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ORIGINAL RESEARCH ARTICLE

Diagnostic PCR-based markers for biotic stress resistance breeding in potatoes (*Solanum tuberosum* L.)

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Abstract

Potato is the third most important crop. Its production and quality is greatly affected by various biotic stresses. In this study, 216 *Solanum tuberosum* subsp. *tuberosum* accessions were screened for late blight, viruses (PVY and PVX) and potato cyst nematodes (PCNs) resistance using robust molecular markers. Solitary or combined late blight resistant genes (*R1*, *Rpi-abpt*, *R3a*, *R3b*) presence was observed in 51 accessions. For PVY resistance, markers STM0003, RYSC3, and YES3-3 A were found in 38, 3, and 1 accession, respectively, and PVX resistance gene markers 1Rx1 and 106Rx2 were found in 19 and 10 accessions, respectively. The STM0003 marker was present in 38 accessions, of which 12 were resistant to PVY and 10 were susceptible. The genotype data on six markers for both the species of PCN showed 45 resistant accessions. A combined analysis of all markers for late blight identified six accessions with all the three resistance genes. Similarly, four accessions, i.e., CP 4226, CP 2049, CP 3696, and CP 3651 had both *Rx1* and *Rx2* genes for PVX resistance, while only one accession, viz., CP 2049 showed the presence of both H1 and HC genes along with the Gpa2 marker for PCN resistance. There were also seven resistance genes found in CP 2049 for late blight (2), PCN (4), and PVX (2), but none of the accession had genes for all four traits. The results revealed that developing potato varieties with combined resistance to late blight, viruses, and PCN requires concerted hybridization between different parental lines.

Keywords DNA markers · *Globodera rostochiensis* · Late blight · Potato virus Y (PVY) · Potato virus X (PVX) · Marker-assisted selection (MAS)

Introduction

Potato (*Solanum tuberosum* L.) is the third most important food crop after wheat and rice, and it is an important source of carbohydrates. Globally, pests and pathogens cause yield losses of up to 17.2% in potatoes (Savary et al. 2019). Among all the biotic stresses, late blight, viruses, and potato cyst nematodes (PCNs) cause heavy yield losses in potatoes. Host resistance is the best strategy to manage the losses caused by these diseases and pests globally. Phenotypic screening of breeding populations for diseases and pests requires specialized facilities, resources, and considerable time. The breeding populations consisting of thousands of plants do not provide sufficient time for phenotypic screening against multiple biotic stresses to select the best accessions. Further, the quarantine pest screening could only be carried out in specific locations. Thus, using robust molecular markers linked to disease and pest resistance genes, make it possible to screen germplasm and identify resistant lines that can be used for breeding and advancing selected progenies.

Phytophthora infestans, which causes late blight of potatoes, is a highly destructive pathogen that affects potatoes throughout the world, including India. This is an airborne, diploid to polyploid, heterothallic pathogen having two mating types (A1 and A2). It causes heavy losses in all potato-growing regions. Late blight is a major problem in Indian hills and plateau regions and complex races have been

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recorded across the country (Sharma et al. 2016). Thus, it becomes necessary to check for the presence of late blight resistance genes in all advanced clones of potatoes before they are released. Several late blight resistance broad-spectrum resistance genes have been mapped in several potato species like *Solanum demissum* (*R1* to *R11*) (Huang et al. 2005; Bradshaw et al. 2006; Hein et al. 2009), S. *bulbocastanum* (*RB/Rpi-blb1, Rpi-blb2, Rpi-blb3, Rpi-bt1, Rpi-apbt*), *S. americanum* (*Rpi-amr1*, *Rpi-amr3*) (Lokossou et al. 2009; Oosumi et al. 2009), S. *pinnatisectum* (*Rpi1, Rpi2* and *Pi-Blatt*) (Yang et al. 2017; Nachtigall et al. 2018), S. *stoloniferum* (*Rpi-sto1, Rpi-sto2*) (Vleeshouwers et al. 2008; Wang et al. 2008), S. *verrucosum* (*Rpi-ver1*) (Chen et al. 2018) etc., and some of these have been used successfully in marker assisted selection (MAS) for variety development. Besides, few more molecular markers linked to late blight resistance genes are available which have tremendous scope for MAS in comparison to time-consuming phenotypic screening (Tan et al. 2010; Yang et al. 2017).

Potato cyst nematodes (PCNs) are soil-inhabiting plant parasitic nematodes of solanaceous species, including potatoes. There are two species of PCN, i.e., *Globodera rostochiensis* and *G. pallida*, which are mainly responsible for economic losses in potatoes. *G. rostochiensis* and *G. pallida* have been subdivided into 5 (*Ro1, Ro2, Ro3, Ro4*, *Ro5*) and 3 (*Pa1, Pa2*, *Pa3*) pathotypes, respectively. Most of these pathotypes at Ooty in Tamil Nadu hills have been identified and confirmed through host differentials (Krishna Prasad 2006). Until now, many PCN resistance genes have been mapped in different potato species, which show resistance to either one or two pathotypes of two PCN species. Resistance genes/quantitative trait locus (QTLs) were found in *S. tuberosum* ssp. *andigena* (*H1*, *H3*, *Gpa2*) (Bakker et al. 2004; J. Bryan et al. 2004), S. *multidissectum* (*H2*) (Strachan et al. 2019), S. *vernei* (*Gpa5, Gpa6*, *Gro6*) (Jacobs et al. 1996; van der Voort et al. 1998), S. *spegazzinii* (*Gro1.2, Gro1.3, Gro1.4*, *Gpa, GpaM1, GpaM2, GpaM3*) (Paal et al. 2004; Caromel et al. 2005)d *tarijense* ($GpaXI^{l}_{tar}$) (Tan et al. 2009). Functional characterization of these resistance genes helped in identifying linked molecular markers which can be used directly in marker-assisted selection (MAS). A list of molecular markers like SCAR (N146, N195, TG689, and 57 R) (Mori et al. 2011; Asano et al. 2012, 2021; Milczarek et al. 2014, 2021), CAPS (TG432), STS (*Gro1-4-1*), and SNP (*HC*) are available which are linked to different PCN resistance genes.

Plant viruses pose another major problem in cultivating potato in sub-tropical plains affecting both its quality and yield. There are more than 50 viruses that affect potato production worldwide (Lal et al. 2021), but six viruses including Potato leaf roll virus (PLRV), Potato Virus Y (PVY), Potato Virus X (PVX), Potato Virus A (PVA), Potato Virus S (PVS), and Potato Virus M (PVM), are most important in terms of distribution and their effect on yields (Ahmadvand et al. 2013). Many resistance genes were mapped in different potato species for potato viruses resistance, which showed extreme and hypersensitive resistance reactions. Extreme resistance genes for PVY have been reported in *S. tuberosum* ssp. *andigenum* (Ry_{adg}) (Kasai et al. 2000), S. *stoloniferum* (Ry_{sto} and Ry_{fsto}) (Flis et al. 2005; Song et al. 2005; Witek et al. 2006), S. *chacoense* (Ry_{chc}) (Sato et al. 2006). Hypersensitive resistance (HR) genes were found in *S. tuberosum* (Ny_{tbr}, *Ny-1* and *Ny-2*) (Celebi-Toprak et al. 2002; Szajko et al. 2014), S. *sparsipilum*(Nc_{spl}) (Moury et al. 2011), and Sarpo Mira (*Ny-Smira*) (Tomczyńska et al. 2014). For PVX resistance, genes were mapped in *S. tuberosum* ssp. *andigenum* (Rx_{adg}), *S. acaule* (Rx_{acl}), *S. chacoense* (Nx_{chc}) and *S. phureja* (Nx_{phu}). All major known strains of these viruses have been reported in India (Krueze et al., 2020). Several studies have shown that the efficiency of potato breeding for resistance to biotic stresses, namely late blight, virus and PCN resistance can be improved by using markers tightly linked to the aforementioned resistance genes (Sharma et al. 2014; Sudha et al. 2016; Bhardwaj et al. 2019; Sood et al. 2022). Keeping this in view, the objectives of the present study were (i) to identify parental potato lines possessing multiple disease resistance genes for exploitation in potato breeding programs and (ii) to test the suitability of several diagnostic markers for disease resistance in potato breeding.

Materials and methods

The plant material used for late blight, viruses and PCN screening consisted of 216 potato accessions maintained at the National Germplasm Repository at Shimla (Himachal Pradesh), India. This collection included exotic potato germplasm accessions imported from other countries, which are currently used as parental lines, Indian potato varieties, and advanced breeding lines (Table S1).

Phenotype data

Every year, 50–100 potato germplasm accessions are screened for late blight, viruses and PCN resistance at the Central Potato Research Institute, Shimla and its regional stations across the country as a part of regular activity. Phenotypic data evaluated at CPRI Shimla, Kufri, Shillong, and Ooty during 2000–2020 on germplasm accessions resistance to late blight, viruses, and PCN was compiled for marker validation (Kumar et al. 2005: unpublished data).

Screening for late blight resistance

Potato accessions were categorized from susceptible to highly resistant by taking four observations at weekly intervals for late blight incidence under natural epiphytotic conditions and converted into area under disease progress curve (AUDPC). Susceptible and resistant controls were planted in each season for comparison and categorization of accessions into various categories from susceptible to highly resistant.

Screening for resistance to potato virus Y (PVY) and potato virus X (PVX)

Plant material was grown in a glasshouse at CPRI, Shimla every year during summer (March-June) from 2000 to 2020 at temperature ranging from 18 to 22 °C. PVY and PVX strains were mechanically inoculated into plants 20 days after emergence as per details provided in previous studies and categorized into susceptible or resistant categories (Sharma et al. 2014; Bhardwaj et al. 2019).

Screening for resistance to potato cyst nematodes (PCNs)

PCN resistance was investigated at two locations, i.e., Shimla and Ooty. Plants were screened for both species of PCN using conventional root-ball approach (Krishna, 2006) under glasshouse conditions at 20–22 °C from 2000 to 2020. Five tubers of each clone were planted in 10 cm diameter pots in soil containing 200–250 cysts per 100 g of soil of the PCN species (*G. rostochiensis* and *G. pallida*), providing 8,000–10,000 eggs and larvae per test tuber. The number of females on the root balls of various cultures was counted after 45–60 days when the females were visible on the root balls. The color of developing females was used to distinguish between two *Globodera* species: white for *G. pallida* and yellow for *G. rostochiensis*. The clones were categorized based on the scheme shown in Table 1.

Table 1 Categories for assessing PCN resistance

Females/root ball	Grade	Resistance*
None	0	HR
1–5 females	1	R
6–20 females	2	MR
21–50 females	3	S
> 50 females	4	HS

*HR: Highly Resistance, MR: Moderately Resistance, R: Resistance, S: Susceptible, HS: Highly Susceptible

Genotyping

Genotyping of the accessions was carried out in the year 2020 at ICAR-CPRI, Shimla. The accessions were grown in pots in the greenhouse and tender leaves were collected for DNA isolation. Total genomic DNA was isolated using the Qiagen Kit protocol (Qiagen, India). The concentration of DNA was determined on 0.8% agarose gel electrophoresis using a known amount of DNA as a reference and using a nanodrop spectrophotometer (Thermo Scientific, US). Details of markers used for each trait screening are presented in Table 2. An Applied Biosystems Thermal Cycler was used to carry out the PCR, performed in 20 µl reaction volume with 2 µl template DNA, 1 µl each of forward and reverse primers, 10 µl of 2x PCR master mix (EmeraldAmp® GT PCR Master Mix) and rest 6 µl of distilled water. The amplified fragments were resolved on 1.2% agarose gel depending on the size of the specific fragment using a horizontal gel electrophoresis system. The screening was done based on the presence or absence of the desired bands.

Results

The results of phenotypic screening and molecular markers linked to various resistance genes for late blight, viruses (PVY and PVX), and PCN were compared for effective use of molecular markers for MAS in potato breeding in India (Table S2). All 216 accessions were examined for the presence of markers/genes conferring resistance to late blight (*R1*, *Rpi-abpt*, *R3a*, *R3b*), PVY (Ry_{adg}, Ry_{sto}, STM0003), PVX (Rx1, 106Rx2) and potato cyst nematodes (TG689, SPUD1636, HC, *Gro-VI-XO2*, GPA2-1, GPA2-2), and the markers results were compared with the phenotype data to evaluate the suitability of molecular markers in potato germplasm screening to various biotic stresses.

Screening for late blight resistance

The phenotypic data of 175 out of 216 accessions was available for late blight disease resistance. There were 89 accessions which showed a resistant phenotype, while 86 accessions displayed a susceptible reaction (Table 3). Genotyping of 216 accessions using LB resistance gene-linked markers revealed 51 accessions with at least one or the other marker present. Of these 51 accessions, phenotype data were available for 23 accessions, which exhibited complete agreement with genotypic data except for one accession CP 2388, which showed susceptible reaction to late blight. Genetic analysis of marker CosA revealed 17 genotypes positive for the fragment of DNA that is diagnostic for the *R1* gene, of which two were resistant to late blight, five

Table 2 Details of the markers used for germplasm screening

Biotic Stress	Marker	Gene	Marker type	Fragment size	Primer sequence	Reference
Late Blight	CosA	*R1*	SCAR	250	F-CTCATTCAAAATCAGTTTTGATC R-GAATGTTGAATCTTTTTGTGAAGG	Gebhardt et al. 2004
	R2	*Rpi-abpt*	SCAR	686	F- ACGGCTTCTTGAATGAA R- GCTCCTGATACGATCCATG	Kim et al. 2012
	R3 1380	*R3a*	SCAR	1380	F-GCTTCCGACATGTATTGATCTCCC R-GGCAGCCACTTCAGCTTCTTACAG	Sokolova et al. 2010
	cLET5E4	*R3b*	CAPS	310	F-CCAGGCATGCTCAATTTGGAGT R-TTCCCTGTTTGGACTACTTGTGGA	Huang et al. 2005
PVY	RYSC3	Ry_{adg}	SCAR	320	F-ATACACTCATCTAAATTTGATGG R-AGGATATACGGCATCATTTTTCCGA	Kasai et al. 2000
	YES3-3 A	Ry_{sto}	STS	341	F-TAACTCAAGCGGAATAACCC R-AAATTCACCTGTTTACATGCTTCTTGTG	Song and Schwarz-fischer 2008
	STM0003	Ry_{sto}	SSR	111	F-GGAGAATCATAACAACCAG R-AATTGTAACTCTGTGTGTGTG	Valkonen et al. 2008
PVX	1Rx1	*Rx1*	SCAR	974	F- GGAGAAATCCTGCAATATAAT R- CGACCGAACTTACATTTTCCC	Ahmadvand et al. 2013
	106Rx2	*Rx2*	SCAR	543	F- GGAGAAATCCTGCAATGTAAC R- CTTGTCAAAGAAAGAAGGCCT	Ahmadvand et al. 2013
G. rostochiensis	Gro-VI-XO2	*GroVI*	SCAR	854	F-CCACCAAACCCATAAAGCTGC R-TGTGAATTGGTATGAATCTGCAACC	Jacobs et al. 1996
	TG689	*H1*	SCAR	141	F-TAAAACTCTTGGTTATAGCCTAT R-CAATAGAATGTGTTGTTTCACCAA	Milczarek et al. 2011
G. pallida	SPUD1636	*Gpa5 QTL*		226	F-GTGCGCACAGGGTAAAACC R-ACCTTAGCGGATGAAAGCC	Bryan et al. 2002
	HC	*RGpS-vrnHC*	SNP	276	F-ACACCACCTGTTTGATAAAAAACT R-GCCTTACTTCCCTGCTGAAG	Sattarzadeh et al. 2006
	GPA2-1	*Gpa2*	STS	1120	F- TTTAGCACGGAATGTGGGGA R- GTTTCCCCATCAAAACTCAC	Asano et al. 2012
	GPA2-2	*Gpa2*	STS	452	F- GCACTTAGAGACTCATTCCA R- ACAGATTGTTGGCAGCGAAA	Asano et al.2012

Table 3 Phenotype information of germplasm accessions on four biotic stresses

Biotic stress	Phenotypic data available	Resistant reaction	Susceptible reaction
Late blight	175	89	86
Potato Virus Y	135	60	75
Potato Virus X	129	26	103
Globodera rostochiensis	199	37	162
Globodera pallida	199	47	152

were moderately resistant, and six were susceptible. Twelve accessions showed the presence of the *Rpi-abpt* gene, of which three were resistant, seven had moderate resistance, and two were susceptible to late blight. Similarly, out 21 accessions that possessed the *R3a* gene, five were resistant, four were moderately resistant and three were susceptible to late blight. Like-wise, twenty-six accessions were found to carry the *R3b* gene, of which five were resistant, eight showed moderate resistance, and four were susceptible to late blight. A combination of two and three late blight resistant genes simultaneously was found in 15 and six accessions, respectively (Fig. 1).

It was observed that genotype-phenotypic concordance for late blight was 50%, 83.3%, 75 and 72.2% for the genes *R1*, *Rpi-abpt*, R_3a and R_3b, respectively, indicating that the *Rpi-abpt* gene was more efficient than other late blight resistance genes for marker-assisted selection. Similarly, the presence of multiple markers/genes such as $R1 + Rpi\text{-}abpt$, $R1 + R_3b$, $R_3a + R_3b$, $Rpi\text{-}abpt + R3a + R_3b$, and $R1 + Rpi\text{-}abpt + R_3a$, showed 100%, 100%, 71.4%, 100%, and 100% concordance, respectively (Table 4). The results clearly revealed that stacking multiple R genes improved the resistance to late blight.

Screening for virus resistance

In case of Potato Virus Y, phenotypic data was available for 135 accessions. Sixty accessions were resistant and 75 were susceptible (Table 3). Resistance screening using markers RYSC3, YES3-3 A, and STM0003 linked to R_{yadg} and R_{ysto} genes demonstrated that 42 accessions contained at least one

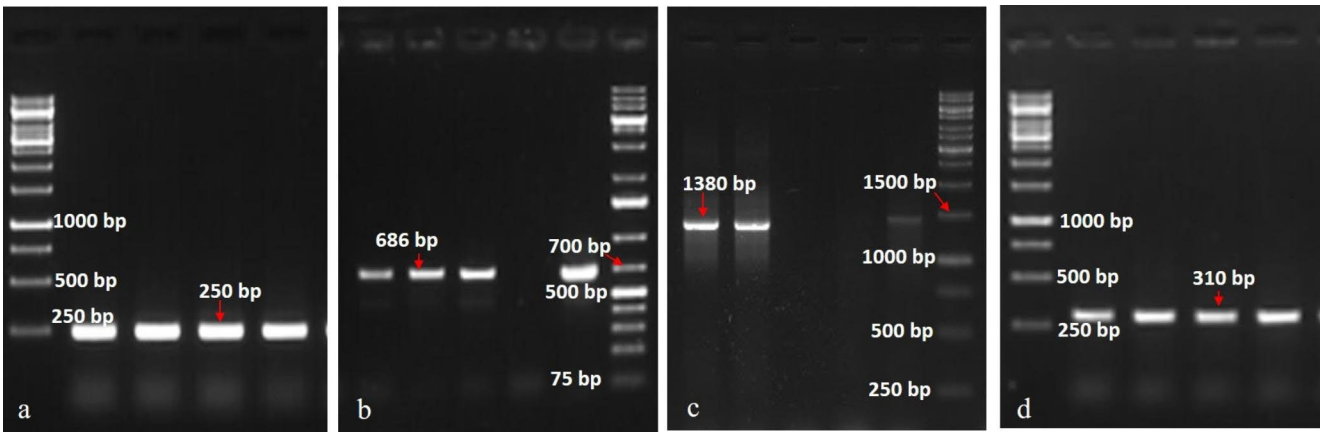

Fig. 1 Agarose gel electrophoresis showing the PCR products of different late blight resistant genes/markers (**a**) CosA marker- 250 bp, 1 kb marker (**b**) R2 marker – 686 bp, 1 kb plus marker (**c**) R3- 1380 bp, 1 kb marker (**d**) cLET5E4 marker-310 bp, 1 kb marker

Table 4 Concordance between genotypic and phenotypic data

Biotic Stress	Gene/Marker	Number of accessions showing presence of band	Phenotypic Reaction		Concordance (%)
			Resistant reaction	Susceptible reaction	
Late blight	R1	14	7	7	50
	Rpi-abpt	12	10	2	83.3
	R3a	12	9	3	75
	R3b	18	13	5	72.2
	R1 + Rpi-abpt	1	1	0	100
	R1 + R3b	1	1	0	100
	R3a + R3b	7	5	2	71.4
	Rpi-abpt + R3a + R3b	1	1	0	100
	R1 + Rpi-abpt + R3a	1	1	0	100
PVY	YES3-3 A	0	-	-	-
	RYSC3	2	1	1	50
	STM0003	22	12	10	54.54
PVX	1Rx1	10	2	8	20
	106Rx2	7	1	6	14.28
G. rostochiensis	TG689	10	6	4	60
	Gro-VI-XO2	16	4	12	25
G. pallida	SPUD1636	0	-	-	-
	HC	4	1	3	24
	GPA2-1	9	4	5	44.44
	GPA2-2	12	6	6	50
	GPA2-1 + GPA2-2	7	3	4	42.85
	HC + GPA2-1 + GPA2-2	1	1	-	100

Note: Concordance was calculated using accessions where both phenotypic and genotypic data was available

PVY resistance gene out of 216 accessions. The YES3-3 A marker was found to be positive for only one genotype (CP 3420) out of 216 accessions. This accession also showed a resistant reaction to PVY, indicating perfect match with the genotype data. The R_{yadg} gene was present in three accessions, one of which (CP 2370) was resistant, one (CP 3256) susceptible, while phenotype data was unavailable for the third accession. Similarly, 38 accessions were positive for the STM0003 marker, out of which 12 were resistant and 10 were susceptible to PVY (Fig. 2). The genotype-phenotype association showed 50% and 54.54% concordance for R_{yadg} and STM0003 markers, respectively (Table 4).

The phenotypic data for Potato virus X was available for 129 out of 216 accessions. Twenty-six accessions showed resistant reactions and 103 were susceptible (Table 3). The marker 1Rx1 was found in 19 accessions, two of which were resistant to PVX and eight were susceptible. The marker 106Rx2 was present in 10 accessions, of which one was resistant to PVX and six were susceptible (Fig. 3). For Potato Virus X, the concordance between genotypic and phenotypic data was 20% and 14.28% for 1Rx1 and 106Rx2

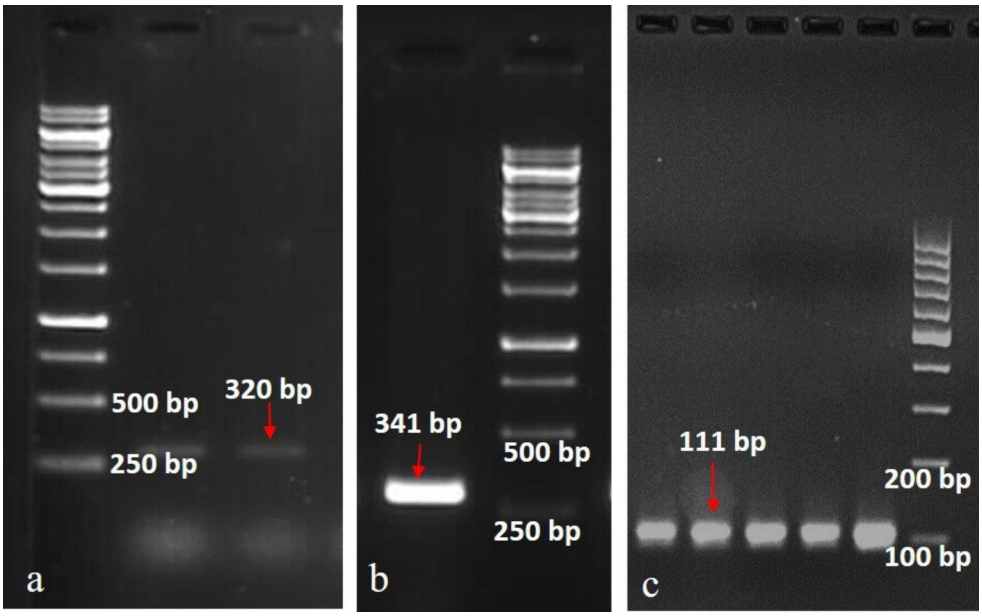

Fig. 2 PCR products of different PVY resistant genes/markers tested on 1.2% agarose gel (**a**) RYSC3 marker – 320 bp, 1 kb marker (**b**) YES3-3 A marker- 341 bp, 1 kb marker (**c**) STM0003 marker- 111 bp, 100 bp marker

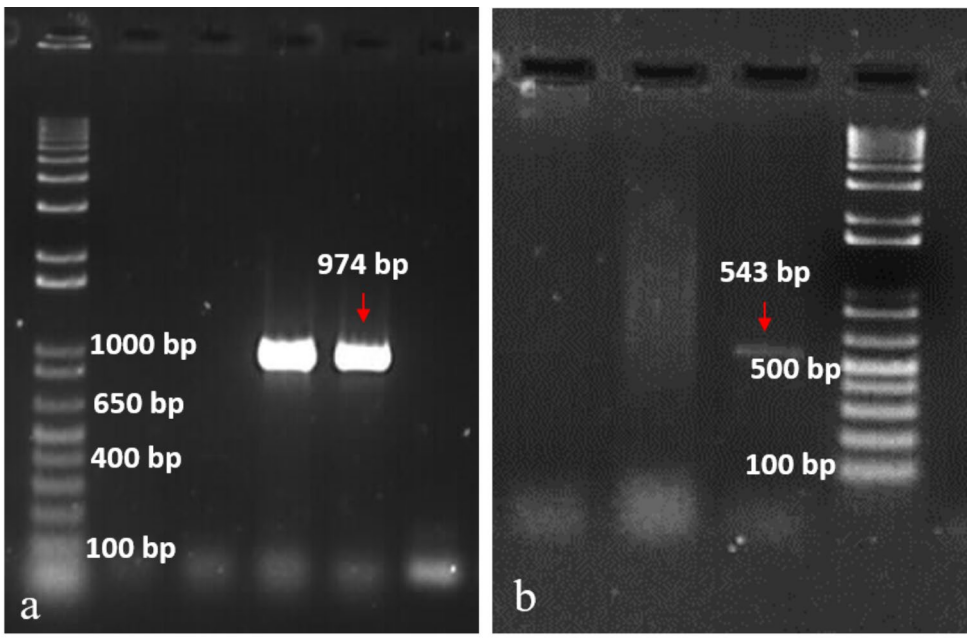

Fig. 3 Agarose gel electrophoresis showing the PCR products of different PVX resistant genes/markers (**a**) 1Rx1 marker of PVX resistant gene Rx2 974 bp, 1 kb plus marker (**b**) 106Rx2 marker of PVX resistant gene Rx2 (Marker – 100 bp plus, desired gene product size-543 bp)

markers, respectively, which indicates poor efficiency of these markers for MAS (Table 4).

Screening for PCN resistance

The phenotypic data was available for 199 accessions on *G. rostochiensis* (Golden cyst nematode), of which 37 lines were resistant and 162 showed susceptible reactions (Table 3). Similarly, 47 accessions showed resistant reaction and 152 displayed susceptible reaction to *G. pallida* (white cyst nematode) out of 199 accessions (Table 3).

Among all the accessions, 45 accessions showed the presence of one or the other PCN resistance markers out of six markers tested for PCN resistance. The presence of PCN resistance markers TG689, HC, Gro-VI-XO2, GPA2-1 and GPA2-2 was found in 12, 4, 17, 10 and 14 accessions, respectively. It was interesting to note that 7 (CP 2030, CP 3036, CP 3696, CP 4226, CP 1317, CP 3651, CP 4179), 1 (CP 2049), and 1 (CP 2049) accessions had a combined presence of two, three and four markers, respectively. Genetic analysis of 216 accessions using the marker TG689 revealed 12 accessions expressing DNA fragments that are diagnostic for the *H1* gene, of which five accessions were resistant to

G. rostochiensis and four were susceptible. A QTL conferring resistance to pathotypes 2 and 3 of *G. pallida* is tightly linked to markers HC and SPUD1636. The marker HC was found in four accessions (CP 2049, CP 2090, CP 2163, and CP 3917), of which one showed a resistant reaction and three showed susceptibility to *G. pallida*. None of the accessions showed the presence of the SPUD1636 marker. Out of 17 accessions that were positive for the Gro-VI-XO2 marker, four showed a resistant reaction, while twelve were susceptible to *G. rostochiensis*. Likewise, the GPA2-1 marker was present in only 10 accessions; four displayed a resistant reaction while five showed a susceptible reaction to *G. pallida*. Further, the presence of GPA2-2 marker was found in 14 accessions, out of which six were resistant and five accessions were susceptible to *G. pallida* (Fig. 4). The concordance of phenotypic and genotypic data was 24%, 44.44%, and 50% for HC, GPA2-1, and GPA2-2 markers, respectively. The combination of two (GPA2-1 + GPA2-2) and three markers (HC + GPA2-1 + GPA2-2) showed 42.85% and 100% concordance of genotype and phenotype data, respectively (Table 4).

In order to identify potential accessions with genes for either of the four traits, we identified 54 potato accessions that possessed at least two genes for resistance to late blight or Potato Virus Y or Potato Virus X or potato cyst nematodes (Table 5). However, based on phenotype reaction, 21 out of these 54 accessions were resistant or moderately resistant and 19 were susceptible to late blight. Similarly, seventeen accessions for PVY, 11 for PVX, 18 for *G. pallida*, and 14 for *G. rostochiensis* were resistant out of these 54 accessions.

Discussion

The screening of potato germplasm and breeding populations for resistance to various biotic stresses require specialized facilities and a considerable amount of time and resources. Moreover, it is a herculean task to screen a large set of germplasm/breeding populations each year for all the major biotic stresses. The best alternative to speed up the screening of breeding populations for late blight, viruses and PCN is MAS using tightly linked molecular markers (Barone 2004). Substituting molecular markers for phenotypic tests can speed up the selection of desirable resistant clones at the seedling stage in early generations itself. Several markers associated with LB, viruses, and PCN resistance have already been identified in potatoes. However, it becomes imperative to see the association of phenotype and genotype data for practical use in the breeding programme in specific regions due to the prevalence of racial diversity of pathogens. Therefore, the molecular markers were used in this study to check their reliability and efficiency. All the markers for late blight resistance used in the study showed a clear and perfect agreement with the phenotype data except one accession, CP 2388. The ability of the late blight pathogen to adapt and mutate rapidly may make the presence of single resistance genes ineffective. So, gene stacking in a single potato cultivar was found to be an effective means of extending the durability of resistance against late blight (Stefańczyk et al. 2020). Our results also showed that stacking multiple R genes improved the resistance to late blight. The results revealed that it is not a single R gene that drives extreme resistance reaction in potatoes; rather, it is the

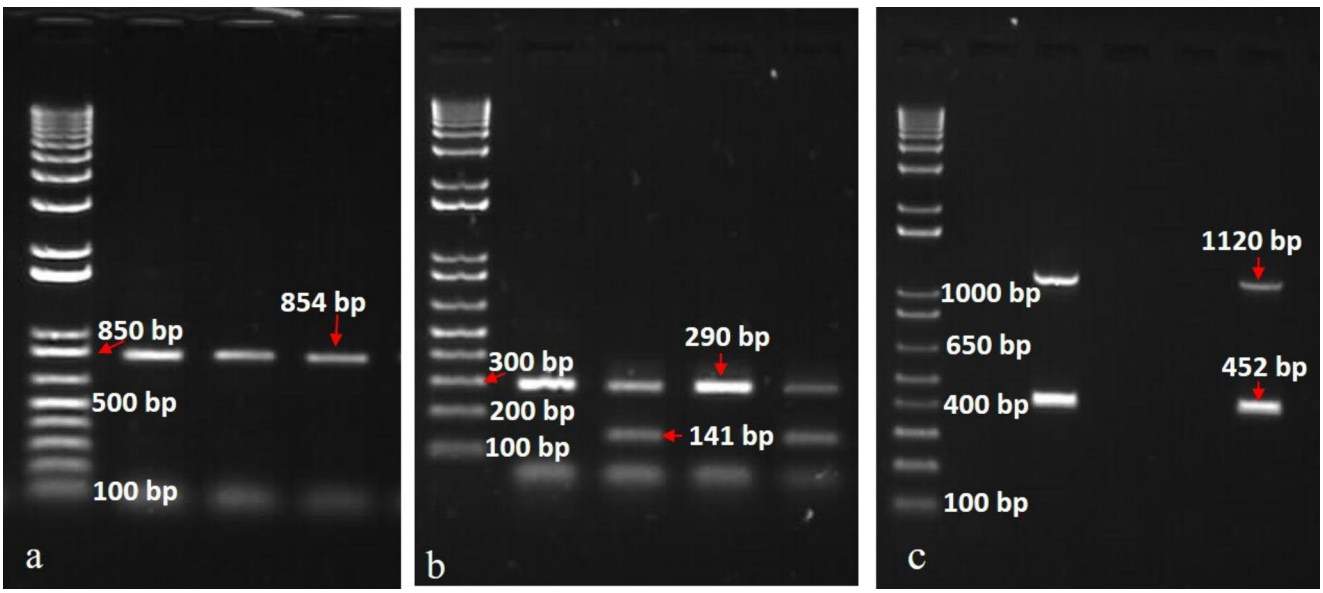

Fig. 4 Agarose gel profile of different PCN resistant genes/markers (**a**) Gro-VI-XO2 marker-854 bp, 1 kb marker (**b**) TG689 marker-141 bp, 1 kb plus marker, BCH internal control-290 bp (**c**) GPA 2-1-1120 bp and GPA 2-2-452 bp, 1 kb plus marker

Table 5 The identified promising accessions (54) with multiple disease resistance genes

Accessions Name	Late blight					PVY				PCN								PVX		
	Genotypic				Phenotypic	Genotypic			Phenotypic	Genotypic						Phenotypic		Genotypic		Phenotypic
	R1	R2	R3a	R3b		YES3-3 A	Ry*adg*	STM0003		TG 689	SPUD 1636	HC	Gro-VI-XO2	GPA2-1	GPA2-2	G PALLIDA	G ROSTO	RX1	RX2	
CP 1137	-	-	+	-	NA	-	-	-	NA	+	-	-	-	-	-	NA	NA	-	-	NA
CP 1151	-	-	-	-	NA	-	-	+	R	-	-	-	-	-	-	R	R	+	-	R
CP 1317	-	-	-	-	S	-	-	-	NA	-	-	-	-	+	+	S	S	-	-	NA
CP 1379	+	-	-	-	S	-	-	-	S	-	-	-	-	-	-	S	S	-	+	S
CP 1405	-	-	-	-	S	-	-	+	S	-	-	-	-	-	-	S	S	+	-	S
CP 1414	-	-	-	-	S	-	-	+	R	-	-	-	+	-	-	S	S	-	-	S
CP 1428	+	+	-	-	MR	-	-	-	S	-	-	-	-	-	-	S	S	-	-	S
CP 1447	-	-	-	-	MR	-	-	-	R	-	-	-	-	-	+	R	S	+	-	R
CP 1454	+	-	-	+	NA	-	-	-	S	-	-	-	-	-	-	S	S	-	-	S
CP 1655	-	-	-	-	NA	-	-	+	NA	-	-	-	-	-	-	S	S	-	+	NA
CP 1659	-	-	-	-	S	-	-	-	S	-	-	-	+	-	-	S	S	-	+	S
CP 1664	-	-	-	-	R	-	-	+	R	-	-	-	+	-	-	R	R	-	-	R
CP 1873	-	-	-	-	S	-	-	+	S	-	-	-	+	-	-	R	S	+	-	S
CP 1889	-	-	-	+	S	-	-	-	S	+	-	-	-	-	-	S	S	-	-	S
CP 1922	-	-	+	+	S	-	-	-	R	-	-	-	-	-	-	S	R	-	-	R
CP 1968	-	-	-	+	MR	-	-	-	R	+	-	-	-	-	-	S	S	-	-	R
CP 1971	+	-	-	-	MR	-	-	-	R	-	-	-	-	-	-	R	R	-	+	R
CP 1990	-	-	-	+	MR	-	-	-	R	+	-	-	-	-	-	S	S	-	-	R
CP 2030	+	-	-	-	MR	-	-	-	R	-	-	-	-	+	+	R	S	+	-	S

Table 5 (continued)

Accessions Name	Late blight					PVY				PCN								PVX		
	Genotypic				Phenotypic	Genotypic			Phenotypic	Genotypic						Phenotypic		Genotypic		Phenotypic
	R1	R2	R3a	R3b		YES3-3 A	Ry*adg*	STM0003		TG 689	SPUD 1636	HC	Gro-VI-XO2	GPA2-1	GPA2-2	G PALLIDA	G ROSTO	RX1	RX2	
CP 2049	-	-	-	+	MR	-	-	-	S	+	-	+	-	+	+	R	R	+	+	S
CP 2059	+	-	-	+	MR	-	-	-	R	+	-	-	-	+	+	R	R	+	-	NA
CP 2090	+	-	-	-	S	-	-	-	S	-	-	+	-	-	-	S	S	-	-	S
CP 2171	-	-	+	+	MR	-	-	-	S	-	-	-	-	-	-	S	S	-	-	S
CP 2314	+	-	+	+	NA	-	-	-	NA	-	-	-	-	-	-	S	S	-	-	NA
CP 2338	-	-	+	+	NA	-	-	-	S	-	-	-	-	-	-	S	S	-	-	S
CP 2370	-	-	-	+	R	-	+	-	R	-	-	-	-	-	-	S	S	-	-	R
CP 2399	-	+	+	+	MR	-	-	-	NA	-	-	-	-	-	-	S	S	-	-	NA
CP 2412	-	-	-	-	S	-	-	+	R	-	-	-	-	-	+	S	S	-	-	S
CP 2418	-	-	+	+	MR	-	-	-	S	+	-	-	-	-	-	MR	R	-	-	S
CP 2927	-	-	-	-	NA	-	-	-	NA	-	-	-	+	-	-	R	S	+	-	NA
CP 3021	-	-	+	+	MR	-	-	-	NA	-	-	-	-	-	-	NA	NA	-	-	NA
CP 3036	-	-	-	-	MR	-	-	-	NA	-	-	-	-	+	+	R	S	+	-	NA
CP 3079	-	-	-	-	S	-	-	+	R	-	-	-	-	+	-	S	S	+	-	S
CP 3091	+	-	-	-	S	-	-	-	R	+	-	-	-	-	-	R	R	-	-	R
CP 3145	-	-	-	-	S	-	-	+	R		-	-	-	-	+	R	S	-	-	R
CP 3182	-	-	-	+	S	-	-	-	NA	+	-	-	-	-	-	R	NA	-	-	S
CP 3256	-	-	+	+	R	-	+	-	S	-	-	-	-	-	-	S	S	-	-	S
CP 3334	-	-	+	+	NA	-	-	-	NA	-	-	-	-	-	-	NA	NA	-	-	S

Table 5 (continued)

Accessions Name	Late blight					PVY				PCN								PVX		
	Genotypic				Phenotypic	Genotypic			Phenotypic	Genotypic						Phenotypic		Genotypic		Phenotypic
	R1	R2	R3a	R3b		YES3-3 A	Ry*adg*	STM0003		TG 689	SPUD 1636	HC	Gro-VI-XO2	GPA2-1	GPA2-2	G PALLIDA	G ROSTO	RX1	RX2	
CP 3420	-	+	-	-	MR	+	-	-	NA	-	-	-	-	-	-	R	S	-	-	NA
CP 3486	-	-	+	+	S	-	-	-	S	-	-	-	-	-	-	R	R	-	-	R
CP 3506	-	-	+	+	NA	-	-	-	NA	-	-	-	-	-	-	NA	NA	-	-	NA
CP 3588	-	-	+	+	R	-	-	-	NA	-	-	-	-	-	-	S	S	-	-	NA
CP 3625	+	-	+	+	NA	-	-	-	NA	-	-	-	-	-	-	S	S	-	-	NA
CP 3636	+	+	+		R	-	-	-	MR	-	-	-	+	-	-	R	R	-	-	S
CP 3639	-	+	+	+	NA	-	-	-	NA	-	-	-	-	-	-	NA	NA	-	-	NA
CP 3641	-	-	-	-	NA	-	-	+	NA	-	-	-	-	-	+	NA	NA	+	-	NA
CP 3651	-	-	-	-	S	-	-	-	NA	-	-	-	-	+	+	NA	NA	+	+	NA
CP 3696	-	-	-	-	S	-	-	-	S	-	-	-	-	+	+	S	R	+	+	S
CP 3718	-	-	+	+	NA	-	+	-	NA	-	-	-	-	-	-	S	S	-	-	NA
CP 3809	+	-	-	-	S	-	-	-	S	+	-	-	-	-	-	R	R	-	-	S
CP 3871	-	-	-	-	MR	-	-	+	NA	-	-	-	+	-	-	S	R	+	-	NA
CP 3917	+	-	+	+	S	-	-	-	NA	-	-	+	-	-	-	S	S	-	-	NA
CP 4179	-	-	-	-	R	-	-	-	R	-	-	-	-	+	+	S	S	+	-	NA
CP 4226	-	-	-	-	NA	-	-	-	NA	-	-	-	-	+	+	S	R	+	+	NA

*MR: Moderately Resistance, R: Resistance, S: Susceptible, NA: Data Not Available

combination of several R genes (horizontal resistance) that drives full resistance.

The accessions (CP 2314, CP 2399, CP 3625, CP 3636, CP 3639, CP 3917) exhibiting the presence of multiple resistance genes to late blight could be used in potato breeding. However, we also found some accessions (CP 1012, CP 1319, CP 1395, etc.) that were resistant to late blight but lacked the presence of any resistance gene in our study. These accessions might have a different source of resistance, resistance genes, or this may be the result of recombination events that separated the markers from resistance alleles.

For PVY, many accessions were resistant in phenotypic screening, but most of them did not show the presence of RYSC3, YES3-3 A, and STM0003 markers. These accessions might contain resistance genes derived from other unidentified sources. On the other hand, 9 accessions despite having PVY resistance genes showed a susceptible reaction. This could be the result of errors in phenotypic screening or genotyping. Additionally, it might be due to the incomplete dominance of the resistant allele in certain resistant backgrounds, as well as the type of marker applied and the type of PVY species tested (Sudha et al. 2016). Our results revealed that the PVX markers (1Rx1 and 106Rx2) examined in our study were ineffective in selecting resistant clones.

Likewise for PCN resistance screening, TG689 linked to the *H1* gene is a robust marker for *G. rostochiensis* resistance and has been used in potato breeding programmes across the continents (Douches et al. 2010; Milczarek et al. 2011). We found 12 accessions positive for TG689 and 17 accessions for the Gro-VI-XO2 marker assay that detected *Gro-VI* QTL for *G. rostochiensis* resistance. As observed earlier, TG689 (60%) concordance with phenotype data was higher than the Gro-VI-XO2 (25%) marker (Milczarek et al. 2011; Sudha et al. 2016). None of the accession was positive for the SPUD1636 marker associated with *S. vernei* derived QTLs (Gpa5 and Gpa6) for *G. pallida* resistance in our study. This calls attention to the importance of the introduction of varieties having SPUD1636. Many studies have shown the robustness of the SPUD1636 marker for *G. pallida* resistance screening (Bryan et al. 2002; Sattarzadeh et al. 2006). Another SNP marker HC, which is a diagnostic marker for the *RGp5-vrnHC* QTL and confers partial resistance to the *Pa2/3* pathotype of *G. pallida* was present in 4 accessions. The GPA2-1 and GPA2-2 markers which confer absolute resistance to one or more pathotypes of *G. pallida* (Asano et al. 2012) were also detected in 10 and 14 accessions, respectively. Overall, a combination of three PCN markers (HC + GPA2-2 + GPA2-2) showed 100% concordance between genotypic and phenotypic data. Due to the genetic complexity of *G. pallida*, it is challenging to deploy resistance genes for effective resistance. However, the availability of two markers that identify a significant portion of the genetic resistance is extremely important for the breeding effort for *G. pallida* resistance. We also found accessions which were marker negative but were resistant to *G. rostochiensis* and *G. pallida*. This indicated the possibility of other resistant genes governing PCN resistance in these accessions.

Overall, we identified 54 potato accessions that possessed at least two genes for resistance to LB or PVY or PVX or PCNs. Similar results were obtained earlier by Sharma et al. (2014) in their study where they identified fourteen elite potato accessions possessing multiple disease resistance genes for LB, PCN and PVY. A single promising accession, CP 2049, was found to carry seven resistant genes for LB (2), PCN (4), and PVX (2) in the present investigation, which must be utilized in the biotic stress breeding programme in India. However, the study also demonstrated that none of the accession had genes for resistance to all four traits together. Therefore, the resistant accessions carrying more than one resistant gene for various biotic stresses should be used as parents to pyramid the genes for all four traits together in a single cultivar.

Conclusion

It has always been the aim of potato breeders to develop varieties that can withstand multiple biotic stresses. Unfortunately, the occurrence of such 'miracle' genotypes remained elusive because of polygenic resistances, tetrasomic inheritances, and insufficient screening techniques. With the help of molecular markers, it is possible to screen large populations for biotic stresses and to select desirable progeny genotypes without the need for extensive phenotypic analyses. The markers used in the present investigation are either tightly linked to the genes or part of the gene and hence less chances of recombination events between the marker and gene of interest. The genetic architecture of potato, being a clonally propagated crop, is fixed in subsequent clonal generations. As a result, the likelihood of success for breeders is very high when they use MAS as a tool to assist them in breeding potatoes.

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Author contribution Conceptualized and designed the experiments: SS and VB. Phenotyping: VK, AKS, SSh and DD. Genotyping: AK,

BS and BD. Experiment material and study faciliation: VB, VK and DK. Wrote the manuscript: VM and SS. Editing: AKT and RS. All authors read and approved the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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