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Categorization of Multitrait Pyramided Genotypes of Rice (*Oryza sativa* L.) into Restorers and Maintainers of Wild Abortive (WA) Cytoplasm under Aerobic Condition

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Abstract In the present study, three CMS lines viz., IR 79156 A, IR 68897A and IR 80555A were crossed with thirteen multitrait pyramided genotypes having different combination of QTLs/genes responsible for traits like water use efficiency, disease resistance (leaf blast and bacterial blight) and root morphological traits. Resulted thirty-nine F_1 hybrids were evaluated for pollen and spikelet fertility. The study indicated high positive correlation (r = >0.8) between spikelet and pollen fertility (%) and hence spikelet fertility (%) was considered as criteria for categorization of multiple trait pyramided genotypes into maintainers, partial maintainers, partial restorers and effective restorers. Out of thirty-nine F_1 hybrids, five were completely sterile and twelve were completely fertile, seven were partial maintainers and fifteen were partial restorers. Among which effective restorers and maintainers can be utilized for development of hybrids under aerobic condition.

Keywords Multiple trait pyramided genotypes, Hybrids, Aerobic, Restorers, Maintainers.

Introduction

Exploitation of heterosis and further development of hybrids in rice under aerobic condition is a potential strategy for feeding ever increasing population of the world. Utilization of heterosis can alone increases 15—20% yield potential in rice [1]. Improvement of grain yield potential, quality and tolerance to biotic and abiotic stress is a great challenge to the breeders with less resource [2]. Limited irrigation water threatens the rice production. To overcome this, it is necessary to switch on for alternate ways of cultivation system with less water and without compromising the yield [3]. Hence, aerobic rice cultivation is a very good choice under scarcity of water. Aerobic rice varieties were showed higher water use efficiency (164%—188%) compared to low land rice varieties [4]. Identification of restorers and maintainers and their use in further breeding program are the initial steps in three line heterosis breeding [5]. Objective of the present study is to utilize the multiple trait pyramided genotypes developed by earlier researchers [6] for different combinations of QTLs/genes controlling traits such as water use efficiency *(qWUE2)*, disease resistance (Bacterial blast*(Xa4, xa5) and Blast (Pi-1))* and root morphological traits (*qRT2*, *qRT7*, *qRT9*). Spikelet fertility was considered to sort out genotypes into different fertility restoration classes. Hence, this investigation provides better opportunity to utilize multiple trait pyramided genotypes as testers in hybridization program.

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Table 1a. Cytoplasmic male sterile (CMS) lines of IRRI origin used for development F1 hybrids.

Sl. No.	CMS lines
1	IR 79156 A
2	IR 68897 A
3	IR 80555 A

ment of Genetics and Plant Breeding, UAS, GKVK, Bengaluru-65).

Materials and Methods

Plant material consists of three CMS lines (Table 1a) of IRRI origin having WA cytoplasmic background and thirteen multiple trait pyramided genotypes (Table 1b) developed at Marker Assisted Selection (MAS) laboratory, University of Agricultural Sciences (UAS), GKVK, Bemgaluru. The experiment was carried out at experimental plots of Department of Genetics and Plant Breeding, UAS, GKVK. Bengaluru. During summer 2015, three CMS lines and thirteen testers were sown ; clipping method of crossing was followed to generate F_1 hybrids by using Line × Tester mating design [7]. Resulted thirty-nine F_1 hybrids were sown in RBD with two replications to evaluate for pollen fertility and spikelet fertility during wet season 2015. All package of practices were followed as per recommendation of rice cultivation under aerobic condition.

Observations on traits such as pollen fertility (%) and spikelet fertility (%) were taken and correlation analysis was done using Microsoft excel statistical package.

Pollen fertility

Spikelet's were collected from F_1 hybrids and their parents before anthesis and fixed in 70% alcohol during 7 AM 8 to AM. Two-three anthers from a spikelet were kept on a clean glass slide and smashed gently using glass rod to release pollens out of anthers. One drop 1.5% acetocarmine stain was added and debris were removed using forceps. Cover slip was used to cover pollen and observed under light microscope (10X). Pollen grains stained red color were considered as fertile, while unstained and shrunken pollen grains as sterile [8]. Observations were recorded by counting total number of stained pollen (fertile) and total number of pollens (fertile + sterile) (Fig. 1a). Recorded observations under five microscopic fields for each sample and mean values were considered for calculation of pollen fertility (%) using the following formula [9].

Table 1b. Multiple trait pyramided genotypes with their QTLs / genes combination developed at MAS lab, UAS, GKVK, Bengaluru and which were used as testers. QTLs/genes of specific traits: *qRT7*, *qRT2*, *qRT9* (Root trait QTLs). *qWUE2* (Water use efficiency QTL). *Xa4*, *Xa5* (Bacterial blight genes). *Pi-1* (Leaf blast gene).

	Testers	
Sl. No.	Genotypes	Pyramided QTLs / genes
1	23-5-55	*qRT7+qWUE2+Xa4+Xa5*
2	23-5-103	*qRT2+qWUE2+Xa4+Xa5*
3	23-5-235	*qRT2+Xa4+Xa5*
4	23-5-236	*qRT7+qWUE2+Pi-1+Xa5*
5	23-5-284	*qRT2+qRT7+Xa4+Xa5*
6	23-5-300	*qRT2+qRT7+qRT9+qWUE2+ Xa4+Xa5*
7	23-5-321	*Xa4+Xa5*
8	23-5-287	*Xa5*
9	23-5-115	*qRT2+qRT7+Xa4+Xa5*
10	23-5-296	*qRT2+qWUE2*
11	23-5-145	*Xa5*
12	23-5-168	*qRT2+qRT7+qRT9+ qWUE2+Xa4*
13	23-5-311	*qRT2+qRT7+qWUE2*

$$\text{Pollen fertility (\%)} = \frac{\text{Total no. of stained pollens} \times 100}{\text{Total no. of pollens}}$$

Spikelet fertility

Punicles of F_1 hybrids and their parents were covered with butter paper cover before anthesis to ensure selfing. After maturity, five panicles were harvested from each line and counted for filled and chaffy spikelet's (Fig. 2b). Spikelet fertility was calculated using the following formula [9].

$$\text{Spikelet fertility (\%)} = \frac{\text{Total no. filled spikelet's per panicle} \times 100}{\text{Total no. of spikelet's per panicle}}$$

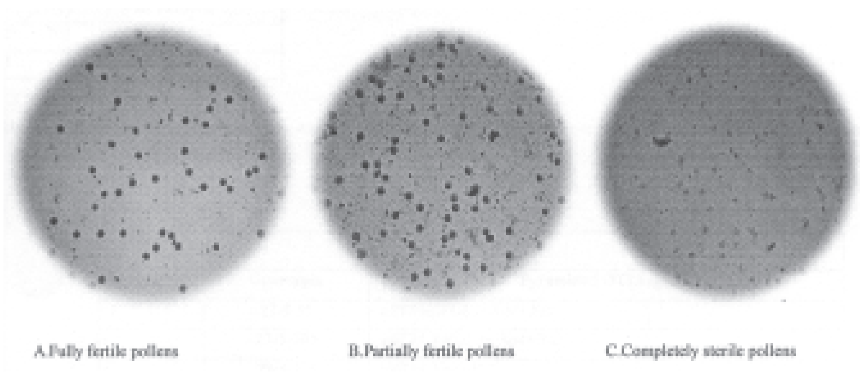

Fig. 1. Different classes of pollen grains under 10X light microscopic field, stained with 1.5% Acetocarmine, round and fully stained pollens were fertile whereas colorless and shrunken pollens were sterile.

Results and Discussion

Pollen fertility and spikelet fertility in the F_1 hybrids ranged from 0% to 97.91% and 0% to 91.49% respectively. In some cases, classification of genotypes into maintaines, partial maintaines, partial restorers and effective restorers based on pollen fertility (%) alone was not matching with classification of same based on spikelet fertility (%), similar results were reported by Das et al. [10]. There was high positive correlation between pollen and spikelet fertility (Table 2a). So we considered only spikelet fertility (%) for classification of multitrait pyramided genotypes into different classes [11, 12]. Based on spikelet fertility (%) genotypes were grouped into four different classes viz., maintainer (M) (0 to 5.00%), partial maintainer (PM) (5.1 to 20.00%), partial restorer (PR) (20.1 to 70.00%) and effective restorer (R) (70.1 to 100.00%) [13].

Out of thirty-nine parental combinations, nineteen genotypes were behaved as effective restorers of fertility and four genotypes as maintainers. Remaining sixteen genotypes behaved as partial restorers and partial maintainers (Table 2b). Out of 13 genotypes involved in crossing with CMS line IR 79156A, eight genotypes (23-5-55, 23-5-236, 23-5-284, 23-5-300, 23-5-321, 23-5-168, 23-5-296 and 23-5-287) were effective restorers and only one (23-5-311) as a maintainer. Among crosses involving CMS line IR80555A, three genotypes (23-5-287, 23-5-311 and 23-5-115) behaved as maintainers and five genotypes (23-5-236, 23-5-

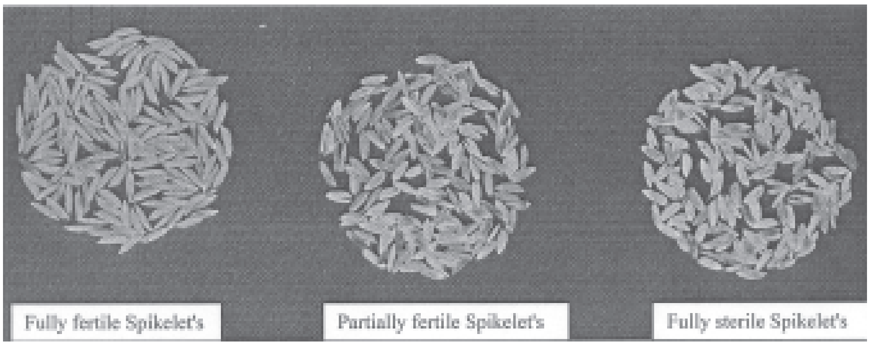

Fig. 2. Representation of different classes of spikelet fertility.

Table 2a. Spikelet fertility (%) and pollen fertility (%) of parents used for hybrids development.

CMS lines	SF(%)	PF (%)
Parents		
IR79156A	0.00	0.00
IR68897A	0.00	0.00
IR80555A	0.00	0.00
Testers		
23-5-55	93.56	97.43
23-5-103	91.21	94.23
23-5-235	89.22	95.55
23-5-236	94.32	93.66
23-5-284	91.57	94.88
23-5-300	90.43	96.64
23-5-321	90.33	97.92
23-5-287	90.12	98.65
23-5-115	90.66	92.23
23-5-296	90.89	95.00
23-5-145	90.23	97.21
23-5-168	90.71	94.87
23-5-311	90.77	94.00
Minimum	89.22	92.23
Maximum	94.32	98.65
Mean	91.17	95.54
SE	0.44	0.57

300, 23-05-55, 23-5-168 and 23-5-296) behaved as effective restorers. There was no maintainer in crosses involving CMS line IR68897A and six effective restorers (23-5-236, 23-5-300, 23-05-55, 23-5-287, 23-5-168 and 23-5-296) have been observed. Relatively high number of effective restorers (8) and maintainers (3) were observed in the crosses involving CMS lines IR79156A and IR80555A respectively. Genotypes were exhibited differential interaction with different CMS lines, which was in agreement with the reports of Virmani et al. [14] and Das et al. [10] Numbers of effective restorers were higher than number of maintainers and similar results were reported by Singh et al. [15] and Sharma et al. [16] Identified eighteen effective restorers and four maintainers can be utilized in further hybridization program.

Conclusion

Present study showed that genetic background of CMS lines is important for fertility restoration. Identified effective restorers and maintainers are well adapted to aerobic condition. So that eighteen effective restorers can be utilized for exploitation of heterosis under aerobic condition. Due to the presence of multitrait pyramided QTLs genes in testers may result in hybrids with high buffering capacity to disease resistance and drought tolerance. Identified four maintainers can be used to develop new CMS lines by back crossing program. Generation of new re-

Table 2b. Classification of multitrait pyramided genotypes into different classes based on pollen fertility (%) and spikelet fertility (%) of F_1 hybrids. SF (Spikelet fertility), PF (Pollen fertility), SF (Stantard error), R (Effective restorer), PR (Partial restorer), PM (Partial maintainer), M (Maintainer).

CMS lines	IR79156A				IR68897A				IR 80555A			
Genotypes	PF (%)	SF (%)	Class	Correlation coefficient (%)	PF (%)	SF (%)	Class	Correlation coefficient (%)	PF (%)	SF (%)	Class	Correlation coefficient (%)
23-05-55	79.2	70	R	93.57	17.4	87.4	R	86.29	87	88.4	R	83.20
23-5-103	51.2	39.2	PR	97.59	22.2	30.2	PR	84.33	26.4	36	PR	93.70
23-5-235	91.6	58.2	PR	90.84	31.5	54.8	PR	90.00	37.4	34.4	PR	91.69
23-5-236	97.5	91.4	R	86.60	74.9	79.6	R	88.07	87	72.3	R	86.57
23-5-284	58.2	71.6	R	87.02	6	22.1	PR	93.42	40.6	12.2	PM	97.36
23-5-300	93.2	81.8	R	80.51	95.2	76.1	R	83.50	93.1	79.4	R	84.08
23-5-321	92.8	81.2	R	99.11	7.6	7.6	PM	97.82	48.6	14	PM	97.54
23-5-287	71.2	73.4	R	90.19	57.7	70.7	R	86.63	0	0	M	–
23-5-115	93.5	16.4	PM	90.79	57.8	69.7	PR	86.65	43.4	3	M	93.31
23-5-296	92.8	84.4	R	94.03	91.8	83.4	R	81.42	94.8	87.6	R	97.30
23-5-145	16.6	23.6	PR	80.19	17.7	32.8	PR	89.34	22	10.5	PM	87.62
23-5-168	89.8	83.2	R	92.83	91.4	75.4	R	76.80	90.2	83.4	R	99.45

Table 2b. Continued.

CMS lines Geno-types	IR79156A PF (%)	SF (%)	Class	Corre-lation coeffi-cient (%)	IR68897A PF (%)	SF (%)	Class	Corre-lation coeffi-cient (%)	IR 80555A PF (%)	SF (%)	Class	Corre-lation coeffi-cient (%)
23-5-311	3.4	3.9	M	88.76	7.6	6.9	PM	96.74	12.8	0	M	–
Minimum	3.4	3.9			6	6.9			0	0		
Maximum	97.5	91.4			95.2	87.4			94.8	88.4		
Mean	71.62	59.87			44.52	53.52			52.56	40.09		
SD	31.06	29.25			34.89	29.55			33.73	36.54		
SE	8.61	8.11			9.68	8.20			9.35	10.13		

sources like maintainers and restorers is a continuous process in hybridization program. Hence, present study provided new resources for development of hybrids in aerobic condition.

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