Identification of Maintainers and Restorers for Development of Medium Slender and Short Grain Aromatic Rice Hybrids

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A B S T R A C T

A study was undertaken to identify maintainers and restorers for their utilization in development of aromatic rice hybrids. The availability of stable cytoplasmic male sterility and fertility restoring system is vital for commercial exploitation of heterosis in rice; it means CMS is a common phenomenon that has been extensively used for production of hybrid seeds in rice crop. Total 60 test crosses were evaluated for identification of restorers and maintainers. These 60 test crosses were attempted with crossing of 20 aromatic rice genotypes (testers) and 3 CMS lines. From these 60 test crosses 8 restorers and 10 maintainers were identified for use in aromatic hybrid rice breeding programme. Most of the genotypes were found partial restorers and partial maintainers. In this study more numbers of maintainers were identified, is a key finding, these identified maintainer will be used to develop fine grain aromatic stable CMS lines. Most of the CMS lines developed so far are long slender or coarse grain non aromatic type. The CMS lines will be developed from this breeding programme are fine grain aromatic type.

Introduction

Rice is the most important staple food crop in the world which plays major role in Indian economy. More than 90% of the world's rice is grown and consumed in Asia. For increased rice production and productivity, development and dissemination of hybrid rice technology has played as major and vital role. The use of cytoplasmic genetic male sterility system for developing hybrids in crops is possible only when effective maintainers and restorers are identified. Use of male sterility system would be appropriate approach for commercial exploitation of heterosis.

CMS system is controlled by the interaction of cytoplasm with nuclear genes (Kaul 1997). Chhattisgarh state has rich genetic diversity of aromatic rice traditional varieties, majority of them having small to medium slender grains.

These rices possessing unique aroma, cooking and eating qualities are consumed locally as a delicacy, therefore these rices fetches very high price in the market. These aromatic local varieties are very tall and late maturing. Due to taller stature they are prone to lodging at the time of booting to maturity stage, which is responsible for its poor yield. Due to late maturity they require high inputs as well more

care for their cultivation. Through the use of hybrid technology it is possible to develop high yielding aromatic medium slender to short grain hybrid varieties. Development of a hybrid in rice using CMS requires identification of a male sterile line (A-line) a maintainer line (B-line) and a restorer line (R-line) (Madhuri *et.al*, 2018). Test cross programmes helps to identify maintainers as well as restorers. Maintainer lines for CMS development and restorer lines use as a male parent in hybrid development programme.

Materials and Methods

Experimental site

The experiment was conducted at Director cum Instructional farm, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh.

Materials

Twenty aromatic rice genotypes including traditional varieties/land races, and farmers' varieties were crossed with three CMS lines (Table-1) in Line x Tester fashion to generated 60 test crosses.

Nursery sowing

The three CMS lines and twenty testers were sown in nursery bed during *Kharif*-2018-2019. Staggered sowing of parent material was done to synchronize the flowering during hybridization. The 21 days old seedlings were transplanted.

Hybridization

Selected 20 testers were crossed with each of the three CMS lines to generate a set of hybrids in a line x tester manner as proposed by Kempthorne (1957). In order to increase the seed setting, the spikelet's of CMS plant that were expected to flower the next day

were clipped one third at the top. The clipped panicles were completely covered with butter paper bags to avoid outside pollen grains. In the next morning (about 8:30 am) panicles from the male parents (testers) were collected during anthesis and were kept in pollination room at temperature of 40^0C under high humidity in glass bottles.

After the completion of anthesis the pollen grains were carefully dusted over clipped spikelets. The pollinated panicles were immediately bagged again and labelled properly mentioning the date and cross combination. In this manner, 60 crosses were attempted for the present study.

The bagged panicles were harvested and separated from mother plants after 30-35 days of hybridization. The hybrid seeds of each cross were collected separately and sun dried. The seeds were treated with bavistin as a precautionary measure against fungal attack before storing in paper bags.

Raising F_1's and parents

During *Rabi* 2018-19, the treated seeds of the 60 hybrids generated during the previous season were carefully germinated in nursery beds. Three CMS lines (A lines/male sterile lines) and their isogenic lines (Maintainer/B lines) along with 20 elite testers (pollen parents) were also sown in nursery beds on the same day.

Planting of F_1's and parents in Kharif 2019

About 21 days old seedlings of 60 Test cross hybrids were planted in Randomized Complete Block Design (RCBD) design with their 23 parents (3 lines along with the isogenic B lines and 20 testers).

The distance between row to row and plant to plant was 20 X 20 cm and keeping single

seedlings per hills in 2.4 meter row length. Transplanting of material was done manually with standard agronomic package practices were adopted to raise ideal crop stand.

Estimation of pollen testing

Testing of pollen fertility of the test cross F_1 was carried out for their fertility or sterility responses. The spikelets (5 to 10) from the just emerged panicle of 3 randomly selected plants were collected in vial containing 70 percent ethanol. With the help of forceps, the anthers from the spikelets were placed on a glass slide containing 2% Iodine Potassium Iodide (IKI) strain. Then the anthers were gently crushed by using needle to release the pollen grains. After removing the debris, a covers lip was put on the slide and observed under microscope. Five panicles of each testcross were covered with butter paper bags to avoid foreign pollen contamination and were harvested at maturity for recording spikelet fertility/sterility observation. The following criteria for classifying the parental lines as maintainers and restorers were used as proposed by Virmani *et al.,* (1997).

Table.A Criteria for classification of restorer and maintainers based on pollen fertility/sterility basis

Category	Pollen fertility (%)	Spikelet fertility (%)
Maintainers	0-1	0
Partial maintainers	1.1-50	0.1-50
Partial restorers	50.1-80	50.1-75
Restorers	>80	>75

Only two characters like pollen sterility percentage and spikelet ferity percentage were recorded to identify maintainers and restorers of CMS lines/test crosses. The following formula were used to calculate pollen sterility and spikelet fertility (%)

Estimation of pollen sterility percentage

Pollen fertility percentage will be calculated by formula: - (Virmani *et al.,* 1997)

$$= \frac{\text{No. of stained pollen}}{\text{Total no. of pollens}} \times 100$$

Estimation of spikelet fertility

Estimation was done on three panicles per plant (two selected at random and one from the main culm) from five randomly selected plants for each test cross hybrid at maturity. Spikelet fertility of hybrids was assessed by taking the count of well filled and chaffy spikelet in each panicle. Spiklet fertility was estimated on B lines

$$= \frac{\text{Number of fertile grains}}{\text{Total number of grains observed}} \times 100$$

Results and Discussion

The establishment of test cross nursery to identify restorers and maintainers is the initial step in three line hybrid rice breeding programme. Results of pollen sterility and fertility are shown in Tables 2. Among the 60 rice genotypes different levels of pollen sterility and fertility were observed.

Such variation in pollen fertility indicated the existence genetic variation in respect of these reproductive traits among the genotypes. The pollen fertility percent of F_1s varied from 0% to 98.5% and spikelet fertility ranged from 0% to 93.31%. Similar observations have been reported by other researchers (Ali *et al.,* 2014, Krishnalatha and Sharma 2012, Sharma *et al.,* 2012). This variation may be due to the pollen fertility–restoring genes differ or their penetrance or expressivity differed with genotypes (Umadevi *et al.,* 2010) or due to existence of modifiers genes.

Table.1 Details of lines and testers used in the present study

S.N.	Female Parent (Lines)	Cytoplasmic source	Source
1	IR 58025A	Wild Abortive (WA)	IRRI, Manila, Philippines
2	CRMS31A	Kalinga	CRRI, Cuttack, Orissa
3	CRMS32A	Kalinga	CRRI, Cuttack, Orissa
Male Parent (Testers)			
1	KALA JEERA	Germplasm,Biodiversity center, IGKV, Raipur	
2	TILKASTURI	Germplasm,Biodiversity center, IGKV, Raipur	
3	DHANIYA PHOOL	Germplasm, Biodiversity center, IGKV, Raipur	
4	KASTURIBHOG	Krishi Vigyan Kendra, Ambikapur	
5	INDRABHES DUBRAJ	Farmers field, Arkar, Dhamtari	
6	CHINNOR	College of Agriculture, Murjhar Farm, raseoni,Balagat	
7	TULSI MOGRA	Germplasm,Biodiversity Center, IGKV, Raipur	
8	KALIKAMOD	Germplasm,Biodiversity Center, IGKV, Raipur	
9	RAMJEERA	Krishi Vigayan Kendra, Janjgir-Champa	
10	DHANIYA DHAN	Krishi Vigyan Kendra, Ambikapur	
11	BASABHOG	Krishi Vigyan Kendra, Ambikapur	
12	SAMUNDCHINI	Krishi Vigyan Kendra, Bilaspur	
13	KALIMUCH	JNKVV, Jabalpur	
14	JAWAPHOOL	Germplasm, Biodiversity Center, IGKV, Raipur	
15	TARUNBHOG	Germplasm, Biodiversity Center, IGKV, Raipur	
16	NAGRI DUBRAJ	Krishi Vigyan Kendra, Dhamtari	
17	JAU PHOOL	Krishi Vigyan Kendra, Ambikapur	
18	DAGRI KAJAR	Dharohar Samiti, Kondagaon	
19	TULSI BHOG	Germplasm, G& PB, IGKV, Raipur	
20	BARHASAL	Germplasm, Biodiversity Center, IGKV, Raipur	

These hybrids were evaluated in *Kharif* 2018 for their spikelet and pollen fertility. All the three CMS lines used in our experiment showed various degrees of spiklet's fertility. Among the 20 parents derived from IR58025A, 2 parents (10%) were found to be restorers and the number of maintainers was also 2 (10%). Six (30%) parents showed partial restorability and 10 (50%) behaved as partial maintainers against this line. Out of 20 parents, 4 parents were (20%) behaved as restorers and 05 (25%) parents as maintainers with CMS line CRMS 31A. Number of parents showing partial restorability was 01 (5%) and that behaving as partial maintainers were 10 (50%) for CRMS 31A. However among 20 parents with CRMS 32A, 3 (15%) parents showed as maintainers and 02 (10%)

parents were identified as restorers. Partial maintainers were 13 (65%) and 02 (10%) parents were behaved like partial restorers. (Figure 1, 2 and 3). Based on the overall F_1's evaluation of 60 crosses derived from all the three CMS lines, 08 (13.3%) crosses were found to have effective restorability (spikelet fertility >75%), while 09 (15%) crosses were partial restorability (50.1 to 75% spikelet fertility), 33 (55%) behaved as partial sterility (0.1 to 50% spikelet fertility) and 10 (16.7%) crosses were showed 100% sterility and tester parents involved in these crosses were considered as maintainer lines (0% spikelet fertility). However using diverse CMS lines in testcrosses can give much validity to such fertility restoration studies (Das *et al.,* 2013 and Prashad *et al.,* 2017).

Table.2 Fertility restoration and other characteristics for identification of restorers and maintainers among 20 testers and 03 CMS lines

S.No.	PARENTS	1	2	3	4	5	6	7	8	9
	IR58025A									
1	KALA JEERA	87	118	10	8	58	76	71	147	51.70
2	TILKASTURI	86	116	6	4	25	35	128	163	21.47
3	DHANIYA PHOOL	86	115	7	5	25	36	137	173	20.81
4	KASTURIBHOG	86	128	7	5	25.7	52	145	197	26.40
5	INDRABHES DUBRAJ	88	79	12	10	98.5	265	19	284	93.31
6	CHINNOR	85	123	8	0	0	0	129	129	0.00
7	TULSI .MOGRA	80	122	13	10	40	54	110	164	32.93
8	KALIKAMOD	78	120	12	9	45	54	91	145	37.24
9	RAMJEERA	81	131	8	6	38	65	131	196	33.16
10	DHANIYA DHAN	84	126	8	5	40	77	147	224	34.38
11	BASABHOG-F$_1$	83	118	13	8	52.5	98	95	193	50.78
12	SAMUNDCHINI	82	128	12	7	50	92	113	205	44.88
13	KALIMUCH	83	112	10	8	45	59	80	139	42.45
14	JAWAPHOOL	83	110	7	6	58.5	94	81	175	53.71
15	TARUNBHOG	85	115	8	5	30	82	158	240	34.17
16	NAGRI DUBRAJ	84	108	10	10	90.6	218	41	259	84.17
17	JAW PHOOL	83	109	7	5	60.5	61	55	116	52.59
18	DAGRI KAJAR	80	108	13	0	0	0	136	136	0.00
19	TULSI BHOG	84	116	14	8	40.6	64	111	175	36.57
20	BARHASAL	84	112	13	7	20	32	145	177	18.08
	CRMS31A									
1	KALA JEERA	83	120	10	0	0	0	143	143	0.00
2	TILKASTURI	75	117	8	5	25.5	27	79	106	25.47
3	DHANIYA PHOOL	79	119	7	5	40.2	54	90	144	37.50
4	KASTURIBHOG	82	116	12	6	30.1	35	133	168	20.83
5	INDRABHES DUBRAJ	87	84	12	12	95.7	224	22	246	91.06
6	CHINNOR	84	107	7	0	0	0	219	219	0.00
7	TULSI .MOGRA	83	108	8	4	45	66	110	176	37.50
8	KALIKAMOD	83	119	9	5	15	23	167	190	12.11
9	RAMJEERA	84	121	8	3	18.7	24	156	180	13.33
10	DHANIYA DHAN	75	117	10	0	0	0	197	197	0.00
11	BASABHOG	82	115	11	7	30.5	44	137	181	24.31
12	SAMUNDCHINI	82	119	10	6	30.2	28	89	117	23.93
13	KALIMUCH	75	111	10	9	65	127	56	183	69.40
14	JAWAPHOOL	83	115	8	0	0	0	227	227	0.00
15	TARUNBHOG	87	128	14	12	81	198	41	239	82.85
16	NAGRI DUBRAJ	87	114	9	8	85	212	31	243	87.24
17	JAW PHOOL	83	120	6	4	25	44	150	194	22.68
18	DAGRI KAJAR	82	100	10	6	15	23	148	171	13.45
19	TULSI BHOG	77	119	11	0	0	0	128	128	0.00
20	BARHASAL	83	121	14	12	85	161	43	204	78.92
	CRMS32A									
1	KALA JEERA	86	117	8	0	0	0	97	97	0.00
2	TILKASTURI	83	110	7	0	0	0	108	108	0.00
3	DHANIYA PHOOL	84	125	8	6	40.5	38	79	117	32.48
4	KASTURIBHOG	78	121	6	6	30.5	43	157	200	21.50

| 5 | INDRABHES DUBRAJ | 87 | 83 | 8 | 8 | 96 | 253 | 25 | 278 | 91.01 |
6	CHINNOR	83	110	6	5	20	25	137	162	15.43
7	TULSI .MOGRA	92	120	9	6	22.5	38	175	213	17.84
8	KALIKAMOD	85	113	8	5	15	19	133	152	12.50
9	RAMJEERA	86	114	6	0	0	0	120	120	0.00
10	DHANIYA DHAN	85	114	8	8	55	83	77	160	51.88
11	BASABHOG	87	119	8	6	18	18	107	125	14.40
12	SAMUNDCHINI	80	113	10	6	15	21	126	147	14.29
13	KALIMUCH	71	109	8	5	50	63	78	141	44.68
14	JAWAPHOOL	81	114	6	4	30.5	41	113	154	26.62
15	TARUNBHOG	82	117	8	6	45	93	113	206	45.15
16	NAGRI DUBRAJ	79	109	10	8	82	221	39	260	85.00
17	JAW PHOOL	87	115	7	5	30	34	98	132	25.76
18	DAGRI KAJAR	84	97	8	6	15	49	237	286	17.13
19	TULSI BHOG	83	112	10	7	15.5	20	116	136	14.71
20	BARHASAL	86	118	12	8	65	83	51	134	61.94

1. Days to 50% Flowering   2. Plant height (cm)   3. No. of Tillers/plant   4. Productive tillers/plant
5. Pollen Fertility (%)   6. Fertile spikelets/Panicle   7.Sterile spikelets/panicle   8. Total Spikelets/Panicle
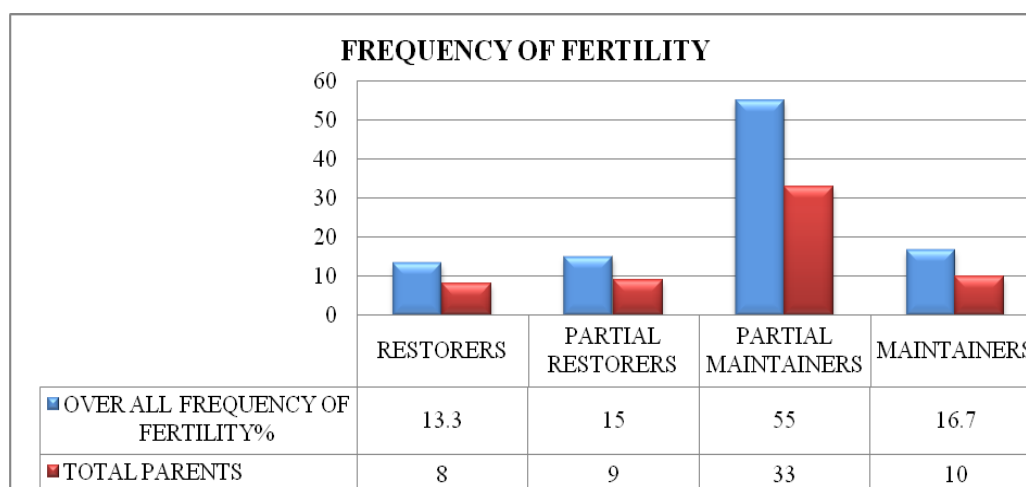9. Spikelet Fertility (%)

Fig.1 Frequency of spikelet fertility of crosses using IR58025A
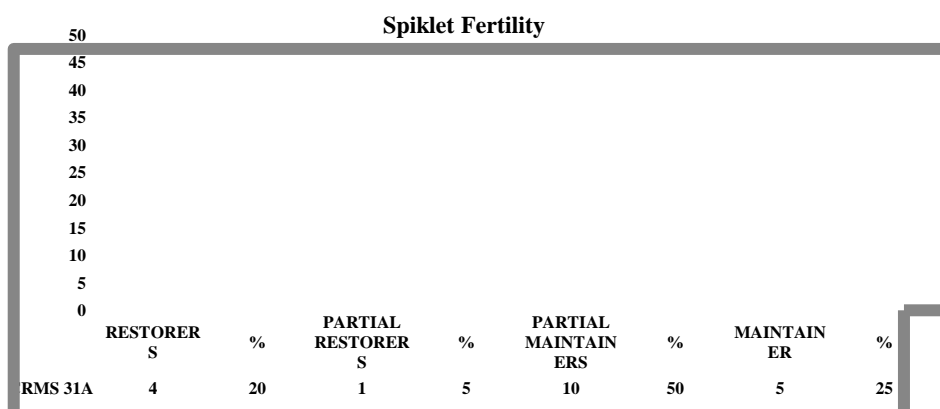
Fig.2 Frequency of spikelet fertility of crosses using CRMS 31A
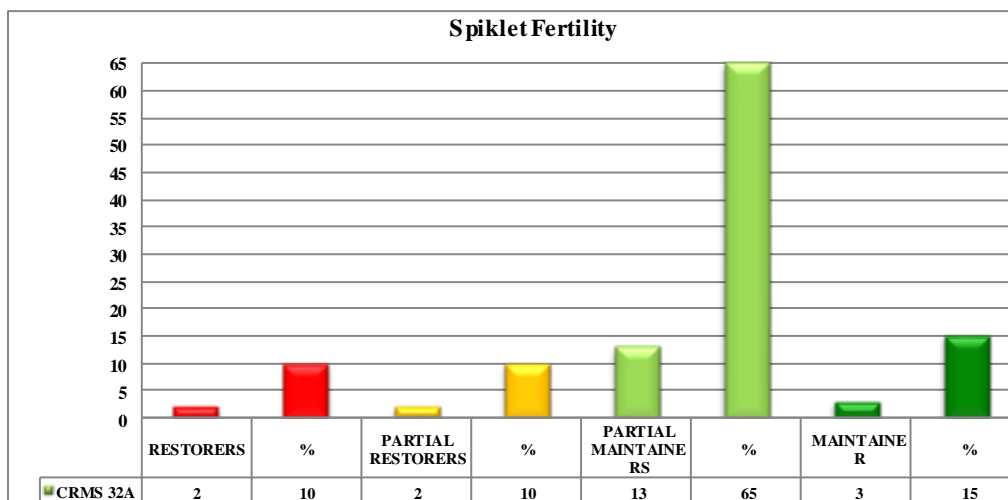
Fig.3 Frequency of spikelet fertility of crosses using CRMS 32A

Fig.4 Frequency of restorers and maintainers

These 08 parents (restorers) can contribute for the development of good hybrids by utilizing the masparental lines. In general the frequency of partial restorers and maintainers were high. But more number of maintainers was identified as compare to restorers. Frequency of restorers and maintainers are presented in Fig.4.

Most interestingly Indrabhesh Dubraj and Nagri Dubraj both were commonly identified as restorers from all the three CMS lines viz. IR 58025A, CRMS 31A and CRMS 32 A. Strong restoration ability of these two parents is due to have two major genes along with

modifier genes. Tarunbhog showed restorability with CMS line CRMS 32A and Barhasal. Whereas Chinnor was identified maintainer from two CMS lines viz. IR 58025A and CRMS 31A. Similarly Kalajeera was showed 100% sterility with CRMS 31A and CRMS 32A. Whereas other parents viz. Dagri Kajar expressed 100% sterility with IR 58025A, Jawaphool and Dhaniya Dhan showed 100% sterility with CRMS 31A. Tilkasturi and Ramjeera expressed 100% sterility with CRMS 32A. The findings of the present investigation revealed that fertility restoration reaction of the genotypes varies with their genetic background. More

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emphasis should be given to utilize popular rice aromatic cultivars in hybrid rice breeding as parental lines to achieve the goal of superior hybrid with better grain quality.

The identified restorers are locally adopted. The identified maintainers can be developed as new members in cytoplasmic male sterile (CMS) family by repeated backcross breeding. This is prerequisite to develop aromatic medium slender and short grain hybrids. The CMS line must be devolved or converted as aromatic short grain type to produce such type of hybrids. Two parents viz. Indrabesh Dubraj and Nagri Dubraj were showed strong restorability with all the three CMS lines, may be due to two or more restorer gene with modifier. The identified restorer lines can be used as pollen parent in developing new commercial aromatic hybrid varieties. New restorer may also be developed through crossing programme which can expand the genetic base of restorer by pyramiding complementary traits from diverse sources according to breeding objectives. Ten aromatic medium slender maintainers identified from this study are one of the significant findings from this study. These maintainers will be converted into aromatic medium slender and short grain CMS lines after recurrent back crossing program which play major role in developing aromatic short grain hybrids.

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