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Genetics of fertility restoration of 'WA' based cytoplasmic male sterility system in rice (*Oryza sativa* L.) using basmati restorer lines

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Abstract

Hybrid technology in basmati rice (*Oryza sativa* L.) is expected to make a quantum increase in production and boost the export of the country. The success in development of basmati hybrids largely depends on availability of effective restorers and precise basic knowledge on the genetics of fertility restoration of such lines. Study using five diverse restorers and Pusa 3A - a 'WA' type cytoplasmic male sterile (CMS) line in basmati background, revealed the fertility restoration to be governed by two major genes with epistatic interactions that differed from cross to cross. Restorers PRR 78 and PRR 73 when crossed with Pusa 3A, segregated in the ratio of 9:3:4 and 1:1:2 in F_2 and BC_1 generations, respectively for pollen and spikelet fertility indicating two major genes with recessive epistasis involved in fertility restoration. Crosses Pusa 3A/PRR 72 and Pusa 3A/IR 42266 showed a segregation ratio of 9:6:1 and 1:2:1 in F_2 and BC_1 generations, respectively, indicating two major genes governing fertility restoration showing epistasis with incomplete dominance. Pusa 3A/IR 48749 gave segregation ratio of 12:3:1 in F_2 and 2:1:1 in BC_1 generation showing digenic dominant epistatic interaction.

Key words : Rice, basmati, genetics, fertility restoration, restorers.

Introduction

Of the various approaches contemplated to break the existing yield barriers in rice to feed the burgeoing population, hybrid technology is considered as one of the promising, sustainable and ecofriendly technologies. Impressive progress and success made by china in this regard has encouraged other rice growing countries to adopt the technology [1]. Presence of exploitable heterosis, availability of dependable cytoplasmic genetic male sterility and fertility restoration system and sound seed production techniques are the pre-requisites for the success of any hybrid rice breeding programme. In the successful development of basmati hybrids, fertility restoration has been a major problem due to the lack of perfect restorers among basmati types. As a remedy, efforts have been made to develop basmati quality restorers using available partial restorers among some basmati types, as a result of which a good number of restorers are now available and hybrids are being developed using them. In the exploitation of heterosis from potential crosses, the level of fertility restoration would likely be the key for added yield advantage. Therefore, a precise understanding of the genetics of fertility restoration is necessary for improving the efficiency and quality of restorers used in basmati hybrid rice breeding.

Though commendable success has been achieved in this direction, yet there are many researchable issues still to be answered to put basmati hybrid breeding programme on a sound footing for making a sustained progress. Of these, the nature of inheritance of the fertility restoring genes is an important aspect where knowledge is lacking, especially in basmati rice. In order to have a well-directed restorer breeding programme, adequate knowledge on genetic control of male fertility restoration in basmati background is necessary. It is also useful for transferring the fertility restoring genes to promising breeding lines to develop improved restorers. Several reports are available on the genetics of fertility restoration of 'WA' CMS lines in rice which reveal that the nature of fertility restoration ranges from dominant monogenic [2, 3], to dominant digenic [4-6] and with different types of gene interactions [6-8]. However, all these reports pertain to non-basmati restorers and no such information is available relating to basmati germplasm. Keeping the foregoing lacunae in view, the present study on male fertility restoration with special reference to basmati hybrids was undertaken.

Materials and methods

In all 278 test crosses involving advanced breeding lines, traditional basmati lines and IRRI restorers, were screened during *kharif* 1998 to identify effective restorers. Among these, five restorers, viz., PRR 72, PRR 78,

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PRR 73, IR 48749-5-3-2-2-2-2 1R and IR 42266-29-4-3-2-2-2-1R were chosen and crossed to basmati cytoplasmic male sterile (CMS) line Pusa 3A (P3A), which is in the background of Pusa Basmati 1 and possesses 'WA' cytoplasm, for the study of genetics of fertility restoration. Of these PRR 72 and PRR 78 were from Indian Agricultural Research Institute (IARI), New Delhi PRR 73 was a breeding line selected from Pak Bas 385; and the other two were from International Rice Research Institute (IRRI), Philippines.

The crosses were made between Pusa 3A and the selected restorers to produce F_1 seed, at Rice Breeding and Genetics Research Centre, Aduthurai, Tamil Nadu, during *rabi* 1998-99. These were grown at IARI, New Delhi, during *kharif* 1999 to produce F_2 seed and simultaneously back crossed to Pusa 3A to generate BC_1, population. The F_1s, F_2s and BC_1s of each cross were planted at IARI, New Delhi during *kharif* 2000, at a spacing of 20 × 15 cm, and having a plant population of 15, 280-300, 150-200 plants per cross in the respective generations for the study on the genetics of fertility restoration. Genetics of fertility restoration was worked out through (i) pollen fertility and (ii) spikelet fertility studies, for which the standard methodologies were followed. Pollen fertility studies were conducted using 1% Iodine Potassium-Iodine (I-KI) solution, where due care was taken about the proper sampling of the plants from F_1, BC_1 and F_2 populations and anthers from four to five randomly chosen spikelets covering the whole panicle (top, middle and bottom). Pollen fertility in per cent was calculated as :

$$\text{Pollen fertility (\%)} = \frac{\text{No. of fertile pollen grains (stained round)}}{\text{Total no. of pollen grains in the microscopic field (i.e. fertile and sterile)}} \times 100$$

Plants were classified into different fertility-sterility group as was done by Chaudhary *et al.* [9] and supported by Govind Raj and Virmani [7]; where, those with more than 60% fertile pollen were grouped as fully fertile, 30-60% fertile pollen as partial fertile, 1-30% fertile pollen as partial sterile and 0% as complete sterile. The goodness of fit for various Mendelian genetic ratios in the F_2s and back cross progenies was tested by Chi square (χ^2) statistic.

For spikelet fertility, one panicle was selected at random from each of the plants in the F_1, F_2 and the back cross population and bagged. At maturity, harvested panicles were counted for number of filled and unfilled spikelets. Spikelet fertility was computed as:

$$\text{Spikelet fertility (\%)} = \frac{\text{No. of filled spikelets in the panicle}}{\text{Total no. of spikelets in the panicle}} \times 100$$

Plants were classified into fully fertile, partial fertile, partial sterile and complete sterile groups according to the classification proposed by Chaudhary *et al.* [9] and supported by Govinda Raj and Virmani [7]. The plants showing > 80% seed setting were grouped under complete fertile, 30-80% under partial fertile, 1-30% under partial sterile and 0% seed setting under complete sterile group. The goodness of fit for the Mendelian segregation ratios was tested by Chi Square (χ^2) test.

Results and discussion

In the present investigation, segregation pattern for pollen and spikelet fertility of crosses involving five genetically diverse restorers and 'WA' type basmati CMS line Pusa 3A, was studied. Data on pollen and spikelet fertility per cent of the F_1 hybrids presented in Table 1, show that pollen fertility ranged between 65 per cent in Pusa 3A/PRR 73 to 76 per cent in Pusa 3A/IR 42266. On the other hand, spikelet fertility ranged between 73 per cent in Pusa 3A/PRR 73 to 82 per cent in Pusa 3A/IR 42266. The results revealed that fertility restoration is under dominant gene control and the degree of restoration varied with the restorers [6]. In general, spikelet fertility count showed 8 to 10% higher values as compared to pollen fertility. IR 42266 showed the best restoring ability with the highest pollen and spikelet fertility counts (76.32% and 81.95%, respectively). On the other hand, PRR 73 showed the lowest pollen fertility of 64.54% and spikelet fertility count of 72.69%.

Table 1. Pollen and spikelet fertility scores in F_1 hybrids

Cross combination	Pollen fertility (%)	Spikelet fertility (%)
1. Pusa 3A/PRR 72	70.56	78.29
2. Pusa 3A/PRR 78	68.43	78.80
3. Pusa 3A/PRR 73	64.54	72.69
4. Pusa 3A/IR 48749	75.84	80.87
5. Pusa 3A/IR 42266	76.32	81.95

*Cross Pusa 3A/PRR 73 was classified as fully fertile on the basis of pollen fertility per cent only.

Fertility restoration in all the crosses in the present investigation was found to be governed by two independent dominant genes, which is well supported from F_2 and BC_1 segregation data (Table 2) and these results are in conformity with the findings of several researchers [7, 10, 11]. The fertility restoring action of one of the genes seemed to be stronger than the other because the presence of one of the genes alone conferred partial pollen fertility i.e. 30-60% (Table 2). Other workers using different CMS-R lines combinations [6–8, 10] obtained similar results. The present investigation, however, suggested that the mode of action of the two genes varied from cross to cross. For instance, crosses Pusa 3A/PRR 72 and Pusa 3A/IR

42266- 29-4-3-2-2-2-1R exhibited epistasis with incomplete dominance (F_2 ratio, 9:6:1); Pusa 3A/PRR 78 and Pusa 3A/PRR 73 recessive gene interaction (F_2 ratio, 9:3:4), and Pusa 3A/IR 48749-5-3-2-2-2-2-1R dominant gene interaction (F_2 ratio 12:3:1). Furthermore, the results of the test crosses involving the same female parent for each cross also confirmed the F_2 segregation ratios and are supported by earlier findings [7, 8, 12]. From the hypothesis put forward for digenic inheritance by Bharaj *et al.* [6], where, assuming that R_1 and R_2 as the two dominant alleles of the two restorer genes, the plants having dominant alleles of the two genes in homozygous or heterozygous condition ($R_1\_R_2\_$) will be fertile. The plants having dominant alleles of one of the two genes in homozygous or heterozygous condition but homozygous recessive alleles of the other gene ($R_1\_r_2$ r_2 or r_1 r_1 $R_2\_$) will behave as partially sterile or partially fertile, and *vice versa*. The plants homozygous for the recessive alleles of both the genes (r_1 r_1 r_2 r_2) will be completely sterile. In the case of epistasis with dominant gene interaction (F_2 ratio 12:3:1 and BC_1 ratio 2:1:1) as observed in the cross Pusa 3A/IR 48749-5-3-2- 2-2-2-1R, the plants having dominant alleles of the two genes in either homozygous or heterozygous condition ($R_1\_R_2\_$) and those having dominant allele of one of the two genes in homozygous or heterozygous condition by homozygous for the other gene ($R_1\_r_2$ r_2 or r_1 $r_1R_2\_$) will be fertile completely, depending on the strength of the gene, thus grouped into one group. This shows the predominance of the stronger gene in its ability to restore fertility.

In crosses Pusa 3A/PRR 78 and Pusa 3A/PRR 73, where the fertility restoration was governed by dominant genes with recessive epistatic interaction (F_2 ratio 9:3:4, BC_1 ratio 1:1:2), the plants homozygous for the recessive alleles of any one of the two genes but homozygous or heterozygous for the dominant alleles of the other gene ($R_1\_r_2r_2$ or $r_1r_1R_2\_$) were sterile depending upon which of the two genes is stronger or weaker. Similarly, in crosses Pusa 3A/PRR 72 and Pusa 3A/IR 42266-29-4-3-2-2-2 1R, where the fertility restoration was governed by dominant genes with semi-dominant epistatic interaction (F_2 ratio 9:6:1, BC_1 ratio 1:2:1), the plants where the recessive gene is allelic for any of the two genes and homozygous or heterozyous for the dominant alleles of the other gene ($R_1\_r_2r_2$ and $r_1r_1R_2\_$) were semi-fertile.

These differences in the type of gene interaction could presumably be due to the influence of female parent and/or a probable variable expression of the weaker gene in different genetic backgrounds. Certain modifier genes could also be responsible for changing the segregation ratio in different generations of study as reported [7, 8].

The analysis based on spikelet fertility (Table 2) gave similar results. Nevertheless, a tendency for high numbers of fertile plant was noticed in the test crosses and most of the F_2 populations. It may be possible that a partially fertile plant (classified based on pollen analysis) could turn out to be fully fertile (by spikelet fertility classification) because relatively few fertile pollen grains are sufficient to effect fertilization. Some plants with lower pollen fertility were graded as higher spikelet fertility types by scores based on spikelet fertility analysis. Therefore, studies based on both pollen and spikelet fertility counts are more precise and reliable than those based on spikelet fertility or pollen fertility alone.

In the present investigation, the genetic ratios were worked out by taking fertility/sterility as discrete qualitative traits, though the role of quantitative component cannot be ruled out. The frequency distribution of seed set percentage was multimodal, suggesting thus, the role of environmental influence. It appears from the present study that fertility restoration is due to the confounding effects of several factors such as number and effectiveness of restorer genes involved, their variable expression, genetic background and above all environmental influence. Similar reports of earlier researchers [5, 7, 13-16] give credence to the present surmises.

The results discussed above revealed that fertility restoration in basmati rice appeared to be governed by two genes with epistatic interaction that differed from cross to cross which shows the possibility of existence of the most appropriate combiantion of the two fertility restoring genes in order to give complete fertility restoration in basmati hybrids. Though the two independently segregating dominant genes present in the restorers PRR 78 and PRR 73 exhibited recessive epistatic interaction in F_2 and testcross populations for male fertility restoration, such type of epistatic interaction is not detrimental to the development of basmati hybrids because the dominant alleles of both the genes present in the F_1 plants will impart complete fertility to the hybrids involving the restorers and CMS lines with 'WA' cytoplasm. We are therefore, very hopeful to breed for promising restorer lines possessing complete fertility restoring ability in basmati germplasm and use them to develop high yielding basmati hybrids when crossed with potential 'WA' type CMS lines also having basmati quality. Studies are also under way to determine the allelic relationships among the restorer genes of the diverse restorers used in order to formulate a pragmatic breeding programme for the development of high yielding basmati hybrids.

Table 2. Segregation pattern for fertility restoration (pollen and spiklet analysis) in crosses involving Pusa 3A(P3A) and five restorers

Cross combination	Generation	Total no. of plants	FF	Segregation pattern no. of plants with fertility reaction			CS	Genetic ratios*	Probability
				PF	SF*	PS			
P3A/PRR72	F_2	286	170 (172)	60 (65)	94 (92)	34 (27)	22 (22)	9:6:1	0.30-0.20 (0.20-0.10)
P3A//P3A/PRR72	BC_1	150	39 (40)	34 (33)	71 (70)	37 (37)	40 (40)	1:2:1	0.80-0.70 (0.80-0.70)
P3A/PRRR78	F_2	290	159 (164)	30 (32)	65 (60)	35 (28)	66 (66)	9:3:4	0.30-0.20 (0.70-0.50)
P3a//P3A/PRR78	BC_1	144	29 (36)	20 (17)	34 (27)	14 (10)	81 (81)	1:1:2	0.50-0.30 (0.20-0.10)
P3A/PRR73	F_2	281	163 (165)	24 (25)	54 (52)	30 (27)	64 (64)	9:3:4	0.70-0.50 (0.70-0.50)
P3A//P3A/PRR73	BC_1	113	30 (31)	13 (14)	32 (31)	19 (17)	51 (51)	1:1:2	0.70-0.50 (0.70-0.50)
P3A/IR-48749	F_2	291	230 (220)	29 (27)	39 (49)	10 (22)	22 (22)	12:3:1	0.10-0.05 (0.70-0.50)
P3A//P3A/IR-48749	BC_1	139	60 (65)	27 (23)	41 (38)	14 (15)	38 (38)	2:1:1	0.30-0.20 0.70-0.50)
P3A/IR-42266	F_2	285	152 (165)	64 (65)	107 (94)	43 (29)	26 (26)	9:6:1	0.20-0.10 (0.10-0.20)
P3A//P3A/IR-42266	BC_1	141	32 (38)	36 (31)	75 (69)	39 (38)	34 (34)	1:2:1	0.80-0.70 (0.90-0.80)

*Genetic ratios were obtained by pooling partial fertile (PF) and partial sterile (PS) plants together into semi-fertile (SF) group. Values in parenthesis represent spikelet fertility data. IR 48749-5-3-2-2-2-2-1R and IR 42255-29-4-3-2-2-2-1R are represented as IR 48749 and IR 42266 in the table respectively.

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