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Original Research Article

EVALUATION OF TEST CROSSES A WAY FORWARD TOWARDS THE IDENTIFICATION OF POTENTIAL RESTORERS & MAINTAINERS FOR THE DEVELOPMENT OF RICE HYBRIDS

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Abstract: *Identification of desirable maintainers and potential restorers in a hybrid rice breeding program is the dire need for the development of potential rice hybrids. Seventeen (17) cytoplasmic male sterile (CMS) lines (15 imported from IRRI and 02 locally developed by RRI, KSK) of rice were crossed with one hundred and sixty (160) testers to identify their genetic nature to behave as restorer or maintainer in the year 2017 and 2018. A total of one hundred and sixty (160) rice test crosses were subjected to pollen and spikelet fertility when crossed with CMS lines. Most of the testers show differential fertility/sterility responses with CMS lines. From these evaluated test crosses thirty-three (33) restorers and thirty-four (34) maintainers (16 Basmati and 18 non-basmati lines) were recognized for further use to develop potential rice hybrids. Four basmati and twenty-nine coarse lines were identified as restorers from the tested genotypes. Most of the genotypes found to be partial restorer (PR) and partial maintainers (PM). Moreover, four promising heterotic combinations/potential restorers were also identified. Six heterotic hybrid combinations had more than 80% spikelet fertility. Potential restorers and maintainers identified in the present exploration could be used to develop high-yielding promising rice hybrids adaptable to the diversified climatic conditions of Punjab, Pakistan.*

Keywords Hybrid rice, restorer, maintainer, testcross, hybrid breeding

Introduction

Rice plays a significant role in the agricultural economy of Pakistan. Pakistan is the 11th largest producer and the 4th largest exporter of rice. Rice accounted for 3.1% of the value added in agriculture and 0.6% of the GDP in Pakistan. Being a main food as well as cash crop, rice holds an important place in the agriculture of Pakistan. During 2019-20, rice 3,034 million hectares increased by 8.0 percent compared to 2,810 thousand hectares last year. The production increased by 2.9 percent to 7.410 million tonnes against 7.202 million tonnes last year (Economic survey of Pakistan, 2019-20). One million ton increase due to surplus production is due to hybrid rice seed technology. The total hybrid rice area under cultivation is 1.700 million acres, while its % share is 23% in Pakistan Hybrid rice area in Sindh & Baluchistan is 1.2 million acres (52%) while 0.03 million acres (6%) in Punjab, Pakistan (Agriculture Statistics of Pakistan, 2019-20).

The yield of rice in Punjab, Pakistan, has plateaued, and there is a dire need to opt for new research innovations like hybrid rice technology to break this stagnant yield barrier. Experience in China (Ma and Yuan 2003) and outside China, in IRRI (Virmani 2003, 2006), 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 specifies that hybrid rice technology offers a feasible option to meet the productivity and food scarcity challenges. Hybrid rice seed production technology is the only innovative solution that can enhance rice productivity to meet the increasing demand, contributing much to food security. Hybrid rice has been introduced and promoted by more than 40 countries around the world, among which India, Bangladesh, Indonesia, Vietnam, the Philippines, and the United States have planted hybrid rice extensively. The cultivated area of hybrid rice reached 20 m/ha with a 2 tons incremental average yield per hectare more than the fine local varieties.

Rice hybrids gave 15-20% more yield advantage over inbred varieties (Islam & Bhuiya, 2010). Due to this, a substantial increase in hybrid production and export

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of non-basmati rice export is encouraging for this sector. High-yielding hybrid rice will increase to 50% of the total area planted with paddy in the next three years, from 25-30%. This will increase the output by 2 million tons.

Development of hybrids rice through cytoplasmic male sterility (CMS) system is only possible when effective parental lines, including maintainers (B) and restorers (R) are identified. CMS lines introduced from China may not be well adapted to a target area. Pollen fertility or spikelet fertility, or both, are considered an index to fix the restoration ability of the lines (Itha *et al*., 2020). Locally adapted maintainers (B) and restorers (R), which depict complete sterility and a high degree of restoration ability of CMS lines along with high combining ability, plays an important role in commercial hybrid rice breeding programme (Anon, 2020). Raghavendra & Hittalmani (2016) observed a higher frequency of restorers (21%) than that of the maintainers (11%) after the assessment of the 6000 test crosses in India. Nevertheless, Ali and Khan (1996) perceived that the maintainers (63%) frequency was much higher than restorers out of 76 test crosses. Akhter *et al*., (2007) and Sabar *et al*., (2007) results also coincide with the findings of Ali and Khan (1996). Hence, the present exploration was commenced to identify maintainer and restorer lines from local rice genotypes against exotic and locally developed cyto-sources (CMS).

Materials and methods

New Fresh test crosses 2016 & 2017

The rice gene pool was appraised to recognize the commercially functional restorers (R) and maintainers (B) in basmati and coarse background at Rice Research Institute, Kala Shah Kaku. For this purpose, ninety-three (93) genotypes/lines of source nursery were transplanted in the field in single row plots of 2.5m length on three different dates i.e., 25.06.2016, 10.07.2016 & 25.07.2016. Recommended crop management practices were adopted. Desirable lines were utilized for making 52 fresh test crosses with ten CMS lines i.e., IR 58025A, IR62829A, IR68275A, IR68280A, SSMS2A, KSK1501A, IR69616A, IR70372A, IR73794A and KSK99404A. At maturity, the seed of a single representative plant from each entry of the source nursery was collected for next season's testing in testcross nursery-2017.

Evaluation of test crosses in test cross nursery 2017 & 2018:

One hundred and four entries (104) comprising the fifty-two (52) test crosses along with their respective pollen parents were sown in test Cross Nursery-2017 (table 1). A single row of ten plants per cross with 22.5 cm spacing on each side transplanted in the field. Recommended agronomic practices and crop protection measures adopted for healthy growth.

During 2018, two hundred and sixteen (216) entries comprising the one hundred and eight (108) test crosses along with their respective pollen parents were sown in test Cross Nursery-2018 (table 2). A single row of twelve plants per cross with 22.5 cm spacing on each side was transplanted in the field. Recommended agronomic practices and crop protection measures were adopted for healthy growth.

Pollen study

During the year's i.e., 2017 and 2018, Pollen sterility of the F_1 was determined by staining pollen grains in 1% potassium iodide-iodine (IKI) solution. At heading, about 25 spikelets of F_1 were collected in the morning just before their blooming and fixed in 70% alcohol. All the anthers from ten spikelets were removed and placed in the stain. Pollen grains were released using a needle. After removing the debris these were observed in a microscope at 10x. The entire slide was scanned, and pollen sterility was counted in three random fields. At maturity, spikelet fertility/sterility was also recorded to confirm the microscopic studies. The criteria for classifying the parental lines as maintainers and restorers were used as proposed by Virmani *et al*. (2003).

Results and discussion

Out of fifty-two (52), fresh test crosses evaluated in the test cross nursery 2017, nine (9) maintainers and ten (10) restorers were categorized based on pollen and spikelet sterility/fertility studies. The rest of the test lines were categorized as partial maintainers and partial restorers. All the maintainers were backcrossed with the recurrent parent for the development of new CMS lines. Out of one hundred (108) fresh test crosses evaluated in the test cross nursery 2018, twenty-five (25) maintainers (table 3) and twenty-three (23) restorers (table 4) were identified based on pollen and spikelet sterility/fertility studies. Rest of the test lines were categorised as partial maintainers and partial restorers. All the maintainers were

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backcrossed with the recurrent parent for the development of new CMS lines. From 160 test hybrids evaluated during both the years (2017 and 2018), 33 restorers, 43 partial restorers, 50 partial maintainers and 34 maintainers were categorized based on pollen and spikelet sterility/fertility studies. The frequency of restorers, partial restorers, partial maintainers and maintainers were 13 %, 35%, 38 % and 14%, respectively.

Four basmati and twenty-nine coarse lines were known as restorers from the tested test crosses. The CMS line(s) used for these restorers are given against each in Table 1. The maximum pollen fertility (95.7%) was observed in the cross of B 6144 with IR58025A. While the minimum pollen fertility (72.5%) was observed in the cross of OP3 with IR69628A. All the CMS lines used carried wild abortive (WA) cyto-sterility source. Among these 12 restorers, Basmati 370 and Basmati 385 are the approved and commercial varieties having good cooking quality traits. Sixteen of these 239 testcrosses (8 Basmati and 8 coarse) were identified as maintainers.

Conclusion

Many hybrids have been released for general cultivation, but a majority of them have been introduced from China and could not acclimatize well under our tropical agro-ecological zone. Hence identification of potential maintainers and restorers for emerging hybrids suitable for our local environmental conditions holds great significance.

Table 1: Testcross nursery- 2017

Sr. No	TC No.	Designation	Sr.No	TC No.	Designation
1	TC1	IR58025A X	109	TC109	IR 73322A/
2	TC2	Khush Basmati	110	TC110	KSK 607
3	TC3	IR58025A X	111	TC111	KSK99404A/
4	TC4	Super Hamalea	112	TC112	Basmati 515
5	TC5	IR58025A X	113	TC113	KSK99404A/
6	TC6	PK1121 Aromatic	114	TC114	Kissan Basmati
7	TC7	IR58025A X	115	TC115	KSK99404A/
8	TC8	5015	116	TC116	Basmati 2000
9	TC9	IR58025A X	117	TC117	KSK99404A/
10	TC10	PK 10198	118	TC118	PK1121 Aromatic
11	TC11	IR58025A X	119	TC119	KSK99404A/
12	TC12	PK 10324	120	TC120	PK8892
13	TC13	IR58025A X	121	TC121	KSK99404A/
14	TC14	PK 9966	122	TC122	BAS385
15	TC15	IR58025A X	123	TC123	KSK99404A/
16	TC16	EL-10	124	TC124	FDM 19
17	TC17	IR62829A X	125	TC125	KSK99404A/
18	TC18	Super Hamalea	126	TC126	Basmati Pak
19	TC19	IR62829A X	127	TC127	KSK99404A/
20	TC20	KSK 609	128	TC128	EL1
21	TC21	IR62829A X	129	TC129	KSK99404A/
22	TC22	KSK 612	130	TC130	Basmati 370
23	TC23	IR68275A X	131	TC131	KSK99404A/
24	TC24	KSK 613	132	TC132	PK10029-13-2-1
25	TC25	IR68280A X	133	TC133	KSK99404A/
26	TC26	PKSub 15-38	134	TC134	33083
27	TC27	IR68280A X	135	TC135	KSK99404A/
28	TC28	Kissan Basmati	136	TC136	5015
29	TC29	IR68280A X	137	TC137	KSK99404A/
30	TC30	PK1121 Aromatic	138	TC138	Basmati 198
31	TC31	IR68280A X	139	TC139	KSK99404A/
32	TC32	KSK 484	140	TC140	PK2021 Aromatic
33	TC33	SSMS2A X	141	TC141	KSK99404A/
34	TC34	PK10198-7-2	142	TC142	Chenab Basmati
35	TC35	SSMS2A X	143	TC143	KSK99404A/

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36	TC36	PK9565-10	144	TC144	FDM24
37	TC37	SSMS2A X	145	TC145	KSK99404A/
38	TC38	PK10299-6-3	146	TC146	Super Gold
39	TC39	SSMS2A X	147	TC147	KSK99404A/
40	TC40	PK10306-15-5	148	TC148	Punjab Basmati
41	TC41	SSMS2A X	149	TC149	KSK99404A/
42	TC42	PK10324	150	TC150	Super Hamalea
43	TC43	SSMS2A X	151	TC151	KSK99404A/
44	TC44	PK9966-10-1	152	TC152	PK10161-1-5-1
45	TC45	SSMS2A X	153	TC153	KSK99404A/
46	TC46	PK10161	154	TC154	PK 386
47	TC47	SSMS2A X	155	TC155	KSK99404/
48	TC48	RRI 4	156	TC156	KSK 704
49	TC49	KSK1501A X	157	TC157	KSK99404A/
50	TC50	PKBB15-177	158	TC158	PKBB-15-1
51	TC51	KSK1501A X	159	TC159	KSK99404A/
52	TC52	PKBB15-184	160	TC160	RRI 3
53	TC53	KSK1501A X	161	TC161	KSK99404A/
54	TC54	PKBB15-173	162	TC162	Super Bas
55	TC55	KSK1501A X	163	TC163	99404A/
56	TC56	PK10299-6-3	164	TC164	PK9966
57	TC57	KSK1501A X	165	TC165	99404A/
58	TC58	PK10324	166	TC166	KSK 624
59	TC59	KSK1501A X	167	TC167	IQS938A/
60	TC60	PK9966	168	TC168	Super Hamalea
61	TC61	IR69616A X	169	TC169	IQS938A/
62	TC62	KSK 601	170	TC170	EL 10
63	TC63	IR69616A X	171	TC171	IQS938A/
64	TC64	RRI-3	172	TC172	KSK 644
65	TC65	IR69616A X	173	TC173	IQS938A/
66	TC66	PK10198-7-2	174	TC174	Basmati 385
67	TC67	IR69616A X	175	TC175	IQS938A/
68	TC68	PK9966-10-1	176	TC176	Bas pAK
69	TC69	IR69616A X	177	TC177	IQS938A/
70	TC70	PK 10324	178	TC178	Super Bas
71	TC71	IR69616A X	179	TC179	IQS938A/
72	TC72	F4 83/732	180	TC180	Chenab Bas
73	TC73	IR70372A X	181	TC181	IR62829A
74	TC74	Kissan Basmati	182	TC182	Basmati 385
75	TC75	IR70372A X	183	TC183	IQS938A/
76	TC76	KSK 484	184	TC184	PK1121 Aromatic
77	TC77	IR73794A X	185	TC185	IQS938A/
78	TC78	PK1121 Aromatic	186	TC186	PK9533-9
79	TC79	IR70372A X	187	TC187	IQS938A/
80	TC80	Punjab Mehran Bas.	188	TC188	Basmati 515
81	TC81	IR73794A X	189	TC189	IQS938A/
82	TC82	Kissan Basmati	190	TC190	KSK434
83	TC83	IR73794A X	191	TC191	IQS938A/
84	TC84	5015	192	TC192	BAS2000
85	TC85	IR73794A X	193	TC193	IQS938A/
86	TC86	KSK 484	194	TC194	KSK 709
87	TC87	IR73794A X	195	TC195	IQS938A/
88	TC88	Super Hamalea	196	TC196	PK 9966
89	TC89	IR73794A X	197	TC197	IQS938A/
90	TC90	PK1121 Aromatic	198	TC198	PK 386

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91	TC91	IR70372A X	199	TC199	IQS938A/
92	TC92	KSK 607	200	TC200	BAS198
93	TC93	99404A X	201	TC201	IQS938A/
94	TC94	Bas 385	202	TC202	Kissan Basmati
95	TC95	99404A X	203	TC203	IQS938A/
96	TC96	PKBB-15-116	204	TC204	DSR10
97	TC97	99404A X	205	TC205	IQS938A/
98	TC98	Bas 198	206	TC206	5015
99	TC99	99404A X	207	TC207	IQS938A/
100	TC100	KSK 434	208	TC208	KSK 643
101	TC101	99404A X	209	TC209	IQS938A/
102	TC102	EL-10	210	TC210	Punjab basmati
103	TC103	99404A X	211	TC211	IQS938A/
104	TC104	KSK 643	212	TC212	IR58025B
105	TC105	KSK4365A/	213	TC213	IQS938A/
106	TC106	Super Hamalea	214	TC214	33083
107	TC107	IR 73322A/	215	TC215	KSK8892A/
108	TC108	Bas 385	216	TC216	PK1121 Aromatic

Table 2: Testcross nursery- 2018

Sr. No.	TC No.	Designation	Sr. No.	TC No.	Designation
1	TC1	IR58025A/	53	TC53	IR73328A/
2	TC2	KSK 611	54	TC54	Kissan Basmati
3	TC3	IR58025A/	55	TC55	IR73328A/
4	TC4	Khush Basmati	56	TC56	Super Hamalea
5	TC5	IR58025A/	57	TC57	IR73328A/
6	TC6	98PP-7	58	TC58	KS 282
7	TC7	IR58025A/	59	TC59	IR73328A/
8	TC8	DGWD	60	TC60	48463
9	TC9	IR58025A/	61	TC61	IR73328A/
10	TC10	IRBB66	62	TC62	KSK434
11	TC11	IR58025A/	63	TC63	IR73328A/
12	TC12	IRBB23	64	TC64	PK386
13	TC13	IR58025A/	65	TC65	IR73328A/
14	TC14	KSK 621	66	TC66	PK1121 Aromatic
15	TC15	IR58025A/	67	TC67	IR73328A/
16	TC16	KSK 613	68	TC68	5015
17	TC17	IR58025A/	69	TC69	IR73328A/
18	TC18	KSK 620	70	TC70	CHENAB BAS
19	TC19	IR58025A/	71	TC71	IR73794A/
20	TC20	KSK 699	72	TC72	PK1121 Aromatic
21	TC21	IR58025A/	73	TC73	IR73794A/
22	TC22	KSK 700	74	TC74	DSR-12-13-14
23	TC23	IR58025A/	75	TC75	IR73794A/
24	TC24	KSK 701	76	TC76	Kissan Basmati
25	TC25	IR58025A/	77	TC77	IR73794A/
26	TC26	KSK 702	78	TC78	Khush Basmati
27	TC27	IR58025A/	79	TC79	IR73794A/
28	TC28	KSK 703	80	TC80	Basmati 385
29	TC29	IR58025A/	81	TC81	IR73794A/
30	TC30	PK15-9	82	TC82	Super Hamalea
31	TC31	IR70372A/	83	TC83	IR73794A/
32	TC32	DGWG	84	TC84	KSK 704
33	TC33	IR70372A/	85	TC85	IR73794A/

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34	TC34	IRBB66	86	TC86	PD-17
35	TC35	IR70372A/	87	TC87	IR73794A/
36	TC36	IRBB23	88	TC88	5015
37	TC37	IR68897A/	89	TC89	IR73794A/
38	TC38	KSK 611	90	TC90	KSK 606
39	TC39	IR68897A/	91	TC91	IR73794A/
40	TC40	Khush Basmati	92	TC92	PKBB 1
41	TC41	IR68897A/	93	TC93	IR73794A/
42	TC42	98PP-7	94	TC94	KSK614
43	TC43	IR68902A/	95	TC95	IR73794A/
44	TC44	KSK 620	96	TC96	KSK 705
45	TC45	IR68902A/	97	TC97	IR75596A/
46	TC46	KSK 621	98	TC98	Kissan Basmati
47	TC47	IR68902A/	99	TC99	IR75596A/
48	TC48	PD 17	100	TC100	Punjab Mehran Basmati
49	TC49	IR68902A/	101	TC101	IR75796A/
50	TC50	Khush Basmati	102	TC102	IRBB66
51	TC51	IR68902A/	103	TC103	KSK4365A/
52	TC52	98PP-7	104	TC104	KSK 620

Table 3: Maintainers (B) identified from Testcross Nursery 2017 & 2018

IR58025A	Khush Basmati
	PK 10324
	KSK 611
	98PP-7
	KSK 600
SSMS 2A	PK9565-10
	PK10299-6-3
	PK10324
KSK1501A	PKBB5-184
	PKBB15-173
	PK1121 Aromatic
	Super Hamlea
IR73794A	Kissan Basmati
	Khush Basmati
	PD-17
IR70372A	IRBB23
IR68897A	KSK 611
	98PP-7
	PD 17
IR68902A	Khush Basmati
	98PP-7
	Super Hamlea
KSK99404A	Basmati 515
	Basmati 370
	PK10029-13-2-1
	Basmati 198

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KSK 716A	PK2021 Aromatic
	PKBB15-116
	KSK 624
	Basmati Pak
	Chenab Bas
	KSK434
	IR58025B

Table 4: Restorers (R) identified from Testcross Nursery 2017 & 2018

IR58025A	DGWD
	IRBB66
	IRBB23
	KSK 613
	KSK 620
	KSK 700
	KSK 702
	KSK 703
	5015
	PK 9966
IR68902A	KSK 621
IR73328A	Kissan Basmati
IR73794A	KSK 703
	5015
	KSK 606
	KSK 614
	Kissan Basmati
IR75596A	Punjab Mehran Basmati
KSK4365A	IRBB66
	KSK 620
IR73322A	KSK 607
KSK99404A	Kissan Basmati
	EL-10
	KSK 643
KSK 716A	KSK 644
	Kissan Basmati
IR62829A	KSK 643
	KSK 612
IR68280A	PK1121 Aromatic
IR69616A	F4 83/732
IR70372A	Kissan Basmati
	PK1121 Aromatic
	Punjab Mehran Basmati

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	5015
	Super Hamalea
	KSK 607

Conflicts of interest

Authors declared no conflict of interest.

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