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CHARACTERIZATION OF ANDIGENA ACCESSIONS FOR BIOTIC STRESS RESISTANCE USING LINKED MOLECULAR MARKERS

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ABSTRACT: In the current study, 72 andigena accessions were screened for resistance to late blight, viruses (PVY and PVX), and potato cyst nematodes (PCNs) using gene-based linked markers. R1 gene was only present in one accession, JEX-A/668. Whereas two accessions, JEX-A/459 and JEX-A/616, showed the presence of the R2 gene. The R3a gene was not present in any of the accessions. The R3b gene was also amplified in the JEX-A/42, JEX-A/107, and JEX-A/459 accessions. In PVY resistance screening, the YES3-3A marker was found in 50 accessions. In comparison, 40 accessions tested positive for the STM0003 marker, which indicates the presence of the *Rysto* gene, whereas 14 accessions tested positive for the RYSC3 marker. In 9 and 20 accessions, respectively, the markers 1Rx1 and 106Rx2, which were connected to the extreme resistance genes Rx1 and Rx2 for PVX resistance, were discovered. Both 1Rx1 and 106Rx2 markers were discovered in six accessions (JEX-A/232, JEX-A/267, JEX-A/638, JEX-A/683, JEX-A/1061, and JEX-A/1092). *Globodera* spp. resistance screening revealed that 17 out of 72 accessions tested positive for the marker TG689. None of the accessions was positive for the SPUD1636 marker. Furthermore, the expected-size bands for the GPA2-1 and GPA2-2 markers, 1120 and 452 bp, respectively, were found in 15 separate accessions. Both markers show the presence of the Gpa2 locus, which almost completely confers resistance to one or more pathotypes of *G. pallida*.

KEYWORDS: Disease Resistance, *Globodera pallida, Phytophthora infestans, Potato,* Marker-Assisted Selection (MAS)

INTRODUCTION

The potato is one of the most important non-cereal food crops in the world. In terms of human consumption, it is the third most important crop after wheat and rice. Since potatoes are propagated asexually in the form of tubers, they are prone to a variety of biotic stresses, such as late blight, PCN, and many viruses (Bhardwaj *et al*., 2019). Every year billion-dollar losses in potatoes are caused by the most destructive disease late blight, which is responsible for the complete destruction of the potato crop in severe cases. Wild potato species have been found to have quantitative or field resistance to late blight. In the past decade, more than 60 late blight-resistant genes were mapped on different potato species (Mangal *et al*., 2022). Resistance genes to late blight were mapped on wild species *S. demissum* (*R1-R11, Rpi-dmsf1*), *S. pinnatisectum* (*Rpi1, Rpi2, Pi-Blatt*), *S. bulbocastanum* (*Rpi-blb1-Rpi-blb3, Rpi-bt1, Rpi-apbt*), *S. hjertingii* (*Rpi-hjt1.1, Rpi-hjt1.2, Rpi-hjt1.3*), *S. chacoense* (*Rpi-chc1, Rpi-chc2*) etc (Paluchowska *et al*., 2022; Tiwari *et al*., 2013; Yang *et al*., 2017). To date, many markers linked to LB resistance genes have been discovered like R1AS linked to R1 gene (Ballvora *et al*., 2002), Q133 (SCAR) for *Rpi-ber1* gene (Tan *et al*., 2010), R2AS for *Rpi-abpt* gene (Kim *et al*., 2012), and SpTG572 (SCAR) for *Rpi2* gene (Yang *et al*., 2017) etc.

Like late blight, viruses too pose a serious problem in potatoes as compared to other diseases because of their vegetative propagation and transmission of viruses from

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tuber to tuber in subsequent generations. PVY resistance genes Ry_{adg} (*S. tuberosum* sp. *andigenum*), Ry_{sto} and Ry_{fsto} (*S. stoloniferum*) and Ry_{hcu} (*S. hougasii*) have been reported to impart extreme resistance to this virus (Cockerham, 1970; Flis *et al*., 2005; Kasai *et al*., 2000; Song *et al*., 2005). Likewise, other genes i.e., Ny_{tbr}, *Ny-1*, Nc_{spl}, Nc_{tbr} and Ny_{dms} were also mapped which provide hypersensitive reaction to PVY (Celebi-Toprak *et al*., 2002; Szajko *et al*., 2008). For PVX extreme (*Rx1, Rx2,* Rx_{adg}, Rx_{acl}) and hypersensitive resistance genes (Nx_{phu}, Nx_{tbr}, Nx_{chc}, *Nb*) were mapped on different wild and cultivated species (Ahmadvand *et al*., 2013; Marano *et al*., 2002; Ritter *et al*., 1991; Tommiska *et al*., 1998). Markers linked to PVX resistance genes are as follows: 1Rx1 for *Rx1* gene, 106Rx2 marker for *Rx2* gene (Ahmadvand *et al*., 2013).

The third major problem in potatoes worldwide is Potato Cyst Nematodes (*Globodera pallida* and *G. rostochiensis*), which cause great losses to agriculture worldwide (Sood *et al*., 2022). Resistance genes to different pathotype of *G. pallida* have been reported in different species like *S. multidissectum* (*H2*), *S. tuberosum* ssp. *andigena* (*H3, Gpa2, GpaIV*$^{s}_{adg}$), *S. vernei* (*Gpa5, Gpa6*), *S. tarijense* (*GpaXI*$^{l}_{tar}$) (Moloney *et al*., 2010; Van Der Voort *et al*., 1997; Strachan *et al*., 2019; Van Der Voort *et al*., 2000). Likewise, resistance genes to different pathotypes of *G. pallida* were mapped in different species like *S. tuberosum* ssp. *andigena* (*H1*), *S. spegazzinii* (*Gro1-4, Gro1.2, Gro1.3, Gro1.4*), *S. vernei* (*Gro 6*) etc (Bakker *et al*., 2004; Jacobs *et al*., 1996; Kreike *et al*., 1993, 1996; Paal *et al*., 2004). Several molecular markers linked to PCN resistance genes have been found, which can be used for marker assisted selection (MAS). Markers linked to known PCN resistance genes are TG689 (SCAR) for *H1* gene, SNP based HC marker for *Gpa5_vrn_QTL*, SPUD1636 for *Gpa5_QTL* and XO2 for *GroVI* gene (Asano *et al*., 2012; Bryan *et al*., 2002; Jacobs *et al*., 1996). It has been demonstrated in several studies that markers tightly linked to the aforementioned resistance genes can improve the efficiency of potato breeding for resistance to biotic stresses (Sharma *et al*., 2014; Sudha *et al*., 2016). Accordingly, the present study was designed to identify potato accessions that possess multiple disease resistance genes that can be exploited in biotic stress resistance potato breeding program.

MATERIALS AND METHODS

The plant material comprising 72 andigena accessions maintained at the ICAR-Central Potato Research Station, Jalandhar, Punjab were used for molecular characterization using late blight, viruses (PVY and PVX) and PCN resistance markers. The collection included exotic potato andigena accessions imported from various countries and used as parental lines for varietal development in India (Table 1).

DNA extraction and PCR assay

Total genomic DNA isolation from young leaves was carried out using a Qiagen Kit. DNA concentration was determined using agarose gel electrophoresis on 0.8% agarose gel and a nanodrop spectrophotometer (Thermo Scientific, US). Molecular characterization of late blight resistance was performed using the CosA, R2, R3 1380, and cLET5E4 markers. Likewise, genetic analysis of viruses (PVY and PVX) resistance was done through RYSC3, STM0003, YES3-3A, 1Rx1, and 106Rx2 markers. The molecular validation for *G. rostochiensis* resistance was carried out using TG689 and Gro-VI-XO2 markers, whereas *G. pallida* resistance was evaluated using HC, SPUD1636, GPA2-1, and GPA2-2 markers. Other details of the markers, marker type, and product size used for screening are presented in Table 2.

A Thermal Cycler Applied Biosystems was used to run PCR performed in 20 µl reaction volume, where 2 µl template DNA, 1 µl each of forward and reverse primers, 10 µl of 2x PCR master mix and rest 6 µl of distilled water. The amplified fragments were resolved

Table 1: List of andigena accessions used for the study

S. No.	Accession Name	Donor No	Source	S.No.	Accession Name	Donor No	Source
1	JEX-A/10	NA	UK	37	JEX-A/379	PI243405	USA
2	JEX-A/14	PI186180	USA	38	JEX-A/380	PI243405	USA
3	JEX-A/15	PI186180	USA	39	JEX-A/390	PI243406	USA
4	JEX-A/19	PI186178	USA	40	JEX-A/457	PI243418	USA
5	JEX-A/21	PI186179	USA	41	JEX-A/459	PI243418	USA
6	JEX-A/26	PI186179	USA	42	JEX-A/468	PI243420	USA
7	JEX-A/30	PI197933	USA	43	JEX-A/498	PI243424	USA
8	JEX-A/42	PI225628	USA	44	JEX-A/506	PI243424	USA
9	JEX-A/45	PI225628	USA	45	JEX-A/513	PI243429	USA
10	JEX-A/58	PI225632	USA	46	JEX-A/539	PI243430	USA
11	JEX-A/76	PI230473	USA	47	JEX-A/595	PI243439	USA
12	JEX-A/79	PI230473	USA	48	JEX-A/612	PI243440	USA
13	JEX-A/93	PI232839	USA	49	JEX-A/616	PI243444	USA
14	JEX-A/107	PI233981	USA	50	JEX-A/617	PI243446	USA
15	JEX-A/122	PI233988	USA	51	JEX-A/638	PI243447	USA
16	JEX-A/132	PI234002	USA	52	JEX-A/668	PI243470	USA
17	JEX-A/164	PI243394	USA	53	JEX-A/683	PI243471	USA
18	JEX-A/189	PI243411	USA	54	JEX-A/705	PI245320	USA
19	JEX-A/197	PI243418	USA	55	JEX-A/707	PI245783	USA
20	JEX-A/198	PI243418	USA	56	JEX-A/708	PI245186	USA
21	JEX-A/199	PI243418	USA	57	JEX-A/763	PI255496	USA
22	JEX-A/202	PI243420	USA	58	JEX-A/801	PI280883	USA
23	JEX-A/215	PI242429	USA	59	JEX-A/804	PI280883	USA
24	JEX-A/216	PI243431	USA	60	JEX-A/827	PI280912	USA
25	JEX-A/232	PI243374	USA	61	JEX-A/865	NA	N/A
26	JEX-A/267	PI243380	USA	62	JEX-A/907	CPC1673	UK
27	JEX-A/274	PI243381	USA	63	JEX-A/912	CIPC700939	PER
28	JEX-A/275	PI243381	USA	64	JEX-A/917	CIPC700939	PER
29	JEX-A/288	PI243385	USA	65	JEX-A/920	CIPC700939	PER
30	JEX-A/296	PI243389	USA	66	JEX-A/947	CIPC701947	PER
31	JEX-A/298	PI243389	USA	67	JEX-A/1038	CIPC702151	PER
32	JEX-A/299	PI243389	USA	68	JEX-A/1046	CIPC702151	PER
33	JEX-A/316	PI243394	USA	69	JEX-A/1061	CIPC701985	PER
34	JEX-A/317	PI243394	USA	70	JEX-A/1081	CIPC702028	PER
35	JEX-A/329	PI243296	USA	71	JEX-A/1092	CIP700116	PER
36	JEX-A/361	PI243403	USA	72	JEX-A/1152	CIP700178	PER

Table 2: Details of the markers used for screening

Biotic stress	Genes	Marker	Marker type	Fragment size (bp)	Primer sequence (5′ to 3′)	Reference
Late blight	*R1*	Cos A	SCAR	250	F-CTCATTCAAAATCAGTTTTGATC R-GAATGTTGAATCTTTTTGTGAAGG	Gebhardt *et al.* (2004)
	Rpi-abpt	R2	SCAR	686	F- ACGGCTTCTTGAATGAA R- GCTCCTGATACGATCCATG	Kim *et al.* (2012)
	R3a	R31380	SCAR	1380	F-GCTTCCGACATGTATTGATCTCCC R-GGCAGCCACTTCAGCTTCTTACAG	Sokolova *et al.* (2010)
	R3b	cLET5E4	CAPS	310	F-CCAGGCATGCTCAATTTGGAGT R-TTCCCTGTTTGGACTACTTGTGGA	Huang *et al.* (2005)
	Ry_{adg}	RYSC3	SCAR	320	F-ATACACTCATCTAAATTTGATGG R-AGGATATACGGCATCATTTTTCCGA	Kasai *et al.* (2000)
	Ry_{sto}	STM0003	SSR	111	F-GGAGAATCATAACAACCAG R-AATTGTAACTCTGTGTGTGTG	Valkonen *et al.* (2008)
PVY	Ry_{sto}	YES3-3A	STS	341	F-TAACTCAAGCGGAATAACCC R-AAATTCACCTGTTTACATGCTTCTTGTG	Song and Schwarzfischer (2008)
	Rx1	1Rx1	SCAR	974	F- GGAGAAATCCTGCAATATAAT R- CGACCGAACTTACATTTTCCC	Ahmadvand *et al.* (2013)
PVX	*Rx2*	106Rx2	SCAR	543	F- GGAGAAATCCTGCAATGTAAC R- CTTGTCAAAGAAAGAAGGCCT	
	Gpa5 QTL	SPUD 1636		226	F-GTGCGCACAGGGTAAAACC R-ACCTTAGCGGATGAAAGCC	Bryan *et al.* (2002)
	Gpa2	GPA2-1	STS	1120	F- TTTAGCACGGAATGTGGGGA R- GTTTCCCCATCAAAACTCAC	Asano *et al.* (2012)
G. pallida	*Gpa2*	GPA2-2	STS	452	F- GCACTTAGAGACTCATTCCA R- ACAGATTGTTGGCAGCGAAA	Asano *et al.* (2012)
	RGp5-vrnHC	HC	SNP	276	F-ACACCACCTGTTTGATAAAAAACT R-GCCTTACTTCCCTGCTGAAG	Sattarzadeh *et al.* (2006)
G. rostochiensis	*H1*	TG 689	SCAR	141	F-TAAAACTCTTGGTTATAGCCTAT R-CAATAGAATGTGTTGTTTCACCAA	Milczarek *et al.* (2011)
	GroVI	Gro-VI-XO2	SCAR	854	F-CCACCAAACCCATAAAGCTGC R-TGTGAATTGGTATGAATCTGCAACC	Jacobs *et al.* (1996)

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. The presence of the band was scored as + (plus) and absence was scored as – (minus).

RESULTS AND DISCUSSION

Marker assisted selection (MAS) has numerous advantages including selection in early generations, reduced load of handling large progenies in advanced generations, speed, efficiency, cost reduction and resources optimization over phenotypic screening in the breeding program (Bhardwaj *et al.*, 2019; Slater *et al.*, 2013). Validated robust markers, previously identified were deployed for late blight, PVY, PVX, and PCN resistance screening of seventy-two andigena accessions. Four late blight, R genes were deployed and it was found that the *R1* gene was found in only one accession JEX-A/668. Two accessions JEX-A/459 and JEX-A/616 showed the presence of the *R2* gene. None of the accessions showed the presence of the R_3a

gene. Likewise, the R_3b gene was amplified in three accessions namely, JEX-A/42, JEX-A/107 and JEX-A/459. Genotyping of 72 accessions using LB resistance gene-linked markers revealed 5 accessions with atleast one or the other resistance gene present in them (Table 3; Fig. 1). Accessions JEX-A/459 exhibited the presence of two genes i.e., *R2* and R_3b simultaneously. All these late blight resistance genes have been introgressed from wild species *Solanum demissum*. A single R gene may not provide complete or extreme resistance to the disease, and thus, it may be necessary to have more than one R gene or a combination of resistant genes to provide total or extreme resistance. As a result, those accessions that show the presence of multiple genes for late blight can be used in the potato breeding program. Since, andigena group is adapted to short-day conditions; late blight disease tends to occur during the later stages of the crop. This may be one of the reasons why very few resistance genes have been developed in these accessions for this disease. In our study, only five accessions out of 72 showed the presence of R genes.

The PVY resistance screening carried out using Ry_{adg} and Ry_{sto} genes linked markers, RYSC3, YES3-3A and STM0003 revealed that the YES3-3A marker was present in 50 accessions (Table 3; Fig. 2). RYSC3 marker was found in 14 accessions while forty accessions were found positive for STM0003 marker indicating the presence of the Ry_{sto} gene in them (Table 3). Genotyping of 72 accessions using PVY resistance gene-linked markers revealed 51 accessions with atleast one or the other marker present in them. Similarly, 29 accessions were found to contain at least two resistant genes, and 12 accessions contained at least three resistant genes for PVY and PVX. Ry_{adg} and Ry_{sto} genes were mapped on *S. tuberosum* sp. *andigenum* and *S. stoloniferum* species respectively which impart extreme resistance to PVY (Cernák *et al*., 2008; Dalla Rizza *et al*., 2006; Kasai *et al*., 2000; Song and Schwarzfischer, 2008). As the andigena accessions have co-evolved in virus-infected hot spots, they are likely to possess virus resistance genes. As a result, more resistance genes for viruses were expected in the collection. These linked markers are very useful for MAS and the introgression of Ry_{adg} and Ry_{sto} genes into potato breeding lines. Valkonen *et al*. (2008) used Ry_{sto} genes in the independent breeding populations with the same PCR-based markers and found that markers specifically detected independent European potato cultivars that express extreme resistance to PVY derived from *S. stoloniferum*. PVY resistance clones can be rapidly selected during early generations of breeding programs using

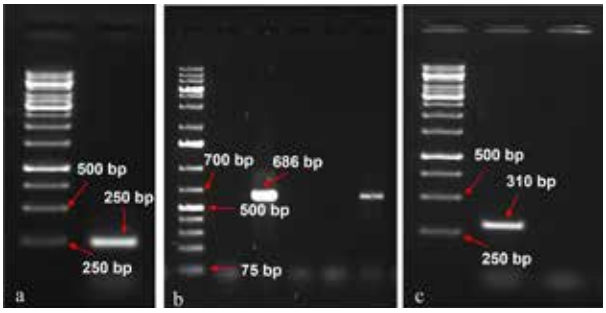


Fig. 1: Agarose gel electrophoresis showing the PCR products of different late blight resistant genes/markers a) CosA marker- 250bp, 1kb marker b) R2 marker – 686bp, 1kb plus marker c) cLET5E4 marker-310bp, 1kb marker

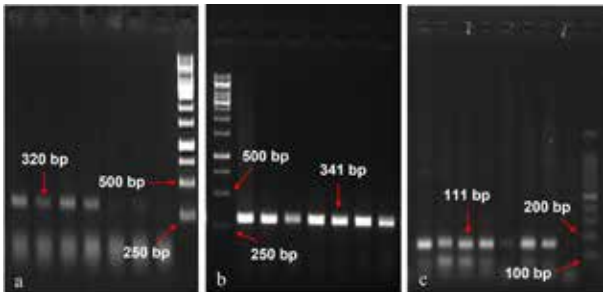


Fig. 2: PCR products of different PVY resistant genes/markers tested on 1.2% agarose gel a) RYSC3 marker - 320bp, 1kb marker b) YES3-3A marker- 341bp, 1kb marker c) STM0003 marker- 111bp, 100bp marker

Table 3: Genotypic data of all andigena accessions used for the study

Accessions	Late blight				Viruses						PCN				Total number of resistance genes
					PVY			PVX							
	R1	R2	R3a	R3b	YES3-3A	RYSC3	STM0003	RX1	RX106	TG 689	HC	Gro-VI-XO2	GPA2-1	GPA2-2	
JEX-A/10	-	-	-	-	+	-	+	-	-	-	-	-	-	-	2
JEX-A/14	-	-	-	-	-	-	-	-	-	+	-	-	-	-	1
JEX-A/15	-	-	-	-	-	-	-	-	-	+	-	-	-	-	1
JEX-A/19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
JEX-A/21	-	-	-	-	+	-	+	-	-	-	-	-	-	-	2
JEX-A/26	-	-	-	-	+	-	+	-	-	-	-	-	-	-	2
JEX-A/30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
JEX-A/42	-	-	-	+	+	-	+	-	+	+	-	-	-	-	5
JEX-A/45	-	-	-	-	+	-	+	-	-	-	-	+	-	-	3
JEX-A/58	-	-	-	-	-	-	-	-	-	-	-	-	+	+	2
JEX-A/76	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
JEX-A/79	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
JEX-A/93	-	-	-	-	-	-	+	-	-	-	-	-	-	-	1
JEX-A/107	-	-	-	+	-	-	-	-	-	-	-	-	-	-	1
JEX-A/122	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
JEX-A/132	-	-	-	-	+	-	+	-	-	-	-	-	-	-	2
JEX-A/164	-	-	-	-	+	-	+	-	-	-	-	-	-	-	2
JEX-A/189	-	-	-	-	+	-	+	-	-	+	+	-	-	-	4
JEX-A/197	-	-	-	-	+	-	+	-	-	-	-	-	-	-	2
JEX-A/198	-	-	-	-	+	-	+	-	-	-	-	-	-	-	2
JEX-A/199	-	-	-	-	+	-	+	-	-	-	-	-	-	-	2
JEX-A/202	-	-	-	-	+	+	+	-	+	+	+	-	+	+	8
JEX-A/215	-	-	-	-	-	-	-	+	-	+	+	-	-	-	3
JEX-A/216	-	-	-	-	+	-	+	-	-	-	-	-	-	-	2
JEX-A/232	-	-	-	-	-	-	-	+	+	-	-	-	+	+	4
JEX-A/267	-	-	-	-	+	-	+	+	+	-	+	-	+	+	7
JEX-A/274	-	-	-	-	+	-	+	-	-	-	-	-	-	-	2
JEX-A/275	-	-	-	-	+	-	+	-	-	-	-	-	-	-	2
JEX-A/288	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
JEX-A/296	-	-	-	-	+	-	-	-	+	+	-	-	+	+	5
JEX-A/298	-	-	-	-	+	-	-	-	+	+	+	-	+	+	6
JEX-A/299	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
JEX-A/316	-	-	-	-	+	-	+	-	-	-	-	+	-	-	3
JEX-A/317	-	-	-	-	+	-	+	-	-	-	-	-	-	-	2
JEX-A/329	-	-	-	-	+	-	-	-	+	-	-	-	-	+	3
JEX-A/361	-	-	-	-	+	-	-	-	+	+	-	-	+	+	5
JEX-A/379	-	-	-	-	-	-	-	-	-	+	-	-	-	-	1

Accessions	Late blight				Viruses					PCN					Total number of resistance genes
					PVY			PVX							
	R1	R2	R3a	R3b	YES3-3A	RYSC3	STM0003	RX1	RX106	TG 689	HC	Gro-VI-XO2	GPA2-1	GPA2-2	
JEX-A/380	-	-	-	-	+	-	+	-	-	-	+	-	-	-	3
JEX-A/390	-	-	-	-	+	+	+	-	-	-	-	+	-	-	4
JEX-A/457	-	-	-	-	+	-	+	-	+	+	+	-	+	+	7
JEX-A/459	-	+	-	+	+	+	+	+	-	-	-	-	-	-	6
JEX-A/468	-	-	-	-	+	+	-	-	+	-	+	-	-	-	4
JEX-A/498	-	-	-	-	+	-	-	-	-	+	+	-	-	-	3
JEX-A/506	-	-	-	-	+	+	+	-	+	+	+	-	+	+	8
JEX-A/513	-	-	-	-	+	-	-	+	-	-	-	-	-	-	2
JEX-A/539	-	-	-	-	+	+	-	-	-	-	-	+	-	-	3
JEX-A/595	-	-	-	-	+	-	-	-	-	-	-	+	-	-	2
JEX-A/612	-	-	-	-	+	-	-	-	-	-	-	+	-	-	2
JEX-A/616	-	+	-	-	+	+	+	-	+	-	-	-	-	-	5
JEX-A/617	-	-	-	-	+	-	+	-	-	+	-	-	-	-	3
JEX-A/638	-	-	-	-	+	-	+	+	+	+	-	+	+	+	8
JEX-A/668	+	-	-	-	-	-	-	-	-	-	-	-	-	-	1
JEX-A/683	-	-	-	-	+	-	+	+	+	+	-	+	+	+	8
JEX-A/705	-	-	-	-	+	-	+	-	+	-	-	-	-	-	3
JEX-A/707	-	-	-	-	+	-	+	-	+	-	-	-	-	-	3
JEX-A/708	-	-	-	-	+	-	+	-	-	-	-	+	-	-	3
JEX-A/763	-	-	-	-	+	-	+	-	-	-	-	-	-	-	2
JEX-A/801	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
JEX-A/804	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
JEX-A/827	-	-	-	-	+	-	-	-	-	-	-	-	-	-	1
JEX-A/865	-	-	-	-	-	-	-	-	+	+	-	-	+	-	3
JEX-A/907	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
JEX-A/912	-	-	-	-	+	+	+	-	-	-	-	-	-	-	3
JEX-A/917	-	-	-	-	-	-	-	-	-	-	-	+	-	-	1
JEX-A/920	-	-	-	-	+	+	+	-	-	-	-	+	-	-	4
JEX-A/947	-	-	-	-	-	-	-	-	-	-	-	+	-	-	1
JEX-A/1038	-	-	-	-	+	-	+	-	-	-	-	-	-	-	2
JEX-A/1046	-	-	-	-	+	+	+	-	-	-	-	-	-	-	3
JEX-A/1061	-	-	-	-	+	+	+	+	+	-	-	-	+	+	7
JEX-A/1081	-	-	-	-	+	+	+	-	-	-	-	-	-	-	3
JEX-A/1092	-	-	-	-	+	+	+	+	+	-	-	-	+	+	7
JEX-A/1152	-	-	-	-	+	+	+	-	+	-	-	-	+	+	6

MAS. Marker 1Rx1 and 106Rx2 which were linked to extreme resistance genes *Rx1* and *Rx2* for PVX resistance, were found in 9 and 20 accessions, respectively. Six accessions (JEX-A/232, JEX-A/267, JEX-A/638, JEX-A/683, JEX-A/1061 and JEX-A/1092) were found to have both 1Rx1 and 106Rx2 markers (Fig. 3).

The screening for resistance to *Globodera* spp. showed that 17 out of 72 accessions were positive for marker TG689 linked with gene *H1* (Table 3). TG689 is a robust marker and has been used in many studies for the selection of the resistance to *Globodera rostochiensis* (Douches *et al.*, 2010 and Milczarek *et al.*, 2011). The marker SPUD1636 associated with the chromosomal segment containing the *S. vernei* derived QTL (*Gpa5* and *Gpa6*) was not amplified in any of the accessions analyzed in the present study. The SNP marker HC was found in 10 accessions, which is a diagnostic marker for *RGp5-vrnHC* QTL and imparts partial resistance to *Pa2/3* pathotype of *G. pallida*. Likewise, twelve accessions showed the presence of

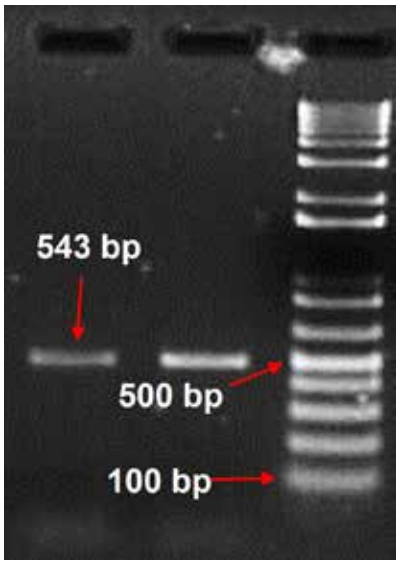


Fig. 3: Agarose gel electrophoresis showing the PCR product of 106Rx2 marker of PVX resistant gene Rx2 (Marker – 100bp plus, desired gene product size-543bp)

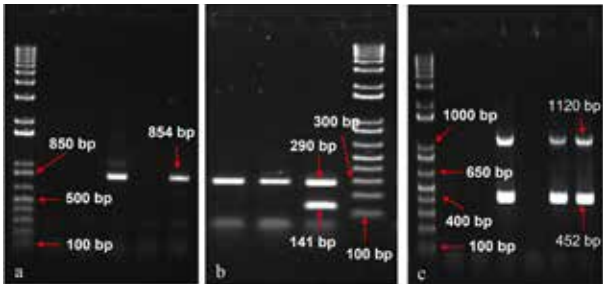


Fig. 4: Agarose gel profile of different PCN resistant genes/ markers a) Gro-VI-XO2 marker-854bp, 1kb marker b) TG689 marker-141bp, 1kb plus marker, BCH internal control-290bp c) GPA 2-1-1120bp and GPA 2-2-452bp, 1kb plus marker

an Gro-VI-XO2 marker which detects *Gro-VI* QTL resistance to *G. rostochiensis*. Further, GPA2-1 and GPA2-2 markers were detected in 15 different accessions with expected-size bands 1120 and 452 bp, respectively. Both markers detect a *Gpa2* locus that nearly confers absolute resistance to one or more pathotypes of *G. pallida* (Asano *et al.*, 2012). Genotyping of 72 accessions using PCN resistance gene-linked markers revealed that 36 accessions with atleast one or the other marker present in them. Similarly, 9 (JEX-A/58, JEX-A/189, JEX-A/215, JEX-A/232, JEX-A/498, JEX-A/865, JEX-A/1061, JEX-A/1092 and JEX-A/1152) accessions were found to contain two resistant genes, three (JEX-A/267, JEX-A/296 and JEX-A/361) accessions contained three resistant genes, and 6 (JEX-A/202, JEX-A/298, JEX-A/457, JEX-A/506, JEX-A/638 and JEX-A/683) accessions contained four resistant genes linked to PCN (Table 3; Fig. 4).

A maximum of eight resistance genes together were identified in four accessions (JEX-A/202, JEX-A/506, JEX-A/638 and JEX-A/683) out of all 72 accessions studied for biotic stresses. Similarly, seven resistance genes were identified in another four andigena accessions (JEX-A/267, JEX-A/457, JEX-A/1061 and JEX-A/1092). Based on molecular markers we identified 53 andigena

accessions possessing at least two genes for resistance to late blight, PVY and/or PCN. A number of studies have shown that markers tightly linked to the genes encoding the resistance to biotic stresses can improve the efficiency of potato breeding for resistance to late blight, virus, and PCN (Bhardwaj *et al*., 2019; Dalamu *et al*., 2023; Mangal *et al*., 2023; Sood *et al*., 2022; Sudha *et al*., 2016).

CONCLUSION

Potato breeders have always strived to develop varieties that are capable of withstanding multiple biotic stresses. Unfortunately, because of the polygenic nature of resistances, tetrasomic inheritances, and inadequate screening techniques to screen large segregating progenies, selection of such 'miracle' genotypes remained mostly elusive. The molecular markers could be used for preliminary screening of large populations for biotic stresses and selection of desirable progeny genotypes without conducting extensive phenotypic testing. In the present study, JEX-A/42, JEX-A/107, JEX-A/459, JEX-A/616, JEX-A/683 were carrying resistance genes for late blight, whereas the genotypes JEX-A/202, JEX-A/506, JEX-A/638, and JEX-A/683 possessed maximum number of resistance genes for PVY, PVX and PCN. The lines identified in the current study through markers will be validated for phenotypic reaction for different biotic stress traits before their utilization in the breeding program.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

ETHICAL STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors

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