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Magnitude of hybrid vigor retained in double haploid lines of some heterotic rice hybrids

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Abstract Sixty one double haploid (DH) lines were evaluated for yield components and yield and compared with the three corresponding hybrids from which they were derived through anther culture. Analysis of a 6×6 diallel experiment led to the selection of these hybrids based on their high heterosis and revealed the nature of gene action of the characters under evaluation. The DH lines along with the hybrids and parents were planted following a simple lattice design with two replications. The results show that in DH lines the values of the characters expressing predominantly additive genetic effects could reach the heterotic level of the hybrids. For characters including yield showing predominantly dominance effects, values in the DH lines were significantly lower than those of the corresponding hybrids. The promising DH lines, however, possess a higher yield potential than the better parents.

Key words *Oryza sativa* L. · Rice · Heterosis Anther culture · Double haploid

Introduction

Heterosis in rice has been successfully exploited in China which has 14 million hectares planted in hybrid rice (Yuan and Mao, 1991). Hybrid rice technology, however, has not been adopted for commercial production by other rice-growing countries even though intensive research in this area has been carried out. One of the main reasons for this is the difficulty in producing hybrid seed due to cytoplasmic male sterility (He Gui-Ting et al. 1987; Toenniessen 1991). This difficulty would be overcome if apomixis could be successfully transferred to rice to provide hybrids with permanently fixed heterosis. While such an achievement is still remote, application of the anther culture technique to highly heterotic hybrids may help in exploiting the advantage of superior gametic genotypes. Theoretically, it is possible to obtain homozygotes with fixed heterosis provided that partial to complete dominance predominates (Sprague and Eberhart 1977). In practice, anther culture applied to hybrid rice has led to the release of several new varieties (Han Hu and Bin Huang 1987). There is, however, a lack of information on the magnitude of heterosis that is retained in the double haploid (DH) line relative to that expressed by the corresponding hybrid from which it is derived.

The study presented here compared the performance of DH lines with that of their corresponding hybrids from which they were developed by anther culture. The findings suggest the possibility of exploiting hybrid vigor in rice through the development of DH lines from anther culture of potential hybrids.

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Materials and methods

Selection of heterotic hybrids and study of gene action

Six improved rice varieties, three developed at the Indian Agricultural Research Institute, 'Pusa 743-1-1', 'Pusa 33'and 'Pusa 205', and three from the International Rice Research Institute, 'IR66', 'IR72' and 'IR74', were crossed following diallel design to develop 15 hybrids. These hybrids along with the parents were planted in a randomized complete block design with three replications. Genetic variance components were evaluated following Jinks and Hayman (1953) and Hayman (1954). Three hybrids namely, 'Pusa 743-1-1'/'IR66', 'Pusa 743-1-1'/'IR72' and 'Pusa 743-1-1'/'IR74' were selected for anther culture based on their high heterosis in grain yield.

Table 1 Mid-parent heterosis (HM) and better parent heterosis (HB) for yield components and yield of the hybrids used for anther culture

Character	Hybrid 1[a]		Hybrid 2[a]		Hybrid 3[a]	
	HB	HM	HB	HM	HB	HM
Days to 50% flowering	–0.8	–2.4*	0.4	–1.0	3.2**	–3.0**
Plant height	–5.6**	–1.9	–4.6**	1.1	1.8	11.5**
Panicle no./plant	–13.4**	–3.6**	–1.6	17.1**	0.7	2.9**
Grain no./panicle	29.7**	33.1**	18.6*	26.8**	14.1	15.9**
1000-grain weight	–11.4**	6.5**	–13.8**	–1.6	–5.0**	10.0**
High density grain	8.5	81.2**	8.6	78.6**	131.2**	178.3**
Harvest index	1.9	9.2**	6.3**	12.8**	–7.4**	12.4**
Grain yield	34.1**	44.7**	51.9**	56.5**	19.0**	34.1**

*, **=Significant at 5% and 1% level, respectively
[a] Hybrid 1='Pusa 743-1-1'/'IR66', Hybrid 2='Pusa 743-1–1'/'IR72', Hybrid 3='Pusa 743-1–1'/'IR7

Development of DH lines

Anther culture of the 3 heterotic hybrids was done at the Biotechnology Center, Indian Agricultural Research Institute, following established techniques for india rice. Sixty one DH lines were obtained, 46 lines derived from 'Pusa 743-1-1'/'IR66', 8 from 'Pusa 743-1-1'/'IR72' and 7 from 'Pusa 743-1-1'/'IR74'. These lines represented the first progeny of distinct DH anther-derived plants.

Field evaluation

The experiment was conducted on the farm of the Indian Agricultural Research Institute, New Delhi, in the wet season of 1991. The test entries, 61 DH lines, 3 corresponding hybrids ('Pusa 743-1-1'/ 'IR66', 'Pusa 743-1-1'/'IR72' and 'Pusa 743-1-1'/'IR74'), and four parents ('Pusa 743-1-1'/'IR66' 'IR72' and 'IR74'), were evaluated in a 9×9 single lattice design with two replications (13 conventional varieties/lines were added to satisfy the required number of treatment being 81). Each plot contained five rows with 25 plants/row. Single seedling was transplanted per hill at 20×20 cm spacing.

Data collection

Observations were recorded on plants in the three middle rows (excluding border plants) for following characters:

- days to 50% flowering: number of days from seeding to the day 50% plants showed panicle exertion
- plant height: in cm, from the ground to the tip of the main panicle at maturity
- panicle number/plant: average number from 5 plants/plot at maturity
- number of filled and unfilled grains/panicle: average number from 5 panicles/plot
- 1000-grain weight: recorded from evenly oven-dried seeds of 5 plants/plot
- proportion of high density grain: the percentage of grains showing specific gravity above 1.2 following the method of Venkateswarlu et al. (1986)
- grain yield: weight of clean and dry grains harvested from the whole plot (excluding border plants) computed at 14% moisture content (in kg/ha)
- biological yield: weight of total biomass including grains after harvesting and drying
- harvest index: ratio of grain yield to biological yield

Data analysis

The analysis of the variance for simple lattice design was performed following Gomez and Gomez (1984). Mean values of the character of test entries were compared using the Least Significant Difference (LSD) test.

Table 2 Estimates of variance components due to additive effect (D) and to dominance effect (H_1), and degree of dominance $(H_1/D)^{1/2}$

Character	D	H_1	$(H_1/D)^{1/2}$
Days to 50% flowering	34.72**	22.88**	0.80
Plant height	61.42**	72.97**	1.09
Panicle no./plant	2.53**	2.12	0.95
Grain no./panicle	91.38	630.77**	2.63
1000-grain weight	12.64**	4.83**	0.62
High density grain	509.16**	916.37**	1.34
Harvest index	28.89**	75.01**	1.61
Grain yield	0.25	83.68**	18.26

*, **=Significant at 5% and 1% level, respectively

Results

Selection of hybrids for anther culture

Out of 15 hybrids, 3 namely, 'Pusa 743-1-1'/'IR66', 'Pusa 743-1-1'/'IR72' and 'Pusa 743-1–1'/'IR74' were selected for anther culture. The heterotic performance of these hybrids expressed as mid-parent heterosis and better parent heterosis is given in Table 1. Mid-parent heterosis for grain yield was highest in 'Pusa 743-1-1'/'IR72' (56.5%) followed by 'Pusa 743-1-1'/'IR66' (44.7%) and 'Pusa 743-1-1'/'IR74' (34.1%). Besides yield, these hybrids showed mid-parent heterosis for more than three yield components, of which grain number/panicle was highly heterotic (Table 1). Better parent heterosis for grain yield was 51.9% in 'Pusa 743-1-1'/'IR72', 34.1% in 'Pusa 743-1-1'/'IR66' and 19.0% in 'Pusa 743-1-1'/'IR74'. The best hybrid ('Pusa 743-1-1'/'IR72') possessed two yield components (grain no./panicle and harvest index) that showed positive better parent heterosis.

Data on estimates of genetic variance (Table 2) showed that both components due to additive and dominance effects were important for days to 50% flowering, plant height, grain weight, high density grain and harvest index. Meanwhile, additive effects predominated for panicle number/plant and dominance effects predominated for grain number/panicle and grain yield. The degree of dominance varied from partial dominance for days to 50% flow-

Table 3 Performance of parents, F_1 hybrid and DH lines derived from 'Pusa 743-1-1'/'IR66'

Character	Mean performance				LSD 5%
	P1[a]	P2[a]	F_1[a]	DH lines (Range)	
Days to 50% flowering	79	89	83	66–97	4
Plant height (cm)	114	102	105	86–127	4
Panicle no./plant	6.7	8.8	9.9	6.3–12.8	2.4
Grain no./panicle	96	94	125	46–102	21
Spikelet sterility (%)	18.8	15.2	16.5	13.6–23.7	3.6
1000-grain weight (g)	29.8	21.9	23.5	18.1–31.6	1.8
High density grain (%)	12.6	63.1	68.5	7.1–68.2	14.9
Harvest index (%)	52.2	51.4	51.8	28.4–54.6	8.5
Grain yield (kg/ha)	5506	5251	7047	2917–6624	702

[a] P1='Pusa 743-1-1', P2='IR66', F_1='Pusa 743-1-1'/'IR66'

Table 4 Performance of parents, F_1 hybrid and DH lines derived from 'Pusa 743-1-1'/'IR72'

Character	Mean performance				LSD 5%
	P1[a]	P2[a]	F_1[a]	DH lines (Range)	
Days to 50% flowering	79	85	81	77–92	4
Plant height (cm)	114	105	108	102–124	4
Panicle no./plant	6.7	9.3	12.4	7.4–12.8	2.4
Grain no./panicle	96	91	138	59–98	21
Spikelet sterility (%)	18.8	14.7	17.1	13.9–23.4	3.6
1000-grain weight (g)	29.8	22.4	26.5	20.4–27.2	1.8
High density grain (%)	12.6	58.4	63.4	15.6–54.7	14.9
Harvest index (%)	52.2	50.0	56.3	33.9–53.4	8.5
Grain yield (kg/ha)	5506	5450	7818	3866–6606	702

[a] P1='Pusa 743-1-1', P2='IR66', F_1='Pusa 743-1-1'/'IR72'

Table 5 Performance of parents, F_1 hybrid and DH lines derived from 'Pusa 743-1-1'/'IR74'

[a]Character	Mean performance				LSD 5%
	P1[a]	P2[a]	F_1[a]	DH lines (range)	
Days to 50% flowering	79	106	85	75–106	4
Plant height (cm)	114	107	112	90–114	4
Panicle no./plant	6.7	10.2	10.8	8.4–12.3	2.4
Grain no./panicle	96	77	121	64–82	21
Spikelet sterility (%)	18.8	18.3	15.4	13.6–22.3	3.6
1000-grain weight (g)	29.8	23.0	29.7	22.8–27.3	1.8
High density grain (%)	12.6	18.9	47.3	12.4–43.6	14.9
Harvest index (%)	52.2	41.4	49.4	36.5–51.5	8.5
Grain yield (kg/ha)	5506	4865	6332	4270–5157	702

[a] P1='743-1-1', P2='IR74', F_1='Pusa 743-1-1'/'IR74'

ering and grain weight, to complete dominance for plant height and panicle number/plant, to overdominance for grain number/panicle, high density grain, harvest index and yield (Table 2).

Evaluation of DH lines

The performance of DH lines and their corresponding hybrids and parents is presented in Tables 3, 4 and 5. It was recorded that there were DH lines showing a shorter duration of growth and shorter plant height than the hybrids and parents. The upper limit of the range of DH lines for panicle number/plant, grain weight, proportion of high density grain and harvest index more or less reached the heterotic level of the hybrids and often exceeded thevalue of the better parents. However, DH lines showed a poorer performance in grain number/panicle than the hybrid.

The DH lines derived from 'Pusa 743-1-1'/'IR66' showed a grain yield ranging from 2917–6624 kg/ha compared to 7047 kg/ha for the hybrid and 5506 kg/ha for the better parent (Table 3). Yield of the DH lines derived from 'Pusa 743-1-1'/'IR72' ranged from 3866–6606 kg/ha against the 7818 kg/ha of the hybrid (Table 4). Range in

Table 6 Percentage increase/decrease in values of promising DH lines compared to F_1 hybrids and better parents (BP)

Line	Panicle no./plant		Grain no./panicle		1000-grain weight		Harvest index		Grain yield	
	BP	F_1	BP	F_1	BP	F_1	BP	F_1	BP	F_1
DH lines derived from Pusa 743-1-1/IR66 (H1)										
A26-2	28.4*	17.7	6.2	−12.4*	1.0	11.1*	3.2	4.0	20.3*	−6.0
A27-2	39.8*	28.1*	2.1	−21.6*	−26.5**	−19.1*	−2.5	−1.7	5.1	−17.8*
A10-2	27.3*	16.7	−18.7	−37.6*	−15.0*	−6.6	0.0	0.0	14.7*	−10.4*
A14-2	7.9	−1.0	−4.2	−26.6*	−1.7	8.1*	−10.5	−9.8	11.1	−13.1*
A53-2	45.4*	33.3*	−8.3	−29.6*	−20.5*	−12.5*	4.6	5.4	12.8*	−11.9*
DH lines derived from Pusa 743-1-1/IR72 (H2)										
A4-2	21.5	−8.9	2.1	−29.9*	−19.1*	−9.0*	2.3	−5.1	19.9*	−15.5*
A1-3	37.6*	3.2	−11.4	−38.4**	−25.2**	−12.4*	5.0	−2.3	19.3*	−16.0*
A1-2	11.8	−16.1	7.3	−35.5**	−11.7*	0.0	−23.0*	−28.6*	12.9*	−20.5*

*,**=Significant at 5% and 1% level, respectively

yield of the DH lines from ‘Pusa 743-1-1’/‘IR74’ was 4270–5157 kg/ha, while the yield of the hybrid was 6332 kg/ha (Table 5).

From 61 distinct DH lines, 5 promising lines from ‘Pusa 743-1-1’/‘IR66’ and 3 from ‘Pusa 743-1-1’/‘IR72’ were identified, while none of DH lines from ‘Pusa 743-1-1’/ ‘IR74’ showed any improvement. The comparison of these promising lines with the hybrids and better parents is given in Table 6. The best DH line from ‘Pusa 743-1-1’/‘IR66’ (A26-2) had a similar yield level as the hybrid thanks to its similar performance for panicle number/plant and harvest index. Its decrease in grain no./panicle was compensated for by the increased value of 1000-grain weight. Relative to the yield of better parent, the yield of this line significantly increased by 20.3%. Other promising DH lines showed yields lower than that of the hybrid by 10.4–17.8%. The best DH lines from ‘Pusa 743-1-1’/‘IR72’ possessed a yield potential that was lower than that of the hybrid by 15.5–20.5% but higher than that of the better parent by 12.0–19.9% (Table 6).

Discussion

Anther culture of potential hybrids makes it possible to develop superior gametic genotypes that possess the vigor and fitness of the hybrid. The advantage of this method is that such heterotic attributes are fixed in the homozygous condition as a consequence of chromosome doubling. Previous studies have shown that it is possible to obtain as desirable genotypes from the anther culture of F_1 rice hybrids as from the F_2 or F_3 population (Li et al. 1978; Liu et al 1980; Yin et al. 1983). It is evident that new rice varieties have been successfully developed from the anther culture of F_1 hybrids as studies have been reported from China (Chen Ying 1986; Loo Shih-wei and Xu Zhi-Hong 1991; Liang Shouyi and Huang Shouyin 1991), Korea (Lee et al. 1989) and Bulgaria (Boyadzhiev 1990). Zapata et al. (1991) identified 6 out of 109 DH lines derived from three hybrids as being promising under saline and nonsaline conditions.

The approach used in this study was to obtain a direct comparison of the DH lines with the hybrids from which they are derived. The results show that the performance of some DH lines is almost comparable to that of the hybrids for the characters days to 50% flowering, plant height, panicle number/plant, 1000-grain weight, high density grain and harvest index (Tables 3, 4 and 5). For this group of characters, the variance component due to additive effects is highly significant (Table 2). All of these characters showed partial/complete dominance except for high density grain and harvest index, which express overdominance. In contrast for for grain number/panicle, none of the DH lines from three hybrids reach the heterotic level of the hybrids. The same trend was also found for grain yield in which all but 1 DH lines possess significantly lower values than the corresponding hybrids. The only DH line from ‘Pusa 743-1-1’/‘IR66’ which has a yield level of similar to that the hybrid (but numerically lower) is also not comparable to the hybrid for grain number/panicle. The study on gene action has revealed that grain no./panicle and grain yield show predominantly dominance effects and possess a very high $(H_1/D)^{1/2}$ ratio (Table 2).

Heterosis can result from partial/complete dominance, overdominance and a combination of these (Comstock and Robinson 1953). Both types of heterosis are likely to involve fixable additive and additive×additive genetic effects, which are fixable in DH lines. In our study,the observation that the performance of DH lines for the first group of characters is comparable to that of the hybrid supports this genetic basis. However, the heterosis associated with overdominance may also involve dominance, additive× dominance and dominance×dominance genetic effects, which are not fixable. Perhaps this is the reason that almost all DH lines cannot reach the heterotic level of the hybrids for the characters grain number/panicle and grain yield, which showed predominantly non-additive genetic effects.

As compared to the better parents, the yield improvement of promising DH lines is encouraging with a yield advantage of up to 20% (Table 6). This improvement is brought about through the combination of desirable traits in DH lines. It is known to date that hybrid rice outyields conventional varieties by 15–20% (Yuan and Virmani 1988).

It is possible to isolate promising DH lines from a relatively small population, as has been demonstrated in this study. In China, var 'Zhonghua No. 1' was selected from 71 DH lines, 'Zhonghua No. 8' and 'No. 9' from 40 DH lines (Chen Ying 1986) and var 'Huayu 15' was selected from 19 pollen-derived plant clusters (Liang Shouyi and Huang Shouyin 1991). Liu et al. (1983) reported that a population of 50–75 pollen-derived lines from each cross was required for efficient selection.

This study suggests that it is possible obtain promising DH lines through the anther culture of highly heterotic hybrids. Although DH lines cannot reach the yield level of the hybrid due to the failure in fixing non-additive genetic effects, this approach would be of some help in improving the yield potential of rice.

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