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EFFICIENCY AND RELIABILITY OF MARKER ASSISTED SELECTION FOR RESISTANCE TO MAJOR BIOTIC STRESSES IN POTATO

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ABSTRACT: Potato is an important food crop of the world. Its production is affected by both biotic and abiotic stresses. Here, in this study we screened 12 advanced generation hybrids including three check varieties for late blight, major potato viruses and Potato cyst nematode. The hybrids were phenol typed for late blight and viruses' resistance. To confirm the resistance, the marker assisted selection was practiced using previously developed resistance markers for all the three biotic stresses. All the hybrids showed resistant reaction to late blight through detached leaf assay as well as whole plant resistance screening. Among checks, Kufri Girdhari was highly resistant whereas, Kufri Himalini was moderately resistant and Kufri Jyoti was susceptible to late blight. The ELISA results for viruses revealed that most of the hybrids were resistant to PVY, PLRV, PVS, PVM, PVA, while all were susceptible to PVX. Late blight resistant genes R_1, R_2 and R_{3a} were present in two, three and six hybrids, respectively. Check variety, Kufri Jyoti was having R_1 gene while Kufri Girdhari contained both R_2 and R_{3a} genes. The RY_{adg} gene for PVY resistance was found in only LBY 18 and RY_{sto} was found in all the hybrids and control varieties. The screening results for PVX gene linked molecular markers revealed that control variety, Kufri Himalini was positive for all four, the advanced hybrids SM/03-23, VMT 5-1, SM/05-75 and LBY18 for three and SM/00-42, SM/08-12 along with control variety, Kufri Girdhari were positive for two markers. All the hybrids showed the presence of SCG17 marker for PVS resistance and NI1127 for PLRV resistance except the control variety, Kufri Himalini for SCG17. The PCN resistance marker, Gro VI-XO2 was present in five hybrids and one check variety, Kufri Himalini, HC was present in six hybrids and two check varieties namely Kufri Jyoti and Kufri Girdhari. The R gene markers screening results were almost in agreement with phenotypic results except few cases. Therefore, we suggest their application in large scale high throughput preliminary screening for disease resistance followed by phenotypic validation of fewer genotypes.

KEYWORDS: MAS, potato hybrids, resistance breeding

INTRODUCTION

Potato is the most important non-cereal food crop of the world. It is 3rd important crop after wheat and rice in terms of human consumption. Development of new resistant varieties with higher productivity and other desirable traits is a continuous process. Being asexually propagated in the form of tubers, potato is prone to many biotic stresses including late blight, and many viruses. Up to 30% of world potato production is lost to these pests and diseases (Oerke *et al*. 1994). Development of new potato hybrids require evaluation from initial breeding stage itself for resistance to diseases. Phenotypic screening for resistance need controlled conditions, appropriate pathogen inoculum as well as disease development in the plants. Marker assisted selection is the best alternative approach for selection and screening of genotypes particularly for traits controlled by one or few genes or major genes governing polygenic traits in any crop. R genes/ linked markers for late blight, PVY, PLRV, PVX, PVM, PVS, PALCV and PCN are available in potato (Ahmadvand *et al*., 2013; Asano *et al*.,

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2012; Ballvora *et al*., 2002; Kasai *et al*., 2000; Takeuchi *et al*., 2008; Song and Schwarz-fischer, 2008). Wild potato species are a valuable genetic pool for finding late blight and other biotic stress resistant genes (Watanbe *et al*., 1995). Eleven resistance (R) genes, named R_1 to R_{11}, were identified in *Solanum demissum* and introduced into *S. tuberosum* (Black *et al*., 1953; Malcolmson and Black, 1966). It is difficult to phenotypically screen all the elite lines and hybrids against resistance to all the diseases. The easiest method is through marker assisted selection where resistance genes/their linked markers are tested for their presence or absence in particular genotypes (Asano and Tamiya, 2016). Through marker assisted selection several hundred to thousand genotypes can be screened in the lab without waiting for main crop season, thus saving both time and resources. However, phenotyping results should exactly match with the gene data in order to utilize the R genes/markers in large scale screening studies. In our study, nine advanced potato hybrids along with three control varieties were subjected to both phenotyping as well as genotyping to establish a correlation among R genes/markers data with actual phenotypic results to diseases for valid conclusions.

MATERIALS AND METHODS

Twelve hybrids including three control varieties were raised in *kharif* 2017 and 2018 at ICAR-Central Potato Research Institute, Shimla. Five plants of each hybrid were raised in pots for screening each against late blight as well as viruses. Out of five plants, three plants were inoculated with particular virus while two were kept as untreated controls.

Late blight screening: The plants were evaluated for late blight reaction using detached leaf assay under lab conditions and whole plant resistance through artificial inoculation in the late blight chambers at ICAR-CPRI, Shimla (Birhman RK and Singh BP (1995); Bhardwaj *et al.* (2013).

Viruses screening: One month old three plants of each genotype were mechanically inoculated withpotato virus Y (PVY), PVX, PVS, PVA, PVM and PLRVusing spray gun procedure. (Fernanda-Northcote 1992; Bhardwaj *et al.* 2015). Two pots of each genotype were kept as uninoculated controls in each case. Leaf samples were taken from inoculated as well as uninoculated plants one month after inoculation for both ELISA and PCR.

Genotyping using R genes/ linked markers: The leaf samples were collected from all the genotypes for DNA isolation. QIAGEN's DNeasy Plant Mini Kit was used for the DNA extraction. The genes/linked markers, PCR condition details as well as reference are given in (**Table 1**). PCR was performed in 20ul reaction volume, where2ul template DNA, 1ul each of forward and reverse primers, 10ul of 2x PCR master mix (EmeraldAmp® GT PCR Master Mix) and rest 6ul of distilled water was used and the reaction was carried out in Thermal cycler Applied biosystems. PCR amplification products were separated on horizontal gel electrophoresis system in 2% (w/v) agarose gels (BioRad, USA).

RESULTS AND DISCUSSION

Resistance to late blight

All the nine advanced hybrids as well as released varieties were observed for late blight reaction one week after inoculation. In both detached leaf assay as well as adult plant resistance, all the advanced hybrids were moderately resistant to resistant whereas control varieties Kufri Jyoti, Kufri Himalini and Kufri Girdhari were highly susceptible, moderately resistant and highly resistant, respectively **(Fig. 1)** and **(Fig. 2)**. All the hybrids showed resistant reaction and showed infection level upto 10 per cent only, while

Table 1. R genes/markers used for genotyping against various biotic stresses in advanced hybrids.

Marker	Gene /QTL	Type	PCR Condition	Size (bp)	Primer sequence (5′-3′)	Reference
Late blight						
R1	R1	AS	94°C,180s; 35 × (94°C, 35s; 60°C, 45s; 72°C, 90s); 72°C, 180s	1400	F-CACTCGTGACATATCCTCACTA R-CAACCCTGGCATGCCACG	Ballvora *et al.* (2002)
CosA	R1	PCR based	94°C, 120s; 35 × (94°C, 20s; 55°C, 30s; 72°C, 90s); 72°C, 180s	210	F-CTCATTCAAAATCAGTTTTGATC R-GAATGTTGAATCTTTTTGTGAAGG	Gebhardt *et al.* (2004)
R2	Rpi-abpt (R2- Ortholog)	PCR based	94°C, 300s; 35 × (94°C, 30s; 54°C, 30s; 72°C, 45s); 72°C, 420s	686	F- GCTCCTGATACGATCCATG R- ACGGCTTCTTGAATGAA	Kim *et al.* (2012)
R3	cLET5E4	CAPS/ HhaI	94°C, 120s; 35 × (94°C, 20s; 55°C, 20s; 72°C, 30s); 72°C, 300s	310	F- CCAGGCATGCTCAATTTGGAGT R-TTCCCTGTTTGGACTACTTGTGGA	Huang *et al.* (2005)
R3a	R3	SCAR	94°C, 180s; 35 × (94°C, 35s; 60°C, 45s; 72°C, 90s); 72°C, 180s	1380	F-GCTTCCGACATGTATTGATCTCCC R-GGCAGCCACTTCAGCTTCTTACAG	Sokolova *et al.*
Potato virus Y						
RYSC3	Ry_{adg}	SCAR	94°C, 300s; 35 × (94°C, 60s; 55°C, 30s; 72°C, 60s); 72°C, 600s	320	F-ATACACTCATCTAAATTTGATGG R-AGGATATACGGCATCATTTTTCCG	Kasai *et al.* (2000)
YES-3A	Ry_{sto}	STS	94°C, 120s; 10 × (94°C, 40s; 61°C, 40s; 72°C, 60s); 30 × (94°C, 40s; 56°C, 40s; 72°C, 60s); 72°C, 300s	341	F-TAACTCAAGCGGAATAACCC R-AAATTCACCTGTTTACATGCTTCTTGTG	Song and Schwarzfischer (2008)
STM003	Ry_{sto}	SSR	94°C, 300s; 35 × (94°C, 60s; 55°C, 30s; 72°C, 60s); 72°C, 600s	111	F-GGAGAATCATAACAACCAG R-AATTGTAACTCTGTGTGTGTG R-GATGAGTCCTGAGTAACGA	Song *et al.* (2005)
PVX						
PVXNb	GM339 PVX (HR)	AS	94°C, 120s; 35 × (94°C, 15s; 54.5°C, 15s; 72°C, 40s); 72°C, 600s	330	F-GGTAGTTGGACGAGCATAT R-CTCACTTTTAGACCAGATTT	Marano *et al.* (2002)
PVXNb	GM637 PVX (HR)	AS	94°C, 120s; 35 × (94°C, 15s; 51.5°C, 15s; 72°C, 40s); 72°C, 600s	220	F-GCAGAAGATCGGATAGCAAAC R-GTAACGAGTTGAAGTTACTGA	Marano *et al.* (2002)
Nb	SPUD237	CAPS/ (*AluI*)	94°C, 120s; 35 × (94°C, 15s; 54.5°C, 15s; 72°C, 40s); 72°C, 600s	250	F-TTCCTGCTGATACTGACTAGAAAACC R-AGCCAAGGAAAAGCTAGCATCCAAG	DeJong *et al.* (1997)
	IPM4	CAPS	94°C, 180s; 35 × (94°C, 30s; 61.5°C, 30s; 72°C, 50s); 72°C, 600s	600	F-GTACTGGAGAGCTAGTAGTGATCA R-ACCACTGGCAAATGGCCATACGA	Bendahmane *et al.* (1997)
PVS						
*PVSNs*SCG17	SCG17	SCAR	94°C, 30s; 10 × (94°C, 15s; 55°C, 25s; 72°C, 105s; 25 × (94°C, 15s; 50°C, 25s; 72°C, 105s); 72°C, 300s	321	F-ACGACCGACACTCAAATTTGTACAAGAAA R-GATGCCCCGACAGAGGAAG	Marczewski *et al.* (2001b)
PLRV						
PLrv.1 (*S. chacoense*)	NI1127	SCAR	93°C, 60s; 35 × (93°C, 30s; 59°C, 15s; 72°C, 90s); 72°C, 600s	1064	F-TAGAGAGCATTAAGAAGCTGC R-TTTTGCCTACTCCCGGCATG	Marczewski *et al.* (2001a)

Marker	Gene /QTL	Type	PCR Condition	Size (bp)	Primer sequence (5'-3')	Reference
Potato cyst nematode						
TG 689	*H1*	SCAR	94°C, 120s; 35 × (94°C, 20s; 55°C, 20s; 72°C, 30s); 72°C, 300s	141	F-TAAAACTCTTGGTTATAGCCTAT R-CAATAGAATGTGTTGTTTCACCAA	Milczarek *et al.* (2011)
HC	GpaVvrn_QTL (HC_QRL	AS	94°C, 300s; 94°C, 60s; 65°C,60s; 72°C, 60s; 6 × (94°C, 30s; 65°C decreasing the annealing temp. to 60°C by 1°C/cycle, 30s; 72°C, 30s); 30 × (94°C, 30s; 60.5°C, 30s; 72°C, 30s); 72°C, 300s	276	F-ACACCACCTGTTTGATAAAAAACT R-GCCTTACTTCCCTGCTGAAG	Sattarzadeh *et al.* (2006)
Gro 1-4-1	Gro 1-4-1	STS	94°C, 600s; 35 × (94°C, 30s; 62°C, 45s; 72°C, 60s); 72°C, 600s	602	F-AAGCCACAACTCTACTGGAG R-GATATAGTACGTAATCATGCC	Asano *et al.* (2012)
Gro VI-XO2	GroVI	SCAR	92°C, 7 min; 25 × (92°C, 1 min, 55°C, 1 min, 72°C, 2 min) 72°C, 5 min	854	F-CCACCAAACCCATAAAGCTGC R-TGTGAATTGGTATGAATCTGCAACC	Jacobs *et al.* (1996)
SPUD 1636	PCA based (Gpa5_QTL)		94°C, 3 min; 94°C, 30s; 65°C, 1 min; 72°C, 1 min; 5 × (94°C, 30s, 65°C decreasing the annealing temperature to 60°C by 1°C per cycle, 30s; 72°C 30s); 24 × (94°C, 30s, 60°C, 30s, 72°C 30s); 72°C, 3 min.	226	F-GTCGGCACAGGGTAAAACC R-ACCTTAGCGGATGAAAGCC	Bryan *et al.* (2002)
TG432	Grp1	CAPS/ Rsal	94°C, 3 min; 35 × (94°C, 30s, 66°C, 45s, 72°C 2 min. 30s) 72°C, 3 min	1900	F-GGACAGTCATCAGATTGTGG R-GTACTCCTGCTTGAGCCATT	Caromel *et al.* (2005)

Kufri Jyoti and Kufri Himalini recorded 60 and 40 per cent disease incidence. In comparison to control varieties all the hybrids were better for resistance to late blight except Kufri Girdhari. Three hybrids viz., SM/03-23, VMT 5-1 and SM/08-11 were at par to Kufri Girdhari for late blight resistance (**Table 2**).

Virus cross-inoculation effect on late blight resistance

The effect of late blight observed on virus inoculated plants was different for different viruses. In case of PVA, SM/03-23 and SM/08-11 showed absolute resistance to late blight which was also observed for check variety Kufri Girdhari. All advanced hybrids except SM/03-23 and SM/08-11 showed increased late blight incidence under cross inoculation. The results were similar in case of other viruses i.e. PVS and PVY inoculation also. Two advanced hybrids viz., SM/03-23 and SM/08-11 however showed resistant reaction to late blight along with check variety Kufri Girdhari under cross inoculation. In case of PVY, highly susceptible variety Kufri Jyoti showed low late blight disease incidence.

ELISA test for resistance to viruses

ELISA test for the hybrids as well as check varieties was conducted and it was observed that all the hybrids and control varieties except SM/09-161 were resistant to PVY. Similarly was the case for PLRV where all hybrids and control varieties showed resistance except SM/00-120. Five hybrids, VMT 5-1, SM/00-120, SM/00-42, SM/08-11, SM/08-12 as well as all the three control varieties were resistant to PVS. For PVA and PVM, all the hybrids as well as control varieties showed resistant reaction. However, all the hybrids as well control varieties were susceptible to PVX. The results revealed that the hybrids VMT 5-1, SM/00-42, SM/08-11 and SM/08-12 were resistant to all viruses except PVX along with all the three control varieties (**Table 2**).

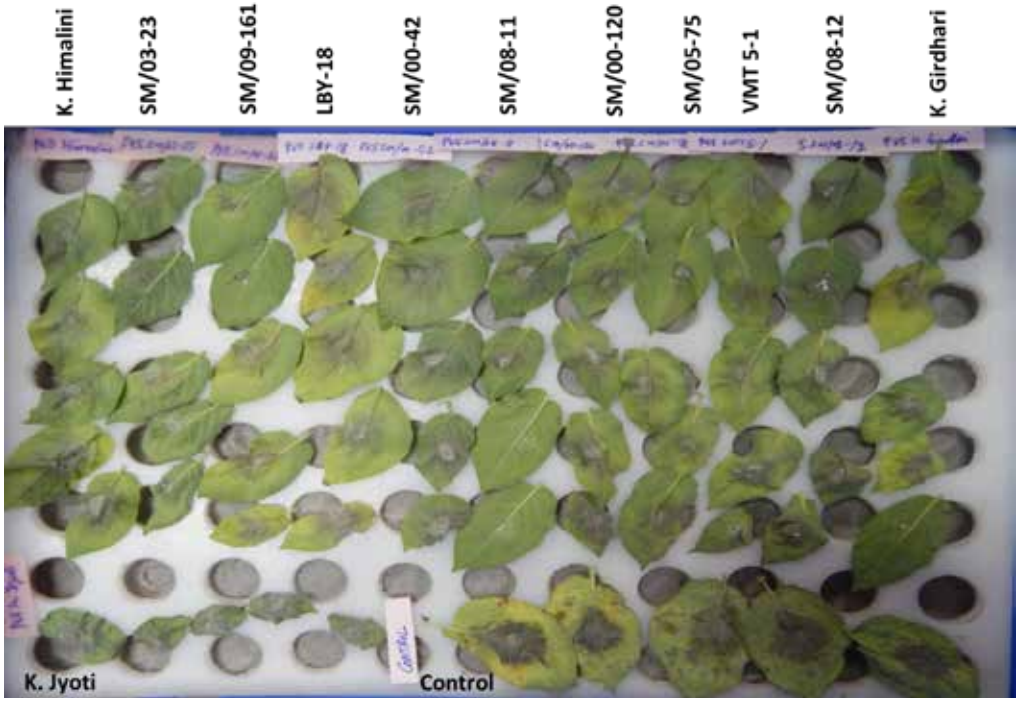

Fig. 1. Detached leaf assay for late blight resistance of advanced potato hybrids.

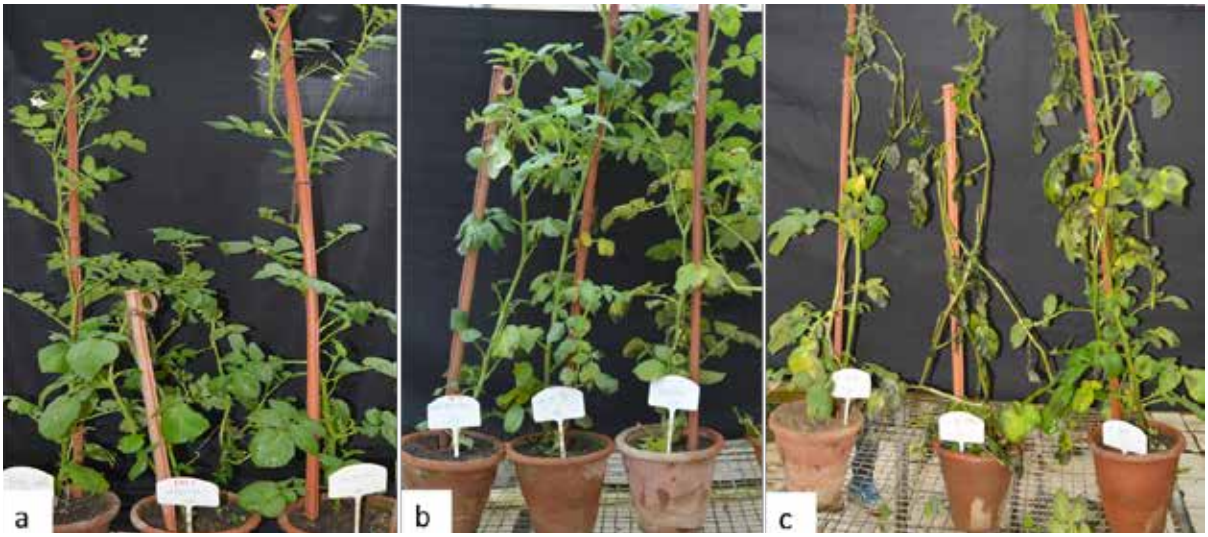

Fig. 2. Adult plant resistanceto late blight a) SM/03-23 b) SM/00-42 c) Kufri Jyoti.

Screening hybrids for R genes

In order to further confirm the results, linked markers previously identified for resistance screening were deployed for late blight, PVY, PVX, PVS, PLRV and PCN in all the twelve genotypes. Five late blight R genes were deployed and it was found that all the twelve genotypes possessed CosA and cLET5E4 (R3 flanking marker) gene. The resistant gene R1 (AS) was present in two hybrids (SM/08-12, SM/00-120) and check

Table 2. Reaction of hybrids to late blight and DAS-ELISA screening for viruses.

Genotype	Late blight	DAS-ELISA					
	Per cent incidence	PVY	PVX	PLRV	PVS	PVM	PVA
SM/00-42	10	-ve	+ve	-ve	-ve	-ve	-ve
SM/03-23	0	-ve	+ve	-ve	+ve	-ve	-ve
SM/09-161	5	+ve	+ve	-ve	+ve	-ve	-ve
SM/00-120	5	-ve	+ve	+ve	-ve	-ve	-ve
SM/08-11	0	-ve	+ve	-ve	-ve	-ve	-ve
VMT 5-1	0	-ve	+ve	-ve	-ve	-ve	-ve
SM/05-75	5	-ve	+ve	-ve	+ve	-ve	-ve
LBY 18	15	-ve	+ve	-ve	+ve	-ve	-ve
SM/08-12	10	-ve	+ve	-ve	-ve	-ve	-ve
Kufri Jyoti	60	-ve	+ve	-ve	-ve	-ve	-ve
KufriHimalini	40	-ve	+ve	-ve	-ve	-ve	-ve
KufriGirdhari	0	-ve	+ve	-ve	-ve	-ve	-ve

variety Kufri Jyoti, R2 gene was present in three hybrids (SM/00-42, VMT-5-1, LBY-18) and check variety Kufri Girdhari, while R3a gene was observed in six hybrids (SM/08-11, VMT-5-1, SM/05-75, SM/08-12, SM/00-42 and SM/03-23) and one check variety Kufri Girdhari (**Fig. 3**). The PVY resistance screening carried out using Ry_{adg} and Ry_{sto} genes linked markers, RYSC3, YES3A and STM003 revealed that Ry_{adg} gene was present in only one hybrid i.e. LBY 18, while all the hybrids and control varieties were found positive for STM003 indicating presence of Ry_{sto} gene in them. None of the genotypes showed YES3A marker band. The screening results for PVX gene linked molecular markers revealed that control variety Kufri Himalini was positive for all four, the advanced hybrids SM/03-23, VMT 5-1, SM/05-75 and LBY18 for three and SM/00-42, SM/08-12 along with control variety Kufri Girdhari were positive for two markers (**Table 3, Fig. 4**). Rest of the hybrids and control variety Kufri Jyoti were positive for one of the four markers. All the hybrids showed the presence of SCG17 marker for PVS resistance and NI1127 for PLRV resistance except the control variety, Kufri Himalini for SCG17 (**Fig. 4**). Out of the six genes screened for PCN resistance in 12 genotypes, the markers Gro VI-XO2 was present in five hybrids (VMT 5-1, SM/05-75, SM/00-120, LBY-18, SM/09-161) and one check variety, Kufri Himalini; HC was present in six hybrids (VMT 5-1, SM/05-75, SM/00-120, SM/00-42, LBY-18, SM/09-161, SM/03-23) and two check varieties namely Kufri Jyoti and Kufri Girdhari; TG 432 was found in four hybrids (SM/08-12, SM/00-42, LBY-18, SM/09-161) and one check variety Kufri Jyoti. None of the genotypes including control varieties had SPUD 1636, Gro 1-4-1 and TG 689 gene linked markers (**Table 3**).

Late blight, which is caused by the oomycete pathogen, *Phytophthorainfestans* (Mont.) de Bary, is considered the most destructive disease affecting potato production worldwide (Stevenson et al. 2001). It is important to screen the hybrids for late blight resistance before their clonal advancement and further testing for tuber and plant traits. In our study, all the hybrids showed resistant reaction to late blight through detached leaf assay as well as whole plant resistance screening. It confirms that the advanced

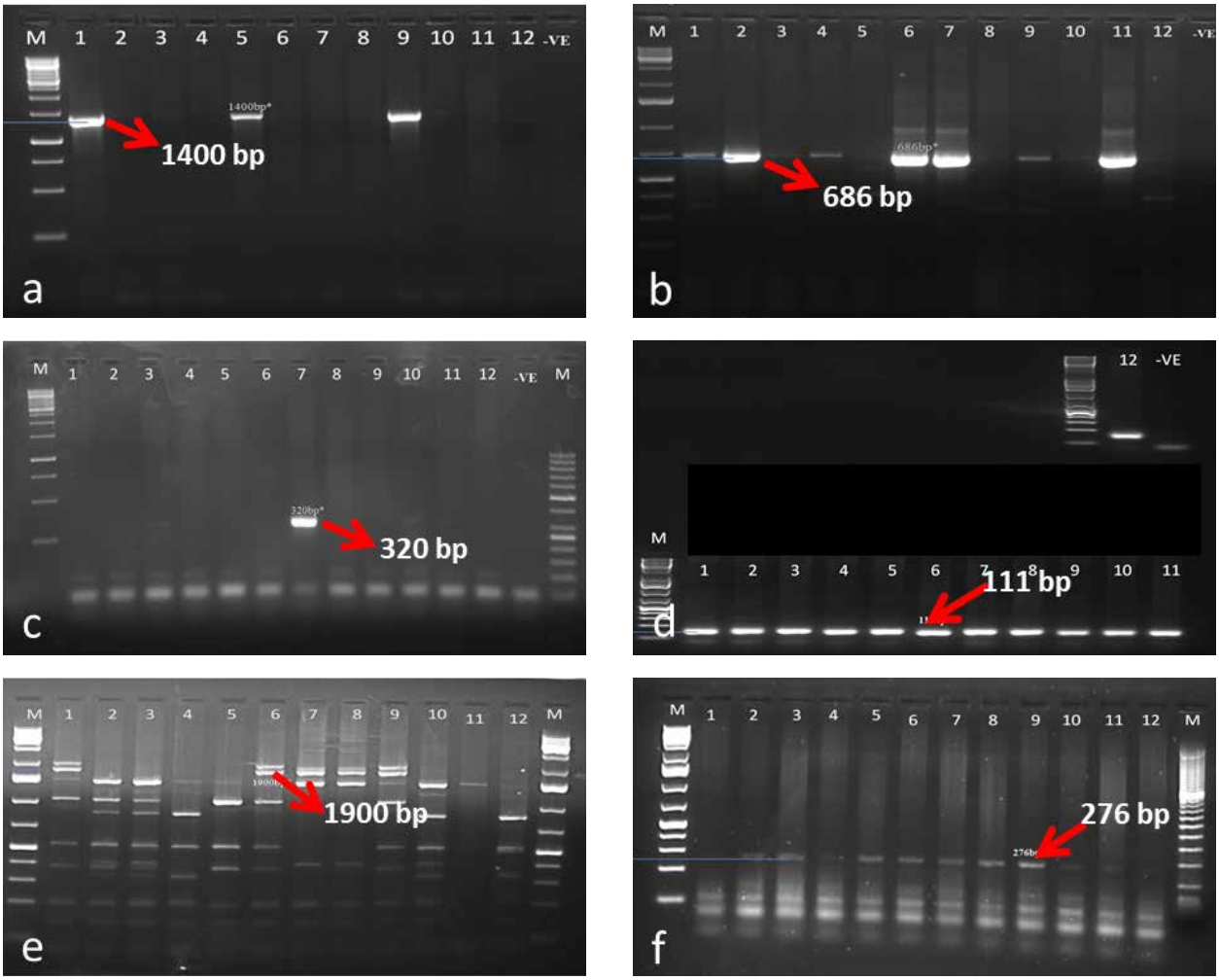

Fig. 3. Electrophoretic profile of different R genes/R gene linked markers in 12 potato genotypes a) R1-Late blight b) R2- Late blight, c) Ryadg-PVY, d) STM003-PVY, e) TG 432-PCN, f) HC-PCN.

Sample details: 1-SM/08-12; 2-VMT 5-1; 3-SM/05-75; 4-SM/08-11; 5-SM/00-120; 6-SM/00-42; 7-LBY 18; 8-SM/09-161; 9-K. Jyoti; 10-SM/03-23; 11-K. Girdhari; 12-K. Himalini

hybrids possess high resistance to late blight, which is the top priority after tuber yield for release of hybrids in potato growing areas of the country. Among checks, Kufri Girdhari was highly resistant whereas, Kufri Himalini was moderately resistant and Kufri Jyoti was susceptible to late blight. Although the results of virus inoculation on late blight cross protection were clear, it need further confirmation and validation through inclusion of highly resistant and susceptible varieties in future studies.

Viral diseases too play a significant role in reducing the yield potentiality of potato. Viral diseases cause heavy losses to the crop and prove a limiting factor in its successful cultivation. In India, occurrence of potato viruses had been noticed more than 60 year ago in Punjab (Mahendra, 1930). Potato Virus X (PVX), Potato Virus Y (PVY), Potato Virus A (PVA), Potato Virus M (PVM), Potato Virus S (PVS), Potato aucuba virus, mop-top virus, potato leaf roll virus (PLRV) and potato apical leaf curl virus (PALCV) are the important

Table 3. Screening of advanced potato hybrids and control varieties using linked markers for late blight, viruses and PCN.

Genotype	Late blight					PVY		PVX			PVS	PLRV	PCN				
	R1AS	R2	R3a	CosR1	cLET5E4	RYSC3	YES 3A	STM003	GM 339	GM 637	SPUD 237	IPM4	SCG 17	NI1127	TG689	HC	Gro 1-4-1	Gro 6-Xo-2	SPUD 1636	TG432
SM/00-42	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve
SM/03-23	-ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
SM/09-161	-ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
SM/00-120	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve
SM/08-11	-ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
VMT 5-1	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve
SM/05-75	-ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve
LBY 18	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve
SM/08-12	+ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve
Kufri Jyoti	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve
KufriHimalini	-ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve
KufriGirdhari	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve

viral diseases in all potato growing zones of India (Uniyal, 2003). Insects (aphid, beetle, leaf hopper and whitefly), fungi and seeds serve as source and vector of virus transmission (Paul and Kumar, 2003). Host resistance is the only successful strategy for containing the viruses. ELISA test is the most reliable method to screen the genotypes for their resistance to various potato viruses. The ELISA results for PVY, PVS, PVA, PVM and PLRV showed that most of the hybrids and control varieties were resistant barring a few in each case. None of the hybrids or control varieties was resistant to PVX. The DAS-ELISA screening results revealed that the four hybrids viz., VMT 5-1, SM/00-42, SM/08-11 and SM/08-12 were resistant to all viruses except PVX.

Genetic markers offer the ability to identify valuable traits in seed populations before those traits are measured in the field. Marker-assisted selection (MAS) often allows for a decrease in the number and extent of field trials and can greatly increase the speed of developing new varieties (Moreau *et al*., 2000; Slater *et al.,* 2013). Consumer preferences change over time, and speeding the development of new varieties would help potato breeders respond to these changes more quickly. Incorporating DNA markers are a cost effective and labour saving alternative to mechanical inoculation for late blight, potato viruses and PCN. MAS for PCN resistance saves over 10% of the cost compared to conventional phenotyping alone (Slater *et al*., 2013).

For late blight resistant reaction and presence of three or more R genes in seven hybrids (SM/08-11, SM/03-23, VMT 5-1, SM/08-12,SM/00-42, SM/00-120 and SM/05-75) and check variety Kufri Girdhari confirmed the resistance of these genotypes to late blight disease. The MAS results for PVY, PVS and PLRV were in agreement for most of the genotypes with the ELISA results except for PVX resistance, where all

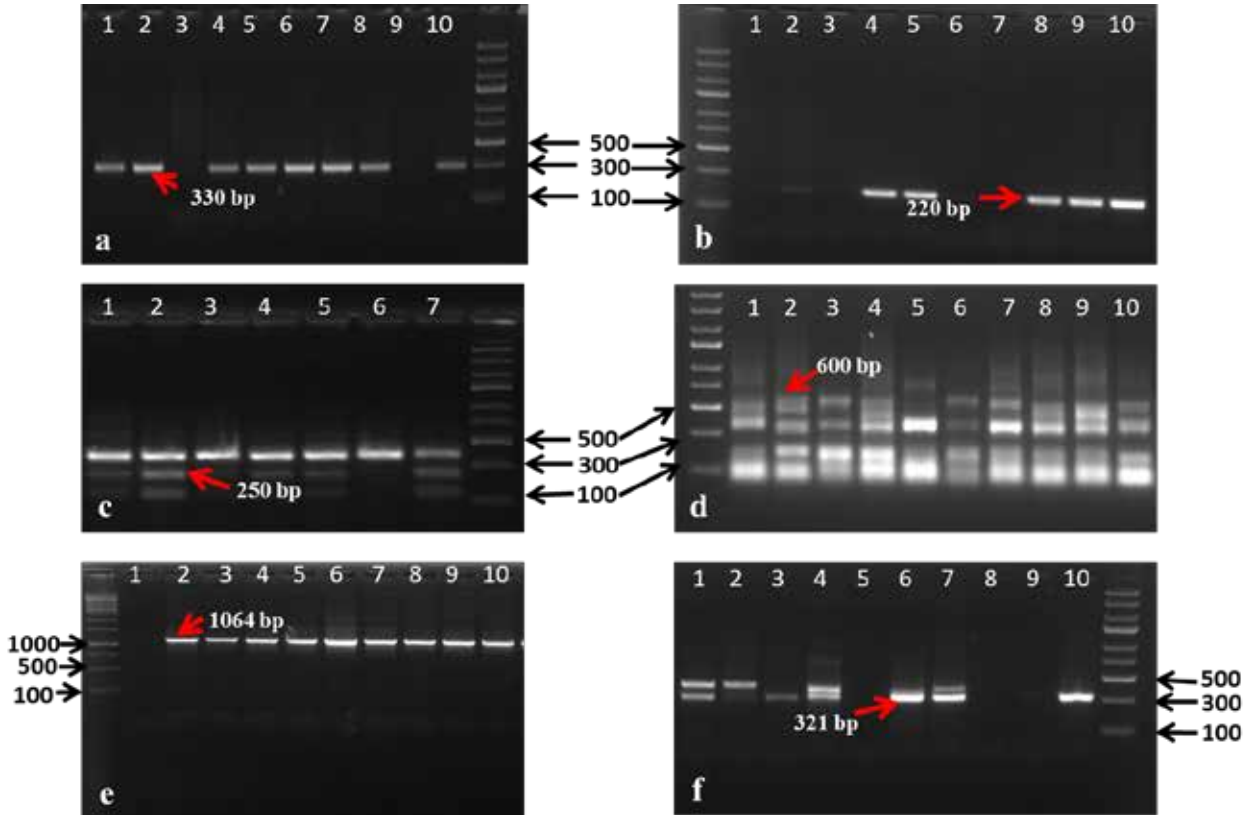

Fig. 4. Electrophoretic profile of different R genes/R gene linked markers in 12 potato genotypes a) GM339, b) GM637, c) SPUD237, d) IPM4-PVX e) NI1127-PLRV f) SCG17-PVS

genotypes showed susceptibility in ELISA test although many of the genotypes were carrying resistant gene linked markers.

In some cases, the genotypes showing presence of marker band for particular R gene showed susceptible reaction in phenotyping. Similarly, the genotypes showing resistant reaction in phenotyping did not show the presence of linked markers to resistant genes for respective virus. The differences in genotyping results in comparison to phenotypic data clearly indicate that there are other unidentified R genes which govern the resistance to these viruses or the markers used for screening does not have tight linkage with the resistant genes. The results also reveal that single R gene may not provide complete or extreme resistance and a series of R genes or interaction of resistant genes could be responsible for resistance. Although many resistant genes have been identified for important potato viruses like RY_{adg} and RY_{sto} for PVY and Rx1 for PVX extreme resistance, they does not seem to work equally for all genotypes and against different virus strains. However, the similar R gene linked markers i.e. RY_{chc} and RX1 have been successfully used in breeding for resistance to PVY and PVX in Japan (Mori *et al*., 2015).

PCN another important pest is a serious threat to potato cultivation in India. Based on the genotypic data of PCN resistance genes, the genotypes SM/09-161, LBY-18, VMT 5-1, SM/05-75, SM/00-120 and SM/00-42 were found to be probable genotypes for PCN resistance. Our results suggest that molecular markers linked to resistant genes for PCN should be essential part of potato

breeding programme. The same technique is being followed in Japan for culling of PCN susceptible genotypes with the use of single dominant gene (H1) that confers nearly complete and durable resistance to pathotypes Ro1 and Ro4 (Asano *et al*., 2012). Moreover, detection of PCN genes using DNA markers is quite beneficial for PCN due to quarantine issues involved in natural screening. Precise DNA markers help breeders identify resistant genotypes without using living nematodes for screening (Asano and Tamiya, 2016).

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