Identification and Introgression of Bacterial Blight Resistance From African Rice (Oryza Glaberrima Steud.)

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Abstract

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the major diseases affecting rice production. In the present study, 31 accessions of *O. glaberrima* were screened for BB resistance consecutively for four seasons and identified 28 stable BB resistant accessions with a mean lesion length of < 3cm. Molecular characterization revealed absence of *xa5*, *xa13*, *Xa21*, *Xa38*, *xa41(t)* and *xa45*, indicating the possibility of presence of novel gene(s) conferring BB resistance. In order to transfer BB resistance from *O. glaberrima*, resistant accession EC861812 was crossed to the susceptible cultivar, IR64 and BC$_2$F$_2$ population developed. Genetic studies in BC$_2$F$_2$ population showed transgressive segregation which indicates that *O. glaberrima* also harbor beneficial alleles for yield related traits. Six resistant BC$_2$F$_2$ plants with mean lesion length of 0.1-0.8 cm were genotyped with 70 parental polymorphic SSR markers which revealed recurrent parent genome 65.1 to 84.5% with average of 67.5%. The BB resistant introgression lines identified in the present study will serve as pre-breeding lines for mapping of BB resistance derived from *O. glaberrima*.

Introduction

Rice (*Oryza sativa* L) (2n = 2x = 24), is the staple food crop that belongs to grass family, Poaceae and is grown in many countries in Asia, Africa and Latin America. It is a major source of calories and is the principal food source for more than half of the world’s population (Khush, 2005). The global demand for rice is expected to be 650 million tonnes by 2050, principally due to the rise in demand in Asian countries (Chukwu *et al*., 2019). Rice production is adversely affected due to several biotic stresses, including bacterial blight (BB) disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Yield loss due to BB is generally in the range of 20–30% across the years. However, under favorable environmental conditions, it can reach up to 80% (Kim, 2018). Developing resistant cultivar is generally regarded as the most effective and economical means of controlling this dreaded disease. Presently at least, 46 genes conferring resistance against various races of *Xoo* have been identified in both cultivated rice and wild relatives of rice (Chukwu *et al*., 2019; Neelam *et al*., 2019; Chen *et al*., 2020). Because of the rapid changes in the virulence pattern of *Xoo* population resulting in emergence of new races, there are reports of resistance breakdown conferred by major resistance genes (Shanti *et al*. 2001). Hence, it is imperative to identify novel genes, which are effective against virulent isolates of the pathogen in order to cope with the dynamic and rapidly evolving pathogen population.

Genus *Oryza* consists of two cultivated species (*O. sativa* L. and *O. glaberrima* steud.) and 22 wild species, which are known to harbor several BB resistance genes. There are at least 12 BB resistance genes have been identified in different species of *Oryza*, namely *Xa21* from *O. longistaminata* (Khush, 1989; Ronald *et al*., 1992), *Xa23* from *O. rufipogon* (Zhang *et al*., 1998; Wang *et al*., 2014), *Xa27* from *O. minuta* (Gu *et al*., 2004), *Xa29* from *O. officinalis* (Tan *et al*., 2004), *Xa30(t)* from *O. nivara* (Jin *et al.*, 2007), *Xa32(t)* from *O. australiansis* (Zheng *et al*., 2009), *Xa33* from *O. nivara* (Kumar *et al*., 2012), *Xa34(t)* from *O. branchyantha* (Ram *et al*., 2010), *Xa35* from *O. minuta* (Guo *et al*., 2010), *Xa38* from *O. nivara* (Cheema *et al*., 2008; Bhasin *et al*., 2012), *Xa41* from *O. barthii* and *O. glaberrima* (Hutin *et al*.,
2015) and Xa45 from *O. glaberrima* (Neelam *et al.*, 2019). Based on the above information, it is clear that different species of *Oryza* are useful resources that can be explored for identification of new sources of BB resistance in rice.

Studies carried out at ICAR-Indian Institute of Rice Research, Hyderabad, Telangana, India revealed consistent BB resistance in several accessions of *O. glaberrima*. This study describes consistent performance of several accessions of *O. glaberrima* with respect to BB resistance, validation of resistance sources using markers specific for major BB resistance genes, transfer of BB resistance from a resistant accession of *O. glaberrima*, viz., EC861812 into the susceptible cultivar IR64 and identification of backcross derived introgression lines possessing BB resistance with considerable recovery of IR64 genome.

**Materials And Methods**

**Plant material**

Thirty one accessions of *O. glaberrima* (Supplementary Table 1) obtained from International Rice Research Institute (IRRI), Philippines were used for studying their reaction against Xoo in the present study. The popular mega-rice variety, IR64 (which is susceptible to BB) was used as the recurrent parent while Improved Samba Mahsuri (possessing three major BB resistance genes, *Xa21, xa13* and *xa5*; Sundaram *et al.,* 2008) was used as resistant check variety.

**Screening of *O. glaberrima* for bacterial blight resistance**

*O. glaberrima* accessions were screened from 2016 to 2019 using a local virulent isolate of Xoo viz., IX-020 (Yugander *et al.,* 2018). The test entries along with recurrent parent and resistant check were raised in mud pots following the required care and fertilizer applications to raise healthy plants. The Xoo strain, IX-020 was grown in modified Wakimoto's culture medium (Laha *et al.,* 2009) and using a 3-day old culture, bacterial suspension (10⁸ cfu/ml) was prepared and used for inoculation. The plants at maximum tillering stage were inoculated by clipping top 2-3 cm of completely developed leaves with sterilized scissors dipped in the bacterial suspension (Kauffman *et al.,* 1973). Disease reactions were recorded following Standard Evaluation System for Rice (IRRI, 2014) and by measuring the lesions length caused by BB on each inoculated leaf, 14 days after inoculation. The mean lesion length (cm) for each plant was calculated based on lesion lengths of five inoculated leaves and used for assessing resistance or susceptibility of individual plants. Based on mean lesion lengths, accessions were classified as resistant (≤3 cm), moderate resistant (3-6 cm), moderate susceptible (6-9 cm) and susceptible (≥9 cm) as per Chen *et al.,* 2000.

**Molecular characterization of BB resistant lines**

BB resistant accessions were characterized for presence/absence of BB resistance genes such as *xa5* (xa5FM), *xa13* (xa13prom), *Xa21* (pTA248), *Xa38* (Oso4g53050) and *xa41(t)* using reported gene-
linked molecular markers (Table 1). Improved Samba Mahsuri (possessing BB resistance genes \textit{xa5}, \textit{xa13} and \textit{Xa27}) and a breeding line PR114-Xa38 (possessing BB resistance gene, \textit{Xa38}) were used as positive check while BB susceptible variety, IR64 was used as negative check. DNA isolation and PCR protocols described in Sundaram \textit{et al.} (2008) and Yugander \textit{et al.} (2018) were adopted for analysis of the target genes.

**Development of backcross populations**

In order to introgress BB resistance, one of the \textit{O. glaberrima} accessions, EC861812 (which was found to be highly resistant to \textit{Xoo} in repeated tests) was crossed with IR64. The resultant \(F_1\)s were backcrossed to the recurrent parent, IR64 to develop \(BC_1F_1\)s. The \(BC_1F_1\) plants were selfed to develop \(BC_1F_2\) population. The \(BC_1F_2\) were evaluated for BB resistance using \textit{Xoo} strain IX-020. Selected BB resistant \(BC_1F_2\) plants were further backcrossed to the recurrent parent, IR64 to develop \(BC_2F_1\)s and then advanced by selfing to develop \(BC_2F_2\) population.

**Estimation of recurrent parent genome in BB resistant introgression lines**

In order to study the extent of recurrent parent (i.e. IR64) background genome recovery in the BB resistant introgression lines derived \textit{O. glaberrima}, SSRs polymorphic between the resistant \textit{O. glaberrima} accession (EC861812) and recurrent parent, IR64 were identified by screening 428 SSR markers spanning over 12 rice chromosomes following the protocol of Sundaram \textit{et al.} (2008). The polymorphic SSRs identified across the 12 chromosomes were then used to genotype BB resistant \(BC_2F_2\) plants to estimate the recurrent parent genome recovery. Genotypic data were analyzed using GGT software (Ralph van Berloo, 2008) to identify the \(BC_2F_2\) plants having maximum recurrent parent genome recovery.

**Evaluation of \(BC_2F_2\) population for yield attributing traits**

Twenty five days old seedlings of selected BB resistant plants identified in \(BC_2F_2\) population were transplanted in replicated mini-plots (2 x 2 m\(^2\) plots) in the experimental plots of ICAR-IIRR, Hyderabad following a spacing of 15 x 20 cm. Data were recorded for eight yield attributing traits viz., days to 50% flowering, plant height, number of tillers per plant, number of productive tillers per plant, panicle length, number of spikelets per plant and spikelet fertility.

**Results**

**Identification of BB resistant accessions of \textit{O. glaberrima}**

\textit{O. glaberrima} accessions were screened repeatedly for four seasons to assess and reconfirm their level of resistance against \textit{Xoo} (Supplementary Table 1). Plants were inoculated with bacterial blight pathogen culture (strain IX-020) and were scored at 14 days after inoculation (DAI) Fig 1(A). In the first experiment, the reactions of the genotypes were scored based on SES scale and in the subsequent experiments, the reactions were recorded by measuring the lesion length. Based on observations of multiple screenings,
out of 31 accessions, 28 accessions showed high level of resistance with lesion length less than 3 cm. The average lesion length among the resistant entries ranged from 0.3 cm (EC # 861795, 861810) to 1.8 cm (EC861811). Two accessions, viz., EC861819 and EC861820 showed a moderate resistance reaction with average lesion length ranging from 3.9-4 cm while EC861804 showed extreme susceptibility with an average lesion length > 30 cm. The cultivar, IR64, exhibited a score of 7 with a lesion length ranging from 7.3-8.2 over the seasons.

**Molecular characterization using reported genes BB resistance**

Thirty accessions of *O. glaberrima* which showed moderate to high level of BB resistance in repeated phenotyping were genotyped for the presence/absence of major BB resistance genes namely *xa*5, *xa*13, *Xa*21, *Xa*38 and *xa*41(t) using gene-specific PCR based markers *xa*5FM, *xa*13prom, pTA248, Oso4g53050-1 and *OssWEET14* promoter markers, respectively. BB resistant rice variety, Improved Samba Mahsuri possessing three BB resistance genes, *xa*5, *xa*13 and *Xa*21 (Sundaram *et al.*, 2008) and breeding line PR114-Xa38 possessing BB resistance gene, *Xa*38 (Bhasin *et al.*, 2012) were used as positive checks while the BB susceptible rice variety IR64 was used as negative check. Presence of resistance allele with respect to different BB resistance genes in the positive checks was revealed through amplification of fragment sizes of 134bp (*xa*5), 450bp (*xa*13), 950bp (*Xa*21), 269bp (*Xa*38) and 489bp (*xa*41), respectively. None of the resistant accessions of *O. glaberrima* showed the presence of the resistant allele for the above mentioned known BB resistance genes (Table 1; Figure 2).

**Development and identification of BB resistant introgression lines**

Among the 31 resistant accessions of *O. glaberrima*, EC861812 consistently showed high level of BB resistance with lesion length of 0.4-1.0 cm across different seasons; hence it was selected as the donor parent (Table 2). The bacterial blight susceptible cultivar, IR64 (used as the female parent and recurrent parent) was crossed to *O. glaberrima* accession (EC861812) during *wet season*, 2016 to produce inter-specific F1 seeds. The seed set was very low (less than 20%), as the F1s were products of inter-specific hybridization. The F1 plants (nine F1 plants) were grown and inoculated with the *Xoo* strain IX-020 during *wet season* of 2017. The mean lesion length of the F1 plants was below 4 cm (Table 3 and Figure 1B). The F1s plants were found to be partially/completely pollen sterile and there was very low seed set on selfing. Hence, the F1 plants were backcrossed with recurrent parent, IR64 to generate BC1F1 seeds. A total of 10 BC1F1 seeds were obtained as the seed set percentage was very low. The BC1F1 seeds were grown during *dry season* of 2017-18 and selfed to produce 206 BC1F2 seeds. High level of spikelet sterility was also observed among plants of BC1F1 population.
During wet season of 2018, BC$_1$F$_2$ population was grown under field conditions and the plants were inoculated with Xoo strain, IX-020 for their reaction to BB. The resistant plants identified in BC$_1$F$_2$ population were further backcrossed to recurrent parent IR64 and 16 BC$_2$F$_1$ seeds were obtained. The seeds of BC$_2$F$_1$ were sown during dry season 2018-19 and inoculated with Xoo isolate (IX-020). Out of 16 BC$_2$F$_1$ plants grown and evaluated for BB resistance, two plants were found to be highly resistant (lesion length ranging from 2.5-3cm), five were moderately resistant (lesion length ranging from 3.3-5.5 cm) and nine plants were moderately susceptible (lesion length ranging from 6.2-8.8cm) (Figure 1D, and Supplementary Table 4). Three resistant/moderately resistant plants of BC$_2$F$_1$ (58*-4-14*-3; 58*-5-6*-1 and 58*-5-6*-3) were further selfed to generate 26 BC$_2$F$_2$ seeds.

During wet season 2019, 26 BC$_2$F$_2$ plants were grown and screened for BB reaction, of which, 17 were resistant (0.1-2.4cm,), and six moderately resistant (3.2-5.4cm), two moderately susceptible (7.1cm and 8.7cm), while one was susceptible (9.7cm) (Figure 1E & F, Table 4 and Supplementary Table 5). The resistant BC$_2$F$_2$ plants were further selfed to generate BC$_2$F$_3$ population.

**Evaluation of BC$_2$F$_2$ population for yield attributing traits**

The mean data of different yield attributing traits (days to 50% flowering, plant height, number of tillers per plant, number of productive tillers per plant, panicle length, number of spikelets per plant and spikelet fertility) of BC$_2$F$_2$ plants are presented in Table 4. Details of plant wise data of different morphological characters are presented in Supplementary Table 6. The mean days to 50% flowering varied from minimum of 75 days (Plant # 58*-5-6*-1-3) to maximum of 88 days (Plant # 58*-4-14*3-6) with mean of 83.61 days. The mean plant height was 83.38 cm and ranged from 70 cm (Plant # 58*-5-6*-1-5) to 101 cm (Plant # 58*-5-6*-1-7). Interestingly, in terms of total tillers per plant, all the BC$_2$F$_2$ plants recorded higher values than both parents IR64 and EC861812. Mean total tillers per plant was 15.96 with ranging from 8 (Plant # 58*-5-6*-1-2, 58*-5-6*-3-8) to 26 tillers (Plant # 58*-4-14*3-7). In case of productive tillers per plant, all the plants recorded higher values than the parents, IR64 and EC861812. The mean productive tillers per plants was 11 and ranged from 4 (Plant # 58*-5-6*-1-3) to 22 (Plant # 58*-5-6*-3-4). The plants showed mean panicle length of 22.48 cm and the values ranged from 19.3 cm (Plant # 58*-5-6*-1-4) to 25cm (Plant # 58*-4-14*3-7, 58*-4-14*3-8). In case of spikelets per panicle, the mean was 101.79 ranging from 56 spikelets (Plant # 58*-5-6*-3-6) to 175 (Plant # 58*-4-14*3-1), while mean spikelet fertility was 32.71% ranging from 14.81% (Plant # 58*-4-14*3-4) to 88.9% (Plant # 58*-4-14*3-8).

**Estimation of recovery of RP genome in BB resistant introgression lines**

Six introgression lines (at BC$_2$F$_2$ generation) showing high level of BB resistance (with lesion length 0.1-0.8 cm) were selected for estimation of recovery of recurrent parent genome. A total of 70 parental
polymorphic SSR markers identified (out of 428 screened) across all the 12 chromosomes were used for genotyping of BB resistant introgression lines. The total number of markers per chromosome ranged from four (Chromosome 9 and 10) to seven (Chromosome 1, 6 and 12) with average of six markers per chromosome (Table 5). The recurrent parent genome recovery percentage among the six selected plants was observed to range from 52.4 (Chromosome 2) to 86.8 (Chromosome 1) with an average of 72.8% (Table 5). The heterozygous component of the genome ranged from 0% (chromosome 4 and 10) to 25.3% (chromosome 6) with an average of 7.98% (Table 5; Plate 4.7). Among the six introgression lines, the recurrent genome per individual line ranged from 65.1% (Plant # 58*-4-14*-3-6) to 84.5% (Plant # 58*-4-14*-3-5) with an average of 67.5% (Table 6; Fig 4 (A)).

**Discussion**

**Novelty of BB resistance in *O. glaberrima***

Identification of novel sources of resistance is imperative to combat menace of bacterial blight disease in rice. African cultivated rice species *O. glaberrima*, has been reported to exhibit high level of resistance to BB resistance (Djedatin *et al.*, 2011; Hutin *et al* 2015; Neelam *et al.*, 2019). In the present investigation, among 31 accessions of *O. glaberrima* evaluated for BB resistance for four seasons, 28 accessions showed very high level of BB resistance with mean lesion length $\leq$ 2 cm. Another two accessions, viz., EC861819 and EC861820 showed moderate resistance with lesion length in the range of 3-4 cm. Molecular characterization of the resistant accessions with gene-specific PCR-based markers revealed that none of the major genes, viz., *xa5, xa13, Xa21, Xa38* and *Xa41* are present in the *O. glaberrima* accessions screened, indicating the possible presence of novel gene(s) associated with BB resistance. Similar to our findings, several studies have reported absence of known BB resistance genes in resistant accessions of *O. glaberrima* (Vikal *et al.*, 2007; Djedatin *et al.*, 2011 and Dossa *et al.*, 2016).

Two novel recessive resistance genes namely *xa41* (Hutin *et al.*, 2015) and *xa45* (Neelam *et al.*, 2019) have been recently identified in *O. glaberrima*. Loss of function of susceptible gene (*OsSWEET14*) due to 18bp deletion in promoter region causes disruption of binding of TAL effectors secreted by *Xoo* which affects the bacterial colonization; hence the mutated *OsSWEET14* gene promoter represents the recessive gene, *xa41* (Hutin *et al.*, 2015). The markers developed for *xa41* gene by Hutin *et al.* (2015) failed to identify the presence of the resistant allele with respect to the gene in the *O. glaberrima* germplasm accessions including EC861812. Another recessive gene, *xa45* identified from *O. glaberrima* (IRGC 102600B) is located on long arm of Chromosome 8 in the same region where, another recessive gene, *xa13* is also present (Neelam *et al.*, 2019). The marker study by Neelam *et al.* (2019) have shown that STS marker with respect to *xa13* co-segregated with *xa45*. Based on these findings, it is most likely that both recessive genes (*xa41* and *xa45*) are not present in the resistant accessions of *O. glaberrima* (including EC861812). Hence, the resistance against BB in the accessions of *O. glaberrima* (including EC861812) is likely to be conferred by novel gene(s).

**Development of BB resistant introgression lines**
Cross incompatibility and spikelet sterility are the two major bottlenecks affecting utilization of *O. glaberrima* for genetic improvement of *O. sativa* cultivars (Hiroko et al., 1962). During inter-specific hybridization between two cultivated species, we have encountered very high level of spikelet sterility in *F*$_1$s and subsequent selfed and backcross generations resulting in segregation distortion, which ultimately affected studies on trait segregation pattern and molecular mapping. Some studies have reported 100% spikelet sterility and partial female fertility in inter-specific cross between two cultivated species of rice due to presence of gamete eliminator and pollen killer (Heuer and Miezan, 2003; Bhatia *et al.*, 2017). There are 10 loci causing hybrid sterility loci (pollen killer or gamete eliminator) between *O. sativa* and *O. glaberrima* (Li *et al.*, 2018). Repeated backcrossing of inter-specific hybrids with the recurrent parent would help in restoring the fertility in the progenies (Sano 1986 and Sano, 1990). In our study, we employed two rounds of backcrossing followed by selfing (*BC*$_1$*F*$_1$-*BC*$_2$*F*$_2$). During each generation of backcrossing and selfing, BB resistant introgression lines were identified by screening against *Xoo* strain IX-020. Six BB resistant introgression lines with recovery of 65.1-84.5% recurrent parent genome identified in *BC*$_2$*F*$_2$ will serve as important novel donor sources for BB resistance to further utilize in mapping and transfer to cultivated varieties for development of durable BB resistance rice cultivars.

**Performance of introgression lines for yield attributing traits**

We also made an attempt to understand the performance of *O. glaberrima* derived introgression lines for yield attributing traits and we found significant favorable variations for days to 50% flowering, maturity, plant height, productive tillers in the *BC*$_2$*F*$_2$ population. We also observed transgressive segregation for yield attributing traits namely plant height, productive tillers, panicle length indicating the beneficial alleles from *O. glaberrima*. However, number of fertile spikelets per panicle was considerably low among the introgression this could be due to high level of sterility observed in the *BC*$_2$*F*$_2$ population. By selecting plants with high spikelet fertility and advancing through selfing, we will be able to able fix the problem of spikelet fertility as we select against spikelet. It is pertinent to note that the linkage drag from *O. glaberrima* is known to affect the performance of introgression lines for yield attributing traits. *O. sativa* cultivars have been successfully utilized for genetic improvement of *O. glaberrima* cultivars in Africa which has resulted in development of NERICA lines, which have shown promising performance for yield attributing traits (Jones *et al.*, 1997). However, *O. glaberrima* genetic resources have not been extensively utilized for genetic improvement of indica cultivars (Sarla and Swamy, 2005). The transgressive segregation observed for yield attributing traits is an indication that *O. glaberrima* also harbor beneficial alleles for yield attributing traits. The utilization of high-through put genomic approaches is indeed essential for gainfully exploring *O. glaberrima* for yield attributing traits in order to achieve genetic gain in indica rice cultivars.

**Conclusion**
Oryza glaberrima is important genetic resource for identification of novel bacterial blight resistance. The BB resistant introgression lines developed from O. glaberrima in the background of elite rice cultivar IR64 are important genetic materials for further mapping of BB resistance. The BC$_2$F$_2$ populations also showed transgressive segregation for yield attributing traits which will pave way for mining O. glaberrima for yield attributing traits in future studies. There are limited efforts have been made to utilize the wealth of O. glaberrima for genetic improvement of indica rice cultivars due to cross incompatibility and high level of spikelet sterility. The findings from our study are in indeed encouraging to extensively use of O. glaberrima for genetic improvement of indica rice cultivars.

Declarations

**Ethical Approval and Consent to participate:** NA

**Consent for publication:** I (Gireesh C), the undersigned, give my consent for the publication of identifiable details, which can include photographs and/or details within the text to be published in the above Journal and Article

**Availability of supporting data:** NA

**Competing interests:** Authors declares that there is no conflict of interest

**Funding:** NA

**Authors' contributions:** CG has designed the experiment. CG, VA and VU were involved in the development of introgression lines. MSA, PS, RMS and GSL were involved in the selection. VA, VU, ChR, MH, VGIL and DA have done the bacterial blight screening and scoring. BM has involved in the data analysis. CG, LVS, IAG, VP and JVR were involved in the preparation and peer review of the manuscript. All authors have reviewed and approved the final manuscript.

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### Tables

#### Table 1. Molecular characterisation of BB resistant accessions of *O. glaberrima* for BB resistance genes

| BB genes | Marker Name | Expected allele size reported in positive check (bp) | Allele size obtained in *O. glaberrima* (EC861812) (bp) | Allele size obtained in other accessions *O. glaberrima* (bp) |
|----------|-------------|--------------------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|
| xa5      | xa5FM       | 134                                                    | 313                                                      | 313                                                      |
| xa13     | xa13prom    | 450                                                    | 220                                                      | 220                                                      |
| Xa21     | pTA248      | 950                                                    | 650                                                      | 650                                                      |
| Xa38     | Oso4g53050-1 | 269                                                    | 317                                                      | 317                                                      |
| Xa41     | Xa41-sweet14 promoter | 489                                               | 507                                                      | 507                                                      |
Table 2. Phenotypic reactions of *O. glaberrima* (EC861812) and IR64 against *Xoo* (IX-020) for four seasons

| Genotype   | Score | Wet season 2016 | Wet season 2017 | Wet season 2018 | Wet season 2019 |
|------------|-------|----------------|----------------|----------------|----------------|
| IR64       | 9     | 7.4            | 8.2            | 7.3            |                |
| EC861812   | 1     | 1.0            | 0.4            | 0.4            |                |

Table 3. Phenotypic reaction of parents and F<sub>1</sub> (IR64/ *O. glaberrima*-EC861812) against *Xoo* strain IX-020

| Genotypes          | Mean Lesion Length (cm) ±SE | Range of lesion length (cm) |
|--------------------|-----------------------------|----------------------------|
| EC861812           | 1.02 ± 0.13                 | 0.7 - 1.5 cm               |
| IR64               | 9.18 ± 1.19                 | 5.5 - 13 cm               |
| F<sub>1</sub> (IR64/EC861812) | 3.68 ± 0.69                 | 2.4 - 6 cm               |

* Disease reaction was based on Chen *et al.* (2000) Resistant (R): 0-3cm; Moderately resistant (MR): 3-6cm; Moderately susceptible (MS): 6-8cm; Susceptible (S): >8cm; NI: not inoculated

Table 4. Descriptive analysis of BC<sub>2</sub>F<sub>2</sub> plants for yield attributing traits

| Characters                     | Mean       | Minimum | Maximum | Skewness |
|--------------------------------|------------|---------|---------|----------|
| Days to 50% flowering          | 83.61±0.76 | 75.00   | 96.00   | 0.79**   |
| Plant height                   | 83.38±1.66 | 70.00   | 101.00  | 0.16**   |
| Total tillers                  | 15.96±1.06 | 8.00    | 26.00   | 0.24**   |
| Productive tillers             | 10.84±0.77 | 4.00    | 22.00   | 0.79**   |
| Panicle length                 | 22.48±0.34 | 17.22   | 25.00   | -0.91*   |
| Spikelet fertility             | 32.71±4.29 | 14.81   | 96.92   | 2.25     |
| Spikelets per panicle          | 101.79±7.88| 56      | 223     | 1.67     |
Table 5. Chromosome wise details of number of markers, genome coverage, marker interval, genome composition, range of introgression and markers showing homozygous donor segment introgression analysis in BC$_2$F$_2$ introgressed lines derived from IR64*2/ O. glaberrima

| Chr. No. | No. of Markers | Genome coverage (Mbp) | Marker interval | Genome composition (%) |
|----------|----------------|----------------------|----------------|------------------------|
|          |                |                      |                | AA         | AB      | BB      |
| 1        | 7              | 20.53                | 2.93           | 86.8       | 0.7     | 12.5    |
| 2        | 6              | 35.5                 | 5.91           | 52.4       | 2.1     | 30.6    |
| 3        | 5              | 31.64                | 6.32           | 81.8       | 5.7     | 12.5    |
| 4        | 6              | 29.1                 | 4.85           | 78.3       | 0       | 17.2    |
| 5        | 6              | 18.93                | 3.15           | 81         | 2.2     | 16.8    |
| 6        | 7              | 21.5                 | 3.07           | 54.7       | 25.3    | 19.4    |
| 7        | 6              | 26.34                | 4.39           | 78.8       | 1.2     | 14.9    |
| 8        | 6              | 28.07                | 4.67           | 73.9       | 3.6     | 18.6    |
| 9        | 4              | 20.89                | 5.22           | 67.5       | 18.1    | 14.3    |
| 10       | 4              | 18.6                 | 4.65           | 84.8       | 0       | 15.2    |
| 11       | 6              | 25.5                 | 4.25           | 61.3       | 24      | 14.7    |
| 12       | 7              | 17.4                 | 2.48           | 72.3       | 12.9    | 14.7    |
| **Total**| **70**         | **294**              | **51.89**      | **72.8**   | **7.98**| **16.78**|
| **Average**| **6**                 | **24.5**            | **4.32**       | **72.8**   | **7.98**| **16.78**|

Note: AA is genome of IR64, BB is genome of O. glaberrima and AB indicate genome of both parents (Heterozygosity).

Table 6. Percent recurrent, donor and heterozygous genome in BC$_2$F$_2$ mapping population derived from IR64*2/ O. glaberrima
| S. No. | Genotypes       | A (%) | B (%) | H (%) |
|--------|-----------------|-------|-------|-------|
| 1      | IR64            | 100   | 0     | 0     |
| 2      | EC861812        | 0     | 100   | 0     |
| 3      | 58*-5-6*-3-1    | 71.9  | 13.3  | 14.8  |
| 4      | 58*-5-6*-3-3    | 71.5  | 11.9  | 14.6  |
| 5      | 58*-5-6*-3-5    | 68.7  | 16.4  | 10    |
| 6      | 58*-5-6*-3-6    | 78.3  | 10.8  | 9.3   |
| 7      | 58*-4-14*-3-5   | 84.5  | 4.6   | 8.1   |
| 8      | 58*-4-14*-3-6   | 65.1  | 12.1  | 16.7  |
| Average|                 | 67.5  | 21.1  | 9.2   |

Note: AA is genome of IR64, BB is genome of *O. glaberrima* and AB indicate genome of both parents (Heterozygosity).

**Figures**
**Figure 1**

See image above for figure legend

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**Fig 1.** Reactions of *O. glaberrima* germplasm (A). F$_1$s (B). BC$_1$F$_2$(C). BC$_2$F$_1$ (D) and BC$_2$F$_2$ (E&F) plants against *Xoo* IX-020; P1-IR64; P2-*O. glaberrima* (EC 861812);

E:$1$-58*-5*-6*-3*-1*; 2-58*-5-6-3-3; 3-58*-5-6*-3-4*; 4-58*-5-6*-5*-5*; 5-58*-5-6*-3-6; 6-58*-5-6*-3-7

F:$1$-58*-5-6*-1-1*; 2-58*-5-6*-1-3*; 3-58*-5-6*-1-4*; 4-58*-5-6*-1-6
Fig 2. Molecular characterisation of BB resistant *O. glaberrima* for known genes.

**L:** 100bp ladder; **Lane 1:** Improved Samba Mahsuri (resistant check for *xa5, xa13, Xa21, Xa38, xa41(t)* and PR114-Xa38 for *Xa38*); **Lane 2:** IR64 (susceptible check); **Lane 3&4:** EC861812(*O. glaberrima*-Bacterial blight resistant); The arrow mark shows the expected band size for positive genotypes carrying resistance genes.

In case of *xa41*, if deletion on promoter region of Sweet14 gene, the expected band size is 489bp in resistant genotypes. In case of susceptible genotype, 507bp allele is expected. But, all genotypes showed 507bp, hence it is clear that *xa41(t)* is not present in *O. glaberrima* accessions.

**Figure 2**

See image above for figure legend
**Fig. 3:** Diagrammatic representation of recovery of recurrent parent genome.

**A:** Graphical representation of genome recovery of recurrent parent among promising introgression lines derived from IR64/O. glaberrima (EC861812).

BC$_2$F$_2$-1: 58*-5-6*-3-1; BC$_2$F$_2$-2: 58*-5-6*-3-3;
BC$_2$F$_2$-3: 58*-5-6*-3-5; BC$_2$F$_2$-4: 58*-5-6*-3-6;
BC$_2$F$_2$-5: 58*-4-14*-3-3; BC$_2$F$_2$-6: 58*-4-14*-3-6

**B:** Pie charts for 12 rice chromosomes depicting the proportion of genome introgression derived from IR64/O. glaberrima (EC861812).

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