MARKER ASSISTED SELECTION INCREASES THE EFFICIENCY OF BREEDING

FOR POTATO VIRUS Y RESISTANCE IN POTATO

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ABSTRACT

Potato Virus Y (PVY) is an important virus of potato due to the non-persistent mode of transmission by aphids causing yield losses. Genetic resistance is the recommended control since insecticides cannot adequately control the spread of PVY by aphids. The gene Ry_{adg} from *S. tuberosum* ssp. *andigena* provides resistance to all strains of PVY. This gene has genetically been mapped to chromosome XI, and linked polymerase chain reaction (PCR) based DNA markers have been identified. This study identified PVY resistant progeny by the use of the molecular sequence-characterized amplified region (SCAR) marker RYSC3. The RYSC3 marker allowed a simple and fast approach to determine if the Ry_{adg} gene was present in the seedling family populations evaluated. The RYSC3 marker identified 16 families with progeny segregating for the Ry_{adg} gene. Progeny segregated 1:1 for PVY resistance, fitting the model simplex (Ryryryry) for the Ry_{adg} allele.

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INTRODUCTION

Potato (*Solanum tuberosum L.*) is a valuable source of nutrition, and supplies the necessary caloric intake of several important nutrients for the growing population of the world (Camire et al. 2009). Potato is ranked fourth in the world for production, behind wheat (*Triticum aestivum)*, rice (*Oryza sativa*), and corn (*Zea mays)* (National Potato Council 2013). Potato breeding programs are essential for the development of new cultivars with the most desired traits meeting grower needs, processor specifications, and consumer demands (Felcher and Douches 2012). Maintaining virus-free seed stock is a major challenge for seed potato growers and potato growers. Potato breeding programs that focus on developing disease resistant cultivars are key to bringing disease incidence under control (Gray et al. 2010).

Numerous viruses infect cultivated potatoes, causing a range of consequences, from yield reduction, to internal tuber defects (Gray et al. 2010). Potato virus Y (PVY) is a single-stranded RNA virus belonging to the family *Potyviridae* (Stevenson et al. 2001), and is a major potato pathogen of great economic concern to both commercial and seed potato producers (Ottoman et al. 2009). PVY can cause significant damage in several species of Solanaceae, but its major economic impact is on potato, reducing crop yields from 10 to 75% (De Bokx and Huttinga 1981). There are three common strains of PVY; PVY^O, PVY^N, and PVY^C (Crosslin et al. 2006), but recombinant strains of PVY have been recently reported; PVY^{NTN} and $PVY^{N:O}$ (Ottoman et al. 2009). Symptoms of PVY include veinal necrosis, leaflet mottling, yellowing of leaflets, leaf drop, dwarfing, and premature death of plants (Gray et al. 2010). Potato cultivar, environmental conditions, time of infection, and virus strain are all factors that influence PVY symptoms (Gray et al. 2010). Necrotic strains of the virus cause tuber symptoms, beginning with formation of protrusions from the tuber surface, which later-become sunken, forming necrotic ring spots that

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make tubers unmarketable (Stevenson et al. 2001). PVY infection in some of the newer North American cultivars, such as Shepody and Russet Norkotah, frequently result in mild, or even latent symptoms, as opposed to the typical foliar symptoms that are observed in more traditional cultivars such as Russet Burbank (Nolte et al. 2004). The lack of symptom expression of PVY despite infection, means that producing certified seed of Russet Norkotah and Shepody can be difficult, because production methods for certified seed potatoes in the United States still rely primarily on visual inspection procedures for detection and rouging of PVY-infected plants (Nolte et al. 2004). PVY is primarily transmitted by aphids, but can also be transmitted mechanically and vegetatively by infected seed (Radcliffe and Ragsdale 2002). Many methods are used to reduce the level of initial PVY inoculum such as planting clean certified seed, eliminating weed reservoirs, and rogueing volunteers early (Gray et al. 2010). Methods used to reduce the spread of PVY by aphids include insecticides, border crops, destruction of volunteers and sources of PVY, and field placement, management, and design (Gray et al. 2010). PVY remains a serious problem affecting the potato industry, and continuing work to identify better control options is necessary.

One method to control and prevent the spread of PVY is to identify potato plants with resistance to PVY (Felcher and Douches 2012). PVY-resistant cultivars are an environmentally friendly and cost-effective control method that can be easily implemented by growers (Ottoman et al. 2009). Unfortunately, there are few cultivars grown widely in North America that express any type of resistance that would significantly reduce virus incidence or transmission (Gray et al. 2010). Marker assisted selection (MAS) is currently being utilized by potato breeding programs to more easily identify plants that carry genes resistant to PVY (Sorri et al.1999). Molecular markers can be an indispensable tool for characterizing PVY resistance resources and providing

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breeders with more detailed information to assist them in selecting parents (Ottoman et al. 2009).

The sequence-characterized amplified region (SCAR) marker, RYSC3 is one of the most

powerful tools in marker-assisted selection for the Ry_{adg} gene (Kasai et al. 2000). The Ry_{adg} gene

derived from *Solanum tuberosum ssp. andigena,* provides resistance against all strains of PVY,

providing an effective means to protect potatoes (Whitworth et al. 2009). A detailed analysis of

the segregation of the Ry_{adg} gene in a family population gives more understanding about the

genetic configuration of the resistant parent with regard to the number of copies of the resistance

gene (Allard 1960). Breeding programs aim to increase PVY resistance by increasing the

frequency of the resistant alleles (Allard 1960). Identification of resistant cultivars by genotype

assessment will allow resistant genotypes to enter a system similar to the late blight fast-track

program used by the North Dakota State University (NDSU) potato improvement team, and

undergo phenotypic testing in the field (Thompson 2012). This research will drive the

development and release of PVY resistant genotypes at NDSU, which will benefit seed and

commercial potato production in the North central regions and beyond.

LITERATURE REVIEW

The Solanaceae family includes some of the most important horticultural crops grown, including tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*), tobacco (*Nicotiana tabacum*), pepper (*Capsicum* spp), and potato (Song et al. 2005). The potato tuber is a starchy, enlarged portion of an underground branch of a stem called a stolon (Thornton and Sieczka 1993). During the last three centuries, the potato has been one of the main side dishes that accompanied meat and milk, and has helped millions of people survive all around the world (Thornton et al.1993). According to the USDA (2012), consumption of one medium russet-skinned potato (approximately one cup) supplies 35 percent of the daily recommended value for vitamin C, 16 percent daily recommended amount of fiber, and 10 percent of the daily requirements of B6. A frequently expressed concern in the ongoing public health debate is the lack of affordability of fresh vegetables, especially those that are nutrient dense. A recent study by Drewnowski and Rehm (2013), reported that potatoes are one of the most nutritional vegetables in the produce aisle, providing one of the better nutritional values per penny than any other raw vegetables, and delivering one of the most affordable sources of potassium of the more frequently consumed vegetables, second only to dry beans. Other studies have determined that potatoes have 75% more food energy per unit area than wheat, and 58% more than rice (Camire et al. 2009). Potatoes provide 54% more protein per production area unit than wheat, and 78% higher than rice (Camire et al. 2009). Currently, there is no other crop that compares to the potato in production of food energy and food value per unit (Camire et al. 2009).

The potato is an important staple food crop grown world-wide across temperate, subtropical, and tropical climates (Camire et al. 2009). China and India are the world's largest potato producing countries, with nearly one third of the world's potatoes harvested (National

Potato Council 2013). The United States ranked fifth in world potato production in 2013, after China, India, the Russian Federation, and Ukraine, producing 17.5 million tonnes (National Potato Council 2013). Advances, such as improved irrigation systems and fertilizers, have allowed growers to produce more potatoes on less land (Thornton and Sieczka 1993). In the 1930s, 141.6 million hectares were planted in the United States; in 2013, 425,729 hectares produced more than one and one-half times the tonnage of the 1930s (USDA 2013). Yields have also increased due to the changes in geographical areas of production, fewer but larger farms, and improved cultural techniques in production (Thornton and Sieczka 1993).

Wild potatoes are widely distributed in the southwestern United States to southern Chile, but the first cultivated potatoes were selected between 6,000 and 10,000 years ago (Spooner and Hetterscheid 2006). These wild species and thousands of indigenous primitive cultivated landrace populations persist throughout the Andes, with a second set of landrace populations on Chiloe Island (Spooner and Hetterscheid 2006). According to Spooner and Hetterscheid (2006) the Chilean populations probably arose from Andean populations that underwent hybridization with the wild species *Solanum tarijense*, found in southern Bolivia or northern Argentina. The first record of potato out of South America is from the Canary Islands in 1562, and the potato swiftly became cultivated in Europe, and then worldwide (Spooner and Hetterscheid 2006). Modern cultivars were created by selection and breeding that allowed more uniform colors and shapes, with improved agronomic qualities such as greater yield and disease resistance (Spooner and Hetterscheid 2006).

Potato breeding

Potato breeding programs evaluate and make selections for numerous traits directed by end use (fresh-market, chip processing, frozen/french fry processing, novelty), what consumers prefer (skin color, flesh color, flavor, shape, culinary quality), and the requirements needed to produce the crop (yield, maturity, disease resistance) (Felcher and Douches 2012). For a new cultivar to be released into the market it must have as many of the desired traits as possible (Felcher and Douches 2012). For example, a new potato chip cultivar must meet grower demands (high yield, disease resistance, early maturing, and produce a light chip color when fried), processor demands (high specific gravity, low levels of reducing sugars, disease resistance, and smooth/round shape), and consumer demands (Felcher and Douches 2012). The selection process takes place in multiple locations, including the field, greenhouse, and laboratory, and requires up to 12 years or more from the initial cross to the release of a new variety (Ortega and Lopez-Vizcon 2012). Trait demands can change over short or long periods of time, thus breeders must preserve and develop a germplasm pool with high frequencies of genes for desirable traits (Felcher and Douches 2012). Furthermore, an adapted virus population may overcome resistance genes introduced into a cultivar by plant breeders after large-scale growing of a cultivar, hence many sources of diverse germplasm are desired by breeders to be able to combat the virus with new resistance genes (Ayme et al. 2006). One way to increase the efficiency of the breeding/selection process for particular characteristics is the use of marker assisted selection (MAS) (Ortega and Lopez-Vizcon 2012).

More than 457,699 hectares of potatoes were grown in the United States in 2013, with a value of more than $3.364 billion (USDA 2013). PVY is responsible for causing significant losses in four main cultivated crops (potato, pepper, tomato, and tobacco), as well as in

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ornamental plants (*e.g.* petunia (*Petunia × hybrida flower*)) (Scholthof et al. 2011). PVY has

become the most economically important disease problem in many areas of the world (Gray et al.

2010). PVY causes a decline in yield and quality, which decreases profitability in commercial

and seed potato production (De Bokx and Huttinga 1981). In seed potato production there are

strict limitations on virus levels for certified seed (Gray et al. 2010). PVY is the leading cause of

rejection of seed lots from certification programs (Gray et al. 2010). Seed fields with high PVY

levels, dependent on field generation (<0.50% presence level in Minnesota, and <0.30%

presence level in North Dakota) are rejected for use as seed by state certification departments,

resulting in significant reduction in crop value, and at times shortages of certified seed,

especially in varieties that are highly susceptible to the disease (Gray et al. 2010, MNDA 2013,

NDSSD 2013). Planting of seed tubers infected with PVY can result in yield losses of up to 75%

(De Bokx and Huttinga 1981). A study by Nolte et al. (2004) compared the effect of various

levels of seedborne PVY inoculum on yield of Russet Burbank, a cultivar that displays typical

mosaic foliar symptoms, and with Russet Norkotah and Shepody, cultivars that show very mild

symptoms as a result of PVY infection. Nolte et al. (2004) determined that seedborne PVY has

virtually the same negative impact on yield for all three cultivars.

PVY

PVY was recognized in potato in 1931, as a member of a group associated with potato

degeneration, a disease known since the 18th century (Smith 1931). PVY has been one of the

most studied plant viruses of all time (Scholthof et al. 2011). PVY belongs to the *Potyvirus*

genus, one of the six genera in the family *Potyviridae* (Shukla and Ward 1998). PVY virions are

filamentous, non-enveloped, flexuous rods, 700-900 nm in length, 11-12 nm in diameter, with

helical symmetry, containing about 6 percent nucleic acid. The potyvirus genome is approximately 10kb long and consists of one single-stranded linear RNA molecule (Scholthof et al. 2011). All potyviruses induce the formation of cylindrical or pinwheel-shaped viral protein inclusion bodies in the cytoplasm of infected cells (Stevenson et al. 2001). These inclusions can be easily identified by light or electron microscopy and are a useful diagnostic characteristic for PVY (Stevenson et al. 2001).

PVY has a worldwide distribution and a large host range, which includes not only cultivated solanaceous species, but also many solanaceous and non-solanaceous weeds (Stevenson et al. 2001). Hairy nightshade is a common weed in the Midwest. It is susceptible to PVY and is attractive to the potato-colonizing aphids, which can serve as sources of infection for seed producers (Cervantes and Alvarez 2011). Other plants in families Chenopodiaceae and Leguminosae are hosts as well (Stevenson et al. 2001). The primary means of PVY transmission in the field is via aphids, yet PVY can also be transmitted mechanically by wounding, grafting, by cutting infected seed, or vegetatively (Radcliffe and Ragsdale 2002). During mechanical transmission (also called sap transmission), PVY is spread through infected plants and healthy plants rubbing, resulting in short distance spread of the virus. This transmission requires mechanical damage by wind or human activity. PVY may be vectored by 50 different aphid species, the majority of which do not depend on potato as their main host and may or may not colonize potato (DiFonszo et al. 1996). PVY is a stylet-borne, non-persistent virus, i.e. there is no latent period between acquiring the virus and the ability to transmit the virus (Cervantes and Alvarez 2011). Once the plant is inoculated by aphids, the virus is translocated to the tubers. Since potatoes are vegetatively propagated by tubers, seed is generally the main source of initial inoculum in an emerging crop, contributing to overall virus incidence (Gray et al. 2010). In

developed countries, growers follow strict guidelines established by seed certification programs in which virus levels in seed production fields are closely monitored (Gray et al. 2010). Seed lots exceeding the virus tolerance levels for certification are rejected and may not be sold as certified seed (Gray et al. 2010).

Aphid vectors

The green peach aphid (*Myzus persicae),* is the most efficient aphid in transmitting PVY (Cervantes and Alvarez 2011). Some insecticides that once controlled the green peach aphid are no longer effective due to resistance (Radcliffe and Ragsdale 2002). Increased use of late blight fungicides that destroy entomopathogenic fungi which control green peach aphid are thought to be a factor in rising green peach aphid populations as well (Radcliffe and Ragsdale 2002). Increasing acreages of green peach aphid host crops, such as canola in potato growing regions, may also be a factor in the growth of green peach aphid populations that have caused higher incidences of PVY (Radcliffe and Ragsdale 2002). Other important aphid vectors include the bird cherry-oat aphid (*Rhopalosiphum padi*), potato aphid (*Macrosiphum euphorbiae*), and the pea aphid (*Acyrthosiphon pisum*) (Davis et al. 2005). In 2000, the soybean aphid (Aphis glycines) was discovered to be a new vector of PVY in North Central regions of the United Sates (Davis et al. 2005). Although individual soybean aphids are inefficient vectors of PVY, large populations of winged aphids that disperse in massive flights in mid to late summer contribute significantly to the spread of PVY (Davis et al. 2005). The soybean aphid feeds exclusively on soybean, but will probe other plants when looking for a suitable food source; this is when the soybean aphid transfers PVY inoculum to healthy potato plants (Davis et al. 2005). Aphids are important to potato producers primarily because they vector devastating viruses, but high aphid

populations can cause direct plant injury and significant yield losses (Radcliffe and Ragsdale 2002).

PVY strains and symptoms

A number of PVY strains have been identified and placed into groups based on their symptomology in potato and tobacco (Sorri et al. 1999). There are three main strains of the virus: PVY^O, PVY^C, PVY^N (Whitworth et al. 2009). First classified in 1931, PVY^O (the "ordinary" strain) is common around potato production areas, causing mild to severe mosaic symptoms, depending on cultivar (Piche et al. 2004). PVY^C (stipple streak strain) produces hypersensitive reactions or mosaic in potato and is not aphid transmitted (Radcliffe and Ragsdale 2002). Appearing in the 1950s, a new tobacco veinal necrosis strain of PVY (PVY^N) was found in Europe and South American countries and has since spread worldwide, eventually being detected in the United States in 1990 (Piche et al. 2004). PVY^N is frequently symptomless or produces mild to severe mosaic symptoms on potato foliage and leaf death in tobacco (Piche et al. 2004). A sub-group serologically related with the necrotic strain PVY^N, known as PVY^{NTN}, causes potato tuber necrotic rings in the tubers of susceptible cultivars such as Yukon Gold (Gray et al. 2010). This strain was first described in Europe in the 1970s, and has become widely dispersed across the globe, being detected in the USA in 1993 (Piche et al. 2004). Recently in the United States and Canada, new strains that have both characteristics of PVY^O and PVY^N have been reported; the new recombinant strain is known as $PVY^{N:O}$ (Crosslin et al. 2006). $PVY^{N:O}$ is transmitted via vegetative propagating of seed and aphids. $PVY^{N:O}$ produces a circular, sunken necrotic lesion on the surface of the tuber (Piche et al. 2004). $PVY^{N:O}$ is among the most common strains currently found in North America (Sagredo et al. 2009). Because foliar

symptoms of PVY$^{N:O}$ are mild, or asymptomatic on some potato varieties, inspections are not a reliable source to identify plants when certifying seed potato lots. Inspectors could test plants that are questionable for virus but that takes time and money, and it allows for the movement of virus during the time of testing. Growers also cannot easily identify and rogue PVY$^{N:O}$ infected plants as a means to reduce the levels of virus inoculum in a field; as a result PVY levels can quickly increase (Gray et al. 2010).

PVY control

Control of PVY includes both direct and indirect approaches. Rouging of infected plants is one direct approach to eliminating PVY, although this is not a reliable method since some genotypes do not show symptoms clearly, especially in the case of asymptomatic/tolerant genotypes (Ottoman et al. 2009). The main indirect PVY control is reducing the level of initial PVY inoculum by the use of limited-generation potato seed (DiFonszo et al. 1996). Limited-generation seed potato production, in combination with certification programs, places restrictions on how long seed can be retained based on the percentage of plants infected with viruses (Gray et al. 2010). Thus, PVY management on seed producing farms must focus on minimizing inoculum in the field and protecting the crop from viruliferous aphids that can introduce and spread existing virus to other healthy plants (Gray et al. 2010). Effective seed certification programs will be the key in reducing initial inoculum in seed potato fields, while crop protection will result from trained and knowledgeable management on the farm (Gray et al.2010). Additional management strategies used by growers to reduce virus availability to aphids and to reduce the potential for aphids to inoculate plants, includes the elimination of weed reservoirs for aphids and virus, the use of border crops to "cleanse" aphids of virus before they enter a potato

field, and the use of chemicals to prevent aphids from feeding on potato plants *e.g.* refined mineral oils, and anti-feedant insecticides (DiFonszo et al.1996). The success of these strategies will depend on the grower's experience with the major aphid species in his geographic region, phenology of aphid flights, and aphid population dynamics (Radcliffe and Ragsdale 2002). Even with accurate application timing, insecticides may prevent the spread of PVY within a potato crop, but they do not act rapidly enough to prevent the transmission of the virus by winged aphids moving into the fields from outside (Stevenson et al. 2001). Mineral oils can be used to reduce the spread of PVY, but they must be applied weekly to protect new foliage, and this has proven to be costly (Stevenson et al. 2001). A more efficient and cost effective strategy to avoid PVY may be resistant cultivars (Scholthof et al. 2011). Planting PVY resistant cultivars is a preferred control method that is environmentally friendly and easy to implement by growers (Ottoman et al. 2009).

Breeding for PVY resistance

PVY resistant cultivars have been developed through genetic transformation and by traditional breeding techniques (Ottoman et al. 2009). Due to the non-acceptance of transgenic potatoes by consumers at this time, traditional breeding is the current means of developing PVY resistant cultivars (Ottoman et al. 2009). Breeding for PVY resistant cultivars using traditional approaches begins with identifying potato clones that are resistant to PVY, followed by introgression of resistant gene(s) into advanced breeding populations (Gray et al. 2010). The traditional way to determine whether potato clones are PVY resistant or susceptible is to inoculate the clones with the virus and evaluate symptoms of viral infection. Classifying clones as either resistant or susceptible can be determined visually by observing plant symptoms, by

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immunological tests such as the enzyme-linked immunosorbent assay (ELISA), and with reverse transcriptase-polymerase chain reaction (RT-PCR) amplification (Ottoman et al. 2009). Grafting and mechanical transmission are common methods for artificially inoculating potato with PVY (International Potato Center 2014). Top grafting requires a PVY-infected scion to be grafted to the non-infected rootstock for virus transmission (International Potato Center 2014). Using mechanical transmission to infect plants with PVY involves lightly dusting the tops of the healthy leaves, then rubbing ground up PVY infected leaf tissue prepared in a buffer solution onto healthy plant leaves (International Potato Center 2014). Successfully obtaining results is very time consuming. Drawbacks to this method include a risk of spreading PVY to non-target susceptible elite breeding lines (Ottoman et al. 2009). It is often difficult to classify clones as PVY resistant or susceptible based on visual evaluations because many times cultivars are asymptomatic (Ottoman et al. 2009). In addition, when screening large segregating populations, ELISA and RT-PCR prove to be time consuming and tedious (Ottoman et al. 2009).

Marker assisted selection

Potato breeding is best described as pedigree breeding where phenotypic selection is based principally on maturity, tuber appearance, and yield. It is executed at the F_1 generation and the genotypes are then established by clonal propagation (Felcher and Douches 2012). Selection may continue over several years to acquire clones with the needed combination of traits the breeder desires (Ortega and Lopez-Vizcon 2012). Developing these traits requires larger plot sizes, more replicates, and testing in numerous, different environments for accurate characterization (Felcher and Douches 2012). This method is unattainable, due to the cost and time constraints to test several thousand single-hill selections for PVY resistance (Ottoman et al.

2009). As a shortcut, breeding programs now use MAS to identify specific genes of interest (Ortega and Lopez-Vizcon 2012). The genetic markers are a sequence of nucleic acids that make up a DNA segment close to the gene of interest (Ortega and Lopez-Vizcon 2012). The genetic markers are located near the DNA sequence of a desired gene (Ortega and Lopez-Vizcon 2012). This linkage helps plant breeders predict whether a plant will have a desired trait (Ortega and Lopez-Vizcon 2012). If plant breeders can find the genetic marker for the gene, it means the desired gene itself may be present (Ortega and Lopez-Vizcon 2012). Molecular markers have been developed that are closely linked with resistance genes to PVY (Whitworth et al. 2009). MAS is an effective option for maximizing selection efficiency to identify progeny resistant to PVY (Sorri et al. 1999).

PVY resistance genes

A number of wild potato species originating in the Andes Mountains of Peru and Bolivia are related to cultivated potato and can be used as sources of resistance to PVY (Kasai et al. 2000; Thornton and Sieczka 1993). Germplasm enhancement, using diverse wild sources of resistance, is of great value in incorporating valuable resistance traits into cultivated potatoes (Kasai et al. 2000). Sources of resistance to PVY have been reported in more than 20 *Solanum* species (Whitworth et al. 2009). In cultivated and wild potato species, two major types of single, dominantly-inherited resistance genes to PVY have been identified, hypersensitive response (Ny, N-genes) and extreme resistance (Ry, R-genes) genes (Song et al. 2005). Hypersensitive response (N-genes) genes prevent virus infection from spreading by programmed cell death (necrosis) at the site of infection. This form of resistance is PVY strain specific (Whitworth et al. 2009). Extreme resistance, not specific to PVY strain, is controlled by R-genes, which reduce

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virus replication in infected cells, thus allowing the plant to defend itself from the virus (Sorri et al. 1999). A viral elicitor that actively triggers cell death and defense reactions through a partly elucidated signal pathway induces extreme resistance (Ayme et al. 2006). There are four known R genes, Ry_{adg}; Ry_{sto}, Ry-f($_{sto}$) and Ry_{chc} confer extreme resistance to PVY and have been mapped successfully (Tiwari et al. 2012).

Gene Ry$_{adg}$

Cockerham (1943) indicated that an examination of *S. tuberosum* ssp. *andigena* was being made in an attempt to find sources of resistance to PVY. In 1970, he reported the presence of resistance genes in *S. chacoense, S. microdontum, S. demissum, S. hougasii,* and *S. stoloniferum*. He did not describe resistance in *S. tuberosum* spp. *andigena.* Munoz et al. (1975) noticed the presence of lines in a selected Andigena population showing a high degree of resistance to PVY in field exposures. These lines had been developed from *S. tuberosum* ssp. *andigena*. Tests were made on the lines of the *S. tuberosum ssp. andigena* and Munoz et al. (1975) determined that extreme resistance to PVY existed in the Andigena germplasm. The resistance gene Ry$_{adg}$, from *S. tuberosum ssp. andigena*, was localized by Hamalainen et al. (1997), on the distal end of chromosome XI by restriction fragment length polymorphism (RLFP) markers TG508, GP125, CD17, and CT168 developed by Tanksley et al. (1992). The marker TG508 was determined to be the closest marker, at a distance of 2.0 CM from the gene (Hamalainen et al. 1997). These four markers have been tested in tetraploid and diploid potatoes and found suitable for MAS for the Ry$_{adg}$ gene (Tanksley et al. 1992). Hamalainen et al. (1998) also established that the Ry$_{adg}$ gene is located on a segment that holds recognized resistance genes to tobacco mosaic virus (TMV). Two amplified sequence-related resistance gene-like

(RGL) fragments, ADG1 and ADG2 were mapped to a region of chromosome XI that is known

to contain the Ry_{adg} gene (Hamalainen et al. 1997, 1998) and other resistance genes (Brigneti et

al. 1997), both of which control extreme resistance to PVY in potato (Sorri et al 1999). Sorri et

al. (1999) developed cleaved amplified polymorphic sequence (CAPS) marker ADG2/Bnv1 that

co-segregates with the Ry_{adg} gene. The PCR-based CAPS marker ADG2/Bbv1, is the first

example of a diagnostic marker for the selection of PVY resistant genotypes in potato, regardless

of genetic background or ploidy level (Sorri et al.1999). The CAPS marker needs little DNA for

analysis and is relatively inexpensive, making it useful for breeding programs for the detection of

the Ry_{adg} gene (Hamalainen et al. 1998). Kasai et al. (2000) followed, by developing sequence

characterized amplified region (SCAR) marker RYSC3 based on nucleotide differences between

the ADG2 fragments from resistant and susceptible lines. The SCAR marker RYSC3 generates a

321 bp fragment in resistant genotypes carrying the Ry_{adg} gene (Kasai et al. 2000). Among the

molecular markers developed for RY_{adg} thus far, the SCAR marker is the most valuable (Kasai et

al. 2000). It offers several advantages including that little DNA is required and scoring the

results is very simple (Kasai et al. 2000). Compared to the CAPS marker, the SCAR marker can

be observed with a single PCR procedure, without subsequent digestion with a restriction

enzyme (Kasai et al. 2000). Another advantage of the SCAR marker is that genomic information

may be obtained directly by analyzing the PCR products (Kasai et al. 2000). Therefore, the

SCAR marker offers the most efficient method for screening a large number of samples in a time

and labor saving manner (Kasai et al. 2000). Furthermore, an extensive study by Whitworth et al.

(2009) demonstrated that the RYSC3 marker could identify clones with resistance to all PVY

strains present in North America.

<center>**Gene Ry$_{sto}$**</center>

Extreme resistance to PVY in S. *stoloniferum* was first reported by Cockerham (1943). A second gene for extreme resistance to PVY, Ry$_{sto}$ gene, is derived from *S. stoloniferum* (Brigneti et al. 1997). Brigneti et al. (1997) mapped Ry$_{sto}$ to the same position as the Ry$_{adg}$ gene on potato chromosome XI using amplified fragment length polymorphism (AFLP) markers M17 and M6 at 0.6 cM distance on both sides of the gene. Later, Song et al. (2005) developed AFLP markers that determined that Ry$_{sto}$ derived from *S. stoloniferum* was mapped on chromosome XII. This was possible because Gebhardt and Valkonen (2001) discovered unreliable pedigree information for the population used by Brigneti et al. (1997). Rizza et al. (2006) discovered that the molecular marker, M45 could recognize both the Ry$_{adg}$ and the Ry$_{sto}$ gene. Valkonen et al. (2008) confirmed that another marker, STM003 was capable of identifying the Ry$_{sto}$ gene in potato. According to Heldak et al. (2007) study, tightly linked CAPS markers STM0003, GP122 $_{718,}$ and GP122$_{406}$ have also been developed for potato, and are thus far the most widely employed because of their reliable detection of the Ry$_{sto}$ gene. Song et al. (2005) developed two sequence-tagged site (STS) markers, YES3-3A and YES3-3B, which have been confirmed for MAS in European cultivars. In most European cultivars, extreme resistance to PVY originates from *S. stoloniferum,* owing to the introgression of the single dominant resistance gene Ry$_{sto}$ into *S. tuberosum* by Ross (1952).

<center>**Gene Ry-f($_{sto}$)**</center>

The Ry-f($_{sto}$) gene has been mapped by using RFLP derived CAPS markers on chromosome XII (Flis et al. 2005). Due to incorrect pedigree information, there has been uncertainty of the previously mapped gene Ry$_{sto}$ (Brigneti et al. 1997), since both genes were

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supposed to have originated from the wild potato species *S. stoloniferum.* An inter-simple

sequence repeat (ISSR) marker UBC 857, three CAPS markers $GP122_{718}$ (EcoRV), $GP204_{800}$

(TaqI), and $GP269_{650}$ (DdeI), and one STS marker $GP81_{400}$ were developed for MAS of the Ry-

f($_{sto}$) gene (Flis et al. 2005). Valkonen et al. (2008) developed the markers $GP122_{718}$ (EcoRV)

and $GP122_{564}$ (EcoRV) for the detection of Ry-f($_{sto}$) in potato. Flis et al. (2005) used the tightly

linked CAPS marker $GP122_{718,}$ and indicated that it was successful for the detection of Ry-f($_{sto}$)

in German and Polish varieties, which possess extreme resistance from *S. stoloniferum*. This

indicated that that the source of Ry-f($_{sto}$) has been widely utilized in various potato breeding

programs and can be monitored be a diagnostic marker via MAS (Flis et al. 2005).

Gene Ry$_{chc}$

The monogenic Ry$_{chc}$ gene originating from *S. chacoense* verifies extreme resistance to

PVY. Using RFLP and random amplified polymorphic DNA (RAPD) marker 38-530 (OPC-01),

Ry$_{cho}$ was mapped to the distal end of chromosome IX by Sato et al. (2006). Since the location of

the Ry$_{chc}$ gene is in a different location from Ry$_{adg}$ and Ry$_{sto,}$ it may possibly belong to another

resistance gene cluster. The RAPD 38-530 marker linked to Ry$_{chc}$ is a useful tool for MAS for the

Ry$_{cho}$ gene in potato (Sato et al. 2006). One of the leading Japanese cultivars, Konafubuki,

showed extreme resistance to PVY (Hosaka et al. 2001). The resistance originally descended

from a wild diploid species S. *chacoense*. Hosaka et al. (2001) successfully used the highly

reproducible RAPD 38-530 marker to screen progeny populations of Konafubuki in his breeding

program. PVY resistance from *S. chacoense* has not been widely utilized in potato breeding,

although some cultivars with the Ry$_{cho}$ gene have been developed (Hosaka et al. 2001).

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Segregation of R genes

A detailed analysis of the segregation of R genes can provide information about the genetic configuration of progeny with regard to the number of copies of the resistance gene and/or the marker (Sagredo et al. 2009). *S. tuberosum* (2n = 4x =48) is an autotetraploid, thus the Ry dominant gene (or marker) may be simplex (1:1, Ryryryry), duplex (5:1, RyRyryry), triplex (∞:0, RyRyRyry), or quadriplex (∞:0, RyRyRyRy) (Whitworth et al. 2009). The segregation ratio is influenced by gametic assortment during the first meiotic division (Allard 1960). The segregation for PVY resistance in progeny derived from the crossing of PVY resistant and susceptible parents depends on the allelic configuration of the resistant parent (Allard 1960). One of the breeding strategies aiming for PVY resistance is to increase the frequency of the resistance genes, allowing for the establishment of duplex parents. This breeding method can create populations that have progeny that are over 75% PVY resistant, making the breeding procedure easier to introgress resistance into advanced selections. Selecting triplex or even quadriplex clones to produce completely resistant progeny is possible. This would enable breeders too only have to make selections based on agronomic traits and end user demands (Ribeiro et al. 2006).

SUMMARY

PVY is a major economic disease agent in most solanaceous crops worldwide. In potato, PVY is responsible for drastic reductions in potato quality and yield, with losses sometimes reaching 80% (De Bokx and Huttinga 1981). It is one of the most important viruses in potato due to the non-persistent mode of transmission via aphid vectors. Genetic resistance is the recommended control method, since insecticides used to control the spread of PVY by aphids is not completely effective (Gray et al. 2010). PVY resistant cultivars would be an ideal disease control method, unfortunately there are few cultivars widely grown in the United States that display any type of resistance (Felcher and Douches 2012). Potato breeding programs that focus on developing PVY resistant cultivars are key to successfully bringing disease incidence under control (Gray et al. 2010). The traditional method used to screen for PVY resistance is to use artificial virus inoculations under controlled conditions and visually determine if clones are PVY resistant (Ottoman et al. 2009). This method is not reliable, because it allows for the escape of susceptible clones, is very tedious and time consuming, and limits screening of large segregating populations (Ottoman et al. 2009). Several R genes for PVY resistance have been identified in wild species and introgressed into the cultivated potato (Tiwari et al. 2012). These include: Ry_{adg} from *S. tuberosum ssp. andigena* (Kasai et al. 2000), Ry_{sto} from *S. stoloniferum* (Brigneti et al., 1997), Ry-f$_{sto}$ from *S. stoloniferum* (Flis et al., 2005), and Ry_{chc} from *S. chacoense* (Tiwari et al. 2012). User-friendly molecular markers have been developed for use in screening segregating populations (Ortega and Lopez-Vizcon 2012). MAS is a tool that can be easily implemented to make the process of identification of resistant clones quicker, simpler, and it eliminates the use inoculation testing, visual assessment, and ELISA testing (Felcher and Douches 2012). The Ry_{adg} gene has been shown to provide extreme resistance to PVY, defined as resistance against all

strains, and it co-segregates with PCR-based, SCAR marker RYSC3 (Kasai et al. 2000). This

marker is suitable for MAS for several reasons: it co-segregates with the trait (PVY resistance), it

requires the use of very little DNA, it is easily scored (presence/absence of a single band), it

takes only a few hours to use, many samples can be analyzed per day, and the cost per sample is

relatively low (Kasai et al. 2000). A more detailed analysis of the segregation of the Ry_{adg} gene

can give more information about the genetic configuration of the progeny with regard to the

number of copies of the resistance gene. Breeding programs aim to increase PVY resistance by

increasing the frequency of the resistant allele, allowing for duplex (RyRyryry) or greater

dosage.

This study aimed to determine if MAS increases the efficiency of developing PVY

resistant cultivars. The objectives of this research were to (i) evaluate NDSU seedling family

populations using the SCAR RYSC3 marker to determine if they are carrying the Ry_{adg} gene, and

(ii) determine the mode of segregation of the Ry_{adg} gene to give more information about the

genetic configuration of the resistant parent(s). The screening was performed in the Potato

Research Laboratory in the Plant Sciences Department at NDSU. This research has the potential

to hasten the development of PVY resistant cultivars using breeding lines developed by the

NDSU potato improvement program and other breeding programs. This research is especially

relevant as new aphid vectors, along with new strains of PVY, have been discovered in the

United States, making cultivars with PVY resistance very desirable to potato growers. PVY is a

significant global disease issue, thus this research will not only benefit the North Dakota and

Minnesota potato industry, but may also positively impact all areas where potatoes are grown.

Developing improved potato cultivars that contain extreme PVY resistance will reduce the need

for alternate disease control methods, such as insecticides which have had limited success, thus

saving the growers money and time, while greatly benefitting the environment.

MATERIALS AND METHODS

Plant materials

Plant materials used in this study included PVY susceptible tetraploid (2n =4x = 48)

potato breeding parental genotypes and cultivars from the North Dakota State University potato

breeding program, and potato breeding lines and cultivars with diverse genetic backgrounds and

the potential to possess PVY resistance. Three Uruguayan potato breeding lines, 95043.11,

90245.1 and 793101.3, originating from *S. tuberosum subsp. andigena*, carry the Ry_{adg} gene, and

were the parents used to introgess PVY resistance in this study (Rizza et al. 2006). A total of 35

cross combinations were made in the greenhouse between PVY susceptible, resistant, and

unknown resistant lines, to create 35 seedling family populations with a total of 740 genotypes

segregating for Ry_{adg} (Table 1). The German cultivar, Romanze (Hansa x Seedling x Desiree),

has varying resistance to potato diseases such as PVA, late blight, powdery scab, and PVY (PVY

strains unknown) (European Cultivated Potato Database 2014). Potato breeding parental

genotypes M2, M3 and M7, originating from a cross with *S. infundibuliforme*, are resistant to

common scab and soft rot; however, information on PVY resistance was unknown (Jansky et al.

2012). The parental breeding line AH66-4 was included in family selection due to its wild

genetic background, though PVY resistance was unknown. The J parental potato breeding lines

were created by Helgeson et al. (1998), by the means of PEG-mediated fusion of leaf cells of *S.

bulbocastanum* (a wild Mexican, diploid species) and *S. tuberosum*; however, information on

PVY resistance was unkown. Parental breeding genotype P2-3 has resistance to PLRV, PVY,

and green peach aphid; P2-3 was identified in the backcross progeny of the non-tuber-bearing

species *S. etuberosum* somatic hybrids (Novy and Helgeson 1994), thus was also used as a

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resistant parent in this study. In a collaborative breeding effort, the true potato seed from bulk pollen was sent from Dr. Kathy Haynes, USDA-ARS potato breeder, and used as a possible PVY resistant parent. The Uruguayan, tetraploid potato breeding line 95043.11, and the tetraploid potato cultivar Russet Burbank were used as controls for extreme resistance and susceptible reaction to PVY infection, respectively (Rizza et al. 2006).

Preparation for template DNA

Total genomic DNA was extracted according to the cetyltrimethylammonium bromide (CTAB) method (Rogers and Bendich 1994). F1 plants were grown from true potato seed under greenhouse conditions. Twenty-five plants per family were selected. As few as four were used when 25 were not available. One young leaf (30 mg) was sampled from each plant being tested. The sample was then transferred into a 1.5 mL Eppendorf tube (Eppendorf Corporation, Hamburg, Germany), dried by a VirTis lyophilizer (SP Scientific, Warminster, PA), and ground into a fine powder using a MM 301 ball mill (Retsch Incorporated, Haan, Germany). CTAB buffer (650 ul 2xCTAB) was added to each tube containing the sample; tubes were incubated in a water bath at $65^{\circ}C$ for 40 minutes. Samples were disrupted violently by shaking every 10 minutes. After incubation, 650 µL of Chloroform, along with 3µl of RNAse (10mg/mL) (Qiagen Corporation, Hilden, Germany) was added, and tubes inverted for 5 minutes. Tubes were centrifuged (Eppendorf centrifuge 5424, Eppendorf Corporation, Hamburg, Germany) (10 min, 14000 rpm), at room temperature; subsequent cell debris, proteins and polysaccharides were collected on the bottom of the chloroform mixture phase. The supernatant (600 µl) containing the DNA was transferred into a new 1.5 mL Eppendorf tube; 300 µL (0.5 volume) of cold iso-

Table 1. Pedigrees of seedling family populations tested for their presence of the SCAR RYSC3 marker associated with the Ry$_{adg}$ gene confering extreme resistance to PVY.

Family Seedling Population	Female	Male
ND113561	90245.1	Dakota Pearl
ND113562B	90245.1	Dakota Trailblazer
ND113563B	90245.1	LBR8
ND113564	90245.1	ND860-2
ND113565C	90245.1	ND7799c-1
ND113566C	90245.1	ND060712C-7
ND113567	95043.11	Dakota Jewel
ND113568	95043.11	AND00272-1R
ND113569	95043.11	ND4659-5R
ND113570	95043.11	ND8555-8R
ND113571CB	95043.11	ND050067cb-1R
ND1265	ATND98459-1RY	95043.11
ND1282	M1	ND7192-1
ND1283C	M2	ND7799c-1
ND1284	M3	Dakota Pearl
ND1285C	M7	ND059846C-4Russ
ND1231B	Romanze	ATND98459-1RY
ND1232B	Romanze	ND8555-8R
ND1233B	Romanze	793101.3
ND1238	AH66-4	AND97279-5Russ
ND1251	AND97279-5Russ	AH66-4
ND1252	AND97279-5Russ	M7
ND122C	Dakota Diamond	M2
ND125B	Dakota Pearl	J138-A12
ND127B	Dakota Trailblazer	M7
ND129AB	Dakota Trailblazer	ND039194AB-1Russ
ND1222C	Ivory Crisp	NDJL21C-1
ND1226	Ivory Crisp	90245.1
ND1228CB	Ranger Russet	ND039194AB-1Russ
ND1230B	Romanze	AND00272-1R
ND113421CB	ND050060Cb-4R	95043.11
ND113434C	ND050132C-6R	95043.11
ND113442C	ND050167c-3R	95043.11
D3258	BD653-4	Bulk Pollen
D3375	BD653-1	Bulk Pollen

propanol was added to the new tube containing the supernatant. The tube was shaken violently for 1 min forming a DNA pellet, centrifuged (2 min, 1000 rpm, at room temperature), and the supernatant decanted. The DNA pellet was washed twice with 500 ul of 70% cold ethanol and allowed to air-dry overnight. The DNA samples were dissolved in 100 µL of sterile water, adjusted to 30 ng/µl and stored in -20˚C until evaluation.

PCR protocol

Polymerase chain reaction (PCR) amplification of the SCAR marker linked to Ry_{adg} was conducted using a Techne-4000 thermocycler (Bibby Scientific, Stafforshire, UK) with primers developed by Kasai et al. (2000) (Table 2), with a final reaction volume of 25 µl. Each reaction contained 12.5 ul 2X GoTaq Green Master Mix (Promega Corporation., Madison, WI), 0.6 ul of each RYSC3 primer (10 uM) (Sigma-Aldrich Corp, The Woodlands, TX), 2.0 ul of each SUS3 primer (10 uM) (Sigma-Aldrich Corporation, The Woodlands, TX), 5.3 ul of sterile water, and 2.0 ul of DNA template. The PCR protocol consisted of an initial denaturation step at 93°C for 9 min., followed by 35 cycles of denaturation at 94°C for 45 s, primer annealing at 55°C for 45 s, and extension at 72°C for 60 s, followed by a final extension at 72°C for 5 min. PCR products were separated by electrophoresis through a 2% agarose gel, stained with ethidium bromide, in 0.5X buffer TBE (TRIS, boric acid, and EDTA), at a voltage of 90 V, for 50 min, then visualized with UV light. Presence of a 321 base pair (bp) band was associated with PVY resistance from Ry_{adg}, and absence indicated association with susceptibility to PVY as in Kasai et al. (2000). The SUS3 marker was used as an internal control to verify a proper PCR reaction. The forward and reverse primer sequences for RYSC3 and SUS3 DNA-based markers, annealing temperatures, and PCR product sizes are listed in Table 2.

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Table 2. Forward and reverse primer sequences for RYSC3 and SUS3 DNA-based markers, annealing temperatures, and PCR product sizes.

Marker[a]	Primer	Forward and reverse primer sequences (5'-3')	Ta[b]	Product Size (bp)[c]	References
RYSC3	3.3.3s	ATACACTCATCTAAATTTGATGG	60°C	(R) 321	Kasai et al.
	ADG23R	AGGATATACGGCATTTTTCCGA		(S) absent	(2000)
SUS3	SUS3-F	CTGCAAGCTAAGCCTGATCTTATTATC	55°C	(P) 600	
	SUS3-R	TTCGGAGTATGGAAAATAGAGATTCACATTCAC			

[a] Names used in this study
[b] Annealing temperature
[c] R band associated with PVY resistance, S band associated with PVY susceptibility, P band associate with control

Analysis

The segregation analysis of the progeny carrying the Ry_{adg} gene was carried out in 16 families with Uruguayan line 90245.1 or 95043.11 as a resistant parent. No other families that produced progeny carrying the Ry_{adg} gene/RYSC3 marker pattern in this study. In an autotetraploid species like potato ($2n = 4x = 48$), if random mating of each one of the four homologous chromosomes is assumed in meiosis, without considering double reduction, the expected ratios in the gametes of a dominant gene or maker are 1:1, in the case of simplex (one copy), respectively (Hackett et al. 1998). The Chi-square goodness of fit test was employed as the statistical indicator of the distinct segregation patterns studied.

RESULTS AND DISCUSSION

A total of 740 plants, representing 35 seedling family populations developed by the North Dakota State University potato improvement team, were evaluated at the genotypic level with the SCAR RYSC3 marker associated with the Ry_{adg} gene. Of the 35 segregating seedling family populations, 16 were found to have progeny segregating for the Ry_{adg} gene (Table 3). No PCR amplification associated with PVY resistance was noted in the remaining 19 families. The 16 families that contained progeny that were positive for the Ry_{adg} gene had Uruguayan parental breeding line 90245.1 or 95043.11 as a parent. These two lines were screened and deemed positive for the Ry_{adg} gene prior to this study (Rizza et al. 2006). Progeny obtained from the family ND1233B (Romanz X 79101.3) were confirmed susceptible which was surprising because Uruguayan line 793101.3 was reported as positive for the RYSC3 marker in Rizza et al. (2006).

The 16 seedling family populations with resistant progeny to PVY were chosen to be evaluated for the segregation of the Ry_{adg} gene. The observed family seedling populations segregating for the Ry_{adg} gene fit the expected ratio for a single dominant gene. No significant deviation from the 1:1 (simplex) was observed (Table 3). This provides convincing evidence that the PVY resistant parents, have simplex allelic configuration for the Ry_{adg} gene.

Knowledge of parental background is important for planning crosses. Molecular markers can be an indispensable tool for characterizing PVY resistance resources and providing breeders with more detailed information to assist them in selecting parents (Ottoman et al. 2009). The parents used in this study were not screened for the PVY resistance gene Ry_{adg} prior to crosses being made, with the exception of parental breeding lines 90245.1, 95043.11, and 793101.3, which were screened by the SCAR RYSC3 marker as reported in Rizza et al. (2006). Breeding

Table 3. Segregation analysis of the RYSC3 marker associated with the RYadg gene for resistance to PVY in seedling family populations with 90245.1 or 95043.11 as a parent.

Family	Source of resistance	Number of plants	RYSC3(+)	RYSC3(-)	Simplex 1:1
ND113561	90245.1	25	14	11	0.36 ns
ND113562B	90245.1	25	13	12	0.04 ns
ND113563B	90245.1	4	2	2	0.00 ns
ND113564	90245.1	25	14	11	0.36 ns
ND113565C	90245.1	25	10	15	1.00 ns
ND1226	90245.1	25	14	11	0.36 ns
ND113566C	90245.1	25	14	11	0.36 ns
ND113567	95043.11	25	13	12	0.04 ns
ND113568	95043.11	25	11	14	0.36 ns
ND113569	95043.11	25	12	13	0.04 ns
ND113570	95043.11	25	16	9	1.96 ns
ND113571CB	95043.11	25	17	8	3.24 ns
ND1265	95043.11	25	14	11	0.36 ns
ND113421CB	95043.11	18	10	8	0.22 ns
ND113434C	95043.11	18	7	11	0.88 ns
ND113442C	95043.11	18	5	13	3.55 ns

The critical value of $\chi 2$ test is 3.84 for *P0.05; ns: non significant

line and cultivar information was collected from, personal communications, research journals

and The European Cultivated Potato Database (Rizza et al. 2006; Jansky et al. 2012; Helgeson et

al. 1998; Novy and Helgeson 1994, and European Cultivated Potato Database 2014). Many of

the parents were previously subjected to field observations for disease susceptibility, but their

resistance to PVY was unknown, with the exception of 90245.1, 95043.11, and 79101.3 (Rizza et

al. 2006). If all the parents used in this study would have been screened with the SCAR RYSC3

marker prior to crosses being made, 382 samples would not have had to have been analyzed

because they did not contain the Ry_{adg} gene. Time, money, and energy was wasted, but this proved to be a perfect example of why parental screening is valuable.

The traditional method of screening progeny by growing plants, artificially inoculating them with virus, followed by ELISA testing, can be used as an accurate selection method, but requires more time and labor, and is more prone to some level of experimental error (Ottoman et al 2009). The use of molecular markers is a practical method for screening numerous samples in a timely and labor saving manner. A molecular PCR based test only requires a small piece of plant tissue (tuber, sprout, or leaf); on the other hand, traditional screening methods require a whole plant (Collard and Mackill 2008). In regard to the time needed to carry out a diagnosis considering, lyophilizing tissue, extracting DNA, PCR, and electrophoresis, molecular testing took a week or less to analyze 200 samples in this study. A high throughput system could shorten that time.

The CTAB DNA extraction method allowed for several samples (100 samples) to be processed daily, with very little starting material required, and it produced very good, consistent PCR results. No loss of samples due to low DNA yield or quality was experienced. The SCAR RYSC3 allele-specific marker chosen for this study, allowed a simple and fast approach to determine if the Ry_{adg} gene was present in the evaluated seedling family populations. Little DNA was required for the PCR reaction, results were obtained after a single PCR procedure, and scoring the results was very straightforward. The internal control marker SUS3, presented a band in all lanes at 600 bp, indicating that the PCR reaction worked properly. When using the RYSC3 marker, the SUS3 control marker is essential to verify if a sample with an absent band at 321 bp is truly susceptible, or absent due to poor DNA (Felcher and Douches 2012). If a band does not appear in the 600 bp lane of the SUS3 control marker, then the sample DNA is considered to be

poor or the PCR reaction did not work properly. In order for the RYSC3 diagnostic and SUS3

control markers to both work properly at the same time, the annealing temperature was lowered

to 55°C from 60°C, and the volume of RYSC3 primer was decreased to 0.6 ul from 2.0 ul,

contrary to Kasai's et al. (2000) protocol. RYSC3 marker evaluations of the positive and

negative controls indicated the presence of bands at 321 bp (associated with the Ry_{adg} gene) for

the positive control 95043.11, and a lack of bands at 321 bp (associated with PVY susceptibility)

for the negative control, Russet Burbank. The PCR amplifications shown in Figure 1. were the

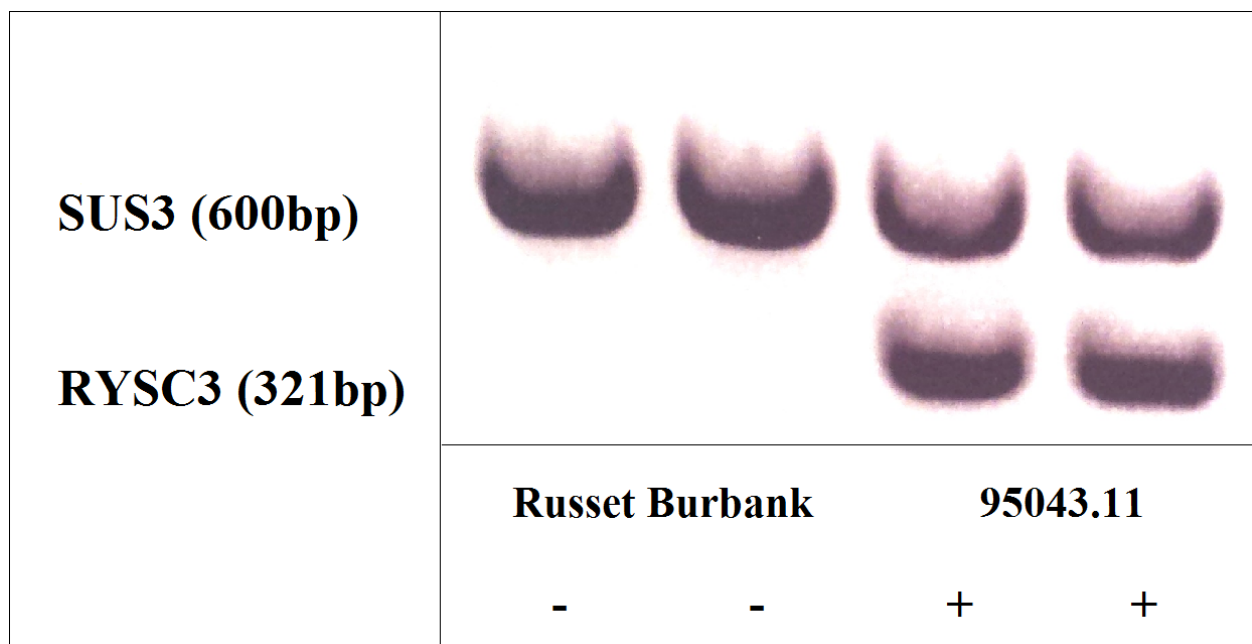expected patterns as seen in Kasai et al. (2000) study.

Figure 1. Gel showing presence or absence of the RYSC3 marker. Lines with a 321 bp band
(such as 95043.11, a positive control known to have the Ry_{adg} gene) are RYSC3 positive and
lines lacking the band (such as Russet Burbank, a negative control known to lack the Ry_{adg} gene)
are RYSC3 negative. The 600 bp band that is present in all lanes is an internal control used to
verify that the PCR reaction worked properly.
- no amplification (susceptible)
+amplification (resistant)

Among the molecular markers developed thus far for the Ry_{adg} gene, the SCAR RYSC3 marker seems to be a very valuable genetic tool to use. The SCAR RYSC3 marker only takes a few hours to use, and the scoring of results was very straightforward (presence or absence of a single band), thus many samples can be analyzed per day. In comparison with the CAPS maker, the SCAR marker results can be revealed by only one PCR procedure, without subsequent digestion with a restriction enzyme, thus saving time and labor. The CAPS marker ADG2 was previously examined when determining which marker to employ for this study (Sorri et al. 1999). It proved troublesome due to uncertainty of PCR conditions unique to the NDSU potato program's laboratory, and multiple and tedious steps in the protocol causing increased opportunity for error, thus was not used.

The evaluation of family seedling populations with SCAR RYSC3 marker allowed for the identification of Ry alleles, so inferences can be made about the genetic constitution of the parental clone. The use of PVY resistant parental lines with multiple copies (duplex, triplex, or quadruplex) of the PVY resistance gene (Ry) is desirable in order to maximize the chances of obtaining PVY resistant progeny. This is interesting because it becomes possible to design superior parents with multiple copies, thus obtaining progeny that are all PVY resistant. Currently, the PCR assays used are not capable of detecting how many Ry alleles are present, so we must depend on progeny testing to determine the number of resistant alleles present in a particular parent. Even so, progeny testing with molecular markers verses screening with artificial inoculations is still quicker, simpler, and more reliable.

Furthermore, pyramiding of several genes for resistance to PVY in parental lines would greatly strengthen the development of PVY resistant progeny (Collard and Mackill 2008). The ability of the PVY virus to overcome two or more effective Ry genes by mutation is considered

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much lower, compared with the resistance controlled by a single Ry gene (Collard and Mackill 2008). Pyramiding may involve the combination of genes from more than two parents (for example Ry$_{adg}$ and Ry$_{sto}$). Pyramiding may be possible through conventional breeding, but it is usually not easy to identify the plants containing more than one gene with conventional screening. MAS can be a reliable way to track both major genes conferring resistance to PVY (Ottoman et al. 2009). Multiple markers for multiple genes may be tested using a single DNA sample without phenotyping (Ottoman et al. 2009).

DNA markers have enormous potential to improve the efficiency and precision of conventional plant breeding (Collard and Mackill 2008). Developing a new variety can take 12 years or more from crossing to release, thus improved early generation breeding strategies such as MAS are needed (Felcher and Douches 2012). Comparing costs of molecular markers and artificial inoculations, markers provide an easier, quicker, cheaper, and more reliable system of screening for the resistance (Collard and Mackill 2008). Thus, it is likely that MAS will gain more influence in potato breeding programs in the future, and will speed up breeding processes considerably (Collard and Mackill 2008). But, why is there such a low impact of MAS in potato breeding today? There are many reasons to support why MAS is not being utilized in plant breeding including: the use of MAS is a relatively new genetic technology (Ortega and Lopez-Vizcon 2012). The first DNA markers were developed in the 1980s, but the user-friendly PCR based markers were not developed until the mid to late 1990s (Collard and Mackill 2008). In the past decade, there has been an influx of published papers describing MAS studies along with the papers on development of new molecular markers (Collard and Mackill 2008). A lot of the time MAS is considered to be a basic "research process" used to develop a variety, thus the explicit details regarding the use of DNA markers is not published (Collard and Mackill 2008). Also,

private seed companies who use MAS do not disclose details of their methodology due to competition with other seed companies (Ortega and Lopez-Vizcon 2012). Another handicap of MAS is that markers can only be applied to certain genetic backgrounds, and therefore, there is still a large amount of resistance that cannot be identified with this procedure (Ortega and Lopez-Vizcon 2012). For example, if there are 40 PVY resistant parents in a university's gene bank, and when screened by the RYSC3 marker, only six of them have the Ry_{adg} gene, 34 genotypes have undiscovered resistance. Additionally, some wild potato lines that can be selected by MAS produce progeny that could be resistant, but have very low agronomic value and high physiological issues, making their success of being selected slim (Ortega and Lopez-Vizcon 2012). Lastly, plant breeders have been making their selections visually without the use of any molecular data for thousands of years. The concepts, specialized equipment, sophisticated techniques, and methods used by molecular geneticists for genotyping may not be understood by plant breeders. Similarly, concepts in plant breeding may not be well understood by molecular geneticists. The misunderstanding between the two sciences ultimately affects the development of new breeding lines (Collard and Mackill 2008).

The next generation of marker technology is approaching (Ortega and Lopez-Vizcon 2012). The efficiency of genotyping is expected to improve, and MAS for polygenic traits that are quick and affordable could be a promising reality (Collard and Mackill 2008). Research funding will be needed to support the development of the new MAS technology, including equipment and training personnel (Ortega and Lopez-Vizcon 2012). This cost could cause more distance between the scientific and breeding communities, private companies and university breeding programs, and developed and developing countries (Ortega and Lopez-Vizcon 2012). When a breeding program is considering the cost of implementing MAS technology and the

benefits, they need to consider what the long term impact of the technology is going to do for their program (Ortega and Lopez-Vizcon 2012). University breeding programs, government agencies, and private companies will need to work and learn together to take advantage of these new technologies to insure a substantial impact on crop improvement, in order to be able to produce enough food for the world's growing population.

CONCLUSION

The SCAR RYSC3 marker successfully indicated the presence of Ry_{adg} gene in the evaluated seedling family populations. Information generated in this study will help the NDSU potato improvement program select parents when breeding for PVY resistance. In the future, parental lines with unknown PVY resistance will be screened with a DNA marker prior to use in a crossing block. In addition, screening for resistance at the seedling level will be performed to allow for rapid identification and development of PVY resistant cultivars. These breeding strategies are the most efficient way to maximize the creation of PVY resistant progeny, resulting in resistant cultivar releases.

Based on this study none of the potato varieties released to date by the NDSU potato improvement program appear to contain alleles associated with PVY resistance at the Ry_{adg} loci. Adding PVY resistance genes from *S. tuberosum* ssp. *andigena* (Ry_{adg}) sources should greatly benefit the NDSU potato improvement program. The application of MAS will allow for the testing of more populations with larger numbers of individuals per population, thus the chances of generating PVY resistant progeny with potential for variety release will be greatly increased. For ongoing research following genetic assessment, resistant genotypes will be used in creating a crossing block for hybridizing, and resistant seedling genotypes will enter a 'fast-track' system to expedite the development of PVY resistant cultivars. NDSU selections identified as resistant to PVY will undergo further phenotype screenings/evaluation in the field in this development process. Although MAS is not used widely in potato breeding programs today, the RYSC3 marker is a practical example of how MAS can be beneficial for the development of new varieties. MAS will almost certainly become one of the most important tools of plant breeders in the future.

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