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Phenotypic and molecular characterization of potato germplasm for potato cyst nematode resistance

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Abstract

Potato cyst nematode (PCN) is considered to be the most devastating pest causing major yield losses in potato worldwide. Resistant potato cultivars would be the most effective strategy to control PCN however, they may not posses enough resistance to all pathotypes of PCN. Therefore breeding programme should focus on developing varieties with horizontal resistance which can be achieved by using parental lines with multiple resistant genes. Hence, the present study was conducted to characterize the phenotypic and genotypic resistance of parental lines against PCN and also an attempt was made to find out the pollen viability of the parental lines to improve the breeding efficiency. Sixty six genotypes were screened phenotypically as well as genotypically against PCN. Out of which, 54 lines were found phenotypically resistant to *G. rostochiensis*, 48 to *G. pallida* and 44 to both the species of PCN. The same set of parental lines was also validated using linked molecular markers. In molecular characterization, the markers linked with *H1* gene (TG689 and 57R) were found in majority of the parental lines screened. Marker SPUD 1636 linked to *GPa5* QTL was positive for three genotypes whereas HC marker (*GPaV*$_{vrn}$ QTL) was positive for 30 parental lines. TG432 linked to *Grp1* gene is positive for 27 parental lines whereas the marker Gro 1-4-1 is not amplified in any of the screened parental lines. Pollen viability of parental lines varied from 15.8 to 93.6%. The results of the study will facilitate breeding of novel resistant varieties by efficient selection of ideal male parent with good pollen potential and multiple resistance to several PCN pathotypes.

Key words: Potato cyst nematode, phenotypic screening, marker assisted selection, resistance genes, pollen viability

Introduction

Potato (*Solanum tuberosum* L.) is the third most important food crop of the world after rice and wheat and mainstays the diet of people in many parts of the world. The present production scenario of potato in our country shows that India stands second after China in production with an estimated production of about 48.53 million tonnes from 2.15 million ha (http://www.agricoop.nic.in/). In general, biotic stresses due to insect pests, nematodes and diseases accounts nearly 37% yield loss throughout the globe of which diseases and nematode parasites alone share 23% (Sasser and Freckman 1987). Among the plant parasitic nematodes, potato cyst nematode (PCN), *Globodera* spp. is one of the major pests of potato crop worldwide (Turner and Evans 2005; Mhatre et al. 2019). PCN comprises of two species (*G. rostochiensis* and *G. pallida*) with eight pathotypes namely, Ro1 to Ro5 of *G. rostochiensis* and Pa1 to Pa3 of *G. pallida* (Krishna Prasad 2008). Both species parasitize potato roots and at the end of life cycle, persists in the soil and plant residues as a tanned and hardened sac filled with eggs called cysts. The cyst protects eggs to survive in adverse environmental conditions for many years. After hatching the second stage juveniles (J2s) of *Globodera* spp. preferentially penetrate the root tips, derive the nutrition (Sheridan et al. 2004) and are responsible for 10-75% yield reduction (Krishna Prasad 2008).

Though there are various options available for the management of PCN, growing resistant cultivar is the most economic, effective and environmentally safe strategy (EPPO/OEPP 2004). Resistant hosts respond to J2 invasion by cell necrosis, disorganization and

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lysis of the syncytium cells which leads to death or further abnormalities thereby limits further multiplication (Trudgill 1991). From Nilgiris and Kodaikanal hills of India three pathotypes of each *G. rostochiensis* (Ro1, Ro2 and Ro5) and *G. pallida* (Pa1, Pa2 and Pa3) have been reported (Krishna Prasad 2006) but resistant cultivars may not possess resistance to all these pathotypes. Therefore, breeding varieties with horizontal resistance should be given prime importance. This can be achieved by using selected parental lines with resistance to different pathotypes. Hence, proper phenotypic as well as genotypic validation of parental lines is the key factor in potato resistance breeding programme. However, phenotyping is laborious and time consuming procedure and may lead to technical errors. These errors in breeding program can be minimized by adopting more rapid and objective method of genetic identification such as marker-assisted selection which holds great promise in plant breeding (Collard and Mackill 2008). Availability of tightly linked molecular markers facilitates identification of germplasm lines carrying resistance genes (Gebhardt and Valkonen 2001). Therefore, molecular markers could be an effective tool for rapid screening of germplasm against pest and diseases (Nadeem et al. 2018). Apart from this, pollen viability of the male parent is the major constraint confronting the potato breeder, which leads to several unsuccessful crosses (Breeze 1921; Stout and Clark 1924). Therefore, the present study was aimed at: (i) to screen the potato germplasm for resistance to PCN, (ii) validate the DNA-based markers known for PCN resistance and, (iii) to study pollen viability of germplasm for its effective utilization in breeding programme.

Materials and methods

Plant materials and morphological characterization

A total of sixty six potato germplasm accessions comprising of 41 parental lines including wild relatives of *Solanum* species from National Active Germplasm Repository of ICAR-Central Potato Research Institute (CPRI), Shimla, 20 advanced selections (OS) developed at ICAR-CPRI Regional Station, Ooty and five popular cultivars maintained at this station were used in present study (Table 2).

Phenotypic screening for PCN resistance was done using root ball technique as per the procedure followed by Krishna Prasad (2006). Five tubers from each cultivars/germplasm/accessions were planted in 10 cm diameter pots in glass house at 20-22^{o}C which had about 500-550 g of soil. The soil used for planting contained a mixed population of both the species of PCN (200-250 cysts 100 g^{-1} soil), providing 8000-10000 eggs and juveniles per test tuber. At 55-60 days after planting the root ball was examined for presence of PCN female and the number was recorded. The two species of *Globodera* were distinguished by the colour of developing females (Yellow: *G. rostochiensis* and White: *G. pallida*). At molecular level the identity of both the species was confirmed using species specific markers (Mulholland 1996). Based on the number of females developed per root ball, the clones were graded following the 0 to 4 scale. The clones showing grade 0-2 are considered to have desirable level of resistance (R) whereas grades 3-4 were designated as susceptible (S) (Krishna Prasad 2006).

Female/root ball	Grade	Remarks
None	0	Immune
1-5 females	1	Highly resistance
6-20 females	2	Moderately resistance
21-50 females	3	Susceptible
> 50 females	4	Highly susceptible

Molecular characterization

DNA extraction and marker assays

Total genomic DNA was extracted from the tender leaves following the standard CTAB method as described by Doyle and Doyle (1990) with minor modifications and further used for molecular assay. DNA concentration was estimated by agarose gel (0.8%) electrophoresis using a known amount of ë DNA as standard followed by nanodrop spectrophotometer (Thermo Scientific, US). Markers which were recommended for selection of genotypes resistant to *Globodera* spp. were selected for PCR assays (Table 1). PCR was performed in a total volume of 20 µl containing 60 ng of template DNA, 1.6 µl dNTPs, 0.3 units Taq DNA polymerase and the related primer pairs, the PCR temperature parameters and primer sequences for amplifying all used markers are described in Table 1. Parameters of PCR were changed for marker HC and SPUD1636 and touchdown PCR was performed. Amplified products were separated using 1.2% agarose gel in 1x TAE buffer and visualized using Gel Documentation System (Bio-Rad, USA). The amplicon size was determined using

Table 1. Details of markers used in the study

Marker	Gene	Type/ enzyme	Size (bp)	Primer sequence (5'-3')	PCR conditions	References
TG689	*H1*	SCAR	141	TAAAACTCTTGGTTATA GCCTAT/CAATAGAATG TGTTGTTTCACCAA	94°C, 2 min; 35x(94°C, 20 s, 55°C, 20 s, 72°C 30 s) 72°C, 5 min.	Schultz et al. 2012
Gro1-4-1	*Gro1-4*	STS	602	AAGCCACAACTCTACT GGAG/GATATAGTACG TAATCATGCC	94°C, 10 min; 35x(94°C, 30 s, 62°C, 45 S, 72°C 1 min.) 72°C, 5 min.	Asano et al. 2012
HC	*GpaV$_{vrn}$ _QTL*	SNP	276	ACACCACCTGTTTGAT AAAAAACT/GCCTTAC TTCCCTGCTGAAG	94°C, 2 min; 94°C, 1 min; 65°C, 1 min; 72°C, 1 min ; 6x(94°C, 30 s, 65°C decreasing the annealing temperature to 60°C by 1°C per cycle, 30 s; 72°C 30 s); 30 x (94°C, 30 s, 60.5°C, 30 S, 72°C 30 s); 72°C, 5 min.	Sattarzadeh et al. 2006
SPUD 1636	*Gpa5 _QTL*	PCR	226	GTCGGCACAGGGTAAAA CC/ACCTTAGCGGATG AAAGCC	94°C, 3 min; 94°C, 30 s; 65°C, 1 min; 72°C, 1 min; 5x (94°C, 30 s, 65°C decreasing the annealing temperature to 60°C by 1° C per cycle, 30 s; 72°C 30 s); 24 x (94°C, 30 s, 60°C, 30 s, 72°C 30 s); 72°C, 3 min.	Bryan et al. 2002
57R	*H1*	SCAR	450	TGCCTGCCTGTCCGA TTTCTGGTTCAGCAAAA GCAAGGACGTG	94°C, 2 min; 35x(94°C, 30 s, 63°C, 15 s, 72°C 1 min.) 72°C, 3 min.	Schultz et al. 2012
TG432	*Grp1*	CAPS/ RsaI	1900	GGACAGTCATCAGATT GTGG/GTACTCCTGCTT GAGCCATT	94°C, 3 min; 35x(94°C, 30 s, 66°C, 45 s, 72°C 2 min. 30 s) 72°C, 3 min.	Caromel et al. 2005

100 bp and 1 kb ladders (Bangalore Genei, India) based on markers used.

Pollen viability

At blooming stage, newly opened flowers or buds which are about to open were collected from each plant and kept in paper bags for pollen viability study. The pollen samples were stained with acetocarmine glycerol jelly technique described by Marks (1954). This test measures the integrity of cytoplasm and red stained pollens shows that the cytoplasm membrane is integral. A drop of acetocarmine glycerol jelly is placed in the centre of the slide and small amount of pollens were mixed in the same. After one minute a slide cover was kept and the mounted slides were observed for staining of pollens. The pollen grains that were fully stained, plump and with well-defined contours were considered as viable, whereas those that were not stained, and/or irregularly shaped were considered as non viable. Four replicates of approximately 250 pollen grains were observed under compound microscope and expressed as the percentage of the viable pollens. CIP, Lima Peru has proposed a scale for pollens viability, accordingly the accession wise data were recorded in the following grades:

Scale	Range	Description
1	0	Sterile (S)
3	<50	Low (L)
5	>50-80	Moderate (M)
7	>80-100	High (H)

Results

Phenotypic screening for PCN

In a resistance breeding programme, phenotypic screening for selection of parents is very crucial and laborious. In the present study, 66 parental lines including germplasm (40 CP accessions), advanced potato hybrid lines (20 OS/E/D/J/R lines), cultivars (5

Kufri cvs.) and wild species *S. vernei* were evaluated for PCN resistance. The species of PCN were identified based on the color of developing females (Fig. 1) from

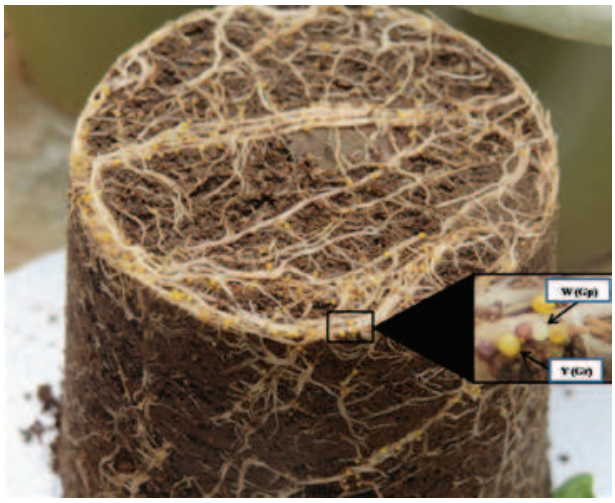

Fig. 1. Root ball technique for PCN screening (Var. Kufri Jyoti) W(Gp) = White females of *G. pallida*; Y(Gr) = Yellow females of *G. rostochiensis*

root ball and also using species specific markers (Fig. 2). The parental lines which favours very fewer (<20) PCN females per root ball were considered as resistant whereas which harboured more than 20 females per root ball were considered as susceptible. Among the tested parental lines, 54 lines (81.81%) were resistant to *G. rostochiensis* and 48 (72.72%) were resistant to *G. pallida* whereas 44 (66.66%) exhibited resistant reaction to both the species of PCN (Table 2). Ten parental lines (CP-2045, CP-1847, CP-2134, CP-2427, OS/06-37, OS/08-277, OS/09-117, OS/10-31, OS/07-63 and OS/06-66) were found resistant only to *G.*

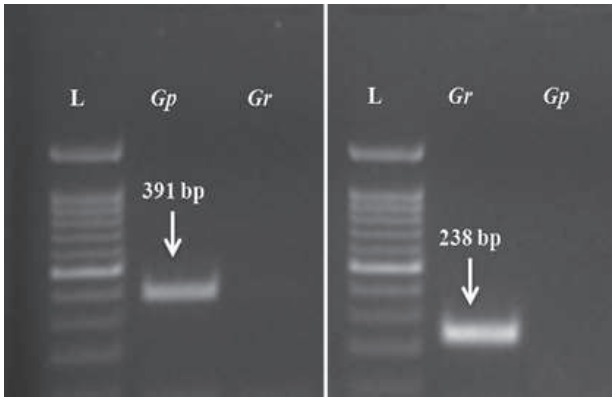

Fig. 2. Molecular validation of PCN species using species specific markers *Gp* = *G. pallida; Gr* = *G. rostochiensis*; L = 100bp Ladder

rostochiensis, 4 parental lines (CP-2340, CP-2384, CP-3182 and OS/01-392) were resistant only for *G. pallida* whereas eight parental lines/cultivars (CP-2373, CP-3181, CP-3715, OS/2000-43, OS/06-31, Kufri Jyoti, Kufri Himalini and Kufri Girdhari) supported more populations of PCN species (> 20 females of each species) and were found susceptible to both the species of PCN. Among the parental lines, CP-2066 showed a strongest resistance response by supporting a negligible population of *G. rostochiensis* (0-1 females/root ball) and *G. pallida* (0-2 females/root ball) followed by CP-3252 (0-3 females of each species per root ball). In contrast, the popular cultivar Kufri Jyoti favoured highest population of both the species of PCN (43 to >125 females per root ball). The susceptible accessions/cultivars were included in the parental lines due to their excellent agronomic traits (potato shape, taste, colour, crop duration and high yielding potential) and late blight resistance thus mainly being used as female parents in breeding programme.

Genotypic screening for PCN

Marker-assisted selection is one of the most important applications of DNA markers since it reduces time and costs with increased efficiency and precision in breeding programme. After phenotypic screening the same set of 66 parental lines were screened for six genes/QTLs conferring resistance to *Globodera* spp. using a set of tightly linked molecular markers. The results depicted that in the current study, H1 gene which imparts resistance for *G. rostochiensis* was validated in 58 out of 66 accessions/cultivars screened by marker TG689 while marker 57R for the same gene was positive for 54 genotypes (Figs.3, 4 and Table 2).

Resistance locus *Grp1*confers resistance to pathotype Pa2/3 of *G. pallida* and Ro5 of *G. rostochiensis* which *was detected using* Cleaved Amplified Polymorphic Sequence (CAPS) marker *TG432 and was amplified* in 27 parental lines which is 40.90% of total lines screened (Fig. 5 and Table 2). Four accessions (CP-2045, 1847, 2134 and OS/06-37) phenotypically susceptible to *G. pallida* and three (CP-2340, 2384 and 3182) susceptible to *G. rostochiensis* were also found to have *Grp 1* locus; these mismatches are probably due to the presence of other PCN pathotypes (Ro1,2,3,4 and Pa1) in soil.

Marker HC and SPUD 1636 are tightly linked with the QTLs conferring resistance to pathotypes, 2 and 3 of *G. pallida*. In this study marker HC could detect 30 parental lines while only 3 by marker SPUD

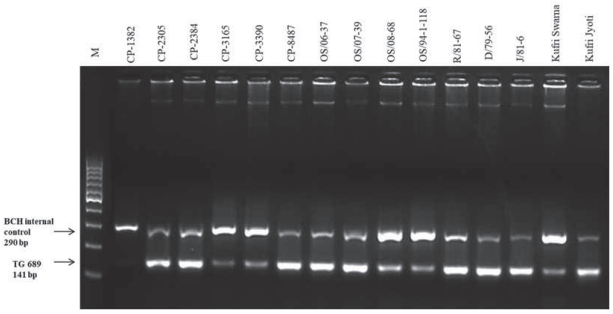

Fig. 3. Detection of *H1* gene through PCR based marker TG689 (141 bp)

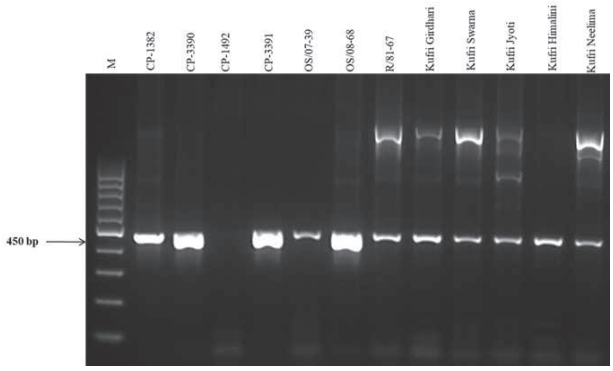

Fig. 4. Detection of *H1* gene through SCAR marker 57R (450bp)

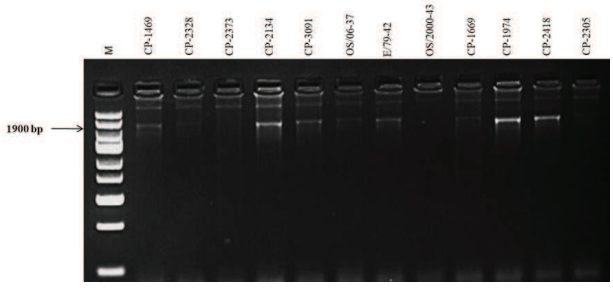

Fig.5. Detection of *Grp1* gene through CAPS marker TG 432 (1900bp)

1636 (Table 2). This shows the efficiency of HC over SPUD 1636 in detecting more number of Pa2/3 resistant accessions. As expected, marker HC was amplified in all four accessions phenotypically resistant only for *G. pallida* whereas it had not amplified in any of the *G. rostochiensis* resistant accession indicating the specificity of the marker. Among the parental accessions only CP-2066 and CP-3252 showed positive response for both HC and SPUD 1636 markers. Other than these two lines SPUD 1636 was positive for PCN resistant cultivar Kufri Swarna as the marker can detect the chromosomal segment carrying the *S. vernei* derived QTL and this is the reason behind positive reaction of Kufri Swarna which is derived from *S. vernei*. In contrast to all the studied markers, Gro1-4-1 marker linked with *S. spegazzinii* derived *Gro 1-4* resistance gene conferring resistance to *G. rostochiensis* was not amplified in any of the screened parental lines (Table 2).

Pollen viability

Pollen potential of the parental lines was estimated by evaluating the pollen viability. Pollen viability partially determines the reproductive success of a species and thereby decides the effectiveness of the parental line in PCN resistant breeding. Significant differences were observed among the studies parental lines in pollen viability. The percentage of pollen viability varied from 15.8 to 93.6%. The highest percentage of viable pollen (93.6%) was recorded in OS/08-277 followed by CP-2373 (93.0%), CP-2328 (90.7%) and OS/01-392 (90.5%). Among the studied accessions, 9 (13.63%) were categorized as the lines with low pollen viability, 33 (50%) with medium pollen viability and 24 (36.36%) with high pollen viability (Table 2). This shows that many of the individuals are having good pollen potential and can be successfully utilized in the breeding programme.

Discussion

The resistance level of plants varies from immune to highly susceptible (Kehr 1966). Generally, potato cyst nematode resistance or tolerance is tested by observing the rate of nematode development using root ball technique (Krishna Prasad 2006; Dalamu et al. 2017). In the present study, phenotypic screening of 66 genotypes showed that 44 (66.66%) were resistant to both the species of *Globodera,* 54 to *G. rostochiensis* (81.81%) and 48 to *G. Pallida* (72.72%). Similar observations with varied responses were also observed with PCN as well as other nematode genera (Dropkin et al. 1969; McClure et al. 1974; Pankaj et al. 1995; Cabasan 2012; Mhatre et al. 2015, 2017).

Phenotypic selections followed by marker assisted validation have great impact in the precision of breeding programme and can be implemented successfully using tightly linked DNA marker (Collard and Mackill 2008). DNA markers have several advantages such as time saving, stability, efficiency, reproducibility and more precise selection of composite traits (Collard et al. 2005; Jena and MacKill 2008). Several major genes/QTLs and their tightly linked

Table 2. Phenotypic and genotypic evaluation of germplasm against potato cyst nematodes (PCN) along with pollen viability assay

S.No.	Genotypes/ varieties	Reaction		Presence (+) or absence (-) of DNA marker (gene/QTL)						Resistance	Pollen viability
		G. rostochiensis	*G. pallida* (*H1*)	TG689 (*H1*)	57R (*Grp1*)	TG432 (*GpaV$_{vrn}$*)	HC (*Gpa5*)	SPUD 1636	Gro 1-4-1 (*Gro1-4*)		
A. *Solanum tuberosum* ssp. *tuberosum* accessions resistant to both the species of PCN											
1	CP-1382	R	R	+	+	+	+	-	-	Ro1,4,5 Pa2/3	80.0 (M)
2	CP-1469	R	R	+	+	+	+	-	-	Ro1,4,5 Pa2/3	46.0 (L)
3	CP-1492	R	R	+	-	-	+	-	-	Ro1,4 Pa2/3	56.0 (M)
4	CP-1515	R	R	-	-	-	-	-	-	-	65.9 (M)
5	CP-1612	R	R	+	+	+	+	-	-	Ro1,4,5 Pa2/3	15.8 (L)
6	CP-1664	R	R	+	+	-	+	-	-	Ro1,4, Pa2/3	72.5 (M)
7	CP-1669	R	R	+	+	+	-	-	-	Ro1,4,5, Pa2/3	58.5 (M)
8	CP-1699	R	R	+	+	-	-	-	-	Ro1,4	20.0 (L)
9	CP-1729	R	R	+	+	-	+	-	-	Ro1,4, Pa2/3	68.4 (M)
10	CP-1843	R	R	+	+	+	+	-	-	Ro1,4,5 Pa2/3	69.7 (M)
11	CP-1869	R	R	+	-	-	+	-	-	Ro1,4, Pa2/3	63.0 (M)
12	CP-1974	R	R	+	+	+	-	-	-	Ro1,4,5, Pa2/3	87.5 (H)
13	CP-1979	R	R	+	+	-	-	-	-	Ro1,4	88.2 (H)
14	CP-2050	R	R	+	+	-	+	-	-	Ro1,4, Pa2/3	57.3 (M)
15	CP-2059	R	R	+	+	+	-	-	-	Ro1,4,5,Pa2/3	80.8 (H)
16	CP-2066	R	R	+	+	+	+	+	-	Ro1,4,5,Pa2/3,Pa2,3	85.0 (H)
17	CP-2290	R	R	+	+	+	-	-	-	Ro1,4,5,Pa2/3	48.4 (L)
18	CP-2305	R	R	+	+	+	+	-	-	Ro1,4,5 Pa2/3	63.2 (M)
19	CP-2328	R	R	+	+	+	+	-	-	Ro1,4,5 Pa2/3	90.7 (H)
20	CP-2358	R	R	+	+	+	+	-	-	Ro1,4,5 Pa2/3	84.2 (H)
21	CP-2417	R	R	+	+	-	+	-	-	Ro1,4, Pa 2/3	62.5 (M)
22	CP-2418	R	R	+	+	+	-	-	-	Ro1,4,5,Pa2/3	88.1 (H)
23	CP-3091	R	R	+	+	+	-	-	-	Ro1,4,5,Pa2/3	71.7 (M)
24	CP-3165	R	R	+	+	-	+	-	-	Ro1,4, Pa2/3	72.3 (M)
25	CP-3252	R	R	+	+	-	+	+	-	Ro1,4, Pa2/3,Pa2,3	44.6 (L)
26	CP-3390	R	R	+	+	+	-	-	-	Ro1,4,5,Pa2/3	82.7 (H)
27	CP-3391	R	R	+	+	-	+	-	-	Ro1,4, Pa2/3	78.7 (M)
28	CP-3872	R	R	+	+	+	-	-	-	Ro1,4,5,Pa2/3	67.0 (M)
29	CP-8487	R	R	+	+	-	-	-	-	Ro1,4	47.3 (L)
30	CP-8487	R	R	+	+	+	+	-	-	Ro1,4,5, Pa2/3	55.1 (M)
31	OS/07-39	R	R	+	+	-	-	-	-	Ro1,4	83.2 (H)
32	OS/08-106	R	R	+	+	-	-	-	-	Ro1,4	84.6 (H)
33	OS/08-124	R	R	+	+	-	-	-	-	Ro1,4	81.2 (H)
34	OS/08-44	R	R	-	-	-	-	-	-	-	74.1 (M)
35	OS/08-68	R	R	+	+	-	-	-	-	Ro1,4	69.3 (M)
36	OS/09-328	R	R	+	-	-	-	-	-	Ro1,4	62.5 (M)
37	OS/94-I-118	R	R	+	+	-	+	-	-	Ro1,4, Pa2/3	72.5 (M)

38	E/79-42 (OS)	R	R	+	+	+	+	-	-	Ro1,4,5 Pa2/3	88.3 (H)
39	D/79-56 (OS)	R	R	+	+	-	+	-	-	Ro1,4 Pa2/3	77.4 (M)
40	J/81-6	R	R	+	+	+	-	-	-	Ro1,4,5,Pa2/3	54.1 (M)
41	R/81-67 (OS)	R	R	+	+	+	+	-	-	Ro1,4,5, Pa2/3	66.6 (M)
B. *Solanum tuberosum* ssp. *tuberosum* accessions resistant to *G. rostochiensis*											
42	CP-2045	R	S	+	+	+	-	-	-	Ro1,4,5,Pa2/3	84.2 (H)
43	CP-1847	R	S	+	+	+	-	-	-	Ro1,4,5,Pa2/3	42.6 (L)
44	CP-2134	R	S	+	+	+	-	-	-	Ro1,4,5,Pa2/3	72.5 (M)
45	CP-2427	R	S	+	+	-	-	-	-	Ro1,4	87.6 (H)
46	OS/06-37	R	S	+	+	+	-	-	-	Ro1,4,5,Pa2/3	85.5 (H)
47	OS/08-277	R	S	+	+	-	-	-	-	Ro1,4	93.6 (H)
48	OS/09-117	R	S	+	+	-	-	-	-	Ro1,4	71.3 (M)
49	OS/10-31	R	S	+	+	-	-	-	-	Ro1,4	70.2 (M)
50	OS/07-63	R	S	+	+	-	-	-	-	Ro1,4	87.1 (H)
51	OS/06-66	R	S	+	+	-	-	-	-	Ro1,4	86.6 (H)
C. *Solanum tuberosum* ssp. *tuberosum* accessions resistant to *G. pallida*											
52	CP-2340	S	R	+	+	+	+	-	-	Ro1,4,5 Pa2/3	46.2 (L)
53	CP-2384	S	R	+	+	+	+	-	-	Ro1,4,5 Pa2/3	62.2 (M)
54	CP-3182	S	R	+	+	+	+	-	-	Ro1,4,5 Pa2/3	56.4 (M)
55	OS/01-392	S	R	-	-	-	+	-	-	Pa2/3	90.5 (H)
D. *Solanum tuberosum* ssp. *tuberosum* accession susceptible to PCN											
56	CP-2373	S	S	-	-	-	-	-	-	-	93 (H)
57	CP-3181	S	S	+	-	-	-	-	-	Ro1,4	57.8 (M)
58	CP-3715	S	S	-	-	-	-	-	-	-	76 (M)
59	OS/2000-43	S	S	-	-	-	-	-	-	88.7 (H)	
60	OS/06-31	S	S	-	-	-	-	-	-	-	82.4 (H)
E. Other *Solanum* accessions resistant to both the species of PCN											
61	*S. verneii* (62-33-3)	R	R	-	-	-	-	-	-	-	62.3 (M)
F. Popular Indian cultivars of *Solanum tuberosum* spp. *tuberosum*											
62	Kufri Swarna	R	R	+	+	-	+	+	-	Ro1,4,Pa2/3, Pa2,3	24.2 (L)
63	Kufri Neelima	R	R	+	+	-	-	-	-	Ro1,4	81.4 (H)
64	Kufri Jyoti	S	S	+	+	-	+	-	-	Ro1,4, Pa2,3	74.3 (M)
65	Kufri Himalini	S	S	+	+	-	+	-		Ro1,4, Pa2,3	78.2 (M)
66	Kufri Girdhari	S	S	+	+	-	+	-	-	Ro1,4, Pa2,3	88.9 (H)
Total Positive		54/66	48/66	58/66	54/66	27/66	30/66	3/66	0/66		

(The grade of PCN reaction is the mean of five replicates, R=Resistant with 0 to 20 females/plant; S=Susceptible with more than 20 females/plant)

markers for PCN resistance have been reported in both wild and cultivated *Solanum* species (Gebhardt and Valkonen 2001). In this study the molecular validation for *G. rostochiensis* resistance was done using TG689, 57R, Gro1-4-1 and TG 432 markers whereas HC, SPUD1636 and TG432 markers was used for *G. pallida* resistance. *H1* is a single dominant gene from *S. tuberosum* ssp. *andigena* which confers resistance to pathotypes Ro1 and Ro4 of *G. rostochiensis* and is linked to the markers TG689 and 57R (Schultz et al. 2012). The results of the study showed that marker TG689 found positive in 87.88% of the studied parental lines whereas 57R was present in 81.82% lines. Among 66 accessions 47 accessions

which showed phenotypic resistance to *G. rostochiensis* were also found positive for both the markers. However, three accessions were found phenotypically positive but genotypically negative for both the markers which indicated the possibility of some other gene(s) involved in the resistance. The results about TG689 were in conformity with Biryukova et al. (2008) and Douches et al. (2010). The present study also revealed that marker TG 689 was present in all the genotypes where 57R was positive. However, the use of 57R leads to a reduction in the number of susceptible recombinants as compared with TG689. These findings are favorable for breeding purposes because it is better to reject susceptible clones than maintaining them for further selection, when they are erroneously classified as resistant.

In contrast to *H1*, no accession was found positive for the Gro1-4-1 marker linked with *S. spegazzinii* derived *Gro 1-4* resistance and our results are in accordance with the earlier findings of Dalamu et al. (2017). Gro1-4-1 is a more specific marker confers nearly absolute resistance to all pathotypes of *G. rostochiensis* and therefore considered as useful in PCN resistance (Asano et al. 2012). However, absence of this gene in our parental line/cultivar resources calls attention to the importance of introduction of genotypes having *Gro1-4*.

Bryan et al. (2002) reported that maker SPUD1636 can detect the chromosomal segment carrying the *S. vernei* derived QTL (*Gpa5*) conferring resistance to *G. pallida* (Pa2 and Pa3). In the present study, SPUD 1636 was positive for three parental lines (CP-2066, CP-3552 and Kufri Swarna) which were also found to have phenotypic resistance against *G. pallida* that shows the presence of resistant QTL *Gpa5*. This is in conformity with the findings of Sudha et al. (2016). Resistance to *G. pallida* can be detected by another SNP marker HC, which is diagnostic for an allele conferring high degree of resistance against *G. pallida* pathotype Pa2/3 and detects $GPaV_{vrn}$-QTL (Sattarzadeh et al. 2006). In present study, HC was amplified in 30 (45.5 %) genotypes of which 27 of the total parental lines were diagnostic to the phenotypic results for *G. pallida* resistance. It is also observed that 20 lines were marker negative but phenotypically resistant to *G. pallida* which attracts the attention for involvement of a novel gene for resistance. Based on the results it can be stated that HC marker is more effective than SPUD 1636 since it identified more number of Pa2/3 resistant accessions. Similar findings were also observed by Schwarzfischer et al. (2009) and Ute (2007) but in a different set of material.

Grp1 locus is responsible for broad-spectrum resistance to *G. rostochiensis* (pathotype Ro5) and *G. pallida* (pathotypes Pa2/3) and is detected by CAPS marker TG432 (Finkers-Tomczak et al. 2009). Rouppe Van der Voort et al. (1998) stated that the resistance to both *G. pallida* and *G. rostochiensis* at the *Grp1* loci is mediated by one or more tightly linked resistance gene(s) probably belonging to the NBS-LRR class. In the present study, 27 parental lines were found positive for the marker TG432 which shows the presence of *Grp1* loci. Since *Grp1* is a single dominant gene conferring resistance to both the species of *Globodera*, the parental lines positive for the marker TG432 were extremely valuable.

After having thorough knowledge about the resistance potential of male parent, it is important to have fertility potential of the same for successful breeding programme. Study of pollen viability and pollen germination provides a reliable male fertility estimate. Hence based on the pollen viability information, suitable parental lines can be selected and used as pollenizer. In our study large variations in pollen viability (15.38%-93.6 %) were observed among the tested lines. Similar results with varied pollen viability were also reported by Breeze (1921) and Larrosa et al. (2012) with different potato varieties and hybrids (*S. gourlayi Hawkes* x *S. tuberosum ssp. tuberosum*) respectively. Pandolfi (1998) and Stout and Clark (1924) reported that the pollen viability is significantly higher in wild species than commercial potato cultivars. In contrast, in our study among the five cultivars, all showed moderate to high pollen viability except Kufri Swarna. Utilization of parental lines possessing multiple resistance genes for PCN and having good pollen viability is required to obtain novel genotypes with durable resistance. The superior germplasm line CP-2066 identified in the present study can be used as an efficient male parent for the development of new PCN resistant cultivars as it exhibited good pollen viability and strong phenotypic resistance against both the species of PCN which was validated by the presence of five (TG689, 57R, TG432, HC and SPUD 1636) out of six tested molecular markers.

Author's contribution

Conceptualization of research (RSu, VB); Experiment designing (RSu, VB, D); Contribution of experimental material (RSu, VB, D); Execution of field/ lab

experiments and data collection (RSu, EPV, PHM, DKL, AB, VB, D, RS); Analysis of data and interpretation (RSu, EPV, PHM, DKL, AB); Preparation of manuscript (RSu, PHM, DKL).

Declaration

The authors declare no conflict of interest.

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