Identification and introgression of novel bacterial blight resistance from *Oryza glaberrima* Steud

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Abstract

Background: Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the major diseases affecting rice production in India and globally. Developing resistant cultivar is generally regarded as the most effective and economical means of controlling this dreaded disease.

Results: A total of 31 accessions of *O. glaberrima* were screened for BB resistance consecutively for four seasons and 28 accessions consistently exhibited high level of resistance with a mean lesion length of < 3cm. Molecular characterization using gene specific markers revealed absence of major resistance genes viz., *xa5*, *xa13*, *Xa21*, *Xa38*, *xa41(t)* and *xa45* in these accessions, indicating the possibility of presence of novel gene(s) conferring BB resistance. In order to transfer BB resistance from *O. glaberrima*, resistant accessions EC861812 showing high level of BB resistance was crossed to the susceptible rice cultivar, IR64 and resultant interspecific hybrid plants were further backcrossed to IR64. Backcrossing was done up to BC₂ generation and during each generation of backcrossing, the plants were evaluated for their BB reaction to identify BB resistant ones. High level of spikelet sterility was observed in F₁ and subsequent backcross generations. BB reactions pattern in F₁ and BC₁F₂ indicated single dominant gene conferring BB resistance in EC861812. We identified six resistant plants at BC₂F₂ (with mean lesion length of 0.1-0.8 cm) which were genotyped with 70 parental polymorphic SSR markers. The recovery of recurrent parent genome among the six resistant lines was found to be 65.1 to 84.5% with average of 67.5%. Interestingly, analysis of BC₂F₂ population for yield attributing traits showed transgressive segregation for plant height, productive tillers, panicle length which indicates that *O. glaberrima* also harbor beneficial alleles for yield related traits.

Conclusion: BB resistant introgression lines derived from *O. glaberrima* identified in the present study will serve as precious pre-breeding resources for development of durable BB resistant elite breeding varieties.

Background

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* (Kush, 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. australiensis* (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 earlier 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,s IR64 and identification of backcross derived introgression lines possessing BB resistance with considerable recovery of IR64 genome.

Material 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 2). Improved Samba Mahsuri (possessing BB resistance genes *xa5*, *xa13* and *Xa21*) and a breeding line PR114-Xa38 (possessing BB resistance gene, *Xa38*) were used as positive check while BB susceptible variety, IR64 was used as negative check. DNA isolation and PCR protocols described in Sundaram *et al.* (2008) and Yugander *et al.* (2018) were adopted for analysis of the target genes.

Development of backcross populations

In order to introgress BB resistance, one of the *O. glaberrima* accessions, EC861812 (which was found to be highly resistant to *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 *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. In order to understand genetics of BB resistance, the inheritance study was carried out in F_1 and BC_1F_2 population and chi square analysis was done to test ratio. Plants with lesion length < 6.0 cm were considered as moderate to highly resistant while lesion length > 6.0 cm as susceptible for purpose of classification during inheritance study.

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 *O. glaberrima*, SSRs polymorphic between the resistant *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 *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 (2×2 m² 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 *O. glaberrima*

O. glaberrima accessions were screened repeatedly for four seasons to assess and reconfirm their level of resistance against *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 *xa5*, *xa13*, *Xa21*, *Xa38* and *xa41(t)* using gene-specific PCR based markers *xa5FM*, *xa13prom*, *pTA248*, *Oso4g53050-1* and *OsSWEET14* promoter markers, respectively. BB resistant rice variety, Improved Samba Mahsuri possessing three BB resistance genes, *xa5*, *xa13* and *Xa21* (Sundaram *et al.*, 2008) and breeding line PR114-*Xa38* possessing BB resistance gene, *Xa38* (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 (*xa5*), 450bp (*xa13*), 950bp (*Xa21*), 269bp (*Xa38*) and 489bp (*xa41*), respectively. None of the 30 accessions of *O. glaberrima* showed the presence of the resistant allele for the abovementioned known BB resistance genes (Table 1; Supplementary Table 2 and Figure 2).

Development and identification of BB resistant introgression lines

Among the 30 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 F_1 plants was below 4 cm (Table 3 and Figure 1B). The F_1 s plants were found to be partially/completely pollen sterile and there was very low seed set on selfing. Hence, the F_1 plants were backcrossed with recurrent parent, IR64 to generate BC_1F_1 seeds. A total of 10 BC_1F_1 seeds were obtained as the seed set percentage was very low. The BC_1F_1 seeds were grown during *dry season* of 2017-18 and selfed to produce 206 BC_1F_2 seeds. High level of spikelet sterility was also observed among plants of BC_1F_1 population.

During *wet season* of 2018, BC_1F_2 population was grown under field conditions and the plants were inoculated with *Xoo* strain, IX-020 for their reaction to BB. Of 203 plants screened in BC_1F_2 population, 152 scored as resistant and 51 as susceptible which is fitting 3:1 single dominant gene inheritance (Supplementary Table 3; Table 4; chi square value 0 P value 1.0). The resistant plants identified in BC_1F_2 population were further backcrossed to recurrent parent IR64 and 16 BC_2F_1 seeds were obtained. The seeds of BC_2F_1 were sown during dry season 2018-19 and inoculated with *Xoo* isolate (IX-020). Out of 16 BC_2F_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, Table 4 and Supplementary Table 4). Three resistant/moderately resistant plants of BC_2F_1 (58*-4-14*-3; 58*-5-6*-1 and 58*-5-6*-3) were further selfed to generate 26 BC_2F_2 seeds.

During *wet season* 2019, 26 BC_2F_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_2F_2 plants were further selfed to generate BC_2F_3 population.

Evaluation of BC_2F_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_2F_2 plants are presented in Table 5. 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_2F_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 BC₂F₂ 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 6). 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 6). 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 6 and Figure 3). 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 7 and Figure 3).

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 ≤ 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*. Interestingly, in our study, presence of resistant allele of *xa13* was not observed in resistant accessions of *O. glaberrima*. Further, F_1 s from our study clearly showed resistance reaction (Figure 1B), while F_1 s from *xa45* gene were susceptible (as the gene is recessive in its inheritance; Neelam *et al.*, 2019). Further, inheritance study showed that single dominant gene conferring resistance in *O. glaberrima* accession (EC861812) unlike *xa41* and *xa45*, which are recessive. 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 Miezán, 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_1F_1 - BC_1F_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_2F_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_2F_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_2F_2 population. By selecting plants with high spikelet fertility and advancing through selfing, we will be able to 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 BC₂F₂ 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 *O. glaberrima* for genetic improvement of indica rice cultivars.

Declarations

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Authors contribution

CG-conceptualized the research program, responsible for overall conduct of research program and manuscript preparation; AV-PhD student carried out the research work; LGS-Inoculation of plant materials for bacterial blight disease; RMS-molecular characterization of rice germplasm; MSA and SP-genetic analysis of BB resistance, MB, ILVG, BM, KKB-genome recovery estimation., LVS, GP and PV- critical manuscript correction

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Availability and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

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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 <i>O. glaberrima</i> (EC861812) (bp)	Allele size obtained in other accessions <i>O. glaberrima</i> (bp)
<i>xa5</i>	xa5FM	134	313	313
<i>xa13</i>	xa13prom	450	220	220
<i>Xa21</i>	pTA248	950	650	650
<i>Xa38</i>	Oso4g53050-1	269	317	317
<i>Xa41</i>	<i>Xa41</i> -sweet14 promoter	489	507	507

Table 2. Phenotypic reactions of *O. glaberrima* (EC861812) and IR64 against *Xoo* (IX-020) for four seasons

Genotype	Score	Lesion length (cm)		
	<i>Wet season 2016</i>	<i>Wet season 2017</i>	<i>Wet season 2018</i>	<i>Wet season 2019</i>
IR64	9	7.4	8.2	7.3
EC861812	1	1.0	0.4	0.4

Table 3. Phenotypic reaction of parents and F₁ (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 – 13.0 cm
F ₁ (IR64/EC861812)	3.68 ± 0.69	2.4 – 6.0 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. Performance of backcross populations derived from IR64 and *O. glaberrima* (EC861812) against *XOO* strain IX-020

Population	No. of resistant plants (lesion length <6cm)	No. of susceptible plants (lesion length >6cm)
BC ₁ F ₂	152	51
BC ₂ F ₁	7	9
BC ₂ F ₂	17	9

Table 5. Descriptive analysis of BC₂F₂ 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 6. 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 BC₂F₂ 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			
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 7. Percent recurrent, donor and heterozygous genome in BC₂F₂ 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

Reactions of *O. glaberrima glaberrima* (A), F₁s(B), BC₁F₂(C), BC₂F₁(D) and BC₂F₂(E&F) plants against Xoo IX-020 ; P1-IR 64 ; 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*-3-5*;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

Figure 2

Molecular characterisation of BB resistant *O. glaberrima* for known genes. L 100bp ladder; Lane 1: Improved Samba Mahsuri (resistant check for say, Ta13, Xa21, Xa38, xer41(t) and PR114-Xa38 for Xa38); Lane 2: 1064 (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 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 2F2-1:58 *-5-6*-3-1;BC 2F2-2:58 *-5-6*-3-3;BC 2F2-3:58 *-5-6*-3-5;BC 2F2-4:58 *-5-6*-3-6;BC 2F2-5:58 *-4-14 *-3-5;BC 2F2-6:58 *-4-14 *-3-6 B: Pie charts for 12 rice chromosomes depicting the proportion of genome introgression derived from IR64/O. glaberrima (EC861812).

Supplementary Files

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