Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(5): 1283-1285
Received: 10-07-2020
Accepted: 16-08-2020

K Parimala
Seed Research and Technology Centre, PJTSAU, Rajendranagar, Hyderabad, Telangana, India

Ch. Surender Raju
Rice Research Centre, Rajendranagar, Hyderabad, Telangana, India

AS Hari Prasad
ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad, Telangana, India

S Sudheer Kumar
PJTSAU, Rajendranagar, Hyderabad, Telangana, India

Corresponding Author:
K Parimala
Seed Research and Technology Centre, PJTSAU, Rajendranagar, Hyderabad, Telangana, India

Evaluation of genotypes for identification of potential restorers and maintainers for development of hybrid rice (*Oryza sativa* L.)

K Parimala, Ch. Surender Raju, AS Hari Prasad and S Sudheer Kumar

Abstract
A study was undertaken for identification of maintainers and restorers to use in hybrid rice breeding programme. In the present study 45 test crosses were developed by crossing three CMS lines with fifteen elite rice genotypes in line x tester design. The resultant 45 test crosses were evaluated and classified as restorers (14), partial restorers (21), partial maintainers (9) and maintainer (1) based on pollen fertility and spikelet fertility percentage. Some of the genotypes exhibited differential fertility reaction with CMS lines. Among the fifteen genotypes tested for their fertility restoration reaction, the genotypes *viz*., RNR-17473, WGL-451 and WGL-3962 were found to be restorers with all the three CMS lines studied. Potential restorers found in the present investigation could be used in hybrid rice breeding programme to develop promising rice hybrids. Only one maintainer identified in this study could be developed as new CMS line by repeated backcross breeding with their respective F_1's.

Keywords: Hybrid rice, test cross, restorer, maintainer

Introduction
Rice is the important staple food crop for more than half of the world's population. The increased demand for rice is expected to exceed production in many countries in Asia, Africa and Latin America. Plateauing trend in the yield of high yielding varieties, declining and degrading natural resources like land and water make the task of increasing rice production quite challenging. It necessitates looking for some innovative technologies to boost up rice production in the country. In this situation, hybrid rice technology is the best feasible and readily acceptable options available to increase the production. Expansion of hybrid rice cultivation area may be an effective and economic way to meet the future rice demands of growing population (Shrivastava *et al*. 2015) [10]. It has a yield advantage of 20-30% over conventional high yielding varieties (Virmani *et al*. 2003) [12]. For the development of viable, adaptable rice hybrids through utilization of cytoplasmic genetic male sterility, the process of identification of maintainers and restorers involving local elite lines has become inevitable. Identification of locally adapted maintainers and restorers which show complete sterility and consistently high degree of restoration of CMS lines would be of great value in commercial hybrid programme, if restoring ability is combined with high combining ability. Parmeshwar Kumar *et al*. (2014) [8], Pankaj Kumar *et al*. (2015) [7], Geeta *et al*. (2016) [3], Rajendra Prasad *et al*. (2017) [5] and Ramesh *et al*. (2018) [9] evaluated the test crosses in rice to identify the restorer and maintainer reaction and reported varying levels of pollen and spikelet fertility percentage. Development of test cross nursery to identify restorers and maintainers is the first step in three line heterosis breeding (Akhter *et al*. 2008) [1]. Keeping in view of the above, the present study was taken up to identify the potential restorers and maintainers to use in hybrid rice development programme.

Material and methods
The material for the present study consists of three CMS lines (IR-80555A, IR-68897A and APMS-6A) and 15 diverse male fertile genotypes and crosses were made in line x tester design. Staggered sowing of parental lines was taken up to attain synchronous flowering and get adequate crossed seed. In a crossing block, 28 days old seedlings of CMS lines and male fertile genotypes were transplanted with a spacing of 20 x 15 cm during rabi 2012-13 at Rice Research Centre, Rajendranagar, Hyderabad. Recommended package of practices and need based plant protection measures were taken up to raise healthy crop. Male sterile lines with just emerged panicles were uprooted and potted into plastic buckets filled with mud and shifted to crossing chamber.

The leaf sheath of the selected panicles was carefully removed. The florets located on the top and at the bottom of the panicle were also removed. Florets expected to open on the next day were used for crossing. Top 1/3rd portion of each floret was clipped previous day evening and covered with butter paper bags. The next day morning pollen from male parents were collected at the time of anthesis and dusted on the bagged panicles of CMS line and labelled. The crossed seeds were collected after seed maturity.

The 45 test crosses developed during rabi 2012-13 were sown during kharif, 2013 and transplanted in 6 meter rows with a spacing of 20 x 15 cm to assess the restorer / maintainer reaction. Pollen fertility test was carried out at flowering stage by collecting the 15-20 spikelets in a vial containing 20% ethanol from five randomly selected plants of each entry. The anthers from spikelets were taken out with the help of forceps and placed on glass slide containing 2% iodine potassium iodide solution. The anthers were gently crushed by using needle to release the pollen grains. After removing the debris, covered with cover slip and observed under microscope to estimate the pollen fertility percentage of test crosses. The pollen fertility percentage was calculated as per the following formula

$$\text{Pollen fertility (\%)} = \frac{\text{Number of fertile pollen grains}}{\text{Total number of pollen grains}} \times 100$$

To estimate the spikelet fertility, three panicles per plant from five randomly selected plants of each test cross were covered with butter paper bags during flowering to avoid pollen contamination. After maturity, the panicles were harvested and threshed. The number of filled and chaffy grains of each panicle were counted separately to estimate the spikelet fertility by using the following formula.

$$\text{Spikelet fertility (\%)} = \frac{\text{Number of filled grains per panicle}}{\text{Total number of grains per panicle}} \times 100$$

Classification of pollen parents as restorers and maintainers were done as per the method proposed by Virmani *et al.* (1997) [13].

Category	Pollen fertility (%)	Spikelet fertility (%)
Maintainers	0-1	0-0.1
Partial maintainers	1.1-50	0.1-50
Partial restorers	50.1-80	50.1-75
Restorers	>80	>75

Results and discussion

The establishment of test cross nursery for identification of restorers and maintainers is the initial step in three-line hybrid rice programme. In the present study, 45 test crosses were developed and evaluated for pollen and spikelet fertility percentage. The performance of the hybrid combinations in test cross nursery for fertility restoration is presented in Table-1. The 45 test crosses were classified as restorers (14), partial restorers (21), partial maintainers (9) and maintainer (1) based on pollen fertility and spikelet fertility percentage. It indicates that fertility restoration reaction of the genotypes varies with their genetic background. A wide range of pollen fertility percentage was noticed which varied from 0.3 % (IR-68897A x RNR-18833) to 90.2 % (IR-68897A x RNR-17473). The spikelet fertility showed a range from 0.5 to 89.2 % and it showed that fertility restoration varied depending on male parent. Among the fourteen restores identified, the crosses *viz*., IR-80555A x RNR-17438, IR-68897A x RNR-17473, IR-68897A x WGL-451, IR-68897A x WGL-3962 and APMS6A x WGL-451 exhibited high spikelet fertility percentage (>80%).

Among the fifteen hybrids produced in combination with IR-80555A, five genotypes considered as restorers, seven genotypes were partial restorers, two were found to be partial maintainer and one genotype (IR-80555A x RNR-18833) showed complete sterility. The test crosses developed with IR-68897A classified as restorers (4), partial restorers (8) and partial maintainers (3). Out of 15 test crosses made with APMS-6A showed that five genotypes were found to be restorers, six were partial restorers and four genotypes exhibited partial maintainer reaction. The variations in fertility restoration of genotypes could be due to differential nuclear cytoplasmic interactions between the testers and CMS lines. Jayasudha and Sharma (2010) [4], Das *et al*. (2013) [2], Geeta *et al*. (2016) [3] and Neha (2017) [6] also reported the differential reaction of the same genotype in restoring the fertility of different CMS lines of same cytoplasmic source.

In the present study, most of the genotypes showed different fertility reaction with different CMS lines except RNR-17473, WGL-451 and WGL-3962 which were found to be restorers with all the three CMS lines. Hence these genotypes can be used as potential restorers to develop high yielding rice hybrids. The genotypes *viz*., RNR-15351, RNR-17420, RNR-17472, RNR-15028 and RNR-17494 exhibited partial restorer reaction with all the CMS lines studied. The genotype, RNR-883 considered as restorer with two CMS lines (IR-68897A and APMS 6A) and the genotype RNR-17438 found to be restorer with CMS lines such as IR-80555A and APMS-6A. The percentage of restorers for CMS lines such as IR-80555A, IR-68897A and APMS-6A was found to be 33.33, 26.67 and 33.33 per cent respectively while only one maintainer for CMS line IR-80555A was observed with a percentage of 6.76 (Fig-1). The study revealed that most of the genotypes were partial restorers for CMS lines *viz*., IR-80555A, IR-68897A and APMS-6A was found to be 46.67, 53.33, 40.00 % respectively. The frequency of partial restorers was higher than that of maintainers. Similar reports of higher frequency of partial restorers and low frequency of maintainers were observed by Veeresha *et al.* (2013) [11] and Geeta *et al*. (2016) [3].

From the study it was evident that percentage of partial restorers were found to be higher compared to other categories. The fertility restoration reaction of the genotypes varied with their genetic background. The identified potential restorers *viz*., RNR-17473, WGL-451 and WGL-3962 which were found restorers with all the three CMS lines can be used as pollen parent in hybrid rice breeding programme. New restorers may also be developed through crossing programme between identified restorers which can expand the genetic base of restorer by pyramiding complementary traits from diverse sources according to breeding objectives. The cross combination (IR-80555A x RNR-18833) identified as maintainer can be developed as new cytoplasmic male sterile line by repeated back cross breeding.

Table 1: Classification of the genotypes based on pollen and spikelet fertility percentage of test crosses in rice

Male fertile line	Cytoplasmic male sterile line								
	IR-80555A			IR-68897A			APMS-6A		
	Pollen fertility (%)	Spikelet fertility (%)	Category	Pollen fertility (%)	Spikelet fertility (%)	Category	Pollen fertility (%)	Spikelet fertility (%)	Pollen fertility (%)
RNR-17473	83.3	79.4	R	90.2	89.2	R	83.4	77.9	R

RNR-15351	63.5	55.8	PR	45	60.9	PR	62.8	55.6	PR
WGL-451	84.4	76.2	R	87.1	83.1	R	81.4	80.1	R
Honne Kattu	57.4	54.8	PR	40.2	45.2	PM	61.7	44.3	PM
RNR-17420	67.6	61.7	PR	65.2	57.8	PR	66.3	71.9	PR
RNR-17472	62.1	64.4	PR	68.3	70.6	PR	49.2	65.8	PR
RNR-15028	68.1	51.0	PR	52.5	58.5	PR	76.1	56.4	PR
RNR-15038	83.1	78.5	R	73.3	72.9	PR	65.1	64.5	PR
WGL-3962	82.4	79.0	R	85.1	83.2	R	82.4	77.2	R
JGL-384	45.0	42.8	PM	1.7	2.4	PM	2.3	6.8	PM
RNR-18833	0.3	0.0	M	1.5	0.5	PM	0.5	0.9	PM
RNR-883	72.5	67.3	PR	83.2	75.1	R	87.5	77.2	R
RNR-17494	75.1	67.4	PR	68.4	52.6	PR	72.9	52.1	PR
Champakali	42.2	35.2	PM	56.9	52.7	PR	25.0	19.3	PM
RNR-17438	87.6	81.3	R	77.5	71.6	PR	86.6	76.4	R

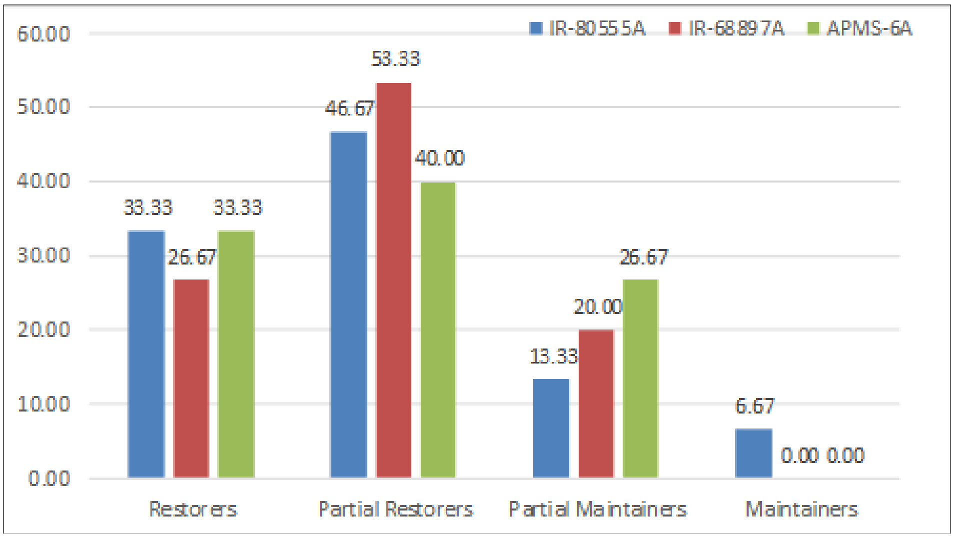

Fig 1: Frequency of different fertility classes based on spikelet fertility

References

1. Akhter M, Zahid MA, Sabar M, Ahmad M. Identification of restorers and maintainers for the development of rice hybrids. Journal of Animal and Plant Science. 2008; 18(1):39-41.
2. Das P, Mukherjee B, Santra CK, Mukhopadhyay, Sankar, Dasgupta T. Evaluation of genotypes for fertility restoring and maintaining behaviours in rice (*Oryza sativa* L.). International Journal of Scientific & Technology Research. 2013; 2:228-32.
3. Geeta Pandey MK, Nautiyal, Priyamvada Chauhan, Pant DP. Evaluation of genotypes for fertility restoring and maintaining behaviour for development of potential rice (*Oryza sativa* L.) hybrids in *tarai* region. Research on Crops. 2016; 17(3):433-438.
4. Jayasudha S, Sharma D. Identification of restorers and maintainers for CMS lines of rice (*Oryza sativa* L.) under shallow low land condition. Electronic Journal of Plant Breeding. 2010; 1(3):311-314.
5. Rajendra Prasad K, Radha Krishna KV, Sudheer Kumar S, Subba Rao LV. Identification of elite restorers and maintainers in rice (*Oryza sativa* L.) based on pollen fertility and spikelet fertility studies. International Journal Current Microbiology Applied Science. 2017; 6(8):2647-2651.
6. Neha Dhirhe, Prabha Chaudhari R, Ram Ambhure, Satyapal Singh, Deepak Sharma. Identification of restorers and maintainers for development of rice (*Oryza sativa* L.) hybrids suitable for aerobic condition. Progressive Research. 2017; 12(3):323-326.
7. Pankaj Kumar, Vinay Kumar Sharma, Bishun Deo Prasad. Characterization of maintainer and restorer lines for wild abortive cytoplasmic male sterility in indica rice (*Oryza sativa* L.) using pollen fertility and microsatellite (SSR) markers. Australian Journal of Crop Science. 2015; 9(5):384-393.
8. Parmeshwar Kumar Sahu, Pooja Sahu, Satyapal Singh, Tarun Singh Patel, Deepak Sharma. Identification of restorers and maintainers for development of rice hybrids using WA and Kalinga sources of CMS lines. Electronic Journal of Plant Breeding. 2014; 5(4):752-755.
9. Ramesh Ch, Damodar Raju Ch, Surender Raju Ch, Rama Gopala Varma N. Spikelet fertility restoration studies for identification of restorers and maintainers in rice (*Oryza sativa* L.). Research Journal of Agricultural Sciences. 2018; 6(4):751-753.
10. Shrivastava A, Mishra DK, Koutu GK, Singh SK, Prakash V, Sohgaura N. Assessment of genetic diversity of restorer and maintainer lines of rice using SSR markers. The Ecoscan. 2015; 9:265-69.
11. Veeresha BA, Hanamaratti NG, Salimath PM, Chetti MB. Identification of restorers and maintainers for development of rice hybrids. Bioinfolet. 2013; 10(2B):602-606.
12. Virmani SS, Mao CX, Hardy B. Hybrid rice for food security, poverty alleviation and environmental protection. Proc. 4th International Symposium on Hybrid Rice, Hanoi, Vietnam, 14-17 May, 2002. Los Baños (Philippines), IRRI, 2003, 407.
13. Virmani SS, Viraktamath BC, Casal CL, Toledo RS, Lopez MT, Manalo JO. Hybrid rice breeding manual. International Rice Research Institute, Philippines, 1997.