

## 22. Identification of a dominant bacterial blight resistance gene from *Oryza nivara* and its molecular mapping

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Rice (*Oryza sativa* L.) is the most important crop of India and is grown all over the country in diverse ecosystems. The productivity of rice is being affected by a number of biotic and abiotic stresses. Among the biotic stresses, bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is an important disease that results in significant yield reduction worldwide. The disease, in its severe form, is known to cause yield losses ranging from 74 to 81% in susceptible cultivars (Srinivasan and Gnanamanickam, 2005). Resistance breeding is considered as the most economical and environmentally safe approach for achieving yield stability. Over 30 BB resistance genes have been identified so far in rice (Khush and Angeles, 1999; Chu et al., 2006a&b) and a few of these like *Xa4* and *Xa7* have been incorporated widely into many high yielding varieties through conventional breeding (Khush et al., 1989). However, widespread cultivation of varieties with single BB resistance gene has led to predominance of *Xoo* races that can overcome the gene (Mew et al., 1992). The deployment of rice cultivars that have multiple BB resistance genes is expected to lead to more durable resistance. Through marker-assisted gene pyramiding, two or more resistance genes like *Xa21*, *xa13*, *xa5* and *Xa4* have been incorporated in the genetic background of elite varieties (Huang et al., 1997; Sanchez et al., 2000; Singh et al., 2001; Joseph et al., 2004; Sundaram et al., 2008). Even though gene pyramid combinations like *Xa21* + *xa13* + *xa5* or *Xa21* + *xa5* or *Xa21* + *xa13* have been observed to possess high level of resistance against multiple isolates of *Xoo*, the durability of resistance in such gene pyramid lines has not been validated so far. In order to enhance the durability of resistance, it is desirable to identify and characterize new genes from wild relatives of rice so that they could be deployed along with *Xa21* or *xa13* or *xa5* in elite rice varieties. Towards this objective, we have identified, characterized and genetically mapped a new BB resistance gene from an accession of *Oryza nivara*.

Fifty-five accessions belonging to *O. nivara* maintained at the Directorate of Rice Research, Hyderabad, were evaluated against five virulent strains of BB pathogen collected from different BB hot-spot locations of India as per the clip inoculation methodology of Kauffman et al. (1973). Six resistant accessions were reevaluated with three hyper virulent strains of the pathogen collected from Pantnagar, Aduthurai and Raipur, India. IRGC105710, an accession of *O. nivara* showed very high level of resistance against all the three pathogen strains. An interspecific cross was then made between *O. nivara* (IRGC105710) and TN1, a BB susceptible cultivar. The F<sub>1</sub> plants were observed resistant when screened with a hyper virulent isolate of BB pathogen collected from Raipur, India (DX133), indicating the possible involvement of dominant gene(s) in governing BB resistance. The F<sub>1</sub> plants were backcrossed to TN1 to generate 90 BC<sub>1</sub>F<sub>1</sub> plants. Forty-two BC<sub>1</sub>F<sub>1</sub> plants were observed BB resistant, while 48 were susceptible, indicating that a single dominant gene could be controlling BB resistance in IRGC105710 ( $\chi^2 = 0.40$ ,  $P = 0.53$ ). A single resistant BC<sub>1</sub>F<sub>1</sub> plant was then selfed to generate a BC<sub>1</sub>F<sub>2</sub> mapping population consisting of 435 plants. When the mapping population was screened with DX133 isolate, 317 BC<sub>1</sub>F<sub>2</sub> plants were observed resistant, while 118 were susceptible. Their genotypes for BB resistance were further examined based on the reactions using BC<sub>1</sub>F<sub>3</sub> plants. As a result, 101 BC<sub>1</sub>F<sub>2</sub> plants were observed homozygous resistant and 216 were heterozygous resistant, and all the 118 susceptible BC<sub>1</sub>F<sub>2</sub> plants produced susceptible progenies (1:2:1,  $\chi^2 = 1.35$ ,  $P = 0.51$ ), demonstrating that a single dominant gene is governing BB resistance in IRGC105710. Allelism tests were carried out with different IRBB lines which carry single dominant BB resistance gene in the genetic background of IR24 (received from IRRI, Philippines) viz., IRBB1, IRBB4, IRBB7, IRBB21 and IRBB26 revealed that the dominant resistance gene present in IRGC105710 is non-allelic to the known dominant BB resistance genes *Xa1*, *Xa4*, *Xa7*, *Xa21* and *Xa26* respectively.

In order to map the dominant BB resistance gene in the *O. nivara* accession, DNA was extracted from the parental lines and individual BC<sub>1</sub>F<sub>2</sub> plants using the procedure of Dellaporta et al. (1983). PCR analysis was performed using 210 SSR markers spread uniformly across the rice genome as per the conditions described by Panaud et al. (1996). Out of 210 SSR markers analyzed, 85 displayed polymorphism among the parental lines

and were used for bulked segregant analysis (Michelmore et al., 1991). In bulked segregant analysis, the SSR marker RM21330 located on chromosome 7 displayed co-segregation not only with the resistant and susceptible bulks but also with the individuals constituting the bulks with a few recombinants. RM21330 along with six other parental polymorphic SSR markers specific for chromosome 7 (RM20866, RM20948, RM21004, RM21177, RM21260 and RM21277) were then analyzed in all the 435 BC<sub>1</sub>F<sub>2</sub> individuals constituting the mapping population for their co-segregation with BB resistance. Fig. 1 displays the segregation pattern of RM21177 in a set of BC<sub>1</sub>F<sub>2</sub> plants. The markers RM21004 and RM21177 were observed to be closest to the BB resistance gene at a genetic distance of 2.0 cM and 4.5 cM, respectively and were flanking the gene on either side. RM20866 and RM20948 were observed to be located on one side of the gene while RM21260, RM21277 and RM21330 were located on the other side of the resistance gene. Fig. 2 displays the linkage map of chromosome 7 showing the genetic position of the linked SSR markers.

Figure 1: Segregation pattern of the SSR marker RM21177 in the BC<sub>1</sub>F<sub>2</sub> population derived from the cross TN1/ *O. nivara* //TN1

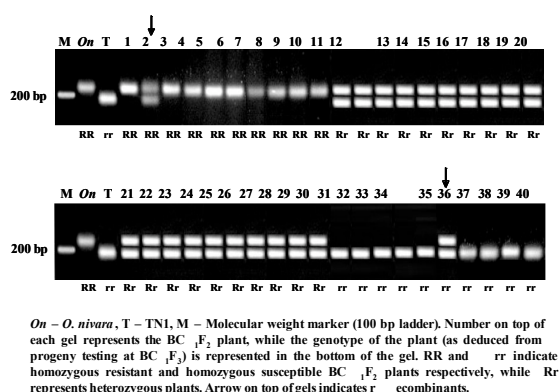
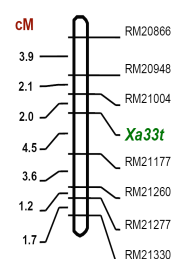


Figure 2: Genetic linkage map of the genomic region in the vicinity of *Xa33t* on chromosome 7



Considering the fact that the *O. nivara* accession IRGC105710 is resistant to many virulent and hypervirulent isolates of *Xoo* and since no other BB resistance gene, particularly from *O. nivara* has been mapped on chromosome 7, it can be assumed that the dominant BB resistance gene present in IRGC105710 could be new. Hence, the gene has been tentatively named as *Xa33t*. Presently, fine mapping analysis and marker-assisted introgression of the gene into the genetic background of an elite fine grained rice variety Samba Mahsuri is in progress.

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