

Pyramiding of biotic and abiotic stress genes in rice

Gitishree Das*, J.N.Reddy, KMDas, H.N. Thatoi, G.J.N. Rao

Central Rice Research Institute, Cuttack-753006, Odisha

ABSTRACT

Rice, the world's major cereal crop, suffers severe yield losses due to biotic stresses like bacterial blight and abiotic stresses like submergence. One of the most effective and reliable method of minimization of the yield losses against these stresses is enhancement of host resistance, an economical and environment friendly approach. With the advances made in the area of molecular markers, pyramiding of genes/QTLs that confer resistance/tolerance against these stresses is possible through marker assisted breeding. This study presents a successful pyramiding of three resistant genes of bacterial blight (*xa5*, *xa13*, *Xa21*) and *Sub1*, a major quantitative trait loci associated submergence tolerance into *Lalat*, a popular genotype using marker assisted selection approach. The gene pyramids exhibited desirable levels of resistance/tolerance against the target stresses thus demonstrating the utility of MAS approach in development of resistant/tolerant cultivars in minimum time with high precision.

Key words: rice, gene pyramid, marker, bacterial blight, submergence

Severe yield losses occur in rice due to diseases like bacterial blight and abiotic stresses like submergence. Bacterial blight caused by *Xanthomonas oryzae pv.oryzae* is of the most serious and widespread diseases on rice in several rice ecologies. As chemical controls proved to be ineffective against the disease, the only way to protect the crop from bacterial blight is the use of resistant varieties (Khush *et al.*, 1989) through incorporation of resistance. More than 31 genes (22 dominant and 9 recessive) for resistance to bacterial blight have been identified (Khush and Brar, 2001) and several of them were transferred into high yielding background using conventional breeding approaches to combat the disease. Tolerance to submergence is an important trait for rainfed lowland rice as it is considered as a weed control strategy for direct seeded conditions in standing water. Since many donors are not available till date, progress was minimal in incorporation of this trait into modern day cultivars.

The emergence of new biotypes/strains/races has resulted in rapid breakdown of the varieties, a consequence of considerable shift in a predominant pathogen races in response to the deployment of resistant varieties (Mew *et al.* 1992) having a single resistance gene. Pyramiding of several genes is a

strategy to prevent/delay the breakdown of resistance. The advances made in the area of molecular markers has made it feasible to pyramid of genes/QTLs that confer resistance/tolerance against several stresses at a time. The present study was an attempt to stack different genes that confer resistance /tolerance against bacterial blight and submergence into *Lalat*, a highly popular indica cultivar using marker assisted backcross breeding approach.

A popular high yielding cultivar *Lalat* was chosen as the recurrent parent. For BB resistance, CRMAS 2621-7-1, a genotype stacked with three BB resistance genes (*xa5*, *xa13* and *Xa21*) in the background of *Lalat* and for submergence tolerance, FR13A (Xu and Mackill, 1996), the well known donor for submergence were chosen. The back cross breeding was practiced and starting from BC₁, screening for the presence/absence of the target gene/QTL was done by PCR (polymerase chain reaction) analysis at each stage using gene specific/tagged markers and the positive plants were backcrossed to the recurrent parent (Fig. 1). The seeds produced were sown first in petri dishes and the seedlings were transferred into pots in the green house and grown till maturity in each generation. The positive BC₃ generation plants were

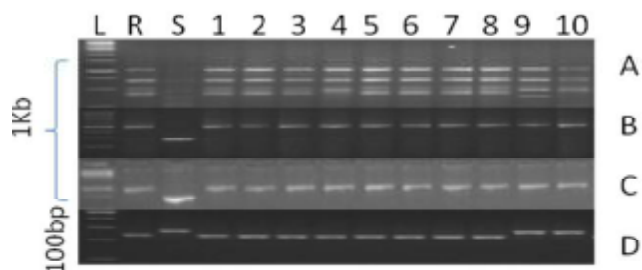


Fig. 1. PCR amplification of *xa5*, *xa13*, *Xa21* and *Sub1* resistance genes in the gene pyramid lines of LALAT.

A. *xa5* with marker RG556 (DraI restriction enzyme)

B. *xa13* with marker *xa13 P*.

C. *Xa21* with marker PTA248

D. *Sub1* with marker SUB1BC2.

L= ladder, R=Resistant, S=Susceptible 1-10 = Pyramid lines

selfed and gene pyramids were isolated in the progenies and selection was based on morphological and molecular characterization. DNA isolation was by CTAB method and the DNA markers employed for gene/QTL linked to BB and submergence and the PCR reactions and amplification products were based on published literature. Markers employed in this study were RG 556 for the linked genes *xa5* (Yoshimura *et al.*, 1995), *Xa13P* for *xa13* (Singh *et al.*, 2011), *pTA 248* for *Xa21* (Ronald *et al.*, 1992) and *SUB1BC2* for *sub 1* gene (Xu *et al.*, 2006).

The gene pyramids were screened under field conditions against a mixture of bacterial blight isolates and the lines was evaluated by visual scoring and measurement of lesion length (LL). Lines showing a LL of < 5cm were scored as resistant and those with > 5 cm were scored as susceptible as per SES scoring system. Twenty one day old seedlings of the same set of lines were subjected to complete submergence for two weeks and the survival rates and the recovery potential of the lines after stress were recorded.

Employing the marker assisted back cross breeding approach, seventy lines were generated in the BC3F3 generation with three BB resistance genes (*xa 5*, *xa13* and *Xa21*) and the *Sub1* QTL in all possible combinations. Most of these pyramid lines displayed higher levels of resistance comparable to the donor parent when screened against BB and ten promising lines were selected for further evaluation. Three lines

like LGP57, LGP56 and LGP58 showed high levels of resistance (Table 1). When these ten pyramids were evaluated against submergence, several of them displayed high levels of tolerance to submergence.

Table 1. Reaction of gene pyramids against BB and submergence

Lalat	Mean lesion Length	Survival percentage
LGP34	3.00±1.47	97.7
LGP41	3.00±1.63	97.5
LGP42	3.38±1.10	93.3
LGP44	4.63±1.88	85.7
LGP52	2.75±1.25	95.0
LGP56	1.28±0.60	100.0
LGP58	1.90±1.06	95.5
LGP59	2.03±0.73	98.7
LGP46	3.38±1.49	0
LGP57	0.60±0.29	0
CRMAS 2621-7-1(DP)	0.38±0.15	98.7
Lalat	12.45±1.33	0
FR13A (R)	NT	100.0
IR42 (S)	NT	0

All results are expressed in mean ± SD, Values ≤ 5.00 = resistant and >5.00 are susceptible. NT - Not tested

The display of enhanced level of resistance in BB pyramid lines is in conformity with the earlier studies on BB gene pyramids (Sanchez *et al.* 2000; Joseph *et al.* 2004; Prashant *et al.* 2006). This enhanced resistance can be attributed to quantitative complementation (Sanchez *et al.* 2000). The transfer of *Sub1* into the gene pyramids enhanced levels of tolerance to submergence. The high levels of resistance/tolerance against both the stresses shown by gene pyramid lines like LGP 52, LGP 56, LGP57 and LGP58 demonstrate the successful transfer and expression of the resistant/tolerant genes/QTL in to desirable genetic background (Table 1).

The availability of PCR based markers has hastened the incorporation of the target QTL without phenotyping at every stage of selection. This success has also validated the marker utility (Reddy *et al.* 1997; Sanchez *et al.* 2000) in the transfer of useful traits with high precision. The development of an improved Lalat with resistance or higher levels of tolerance to a major biotic stress (BB) and an abiotic stress (submergence)

will greatly help in realizing higher rice production through stabilization of yield through minimization of losses.

REFERENCES

- Joseph M, Gopalakrishnan S, Sharma RK, Singh VP, Singh AK, Singh NK, Mohapatra T 2004. Combining bacterial blight resistance and basmati quality characteristics by phenotypic and molecular marker assisted selection in rice. *Mol Breed* 13:377-387.
- Khush GS, Brar DS 2001. Rice genetics from Mendel to functional genomics. In: *Rice Genetics IV*, (Khush G.S; Brar D.S. and Hardy, B., eds.), Proc. IV Intern. Rice genetics Symp., 22-27, October 2000, Intern. Rice Res. Inst., Philippines, Science Publishers, India. pp3-25.
- Khush GS, Mackill DJ and Sidhu GS 1989. Breeding rice for resistance to bacterial blight. In: *Bacterial blight of rice*. Proc. Intern. Workshop on Bacterial Blight of Rice, 14-18, March 1988, Intern. Rice Res. Inst., Manila, Philippines. pp. 207-217.
- Mew TW, Vera Cruz CM, Medalla ES 1992. Changes in race frequency of *Xanthomonas oryzae* pv. *oryzae* in response to the planting of rice cultivars in the Philippines. pp. 207-217.
- Prashant S, Panchbhai AN, Priti D, Vaishali N, Panchbhai SD, Usha BZ, Azhakananadam K, Char BR 2006. Evolution of bacterial blight resistance in rice lines carrying multiple resistance genes and Xa21 transgenic lines. *Current Science* 90: 818- 824.
- Ronald PC, Albano B, Tabien R, Abenes L, Wu K, McCouch SR and Tanksley SD 1992. Genetic and physical analysis of the rice bacterial blight disease resistance locus, Xa21. *Mol. Gen. Genet.* 236: 113-120.
- Reddy JN, Baraoidan MR, Bernardo MA, George MLC and Sridhar R 1997. Application of marker-assisted selection in rice for bacterial blight resistance gene, Xa21. *Cur Sci.* 1997.73:10.
- Sanchez AC, Brar DS, Huang N, Li Z and Khush GS 2000. Sequence Tagged Site marker-assisted selection for three bacterial blight resistance genes in rice. *Crop. Sci.* 40: 792-797.
- Singh AK, Gopalakrishnan S, Singh VP, Prabhu KV, Mohapatra T, Singh NK, Sharma TR, Nagarajan M, Vinod KK, Singh D, Singh UD, Chander S, Atwal SS, Seth R, Singh VK, Ellur RK, Singh A, Anand D, Khanna A, Yadav S, Goel N, Singh A, Shikari AB, Singh A, Marathi B, 2011. Marker assisted selection: a paradigm shift in Basmati breeding. *Indian Journal of Genetics and Plant Breeding* 71(2) Special Issue : 1-9.
- Singh S, J S Sidhu, N Huang, Y. Vkal and Z. Li *et al.*, 2001. Pyramiding three bacterial blight resistance genes (Xa5, Xa13 and Xa21) using marker assisted selection into indica rice cultivar PR106. *Theor. Applied Genet.*, 102:1011-1015
- Xu K, Mackill DJ. A major locus for submergence tolerance mapped on rice chromosome 9. *Mol Breed.* 1996:2:219-24.
- Xu K, Xu X, Fukao T, Canlas P, Rodriguez RM, Heuer S, Ismail MA, Serres JB, Ronald PC and Mackill JD 2006. Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice. *Nature Publishing Group* 442:705-708.
- Yoshimura S, Yoshimura A, Iwata N, McCouch SR, Abenes A, Baraoidan MR, Mew TW and Nelson RJ 1995. Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers. *Mol. Breed.* 1: 375-387