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Short Communication

Differential fertility restoration of restorer genes to WA-cytoplasmic male sterility system in rice (*Oryza sativa* L.)

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The present investigation reports the influence of residual genetic background in restoration ability of the restorers. In a systematic set of crosses involving five WA-cms and ten restorer lines evaluated for combining ability P1292 recorded significantly poor while PRR72 significantly superior general combining ability for spikelet fertility. The cross combinations involving WA-cms line PMS2A and these two restorers constituted the basic experimental material for genetic analysis of fertility restoration. The F_1 plants of these crosses were backcrossed to the cms line to develop testcross (BC_1F_1) progenies. The F_1 , F_2 and BC_1F_1 plants were transplanted in the field using single seedling per hill at the Indian Agricultural Research Institute, New Delhi to study the inheritance of fertility restoration. Allelic test was conducted for fertility restorer genes present in two restorer lines. The F_1 plants from the cross between PRR72 and P1292 were testcrossed to the cms PMS2A to develop three way testcross ($TWCF_1$) progeny for allelic relationship studies. Pollen and spikelet fertility of the plants were assessed in each case and plants were classified as fully fertile (FF), semi fertile (SF), semi sterile (SS) or completely sterile (CS). For estimation of pollen fertility, anthers of three randomly taken spikelets representing lower, middle and top portion of the panicle were smeared in 1% I_2-KI solution and examined under a light microscope. The pollen grains were classified as sterile or fertile based on their staining behaviour and shape [1]. Based on pollen fertility analysis, plants were classified as FF (>60%), SF(31-60%), SS(1-30%) or CS (<1%). Similarly, with respect to spikelet fertility, all the plants were classified as FF (>70%), SF (41-70%), SS(1-40%) or CS(<1%). Chi-square analysis was employed to test the goodness of fit of the genetic hypothesis. Presence or absence of SS and CS plants in $TWCF_1$ was used to indicate the absence or presence of identical restorer genes in the restorer lines [2].

The analysis of pollen and spikelet fertility in F_2 population of the cross between PMS2A and PRR72 revealed digenic mode of inheritance of fertility restoration (Table 1). The frequency of FF, SF plus SS and CS plants fitted well to 9:6:1 F_2 segregation ratio displaying an epistasis with incomplete dominance. The fertility restoring action of one (*Rf1*) of the two genes seemed to be stronger than the other (*Rf2*) because the presence of *Rf1* alone conferred semifertility, though *Rf1* and *Rf2* appeared to have additive effects in imparting fertility restoration. The inference derived from segregation pattern of plants in F_2 population was also confirmed by 1:2:1 segregation ratio of FF, SF plus SS and CS plants in BC_1F_1 (Table 2). These results are in accordance with the earlier reports suggesting an epistasis with incomplete dominance as the genetic basis of fertility restoration in WA-cms lines [2-4].

The segregation pattern for pollen and spikelet fertility in F_2 population of the cross involving PMS2A with P1292, a restorer line isolated from basmati background, corresponded to a trigenic segregation ratio of 27:30:7 for FF, SF plus SS and CS plants indicating that the presence of three dominant genes imparted normal fertility in the segregating plants (Table 1). The segregation pattern could, however, be explained on the basis of involvement of two fertility restorer genes. Assuming that two dominant genes, one with strong (*Rf1*) and another with weak (*Rf2*) effect conferred fertility in the plants and one dominant gene allowed the expression of weak restorer gene, the restoration pattern is expected to segregate into 27:30:7 F_2 ratio as mentioned above. The observed segregation pattern showed a close correspondence with the segregation ratio expected on the basis of proposed hypothesis. Thus, it appeared that the plants homozygous for the recessive allele *rfe* of the gene (*Rfe*) which allowed the expression of *Rf2* will be sterile even with

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Table 1. Segregation pattern for pollen (P) and spikelet (S) fertility restoration in F_2 populations

Cross		Number of plants				Genetic* Ratio	Probability
		FF	SF	SS	CS		
PMS2A × PRR72	P	129	40	38	17	9:6:1	0.50-0.70
	S	135	46	26	17	9:6:1	0.20-0.30
PMS2A × P1292	P	90	33	53	16	27:30:7	0.20-0.30
	S	84	37	52	19	27:30:7	0.80-0.90

*Segregation pattern by pooling SF and SS plants together

homozygous or heterozygous condition for *Rf2*. From the segregation ratio as noticed in BC_1F_1, it was evident that *Rfe* was contributed by the genetic background of cms line since FF, SF plus SS and CS plants appeared in 1:2:1 ratio (Table 2). Earlier study indicated trigenic complementary interaction between three dominant genes as the basis of fertility restoration in crosses involving WA-cms lines and basmati breeding lines [5]. In the present study, though trigenic ratio was obtained, inheritance pattern could be explained on the basis of involvement of two fertility restorer genes (*Rf1* and *Rf2*), whereas the presence of third gene (*Rfe*) was required to allow the expression of *Rf 2* since restorer line seemed to possess *rfe* which acted as an inhibitor of *Rf2*.

The pollen and spikelet fertility analyses in $TWCF_1$ did not reveal the presence of CS plants (Table 2). Appearance of SS plants in addition to FF and SF plants, however, suggested that PRR72 and P1292 possessed non-allelic strong (*Rf1*) and allelic weak (*Rf2*) restorer genes. The inference borne out was in perfect agreement with the distribution pattern of pollen and spikelet fertility which indicated the presence of SS plants in $TWCF_1$. Such plants are expected to appear only in the case independent of segregation of non-allelic that is, different *Rf1* existing in the restorers.

Interestingly, the mode of action of *Rf 2* in these restorers was different even in crosses involving the same cms line. The inheritance pattern in PMS2A × P1292 (*Rf1 rf1 Rf2 rf 2 Rfe rfe*) clearly indicated that *Rf 2* was able to express only in the presence of *Rfe* which allowed its expression and homozygous condition for *rfe* at this locus acted as its inhibitor. The segregation pattern in BC_1F_1 of this cross further indicated that P1292 possessed *rfe* while cms line contributed *Rfe*. Analysis of segregation pattern in PMS2A × PRR72 (*Rr1 rf1 Rf2 rf2 Rfe Rfe*) also led to the same conclusion since *Rf2* appeared to be capable of its own expression in this case. Apparently therefore, the differential mode of action of *Rf 2* could be due to the presence of *rfe* in the genetic background of restorer. The allelic status at this locus is expected to influence the expression of restorer genes(s) and consequently the level of restoration in crosses with different cms lines. Genetic analysis of fertility restoration in the present study, therefore indicated that, in addition to diversity of restorer gene(s), residual genetic background of restorer lines could also be responsible for their differential restoration ability.

Table 2. Segregation pattern for pollen (P) and spikelet (S) fertility restoration in testcross (BC_1F_1) and three-way testcross ($TWCF_1$) progenies

Cross		Number of plants				Genetic* ratio	Probability
		FF	SF	SS	CS		
PMS2A × (PMS 2A × PRR72)	P	23	19	20	14	1:2:1	0.30-0.50
	S	25	23	14	14	1:2:1	0.10-0.20
PMS2A × (PMS 2A × P1292)	P	16	12	11	9	1:2:1	0.30-0.50
	S	12	17	9	10	1:2:1	0.70-0.80
PMS2A × (PRR72 × P1292)	P	41	15	4	0	-	-
	S	36	20	4	0	-	-

*Segregation pattern by pooling SF and SS plants together

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