N and K indicate Nipponbare and Kasalath alleles increased the values, respectively.
e): Estimates obtained from a multiple QTL model.

at 15ºC for 4 days to represent LTG. Five putative QTLs, qLTG-2, qLTG-4-1, qLTG-4-2, qLTG-5 and qLTG-11, were detected on chromosomes 2, 4 (2 regions), 5 and 11, respectively (Table 1). In the case of qLTG-2, qLTG-4-1 and qLTG-11, Kasalath alleles increased LTG, while Nipponbare alleles increased it in the case of qLTG-4-2 and qLTG-5. Three putative QTLs, qLTG-2, qLTG-4-1 and qLTG-11 should be very useful for improving LTG in *japonica* rice varieties.

Screening of rice genetic resources for seed longevity

Rapid seed deterioration is a serious problem for direct seeding culture in rice[22]. Genetic resources were screened for seed longevity to improve this trait in rice breeding programs. A total of 162 varieties from all over the world were used. The seeds were stored for 12 months at 30ºC in a drying machine and then kept in air-tight containers over a saturated potassium chromate (K_2CrO_4) solution to maintain the attained seed moisture content at 15–16% equilibrium[3] for 2 months. Subsequently, seeds were germinated at 25ºC for 7 days and seed longevity was determined by the germination percent. The results of screening showed that the varieties originating from India had long seed longevity and Japanese varieties had poor seed longevity (Fig. 2). The varieties originating from India should be suitable as parents for genetic analysis and breeding of seed longevity.

Mapping QTLs controlling seed longevity

For MAS of seed longevity, QTLs controlling this trait were identified using the same BILs with genotype data of RFLP markers in the QTL analysis of LTG. On the other hand, the relationship between seed dormancy and seed longevity is not known. Many studies have been performed to answer this question but the results have not been consistent[3,5,16,19]. We attempted to clarify the relationship between seed dormancy and seed longevity by comparing the chromosomal location of QTLs for seed longevity and seed dormancy. The seeds harvested at 40 days after heading were germinated at 25ºC for 7 days. The germination percent was used to determine the degree of seed dormancy. Then, seeds of each BIL were kept for 12 months at 30ºC in dry conditions to promote loss of viability. To measure seed longevity, we performed an additional aging treatment for 2 months at 30ºC maintaining seeds at 15% moisture content. We measured germination percent of these treated seeds at 25ºC for 7 days to estimate the degree of seed longevity. Three putative QTLs for seed longevity, qLG-2, qLG-4 and qLG-9, were detected on chromosomes 2, 4 and 9, respectively. Kasalath alleles increased the seed longevity at these QTLs. The largest effect occurred with qLG-9 and explained 59.5% of total phenotypic variation in BILs. Otherwise, 5 putative QTLs for seed dormancy, qSD-1, qSD-3, qSD-5, qSD-7 and qSD-11, were detected on chromosomes 1, 3, 5, 7 and 11, respectively (Table 2). Based on the comparison of chromosomal location of QTLs for seed longevity and seed dormancy, these traits seem to be controlled by different genetic factors.

Introduction of accelerated coleoptile growth by backcross breeding into Japanese elite rice

To develop rice varieties with stable seedling establishment at low temperature, it is necessary to introduce

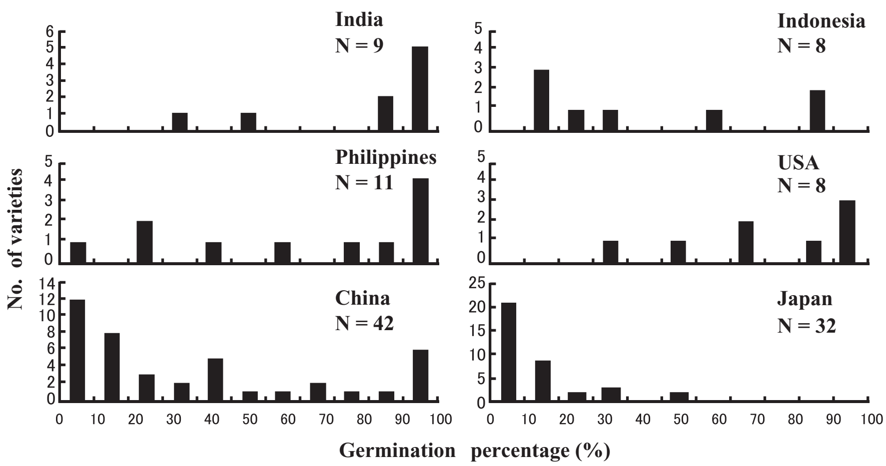

Fig. 2. Frequency distributions of seed longevity in introduced varieties from various rice growing areas of the world
N: Population size.

Table 2. Putative QTLs of seed dormancy and longevity

	QTL	NML[a)]	Chromosome	GLM/SAS Probability	MAPMAKER/QTL			
					LOD	% Variation[b)]	AE[c)]	DPE[d)]
Seed dormancy	qSD-1	R1613	1	0.0006	2.626	11.6	14.5	K
	qSD-3	C25	3	0.0014	2.436	11.3	13.7	K
	qSD-5	R1838	5	0.0001	3.099	13.6	–13.6	N
	qSD-7	R1357	7	0.0076	1.490	6.8	–10.7	N
	qSD-11	C189	11	0.0018	2.118	9.7	13.3	K
					Total[e)]	41.1		
Seed longevity	qLG-2	C1470	2	0.0007	2.809	13.4	14.4	K
	qLG-4	R514	4	0.0012	2.428	11.6	15.0	K
	qLG-9	R79	9	<0.0001	13.883	59.5	25.5	K
					Total[e)]	68.2		

a): Nearest marker locus of putative QTLs.
b): Phenotypic variation explained by each QTL.
c): Additive effects (1/2 weight) of Kasalath allele by arc sine of germination percentage.
d): Direction of phenotypic effect. N and K indicate Nipponbare and Kasalath alleles increased the values, respectively.
e): Estimates obtained from a multiple QTL model.

accelerated coleoptile growth into Japanese elite varieties[15]. Coleoptile growth at low temperatures of European varieties was faster than that of Japanese varieties. Hence, undesirable traits of European varieties, for example, low yielding ability and bad grain appearance, have made it difficult to improve coleoptile growth in rice breeding programs in Japan. The objective of this study was to confirm the efficiency of backcross breeding to introduce accelerated coleoptile growth into *japonica* elite varieties from European varieties to develop rice varieties for direct seeding culture under cool temperature conditions.

A near-isogenic line for coleoptile growth, Satukei 00019 was bred by a backcross between Kitaibuki, a Japanese variety, as the recurrent parent and Arroz da Terra, a European variety, as the donor parent. Satukei 00019 had more rapid coleoptile growth and higher seedling establishment percentage than Kitaibuki in a direct seeding culture test conducted in submerged fields (Table 3). From the results, a large contribution of rapid coleoptile growth to seedling establishment in direct seeding culture in submerged fields under cool conditions and the efficiency of the backcross method to introduce accelerated coleoptile growth were confirmed.

Table 3. Coleoptile length and seedling establishment rates of Satukei 00019, Kitaibuki and Arroz da Terra

Variety	Coleoptile length[a)] (cm)	Seedling establishment percentage[b)] (%)
Satukei 00019	23.6	76.4
Kitaibuki	11.4	57.6
Arroz da Terra	27.2	70.2
$LSD_{0.05}$	3.6	16.4

a): Coleoptile length at 15ºC for 7 days under tap water.
b): In direct seeding culture test in a paddy field at the National Agricultural Research Center for Hokkaido Region in 2000.

Discussion

Currently, the parents utilized for improvement of LTG in Japanese rice breeding programs have been only European varieties, for example, Italica Livorno and Arroz da Terra[7]. Based on the results of a re-assessment of rice genetic resources for LTG after removing the effect of seed dormancy, we found *indica* varieties originating from India, China and Korea from the varietal group previously estimated as low LTG with high LTG. Particularly, Kasalath, an *indica* variety originating from India, had high LTG similar to the level of Italica Livorno and Arroz da Terra. In this study, we detected 3 putative QTLs (qLTG-2, qLTG-4-1 and qLTG-11) for which the Kasalath alleles increased LTG. These alleles should be very useful to improve LTG in *japonica* rice varieties.

In this study, qLG-9, with a large effect on seed longevity was detected and explained 59.5% of total phenotypic variation in BILs. The Kasalath allele in the case of qLG-9 will be useful for improving seed longevity. MAS will be a helpful method to select plants with qLG-9 because the phenotyping for seed longevity is not easy due to variable environmental conditions during seed development, seed processing, and storage. In addition, it takes a long time to lose seed viability. Recently, genes at QTLs have been isolated for fruit size and yield related traits in tomato[2] and heading date in rice[21] by the map-based strategy. The QTLs, qLG-9, with the largest effect, should be an appropriate target of map-based cloning. The isolation of genes at QTLs for seed longevity will be necessary to understand genetic mechanisms of this trait and help to establish a new genetic diagnosis for seed longevity to predict regeneration intervals of rice accessions stored in genebanks.

Besides coleoptile growth, other factors affect seedling establishment, such as, LTG[19], the ability of the seminal root to penetrate soil[4], resistance to oxygen deficiency[17], and the rate of increase in leaf chlorophyll content[22]. The complex interactions among these factors seem to affect seedling establishment. Therefore, the separation of these complex effects is necessary to elucidate each factor. Near-isogenic lines for a target factor would be useful to identify the effect of each factor. The seedling establishment percentage of Satukei 00019, a near isogenic line of Kitaibuki for accelerated coleoptile growth, was superior to that of Kitaibuki. From the results presented here, a large contribution of rapid coleoptile growth to seedling establishment in direct seeding culture in submerged fields under cool conditions was confirmed. In 2001, Satukei 00019 was named Hokkai PL8 and distributed to breed new varieties for direct seeding culture as a parental line. This line will also be useful to elucidate genetic and physiological mechanisms of coleoptile growth.

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