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Evaluation of QTL Related SSR Marker Universality in Korean Rice Breeding Populations

Moon-Tae Song*†, Jeom-Ho Lee*, Sang-Bok Lee*, Ja-Hwan Ku*, Youn-Sang Cho*, Myung-Hee Song*, Sung-Ho Choi*, and Hung-Goo Hwang*

**National Crop Experiment Station, Rural Development Administration, Suwon, 441-100, Korea*

ABSTRACT : If a quantitative trait loci (QTL) marker identified in a population is applicable to different populations (marker universality), this will not only reduce the labor and cost in marker assisted selection (MAS), but accelerate the application of molecular markers to real breeding programs. Present study aims to evaluate the defined QTL related markers from a population to a different breeding population for the MAS. Four rice breeding populations were subjected to seventy-five simple sequence repeat (SSR) markers which were already identified for their polymorphism information content (PIC) in the parents of the crossings. Among them, eight markers were evaluated for their correlation between presence of marker alleles and phenotypic expression in breeding populations. A reasonable level of polymorphism for the mapped markers originated from any sources of rice accessions was observed between crosses of any sources (marker repeatability). However, correlation between presence of markers and expression of the traits in rice breeding populations was not significant except for minor portion of traits and markers examined (failure of marker universality). In the present study, various strategies were discussed to develop new markers with universality of breeding application.

Keywords : rice, molecular marker, simple sequence repeat (SSR), quantitative trait loci (QTLs), marker-assisted selection (MAS)

Marker and QTL information obtained from a segregating population can be used to design efficient breeding strategies. In recent years major advances in marker availability and statistical methods for assessing marker-trait correlation have been achieved (Lander and Bostein 1989, Jansen and Stam 1994, Falconor and Mackay 1996). MAS has been advocated as a useful tools for rapid genetic advancement in the case of quantitative traits (Lande and Thompson 1990, Knapp 1994, 1998). In contrast to disease and insect resistance, many important traits in crop plants show continuous phenotypic variation known QTL. Since Paterson *et al.* (1988) described genetic dissection of several quantitative traits into single Mendelian factors in tomato, many QTLs have been identified using DNA markers in various crops, such as tomato (Paterson *et al.*, 1991; deVicentre and Tanksley, 1993) and maize (Edward *et al.*, 1992, Stubber *et al.*, 1992).

In rice, QTL analysis with DNA markers, based on a well saturated genetic linkage map (Saito *et al.*, 1991, Causse *et al.*, 1994; Kurata *et al.*, 1994; Yano and Sasaki, 1997, McCouch *et al.*, 1998; Cho *et al.*, 2000), has been employed to detect genomic regions associated with several traits exhibiting complex inheritance. Major advances in rice QTL mapping is well summarized in the review paper by Mackill and Junjian (2001).

Presently, practical application of MAS in rice breeding is limited although the availability of comprehensive molecular linkage maps, tight linkage of target genes with molecular markers, and rapid development of polymerase chain reaction (PCR)-based DNA markers have facilitated the employment of the techniques. The limit is originated from technical aspects such as unavailability of tight linkage of markers to target traits, an efficient means of screening large populations for the molecular markers, and screening technique with high reproducibility across laboratories (Mohan *et al.*, 1997). In addition to technical difficulties, one of the major problems in using molecular marker technology in breeding programs is the cost involved.

The expense includes not only the materials and supplies for MAS with defined molecular markers, but also includes the cost of developing new markers for target traits in designed breeding programs (Mohan *et al.*, 1997). If a QTL marker identified in a population is applicable to a different populations (marker universality), this will greatly reduce the labor and cost in MAS in future, and accelerate the application of molecular markers to real breeding programs (Dudley, 1993). Whether a marker allele accurately identified as being associated with a favorable QTL allele in a population can be used to select for that favorable QTL allele in a different population depends on presence of segregation of the markers and the QTL in the second population.

†Corresponding author: (Phone) +82-31-290-6793 (E-mail) mtsong@rda. go.kr
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Additionally whether the linkage phase between the marker and the QTL allele is the same in both the populations, and genotypes of other QTL if epistasis is present is another consideration.

In this paper we describe an experiment using SSR markers associated with several important traits in rice breeding programs to be applied to four different breeding populations derived from genetically representative crossing combinations such as crossings between Tongil cultivars and japonica cultivars. The focus of the experiment lies on the possibilities of universal application of mapped marker developed in a population to different breeding populations.

MATERIALS AND METHODS

Plant materials and traits evaluation

Four rice-breeding populations were used in this experiment (Table 1). Three of them were derived from crosses between same subspecies of rice, SR25225, F_2 population and SR21775, F_7 population between japonica cultivars and SR26061, F_2 population between Tongil cultivars, which were developed from crosses between japonica and indica cultivars. The remaining one was SR23033; F_6 population derived from crossing between different subspecies of rice, Tongil and japonica cultivars. One hundred individual plants in F_2 generation and one hundred breeding lines in F_6 and F_7 generation (25 individual plants in each breeding lines) were planted in 1991 summer crop seasons for field evaluation. Measurements were taken for heading date, plant height, number of panicle, panicle length, grain number per panicle, and grain filling rate. The same sets of plant materials were subjected to cold water treatment (17°C) at seedling stage for their evaluation on cold tolerance at nursery of plants.

DNA extraction

Genomic DNA was isolated from green leaves of young seedlings of the same plant materials used in field evaluation. Each sample comprising of five to eight seedlings for advanced breeding line and leaf tissues of 30-day-old F_2 individual plants was processed according to method of Song *et al.*, (2002) until the crude-DNA precipitation step. The crude DNA was then spooled-out, washed twice with 70% ethanol, dissolved in 3-5 ml TES (10 Mm EDTA, 5M NaCl, pH 8.0) containing 20 μg/ml RNAse-A, incubated at 37°C for 30 min and extracted with chloroform:iso-amyl alcohol (24:1, v/v). The DNA was then re-precipitated with two volumes of chilled absolute ethanol. The purified DNA was finally dissolved in TE (10 mM Tris, 0.1 Mm EDTA, pH 8.0) buffer and checked for its quality and quantity.

SSR markers

A total of seventy-five SSR markers were used in this experiment (Table 2). QTL related SSR markers used in this experiment were originated from the QTL studies with recombinant inbred lines of crossing between japonica and wild rice (Hwaseong/Rufipogon: HR), between Tongil and japonica (Milyang23/Stejarhee45 : MS, Milyang23/Kiho : MK, and Milyang23/Stejarhee45//Milyang23 : MR), and be- tween japonicas (Suwon365/Choocheong: SC), respectively (Kang *et al.*, 1998; Suh *et al.*, 2002; Jeong, 2001; Kwon *et al.*, 2000). One hundred thirty five alleles were reported from these seventy-five SSR markers. Their linkage to quantitative traits, location in chromosome, phenotypic variation, and allele effects are shown in Table 2.

PCR conditions and separation of SSR alleles

For SSR amplification, each 20 μl amplification reaction mixer consisted of 10 mM of Tris-HCl (pH 9.0), 50 mM of KCl, 0.1% Triton-100, 2 mM of $MgCl_2$, 0.1 mM of dNTPs, 200 nM of primers, 0.5 units of Taq polymerase, and 20 ng of genomic DNA was used. All fragments were amplified using the following PCR profile: 5 min at 95°C, followed by 45 s at 94°C, 60 s at 55°C, and 2 min at 72°C for 35 cycles, and 5 min at 72°C for a final extension. After 35 cycles a final extension step was performed at 72°C for 5 min. PCR amplifications were carried out using a GeneAmp 9700 cycler (Perkin Elmer, Weiterstadt).

PCR fragments were separated on denaturing polyacrylamide gels consisting of 10% polyacrylamide (AA:BIS= 19:1) and 7 M urea in 0.5 M TBE buffer. To this end PCR

Table 1. Plant materials with different combination of genetic background and generation advancement.

Crossing nomenclature	Parents		Ecotype of parents	Generation
	Female	Male		
SR25225	Choocheng	Daean	Japonica/Japonica	F_2
SR26061	Namcheon/Dasan	Anda	Tongil/Tongil	F_2
SR21775	Suwon420	Ilpum	Japonica/Japonica	F_7
SR23033	Dasan	Jungan	Tongil/Japonica	F_6

Table 2. SSR markers and their marker information used for this experiment.

Marker	Origin†	Trait	QTLs	Chr. No.	PV‡	Allele Effect
OSR019	SC	Heading	qDTH-6	6	4.2	2.0
OSR020	MS	Cold tolerance	qNSR12	12	1.3	3.0
OSR020	MS	Cold tolerance	qSFR12	12	0.5	2.8
OSR020	SC	Fertility	qFER-12	12	5.8	4.0
OSR020	SC	KI197	qKI197-3	12	-	-
OSR023	MS	Culm length	qCL1	1	3.9	1.3
OSR023	MS	Cold tolerance	qPE1	1	5.5	0.5
OSR026	SC	KJ301	qKJ301-4	2	-	-
OSR027	MS	Heading	qDH1	1	3.9	1.3
OSR030	MS	Grain no.	qNS8	8	1.9	8.6
OSR032	SC	Grain length	qGL-12	12	7.7	0.1
OSR032	SC	KI197	qKI197-4	12	-	-
OSR032	SC	KJ301	qKJ301-6	12	-	-
OSR032	SC	Grain width/length ratio	qLWR-12	12	12.5	0.1
RM001	HR	Grain weight	qGW-1-1	1	9.4	1.6
RM001	MS	Grain number	qNS1	1	5.1	7.5
RM003	HR	Heading	qDHT-6-1	6	35.6	-8.9
RM003	HR	Grain yield	qYI-6-1	6	9.9	-60.1
RM004	MK	Gain type	qPL-12	12	-	-
RM004	MK	Panicle length	qPL-12	12	-	-
RM005	MR	Gain number	qSP-1	1	12.2	14.4
RM005	MR	Grain yield	qYI-1	1	3.7	1.9
RM007	MK	Panicle number	qNOP-3	3	-	-
RM010	MK	Awn	qAWN-7	7	-	-
RM011	MR	Grain number	qSP-7	7	5.1	10.0
RM013	HR	Panicle length	qPL-5-1	5	8.2	1.3
RM013	MR	Grain yield	qTI-5	5	4.1	2.3
RM016	MS	Culm length	qCL3	3	0.8	-8.9
RM017	MK	Hardness	qHARDN-12	12	-	-
RM017	MK	Adhesive	qADH-12	12	-	-
RM017	MK	SH	qSH-12	12	-	-
RM018	HR	Grain yield	qYI-7-1	7	7.9	-69.9
RM019	HR	Grain weight	qGW-12-1	12	8.9	1.8
RM019	MK	Panicle number	qNOP-12	12	-	-
RM020	MS	Cold tolerance	qGYR12	12	5.6	1.7
RM020	HR	Grain weight	qGW-11-3	11	8.6	1.7
RM020	MK	Hardness	qHARDN-11	11	-	-
RM021	MK	White core	qWC-11	11	-	-
RM021	MS	Culm length	qCL11	11	0.5	2.4
RM022	MR	Heading	qDTH-3	3	3.8	1.4
RM025	HR	Grain fertility	qFER-8-1	8	15.4	-7.3
RM025	MS	Grain number	qNS8	8	1.9	8.6
RM026	MR	Grain weight	qGW-5	5	5.3	-0.6
RM030	HR	Grain weight	qGW-6-1	6	5.4	1.0
RM030	HR	Panicle number	qNP-6-1	6	4.9	-0.8
RM030	HR	Panicle length	qPL-6-2	6	5.5	0.7
RM031	MS	Culm length	qCL5	5	1.3	2.7
RM032	MK	Panicle length	qPL-8	8	-	-
RM032	MK	Grain width	qGT-2	2	-	-
RM034	HR	Grain fertility	qFER-1-1	1	14.5	8.3
RM034	HR	Grain yield	qYI-1-1	1	6.0	56.8
RM034	MR	Heading	qDTH-1	1	8.1	-1.7
RM036	MK	Grain length	qGL-3	3	-	-
RM036	MS	Culm length	qCL3	3	0.8	-8.9
RM040	MK	Grain length	qGL-11	11	-	-
RM047	MK	Grain number	qGPP-11	11	-	-
RM047	SC	Heading	qDTH-7	7	9.1	-3.0

Table 2. continued

Marker	Origin	Trait	QTLs	Chr. No.	PV	Allele Effect
RM047	SC	TOYO Value	qTOYO-7	7	8.8	-3.20
RM050	HR	Grain yield	qYI-6-2	6	8.3	-54.9
RM051	HR	Grain fertility	qFER-7-1	7	4.4	-4.4
RM052	MK	White core	qWC-8	8	-	-
RM070	HR	Culm length	qCL-7-1	7	4.6	20.4
RM080	MK	Brown rice ratio	qBGR-8	8	-	-
RM084	MK	Panicle number	qNOP-1	1	-	-
RM153	SC	Grain length	qGL-5	5	5	0.1
RM153	SC	Culm length	qPL-5	5	4.2	0.5
RM163	MS	Cold tolerance	qCLR5	5	3.9	-1.8
RM163	MS	Grain number	qNS5	5	3.8	-5.6
RM164	MR	Heading	qDTH-5	5	10.6	-2.1
RM167	HR	Grain fertility	qFER-11-1	11	10.9	-6.4
RM167	HR	Grain weight	qGW-11-2	11	10.8	-1.5
RM167	MR	Panicle number	qPN-11	11	3.5	-0.8
RM202	HR	Grain number	qSPP-11-1	11	5.6	8.2
RM202	MR	Grain fertility	qFE-11	11	3.9	6.3
RM206	HR	Grain weight	qGW-11-1	11	10.2	-1.4
RM206	MR	Grain yield	qYI-11	11	6.5	5.0
RM206	MS	Culm length	qCL11	11	0.5	2.4
RM206	SC	Grain number	qSPP-11	11	4.3	10.3
RM207	SC	KJ301	qKJ301-2	2	-	-
RM210	HR	Heading	qDH-8-1	8	6.2	-5.1
RM213	SC	TOYO value	qBD-2	2	4.2	-7.3
RM213	SC	Consistency	qCON-2	2	6.1	1.7
RM213	SC	KJ301	qKJ301-1	2	-	-
RM214	HR	Grain number	qSPP-7-1	7	6.1	11.0
RM214	SC	Grain length	qGL-7	7	4.2	0.1
RM215	HR	Panicle number	qNP-9-1	9	4.8	-0.8
RM215	HR	Panicle length	qPL-9-1	9	10.8	1.0
RM216	HR	Grain number	qSPP-10-1	10	12.6	13.9
RM216	HR	Heading	qDTH-10-1	10	6.0	4.5
RM216	HR	Grain weight	qGW-10-1	10	7.4	-1.4
RM217	HR	Panicle length	qPL-6-1	6	6.9	-0.6
RM221	HR	Grain number	qSPP-2-1	2	4.8	7.8
RM226	HR	Panicle number	qNP-4-1	4	4.7	0.8
RM226	SC	Grain fertility	qFER-4	4	5.3	-3.70
RM227	HR	Grain fertility	qFER-3-1	3	11.1	-8.6
RM227	HR	Grain yield	qYI-3-1	3	7.4	-75.1
RM231	HR	Grain fertility	qFER-3-2	3	5.7	-6.2
RM232	HR	Heading	qDTG-3-1	3	7.5	4.4
RM232	HR	Panicle length	qPL-3-1	3	11.9	1.0
RM233	HR	Grain weight	qGW-5-1	5	5.9	-1.2
RM233	HR	Grain number	qSPP-5-1	5	5.7	8.6
RM233	MS	Culm length	qCL5	5	1.3	2.7
RM235	MK	Cold tolerance	Cold24-12	12	-	-
RM241	HR	Panicle length	qPL-4-1	4	5.5	-0.6
RM241	MK	Awn	qAWN-4	4	-	-
RM241	MK	Fat	qFAT-4	4	-	-
RM241	MR	Panicle number	qPN-4	4	4.9	0.8
RM241	SC	Grain width/length	qLWR-4	4	4.4	-0.03
RM242	HR	Heading	qDTH-9-1	9	9.5	4.8
RM242	HR	Grain number	qSPP-9-1	9	7.9	9.6
RM243	MK	Panicle number	qNOP-1	1	-	-
RM247	HR	Culm length	qCL-12-1	12	5.1	23.1
RM247	MS	Cold tolerance	qGYR12	12	5.6	1.7
RM247	MS	Cold tolerance	qNSR12	12	1.3	3.0
RM247	MS	Cold tolerance	qSFR12	12	0.5	2.8
RM249	HR	Grain fertility	qFER-5-1	5	8.1	-5.4

Table 2. continued

Marker	Origin	Trait	QTLs	Chr. No.	PV	Allele Effect
RM249	HR	Grain yield	qYI-5-1	5	7.1	-54.6
RM250	HR	Grain weight	qGW-2-2	2	11.4	-1.5
RM251	HR	Grain weight	qGW-3-1	3	10.6	-1.5
RM251	MK	Callus restorability	qACF-3	3	10.0	-4.6
RM253	MR	Culm length	qCL-6	6	3.1	9.6
RM253	MR	Grain weight	qGW-6	6	6.6	1.0
RM254	HR	Grain fertility	qFER-11	11	10.6	-5.7
RM256	HR	Grain weight	qGW-8-1	8	11.0	2.1
RM257	MK	Grain fertility	qPRG-9	9	-	-
RM257	MR	Heading	qDTH-9	9	8.3	2.2
RM258	MK	Grain length	qGL-10	10	-	-
RM270	MS	Culm length	qCL12	12	3.4	3.9
RM270	MS	Cold tolerance	qPE12	12	5.2	0.6
RM277	MS	Culm length	qCL12	12	3.4	3.9
RM277	MS	Cold tolerance	qPE12	12	5.2	0.6
RM305	MS	Cold tolerance	qCLR5	5	3.9	-1.8
RM305	MS	Grain number	qNS5	5	3.8	-5.6
RM315	MS	Cold tolerance	qPE1	1	5.5	0.5
RM323	MS	Grain number	qNS1	1	5.1	7.5

†HR, Hwaseong/Rufipogon; MS, Milyang23/Stejarhee45; MK, Milyang23/Kiho; SC, Suwon365/Choocheong; MR; Milyang23/Stejarhee 45//Milyang23;
‡PV, Phenotypic variation; Chr. No. Chromosome position of markers

reactions were mixed with equal volumes of loading buffer (formamide containing 0.8 mM EDTA and traces of bromophenol blue and xylene cyanol), denatured at 95 C for 5 min and snap cooled on ice. Afterwards, samples were loaded on pre-heated Sequi-Gen GT Sequencing Cells (Bio Rad, Munich), that were run at 1,800 V for 2.5 up to 4 h, depending on the fragment sizes to be separated. After the run, the fragments were visualized by silver staining. For this, the gel was fixed for 5 min in fixative (10% ethanol, 5% acetic acid), rinsed in deionized water, stained for 15 min in 0.3% (w/v) silver nitrate. Rinsing was followed again in deionized water, and developed for approximately 15 min until the bands became visible in 1.5 g/l of NaOH, 4 ml/l of 37% formaldehyde and 85 g/l of NaBH. Scoring was done by visual inspection.

Data analysis

Polymorphism between the parental materials was surveyed for seventy-five mapped SSR markers in Table 2. From these, SSR markers showing polymorphism between a specific crossing combination were chosen for their survey in advanced breeding populations. A simple linear regression was used to establish whether or not there is a significant difference in means of traits between female parent and male parent markers. Marker genotypes were coded as 1 as female parent type, or 2 as male parent type in the case of advanced generation. The quantitative trait phenotypes are then regressed on this coded "X" variable. If the slope is significant, linkage between the marker and a QTL is thought to be significant and t-test were conducted for the markers with significant at or higher than 0.05 probability.

RESULTS AND DISCUSSION

Polymorphism of SSR markers in different cross combinations

Of the 75 SSR primers examined, three SSR primers of RM030, RM202, and RM333 were shown as representative example (Fig. 1). The eight cultivars contained in this experiment were three crossing parents classified as Tongil (Namcheon, Dasan, Anda), and five japonicas (Choocheong, Daean, Ilpum, Suweon420, and Jungan). Band separation within and between subspecies was different depending on SSR primers used. For example, band separation between subspecies was recorded although within subspecies was not observed in case of RM030. In contrast, as appeared in case of RM333, band separation was observed within as well as between subspecies. All together the tendency with a clear distinction between subspecies and a less distinction within subspecies were observed for most of the primers examined.

Comparison of DNA polymorphism between parents involved in crosses of each population was conducted with seventy-five mapped SSR markers originated from various sources of rice mapping populations such as between wild rice and japonica cultivars. Some of the markers showed duplicated QTL detection depending on cross combinations

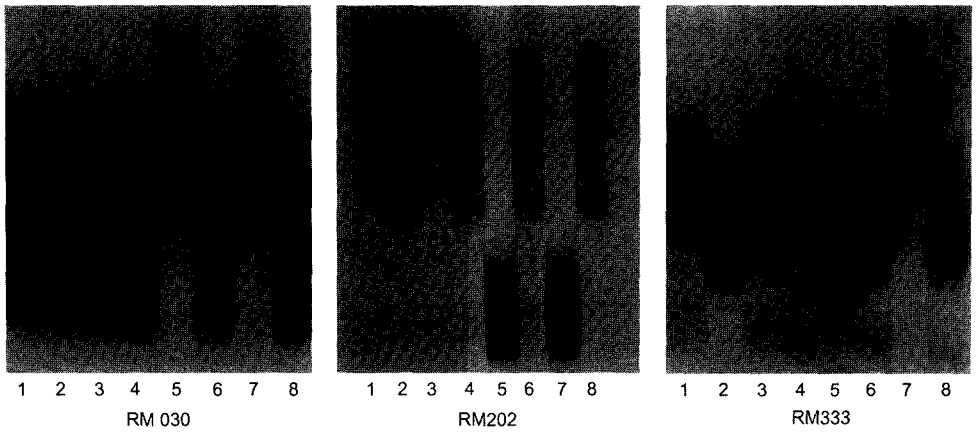

Fig. 1. SSR bands produced by RM030, 020, 333.1, Choocheong (japonica); 2, Daean (Tongil); 3,Ilpum (japonica); 4,Suweon420 (japonica); 5,Namcheon (Tongil); 6,Anda- (Tongil); 7,Dasan (Tongil); 8,Joongan (japonica).

Table 3. Different polymorphism existing in different cross combinations.

Crossing Name	Polymorphic rate by different primer origin (%)				
	HR† (47)	MS (28)	MK (25)	SC (20)	MR (15)
SR25225	34	32	36	50	33
SR26061	98	93	96	95	80
SR21775	28	43	24	80	53
SR23033	94	93	84	95	80

†HR, Hwaseong/Rufipogon; MS, Milyang23/Stejarhee45; MK, Milyang23/Kiho;SC, Suwon365/Choocheong; MR, Milyang23/Stejarhee 45//Milyang23
*Numbers in ( ) indicates total numbers of the SSR markers examined.

used for QTL analysis and a total of one hundred thirty five QTLs were recorded (Table 2). In polymorphism survey (Table 3), the crosses used Tongil cultivars as parent (SR26061 and SR23033) showed more polymorphism than crosses used japonica cultivars only (SR25225 and SR21775). Crosses that used Tongil cutivars used as parent, even in the case of crosses between Tongil cultivars showed more than 80% polymorphism compared to that of the japonica crosses which were approximately 20-40%. This is plausible that although indica germplasm introduction in Korean rice breeding programs has been limited, genetic diversity of Tongil cultivars is still more than that of japonica cultivar used for Korean rice breeding programs as reported by Song et al (2002). They showed that cluster analysis for the 60 rice accessions used for Korean breeding parents was performed on similarity coefficient matrices calculated from SSR markers to estimate their genetic distance. The similarity coefficients ranged from 0.70 to 1.0 to separate the accessions into two broad groups; japonica group (group I) and non-japonica group which included both Tongil and indica accessions (Group II). In the japonica group, all the accessions were resolved into a single minor group. However, no conspicuous separation between indica and Tongil in non-japonica group was observed. It was concluded that the genetic distance within indica cultivars and within Tongil cultivars were farther than the within japonica cultivars and was consistent with other results considering that several closely related japonica cultivars were included in their experiments (Mackill, 1995; Yang *et al.*, 1994; Zhang *et al.*, 1992).

With regard to relationship between marker origin and breeding population, low polymorphism was found in SSR markers from japonica cultivars (as low as 24% in SR-21775) with only one exception in all eight parents examined. Even there was no significance difference in polymorphism of marker from wild rice. This is consistent with other result by Panaud et al. (1996) in which they found that most SSR primers amplified PCR products in *O. rufipogon* (89%) and *O. nivara* (88%). They also found that even in case of low levels of null alleles in these species, the null alleles could arise from point mutation in one or both of the primer sites. The low percentage of null alleles in wild relatives of rice implied that most SSR primers developed for rice could be employed with *O. rufipogon* and *O. nivara*, the close relatives of *O. sativa*. The primers capable of being amplified in wild relatives, it was also noted that the band sizes of PCR products of wild relatives were outside the range of those amplified from *O. sativa*, suggesting that those alleles identified in wild relatives might be unique and different from

those detected in *O. sativa*.

One interesting exception was that the markers originated from cross between japonicas (SC: Suwon365/Choocheong) showed a significant higher polymorphism in japonica populations (SR25225 and SR21775) than between crosses with Tongil cultivars. This is not consistent with other data from this study and earlier studies. In this study DNA polymorphism between genetically distant cultivars was usually higher than those of between genetically closer cultivars. Virks *et al.* (2000) showed same tendency of phenomena when they examined genetically different cultivars with the use of the specific mapped markers associated with specific agronomic characters resulting the higher polymorphism between related cultivars. This may be due to the fact that, instead of using randomly chosen SSR markers, using mapped or specially designed SSR markers specific for the designed regions of markers could detect the polymorphism in that region of interest. Otherwise, polymorphism can not be detected because of its absence in the genome in cultivars with genetically large distance.

Correlation between presence of markers and expression of the traits in rice breeding populations

Among seventy-five SSR markers examined, eight SSR markers associated with nine QTLs were chosen for correlation between their presence of alleles and phenotypic expression which were evaluated in field (grain weight, fertility, culm length) and in greenhouse (cold tolerance). Each of 100 hundred breeding lines from F_7 generation of SR21775 and F_6 generation SR23033 was evaluated for segregating pattern of alleles, whose representative banding pattern appeared in Fig. 2.

A simple linear regression was used to establish whether or not there is a significant correlation between means of traits evaluated and marker genotypes for male parent as 1 and female parent 2. The quantitative trait phenotypes were then regressed on this coded "X" variable. If the slope was significant, linkage between the marker and a QTL was thought to be significant. A three-genotype system (in case of F_2 generation) was not considered in this study because insufficient information was available for interpreting the dominance and additive effect of markers evaluated (Haley and Knott, 1992). Most of the markers (averaged more than 95%) followed one of the band patterns of either male or female parents (Fig. 2). An ignorable portion of heterozygosity in band profile was detected in our study as expected in case of using advanced generation lines as experimental materials.

Correlation between existence of markers and expression

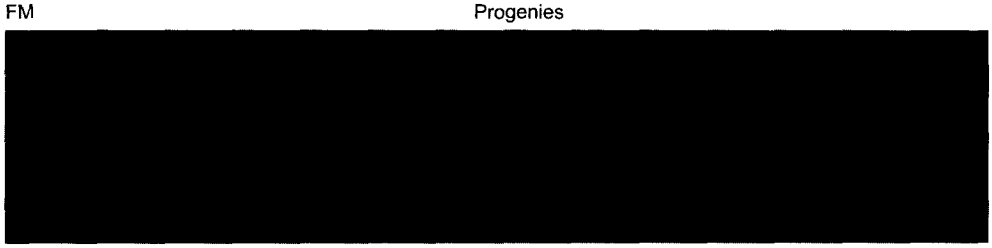

Fig. 2. Segregating DNA band pattern by RM 221 in the population of SR21775. F, female parent; M, male parent

Table 4. Correlation between existence of alleles of markers and agronomic performance.

Trait	Marker	Origin	QTLs	Chromosome number	Phenotype Variation	Allel Effect	T-test value	
							SR21775	SR23033
Grain weight	RM167	HR	QGW-11-2	11	10.8	-1.5	NE	0.11^{ns}
Grain weight	RM206	HR	QGW-11-1	11	10.2	-1.4	3.68^{***}	0.56^{ns}
Cold tolerance	OSR20	MS	QNSR-12	12	1.3	3.0	2.35^{*}	0.49^{ns}
Fertility	OSR20	SC	QFER-12	12	0.5	2.8	0.67^{ns}	NE
Cold tolerance	RM247	MS	QGYR-12	12	5.6	1.7	NE	1.21^{ns}
Cold tolerance	RM315	MS	qPE-1	1	5.5	0.5	NE	0.35^{ns}
Culm length	RM247	HR	QCL-12-1	12	5.1	23.1	NE	1.10^{ns}
Culm length	RM277	MS	QCL-12	12	3.4	3.9	NE	0.10^{ns}
Grain fertility	RM202	MR	QFE-11	11	3.9	6.3	NE	0.91^{ns}

*indicates significance at P= 0.05; ***indicates significance at P=0.001; ns indicates no significance at P=0.05; † NE: not evaluated

of the traits in rice breeding populations was not significant in sixteen cases from the eighteen cases examined (Table 4). This is expected from the reports that whether a marker allele accurately identified as being associated with a favorable QTL allele in one F_2 population could be used to select for that favorable QTL allele in a different F_2 population would depend on (1) the presence of segregation of the marker and the QTL in the second population, (2) whether the linkage phase (coupling or repulsion) between the marker and the QTL alleles is the same in both populations, and (3) genotypes of the QTL are heterozygous and the same linkage phase in the second population as in the first and epistasis is not important, then associations identified in one population may be used for selection in a second population. This is a very difficult requirement for a researcher to identify specific DNA markers to meet all criterions described above (Mohan *et al.*, 1997). Additionally, there are possibilities that the QTLs evaluated here were influenced by several loci rather than one locus evaluated in this study. In the latter case, the minor loci effect would be masked by other loci with larger allele effects which might be happened in our experiment for which a specific experimental design and examination needed.

However, one finding that the markers for two cases from eighteen cases examined showing significant correlation between existence of markers and expression of the traits were from japonica origin (in this case from Hwaseong in HR and stejarhee 45 in MS) would be useful in rice breeding. Ni *et al.* (2001) found that information on genetic diversity of rice subspecies for specific genomic regions would be quite useful for rice breeding programs. A major application of this work is to determine the feasibility of mapping genes within the japonica subspecies, and in particular, within the temperate japonica group, to which most California cultivars belong. Some traits such as cooking quality cannot be accurately measured in wide crosses, where the grain quality requirements are completely divergent. Their data indicated that it should be possible to obtain adequate polymorphism in crosses between California cultivars and premium quality Japanese cultivars to map traits of interest with simple inheritance. This situation could be applied to Korean rice breeding programs as discussed in conclusion.

In conclusion, results from the present study compromised with other studies were summarized to three points in view on the marker universality in rice breeding. The first finding was a reasonable level of polymorphism for the mapped markers originated from any sources of rice accessions was observed in between crosses of any sources with the additional benefit of higher polymorphism by sharing same rice subspecies ensuring a higher polymorphism (mark repeatability). The other one was correlation between existence of markers and expression of the traits in rice breeding populations was not significant except for minor portion of the markers such as grain length with RM206 implying the failure in universality of marker application in MAS breeding (marker universality). However, the third point that even two cases from eighteen cases examined showed significant correlation between existence of markers and expression of the traits were from japonica origin, could not be ignored considering no clear solution other than usual methods used in this experiment as defined.

For these in consideration, further strategies to develop markers in the points of marker repeatability and universality maybe suggested. Use of the genetically related crossing parent for their marker and QTL typing is recommended if the materials are adequate for the two requirements. In this case a low polymorphism because of their genetic distance could be problematic for which the assurance of higher polymorphism and many available SSR markers would be overcome. However, this suggestion would be applicable for markers for short-term breeding plans such as japonica/japonica crossing projects. The story would be different for the long-term breeding programs such as japonica/indica projects. One additional caution should be taken that insurance of genetic diversity to avoid genetic vulnerability of the designed germplasm pools such as Korean rice breeding germplasm might be evaded by using this method as discussed by Song *et al.* (2002). In their experiments, among 60 cultivars examined four most popular rice cultivars grown in Korea was concentrated in a specific clustering area because of sharing of the same cultivars for one of their crossing parents because of breeder's preference to the limited use of elite germplasm for crossing parents repeatedly to follow social demands.

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