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

Authors: Nie, Xianzhou, Lalany, Fayruza, Dickison, Virginia, Wilson, Donna, Singh, Mathuresh, et al.

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SHORT COMMUNICATION

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

Xianzhou Nie, Fayruza Lalany, Virginia Dickison, Donna Wilson, Mathuresh Singh, David De Koeyer, and Agnes Murphy

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 Ry_{sto}, were detected in 'Barbara' and its three descendants that exhibit ER to PVY; RYSC3, a marker linked to Ry_{adg}, was detected in breeding clones NY121 and NY123. Assessment of RYSC3 as a marker for selection for Ry_{adg}-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 Ry_{sto} chez le cultivar Barbara et trois de ses descendants illustrant une résistance peu commune au PVY ainsi que le marqueur RYSC3, lié à Ry_{adg}, chez les clones NY121 et NY123. L'évaluation du marqueur RYSC3 pour la sélection de la résistance extrême médiée par Ry_{adg} a confirmé son efficacité pour l'identification des variétés résistant exceptionnellement au PVY. [Traduit par la Rédaction]

Mots-clés : pomme de terre, marqueur moléculaire, sélection assistée 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 *R* 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, Ry_{adg}, Ry_{sto}, and Ry_{chc}, which were derived from *Solanum tuberosum* ssp.

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X. Nie, V. Dickison, D. Wilson, and A. Murphy. Fredericton Research and Development Centre, Agriculture and Agri-Food Canada, 850 Lincoln Road, Fredericton, NB E3B 4Z7, Canada.

F. Lalany. Fredericton Research and Development Centre, Agriculture and Agri-Food Canada, 850 Lincoln Road, Fredericton, NB E3B 4Z7, Canada; Department of Biochemistry and Microbiology, 3800 Finnerty Road, Victoria, BC V8P 5C2, Canada.

M. Singh. Agricultural Certification Services, Fredericton, NB E3B 8B7, Canada.

D. De Koeyer. Fredericton Research and Development Centre, Agriculture and Agri-Food Canada, 850 Lincoln Road, Fredericton, NB E3B 4Z7, Canada; International Institute of Tropical Agriculture, PMB 5320, Oyo Road, Ibadan 200001, Oyo State, Nigeria.

Corresponding author: Xianzhou Nie (email: xianzhou.nie@agr.gc.ca).

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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.

			Molecular markers				Parents	
Clone/ selection	Country of origin	Response to PVY infection (phenotype)	YES3-3A (Ry_{sto})	YES3-3B (Ry_{sto})	GP122718 (Ry_{sto})	RYSC3 (Ry_{adg})	Female	Male
A11272-02	Canada	ER	–	–	–	–	A794	MDR Bulk
AC Chaleur	Canada	S	–	–	–	–	Belleisle	N457
Barbara	Germany	ER	+	+	–	–	MPI64.956/68	AM62.740
Bison	USA	S	–	–	–	–	ND4652-4 R	ND5124-1 R
Cupids	Canada	S	–	–	–	–	N150-3	Wauseon
F00069	Canada	ER	+	+	–	–	Barbara	Cupids
F07058	Canada	S	–	–	–	–	F87084	Rochdale Gold-Dorée
F07059	Canada	S	–	–	–	–	F87084	Rochdale Gold-Dorée
F07060	Canada	ER	–	–	–	–	F87084	Rochdale Gold-Dorée
F08086	Canada	ER	+	+	–	–	Barbara	Monalisa
F08087	Canada	ER	+	+	–	–	Barbara	Monalisa
F79070	Canada	ER	–	–	–	–	Y66-13-636 (Agitato)	F58050
F86028	Canada	ER	–	–	–	–	F65059	Y66-136-36 (Agitato)
F87084	Canada	ER	–	–	–	–	S62-47-1	Cupids
G7815-9Y	Canada	S	–	–	–	–	G7410-2Y	MPI.74.2Y
Green Mountain	USA	S	–	–	–	–	Dunmore	Excelsior
NY121	USA	ER	–	–	–	+	N43-288	E74.7
NY123	USA	ER	–	–	–	+	*S. tuberosum*	*S. berthaultii*
Shepody	Canada	S	–	–	–	–	Bake King	F58050

Note: S, susceptible; ER, extreme resistant; –, absence; +, presence. Clones/selections originated in Canada were bred and selected by AAFC's potato breeding program.

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 GP122 for Ry_{sto} (Flis et al. 2005; Song and Schwarzfischer 2008; Valkonen et al. 2008), and the sequence characterized region (SCAR) marker RYSC3 for Ry_{adg} (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 $GP122_{718}$. 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/clones 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^{O} 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

405 nm (A_{405}) is three times the negative (healthy) control with a reading ≥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′-TAACTC AAGCGGAATAACCC-3′) and 3R (5′-AATTCACCTGT TTACATGCTTCTTGTG-3′) for YES3-3A; 3F, and 3B (5′-CATGAGATTGCCTTTGGTTA-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, and 30 cycles of 40 s at 94 °C, 40 s at 53 °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) containing 1× Gel Red (Biotium, Inc., Hayward, CA) and a 10% Novex TBE polyacrylamide gel (PAG) (Invitrogen, Carlsbad, CA) (Fulladolsa et al. 2015), respectively. The PAG was stained with 1× SYBR® Gold nucleic acid stain (Life Technologies, Gaithersburg, MD) and visualized on the BioSpectrum® Imaging System™ (UVP, Inc., Upland, CA), whereas the agarose gel was visualized directly on the imaging system. The presence and absence of the YES3-3A and YES3-3B markers at 341 and 284 bp (Song and Schwarzfischer 2008), respectively, were recorded.

For detection of marker $GP122_{718}$, PCR with primers GP122F (5′-TATTTTAGGGTACTTCTTTCTTA-3′) and GP122R (5′-GCACTCAATAGCCCTTCTT-3′) was performed as described by Flis et al. (2005). A total of 40 cycles of amplification were performed, each included 20 s at 93 °C, 25 s at 53 °C, and 60 s at 72 °C. A final extension of 5 min at 72 °C was then carried out. Thereafter, 5 μL of the PCR products were separated on a 2% agarose gel containing 1× Gel Red (Biotium, Inc., Hayward, CA) by gel electrophoresis, and visualized on the BioSpectrum® Imaging System™. After determining the presence of a single amplicon of 718 bp, the remaining PCR product was restricted with 100 U *Eco*RV (New England Biolabs, Beverly, MA) at 37 °C for 1 h. Thereafter, the mix was fractionated by electrophoresis on a 2% agarose gel, and visualized on the BioSpectrum® Imaging System™. The presence and absence of the marker $GP122_{718}$ (i.e., the EcoRV-nondigestible fragment at 718 bp (Flis et al. 2005)) was recorded.

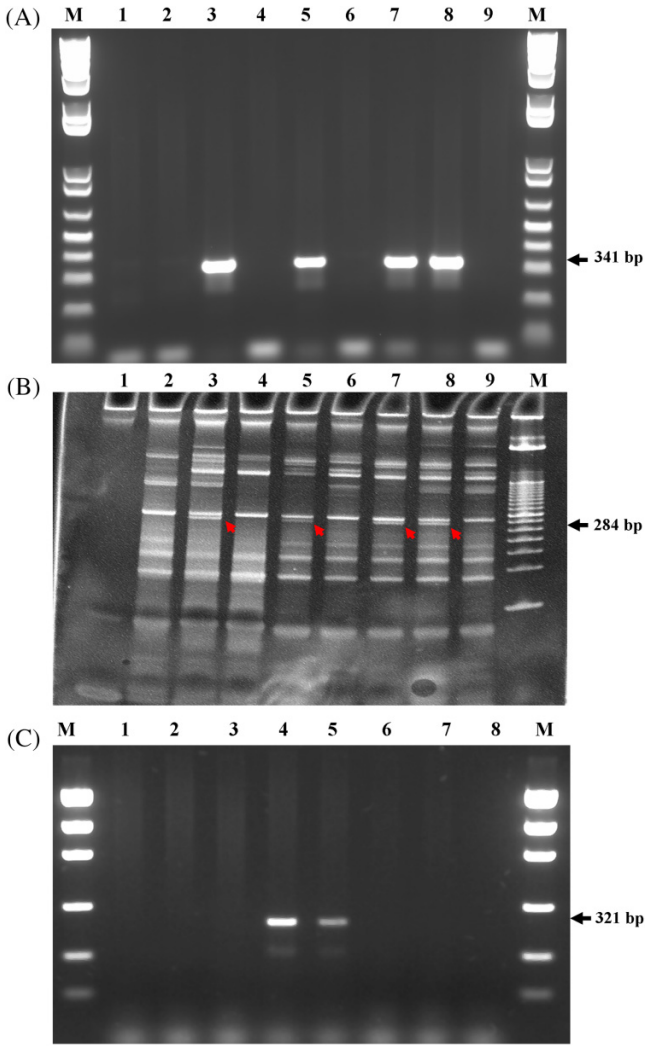
For detection of marker RYSC3, PCR with the primers 3.3.3 s (5′-ATACACTCATCTAAATTTGATGG-3′) and ADG23R (5′-AGGATATACGGCATCATTTTTCCGA-3′) was performed as described by Kasai et al. (2000). A total of 35 cycles of amplification, each included 45 s at 94 °C, 45 s at 60 °C, and 60 s at 72 °C, were performed. 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 PVY^O), *Nc* (specific to PVY^C), and *Nz* (specific to PVY^Z) (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 PVY^O. Indeed, in a previous report, F87084, one of the 11 with ER to PVY^O, exhibited immunity to all tested PVY strains including PVY^O, PVY^N, $PVY^{N:O}$, and PVY^{NTN} (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 PVY^O 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 $GP122_{718}$, a marker whose presence has been observed in many European cultivars carrying Ry_{sto} (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 $GP122_{718}$ 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,

Fig. 1. Detection of YES3-3A, YES3-3B and RYSC3 markers in potato breeding clones and advanced selections. (A) Detection of marker YES3-3A. After completion of PCR using primers developed by Song and Schwarzfischer (2008), the PCR products were subjected to 1.4% agarose gel electrophoresis. Lanes 1–9: G7815-9Y, Shepody, F00069, Green Mountain, F08087, F87084, F08086, Barbara, and NY121, correspondingly; lane M, 1 Kb Plus DNA Ladder (Invitrogen). (B) Detection of YES3-3B. After completion of PCR using primers developed by Song and Schwarzfischer (2008), the PCR products were subjected to Novex TBE gel (10% polyacrylamide gel) electrophoresis for high resolution (Fulladolsa et al. 2015). The marker of 284 bp is indicated by arrows. Lanes 1–9: G7815-9Y, Shepody, F00069, Green Mountain, F08087, F87084, F08086, Barbara and AC Chaleur, correspondingly; lane M, 50 bp DNA Ladder (Invitrogen). (C) Detection of marker RYSC3. After completion of PCR using primers developed by Kasai et al. (2000), the PCR products were subjected to 2% agarose gel electrophoresis. Lanes 1–8: F07058, Bison, A11272-02, NY121, NY123, F87084, F02010 and negative control (no genomic DNA), correspondingly; lane M, Low Mass DNA Ladder (Invitrogen).



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 Ry_{sto} marker YES3-3B and the Ry_{adg} marker RYSC3. Moreover, of the six resistant clones/cultivars that did not carry either YES3-3B or RYSC3, two ('Brodick' and 'Teena') likely carry Ry_{sto} and two (CHC 39-7 and CHC 40-3) are *S. chacoense* clones carrying Ry_{chc} (Fulladolsa et al. 2015). In this study, the Ry_{sto} markers YES3-3A, YES3-3B, and $GP122_{718}$ were absent in F87084 as well as its descendant F07060, even though F87084 was thought to possess Ry_{sto} 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 $GP122_{718}$ in Barbara and its descendants. Nevertheless, it cannot rule out the possibility that Ry_{sto} in Barbara and Ry_{sto} (i.e., $Ry\text{-}f_{sto}$) 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.

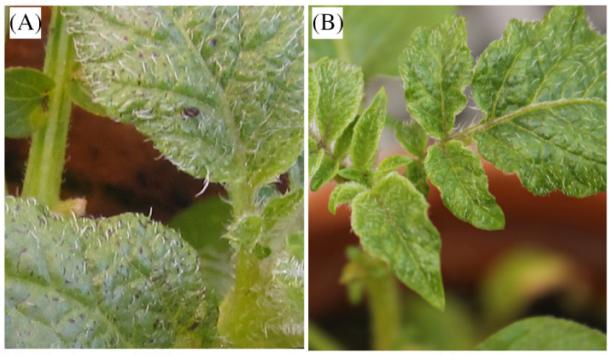
To assess the efficacy of RYSC3 as a marker for marker-assisted selection of Ry_{adg}-mediated ER, six advanced selections (16319-01, 16319-02, 16319-05, 16319-08, 16320-01, and 16320-04) derived from NY121 with unknown phenotypes to PVY infection were tested. Four selections (16319-01, 16319-02, 16319-05, 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

Table 2. Validation of the efficacy of RYSC3 for marker-assisted selection of Ry_{adg}-conferred extreme resistance to *Potato virus Y* (PVY) in potato advanced selections.

Advanced selection (female × male parents)	RYSC3 marker	Symptoms on emerging leaves after top graft-inoculation with PVY	ELISA (A_{405}) with PVY antibody	Phenotype
16319-01 (NY121 × F87031)	+	Necrotic spots	0.007	ER
16319-02 (NY121 × F87031)	+	Necrotic spots	0.007	ER
16319-05 (NY121 × F87031)	+	Necrotic spots	0.008	ER
16319-08 (NY121 × F87031)	−	Mosaic	2.161	S
16320-01 (NY121 × V1002-2)	−	Mosaic	2.462	S
16320-04 (NY121 × V1002-2)	+	Necrotic spots	0.003	ER

Note: +, presence; −, absence; ER, extreme resistant; S, susceptible. A sample is considered positive in ELISA when A_{405} is three times of the negative (healthy) control with a reading ≥0.100.

Fig. 2. Response of advanced selections derived from potato breeding clone NY121 to graft-inoculation with *Potato virus Y* strain NTN (PVY^{NTN}). (A) Necrotic spots on emerging leaves after graft-inoculation with PVY^{NTN} in a representative selection in which RYSC3 marker is present. The necrosis indicates a hypersensitive-like response triggered by PVY from the attached scion at approximately 10 d post-graft inoculation (dpi). (B) Development of mosaic symptoms on emerging leaves after graft-inoculation with PVY^{NTN} in a representative selection in which the RYSC3 marker is absent at 10 dpi. The mosaic symptoms indicate the systemic infection with PVY on the plant. ELISA results (A_{405}) with PVY antibody were obtained from the corresponding plants above.



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_{adg}-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_{adg} are desired.

In summary, the presence/absence of four molecular markers reported to be linked to two *Ry* genes, Ry_{sto} and Ry_{adg}, was analyzed in potato breeding clones and advanced selections in AAFC's potato breeding program for the first time. The Ry_{adg} marker RYSC3 and the Ry_{sto} markers YES3-3A and YES3-3B were detected in two and four breeding clones/selections, respectively, indicating the existence of Ry_{sto} and Ry_{adg} 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.

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