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# Identification and marker assisted introgression of blast resistance gene into the parental line of rice hybrid<sup>+</sup>

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Abstract: Rice (Oryza sativa L.) is the most widely cultivated and consumed staple food crops for 10 billions of people in the globe. This crop severely affected by fungal disease called blast, caused by 11 a Magnaporthe oryzae (synonymous: Pyricularia oryzae), is the most dangerous disease and often 12 causes severe reduction of about 80% of rice yield under conducive conditions. One dominant (Piz) 13 was introgressed into TNAU CMS 2B through marker assisted breeding. This study identified 22 14 gene introgressed individuals in first generation of backcross population through foreground selec-15 tion, indicating these could be promising genetic resources for breeders to use in their breeding 16 programme. 17

Keywords: molecular markers; gene; rice disease

## 1. Introduction

Rice is the most important and widely consumed staple food crops worldwide. The 21 rising demand of rice is increasing day by day. To fulfill the future requirement of food 22 grain production, we must produce more than 40% by 2030 [1]. Rice blast is an important 23 fungal disease caused by Magnaporthe oryzae, is considered most significant and destruc-24 tive disease in the world [2,3] and thus led 35-50% yield loss globally [4]. Agriculture 25 sector greatly threatened by climatic shocks, an emerging of new pathotypes of biotic 26 stresses poses serious challenges to scientific community more particularly rice breeders, 27 who like to double the rice production by incorporating diverse resistance genes against 28 both biotic and abiotic stresses [5]. The most effective and efficient way to introgress re-29 sistance genes into the desirable genetic background in the breeding programs through 30 marker assisted selection (MAS) [6-11]. The CORH 03 is a popular rice hybrid released by 31 Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, having the charac-32 teristic features of medium duration quality with high yielding potential and, non-sticky 33 and non-aromatic nature. It has occupied large areas under cultivation in rice growing 34 districts in the state, where blast disease is serious concern. The primary goal of the pre-35 sent study was to introgress the  $Pi_z$  blast resistance gene into the maintainer line of the 36 popular rice hybrid through MAS. 37

# 2. Materials and methods

TNAU CMS 2B is a stable maintainer line of TNAU CMS 2A, which is female parental 39 line of CORH 03 hybrid, were used as a recipient/recurrent parent. The parent Zenith was 40 used as a donor source for one dominant gene ( $Pi_z$ ) for blast resistance. The hybrid F<sub>1</sub> was 41 developed by crossing between TNAU CMS 2B and Zenith, and the resistance gene carrying individuals in the agronomically superior background were identified by enabling 43 closely linked simple sequence repeats (SSR) marker RM 549. Furthermore, the identified 44

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Genomic DNA was isolated from the fresh leaf tissue of parents and their BC<sub>1</sub>F<sub>1</sub> in-7 dividuals using Cetyltrimethylammonium bromide (CTAB) method of Doyle and Doyle 8 [12]. The Polymerase chain reaction (PCR) amplification was carried out in thermal cycler 9 (Bio Rad Laboratories, California) programmed for SSR primer; initial denaturation at 10 94°C for 5 min, 35 cycles of denaturation for 1 min, primer annealing at 55°C for 1 min 11 approximately and extension at 72°C for 1 min followed by additional extension of 7 min 12 at 72°C. The amplified PCR products were separated in polyacrylamide gel electrophore-13 sis, and subsequently bands were visualized after silver nitrate staining. 14

The isolated blast lesions placed on prune juice agar medium, then incubated for 16 seven days at room temperature (28±2 °C) and single spore isolation method used to harvest pure culture of conidia, which was further maintained in Potato Dextrose Agar Slant. 18 The spores with the concentration of 10<sup>6</sup> spores/ml were sprayed in the blast nursery. The 19 scoring was done on 21 days after inoculum spray with the 1-9 scale as per Standard Evaluation System of the Directorate of Rice Research, India (SES, DRR 2011-2012) (Table 1). 21

Table 1. The standard evaluation scale for blast disease in rice (2011–2012).

Score	Description (Blast affected lesion area, %)
1	Tiny brown specks of pinhead size on plants without sporulation
2	About 1-2 mm small roundish to slightly elongated necrotic grey spots and
2	lesions are mostly found on the lower leaves
2	Similar to score 2, and also significant number of lesions on upper side of
3	leaves
4	<2%
5	2-10%
6	11-25%
7	26-50%
8	51-75%
9	> 75%

#### 3. Results

A total of 48 plants in BC<sub>1</sub>F<sub>1</sub> generation of cross from TNAU CMS 2B × (TNAU CMS 2B × 25 Zenith), were subjected to PCR analysis using closely linked foreground DNA marker 26 identified 22 gene introgressed individuals (Figure 1). Almost all gene introgressed indi-27 viduals identified in this study were in heterozygous condition. Of these, one individual 28 (plant no. 5) was tagged based on their introgression of genes along with morphological character-29 istics with high single plant yield (28.89g) would be advanced next cycle of breeding. In addition, 30 plant number 23 (26.13g), 25 (24.83g), 2 (23.49g), and 24 (23.20g) ranked second, third, fourth and 31 fifth high yielding with blast resistance gene introgressed background (Table 2). 32



**Figure 1.** Identification of  $Pi_z$  blast resistance gene in BC<sub>1</sub>F<sub>1</sub> cross of TNAU CMS 2B × 34 (TNAU CMS 2B × Zenith). 35

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Plant No	Single plant yield (SPY, g)
1	22.63
2	23.49
3	21.65
4	19.82
5	28.89
6	16.51
7	18.14
8	22.70
13	18.95
14	17.54
15	19.29
16	18.83
17	17.73
18	18.79
19	22.41
21	24.31
23	26.13
24	23.20
25	24.83
26	21.53
27	18.16
29	21.89
30	21.71
35	21.00

 

 Table 2. Single plant yield of gene introgressed progenies of BC1F1 cross of TNAU CMS 2B × Zenith.
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#### 4. Discussion

Marker assisted foreground selection in the cross TNAU CMS 2B × (TNAU CMS 2B × 4 Zenith) identified 22 individuals had blast resistance gene Piz. Of these 12 individuals had 5 more than 20g of single plant yield. The plant number 5 was identified as most promising 6 individual could be further advanced to obtain homozygous condition using marker as-7 sisted backcross breeding. Several researchers have been also introgressed blast resistance 8 genes into their breeding lines like Jinyou 402 and Jinyou 407, CO 39, and PRR78 (R) [4, 9 13,14]. Earlier studies have been found blast resistance gene Piz introgressed lines showed 10 wider spectrum of disease resistance. The gene introgressed individual identified had also 11 found to have increased grain yield which could be utilized as donor parent in future by 12 means of gene introgression with an increased grain yield. 13

#### 5. Conclusions

The stabilized pyramided lines with high yielding background will be used as genetic15stocks for biotic stress resistance breeding program and as improved parental lines in16hybrid rice breeding.17

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