Full Length Research Article
Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 24/02/2014;
Date Revised: 20/10/2016;
Date Published Online: 25/02/2017;

Authors' Affiliation:
Department of Biological sciences University of Sargodha sub campus Bhakkar - Pakistan

***Corresponding Author:**
Musarrat Ramzan
Email:
marain79@gmail.com

How to Cite:
Ramzan M, Ahmed R, Kauser N, Shah AA, Saba R, Hussain I, Fayyaz S, Khan S (2017). Confirmation of root-knot nematode resistant gene *Rmi1* using SSR markers. Adv. Life Sci. 4(2): 55-59.

Keywords:
Rmil gene, Soybean, Root-knot nematodes, SSR markers, Pakistan

Open Access

Confirmation of root-knot nematode resistant gene *Rmi1* using SSR markers

Musarrat Ramzan*, Rifat Z. Ahmed, Naheed Kauser, Anis Ali Shah, Rabia Saba, Iqtidar Hussain, Shahina Fayyaz, Saifullah Khan

Abstract

Background: The Root Knot Nematode (RKN) is a serious economic threat to various cultivated crops worldwide. It is a devastating pest of soybean and responsible to cause severe yield loss in Pakistan. The cultivation of resistant soybean varieties against this pest is the sustainable strategy to manage the heavy loss and increase yield. There is an utmost need to identify RKN resistant varieties of soybean against cultivated in Pakistan. The presented study is an attempt to identify and confirm the presence of resistant gene *Rmi1* in soybean.

Method: Molecular studies have been done using Simple Sequence Repeat (SSR) marker system to identify resistant soybean varieties against Root Knot Nematode (RKN) using fifteen (15) indigenous cultivars and four (4) US cultivars. DNA was isolated, purified, quantified and then used to employ various SSR markers. The amplified product is observed using gel documentation system after electrophoresis.

Results: Diagnostic SSR markers Satt-358 and Satt-492 have shown the presence of *Rmi1* gene in all resistance carrying genotypes. Satt-358 amplified the fragment of 200 bp and Satt-492 generated 232 bp bands in all resistant genotypes. This study confirmed the *Rmi* gene locus (G248A-1) in all internationally confirmed resistant including six (6) native varieties.

Conclusion: These investigations have identified six (6) resistant cultivars revealing the effective and informative sources that can be utilized in breeding programs for the selection of RKN resistance soybean genotypes in Pakistan.

How to Cite **Ramzan M, Ahmed R, Kauser N, Shah AA, Saba R, Hussain I, Fayyaz S, Khan S (2017). Confirmation of root-knot nematode resistant gene *Rmi1* using SSR markers. Adv. Life Sci. 4(2): 55-59.**

Introduction

Southern root-knot nematode *Meloidogyne incognita (Mi)* is a serious pest of soybean or *Glycine max* (L.) and responsible to cause severe yield loss of soybean crop worldwide, particularly in the southern USA and Pakistan [1,2]. The most successful method for controlling the yield losses is the use of *Mi* resistant soybean genotypes. DNA marker assisted screening of genotypes is known to accelerate the identification of root-knot nematode resistant soybean cultivars. A major quantitative loci (QTL) near the top of the linkage group O (LG-O) confers resistance to *Mi* and can be identified by DNA markers [1]. Several molecular markers have been used to assess genetic diversity and also to locate/confirm the resistant genes against nematodes including SSR markers, refer to tandem repeat sequences of variable length, and exhibit a high degree of allelic distinction [3,4]; result is abundant and highly reproducible [5]. In soybean, various scientists have identified the locus of resistance to *Mi nematode* and several recent studies have confirmed this resistance locus against *Mi* in soybean [6,7]. Tamulonis *et al.*, found that resistance was quantitatively inherited with seed character conferring the resistance locus/ primer G248A-1 linked with *Mi* resistance in soybean by applying several SSR markers [7].

The objective of this study was to determine the presence of *Mi* resistant gene present on linkage group O (LG-O) of Pakistani soybean cultivars by applying reported microsatellite markers.

Methods

Collection of plants materials

Total nineteen (19) varieties of soybean were used for present study which includes fifteen (15) Pakistani soybean genotypes and four US cultivars. These indigenous fifteen soybean cultivars were previously screened against *Meloidogyne incognita* nematode (*Mi*) in green house and characterized on the basis of resistance and susceptibility to *Mi* in pathogenicity test (unpublished data).

DNA isolation

Total genomic DNA was isolated according to the modified and optimized CTAB method reported by Doyle and Doyle [8].

Estimation of quality and purity of DNA

Quantification and purity of the DNA was estimated as described by Sambrook and Russell (2001). DNA concentration was measured in spectrophotometer using 1:100 dilutions. Readings were noted for each DNA sample at wavelength 260nm and 280nm. DNA concentration in the sample was calculated by the following formula,

DNA concentration (µg/ml) = A 260×50×dilution factor (100)

Simple Sequence Repeats (SSRs) analysis

On the basis of the genetic linkage map of soybean [9], three SSR markers (Satt_358, Satt_132 and Satt_492) located near *Mi* resistance QTL on LG-O [10] were selected for this study. Primer sequences for each SSR were obtained from SoyBase, a USDA-sponsored genome database (http://soybase.org/ssr.html). All primers were synthesized by Integrated DNA Technologies, Inc, USA.

DNA amplification using SSRs

DNA amplification reactions were carried out using 3 pairs of SSR primers previously developed for soybean (USDA). Polymerase chain reactions were performed in 20µl reaction mixture containing 8ul PCR reaction mix (Promega) ,the PCR reaction mix contains PCR buffer, 25mM MgCl, 200mM each dNTPs, 1.5 units of Taq polymerase, 2µl (05 picomole/µl)of each reverse and forward primers and 3 ul (50ng/µl) genomic DNA. The PCR amplification programme was as follow: initial denaturation 94^{0}C for 03 min, denaturation 35 cycles with 94^{0}C for 30 Sec, annealing 46^{0}C for 01 min, extension 72^{0}C for 01 min and final extension was set 72^{0}C for 05 min. PCR was performed using 96 well Eppendorff Master gradient cycler. The amplified product stored at 4°C for Metaphore agarose gel electrophoresis.

Metaphor agarose (Ultra high-resolution agarose)

Metaphor agarose gel was prepared by addition of 1 mg metaphor agarose and 1mg agarose in chilled 1x TBE (pH 8) buffer. This mixture was melted in a microwave until the agarose dissolved completely and the solution became clear. Ethidium bromide 5µl (0.05µg/ml) was mixed properly in the agarose solution. Once the molten gel solidified after being poured into a cast, it was kept at 4°C for 20 minutes before use to obtain a good resolution. The electrophoresis was performed in large submarine units (Thermo EC-320) at 60V for 2hr

(Thermo EC, EC-250-90). Then gels were removed from the electrophoresis tank and placed in gel documentation system (UV Tech™, UK), the gel images were captured. The size of PCR products were determined by DNA ladder (Fermentas). 100bp ladder (Life Technologies™) used as molecular size marker or gene ruler on each gel.

Data analysis

Amplified PCR products of microsatellite were scored qualitatively for presence and absence for each marker allele-genotype combination. Data were entered into a binary matrix as discrete variables, 1 for presence and 0 for absence of the character. Polymorphism information content (PIC) value of a marker was calculated according to formula [11]. Only major bands consistently amplified were scored and faint bands were not considered. All computations were carried out using the NTSYS-PC, version 2.2 packages [12].

Results

For this study 3 SSR markers (Satt_358, Satt_132 and Satt_492) were selected based on the integrated soybean genetic linkage map. These markers are reported to be located near the G248 A-1 diagnostic marker for *Mi*-resistance. Satt _358 exhibits 200 bp amplified band in *Mi* resistant homozygous genotype, 200/192 bp bands in heterozygous *Mi* resistant genotypes and 192 bp band in *Mi* susceptible genotypes. Satt_132 is reported to amplify characteristic 238 bp band in *Mi* resistant cultivars whereas in susceptible cultivars it could be 236, 246, 248, 250 or 252 bp band. Satt_492 is reported to amplify 232 bp bands in resistant as well as susceptible soybean cultivars.

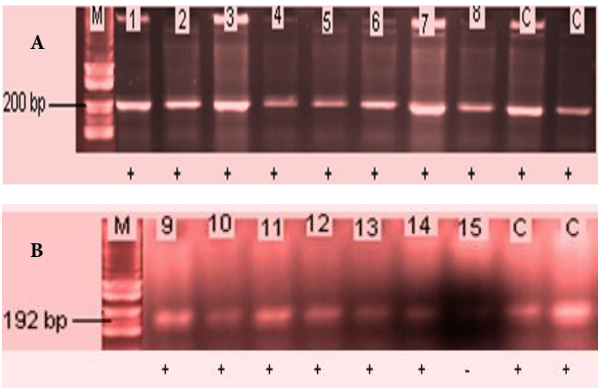
In PCR results of US *Mi* resistant soybean genotypes Forrest and Gregg and US *Mi* susceptible genotypes Lee and Bossier were used as control and confirmed a major resistant *Mi locus* gene near diagnostic markers (G248 A-1) with Satt_358 and Satt_132 markers which amplified expected band size (similar to the resistant genotype). This confirmed the presence of G248 A-1 on the linkage group of O in the positive tagged genotypes.

The entire studied soybean cultivars with Satt_358 marker amplified either 200 bp fragment or 192 bp band except one cultivar NARC-1 which did not show any amplified band. 10 cultivars showed 200 bp band and 7 cultivars exhibited 192 bp band. The microsatellite marker Satt_132 amplified 238 bp except in Rawall-1genotype.

Among susceptible cultivar 250 bp fragment was amplified in six genotypes and absent in PR-142 genotype while these entire bands were also present in reported resistant and susceptible cultivars. For Satt_492 marker, all studied genotypes showed 2 banding patterns except NARC-3, Ajmeri and NARC-2. 7 genotypes showed 232 bp fragments whereas 9 genotypes exhibited 250 bp band fragments (Table 1; Fig. 1).

Genotypes	Band size(bp)			
	Rmi1	**SSR Satt_358**	**SSR Sat_132**	**SSR Satt_492**
High level of *Mi* resistance				
Gregg	+	200	238	232
Forrest	+	200	238	232
AGS-109	+	200	238	250
AGS-08	+	200	238	250
AGS-09	+	200	238	250
95086	+	200	238	232
NARC-3	+	200	238	-
Rawall-I	+	200	-	250
Moderate level of *Mi* resistance				
NARC-4	+	200	238	232
AJMERI	+	192	238	-
NARC-5	+	200	-	250
***Mi* susceptible**				
Bossier	-	192	236	232
Lee	-	192	250	232
AGS-20	-	192	250	232
PR-142	-	192	-	250
FS-85	-	192	250	250
NARC-1	-	-	250	250
NARC-2	-	192	250	-
William-82	-	192	250	250

Table 1: *Mi* reaction and PCR amplified band sizes at three SSR markers associated with *Rmi1* QTL resistance gene in 28 soybean genotypes.



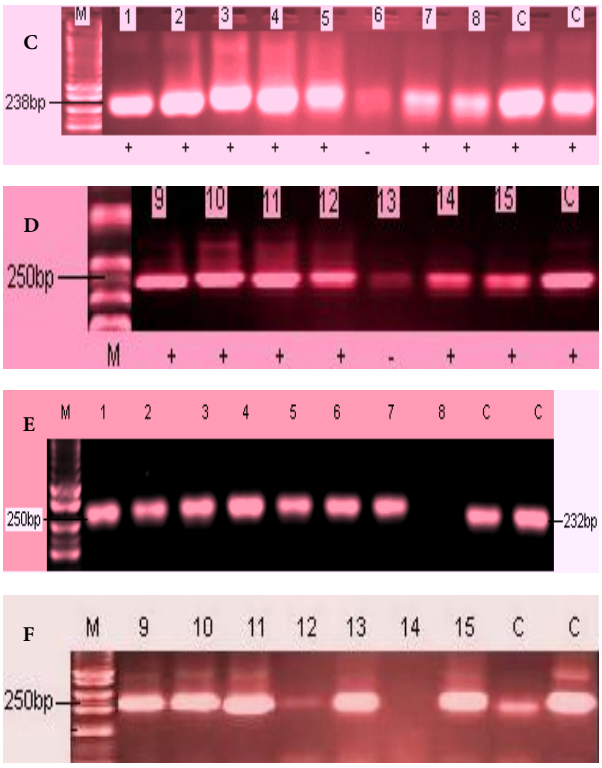


Figure 1 (A-F): Banding pattern showing the presence and absence of *Rmi1* gene in genotypes of soybean amplified 200 (A and B), 238 (C and D) and 250 & 232 (E and F) bp size of band. M= 50 bp molecular marker, + sign represent presence of band whereas – sign indicates absence of *Rmi1* gene. Line is showing amplified gene. C= indicate positive control USDA genotypes (Forrest and Gregg respectively) and susceptible genotypes of USDA.

Discussion

In the present study microsatellite marker technique was utilized to screen indigenous soybean germ plasm for *RMi* resistance. SSR markers Satt_358 and Satt_132 have been reported to identify resistant genotypes whereas Sat_492 has been found to be less effective. Bo *et al.* and Li *et al.* used six SSR markers flanking the G248 A-1 locus on LG-O to track the inheritance of this locus in different soybean lines [1,10]. Bo *et al.*, also proved in his study that *Mi* resistance in soybean cultivars was due to the presence of *Rmi1* gene on QTL (G248 A-1) on LG-O. They reported strong evidence of co-segregation of RKI resistance and a 200 bp band at Satt_358 marker.

Results of pathogenicity test (data not shown) were relevant to this molecular analysis. Co-descent analysis of markers and phenotype (pathogenicity) showed that *Mi*-resistant cultivars possess a 200 bp band at Satt_358 and a 238 bp at Sat_132 against southern root-knot nematode (*Meloidogyne incognita*). The relationship between *Mi* reaction and Satt_358 and Satt_132 genotypes was previously described, where *Mi* resistant cultivars inherited a 200 bp (Satt_358) and 238 bp (Satt_132) band and *Mi*-susceptible cultivars inherited a 192 bp band at Satt_358 (Table 1) [1]. In the present study all tested *Mi*-resistant cultivars exhibited the 200 bp band for Satt_358 which is in accordance with previous reports [2,7].

Sat_492 is another closest microsatellite marker to *Rmi1* gene on LG-O and located near the *RMi* locus (G248 A-1 locus) on the linkage group O [9]. Tamulonis *et al.*, reported a major G248 A-1 diagnostic locus which was indicated in the Satt_492 to Satt_358 interval was 3.1cM [2]. Results from *Mi* reactions differ from Satt_492 in 5 out of 7 susceptible genotypes which showed 250 bp band except Ajmeri and NARC-2 genotypes while this 250 bp band is also present in indigenous resistant genotypes except one genotype soybean 95086 which contain 232 bp band, the positive control genotypes exhibited 232 bp band.

RMi allele present in all resistant genotypes except 95086 and susceptible genotypes. The marker Satt_492 was not as strongly associated with *Mi* resistance as Satt_358 or Sat_132 (Table 1). During our *Mi*-screening experiment at molecular level, we noticed that 95086 did not exhibit any resistance related band whereas it shows the resistance against *Mi* during pathogenicity test at green house. This variation in 95086 cultivar contrasting to other tested genotypes might be due to parentage that both have homozygous alleles for this gene.

Genotype 95086 possesses a 200 bp (Satt_358), 238 bp (Sat_132) and 232 bp at Satt_492. On the basis of these results, we concluded that there are two ancestral sources of *Mi* resistance in this indigenous cultivar. Therefore, in conclusion the tight linkage of both Satt_358 and Sat_132 to the diagnostic marker G248 A-1 on LG-O in studied soybean cultivars indicates that selection for the *Mi*-resistant allele by any of these markers should be highly effective in identifying *Mi*-resistant plants / genotypes. Therefore, indigenous resistant germplasm can be effectively used in developing breeding strategies for the selection of *Mi*-resistant plants.

It is interesting that all *Mi*-susceptible cultivars possess a 192 bp band at Satt_358 diagnostic marker and 250 bp band at Sat_132. These results strongly support the association of Satt_358 and Satt_132 markers with the G248 A-1 locus for *Mi* resistance. Thus, the *Mi*-

reaction of these genotypes was predicted on the basis of the presence or absence of the 200 bp and 238 bp allele for *Mi*-resistance at Satt_358 and at Satt_132, respectively.

In present study utilizing SSR markers, the efforts were extended to screen the targeted resistance gene against southern root knot nematode (*M. incognita*) located on LG-O, this result is an approach, focusing on the fixing of resistance gene in high yielding genotypes of indigenous soybean germplasm.

References

1. Ha B-K, Bennett JB, Hussey RS, Finnerty SL, Boerma HR. Pedigree analysis of a major QTL conditioning soybean resistance to southern root-knot nematode. Crop Science, (2004); 44(3): 758-763.
2. Tamulonis J, Luzzi B, Hussey R, Parrot W, Boerma H. DNA markers associated with resistance to Javanese root-knot nematode in soybean. Crop Science, (1997); 37(3): 783-788.
3. Panaud O, Chen X, McCouch S. Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). Molecular and General Genetics, (1996); 252(5): 597-607.
4. Temnykh S, Park WD, Ayres N, Cartinhour S, Hauck N, *et al.* Mapping and genome organization of microsatellite sequences in rice (Oryza sativa L.). Theoretical and Applied Genetics, (2000); 100(5): 697-712.
5. Gutierrez M, Patto MV, Huguet T, Cubero J, Moreno M, *et al.* Cross-species amplification of Medicago truncatula microsatellites across three major pulse crops. Theoretical and Applied Genetics, (2005); 110(7): 1210-1217.
6. Luzzi B, Boerma H, Hussey R. Resistance to three species of root-knot nematode in soybean. Crop Science, (1987); 27(2): 258-262.
7. Tamulonis J, Luzzi B, Hussey R, Parrott W, Boerma H. RFLP mapping of resistance to southern root-knot nematode in soybean. Crop Science, (1997); 37(6): 1903-1909.
8. Doyle JJ. Isolation of plant DNA from fresh tissue. Focus, (1990); 1213-15.
9. Cregan P, Jarvik T, Bush A, Shoemaker R, Lark KG, *et al.* An integrated genetic linkage map of the soybean genome. Crop Science, (1999); 39(5): 1464-1490.
10. Li Z, Jakkula L, Hussey R, Tamulonis J, Boerma H. SSR mapping and confirmation of the QTL from PI96354 conditioning soybean resistance to southern root-knot nematode. Theoretical and Applied Genetics, (2001); 103(8): 1167-1173.
11. Meehan D, Xu Z, Zuniga G, Alcivar-Warren A. High frequency and large number of polymorphic microsatellites in cultured shrimp, Penaeus (Litopenaeus) vannamei [Crustacea: Decapoda]. Marine Biotechnology, (2003); 5(4): 311-330.
12. Rohlf F. NTSYS-PC 2.02. Numerical taxonomy and multivariate analysis system Applied Biostatistics, Setauket, New York, (1998).