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Short Communication

Molecular mapping of rice root-knot nematode (*Meloidogyne graminicola*) resistance gene in Asian rice (*Oryza sativa* L.) using STMS markers

Priyank Hanuman Mhatre[1]*, Pankaj, Anil Sirohi, Ashok Kumar Singh[2], Ranjith K. Ellur[2], Prolay Kumar Bhowmick[2] and Vivek Kumar Singh[2]

Division of Nematology; [2]Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110 012

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Abstract

Rice root-knot nematode, *Meloidogyne graminicola* is one of the major pests of rice cropping system and is responsible for considerable yield reductions. Out of various management options tried so far, the resistance against *M. graminicola* in rice could be the most valuable in alleviating this problem. In present study a rice cultivar Abhishek exhibited a strong resistance with least number of galls (2galls/plant) and a kind of necrotic browning of roots which is typical of R-gene mediated resistance response. In order to map the gene governing resistance to root-knot nematode, the cultivar Abhishek was crossed with the Bangla Patni, a highly susceptible genotype to generate F_2 mapping population. Using bulked segregant analysis, a marker *HvSSR10-21* was identified to be putatively linked with the resistant locus in cv. Abhishek with significant LOD score. The significant LOD score value indicates the linkage between identified marker and the resistant locus against *M. graminicola*. We designate this gene as *Mg1*(t).

Key words: *Meloidogyne graminicola*, resistance, molecular marker, mapping, Buked Segregant Analysis

Rice (*Oryza sativa* L.) is a staple food and the major source of calories to the world population (Fageria 2007; Zeigler and Barclay 2008). In India the projected annual rice requirement is about 112 MT in the year 2020 (Mahajan et al. 2012), which is about 10 MT more than the current rice production (FAOSTAT, 2014). This target is attained by enhancing the productivity potential of rice and reducing the yield losses due to several biotic and abiotic stresses. Among biotic stresses nematodes are major limiting factor for successful production of rice and among nematodes rice root-knot nematode, *Meloidogyne graminicola* (Golden and Birchfield 1965) is one of the major pests of the rice-wheat cropping system, and is responsible for considerable yield reductions up to 20-80% in rice. To avoid this huge loss, nematode management is of prime importance and among the several methods available host plant resistance is one of the best management option (Atkinson et al. 2003). Resistant plant suppresses nematode penetration, development and reproduction (Trudgill 1991; Cabasan et al. 2012). The resistance to *M. graminicola* has been already identified in two species of African rice species; *Oryza glaberrima* and *O. longistaminata* (Soriano et al. 1999) but the information about resistance and the mechanism involved against *M. graminicola* is limited in *O. sativa*, the Asian rice (Mhatre et al. 2015).

In the present study, 64 cultivars and landraces of Asian rice (*Oryza sativa*) were evaluated for resistance against rice root-knot nematode on the basis of gall index. The study showed a large variation in susceptibility and sensitivity to *M. graminicola* infection among the rice cvs./landraces examined. Out of the total 64 genotypes examined, seven showed resistant to tolerant reaction with less number of galls/plant and among these, Abhishek exhibited strong resistant response with least number of galls (2 galls/plant) and necrotic browning of roots. On the contrary, 100.75 healthy galls/plant were observed in the cv. Bangla Patni (Fig. 1). Based on these contrasting characters, genotypes Abhishek and Bangla Patni were selected as resistant and susceptible parent, respectively in the present study (Mhatre et al. 2015).

*Corresponding author's e-mail: priyank.iari@gmail.com
[1]Present address : Division of Plant Protection, ICAR-Central Potato Research Station, Ooty, The Nilgiris 643 004, Tamil Nadu
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Fig. 1. Root system at 20 days after inoculation (left) healthy roots of resistant parent Abhishek and (right) heavily galled roots of susceptible parent Bangla Patni

In order to map the gene governing resistance to root-knot nematode, the cultivar Abhishek was crossed with the highly susceptible genotype Bangla Patni to generate F_2 population for mapping. Out of 419 STMS markers used in parental polymorphic survey showed polymorphism between *M. graminicola*-resistant (350 bp) and susceptible (370 bp) parents. These parental polymorphic SSR markers were used for Bulked Segregant Analysis (BSA), a method for rapidly identifying markers linked to any specific gene or genomic region. Michelmore et al. (1991) discovered and used bulked segregant analysis for the first time to identify random amplified polymorphic DNA (RAPD) markers in lettuce linked to a resistance gene against downy mildew. BSA was used to map several nematode resistance genes *viz*., *Rk*, *Me3* and *4*, *Mi3*, *MI-HT* etc. (Djian-Caporalino et al. 2001; Yaghoobi et al. 2005; Wang et al. 2013). In the present study we used BSA to map the resistance gene in Asian rice cv. Abhishek against *M. graminicola.*

A total of 150 F_2 plants from the cross between Abhishek and Bangla Patni were evaluated against *M. graminicola* infection using gall index. On the basis of phenotyping, the resistant and susceptible bulks were generated by pooling the DNA of 10 most resistant and 10 most susceptible plants. Bulked Segregant Analysis was performed using 94 parental polymorphic SSR markers. Out of these, one marker *HvSSR10-21* from chromosome 10 was identified to be putatively linked to *M. graminicola* resistance gene based on its ability to differentiate the resistant parent, susceptible parent, resistant bulk and susceptible bulk. The entire F2 population was genotyped using *HvSSR10-21*. The Chi-square analysis of the resistant reaction (phenotyping) and the linked marker (genotyping) showed that the resistance gene as well as the marker *HvSSR 10-21* segregated in the expected Mendelian ratio (3:1) with high level of significance i.e. P=0.05 (Tables 1 and 2).

Linkage map was constructed using software MAPMAKER version 3.0 (Lander et al. 1987) with a minimal LOD score of 3.0 and a maximal genetic distance of 30.0 centiMorgan (cM). The result of linkage analysis suggested that the genetic distance between *HvSSR 10-21* and the rice root-knot nematode resistance gene was 18.1 cM with the LOD score of 8.5. The significant LOD score value indicates the linkage between identified marker and the R-gene against *M*. *graminicola*. Previously six quantitative trait loci (QTLs) have been identified for partial resistance

Table 1. F_2 segregation from the cross between Abhishek and Bangla Patni tested against *M. graminicola* at 20 days after inoculation of plants growth

Phenotyping	Observed frequency	Expected frequency	d.f.	Expected ratio	χ^2 value	P value
R	117	112.5	1	3:1	0.9867	0.3206^{ns}
S	32	37.5				

R= Resistant plants; S= Susceptible plants; ns=non-significant; d.f.= Degrees of freedom

Table 2. Segregation of co-dominant SSR marker *HvSSR 10-21*on F2 from cross Abhishek and Bangla Patni

Genotyping	Observed frequency	Expected frequency	d.f.	Expected ratio	χ^2 value	P value
A	31	33.75	2	1:2:1	0.6148	0.7354^{ns}
H	72	67.50				
B	72	33.75				

A=Homozygous for Abhishek allele; B=Homozygous for Bangla Patni allele; H=Heterozygous; ns=non significant; d.f.=Degrees of freedom

to rice root-knot nematode, *M. graminicola* (Shrestha et al. 2007) but till date, there is no report of dominant R-gene against *M. graminicola* and to our knowledge, this is the first attempt to map a resistance gene against *M. graminicola*, using Bulked Segregant Analysis and the resistant locus is tentatively designated as *Mg1*(t).

The cv. Abhishek is a short duration, high yielding variety with multiple disease and pest resistance attributes (Anon. 2011a; Anon. 2011b). Our study added new information about nematode resistance to cv. Abhishek. As in Asia the maximum farmers are small land holder so an improved cultivar like Abhishek with multiple disease resistance and good agronomic traits can be a more practical mean for sustained agricultural production. In future, we have to look forward for the fine mapping of identified locus using several markers available in the identified location and cloning of resistant locus for its further utilization in marker assisted selection (MAS) for varietal improvement of rice against rice root-knot nematode.

Author's Contribution

Conceptualization of research (P, AS, AKS); Designing of the experiments (P, AKS); Contribution of experimental material (AKS, PKB); Execution of field/ lab experiments and data collection (PHM, P, AS, AKS, RKE, PKB, VKS); Analysis of data and interpretation (PHM, P, RKE); Preparation of manuscript (PHM, P, RKE).

Declaration

The authors declare no conflict of interest.

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