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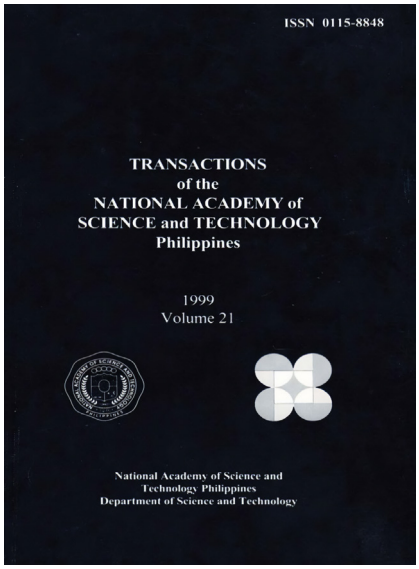

Applications of Molecular Marker Technology in the Philippine Rice Breeding

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APPLICATIONS OF MOLECULAR MARKER TECHNOLOGY IN THE PHILIPPINE RICE BREEDING

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ABSTRACT

There is an urgent need to increase the genetic yield potential of rice since past yield increases have been negated by unabated population growth and reduction in rice hectarage in many countries including the Philippines. Molecular marker technology promises to speed up and improve the overall efficiency of plant breeding and, hence, the attainment of higher yield potential. This paper describes efforts at PhilRice to use molecular marker-aided approaches for breeding direct-seeded and hybrid rice varieties, considered 'new frontier' varietal types for the next millennium.

For hybrid rice breeding, the nuclear genome diversity of 22 cytoplasmic male-sterile (CMS) lines used as female parents for breeding hybrids in the Philippines were assayed at loci amplified by 20 microsatellite or simple-sequence repeat (SSR), 25 RAPD, and 10 amplified fragment length polymorphism (AFLP) primers or primer combinations. A high degree of polymorphism was detected in the CMS lines. Microsatellites and AFLPs appeared to be the most suited for DNA fingerprinting. Cluster analysis based on 222 molecular markers classified the CMS lines into nine groups. The groupings could guide hybrid rice breeders in developing genetically diverse and heterotic rice hybrids.

To determine the utility of markers for predicting heterosis or hybrid vigor, the relationship of SSR heterozygosity and heterotic potential was studied for eight traits in 48 rice hybrids derived from 5 CMS and 10 male parents. Based on 43 microsatellite loci, the CMS and male parents clustered into 2 and 8 groups, respectively, at 75% level of genetic similarity. Microsatellite heterozygosity (based on all the markers used) and heterotic performance were significantly correlated for the number of tillers per plant and LAI when all F_1's were used in the analysis. Significant correlation were

observed for maturity and number of tillers per plant between SSR polymorphism and heterosis relative to the male parent when only hybrids with positive heterosis for each trait were analyzed. Significant negative correlations were observed between heterozygosity and heterosis for maturity, harvest index, and grain yield, relative to the check varieties. Correlations for other traits were insignificant. The relationship of molecular marker heterozygosity and heterosis, therefore, appears to be complex and could vary with the traits and germplasm studied.

For breeding direct-seeded rice, 25 quantitative trait loci (QTL) underlying seedling vigor (SV), one most important traits in direct-seeded rice culture, have been mapped through interval and single-point analyses to different rice chromosomes using restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers. To identify high-SV donors in local germplasm, 37 RAPDs flanking SV QTL were used as diagnostic probes to genotype 49 cultivars at 11 SV QTL. High-SV genotypes were identified in addition to known SV genetic donors and cluster analysis discriminated the 49 cultivars into eight major clusters. Newly identified high-SV genotypes such as Aus 257, Vandana, Dular WB, CG-14, CG-17, CG-20 and UG-20, that could possess alternative SV QTL alleles, have been used to develop crosses designed to concentrate favorable SV alleles in new populations that could be subjected to marker-aided selection.

Keywords: Molecular markers, breeding, direct seeding, hybrid, rice

INTRODUCTION

Molecular marker technology provides a very powerful tool for genetic analysis at the DNA level. DNA-based markers such as simple-sequence repeats (SSR) or microsatellites, amplified fragment length polymorphism (AFLP), and random amplified polymorphic DNA are randomly distributed across the rice genome (Panaud et al., 1996; Mackill et al, 1996; Redoña et al., 1996). Furthermore, all three types of markers are polymerase chain reaction (PCR)-based and does not require the use of radioisotopes, a limiting factor in developing country-laboratories lacking radioactive containment and waste disposal facilities.

Plant breeding programs have begun using molecular markers to addressing specific and applied breeding goals. Breeding areas where molecular markers have already been used include QTL analysis, marker-assisted selection, and genetic diversity analyses. In breeding direct-seeded rice varieties, QTLs have been mapped for seedling vigor (Redoña and Mackill, 1996a, b and c) and submergence tolerance (Xu and Mackill, 1996). In hybrid rice breeding, molecular markers were also useful in determining the relationship between genetic diversity and hybrid performance (Zhang et al., 1995; Xiao et al., 1996). Tagging economically-important genes with molecular markers will facilitate their transfer of gene(s) into elite breeding lines and cultivars and pave the way for map-based or positional cloning.

Table 1. Major features of different types of molecular markers.

Property	AFLPs	RAPDs	SSRs
Inheritance	Dominant	Dominant	Codominant
Genomic Distribution	Random	Random	Random
Technical ease	Difficult	Easy	Medium
Radioisotopes required	None	None	None
Fine mapping suitability	High	Low	Medium
Resolution	High	Medium	High
No. of loci per reaction	50+	8	5+
Clonability	Yes	Yes	Yes
No. of markers	Unlimited	~10000	~2000

MOLECULAR MARKER TECHNOLOGY AND HYBRID RICE VARIETAL DEVELOPMENT

Genetic Characterization of CMS Lines

Hybrid rice breeding in the Philippines is predominantly based on the cytoplasmic-genetic male sterility (CMS) or three-line system. However, concerns over the genetic vulnerability of the limited number of commercially usable CMS lines have been raised previously. Maintaining genetic diversity in CMS germplasm would reduce risks associated with genetic uniformity and could also facilitate the development of heterotic pools and combinations. Although various CMS sources have been used in developing CMS lines, there is limited information confirming the distinctness of each CMS source (Virmani and Banghui, 1988). Investigations based on agronomic traits, cross-pollinating ability, and restoration and maintaining relationships revealed differences in the CMS lines used for breeding rice hybrids in the Philippines (Xu et al., 1995). Molecular markers such as RAPD, microsatellites, and AFLP, however, allow for a more comprehensive characterization of genetic diversity in germplasm pool. These marker types were, therefore, used to assess the nuclear genome variation of CMS lines used in breeding F_1 hybrids at PhilRice and to determine the genetic relationships among the CMS lines based on molecular data.

Twenty-two CMS lines were studied including 16 developed at/or introduced through the International Rice Research Institute (IRRI) and provided by Dr. S. S. Virmani, four from Yunnan Agricultural University (YAU), China provided by Professor Li Zhengyou, and two developed at PhilRice (Figure 1). IRRI CMS lines mostly belong to the CMS-WA types while those from YAU are CMS-ZTB types. DNA extraction from 8-week old plants followed the CTAB procedure (Murray and Thompson, 1980). PCR reactions were run on a PTC-100 thermocycler

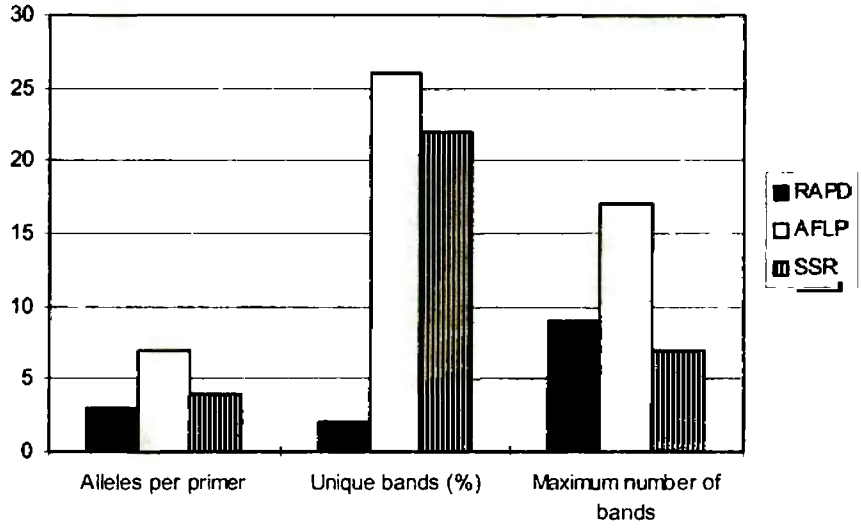


Figure 1. Molecular genetic variation detected by the three types of markers used in determining the genetic diversity of CMS lines at PhilRice.

using 22 SSR primers, 30 random 10-mers, and 10 +3/+3 AFLP primer combinations. To widen the genomic coverage of marker assays, RAPD primers, RM pairs and AFLP primer combinations that produced bands mapped to different rice chromosomes (Redoña and Mackill, 1996; Panaud et al., 1996; Mackill et al., 1996) were used. RAPD products were electrophoresed on 2% agarose gels and visualized under UV light after ethidium bromide staining. PCR products for microsatellite and AFLP analyses were ran on 6% (w/v) polyacrylamide denaturing gels and visualized using Silver Sequence^{TM} DNA staining reagents. Binary scoring was based on presence or absence of bands using only non-redundant information. Similarity coefficients were derived using the Dice method, and were utilized in cluster using the unweighted pair-group method (UPGMA) and the computer software NTSYS-Pc (Rohlf, 1990).

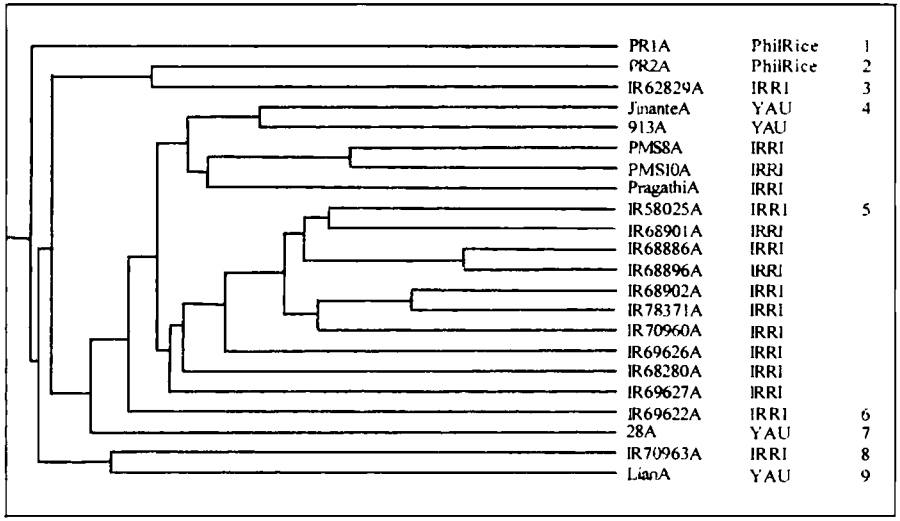
RAPDs, SSRs, and AFLPs effectively detected molecular genetic variation among the CMS lines. Seventy-seven of the 79 SSR alleles (97%) were polymorphic with the number of alleles ranging from 2 to 7 and averaging 3.8 per primer (Figure 1). The 30 RAPD primers amplified 83 polymorphic bands, ranging from 1 to 9 and averaging 2.7 per primer. For AFLPs, 72 bands showed polymorphism with the number of polymorphic bands ranging from 3 to 17 and averaging 7.2 per primer combination. Molecular genetic variation, therefore, was existent in the CMS germplasm assayed. SSRs and AFLPs were more effective in detecting unique alleles than RAPD with 17 SSR alleles (22%), 19 AFLPs (26%), and two

RAPDs (2%) amplified in only one CMS line. Comparing these markers, Powell et al. (1996) noted that SSRs had the highest expected heterozygosity and AFLPs had the highest number of loci simultaneously assayed per experiment. The unique AFLPs, SSRs and RAPDs could be useful as diagnostic tools for varietal identification or DNA fingerprinting of CMS lines.

Diversification of cytoplasm is being resorted to by hybrid rice breeding programs using the CMS system in order to reduce genetic vulnerability. Most of the IRRI lines used possess the WA cytoplasm derived from *Oryza sativa f. spontanea.* With the exception of IR69627A, these lines had at least 50% of their SSR alleles in common with IR58025A, a line that has been used extensively to develop hybrids in the tropics (Table 2). Cluster analysis based on the three types of markers separated the lines into 9 groups with a major group, consisting of 10 IRRI CMS lines, included IR58025A (Figure 2). CMS lines from China and PhilRice, aside from having a different cytoplasmic base, appeared to have a different nuclear genetic base than IRRI lines that mostly possess the wild-abortive cytoplasm derived from *Oryza sativa f. spontanea.* Results indicate, however, that CMS lines with the same cytoplasmic base could also possess nuclear genome diversity. Hence, molecular characterization of both cytoplasmic and nuclear genomes should be more effective in overall genetic diversity assessment of a hybrid rice germplasm pool.

Table 2. Percentage microsatellite alleles shared between IR58025A or IR62829A and the CMS lines.

CMS Line	% Allele shared w/ IR58025A	% Allele shared w/ IR62829A	CMS Line	% Allele shared w/ IR58025A	% Allele shared w/ IR62829A
28A	45.5	45.5	IR70960A	77.3	54.5
IR58025A	100.0	54.5	IR70963A	50.0	54.5
IR62829A	54.5	100.0	IR78371A	68.2	50.0
IR68280A	59.1	59.1	913A	50.0	50.0
IR68886A	81.0	54.5	Jinante A	50.0	40.9
IR68896A	77.3	54.5	Lian A	52.4	45.5
IR68901A	63.6	59.1	PMS8A	54.5	50.0
IR68902A	68.2	50.0	PMS10A	68.2	54.5
IR69622A	72.7	50.0	PR1A	27.3	31.8
IR69626A	59.1	50.0	PR2A	50.0	63.6
IR69627A	40.9	45.5	Pragathi A	59.1	40.9



Based on 77 SSRs, 83 RAPDs and 77 AFLPs. Numbers indicate groupings (top to bottom).

Figure 2. Molecular genetic variation detected by the three types of markers used in determining the genetic diversity of CMS lines at PhilRice.

Prediction of Heterosis using Microsatellite Markers

The power of molecular markers to assess the level of genetic diversity between two parents have generated considerable interest in their utility for predicting hybrid performance in crop breeding programs. Short tandem repeats or microsatellite DNA sequences offer a reliable and effective means of assessing genetic variation in many crops including rice (Xiao et al., 1996). Several studies have reported variable results on the relationship between marker distance and F_1 performance. However, Zhang et al. (1995) noted that despite the inconsistency in the correlations of molecular divergence and performance of the F_1 hybrid, genetic distances based on marker genotypes are in close agreement with pedigree information and can unambiguously resolve lines into their respective heterotic groups.

Fifteen rice varieties used in breeding hybrids in the Philippines, including five cytoplasmic-genetic male sterile (CMS) lines and ten restorer lines, were utilized to develop a partial diallel set of crosses. Eight of these were from the Philippines, five were from China and one each came from Vietnam and IRRI. The CMS lines were earlier shown to be genetically diverse based on 222 AFLP, SSR and RAPD markers (Redoña et al, 1998). The 48 F_1 hybrids generated and their male parents were planted in a field following a randomized complete block design with 2 replications. Two (2) check varieties, an inbred PSBRc28 and a hybrid PSBRc72H or Mestizo, were included to investigate the standard heterotic performance of the hybrids. Three to five plants were examined for eight vegetative

and reproductive characters namely plant height, maturity, leaf area index (LAI), root length, root weight, number of productive tillers, harvest index and grain yield per plant. DNA extraction, SSR assays, and cluster analysis were as described previously. Marker heterozygosity of the F_1's or the genetic distance between the parents was measured as the percentage difference of marker genotypes between the two parents of each cross combination.

Based on 43 microsatellite loci, the CMS and male parents clustered into 2 and 8 groups, respectively, at 75% level of genetic similarity. The hybrids derived from the crosses among these parents showed very low to intermediate heterosis (superiority over the male parent) for plant height, maturity, harvest index, and grain yield, whereas heterosis was high for LAI, root length, root weight, and number of productive tillers. Microsatellite allele heterozygosity (based on all the markers used) and heterotic performance were significantly correlated for the number of tillers per plant and LAI when all F_1's were used in the analysis. Significant correlation were observed for maturity and number of tillers per plant between SSR polymorphism and heterosis relative to the male parent when only hybrids with positive heterosis for each trait were analyzed. Significant negative correlations were observed between heterozygosity and heterosis for maturity, harvest index, and grain yield, relative to the check varieties. Correlations for other traits were insignificant.

The materials used in the study represents the breadth of genetic diversity currently being used to develop Philippine rice hybrids. Microsatellite DNA analysis effectively detected the presence of genetic variation in these materials. This diversity can be exploited to develop hybrids with wide genetic base thereby avoiding genetic uniformity. Breeding superior hybrids would be greatly expedited if heterotic performance can be predicted based on marker data. However, in this study, the relationship of parental genetic diversity based on all the markers used (general heterozygosity) with heterotic performance of the hybrids was generally low for the traits measured. Of the eight traits analyzed, marker-heterosis correlations were highest for LAI and the number of productive tillers. Negative and/or insignificant correlations were observed for grain yield and harvest index. Hence, it appears that for this set of germplasm, molecular marker data may not be useful for heterosis prediction. The relationship between molecular DNA divergence and heterosis appears to be complex and variable as has been reported in other studies using different germplasm. It has been suggested that the use of DNA markers closely linked to specific traits to determine specific heterozygosity may be more effective for predicting heterotic performance (Zhang et al., 1996). However, this would require prior knowledge of QTL for traits of interest in the germplasm being assayed, information that may not be readily available in many hybrid breeding programs in the tropics.

MOLECULAR MARKER TECHNOLOGY AND INBRED RICE VARIETAL DEVELOPMENT

Marker-aided Genetic Characterization of Cultivars for Direct Seeding

Direct seeding is the main cultural practice of farmers in at least three major rice-growing season in the Philippines. Farmers realize more profit using direct seeding due to higher yields and lower hired labor requirement (Francisco, 1997). Current Philippine rice varieties, however, have been bred and selected for under transplanted culture. Hence, there is a need to develop varieties and management practices specifically adapted to direct seeding to maximize productivity under this rice culture.

Seedling vigor (SV), or a plants ability to emerge rapidly from soil or water (Heydecker, 1960), is one of the most important traits under direct seeding. In rainfed areas, vigorous seedling growth provides the rice plant the competitive advantage against weeds (De Datta, 1986) and minimizes damage caused by poor water control (Nanda and Coffman, 1979). Long shoots, roots, mesocotyls, and coleoptiles have been associated with good SV in rice (Peterson et al., 1978; Redoña and Mackill, 1996a; Turner et al., 1982; Dilday et al, 1990). Genetic variation for these traits have been observed and molecular markers have been used to identify quantitative trait loci (QTL) for SV-related characters (Redoña and Mackill, 1996b; 1996c). However, there is a limited information on SV performance of germplasm used in many tropical rice breeding programs.

Forty-nine promising parental cultivars for direct-seeded rice breeding were aanalyzed for SV performance using controlled laboratory tests and and in molecular marker assays designed to identify high-SV donors. The three major rice varietal groups – indica, japonica, and javanica, were represented and two high-SV donors, Italica Livorno (IL) and Black Gora (BG), used in previous SV studies (Redoña and Mackill, 1996b; 1996c), were also included. SV-related traits- length of shoots, roots, coleoptile, and mesocotyl, were measured on 10-day old seedlings in a slant board test, a laboratory technique for SV screening (Jones and Peterson, 1976), using a constant temperature of 25°C. Three replications were used and SV traits were measured on 20 plants per cultivar per replication, DNA extraction from leaf tissue samples and PCR assays were as described earlier. A total of 59 random amplified polymorphic DNA (RAPD) primers were used. Thirty-nine of these produced bands that mapped to SV QTL positions in seven chromosomes (Redoña and Mackill, 1996c). The other 20 primers were randomly selected in order to provide a basis for comparison with mapped RAPDs. SV phenotypic data were analyzed using simple computer macros and SAS procedures (SAS Institute, 1989). Binary scoring for the 39 previously mapped RAPDs, identified based on DNA fragment size, and the 70 bands from the 20 randomly-selected primers and cluster analysis were as described earlier.

Highly significant differences were observed among the 49 cultivars for all four SV traits measured- length of shoots, roots, coleoptile and mesocotyl. All

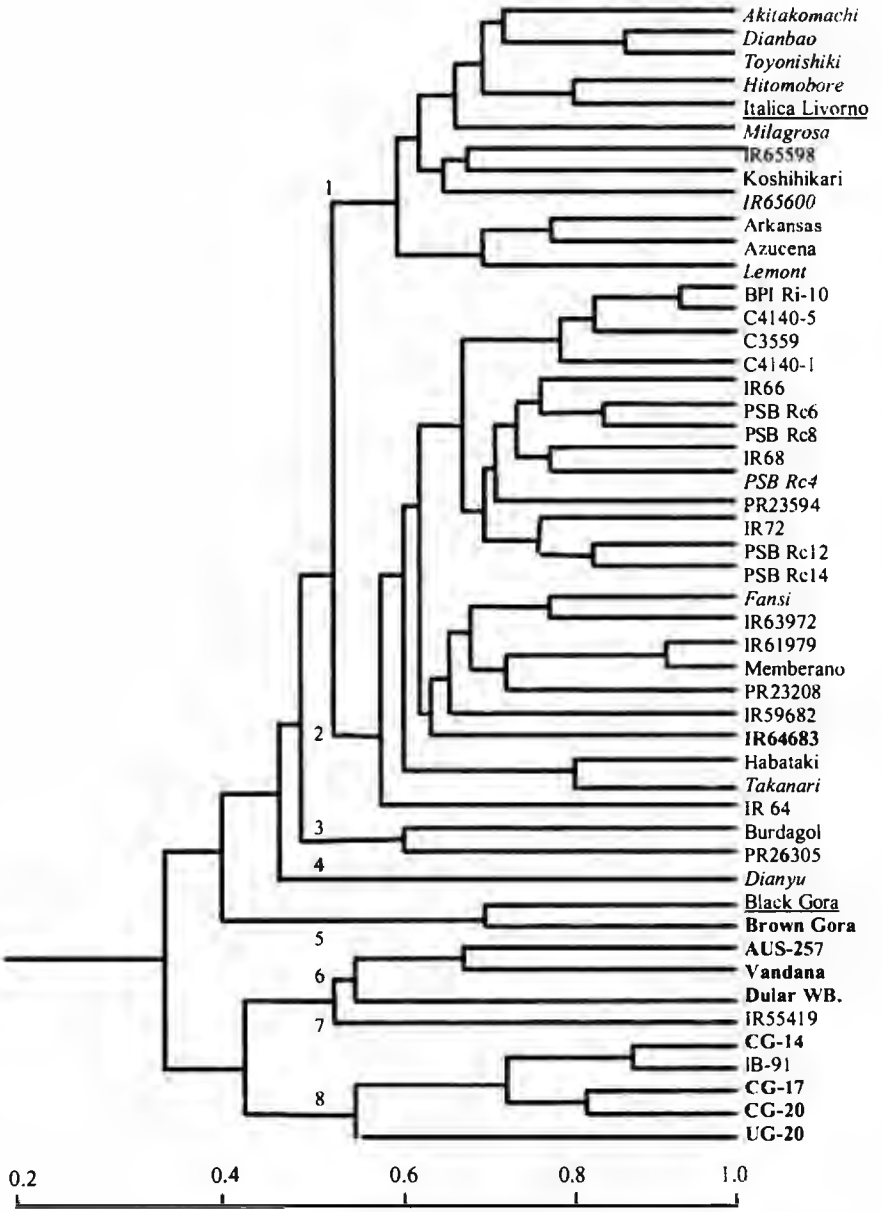


Figure 3. Grouping of cultivars based on genetic similarity coefficients derived using RAPDs bordering regions influencing seedling vigor. Main clusters are marked from I to 8, with high-SV cultivars indicated in bold, low-SV in italics, and known donors underlined.

Table 3. Correlation between parental genetic distance and heterosis for eight traits in the 48 F_1 hybrid combinations.

a) 1998 WS

Trait	*Male parent*		*PSBRc28*		*PSBRc72H*	
	I[a]	II[b]	I[a]	II[b]	I[a]	II[b]
Plant height	0.14	0.39	-0.18	-0.10	-0.18	0.14
Maturity	-0.05	-0.60*	-0.07	0.35	-0.07	0.45*
Leaf area index	0.34*	0.45**	-0.26	0.02	-0.26	-0.15
Root length	0.05	-0.07	0.13	0.07	0.13	0.14
Root weight	0.16	0.13	0.06	0.00	0.06	0.04
No. of tillers/plant	0.37*	0.43**	-0.05	-0.07	-0.05	-0.02
Harvest index	-0.31*	0.22	-0.38**	none	-0.38**	0.07
Yield	-0.12	0.09	-0.29*	-0.23	-0.29*	-0.23

b) 1998 DS

Trait	*Male parent*		*PSBRc28*		*PSBRc72H*	
	I[a]	II[b]	I[a]	II[b]	I[a]	II[b]
Plant height	-0.40**	-0.00	-0.04	0.26	-0.05	0.54
Maturity	-0.20	0.10	-0.40**	0.29	-0.40**	none
Leaf area index	0.10	0.41	-0.10	-0.20	-0.14	-0.20
Root length	-0.10	-0.30	-0.04	-0.22	-0.04	-0.04
Root weight	-0.10	0.06	-0.05	0.31	-0.05	0.34
No. of tillers/plant	0.17	-0.10	0.11	0.12	0.11	0.12
Harvest index	-0.10	0.46	-0.20	-0.10	-0.21	-0.11
Yield	-0.30	-0.30	-0.30	-0.20	-0.31	none

c) Average of 1998 WS & DS

Trait	*Male parent*		*PSBRc28*		*PSBRc72H*	
	I[a]	II[b]	I[a]	II[b]	I[a]	II[b]
Plant height	-0.15	0.01	-0.10	0.24	-0.10	-0.19
Maturity	-0.08	0.27	-0.15	-0.19	-0.15	none
Leaf area index	0.39**	0.45*	-0.17	0.11	-0.21	-0.12
Root length	0.10	-0.06	0.10	-0.12	0.10	0.02
Root weight	0.13	0.05	0.05	0.03	0.05	0.002
No. of tillers/plant	0.41**	0.40*	0.04	0.03	0.03	0.21
Harvest index	-0.26	0.77*	-0.26	none	-0.29*	-0.66
Yield	-0.30*	-0.03	-0.29*	-0.43	-0.30*	-0.81

[a] Correlations based on 48 F_1 hybrid combinations.
[b] Correlations based on F_1 hybrid combinations with positive heterosis.

traits exhibited continuous distribution indicating quantitative inheritance for SV in rice. Several cultivars gave consistently superior or inferior performance for most SV parameters. The aus cultivar Black Gora (BG) was among the 10 most vigorous for all four SV parameters. However, other high-SV donors were also identified including CG-17, CG-14, CG-20, AUS-257, UG-20, and Brown Gora, that were among the 10 most vigorous cultivars for three of four SV traits. Cluster analysis, based on genetic similarities at all 39 RAPD loci flanking SV QTL, discriminated the 49 cultivars into 8 major groups with one cluster composed of 23 or 46% of the test cultivars (Figure 3). The clustering patterns did not strictly follow traditional bases for grouping based on subspecific classification, pedigree, and/or geographical origin. For example, while japonicas formed cluster 1, indicas, japonicas and javanicas grouped together in cluster 2, and cultivars from Japan, USA, China, and the Philippines formed cluster 1.

The clustering of cultivars based on markers bordering SV QTL appeared to have some relationship with actual SV performance based on phenotypic data. For example, clusters 6 and 8 consisted mostly of cultivars with high SV, while cluster 1 was composed mainly of cultivars with low-SV cultivars. This suggests that classification based on marker genotypes at SV QTL may be reflective of actual SV phenotypic performance. High-SV cultivars belonging to different clusters may possess alternative SV alleles. Three major groups of SV donors could be discerned- the first group composed of Black Gora and Brown Gora, the second comprised of Aus 257, Vandana and Dular WB, and the third group composed of CG-14, CG-17, CG-20 AND UG-20. IL clustered differently from the other high SV cultivar BG. Redoña and Mackill (1996d) suggested that different sets of SV alleles may be present in these SV genetic donors. Crosses between high-SV cultivars from different clusters should provide broader genetic SV variability in the resulting breeding populations that could be exploited in breeding varieties for direct-seeded culture. Such crosses have, therefore, been generated at PhilRice's breeding program.

Genetic Diversity Analysis of Progenitor Cultivars of Modern Rice Varieties using AFLPs

In the Philippines, very few studies have been conducted on the genetic diversity of varieties approved for commercial release and their progenitor cultivars. Results from these few studies indicate that the genetic base of Philippine modern varieties seem to be relatively narrow (de Leon, 1994; Caldo, 1996). Information on the genetic relatedness or diversity of modern Philippine rice varieties can influence the direction of PhilRice's breeding program.

AFLP markers are among the most promising genetic markers for genetic diversity studies in rice (Mackill et al., 1996). Eighty land race progenitors of modern Philippine varieties used by Caldo (1996) and assayed with RAPD and microsatellite markers by Sebastian et al. (1998a) were used in this study. DNA extraction and AFLP procedures as well as cluster analysis were as earlier described.

Using one selective nucleotide in the first amplification and 14 Eco R1-Mse1primer combinations with three selective nucleotides, a total of 110 major AFLPs were detected among the 80 landrace progenitors evaluated (Table 4). The average number of polymorphic loci per primer combination was 8 loci and the range was from 4 to 13 loci. These results were comparable to those obtained by Mackill et al. (1995), also using Eco R1- Mse1 primer combinations, who reported an average of eight AFLPs per primer combination.

Cluster analysis produced two major clusters (Figure 4) at the highest fusion level, designated as Cluster 1 and Cluster 2, with the latter including most of the test materials. Cultivars in Cluster 1 included many cultivars from the southern US rice belt that have been previously classified by RAPD analysis as belonging to the tropical japonica or javanica subspecies (Mackill, 1995). Cluster 1 also includes traditional Philippine cultivars such as the premium-quality rices Milagrosa and Azucena. It is interesting to note that based on isozyme analysis (Mallik et al., 1995), most traditional Philippine varieties have also been classified as tropical japonicas. Results of this AFLP study lend support to this observation. Also in Cluster 1 are known tropical japonica cultivars from Africa such as OS4. Cluster 1, therefore, may represent the tropical japonica group among the cultivars assayed. Grouping patterns for cultivars in Cluster 2 based on geographical origin, on the other hand, cannot be easily discerned. However, cultivars originating from India, a known center of diversity for the indica subspecies, belonged to Cluster 2. Two major subclusters could be discerned at next highest fusion level. One subcluster included the wild species *Oryza nivarra* from India that, like cultivated rice, also possesses the AA genome. Clustering patterns of cultivars from the same country were not clear-cut. For example, the Chinese cultivars generally clustered close to each other while the Philippine traditional cultivars *Palawan* and *Kinampupoy* clustered far apart within Cluster 2.

Also evident in the dendogram were the close clustering of different accessions of the same cultivar. This implies that these accessions may actually be just one genotypic sample. AFLPs, therefore, appear to be an effective way for checking duplications of entries conserved in gene banks, thereby, saving a lot of resources that would otherwise be needed for germplasm storage and rejuvenation.

CONCLUSION

Molecular marker technology is beginning to be used in breeding both inbred and hybrid rice varieties in the Philippines. In addition to the above studies, other molecular marker applications are being conducted at PhilRice by different research groups. Among these are mapping studies for rice tungro virus resistance (Sebastian, 1996) and blast resistance (Tabien, 1998) and tagging of thermosensitive genetic male sterility (TGMS) genes using microsatellite markers. All these studies aim to facilitate the transfer of the target genes through marker-based selection into elite breeding lines both for inbred and hybrid rice breeding. Molecular markers

Table 4. AFLP primers used to screen the progenitor cultivars and the number of polymorphic loci detected per primer combination.

A. First Amplification
1. Eco +1 : 92R11 (A)
2. Mse +1 : 92H18 (A)

B. Second Amplification

	EcoRI +3	*Sequence*	*Mse +3*	*Sequence*	*# of bands*
1	92SO3 (A)	5' -GACTGCGTACCAATTCAAG-3'	92GO5 (A)	5' - GATGAGTCCTGAGTAAAGG -3'	7
2	92SO6 (A)	5' -GACTGCGTACCAATTCAGA -3'	92F38 (A)	5' - GATGAGTCCTGAGTAAACC - 3'	4
3	92SO5 (A)	5' -GACTGCGTACCAATTCACA -3'	92GO7 (A)	5' - GATGAGTCCTGAGTAAAGT -3'	6
4	92SO6 (A)	5' -GACTGCGTACCAATTCAGA -3'	92GO6 (A)	5' - GATGAGTCCTGAGTAAAGC -3'	5
5	93B11 (A)	5' -GACTGCGTACCAATTCACC -3'	92G10 (A)	5' - GATGAGTCCTGAGTAAATA -3'	9
6	93B11 (A)	5' -GACTGCGTACCAATTCACC -3'	92GO3 (A)	5' - GATGAGTCCTGAGTAAAAT -3'	8
7	92SO3 (A)	5' -GACTGCGTACCAATTCAAG-3'	92GO8 (A)	5' - GATGAGTCCTGAGTAAACG-3'	9
8	92SO6 (A)	5' -GACTGCGTACCAATTCAGA -3'	92GO5 (A)	5' - GATGAGTCCTGAGTAAAGG -3'	9
9	92SO3 (A)	5' -GACTGCGTACCAATTCAAG-3'	92GO3 (A)	5' - GATGAGTCCTGAGTAAAAT - 3'	13
10	92SO7 (A)	5' -GACTGCGTACCAATTCATA -3'	92G10 (A)	5' - GATGAGTCCTGAGTAAATA -3'	4
11	93B11 (A)	5' -GACTGCGTACCAATTCACC -3'	92G11 (A)	5' - GATGAGTCCTGAGTAAATG -3'	8
12	92SO6 (A)	5' -GACTGCGTACCAATTCAGA -3'	92GO8 (A)	5' - GATGAGTCCTGAGTAAACG-3'	4
13	92SO3 (A)	5' -GACTGCGTACCAATTCAAG-3'	92GO6 (A)	5' - GATGAGTCCTGAGTAAAGC -3'	12
14	92SO3 (A)	5' -GACTGCGTACCAATTCAAG-3'	92G11 (A)	5' - GATGAGTCCTGAGTAAATG -3'	12

Total number of AFLPs 110

Average number of AFLPs per gel 8

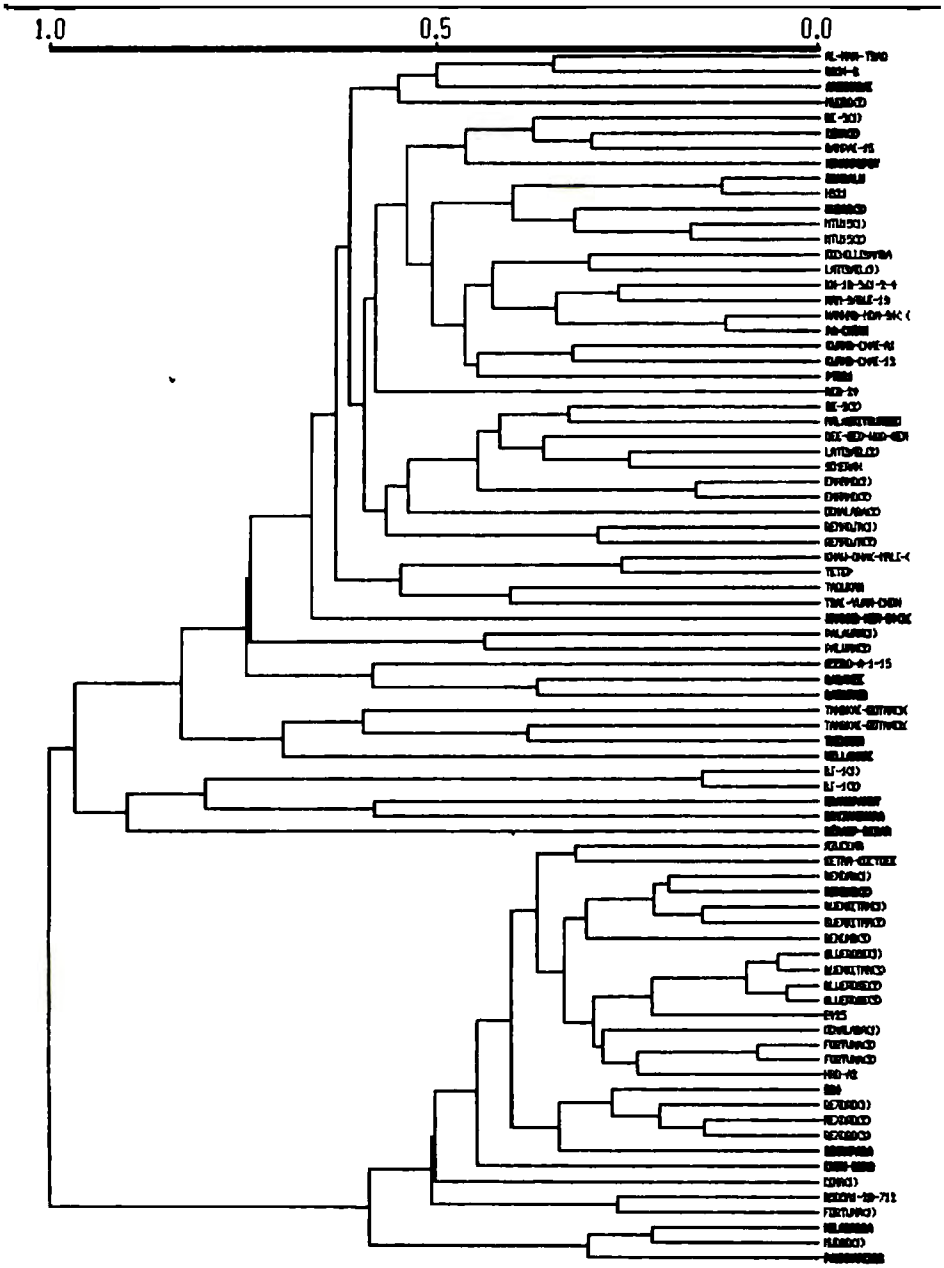


Figure 4. Molecular genetic variation detected by the three types of markers used in determining the genetic diversity of CMS lines at PhilRice.

associated with desired genes or QTL could be useful in various stages of varietal development: (a) markers may be utilized in screening parental materials and breeding lines for desired genotypes at marker loci in order to introgress and/or pyramid favorable genes/QTL alleles in new populations, (b) advanced breeding lines derived from mapping populations that are recombinant for the greatest number of favorable genes/alleles at QTL may be selected and used directly as cultivars or indirectly as parental materials, (c) recombinant lines may be used in backcrosses to other cultivars and selection of favorable genes/alleles at QTL based on marker genotypes may be undertaken (Redoña and Mackill, 1996c). However, cheaper, technically simpler, and readily available marker technologies may still need to be developed for markers to find large-scale breeding applications. This is especially true in the Philippines where the use of molecular markers are currently limited to a few well-equipped and adequately-financed research institutions with trained technical manpower.

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