

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

Ajitha V

Acharya N. G. Ranga Agricultural University

Udaya V

Acharya N. G. Ranga Agricultural University

Anantha M S

ICAR-Indian Institute of Rice Research

Laha G. S

ICAR-Indian Institute of Rice Research

Sundaram R M

ICAR-Indian Institute of Rice Research

Senguttuvel P

ICAR-Indian Institute of Rice Research

Muralidhara B

ICAR-Indian Institute of Rice Research

Ishwarya Lakshmi V G

Acharya N. G. Ranga Agricultural University

Aleena Dasari

ICAR-Indian Institute of Rice Research

Honnappa M

ICAR-Indian Institute of Rice Research

Raveendra Ch

ICAR-Indian Institute of Rice Research

Irfan Ahmad Ghazi

University of Hyderabad

Subbarao L V

ICAR-Indian Institute of Rice Research

Padma V

Acharya N. G. Ranga Agricultural University

Ramana J V

Acharya N. G. Ranga Agricultural University

Gireesh C (✉ giri09@gmail.com)

Research Article

Keywords: Rice, *Oryza glaberrima*, Bacterial blight, Resistance genes, Introgression lines, yield

Posted Date: January 17th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1246576/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

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 BC₂F₂ population developed. Genetic studies in BC₂F₂ population showed transgressive segregation which indicates that *O. glaberrima* also harbor beneficial alleles for yield related traits. Six resistant BC₂F₂ 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. 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 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 (10^8 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 *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 F₁s were backcrossed to the recurrent parent, IR64 to develop BC₁F₁s. The BC₁F₁ plants were selfed to develop BC₁F₂ population. The BC₁F₂ were evaluated for BB resistance using *Xoo* strain IX-020. Selected BB resistant BC₁F₂ plants were further backcrossed to the recurrent parent, IR64 to develop BC₂F₁s and then advanced by selfing to develop BC₂F₂ 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 *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 BC₂F₂ plants to estimate the recurrent parent genome recovery. Genotypic data were analyzed using GGT software (Ralph van Berloo, 2008) to identify the BC₂F₂ plants having maximum recurrent parent genome recovery.

Evaluation of BC₂F₂ population for yield attributing traits

Twenty five days old seedlings of selected BB resistant plants identified in BC₂F₂ population were transplanted in replicated mini-plots (2 x 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 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 F_1 seeds. The seed set was very low (less than 20%), as the F_1 s were products of inter-specific hybridization. The F_1 plants (nine F_1 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, BC₁F₂ 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 BC₁F₂ population were further backcrossed to recurrent parent IR64 and 16 BC₂F₁ seeds were obtained. The seeds of BC₂F₁ were sown during dry season 2018-19 and inoculated with *Xoo* isolate (IX-020). Out of 16 BC₂F₁ 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 BC₂F₁ (58*-4-14*-3; 58*-5-6*-1 and 58*-5-6*-3) were further selfed to generate 26 BC₂F₂ seeds.

During *wet season* 2019, 26 BC₂F₂ 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 BC₂F₂ plants were further selfed to generate BC₂F₃ population.

Evaluation of BC₂F₂ 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 BC₂F₂ 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 BC₂F₂ 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 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 ≤ 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 F₁s 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 (BC₁F₁-BC₂F₂). 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 BC₂F₂ 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 BC₂F₂ 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 BC₂F₂ 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 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.

Acknowledgement: Authors acknowledge the research support received from ICAR-Indian Institute of Rice Research for carrying out the research. The authors also acknowledge the *Oryza glaberrima* accessions used in the study were obtained from International Rice Research Institute, Philippines.

References

1. Bhasin H, Bhatia D, Raghuvarshi S, Lore J S, Sahi G K, Kaur B, Vikal Y and Singh K (2012) New PCR-based sequence tagged site marker for bacterial blight resistance gene *Xa38* in rice. Molecular Breeding 30: 607-611
2. Bhatia D, Joshi S, Das A, Vikal Y, Sahi G K, Neelam K, Kaur K and Singh K (2017) Introgression of yield component traits in rice (*Oryza sativa* ssp. *indica*) through interspecific hybridization. Crop Science 57: 1-17

3. Cheema K, Grewal N, Vikal Y, Sharma R, Lore J, Das A and Singh K (2008) A novel bacterial blight resistance gene from *O. nivara* mapped to 38 kb region on chromosome 4L and transferred to *O. sativa* L. *Genetics Research* 90(5): 397-407
4. Chen S, Wang C, Yang J, Chen B, Wang W, Su J, Feng A, Zeng L and Zhu X (2020) Identification of the novel bacterial blight resistance gene *Xa46(t)* by mapping and expression analysis of the rice mutant H120. *Scientific Reports* 10:12642
5. Chen S, Lin X H, Xu C G and Zhang Q (2000) Improvement of bacterial blight resistance of 'Minghui 63', an Elite Restorer Line of Hybrid Rice, by Molecular Marker-Assisted Selection. *Crop Science* 40: 239-244
6. Chukwu S C, Rafii M Y, Ramlee S I, Ismail S I, Hasan M M, Oladosu Y A, Magaji U G, Akos I and Olalekan K K (2019) Bacterial leaf blight resistance in rice: a review of conventional breeding to molecular approach. *Journal of Molecular Biology*
7. Djedatin G, Ndjiondjop M N, Mathieu T, Vera Cruz C M, Sanni A, Ghesquière A and Verdier V (2011) Evaluation of African cultivated rice *Oryza glaberrima* for resistance to bacterial blight. *Plant Disease* 95: 441-447
8. Dossa G S, Oliva R, Maiss E, Vera Cruz C and Wydra K (2016) High temperature enhances the resistance of cultivated African rice, *Oryza glaberrima*, to bacterial blight. *Plant Disease* 100: 380-387
9. Gu K, Tian D, Yang F, Wu L, Sreekala C, Wang D, Wang G L, Yin Z (2004) High-resolution genetic mapping of *Xa27(t)*, a new bacterial blight resistance gene in rice, *Oryza sativa* L. *Theoretical and Applied Genetics* 108:800-807
10. Guo S B, Zhang D P and Lin X H (2010) Identification and mapping of a novel bacterial blight resistance gene *Xa35(t)* originated from *Oryza minuta*. *Scientia Agricultura Sinica* 43:13
11. Heuer S and Miezán K M (2003) Assessing hybrid sterility in *Oryza glaberrima* × *O. sativa* hybrid progenies by PCR marker analysis and crossing with wide compatibility varieties. *Theoretical and Applied Genetics* 107(5):902-9
12. Hiroko M, Hinata K and Oka H I (1962) Comparison between two cultivated rice species *Oryza sativa* L. and *Oryza glaberrima* Steud. *Japanese Journal of Breeding* 12: 153-165
13. Hutin M, Sabot F, Ghesquière A, Koebnik R and Szurek B (2015) A knowledge-based molecular screen uncovers a broad spectrum *OsSWEET14* resistance allele to bacterial blight from wild rice. *Plant Journal* 84: 694-703
14. Jin X W, Wang C L, Yang Q, Jiang Q X, Fan Y L, Liu G C and Zhao K J (2007) Breeding of near-isogenic line CBB30 and molecular mapping of *Xa30(t)*, a new resistance gene to bacterial blight in rice. *Scientia Agricultura Sinica* 40:1094-1100 (Chinese with English abstract)
15. Jones M P, Dingkuhn M, Aluko G K and Semon M (1997) Interspecific *Oryza sativa* L. × *O. glaberrima* Steud. progenies in upland rice improvement. *Euphytica* 94: 237-246
16. Kauffman H E, Reddy P K, Hiesh S P Y and Merca S D (1973) An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. *Plant Disease Reports* 57: 537-541

17. Khush G S, Mackill D J and Sidhu G S (1989) Breeding rice for resistance to bacterial blight. Proceedings of the international workshop on bacterial blight of rice. International Rice Research Institute, Los Banos, Phillipines 207-217
18. Khush G S (2005) What will it take to feed 5.0 billion rice consumers in 2030. *Plant Molecular Biology* 59: 1-6
19. Kim S M (2018) Identification of novel recessive gene *Xa44(t)* conferring resistance to bacterial blight races in rice by QTL linkage analysis using an SNP chip. *Theoretical and Applied Genetics* 131: 2733-2743
20. Kumar N P, Sujatha K, Laha G S, Srinivasa Rao K, Mishra B, Viraktamath B C, Hari Y, Reddy C S, Balachandran S, Ram Tejaswi, Sheshumadhav M, Shobha Rani N, Neeraja C N, Ashok Reddy G, Shaik Hassen and Sundaram R M (2012) Identification and Fine-Mapping of *Xa33*, a Novel Gene for Resistance to *Xanthomonas oryzae* pv. *oryzae*. *Phytopathology* 102: 222-228
21. Laha G S, Reddy C S, Krishnaveni D, Sundaram R M, Prasad S M, Ram T, Muralidharan K and Viraktamath B C (2009) Bacterial blight of rice and its management. Technical Bulletin No.41. Directorate of Rice Research (ICAR), Rajendranagar, Hyderabad-500 030, A.P., India. 37
22. Li J, Zhou J, Xu P, Deng X, Deng W, He M, Yang Y, Zhang Y and Tao D (2018) Neutral alleles at hybrid sterility loci of *Oryza glaberrima* from AA genome relatives in Genus *Oryza*. *Breeding science* 68(3): 343–351
23. Neelam K, Mahajan R, Gupta V, Bhatia D, Gill B K, Komal R, Lore J S, Mangat G S and Singh K (2019) High-resolution genetic mapping of a novel bacterial blight resistance gene *xa-45(t)* identified from *Oryza glaberrima* and transferred to *Oryza sativa*. *Theoretical and Applied Genetics* 133(3): 689-705
24. Ralph van Berloo (2008) GGT: Software for the display of graphical genotypes. *Journal of Heredity* 90(2)
25. Ram T, Laha G S, Gautam S K, Deen R, Madhav M S, Brar D S and Viraktamath B C (2010) Identification of a new gene introgressed from *Oryza brachyantha* with broad-spectrum resistance to bacterial blight of rice in India. *Rice Genetics Newsletter* 25:57-58
26. Ronald P C, Albano B, Tabien R, Abenes L, Wu K S, McCouch S and Tanksley S D (1992) Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21*. *Molecular Genetics and Genetics* 236:113-120
27. Sano Y (1986) Sterility barriers between *Oryza sativa* and *O. glaberrima*. In: International Rice Research Institute-IRRI (ed.) *Rice Genetics*, IRRI, Manila.109-118
28. Sano Y (1990) The genic nature of gamete eliminator in rice. *Genetics* 125: 183–191
29. Sarla N and Swamy B P M (2005) *Glaberrima*: A source for the improvement of *Oryza sativa*. *Current Science* 89(6)
30. Shanti M L, George M L C, Cruz C M V, Bernardo M A and Nelson R J (2001) Identification of resistance genes effective against rice bacterial blight pathogen in Eastern India. *Plant Disease* 85:506-512

31. Sundaram R M, Vishnupriya M R, Biradar S K, Laha G S, Reddy G A, Shoba Rani N, Sarma N P and Sonti R V (2008) Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite *indica* rice variety. *Euphytica* 160: 411-422
32. Tan G X, Ren X, Weng Q M, Shi Z Y, Zhu L L and He G C (2004) Mapping of a new resistance gene to bacterial blight in rice line introgressed from *Oryza officinalis*. *Acta Genetica Sinica* 31:724-729 (Chinese with English abstract)
33. Vikal Y, Das A, Patra B, Goel R K, Sidhu J S and Singh K (2007) Identification of new sources of bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) resistance in wild *Oryza* species and *O. glaberrima*. *Plant Genetic Resources: Characterization and Utilization* 5(2); 108-112
34. Wang C, Fan Y, Zheng C, Qin T, Zhang X and Zhao K (2014) High-resolution genetic mapping of rice bacterial blight resistance gene Xa23. *Molecular Genetics and Genomics*
35. Yugander A, Sundaram R M, Kuldeep Singh, Ladhalakshmi D, Subba Rao L V, Sheshu Madhav M, JyothiBadri Srinivas Prasad M and Laha G S (2018) Incorporation of the novel bacterial blight resistance gene *Xa38* into the genetic background of elite rice variety Improved Samba Mahsuri. *Plos one* 13(5): e0198260
36. Zhang Q, Lin S C, Zhao B Y, Wang C L, Yang W C, Zhou Y L, Li D Y, Chen C B and Zhu L H (1998) Identifying of a new gene for resistance to bacterial blight from *O. rufipogon*. *Rice Genetics Newsletter* 15:138-142
37. Zheng C K, Wang C L, Yu Y J, Liang Y T and Zhao K J (2009) Identification and molecular mapping of *Xa32(t)*, a novel resistance gene for bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) in rice. *Acta Agronomica Sinica* 35:1173-1180 (Chinese with English abstract)

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 cm
F ₁ (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 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 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 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 6. 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

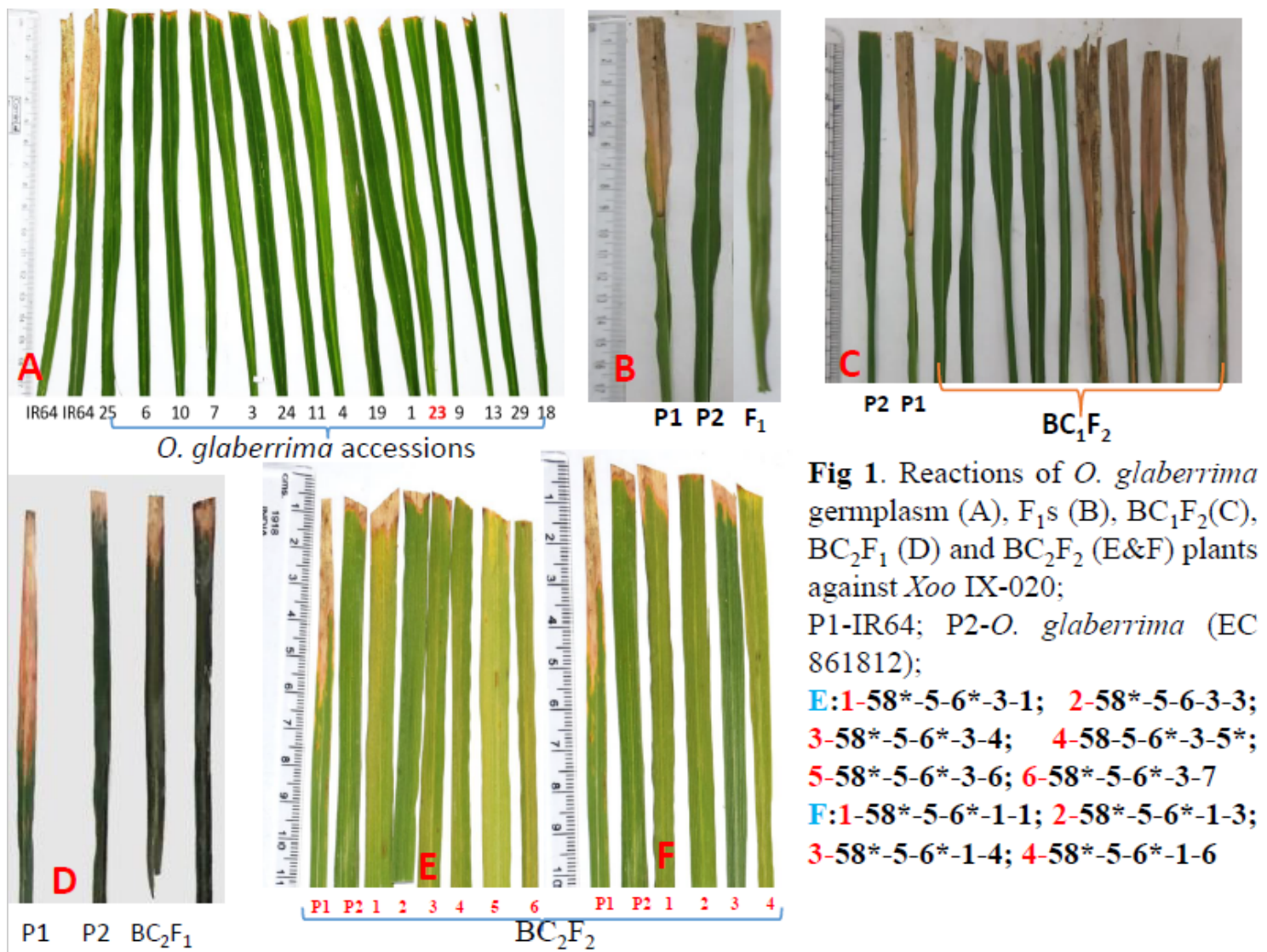


Figure 1

See image above for figure legend

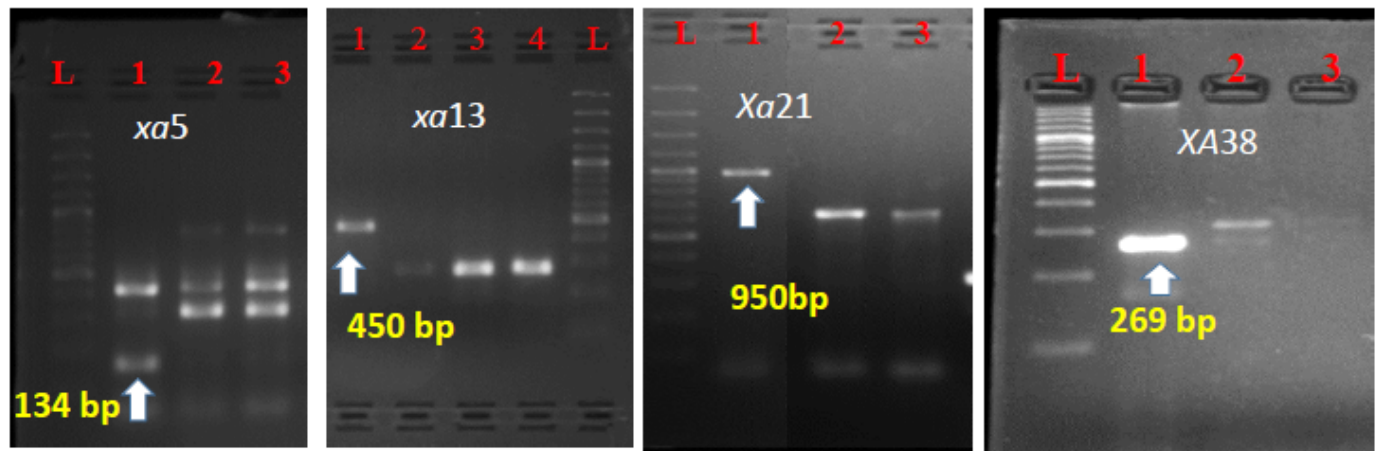


Fig 2. Molecular characterisation of BB resistant *O. glaberrima* for known genes.

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

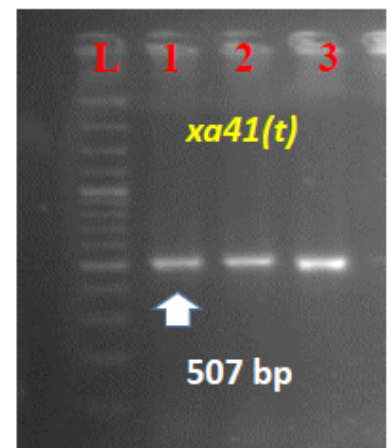


Figure 2

See image above for figure legend

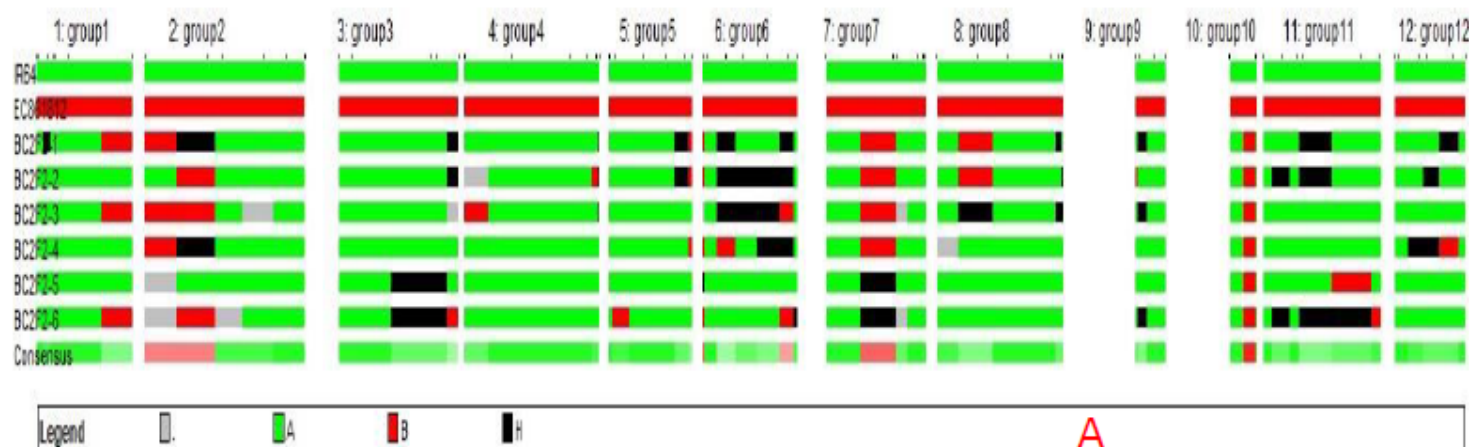


Fig. 3: Diagrammatic representation of recovery of recurrent parent genome.

A: Graphical representation of genome recovery of recurrent parent among promising introgression lines derived from IR64/*O. glaberrima* (EC861812).
BC₂F₂-1: 58*-5-6*-3-1; **BC₂F₂-2:** 58*-5-6*-3-3;
BC₂F₂-3: 58*-5-6*-3-5; **BC₂F₂-4:** 58*-5-6*-3-6;
BC₂F₂-5: 58*-4-14*-3-5; **BC₂F₂-6:** 58*-4-14*-3-6

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

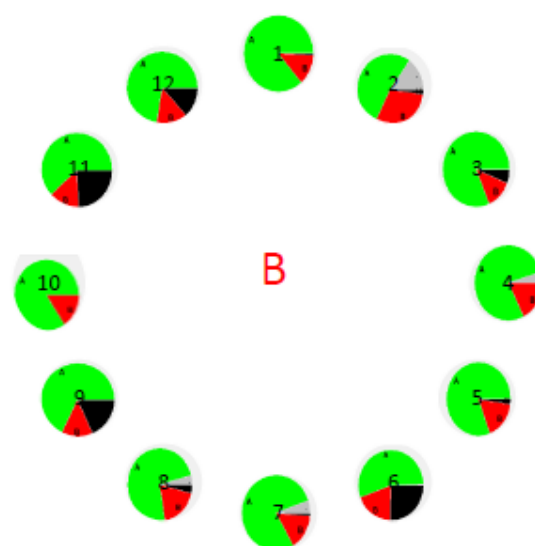


Figure 3

See image above for figure legend

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplemetnarytables.doc](#)