Detection of molecular markers linked to Ry genes in potato germplasm for marker-assisted selection for extreme resistance to PVY in AAFC’s potato breeding program

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Source: Canadian Journal of Plant Science, 96(5) : 737-742

Published By: Canadian Science Publishing

URL: https://doi.org/10.1139/cjps-2015-0335
Detection of molecular markers linked to Ry genes in potato germplasm for marker-assisted selection for extreme resistance to PVY in AAFC's potato breeding program

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Abstract: Molecular markers reported to be linked to extreme resistance (ER) against Potato virus Y (PVY) were evaluated in potato germplasm. YES3-3A and YES3-3B, markers linked to Rysto, were detected in 'Barbara' and its three descendants that exhibit ER to PVY; RYSC3, a marker linked to Ryadg, was detected in breeding clones NY121 and NY123. Assessment of RYSC3 as a marker for selection for Ryadg-mediated ER validated its efficacy in identification of selections with ER to PVY.

Key words: potato, molecular marker, marker-assisted selection, PVY resistance gene.

Résumé : Les auteurs ont évalué des marqueurs moléculaires prétendument associés à une résistance extrême au virus Y de la pomme de terre (PVY) sur du matériel génétique de pomme de terre. Ils ont décelé les marqueurs YES3-3A et YES3-3B associés à Rysto chez le cultivar Barbara et trois de ses descendants illustrant une résistance peu commune au PVY ainsi que le marqueur RYSC3, lié à Ryadg, chez les clones NY121 et NY123. L’évaluation du marqueur RYSC3 pour la sélection de la résistance extrême médie par Ryadg a confirmé son efficacité pour l’identification des variétés résistant exceptionnellement au PVY. [Traduit par la Rédaction]

Mots-clés : pomme de terre, marqueur moléculaire, sélectionassistée par marqueur, gène de résistance au PVY.

Introduction

Potato virus Y (PVY) is one of the most economically important pathogens affecting potato production worldwide (Singh et al. 2008). Transmitted through the use of infected seed-tubers and by aphids in a non-persistent manner, PVY can cause both quality degradation and up to 90% yield reduction, depending on potato cultivars and virus strains (Nie et al. 2012, 2013). Breeding of PVY resistant potato cultivars is one of the most effective strategies for disease management. Two types of resistance, namely hypersensitive resistance (HR) and extreme resistance (ER), have been recognized in potato (Cockerham 1970; Singh et al. 2008; Nie et al. 2015). The former is conferred by N genes; and the latter is strain-nonspecific and is conferred by K genes (Singh et al. 2008; Nie et al. 2015). It is noteworthy that HR-conferred by most N genes is strain specific (Singh et al. 2008; Nie et al. 2015) with the exception of Ny-1 and Ny-2 (Szajko et al. 2008, 2014). Three R genes, Ryadg, Rysto, and Ry chc, which were derived from Solanum tuberosum ssp.
andigena, S. stoloniferum, and S. chacoense, respectively, have been identified in potato germplasm and are used for breeding potato cultivars with ER against PVY (Fulladolsa et al. 2015).

The use of genetic markers for the selection of cultivars with desirable traits has proven to be time and cost efficient in plant breeding (Xu and Crouch 2008). However, only markers that are tightly linked to the desired gene have the potential for increasing selection efficiency. Several markers linked to ER against PVY in potato have been reported. These markers include the sequence tagged site (STS) markers YES3-3A and YES3-3B as well as cleaved amplified polymorphism (CAPS) marker GP122718 for Ry<sub>sto</sub> (Flis et al. 2005; Song and Schwarzfischer 2008; Valkonen et al. 2008), and the sequence characterized region (SCAR) marker RYSC3 for Ry<sub>adg</sub> (Kasai et al. 2000).

Agriculture and Agri-Food Canada’s potato breeding program in Fredericton is one of the major potato breeding programs in North America. Breeding for cultivars with superior traits including improved disease resistance is an aim of the program. Potato germplasm with different genetic background and traits have been obtained through material exchange and germplasm enhancement for over six decades. These efforts have, on one hand, enriched the genetic diversity of potato germplasm in the breeding program. But on the other hand, they also have complicated the identification of the Ry gene type and origin in PVY-resistant germplasm, thus hindering the utilization of Ry markers developed elsewhere for marker-assisted selection in the breeding program.

In this study, we tested 19 breeding clones/advanced selections for their response to PVY infection and the correlation to markers RYSC3, YES3-3A, YES3-3B, and GP122718. The occurrence of the Ry gene type and the effectiveness of the markers for marker-assisted selection of potato clones with ER are discussed.

### Materials and Methods

Nineteen breeding clones and advanced selections were used for genotype and phenotype analysis (Table 1). The materials were maintained at Fredericton Research and Development Centre, Agriculture and Agri-Food Canada (AAFC), Fredericton, Canada.

Resistance to PVY was evaluated by mechanical and graft inoculations with PVY<sup>0</sup> as described in De Jong et al. (2001) and Nie et al. (2015). Plants that could not be infected by PVY even after graft-inoculation were considered to have ER.

ELISA with PVY polyclonal antibody (Adgen Phytodiagnostics — Neogen, Scotland, UK) was performed according to the manufacturer’s instructions. A sample is considered positive when absorbance at

### Table 1. Response of potato breeding clones and advanced selections to Potato virus Y (PVY) infection and the presence/absence of molecular markers to Ry genes in the germplasm.

<table>
<thead>
<tr>
<th>Clone/selection</th>
<th>Country of origin</th>
<th>Response to PVY infection (phenotype)</th>
<th>YES3-3A (&lt;em&gt;Ry&lt;sub&gt;sto&lt;/sub&gt;&lt;/em&gt;)</th>
<th>YES3-3B (&lt;em&gt;Ry&lt;sub&gt;sto&lt;/sub&gt;&lt;/em&gt;)</th>
<th>GP122718 (&lt;em&gt;Ry&lt;sub&gt;sto&lt;/sub&gt;&lt;/em&gt;)</th>
<th>RYSC3 (&lt;em&gt;Ry&lt;sub&gt;adg&lt;/sub&gt;&lt;/em&gt;)</th>
<th>Parents</th>
</tr>
</thead>
<tbody>
<tr>
<td>A11272-02</td>
<td>Canada</td>
<td>ER</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>A794</td>
</tr>
<tr>
<td>AC Chaleur</td>
<td>Canada</td>
<td>S</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>MDR Bulk</td>
</tr>
<tr>
<td>Barbara</td>
<td>Germany</td>
<td>ER</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>N457</td>
</tr>
<tr>
<td>Bison</td>
<td>USA</td>
<td>S</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND4652-4 R</td>
</tr>
<tr>
<td>Cupids</td>
<td>Canada</td>
<td>S</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND5124-1 R</td>
</tr>
<tr>
<td>F00069</td>
<td>Canada</td>
<td>ER</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>N150-3</td>
</tr>
<tr>
<td>F07058</td>
<td>Canada</td>
<td>S</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Cupids</td>
</tr>
<tr>
<td>F07059</td>
<td>Canada</td>
<td>S</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>F87084</td>
</tr>
<tr>
<td>F07060</td>
<td>Canada</td>
<td>ER</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Rochdale Gold-Dorée</td>
</tr>
<tr>
<td>F08086</td>
<td>Canada</td>
<td>ER</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>Barbara</td>
</tr>
<tr>
<td>F08087</td>
<td>Canada</td>
<td>ER</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>Monalisa</td>
</tr>
<tr>
<td>F79070</td>
<td>Canada</td>
<td>ER</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>F86028</td>
</tr>
<tr>
<td>F86028</td>
<td>Canada</td>
<td>ER</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Y66-13-636 (Agitato)</td>
</tr>
<tr>
<td>F87084</td>
<td>Canada</td>
<td>ER</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>S62-47-1 Cupids</td>
</tr>
<tr>
<td>G7815-9Y</td>
<td>Canada</td>
<td>S</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>G7410-2Y</td>
</tr>
<tr>
<td>Green</td>
<td>USA</td>
<td>S</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Dunmore Excelsior</td>
</tr>
<tr>
<td>Mountain NY121</td>
<td>USA</td>
<td>ER</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>N43-288 E74.7</td>
</tr>
<tr>
<td>NY123</td>
<td>USA</td>
<td>ER</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>S. &lt;em&gt;tuberosum&lt;/em&gt; S. &lt;em&gt;berthaultii&lt;/em&gt;</td>
</tr>
<tr>
<td>Shepody</td>
<td>Canada</td>
<td>S</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Bake King F58050</td>
</tr>
</tbody>
</table>

Note: S, susceptible; ER, extreme resistant; −, absence; +, presence. Clones/selections originated in Canada were bred and selected by AAFC’s potato breeding program.
405 nm ($A_{405}$) is three times the negative (healthy) control with a reading $\geq 0.100$ (Nie et al. 2015). Total genomic DNA was isolated from fresh leaf tissue of each potato clone using the Qiagen DNeasy® Plant Mini Kit (Mo Bio Laboratories, Inc., Carlsbad, CA) according to the manufacturer’s protocol. The genomic DNA was then subjected to PCR with desired primers for the target markers. Each PCR reaction contained 50 μl PCR mix, including 50 ng total genomic DNA, 1x GeneAmp® PCR Buffer II (Applied Biosystems, Foster City, CA), 1.5 mM MgCl$_2$, 0.25 μM each of the primers, 0.1 mM each of the dNTPs, and 50 U AmpliTaq® Gold DNA polymerase (Applied Biosystems).

For detection of YES3-3A and YES3-3B markers, PCRs with respective primer pairs [3F (5′-TAATCT AAGCGGAATAACC-3′) and 3R (5′-AATTCACTGT TTACATGCTTCTTG3′-3′) for YES3-3A; 3F, and 3B (5′-CATGAGATTTGCCTTGTGTTA-3′) for YES3-3B] were carried out as described by Song and Schwarzfischer (2008). A 40-cycle amplification was performed, which included 10 cycles of 40 s at 94 °C, 40 s at 55 °C, and 60 s at 72 °C. A final extension at 72 °C was performed for 5 min. The PCR products of YES3-3A and YES3-3B were separated on a 1.4% agarose gel (Song and Schwarzfischer 2008), respectively, were characterized as susceptible to PVY as the virus could not be detected by ELISA after initial mechanical inoculation and follow-up graft-inoculation. These clones were Barbara, F00069, F08086, F08087, F79070, F86028, F87084, A11272-02, F07060, NY121, and NY123. Unlike the hypersensitive resistance conferred by most N genes including Ny (specific to PVYO), Nc (specific to PVYc), and Nz (specific to PVYz) (Singh et al. 2008; Nie et al. 2015), the ER exhibited in these clones is likely to be strain nonspecific even though they were only assessed for resistance to PVY5. Indeed, in a previous report, F87084, one of the 11 with ER to PVYO, exhibited immunity to all tested PVY strains including PVYO, PVYN, PVYN0, and PVYNTN (Nie et al. 2015). Eight clones including AC Chaleur, Cupids, G7815-9Y, Green Mountain, Shepody, F07058, F07059, and Bison were characterized as susceptible to PVY infection (Table 1) as they were readily infected with PVYO after mechanical inoculation. It is noteworthy that potato clones/cultivars commonly used as breeding materials for PVY resistance in the tetraploid potato breeding program in Fredericton have no S. chacoense background according to the available pedigrees/records (data not shown), and the ER exhibited in the materials is likely derived from S. stoloniferum and (or) S. tuberosum ssp. andigena.

The YES3 markers (i.e., YES3-3A and YES3-3B) were detected in ‘Barbara’ (Table 1; Figs. 1A and 1B), consistent with previous studies by Song and Schwarzfischer (2008). These markers were also detected in three advanced selections (F00069, F08086, and F08087) in which Barbara was the female parent (Table 1). The RYSC3 was detected in breeding clones NY121 and NY123 (Table 1; Fig. 1C), consistent with studies by Kasai et al. (2000). The marker GP122718, a marker whose presence has been observed in many European cultivars carrying R$_{stv}$ (Flis et al. 2005; Song et al. 2005; Valkonen et al. 2008), was not detected in any of the materials tested. It is particularly interesting that GP122718 was not detected in Barbara, consistent with the report by Song et al. (2005), but contradictory to that by Flis et al. (2005). All the breeding clones and advanced selections that were phenotyped as susceptible to PVY were free of the tested markers. Nevertheless, several breeding clones/advanced selections including A11272-02, F07060, F79070, F86028, and ADG23R (5′-AGGATATACGGCATCATTTTCCGA-3′) was performed as described by Kasai et al. (2000). A total of 35 cycles of amplification, each included 45 s at 94 °C, 45 s at 60 °C, and 60 s at 72 °C, were performed. A final extension followed at 72 °C for 5 min. PCR products were separated by gel electrophoresis on a 2% agarose gel containing 1× Gel Red, and visualized on the BioSpectrum® Imaging System™. The presence and absence of the RYSC3 marker at 321 bp (Kasai et al. 2000) was recorded.

### Results and Discussion

Of the 19 potato breeding clones and advanced selections assessed, 11 were characterized as extremely resistant to PVY as the virus could not be detected by ELISA after initial mechanical inoculation and follow-up graft-inoculation. These clones were Barbara, F00069, F08086, F08087, F79070, F86028, F87084, A11272-02, F07060, NY121, and NY123. Unlike the hypersensitive resistance conferred by most N genes including Ny (specific to PVYO), Nc (specific to PVYc), and Nz (specific to PVYz) (Singh et al. 2008; Nie et al. 2015), the ER exhibited in these clones is likely to be strain nonspecific even though they were only assessed for resistance to PVY5. Indeed, in a previous report, F87084, one of the 11 with ER to PVYO, exhibited immunity to all tested PVY strains including PVYO, PVYN, PVYN0, and PVYNTN (Nie et al. 2015). Eight clones including AC Chaleur, Cupids, G7815-9Y, Green Mountain, Shepody, F07058, F07059, and Bison were characterized as susceptible to PVY infection (Table 1) as they were readily infected with PVYO after mechanical inoculation. It is noteworthy that potato clones/cultivars commonly used as breeding materials for PVY resistance in the tetraploid potato breeding program in Fredericton have no S. chacoense background according to the available pedigrees/records (data not shown), and the ER exhibited in the materials is likely derived from S. stoloniferum and (or) S. tuberosum ssp. andigena.

The YES3 markers (i.e., YES3-3A and YES3-3B) were detected in ‘Barbara’ (Table 1; Figs. 1A and 1B), consistent with previous studies by Song and Schwarzfischer (2008). These markers were also detected in three advanced selections (F00069, F08086, and F08087) in which Barbara was the female parent (Table 1). The RYSC3 was detected in breeding clones NY121 and NY123 (Table 1; Fig. 1C), consistent with studies by Kasai et al. (2000). The marker GP122718, a marker whose presence has been observed in many European cultivars carrying R$_{stv}$ (Flis et al. 2005; Song et al. 2005; Valkonen et al. 2008), was not detected in any of the materials tested. It is particularly interesting that GP122718 was not detected in Barbara, consistent with the report by Song et al. (2005), but contradictory to that by Flis et al. (2005). All the breeding clones and advanced selections that were phenotyped as susceptible to PVY were free of the tested markers. Nevertheless, several breeding clones/advanced selections including A11272-02, F07060, F79070, F86028,
and F87084 were free of the tested markers even though they were extremely resistant to PVY (Table 1), suggesting that more markers are needed in order for these materials to be effectively used in marker-assisted selection for PVY resistant cultivars.

The absence of existing Ry markers is not uncommon in PVY resistant potato germplasm. In a recent report by Fulladolsa et al. (2015), six out of 19 PVY resistant cultivars/clones were free of the Rysto marker YES3-3B and the Ryadg marker RYSC3. Moreover, of the six resistant clones/cultivars that did not carry either YES3-3B or RYSC3, two (‘Brodick’ and ‘Teena’) likely carry Rysto and two (CHC 39-7 and CHC 40-3) are S. chacoense clones carrying Rychc (Fulladolsa et al. 2015). In this study, the Rysto markers YES3-3A, YES3-3B, and GP122718 were absent in F87084 as well as its descendant F07060, even though F87084 was thought to possess Rysto based on the available pedigree (De Jong et al. 2001). The disassociation between ER to PVY and the tested markers in these materials could be attributed to historic recombination events which occurred during germplasm development. This might also explain the presence of YES3-3B and the absence of GP122718 in Barbara and its descendants. Nevertheless, it cannot rule out the possibility that Rysto in Barbara and Rysto (i.e., Ry-sto) in cultivars that were reported by Flis et al. (2005) might have been derived from different accessions of S. stoloniferum. This hypothesis could also be true for F87084 and other clones that are extremely resistant to PVY infection but lack the tested markers.

To assess the efficacy of RYSC3 as a marker for marker-assisted selection of Ry-adg-mediated ER, six advanced selections (16319-01, 16319-02, 16319-05, 16319-08, 16320-01, and 16320-04) derived from NY121 with unknown phenotypes to PVY infection were tested. Four selections (16319-01, 16319-02, 16319-05, and 16320-04) tested positive and two (16319-08 and 16320-01) tested negative with the marker (Table 2). The selections were then subject to graft-inoculation with PVY NTN (Nie et al. 2015). Approximately 10 d post top-graft inoculation (dpi) with PVY NTN-infected scions, emerging leaves of 16319-01, 16319-02, 16319-05, and 16320-04 (rootstocks) developed necrotic spots (Fig. 2A), indicating the triggering of hypersensitive-like response in the plants by PVY from the attached scions. A similar phenomena has been observed in plants bearing a corresponding R gene to a specific virus such as PVA (Nie and Singh 2001) and PVY (Nie et al. 2015). Indeed, ELISA tests of these leaves did not detect any detectable level of PVY (Table 2), demonstrating ER to PVY in these selections. In contrast, mosaic symptoms were observed in the emerging leaves of 16319-08 and 16320-01 at 10 dpi (Fig. 2B). Moreover, PVY was readily detected in these selections by ELISA (Table 2), indicating susceptibility to PVY infection. These results demonstrate that the presence or absence
of RYSC3 is in agreement with the ER or susceptibility to PVY infection in these selections. In spite of the small sample size, this assessment, together with studies by others (e.g., Ottoman et al. 2009), shows the effectiveness of the marker RYSC3-assisted selection for Ry<sub>adg</sub>-conferred ER, providing the marker is present in at least one of the two parents. It is notable that in the study by Ottoman et al. (2009), a low level of discrepancies (3.6%) between phenotype and genotype was observed in a segregating population, suggesting that new markers with a higher linkage to Ry<sub>adg</sub> are desired.

In summary, the presence/absence of four molecular markers reported to be linked to two Ry genes, Ry<sub>sto</sub> and Ry<sub>adg</sub>, was analyzed in potato breeding clones and advanced selections in AAFC’s potato breeding program for the first time. The Ry<sub>adg</sub> marker RYSC3 and the Ry<sub>sto</sub> markers YES3-3A and YES3-3B were detected in two and four breeding clones/selections, respectively, indicating the existence of Ry<sub>sto</sub> and Ry<sub>adg</sub> in potato germplasm in the program, thus paving the foundation for their utilization in marker-assisted selection of cultivars with extreme resistance to PVY. New markers are needed for germplasm that is extremely resistant to PVY but is free of the existing markers.

**Acknowledgements**

We thank Dr. Helen Tai for valuable comments on the manuscript prior to submission and Darcy Sutherland for technical assistance. We also acknowledge the assistance of the AAFC field staff at Benton Ridge for the cultivation of the test materials. This research was funded by AAFC under the peer-reviewed projects No. 1110 and No. 1199.

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