Jurnal Agronomi Indonesia
(Indonesian Journal of Agronomy)

ISSN 2085-2916 | e-ISSN 2337-3652
December 2023, *51*(3):449-458
DOI: https://dx.doi.org/10.24831/jai.v51i3.51194

Research Article

Confirmations on gene introgression events and hybridity for BLB resistance and yield in rice

Muh Aswad Ashan [1], Reflinur [2], and Sintho Wahyuning Ardie [3,*]

[1] Plant Breeding and Biotechnology Study Program, Graduate School of IPB University (Bogor Agricultural University), Jl. Meranti, Kampus IPB Dramaga, Bogor 16680, INDONESIA
[2] Research Center for Genetic Engineering, Research Organization for Life Science and Environment, National Research and Innovation Agency, Jl. Raya Bogor Km 46, Cibinong, Bogor 16911, INDONESIA
[3] Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University (Bogor Agricultural University), Jl. Meranti, Kampus IPB Dramaga, Bogor 16680, INDONESIA
* Corresponding author (✉ sintho_wa@apps.ipb.ac.id)

ABSTRACT

A superior variety of rice with high yield and resistance to diseases is expected to meet the increasing demand for this one of the most important carbohydrate sources worldwide. Bacterial leaf blight (BLB) caused by Xanthomonas oryzae pv. oryzae (Xoo) is one of the major biotic stresses limiting rice production. Combining BLB resistance and high-yield traits is possible through gene pyramiding. Introgression of targeted traits in the parental genotypes and hybridity confirmation in the F_1 generation are important initial steps in gene pyramiding. This study aimed to confirm the presence of targeted genes in parent genotypes using specific molecular markers and to confirm the hybridity of F_1 plants generated from three crosses combinations of Inpari 32 (as the female parent) and three Code-qTSN4 lines (as the male parents) using SSR markers. This study successfully confirmed the introgression of BLB-resistance genes in Inpari 32 (Xa4, Xa7, and Xa21) and in Code-qTSN4 (Xa4, Xa7). The introgression of yield-related QTL, qTSN4, was confirmed in the three Code-qTSN4 lines (A10-1, B12-2, and A16-5) by RM17483 marker. Five SSR markers (RM5, RM55, RM105, RM223, and RM561) successfully confirmed the hybridity of F_1 derived from Inpari 32xA10-1 (five individuals), Inpari 32xB12-2 (one individual), Inpari 32xA16-5 (two individuals). These confirmed hybrids can be further evaluated in the F_2 generation.

Keywords: Gene pyramiding; molecular marker; SSR; *qTSN4*; *Xoo*.

Edited by:
Maryati Sari
IPB University

***Received*:**
2 November 2023
***Accepted*:**
21 December 2023
***Published online*:**
29 December 2023

Citation:
Ashan, M. A. Reflinur, & Ardie, S. W. (2023). Confirmations on gene introgression events and hybridity for BLB resistance and yield in rice. *Jurnal Agronomi Indonesia (Indonesian Journal of Agronomy)*, *51*(3), 449-458

INTRODUCTION

Rice is the primary staple food for half of the globe's population, especially in China, India, Indonesia, Japan, and other Southeast Asian countries. However, there are abiotic and biotic factors that impact rice cultivation and significantly limit its production (Dar et al., 2021; Singh et al., 2020).

Bacterial leaf blight (BLB) disease caused by the bacterium *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the biotic factors that causes a significant decrease in rice production. The disease was reported to have become an epidemic in several locations of rice cultivation in many countries including Indonesia (Naqvi, 2019). The *Xoo* pathogen that infects rice plants produces bacterial droplet blight, which is the initial cause of a decrease in grain yield (Chukwu et al., 2019). BLB disease was recorded to reduce rice production by around 20%-30% (Saha et al., 2015). Therefore, the development of a high-

yielding variety resistant to several BLB strains in rice breeding programs is expected to meet the constantly high demand for rice worldwide (Arunakumari et al., 2016).

At present, a total of 46 genes (*Xa1-Xa46*) involved in rice resistance to various *Xoo* strains have been reported (Fiyaz et al., 2022). Developing new promising rice lines possessing durable resistance against BLB disease by pyramiding several desirable BLB resistance genes is essential in long-term BLB management and anticipating the emergence of a broader population of *Xoo* (Ramalingam et al., 2020). In addition to BLB resistance, attempts to meet the continuously increasing rice demand should include yield improvement. A previous study reported the *qTSN4* (total spikelet number) locus as a major QTL associated with increasing the number of rice grains (Fujita et al., 2012). Pyramiding of major genes/QTL related to BLB resistance and yield is expected to create superior rice varieties resistant to BLB with high yield.

Introgression major gene/QTL associated with resistance to BLB and high yield can be applied in popular superior rice varieties. Ciherang is the most popular variety in Indonesia and occupied more than 40% of the rice fields in the country by 2011 (Anas et al., 2022) with potential productivity of 7.05 tons ha^{-1} (Satoto et al., 2016). Ciherang variety is therefore used as a genetic background for creating many superior varieties in rice breeding, one of which is Inpari 32.

Inpari 32 was generated from the crossing of the Ciherang rice variety and the rice line IRBB64. The IRBB64 rice line was known to have several BLB resistance genes, namely *Xa4, Xa5, Xa7,* and *Xa21* (Khoshkdaman et al., 2014). Further improvement of Inpari 32 can be done by crossing this variety with a high-yielding rice variety. Code is a high-yielding rice variety (7.5 tons ha^{-1}) carrying BLB resistance genes *Xa4* and *Xa7*, where the BLB resistance gene originated from IRBB64 and IRBB7 (Fatimah et al., 2014; Suryadi et al., 2016). Code variety was improved by inserting *qTSN4*, a locus that regulates the total spikelet number and early maturity, and resulted in a Code-qTSN4 line (Tasliah et al., 2015) which was further evaluated by molecular analysis and adaptation assessment (Tasliah et al., 2019). Crossing Inpari 32 and Code-qTSN4 is expected to generate superior rice variety with higher yield and better resistance to BLB.

Confirmation of the presence of target genes in the parental genotypes as well as hybridity confirmation of the progenies are crucial stages in a gene pyramiding strategy (Suarez-Gonzalez et al., 2018). Molecular marker is a swift and accurate method to verify the presence of target genes and to confirm hybridity (Miah et al., 2013; Chukwu et al., 2019). This study aimed to confirm the presence of major genes/QTLs linked to BLB resistance and yield in the Inpari 32 variety and the Code-qTSN4 lines and to confirm hybridity in the F_1 generation using molecular markers.

MATERIALS AND METHODS

Confirmation of the presence of xa/Xa genes and qTSN4 in parental genotypes

The *Xa4*, *Xa5*, *Xa7*, and *Xa21* BLB resistance genes were introgressed into the Ciherang variety by crossing it with IRRBB64, resulted in Inpari 32 (Khoskadman et al., 2014). The *qTSN4* locus was introgressed into Code variety by crossing it with the IR64-NIL-qTSN4 resulting in Code-qTSN4 line (Tasliah et al., 2015). The genetic materials used as parental genotypes in this study were Inpari 32 (as the female parent) and three backcross Code-qTSN4 lines (as the male parent) as shown in Table 1.

Table 1. The genetic material used as parental genotypes in rice crosses for pyramiding genes for BLB resistance and high-yield.

Line/Variety	Genetic background	Reference
Inpari 32	Ciherang x IRBB64 (as female parent)	IAARD (2020)
A10-1	BC_1F_5 Code x *qTSN4* (as male parent)	Tasliah et al. (2019)
A16-5	BC_1F_5 Code x *qTSN4* (as male parent)	Tasliah et al. (2019)
B12-2	BC_2F_4 Code x *qTSN4* (as male parent)	Tasliah et al. (2019)

Total genomic DNA was isolated from the leaves of seedlings at 14 days after planting (DAP) using the modified CTAB method of Aboul-Maaty and Oraby (2019). DNA concentration was estimated using NanoDropTM2000 (Thermo Scientific, USA), while DNA purity was estimated based on the absorbance ratio values A_{260}/A_{280} and A_{260}/A_{230}. The PCR reaction was carried out with a total volume of 10 µL with a composition of 2 µL of 20 ng µL^{-1} genomic DNA, 0.5 µL of forward and reverse primers with a concentration of 10 µM, 5 µL of 2x MyTaqTM HS Red Mix, and 2.5 µL of sterile distilled water. Primers used to confirm the presence of BLB resistance-related genes (*Xa4, xa5, Xa7, Xa21*) and *qTSN4* in the parental genotypes are shown in Table 2.

Table 2. List of primers to confirm the presence of *xa/Xa* genes and *qTSN4* in the parental rice genotypes.

Marker	Primer	Gene/ QTL	Primer sequence (5'-3')	Amplicon (bp)	Reference
STS	MP	*Xa4*	F:ATCGATCGA TCTTCACGAGG	120 (Susceptible)	Ma et al. (1999)
			R:TCGTATAAAAG GCATTCGGG	150 (Resistant)	
	M5	*Xa7*	F:CGATCTTACTGGCTCTGCAACTC TGT	294 (Resistant)	Porter et al. (2003)
			R:GCATGTCTGTGTCGATTCGTCCG TACGA	1,170 (Susceptible)	
	pTA248	*Xa21*	F:AGACGCGGAAGGGTGGTTCCCG GA	700 (Susceptible)	Chunwongse et al. (1993)
			R:AGACGCGGTAATCGAAAGATGA AA	960 (Resistant)	Huang et al. (1997)
SNP	RG556/ DraI	*xa5*	F:TAGCTGCTGCCG TGCTGCGC	950 (Susceptible)	Huang et al. (1997)
			R:AATATTTCAGTGT GCATCTC	450 (Resistant)	
SSR	RM17483	*qTSN4*	F:TAGCTTCGGTTCTTGATCGTTGG	157	Fujita et al. (2012)
			R:AAACAGATTGCTCACCACCTTGG		Tasliah et al. (2015)

The PCR program used initial denaturation at 95°C for 5 min, followed by 35 cycles consisting of denaturation at 94°C for 30 sec, annealing at 55°C for 1 min, and elongation at 72°C for 1 min. The PCR reaction ended with a final elongation process for 7 min at 72°C. Amplicons generated by the STS and SNP markers were visualized by electrophoresis in a 1.5% (w/v) agarose gel at a voltage of 100 V for 45 min in a 0.5x TBE buffer. The electrophoresis gel was then immersed in 0.5 µg mL^{-1} EtBr solution for 60 sec and washed with distilled water for 5 min before being visualized using a UV transilluminator (Gel Doc EZTM, Bio-Rad, USA). Meanwhile, amplicons produced by the SSR marker were analyzed by electrophoresis in a 7% polyacrylamide gel at a voltage of 80V for 110 min in 1x TBE buffer, following the electrophoresis method described by Mesapogu et al. (2013).

Hybridity confirmation of F_1 plants generated from three crossing combinations of Inpari 32xCode-qTSN4

Three crossing combinations were developed by crossing Inpari 32 (as the female parent) with three Code-qTSN4 lines (A10-1, B12-2, and A16-5). Flowering synchronization of the female and male parents was done by adjusting the planting time. The emasculation of female flowers was conducted one day before pollination to minimize the occurrence of self-pollination following the artificial hybridization method for self-pollinated plants (Sha, 2013). Six F_1 seeds from each cross combination were sown in a 14 cm x 14 cm pot containing planting medium with 200 g per pot manure, urea 300 kg ha^{-1} (42 g per pot), SP-36 150 kg ha^{-1} (2.1 g per pot) and KCl 75 kg ha^{-1} (1.05 g per pot).

Hybridity confirmation used simple sequence repeats (SSR) markers. Screening for SSR markers to confirm hybridization was carried out by amplifying the genomic DNA of the parental genotype using 21 markers (Table 3). Total genomic DNA was isolated from the leaves of seedlings at 14 DAP using a modified CTAB method of Aboul-Maaty and

Oraby (2019). The PCR reaction was carried out with a total volume of 10 µL with a composition of 2 µL of 20 ng µL^{-1} genomic DNA, 0.5 µL of forward and reverse primers with a concentration of 10 µM, 5 µL of 2x MyTaqTM HS Red Mix, and 2.5 µL of sterile distilled water. The steps of the PCR program followed its references in Table 3. Amplicons were analyzed by electrophoresis in a 7% polyacrylamide gel (80 V for 110 min), following the polyacrylamide gel electrophoresis of Mesapogu et al. (2013) method.

Table 3. SSR markers to screen polymorphic in the parental genotypes to confirm hybridity of the F_1 progenies.

Primer		Primer sequence (5' to 3')	Reference
RM5	F:	TGCAACTTCTAGCTGCTCGA	Masuduzzaman et al. (2016)
	R:	GCATCCGATCTTGATGGG	
RM19	F:	CAAAAACAGAGCAGATGAC	Ashraf et al. (2016)
	R:	CTCAAGATGGACGCCAAGA	
RM55	F:	CCGTCGCCGTAG TAGAGAAG	Masuduzzaman et al. (2016)
	R:	TCCCGGTTATTTTAAGGCG	
RM105	F:	GTCGTCGACCCATCGGAGCCAC	Masuduzzaman et al. (2016)
	R:	TGGTCGAGGTGGGGATCGGGTC	
RM161	F:	TGCAGATGAGAAGCGGCGCCTC	Masuduzzaman et al. (2016)
	R:	TGTGTCATCAGACGGCGCTCCG	
RM202	F:	CAGATTGGAGATGAAGTCCTCC	Adegbaju et al. (2015)
	R:	CCAGCAAGCATGTCAATGTA	
RM215	F:	CAAAATGGAGCAGCAAGAGC	Nachimuthu et al. (2015)
	R:	TGAGCACCTCCTTCTCTGTAG	
RM223	F:	GAGTGAGCTTGGGCTGAAAC	Lang et al. (2017)
	R:	GAAGGCAAGTCTTGGCACTG	
RM230	F:	GCCAGACCGTGGATGTTC	Grimm et al. (2013)
	R:	CACCGCAGTCACTTTTCAAG	
RM254	F:	AGCCCCGAATAAATCCACCT	Rejeth et al. (2020)
	R:	CTGGAGGAGCATTTGGTAGC	
RM287	F:	TTCCCTGTTAAGAGAGAAATC	Nachimuthu et al. (2015)
	R:	GTGTATTTGGTGAAAGCAAC	
RM413	F:	GGCGATTCTTGGATGAAGAG	Nachimuthu et al. (2015)
	R:	TCCCCACCAATCTTGTCTTC	
RM431	F:	TCCTGCGAACTGAAGAGTTG	Masuduzzaman et al. (2016)
	R:	AGAGCAAAACCCTGGTTCAC	
RM464	F:	AACGGGCACATTCTGTCTTC	Goswami et al. (2017)
	R:	TGGAAGACCTGATCGTTTCC	
RM474	F:	AAGATGTACGGGTGGCATTC	Nachimuthu et al. (2015)
	R:	TATGAGCTGGTGAGCAATGG	
RM528	F:	GGCATCCAATTTTACCCCTC	Ngangkham et al. (2019)
	R:	AAATGGAGCATGGAGGTCAC	
RM561	F:	GAGCTGTTTTGGACTACGGC	Zhang et al. (2022)
	R:	GAGTAGCTTTCTCCCACCCC	
RM3843	F:	CCAGATCATCCAGGCATAACATCACC	Ngangkham et al. (2019)
	R:	CGGCGCTGGTAAACTCCATTCC	
RM23835	F:	TTCCGCTGTTTCTCTTCTTGTGC	Goswami et al. (2017)
	R:	CTGGTTCTGCTGGTTCTGTAGTTGG	
M23865	F:	TCATCCCATTCTCTTCCTCACC	Goswami et al. (2017)
	R:	CATACGGCCATACAAATGAACC	
RM23869	F:	GGCATATTCGTGTTGTCCTCACC	Goswami et al. (2017)
	R:	GCCACGCGTACCTGAGATATGG	

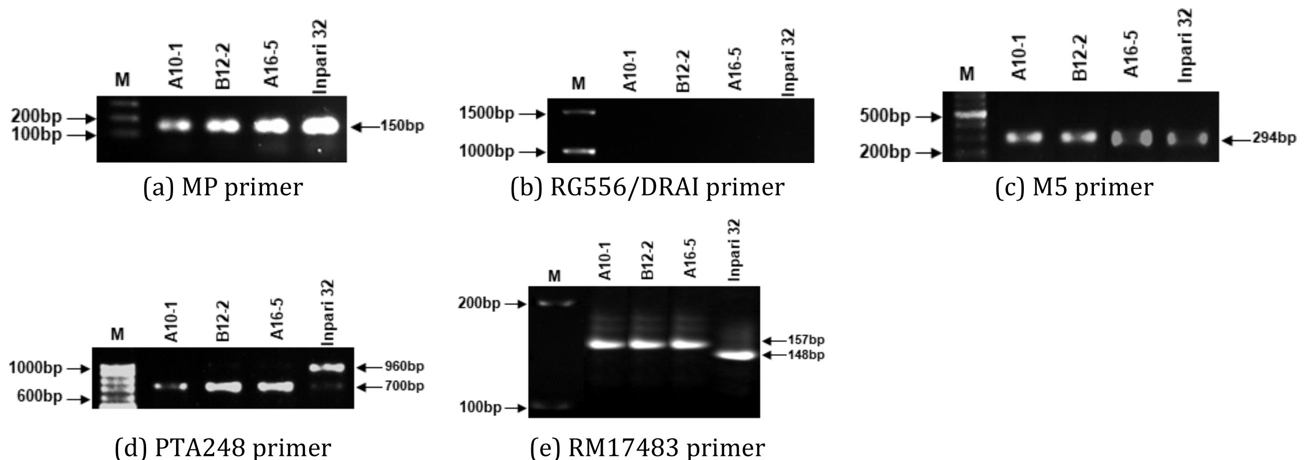
RESULTS AND DISCUSSION

Confirmation of the presence of xa/Xa genes and qTSN4 in parental genotypes

The Inpari 32 variety was expected to have the BLB resistance genes (*Xa4, xa5, Xa7*, and *Xa21*) from the IRBB64 line as the male parent (Khoshkdaman et al., 2014). However, the successful introgression of these resistance genes has not been confirmed in present

research. Confirmation of successful introgression of the BLB resistance gene in the Inpari 32 variety is essential before using this variety as a female parent to pyramid the BLB resistance genes and yield (*qTSN4*). Introgression of the *Xa4* gene in the parental genotype is confirmed using MP primers which produced approximately 120 bp amplicon in the susceptible genotype and approximately 150 bp amplicon in the resistant genotype (Ma et al., 1999; Wang et al., 2020; Djedatin et al., 2022). Our result showed that the MP primer produced approximately 150 bp amplicons in the parental genotypes (A10-1, B12-2, A16-5, and Inpari 32) as shown in Figure 1a. This result confirmed the presence of *Xa4* in all parental genotypes.

RG556/DRAI was used to confirm the presence of *xa5* since the RG556 primer was reported to produce 1,000 bp and 1,500 bp amplicons in the rice genome (Huang et al., 1997). The PCR product was further digested with the Dra I enzyme to produce a specific amplicon polymorphism (SAP), estimated at 390+410 bp in the resistant genotype and 320+450 bp in the susceptible genotype (Huang et al., 1997). However, this study failed to detect amplicons in the parental genotypes after many repeated experiments (Figure 1b). A previous study reported that the *xa5* gene was also not detected using six RFLP markers (one of which was RG556) in 61 F_2 individuals resulting from the IR24xIRBB5 cross (Yoshimura et al., 1995). This indicates that the presence of *xa5* in the parental genotypes in this study needs to be confirmed using different markers.



(a) MP primer (b) RG556/DRAI primer (c) M5 primer

(d) PTA248 primer (e) RM17483 primer

Figure 1. Electropherogram of parental genotypes to confirm the presence of genes of *Xa4* (a), *xa5* (b), *Xa7* (c), *Xa21* (d), and *qTSN4* (e). M: Marker DNA ladder 100 bp.

The M5 primer was used to confirm the presence of the *Xa7* gene. This marker was developed from the mapping of a cross-population between IR24 (susceptible) and IRBB7 (resistant) (Ogawa et al., 1991). Primer M5 has a distance of ≤ 27 kb from *Xa7*, and it amplified a 1,170 bp amplicon in IR24 (susceptible) and a 294 bp amplicon in IRBB7 (resistant) (Porter et al., 2003). The presence of the Xa7 gene in the parental genotypes (A10-1, B12-2, A16-5, and Inpari 32) was confirmed by the presence of an approximately 294 bp amplicon (Figure 1c).

The presence of the *Xa21* gene was confirmed using the PTA248 primer which was developed from the RAPD marker, RAPD248, and further designed as a restriction fragment length polymorphisms (RFLP) linked to *Xa21* (Ronald et al., 1992). The PCR product from this primer produces a 900 bp amplicon in the resistant genotype and a 700 bp amplicon in the susceptible genotype (Chunwongse et al., 1993). The PTA248 was used to identify the presence of the *Xa21* gene in the development of gene-pyramid lines with durable bacterial blight resistance (Kumar et al., 2016). In our study, the 900 bp of Inpari 32 confirmed the presence of the *Xa21* resistance gene, while the 700 bp amplicon of A10-1, B12-2, and A16-5 confirmed the presence of the *Xa21* susceptible gene (Figure 1d).

The presence of *qTSN4* was confirmed using primer RM17483 developed from mapping NIL YTH326[YP9]. The qTSN4[YP9]-related RM17483 is located between RM3424 and RM17492 with a distance of 398 kbp (Fujita et al., 2009). The RM17483

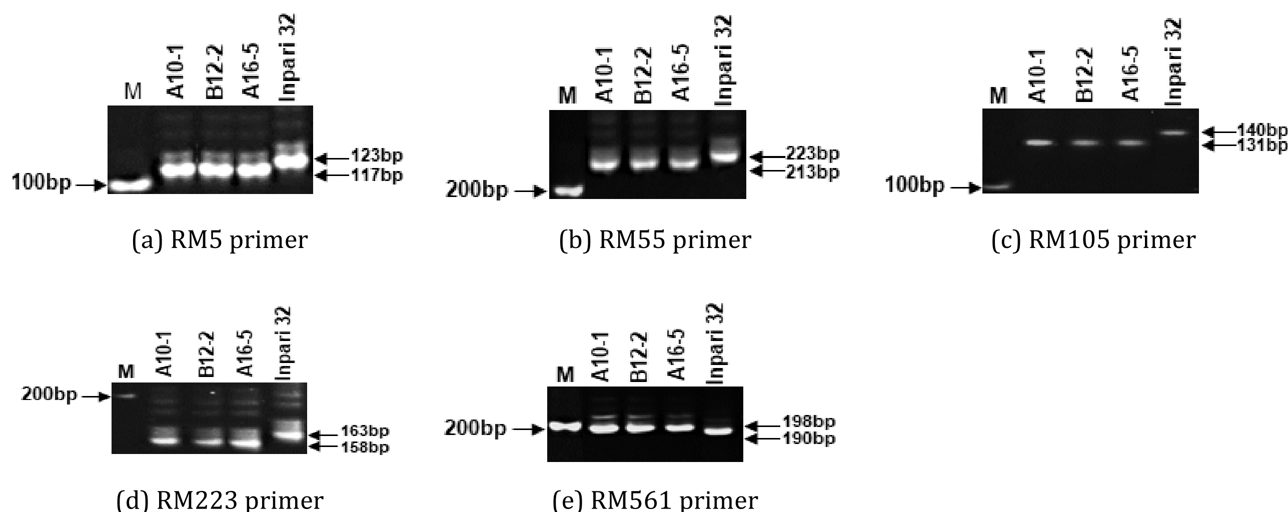
generated PCR product (Figure 1e) showed an amplicon of 157 bp, confirming the successful introgression of *qTSN4* in Code-qTSN4 lines A10-1, B12-2, and A16-5 (Fujita et al., 2009, 2012; Tasliah et al., 2015; Tasliah et al., 2019). An approximately 148 bp amplicon was observed in Inpari 32 (Figure 1e). The identity of this amplicon and its effect on yield needs to be investigated further.

Crossing between Inpari 32 and three Code-qTSN4 lines was carried out after the presence of the BLB resistance gene and *qTSN4* in the parental genotypes was confirmed. Hybridity confirmation in F_1 is a crucial stage in self-pollinated plants since the occurrence of self-pollination after artificial hybridization is often observed (Sha, 2013). Seeds resulting from self-pollination cannot be physically distinguished from seeds resulting from cross-pollination. The Grow-Out-Test (GOT), a conventional method to test hybrid purity, is considered less accurate, time-consuming, and laborious (Bora et al., 2016). Therefore, hybridity confirmation utilizing molecular markers can be done by comparing the banding patterns of F_1 plants and their parents (Deshmukh et al., 2013). Screening for simple sequence repeat (SSR) markers to confirm hybridization was carried out by amplifying the genomic DNA of the parental genotypes using 21 markers. The screening results showed that 15 markers were monomorphic, one marker showed no amplicon, and five markers were polymorphic (Table 4).

Table 4. Screened SSR polymorphic markers in the parental genotypes to confirm hybridity of the F_1 progenies.

Polymorphic		Monomorphic	
Primers	Amplicon (bp)	Primers	Amplicon (bp)
RM5	A10-1 (117)	RM19	226
	B12-2 (117)	RM161	187
	A16-5 (117)	RM202	189
	Inpari 32 (123)	RM215	148
RM55	A10-1 (213)	RM230	257
	B12-2 (213)	RM254	165
	A16-5 (213)	RM287	118
	Inpari 32 (223)	RM413	79
RM105	A10-1 (131)	RM431	251
	B12-2 (131)	RM464	(no band)
	A16-5 (131)	RM474	252
	Inpari 32 (140)	RM528	232
RM223	A10-1 (158)	RM3843	100
	B12-2 (158)	RM23835	194
	A16-5 (158)	RM23865	149
	Inpari 32 (163)	RM23869	174
RM561	A10-1 (198)		
	B12-2 (198)		
	A16-5 (198)		
	Inpari 32 (190)		

Therefore, only five polymorphic SSR markers were further used to confirm the hybridity of the F_1 seedlings, which can differentiate the female parent (Inpari 32) from the male parents (A10-1, B12-2, A16-5). The RM5 marker produced an approximately 117 bp amplicon in the male parental genotypes and 123 bp in Inpari 32 (Figure 2a). The RM55 marker produced an approximately 213 bp amplicon in the male parental genotypes and 223 bp in Inpari 32 (Figure 2b). The RM105 marker produced an approximately 131 bp amplicon in the male parental genotypes and 140 bp in Inpari 32 (Figure 2c). The RM223 marker produced an approximately 158 bp amplicon in the male parental genotypes and 163 bp in Inpari 32 (Figure 2d). The RM561 marker produced an approximately 198 bp amplicon in the male parental genotypes and 190 bp in Inpari 32 (Figure 2e).

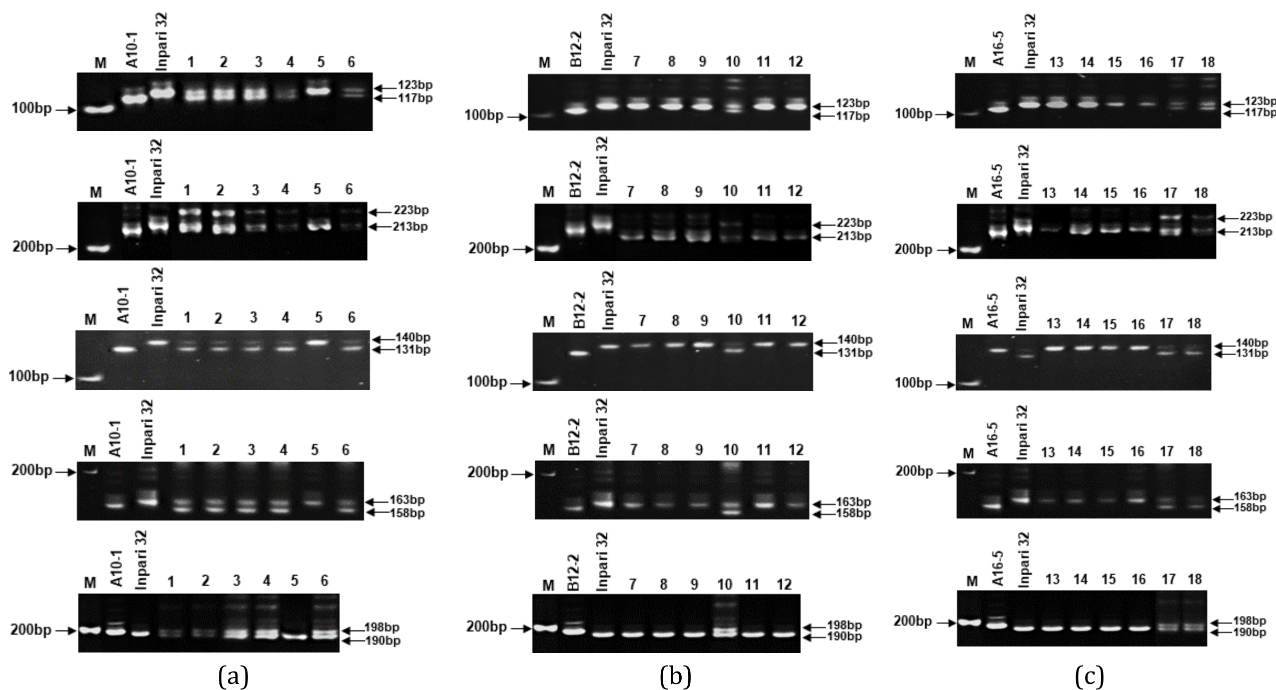


(a) RM5 primer (b) RM55 primer (c) RM105 primer

(d) RM223 primer (e) RM561 primer

Figure 2. Electropherogram of parental genotypes with SSR Markers RM5 (a), RM55 (b), RM105 (c), RM223 (d), and RM561 (e). M: Marker DNA ladder 100 bp.

Polymorphic SSR primers verified that F_1 plants number 1, 2, 3, 4, and 6 from the Inpari 32xA10-1 (Figure 3a), F_1 plant number 10 from the Inpari 32xB12-2 (Figure 3b), and F_1 plants number 17 and 18 from the Inpari 32xA16-5 (Figure 3c) as hybrids. These F_1 plants showed bands from both parents (Inpari 32 and the Code-qTSN4 lines). Amplification of other F_1 plants using the five SSR markers only produced the same amplicon as Inpari 32 as the female parent, indicating the occurrence of self-pollination. This study showed that the Inpari 32xA10-1 resulted in the most hybrids compared to the other two cross combinations. The verified F_1 hybrids are recommended to be evaluated further in the F_2 generation.



(a) (b) (c)

Figure 3. Electropherogram of F_1 Inpari 32xA10-1(a), F_1 Inpari 32xB12-2 (b), F_1 Inpari 32xA16-5 (c) using SSR markers RM5, RM55, RM105, RM223, and RM561, respectively. M: Marker DNA ladder 100bp.

CONCLUSIONS

The presence of *the Xa4, Xa7,* and *qTSN4* was confirmed in Code-qTSN4 lines (A10-1, B12-2, and A16-5), while the presence of *Xa4, Xa7,* and *Xa21* was confirmed in Inpari 32. The RG556/DRAI marker failed to verify the presence of *xa5* in this study. Five F_1 plants derived from the Inpari 32xA10-1 cross, one F_1 plant from the Inpari 32xB12-2 cross, and two F_1 plants from the Inpari 32xA16-5 cross were confirmed as hybrids by five SSR markers and can be continued to be evaluated further in the F_2 generation.

ACKNOWLEDGEMENTS

The authors thank to National Research and Innovation Agency Indonesia (BRIN), PT. Syngenta Seed Indonesia, PT. Wilmar Padi Indonesia, and PT. Wilmar Benih Indonesia for encouraging research. .

REFERENCES

[IAARD] Indonesian Agency for Agricultural Research and Development. (2020). Description of superior rice varieties. (In Indonesian.). Indonesian Agency for Agricultural Research and Development Ministry of Agriculture of Republic Indonesia.

Aboul-Maaty, N. A., & Oraby, H. A. (2019). Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. *Bulletin of the National Research Centre*, *43*(1), 1–10. doi: 10.1186/s42269-019-0066-1.

Adegbaju, M. S., Akinyele B. O., Akinwale M. G., Igwe D., & Osekita, O. (2015). Molecular characterization and genetic diversity analysis of rice varieties and landraces based on SSR markers. *International Journal of Research Studies in Biosciences, 3*(10), 54–65.

Anas, Damayanti, F., Kadapi, M., Carsono, N., & Sari, S. (2022). The shifting genetic diversity pattern of Indonesian rice improved varieties from 1943-2019 based on historical pedigree data. *Biodiversitas, 23*(9), 4649–4656. doi: 10.13057/biodiv/d230931.

Arunakumari, K., Durgarani, C. V., Satturu, V., Sarikonda, K. R., Chittoor, P. D. R., Vutukuri, B., Laha, G. S., Nelli, A. P. K., Gattu, S., Jamal, M., Prasadbabu, A., Hajira, S., & Sundaram, R. M. (2016). Marker-assisted pyramiding of genes conferring resistance against bacterial blight and blast diseases into Indian rice variety MTU1010. *Rice Science, 23*(6), 306–316. doi: 10.1016/j.rsci.2016.04.005.

Ashraf, H., Husaini, A. M., Bhat, M. A., Parray, G.A, Khan, S., & Ganai N. A. (2016). SSR based genetic diversity of pigmented and aromatic rice (*Oryza sativa* L.) genotypes of the western Himalayan region of India. *Physiology and Molecular Biology of Plants, 22*(4), 547–555. doi: 10.1007/s12298-016-0377-8.

Bora, A., Choudhury, P. R., Pande, V., & Mandal, A. B. (2016). Assessment of genetic purity in rice (*Oryza sativa* L.) hybrids using microsatellite markers. *3 Biotech, 6*(1), 1–7. doi: 10.1007/s13205-015-0337-y.

Chukwu, S. C., Rafii, M. Y., Ramlee, S. I., Ismail, S. I., Hasan, M. M., Oladosu, Y. A., Magaji, U. G., Akos, I., & Olalekan, K. K. (2019). Bacterial leaf blight resistance in rice: a review of conventional breeding to molecular approach. *Molecular Biology Reports, 46*(1), 1519–1532. doi: 10.1007/s11033-019-04584-2.

Chunwongse, J., Martin, G. B. & Tanksley, S. D. (1993). Pre-germination genotypic screening using PCR amplification of half-seeds. *Theoretical and Applied Genetics, 86*(6), 694–698. doi: 10.1007/BF00222658.

Dar, M. H., Bano, D. A., Waza, S. A., Zaidi, N. W., Majid, A., Shikari, A. B., Ahangar, M. A., Hossain, M., Kumar, A., & Singh, U.S. (2021). Abiotic stress tolerance-progress and pathways of sustainable rice production. *Sustainability, 13*(4), 1–19. doi: 10.3390/su13042078.

Deshmukh, U. C., Saxena R. R., Xalxo, M. S., Sharma, D., & Verulkar, S. B. (2013). Hybrid purity testing in rice (*Oryza sativa* L.) using microsatellite markers. *Electronic Journal of Plant Breeding, 4*(1), 1021–1026.

Djedatin, G., Nanoukon, C., Missihoun, A., Lomou, M., Sedah, P., & Agbangla, C. (2022). Molecular identification of *xa4* resistance gene to *Xanthomonas oryzae* pv. *oryzae* in cultivated rice in Northwest Benin. *Asian Asian Research Journal of Agriculture, 15*(4), 11–22. doi: 10.9734/arja/2022/v15i430162.

Fatimah, Priyatno, T. P., Fadlillah, S. H., Hermanto, Baroya, M., Mahrup, Wawan, Sasongko, D., Suryadi D., & Kadir, T. S. (2014). Isolation and disease assessment of *Xanthomonas oryzae* pv. *oryzae* from Java Island and pathogenic assay on near isogenic lines with different resistant genes. *Indonesian Journal of Biology, 10*, 237–245.

Fiyaz, R. A., Shivani, D., Chaithanya, K., Mounika, K., Chiranjeevi, M., Laha, G. S., Viraktamath, B. C., Rao, L. V. S., & Sundaram, R. M. (2022). Genetic improvement of rice for bacterial blight resistance: Present status and future prospects. *Rice Science, 29*(2), 118–132. doi: 10.1016/j.rsci.2021.08.002.

Fujita, D., Santos, R. E., Ebron, L. A., Telebanco-Yanoria, M. J., Kato, H., Kobayashi, S., Uga, Y., Araki, E., Takai, T., Tsunematsu, H., Khush, T. I., Brar, D. S., Fukuta, Y., & Kobayashi, N. (2009). Development of introgression lines of an Indica-type rice variety, IR64, for unique agronomic traits and detection of the responsible chromosomal regions. *Field Crops Research, 114*(2), 244–254. doi: 10.1016/j.fcr.2009.08.004.

Fujita, D., Tagle, A. G., Ebron, L. A., Fukuta, Y., & Kobayashi, N. (2012). Characterization of near-isogenic lines carrying QTL for high spikelet number with the genetic background of an indica rice variety IR64 (*Oryza sativa* L.). *Breeding Science, 62*(1), 18–26. doi: 10.1270/jsbbs.62.18.

Goswami, S., Kar, R. K., Paul, A. & Dey, N. (2017). Genetic potentiality of indigenous rice genotypes from Eastern India with reference to submergence tolerance and deepwater traits. *Current Plant Biology, 11*(12), 23–32. doi: 10.1016/j.cpb.2017.10.002.

Grimm, A., Fogliatto, S., Nick, P., Ferrero, A., & Vidotto, F. (2013). Microsatellite markers reveal multiple origins for Italian weedy rice. *Ecology and Evolution, 3*(14), 4786–4798. doi: 10.1002/ece3.848.

Huang, N., Angeles, E. R., Domingo, J., Magpantay, G., Singh, S., Zhang, G., Kumaravadivel, N., Bennett, J., & Khush, G. S. (1997). Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. *Theoretical and Applied Genetic, 95*(3), 313–320. doi: 10.1007/s001220050565.

Khoshkdaman, M., Ebadi, A. A., Majidi-Shilsar, F., & Dariush, S. (2014). Preliminary evaluation of resistance genes in rice against bacterial leaf blight in Guilan Province—Iran. *Agricultural Sciences, 5*(2), 94–98. doi: 10.4236/as.2014.52012.

Kumar, V. A., Balachiranjeevi, C. H., Naik, S. B. Rambabu, R., Rekha, G., Harika, G., Hajira, S. K., Pranathi, K., Anila, M., Kousik, M., Kumar, S. V., Yugander, A., Aruna, J. Kumar, T. D., Rao, K. V. S., Prasad, A. S. H., Madhav, M. S., Laha, G. S., Balachandran, S. M., Prasad, M. S., Viraktamath, B. C., Babu, V. R. & Sundaram, R. M. (2016). Development of gene-pyramid lines of the elite restorer line, RPHR-1005 possessing durable bacterial blight and blast resistance. *Frontiers in Plant Science, 7*, 1195. doi: 10.3389/fpls.2016.01195.

Lang, N. T., Phuoc, N. T., Ha, P. T. T. & Buu, B. C. (2017). Identifying QTLs associated and marker-assisted selection for salinity tolerance at the seedling, vegetative and reproductive stages in rice (*Oryza sativa* L.). *International Journal of Environment, Agriculture and Biotechnology, 2*(6), 2927–2935. doi: 10.22161/ijeab/2.6.20.

Ma, B., Wang, W., Zhao, B., Zhou, Y., & Zhu, L. Z. W. (1999). Studies of PCR marker for the bacterial blight resistance gene *Xa-4. Hereditas, 21*(3), 9–12.

Masuduzzaman, A., Haque, M., Ahmed, M. E., & Mohapatra, C. K. (2016). SSR marker-based genetic diversity analysis of tidal and flood prone areas in rice (*Oryza sativa* L.). *Journal of Biotechnology & Biomaterials, 6*(3), 241-250. doi: 10.4172/2155-952x.1000241.

Mesapogu, S., Jillepalli, C. M., & Arora, D. K. (2013). Agarose gel electrophoresis and polyacrylamide gel electrophoresis: methods and principles. *Analyzing Microbes* (pp. 73–91). Springer.

Miah, G., Rafii, M. Y., Ismail, M. R., Puteh, A. B., Rahim, H. A., Islam, K. N., & Latif, M. A. (2013). A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. *International Journal of Molecular Sciences, 14*(11), 22499–22528. doi: 10.3390/ijms141122499.

Nachimuthu, V. V., Raveendran, M., Duraialaguraja, S., Sivakami, R., Pandian, B. A., Ponniah, G., Gunasekaran, K., Swaminathan, M., Suji, K. K., & Sabariappan R. (2015). Analysis of population structure and genetic diversity in rice germplasm using SSR markers: An initiative towards association mapping of agronomic traits in *Oryza sativa*. *Rice, 8*(1), 1–24. doi: 10.1186/s12284-015-0062-5.

Naqvi, S.A.H. (2019). Bacterial leaf blight of rice: an overview of epidemiology and management with special reference to Indian sub-continent. *Pakistan Journal of Agricultural Research, 32*(1), 59–72. doi: 10.17582/journal.pjar/2019/32.1.59.72.

Ngangkham, U., Dash, S., Parida, M., Samantaray, S., Nongthombam, D., Yadav, M. K., Kumar, A., Chidambaranathan, P., Katara, J. L., Patra, B. C., & Bose, L. K. (2019). The potentiality of rice microsatellite markers in assessment of cross-species transferability and genetic diversity of rice and its wild relatives. *3 Biotech, 9*(6), 1–19. doi: 10.1007/s13205-019-1757-x.

Ogawa, T., Yamamoto, T., Kush, G. S., & Mew, T. (1991). Breeding of near-isogenic of rice with single genes for resistance to bacterial blight pathogen (*Xanthomonas campestris* pv. *oryzae*). *Japanese Journal of Breeding, 41*, 523–529.

Porter, B. W., Chittoor, J. M., Yano, M., Sasaki, T., & White, F. F. (2003). Development and mapping of markers linked to the rice bacterial blight resistance gene *Xa7*. *Crop Science, 43*(4), 1484–1492. doi: 10.2135/cropsci2003.1484.

Ramalingam, J., Raveendra, C., Savitha, P., Vidya, V., Chaithra, T. L., Velprabakaran, S., Saraswathi, R., Ramanathan, A., Pillai, M. P., Arumugavhamy, S., & Vanniarajan, C. (2020). Gene pyramiding for achieving enhanced

resistance to bacterial blight, blast, and sheath blight diseases in rice. *Frontiers in Plant Science, 11*, 1–12. doi: 10.3389/fpls.2020.591457.

Rejeth, R., Manikanta, C. L. N., Beena, R., Stephen, R., Manju, R. V., & Viji, M. M. (2020). Water stress mediated root trait dynamics and identification of microsatellite markers associated with root traits in rice (*Oryza sativa* L.). *Physiology and Molecular Biology of Plants, 26*(6), 1225–1236. doi: 10.1007/s12298-020-00809-y.

Ronald, P. C., Albano, B., Tabien, R., Abenes, L., Kung-Sheng, W., McCouch, S., & Tanksley, S. D. (1992). Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21*. *Molecular Genetics and Genomics, 236*(1), 113–120. doi: 10.1007/BF00279649.

Saha, S., Garg, R., Biswas, A., & Rai, A. B. (2015). Bacterial diseases of rice: an overview. *Journal of Pure and Applied Microbiology,* 9(1), 725–736.

Satoto, Rumanti, I. A., & Widyastuti, Y. (2016). Yield stability of new hybrid rice across locations. *Agrivita, 38*(1), 33–39. doi: 10.17503/agrivita.v38i1.675.

Sha, X. 2013. Rice artificial hybridization for genetic analysis. In: Yang, Y., editor, *Methods in molecular biology*. Humana Press. p. 1–12

Singh, P., Verma, R. L., Singh, R. S., Singh, R. P., Singh, H. B., Arsode, P., Kumar, M., Singh, P. K., Rakshit, A., Singh, H. B., Singh, A. K., Singh, U. S., & Fraceto, L. (2020). Biotic stress management in rice (*Oryza sativa* L.) through conventional and molecular approaches. In: Rakshit, A., Singh, H.B., Singh, A.K., Singh, U.S., & Fraceto, L., editors. *New frontiers in stress management for durable agriculture*. Springer Singapore, Singapore. p. 609–644.

Suarez-Gonzalez, A., Lexer, C. & Cronk, Q. C. B. (2018). Adaptive introgression: A plant perspective. *Biology Letters, 14*(3), 20170688. doi: 10.1098/rsbl.2017.0688.

Suryadi, Y., Samudra, I. M., Priyatno, T. P., Susilowati, D. N., Lestari, P., Fatimah, & Kadir, T. S. (2016). Determination of pathotypes from Indonesian *Xanthomonas oryzae* Pv. *oryzae* population causing bacterial leaf blight and their reactions on differential rice. *Makara Journal of Science, 20*(3), 109-118. doi: 10.7454/mss.v20i3.6241.

Tasliah, Ma'sumah, & Prasetiyono, J. (2019). Molecular analysis and adaptation test of Code- qTSN4 and Code-qTDH8 rice science. (In Indonesian). *Journal AgroBiogen, 15*(1), 11–22.

Tasliah, T., Ma'sumah, M., Trijatmiko, K. R., & Prasetiyono, J. (2015). Molecular analysis and agronomic performance of BC_1F_1 crosses Code x qTSN4 and Code x qDTH8. (In Indonesian). *Journal AgroBiogen, 11*(1), 17. doi: 10.21082/jbio.v11n1.2015.p17-24.

Wang, S., Liu, W., Lu, D., Lu, Z., Wang, X., Xue, J., & He, X. (2020). Distribution of bacterial blight resistance genes in the main cultivars and application of *Xa23* in rice breeding. *Frontiers in Plant Science, 11*, 1–10. doi: 10.3389/fpls.2020.555228.

Yoshimura, S., Yoshimura, A., Iwata, N., McCouch, S. R., Abenes, M. L., Baraoidan, M. R., Mew, T. W., & Nelson, R. J. (1995). Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers. *Molecular Breeding, 1*(4), 375–387. doi: 10.1007/BF01248415.

Zhang, Y., He, Q., Zhou, X. Zheng, S., Wang, Y., Li, P., & Wang Y. (2022). Genetic diversity and population structure of 93 rice cultivars (lines) (*Oryza sativa* Xian group) in Qinba in China by 3 types of genetic markers. *BMC Genomics, 23*(1), 1–16. doi: 10.1186/s12864-022-08707-1.

Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of the publisher(s) and/or the editor(s).

Copyright: © 2023 by the authors. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).