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RESEARCH

Allele Profiling of Bacterial Blight Resistance Genes *Xa4*, *Xa38*, and *Xa21* in Selected Sri Lankan Rice Germplasm

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ABSTRACT

In rice, resistance to bacterial blight (BB) is conveyed by several *Xa* genes, among which *Xa4*, *Xa21* and *Xa38* convey durable resistance. Most Sri Lankan rice germplasm is uncharacterized for the alleles carried at *Xa* genes. Knowledge on the allele profile of major *Xa* genes of rice accessions/varieties is essential for making informed decisions in rice breeding programs. In the current study, the allele profiles of 42 Sri Lankan rice accessions/varieties were developed targeting three *Xa* genes (*Xa4*, *Xa21*, and *Xa38*) using intragenic/linked markers amplifying known resistance/susceptible alleles. According to allele profiles, the rice accessions/varieties were grouped into 11 allele-haplotypes. The varieties, Bg 250, Bg 251 and At 354 carried resistance alleles at *Xa4*, *Xa21*, and *Xa38* (H1 and H3). Most other accessions/varieties reported either one (*Xa4*- H8 and *Xa38*- H10) or two resistance alleles (*Xa4* and *Xa21*- H2; *Xa4* and *Xa38*- H9; *Xa21* and *Xa38*- H11). Three allele-haplotypes were reported with a novel allele at *Xa4* (H4, H5, and H6). *Ma wee* and *Kuru wee* reported susceptible alleles at all three *Xa* genes (H7). A significant association ($p \leq 0.05$) between the *Xa* allele-haplotypes and the BB disease response was not observed. To achieve durable BB disease resistance in rice, it is recommended to introgress resistance alleles of the major *Xa* genes when releasing rice varieties, for which the reported allele profile of *Xa4, Xa21* and *Xa38* genes in the selected panel of Sri Lankan rice varieties/accessions will be of great importance.

INTRODUCTION

Rice is a major cereal crop known to provide the main caloric requirement of approximately 2/3rd of the world's population. It is predicted that the world population will reach 9.5 billion by the year 2050 (FAO, 2009), hence, feeding the increasing number of heads will be a main challenge in the rice sector. The final yield and quality of rice depends on the genetic potential, plant vigor, environment, and pre- and post- harvest practices. To meet the demand, improving both yield and quality attributes, and production of a climate resilient rice crop tolerant to major abiotic stresses, and resistant to pests become a key target in rice breeding programs globally.

Biotic and abiotic factors are major causes of yield and quality losses in rice. Rice is susceptible to many diseases; among them bacterial blight (BB) is one of the devastating diseases which cause severe damages in rice fields all over the world. In an epidemic, BB has been reported to cause significant economic losses in both yield and quality, leading to yield losses up to 80% in some regions (Wonni et al., 2016; Dilla-ermita et al., 2017). The gram-negative bacteria *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is the causal agent of BB and under high humidity and mild temperatures the pathogen is known to develop and spread faster in rice fields. Controlling BB in rice is one of the major challenges faced by rice farmers all over the world. There is no specific regime of chemicals that can be effectively used to control BB (Song et al., 2016; Kumar et al., 2020). Though antibiotics have been reported as a control strategy, it is considered neither as a feasible, nor cost effective disease management option. As a long-term, stable disease management strategy plant breeders try to incorporate gene-bound resistance against BB into newly released rice varieties (Lang et al., 2010; Perumalsamy et al., 2010; Lua et al., 2014; Kim et al., 2015).

Nearly 45 *Xa* genes have been reported so far and their combined action is known to deliver resistance against BB (Fiyaz et al., 2022). The relative contribution of resistance from each *Xa* gene for mitigation of disease development of BB is different; some exert major effects, some minor effects, some specific effects for a few *Xoo* strains and some work against a broader range of *Xoo* strains (Akhtar et al., 2008; Fred et al., 2016). The *Xa4* (*Os11g0691280*; Chr11: 27668321..27675334), *Xa21* (*Os11g0569733*; Chr11: 21273533..21277443), and *Xa38* (*Os04g0621500*; Chr4: 31576517..31583927) are a few of the known major BB resistance genes which deliver high, durable and stable resistance (Huang et al., 1997; Sun et al., 2003; Sabar et al., 2016; Zhang et al., 2017; Xu et al., 2019). Molecular marker-assisted selection (MAS) can be used to introgress the resistance alleles of these *Xa* genes into elite rice varieties. In previous studies, intragenic markers *Xa4_F/R* and *ABUOP0001_F/R* were used to track BB resistance alleles of genes *Xa4* and *Xa21*, respectively (Hajira et al., 2016; Yap et al., 2016; Nanayakkara et al., 2020). For the gene *Xa38,* the linked marker *Os04g53050-1_F/R* was recommended (Bhasin et al., 2012).

Rice germplasm around the world is known to be diverse in its genetic potential with respect to BB response. Sri Lanka has a diverse collection of rice germplasm, accounting to approximately 1,500 rice accessions (Withanawasam., 2017). However, the allelic diversity of the *Xa* genes in Sri Lankan rice germplasm remains mostly unknown. Identification of existing alleles will enable the rice breeders to make informative decisions during parental selection for varietal development and other pre-breeding activities. The current study was conducted to profile the known resistance/susceptible alleles carried at *Xa4, Xa21,* and *Xa38* BB resistance gene loci as an allele-haplotype in a core rice panel of 42 rice accessions/varieties comprising of Sri Lankan traditional rice accessions (SLTA) and Sri Lankan newly improved rice varieties (SLNIV).

METHODOLOGY

Bacterial blight disease responses of the core rice panel

The BB responses of 42 Sri Lankan rice accessions/varieties (16 SLTAs and 26 SLNIVs) from the *Yala* season of 2017 was previously reported in Nanayakkara et al. (2020). In the current study, the estimated median score (EMS) reported for these 42 Sri

Lankan rice accessions/ varieties were used for further analysis under the Creative Commons Attribution License (Nanayakkara et al., 2020; Supplementary Table 1- Column G).

Identification of alleles carried at BB resistance genes *Xa4*, *Xa21* and *Xa38* in the core rice panel

The primer pairs *Xa4_F/R* (*Xa4*; Yap et al., 2016) and *Os04g53050-1_F/R* (*Xa38*; Bhasin et al., 2012) were used for PCR amplification of the previously reported resistant/susceptible alleles carried by the 42 Sri Lankan rice accessions/varieties. The PCR amplification was done using a CT1000 thermal cycler (Bio-Rad Laboratories, Inc., California, USA) with a 15 µL final PCR reaction volume that included 150 ng of template DNA, 1× GoTaq® Green master mix (Promega Corporation, Maddison, USA), 0.125 µM primer concentration and 1 mg/mL bovine serum albumin (Promega Corporation, Maddison, USA). In the no-template control, the template DNA was replaced with nuclease-free water (Promega Corporation, Maddison, USA). The template DNA was initially denatured at 94 °C for 5 min, followed by 35 cycles of PCR amplification: 94 °C denaturation for 30 s, annealing at a primer-specific annealing temperature (58 °C for *Xa4_F/R* and 56 °C for *Os04g53050-1_F/R*) for 30 s and a primer extension at 72 °C for 30 s. The PCR cycle was completed with an additional primer extension step of 72 °C for 5 min. The amplified PCR products were resolved on a 3% agarose gel (Sigma-Aldrich®, Merck KGaA, Darmstadt, Germany) pre-stained with 5% ethidium bromide and was visualized using a gel documentation system (UVCI-1100, Major Science, USA). The alleles were called resistant and susceptible by comparison to previously reported fragment sizes (Table 01). The newly reported alleles in the core panel were noted separately. For the *Xa21* gene the allele calls for marker *ABUOP0001* previously reported for the 42 rice accessions were extracted from Nanayakkara et al. (2020) (Supplementary Table - 1; Column E) under the Creative Commons Attribution License.

Data analysis

A cluster analysis was done using the allelic scores of *Xa4, Xa21* and *Xa38* based on the Euclidean distance coefficient and unweighted pair group method with arithmetic means (UPGMA; Sokal & Michener., 1958) using Past, (v4.13). The EMS values extracted from Nanayakkara et al. (2020) were recategorized based on Acharya and Sujiata, (2021). A Kruskal-Walis test was performed to compare the EMS values of the identified haplotypes in Minitab 17 (Minitab, Inc.; www.minitab.com).

Table 1. Details of the DNA markers used to amplify the alleles of *Xa4* and *Xa38* bacterial blight resistance genes in the core rice panel

Xa Gene	Marker name	Primer sequence (5` to 3`)	Expected band size of resistant allele (R allele) and susceptible alleles (S allele)	Type	Source
Xa4	*Xa4 F/R*	F:GCAGCACCATCTCC ATCGTTTC R:CTGCTATAAAAGGC ATTCGGGTCTC	R allele: 217 bp S allele: 198 bp	Intragenic	Yap et al., 2016
Xa38	*Os04g53 050-1*	F:TCTTCTATTGCTAA CATTGGTG R:TCGCATTCATTTTC AGAG	R allele:269 bp S allele: 317 bp	Linked (designed based on the gene *Os04g53050* which co-segregates with *Xa38* gene)	Bhasin H. et al., 2012

RESULTS AND DISCUSSION

Bacterial blight is a major rice disease prevailing in Sri Lanka. Therefore, introgression of genetic resistance against BB to elite varieties destined for wider cultivation is an effective and eco-friendly control strategy. For most Sri Lankan rice accessions the alleles carried at major BB resistance genes are not available, except for those reported for *Xa21* in Nanayakkara et al. (2020). Knowledge on the alleles carried at major BB resistance genes is important for rice breeders as well as for pre-breeders working on BB resistance. Here we report, the resistance/susceptible alleles carried at the major BB resistance genes *Xa4* and *Xa38* for a panel of 42 Sri Lankan rice accessions/varieties and the association of BB disease responses to allele-haplotype of three major BB resistance genes *Xa4, Xa38* and *Xa21* (extracted from Nanayakkara et al. (2020).

In the current study, the disease categorization reported in Acharya and Sujiata, (2021) was modified further to categorize the rice accessions/varieties according to the BB disease response expressed. Accordingly, the 42 Sri Lankan rice accessions/varieties were categorized as resistant (EMS of 1 and 2), moderately resistant (EMS of 3 and 4), moderately susceptible (EMS of 5 and 6), susceptible (EMS of 7 and 8) and highly susceptible (EMS of 9). Compared to Koch, (1989) adopted in Nanayakkara et al. (2020), the current categorization is reflective of a broader disease response spectrum and hence, is more appropriate for deducing the association of disease responses to the allele-haplotypes.

Accordingly, 11 rice accession/varieties (SLTAs - *Kuru wee, Mada el* and *Mahakuru wee*; SLNIVs - Bg 250, Bg 251, Bg 352, Bg 360, At 307, At 354, Bw 367, and Ld 253) were categorized as resistant (EMS 1 and 2; Supplementary Table 1). Further, an additional 23 rice accessions/varieties (SLTAs - 8 and SLNIVs - 15) were categorized as moderately resistant (EMS 3 and 4; Supplementary Table 1). Under the susceptible disease response spectrum three rice accession/varieties (SLTAs - *Dik wee* and *Kattaran*; SLNIV - Bw 372) were categorized as moderately susceptible (EMS 5 and 6; Supplementary Table 1), five rice accession/varieties (SLTAs - *Suwadel* and *Yakada wee*; SLNIV - Bg 301, Bg 358, and Ld 365) were categorized as susceptible (EMS 7 and 8; Supplementary Table 1).

The BB disease responses are driven by the cumulative effect of the resistance/susceptible alleles of *Xa* genes in a particular rice accession/variety. In the current study, the alleles of two major *Xa* genes, namely *Xa4* and *Xa38* were identified for 42 Sri Lankan accessions/varieties (Supplementary Table 1) and the alleles of *Xa21* for the same rice panel were extracted from Nanayakkara et al. (2020). Based on the alleles reported for these three BB disease resistance genes *Xa4, Xa21* and *Xa38,* 11 allele-haplotypes (H1-H11) were deduced in the selected core panel of 42 Sri Lankan rice accessions/varieties (Figure 1).

With respect to the three *Xa* genes *Xa4*, *Xa21* and *Xa38*, only SLNIVs At 354, Bg 250, and Bg 251 (in a heterozygous state at the *Xa38* locus) carried resistance alleles at all three loci (Figure 1 - H1 and H3; Supplementary Table 1). None of the studied SLTAs reported resistant alleles at all three of these *Xa* genes. According to Supplementary Table 1, varieties Bg 250, Bg 251 and At 354 reported a resistant disease response. However, it is noteworthy to mention that according to the varietal descriptors, Bg 250 and Bg 251 are reported as moderately resistant and the variety At 354 was reported as susceptible (https://doa.gov.lk/rrdi_rice_varities/). Given the fact that BB is a quantitative trait which is highly dependent on the environment (Burt et al., 2014; Han et al., 2014) and due to the strain-specific resistance delivered by *Xa* genes, such variations could be expected when comparing responses from different seasons and experimental locations.

In the core panel, SLTAs *Kuru wee* and *Murungakayan* carried the susceptible alleles at all three genes *Xa4*, *Xa21* and *Xa38* (Figure 1 - H7, Supplementary Table 1). However, *Kuru wee* and *Murungakayan* reported a resistant and moderately resistant BB disease response, respectively (Supplementary Table 1). Being traditional Sri Lankan accessions,

these accessions could be carrying unique genetic make-up, with potentially novel QTL/genes that are responsible for resistant BB disease responses, hence, it is recommended to carry out QTL mapping studies to deduce the *Xa* genes responsible for the observed BB resistant/moderately resistant disease response, despite carrying a susceptible allele-haplotype at the major *Xa* genes *Xa4*, *Xa21* and *Xa38*

With respect to other *Xa* gene combinations, SLNIVs Bg 305, Bg 352, Bg 358, Bg 360, Bg 366, Bg 369, At 362, At 373, Bw 367, Bw 14-509 and Ld 365, and SLTA *Yakada wee* reported carrying resistance alleles at the two *Xa* genes, *Xa4* and *Xa38* (Figure 1 - H9, Supplementary Table 1). These varieties exerted a disease response of resistant (Bg 352, Bg 360 and Bw 367), moderately resistant (Bg 305, Bg 366, Bg 369, At 362, At 373 and Bw 14-509) and susceptible (Bg 358, Ld 365 and *Yakada wee*) (Supplementary Table 1). Furthermore, SLNIV Ld 371 was the only variety to carry the resistance allele combination of genes *Xa21* and *Xa38* (Figure 1 - H11, Supplementary Table 1) and reported a moderately resistant BB disease response (Supplementary Table 1). The SLTA *Mahakuru wee* reported a resistant allele combination for *Xa4* and *Xa21* (Figure 1 - H2, Supplementary Table 1) and a resistant disease response (Supplementary Table 1).

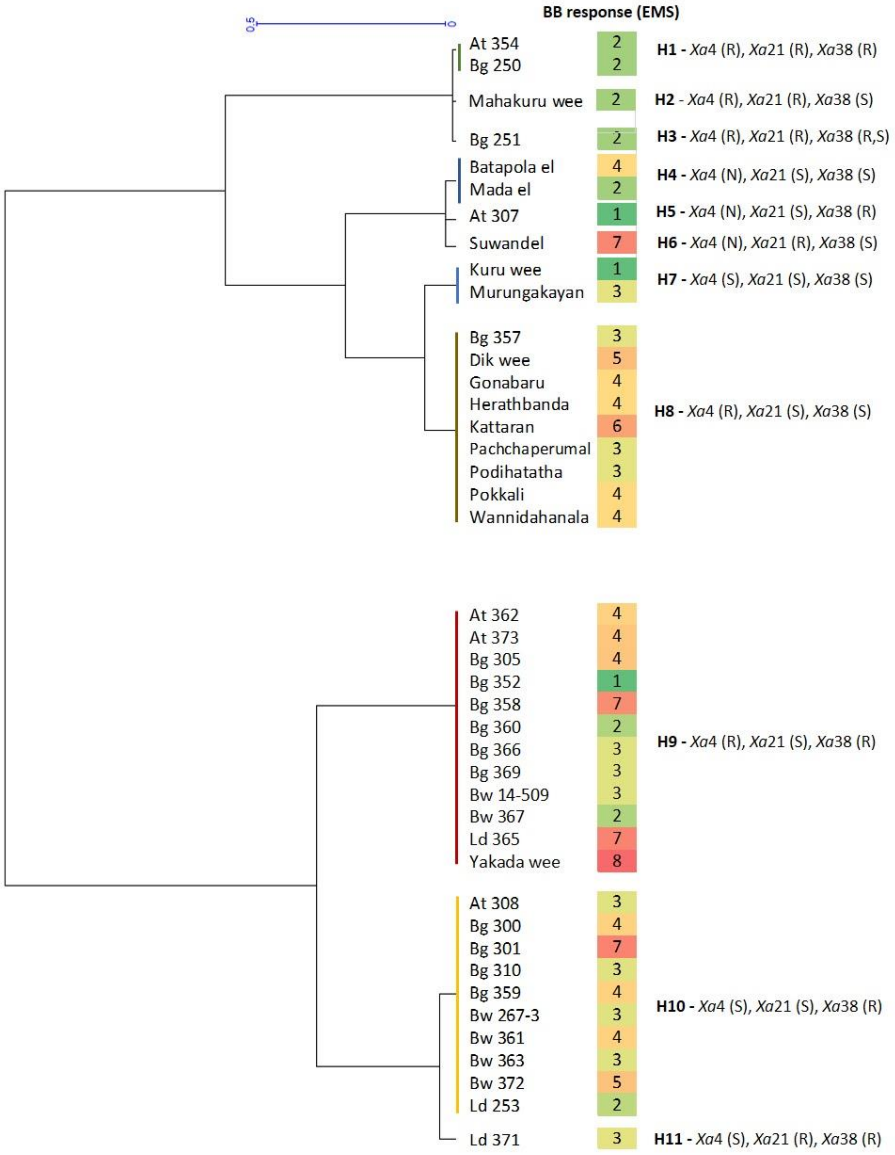

Figure 1: Allele-haplotypes identified for bacterial blight disease resistance genes *Xa4, Xa21* and *Xa38* in a core panel of 42 Sri Lankan rice accessions/varieties

The SLTAs *Pokkali*, *Dik wee*, *Herath Banda*, *Kattaran*, *Wannidahanala*, *Podihatatha, Pachchaperumal*, *Gonabaru*, and SLNIV Bg 357 reported only the known resistance allele at *Xa4* (Figure 1 - H8, Supplementary Table 1). Furthermore, *Mada el, Batapola el*, *Suwandel* and At 307 reported a new allele for *Xa4*. In previous research, only the resistance allele 217 bp and susceptible allele 198 bp was reported for *Xa4* (Yap et al., 2016). The novel allele detected has a fragment size which is in between the previously reported alleles (approximately 225 bp) on a 3% agarose gel. Therefore, it is recommended to size it through a capillary gel electrophoresis approach and validate its association to the BB disease response. Nevertheless, the detection of such novel alleles indicates the existence of a unique genetic diversity among the Sri Lankan rice germplasm.

The SLNIVs Bg 300, Bg 301, Bg 310, Bg 359, At 308, Bw 267-3, Bw 361, Bw 363, Bw 372, and Ld 253 accessions reported only the known resistance allele at *Xa38* (Figure 1 - H10, Supplementary Table 1). In addition, At 307 reported the resistance allele of *Xa38* with the novel allele at *Xa4* with an unknown association to BB disease response (Figure 1 - H5, Supplementary Table 1). Similarly, the SLTA *Suwandel* reported only the known resistance allele at the *Xa21* (Figure 1 - H6; Supplementary Table 1), along with the novel allele at *Xa4.*

Based on Kruskal–Wallis test no single allele-haplotype (H1 - H11) was significantly ($p \leq 0.05$) associated with a BB disease response expressed as EMS. The inability to find a clear association between the allele haplotypes of these *Xa* genes and the BB disease response could well be because of the additive effect of other known *Xa* genes in play and novel *Xa* genes responsible for resistance/susceptibility which are yet to be identified in this unique germplasm. Therefore, it is worthy to further expand the screening of alleles to cover other known major *Xa* genes and to conduct QTL mapping to identify novel genomic regions associated with BB resistance/susceptibility.

Given the severity of the impact of BB in rice production in countries such as Sri Lanka where rice is the main staple food of majority of the people, identification of the accessions that carry resistance alleles gives an important perspective when selecting rice accessions in breeding programs. In this regard, the findings of the current study are very much important for the Sri Lankan rice breeders to make informative decisions with respect to development of rice varieties deploying genetic resistance to mitigate BB in rice cultivation. Further, the study reveals opportunities for pre-breeders to explore the genomes of the mostly uncharacterized Sri Lankan rice germplasm to discover novel QTL related to BB disease resistance. It is a known fact that a single *Xa* gene cannot deliver the expected durable resistance against BB given the existence of several pathogen strains and dependance on the environmental conditions in the cultivation areas. Based on our observations, it is recommended to introgress resistant allele-haplotypes (considering the major *Xa* genes such as *Xa4*, *Xa21*, *Xa38* and other) through gene pyramiding using MAS when releasing modern varieties.

CONCLUSIONS

The current study reports 11 allele-haplotypes (H1 - H11) in a core panel of 42 Sri Lankan rice accessions/varieties considering three major *Xa* genes *Xa4, Xa21* and *Xa38* delivering resistance against BB in rice. Only three SLNIVs (Bg 250, Bg 251 and At 354) carried the resistance alleles for all the three *Xa* genes under consideration. While most accessions/varieties reported one or more combinations of resistance alleles for these major *Xa* genes, accessions/varieties *Murungakayan* and *Kattaran* reported susceptible alleles for all three *Xa* genes. None of the identified allele-haplotypes showed a significant association ($p \leq 0.05$) to any of the BB disease response categories (EMS 1 - 8), indicating additional genes at play.

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REFERENCES

Acharya, S., (2021). Metacognitive skills and academic achievement of higher secondary school students. Pedagogy of Learning, 7 (4), 30-36. Available at: http://pedagogyoflearning.com. DOI: 10.46704/pol.2021.v07i04.004

Akhtar, M.A., Abdulrafi, A. and Hameed, A. (2008). Comparison of methods of inoculation of *Xanthomonas oryzae pv.oryzae* in rice cultivars. *Pakistan Journal of Botany*, 40(5), 2171-2175.

Bhasin, H., Bhatia, D., Raghuvanshi, S., Lore, S.J., Sahi, K.G., Kaur, B., Vikal, Y., and Singh, K. (2012). New PCR-based sequence-tagged site marker for bacterial blight resistance gene Xa38 of rice. *Molecular Breeding*, 30, 607–611.

Burt, T.P., and Weerasinghe, K.D.N., (2014). Rainfall distributions in Sri Lanka in time and space: an analysis based on daily rainfall data. *Journal of Climate*, 2(4), 242-263. doi.org/10.3390/cli2040242.

Dilla-Ermita, C.J., Tandayu, E., Juanillas, V.M., Detras, J., Lozada, D.N., Dwiyanti, M.S., Cruz, C.V., Mbanjo, E.G.N., Ardales, E., Diaz, M.G., Mendioro, M., Thomson, M.J. and Tobias. (2017). Genome-wide association analysis tracks bacterial leaf blight resistance loci in rice diverse germplasm. *Rice*, 10:8. doi.10.1186/s12284-017-0147-4.

FAO, (2009). State of the world's forests 2009. Rome.www.fao.org/docrep/011/i0350e/i0350e00.htm.

Fiyaz, R.A., Shivani, D., Chaithanya, K., Mounika, K., Chiranjeevi, M., Laha, G.S., Viraktamath, B.C., Subba Rao, L.V., and Sundaram, R.M., (2022). Genetic Improvement of Rice for Bacterial Blight Resistance: Present Status and Future Prospects. *Rice Science*, 29(2), 118-132.

Fred, A.K., Kiswara, G., Yi, G., and Kim, K., (2016). Screening rice cultivars for resistance to bacterial leaf blight. *Journal of Microbial Biotechnology*, 26(5), 938–945. doi.org/10.4014/jmb.1510.10016.

Han, X., Yang, Y., Wang, X., Zhou, J., Zhang, W., Yu, C., Cheng, C., Cheng, Y., Yan, C., and Chen, J., (2014). Quantitative trait loci mapping for bacterial blight resistance in rice using bulked segregant analysis. *Journal of Molecular Sciences,* 15, 11847-11861. doi:10.3390/ijms150711847.

Hajira, S.K., Sundaram, R.M., Laha, G.S., Yugander, A., Balachandran, S.M., Viraktamath, B.C., Sujatha, K., Balachiranjeevi, C.H., Pranathi, K., Anila, M., Bhaskar, S., Abhilash, V., Mahadevaswamy, H.K., Kousik, M., Dilip Kumar, T., Harika, G., and Rekha, G., (2016). A Single-Tube, Functional Marker-Based Multiplex PCR Assay for Simultaneous Detection of Major Bacterial Blight Resistance Genes Xa21, xa13 and xa5 in Rice. *Rice Science*, 23(3), 144-151.

Huang, N., Angeles, E.R., Domingo, J., Magpantay, G., Singh, S., Zhang, G., Kumaravadivel, N., Bennett, J., and Khush, G.S., (1997). Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. *Theoretical Applied Genetics*, 95, 313-320. doi.org/10.1007/s001220050565.

. Kim, S.M., Suh, J.P., Qin, Y., Noh, T.H., Russell, F., Reinke, and Kshirod, K. J., (2015). Identification and fine-mapping of a new resistance gene, *Xa40*, conferring resistance to bacterial blight races in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, 10(2), 1933–1943. doi.org/10.1007/s00122-015-2557-2.

Kumar, A., Kumar, R., Sengupta, D., Das, S.N., Pandey, M.K., Bohra, A., Sharma, N.K., Sinha, P., Sk, H., Ghaz, I.A., Laha, G.S., and Sundaram, R.M., (2020). Deployment of

Genetic and Genomic Tools Toward Gaining a Better Understanding of Rice-*Xanthomonasoryzae* pv. *oryzae* Interactions for Development of Durable Bacterial Blight Resistant Rice. *Frontier Plant Science,* 11,1152.

Lang, J. M., Hamilton, J. P., Diaz, M. G. Q., Sluys, M. A.V., Burgos, M. R. G., Cruz, C.M.V., Buell, C. R., Tisserat, N. A., and Leach, J. E., (2010). Genomics-based diagnostic marker development for *Xanthomonas oryzae pv. oryzae* and *X. oryzae pv. Oryzicola*. *Plant Disease*, 94,311-329. doi.org/10.1094/PDIS-94-3-0311.

Lau, W., Pana, L., Zhaob, H., Jiac, Y., Wangd, Y., Yua, X., and Wanga, X., (2014). Molecular detection of *Xanthomonas oryzae pv. oryzae*, *Xanthomonas oryzae pv. oryzicola*, and *Burkholderia glumae* in infected rice seeds and leaves. *The Crop Journal*, 2, 398-406. doi.org/10.1016/j.cj.2014.06.005.

Nanayakkara, D., Edirisingha, I., Dissanayake, L., Weerasinghe, D., Suriyagoda, L., Herath, V., Perera, C., and Jayatilake, D., (2020). A novel intragenic marker targeting the ecto-domain of bacterial blight resistance gene *Xa21* for marker-assisted selection in rice, *Journal of Crop Improvement*, 34:6, 824-841. DOI:10.1080/15427528.2020.1771643

Nanayakkara, N. H. L. D. L. D., Herath, V., and Jayatilake, D.V., (2018). Haplotype diversity analysis of bacterial leaf blight resistance gene *Xa21* in rice. *Tropical Agricultural Research*, 30, 56–68. doi: 10.4038/tar.v30i1.8278.

Perumalsamy, S., Bharani, M., Sudha, M., Nagarajan, P., Arul, I., Saraswathi, R., Balasubramanian, P., and Ramalingam, J., (2010). Functional marker-assisted selection for bacterial leaf blight resistance genes in rice (*Oryza sativa L.*). *Plant Breeding*, 129, 400—406. doi.org/10.1111/j.1439-0523.2009.01705.x.

Sabar, M., Bibi, T., Farooq, H.U., Haider, Z., Naseem, I., Mahmood, A., and Akhter, M., (2016). Molecular screening of rice (*Oryza sativa L.*) germplasm for *Xa4*, *xa5* and *Xa21* bacterial leaf blight (BLB) resistant genes using linked marker approach. *African Journal of Biotechnology*, 15(41), 2317-2324. doi: 10.5897/AJB2016.15612.

Sokal, R.R. and Michener, C.D., (1958). A Statistical Method for Evaluating Systematic Relationships. University of Kansas Scientific Bulletin, 38: 1409–1438

Hur, Y.J., Cho, J.H., and Park, H.S., (2016). Pyramiding of two rice bacterial blight resistance genes, *Xa3* and *Xa4*, and a closely linked cold-tolerance QTL on chromosome 11. *Theoretical Applied Genetics,* 129, 1861–1871.

Sun, X., Yang, Z., Wang, S., and Zhang, Q., (2003). Identification of a 47-kb DNA fragment containing *Xa4*, a locus for bacterial blight resistance in rice. *Theoretical Applied Genetics*,106, 683–687. Doi 10.1007/s00122-002-1117-8.

Withanawasam, D.M., (2107). Heritage of heirloom rice varieties of Sri Lanka. 14pp.

Wonni, I., Hutin, M., Ouédrago, L., Somda, I., Verdier, V., and Szurek, B., (2016). Evaluation of elite rice varieties unmasks new sources of bacterial blight and leaf streak resistance for Africa. *Journal of Rice Research*, 4, 1. doi.org/10.4172/2375-4338.1000162.

Xu, Z., Xu, X., Gong, Q., Li, Z., Li, Y., Wang, S., Yang, Y., Ma, W., Liu, L., Zhu, B., Zou, L., and Chen, G., (2019). Engineering broad-spectrum bacterial blight resistance by simultaneously disrupting variable TALE-binding elements of multiple susceptibility genes in rice. *Molecular Plant*, 12(11), 1434-1446. doi.org/10.1016/j.molp.2019.08.006.

Yap, R., Hsu, Y.C., Wu, Y.P., Lin, Y.R., and Kuo, C.W., (2016). Multiplex PCR genotyping for five bacterial blight resistance genes applied to marker-assisted selection in rice (*Oryza Sativa*). *Plant Breeding*, 135, 309–317. doi:10.1111/pbr.12368.

Zhang, F., Wu, Z.C., Wang, M. M., Zhang, F., Dingkuhn, M., and Xu, J. L., (2017). Genome-wide association analysis identifies

resistance loci for bacterial blight in a diverse collection of *indica* rice germplasm. *PLoS ONE*, 12(3), doi.org/10.1371/journal.pone.0174598.

Supplementary Table 1: Details of estimated median score (EMS), disease response and alleles carried at *Xa4, Xa21* and *Xa38* for 42 Sri Lankan rice accessions/ varieties

Category	Variety/Acession	Estimated Median Score (EMS)*									Disease response	Allele**		
		1	2	3	4	5	6	7	8	9		*Xa4*	*Xa21****	*Xa38*
Sri Lankan Newly Improved Varieties	At 307	1									Resisitant	N	S	R
	At 308			3							Moderately resisitant	S	S	R
	At 354		2								Resisitant	R	R	R
	At 362			3							Moderately resisitant	R	S	R
	At 373				4						Moderately resisitant	R	S	R
	Bg 250			3							Moderately resisitant	R	R	R
	Bg 251		2								Resisitant	R	R	R/S
	Bg 300			3							Moderately resisitant	S	S	R
	Bg 301							7			Susceptible	S	S	R
	Bg 305				4						Moderately resisitant	R	S	R
	Bg 310			3							Moderately resisitant	S	S	R
	Bg 352	1									Resisitant	R	S	R
	Bg 357			3							Moderately resisitant	R	S	S
	Bg 358							7			Susceptible	R	S	R
	Bg 359			3							Moderately resisitant	S	S	R
	Bg 360		2								Resisitant	R	S	R
	Bg 366			3							Moderately resisitant	R	S	R
	Bg 369			3							Moderately resisitant	R	S	R
	Bw 14-509			3							Moderately resisitant	R	S	R
	Bw 267-3			3							Moderately resisitant	S	S	R
	Bw 361			3							Moderately resisitant	S	S	R
	Bw 363			3							Moderately resisitant	S	S	R
	Bw 367			3							Moderately resisitant	R	S	R
	Bw372					5					Moderately susceptible	S	S	R
	Ld 253		2								Resisitant	S	S	R

	Ld 365				7	Susceptible	R	S	R
	Ld 371		3			Moderately resisitant	S	R	R
Sri Lankan Traditional Acessions	Batapola el			4		Moderately resisitant	N	S	S
	Dik wee				5	Moderately susceptible	R	S	S
	Gonabaru			4		Moderately resisitant	R	S	S
	Herath banda			4		Moderately resisitant	R	S	S
	Kattaran				6	Moderately susceptible	R	S	S
	Kuru wee	1				Resisitant	S	S	S
	Mada el		3			Moderately resisitant	N	S	S
	Mahakuru wee	2				Resisitant	R	R	S
	Murungakayan		3			Moderately resisitant	S	S	S
	Pachchaperumal		3			Moderately resisitant	R	S	S
	Podi hatatha		3			Moderately resisitant	R	S	S
	Pokkali			4		Moderately resisitant	R	S	S
	Suwadel				7	Susceptible	N	R	S
	Wanni dahanala		3			Moderately resisitant	R	S	S
	Yakada wee				8	Susceptible	R	S	R

* EMS extracted from Nanayakkara et al. (2020)

** R - Resistant allele; S- Susceptible allele; N- New allele

*** *Xa21* allele scores extracted from Nanayakkara et al. (2020)