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Short communication

Validation of potato cyst nematode resistant genotypes through molecular markers

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ABSTRACT

Forty-two potato genotypes exhibiting phenotypic resistance against Potato Cyst Nematode (PCN) species *Globodera rostochiensis (*golden) and *Globodera pallida* (white) were screened for molecular validation using tightly linked genetic DNA markers. *H1* and *GroV1 gene* conferring resistance against golden nematodes *were* validated by TG 689 & 57R and X02 markers, respectively. Similarly, resistance against *Globodera pallida* governed by *QTL GpaVvrn* and *QTLs Gpa5, Gpa6*, was confirmed by corresponding markers HC and SPUD1636. On the basis of phenotypic and genotypic confirmation, this study identifies CP 1843, 1879 and JEX/A 267 as elite potato genotypes that can be utilized as parental lines for introgression of resistant genes against both PCN species.

Key words: Genotypes, potato cyst nematode, marker-assisted breeding, *QTLs,* markers.

Potato cyst nematode (*Globodera* spp.) is a global pest. The annual yield loss due to this pest range from 20% to 70% (Oerke *et al*., 6). Resistance against Potato cyst nematode (PCN) is mainly derived from *Solanum tuberosum* ssp. *andigena*, *Solanum vernei* and *Solanum spegazzini* (Milczarek *et al*., 5). In India, Potato cyst nematode was first reported in year 1961 from Nilgiri hills of Tamil Nadu (Jones, 2). Considering its economic importance domestic quarantine (embargo) was imposed. At present the pest is restricted to the areas of Tamil Nadu and Kerala (Krishna Prasad, 4). Recent surveys in the Nilgiris revealed PCN presence is in high intensities (>51 cysts/100 cc soil). In India both species are prevalent and the pathotypes available are *Ro1*, *Ro2* and *Ro5* of *Globodera rostochiensis* and *Pa1*, *Pa2* and *Pa3* of *Globodera pallida* (Krishna Prasad, 3). Being a quarantined pest and difficulty in management, resistance breeding is the most sustainable strategy. In India, the first potato cyst nematode resistant variety bred through conventional breeding was Kufri Swarna released in 1985 with *Solanum vernei* as the source of resistance. It has resistance against pathotypes *Ro1* and *Pa2*. However, continuous cultivation of specific resistant varieties leads to build up of other virulent pathotypes. Recently, another *S. vernei* derived resistant hybrid 'OS/93-D-204' has been bred and released as a variety 'Kufri Neelima' for Nilgiri hills. It is resistant to pathotypes *Ro1* and *Pa2*. Development of resistant varieties through conventional breeding is laborious and time consuming. In order to hasten the resistance breeding programme, interventions of molecular markers have a vital role. It provides information about the prevalence of resistance genes in population and facilitates combining/ pyramiding of multiple resistance genes. Keeping this in view the aim of present study was to evaluate the usefulness of different PCR based markers linked to PCN resistance genes for rapid screening of Indian potato genotypes (cultivars and breeding lines).

The PCN resistant potato cultivars and breeding lines are maintained under tissue culture of National Active Germplasm Repository of CPRI, Shimla. A total of 42 breeding lines of potato including 8 exotic varieties were grouped according to phenotypic traits into three categories, *i.e.* group I including genotypes resistant against *G. rostochiensis*, group II for *G. pallida* resistant genotypes and group III of combined resistance. The total genomic DNA was extracted from tissue culture raised plantlets by extraction kit (Sigma Aldrich) and was amplified in DNA thermal cycler (Applied Biosystems) using markers TG689 and 57 R (*H1* gene, chromosome V); Gro 1.4.1 (*Gro 1.4* gene, chromosome III); X02 (*GroVI* gene, chromosome V); SPUD 1636 (*QTLs Gpa5* and *Gpa6,* chromosome V) and HC (*GpaVvrn,* chromosome V). The components of the PCR reaction mixture are: 1X Tris buffer with 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 U *Taq* polymerase, 1 µM primer and 20 ng template DNA for each reaction with final volume of 20 µl. The PCR conditions for the different markers are followed as per protocol given by Milczarek *et al*. (5), Asano *et al.* (1) and Schultz *et al*. (7). The annealing temperature of each marker was standardized by gradient PCR

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(Biorad 100). The amplified products were resolved on a 2.0% agarose gel at a constant voltage of 60 V for 150 min. using a horizontal gel electrophoresis system.

Several major genes are known to be associated with PCN resistance in both wild and cultivated *Solanum* species. Out of which five major genes/ QTLs have been validated in the present study by a set of linked molecular markers in known phenotypically resistant genotypes. The result depicted that the markers TG689, 57R and X02 for *G. rostochiensis* did not amplify in any of the *G. pallida* resistant genotypes. Similarly, the markers HC and SPUD1636 for *G. pallida* resistance did amplify in any of the *rostochiensis* resistant genotypes as expected (Table 1).

H1 gene for *G. rostochiensis* resistance was validated in all the genotypes screened by marker 57R while the marker TG 689 for the same gene amplified in 28 out of 33 genotypes (Table 1, Fig. 1&2). *GroV1* gene was present in 15 genotypes depicting that the source of resistance is derived from *S. vernei*. Genotypes CP 1598, 1669, 1670, 1671, 1720, 1843, 2290, 2329, 2339, 3209, JEX/A 164, 413, 622, 780 and 911 contain both *H1* and *GroV1* genes. Gene *Gro 1.4* derived from *S. spegazzinii* was present in none of the genotypes. Our results are in accordance to Schultz *et al*. (7) who reported that marker 57R is the most common in *G. rostochiensis* resistant genotypes. The marker TG 689 also holds promise for selection of *G. rostochiensis* resistant genotypes. However, this marker did not amplified in all resistant genotypes which were similar to Milzareck *et al*. (5) results of occurrence of resistant and “marker-negative” individuals. *Gro 1.4* gene did not amplify in any of the genotypes, necessitating validation of this marker in wider germplasm pool for broadening the genetic base.

Table 1. Genotypic profile of potato genotypes resistant against *G. rostochiensis* (Group I), *G. pallida* (Group II) and both species (Group III).

Group	Genotype	Genes/ QTLs					
		H1		*GroVI*	*GpaVvrn*	*Gpa5, Gpa6*	*Gro1-4*
Group I	*Solanum tuberosum* ssp. *tuberosum*	TG 689	57R	X02	HC	SPUD1636	Gro 1-4-1
	CP 1598	+	+	+	-	-	-
	CP 1669	+	+	+	-	-	-
	CP 1670	+	+	+	-	-	-
	CP 1671	+	+	+	-	-	-
	CP 1720	+	+	+	-	-	-
	Solanum tuberosum ssp. *andigena*						
	JEX/A 132	+	+	-	-	-	-
	JEX/A 164	+	+	+	-	-	-
	JEX/A 413	-	+	+	-	-	-
	JEX/A 622	+	+	+	-	-	-
	JEX/A 780	+	+	+	-	-	-
	JEX/A 911	-	+	+	-	-	-
	Exotic varieties						
	Reba/ CP 4362	+	+	-	-	-	-
	Pike/ CP 4364	+	+	-	-	-	-
	Andover/ CP 4367	+	+	-	-	-	-
	Salem/ CP 4363	+	+	-	-	-	-
	Keuka Gold/ CP 4365	+	+	-	-	-	-
	Eva/ CP 4366	-	+	-	-	-	-
	Lehigh/ CP 4360	+	+	-	-	-	-
	Marcy/ CP 4361	+	+	-	-	-	-
	Genotypes showing positive results	16/19	19/19	10/19	-	-	-

Contd...

Table 1 Contd...

Group	Genotype	Genes/ QTLs					
		H1		*GroVI*	*GpaVvrn*	*Gpa5, Gpa6*	*Gro1-4*
Group II	*Solanum tuberosum* ssp. *tuberosum*						
	CP 3303	-	-	-	-	-	-
	CP 3305	-	-	-	-	-	-
	CP 3306	-	-	-	-	-	-
	CP 3307	-	-	-	-	-	-
	CP 3308	-	-	-	-	-	-
	Solanum tuberosum ssp. *andigena*						
	JEX/A 79	-	-	-	-	-	-
	JEX/A 506	-	-	-	-	-	-
	JEX/A 712	-	-	-	-	-	-
	JEX/A 877	-	-	-	-	-	-
	Genotypes showing positive results	-	-	-	0/9	0/9	-
Group III	*Solanum tuberosum* ssp. *tuberosum*						
	CP 1843	+	+	+	-	+	-
	CP 1879	+	+	-	+	-	-
	CP 2059	+	+	-	-	-	-
	CP 2290	+	+	+	-	-	-
	CP 2329	+	+	+	-	-	-
	CP 2339	+	+	+	-	-	-
	CP 2417	+	+	-	-	-	-
	CP 3181	+	+	-	-	-	-
	CP 3209	+	+	+	-	-	-
	CP 3534	+	+	-	-	-	-
	Solanum tuberosum ssp. *andigena*						
	JEX/A 121	+	+	-	-	-	-
	JEX/A 216	-	+	-	-	-	-
	JEX/A 267	+	+	-	+	-	-
	JEX/A 708	-	+	-	-	-	-
	Genotypes showing positive results	12/14	14/14	5/14	2/14	1/14	0/14

+ = Presence of gene; - = Absence of gene

For *G. pallida* resistance, *QTL GpaVvrn* was present in two genotypes, while *QTLs Gpa5* and *Gpa6* were present in only one genotype (Table 1), which was validated by markers HC and SPUD1636, respectively. The other resistant genotypes could not amplify the markers confirming presence of resistance source other than *S. vernei.* Genotypes CP 1843, 1879 and JEX/A 267 contain resistance genes against both *G. rostochiensis* and *G. pallida*. Thus, these genotypes serve as valuable source of breeding material for pyramiding PCN resistance genes.

In conclusion, detection of resistance genes by DNA based markers may complement the phenotypic evaluation. 57R is the most robust marker for identification of *G. rostochiensis* resistant genotypes. Furthermore, there is a need for validation of these tightly linked markers in wider population and development of new markers suitable for characterisation of Indian PCN resistant genotypes.

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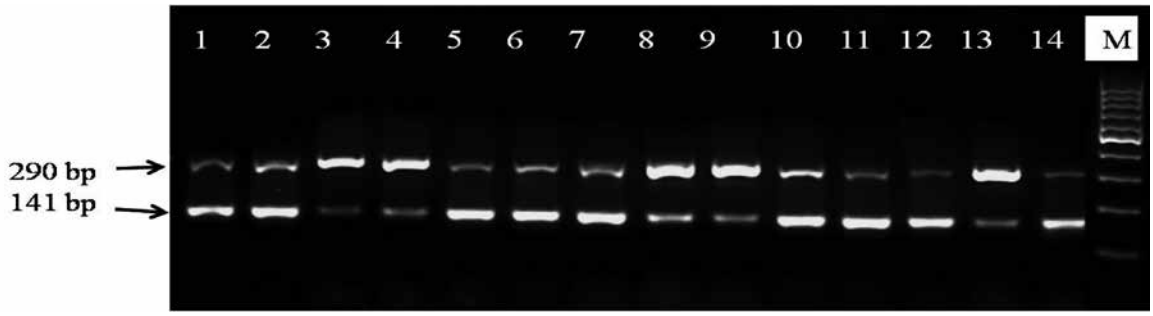

Fig. 1. DNA profile of different potato genotypes M : 100 bp DNA ladder, 1-14 positive samples of TG 689.

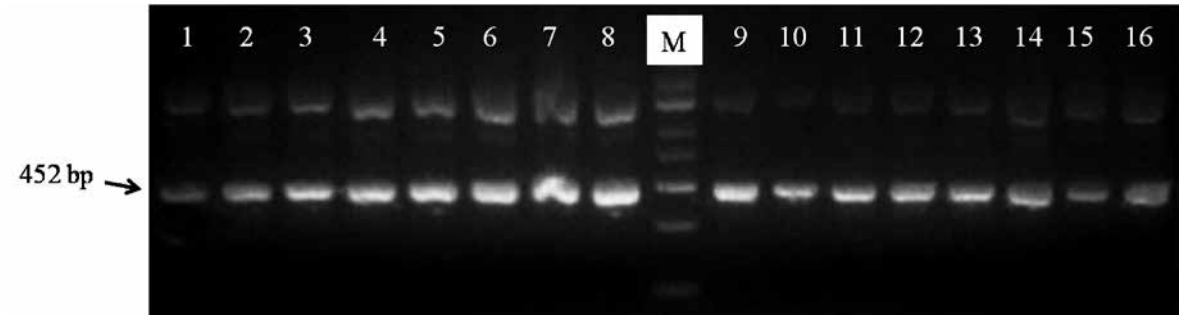

Fig. 2. DNA profile of different potato genotypes M : Express DNA ladder, 1-16 positive samples of 57R.

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