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Multiple DNA Markers for Evaluation of Resistance against *Potato virus Y*, *Potato virus S* and *Potato leafroll virus*

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Abstract

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Molecular markers within or close to genes of interest play essential roles in marker-assisted selection. PCR-based markers have been developed for numerous traits in different plant species including several genes conferring resistance to viruses in potato. In the present work, rapid and reliable approaches were developed for the simultaneous detection of *Ryadg* and *Ry-fsto*, *Ns*, and *PLRV.*1 genes conferring resistance to *Potato virus Y*, *Potato virus S* and *Potato leafroll virus*, respectively, on the basis of previously published and newly modified markers. The sequence characterized amplified region (SCAR) markers for *Ryadg*, *Ns* and *PLRV1* and the newly modified cleaved amplified polymorphic sequences (CAPS) marker for *Ry-fsto* were amplified in one PCR reaction which could simply characterize *Ryadg* and *PLRV.1* resistance. Additional digestion of amplicons with *Eco*RV and *Mfe*I for genotyping the *Ry-fsto* and *Ns* resistance genes, respectively, was needed. The effectiveness of genotyping in triplex and tetraplex PCRs was tested on 35 potato varieties used for potato seed production and breeding programs.

Keywords: multiplex genotyping; *Solanum tuberosum*; virus resistance

Viruses are an important group of plant pathogens affecting potato production worldwide and specifically they have a huge impact on potato seed production industries. Breeding for resistance is suggested as the most effective and environmentally safe strategy to manage plant pathogens including potato viruses (Swiezynski 1994). Among several breeding techniques, marker-assisted selection (MAS) is supposed to be the best one in terms of time, cost, labour and reproducibility of results (Xu & Crouch 2008).

Several DNA markers linked to several resistance genes conferring resistance against potato viruses have already been developed (Tiwari *et al*. 2012; Ramakrishnan *et al*. 2015). Some of these markers including SCAR-RYSC3 (Kasai *et al*. 2000; Lopez-Pardo *et al*. 2013), CAPS-GP122$_{718}$ (Flis *et al*. 2005), CAPS-SCG17$_{448}$ (Marczewski *et al*. 2001a) and SCAR-Nl27$_{1164}$ (Marczewski 2001b) were linked to the resistance genes *Ryadg* and *Ry-fsto* (against *Potato virus Y*, PVY), *Ns* (*Potato virus S*, PVS) and *PLRV.1* (*Potato leafroll virus*, PLRV), respectively. Genes *Ryadg*, *Ry-fsto*, *Ns* and *PLRV.1* were mapped on potato chromosomes XI (Brigenti *et al*. 1997), XII (Flis *et al*. 2005), VIII (Marczewski *et al*. 2002) and XI (Marczewski *et al*. 2001b), respectively. PCR amplification of 1164 and 321 nucleotide (nt) fragments related to SCAR-Nl27$_{1164}$ and SCAR-RYSC3 are informative for the presence of resistance QTL/gene *PLRV.1* and *Ryadg*, respectively (Kasai *et al*. 2000; Marczewski *et al*. 2001b). The amplified fragments of 718 and 448 nt belonging to markers CAPS-GP122$_{718}$ and SCAR-SCG17$_{448}$ demand further subjection to *Eco*RV and *Mfe*I restriction endonucleases, respectively, to identify the presence of dominant (resistant) or recessive (susceptible)

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alleles of the *Ry-fsto* and *Ns* genes (Marczewski *et al*. 2001a; Flis *et al*. 2005).

Nucleic acid-based detection methods are used routinely in plant sciences for crop improvement and improving resistance against pathogens (Poczai *et al*. 2013).

In the present work, we developed a robust reaction mixture for simultaneous detection of markers linked to *Ryadg*, *Ry-fsto*, *Ns* and *PLRV.1* genes/QTL in multiplex PCRs. Previously published primer pairs for each of *Ryadg* (Kasai *et al*. 2000), *Ns* (Marczewski *et al*. 2001a) and *PLRV.1* (Marczewski *et al*. 2001b) genes/QTL and newly modified primers for *Ry-fsto* ($GP122_{718}$-F: TATTTTAGGGGTACTTCTTTCTTA; $GP122_{718}$-R: GCACTCAATAGCCCTTCTT) gene (Flis *et al*. 2005; this work) were applied. The following potato cultivars were used for development and validation of reaction mixtures for simultaneous detection of all four genes in one PCR tube: Agria, Almera, Arinda, Boren, Fontane, Lenora, Marfona, Markies, Picasso, Ramos, Santana, Satina, Savalan, Sinora, Lady Rosetta, Impala, Daifla, Hermes, Desiree, Granola, Diamant, Florida, Emeraude, Marabel, Arnova, Costanera, Chanchan, Perricholi, Rabadina, Oceania, Early valley, Purple valley, Bora valley and Juice valley. Genomic DNA from leaf or tuber tissues was extracted according to Saghai-Maroof *et al*. (1984).The reagents for multiplex PCR amplification of all markers in 50 µl reaction mixtures were included as follows: 5 µl PCR buffer (10×), 5 µl $MgCl_2$ (25 mM), 4 µl dNTP (2 mM), 0.5, 0.5, 1 and 1 µl from 10 pm stocks of each of forward and reverse primers of CAPS-$SCG17_{448}$, SCAR-$Nl27_{1164}$, CAPS-$GP122_{718}$ and SCAR-RYSC3 markers, respectively, 0.4 µl *Taq* DNA polymerase, 4 µl of DNA (20 ng) template. Amplifications of markers were carried out in an Eppendorf thermal cycler (Eppendorf AG, Germany) with a program consisting of an initial denaturation of DNA at 94°C for 3 min, 35 cycles at 94°C for 40 s, 59°C for 40 s, 72°C for 50 s and final extension at 72°C for 5 min. This step was sufficient to characterize the presence of *Ryadg* gene and *PLRV.1* QTL in potato cultivars (Figure 1). However, the other markers demand further subjection to *EcoR*V or *Mfe*I restriction endonucleases to identify the presence of dominant or recessive alleles (Figure 1).

Triplex PCR products from CAPS-$GP122_{718}$, CAPS-$SCG17_{448}$ and SCAR-$Nl27_{1164}$ markers (Figure 2) were subjected to *EcoR*V and *Mfe*I restriction enzymes at 37°C in a total volume of 20 µl (Figures 1 and 2). As shown in Figure 2, subjection of amplified fragments of all three markers to *EcoR*V restriction enzyme cleaved CAPS-$GP122_{718}$ and SCAR-$Nl27_{1164}$ markers to about 434, 284 nt and 620, 544 nt fragments, respectively, but it did not cleave CAPS-$SCG17_{448}$ fragment. Subjection of PCR fragments of all three markers to *Mfe*I partially cleaved CAPS-$SCG17_{448}$ marker to 251 and 147 nt fragments only in cultivars Sante, Oceania, Rabadina, Bora valley and Juice valley (Figure 2).

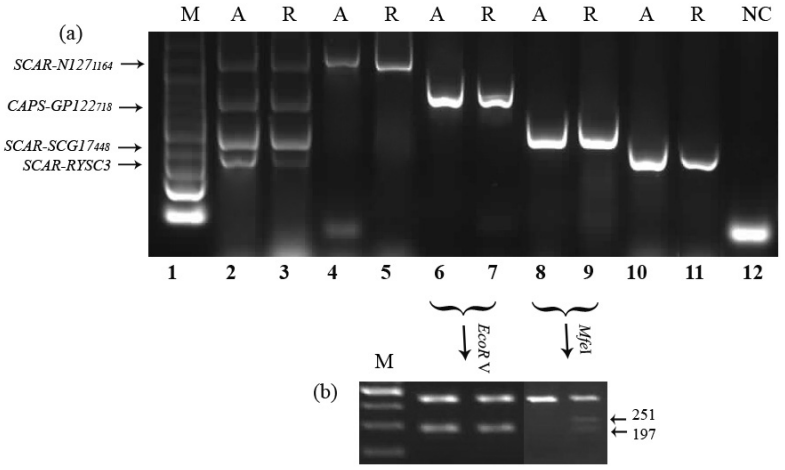

Figure 1. Simplex (lanes 4–11) and tetraplex (lanes 2–3) amplification of markers SCAR-$Nl27_{1164}$ (lanes 4–5), SCAR-$SCG17_{448}$ (lanes 8–9), SCAR-RYSC3 (lanes 10–11) and CAPS-$GP122_{718}$ (lanes 6–7) linked to the *PLRV.1* and *Ns*, *Ryadg* and *Ry-*f_{sto} genes conferring resistance against PLRV, PVS and PVY, respectively, in potato cultivars
(a): M – 100 bp molecular size marker; A – cv. Agria; R – cv. Rabadina; NC – negative control; (b): PCR fragments of CAPS-$GP122_{718}$ and SCAR-$SCG17_{448}$ were subjected to digestion with *EcoR*V and *Mfe*I, respectively, for characterization of recessive alleles (lanes 6–8) or heterozygosity (lane 9)

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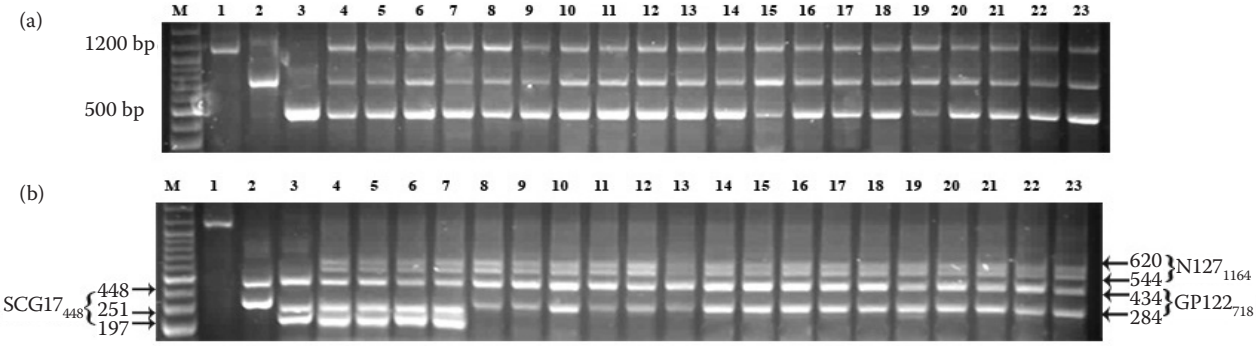

Figure 2. Simultaneous amplification of SCAR-Nl27$_{1164}$, CAPS-GP122$_{718}$ and SCAR-SCG17$_{448}$ markers in potato cultivars by triplex PCR assay (a) and co-subjection of PCR products to *Eco*RV and *Mfe*I restriction endonucleases (b) simplex (lanes 1, 2, 3) and triplex (lane 4) amplification of markers and subjection to *Eco*RV-*Mfe*I endonucleases in cv. Sante are shown; triplex amplification and *Eco*RV-*Mfe*I subjection of markers in other cultivars are shown by numbers on lanes: 5 – cv. Oceania, 6 –cv. Rabadina, 7 –cv. Bora valley, 8 – cv. Early valley, 9 – cv. Agria, 10 – cv. Almera, 11 – cv. Arinda, 12 – cv. Boren, 13 – cv. Fontane, 14 – cv. Lenora, 15 – cv. Marfona, 16 – cv. Markies, 17 – cv. Picasso, 18 – cv. Diamant, 19 – cv. Impala, 20 – cv. Marabel, 21 – cv. Arnova, 22 – cv. Granola, 23 – cv. Desiree; M indicates molecular size marker 100 bp

The efficiency of the studied markers in detecting *Ryadg* (Kasai *et al.* 2000; Ottoman *et al.* 2009; Ortega & Lopez-Vizcon 2012), *Ry-fsto* (Flis *et al.* 2005), *Ns* (Marczewski *et al.* 2001a) and *PLRV.1* (Marczewski *et al.* 2001b) is highly validated in several potato cultivars including cultivars Agria, Sante, Desiree and Granola, breeding clones and in their descendants. We have not access to the phenotypes of resistance/susceptibility to PVY, PVS and PLRV of other cultivars studied in this work to further validate the efficiency of these markers. However, the developed multiplex reaction mixture and newly modified primers for the *Ry-fsto* gene could be applied for characterizing any of these genes alone or in combination with other genes/QTL tested in this work in MAS for potato breeding for PVY, PLRV and PVS resistance.

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