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SHORT COMMUNICATION

A multiplex PCR approach to simultaneously genotype potato towards the resistance alleles *Ry-f_{sto}* and *Ns*

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Abstract A simple and robust multiplex PCR approach was developed for detection of the alleles *Ry-f_{sto}* and *Ns* conferring resistance of potato to *Potato Virus Y* (PVY) and *Potato Virus S* (PVS), respectively. Cleaved amplified polymorphic sequence (CAPS) markers $GP122_{564}$ linked to *Ry-f_{sto}* and $SC811_{260}$ linked to *Ns* were amplified in one PCR reaction and identified after simultaneous digestion of the amplicons with restriction enzymes *Eco*RV and *Mbo*I. Effectiveness of this procedure for marker-assisted selection was confirmed in 55 potato cultivars.

Keywords MAS · Multiplex PCR · Potato · PVS · PVY · Resistance

Potato Virus Y (PVY) and *Potato Virus S* (PVS) are pathogens that occur worldwide in potato (*Solanum tuberosum* ssp. *tuberosum*) crops (Brunt 2001). Breeding of resistant potato cultivars is the most effective and environmentally safe strategy to achieve protection against Potato viruses (Świeżyński 1994). From the 1930s to the present wild *Solanum* species have been widely used as sources of resistance genes in cultivated potato breeding programs (Valkonen et al. 1996). The gene *Ns* derived from *Solanum tuberosum* ssp. *andigena* conferred resistance to PVS in potato (Marczewski et al. 1998), whereas *S. stoloniferum* was a donor of the gene *Ry-f_{sto}* responsible for extreme resistance of potato to PVY (Flis et al. 2005). Potato plants possessing *Ry-f_{sto}* and/or *Ns* remained symptomless after mechanical inoculation. *Ns* and *Ry-f_{sto}* have been mapped on potato chromosomes VIII (Marczewski et al. 2002) and XII (Flis et al. 2005), respectively. The development of DNA markers linked to desired traits, including disease resistance genes, becomes more and more helpful for marker-assisted selection in potato breeding programs (Barone 2004). An inter-simple sequence repeat (ISSR) marker $UBC811_{660}$ was applied to identify the allele *Ns* in cultivars resistant to PVS (Marczewski 2001). A cleaved amplified polymorphic sequence (CAPS) marker $GP122_{718}$ was successfully used to detect several Polish and German cultivars expressing extreme resistance to PVY (Flis et al. 2005). $GP122_{718}$ and $UBC811_{660}$ have been cloned and sequenced according to Marczewski et al. (2006). $UBC811_{660}$ (accession DQ415915) did not share significant homology to any sequences deposited in data bases. *Eco*RV recognition site, being diagnostic in

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detection of the allele *Ry-f_{sto}* (Flis et al. 2005), was found for all seven randomly chosen bacterial clones, possessing a 614 bp marker fragment of GP122 (DQ151984-91). In contrast, none of seven 614 bp inserts (DQ151992-97) amplified in a PVY-susceptible plant contained *Eco*RV site.

Here, we report a multiplex PCR approach for detection of the alleles *Ry-f_{sto}* and *Ns*. New PCR primers were designed as previously described (Marczewski et al. 2006) to amplify a 564 bp fragment of GP122 and a 454 bp fragment, designated as $SC811_{454}$, for the *Ns*-linked marker. The PCR amplification was performed in 20 µl of 20 mM Tris–HCl pH 8.4, 50 mM KCl, 2.5 mM $MgCl_2$, 0.1 mM of each deoxynucleotide, 0.2 µM of each primer (f: 5′-TATTTTAGGGGTACTT-CTTTCTTATGTT-3′; r: 5′-CTGTCAAAAAA-ATTCACTTGCATAACTAC-3′) for GP122 and 0.25 µM of each primer (f: 5′-CGAACAAAAT-ACGTAATGCATTGAATAA-3′; r: 5′-GACCT ATATCAGTCCCTTCTAATCCACTAT-3′) for $SC811_{454}$, containing 1 U *Taq* DNA Polymerase (Invitrogen, Carlsbad, CA, or Fermentas, St. Leon-Rot,) and 30 ng of genomic DNA as a template. A total genomic DNA was isolated using classical CTAB- method (Weigel and Glazebrook 2002) or as described in Flis et al. (2005). The PCR parameters were: 94°C for 60 s followed by 30 cycles of 93°C for 15 s, 56°C for 20 s, 72°C for 60 s and a final extension time of 5 min at 72°C. Electrophoretic conditions were used as previously described (Flis et al. 2005). The amplicons produced by the multiplex PCR were digested simultaneously with the restriction enzymes *Eco*RV and *Mbo*I.

Fifty-five potato cultivars bred in Poland and established for resistance level to PVY and PVS were tested. Nineteen PVY-extreme resistant cultivars listed in Flis et al. (2005) and five newly evaluated: Danusia, Jasia, Sonda, Ślęza and Ursus, possessed the gene *Ry-f_{sto}*. Among these, Barycz, Klepa, Meduza and Omulew carried both *Ry-f_{sto}* and *Ns*. Out of 23 cvs indicating hypersensitivity or susceptibility to PVY (Flis et al. 2005) only cv. Neptun was known to carry the gene *Ns* (M. Chrzanowska, personal communication). The other 22 and the following eight cvs: Bartek, Czapla, Ekra, Osa, Tara, Tokaj, Wawrzyn and Zebra are known to lack *Ry-f_{sto}* and *Ns*. The

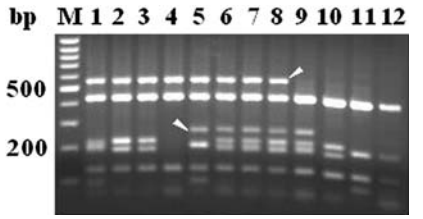

Fig. 1 Multiplex PCR for detection of CAPS markers $GP122_{564}$ linked to *Ry-f_{sto}* and $SC811_{260}$ linked to *Ns*. Cultivars having *Ry-f_{sto}*: Alicja, Anielka, Beata and Nimfy are shown in lanes 1–4, respectively. Lanes 5–8 show cultivars possessing both *Ry-f_{sto}* and *Ns*: Barycz, Klepa, Omulew and Meduza. Lane 9: cv. Neptun with *Ns*. Lanes 10–12 show cvs Tokaj, Wawrzyn and Zebra, that are known to lack *Ry-f_{sto}* and *Ns*. Lane M contains the 100 bp DNA ladder. $GP122_{564}$ and $SC811_{260}$ are indicated by arrows

presence of the 564 bp *Eco*RV restriction fragment of the GP122 amplicon in all 24 PVY-extreme resistant cultivars indicated that the sequence $GP122_{564}$ linked to *Ry-f_{sto}* did not possess a restriction site for *Mbo*I. On the other hand, *Mbo*I-digested $SC811_{454}$ revealed an approximately 260 bp informative restriction fragment, $SC811_{260}$, which was useful for tagging the locus *Ns* in the reference population Ns (Marczewski et al. 1998). $SC811_{260}$ was found in all five cultivars resistant to PVS and was not observed in 30 cultivars which lacked *Ry-f_{sto}* and *Ns*. *Eco*RV did not interfere with identifying of $SC811_{260}$.The genotyping of 12 cultivars is shown in Fig. 1.

The genes *Ns* and *Ry-f_{sto}* were introgressed into many European potato gene pools (Flis et al. 2005; Marczewski et al. 1998). Hence, this method provides a tool for the simultaneous tagging of these resistance loci in various breeding programs. To our knowledge it is the first example of the simple and reproducible multiplex PCR that can considerably simplify screening of potato for PVY and PVS resistance through marker-assisted selection.

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