

PHENOTYPIC EVALUATION OF ADVANCED BREEDING LINES FOR RESISTANCE AGAINST BACTERIAL LEAF BLIGHT DISEASE IN RICE

ABSTRACT

In the present study, one of the major bacterial blight (BB) resistance gene *Xa21* was introgressed into an Indian rice variety MTU1010 NIL. Breeding line of Akshayadhan NIL possessing *Xa21* was used to transfer the target trait into the susceptible parent MTU1010 NIL, which is highly susceptible to BB which limits its spread to the disease endemic areas. Hence, an attempt was made to incorporate BB (*Xa21*) resistance gene from breeding line of Akshayadhan NIL which is highly resistant to the rice bacterial blight caused by the pathogen, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). The visual scoring of bacterial blight by using the SES scale (IRRI, SES 2014) in the advanced breeding lines and the resultant resistant progenies were selected. This work demonstrates the successful targeted introgression of a bacterial blight dominant gene *Xa21* into a most popular rice variety, MTU1010 NIL, a short duration, high yielding, long slender rice variety occupied maximum area in India particularly during dry-season. In this study, exemplifies the improvement of the targeted popular variety MTU1010 NIL for the target trait.

Key Words: Bacterial Blight, MTU1010 NIL, *Xa21*, *Xoo*, Akshayadhan

INTRODUCTION

Rice is the principal staple food crop of the world and rice production has so far kept pace with the growing population, predominantly due to cultivation of high-yielding, high-input demanding, and semi-dwarf varieties (Gnanamanickam, 2009). However, in recent years the introduction of semi-dwarf rice varieties and the large-scale use of inputs like fertilizers and insecticides have changed the aspects of pests and diseases of rice and increasing their incidence significantly. MTU1010, a short duration rice variety released in 2000 derived from the cross Krishnaveni/IR64, is extremely popular and has been planted for many years in a minimum of one million hectares. This variety possesses resistance against brown plant hopper with long slender grains and is highly susceptible to bacterial blight which limits its spread to areas where the disease is endemic. Most devastating disease in rice is bacterial blight caused by *Xanthomonas oryzae* pv. *Oryzae* throughout Asia and they are endemic to several rice growing states in Telangana of India (Production Oriented Survey, DRR, 2008). Bacterial blight (BB) can cause yield loss as high as 50% or more. Sometimes, the disease may occur at different period of growth stages causing severe

loss to rice crop (POS, 2008). In the absence of effective chemicals or any other methods of control agents against BB pathogen (Devadath *et al.*, 1989), development of durable, broad-spectrum resistant varieties has been encouraged (Jena and Mackill, 2008, Kumar *et al.*, 2014, Sundaram *et al.*, 2014). Breeding for host-plant resistance is considered as the most economical and eco-friendly strategy for management of pests and diseases of crop plants and achieving yield stability. Molecular markers can accelerate resistance breeding efforts, as segregating plants can be selected on the basis of molecular marker alleles instead of their phenotypes and introgression of multiple resistance genes or gene pyramiding can be tracked easily in a population (Sundaram *et al.*, 2014). Till date, 42 BB resistance (both dominant and recessive) genes have been identified (Bhasin *et al.*, 2012; Natraj Kumar *et al.*, 2012) and designated these in a series from *Xa1* to *Xa42* (Yang *et al.*, 1998; Sun *et al.*, 2004; Gu *et al.*, 2004; Cheema *et al.*, 2008; Kim *et al.*, 2015). Of these, *Xa21*, a major resistance gene, originally introgressed from *Oryza longistaminata* was observed to confer resistance to most Indian isolates of the bacterial pathogen. The gene has also been reported to confer durable resistance to the pathogen across many parts of the world including India (Sundaram *et al.*, 2014).

MATERIALS AND METHODS

Plant Material

Breeding lines of MTU1010, a susceptible variety to bacterial blight and Akshayadhan carrying BB resistant gene *Xa21* was used to transfer resistant gene into MTU1010 NIL. The fifty advanced breeding lines were screened for bacterial blight resistance as per SES scale, 2013.

Isolation and characterization of the bacterial blight pathogen

Virulent race of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) was isolated from the infected leaf samples on modified Wakimoto's medium which is collected from Hyderabad, Telangana were maintained on Hayward's agar media at 28°C for 96 hours. After incubation period, the bacterial cells were harvested and diluted with 10ml of sterile distilled water to get a final concentration of approximately 10^{8-9} cfu/ μ l (Preece., 1982). The pathogenicity of the bacterial pathogen was confirmed on susceptible rice variety MTU1010.

Screening of Advanced breeding lines

Fifty advanced breeding lines along with parents were screened in the field for bacterial blight disease resistance using virulent race of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). The bacterial pathogen was multiplied on modified Wakimoto's medium at $28 \pm 1^\circ\text{C}$. Advanced breeding lines were inoculated with bacterial suspension at maximum tillering stage (55 days after transplanting) by following leaf clipping method described by Kauffman *et al.* (1973). Inoculation was carried out by clipping the tip (about 1 to 2 cm) of

fully expanded uppermost leaf with scissors dipped into the inoculum. Ten leaves were inoculated per plant and disease reaction was scored after 15 days after inoculation. The Lines were categorized as resistant (lesion length \leq 4 cm), moderately resistant (lesion length 4.1-8 cm) or susceptible (lesion length $>$ 8 cm) (Shanti *et al.*, 2001).

RESULTS

Phenotyping for bacterial blight resistance gene in Advanced breeding lines

A total 50 advanced breeding lines along with parents were screened using bacterial cultures of a virulent local isolate of the bacterial blight pathogen, DX-020. The advanced breeding lines were inoculated with bacterial culture at maximum tillering stage by following leaf clip inoculation method of kaufman *et al.* (1973). Along with the breeding lines, resistant checks, Improved Samba Mahsuri and Akhayadhan (NIL) and susceptible parent MTU1010 NIL were also screened for bacterial blight resistance under field conditions. The lesion length on leaves was measured at 15 days after inoculation. Scoring was done as per SES scale, IRRI, 2013. Out of 50 advanced breeding lines evaluated against BB, 34 breeding lines *viz.*, VSR-1, VSR-2, VSR-3, VSR-4, VSR-5, VSR-6, VSR-13, VSR-14, VSR-15, VSR-17, VSR-18, VSR-19, VSR-20, VSR-21, VSR-22, VSR-23, VSR-24, VSR-25, VSR-26, VSR-27, VSR-28, VSR-29, VSR-30, VSR-31, VSR-33, VSR-34, VSR-35, VSR-36, VSR-42, VSR-44, VSR-45, VSR-47, VSR-48 and VSR-50 were found resistance with disease score of 1. Thirteen breeding lines *viz.*, VSR-7, VSR-8, VSR-9, VSR-10, VSR-11, VSR-12, VSR-16, VSR-33, VSR-37, VSR-38, VSR-40, VSR-41 and VSR-46 were found moderately resistant to BB pathogen with disease score 3, remaining four breeding lines *viz.*, VSR-32, VSR-39, VSR-43 and VSR-49 were found susceptible to BB pathogen with disease score 7. Susceptible check MTU1010 showed high susceptibility to the disease with a disease score of 9 and resistant checks Improved Samba Mahsuri and Akshayadhan showed highly resistant reaction to the disease respectively with a disease score of 1 in the Table 1.

DISCUSSIONS

Gene pyramiding with two or more genes is successful as per the studies Sundaram

et al. (2008) and Rekha *et al.* (2018), but a single major BB resistance gene like *Xa21* have shown the desired level of resistance against the known isolates of BB pathogen. Similar results of single gene conferring resistance was observed with Hari *et al.* (2013), Balachiranjeevi *et al.* (2015), Abhilash *et al.* (2016) and Laxmi Prasanna *et al.* (2018) and the known isolates of BB pathogen confers good level of resistance. In the present study, the improved breeding lines for bacterial blight with major dominant gene is expected to show high level of resistance against the disease bacterial leaf blight in Telangana and South Indian States, as the *Xa21* gene are known to be effective in these locations (Aruna kumari *et al.*, 2016 and Laxmi Prasanna *et al.*, 2018).

CONCLUSIONS

Results from mean disease score show similar trend for resistant, moderately resistant, moderately susceptible and susceptible genotype as obtained through SES scale visual scoring. In final words, through this study, out of 50 breeding lines, 33 breeding lines are resistant to bacterial leaf blight. These breeding lines can be utilized in various breeding programmes for bacterial blight resistance.

Table 1. Screening details Advanced breeding lines for BB resistance and scoring details as per IRRI-SES scale (IRRI 2013)

Parents and Checks	Reaction against BB	
	DX020	
	Score	R/MR/S
MTU1010 NIL	9	S
Akshayadhan NIL	3	R
ISM (Resistant Check)	1	R
Improved breeding lines	Score	R/MR/S
VSR 1	1	R
VSR 2	1	R
VSR 3	1	R
VSR 4	1	R
VSR 5	1	R
VSR 6	1	R
VSR 7	3	MR
VSR 8	3	MR
VSR 9	3	MR
VSR 10	3	MR
VSR 11	3	MR
VSR 12	3	MR
VSR 13	1	R
VSR 14	1	R
VSR 15	1	R
VSR 16	3	MR
VSR 17	1	R

VSR 18	1	R
VSR 19	1	R
VSR 20	1	R
VSR 21	1	R
VSR 22	1	R
VSR 23	1	R
VSR 24	1	R
VSR 25	1	R
VSR 26	1	R
VSR 27	1	R
VSR 28	1	R
VSR 29	1	R
VSR 30	1	R
VSR 31	1	R
VSR 32	7	S
VSR 33	1	R
VSR 34	1	R
VSR 35	1	R
VSR 36	1	R
VSR 37	3	MR
VSR 38	3	MR
VSR 39	7	S
VSR 40	3	MR
VSR 41	3	MR
VSR 42	1	R
VSR 43	7	S
VSR 44	1	R
VSR 45	1	R
VSR 46	3	MR
VSR 47	1	R
VSR 48	1	R
VSR 49	7	S
VSR 50	1	R

REFERENCES

Abhilash, K.V., Balachiranjeevi, C.H., Bhaskar, N.S., Rambabu, R., Rekha, G., Madhav, K.R., Vijay, S., Pranathi, K., Harika, G., Mahadevaswamy, H.K., Anila, M., Hajira, S.K., Yugander, A., Hariprasad, A.S., Madhav, M., Laha, G.S., Balachandran, S.M., Sundaram, R.M and Prasad, M.S. 2016. Marker-assisted introgression of bacterial blight and blast resistance genes into RPHR 1005, restorer line of the popular rice hybrid, DRRH-3. *Journal of Plant Biochem Biotechnol.* 25 (4): 400–409

- Balachiranjeevi, C.H., Bhaskar, N.S., Kumar, V.A., Harika, G., Swamymahadev, H.K., Hajira, Sk., Kale, R.R., Koushik, M.B.V.N., Bhadana, V.P., Hariprasad, A.S., Laha, G.S., Balachandran, S.M., Madhav, M.S., Fiyaz, A.R., Viraktamath, B.C., Swamy, B.P.M., Ali, J and Sundaram, R.M. 2018. Marker-assisted pyramiding of two major, broad-spectrum bacterial blight resistance genes, *Xa21* and *Xa33* into an elite maintainer line of rice, DRR17B. *PloS One*. 13 (10): e0201271.
- Bhasin, H., Bhatia, D., Raghuvanshi, S., Lore, J.S., Sahi, G.K and Kaur, B. 2012. New PCR-based sequence-tagged site marker for bacterial blight resistance gene *Xa38* of rice. *Molecular Breeding*. 30: 607-611.
- Cheema, K.K., Grewal, N.K., Vikal, Y., Sharma, R., Lore, J.S., Das, A and Bhatia, D. 2008. A novel bacterial blight resistance gene from *Oryza nivara* mapped to 38 kb region on chromosome 4 and transferred to *Oryza sativa* L. *Genetical Research*. 90:111.
- Devadath, S. 1989. Chemical control of bacterial blight of rice. In Bacterial blight of rice. International Rice Research Institute, Manila, Philippines. 89-98.
- Gnanamanickam, Rice and its importance to human life Prog Biol Con, 8 (2009), pp. 1-11.
- Gu, K., Tian, D., Yang, F., Wu, L., Sreekala, C and Wang, D. 2004. High-resolution genetic mapping of *Xa27(t)*, a new bacterial blight resistance gene in rice, *Oryza sativa* L. *Theor Appl Genet*. 108:800–807.
- Hari, Y., Srinivasrao, K., Ramesha, M.S., Virakthmath, B.C., Laha, G.S., Balachandran, S.M., Prasad, M.S., Reddy, C.S., Prasad, A.S.H., Natarajkumar, P., Sujatha, K and Sundaram, R.M. 2013. Improvement of maintainer line (IR58025B) for bacterial blight (BB) and blast resistance through marker assisted breeding. *Plant Breeding*. 132 (6).
- IRRI, 1996. Standard Evaluation System for Rice. 4th Edn., International Rice Research Institute, Manila, Philippines.
- Jena, K.K and Mackill, D.J. 2008. Molecular markers and their use in marker-assisted selection in rice Crop Sci. 48 (4): pp. 1266-1276.
- Kauffman, H.E., Reddy, A.P.K., Hsieh, S.P.Y and Merca, S.D. 1973. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. *Plant Dis. Rep.* 56: 537-540.
- Kim, S.M., Suh, J.P., Qin, Y., HNoh, T., Reinke, R.F and Jena, K.K. 2015. Identification and fine mapping of a new resistance gene, *Xa40*, conferring resistance to bacterial blight races in rice (*Oryza sativa* L.). *Theor Appl Genet*. doi: 10.1007/s00122-015-2557-2.
- Kumar, A., Dasgupta, P and Kumar, R. 2014. Emerging opportunities and challenges in rice production Pop Kheti, 2 (2), pp. 6-11.
- Laxmi Prasanna, B., Dangi, K.S., Sundaram, R.M., Damodar Raju, C.H., Jagadeeshwar, R., Rekha, G and Sinha, P. 2018. Screening of breeding lines of MTU1010 for their resistance against bacterial blight and blast. *International Journal of Current Microbiology and Applied Sciences*. 7(7): 4077-4084.

- Natraj kumar, P, Sujatha K, Laha GS, Srinivasarao K, Mishra B, Viraktamath BC, *et al.*, 2012. Identification and fine-mapping of *Xa33*, a novel gene for resistance to *Xanthomonas oryzae* pv. *Oryzae*. *Phyto Path.* 102, 222-228.
- Preece, T.F., Rhodes, M.E and Skinner, F.A. 1982. Progression of bacterial disease within plants. Bacterial and plants, eds Academic Press, London. PP: 71-83.
- Rekha, G., Kumar, A.V., Virakthamath, B.C., Pranathi, K., Koushik, M.B.V.N., Prasanna, L.B., Backiyalakshmi, C., Sinha, P., Ravindra, R.K., Bhaskar, S., Hajira, S.K., Balachiranjeevi, C.H., Swapnil, K., Rambabu, R., Harika, G., Punniakoti, E., Anila, M., Dilip kumar, T., Yugander, A., Chaitra, K., Praveen, M., Prasad, M.S., Laha, G.S., Neeraja, C.N., Giri, A., Subbarao, L.V., Babu, R.V and Sundaram, R.M. 2017. Improvement of blast resistance of the popular high yielding medium slender-grain type, bacterial blight resistant rice variety, improved Samba Mahsuri by marker assisted breeding. *Journal of Plant Biochemistry and Biotechnology.* 27: 463-472.
- Ronald, P.C., Albano, B., Tabien, R., Abenes, M.L.P., Wu, K.S and McCouch, S.R. 1992. Genetic and physical analysis of the rice bacterial blight disease resistance locus *Xa21*. *Mol. Gen. Genet.* 236: 113-120.
- Shanti, M.L., George, M.L.C., Cruz, V.C.M., Bernando, M., Nelson, R.J and Leung, H. 2001. Identification of resistance genes effective against rice bacterial leaf blight pathogen. *Plant Dis.* 85: 506-512.
- Sundaram, R.M., Subhadeep, C., Oliva, R., Laha, G.S., Jan, E. L., Ramesh, V and Sonti. 2014. Update on Bacterial Blight of Rice: Fourth International Conference on Bacterial Blight. *Rice.* 7: 12.
- Sundaram, R.M., Vishnupriya, M.R., Biradar, S.K., Laha, G.S., Reddy, A.G and Rani, N.S. 2008. Marker assisted introgression of bacterial blight resistance in SambaMahsuri, an elite indica rice variety. *Euphytica.* 160: 411-422.
- Sun, X., Cao, Y., Yang, Z., Xu, C., Li, X., Wang, S and Zhang Q. 2004. *Xa26*, a gene conferring resistance to *Xanthomonas oryzae* pv. *oryzae* in rice, encodes an LRR receptor kinase-like protein. *Plant J.* 37: 517-527.
- Yang, Q., Saito, K., Yang, P., Wang, Q., Sunohara, Y and Zheng, F. 2001. Molecular mapping of a new blast resistance gene *Pi25(t)* possessed in a Japonica rice cultivar, *Oriza sativa* L. cv. Yunxi. 2:49-55. In proc general meeting Rice blast in china Kunming.