



Effect of planting time on outcrossing percentage in CMS line seed production of rice

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Commercial exploitation of hybrid rice depends on the identification of suitable heterotic combinations; their stable cytoplasmic male sterile (CMS), maintainer, and restorer lines and economically viable seed production technology; seed distribution infrastructure; and other factors. Although several heterotic combinations with stable CMS lines of IRRI origin were developed at the ARS, seed production is yet to be perfected. A higher outcrossing rate is one of the major factors contributing to higher seed yield in hybrid rice. Seed yield depends on several cultural and environmental factors such as planting time and weather parameters from flowering to maturity. This study identifies suitable seasons for effective and economically viable hybrid seed production.

A trial was carried out at monthly intervals from 1 November 1993 to 1

October 1994 at ARS following standard seed production practices. IR62829A was sown on the first of every month followed by staggered sowing of IR62829B at 2-d intervals, i.e., on the fifth and seventh of every month. Twenty-eight-day-old seedlings were transplanted in each plot of 40 m² consisting of 24 rows with a row ratio of 4A: 2B lines and a spacing of 15 × 20 cm. During transplanting, seedlings of B lines sown during different dates (D₁ and D₂) were planted alternately. Two rows of B lines were planted at the end of each set as border rows. At initial flowering, flag leaves of CMS lines were clipped from the top half to one-third to facilitate free movement of pollen. Supplementary pollination techniques such as pulling a long nylon rope (5 mm diam) back and forth every 30 min or shaking the pollen parent with a bamboo pole were followed at peak anthesis for about a week to increase outcrossing percentage.

Data on yield, yield components, and daily meteorological information for 10 d from the initial flowering date are presented for each set of sowing dates (see table). A simple correlation was estimated between outcrossing percentage, seed yield, and weather parameters.

Results revealed that June flowering (April sowing) was the most favorable time, followed by August and September flowering periods. An increasing trend was observed between wind velocity and outcrossing percentage ($r = 0.95^{**}$) and seed yield ($r = 0.97^{**}$). In this study, maximum temperature (cv 6.7%), minimum temperature (cv 13.3%), and relative humidity (cv 5.2%) were less variable than wind velocity (cv 56.5%). Both maximum and minimum temperatures within a range of 28.9/19.6 °C to 36.2/29.4 °C, however, were not correlated with outcrossing ($r = 0.27, 0.56$) and seed yield ($r = 0.08, 0.56$). Relative

Weather parameters affecting outcrossing percentage in CMS line seed multiplication.

Month of sowing	Initial flowering	Outcrossing (%)	Seed yield (kg ha ⁻¹)	Temperature (%)		Relative humidity (%)	Wind velocity (m s ⁻¹)
				Max	Min		
June	23 Aug 93	22.8	819	31.9	27.5	85.0	2.20
July	17 Sep 93	12.4	408	30.9	26.2	91.7	1.60
Aug	15 Oct 93	9.5	327	32.9	27.3	92.3	0.60
Sep	23 Nov 93	11.9	374	31.4	21.7	88.3	0.90
Oct	24 Dec 93	15.0	492	28.9	19.9	86.1	1.40
Nov	31 Jan 94	11.8	347	30.5	19.6	90.6	0.70
Dec	3 Mar 94	12.3	434	33.2	21.7	89.5	0.80
Jan	24 Mar 94	13.5	459	33.4	22.8	91.9	0.98
Feb	28 Apr 94	15.4	498	36.2	25.8	78.3	1.40
Mar	24 May 94	17.9	514	35.8	27.1	80.0	1.70
Apr	22 June 94	24.6	912	34.9	29.4	86.2	3.14
May	19 July 94	23.3	861	32.2	25.8	87.3	3.08
Mean		15.9	554	32.7	24.6	87.3	1.54
	"t" calculated	1.37	0.29				

humidity was negatively correlated with outcrossing ($r = -0.47$) and seed yield ($r = -0.5$). The study indicates that seed production can be taken up in areas of artificial irrigation with tube wells or bore wells during summer months (April and May) without hindering the normal crop season.

The outcrossing potential in CMS lines, however, was higher in semiarid zones such as Warangal (43.9%) and Palem (40.2%) than in a humid zone such as Maruteru (19.4%) in Andhra Pradesh. This may be due to the relatively higher humidity (80-90%) and low wind velocity

(mean 1.54 m s^{-1}) prevailing at ARS, Maruteru. Seed production of hybrid rice may thus be more efficient and economical in semiarid zones of the country.

Association between simple sequence repeat (SSR) marker diversity, pedigree record, quantitative trait variation, and hybrid performance in rice

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Knowledge of genetic diversity among prospective parental lines is important for the success of a hybrid breeding program. Genetic diversity is usually measured using pedigree information, plant phenotypic data, and molecular markers. In this study, we determined the relationship between various genetic diversity measures and hybrid performance in rice.

The materials used in this study were 37 maintainer and 44 restorer lines of the WA-CMS (wild abortive-cytoplasmic male sterile) system, which represented germplasm of different origins (IRRI, Philippines, and China) used in our tropical hybrid rice breeding program. The pedigrees of these parental lines could be traced back to ultimate ancestors that had no known pedigree information based on published pedigree records (IRRI 1985, 1995) and PhilRice technical notes. Of these lines, 10 maintainer and 18 restorer lines were randomly selected to produce 34 F_1 hybrids in 1997. The hybrids and their parents were evaluated at three field locations in the Philippines from the 1997 dry season to 1998 dry season.

Coefficients of coancestry were calculated for the parental lines using the pedigree information. D^2 estimates were

derived using data on 11 quantitative characters: days to flowering, flag leaf length and width (cm), plant height (cm), number of productive tillers, panicle length (cm), 100-grain weight (g), grain yield per plant (g), grain length and width (mm), and panicle weight (g). SSR assays with 37 primer pairs were made at PhilRice's Genetic Laboratory. Nei and Li (1979) coefficients were then derived. Correlation analysis between various diversity measures and hybrid performance or midparent heterosis on six measured traits (plant height, grain yield, total plant weight, 100-grain weight, grain length and width) was performed.

In the analysis of variance across three locations, significant to highly significant differences were observed for all quantitative traits under investigation, except for flag leaf width. The mean number of alleles per SSR locus was 4.24 ± 1.71 , ranging from 2 to 9. There was one locus where more than 5 alleles were resolved.

There was no or poor correlation between different measurements of diversity and the pedigree records for both B and R lines (Table 1). These results suggested that the methods for measuring

genetic diversity are not consistently associated with each other. This confirms previous reports in durum wheat (Autrique et al 1996) and barley (Shut et al 1997).

No correlation was observed between the diversity measures based on all SSR markers and F_1 performance or midparent heterosis for all quantitative traits measured (Table 2), indicating that molecular diversity in a random set of SSR markers is not useful in predicting midparent heterosis. Prediction power should be improved if selected markers are linked to quantitative trait loci affecting heterosis. Genetic diversity measured by the pedigree-based coefficient of coancestry was significantly correlated with the F_1 mean performance but not with midparent heterosis for highly heritable traits, including 100-grain weight, grain length, and plant height. Therefore, pedigree information can be useful in tracing genes of additive action. D^2 estimates based on quantitative trait differences between parents were significantly associated with F_1 performance for plant height and midparent heterosis for total plant weight, plant yield, and 100-grain weight.

Table 1. Correlation coefficients among genetic diversity measures in maintainer (B) and restorer (R) lines of rice.^a

	Quantitative trait	SSR
Pedigree record	-0.061 (B) 0.216***(R)	0.092* (B) 0.033 (R)
Quantitative trait		0.025 (B) 0.047 (R)

^a*, ** indicate significance levels of $P \leq 5\%$ and 1% , respectively.

Table 2. Correlation between hybrid performance (F_1), midparent heterosis (H_{MP}), and different diversity measures of parental lines.^a

Basis for diversity	Plant height (cm)	Total plant weight (g)	Grain yield per plant (g)	100-grain weight (g)	Grain length (mm)	Grain width (mm)
SSR markers						
F_1	-0.03	-0.02	0.07	-0.04	-0.01	0.09
H_{MP}	0.30	-0.06	0.06	0.06	0.05	0.01
Pedigree record						
F_1	0.34*	0.19	-0.07	0.43**	0.43**	-0.26
H_{MP}	0.30	0.14	0.10	0.01	0.26	0.05
Quantitative traits						
F_1	0.40*	-0.03	-0.18	0.16	0.05	0.32
H_{MP}	0.12	0.38*	0.40*	0.42*	0.23	0.01

^a*, ** indicate significance levels of $P \leq 5\%$ and 1% , respectively.

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Epistatic QTLs affecting hybrid breakdown in recombinant inbred populations derived from indica-japonica crosses

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Inbreeding depression (ID), the depressive effect on the expression of traits, arises primarily from increasing homozygosity in outcrossing species. Heterosis (H), the superiority of F_1 performance relative to parental performance, is associated with heterozygosity (Allard 1960, Filho 1999). In quantitative genetic theory, ID and H are due to nonadditive gene actions and are considered as two aspects of the same phenomenon (Mather and Jinks 1982). Recently, Li et al (1997) suggested that hybrid breakdown (HB) in rice is part of ID due largely to additive epistasis. In this study, we examined HB in a recombinant inbred (RI) population of rice and mapped main-effect and epistatic quantitative trait loci (QTLs) associated with grain yield and

biomass, which appeared to shed some light on the genetic basis of HB in rice.

A set of 254 F_{10} recombinant inbred lines (RILs) derived from a cross between Lemont (japonica) and Teqing (indica) was used. The parents and RILs were evaluated for grain yield (GY) and biomass (BY) per plant in a replicated trial conducted at CNRRI in 1996. Genotyping was conducted at Texas A&M University, USA. A complete linkage map of 182 markers spanned 1,918.7 cM and covered 12 rice chromosomes with an average interval of 11.3 cM between markers (Li et al 1999). The values of HB (RILs – MP) for GY and BY were used as input data, where MP was the mid-parent value. A mixed linear model was used for mapping epistatic QTLs using

QTLMAPPER v. 1.0 based on a threshold of $LOD > 2.5$ (Wang et al 1999).

The mean HB of the RILs was -2.7 t ha^{-1} (-54.6%) and -3.7 t ha^{-1} (-37.7%), ranging from -4.5 t ha^{-1} (-90.8%) to 2.5 t ha^{-1} (50.8%) for GY and from -7.6 t ha^{-1} (-78.4%) to 4.1 t ha^{-1} (42.2%) for BY.

The segregation of the RILs for BY and GY could be largely explained by four main-effect QTLs and seven pairs of epistatic loci (see table). Four main-effect QTLs affecting both GY and BY were mapped to chromosomes 2, 3, and 9, which unlikely contributed to HB since the effects of two alleles at each QTL tend to cancel each other. On the other hand, all but one pair of the epistatic QTLs had significant positive epistatic effects on GY

and/or BY. According to Mather and Jinks (1982), this indicated that most interactions between alleles from the same parents generally resulted in increased GY and/or BY, whereas those between alleles from different parents resulted in reduced GY and BY. These results indicated that disharmonic interactions between the japonica (Lemont) alleles and the indica (Teqing) alleles at these epistatic loci were largely responsible for HB observed in the RI population.

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Main-effect QTLs and digenic epistatic loci affecting hybrid breakdown of biomass (BY, in t ha⁻¹) and grain yield (GY, in t ha⁻¹) in the Lemont/Teqing RILs of rice.

Trait	Chromosome	Marker interval <i>i</i>	Chromosome	Marker interval <i>i</i>	LOD	a_i^a	a_j	a_{ij}
BY	2	C624x–G45			2.65	1.08***		
GY					2.26	0.55***		
BY	3	C515–RG348			9.81	-1.86***		
GY					8.37	-0.84***		
BY	3	G249–RG418			5.50	-1.54***		
GY					5.87	-0.82***		
BY	9	RG451–RZ404			3.53	-1.03***		
GY					2.29	-0.43***		
BY	5	g11–Y1049	11	L457b–G2132b	3.80	-	-	0.76***
GY					4.66	-	-	0.44***
BY	1	R210–RZ382	5	RG556–g11	2.58	-	-	0.42**
GY					3.98	-0.29*	-	0.31***
BY	7	G20–RG30	12	G402–RG20q	5.10	-0.73**	-	0.65***
GY					2.52	-0.29*	-	0.23**
BY	8	CSU754–G104	10	CDO98–RG752	2.86	-	-0.56*	0.52***
GY					3.95	-	-0.42**	0.28***
BY	2	RG654–RG256	9	CDO82–CDO226b	4.24	-	-0.68**	0.51***
GY					2.66	-	-	0.23**
BY	1	RZ382–RG532	11	RG1109–RZ537b	2.50	-0.57*	-	-0.54**

^a a_i and a_j were the main effects of the epistatic loci associated with the Lemont allele, and a_{ij} was the epistatic effect between loci *i* and *j*. *, **, *** represent the significance levels of *P* < 0.05, 0.001, and 0.0001, respectively.

Molecular mapping of quantitative trait loci (QTLs) associated with whitebacked planthopper in rice

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The whitebacked planthopper (WBPH), *Sogatella furcifera* (Horvath) (Homoptera: Delphacidae), has emerged as a major pest of rice in India and other Asian countries. Classical genetic analysis of selected rice accessions resulted in the

identification of five major genes: *Wbpb 1* (Sidhu et al 1979), *Wbpb 2* (Angeles et al 1981), *Wbpb 3* and *Wbpb 4* (Hernandez and Khush 1985), and *Wbpb 5* (Wu and Khush 1985). These genes have been incorporated into improved germplasm to

broaden the genetic base for resistance to WBPH. The need to identify gene loci conferring durable resistance to insect pests, however, led to the molecular marker-based QTL (MQTL) analysis involving 96 doubled-haploid lines derived from IR64/Azucena.

Parents and 96 doubled-haploid lines were evaluated by screening at the seedling and maturity stage. For seedling screening, the mass screening method was adopted. Pregerminated seeds of test lines were sown 3 cm apart in 20-cm rows in 50 × 50 × 10-cm wooden boxes. Each line was planted in a replication across the width of the seedbox with 15 plants per row. One row each of the susceptible check, TN1, and the resistant check, PTB33, was sown at random in all seedboxes. Ten days after seeding, each seedling was exposed to 5-8 first- to third-instar WBPH nymphs. After infestation, the wooden seedboxes with seedlings were covered with wire-mesh wooden cages. Test plants were observed daily for WBPH damage. A damage rating of test plants was made by row when 90% of the plants in the susceptible check row were killed.

For mature plants, the pot screening method was adopted. Pregerminated seeds of each test line and TN1 were sown in 16-cm-diam pots. Thirty days after sowing, seedlings were thinned to 2 pot⁻¹ and covered with cylindrical mylar film cages (13 × 90 cm). Two treatments using first-instar nymphs were used for the 35-d-old plants: 0 and 50 pot⁻¹. Treatments were replicated twice.

At 32 d after infestation, visual plant damage ratings were taken on all test lines and the WBPH progenies were collected in glass vials with a suction device. Insects were dried in an oven at 50 °C for 60 h and weighed. Infested and uninfested test plants (both shoots and roots) were removed from the pots and air-dried for 3 h, dried in an oven at 50 °C for 70 h, and weighed. From the data, tolerance parameters such as functional plant loss index (FPLI), tolerance index (TI), antibiosis index (AI), and plant dry weight loss per milligram of WBPH dry weight produced (PDLOSS) were computed. Phenotypic data gathered for various parameters were subjected to QTL analysis using marker data for IR64/Azucena doubled-haploid populations. The results of interval mapping indicated the presence of a major QTL on chromosome 11 for the

$$\text{PDLOSS} = \frac{\text{Dry weight of uninfested plant (mg)} - \text{dry weight of infested plant (mg)}}{\text{Dry weight of WBPH progeny produced on the test line (mg)}}$$

phenotypic values of PDLOSS. Data on PDLOSS were obtained using the formula above (Panda and Heinrichs 1983).

The phenotypic value of this parameter varied greatly between the parents (221.6 for IR64 and 377.0 for Azucena). The doubled-haploid lines ranged from 23.3 to 201.3 and had a mean value of 248.0. TN1 had a value of 98.1. The frequency distribution of the phenotypic values is given in Figure 1. The skewed nature of the distribution indicated the qualitative nature of the trait. The heritability estimate was high for this parameter (72%). The QTL mapped on chromosome 11 was flanked by markers RG103 and RG167 and explained the phenotypic variance of 79% with a LOD score of 7.31 (Fig. 2). No QTL could be detected when phenotypic data of other parameters were used. The single-marker analysis for the markers identified for PDLOSS also indicated the association of markers with QTLs. The single-marker analysis for the markers in between and outside the interval of RG103-RG167 indicated no marker-QTL association, suggesting the possibility of two QTLs for the trait (see table). The analysis of the phenotypic effect of QTLs identified for WBPH resistance indicated that IR64

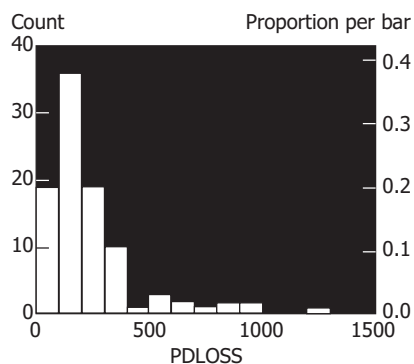


Fig. 1. Frequency distribution among doubled-haploid lines of IR64/Azucena.

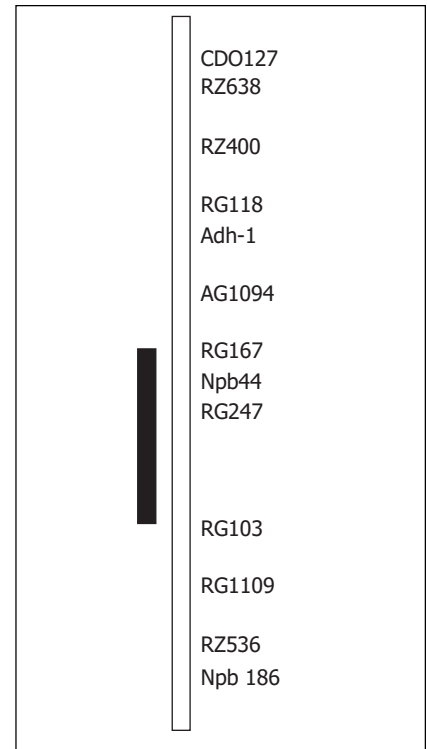


Fig. 2. Marker interval indicating the presence of a QTL for PDLOSS.

contributed more toward resistance. We anticipate identifying newer QTLs when additional phenotypic screening methods are used for screening rice varieties for WBPH resistance.

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Putative QTLs identified for PDLOSS based on single-marker analysis.^a

Marker	No. of individuals with		Mean of PDLOSS for DHLs ^b with		F value
	IR64 allele	Azucena allele	IR64 allele	Azucena allele	
RG1094	76	16	242.21	287.65	0.540
RG167	78	12	203.06	339.99	5.600 ^c
Npb44	78	12	247.06	269.27	0.101
RG247	78	12	237.59	269.27	0.213
RG103	52	38	209.17	312.37	4.780 ^c
RG1109	76	16	242.21	287.65	0.540

^aThe markers present in between RG167 and RG103 do not show linkage. ^bDHL = doubled-haploid line. ^cSignificant at 1% level.

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notes

Starting this issue, IRRN will feature useful and interesting listservers and sites on the World Wide Web on rice and other related or relevant topics.

The Food and Agriculture Organization of the United Nations operates a list server that sends reports on the current food situation in several different countries around the world. These reports are not restricted to rice; they cover the food situation in general. Typically, the reports are issued in response to crisis situations.

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- Analyses of the world's agricultural situation
- Information on fertilizers and environment
- Information on fertilizer trade
- Links to IFA member companies in all parts of the world
- Events such as meetings