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MOLECULAR CHARACTERIZATION OF POTATO PARENTAL LINES FOR ENHANCED RESISTANCE TO POTATO CYST NEMATODES

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ABSTRACT: Potato cyst nematodes (*Globodera pallida* and *G. rostochiensis*), are major pests that significantly reduce potato yields worldwide. Developing potato varieties resistant to cyst nematodes is essential to sustain potato production worldwide especially in temperate areas. In this study, we did molecular characterization of 85 potato accessions using linked markers/genes to PCN resistance. We used three QTLs/gene-linked markers for each species: *G. rostochiensis* (*H1*, *GroVI*, and *Grp1*) and *G. pallida* (*Gpa2*, *Gpa5*, *GpaIV^{s}adg*). The screening results revealed diverse resistance levels among the accessions based on presence and absence of the marker bands. For *G. pallida*, 22 accessions carried both Gpa2-1 and Gpa2-2 markers, while only three showed the presence of *SPUD1636* marker associated with *Gpa5*. Most accessions, except one, tested positive for the Contig237 marker linked to *GpaIV^{s}adg*. Likewise, for *G. rostochiensis*, 21 accessions had the *H1* gene marker TG689, while 14 showed the presence of 57R marker. The *GroVI* markers U14 and X02 were found in 33 and 57 accessions, respectively, and the *Grp1* marker TG432 was present in 12 accessions only. Notably, two accessions, CP4052 and CP4057, exhibited seven resistance markers, making them prime candidates for breeding programs aimed at developing durable PCN resistant potato varieties. The use of molecular markers improves the efficiency of selecting resistant plants and is more cost-effective and quicker than traditional methods. This approach helps in early identification of parental lines with multiple resistance genes, aiding in gene stacking and enhancing the overall breeding process for PCN-resistant potatoes.

KEYWORDS: *Globodera rostochiensis*; *Globodera pallida*; Molecular Characterization; PCN-Resistant Genotypes; PCR Amplification; Resistance Genes

INTRODUCTION

Potatoes hold immense significance as a non-grain food crop on a global scale. They exhibit substantial nutritional value and remarkable productivity, making them highly advantageous in less developed nations (Islam *et al.,* 2022). Similar to numerous other agricultural crops, potatoes face difficulties from a variety of pests and diseases. Among these, the presence of potato cyst nematodes (PCN) poses a significant challenge to this crop, such as yellowing of the leaves, wilting, stunted growth, resulting in substantial yield losses annually (Meiyalaghan *et al.,* 2018; Varandas *et al.*, 2020).

Two commercially significant PCN species, namely *Globodera rostochiensis* (golden cyst nematode) and *G. pallida* (pale cyst nematode), are responsible for significant losses in potato yields worldwide (Gavrilenko

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et al., 2021). They can cause yield losses ranging from 19% to 80%, which depends on the potato variety, environmental conditions, and nematode population levels in the field. PCN can be traced back to the Andes region in South America and were introduced to Europe in the 1850s after severe Great Irish Famine (1840s) and now expanded their reach to at least 80 countries. The first detection of *G. rostochiensis* occurred in India (Ooty in Nilgiri hills) in 1961 (Prasad, 1996), while the finding of *G. pallida* occurred in 1996 (Spychalla & De Jong, 2024). Many regions of the world have imposed stringent quarantine laws in an effort to control and stop the spread of PCN once it has been detected in the field (Price *et al.*, 2021). Today, 5 pathotypes of *G. rostochiensis* (*Ro1*, *Ro2*, *Ro3*, *Ro4*, and *Ro5*) and 3 pathotypes of *Globodera pallida* (*Pa1*, *Pa2*, and *Pa3*) are known in the world *(Bairwa et al.,* 2023). As of right now, there are no chemicals on the market that are intended specially to regulate PCN except some generic nematicide.

The best way to manage PCN is to make use of host resistance (Price *et al.*, 2024). Different levels of resistance to PCN have been found in a large number of genes/QTLs and by introducing these genes/QTLs into the cultivars, potatoes with resistance levels to PCN can be produced effectively (Islam *et al.*, 2024). Since 1948, genes for resistance to PCN have been discovered in a range of wild potato species. A few genes have been introgressed in breeding lines and cultivars (Gebhardt & Valkonen, 2001). The potato has many loci of resistance to PCN on chromosomes III (*Gro1.4_QTL*), IV (*Gpa4_QTL*), V (*Gpa_QTL*, *GpaM1*, *Gpa5*, *H1*, and *Grp1_QTL*), IV (*GpaM2*), VII (*Gro1-4*), IX (*Gpa6 QTL*), X (*Gro1.2 QTL*), IX (*GpaXI* and *Gro1.3 QTL*) and XII (*GpaM3* and *Gpa2*) (Barone *et al.*, 1990; Pineda *et al.*, 1993; Gebhardt *et al.,* 1993; Kreike *et al.,* 1993, 1994, 1996; Leister *et al.,* 1996; Jacobs *et al.,* 1996; Rouppe Van Der Voort *et al.*, 1997, 1998; Bradshaw *et al.,* 1998; Caromel *et al.,* 2003, 2005; Adillah Tan *et al.*, 2009). The loci *Gpa*, *Gpa2*, *Gpa4*, *Gpa5*, *Gpa6*, *GpaXI*, *GpaM1*, *GpaM2*, and *GpaM3* provides resistance to *G. pallida*. The genes/QTLs *H1*, *GroVI*, *Gro1*, *Gro1.2*, *Gro1.3*, and *Gro1.4* provides resistance to *G. rostochiensis*. *H1* gene mapped from *Solanum tuberosum* ssp. andigena (CPC1673) located on distal end of long arm of chromosome 5 show hypersensitive reaction to *G. rostochiensis* pathotype Ro1 and Ro4. Molecular marker linked to these above resistance genes helped in the identification of lines having these genes. SCAR marker N146, N195, TG689, 57R linked to *H1* gene (Asano *et al.,* 2012; Mori *et al.,* 2011) SPUD1636 for *Gpa5* (Bryan *et al.,* 2002), TG432 for *Grp1*, X02 for *GroVI* (Jacobs *et al.,* 1996), Contig237 for *GpaIV*^{s}adg etc. were developed which can be used in the marker-assisted selection (MAS). The *H1* resistance gene has proven to be durable for more than 50 years against *G. rostochiensis* (Price *et al.,* 2024).

While bioassays provide the most conclusive evidence of resistance, MAS is a considerably more pragmatic approach for detecting resistant clones in the initial phases of the breeding pipeline (Spychalla & De Jong, 2024). Evaluating the breeding material's phenotype is a lengthy process that is influenced by environmental conditions and necessitates the preliminary vernalization of tubers (Ivanova-Pozdejeva *et al.,* 2023). Studies have demonstrated that genetic marker analysis is over ten times more cost-effective than artificial inoculation with PCN. Therefore, MAS for PCN-resistant cultivars is a primary goal in numerous potato breeding programmes. Nonetheless, there has been some observed decrease in the correlation between the predicted marker allele and the resistance phenotype. DNA markers have made it possible to identify complex loci containing several resistance genes (Totsky

et al., 2021). For cultivars with an unknown source of resistance, markers can be used to identify genes within their genomes. When it comes to potato breeding, MAS utilizes a more cost-effective selection technique compared to traditional phenotypic screening in the field (Slater *et al*., 2020). Molecular markers can be employed in selecting parent plants to enhance the efficient use of existing potato germplasm. Additionally, it can facilitate the advancement of cultivars harbouring numerous resistance genes, a process known as gene stacking or pyramiding (Bhardwaj *et al*., 2019). Determining the pedigrees of specific varieties can be challenging at times, primarily because the parental forms that were utilized in the early stages of breeding are often obscure. Consequently, it is challenging to ascertain the specific wild species from which resistance to PCN was acquired. DNA markers can be used to trace the introgression of genetic material from wild Solanum species to cultivated potatoes. In this study our objective was to perform an initial molecular screening on the existing germplasm and parent material in a potato breeding programme.

MATERIAL AND METHODS

Experimental material and site

The plant material comprising 85 potato germplasm [parental lines for biotic stress (late blight, virus and PCN) resistance breeding programme maintained at the ICAR-Central Potato Research Institute (CPRI), Shimla, Himachal Pradesh, India, were used for molecular characterization using PCN resistance genes linked markers. The collection comprised exotic potato accessions imported from various countries, advanced breeding lines, and indigenous developed potato varieties (Table 1).

Isolation, quantification and PCR amplification of genomic DNA

The total genomic DNA isolation from young leaves (50 to 100 mg of leaf tissue) was carried out using a Qiagen's DNAeasy Plant Mini Kit. DNA concentration was determined using agarose gel electrophoresis on 0.8% agarose gel and a nanodrop spectrophotometer (Thermo Scientific, US). *G. pallida* resistance was carried out using SPUD1636, Gpa2-1, Gpa2-2, and Contig237 markers, whereas *G.*

Table 1. List of potato genotypes used for the molecular characterization.

S. No.	Genotype	Source (Variety/clone number)	S. No.	Genotype	Source (Variety/clone number)
1.	CP1911	USA (B 6558-16)	44.	CP1971	NET (SATURNA)
2.	CP3774	Peru (CIP 393385.39)	45.	Kufri Neelkanth	Biofortified variety (ICAR-CPRI)
3.	CP3771	Peru (CIP 393371159)	46.	CP4630	Processing variety, Kufri Chipsona-4 (ICAR-CPRI)
4.	HR9-5	Advanced breeding clone for late blight resistance (ICAR-CPRI, Shimla)	47.	CP4105	Late blight resistant variety, Kufri Girdhari (ICAR-CPRI)
5.	CP4311	Denmark (SARPO MIRA)	48.	CP4070	Hill variety, Kufri Himalini (ICAR-CPRI)
6.	LBY-24	Advanced breeding clone for late blight resistance (ICAR-CPRI, Shimla)	49.	CP3400	Table purpose variety, Kufri Jawahar (ICAR-CPRI)
7.	CP4039	Peru (MARIELA × XY9)	50.	MP/09-68	Advanced breeding clone for processing purpose (ICAR-CPRI, Shimla)
8.	CP2186	USA (Norchip)	51.	CP2350	Canada (GEMSEG)
9.	CP3773	Peru (CIP 393371.58)	52.	CP4087	Netherlands (CMK 1997-022-017)

S. No.	Genotype	Source (Variety/clone number)	S. No.	Genotype	Source (Variety/clone number)
10.	CP4046	Peru (C 91.612 × C 92.044)	53.	CP2416	Peru (CIP 720124)
11.	LBY-15	Advanced breeding clone for late blight resistance (ICAR-CPRI, Shimla)	54.	CP2372	Peru (CIP 379706.34/LT-9)
12.	CP4505	Peru (CIP 397079.6)	55.	CP3180	Polland (ATOL)
13.	CP4494	304387.17	56.	CP2067	Peru (CIP 573275/ ASN69-1)
14.	CP4052	Peru (CIP 397029.21)	57.	CP4085	Netherlands (MELODY)
15.	SM/92-338	Bacterial wilt resistant advanced clone (HB/82-372/JEX/C-166 (Kufri Pukhraj))	58.	MP/09-73	Advanced breeding clone for processing purpose (ICAR-CPRI, Shimla)
16.	CP4042	Peru (394611.112)	59.	CP3134	Peru (CIP 720136/ SANTA CECILIA)
17.	HR9-3	Advanced breeding clone for late blight resistance (ICAR-CPRI, Shimla)	60.	CP3640	Canada (A84420-5)
18.	CP3402	Early bulking variety, Kufri Pukhraj (ICAR-CPRI)	61.	CP2419	Peru (CIP 720131/ MANTIQUEIRA)
19.	CP4057	Peru (CIP397079.6)	62.	CP1945	USA (K 194-3)
20.	Farida	Netherlands	63.	CP3646	Canada (KENNEBECK)
21.	CP4437	Ivory russet	64.	CP1917	USA (B 6581-4)
22.	Atlantic	USA	65.	CP3647	Canada (SHEPODY)
23.	CP3702	Innovator	66.	CP2294	Peru (CIP 377369.7/P-4)
24.	CP4300	USA (FL2215)	67.	CP4500	Peru (CIP 393073.197)
25.	CP4433	Colomba	68.	CP2379	Peru (CIP 575049/ CEW-69-1)
26.	CP4301	USA (FL2221)	69.	MP/2K-424	Advanced breeding clone for processing purpose (ICAR-CPRI, Shimla)
27.	Heraclea	Netherlands	70.	CP4075	USA (FL 1533)
28.	Navigator	-	71.	CP2067	Peru (CIP 573275/ ASN69-1)
29.	CP3920	CHN (Santana)	72.	HT/97-727	Advanced breeding clone for Heat tolerance (ICAR-CPRI, Shimla)
30.	CP4430	VMT 5-1	73.	CP1940	USA (K 85-6)
31.	CP4175	Peru (CIP 397006.18)	74.	CP2418	Peru (CIP 720130/ CHIQUITA)
32.	CP3636	Peru (CIP 801020/ KAGIRI)	75.	CP1909	USA (B 6532-10)
33.	CP2011	Mexico (CIP676082)	76.	CP1980	GFR (ANETT)
34.	CP4045	Peru (CIP 395112.6 (391686.15 × 393079.4))	77.	CP4398	Peru (CIP 304394.56)
35.	CP3470	Peru (CIP 385280.2/ XY.3)	78.	CP4439	Netherlands
36.	MS/08-1148	Advanced clone of Kufri Surya × CP3125 cross combination	79.	CP2379	Peru (CIP 575049/ CEW-69-1)
37.	CP2379	Peru (CIP 575049/ CEW-69-1)	80.	CP1717	France (ROSEVAL)
38.	SM/95-43	Advanced breeding clone for late blight resistance (ICAR-CPRI, Shimla)	81.	QBA/92-4	Advanced breeding clone for processing purpose (ICAR-CPRI, Shimla)
39.	CP4496	Peru (CIP 390478.6)	82.	CP2069	Peru (CIP 800144/ DTO-2)
40.	CP4043	Peru (CIP 395017.229 (393085.13 × 392639.8))	83.	CP4568	USA (LR)
41.	CP2370	Peru (CIP 378711.5/ MUZIRANZARA)	84.	MP/98-31	Advanced breeding clone for processing purpose (ICAR-CPRI, Shimla)
42.	CP4047	Peru (CIP 395193.6 (C 91.612 × C 92.030))	85.	CP3173	Polland (ELBA)
43.	CP4084	NET (CYCLOON)			

rostochiensis resistance was evaluated using TG689, 57R, U14, XO2, and TG432 markers. PCR amplification was performed in 20 µl reaction comprising: 10 µl of EmeraldAmp GT PCR Master Mix (2x Premix), 1 µl each of forward and reverse primers, 6 µl of nuclease free water and 2 µl of template DNA. Details of the markers, marker type, product size, and PCR conditions used for screening are given in Table 2.

Electrophoresis and gel documentation of amplified DNA

The amplified DNA products were separated using horizontal gel electrophoresis in an agarose gel containing ethidium bromide (10 mg/µl). The gel was run at 70 mA for 2 hours in 1× TBE buffer (pH 8.0) and visualized using a gel documentation system (BioSpectrum® Imaging System™, UK).

Data analysis

The screening was conducted by checking for the presence or absence of the desired bands. The presence of the band was scored as plus (+) and absence was scored as minus (-) and total number of genes amplified genotype wise are given in Table 3.

RESULTS AND DISCUSSION

In total, 85 potato accessions were characterized for the presence of genes/QTLs for resistance to PCN (*Gpa2*, *Gpa5*, *GpaIV^{s}adg*, *H1*, *GroVI* and *Grp1*) using specific marker assays (Gpa2-1, Gpa2-2, SPUD1636, Contig237, TG689, 57R, U14, X02, and TG432) (Table 1). The results obtained with molecular markers developed for the different resistant genes/QTLs are presented below and in Table 3.

Molecular characterization of genotypes for *G. pallida* resistance

Screening 85 accessions with the marker Gpa2-1 (fragment size of 1120 bp) and Gpa2-2 (452 bp) revealed 22 accessions positive for both the DNA markers that is diagnostic for the *Gpa2* resistant locus (Fig. 1). Both markers were amplified in PCR conditions recommended by Asano *et al.*, (2012). The locus *Gpa2* identified in *S. tuberosum* ssp. andigena CPC1673 was mapped on distal end of chromosome 12 which confers resistance to *Globodera pallida* (Pa2) (Rouppe Van Der Voort *et al.*, 1997).

Only three accessions (Atlantic, CP4439 and Heraclea) showed the presence of SPUD1636, marker linked to the QTL *Gpa5.* This call attention to the importance of

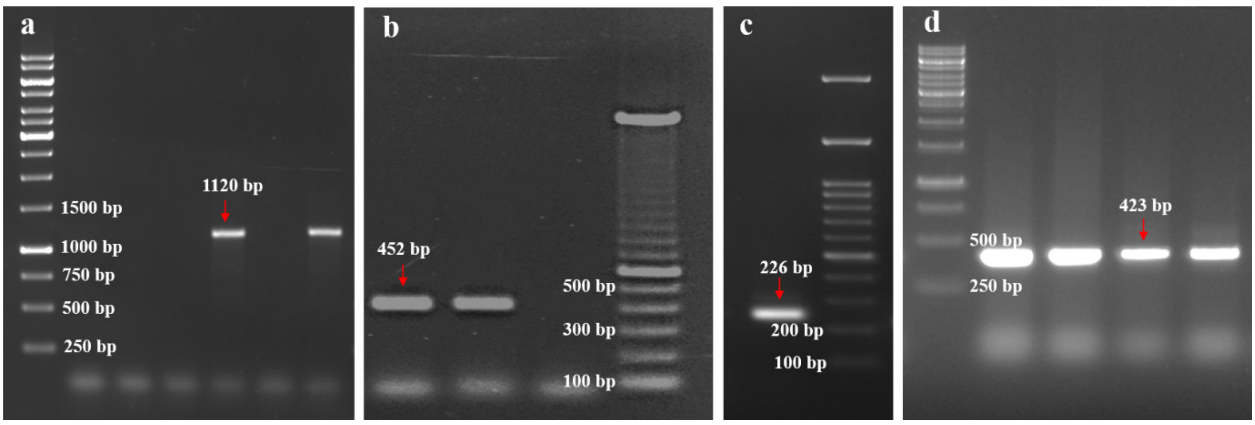

Fig. 1. Agarose gel electrophoresis showing amplification of different PCN resistant genes. a) Gpa2-1 marker-1120 bp, 1 kb ladder; b) Gpa2-2 marker-452 bp, 100 bp ladder; c) SPUD1636 marker-226 bp, 100 bp ladder; and d) Contig 237 marker-423 bp, 1 kb ladder.

Table 2. Details of the molecular markers used for characterization.

Potato cyst nematodes	Genes/ QTL	Marker	Marker type	Fragment size (bp)	Primer sequence (5' to 3')	PCR conditions	Reference
G. pallida	Gpa2	Gpa2-1	STS	1120	F- TTTAGCACGGAATGTGGGGA R- GTTTCCCCATCAAAACTCAC	94°C-10 min, (94°C-45 s, 60°C-1 min, 72°C-1 min) 35 cycles, 72°C-5 min	Asano *et al.*, 2012
		Gpa2-2	STS	452	F- GCACTTAGAGACTCATTCCA R- ACAGATTGTTGGCAGCGAAA	94°C-10 min, (94°C-45 s, 60°C-1 min, 72°C-1 min) 35 cycles, 72°C-5 min	Asano *et al.*, 2012
	Gpa5	SPUD 1636	PCR	226	F-GTGCCGCACAGGGTAAAACC R-ACCTTAGCGGATGAAAGCC	94°C-3 min, 94°C-30 s, 65°C-1 min, 72°C-1 min, (94°C, 30 s, 65°C decreasing the annealing temp. to 60°C by 1°C per cycle, 30 s; 72°C 30 s) 5 cycles, (94°C, 30 s, 60°C, 30 s, 72°C 30 s) 24 cycles; 72°C, 3 min	Bryan *et al.*, 2002
	GpaIV^{s} adg	Contig 237	CAPS/ TaqI	423	F- GCAGTCCTAATTGCACGTAACA R- CTTACTTGGGCAACCCAGAAT	94°C-10 min, (94°C-1 min, 54°C-1 min, 72°C-1 min) 35 cycles, 72°C-5 min	Asano *et al.*, 2012
G. rostochiensis	H1	TG689	SCAR	141	F-TAAAACTCTTGGTTATAGCCTAT R-CAATAGAATGTGTTGTTTCACCAA	94°C-2 min, (94°C-20s, 55°C-20s, 72°C-30s) 35 cycles, 72°C-5 min	Milczarek *et al.*, 2011
		57R	SCAR	450R	F-TGCCTGCCTCTCCGATTTCT R-GGTTCAGCAAAAGCAAGGACGTG	95°C-3 min, (94°C-30 s, 63°C-20 s, 72°C-1 min) 35 cycles, 72°C-3 min	Milczarek *et al.*, 2014
	GroV1	U14	SCAR	366	F-GGGCTTGTATAAGACCTCCGAGAGG R-CCCTTCCTTGGGTAGTTTGAGCG	92°C-7 min, (92°C-1 min, 57°C-1 min, 72°C-2 min) 25 cycles, 72°C-5 min	Jacobs *et al.*, 1996
		XO2	SCAR	854	F-CCACCAAACCCATAAAGCTGC R-TGTGAATTGGTATGAATCTGCAACC	92°C-7 min, (92°C-1 min, 55°C-1 min, 72°C-2 min) 25 cycles, 72°C-5 min	Jacobs *et al.*, 1996
	Grp1	TG432	CAPS/ RsaI	1900	F-GGACAGTCATCAGATTGTGG R-GTACTCCTGCTTGAGCCATT	94°C-3 min, (94°C-30 s, 66°C-45 s, 72°C-2 min) 35 cycles, 72°C-5 min	Asano *et al.*, 2012

Table 3. Evaluation of genotypes based on molecular markers.

Accession Number	Markers linked to *G. pallida* resistance genes				Markers linked to *G. rostochiensis* resistance genes					Total No of marker
	Gpa2_ QTL-Gpa2-1	Gpa2_ QTL-Gpa2-2	Gpa5-SPUD 1636	Contig237	H1-TG689	H1-57R	GroVI_ Ro1-U14	GroV1 -xo2	Grp1_ QTL-TG432	
CP-1911	-	-	-	+	-	-	-	+	-	2
CP-3774	-	-	-	+	-	-	-	-	-	1
CP-3771	+	+	-	+	-	-	-	-	+	4
HR9-5	+	+	-	+	-	-	-	+	+	5
CP-4311	-	-	-	+	-	-	+	+	+	4
LBY-24	+	+	-	+	-	-	+	-	-	4
CP-4039	-	-	-	+	-	-	+	+	-	3
Norchip	+	+	-	+	-	-	-	+	-	4
CP-3773	-	-	-	+	-	-	+	+	-	3
CP-4046	-	-	-	+	+	+	+	+	-	5
LBY-15	-	-	-	+	-	-	-	-	-	1
CP-4505	+	+	-	+	-	-	-	-	-	3
CP-4494	-	-	-	+	-	-	-	+	-	2
CP-4052	+	+	-	+	+	+	+	+	-	7
SM/92-338	-	-	-	+	-	-	+	+	-	3
CP-4042	-	-	-	+	+	+	-	+	-	4
HR9-3	+	+	-	+	-	-	-	+	-	4
K.Pukhraj	-	-	-	+	-	-	+	+	-	3
CP-4057	+	+	-	+	+	+	+	+	-	7
Farida	-	-	-	+	-	+	-	+	-	3
Ivory russet	+	+	-	+	-	+	+	+	-	6
ATL	-	-	+	+	+	-	-	+	-	4
Innovator	-	-	-	+	-	-	-	+	-	2
FL-2215	-	-	-	+	+	-	-	+	-	3
Colomba	-	-	-	+	+	-	-	+	-	3
FL-2221	+	+	-	+	+	-	-	+	-	5
Heraclea	-	-	+	+	+	-	-	+	-	4
Navigator	-	-	-	+	+	-	-	+	-	3
Santana	-	-	-	+	-	-	-	-	-	1
VMT 5-1	-	-	-	+	-	-	+	+	-	3
CP-4175	+	+	-	+	-	-	+	-	-	4
CP-3636	-	-	-	+	-	-	-	+	-	2
CP-2011	-	-	-	+	-	-	-	+	-	2
CP-4045	+	+	-	+	-	-	-	-	-	3
CP-3470	+	+	-	+	-	-	+	-	-	4
MS/08-1148	-	-	-	+	-	-	-	+	-	2
CP-2379	-	-	-	+	+	-	-	-	-	2

Accession Number	Markers linked to *G. pallida* resistance genes				Markers linked to *G. rostochiensis* resistance genes					Total No of marker
	Gpa2_ QTL-Gpa2-1	Gpa2_ QTL-Gpa2-2	Gpa5-SPUD 1636	Contig237	H1-TG689	H1-57R	GroVI_ Ro1-U14	GroV1 -xo2	Grp1_ QTL-TG432	
SM/95-43	-	-	-	+	-	-	-	-	-	1
CP-4496	+	+	-	+	-	-	+	+	-	5
CP-4043	-	-	-	+	-	-	+	-	-	2
CP-2370	+	+	-	+	-	-	-	+	-	4
CP-4047	+	+	-	+	-	-	-	+	-	4
CP-4084	-	-	-	+	-	-	+	+	-	3
K. Neelkanth	-	-	-	+	-	-	+	-	-	2
CP4630 (K. Chipona-4)	-	-	-	+	+	-	-	+	-	3
CP4105 (K. Girdhari)	-	-	-	+	-	-	+	-	-	2
CP4070 (K. Himalini)	-	-	-	+	-	-	+	+	-	3
CP3400 (K. Jawahar)	-	-	-	-	+	-	-	+	-	2
MP/09-68	-	-	-	+	-	-	+	-	-	2
CP-2350	-	-	-	+	-	-	+	-	-	2
CP-4087	+	+	-	+	+	+	-	-	+	6
CP-2416	-	-	-	+	-	-	-	+	-	2
CP-2372	-	-	-	+	-	-	-	-	+	2
CP-3180	-	-	-	+	-	-	+	+	-	3
CP-2067	-	-	-	+	-	-	+	-	-	2
CP-4085	-	-	-	+	-	-	+	+	-	3
MP/09-73	-	-	-	+	-	-	-	+	-	2
CP-3134	-	-	-	+	+	+	+	-	-	4
CP-3640	-	-	-	+	-	-	+	+	-	3
CP-2419	-	-	-	+	+	+	-	+	+	5
CP-1971	-	-	-	+	-	-	-	+	-	2
CP-3646	-	-	-	+	-	-	-	-	-	1
CP-1917	+	+	-	+	+	-	-	-	-	4
CP-3647	-	-	-	+	-	+	+	+	+	5
CP-2294	-	-	-	+	-	-	+	+	-	3
CP-4500	-	-	-	+	-	-	-	-	-	1
CP-2379	-	-	-	+	-	-	-	+	-	2
MP/2K-424	-	-	-	+	-	-	-	+	+	3
CP-4075	+	+	-	+	+	-	+	+	-	6
CP-2067	-	-	-	+	-	+	-	-	-	2
HT/97-727	-	-	-	+	-	-	+	+	-	3
CP-1940	-	-	-	+	-	-	+	-	-	2
CP-2418	+	+	-	+	+	+	-	+	-	6
CP-1909	-	-	-	+	-	-	-	+	-	2
CP-1980	+	+	-	+	-	-	-	-	-	3

Accession Number	Markers linked to *G. pallida* resistance genes				Markers linked to *G. rostochiensis* resistance genes					Total No of marker
	Gpa2_ QTL-Gpa2-1	Gpa2_ QTL-Gpa2-2	Gpa5-SPUD 1636	Contig237	H1-TG689	H1-57R	GroVI_ Ro1-U14	GroV1 -xo2	Grp1_ QTL-TG432	
CP-4398	-	-	-	+	-	-	-	+	-	2
CP-4439	-	-	+	+	-	-	+	+	-	4
CP-2379	-	-	-	+	-	-	-	+	-	2
CP-1717	-	-	-	+	-	-	-	-	+	2
QBA/92-4	-	-	-	+	-	-	-	+	+	3
CP-2069	-	-	-	+	+	-	+	+	-	4
LR/4568	-	-	-	+	-	+	-	-	-	2
MP/98-31	-	-	-	+	-	-	-	+	-	2
CP-3173	+	+	-	+	-	-	-	+	+	5
CP-1945	-	-	-	+	+	+	-	+	+	5
Total number of breeding germplasm with markers	22	22	03	85	21	14	33	57	12	

introduction of varieties having SPUD1636. *Gpa5* locus maps to chromosome 5 which acts additively with *Gpa6* locus (chromosome 9) to confer durable resistance to *G. pallida*. (Bryan *et al*., 2002a) developed PCR-based marker, SPUD 1636 (Fig. 1) from AFLP marker which can detect the *Gpa5* locus and detect the chromosomal segment carrying the *S. vernei* derived QTL conferring resistance to *G. pallida*.

Except one variety Kufri Jawahar, all the accessions were positive for the Contig237 marker, linked to *GpaIV^{s}adg* resistance locus. This indicates that this QTL has been widely used in resistant breeding programme. QTL mapping of resistance to *G. pallida* allocated a QTL (*Gpa4*) to chromosome IV (Bradshaw *et al*., 1998; Gebhardt & Valkonen, 2001). SNP based marker Contig237 (Fig. 1) is linked to *GpaIV^{s}adg*, imparting resistance against *G. pallida* pathotype Pa2/3 (Moloney *et al.,* 2010).

For molecular screening purposes we have used 5 markers linked to *G. pallida* resistance genes (4 exclusively for *G. pallida* and one for both PCN species). In the present study, out of all accessions four accessions (CP3771, HR9-5, CP4087 and CP3173) exhibited presence of 4 marker (except SPUD1636) linked to *G. pallida* resistance genes. Likewise, 18 accessions were positive for either 3 markers. One accession (Kufri Jawahar) was found having no resistance gene linked to *G. pallida*. In our previous study we have found multiple accessions showing the presence of 2 or more marker for *G. pallida* resistance (Mangal *et al*., 2023). To search for potato cultivars bred in Russia with multiple resistance to PCN, Gavrilenko *et al*., (2021) used marker associated with four loci: *Gpa2*, *GpaVvrn_QTL*, *GpaVs spl_QTL*, *Grp1_QTL* and found several domestic cultivars with multiple PCN resistance. Dalamu *et al*., (2017) used two markers (HC and SPUD1636) which were linked to *G. pallida* resistance genes/QTL and observed many genotypes having both markers together.

Molecular characterization of genotypes for *G. rostochiensis* resistance

In our study, applying the specific primers TG689 and 57R for the resistance

gene *H1* gave the presence of expected product size for 21 and 14 accessions respectively out of 85 tested accessions (Fig. 2). The *H1* gene was derived from *S. tuberosum* ssp. andigena (CPC1673) in 1952 which confers nearly complete or durable resistance to *G. rostochiensis* pathotypes Ro1 and Ro4 (Gebhardt *et al.*, 1993) and has been extensively introgressed into commercial potato cultivars. According to Finkers-Tomczak *et al.* (2011) the molecular marker 57R was found to be closely linked to *H1* in a cross between the diploids SH83–92-488 and RH89–039-16. Similarly, marker TG689 has been utilized for *H1* PCN resistance screening, demonstrating a high congruence between the marker assay and the PCN-resistance phenotype (Biryukova *et al.*, 2008). Milczarek *et al.* (2014) found that the 57R and TG689 markers showed over 90% agreement with phenotypic tests, confirming their effectiveness in selection but the use of 57R leads to a reduction in the number of susceptible recombinants as compared with TG689. This is advantageous for breeding purposes because it is preferable

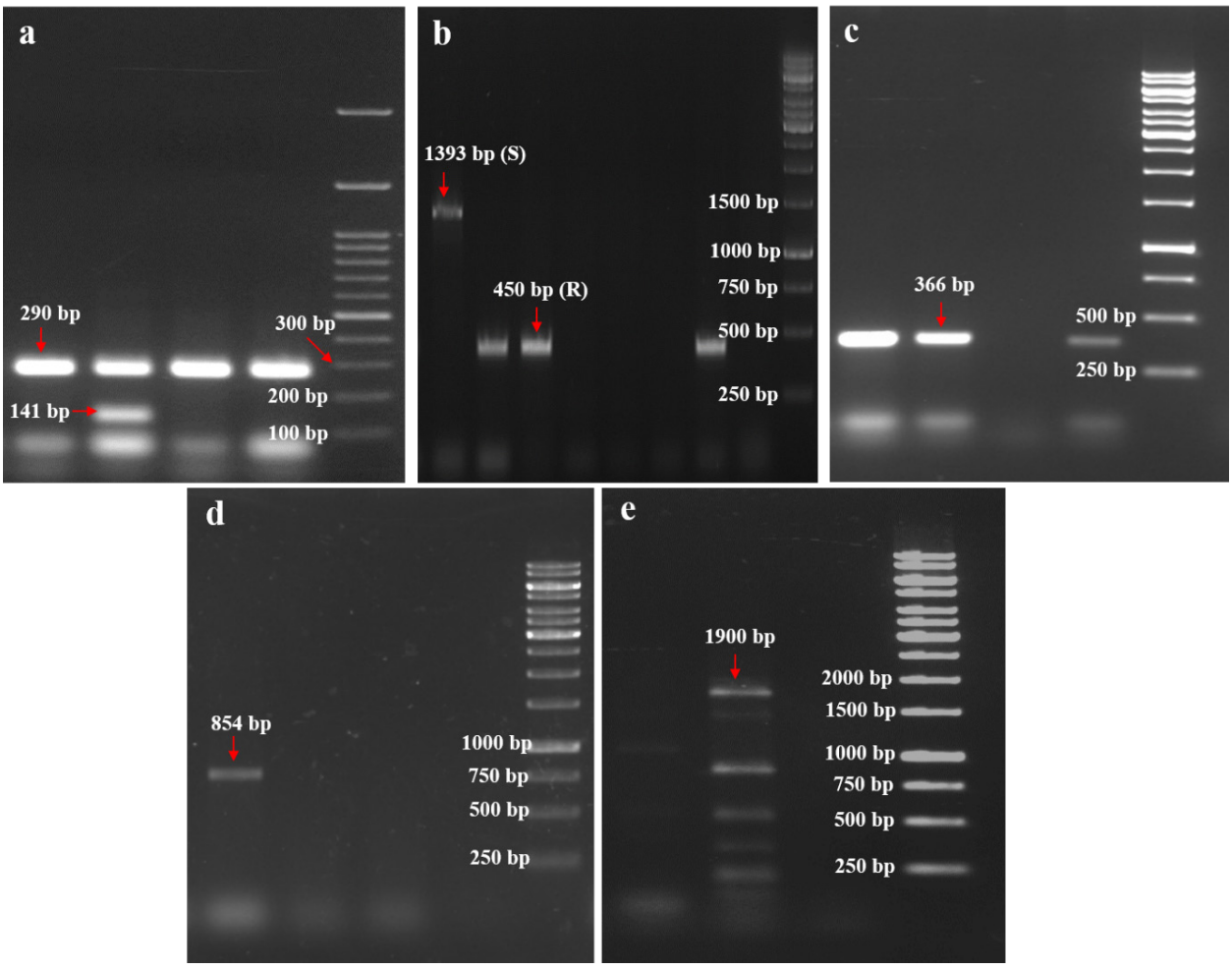

Fig. 2. Agarose gel electrophoresis showing amplification of different PCN resistant genes. a) TG689 marker-141, BCH (positive control)-290 bp, 100 bp ladder; b) 57R marker-450 bp (Resistant) and 1393 bp (Susceptible), 1kb ladder; c) U14 marker-366 bp, 1 kb ladder; d) XO2 marker-854 bp, 1 kb ladder; and e) TG432 marker-1900 bp, 1kb ladder.

to discard susceptible clones early on rather than mistakenly classify them as resistant and maintain them for further selection. Similarly, Schultz *et al.* (2012) used these markers in Australian and Scottish potato breeding programmes, Park *et al.* (2018) in New York breeding clones, and Whitworth *et al.* (2018) in advanced breeding population and observed same results. Zoteyeva *et al.* (2020) identified the good correlation of the marker 57R with resistance to PCN in the screening of interspecific potato hybrids. Ivanova-Pozdejeva *et al.* (2023) compared the applicability of *H1* gene markers TG689 and 57R in Estonian cultivars and other breeding clones and found 57R marker suitable for implementing genetic testing for nematode resistance. Meiyalaghan *et al.* (2018) designed and used two probe-based HRM markers (57R_1P and TG689_1P) for identifying resistance to *G. rostochiensis* pathotype Ro1 in 155 potato accessions. From this study they concluded that the HRM markers were more effective in screening as compared to conventional SCAR (57R and TG689) marker and found poor transferability for the SCAR markers. Totsky *et al.* (2021) screened GenAgro potato collection of ICG SB RAS using known diagnostic PCR markers and found 57R, a highly reliable marker and also suggests the need to use this marker when selecting samples resistant to PCN. Marker TG689 was successfully amplified in 47 out of 60 resistant cultivars (Milczarek *et al.*, 2011b). Additional studies have confirmed that 57R is a valuable marker, demonstrating over 90% concordance in various analyses. Dalamu *et al.*, (2023) screened 94 native potato accessions and observed 3 and 5 accessions positive for TG689 and 57R, respectively. Galek *et al.* (2011) demonstrated that testing for the presence of *H1* gene is highly useful before conducting bioassays, as the marker is much simpler to utilize.

Marker U14 and X02 linked with the gene *GroVI* was amplified in 33 and 57 accessions, respectively (Fig. 2). The *S. vernei* derived *GroV1* locus (long arm of chromosome 5) was found in the same region of the potato genome as the *H1* nematode resistance locus (Jacobs *et al*., 1996). Two reported SCAR markers were developed by converting the RAPD markers X02 and U14, which flank *GroVI*, generating amplicons of 854 bp and 366 bp, respectively (Gartner *et al*., 2021; Jacobs *et al*., 1996). The DNA marker of gene *GroVI* can be used as an indicator of the presence of genetic material of *S. vernei* in potato varieties. Bhardwaj *et al*. (2019) found 6 genotypes positive for X02 marker out of 12 genotypes studied.

CAPS marker TG432 (linked to *Grp1* locus located on short arm of chromosome V) was detected in 12 accessions out of 85 tested accessions with broad spectrum resistance levels to both *G. rostochiensis* (Ro5) and *G. pallida* (Pa2/3) pathotype (Finkers-Tomczak *et al*., 2009; Rouppe Van Der Voort *et al*., 1998).

For molecular screening of accessions having *G. rostochiensis* resistance genes we have used 5 markers (4 exclusively for *G. rostochiensis* and one for both PCN species). Out of all accessions six accessions (CP4046, CP4052, CP4057, CP2419, CP3647 and CP1945) exhibited presence of either 4 markers linked to *G. rostochiensis* resistance genes. Likewise, 8 accessions were positive for either 3 markers. Ten accessions were found having no resistance gene linked to *G. rostochiensis*. In our previous study we have found multiple accessions showing the presence of 2 or more marker for *G. rostochiensis* resistance (Mangal *et al*., 2023). Out of 60 *G. rostochiensis* resistant cultivar screened, Milczarek *et al.*, (2011) found 18 cultivars which exhibited presence of at least two marker linked to resistance genes.

Combined resistance to both PCN species

In the present investigation total nine markers linked to PCN were used for molecular screening purpose. Based on molecular markers analyses we have identified two accessions CP4052 and CP4057 which exhibited presence of seven resistance markers. Four accessions (Ivory russet, CP4087, CP4075 and CP2418) showed the presence of either 6 markers. Likewise, seven, sixteen, twenty-two, twenty-seven, and seven accessions showed presence of 5, 4, 3, 2 and 1 markers, respectively. Similarly, Dalamu *et al*. (2017) identified elite potato genotypes CP1843, CP1879, and JEX/A-267 that can be utilized as parental lines for introgression of multiple resistant genes against both PCN species. Sharma *et al*. (2014), Slater *et al*. (2016), Bhardwaj *et al*. (2019), Mangal *et al*. (2023) used different markers in their breeding programme linked to PCN resistance genes and found genotypes having multiple resistance genes.

CONCLUSION

PCN are a significant global pest. Breeding potato cultivars with resistance to these pests is the most sustainable method of control. Before choosing parents for PCN resistance breeding programme, their molecular characterization with resistance genes linked marker will improve the efficiency of breeding programme. It will not only the save the time but it will help in the identification of parental lines having multiple resistance genes. This strategy is particularly valuable for resistance breeding against *G. pallida* due to the absence of any identified single dominant gene that provides high levels of resistance against this species. The genotypes identified in the current study having maximum resistance genes can be used in the hybridization programme. These genotypes also can be used for cultivar development after evaluation of their agronomic and yield characters. While using DNA markers for only a few genes cannot entirely replace extensive laboratory testing, it does provide a simple and reliable method for selecting potato forms resistant to this parasite in a shorter time. This approach significantly reduces the number of genotypes in the sample for further breeding. Various researchers have used different markers linked to PCN resistance genes in their breeding programmes, resulting in improved selection efficiency. They recommend elite potato genotypes that can be used as parental lines for the introgression of resistance genes against both PCN species.

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