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SHORT COMMUNICATION

Application of Marker Assisted Selection for *Potato Virus Y* Resistance in the University of Wisconsin Potato Breeding Program

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Abstract *Potato virus Y* (PVY) is responsible for the majority of seed potato lot rejections in North America. Some commercial cultivars and breeding lines have major genes for PVY resistance, but their use in North American breeding programs has been limited. Marker assisted selection of PVY resistance can increase the efficiency of identifying resistant plants and integrating resistance into new cultivars. We used markers RYSC3 and YES3-3B, linked to genes Ry_{adg} and Ry_{sto}, respectively, to screen 46 breeding clones and cultivars, and identified 19 resistant clones. Resistant parents were crossed with susceptible parents with good market and agronomic traits and molecular marker analysis of each family showed a 1:1 segregation ratio. The persistence of this segregation ratio over 2 years of selection and the lack of major linkage drag as assessed by a breeder rating indicated that there was no evidence of an association of PVY resistance with undesirable traits.

Resumen El virus Y de la papa (PVY) es responsable por la mayoría de los rechazos de los lotes de papa para semilla en Norteamérica. Algunas variedades comerciales y líneas de mejoramiento tienen genes mayores para resistencia al PVY, pero es limitado su uso en programas de mejoramiento de Norteamérica. La selección asistida con marcadores de resistencia al PVY puede aumentar la eficiencia en la identificación de plantas resistentes y en la integración de la resistencia a nuevas variedades. Nosotros usamos los marcadores RYSC3 y YES3-3B, ligados a los genes Ryadg y Rysto, respectivamente, para evaluar 46 clones y variedades, e identificamos 19 clones resistentes. Se cruzaron padres resistentes con susceptibles de buenas características agronómicas y de mercado, y el análisis de marcadores moleculares de cada familia mostró una relación de segregación de 1:1. La persistencia de esta relación de segregación en dos años de selección, y la carencia de ligamiento mayor de arrastre evaluada por calificación de un fitomejorador, indicaron que no hubo evidencia de una asociación de la resistencia del PVY con caracteres no deseables.

Keywords Marker assisted selection · Potato virus Y · Resistance · Solanum tuberosum · Potato breeding

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Introduction

Potato breeding in North America has traditionally been done by crossing diverse, desirable tetraploid parents with complementary traits, selected based on their phenotype, followed by multiple cycles of progeny selection by breeder merit. To paraphrase Carputo and Frusciante (2011), F1 progeny are grown in single-hill plots and the first generation is cultivated, screened, and selected clones are further evaluated for the next seven to eight seasons in increasingly sophisticated field and laboratory trials, with a decreasing number of clones each year. Multiple viruses are common in potato. Vegetative propagation of tubers, which is necessary for subsequent selection cycles, leads to an increase in virus incidence in breeding program clones.

In recent years, *Potato virus Y* (PVY) has become a serious problem in potato. PVY is primarily managed through seed

certification, which limits virus incidence in planting materials to levels below a threshold that causes significant yield losses (Frost et al. 2013; Karasev and Gray 2013). Wisconsin seed certification data for 2002–2012 show that 92 % of rejections caused by plant diseases was due to PVY (Frost et al. 2013). The most effective way to control PVY incidence is by the use of resistant cultivars. However, North American breeding programs have made limited efforts to include virus resistance as a selectable trait and limited testing for virus susceptibility has led to the release and widespread acceptance of cultivars that do not express virus symptoms clearly (Gray et al. 2010). These symptomless carriers are problematic for the appropriate diagnosis of virus incidence during field inspections performed by certification agents, reducing the effectiveness of the certification process. Emerging recombinant PVY strains, which often show mild symptoms, also make it difficult to visually identify infected plants. Additionally, necrotic strains of the virus can induce potato tuber necrotic ringspot disease (PTNRD), which can severely affect tuber quality in numerous cultivars making them unmarketable (Kerlan 2006; Karasev and Gray 2013). Proper diagnosis of PVY infection requires the use of serological methods or polymerase chain reaction (PCR) to test breeding materials, which can be tedious, time-consuming and expensive. However, a molecular marker could be used more easily to predict the response to viral infection (Ottoman et al. 2009) by identifying a unique DNA region linked to resistance.

Marker-assisted selection consists of the use of DNA markers in plant breeding and can increase its efficiency and precision. Markers can be used to detect allelic variation in genes conferring traits of interest, characterize genetic resources, and provide information to assist in parental selection (Collard and Mackill 2008), which is an important part of conventional potato breeding. In potato, there is an increasingly widespread availability of molecular markers linked to different traits (Barone 2004) and marker-assisted selection can be useful for the introgression of resistance or simultaneous selection of plants with several traits (Solomon-Blackburn and Barker 2001b). Gebhardt et al. (2006) showed that marker-assisted selection for major genes of resistance to pathogens is efficient for combining those traits in breeding lines and cultivars. Ottoman et al. (2009) used markers for selecting PVY resistant clones and suggest they are efficient tools for reducing the number of PVY susceptible clones retained for further field evaluations, while increasing the chances of generating PVY resistant cultivars.

There are two types of genetic resistance to PVY identified in potato, hypersensitive resistance and extreme resistance (Halterman et al. 2012). Hypersensitive resistance is usually strain-specific, expressed by local necrotic lesions that prevent the spread of viral infection (Solomon-Blackburn and Barker 2001a), and is conferred by genes including Ny_{trb} and Nc_{spl} on chromosome IV (Celebi-Toprak et al. 2002; Moury et al. 2011), and *Ny-1* on chromosome IX (Szajko et al. 2008). Extreme resistance is asymptomatic and results in little to no detectable virus and confers resistance to several strains (Solomon-Blackburn and Barker 2001a; Halterman et al. 2012). Several genes for extreme resistance to PVY have been found and markers have been developed for their detection. These include: molecular markers ADG2 *BbvI*, RYSC3 and RYSC4 for detection of Ry_{adg} from *Solanum tuberosum* ssp. *andigena*, on chromosome XI (Sorri et al. 1999; Kasai et al. 2000); 38–530 and CT220 for Ry_{chc} from *Solanum chacoense*, on chromosome IX (Hosaka et al. 2001; Sato et al. 2006); GP122, STM003, and YES3-3B for Ry_{sto} from *Solanum stoloniferum*, on chromosome XII (Song et al. 2005; Song and Schwarzfischer 2008; Valkonen et al. 2008).

To address the need for PVY resistance in Wisconsin cultivars and the general disconnect between PVY resistance breeding and potato pathology (Karasev and Gray 2013), we screened parental clones for resistance in the greenhouse and used molecular markers to identify putative resistant donors, and developed breeding populations carrying resistance to PVY. We present the results of the inoculation assays and molecular marker screening and discuss the usefulness of marker-assisted selection in a conventional selection-based program.

Materials and Methods

Plant Material

Forty-six breeding clones and cultivars were used for phenotype and genotype analyses (Table 1). Cultivars Eva, NY121, Tacna, White Lady and Snowflake, carrying markers for Ry_{adg} or Ry_{sto}, were crossed with select susceptible cultivars. F1 progeny were grown from seed in the greenhouse at the Rhinelander Agricultural Research Station (RARS), Rhinelander, WI to produce one small seedling tuber per plant. The seedling tubers were then planted in the field at RARS in 2011 (field year 1). Additionally, a second set of F1 progeny from crosses White Lady x Nicolet, White Lady x Tundra, and Tacna x (Superior x Silverton) were planted at UW-Madison Walnut Street Greenhouse (WSGH). Tissue was collected from each plant grown at WSGH to use for marker screening (Table 2).

Clone Selections

Selections of field year 1 clones grown at RARS (2011) were made based on Verticillium wilt resistance and maturity, assessed during the growing season, and tuber uniformity and yield. Selected clones were planted as 8-hill plots in 2012 (field year 2). 10 weeks after planting, field year 2 clones were rated on a scale of 1 to 5 for Verticillium wilt severity (1:

Table 1 Analysis of genotype and PVY susceptibility phenotype in 46 potato breeding clones and cultivars of the University of Wisconsin Potato Breeding Program

Breeding clone	Phenotype[b]	RYSC3[c]	YES3-3B[c]	PVY resistance gene reported previously	Origin
Brodick	Resistant	A	A		UK
CHC 39-7	Resistant	A	A		USA
CHC 40-3	Resistant	A	A		USA
Kankan	Resistant	A	A		Hungary
PNT	Resistant	A	A		USA
Teena	Resistant	A	A		UK
A96949-4	Resistant	A	P		USA
A96953-13	Resistant	A	P		USA
Cyclamen	Resistant	A	P	Ry_{sto} (Heldák et al. 2007)	Hungary
Daisy	Resistant	A	P		France
EHR	Resistant	A	P		USA
PA92A08-17	Resistant	A	P		USA
Pushkinets	Resistant	A	P		Russia
Snowflake	Resistant	A	P	Ny_{tbr} (Solomon-Blackburn and Barker 2001a, b)	USA
Stobrawa	Resistant	A	P		Poland
W8946-1 Rus[a]	Resistant	A	P		USA
White Lady	Resistant	A	P	Ry_{sto} (Heldák et al. 2007)	Hungary
A93575-4	Resistant	P	A	Ry_{adg} (Ortega and Lopez-Vizcon 2012)	USA
Tacna	Resistant	P	A	Ry_{adg} (Ortega and Lopez-Vizcon 2012)	Peru
Allegany	Susceptible	A	A	Ny_{adg} (Valkonen et al. 1994)	USA
AWN86524-5	Susceptible	A	A		USA
Carola	Susceptible	A	A		Germany
Divina	Susceptible	A	A		Holland
Fabula	Susceptible	A	A		Holland
Goliath	Susceptible	A	A		Holland
Iris	Susceptible	A	A		Germany
Keuka Gold	Susceptible	A	A		USA
Meduza	Susceptible	A	A		Poland
Monona	Susceptible	A	A		USA
MX750660	Susceptible	A	A		USA
Nicola	Susceptible	A	A		Germany
O9BM164	Susceptible	A	A		
Oktiabronok	Susceptible	A	A		Russia
Ranger Russet	Susceptible	A	A		USA
Roclas	Susceptible	A	A		Romania
Russet Sebago	Susceptible	A	A		USA
Satina	Susceptible	A	A		Germany
Stirling	Susceptible	A	A		UK
Tara	Susceptible	A	A		Poland
W2230[a]	Susceptible	A	A		USA
W6002-IR[a]	Susceptible	A	A		USA
W8397-1[a]	Susceptible	A	A		USA
W8405-IR[a]	Susceptible	A	A		USA
W8639-5[a]	Susceptible	A	A		USA
Torridon	Susceptible	A	A	Ny_{tbr} or Ny_{chc} (Solomon-Blackburn and Bradshaw 2007)	UK
Talovsky	Susceptible	A	A		Russia

[a] Developed and maintained by the UW-Potato Breeding Program. Other clones were maintained but not developed by the program

[b] Phenotype was determined by mechanical inoculations

[c] *RYSC3* marker for Ry_{adg}, *YES3-3B* marker for Ry_{sto}, *A* marker absent, *P* marker present

Table 2 F1 progeny grown in the greenhouse showed a 1:1 genotypic segregation ratio, indicating that resistant parents carried a single dominant simplex allele for PVY resistance

Cross	Marker	Segregation ratio present:absent	Chi-square statistic	*P*-value
White Lady × Nicolet	YES3-3B	92:99	0.257	0.613^{NS}
White Lady × Tundra	YES3-3B	52:51	0.010	0.921^{NS}
Tacna × (Superior × Silverton)	RYSC3	28:32	0.267	0.606^{NS}

^{NS} Non-significant values at $P>0.05$

no symptoms; 1.5: 1–10 % foliar wilting, necrosis/chlorosis; 2: 11–20 %; 2.5: 21–30 %; 3: 31–40 %; 3.5: 41–60 %; 4: 61–80 %; 4.5: 80–95 %; 5: 96–100 %, or dead plant). One week after flowering, field year 2 clones were rated for late vigor on a scale of 1 to 5 (1: most vigorous, usually complete canopy closure; 5: least vigorous, usually stunted plants), a relative measure among all evaluated families. An overall tuber breeder rating was also assigned to field year 2 clones, using a scale of 1 to 5 (1: uniform size and shape; 5: deformed tubers showing external defects such as growth cracks, knobs or other tuber deformities). Tissue was collected from each field year 2 clone to use for marker screening (Table 3).

Mechanical Inoculations and PVY Testing

Sprouts of the 46 breeding clones and cultivars were planted in 6-in. pots and plants were maintained at WSGH. PVY inoculum was prepared by macerating PVY^{O}-infected *Nicotiana tabacum* L. 'Xanthi' leaves in 0.01 M potassium phosphate buffer (1:10 *w/v*). Rub inoculations were performed on two plants per clone, 4 weeks after planting. Two weeks post-inoculation, chemiluminescent dot-blot immunoassays (Fulladolsa Palma et al. 2013) and enzyme-linked immunosorbent assays (PVY PathoScreen® Kit, Agdia, Inc., Elkhart, IN) were used to detect the virus. Plants that were negative for PVY infection were inoculated again and the serological assays were repeated after 2 weeks. On those plants that were negative for the second time, inoculation and detection assays were repeated once more.

Table 3 Field year 2 clones from seven families (grown at RARS in 2012) showed similar number of plants carrying and not carrying markers linked to PVY resistance after one round of selection

Cross	Marker	Segregation ratio present:absent	Chi-square statistic	*P*-value
White Lady × Nicolet	YES3-3B	18:09	3.000	0.083^{NS}
White Lady × Tundra	YES3-3B	20:18	0.105	0.745^{NS}
White Lady × K3206-1	YES3-3B	20:20	0.000	1.000^{NS}
Snowflake × W2717-5	YES3-3B	18:18	0.000	1.000^{NS}
Eva × Nicolet	RYSC3	14:17	0.290	0.590^{NS}
Eva × Tundra	RYSC3	9:10	0.053	0.819^{NS}
NY121 × AF84-4	RYSC3	6:7	0.077	0.781^{NS}

^{NS} Non-significant values at $P>0.05$

Plant DNA Isolation, PCR Conditions, and Electrophoresis

Genomic DNA was isolated by macerating two 5-mm^2 sections of young leaf tissue in 400 μL of extraction buffer (200 mM Tris–HCl (pH 7.5), 250 mM NaCl, 25 mM EDTA, 0.5 % SDS) in a 1.7 mL microcentrifuge tube. Samples were vortexed and incubated at room temperature for 1 h. They were then centrifuged at 12000 rpm for 10 min and the supernatant was transferred to a new tube. A volume of 300 μL of 2-propanol was added to the supernatant and centrifuged at 12000 rpm for 10 min. The supernatant was discarded and the pellet was washed with 600 μL of ethanol. The samples were centrifuged at 12000 rpm for 5 min and the supernatant was discarded. The pellet was resuspended in 50 μL of TE buffer (10 mM Tris–HCl (pH 7.5), 1 mM EDTA (pH 7.5) and stored at −20 C.

Polymerase chain reaction (PCR) amplification of the Ry_{adg} SCAR marker RYSC3 (Kasai et al. 2000) was performed on a Techne TC-512 thermal cycler (Bibby Scientific Ltd, Duxford, Cambridge, UK) in a 25 μL reaction containing 12.5 μL PCR Master Mix (Promega, Madison, WI), 2.5 μL of primers 3.3.3 s (5′ ATACACTCATCTAAATTTGATGG 3′) and ADG23R (5′ AGGATATACGGCATCATTTTTC CGA 3′), and 2 μL of DNA (4 to 200 ng). The PCR program consisted of 93 C for 9 min followed by 35 cycles of 94 C for 45 s, 60 C for 45 s, 72 C for 60 s and a final extension at 72 C for 5 min. PCR products were separated on a 2 % agarose gel. Presence of a 321 bp fragment was diagnostic of Ry_{adg}.

The Ry_{sto} STS marker YES3-3B (Song and Schwarzfischer 2008) was amplified in a 25 μL reaction containing 12.5 μL PCR Master Mix (Promega, Madison, WI), 2 μL of primers 3 F (5′ TAACTCAAGCGGAATAACCC 3′) and 3B (5′ CATGAGATTGCCTTTGGTTA 3′), and 2 μL of DNA (4 to 200 ng). The PCR protocol consisted of 94 C for 2 min, 10 cycles of 94 C for 40 s, 55 C for 40 s, 72 C for 60 s, 30 cycles of 94 C for 40 s, 53 C for 40 s, 72 C for 60 s, and a final extension at 72 C for 5 min. PCR products were separated on an 8 % polyacrylamide gel and presence of a 284 bp fragment was diagnostic of Ry_{sto}.

Results and Discussion

Phenotype and Genotype of Breeding Clones and Cultivars

The PVY susceptibility phenotype of 46 breeding clones and cultivars was determined by mechanically inoculating plants and performing serological tests 2 weeks post-inoculation (Table 1). We found 19 resistant clones; two carried the RYSC3 marker for Ry_{adg} and 11 carried YES3-3B for Ry_{sto}. Markers were not present in susceptible clones. RYSC3 was highly correlated with extreme resistance conferred by Ry_{adg} in 100 % of plants used to validate the marker (Kasai et al. 2000). YES3-3B was derived from an AFLP marker reported to be tightly linked (LOD>3.0) to Ry_{sto} (Song et al. 2005). Four evaluated, resistant cultivars were previously reported to carry Ry_{adg} or Ry_{sto} (Table 1). Hypersensitive resistance genes Ny_{tbr} or Ny_{chc} have also been reported to be found or suggested to be present in the pedigrees of cultivars Snowflake, Allegany, and Torridon (Table 1), but this was not further examined.

The markers were absent from six resistant clones (Table 1). CHC 39–7 and CHC 40–3 are *S. chacoense* clones (NRSP-6 PI 275138 and PI 320285, respectively) and PNT is a *S. pinnatisectum* clone (NRSP-6 PI 253214), which are wild relatives of potato with PVY resistance (Cai et al. 2011); Brodick and Teena are Scottish cultivars with Snowflake in their pedigree and likely carry Ry_{sto}. Their resistance could also be derived from the maternal great-grandparent Pentland Crown, a cultivar carrying the Ny_{tbr} gene for hypersensitive resistance to PVY (Solomon-Blackburn and Bradshaw 2007). The female parent of cultivar Kankan includes not only *S. stoloniferum*, but also *S. demissum*, which has been reported to be resistant to PVY (Cockerham 1970). Other possible explanations for the absence of the YES3-3B marker in Brodick, Teena, and Kankan include recombination events between the marker and the gene of interest, differences in the background of the populations on which the markers were developed so that the polymorphisms are not present in the mentioned cultivars, or a germplasm handling and labeling error at some point during acquisition or maintenance of these clones.

Evaluation of Segregating Families Using Markers

Breeding clones White Lady, Snowflake, and Tacna were selected based on the presence of molecular markers YES3-3B and RYSC3 (Table 1) and used as parents in crosses with other susceptible clones with good market and agronomic traits, such as high chipping quality, scab resistance, and late blight resistance. Eva and NY121, which have been reported to carry Ry_{adg} (Kasai et al. 2000; Plaisted et al. 2001; Baldauf et al. 2006) and the RYSC3 marker (Sagredo et al. 2009), were also used as resistant parents. We genotyped 191 F1 progeny from the cross White Lady × Nicolet, 103 from White Lady × Tundra, and 60 from Tacna × (Superior × Silverton). Chi-square analyses were performed using R statistical software version 2.15.3 (R Core Team 2013) to determine if Mendelian segregation had occurred (Table 2). All populations showed a 1:1 segregation ratio (α=0.05), expected for populations derived from a single dominant simplex (Rrrr) crossed with a nulliplex (rrrr), as has been previously described for White Lady (Solomon-Blackburn and Mackay 1993; Cernák et al. 2008; Kuhl 2011).

Field year 2 clones from seven families were also genotyped and Chi-square analyses showed that the segregation ratio remained constant after the first round of selection occurred at RARS during field year 1 (Table 3). In addition, field year 2 clones were rated for late vigor and Verticillium wilt severity, and were given a tuber breeder rating during harvest. These traits were used to select clones that would be evaluated in future field trials. A Mann–Whitney *U* test was performed on the ratings of the three selection criteria given to each individual clone. No significant differences (α=0.05) in late vigor or Verticillium wilt severity were found between genotypes within each family (data not shown). Similarly, no significant differences (α=0.05) in tuber breeder rating were found between genotypes within or among the families derived from White Lady × Nicolet and White Lady × Tundra crosses (data not shown). These result indicated no evidence of linkage drag with regard to plant vigor, Verticillium wilt severity or tuber breeder rating, associated with markers linked to Ry_{sto} or Ry_{adg}.

Although resistance is the most effective method for control of infection by pathogens, viral or other, the question of whether there is a fitness cost to the plant in the absence of disease is controversial (Burdon and Thrall 2003) and research in this area has been limited (Brown 2002). For example, Ayala et al. (2001) found that resistance to *Barley yellow dwarf virus* in wheat recombinant lines did not have positive or negative effects on crop yield or quality. In contrast, Le Gouis et al. (1999) found that winter barley lines carrying the *ym4* (*rym4*) gene for resistance to *Barley mild mosaic virus* and strain 1 of *Barley yellow mosaic virus* were lower yielding than susceptible lines by an average of 4 % in four out of eight trials. Marker-assisted selection and cisgenics have been applied to precision breeding, reducing linkage drag, especially in vegetatively propagated crops such as potato and apple (Jacobsen and Schouten 2007; Collard and Mackill 2008). Because the sources of resistance used in this study are advanced breeding clones and commercially available cultivars crossed with others of similar market class, linkage drag is likely to be low. Marker-assisted selection can be further used in breeding resistant potato varieties with different agronomic and marketable traits. Using modern techniques, such as single nucleotide polymorphism analysis and whole genome sequencing, further development of markers linked to

genes of interest can increase the molecular tools available, improving breeding precision and the introgression of traits from wild relatives of potato.

As discussed by Ottoman et al. (2009), the use of MAS for PVY resistance can reduce the time and cost of disease screening, and increase the efficiency of genotyping. Here, we report the results of the application of MAS in the first stages of a conventional potato breeding program in Wisconsin. Few other published examples (Kasai et al. 2000; Flis et al. 2005; Gebhardt et al. 2006; Rizza et al. 2006; Witek et al. 2006; Sagredo et al. 2009; Whitworth et al. 2009; Ortega and Lopez-Vizcon 2012) provide experimental data on the presence of molecular markers linked to PVY resistance in cultivars commonly used as parental materials in breeding programs across the globe. This information is useful for the adoption of MAS and the incorporation of PVY resistance in future cultivar releases, offering the seed potato industry the possibility of significantly reducing the effects of their number one disease concern.

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