

Pollen and spikelet fertility analysis in rice crosses involving WA cytosteriles

S. Murugan* and J. Ganesan

Department of Agricultural Botany, Faculty of Agriculture, Annamalai University, ANNAMALAINAGAR (T.N.) INDIA

ABSTRACT

Three cytoplasmic malesterile lines viz., IR58025A (L₁), IR 62829 A (L₂), V20 A (L₃) of wild abortive (WA) source were crossed as 'lines' with fourteen genotypes as 'testers' to get 42 hybrids. The 42 hybrids obtained were subjected to pollen and spikelet fertility analysis. Six testers viz, IR72, IR24, Duansan, ARC 11353, IR 54 742-2-22-19-3 and IET 13153 were identified as restorers for the three CMS lines.

Key words : Pollen, Spikelet, Fertility.

INTRODUCTION

The use of cytoplasmic genetic male sterility system in developing hybrids in crops is possible only when effective restorers are identified. In rice, effective restorers have been identified among cultivated varieties and elite breeding lines for various CMS systems. Pollen or spikelet fertility or both have been used as an index to fix the restoration ability of the lines (Sutaryo, 1989). Identification of new restorers is a continuous process in hybrid rice development programme in order to increase heterosis and reduce genetic vulnerability due to plasmon uniformity. The present study was undertaken to find out the fertility restoration ability of the fourteen testers on the three WA cytoplasm.

MATERIALS AND METHODS

Three cytoplasmic male sterile lines viz., IR58025 A (L₁), IR 62829 A (L₂) and V20 (L₃) and fourteen testers IR 72 (T₁), IR 24 (T₂), Duansan (T₃), ARC 11353 (T₄), IR 54742-22-19-3 (T₅), IET 13153 (T₆), IR20 (T₇), IET 1444 (T₈), ADT 36 (T₉), TKM 9 (T₁₀), ASD 16 (T₁₁), ADT 39 (T₁₂), CO37 (T₁₃) and Co41 (T₁₄) were raised in a crossing block during Navarai 2000 (Jan-May). Three weekly sowings were taken to synchronize the flowering. Crosses were effected in line x tester fashion to get 42 hybrids. The one third of the tip of the spikelets in the panicle of CMS lines were clipped off using scissors during early morning hours (6.30 AM) and the immature spikelets were removed. The panicles were covered with butter paper cover. At the time of anther dehiscence, panicle from desired male parent was collected and inserted through the top of the cover and brushed over the clipped off spikelets of CMS lines to effect pollination. The F₁ generation of all the crosses were raised during kuruvai 2001 (June-July) in a test cross nursery. Each entry was planted in a single row of 20 plants with a spacing of 20 cm x 20 cm. All the plants in each entry were scored for pollen and spikelet fertility. The anthers were crushed in one per cent iodine – potassium iodide solution and observed under light microscope. The countings were taken in five fields per slide. The completely stained spherical pollens were considered fertile, while, the unstained, partially stained and shriveled pollens were taken as sterile (Young et al., 1983).

$$\text{Pollen fertility \%} = \frac{\text{Number of stained round pollen}}{\text{Total number of pollen counted}} \times 100$$

The pollen fertility status was fixed as under, following IRRI (1986) and govindaraj and Virmani (1988) classification.

Pollen fertility per cent	Fertility status
60-100.00	Fertile
30-59.99	Partially fertile
1-29.99	Partially sterile
0-0.99	Sterile

*Author for correspondence

The spikelet fertility was calculated as the proportion of fully developed grains to the total number of spikelets on selfed primary panicle of each selected plant. The range of spikelet fertility was fixed as under (Govindaraj and Virmani, 1988).

Spikelet fertility per cent	Fertility status
80-100.00	Fertile
30-79.99	Partially fertile
1-29.99	Partially sterile
0-0.99	Sterile

RESULTS AND DISCUSSION

The pollen fertility per cent of hybrids was found to vary from 19.7 (L₂ x T₁₄) to 97.80 (L₁ x T₂). The hybrids produced by using the three male sterile lines with testers T₁ to T₆ showed high fertility status (> 60 per cent) thereby classifying the male parents as restorers. The testers T₁₀, T₁₂ and T₁₃ showed partial restoration with the three CMS lines used, whereas T₁₄ showed partial maintenance reaction. The remaining testers T₇, T₈, T₉ and T₁₁ showed restoration / partial restoration with one line and partial maintenance with other line (Table 1). This kind of differential reaction of the same genotype in restoring the fertility of different CMS lines of same cytoplasmic source was reported by Virmani (1987), Leenakumari *et al.* (1994).

Table 1: Pollen fertility (per cent) of F₁ hybrid in test cross nursery

Lines/testers	L ₁	L ₂	L ₃
T ₁	94.67(R)	92.82(R)	91.36(R)
T ₂	97.08(R)	87.65(R)	90.86(R)
T ₃	84.78(R)	86.74(R)	94.52(R)
T ₄	93.60(R)	87.75(R)	87.00(R)
T ₅	95.60(R)	88.91(R)	87.21(R)
T ₆	91.78(R)	86.53(R)	92.78(R)
T ₇	45.00(PR)	32.49(PR)	26.31(PM)
T ₈	51.67(PR)	58.74(PR)	70.38(R)
T ₉	23.60(PM)	84.74(R)	54.89(PR)
T ₁₀	54.61(PR)	59.81(PR)	50.75(PR)
T ₁₁	41.43(PR)	58.16(PR)	27.71(PM)
T ₁₂	41.43(PR)	58.16(PR)	44.31(PR)
T ₁₃	31.52(PR)	53.86(PR)	40.71(PR)
T ₁₄	21.30(PM)	19.70(PM)	24.30(PM)

FM : Partial maintainer
 PR : Partial restorer
 R : Restorer

And Hariprasanna *et al.* (2005). This could be due to differential nuclear cytoplasmic interactions between the testers and CMS lines.

The spikelet fertility of F_1 s ranged from 23.68 to 94.13 percent (Table 2). The tester T_1 to T_6 showed spikelet fertility restoration with all the three CMS lines. The tester T_9 also exhibited restoration (81.74 per cent) with L_2 , but behaved as partial restorer with L_1 (38.49 per cent) and L_3 (70.59 per cent). The remaining crosses revealed partial restoration except $L_3 \times T_7$, $L_3 \times T_{11}$, $L_2 \times T_{14}$ and $L_3 \times T_{14}$ which showed partial maintenance reaction. None of the testers behaved as complete maintainer with the three CMS lines.

There were instances in which the classification of testers

Table 2 : Spikelet fertility (per cent) of F_1 hybrid in test cross nursery

Lines / Trsters	L_1	L_2	L_3
T_1	92.1(R)	89.61(R)	94.13(R)
T_2	92.45(R)	86.42(R)	91.28(R)
T_3	86.30(R)	81.78(R)	91.64(R)
T_4	91.70(R)	88.49(R)	89.73(R)
T_5	94.33(R)	87.61(R)	88.31(R)
T_6	93.71(R)	82.67(R)	90.38(R)
T_7	58.48(PR)	34.71(PR)	29.65(PM)
T_8	60.51(PR)	64.38(PR)	70.61(PR)
T_9	38.49(PR)	81.74(R)	70.61(PR)
T_{10}	50.35(PR)	60.91(PR)	49.73(PR)
T_{11}	63.45(PR)	54.23(PR)	23.68(PM)
T_{12}	52.47(PR)	60.74(PR)	50.89(PR)
T_{13}	42.73(PR)	40.12(PR)	37.65(PR)
T_{14}	4.75(PR)	24.37(PM)	26.15(PM)

PM : Partial maintainer
PR : Partial restorer
R : Restorer

based on pollen fertility did not correlate with the classification based on spikelet fertility. For example, IET 1444 with IR 62829 A was categorized as partial restorer by spikelet fertility analysis and as restorers by pollen fertility analysis. Such non correlation between pollen fertility and spikelet fertility was reported by Oinam and Kaushik (1993). The genotype TKM 9 identified as partial restorer in this

location has been reported to be a restorer at Faizabad, and maintainer at Coimbatore (DRR, 1997). Such a differential reaction of pollen parents at different locations may be attributed to genic x environmental interaction or due to some minor differences in the genetic constitution of the parents maintained at different centers. For fertility to express the restorers should have the whole spectrum of major and minor genes. The tester T_1 to T_6 , T_8 and T_9 could be utilized in heterosis breeding after testing their combining ability and heterosis.

REFERENCES

- DRR. (1997).** Development and use of hybrid rice technology. Final report of hybrid rice project (1991-1996). Directorate of Rice Research, Rajendranagar, Hyderabad, India.
- Govindaraj, K. and S.S.Virman. (1988).** Genetics of fertility restoration of 'WA' type cytoplasmic male sterility in rice. *Crop. Sci.*, **28**: 787-792.
- Hariprasanna, K., F.U. Zaman and A.K. Singh. (2005).** Identification of versatile fertility restorer genotypes for diverse CMS lines of rice (*Oryza sativa* L.). *Oryza*, **42(1)** : 20–26.
- IRRI. (1986).** Annual report 1985. Int. Rice Res. Inst. Manila, Philippines.
- Leenakumari, S., M.Mahadevappa and A.Mohan. (1994).** Restorers for cytoplasmic male sterile lines derived from MS 577A. *Int. Rice Res. Newsl.*, **19(1)**: 5-6.
- Oinam, G.S. and R.P. Kaushik (1993).** Identifying maintainer and restorers lines for hybrid rice in Himachal pradesh (H.P), *India. Int. Rice Res. Notes*, **18**: 7-8.
- Sutaryo, B. (1989).** Evaluation of some F_1 rice hybrids developed using MB 365 A as CMS line. *Int. Rice. Res. Newsl.*, **14**: 7-8.
- Virmani, S.S. (1987).** hybrid rice breeding in: Feistifer WP, Kelley AT (eds). Hybrid seed production of selected cereal, oil and vegetable crops. *FAO Plant Prot. Pap.* **82**: 35-53.
- Young, S.S. Virmani and G.S. Kush (1983).** Cytogenetic relationship among cytoplasmic genic male sterile, maintainer and restorer lines of rice. *Philippines J. Crop Sci.*, **8(3)**: 119-124.

Received : August, 2005; Accepted : January, 2006