23. Inheritance of bacterial blight resistance in two accessions of wild rice, *Oryza nivara*

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Bacterial blight (BB) of rice caused by *Xanthomonas oryzae* pv *oryzae* (*Xoo*) is one of the most economically serious diseases of lowland irrigated rice and can cause yield losses up to 50% (Gnanamanickam et al. 1999). It is the major disease in Punjab state of India and resistance breeding is the most economical and environmentally safe approach for achieving yield stability (Khush 1993). Extensive cultivation of rice in Punjab began only in 1970s but variability in the pathogen seems very high (Singh et al. in preparation). Currently, more than 20 genes (*Xa*1 to *Xa*27) conferring host resistance against various strains of *Xoo* have been identified (Lin et al. 1996, Zhang et al. 1998, Khush and Angeles 1999, Chen et al. 2002, Yang et al. 2003, Gu et al. 2003) and the majority of these are from cultivated *indica* rice with *Xa*4 being most widely used gene in the Indian sub-continent (Vikal et al. 2004). Two genes *Xa*21 and *Xa*23 have been identified from two related 'A' genome wild species, *O. longistaminata* and *O. rufipogon,* respectively (Khush et al. 1990, Zhang et al. 1998), while *Xa*27 is from a distantly related species, *O. minuta* (Gu et al. 2004). None of the known *Xa* genes (*Xa*1 to *Xa*21) so far tested is effective individually against all the pathotypes of *Xoo* prevalent in Punjab (our unpublished results). The narrow genetic base of cultivated rice will cause vulnerability to BB because of an increased frequency of newly evolved pathotypes of greater virulence. Pyramiding of known R genes or scouting of new genes with a wider resistance spectrum, are the alternatives for breeding varieties with durable resistance.

O. nivara (Sharma et Shastry), the progenitor of *O. sativa* subspecies *indica* (Yamanaka et al. 2003), is widely distributed in South and South East Asia (Vaughan and Morishima 2003). So far no BB resistance gene has been designated from this species. We evaluated 125 accessions of *O. nivara* against six *Xoo* pathotypes prevalent in Punjab state of India over a period of three years (Vikal et al. in preparation) and a few accessions showed resistance to all the pathotypes indicating that they may carry some novel gene for broad resistance or several genes that together confer resistance to all six pathotypes. Two accessions, IRGC 81825 (obtained from IRRI, Philippines) and CR 100428 (obtained from CRRI, Cuttak), both originating from India, were resistant to all the six pathotypes and were used for studying the inheritance of BB resistance and its transfer to cultivated rice *O. sativa* cv PR114. The F_1 of the cross

PR114/ *O. nivara* acc. 81825 showed a resistant reaction whereas F_1 of the cross PR114/ *O. nivara* acc. 100428 showed a susceptible reaction indicating that the resistance in acc. 81825 is dominant and that of acc. 100428 is recessive. Here we report the inheritance of resistance in these two accessions using F_2 populations.

O. sativa cv PR114 and *O. nivara* accessions IRGC 81825 and CR 100428 were used for studying inheritance of BB resistance in *O. nivara*. PR114 is resistant to five pathotypes but susceptible to one pathotype PbXO7 (Singh et al. in preparation) and was used as female parent. The F_2 populations (>900 plants in each cross) were planted in the field with row-to-row and plant-to-plant spacing of 30 x 20 cm. The crop was raised following standard cultural practices. Both the parents and the F_1s were planted along with the F_2 populations.

Pathotype PbXO7 is newly evolved and is the most virulent among six pathotypes. This pathotype was used for inoculating the parents and the F_2 populations. The culture of PbXO7 was revived from stock cultures on PSP medium (200g potato, 10g sucrose, 5g peptone and 20g agar in one litre) for 48 hours at 28^{o}C. The inoculum was prepared by suspending the bacteria in distilled water and adjusting the optical density to 1.0 (590nm) to give a concentration of approximately $1x10^9$ cells/ml. The plants were inoculated by clipping tips of 5-10 fully opened leaves at the maximum tillering stage (approximately 45 days after transplanting) as per Kauffman et al. (1973). Disease reaction (lesion length in cm) was scored 14 days after inoculation. Plants having lesion lengths of more than 5cm were considered as susceptible and the ones with lesion lengths less than 5cm were considered as resistant. Chi-square (χ^2) test was used for testing goodness of fit for number of gene(s) segregating in the population.

The pathotype PbXO7 shows virulent reactions on *Xa*1, *Xa*2, *Xa*3, *Xa*4, *xa*5, *Xa*7, *xa*8, *Xa*10, *Xa*11, *xa*13 and *Xa*14, whereas *Xa*21, shows moderate resistance to this pathotype. The two accessions showed resistance against all the six pathotypes for three consecutive years (Fig. 1). The F_1s of both the interspecific crosses showed normal pollen fertility and seed set, hence any distortion due to sterility was not expected in these crosses. This was confirmed by testing the population with a set of microsatellite markers (data not shown). Reaction of F_1s obtained from crosses PR114/*O. nivara* acc. 81825 and PR114/*O. nivara* acc. 100428 showed resistant and susceptible reactions, respectively, against pathotype PbXO7, suggesting that resistance in acc. 81825 was dominant and that of acc. 100428 was recessive. Segregation patterns of the F_2 populations are presented in Table 1. The F_2 population of the cross PR114/*O. nivara* acc. 81825 segregated in 3 resistant: 1 susceptible ($\chi^2 = 0.052$), thereby confirming that BB resistance in the accession 81825 is governed by a single dominant gene. Similarly, the F_2 population

Table 1. Disease reaction of parents, F_1s and F_2 populations of crosses PR114/*O. nivara* acc. 81825 and PR114/*O. nivara* acc. 100428 against *Xoo* pathotype PbXO7

Crosses	Lesion length (cm)			No. of F_2 plants			χ^2 (expected ratio)	P value
	PR114	*O. nivara*	F_1	Resistant	Susceptible	Total		
PR114/ *O. nivara* acc. 81825	21.3	0.5	2.0	689	233	922	0.052 (3:1)	0.7 – 0.9
PR114/ *O. nivara* acc. 100428	21.3	1.5	26.0	243	724	967	0.005 (1:3)	0.9 – 0.95

Fig. 1. BB reaction of *O. nivara* acc. IR81825 to five pathotypes of *Xoo* (leaves 1-5) and PR114 to pathotype PbXo7 (leaf 6).

of the cross PR114/*O. nivara* acc. 100428 segregated in 1 resistant: 3 susceptible ($\chi^2 = 0.005$), thereby confirming that BB resistance in the accession 100428 is governed by single recessive gene. Both genes

are expected to be novel because of their effectiveness against all the six pathotypes of *Xoo* prevalent in Punjab. We are now mapping these genes using SSR markers and also transferring these genes into cultivated rice background.

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