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Short Communication

Molecular characterization of potato virus Y resistance in potato (*Solanum tuberosum* L.)

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Abstract

Amongst viruses, *Potato virus Y* (PVY) is the most prevalent virus affecting potato productivity. Resistance genes (*Ry*) in *Solanum tuberosum* subsp. *andigena* (Ry_{adg}) and *Solanum stoloniferum* (Ry_{sto}) confer extreme resistance (ER) to PVY. SCAR marker RYSC3 for Ry_{adg} gene and SSR marker STM0003 for Ry_{sto} gene were used to screen 119 potato genotypes including 44 Indian varieties. These genotypes were also challenge inoculated with PVY^{o} strain and confirmed for ER by DAS-ELISA. Gene Ry_{adg} was present in 11 and Ry_{sto} in 22 genotypes, while 3 genotypes possessed both the genes. The statistical analysis indicated that among the two tested markers, RYSC3 is better diagnostic of PVY resistance. The identified genotypes can serve as potential source for future virus resistance breeding programs.

Key words: Potato virus Y, MAS, extreme resistance gene, *S. tuberosum, S. stoloniferum*

Potato (*Solanum tuberosum* L.) is third most important food crop in the world after rice and wheat. It is prone to a number of biotic and abiotic stresses, out of which late blight followed by viruses is most rampant. More than 30 different viruses affect potato crop worldwide, among which potato virus Y (PVY), a member of the *potyvirus* group is the most active often causing yield reduction up to 80% in combination with other viruses like PVX and PLRV (Jeffries et al. 2006). Virus is transmitted by aphids in a non-persistent manner and can cause infection rapidly making it very difficult to manage through chemical means. Since potato crop is mostly propagated through tubers, PVY infection *via* seed can spread far and wide across generations, thus necessitating the need to develop robust virus free seed production programme, which currently is restricted to developed countries while the situation is grim in many developing countries, where the healthy seed production programme either does not exist or is inadequate. In India, potatoes are mostly grown under sub-tropical climate that favour the proliferation of viruses because of the congenial conditions prevalent for vectors as well as their expression and thus limit the production of healthy seed. So, the development and use of virus resistant varieties is the only plausible alternative to tackle the virus spread. Introgression of PVY resistance using molecular markers offer an ideal strategy for faster potato breeding. Out of several genes identified for PVY resistance in wild species Ry_{adg} and Ry_{sto} genes confer extreme resistance (ER) to PVY *i.e.* against all strains with no symptoms and localised necrosis after graft inoculation in plants (Videl et al. 2002). Thus, indirect selection of resistant genotypes through molecular markers at the seedling stage can be useful in hastening potato breeding (Sharma et al. 2013). The current investigation was undertaken to screen the tetraploid potato germplasm including Indian potato cultivars employing SCAR and SSR marker linked to Ry_{adg} and Ry_{sto} genes, respectively and confirmation of resistance by mechanical inoculation followed by double antibody

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sandwich enzyme-linked immunosorbent assay (DAS-ELISA). We could identify some elite parental lines that can be exploited for transferring the virus resistance into new potato cultivars.

Plant materials consisted of 119 tetraploid potato accessions (Indian and exotic lines) available in the repository of Central Potato Research Institute (CPRI), Shimla, Himachal Pradesh, India. The material was raised in earthen pots (5 tubers per accession) during summer seasons (April-August) of 2009 and 2010 in the glass house at CPRI, Shimla. The plants were mechanically inoculated with the PVY^{o} strain using spray gun (Fernanda-Northcote 1992). The plants were periodically observed for symptoms development and after one month of inoculation, the plants were tested by DAS-ELISA. The PCR reactions volume was set to 20µl reaction containing 10x tris buffer B, 25mM $MgCl_2$, 2.5mM each dNTPs, 1µM of each specific primer, 3 unit/µl Taq polymerase and 25ng/µl genomic DNA. PCR amplifications of SCAR marker RYSC3 linked to Ry_{adg} gene (Kasai et al. 2005) and SSR marker STM0003 linked to Ry_{sto} gene (Song et al. 2005) were carried out in C-1000 Thermal Cycler (Bio-Rad, USA). The PCR reaction cycle for the SCAR marker RYSC3 consisted of an initial denaturation step at 94°C for 300 s, followed by 35 cycles of denaturation at 94°C for 60 s, primer annealing at 55°C for 30 s, and extension at 72°C for 60 s, followed by a final extension at 72°C for 300 s. PCR amplification products were separated on horizontal gel electrophoresis system in 2% (w/v) agarose gels (Bio-Rad, USA). Presence of single band of 320 bp was associated with Ry_{adg} gene. The PCR reaction cycle for the SSR marker STM0003 consisted of an initial denaturation step at 94°C for 300 s, followed by 30 cycles of denaturation at 94°C for 45 s, primer annealing at 55°C for 45 s, and extension at 72°C for 60 s, followed by a final extension at 72°C for 300 s. The PCR product was analysed using chip based automated electrophoresis system MultiNA (Shimadzu, Biotech, Japan). Specific allele peak of 111$\pm$ 5bp size was found to be associated with Ry_{sto} gene. Statistical analysis was done using SPSS software 9.0.

Among tested lines, 61 showed PVY resistance through ELISA testing and 58 lines were susceptible to PVY infection (Tables 1 and 2). Out of the 61 phenotypically resistant lines, 36 possessed either Ry_{adg} (11) or Ry_{sto} (22) and 3 lines possessed both the genes for resistance to PVY. Thus, a clear relationship can be observed among the genotypes possessing any of these resistance genes and their PVY resistance. Besides, 34 accessions comprising both indigenous and exotic genotypes were resistant to PVY infection through ELISA test when challenge inoculated with PVY^{0} strain but did not possess either Ry_{adg} or Ry_{sto} genes. Whitworth et al. (2009) have reported a similar observation that Ry_{adg} gene from *Andigena* imparts resistance against all strains of PVY but some resistant potato genotypes can also possess some unidentified *R*-genes. These accessions seem to possess different gene for PVY resistance derived from wild or cultivated potato species other than *Solanum tuberosum* ssp. *andigena* and *Solanum stoloniferum*. These accessions found resistant to PVY but lacking both Ry_{adg} and Ry_{sto} genes may be screened to confirm the presence of other PVY resistance genes from more diverse sources through their linked markers to reveal their actual source of PVY resistance. In present study, we could find 9 such accessions, where the genotypes were susceptible to PVY infection despite the presence of PVY resistance gene Ry_{sto} through tightly linked marker gene STM0003. Such discrepancy between presence of gene for PVY resistance and susceptibility of genotype to PVY infection can be attributed to errors in ELISA/PCR assays or recombination (Ortega and Lopez-Vizeon 2012) or low variance explained by the tested marker. Further, correlation between presence of the marker and phenotypic resistance was tested with non-parametric, Mann-Whitney and One-way ANOVA tests. When using presence or absence of a single marker as grouping variable, presence of the Ry_{adg} gene with RYSC3 marker was significantly correlated with resistance with $q < 0.005$ and amount of variance (R^2) =12.68%, while the marker STM0003 was not significantly correlated with resistance with $q > 0.005$ and R^2=1.73% only. Therefore, among the two tested markers, RYSC3 is better diagnostic of PVY resistance. Sixty one diverse potato accessions having resistance to PVY were identified, which can serve as valuable source for PVY resistance in potato breeding. Marker assisted selection for PVY resistance has been adopted at limited scale for parental line screening as well as clonal selection in tetraploid

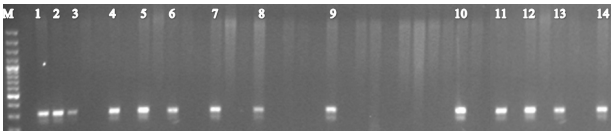

Fig. 1. Gel image of 14 positive genotypes with Ry_{adg} gene as amplified using RYSC3 SCAR marker with 320 bp in 2.5% agarose gel. Lane 1 marker

Table 1. Potato germplasm screened for Ry_{adg} and Ry_{sto} genes and their ELISA testing for PVY resistance

Origin	Markers (gene)		Genotype	
(Ry_{adg})	RYSC3 (Ry_{sto})	STM0003	Resistant	Susceptible
Indigenous	+	+	K. Alankar, K. Jawahar	
germplasm	+	-	K. Chipsona-1, K. Himsona, MP/97-625, MP/97-699, MP/97-921, MP/04-578	
	-	+	K. Frysona, K. Giriraj, K. Megha, B-420 (2), QB/A-9-120, MP/92-35, MP/97-1008, MP/99-1189	K. Arun, K. Jeevan, K. Kundan, K. Red
	-	-	K. Anand, K. Chamatkar, K. Chipsona-2, K. Chipsona-3, K. Girdhari, K. Kanchan, K. Khyati, K. Lalima, K. Shailja, K. Sutlej, K. Swarna, A/98-98, HR 9-5, JEX/A-695, JEX/A-785, MP/98-31, MP/99-322, MP/99-406, MP/01-572, MP/04-816, MP/05-41	K. Ashoka, K. Badshah, K. Bahar, K. Chandramukhi, K. Dewa, K. Himalini, K. Jyoti, K. Khasigaro, K. Kuber, K. Lauvkar, K. Muthu, K. Kumar, K. Naveen, K. Neela, K. Pukhraj, K. Pushkar, K. Sadabahar, K. Safed, K. Sheetman, K. Sherpa, K. Sindhuri, K. Surya, HT/97-727, JEX/A-663, MP/97-637, MP/98-42, MP/98-172, MP/2K-424, MP/2K-1058, MP/02-472, MP/05-05, MP/05-142, MP/05-180,VMT 5-3
Exotic germplasm				
Peru	+	+	CP 4038	
	+	-	CP 2058, CP 3771, CP 4039, CP 4046, CP 4047	
	-	+	CP 2067, CP 2294	CP 2407, CP 4051
	-	-	CP 2064, CP 2066, CP 2175, CP 3198, CP 3359, CP 4042, CP 4043 CP 4058	CP 2372, CP 2378, CP 4041, CP 4044, CP 4045, CP 4049, CP 4050, CP 4057,
USA	-	+	CP 1765, CP 1945, CP 2186	CP 1748, CP 1920
	-	-	CP 1798, CP 1911	CP 1371, CP 1390
The Netherlands	-	+		CP 1780
	-	-		CP 1404
Germany	-	-		CP 1456, CP 1588
France	-	-	CP 1717	
Mexico	-	-		CP 2011
Poland	-	-	CP 3173	
Canada	-	-	CP 3594, CP 3644	

+/-: Presence/Absence of marker; K = Kufri

Table 2. Frequency distribution of PVY resistance among different potato genotypes screened for Ry_{adg} and Ry_{sto} genes and marker-trait associations

PVY Resistance			Accessions type	Accessions (No)
RYSC3	STM003	PVY resistance through ELISA		
+	+	R	Exotic Lines	1
			Indigenous Varieties	2
			Hybrids and Andigena	0
+	-	R	Exotic Lines	5
			Varieties	2
			Hybrids and Andigena	4
-	+	R	Exotic Lines	5
			Varieties	3
			Hybrids and Andigena	5
-	-	R	Exotic Lines	13
			Varieties	11
			Hybrids and Andigena	10
-	+	S	Exotic Lines	5
			Varieties	4
			Hybrids and Andigena	0
-	-	S	Exotic Lines	15
			Varieties	22
			Hybrids and Andigena	12
			Total accessions	119

potato breeding programmes (Ottoman et al. 2009; Sagredo et al. 2009; Kaushik et al. 2013). Incorporation of marker based selection strategy in breeding programme for PVY resistance in potato based on *Solanum tuberosum* ssp. *andigena* and *Solanum stoloniferum* genes (Valkonan et al. 2008) have been advocated by earlier workers. Although the method is not infallible in its ability to dissect all the factors underlying resistance to PVY, yet it helps in rapid selection of PVY resistance clone in early generation of breeding programme. However, we have to be cautious in our approach in relying absolutely on marker based selection of genotypes for PVY resistance. There is urgent need to excavate more resistance genes and divergent sources other than S*olanum tuberosum* ssp. *andigena* and *Solanum stoloniferum* against PVY coupled with advanced serological techniques to identify resistant parents and progenies for precise PVY resistance breeding.

References

Fernanda-Northcote E. N. 1991. Potato seedling screening for resistance to potato viruses X and Y by the mass inoculation technique with spray gun. *CIP Research Guide* International Potato Center, Lima, Peru, **37**: 12 pp.

Jeffries C., Barker H. and Khurana S. M. P. 2006. Viruses and viroids. *In*: Handbook of Potato Production, Improvement and Post harvest Management. Gopal, J. and Khurana, S.M.P. (eds), Food Product Press, New York, 387-389.

Kasai K., Morikawa Y., Sorri V. A., Valkonen J. P. T., Gebhardt C. and Watanabe K. N. 2000. Development of SCAR markers to the PVY resistance gene *Ryadg* based on a common feature of plant disease resistance genes. Genome, **43**: 1-8.

Kaushik S. K., Sharma R., Garg I. D., Singh B. P., Chakrabarti S. K., Bhardwaj V. and Pandey S. K. 2013. Development of a triplex (YYYy) parental potato line with extreme resistance to potato virus Y (PVY) using marker assisted selection (MAS). J. Hortic. Sci. Biotech., **88**: 580-584.

Ortega F. and Lopez-Vizcon C. 2012. Application of molecular marker-assisted selection (MAS) for disease resistance in a practical potato breeding programme. Potato Res., **55**: 1-13.

Ottoman R. J., Hane D. C., Brown C. R., Yilma S., James S. R., Mosley A. R., Crosslin J. M. and Vales M. I. 2009. Validation and implementation of marker-assisted selection (MAS) for PVY resistance (Ry_{adg} gene) in a tetraploid potato breeding program. Amer. J. Potato Res., **86**: 304-314.

Sagredo D. B., Mathias R. M., Barrientos P. C., Acuña B. I., Kalazich B. J. and Rojas J. S. 2009. Evaluation of a SCAR RYSC3 marker of the Ry_{adg} gene to select resistant genotypes to *Potato virus Y* (PVY) in the INIA potato breeding program. Chilean J. Agri. Res., **69**: 305-315.

Sharma Reena, Kaushik S. K., Bhardwaj Vinay, Sharma S., Bhat A. K. and Singh B. P. 2013. Molecular characterization of potato genotypes for late blight resistance. Potato J., **40**: 164-172.

Song Y. S., Hepting L., Schweizer G., Hartl L., Wenzel G. and Schwarzfischer A. 2005. Mapping of extreme resistance to PVY (Ry_{sto}) on chromosome XII using anther-culture-derived primary dihaploid potato lines. Theor. Appl. Genet., **111**: 879-887.

Valkonen J. P. T., Wiegmann K., Hamalainen J. H., Marczewski W. and Watanabe K. N. 2008. Evidence for utility of the same PCR-based markers for selection of extreme resistance to Potato virus Y controlled by Ry_{sto} of *Solanum stoloniferum* derived from different sources. Ann. Appl. Biol., **152**: 121-130.

Vidal S., Cabrera H., Andersson R. A., Fredriksson A. and Valkonen J. P. T. 2002. *Potato gene Y-1* is an N gene homolog that confers cell death upon infection with Potato virus Y. Mol. Plant Microbe Interact, **15**: 717-727.

Whitworth J. L., Novy R. G., Hall D. G., Crosslin J. M. and Brown C. R. 2009. Characterization of broad spectrum potato virus Y resistance in a *Solanum tuberosum* ssp. *andigena*-derived population and select breeding clones using molecular markers, grafting and field inoculations. Amer. J. Potato Res., **86**: 286-96.