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Hybrid Rice Research and Development in Pakistan

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Abstract: Having convinced of the potential of hybrid rice, Rice Research Institute, Kala Shah Kaku began a research project with the objectives to evaluate local and exotic genetic material for their use in hybrid rice breeding programme; to evaluate existing hybrids for yield, adaptability and grain quality and to initiate hybrid rice breeding for the development of parental lines. From 1993 to 2006, 800 exotic rice hybrids were evaluated for yield, adaptability and heterosis. Heterosis of hybrids varied from 8 to 142% over local check variety KS 282. A number of exotic hybrids out yielded the check variety but could not be popularized due to their poor cooking qualities. Six hundred eight test crossed were evaluated. The Institute has 22 CMS lines, 38 restorers and 52 maintainers from 608 testcrosses. Local germplasm have the more frequency of maintainers than restorers. IR58025A, IR79156A, IR68897A, IR68886A, IR73328A, IR75596A, IR70369A and IR79128A were selected for the development of hybrid rice on the basis of their better floral and agronomic characteristics. So far, ten rice hybrids have been developed and evaluated for yield and grain quality characteristics. Highest seed yield of > 1.24 t ha^{-1} was recorded in row ratio 2:10. These accomplishments have encouraged the rice scientists and the policy makers to develop and use hybrid rice technology in Pakistan.

Key words: Hybrid rice, restorers, maintainers, basmati, Pakistan

INTRODUCTION

Rice has a special significance in Asia, where about 90% of the rice is produced and consumed as a staple food. Considering the increasing requirement because of population increase on the one hand and decreasing land and water resources available for rice cultivation on the other, it is serious to develop and use rice technologies that will result in higher yields. Experience in china (Ma and Yuan, 2003) and outside China, in IRRI (Virmani, 2003), India (Mishra *et al*., 2003), Vietnam (Hoan and Nghia, 2003), the Philippines (Redona *et al*., 2003), Bangladesh (Julfiquar and Virmani, 2003) and several other countries clearly indicates that hybrid rice technology offers a viable option to meet this challenge. Hybrid rice varieties have clearly shown a 1-1.5 t ha^{-1} yield advantage over semidwarf inbred high-yielding varieties (HYVs) in farmers fields in china and other countries.

The increased yield of rice hybrids alone does not ensure profitability to farmers if their grain quality is not acceptable and if they fetch a low price in the market. Khush *et al*. (1988) studied this subject intensively and concluded that hybridity per se did not harm grain quality in terms of physical and chemical characteristics as long as both parents possess acceptable grain quality, hybrid rice breeding programs must give emphasis (if they have not done so in the past) to the critical evaluation of parental lines and hybrids for grain quality before these are released for commercialization.

Hybrid rice breeding programme at Rice Research Institute, Kala Shah Kaku was started in 1999. In 2000, this programme was strengthened by the Government with the approval of a research project entitled Development of Hybrid Rice in the Punjab. Hybrid rice is becoming popular in Pakistan due to the yield advantage over inbred varieties. Production of Basmati hybrids was also a part of this programme. The development of genetic tools essential for production of hybrid rice Cytoplasmic Male Sterile (CMS), maintainer and restorer lines- were initiated at the Institute (Akhter *et al*., 2006).

Expression of floral traits influencing out crossing was generally better in dry season than in winter season (Seetharamaah *et al*., 1993). McWilliam *et al*. (1995) found high frequency of restorers (21%) than was the maintainers (11%) from the evaluation of the 6000 testcrosses in India. On the other hand, less restorer and higher maintainer frequency was observed in the local germplasm of Pakistan (Sabar and Akhter, 2003). Ali and Khan (1996) also observed that frequency of the maintainers (63%) was much higher than that of restorers

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among 76 hybrids tested. Nanda and Virmani (2000) observed that IR58025A is the only reliable CMS line, being used for the development of commercial rice hybrids in India.

The objective of this study were to evaluate local and exotic genetic material for their use in hybrid rice breeding programme; to evaluate existing hybrids for yield, adaptability and grain quality and to initiate hybrid rice breeding for the development of parental lines..

MATERIALS AND METHODS

The establishment of testcross nursery to identify restorers and maintainers is the first step in three-line heterosis breeding. For this purpose, a source nursery comprising of uniform rice genotypes was transplanted on three different dates i.e., June 20th, July 5th and July 20th each year. Standard agronomic and plant protection measures were adopted during the years. From kharif 2000 to 2004, 608 test crosses were made among different elite lines and cms lines to categorize the existing germplasm as a restorer or maintainer line. Data on the agronomic characters of the potential restorers and maintainers were collected from 5 selected plants. Pollen studies were carried out for their fertility/sterility of testcross F_1 plants. For the purpose, 15-20 spikelets from the just emerged panicles of 3 randomly selected plants were collected in a vial containing 70% ethanol. All the anthers from 6 spikelets were taken out with the help of a forceps and placed on a glass slide with a drop of 1% Iodine Potassium Iodide (IKI) stain. the pollen grains were gently crushed with a needle to release the anthers. After removing the debris, a cover glass was placed and the slide was observed under the research microscope. The criteria for classifying the parental lines as maintainers and restorers were used as proposed by Virmani *et al*. (1997).

From 1993 to 2006 different IRRI hybrids received in the form of International Hybrid Rice Observational Nursery (IRHON) were evaluated for yield and other economic characteristics. Augmented in Randomized Complete Block Design has been adopted. Hybrids were transplanted with the spacing of 20×20 cm with one seedling per hill. The recommended plot size was 5×1.4 m for each entry. Standard agronomic and plant protection measures were adopted. Fertilizers at the rate of 170-100-0 NPK kg ha^{-1} were applied. Chemical protection against diseases and insect pests was need-based level. The data on heading days, plant height, tillers per plant, phenotypic acceptability, grain yield and cooking quality were collected in each year. The same procedure was adopted for testing of local hybrids.

For hybrid seed production, the restorer (Basmati 385) was seeded 3 times with intervals of three days between seeding. CMS (A) line i.e., IR 58025A was seeded 21 days after second seeding of restorer. Accordingly 30 days old nursery of these parents were transplanted in the field on their scheduled/repeated dates. Six row ratio treatments considered were as follows.

S. No.	Row ratio (R:A)	Field layout
1.	2:6	Two Restorer+Six CMS lines
2.	2:8	Two Restorer+Eight CMS lines
3.	2:10	Two Restorer+Ten CMS lines
4.	3:8	Three Restorer+Eight CMS lines
5.	3:10	Three Restorer+Ten CMS lines
6.	3:12	Three Restorer+Twelve CMS lines

Row length per treatment was 15 m. Plant to plant distance was 15 cm in all the three seeding of restorers. However, cms line seeding distance was spaced at 30×15 CMS. A space isolation of 50 m was provided for seed production plot to avoid the foreign pollen interception. To ensure purity of hybrid seed, roughing was done during different growth stages. At booting stage with the help of a sharp sickle ½ or 2/3 of the A line's flag leaf was removed from just above the flag leaf joint with the tiller. Two applications of GA_3 spray were made at the rate of 3 g/1000 m^2, the first when 15-20% of the tillers started heading and the second 2 days after first spray. A ULV/Knapsack sprayer was used for this purpose. A rope was used at the time of flowering for supplementary pollination. This operation was done 3-4 times daily at peak anthesis for ten days (Virmani *et al*., 1997). Restorer parent was harvested first. A line seed was harvested and threshed separately.

For seed multiplication of cms line (A), IR58025A and IR73328A were seeded on 20.05.2006. The seeding of respective maintainer (B) lines, were done on 22.05.2006 and 25.05.2006 to prolong the pollen availability period. The CMS lines along with their maintainer lines were transplanted in the field accordingly with plant spacing of 20×20 cm. At flowering, supplementary pollination by shaking maintainer lines with the help of a rope was carried out to ensure the proper pollination for having good seed setting of CMS line.

For evaluation of cms lines, twenty two Cytoplasmic Male Sterile (CMS) lines and their respective maintainer lines were transplanted in the field on 15.07.2005 in rows of 30 plants spaced at 22.5 cm from each side At flowering, all CMS lines were crossed with their respective maintainers to produce the nucleus seed of CMS lines as well. CMS lines were evaluated for pollen sterility (%), Out Crossing Rate (OCR) and agronomic traits i.e., Plant height, No. of tillers/plant, spikelets per panicle and days to 50% flowering.

The general reference for data collection was the Standard Evaluation System for Rice (Anonymous, 1996).

RESULTS AND DISCUSSION

Evaluation of genetic material: Out of 608 test crosses, 38 lines were identified as restorers. Among these restorer lines, 16 belong to Basmati group while the rest fall in coarse rice (Table 1). Similarly 52 maintainers were found from the tested test crosses. Out of these maintainer lines, 26 lines were Basmati and the rest were coarse lines (Table 2). The frequency of restorers and maintainers were 6 and 9% respectively amongst the tested genotypes. A similar results were also found by Ali and Khan (1996) Sabar and Akhter (2003) and Virmani and Kumar (2004) but are in contradict with the findings of McWilliam *et al.* (1995). Important characteristics like plant height, productive tillers per plant, maturity days and number of grains per panicle of potential restorers are given in Table 3.

Table 1: Elite genotypes identified as restorers

Genotypes	Group	CMS line (s) used.
TN 1	Coarse	IR58025A
Super fine	Coarse	SSMS2A
Shaheen Basmati	Basmati	IR68280A, IR58025A
PK3699-43	Coarse	IR58025A,
PK 178-2	Coarse	IR69617A
PK 1399	Coarse	IR58025A
OL-20	Basmati	IR58025A
OL-14	Basmati	IR58025A
LG-97	Coarse	IR58025 A, IR 68280A
LG-41	Coarse	IR58025A
LG-275	Coarse	IR68280A
LG-25	Coarse	IR68280A
LG-202	Coarse	IR58025A
LG16	Coarse	IR58025A
LG141	Coarse	R58025A
IRRI-GP 275	Coarse	IR58025A
IRRI-GP 2	Coarse	IR58025 A
IR74	Coarse	IR58025A
IR72	Coarse	IR58025A
IR 2053	Coarse	IR70369A, IR 69616A
IR 184	Coarse	IR69628A
Indian Basmati	Basmati	IR58025A
Basmati Pak	Basmati	IR68280A, IR 69617A
Basmati 385	Basmati	SSMS-1A, SSMS-2A, IR68280A, IR58025A, IR68275A, IR69628A, IR70372A, IR 69617A,
Basmati 370	Basmati	IR58025A, IR73321A,IR70372A,
99722	Coarse	IR67683A
98PP3	Basmati	IR69628A
96407	Coarse	IR58025A
60001	Basmati	IR69628A
49931	Basmati	IR70369A
485-4	Coarse	IR70362A, IR 69616A
48514-99	Coarse	IR58025A
48414	Basmati	IR58025A
45287	Basmati	IR58025A
4266	Basmati	SSMS2A
40265	Basmati	IR 69616A
33897-1	Basmati	IR68280A, IR70369A
33608	Basmati	IR68280A, IR 69616A, SSMS-2A

Table 2: Elite genotypes identified as maintainers

Genotypes	Group	CMS line(s) used.
00518-1	Basmati	IR58025A
00518-2	Basmati	IR58025A
1053-2-2	Basmati	IR 69616A
33797-1	Coarse	IR58025A, IR70362A
4029-3	Basmati	IR68280A
4291	Basmati	SSMS2A, IR68280A, IR69617A
4365	Basmati	SSMS2A, IR70369A,
47456	Basmati	IR58025A
4991	Basmati	IR 69616A
52773-2	Basmati	IR68280A
52799	Basmati	IR68280A
96408	Basmati	SSMS2A, IR69628A
97409	Basmati	IR68280A
97502	Basmati	SSMS2A
98313	Basmati	IR68280A
98801	Coarse	IR58025A
98PP17	Basmati	IR70372A
98PP47	Basmati	IR68280A
98PP50	Basmati	SSMS2A, IR69628
99509	Basmati	IR58025A, IR67684A, IR 69616A
99722	Coarse	IR 69616A
99723	Coarse	IR58025A
Basmati 198	Basmati	IR 69616A
Basmati 2000	Basmati	IR69628A, IR68280A, IR 69616A,
CB 268-02	Basmati	IR70372A, IR58025A
DR60	Coarse	IR58025A, IR69628A, IR70959A
DR83	Coarse	SSMS2A
IR 55186-46-4-3-2	Coarse	IR58025A
IR36	Coarse	SSMS2A, IR70369A, IR69628A, IR 69616A
IR6	Coarse	IR58025A, SSMS1A, SSMS2A, IR70959A
IR60	Coarse	IR68885A,
IR64	Coarse	IR58025A, SSMS2A
IR9	Coarse	SSMS2A, IR70369A, IR69628A, IR70362A, IR58025A
Kernal local	Basmati	IR58025A, IR69628A
KS282	Coarse	SSMS2A
LG116	Coarse	IR68280A
LG176	Coarse	IRS8025A
LG184	Coarse	IR68280A
LG-25	Coarse	IR58025A
LG60	Coarse	IR68280A
LG78	Basmati	IR68280A, IR70372A
OP56/99	Basmati	SSMS2A
PARC 154	Coarse	IR58025A
PARC 97	Coarse	IR58025A
PK 3717-12	Coarse	IR70372A
PK 3849-18	Coarse	IR58025A, IR70372A
PK578-20-1-2-1-1	Coarse	IR58025A
PK939-4-1-6	Coarse	IR58025A
Shadab	Coarse	IR58025A
Shaheen Basmati	Basmati	IR58025A, IR70959A
Shua 92	Coarse	IR58025A, IR 69616A
Super Basmati	Basmati	IR69628A, IR68280A, SSMS2A, IR70369 A, IR75606A

Table 3: Characteristics of potential restorers

Designation	Plant height (cm)	Tillers/plant	Grains/panicle	Maturity days
Basmati 385	124	16	139	108
96407	103	14	98	95
TN1	128	19	126	105
68926	80	18	107	88
PK3699-43	93	14	130	103
Shaheen Basmati	132	16	115	100
LG 202	108	13	86	100
OL14	128	12	115	104
40265	126	16	147	111
OL20	130	14	116	100
LG22	104	13	108	105
KSK 433	106	24	110	102
KSK 438	70	24	104	97
33608	125	17	145	105
99722	119	15	68	103
IR72906-24-1-3-1R	85	18	91	100
IR72998-93-3-3-2R	68	19	75	90
IR73014-59-2-2-2R	81	14	60	97

Table 4: Performance of exotic rice hybrids

Hybrids	Yield ($t\ ha^{-1}$)	Standard heterosis % (over KS282)	Heading days	Height (cm)	Tillers per plant	Phenotypic acceptability	Cooking quality
IR76715H (IR68897A/RP2087-194-1-2-2R)	11.76	142	120	118	23.0	3	<KS 282
IR76712H (IR68897A/IR68427-8-3-3-2R)	11.34	133	103	106	15.6	3	<KS 282
IR79118H (IR73328A/IR69702-78-3-3R)	10.82	123	96	112	16.2	3	<KS 282
IR68284H (IR58025A/IR34686-179-1-2-1R)	10.61	118	104	110	15.0	3	<KS 282
IR75587H (IR68899A/IR62162-184-3-1-3-2R)	9.74	100	101	104	14.0	3	<KS 282
IR80641H (IR 75596A/IR73014-259-2-2-2R)	8.75	80	109	113	23.2	5	<KS 282
IR64616H (IR68829A/IR29723-143-3-2-1R)	8.16	68	103	97	22.0	3	<KS 282
IR73410H (IR69626A/IR46R)	7.37	51	102	117	20.0	1	<KS 282
IR75583H (IR68897A/IR62653-8-3-3R)	7.26	50	119	116	18.0	3	<KS 282
IR68284H	7.17	48	104	110	15.0	3	<KS 282
IR58025A/IR65509-22-1-2-1R	6.93	42	98	108	16.6	3	<KS 282
IR81971H (IR73827-23S/IR73759-128-1-3-3-1-1)	6.89	29	107	116	15.6	5	<KS 282
IR82386H (IR79125A/IR73885-1-4-3-2-1-10R)	6.73	28	74	98	12.6	5	<KS 282
IR68284H (IR58025A/IR34686-179-1-2-1R)	6.44	33	104	110	12.0	7	<KS 282
IR80785H (IR70959A/IR68427-17-2-3-1R)	6.38	31	102	84	12.0	7	<KS 282
IR58025 A/IR65507-58-1-3-1R	6.19	26	98	109	17.0	3	<KS 282
IR81247H (IR 68897A/IR69702-57-1-1R)	6.13	26	116	117	18.6	5	<KS 282
IR81948H (IR 68897A/IR73004-142-2-3R)	5.45	12	111	125	16.8	5	<KS 282
IR83212H (IR 79156A/IR75282-58-1-2-3 R)	5.23	8	64	127	10.0	3	=KS 282
KS282 (Local check)	4.85	-	72	105	13.0	1	Good

Evaluation of exotic rice hybrids: From 1993 to 2006, 800 hybrids were evaluated for yield, adaptability and standard heterosis. Heading days, height, tillers, phenotypic acceptability and cooking of hybrids were studied from 1996 to 2006 (Table 4). Standard heterosis of hybrids varied from 8 to 142% over KS 282 (local check variety). Maximum yield of 11.76 $t\ ha^{-1}$ was given by the hybrid IR76715H, while the lowest yield of 5.23 $t\ ha^{-1}$ was given by the IR83212H. The present data depicted that there were rice hybrids those have higher yield than

Table 5: Performance of promising local rice hybrids

Hybrid/ variety	Plant height (cm)	Tiller/ plant	Mat. days	Grains/ panicle	1000 Grain weight	Spikelet fertility (%)	Paddy yield t ha^{-1}	Standard heterosis (%)	Cooking
LH1	117	19	102	223	22.7	85	5.06	+20	= Basmati 385
LH10	102	21	92	137	23.1	78	5.27	+14	<KS 282
LH15	106	14	94	168	24.8	80	5.60	+ 21	= KS 282
Bas.385 (check for LH1)	136	17	104	149	21.2	90	4.20	-	Good
KS 282 (check for LH10 and LH15)	96	13	90	119	25.0	85	4.60	-	Good

+ increase over check

Table 6: CMS Lines and their characteristics

Designation	Plant height (cm)	Tillers /plant	50% flowering days	Spikelet per panicle	OCR (%)	Pollen sterility (%)
IR58025A	74	12.7	93	116	30	100
IR62829A	62	15.0	88	141	38	100
IR68275A	75	16.7	100	137	17	100
IR68280A	71	21.7	104	133	20	100
IR68885A	76	18.0	94	73	28	100
IR68886A	80	19.0	102	89	37	100
IR68897A	82	24.0	87	79	46	100
IR68902A	77	13.7	99	95	15	100
IR69616A	73	11.7	105	170	29	100
IR69626A	80	15.3	83	112	18	100
IR70369A	79	30.0	87	144	40	100
IR70372A	85	14.7	119	120	27	100
IR70959A	89	19.0	91	145	15	100
IR72788A	72	21.3	98	107	23	100
IR73322A	80	21.0	101	146	19	100
IR73328A	84	17.0	93	130	31	100
IR73794A	73	16.7	102	101	25	100
IR75596A	56	23.0	84	86	30	100
IR75606A	94	14.3	105	96	16	100
IR79128A	60	18.0	87	68	32	100
IR79156A	72	24.4	77	101	26	100
SSMS2A	81	13.0	105	85	30	100

approved check variety i.e., KS 282. Ali (1998), Ali and Khan (1995) and (Virmani and Kumar (2004) also found similar results. Cooking quality of the out yielding hybrid was recorded as poor compared to the check variety. These results are in accordance with the findings of Singh *et al.* (2000) and Khush *et al.* (1988).

Heading days of the tested hybrids ranged from 64 to 120 days compared to check variety which has about 72 days (Table 4). Early maturing hybrid was IR83212H while the late maturing hybrid was IR76715H. The tested hybrid IR 80785H obtained the shortest height (84 cm), while the tallest hybrid was IR 83212 H with the plant height of 127 cm. The check variety obtained the height of 105 cm. Regarding tillers per m^2, most of the hybrids had more number of tillers than the check variety (KS 282).

IR 73410H exhibited excellent crop stand and grain appearance having rank No. 1 for phenotypic acceptability, while two hybrids had rank No. 7 for the same trait. Cooking quality of the out yielding hybrid was recorded as poor compared to the check variety except IR 83212H. These results are in accordance with the findings of Singh *et al.* (2000) and Khush *et al.* (1988). Grain quality characteristics are very important parameters for the determining consumer favorable reception for any hybrid. It has been found that the grain quality of hybrids in not comparable to that of premier quality varieties such as basmati 385 and KS 282. Since most of the out yielding hybrids have inferior cooking quality and poor phenotypic acceptability, therefore, there is a dire need to develop local parental lines for the development of rice hybrids having high yield, good adaptability and acceptable cooking and eating quality.

Evaluation of new rice hybrids: In Basmati group, LH-1 was the only local test hybrid. LH-1 gave 20% standard Heterosis for paddy yield over the check variety Basmati 385 (Table 5). The maturity days of LH-1 were at par with Basmati 385. In coarse group, 2 rice test hybrids LH-10 and LH-15 performed better than the check variety KS282. Standard Heterosis for paddy of LH-15 and LH-10 was 21 and 14%, respectively. Plant height and maturity days were at par with KS282.

Evaluation of CMS lines: Morphological characteristics of these 22 CMS lines were recorded. Complete pollen sterility was observed in all lines. All CMS lines were also evaluated for agronomic traits (Table 6). Semi dwarf plant height was observed in all lines except IR75606A which had intermediate height. Better tillering ability was observed in all CMS lines. The range of spikelets per panicle was 68-170. IR 69616A was remained on the top with 170 spikelets/panicle whereas IR 79128A was remained at the bottom. Six CMS line were late in maturity i.e.,>100 days for 50% flowering. IR 68897A gave maximum out crossing rate i.e., 46% amongst all the cms lines followed by IR 70369A having 40% out crossing rate.

On the basis of the traits evaluated, IR58025A, IR79156A, IR68897A, IR68886A, IR73328A, IR75596A, IR70369A and IR79128A can be recommended for hybrid rice seed production on the basis of their better floral and agronomic characteristics. For the development of new local cms lines forty six maintainers are in CMS conversion programme. These are 3, 3, 6 and 34 lines, in BC_5, BC_3, BC_2 and BC_1, respectively.

Hybrid rice seed production: Highest seed yield of >1.24 t ha^{-1} was recorded in row ratio 2:10. However, the seed yield from 0.80-1.10 t ha^{-1} was produced by row ratios 2:12, 3:10 and 3:12. Lowest hybrid seed yield was produced in row ratio 3:8. These findings were also observed by Ali *et al*. (2005). Better synchronization and more number of tillers of the restorers and CMS line can produce > 3.0 t ha^{-1}.

CONCLUSION

The study was a good start to categorize the local germplasm for hybrid rice research studies. Basmati 385, 99722, 40265, PK3699-43 and LG22 were identified as potential restorers. IR58025A, IR79156A, IR68897A, IR68886A, IR73328A, IR75596A, IR70369A and IR79128A were selected for the development of hybrid rice on the basis of their better floral and agronomic characteristics. For hybrid seed production, the row ratio 2:10 (Restorer: CMS) gave the maximum seed yield i.e., >1.5 t ha^{-1}. So far, ten rice hybrids have been developed and evaluated for yield and grain quality characteristics. Rice hybrids having higher magnitude of heterosis, better grain quality and resistance to major pests and diseases are needed to be developed. Top priority has to be given to maintain the purity of parental lines and to produce high quality hybrid seed. Involvement of seed agencies from private sector will be crucial to meet the increased demand for hybrid seed.

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