Improvement of bacterial blight resistance of the popular variety, Nellore Mahsuri (NLR34449) through marker-assisted breeding

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Abstract. To combat the dreaded diseases in rice like bacterial blight (BB) and blast, host plant resistance has been advocated as the most suitable and sustainable method. Through the present study, we have successfully incorporated three major BB resistance genes, namely Xa21, xa13 and xa5 into NLR3449, a high yielding, blast resistant, fine-grain type, popular rice variety through marker-assisted backcross breeding. Foreground selection was carried out using polymerase chain reaction based, gene-specific markers, namely pTA248 (Xa21), xa13prom (xa13) and xa5FM (xa5) at each generation of backcrossing, while 127 polymorphic SSR markers spanning on 12 chromosomes were used for background selection and backcrossing was limited to two rounds. At BC2F1 generation, a single plant (NLR-87-10) with 89.9% recovery, possessing all the three BB resistance genes was forwarded to BC2F2 generation. A solitary BC2F2 plant, namely NLR-87-10-106 possessing all the three resistance genes and 96% genome recovery was identified and advanced through selfing until BC2F4 generation by adopting pedigree-method of selection. Three best BC2F4 lines, possessing high level of resistance against BB and blast, and equivalent or superior to NLR 34449 in terms of yield, grain quality and agro-morphological traits were identified and advanced for multi-location trials.

Keywords. NLR 34449 rice variety; rice; bacterial blight; blast; marker-assisted backcross breeding.

Introduction

Rice is an important food crop that serves as a major carbohydrate and energy source for nearly half of the world’s population (Van Nguyen and Ferrero 2006). In India, it is grown throughout the year in a variety of agro-ecosystems, which include irrigated, rain-fed, deep-water and hills

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(Sundaram et al. 2009). We need to produce at least 40% more rice by 2030 to feed the growing population and there are many challenges in meeting the food security requirements for the future (Khush 2005). The challenges include dwindling natural resources and adverse effects from a rapidly changing climate and a constant battle against new existing as well as emerging pathogens and pests (Pradhan et al. 2015). Rice is affected by many diseases and among them, bacterial blight (BB) caused by Xanthomonas oryzae
The rice variety NLR34449, popularly known as Nellore Mahsuri, was released from Rice Research Station, Acharya N.G. Ranga Agricultural University, Nellore, India. It is popular among the rice farmers of south India, as it is high yielding, possesses highly desirable medium slender (MS) grain type, has excellent blast resistance, is of short duration (125 days), possesses nonlodging plant type and is highly amenable for machine harvesting. It is highly preferred by the farmers of Andhra Pradesh and other south Indian states Nellore rice (kvknellore-angrau.org). Despite its high yield and blast resistance, the variety is highly susceptible to BB disease, which limits its production and adoption significantly in BB endemic areas of south India. Keeping these points in view, through the present study, we have transferred three major BB resistance conferring genes, namely Xa21, xa13 and xa5 from an elite, highly-popular fine grain, BB resistant variety Improved Samba Mahsuri (ISM) into the genetic background of NLR34449 through marker-assisted backcross breeding (MABB).

### Material and methods

#### Plant material

NLR34449 (popularly known as Nellore Mahsuri), a high yielding, medium slender, blast resistant and short duration variety (125 days) developed from the cross IR 72/BPT 5204 in the year 2009 was used as the recurrent parent. Improved Samba Mahsuri (ISM), a high yielding, medium-slender grain type, BB resistant variety developed by ICAR-Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, India and CSIR-Centre for Cellular and Molecular Biology (CSIR-CCMB), Hyderabad, India, possessing three major BB resistance genes, namely Xa21, xa13 and xa5 (Sundaram et al. 2008) was used as the donor parent for BB resistance.

#### MABB strategy

NLR34449 was crossed with ISM during dry season 2016–2017. F1s developed from the above mentioned cross were confirmed for their heterozygosity using target resistance gene specific markers, namely, pTA248 specific for Xa21 (Ronald et al. 1992), xa13prom specific for xa13 (Hajira et al. 2016) and xa5FM specific for xa5 (Hajira et al. 2016). True F1s thus identified were then backcrossed with the recurrent parent, NLR34449 to generate BC1F1s. They were subjected to foreground selection using genespecific markers and positive plants (i.e., plants which are heterozygous for all the three target BB resistance genes) were then analysed for the recurrent parent genome (RPG) recovery through background selection using a set of 127 SSR parental polymorphic markers evenly distributed across the 12 rice chromosomes (listed in table 1, a&b in electronic supplementary material at http://www.ias.ac.in/jgenet/) to identify those plants with maximum RPG recovery. A single BC1F1 plant positive for all three target genes and with maximum RPG recovery was selected and

| Generation | Total no. of plants analysed | Total no. of positive plants to all three target genes (Xa21, xa13, xa5) | Per cent of recurrent parent genome (RPG) recovery | Selected plant with maximum RPG% |
|------------|-----------------------------|------------------------------------------------|-----------------------------------------------|---------------------------------|
| 1          | F1                          | 95                                             |                                               | NLR-87                          |
| 2          | BC1F1                       | 175                                            | 20                                            | NLR-87-10                       |
| 3          | BC2F1                       | 105                                            | 13                                            | NLR-87-106                      |
| 4          | BC2F2                       | 318                                            | 5                                             | NLR-87-106-40-50                |
| 5          | BC2F4                       |                                                |                                               | (i) NLR-87-10-106-40-50         |

The true F1s obtained from the cross between NLR34449 × ISM were backcrossed with NLR34449. The best BC1F1 plant was identified through foreground and background selection was backcrossed with NLR34449 to generate BC2F1s. The best BC2F1 plant (identified through foreground and background selection was selfed to produce BC3F1s. Selected homozygous BC2F2 plant with maximum RPG% was selfed and advanced through pedigree method of breeding to BC3F4 generation.
backcrossed with NLR34449 to generate BC2F1s. Marker-assisted foreground and background selection was repeated as mentioned above among the BC2F1 plants and a single BC2F1 plant possessing the target resistance genes and also with maximum recurrent genome recovery were selfed to develop BC2F2s. Finally, a single BC2F2 plant possessing *Xa21*, *xa13* and *xa5* in homozygous condition along with maximum RPG and closely resembling NLR34449 (based on morphological features) was identified and advanced through pedigree method of breeding till BC2F4 (figure 1). The extent of donor parent segment introgression among the selected backcross derived plants at BC2F4 generation was assessed utilizing the software tool, Graphical Genotype v 2.0 (Van Berloo, 1999) and the BC2F4-5 lines were also further evaluated for BB resistance and key agromorphological traits.

**Phenotypic screening for BB and blast resistance**

**BB screening:** Thirty-day old seedlings of the improved breeding lines of NLR34449 at BC2F4 generation were transplanted in the main field of experimental farm of ICAR-IIRR located at Rajendranagar, Hyderabad, India to assess their resistance against BB during wet season 2019. IX0-20, a local virulent isolate (collected from Hyderabad, Telangana, India) of *Xanthomonas oryzae* pv. *oryzae* was used for screening of BB resistance of the breeding lines. Inoculation was done at maximum tillering stage following leaf clip method of Kauffman *et al.* (1973) by clipping the leaf tip (about 1–2 cm) of the uppermost leaf with scissors dipped into the inoculum. Symptoms were measured at 15 days after inoculation based on the IRRI SES (IRRI 2013) for their resistance/susceptibility.

**Screening for blast resistance**

During dry season 2019–2020, gene pyramided lines possessing *Xa21*, *xa13* and *xa5* at BC2F5 generation were analysed for their resistance against blast in uniform blast nursery along with the susceptible (HR12) and resistant (C101A51 and Tetep) checks using a local isolate, SPI-40 of the blast pathogen, *Magnaporthe oryzae* as per the protocol described in Mohan *et al.* (2011). After 15 days of inoculation, the lines were scored for their resistance/susceptibility to the disease based on IRRI-SES scale, 1996 (IRRI 1996).

**Screening of the improved lines for agro-morphological characters**

During dry season 2020, 30-day old seedlings of the recurrent parent NLR34449 and donor parent ISM along with five best backcross derived lines at BC2F5 generation, possessing BB resistant genes in the genetic background of NLR34449 were transplanted in the main field of experimental farm of ICAR-IIRR located at Rajendranagar, Hyderabad, India, at a spacing of 15 × 20 cm in 2 m² plots in three replications and the field was applied with the recommended dose of NPK fertilizers (@ 220:70:80 kg/ha). Phenotypic data was collected for the selected plants for key agro-morphological traits, namely plant height (cm), days to 50% flowering (DDF), panicle length (cm), number of productive tillers per plant, panicle exertion, grain yield per 33 plants (i.e. per m²; in g), number of grains per panicle, grain type, L/B ratio, 1000-grain weight (g) among 33 plants for each replication (*n* = 3) as explained in Abhilash *et al.* (2016). The data was statistically analysed as per the procedure described in Freeman *et al.* (1973). Least significance difference (LSD) values at 5 to 7 per cent level of significance and coefficient

![Figure 1](attachment:crossing_scheme.png)

**Figure 1.** Outline of the crossing scheme. Nellore Mahsuri (NLR34449) was crossed with ISM possessing BB resistant genes *Xa21*, *xa13*, *xa5* to build BB resistance through stepwise MABB strategy.
of variation (CV) were calculated using standard errors of mean (S.Em. ±) using Microsoft Excel package. The software package, R studio (R Core 2016) was used for analysis of variance (ANOVA) to determine significant variation among the improved breeding lines of NLR34449.

Results

Marker-assisted introgression of Xa21, xa13 and xa5 into the genetic background of NLR34449

Ninety-five F1 plants, which were derived from the cross NLR34449 × ISM were confirmed for their hybridity with respect to the three target BB resistance genes, i.e. Xa21, xa13 and xa5 with the help of gene-specific markers, namely pTA248, xa13prom and xa5FM, respectively during wet season 2017. A total of 80 F1 plants were found to be heterozygous for all the three target BB resistant genes. These plants (i.e. Xa21xaxa21, xa13xa13 and xa5xa5) were used as pollen donors to develop BC1F1s. Among 175 BC1F1s, 21 plants were found to be heterozygous for all the three target BB resistant genes (i.e. Xa21, xa13 and xa5). These 21 plants were analysed to assess the recovery of the RPG using a set of 127 parental polymorphic primers (table 1, a&b in electronic supplementary material) and the analysis revealed that a single BC1F1 plant (NLR-87) had the highest RPG recovery of 78.5%. This plant, NLR-87, was then used as a pollen donor to produce BC2F1s. A total of 105 BC2F1 plants were raised and evaluated through foreground selection with gene-specific markers and 13 were found to be positive for all the three target BB resistant genes and they were subjected for background genome recovery analysis with parental polymorphic SSR markers and a single plant (NLR-87-10) with maximum RPG re-recovery (89.9%) was identified. This plant was selfed to produce BC2F2 plants. A total of 425 BC2F2 plants were grown in the field and subjected to phenotypic screening with a local isolate of Xoo. Ixo-20 at maximum tillering stage to screen for their resistance against BB. A total of 359 plants were found to be resistant.

When the phenotypically resistant plants were subjected to marker-based analysis with the help gene-specific markers, a total of five plants were identified to be homozygous for all the three target resistant genes (figure 2). A solitary plant (NLR-87-10-106) which was homozygous resistant to all the three target resistant genes and possessing maximum recovery of NLR34449 genome (94.6%) was identified and advanced further through pedigree method of breeding to BC3F2 generation. The details of number of plants screened and selected in each generation of backcrossing are given in table 1. A set of five promising BC3F2 lines, namely NLR-87-10-106-40-50, NLR-87-10-106-41-51, NLR-87-10-106-42-52, NLR-87-10-106-43-53, NLR-87-10-106-44-54 identified to be identical to the recurrent parent NLR34449 with respect to agro-morphological traits and they were then subjected to analysis of their resistance against BB, blast, yield and also for agro-morphological traits.

The five selected BC2F4 lines were further analysed to assess the extent of linkage drag around the target BB resistant genes i.e., Xa21, xa13 and xa5. This analysis revealed that for Xa21 gene located on Chr. 11L, a segment of 0.3 Mb was introgressed at both proximal and distal ends from the donor parent genome in the best BC2F4 plant (NLR-87-10-106-40-50). Thus, in total, a segment of 0.6 Mb was introgressed from the donor parent with respect to the genomic region in the vicinity of Xa21 (figure 3a). With respect to xa13 gene located on Chr. 8L, a segment of 0.2 Mb was introgressed at proximal end, while 0.3 Mb introgressed at distal end from the donor parent genome in the best BC3F2 plant (NLR-87-10-106-40-50). Thus, in total, a segment of 0.5 Mb was introgressed from the donor parent with respect to the genomic region in the vicinity of xa5 (figure 3c). All the five selected introgressed BC2F4 lines were subjected to screening for BB and blast resistance, and also evaluated for their agro-morphological traits.

Assessment of BB and blast resistance in the improved lines of NLR34449

The donor parent ISM, RPBio- Patho-1 and RPBio- Patho-2 were observed to be highly resistant to the disease with a lesion length ranging from 0.1 to 1.7 ± 0.3 (score 1). The recurrent parent, NLR34449 was observed to be highly susceptible to the disease with a lesion length of 8.7 ± 0.0 (score 9) when screened with IXO-20. All the five selected BC2F4 were observed to be resistant to BB disease, showing a lesion length of 0.1–1 cm, which was similar to the donor parent (i.e. ISM) (figure 4a; table 2). With respect to blast disease, the susceptible check, HR12 and the parent, donor ISM were highly susceptible to the disease, with a score of 9, while the resistant check, Tetep and the recurrent parent, NLR34449 were found to be resistant to the disease with a score of 1 and 3, respectively. The selected BC2F4 lines were observed to show resistance reaction against blast disease with a disease score of 2–4 (figure 4b; table 2).

Evaluation of agro-morphological traits and data analysis in the improved lines of NLR34449

Among the improved lines, the BC2F4 line, NLR-87-10-106-44-54 displayed better attributes with respect to most of the agro-morphological traits when compared to the recurrent
parent, Nellore Mahsuri. All the introgressed lines were slightly taller than the recurrent parent, Nellore Mahsuri and shorter than donor parent, ISM, except a single line NLR-87-10-106-42-52 (75.5 ± 0.9 cm) which was observed to be slightly shorter than recurrent parent (table 3). The introgressed lines were found to perform equivalent or better than both the recurrent as well as donor parents in terms of panicle length, number of grains per panicle, 1000 grain weight and grain yield per plant with good panicle exertion (figure 5; table 3). Two improved lines, NLR-87-10-106-40-50 and NLR-87-10-106-42-52 recorded grain yield per plant (24.1 ± 0.6 g) equivalent to the recurrent parent (table 3).
For genetic parameters such as genotypic coefficient of variance (GCV) and phenotypic coefficient of variance (PCV), lower values (0–10%) was recorded in days to 50% flowering (DFF), plant height (PH), panicle length (PL), number of grains per panicle (NGP), 1000 grain weight and L/B ratio and moderate values (10–20%) for productive tillers, grain yield per plant. Values for heritability in broad sense was noticed to be moderate (>50%) in days to 50% flowering (DFF), plant height, panicle length, number of grains per panicle, L/B ratio and to be high for remaining traits (>60%). For genetic advance in per cent of mean (GAM) lower values were (0–10%) observed in days to 50% flowering (DFF), plant height, panicle length, L/B ratio and moderate (10–20%) in number of grains per panicle, 1000 seed weight and higher (>20%) with respect to the remaining traits (table 3).

Table 2. Reaction of selected improved lines of NLR34449 after inoculation with bacterial blight and blast pathogen.

| Parents and checks | Reaction against BB | Reaction against blast |
|--------------------|---------------------|------------------------|
|                    | IXO-20 Score R/S    | SP140 Score R/S        |
| ISM                | 0.0 ± 0.0 R         | 9 S                    |
| NLR 34449          | 8.7 ± 0.0 S         | 3 R                    |
| RPBio- Patho 1     | 1.3 ± 0.3 R         | 2 R                    |
| RPBio- Patho 2     | 1.7 ± 0.3 R         | 1 R                    |
| TN1                | 8.7 ± 0.3 S         | – –                    |
| HR12               | 1 ± 0.3 R           | 9 S                    |
| NLR-86-10-106-40-50| 1 ± 0.3 R           | 3 R                    |
| NLR-86-10-106-41-51| 1 ± 0.3 R           | 3 R                    |
| NLR-86-10-106-42-52| 1 ± 0.3 R           | 3 R                    |
| NLR-86-10-106-43-53| 1 ± 0.3 R           | 4 R                    |
| NLR-86-10-106-44-54| 1 ± 0.3 R           | 3 R                    |

R, resistant; S, susceptible.

Discussion

Rice yield and productivity drastically reduced due to two major diseases, namely BB and blast (Ou 1985; Srinivas Prasad et al. 2009). Development of improved versions of crop varieties through introgression of resistance genes with the help of molecular markers has been well demonstrated with respect to blast disease (Manu et al. 2012; Hari et al. 2013; Madhavi et al. 2013; Ratna Madhavi 2016; Ashkani et al. 2014; Rekha et al. 2018) and BB (Joseph et al. 2004; Sundaram et al. 2008, 2009). Until date, at least 45 BB resistance genes (Neelam et al. 2019; Sundaram et al. 2014) have been identified. Among them, the major, dominant, broad spectrum BB resistance gene, *Xa21* (originally derived from *O. longistaminata*), located on Chr. 11, a major recessive gene located on chromosome 8, *xa13* another major recessive gene located on chromosome 5, *xa5* are...
Table 3. Evaluation of agro-morphological characters along with parents under field conditions

| Plant identity | Days to 50% flowering (DFF) | Mean plant height (cm) | No. of productive panicles/plant | Panicle length (cm) | Number of grains per panicle | Grain yield per plant (g) | 1000 seed weight (g) | L/B ratio | 1000 seed weight | Grain type | Panicle exertion |
|----------------|-----------------------------|------------------------|----------------------------------|---------------------|-----------------------------|---------------------------|------------------------|-----------|-----------------|------------|----------------|
| 1 NLR34449     | 95.0 ± 0.3                  | 77.0 ± 0.3             | 13.7 ± 0.3                       | 22.0 ± 0.6          | 242.0 ± 1.5                 | 24.0 ± 0.3                | 14.1 ± 0.6             | 2.70 ± 0.0 | MS              | FE         |                |
| 2 RPBio-226    | 101.0 ± 1.2                 | 84.0 ± 0.6             | 12.3 ± 0.9                       | 19.2 ± 0.6          | 225.0 ± 1.2                 | 17.1 ± 1.7                | 12.5 ± 0.7             | 2.70 ± 0.0 | MS              | PE         |                |
| 3 NLR-87-10-106-40-50 | 97.3 ± 0.3 | 77.7 ± 0.4             | 17.0 ± 0.0                       | 22.5 ± 0.6          | 268.0 ± 1.2                 | 24.1 ± 0.2                | 14.3 ± 0.2             | 2.70 ± 0.0 | MS              | FE         |                |
| 4 NLR-87-10-106-41-51 | 101.0 ± 0.6 | 78.2 ± 0.9 | 16.0 ± 0.6                       | 22.1 ± 0.3          | 273.0 ± 0.9                 | 24.7 ± 0.4                | 14.2 ± 0.2             | 2.70 ± 0.0 | MS              | FE         |                |
| 5 NLR-87-10-106-42-52 | 99.0 ± 0.6 | 75.5 ± 0.9             | 16.3 ± 0.3                       | 22.5 ± 0.3          | 272.0 ± 0.9                 | 24.1 ± 0.6                | 14.5 ± 0.1             | 2.70 ± 0.0 | MS              | FE         |                |
| 6 NLR-87-10-106-43-53 | 99.0 ± 0.3 | 77.7 ± 0.6             | 16.7 ± 0.9                       | 23.0 ± 0.4          | 263.0 ± 1.5                 | 26.8 ± 0.5                | 15.0 ± 0.1             | 2.70 ± 0.0 | MS              | FE         |                |
| 7 NLR-87-10-106-44-54 | 94.0 ± 0.9 | 79.0 ± 0.6             | 17.7 ± 0.3                       | 23.8 ± 0.3          | 280.0 ± 0.9                 | 28.8 ± 0.3                | 16.1 ± 0.1             | 2.70 ± 0.0 | MS              | FE         |                |
| Mean           | 97             | 78.04                  | 15.67                            | 22.15               | 260                         | 24.23                     | 14.37                  | 2.68       |                 |            |                |
| CV (%)         | 2.7            | 3.7                    | 6.5                              | 5.45                | 5.61                        | 5.50                      | 5.08                   | 2.61       |                 |            |                |
| F_cal value    | 3.14**         | 3.5**                  | 10.63**                          | 4.36**              | 5.56**                      | 21.94**                   | 6.42**                 | 3.02**     |                 |            |                |
| GCV            | 2.27           | 3.36                   | 11.75                            | 5.77                | 6.91                        | 14.53                     | 6.83                   | 2.14       |                 |            |                |
| PCV            | 3.51           | 4.99                   | 13.46                            | 7.94                | 8.9                         | 15.53                     | 8.51                   | 3.38       |                 |            |                |
| h^2b (%)       | 41.69          | 45.37                  | 76.25                            | 52.84               | 60                          | 87.47                     | 64.36                  | 40.25      |                 |            |                |
| LSD@5%         | 4.67           | 5.12                   | 1.83                             | 2.15                | 26.01                       | 2.37                      | 1.3                    | 0.12       |                 |            |                |
| GAM@5%         | 3.02           | 4.66                   | 21.14                            | 8.64                | 11.06                       | 27.99                     | 11.28                  | 2.8        |                 |            |                |

CV, coefficient of variation; PCV, phenotypic coefficient of variance; GCV, genotypic coefficient of variance; h^2b, broad sense of heritability; F_cal, F test calculated; LSD, least significant difference; GAM, genetic advance in % of mean; MS, medium slender; FE, full exserted; PE, partially exserted.
known to confer broad spectrum and durable resistance against the disease (Sundaram et al. 2008, 2009; Lalitha et al. 2013; Pradhan et al. 2015; Ramalingam et al. 2017; Rekha et al. 2018).

NLR34449, popularly known among farmers as Nellore Mahsuri, was released from Rice Research Station, Acharya N.G. Ranga Agricultural University (ANGRAU), Nellore. This variety is popular among the farmers, as it is high yielding, semi-dwarf in plant stature, blast resistant, early maturing (120 days) and is nonlodging type (suitable for machine harvesting). It is highly preferred by rice farmers in the states of Andhra Pradesh, Telangana and other south Indian states (http://www.kvknellore-angrau.org/index.php). Further, it possesses medium-slimmer grain type, which is highly desirable among the rice consumers in south India and varieties with medium-slimmer grain type like Samba Mahsuri, Sona Mahsuri etc. are getting increasingly popular across all the rice growing areas of India. As NLR34449 is highly susceptible to BB disease, we attempted to improve NLR34449 for durable BB resistance by using marker-assisted introgression of three BB resistance genes *Xa21*, *xa13* and *xa5*.

In rice molecular breeding programmes, molecular markers that are tightly linked to target genes/QTLs are being utilized to overcome the limitations of conventional phenotype based breeding and improve the selection efficiency of target genes or traits in backcross breeding programmes (Jena and Mackill 2008). This is done through the process of ‘foreground selection’ where markers are used alone or in combination with phenotypic screening in selection for the target trait(s) (Hospital and Charcosset 1997). In the present study, the codominant markers, pTA248 (Ronald et al. 1992), *xa13*prom and *xa5*FM (Hajira et al. 2016) were used to screen for the presence of three BB resistance genes, namely *Xa21*, *xa13* and *xa5*, respectively and all the three markers are reported to be functional markers or very closely linked to the respective genes, with no chance or very little chance for recombination (Hajira et al. 2016). These markers were also successfully used by Ramalingam et al. (2017) and Rekha et al. (2018). The strategy of combining genotyping with phenotyping for BB resistance in the later generations of backcrossing/selection adopted in this study at BC$_2$F$_2$ generation was reported earlier in the study of Hari et al. (2011, 2013), where a stringent phenotypic screening initially followed by selective genotyping of resistant plants has been reported to save time and resources. This is because; marker-assisted selection was done only with plants that exhibited phenotypic resistance against the targeted stress.

MABB strategy is very helpful in reducing the number of backcrosses needed for complete recovery of the RPG. Even though there are reports which suggest that a minimum of three to four backcrosses are required for near complete recovery of RPG (Bai et al. 2006; Hasan et al. 2015a; Sundaram et al. 2008, 2009). Our recent experiences and also other studies suggest that by adopting a stringent MABB strategy, the number of backcrosses can be limited to just two, and still we can maximize the RPG recovery (Singh et al. 2001; Basawaraj et al. 2010; Miah et al. 2015; Abhilash et al. 2017; Rekha et al. 2018; Swathi et al. 2019).

Marker-assisted background selection in the early backcross generations has been advocated for a quick recovery of the RPG (Chen et al. 2001 and Joseph et al. 2004). Polymorphic microsatellite markers are usually utilized for background selection to assess the recovery of the RPG and also to shorten the number of backcross generations (Hospital and Charcosset 1997) to estimate the amount of RPG contribution. Similar approach has been adopted in earlier studies also (Anila et al. 2014; Abhilash Kumar et al. 2015; Balachiranjeewi et al. 2015; Bhaskar et al. 2015; Mahadevaswamy et al. 2018; Rekha et al. 2018).

The donor parent and recurrent parent used in the present study have the same background (i.e. Samba Mahsuri); hence backcrossing was restricted to two rounds. The RPG recovery was 78.5% (plant # NLR-87) in the best BC$_1$F$_1$ plant, while the value was 88.5% (plant # NLR-87-10) in the best BC$_1$F$_1$ plant. In BC$_2$F$_2$ generation, the RPG was observed to be 94.6% (line # NLR-87-10-106) and 96% (line # NLR-87-10-106-44-54) in the selected best BC$_2$F$_2$ lines. The estimation of linkage drag of donor segment in the best BC$_2$F$_2$ lines (plant # NLR-87-10-106-44-54) using graphical genotyping analysis (GGT) revealed that the extent of donor parent genome is restricted to 0.6 Mb with respect to *Xa21* on chromosome 11, 1.3 Mb with respect to *xa13* on chromosome 8 and 0.5 Mb with respect to *xa5* on chromosome 5, highlighting very high recovery of the genome of Nellore Mahsuri genome (up to 94.6%) at BC$_2$F$_2$ (NLR-87-10-106). Similar results were observed in earlier studies where *Xa21*, *xa13* and *xa5* genes were introgressed into Samba Mahsuri, Jalmagna, where a recovery of 97% and 94.6%, respectively was documented (Pradhan et al. 2015; Sundaram et al. 2008, respectively). Few other studies also reported similar observations (Abhilash Kumar et al. 2016, 2017; Balachiranjeewi et al. 2015, 2018; Basawaraj et al. 2010; Ahmed et al. 2015, Hasan et al. 2015b; Rekha et al. 2018; Shanti et al. 2010; Swathi et al. 2019).

Phenotypic screening of the improved versions of the NLR34449 against BB disease revealed that all the lines are resistant to the disease with a score of 1. Further, they were also resistant to blast with a score of 2-4 (figure 4; table 2). The results are in agreement with earlier reports, where the BB resistance genes, *Xa21*, *xa13* and *xa5* were deployed either singly or in combination (Yoshimura et al. 1995; Huang et al. 1997; Dokku et al. 2013a, b; Gitishree and Rao 2015; Sanchez et al. 2000; Singh et al. 2001; Sundaram et al. 2008) and the derived lines showed resistance against the disease. The objective of the present study was to develop pyramided lines of NLR3449 with durable resistance against BB. In earlier studies, introgressed lines possessing two or more genes against BB were observed to possess higher level of resistance, possibly due to quantitative complementation (Sanchez et al. 2000; Sundaram et al. 2008). Cultivation of breeding lines of NLR34449
possessing the three resistance genes in BB endemic areas may be beneficial to farmers and also the resistance is expected to be more durable, as three resistance genes with distinct functions (Sundaram et al. 2009) have been deployed. There have been various reports, where a single major blast resistance gene like Pi1 or Pi2 have shown the desired level of resistance against the disease (Fu et al. 2012). Similar to the recurrent parent, NLR34449, which is highly resistant to blast disease, all the improved lines showed resistance against blast disease. Our preliminary analysis has indicated the possibility of presence of the major blast resistance gene, Pi1 in NLR34449, based on marker analysis (data not shown) and resistance in the improved breeding lines of NLR34449 could possibly be due to the presence of Pi1. The results observed in the present study with respect to resistance against BB and blast are in correspondence with the results obtained in earlier reports (Hari et al. 2013; Balachiranjeevi et al. 2015; Abhilash et al. 2016; Rekha et al. 2018), where no negative interaction was observed between blast and BB resistance genes. The improved breeding lines with BB resistance using NLR34449 as recurrent parent are expected to show high level of resistance against both the diseases. These two diseases are known to be widely prevalent and limiting the rice production in the Indian state of Andhra Pradesh (also known as rice bowl of south India) and also other Indian states (Aruna Kumari et al. 2016).

Most of the improved lines were observed to be equivalent to NLR34449 in terms of grain yield and other agromorphological characters and some of them were found to be superior to NLR34449 with respect to yield (table 3), and all the lines were showing good panicle exsertion with plant height equivalent to NLR34449 and hence are nonlodging (figure 5). Further, all the selected improved lines, possessing BB and blast resistance were observed to be retaining the medium-slender grain type and fine-grain quality attributes of the NLR34449. It can be expected that the BB and blast resistant lines of NLR34449 developed through present study ensure that the farmers get a high price in the market, similar to NLR34449 of Samba Mahsuri as they are identical in terms of grain quality and yield, plus the added advantage of resistance against two major diseases. One of the significant achievement of this study was the complete recovery of the yield and yield related traits along with grain quality features in the improved lines of NLR34449 possessing BB and blast resistance. Combining phenotype-based selection with MAS in the present study was helpful in not only recovering good features of the recurrent parent, but also in selection of superior lines (with better yield and panicle exsertion) as compared to the recurrent parent, NLR34449.

In conclusion, the improved versions of Nellore Mahsuri possessing BB and blast resistance, developed in the present study may offer a distinct advantage to farmers of NLR34449, whose fields are affected by both BB and blast. Further, cultivation of such improved backcross derived lines of NLR34449 possessing resistance against BB and blast could help to improve rice production in the disease endemic areas in many states of India, where fine-grain type varieties like NLR34449, Samba Mahsuri, HMT Sona etc. are preferred. Further, the improved lines of NLR34449 developed in this study can also be used as donors to transfer BB and blast resistance into other genetic backgrounds as they possess high yield and medium-slender grain type. Among the improved lines of NLR 34449, the backcross derived line # NLR-87-10-106-44-54 possessing Xa21, xa13 and xa5 showed very good phenotype and better panicle exsertion along with high yield and diseases resistance and hence it has been identified as one of the promising lines (table 3). This line is being nominated for AICRIP trials and state trials for possible commercial release to farmers. The data and information generated from the study are available in our laboratory as hard and soft copies and can be shared based on request.

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Author Contributions

AD carried out the present work and developed the improved versions of NLR34449 with BB and blast resistance, and drafted the manuscript. PV, BSM, LGS, SLV, SRM provided the infrastructural facilities for doing research. The authors would also like to thank Indian Council of Agriculture Research and Department of Biotechnology for providing all the necessary facilities to carry out the research work.

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