



RESEARCH ARTICLE

DEVELOPMENT OF RESTORER LINES OF RICE (*ORYZA SATIVA L.*) RESISTANT TO FOR BACTERIAL BLIGHT THROUGH MARKER AIDED PEDIGREE SELECTION (MAPS)

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ABSTRACT

Biotic stresses that rob the yield advantage imparted through heterosis breeding can be retained by developing parental lines with inbuilt resistance. Bacterial leaf blight (BB) is one of prime diseases of rice, causing huge yield losses throughout the world. Here we have successfully demonstrated development of restorer lines with inbuilt resistance to bacterial leaf blight by Marker Aided Pedigree Selection (MAPS) - phenotypic selection, phenotyping and marker aided selection in the segregating generations for selecting desirable phenotype with target genotype of resistance genes. Phenotyping for bacterial leaf blight was conducted in F₂ population developed from crossing KPG OS-722R with one donor KCMS2001 and 148 agronomically superior plants from putatively resistant plants were selected and genotyped for presence of *Xa21* and *xa13* genes by using linked markers pTA248 and *xa13* promo, respectively. A total of 24 plants out of 148 plants were genotyped, selected and advanced to next generation. Out of 24 F₃ progenies grown, 14 agronomically superior progenies were identified and one plant from each progeny was selected. Phenotyping for BB was conducted on 14 F₄ lines under field conditions and 11 resistant lines were selected. After evaluation of per se performance and restoration ability of 11 lines, a total of three lines with strong resistance to BB and superior agronomic traits were selected. This work clearly demonstrates development of inbuilt resistant new restorer lines for BB with a lot of saving in time and other resources.

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INTRODUCTION

Bacterial leaf blight (BB) (caused by *Xanthomonas oryzae p v. Oryzae, xoo*) of rice is one of most destructive diseases of rice, resulting in huge yield losses across the world every year (Mew 1987). It is having potentiality to reduce yield levels by 74 -81% (Srinivasan and Gnanamanickam, 2005). The most effective, efficient and eco-friendly way to tackle this destructive disease is deployment of genetically enriched resistant cultivars (Khush et al., 1989). As on date, more than 40 genes were identified from cultivated rice and wild rice (Zhang 2005; Chun et al., 2012; Bhasin et al., 2012; Natraj Kumar et al., 2012; Hari et al., 2013; Balachiranjeevi et al., 2015). Out of these, 27 are dominant and 11 are recessive (NinoLui et al., 2006; Singh et al., 2007; Natraj Kumar et al., 2012). Six resistance genes, *Xa21*, *Xa1*, *xa5*, *xa13*, *Xa26* and *Xa27* have already been cloned and six resistance genes have

been physically mapped (*Xa2*, *Xa4*, *Xa7*, *Xa30*, *Xa33* and *Xa38*) (Chu et al., 2006; Bhasin et al., 2012). Pyramiding of resistance genes is time-consuming and difficult through conventional breeding and it even more difficult if resistance gene is recessive in nature. Molecular markers closely linked to disease / race specific resistance genes enables selection of minimum number of desired segregants with two or more resistance genes through marker aided selection (Abenes et al., 1993; Yoshimura et al., 1995; Zhang et al., 1996; Huang et al., 1997; Sanchez et al., 2000; Davierwala et al., 2001; Singh et al., 2001; Joseph et al., 2004; Leung et al., 2004; Perez et al., 2008; Sundaram et al., 2008, 2009; Basavaraj et al., 2010; Hari et al., 2011, 2013; Balachiranjeevi et al., 2015). Many workers reported that marker assisted selection is more effective and economical than the conventional phenotype based breeding (Sanchez et al., 2000; Chen et al., 2001; Singh et al., 2001; Cao et al., 2003; Narayanan et al., 2004; Sundaram et al., 2008, 2009; Chen et al., 2009; Zhan et al., 2012). BB resistance gene, *Xa21* is one of major gene which originally introgressed from the wild rice, *Oryza*

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longistaminata (Ronald *et al.*, 1992; Song *et al.*, 1995) and confers broad spectrum resistance against a wide range virulent BB pathogen races in India and abroad. A number of researchers have successfully used *Xa21* gene for development/ improvement BB resistant varieties (Joseph *et al.*, 2004; Gopalakrishnan *et al.*, 2008; Sundaram *et al.*, 2008, 2009; Pandey *et al.*, 2013) and parental lines of hybrids in rice (Chen *et al.*, 2001; Basavaraj *et al.*, 2010; Hari *et al.*, 2011, 2013; Balachiranjeevi *et al.*, 2015) by using a closely linked PCR based marker pTA248 in India and other countries. A recessive bacterial blight resistance gene *xa13* was first reported in the rice variety BJI and subsequently it was mapped on long arm of chromosome 8 (Ogawa *et al.*, 1987; Zhang *et al.*, 1996). Very tightly linked markers are available and successfully utilized by many workers for marker assisted selection (Sundaram *et al.*, 2014). BB resistance gene combination, *Xa21* + *xa13*, is known to be very effective across India (Joseph *et al.*, 2004; Gopalakrishnan *et al.*, 2008). The present study mainly undertaken for development of restorer lines with BB resistance genes, *Xa21* and *xa13* through marker aided pedigree breeding. The newly developed restorer lines can be used for development of bacterial blight resistant hybrids. The newly developed lines will also serve as valuable genetic stocks in future breeding programs.

MATERIALS AND METHODS

Plant material and methods

Plant material consisting of one donor parent KRG- 2001 and one restorer line KPGOS-722 were used as parents for development of bacterial blight resistant restorer lines. Donor parent consist of two resistant genes against bacterial blight in homozygous condition-one dominant gene *Xa21* and another recessive gene *xa 13*. Donor parent is in restorer background for WA cytoplasm. KPGOS-722 is a popular restore line of three line rice hybrids but susceptible to BB and it is good general combiner. Two molecular markers - pTA248 and *xa13* promo which are very closely linked to *Xa21* (pTA-248) and *xa13* (*xa13* promo) genes, respectively, were used for selection of resistant offspring in the segregating populations for development of resistant restorer lines. In addition to these, Taichung Native 1 (TN1) and KRG-2001 were used as susceptible and resistant checks for BB screening respectively.

Marker-assisted pedigree breeding for BB resistance

Marker aided pedigree breeding (Figure 1) was adopted for development of biotic stress resistant restorer lines. After parental polymorphism survey with linked molecular markers-pTA-248 and *xa13* promo, a breeding cross was generated during wet season 2012 by crossing KPGOS-722R (susceptible for BB) with donor parent- KRG2001 and F₁ was grown during dry season 2012-13 for obtaining F₂ seed. Hybridity of F₁ was confirmed by linked markers. True F₁ plants were selfed to generate F_{1:2} seed. F₂ population consisting of ~1500 plants was grown during Kharif 2013 along with donor parent and TN-1. F₂ population was first phenotyped along with resistant and susceptible checks by using local BB inoculum at maximum tillering stage as described by Kauffman *et al.* (1973), and putative resistant plants with good agronomic traits were selected and genotyped for presence of target genes *Xa21* and *xa13* with linked molecular markers- pTA-248 and *xa13* promo, respectively. Homozygous plants for *Xa21* and heterozygous/ homozygous

plants for *xa13* were selected and selfed to generate F₃s (Figure 4). In F₃ generation plants with good agronomic features and which were double positive for both genes-*Xa21*+*xa13* were selected among the superior progenies and selfed to generate F₄s. In F₄ generation phenotyping for BB was performed and resistant lines were selected for further evaluation of agronomic characters and restoration ability.

WS-Wet season DS-Dry season

DNA was isolated from the parents and segregating populations by following the protocol of Zheng *et al.*, (1995). The PCR-based STS marker pTA248 (Ronald *et al.*, 1992) and *xa13* promo ((specific for *xa13*; Sundaram *et al.*, 2011) were used to identify the allelic status of *Xa21* and *xa13* in parents and segregating materials. PCR was performed using 1 U of Taq DNA polymerase (Bangalore Genei, Bangalore, India) and 1X PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM MgCl and 0.01 mg/ml gelatin), 5 Pico moles of each primers, 0.05 mM dNTPs and 50 ng template DNA in 25 µl reaction volume with a thermal profile of 94°C for 5 min (initial denaturation), followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72 °C for 1 m and a final extension of 7 min at 72 °C. The amplified product of pTA248 (*Xa21*) was electrophoretically resolved on a 1.5 % Seakem LE agarose gel (Lonza, Rockland, ME, USA); the amplicons of (*xa13*) were loaded in 2 % agarose. Seakem LE agarose gels containing 0.5 mg/ml of ethidium bromide in 0.5X TBE buffer and imaging was done under UV.

Screening for Bacterial blight resistance

Local virulent isolate of BB was used for screening of donor parent, susceptible parents and segregating populations (F₂s and F₄s) under field conditions. Plants were clip-inoculated with a bacterial suspension of 10⁹ cfu/ml at maximum tillering stage (45–55 days after transplanting) through the methodology of Kauffman *et al.*, (1973). Approximately 5–10 leaves were inoculated per plant and disease reaction was scored 14 days after inoculation. Disease score was calculated as per IRRI standard evaluation system (IRRI-SES) scale (IRRI 2013).

Evaluation of restoration ability of newly developed biotic resistant restorers

Selected newly developed BB resistant F₅ lines and their parent (KPGOS-722R) were crossed to CMS line IR58025A during dry season 2014-15. F₁s along with four commercial hybrid and varietal checks (US-312, MTU-1010, PHB-27P31, and KRH-2) were planted for evaluation of restoration ability during Kharif 2015 at Rice Research farm, Kaveri Seed Company Limited at a spacing of 20 x 15 cm in a two row plot of 12 plants per row in an augmented design. Days to 50% flowering (DFF), plant height (PH), No of panicles per plant (PN), panicle length (PL), total spikelets per plant (TSP), % Pollen sterility/ fertility, spikelet fertility (SF%), and yield per plant (YPP) were recorded on five random plants in the plot. The pollen sterility and spikelet fertility were recorded at flowering and maturity stages, respectively. At the time of flowering, at least 15 spikelets were taken from labeled panicles of each plant and fixed in 70 per cent ethanol. Pollen sterility/ fertility were determined by staining pollen with 1 % Iodine potassium iodide (I₂KI solution; 1 g iodine, 2 g KI, dissolved in 300 ml distilled water).

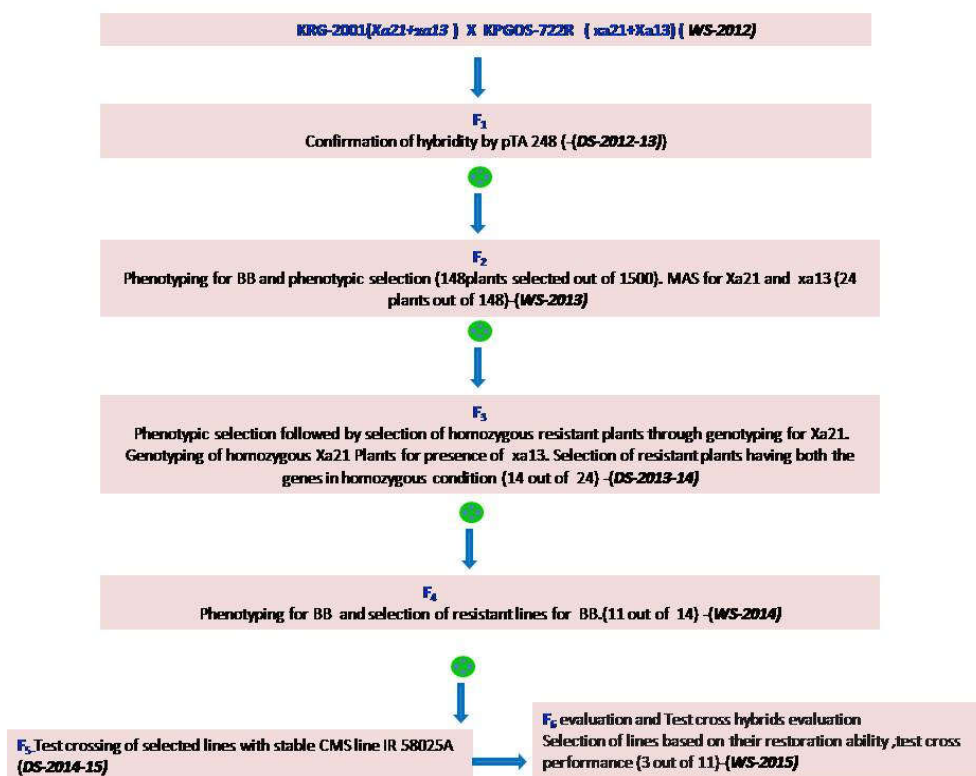


Fig.1 Flow chart of marker assisted pedigree selection (One breeding cross, PST-1205 was taken as example)

Fig.1. Flow chart of marker assisted pedigree selection (One cross combination is taken as example) WS-Wet season, DS-Dry season

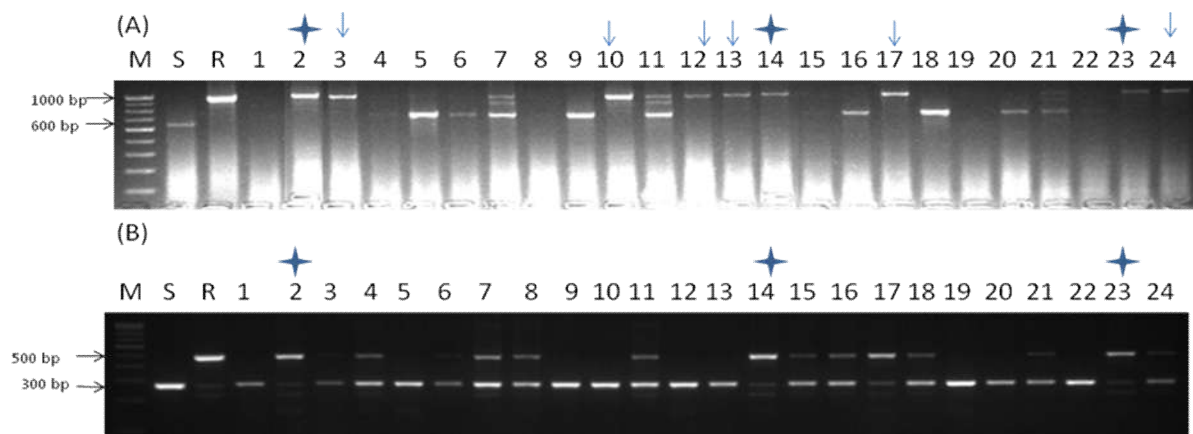


Figure 2. Foreground selection for BB genes in selected F2s for BB with linked markers pTA 248 and xa13 promo for presence of Xa21 and xa13 respectively –M-Molecular weight marker (100 bp ladder), SP-Susceptible parent, R-Donor parent. Numbers on the top of each gel are F3 plant numbers. (A) gels is genotyping of selected F2's with p TA 248 for Xa21 and. (B) gels is genotyping of selected F2's with xa13 promo for xa13. Arrows indicates

All round and dark pollen grains were counted as fertile and poorly stained irregular pollen grains were counted as sterile and Pollen sterility/ fertility was expressed in percentage. At the time of maturity, bagged and labeled panicles were harvested, numbers of filled grains and unfilled grains were counted and spikelet fertility was recorded in percentage.

Screening of F6 lines for agro-morphological characteristics

Twenty five days-old seedlings of the selected F6 lines along with parents were transplanted at Rice Research farm, Kaveri Seed Company Limited, Gowraram, Wargal (M), Medak, Telangana, India, at a spacing of 20 x 15 cm in 5.4 m² plot size with two replications during wet season 2015. Standard

agronomic practices were followed to raise a healthy crop. Days to 50% flowering (DFF), plant height (PH), No of panicles per plant (PN), panicle length (PL), spikelet fertility (SF%), and grain yield per plot (GYPP) were recorded on five random plants in the plot

RESULTS

Development of BB resistant restorer lines through Marker Assisted Pedigree Selection (MAPS)

One breeding cross (KRG2001/KPGOS-722R, coded as PST-1205) was obtained by crossing susceptible parents -KPGOS-722R to the resistant parent - KRG2001 with *Xa21* and *xa13* in homozygous condition. The hybridity of F₁s were confirmed

by using pTA248, the co-dominant marker specific for *Xa21*. True F₁s were selfed to generate F₂ generation seed. F₂ population consisting of ~1500 plants was raised and phenotyped for BB at maximum tillering stage with local isolate (Hyderabad, Telangana). Based on phenotyping observations and agronomic observations, 148 plants were selected for foreground selection of *Xa21* and *xa13*. Foreground selection data was presented in Table 1. Foreground selection of selected F₂ plants identified three double positive plants for *Xa21* and *xa13* (Fig. 2). A total of 24 F₂ plants were selected having different gene combinations along with three double positive plants and advanced to F₃ generation. In F₃ generation, fourteen plants were selected which are agronomically superior and double positive for both genes and were selfed to generate F₄ generation seed.

Evaluation of BB resistance of the selected F₄ lines

A total of 14 selected F₄ lines were phenotyped for BB under field conditions using local isolate (Hyderabad, Telangana). In BB screening, % DLA ranged from 1.36±0.09 (PLS-1850) to 52.00±6.36 (TN-1). Donor parent, KRG-2001 showed a score of 2.88±0.14 and susceptible parents- KPGOS-722R showed a score of 9.00±0.70. For F₄ lines, it varied from 1.36±0.09 (PLS-1850) to 7.71±0.14 (PLS-1863). The details of results of screening of the F₄ lines against BB are given in Table 2.

Table 1. Genotypes identified by linked molecular markers pTA248 and xa13 promo in F₂ population

S. No.	Genotype	Phenotypic expression	KRG2001/KPGOS-722R (PST-1205)
1	<i>Xa21 xa21 Xa13 xa13</i>	R	35 (6)
2	<i>Xa21 Xa21 Xa13 Xa13</i>	R	8
3	<i>Xa21 xa21 Xa13 Xa13</i>	R	15
4	<i>Xa21 Xa21 Xa13 xa13</i>	R	28 (10)
5	<i>Xa21 xa21 xa13 xa13</i>	R	9 (5)
6	<i>Xa21 Xa21 xa13 xa13</i>	R	3 (3)
7	<i>xa21 xa21 Xa13 Xa13</i>	S	24
8	<i>xa21 xa21 Xa13 xa13</i>	S	19
9	<i>xa21 xa21 xa13 xa13</i>	S	7
Total			148(24)

* All figures in parenthesis of table indicates the number of plants forwarded to next generation from a particular combination

Table 2. Phenotyping of F₄s for Bacterial blight

S.No	Line	% DLA	Scale	Reaction
KRG-2001/KRGOS-722 (PST-1205)				
1	PLS-1848	2.91±1.19	1	R
2	PLS-1850	1.36±0.09	1	R
3	PLS-1856	1.50±1.11	1	R
4	PLS-1858	3.85±1.71	1	R
5	PLS-1859	4.11±1.43	1	R
6	PLS-1861	7.81±0.08	3	MR
7	PLS-1863	7.71±0.14	3	MR
8	PLS-1879	2.65±0.05	1	R
9	PLS-1882	1.81±0.02	1	R
10	PLS-1886	6.58±0.01	3	MR
11	PLS-1891	5.71±1.34	1	R
12	PLS-1892	2.90±0.11	1	R
13	PLS-1893	2.10±0.48	1	R
14	PLS-1894	7.33±2.20	3	MR
15	KRG-2001	2.88±0.14	1	R
16	KRGOS-722	9.00±0.70	3	MR
17	TN-1	52.00±6.36	9	HS

Screening for agro-morphological characteristics

A total of 11 F₆ lines were evaluated for agronomic traits along with parents-KPGOS-722R and KRG-2001 during *Kharif* 2015

at Rice Research farm, Kaveri Seed Company Limited and results are given in Table 3. Days to 50% flowering ranged from 95.50±0.70 days (PLS-1859) to 108.00±1.41 days (PLS-1891). Plant height ranged from 87.80±4.94 cm (PLS-1882) to 99.70±4.10 cm (PLS-1891). Productive tillers per plant ranged from 7.05±0.35 (PLS-1859) to 8.90±0.56 (PLS-1893). Panicle length ranged from 24.20±0.56 cm (PLS-1891) to 27.45± 0.49 cm (PLS-1892). Total number of spikelets per panicle ranged from 205.67±7.09 (PLS-1856) to 268.00±10.53 (PLS-1882). Spikelet fertility ranged from 86.30±1.05 (PLS-1879) to 95.70±1.11% (PLS-1850). Grain yield per plot ranged from 1.34±0.08 Kg (PLS-1856) to 5.02±0.15 Kg (PLS-1859). All except one line possess Medium slender grain type and PLS-1891 is short bold grain type line (Table 3).

Evaluation of restoration ability of newly bred biotic resistant restorer lines

Eleven test cross hybrids of newly developed biotic stress restorer lines along with their parental hybrid combinations and commercial checks (US-312, PHB-27P31, KRH-2 and MTU-1010) were evaluated for their restoration ability and other agronomic traits as described in materials and methods. Data on agronomic traits were presented in table 3. Test cross hybrids showed spikelet fertility (SF)% range of 80.27±2.11 (IR-58025A X PLS-1856) to 91.00±4.25 (IR-58025A X PLS-1893) and pollen fertility ranges from 75.89±1.49 (IR-58025A X PLS-1879) to 95.87±3.09 (IR-58025A X PLS-1882). Among other agronomic characters, days to 50% flowering ranged from 90 days (IR-58025A X PLS-1856) to 102 days ((IR-58025A X PLS-1892). For gain yield per plant a combination IR58025A X PLS-1882 recorded an yield advantage of 53.82% over MTU-1010 and 29.85% over best hybrid check PHB-27P31. Plant height ranged from 92.67±3.05 cm (IR58025A X PLS-1886) to 107.33± 2.51 cm (IR58025A X PLS-1892), No of panicles ranged from 7.67±1.52 (IR58025A X PLS-1894) to 12.67±1.52 (IR58025A X PLS-1859), pollen fertility was ranged from 75.89±1.49 % (IR58025A X PLS-1879) to 95.87±3.09 % IR58025A X PLS-1882), panicle length ranged from 25.42±1.03 (IR58025A X PLS-1850) to 29.00±0.5 cm IR58025A X PLS-1859), spikelet fertility was ranged from 80.27±2.11 % (IR58025A X PLS-1856) to 91.00±4.25 % (IR58025A X PLS-1891), grain yield per plant ranged from 20.33±4.93 g (IR58025A X PLS-1891) to 35.33±3.21 g (IR58025A X PLS-1859). All hybrid combinations except three showed high grain yield over PHB-27P31 and all but one hybrid combinations showed high grain yield over MTU-1010.

DISCUSSION

Sustainability of widely adapted hybrids, otherwise susceptible to pest and diseases can be increased with introgression of suitable resistance genes into the parental lines through marker assisted back cross breeding. But it is a time consuming and sometimes exact reconstitution of hybrid may not be possible due to several reasons. Marker assisted pedigree selection (MAPS) which integrates phenotypic selection and marker assisted selection helps in development of parental lines with desirable agro-morphological traits and target resistance genes. In the present study, marker assisted pedigree selection (MAPS) was used to develop inbuilt disease resistant restorer lines of hybrid rice for bacterial leaf blight with *Xa21* and *xa13* genes. Since we already developed cytoplasmic male sterile (CMS) lines with *Xa21* and *xa13* gene combinations (unpublished), targeted

both genes—one dominant (*Xa21*) and one recessive (*xa13*) genes for development of the restorer lines for functional expression of trait phenotype conferred by *xa13* in the hybrids developed by using the newly developed resistant restorer lines. Li *et al.*, (2001) suggested that a combination of R genes that have different and distinct mode of defensive pathways gives high level and more durable resistance. The resistant gene-*xa13*, is completely recessive, has no residual effects against the virulent races, and is known to show more pronounced race specificity and considered effective against many Indian races of *Xoo*. The resistant gene- *Xa21* shows complete dominance against the avirulent *Xoo* races and has large residual effects against virulent ones and acts independently and cumulatively along with other genes (Li *et al.*, 2001) and has a mode of action which is distinct from *xa13* (Sundaram *et al.*, 2008) and *Xa21 + xa13* gene combination is known to be very effective across India (Joseph *et al.*, 2004; Gopalakrishnan *et al.*, 2008). Hence selected *Xa21 + xa13* gene combination for development of restorer lines. Several workers earlier has been successfully deployed marker assisted selection (MAS) for improving biotic stress resistance against BB, blast and brown plant hopper in rice (Narayanan *et al.*, 2002; Joseph *et al.*, 2004; Sundaram *et al.*, 2008, 2009; Basavaraj *et al.*, 2010; Hari *et al.*, 2011, 2013; Singh *et al.*, 2012; Zhan *et al.*, 2012; Balachiranjeevi *et al.*, 2015; Arunakumari *et al.*, 2016). Earlier, Zhan *et al.*, 2012 used similar strategy of marker assisted selection and pedigree selection for pyramiding of *Pi25+Xa21+xa13+xa5* genes and developed a novel restorer line R8012 with strong restorer ability. In this study they have used two parents, one of them is having resistance genes for blast and another having resistance genes for BB. Very recently Arunakumari *et al.*, (2016) introgressed *Xa21+ xa13* along with *Pi54* into Indian popular rice variety MTU-1010 by using marker assisted pyramiding.

In the present study one restorer line-KPGOS-722R with strong restorability, good agronomic performance and good combining ability were used as male parents and KRG-2001 possessing two resistant genes –*Xa21+xa13* in homozygous condition in the restorer back ground with good agronomic performance was used as female parent. F₂'s were simultaneously phenotyped for BB and evaluated for phenotypic selection. Putative resistant plants with desirable traits suitable for restorer lines were genotyped for presence of *Xa21+ xa13*. Abenes *et al.* (1993) followed similar strategy for identification of F₂ plants consisting of BB genes after phenotyping for BB. Dual selection strategy –phenotyping for BB and phenotypic selection for desirable traits in putative resistant plants of F₂ population dramatically reduced the number of plants to be genotyped for presence of target genes. This enables, marker assisted pedigree selection (MAPS) strategy can be applied in wider scale for breeding of new inbuilt resistant parental lines of hybrid rice. We have selected plants with different gene combinations—Homozygous/Heterozygous conditions for both genes- *Xa21* and *xa13* or one gene as we can select homozygous plants for both genes in the next generation (F₃). A total of 14 plants were selected from best F₃ progenies based on genotyping data (double positive plants for both *Xa 21* and *xa13*) and agronomic characters desirable for maintainer lines and advanced to F₄ generation.

Phenotyping of 14 F₄ lines selected based on genotyping data showed that 10 lines are resistant, 4 lines are moderately resistant (Table 3). It is expected that all lines developed by using a resistant donor parent should have equal resistant

levels to that of donor parent but we found that some of lines are moderately resistant (Table 3) suggesting that background effect of lines in which they are present (Zhang *et al.*, 2006; Huang *et al.*, 2012 ; Xu *et al.* 2012). In hybrid rice breeding test cross performance of a line with a tester (a CMS line in the study) is most crucial than the line per se performance even though it is important to some extent in commercial hybrid breeding. In the present study, some of lines per se performance was not good in comparison to parents from which they were developed but in combination with a tester- CMS line IR 58025A they showed good level of yield superiority. For example PLS-1850 showed poor per se performance of - 43.44% in comparison to their respective parents but test cross performance of PLS-1850 with IR 58025A was very good with +42.20% in comparison to parental hybrid combinations with IR 58025A (IR 58025A x KPGOS-722R). Above hybrid combination (IR 58025A x PLS-1850) also showed high level of yield advantage over checks MTU-1010 and PHB 27P31 (Table 4). Best lines suitable as parents are those which have good per se performance and test cross performance because there are some practical per se performance requirements for parents as a part of commercially viable hybrid combinations. Some of the developed lines in the present study are having good per se performance and test cross performance Viz., PLS-1859. PLS-1859 is ranked 3rd in the per se performance and 1st in test cross performance. It showed a yield advantage of 63.05% and 37.64% over MTU-1010 and PHB-27P31 respectively.

Based on the test cross performance, per se performance and resistance to BB, a total of three lines - PLS-1859, PLS-1882, and PLS-1892 were selected from 14 lines developed in the present study. These lines possess strong resistance to BB, medium slender grain type and medium maturity (except-PLS-1859 which is a medium early duration line; Table 3) are being used for development of BB resistant hybrids across durations for medium slender grain type rice hybrids by using CMS lines of different durations. The present study clearly demonstrates that inbuilt resistant parental lines for pest and diseases can be developed depending upon availability of tightly linked molecular markers for target pest and diseases by using marker assisted pedigree selection as tool in regular breeding programs.

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