Indian J. Genet. Plant Breed, (2022); 82(4): 474-479
doi: 10.31742/ISGPB.82.4.11
ISSN: 0975-6906
www.isgpb.org

RESEARCH ARTICLE

The efficacy of molecular markers-linked to Ry_{adg} gene in potato (*Solanum tuberosum* L.) genotypes

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Abstract

Potato virus Y (PVY) is one of the most important viruses in potato production. The use of resistant varieties is the most effective method to control this virus and, in potato breeding programs, Ry_{adg} is one of the most widely used resistance genes against PVY. Although several molecular markers linked to Ry_{adg} have been developed; they have not been compared with each other. In this study, five molecular markers RYSC3, ADG2 BbvI, ADG1, ADG2 and M45, developed earlier, have been evaluated in potato genotypes inoculated with PVY. In addition, markers GP-122_{718}, GP-122_{564} and YES 3-3B for Ry_{sto} and RY186 for Ry_{chc} were used to support the results of the markers related to the Ry_{adg} gene. All markers yielded DNA fragments when potato genotypes were tested, but only the M45 marker was compatible with results of the bioassay. The results of present study showed that the predictive nature of the molecular markers can change according to the genetic background of the potato cultivar/line and may be incorrect with some genotypes.

Keywords: Bioassay, breeding, marker, PVY, *Solanum tuberosum*.

Introduction

Potato is the third most important food crop in the world and is planted on 17.6 million ha with a yield of 368 million tonnes (FAOSTAT 2018). It is commonly attacked by viral pathogens and its production is constrained by viruses worldwide (Nie et al. 2015). Potato virus Y (PVY) is one of the most important viruses causing huge losses in potato (Scholthof et al. 2011). PVY strains induce mosaic, leaf mottling, necrosis, crinkling, tuber necrosis, dwarfing of plants and cause yield losses of 10–80% in potato, depending on cultivar, virus strain and environmental conditions. The virus is transmitted through the use of infected tubers and aphids in a non-persistent manner (Loebenstein and Gaba 2012; Nie et al. 2013, 2015).

The control of the virus is based on different management methods. The use of resistant plants is one of the most important management practices (Kang et al. 2005); that is also environmentally friendly, cost-effective and easy to implement by growers (Ottoman et al. 2009). Potato genotypes have normally two types of resistance against PVY i.e., hypersensitive resistance and extreme resistance. Hypersensitive resistance conferred by *N* genes is defined as death of some cells (necrosis) at the infection site, which inhibits infection spread to other cells. Extreme resistance mediated by *Ry* genes prevents virus multiplication at early stages and normally doesn't cause cell death at the infection area (Solomon-Blackburn and Barker 2001; Heldák et al. 2007).

Several resistance genes against PVY have been identified in wild potato species. Ry_{adg} in *Solanum tuberosum* subsp. *andigena* is known to provide a high level of resistance against all strains of PVY (Barker 1997; Hämäläinen et al. 1997; Whitworth et al. 2009). It has been genetically localized on chromosome XI (Hämäläinen et al. 1997) and is a single dominant gene (Tiwari et al. 2012). Several molecular markers linked to Ry_{adg} have been developed for potato breeding (Hämäläinen et al. 1998; Sorri et al. 1999; Kasai et al. 2000; Herrera et al. 2018). These molecular markers have

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How to cite this article: Kahveci E., Özkaynak E. and Devran Z. 2022. The efficacy of molecular markers-linked to *Ryadg* gene in potato (*Solanum tuberosum* L.) genotypes. Indian J. Genet. Plant Breed., **82**(4): 474-479.
Source of support: STRC, Turkey (TEYDEP Project 3110172)
Conflict of interest: None.
Received: July 2022 **Revised:** Oct. 2022 **Accepted:** Nov. 2022

been widely used for screening of PVY resistance in potato breeding programs (Kasai et al. 2000; Heldak et al. 2007; Ottoman et al. 2009; Whitworth et al. 2009; Herrera et al. 2018; Slater et al. 2020).

Potato has a large genetic diversity due to the tetraploid structure of the genome. Therefore, molecular markers may give dissimilar results in potato varieties with different genetic backgrounds. The literature revealed that the molecular markers which are tightly linked to the Ry_{adg} gene have been developed but there are limited studies on comparing them with each other in potato genotypes tested with PVY. Therefore, a study was conducted to determine the efficacy of these molecular markers in potato genotypes for potato breeding programs.

Materials and methods

Plant material

A total of twenty-two advanced breeding lines of potato were used for the biological assay and screening of molecular markers in the study (Table 1).

Plant propagation

Virus-free single node cuttings from advanced potato lines were grown in sterilized sand and then propagated in Petri dishes containing MS medium with 3% sucrose and 0.7% agar. Potato cultures were placed in a growth chamber with a 16 h photoperiod at 24 ± 1°C. Subsequently, *in-vitro* plants were transplanted into vials containing a sterile mixture of peat moss and perlite in a growth chamber. The plants were irrigated until they rooted and transplanted into plastic pots in a growth chamber.

Biological assay

An isolate of PVY^{O} was multiplied in *Nicotiana tabacum* cv. *xanthii* plants. Infected tobacco leaves were homogenized in phosphate buffer (0.01 M, pH 7.0) with freshly added 0.2% sodium sulphite. After adding carborundum powder (600 mesh) the two uppermost leaves of potato plants at about six-leaf stage, which had been incubated in the dark on the previous day, were inoculated. The second inoculation was performed in a similar manner 3–7 days later. After inoculation, the plants were incubated in a growth chamber at 24°C (day) and 22°C (night) with a 16 hours light/8 hours dark photoperiod for 45 days. Each genotype was replicated 12 times and the experiment was repeated two times. Also, six plants for each genotype were mock inoculated. The plants were observed every two days for 45 days, and DAS-ELISA was carried out using polyclonal

Table 1. Potato advanced breeding lines used in the present study

Genotype	Characteristic features
P1	Strong plant, early and oval-long big size tubers
P2	Strong plant, oval tubers and high yield
P3	Strong plant, high yield, long tubers and French Fry
P4	Oval-long tubers, high yield and pink eyed tubers
P5	Early, oval-long tubers, and French Fry
P6	Yellow skin and flesh colour, high yield and French Fry
P7	Strong plant, good tuber shape, big tuber size and oval-long tubers
P8	Early, very high yield, medium size tubers and good tuber shape
P9	Early, high yield and oval-round tubers
P10	Early and oval-round tuber shape
P11	Medium late, very high yield, good tuber shape, Fresh Market and PVX
P12	Good tuber shape, super yellow flesh colour, Fresh Market and PVX
P13	Medium-late, round-oval tubers, high yield Fresh market and PVX
P14	Dark yellow skin, medium-early, Fresh market and French Fry and PVX
P15	Medium early, oval-round tubers, Fresh market and French Fry and PVX
P16	Medium-early, standard-round tubers, very high yield, Fresh Market and PVX
P17	Medium-early, high yield and quality, oval tubers and PVX
P18	Medium-early, medium size tubers, good tuber shape, high yield and PVX
P19	Medium-early, oval-long tubers and Fresh market
P20	Early, oval-round tubers and crisp processing
P21	Brown tuber skin colour, long tubers and French fry
P22	Very high yield and medium size-oval tubers

Table 2. Primer sequences of markers for the Ry_{adg}, Ry_{sto} and Ry_{cho} genes in potato.

Gene	Marker	Primer	Primer Sequences (5'-3')	Reference
Ry_{adg}	RYSC3	3.3.3s ADG23R	ATACACTCATCTAAATTTGATGG AGGATATACGGCATCATTTTTCCG	Hamalainen et al. (1998) Kasai et al. (2000)
Ry_{adg}	ADG2 BbvI	ADG2-F ADG2-R	ATACTCTCATCTAAATTTGATGG ACTGAACAGCATCATGTTCAAG	Ottoman et al. (2009)
Ry_{adg}	ADG1	3.3.13s 3.3.13as	CACACTCTCGTATCAGTTTGA ATTTAATAGCGTGACAGTCAAC	Hamalainen et al. (1998)
Ry_{adg}	ADF2	3.3.3s 3.3.3as	ATACTCTCATCTAAATTTGATGG ACTGAACAGCATCATGTTCAAG	Sorri et al. (1999)
Ry_{adg}	M45	M45F1 M45R1	TGGAGTATTTGGATCTAAGGG AACACATAAGGAGCGAT	Herrera et al. (2018)
Ry_{sto}		GP-122_{718}	TATTTTAGGGTACTTCTTTCTTA GCACTCAATAGCCCTTCTT	Flis et al. (2005)
Ry_{sto}		GP-122_{564}	TATTTTAGGGGTACTTCTTTCTTATGTT CTGTCAAAAAAATTCACTTGCATAACTAC	Witek et al. (2006)
Ry_{sto}	YES-3B	3F 3B	TAACTCAAGCGGAATAACCC CATGAGATTGCCTTTGGTTA	Song&Schwarzfischer (2008)
Ry_{cho}	RY186	RY186-11 RY186-12	TGGTAGGGATATTTTCCTTAGA GCAAATCCTAGGTTATCAACTCA	Takeuchi et al. (2008)

antibodies according to the manufacturer's instructions (Loewe Biochemica GmbH, Sauerlach, Germany). The samples having an absorbance value more than two times the negative controls were considered positive for PVY virus (Ottoman et al. 2009).

DNA isolation and molecular markers

Plant genomic DNA was isolated from young leaves of potato lines using a Wizard Magnetic Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Molecular markers for the Ry_{adg} gene and their primer sequences are listed in Table 2. In addition, molecular markers linked to Ry_{sto} and Ry_{chc} were used to compare results and check for the presence of any additional resistance genes. All primers were purchased from İontek (İstanbul, Turkey). PCR amplification was performed in a total volume of 25 µL containing 2.5 µL 10X PCR buffer, 0.2 mM dNTP, 0.4 mM of each primer, 2 mM $MgCl_2$, 20 ng of template DNA, and 1 unit taq DNA polymerase (Vivantis, Selangor DE, Malaysia). PCR amplification was carried out using a thermocycler (PTC-200, MJ Research, USA). The cycling conditions were as follows: 94°C for 3 minutes, 35 cycles of denaturation at 94°C for 30 s; annealing at 45°C for 30 s (ADG2 BbvI), 50°C for 30 s (ADG2), 52°C for 30 s (RYSC3and M45), 55°C for 30 s (RY186), 54°C for 30 s (ADG1 and YES3-3B), or 56°C for 30 s (GP122_{718} and GP122_{564}); and elongation at 72°C for 1 minutes, with 72°C for 7 minutes as a final extension. The PCR products were separated on a 2% agarose gel containing TAE buffer at 110 V for 2 hours and visualized under UV light after staining with ethidium bromide.

Results

The potato seedlings were inoculated and then evaluated visually for resistance and finally the presence/absence of the PVY virus was confirmed by ELISA. Eight genotypes (advanced breeding line) out of 22 were resistant and 14 were susceptible (Table 3). For molecular screening for the Ry_{adg} gene, DNA isolated from all the genotypes was amplified with primers for the markers RYSC3, ADG2 BbvI, ADG1, ADG2 and M45. In addition, the markers GP-122_{718}, GP-122_{564} and YES3-3B for Ry_{sto} and RY186 for Ry_{chc}, were used for comparison as well as checking for other sources of PVY resistance (Table 2). All the markers utilized earlier in various studies gave DNA fragments. However, only the M45 marker was completely in accordance with the results for resistance from the biological assay in potato genotypes tested for Ry_{adg}; all other markers had significant discrepancies (Table 3).

Discussion

Potato is one of the most important crops for human nutrition worldwide however, many pests and pathogens restrict potato production. PVY is one of the most important viruses causing yield losses in potato growing areas. Virus-free or certified seeds are one of the most effective management strategies to reduce the impact of PVY in conjunction with appropriate agronomic practices. Also, resistant cultivars would be very beneficial in managing crop loss caused by PVY. Thus, determination of resistance is necessary for development of resistant potato genotypes. Resistance in plants is typically determined with bioassays but can also be achieved by deployment of molecular markers. Performing bioassays are space and time-

Table 3. Results of bioassay, ELISA and molecular markers used in this study for screening PVY resistance

			Ry_{adg} Markers					Ry_{sto} Markers			Ry_{chc} Marker
Genotype	Phenotype	DAS-ELISA	RYSC3	ADG BbvI	ADG1	ADF2	M45	GP-122$_{718}$	GP-122$_{564}$	YES-3B	RY186
P1	S	1	+	+	+	+	-	-	-	-	-
P2	S	1	+	+	+	+	-	-	-	-	-
P3	S	1	+	+	+	+	-	-	-	-	-
P4	S	1	+	+	+	+	-	-	-	-	-
P5	S	1	+	+	+	+	-	-	-	-	-
P6	S	1	+	+	+	+	-	-	-	-	-
P7	S	1	+	+	+	+	-	-	-	-	-
P8	S	1	+	+	+	+	-	-	-	-	-
P9	S	1	+	+	+	+	-	-	-	-	-
P10	S	1	+	+	+	+	-	-	-	-	-
P11	R	0	-	+	+	+	+	-	-	-	-
P12	R	0	-	+	+	+	+	-	-	-	-
P13	R	0	-	+	+	+	+	-	-	-	-
P14	R	0	-	+	+	+	+	-	-	-	-
P15	R	0	-	+	+	+	+	-	-	-	-
P16	R	0	-	+	+	+	+	-	-	-	-
P17	R	0	-	+	+	+	+	-	-	-	-
P18	R	0	-	+	+	+	+	-	-	-	N
P19	S	1	-	+	+	+	-	-	-	-	-
P20	S	1	-	+	+	+	-	-	-	-	N
P21	S	1	-	+	+	+	-	-	-	-	-
P22	S	1	-	+	+	+	-	-	-	-	-

S = Susceptible, R = Resistant, 1 = Positive reaction, 0 = No reaction, - = Marker absent, + = Marker present and N = No amplification

consuming as they need to be conducted in greenhouse or field conditions. However, molecular markers that are tightly linked to resistance genes can be used without special facilities for biological evaluation and are not influenced by growth stages or growing conditions (Mori et al. 2011; Fullodolsa et al. 2015). Additionally, molecular markers may reduce costs and increase the precision and efficiency of the selections (Peleman and van der Voort 2003; Barone 2004; Fullodolsa et al. 2015). In the present study, the performance of several molecular markers linked to the Ry_{adg} gene in potato was assessed. The data generated from the RYSC3 assay, developed by Kasai et al. (2000) was not in accordance with the biological assay data. However, previous studies reported that the marker has been widely applied to develop potato genotypes resistant to PVY (Gebhardt et al. 2006; Rizza et al. 2006; Bhardwaj et al. 2007; Heldáket al. 2007; Whitworth et al. 2009; Tiwari et al. 2013a,b). Sagredo et al. (2009) investigated the effectiveness of the RYSC3 marker for selecting PVY-resistant genotypes carrying the Ry_{adg} gene in a potato breeding programme. Within the group of 71 progenitors, 30 plants had some kind of resistance to the virus, of which 17 were carriers of the RYSC3 marker. Slater et al. (2020) used the marker RYSC3 for marker-assisted selection (MAS) on Australian germplasm and determined the degree of correlation between the resistance phenotype and the marker. In another study, Hämäläinen et al. (1998) identified two markers viz., ADG1 and ADG2 which are closely linked to Ry_{adg} gene. However, in the present study, these molecular markers did not give results that were consistent with the biological assay. Sorri et al. (1999) reported that the ADG2 Bbvl marker is applicable for selection of Ry_{adg} in potato at different ploidy levels and in diverse genetic backgrounds. However, this was not the case in our study, indicating that genetic background of the potato cultivar/line may effect performance of this marker. The M45 marker was developed and validated on potatoes with known presence/absence of the Ry_{adg} gene (Herrera et al. 2018). Slater et al. (2020) screened this marker on 13 Australian germplasms and determined 12 of them to be resistant, although phenotypic screening showed that 1 of

these 12 germplasms was susceptible. In the present study, the M45 marker was the only marker that was in complete agreement with the bioassay results.

Molecular markers closely linked to Ry_{sto} and Ry_{chc} were used for comparison and to confirm that there were no other resistance genes. GP-122_{718} and GP-122_{564}, markers were developed for detection of $Ry\text{-}_{fsto}$ (Flis et al. 2005; Witek et al. 2006) and Song and Schwarzfischer (2008) reported that the YES3-3B marker for Ry_{sto} was detected in potato genotypes that were resistant PVY. The RY186 marker for Ry_{chc} was developed by Takeuchi et al. (2008). In the present study, the negative results for the four markers of Ry_{sto} and Ry_{chc} with resistant genotypes indicate the absence of these resistance genes and strengthen the assertion that M45 is a marker for Ry_{adg} resistance except for M75 marker. Since the present findings of the Ry_{adg} gene are not completely in agreement with the phenotypic results of the biological assay, it is concluded that the markers used are not tightly linked to the R gene or unique genotype. Thus, the results indicated that the genetic background of a potato cultivar/line determines the validity and accuracy of the molecular markers. Therefore, markers of interest should be analysed for their applicability in breeding material before routine use.

Authors' contribution

Conceptualization of research (EK, Eë, ZD); Designing of the experiments (EK, ZD); Contribution of experimental materials (EK, Eë, ZD); Execution of field/lab experiments and data collection (EK, Eë, ZD); Analysis of data and interpretation (EK, Eë, ZD); Preparation of manuscript (EK, Eë, ZD).

Acknowledgement

Authors gratefully acknowledge the financial support of the Scientific and Technological Research Council of Turkey (TEYDEP Project 3110172). The authors thank M.Y. Genetik Tarım Teknoloji Laboratuvar staff for technical support in laboratory.

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