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IDENTIFICATION OF RESTORERS AND MAINTAINERS FOR DIFFERENT WILD ABORTIVE CYTOPLASMIC MALE STERILE LINES IN RICE (*ORYZA SATIVA* L.)

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Abstract

F_1 hybrids produced by crossing 40 different rice genotypes with 2 CMS lines (IR79156A and Pusa6A) behaved differently with regard to pollen fertility. Out of the 80 F_1 hybrids, 21 were completely sterile and 37 completely fertile. The remaining 22 hybrids expressed varying degrees of fertility, within the range of 13 partial restorers and 9 partial maintainers. Sixteen genotypes *viz.*, IET 21519, IET 22218, IET 22228, IET 22202, IET 20524, IET 21542, Vardhan, Akshaya Dhan, Rajendra Kasturi, Sarjoo 52, HUR81, BPT 5204, RPBIO226, Type3, MTU7029 and Dantaswari were found to be common effective restorers for both the CMS lines (IR 79156A and Pusa6A). Genotypes NDR97 and Pant Dhan12 were found to be effective restorer for only IR79156A whereas, IET22251, IET21528 and Khutadhan were effective restorer for CMS line Pusa6A. Pusa Basmati 1460 and Pant Dhan4 produced sterile hybrids when crossed with IR79156A. IET 22225, Krhani and HUR52 produced sterile hybrids when crossed with Pusa6A. Eight genotypes (IET22237, HUR3022, HUR 105, Pant Sugandh Dhan-17, Nagina22, Anjali, IDR763 and Vandana) produced sterile hybrids when crossed with both the CMS lines, (IR79156A and Pusa6A). These genotypes may be used for development of new male sterile lines.

Introduction

Rice contributes nearly 43% of the total food grain production and 46% of total cereal production in India. In India, rice is grown in 42.86 million hectare area with production of 104.32 million tons and productivity of 3.59 t/ha, respectively (Foreign Agriculture Services/USDA, Office of Global analysis, April, 2012). To meet the rising demand of ever-growing population, India has to enhance her rice production up to 135 million tons by 2020. Among the innovative genetic options available, hybrid rice technology is one of the strongest tools. Use of diversified and locally adapted CMS lines as well as restorer lines will increase the cytoplasmic diversification and further help to prevent genetic vulnerability due to the use of a single CMS source (Pradhan *et al*. 1992). The restorers and maintainers for WA cytoplasm were reported earlier by Rosamma and Vijayakumar (2005), Sabar *et al*. (2007), Akhter *et al*. (2008) Das *et al.* (2012), Khan *et al*. (2012), Krishnalatha and Sharma (2012), Sharma *et al*. (2012), Soni *et al.* (2012), Singh *et al.* (2013) and Bhati *et al.* (2014). Hence, the present investigation was undertaken with an objective to identify the different restorers and maintainers for three CMS lines from among the local and high yielding rice genotypes.

Materials and Methods

Eighty F_1s and their parental lines with two CMS lines (IR79156A and Pusa6A) were grown in single row of 3.0 m with three replications in randomized block design with spacing of 20 × 15 cm during Kharif 2013 at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras

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Hindu University, Varanasi, India. The investigation was undertaken to estimate pollen and spikelet fertility to identify restorers and maintainers. The purpose of identification of maintainers was to isolate locally adopted genotypes which may be converted into new CMS lines through repeated backcross breeding. While the purpose of identification of restorers was to find out suitable fertility restorers among the locally adapted varieties for the existing CMS lines as a short term strategy for hybrid breeding programme. The pollen and spikelet fertility percentage were used as the fertility index (Babu 2010).

For pollen fertility, spikelets were collected from the panicle at the flowering stage. Mature anthers from 5 randomly selected spikelets were squashed, smeared and stained with 1 per cent iodine potassium iodide (IKI) solution and examined under light microscope. Pollen grains were counted at three different spots in the microscopic field. Stained, well-filled and round pollen grains were counted as fertile (viable), while unstained, shriveled and empty pollen grains were considered as sterile (non viable). Pollen viability was calculated and expressed in percentage as:

$$\text{Pollen fertility (\%)} = \frac{\text{No. of stained pollen grains}}{\text{No. of total pollen grains}} \times 100$$

For spikelet fertility, well-developed filled grains were counted from 5 randomly selected panicles for each test entry in each replication and expressed in percentage. The following formula was used for computing fertility percentage:

$$\text{Spikelet fertility (\%)} = \frac{\text{No. of filled spikelets}}{\text{No. of total pollen spikelets}} \times 100$$

Results and Discussion

Out of the 80 F_1 hybrids having CMS lines with wild abortive cytoplasm, 37 were completely fertile and 21 completely sterile. The remaining 22 hybrids expressed varying degrees of fertility. Thirteen of them were partial restorer and the remaining 9 were partial maintainer (Tables 1 - 4). Total 16 genotypes *viz.*, IET 21519, IET 22218, IET 22228, IET 22202, IET 20524, IET 21542, Vardhan, Akshaya Dhan, Rajendra Kasturi, Sarjoo 52, HUR81, BPT 5204, RPBIO226, Type3, MTU7029 and Dantaswari were found to be common effective restorers for both the CMS lines (IR 79156A and Pusa6A) (Fig. 1). Genotypes NDR97 and Pant Dhan12 were found to be effective restorer for only IR79156A whereas, IET22251, IET21528 and Khuta dhan were found to be effective restorer for CMS line Pusa6A. These restorer genotypes may be tested for heterosis for development of new rice hybrids. Pusa Basmati 1460 and Pant Dhan 4 produced sterile hybrids when crossed with IR79156A. IET 22225, Krhaniand HUR52 produced sterile hybrids when crossed with Pusa6A. Eight genotypes (IET 22237, HUR 3022, HUR 105, Pant Sugandh Dhan17, Nagina22, Anjali, IDR763 and Vandana) produced sterile hybrids when crossed with both the CMS lines (IR79156A and Pusa6A). These genotypes may be used for development of new male sterile lines. The study concluded that the frequency of potential restorers is higher in number than the frequency of maintainers. The similar results were also reported by Rosamma and Vijayakumar (2005), Sharma *et al*. (2012), Soni *et al.* (2012), Singh *et al.* (2013) and Bhati *et al.* (2014) in rice. The pollen as well as spikelet fertility are highly influenced by environmental conditions. Thus a partial restorer in one environment may behave like complete restorer at another environment. Similarly, the partial or weak maintainer lines under the environment favoring pollen sterility may behave like maintainer lines Virmani *et al.* (2003). However these lines were also tested during Rabi 2012 - 2013 at CRRI, Cuttack, Odisha, India revealed that eight

Table 1. Per cent pollen and spikelet fertility of 40 genotypes involving two cytoplasmic male sterile lines.

Sl. No.	Genotypes	IR 79156A		Pusa 6A	
		PF	SF	PF	SF
1.	IET - 20924	65.81	61.22	35.85	30.54
2.	IET - 21519	88.43	86.71	78.61	76.32
3.	IET - 22218	87.56	85.91	82.92	81.36
4.	IET - 22251	55.96	44.00	83.17	77.79
5.	IET - 20935	45.22	36.90	63.92	57.21
6.	IET - 20556	64.79	55.92	23.11	12.95
7.	IET - 22228	86.60	84.17	78.80	77.24
8.	IET - 22225	23.77	17.96	0	0.00
9.	IET - 22202	85.67	83.23	82.94	81.18
10.	IET - 21528	29.90	22.24	65.65	58.54
11.	IET - 22237	0	0	0	0
12.	IET - 20524	80.83	78.11	80.76	78.89
13.	IET - 21542	84.01	81.88	83.64	81.54
14.	Vardhan	86.70	84.64	87.84	86.01
15.	Akshaya Dhan	80.59	79.30	86.89	84.65
16.	HUR - 3022	0.00	0.00	0.00	0.00
17.	HUR - 105	0.00	0.00	0.00	0.00
18.	HUBR - 2-1	65.72	55.79	39.69	32.43
19.	Rajendra Kasturi	88.24	85.56	86.63	84.77
20.	Sarjoo - 52	82.65	81.27	85.05	83.32
21.	NDR - 359	28.2	21.77	66.47	54.57
22.	NDR - 97	81.15	79.04	59.49	55.48
23.	Adam Chini	72.47	66.9	70.67	59.24
24.	Pusa Basmati - 1460	0	0	28.41	31.44
25.	Pant Dhan- 4	0	0	25.07	19.62
26.	Pant Dhan -12	83.08	77.76	45.93	37.76
27.	Pant Sugandh Dhan-17	0	0	0	0
28.	Khutadhan	66.00	57.58	81.25	78.35
29.	Nagina - 22	0	0	0	0
30.	Krhani	25.4	14.56	0	0
31.	HUR - 8-1	82.76	80.13	83.34	81.34
32.	BPT - 5204	78.60	76.52	81.91	79.79
33.	RPBIO-226	81.31	79.84	87.58	86.40
34.	HUR - 5-2	68.00	63.58	0	0
35.	Anjali	0	0	0	0
36.	IDR - 763	0	0	0	0
37.	Type - 3	81.40	78.41	86.05	84.00
38.	MTU - 7029	80.54	79.27	89.83	88.08
39.	Vandana	0	0	0	0
40.	Danteswari	81.29	78.44	85.33	84.08

PF = Pollen fertility (%); SF = Spikelet fertility.

Table 2. Fertility classification of 40 genotypes for two cytoplasmic male sterile (CMS-WA) lines.

Sl. No.	Genotypes	IR 79156A	Pusa 6A
1.	IET - 20924	PR	PM
2.	IET - 21519	R	R
3.	IET - 22218	R	R
4.	IET - 22251	PR	R
5.	IET - 20935	PM	PR
6.	IET - 20556	PR	PM
7.	IET - 22228	R	R
8.	IET - 22225	PM	M
9.	IET - 22202	R	R
10.	IET - 21528	PR	R
11.	IET - 22237	M	M
12.	IET - 20524	R	R
13.	IET - 21542	R	R
14.	Vardhan	R	R
15.	AkshayaDhan	R	R
16.	HUR - 3022	M	M
17.	HUR - 105	M	M
18.	HUBR - 2-1	PR	PM
19.	RajendraKasturi	R	R
20	Sarjoo- 52	R	R
21	NDR - 359	PM	PR
22	NDR - 97	R	PR
23	Adam Chini	PR	PR
24	Pusa Basmati - 1460	M	PM
25	Pant Dhan- 4	M	PM
26	Pant Dhan -12	R	PR
27	Pant Sugandh Dhan-17	M	M
28	Khutadhan	PR	R
29	Nagina - 22	M	M
30	Krhani	PM	M
31.	HUR - 8-1	R	R
32.	BPT - 5204	R	R
33.	RPBIO-226	R	R
34.	HUR - 5-2	PR	M
35.	Anjali	M	M
36.	IDR - 763	M	M
37.	Type - 3	R	R
38.	MTU - 7029	R	R
39.	Vandana	M	M
40.	Danteswari	R	R

M = Maintainer; R = Restorer; PM = Partial maintainer; PR = Partial restorer.

Table 3. Means of spikelet and pollen fertility of parents (only full restorers) in rice.

Sl. No.	Genotypes	Pollen fertility (%)	Spikelet fertility (%)
	Lines		
1.	IR 79156A	0	0
2.	Pusa 6 A	0	0
	Testers		
1.	IET 21519	90.94	89.07
2.	IET 22218	88.48	86.20
3.	IET 22228	88.30	86.60
4.	IET 22202	90.07	87.02
5.	IET 20524	88.52	86.86
6.	IET 21542	91.00	89.59
7.	Vardhan	89.76	87.58
8.	AkshayaDhan	88.25	86.14
9.	RajendraKasturi	90.05	88.16
10.	Sarjoo- 52	86.00	84.20
11.	HUR-8-1	86.00	84.23
12.	BPT 5204	90.17	87.88
13.	RPBIO-226	87.99	86.15
14.	Type-3	85.61	82.37
15.	MTU-7029	87.90	85.28
16.	Danteswari	86.71	83.66
Grand mean		88.48	86.31
SE(m)		1.064	1.349
CD (5%)		2.173	2.755
CD (1%)		2.926	3.709

Table 4. Means of spikelet and pollen fertility of F_1 hybrids (only full restorers) in rice.

Sl. No.	F_1	Pollen fertility (%)	Spikelet fertility (%)
1.	IR 79156A × IET 21519	88.43	86.71
2.	IR 79156A × IET 22218	87.56	85.91
3.	IR 79156A × IET 22228	86.60	84.17
4.	IR 79156A × IET 22202	85.67	83.23
5.	IR 79156A × IET 20524	80.83	78.11
6.	IR 79156A × IET 21542	84.01	81.88
7.	IR 79156A × Vardhan	86.70	84.64
8.	IR 79156A × Akshaya Dhan	80.59	79.30
9.	IR 79156A × Rajendra Kasturi	88.24	85.56
10.	IR 79156A × Sarjoo- 52	82.65	81.27
11.	IR 79156A × HUR-8-1	82.76	80.13
12.	IR 79156A × BPT 5204	78.60	76.52
13.	IR 79156A × RPBIO-226	81.31	79.84
14.	IR 79156A × Type-3	81.40	78.41

(Contd.)

(Contd.)

Sl. No.	F_1	Pollen fertility (%)	Spikelet fertility (%)
15.	IR 79156A × MTU-7029	80.54	79.27
16.	IR 79156A × Danteswari	81.29	78.44
17.	Pusa 6A × IET 21519	78.61	76.32
18.	Pusa 6A × IET 22218	82.92	81.36
19.	Pusa 6A × IET 22228	78.80	77.24
20.	Pusa 6A × IET 22202	82.94	81.18
21.	Pusa 6A × IET 20524	80.76	78.89
22.	Pusa 6A × IET 21542	83.64	81.54
23.	Pusa 6A × Vardhan	87.84	86.01
24.	Pusa 6A × Akshaya Dhan	86.89	84.65
25.	Pusa 6A × Rajendra Kasturi	86.63	84.77
26.	Pusa 6A × Sarjoo- 52	85.05	83.32
27.	Pusa 6A × HUR-8-1	83.34	81.34
28.	Pusa 6A × BPT 5204	81.91	79.79
29.	Pusa 6A × RPBIO-226	87.58	86.40
30.	Pusa 6A × Type-3	86.05	84.00
31.	Pusa 6A × MTU-7029	89.83	88.08
32.	Pusa 6A × Danteswari	85.33	84.08
Grand mean		83.91	81.95
SE(m)		1.054	1.270
CD (5%)		2.108	2.540
CD (1%)		2.804	3.378

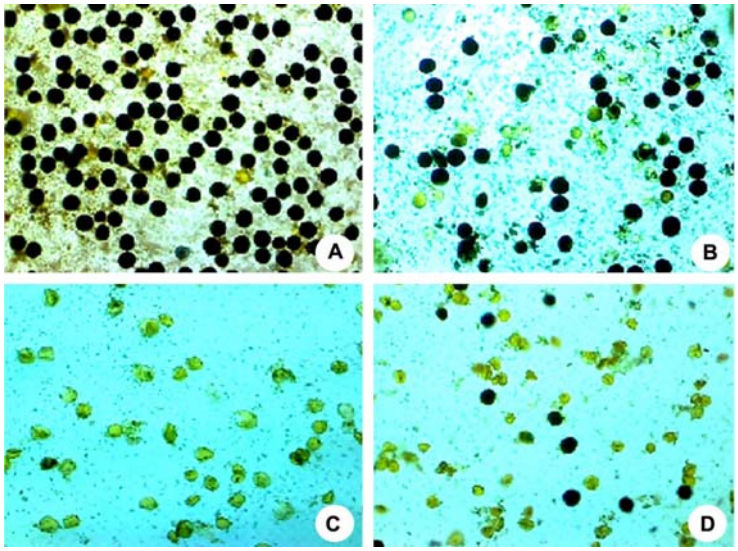

Fig. 1. Differential staining of rice pollen grains of hybrids stained using 1 per cent iodine potassium iodide (IKI) solution and examined under light microscope. Round dark stained- fertile pollen and light stained with irregular shape- sterile pollen. (A) full restorer type, (B) partial restorer type, (C) full maintainer type and (D) partial maintainer.

genotype IET-20524, Vardhan, Akshaya Dhan, Rajendra Kasturi, HUR81, RPBIO226, Type 3 and Dantaswari were found partial restorer but similar genotypes were found complete fertile when crosses with both the CMS lines during Kharif 2013 at BHU, Varanasi, India. Similarly, five genotype HUR105, Pant Sugandh Dhan17, Anjali, IDR-763 and Vandana were found partial maintainer at CRRI, Cuttack but similar genotype were found maintainer at BHU, Varanasi due to environment effect (Bhati *et al.* 2014). The lines that restore fertility between 30 and 59 per cent can be safely rejected from the breeding programme. The study suggests that the new CMS lines could be developed by using newly identified maintainers (IET 22237, HUR3022, HUR 105, Pant Sugandh Dhan17, Nagina22, Anjali, IDR763, Vandana, Pusa Basmati 1460, Pant Dhan4, IET 22225, Krhani and HUR52) as these lines produced full sterile hybrids with CMS lines which could be converted into new CMS lines through repeated backcrossing. New hybrids could be developed by using the identified restorers (IET 21519, IET 22218, IET 22228, IET 22202, IET 20524, IET 21542, Vardhan, Akshaya Dhan, Rajendra Kasturi, Sarjoo 52, HUR81, BPT 5204, RPBIO226, Type3, MTU-7029, Dantaswari, NDR97, Pant Dhan12, IET22251, IET21528 and Khutadhan) as they have produced full fertile hybrids. These results are in conformity with the findings of Gautam and Singh (2004), Rosamma and Vijayakumar (2005), Sabar *et al*. (2007), Akhter *et al*. (2008) Das *et al.* (2012), Khan *et al*. (2012), Krishnalatha and Sharma (2012), Sharma *et al*. (2012), Soni*et al.* (2012), Singh *et al.* (2013) and Bhati *et al.* (2014).

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