Research Article

Pollen fertility Vs Spikelet fertility in F2 of a CMS based hybrids in rice (*Oryza sativa L.*) under Aerobic condition

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Abstract :
Identification of maintainer and restorers from rice germplasm through test crossing is the initial step in three line heterosis breeding. Maintainer lines can be converted into cytoplasmic male sterile lines to utilize in development of rice hybrids. Restorer lines can be used as males to produce F_1 hybrids. Three hundred F_2 plants of the cross KCMS 26A X IET 19886 were grown under aerobic situation and fifty randomly selected plants were analyzed for per cent pollen fertility (using one per cent I-KI solution) and per cent spikelet fertility. Based on the per cent pollen fertility (PF %) the individual plants were grouped as effective restorer (>96% PF), partial restorer (21-95% PF), partial maintainer (6-20% PF) and maintainer (<5%PF) lines. Based on spikelet fertility (SF %) individual plants were grouped as effective restorer (>80% SF), partial restorer (21-79% SF), partial maintainer (1-20% SF) and maintainer (<1%SF). There was strong evidence for relationship between pollen fertility and spikelet fertility. Potential restorer is one, that would produce high per cent of seed set in F_1 while, a potential maintainer would produce 100 per cent sterile pollen in F_1. The results in the current study with F_2 progenies of a cross involving male sterile line indicated that spikelet fertility and pollen fertility are the potential traits in identification of restorer and maintainer lines, respectively.

Key words:
Maintainer, restorer, fertility restoration, pollen fertility, aerobic hybrid

Introduction

Ever growing human population demands more and more rice has to be produced from lesser land and lesser inputs (Sabar and Akhter, 2003) due to increased pressure on these resources. Future food security of major rice growing countries lies in the development of hybrid rice varieties which has potential to increase production and productivity. Rice hybrids released in the country have shown 15-20 per cent higher yield potential than inbred varieties. The procedure for developing rice hybrids is quite distinct from those employed for breeding conventional varieties. The cytoplasmic genetic male sterile system involving a CMS source, a maintainer and a restorer is extensively being used in the production of commercial rice hybrids (Virmani *et. al,* 1997). In hybrid rice breeding the establishment of test cross nursery to identify restorer and maintainer is the first step. Maintainers and restorers are identified based on the pollen and spikelet fertility. The objective of this study was to evaluate the potential trait in identifying maintainers and restorers useful in three line rice heterosis breeding.

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Material and methods

Three hundred F_2 plants derived from a CMS based cross KCMS 26A / IET 19886 were grown during kharif 2009 under aerobic condition at UAS, GKVK, Bengaluru. The crop was maintained by dry sowing and irrigation was provided once in 5-7 days as per the standard package of practices for aerobic rice. Other agronomic and plant protection measures were also adopted during crop growth period. Pollen studies were carried out on fifty (A1-A50) randomly selected F_2 plants for their fertility/sterility. For each randomly selected plant three spikelets each from top, middle and bottom of the main panicle were collected and pollen grains were squeezed out from the entire anther on a clean glass slide and stained with one per cent Iodine Potassium Iodide (I-KI) stain. The slides were examined under microscope at a magnification of 40X. Round well filled and deeply stained pollen grains were counted as fertile and shriveled and lightly stained were counted as sterile. Pollen fertility was expressed in percentage as given below.

$$\text{Pollen fertility (\%)} = \frac{\text{Total number of fertile pollen grains in 5 microscopic fields}}{\text{Total number of pollen grains in 5 microscopic fields (fertile + sterile)}}$$

Spikelet fertility was assessed at seed maturity stage from two panicles from each selected plant and expressed in percentage by taking the actual count of filled seeds to the total number of spikelets in a panicle.

$$\text{Spikelet fertility (\%)} = \frac{\text{Number of filled spikelets}}{\text{Total number of spikelets}}$$

Result and discussion

Pollen fertility:

Pollen fertility is one of the important traits in three line heterosis breeding especially at test cross nursery stage which is a first step. In the present study pollen fertility showed significant correlation with spikelet fertility. The plants A21, A38, A13, A41 and A1 showed high pollen fertility of 100, 96.2, 94, 93.8 and 86.46 per cent, respectively but their spikelet fertility was not in the range of restorer. Pollen fertility is a genetically controlled trait and is less influenced by environment; however spikelet fertility is influenced by environmental factors like nutrition. Therefore pollen fertility alone may not be an efficient trait to identify restorers. On the contrary, in plants like A6, A44, A4, A18 and A16, per cent pollen sterility was well correlated with per cent spikelet sterility. This result shows that pollen sterility could be used as a potential trait in identification of maintainers rather than restorers.

Spikelet fertility:

Yield of heterotic F_1 depends on number of filled spikelets which is an important yield attributing trait. In the present study plants like A7, A9, A20, A27 and A1 recorded higher spikelet fertility of 83.6, 79.87, 77.65, 77.2 and 74.14 per cent besides pollen fertility of 70.3, 67.6, 53, 83.93, and 54.1 per cent, respectively. Even the plants with medium pollen fertility per cent (40-60%) showed high spikelet fertility. This could be mainly because each spikelet may receive many pollen grains but one fertile pollen would be sufficient to fertilize the spikelet (Joshi *et al*. 2003). Hence for identification of a restorer line spikelet fertility is potential trait than pollen fertility.

Pollen fertility and spikelet fertility traits of the hybrids can be used for identifying maintainers and restorers (Virmani *et al*. 1997; Sarial and Singh 2000 and Sabar and Akhter, 2003). In test cross nursery, pollen fertility of the test cross can be used for identification of maintainers in early stage itself i.e. at flowering, so that the genotype can be utilized in conversion program through backcrossing in the same crop season.

The result indicated that pollen fertility is a potential trait for identification of maintainer lines and the spikelet fertility for identification of restorers in early stage of flowering and can be effectively applied for three line heterosis breeding in rice.

References

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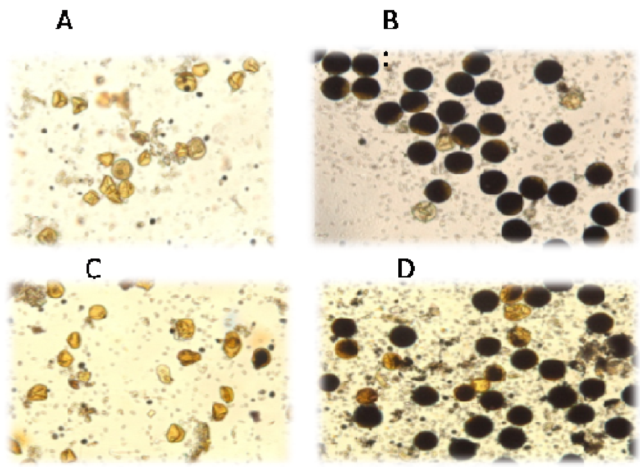

Figur 1:differential staining of rice pollen graines of selected F_2 progenies of hybrid cross stained using I-KI solution: Round dark stained –fertile pollen and light stained with irregular shape- sterile pollen. A: maintainer type reaction B: restorer type reaction,C: partial maintainer type, D: partial restorer type.

Table 1. Mean and range of pollen fertility, spikelet fertility in fifty F_2 plants of cross KCMS26A X MLTK-12 in rice

Character	Mean	Minimum	Maximum	Std. Dev.
Pollen fertility (%)	43.40	0	100	29.67
Spikelet fertility (%)	36.01	0	83.6	25.87
Days to 50% flowering(DFF)	103.77	92	120	6.39

Figure 2: Frequency distribution of A: spikelet fertility (SFT), B: pollen fertility (PFT) C: F_2 cross of KCMS26A X MLTK-12 in rice

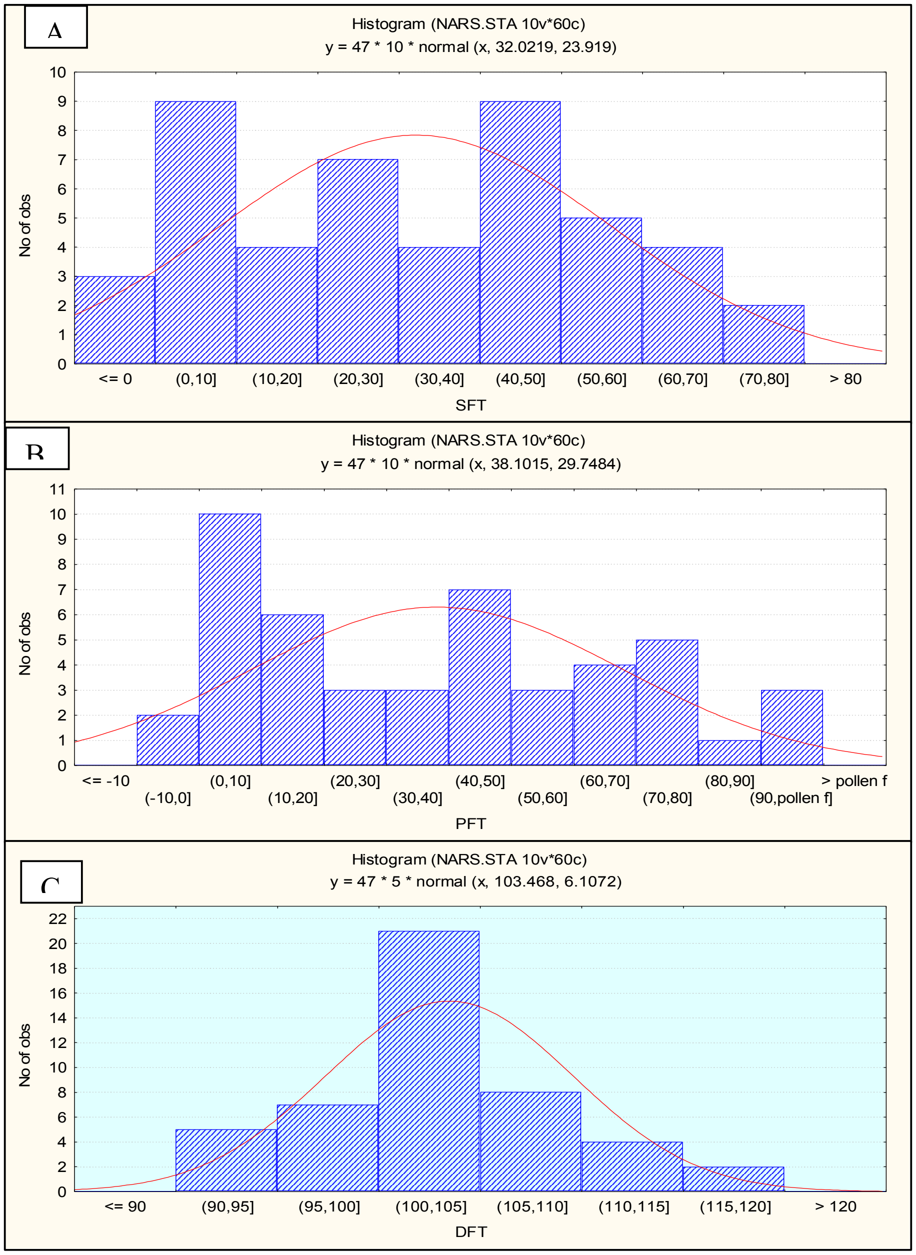

Table 2. Top 10 Fertile plants based on pollen fertility and spikelet fertility

Plant no	Pollen fertility %	Plant no	Spikelet fertility %
A21	100	A7	83.60
A38	96.2	A9	79.87
A13	94	A20	77.65
A41	93.8	A27	77.20
A5	86.46	A1	74.14
A27	83.98	A23	67.27
A15	76.96	A38	63.33
A35	76	A50	62.50
A24	75.68	A41	60.07
A8	75	A8	59.57

Table 3. Top 10 Sterile plants plants based on pollen fertility and spikelet fertility

Plant no	Pollen fertility %	Plant no	Spikelet fertility %
A6	0	A6	0.00
A44	0	A26	0.00
A4	0.2	A44	0.00
A18	0.9	A18	0.37
A16	2.4	A4	0.50
A29	2.56	A36	0.63
A26	3.93	A45	0.67