Indian J. Hort. 70(3), September 2013: 373-377

Identification of host gene conferring resistance to *Potato virus Y* using *Ry* gene-based molecular markers

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ABSTRACT

Potato (*Solanum tuberosum* L.) is the third most important food crop of the world and vegetatively propagated mainly through tubers. Tuber yield mainly depends on the quality of potato 'seed tubers' especially free from viral diseases, otherwise it proliferates over the successive clonal generations. More than 30 different viruses infect potato, and *potato virus* Y (PVY) is the most important one in the north-western/ north-eastern parts and plateau regions of India. So, in this study breeding for virus resistance, seventy-one exotic potato germplasm were evaluated for the host-resistance gene using molecular makers linked to the *Ry* genes. Germplasm were also examined for the extreme resistance to PVY by *Ry* gene-linked markers and confirmatory results of resistance by ELISA testing. Among all, 26 germplasm (group I) showed the presence of Ry_{adg} gene as revealed by SCAR (RYSC3) and CAPS (ADG2/*Bbv*I) markers and also exhibited resistance to PVY by ELISA testing after mechanical inoculation of PVY^{O} isolate. This group I germplasm appear to be future research materials for the introgression of resistant gene Ry_{adg} derived from *S. tuberosum* spp. *andigena* using marker-assisted selection (MAS) in the Indian potato breeding. In addition, 45 germplasm (group II) did not amplify any of the markers of *Ry* genes available to date derived from wild *Solanum* species and their resistance/susceptibility is also not known. Thus, these research findings highlighted the use of the Ry_{adg} gene-based molecular markers to accelerate the potato breeding through MAS.

Key words: MAS, molecular markers, potato, PVY resistance, *Ry* gene.

INTRODUCTION

Potato (*Solanum tuberosum* L.; 2*n* = 4*x* = 48) is the most important non-cereal food crop of the world on accounts of high yield potential and rich nutritive values. Yields depend to a great extent on the quality of potato 'seed tubers', mainly absence of viral diseases. Viruses are the first and foremost problem in seed potato. Once the virus infects a potato clone, it is difficult to eradicate from seed lots. Consequently, disease becomes perpetual and proliferates over the successive clonal generations. Vegetative propagation of potato enables viruses to persist from one to next generation, resulting in continuous decline in productivity. Though more than 30 different viruses infect potato, potato virus Y (PVY) (genus *Potyvirus*) is the most important in the north-western/ north-eastern parts and plateau regions of India often causing yield reduction up to 80% in combination with other viruses. The *potyvirus* genus is currently the largest of the plant virus groups and is thought to be one of the most destructive groups of plant viruses affecting potato crop (Khurana, 5).

To surmount these problems, use of quality seeds or virus resistant cultivars is one of the alternatives. Hence, from breeding point of view, *Ry* (extreme resistant gene to PVY) genes are most suitable for development of virus resistant cultivars. The *Ry* genes have been identified in many *Solanum* species such as *S. tuberosum* ssp. *andigena* and *S. stoloniferum* (Cockerham, 2). The properties of extreme resistance and race non-specificity of the Ry_{adg} gene have remarkably made it an ideal source of PVY resistance for the potato breeding programs. During the past decade, numerous investigations have established that molecular markers, *viz.*, SCAR (Sequence characterized amplified region) (Kasai *et al.*, 4) and CAPS (Cleaved amplified polymorphic sequence) (Sorri *et al.*, 10), derived from resistant parent and progenies linked to Ry_{adg} gene and persist over the generations. These molecular markers facilitated selection of PVY resistant progenies in early segregating population at the seedling stage and are useful tools for faster potato breeding. This paper focuses on the utilization of the present-day available molecular markers as reviewed by Tiwari *et al.* (12) to uncover host-resistance genes and also to validate PVY extreme resistance in the exotic potato germplasm. The confirmatory results of PVY resistance by mechanical inoculation followed by double antibody sandwich enzyme-linked immunosorbent assay (das-ELISA) of the *Ry* gene containing genotypes are also reported here.

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MATERIALS AND METHODS

Seventy-one exotic potato germplasm were received from International Potato Centre, Lima-Peru under CIP-CPRI (ICAR) collaborative projects on biotic and abiotic stresses. Germplasm were grown in earthen pots filled with the mixture of sieved-soil, farm-yard manures and sands (2:1:1) during summer season (April-August) in the glasshouse at CPRI, Shimla. Plants were watered regularly and fertilized to maintain good health. An advance potato hybrid clone LBY-26 was used as a positive control for PVY resistance, whereas commercial potato cv. Kufri Chandramukhi was used as PVY susceptible control in this study.

Plant DNA was isolated from 100 mg leaves collected from fresh *in vitro* sub-cultures using the GenElute Plant Genomic DNA MiniPrep Kit (Sigma-Aldrich, St. Louis, USA). DNA quality and quantity were determined with NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, USA), and quality was also assessed on 0.8% (w/v) agarose gel. The isolated genomic DNA was used for DNA analyses. Host resistant genes for PVY were determined using available molecular markers of the different wild *Solanum* species following PCR amplification of the *Ry* genes as described previously by other researchers reviewed in Tiwari *et al.* (12). Out of many molecular markers for host resistant genes for PVY, the SCAR marker RYSC3 Kasai *et al.* (12) and CAPS marker ADG2/*Bbv*I Sorri *et al.* (10), linked to the Ry_{adg} gene showing their presence in our samples were used in this study for the detection of resistant plants. All the procedures for DNA analysis such as PCR amplification, gel documentation, scoring of bands; and serological testing (ELISA) of virus infection by artificial inolculation were performed as described in Tiwari *et al.* (13).

RESULTS AND DISCUSSION

A total of 71 exotic potato germplasm were evaluated for host resistance gene and extreme resistance to PVY through molecular markers. The Ry_{adg} markers analyses indicated the presence of linked genes associated with 26 germplasm (group I) as revealed by both the markers RYSC3 (Fig. 1) and ADG2/*Bbv* I (Fig. 2). The gene Ry_{adg} conferring extreme resistance to PVY was derived from one of the cultivated potatoes, *S. tuberosum* spp. *andigena*. In the serological study, confirmatory result of PVY resistance of the 26 germplasm was also determined through ELISA (Table 1). Twenty-six germplasm showed resistant response to PVY when tested by mechanical inoculation and screening by DAS-ELISA after 25 days of inoculation of PVY^{o}. The ELISA readings (A_{405} nm) of susceptible control after 25 days of mechanical inoculation were more than double (> 0.06) from that of the healthy control (0.03). Resistant germplasm displayed resistance to PVY consisting of the absence of virus symptoms and lower absorbance value by ELISA than threshold value of healthy control (0.03), consequently they confirmed as resistant germplasm. Variations in absorption values at A_{405}

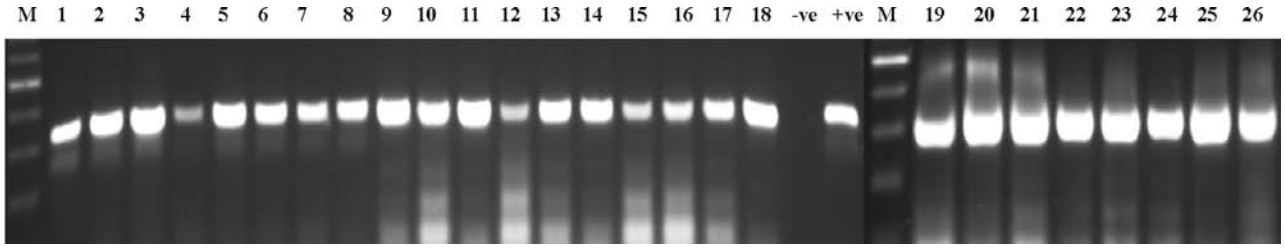

Fig. 1. DNA amplification using SCAR marker RYSC3 at 321 bp in PVY resistant potato germplasm from left to right (Sl. Nos. 1-26 of the Table 1); -ve = Kufri Chandramukhi (susceptible); +ve = LBY-26 (resistant); M = 100 bp ladder.

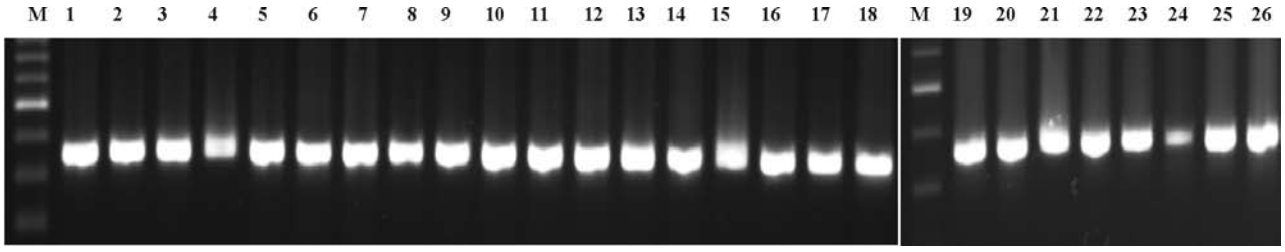

Fig. 2. DNA amplification using CAPS marker ADG2/*Bbv*I at 355 bp in PVY resistant potato germplasm from left to right (Sl. Nos. 1-26 of Table 1); M = 100 bp ladder.

Table 1. PVY resistant/susceptible potato germplasm revealed by the presence of Ry_{adg} gene-based molecular markers and serological assay by ELISA testing.

S. No.	Germplasm	ELISA testing for PVY infection	Molecular marker of Ry_{adg} gene	
			RYSC3	ADG2/ *Bbv* I
Group I				
	CP 4038	R	+	+
	CP 4039	R	+	+
	CP 4046	R	+	+
	CP 4047	R	+	+
	CP 4048	R	+	+
	CP 4053	R	+	+
	CP 4164	R	+	+
	CP 4166	R	+	+
	CP 4167	R	+	+
	CP 4168	R	+	+
	CP 4174	R	+	+
	CP 4175	R	+	+
	CP 4177	R	+	+
	CP 4179	R	+	+
	CP 4181	R	+	+
	CP 4184	R	+	+
	CP 4195	R	+	+
	CP 4196	R	+	+
	CP 4198	R	+	+
	CP 4202	R	+	+
	CP 4203	R	+	+
	CP 4205	R	+	+
	CP 4206	R	+	+
	CP 4208	R	+	+
	CP 4210	R	+	+
	CP 4211	R	+	+
Group II				
	CP-4040	nd	-	-
	CP-4041	nd	-	-
	CP-4042	nd	-	-
	CP-4043	nd	-	-
	CP-4044	nd	-	-
	CP-4045	nd	-	-
	CP-4049	nd	-	-
	CP-4050	nd	-	-
	CP-4051	nd	-	-
	CP-4052	nd	-	-
	CP-4054	nd	-	-
	CP-4055	nd	-	-
	CP-4056	nd	-	-
	CP-4057	nd	-	-
	CP-4058	nd	-	-
	CP-4169	nd	-	-
	CP-4170	nd	-	-
	CP-4171	nd	-	-
	CP-4172	nd	-	-
	CP-4173	nd	-	-
	CP-4176	nd	-	-
	CP-4178	nd	-	-
	CP-4180	nd	-	-
	CP-4183	nd	-	-
	CP-4185	nd	-	-
	CP-4186	nd	-	-
	CP-4187	nd	-	-
	CP-4188	nd	-	-
	CP-4189	nd	-	-
	CP-4190	nd	-	-
	CP-4191	nd	-	-
	CP-4192	nd	-	-
	CP-4193	nd	-	-
	CP-4194	nd	-	-
	CP-4197	nd	-	-
	CP-4199	nd	-	-
	CP-4200	nd	-	-
	CP-4201	nd	-	-
	CP-4204	nd	-	-
	CP-4207	nd	-	-
	CP-4209	nd	-	-
	CP-4212	nd	-	-
	CP-4213	nd	-	-
	CP-4214	nd	-	-
	CP-4215	nd	-	-

Contd...

Contd...

R/S : Resistance/Susceptible; +/− : presence/absence; nd: not determined

nm of ELISA readings indicated the allelic variants in term of plex doses (simplex/duplex/triplex/tetraplex) of the Ry_{adg} gene for PVY resistance in the germplasm. Thus, our results indicated the presence of only Ry_{adg} in the PVY resistant germplasm of the group I. Other Ry_{adg} markers ($ADG1_{356}$ and $ADG2_{354}$) did not amplify the desired amplicons in our plant materials. Besides, all other molecular markers for the genes Ry_{sto}/$Ry\text{-}f_{sto}$ and Ry_{chc} as reviewed by Tiwari *et al.* (12), derived from the wild potato species did not produce any amplicons in the 71 germplasm samples. This indicated that the resistance sources of the group I germplasm were derived from *S. tuberosum* spp. *andigena* and not from the *S. stoloniferum* and *S. chacoense*. Whereas, group II consisting of 45 potato germplasm failed to amplify *Ry* gene from either of the available marker of Ry_{adg}, Ry_{sto}, $Ry\text{-}f_{sto}$ and Ry_{chc} used in the study. Hence, group II germplasm were not evaluated by serological assay and so resistance/ susceptibility of these 45 germplasm is unknown here.

It is evident from the present study that presence of Ry_{adg} gene and extreme resistance in the group I germplasm was derived from *S tuberosum* spp. *andigena*. It could be inferred therefore that SCAR and CAPS markers may be used for marker-assisted selection (MAS) of progenies derived from the crosses involving these germplasm in the Indian potato breeding programme. The CAPS marker, ADG2/ B*bv*I, and SCAR marker, RYSC3, have been well documented for PVY resistance through MAS in potato by many researchers worldwide. Ottoman *et al.* (6) successfully screened a full-sib tetraploid population segregating for Ry_{adg} using SCAR marker (RYSC3), and CAPS marker (ADG2/B*bv*I). RYSC3 marker has been validated by many researchers to select genotype carrying Ry_{adg} in breeding potatoes for PVY resistance (Heldák *et al.*, 3; Rizza *et al.*, 7; Sagredo *et al.*, 8; Whitworth *et al.*, 15). Therefore, the SCAR marker RYSC3 is a powerful tool in MAS for the gene Ry_{adg} in potato breeding.

The practical application of molecular markers developed for the mapped genes in potato breeding programs has been progressing at a slower speed in the India and abroad. Published examples are mainly limited to diploid material and a small number of genes, *i.e.*, Ry_{adg} (Sorri *et al.*, 10; Kasai *et al.*, 4) for extreme resistance to PVY, *Ns* for PVS resistance, *Gro1* for resistance to *G. rostochiensis* and *Sen1* for resistance to potato wart are reviewed in Tiwari *et al.* (12). Though molecular markers have been identified for PVY resistance from *S. stoloniferum* (Song *et al.*, 9; Valkonen *et al.*, 14) and *S. tuberosum* ssp. *andigena* (Sorri *et al.*, 10; Kasai *et al.*, 4; Rizza *et al.*, 7) but their application in breeding programme is still limited to a few instance. However, the SCAR and CAPS markers linked to the Ry_{adg} gene have been used in a comparatively wider scale in potato breeding for virus resistance. The ability to check for the presence of Ry_{adg} gene through molecular markers before hybridizing parents or before the field screening of progeny can increase selection efficiency for PVY resistance in potato breeding. Before relying on a specific set of markers in a breeding program, it would be important to test the markers in germplasm with known resistant species background and to confirm their resistance responses to the various PVY strains. On the other hand, absence of *Ry* gene in group II germplasm necessitate the identification of new markers linked with other PVY resistant genes derived from wild *Solanum* species and also through serological assay.

To conclude, among molecular markers available till date in the potato breeding for PVY resistance, Ry_{adg} gene reveal effectiveness in identifying potato germplasm accessions possessing extreme resistance to PVY. This finding supports the use of molecular markers for faster potato breeding to accelerate the development of PVY resistant cultivars/clones by the pyramiding of genes. Further, quantitative real time PCR analysis of the group I germplasm may reveal the Ry_{adg} allelic variants of plex doses (simplex/duplex/ triplex/tetraplex) for PVY resistance. In future, a survey of more diverse potato germplasms possessing resistant gene(s) is required for validation of new molecular markers. Since, more virus resistance genes are being tagged, it is expected that the MAS would necessarily hasten the development of new potato cultivars with PVY resistance gene.

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Received: March, 2012; Revised: February, 2013;
Accepted: April, 2013