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Development and evaluation of restorers and their relationship with heterosis in rice (*Oryza sativa* L.)

C. H. M. Vijayakumar, M. Ilyas Ahmed, B. C. Viraktamath, M.S. Ramesha and B. Mishra

Directorate of Rice Research, Rajendranagar, Hyderabad 500 030

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Abstract

One hundred and sixteen restorers whose hybrid combinations had been evaluated in the national testing were critically evaluated in the field for two years. Those were grouped as high, medium and low based on Duncan's Multiple Range Test (DMRT) and mean and standard deviation of joint scores. More than 75% of the entries were found in the same groups in both the years indicating a very high reproducibility and repeatability of the method of grouping. Only 50 restorers out of 116 were found to give heterotic hybrids with an yield advantage of 1.0 t ha^{-1} or more over the best inbred check variety. Majority of the restorers (>86%) showing heterosis were from the medium group emphasizing the need to select restorers from this group in order to develop heterotic hybrids. In another experiment, 28 F$_6$ lines derived from a CMS based hybrid IR 62829A/ WGL 3962 were crossed with IR 62829A to assess the potential of newly developed iso-cytoplasmic restorers. The F$_1$ crosses were evaluated along with the original hybrid, IR 62829A/ WGL 3962. Results indicated that only 32% of the restorers were able to restore the complete fertility in hybrids implying that the restoration is governed by more than one gene. Nearly 14% of the crosses involving new restorers showed significant heterosis over the original hybrid from which they were derived indicating that there were heritable genetic factors responsible for heterosis. Selection of restorers based on the joint scores of a number of dependent traits appears to be a sound criteria to develop heterotic rice hybrids.

Key words : Rice, selection criteria for restorers, heterosis, iso-cytoplasmic restorers

Introduction

The genetic gains in yield and yield stability offered by heterosis have prompted the use of hybrids in several crops. Heterosis in rice has been known since 1926 [1]. China was the first country to commercially exploit heterosis in rice. The magnitude of heterosis depends on the choice of appropriate parental lines. Selection of suitable donors to improve parents for enhancing heterosis and to predict the performance of hybrids based on the parents has always been a primary objective in all hybrid crop breeding programs including rice. As in other crops, where cytoplasmic genetic male sterility has been used to develop hybrids, in rice too, only a few CMS lines could be utilized for the development of commercial hybrids. This underscores the need for laying greater emphasis on the selection of male parents (restorers). Several methods *viz.*, *per-se* performance, combining ability, Mahalanobis's generalized distance etc., were employed utilizing pedigree information, morphological traits, biochemical data and DNA based markers to study the relationship between genetic diversity among parents and heterosis [2,3] and for selecting the prospective parents, but the results have not been consistent. Unlike in Maize, there are no recognized heterotic groups in rice. Therefore, development of method(s) to choose potential parents before making all possible crosses and their field evaluation could improve efficiency of hybrid breeding to a great extent.

The genetic mechanisms underlying heterosis are complex and not understood clearly. However, certain molecular studies [4-7] have identified alleles responsible for significant reduction or enhancement in the level of heterosis. Here, we report on the relationship between distribution pattern of male parents (restorers) and heterosis and also on the possible inheritance of gene(s) responsible for heterosis from the heterotic hybrid to its progenies down the selfing generations.

Materials and methods

The materials for the first set of experiment comprised 116 known restorers (of 'WA' cytoplasmic male sterility system) whose hybrid combinations have been evaluated over years in the national testing at various network centers in India. The 116 restorers were critically evaluated in RBD with two replications during wet seasons of 1995 and 1996 at Directorate of Rice Research (DRR) farm, Rajendranagar, Hyderabad. Each entry was planted in a single row of 3m length and with a spacing of 20cm between rows and 15cm between plants. At maturity, grain yield per m2 and observations on several yield traits were recorded on five randomly selected plants. The data collected on eight traits *viz.*, plant height (HT), number of panicles plant^{-1} (PN), panicle length (PL), number of fertile spikelets (FS), spikelet fertility percent (SFP), 100 grain weight (TW), grain yield per plant (GYP) and plot yield per m^2 (PY m^{-2}) were used to classify the restorers. Initially, overlapping groups of restorers were obtained for each trait based on DMRT (Duncan's Multiple Range Test). Then, a joint score over seven traits (involving either GYP or PY m^{-2} in combination with other traits) was computed for each line following a method as detailed by Arunachalam and Bandyopadhyay [8]. Using mean and standard deviation of joint scores, three groups: high, medium and low were made. The data on the evaluation of hybrids in national testing program available at DRR from 1990 to 1998 was utilized to identify the promising restorers (whose hybrid combinations showed commercial yield heterosis against the highest yielding inbred check variety in the trial). The percentage of promising restorers was calculated for each group.

The materials for the second set of experiments comprised a CMS based hybrid, IR 62829A/ WGL 3962R and 28 F$_1$ crosses made between F$_6$ recombinant inbred lines (derived from IR 62829A/ WGL 3962R by pedigree method) and their female parent, IR 62829A. All the F$_1$ crosses including the original hybrid (IR 62829A/ WGL 3962R) were evaluated in RBD during wet season 1997. Each entry was planted in 3 rows of 3m length with a spacing of 20 × 15 cm. Data on yield, yield traits and spikelet fertility were collected on five randomly selected plants in each entry. The data on grain yield and spikelet fertility were utilized to identify heterotic hybrids.

Results and discussion

The distribution of restorers based on DMRT followed by joint scoring is presented in Table 1. The distribution pattern was compared for two years and between GYP and PY m^{-2} in order to examine the reproducibility of the results over years. To validate the results for the large sample size, i.e. PY m^{-2} was used in place of GYP in combination with other 6 traits which were common to both GYP and PY m^{-2}. Majority (69-74%) of the restorers were found to be in medium group followed by 13.8-16.4% in high category and 12.1-14.7% in the low group. The differences between years and between GYP and PY m^{-2} were marginal for the distribution percentages. These results have confirmed our earlier findings [9], where 29 parents were used for the study. When GYP was used for the analysis, nearly 75% of the restorers that were found in medium group in 1995 also appeared in the same group in 1996. While, it was slightly less (67.44%) when PY m^{-2} was used for the analysis and these differences are expected as the sample size is increased and such differences could be reduced to minimal by proper experimentation and reducing the errors in measurement of traits. With regard to the high and low groups the percentage of common restorers reduced drastically. This is because, interchanging of groups is common for the genotypes which occur on either borders of the medium group. This was common for both the traits, because slight variations in estimates of standard deviation could influence the genotypes to move in or out of medium group, but such inter-change never occurred between high and low groups.

Table 1. Distribution of 116 restorers into different categories and their comparison between years

Group	GYP			PY/m^2		
	1995	1996	% common	1995	1996	% common
High <(m−σ)	17 (14.7)	16 (13.8)	47.06	16 (13.8)	19 (16.4)	31.25
Medium >(m−σ) ≤(m+σ)	82 (70.7)	85 (73.3)	74.39	86 (74.1)	80 (69.0)	67.44
Low >(m+σ)	17 (14.7)	15 (12.9)	29.41	14 (12.1)	17 (14.7)	21.43
m	0.61	0.57	-	0.61	0.58	-
σ	0.11	0.13	-	0.11	0.13	-

Figures in parenthesis indicate percentages

The promising restorers along with their status of grouping are presented in Table 2. Out of 116 restorers, only 50 showed standard heterosis with an yield advantage of 1.0 t ha^{-1} or more over the highest yielding variety in the national testing at one or more locations. Of these 50, as many as 36 restorers appeared in the same group during both the years of testing indicating 72% reproducibility. These 36 include 31 from the medium group alone, followed by four from high group and one from low group, accounting for 86.11, 11.11 and 2.78% respectively. These findings based on two years data are similar to our earlier observations [9], where 7 out of 8 of the promising restorers were from medium group. A careful examination of the hybrids evaluation data (Table 2) further revealed that most of the hybrids that exhibited heterosis in the multilocation/year tests had their restorers from the medium group. Although the restorers found in high category gave hybrid combinations with standard heterosis, their frequency was low. The restorers found in high category were mostly superior in terms of their per-se performance (Table 3). The mean values of high category for all the traits were always higher followed by medium group. This would mean that most of the lines which are selected based on their per-se performance need not show heterosis in their hybrids. Contrary to our earlier results, there was one restorer (IR 53901) belonging to low group which showed standard heterosis.

Table 2. Promising restorers whose hybrid combinations showed standard heterosis in national testing program

Designation	No. of times found heterotic	Overall Group Status	
		1995	1996
BR 168-2B-23R	3	M	M
ARC 11353	3	M	M
IR 13419	4	M	M
PAU 1126-15	1	M	M
PAU 1106-6	2	M	M
PAU 1106-5	5	M	M
PR 103	1	M	M
IR 10198	6	M	M
IR 29723-143	17	M	M
IR 25912-81	1	M	M
IR 21567-18	7	M	M
IR 32419-28	2	M	M
IR 32358-90	2	M	M
IR 34686-179	5	M	M
IR 31802	3	M	M
IR 35366-62	3	M	M
Suwean 318	1	M	M
WGL 3962	1	M	M
IR 46	11	M	M
IR 47310-94	2	M	M
IR 42686-C2	2	M	M
IR 49461-128	4	M	M
IR 48563-44	1	M	M
IR 59566-157	1	M	M
IR 58110-144	1	M	M
IR 54883-100	2	M	M
IR 53915-51	1	M	M
IR 53970-96	1	M	M
IR 53964-39	1	M	M
IR 72	4	M	M
IR BB-7	1	M	M
IR 54969-41	3	H	H
IR 54742-22	13	H	H
RP 1057-393-1R	2	H	H
IR 32809-314	5	H	H
IR 53901	2	L	L
Pusa 150	2	M	L
IR 13603	4	H	M
IR 40750-82	5	H	M
IR 48725-B-B	8	M	H
IR 50404-57	1	L	M
IR 51078-33	2	L	M
IR 54056-64	5	H	M
IR 9761-19	2	M	L
Swarna	2	M	H
Vajram	5	M	H
IR 37721-90	2	H	M
IR 36	1	M	H
IR 39323-18	2	L	M
IR 44675-101	1	L	M

H = High; M = Medium; L = Low

Table 3. Mean comparison of three categories of restorers for various traits

Trait	1995			1996		
	High	Medium	Low	High	Medium	Low
HT	105.56	95.13	85.56	99.14	90.42	83.40
PN	7.92	7.84	7.71	9.00	8.52	8.26
PL	26.02	25.25	24.95	24.84	23.35	21.67
FS	133.28	112.40	94.10	126.53	106.65	86.54
SFP	90.10	85.84	82.51	89.51	86.42	83.19
TW	2.62	2.44	2.23	2.54	2.42	2.12
SYP	20.89	15.60	13.32	20.17	15.22	10.79
PY/m^2	608.60	521.76	461.66	562.82	411.59	270.07

Table 4. Performance comparison of newly developed iso-cytoplasmic restorers for heterotic potential with their original hybrid (IR62829A/WGL 3962)

Particulars	Test hybrids			Performance of the original hybrid (IR 62829A/ WGL 3962R)
	No.	%	Range for trait	
Hybrids showing normal spikelet fertility (%)	9	32.1	75.7-91.7	73.00
Hybrids showing yield advantage over the original (g/plant)	5	17.8	14.4-29.9	12.37
Hybrids showing significant heterosis (g/plant)	4	14.3	23.1-29.9	

The results on the heterotic performance of new iso-cytoplasmic restorers in comparison to their original hybrid, IR 62829A/ WGL 3962 are presented in Table 4. It is clear from the table that only 32% (9 out of 28) of the lines restored normal fertility in their hybrids, while the remaining 68% of them showed partial fertility in their hybrids. This implies that the fertility restoration is controlled by more than one gene, since normal fertile plants were selected in all the segregating generations to develop new lines. If the fertility restoration was monogenically controlled, then it is expected that all the crosses would restore normal fertility, because the plants were already selected in the WA cytoplasm background. The available evidences [10-14] show that fertility restoration of WA cytoplasm is controlled by two dominant genes Rf-3 (Rf-WA-1) and Rf-4 (Rf-WA-2) located on chromosomes 7 and 10 respectively [15]. Interestingly, occurrence of 32% restorers among the derived lines is still higher than the normal frequency observed in conventional test crosses involving varieties and elite breeding lines which ranges from 20-25%. Nearly 17% of the hybrid combinations (5 out of 28) tested showed yield advantage over the original hybrid, IR 62829A/ WGL 3962R. However, one of them was not significantly different, thus 14% (4 out of 28) of the crosses showed significantly higher yield over the original hybrid. These observations have many implications in hybrid breeding. Firstly, the isolation of restorers from CMS based heterotic hybrids otherwise called iso-cytoplasmic restorers can be considered as one of the effective methods for the development of new restorers with heterotic potential. However, it would be wise to utilize such restorers for crossing with different CMS lines in order to reduce uniformity and to enhance the heterosis. Secondly, the observation that the new restorer lines are more heterotic compared to the original hybrid indicates that the gene or gene combinations responsible for heterosis were inherited from the hybrid to their progeny down the selfing generations.

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