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Hybridity estimation of F1s of rice (*Oryza sativa* L.) using microsatellite markers

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Abstract
The present investigation was undertaken to assess the hybridity of F1 plants derived from the crossing of TCDM-1 X IR64 (Cross 1), CG Dev bhog X Tetep (Cross 2). In these set of crosses TCDM-1 and CG Dev bhog are two well-known popular blast susceptible varieties with good grain and aromatic quality. IR64 and Tetep are also two well-known blast resistant variety used as check in most of the blast screening experiments. Six microsatellite markers were used for confirming the hybridity of selected F1 plants from each set of crosses. Polymorphism survey was conducted between the parents of the all the two crosses revealed that three of them were polymorphic. The screening of F1s with polymorphic markers showed heterozygous result, thereby confirming hybridity in all the F1s confirming the hybridity.

Keywords: Hybridity, estimation, microsatellite, *Oryza sativa* L.

Introduction
Rice (*Oryza sativa* L.) one of the most important and demanding staple food on earth, is a self-pollinating diploid crop. Hybrid rice stands with an important avenue for increasing production of rice for the growing population. Hybrid rice production provides a broad platform to the scientist of private as well as other areas to make a beneficial outcome (Spielman *et al*., 2013) [8]. The true hybrid cross F1s are utilized for developing various generation of hybrids through different breeding methods. Therefore process of crossing should be so accurate that there should be no chance of error in the production of F1 seeds in a good quantity. After the procedure of crossing the confirmation of F1 hybridity is another most vital process in breeding. It helps in the assessment of desired purity and highlights the difference in parents and progeny. For testing this hybridity through conventional method it is very time consuming and many a times fails to identify true genotypes (Sundaram *et al*., 2008) [9] as most of the morphological expressions are dependent on environmental factors. On the other side Marker Assisted Selection plays a very crucial role in today's era to estimate an accurate research outcome in a short time period and without any environmental hindrance (Pallavi *et al*., 2011) [7]. Among the molecular markers SSR markers which are co-dominant in nature are the best suited markers to evaluate the hybridity and purity (Sundaram *et al*., 2008; Kannan *et al*., 2017; Sharma *et al*., 2004) [9, 3, 6]. The present investigation is on the hybridity testing of the F1s using Marker Assisted Selection which is further utilized in backcross method to establish an improved blast resistance rice variety.

Materials and Methods
The study involves development of two different sets of crosses and from them two different sets of F1 population. In the first set of cross TCDM-1 a popular variety holding good grain quality and aroma is improved in this experiment with a well-known blast resistance variety as a donor parent to establish blast resistance in it. In the second set of cross CG Devbhog which is also a popular aromatic variety of Chhattisgarh is improved for blast resistance with a popular blast resistant variety Tetep as a donor parent. Both the blast resistance variety holds the identified genes of blast resistance (Pi1, Pikh, and Pi33) against the prevalent race of *Magnaporthe oryzae* using Marker Assisted Selection. In this experiment pot cultivation was favoured and further crossing procedure was carried out in it. The female parent were emasculated and covered with butter paper cover to avoid any other cross pollination contamination, the male parents pollens were dusted over the recipient female parent's emasculated panicles and covered them again with butter papers.

Just after 10-12 days seed development was noticed and seeds were harvested after 30-35 days when they were properly matured. The F1 seeds were properly dried and stored at low temperatures. The seeds were sown after few months to raise the F1 population from both the sets.

Fig 1: Crossing procedure and production of F1 seeds

he genomic DNA of parents from both the cross set and from two different sets of F1 was isolated with CTAB method. The quality and quantity of DNA was estimated using Agrose gel electrophoresis and spectrometric method (Nanodrop). Marker screening survey was undertaken using random SSR markers in which three RM markers were showing heterozygous result among the parents and the F1 hybridity was also confirmed using this markers. DNA amplification was carried out using Polymerase chain reaction (PCR) tubes containing 10 µl of master mix. Amplification reaction was carried out in Veriti 96 well thermal cycler (Applied Biosystems).

Table 1: Linked marker gene chromosome locus PCR product size annealing temp

S.no	Linked marker	Gene	Chromosome locus	PCR product size	Annealing temp
1	RM72	Pi33	8	166bp	56˚C
2	RM206	PiKh	6	147bp	52˚C
3	RM224	Pi1	11	157bp	58˚C

Result and Discussion

Use of marker Assisted Selection for the confirmation of hybridity of the crossed material using RM markers is a very precise method. In the present study the best F1 plants were screened with foreground markers to identify the true and best F1 plants carrying the gene of interest. The twelve plants showed gene positive result with RM 72 (TCDM-1 X IR64) and five gene positive plants with RM 224 from the second cross of (Chhattisgarh Devbhog X Tetep) and sixteen plants showing gene positive result with RM 206, which shows these F1s were tightly linked with blast resistance markers showing the presence of desired blast resistant gene.

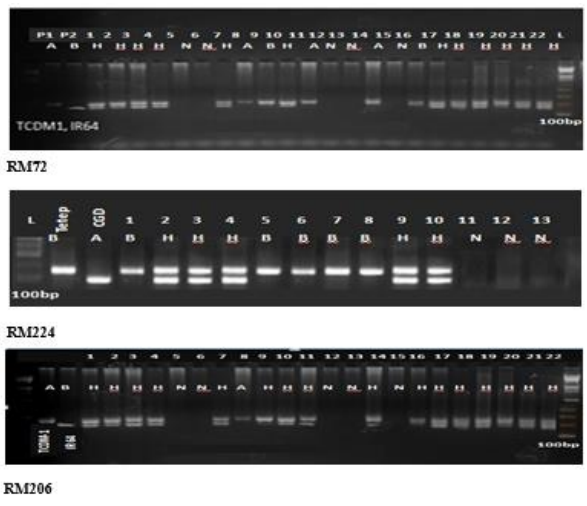

Fig 2: Gel images showing the true F1 to utilize for further breeding program.

In fig.2. “A” is recipient parent, “B” is donor parent and “H” is heterozygous true F1.

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