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

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**Abstract**


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 *PLRV.1* 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 *EcoRV* and *MfeI* 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 *EcoRV* and *MfeI* restriction endonucleases, respectively, to identify the presence of dominant (resistant) or recessive (susceptible)
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: TATTTTAGGGGTACTTCTTTA; 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, Rams, 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 MgCl₂ (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 EcoRV or MfeI 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 EcoRV and MfeI 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 EcoRV 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 these markers to MfeI 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).
The efficiency of the studied markers in detecting \textit{Ryadg} \cite{Kasai et al., 2000; Ottoman et al., 2009; Ortega \\& Lopez-Vizcon 2012}, \textit{Ry-fsto} \cite{Flis et al., 2005}, \textit{Ns} \cite{Marczewski et al., 2001a} and \textit{PLRV.1} \cite{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 \textit{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.

\textbf{References}


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