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Marker-Assisted Selection for the Broad-Spectrum Potato Late Blight Resistance Conferred by Gene *RB* Derived from a Wild Potato Species

Lara M. Colton, Horia I. Groza, Susan M. Wielgus, and Jiming Jiang*

ABSTRACT

Potato (*Solanum tuberosum* L.) late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is one of the most damaging diseases in any crop. Deployment of resistant varieties is the most effective way to control this disease. However, breeding for late blight resistance has been a challenge because the race-specific resistance genes introgressed from wild potato *S. demissum* Lindl. have been short lived and breeding for "horizontal" or durable resistance has achieved only moderate successes. We previously demonstrated that the high-level late blight resistance in a wild potato relative, *S. bulbocastanum* Dunal subsp. *bulbocastanum*, is mainly controlled by a single resistance gene *RB*. Transgenic potato lines containing the *RB* gene have showed strong late blight resistance, comparable to the backcrossed progenies derived from the somatic hybrids between potato and *S. bulbocastanum*. Here we report the development of a polymerase chain reaction-based DNA marker for tracking the *RB* gene in breeding populations derived from the potato × *S. bulbocastanum* somatic hybrids. Several marker-positive breeding lines showed the expected late blight resistance in greenhouse evaluations. Our results demonstrate that marker-based selection will allow us to effectively transfer the *RB* gene into potato using traditional breeding methods, an alternative to deploying the *RB* gene through genetic transformation.

Late blight caused by *P. infestans* is the most serious threat to potato production worldwide (Duncan, 1999; Garelik, 2002). Late blight starts as dark, water-soaked lesions present on the leaf surface that rapidly spread over the foliage. The devastation caused by *P. infestans* was first noticed during the middle 19th century Irish potato famine, which resulted in one million deaths. Late blight is still the most serious problem of cultivated potatoes and may cause complete tuber loss in susceptible germplasm (Ojiambo et al., 2000). Current potato production practices use expensive fungicide applications and intensive cultural practices to control *P. infestans* outbreaks. However, due to the constant genetic shifts in *P. infestans* populations and the decrease in fungicide effectiveness, this pathogen can cause significant losses in potato (Fry and Goodwin, 1997; Garelik, 2002).

The most effective and environmentally friendly way to prevent widespread devastation by late blight is to incorporate natural resistance into potato cultivars. Since the middle 19th century, there has been extensive selection and breeding for late blight resistance. Many current potato cultivars contain resistance derived from *S. demissum* ($2n = 6x = 72$), *S. andigena* Hawkes ($2n = 4x = 48$), and other wild species. Most of the resistance obtained from these wild species, mainly from *S. demissum,* belong to the "vertical resistance" type. In vertical resistance, plants containing a specific resistance gene (R gene) or virulence gene will interact with the corresponding avirulence gene found in the pathogen, which is known as the "gene-for-gene" concept (Flor, 1971; Hammond-Kosack and Jones, 1996; Keen, 2000; Leister, 2000). Potatoes containing these R genes are only effective in preventing the development of late blight if the invading *P. infestans* race contains the corresponding avirulence gene. The R gene–mediated resistance is often short lived and is rapidly overcome by new strains of the late blight pathogen. A total of 11 R genes, all from *S. demissum,* have been characterized in potato (Black et al., 1953; Malcolmson and Black, 1966).

In the 1970s potato breeding with emphasis on vertical resistance was replaced by breeding for "horizontal resistance" (Wastie, 1991). Horizontal resistance is thought to be polygenic or a quantitative trait that confers resistance to multiple races of a particular pathogen (Agrios, 1997). The mechanisms of horizontal resistance have not been well understood. Horizontal resistance is believed to be much more durable than vertical resistance due to the interaction of many genes which recognize different races of the same pathogen. Durable or horizontal resistance is described as being effective during prolonged and widespread use in an environment conducive to the disease (Johnson, 1984). However, horizontal resistance is difficult to breed for due to its polygenic nature and poorly understood mechanisms of action.

Many wild potato species coexist in the same habitat as the late blight pathogen and have developed mechanisms for survival along with the pathogen. *Solanum bulbocastanum* ($2n = 2x = 24$), a diploid species native to Mexico, has previously been characterized as possessing durable resistance against *P. infestans*, even under high disease pressure (Niederhauser and Millis, 1953; van Soest et al., 1984). *S. bulbocastanum* shows a general suppression, but not total elimination, of late blight symptoms. The resistance present in *S. bulbocastanum* is effective against all the known races of *P. infestans.* Surprisingly, the late blight resistance in the *S. bulbocastanum* clone PT29 was mapped to a single locus on chromosome 8 (Naess et al., 2000). This single gene, *RB*, has been previously cloned and transformed into Katahdin, a highly susceptible potato cultivar. Katahdin plants transformed with the *RB* gene showed broad-spectrum resistance against a number of *P. infestans*

Department of Horticulture, University of Wisconsin-Madison, Madison, WI 53706, USA. Received 3 Feb. 2005. *Corresponding author (jjiang1@wisc.edu).

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677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: GM, genetically modified; kb, kilobase; LRR, leucine-rich repeat; PCR, polymerase chain reaction; RGA, resistance gene analog.

strains in both greenhouse and field tests (Lozoya-Saldana et al., 2005; Song et al., 2003). These results show that it should be possible to develop resistant varieties by introgression of the *RB* gene from *S. bulbocastanum* into cultivated potato, presumably by either transgenic or traditional breeding methods.

Somatic hybrids between potato and *S. bulbocastanum* clone PT29 were developed. Several backcrossed progenies (BC1 and BC2) derived from these hybrids have been extensively tested in the field, demonstrating remarkably high resistance to late blight (Helgeson et al., 1998). These backcrossed progenies have been widely used as parents in many potato breeding programs in the United States. Four BC1 and three BC2 lines have been used as parents in the Wisconsin Potato Breeding program to generate a large number of new breeding lines. However, it has been difficult to effectively track the *RB* gene in breeding populations that have not been exposed to late blight inoculation. To alleviate this problem, we have developed a polymerase chain reaction (PCR)-based molecular marker for tracking the gene *RB* in breeding populations. We demonstrate that this marker can be used effectively for marker-assisted selection of the *RB*-mediated late blight resistance.

MATERIALS AND METHODS

Plant Materials

Plants used for PCR marker development include several independent transgenic Katahdin lines in which resistance or susceptibility to *P. infestans* had previously been determined (Song et al., 2003). The late blight resistance of several backcross progenies derived from the potato × *S. bulbocastanum* somatic hybrids have been characterized by extensive greenhouse and field tests (Helgeson et al., 1998). These backcross progenies, including resistant lines J101K6, J101A7, J101A32, J101A45, and J101K6A22 and susceptible lines J101A20 and J101A39, were used for marker development. The breeding lines used in marker-assisted selection were developed from crosses using potato varieties or advanced selections as the male parent and one of the resistant BC1 progenies (J101K6, J101K27, J103K7, J103K8) or BC2 progenies (J101K6A19, J101K6A21, J101K6A22) derived from the potato × *S. bulbocastanum* somatic hybrids as the female parent. Eight unselected populations (WTS families 1212, 1214, 1216, 1217, 1218, 1219, 1220, and 1221) were received from Dr. Joseph Pavek (USDA-ARS, Aberdeen Research and Extension Center, Idaho).

DNA Isolation and PCR

Potato tubers were stored at room temperature until sprouting was observed. DNA was isolated from the resulting shoots using the Qiagen Plant Dneasy Kit according to the manufacturer's instructions (Qiagen, Valencia, CA). PCR was performed at least three times on each sample using the PCR marker for gene *RB*. The marker consists of primer 1: 5′CACGAGTGCCCTTTTCTGAC and primer 1′: 5′ACAATTGAATTTTTAGACTT. The 25-μL PCR reactions were composed of approximately 50 ng DNA, 25 μmol of each dNTP, 1.5 nmol of each primer, 1 unit of Taq polymerase (Promega, Madison, WI), and buffer with 15 m*M* $MgCl_2$. The PCR was performed by the following protocol: 7 min at 95°C; 38 cycles of 20 s at 95°C, 20 s at 50°C, and 2 min at 72°C; followed by a final extension step of 7 min at 72°C. PCR products were separated on a 1.2% agarose gels in 1× Tris-borate EDTA and visualized and photographed with the Gel Doc 2000 (BioRad, Richmond, CA) under UV light after staining with ethidium bromide.

Late Blight Resistance Evaluation in Greenhouses

To confirm that the presence of the PCR marker correlates with the late blight resistance phenotype, we evaluated the late blight resistance of seven marker-positive and seven marker-negative breeding lines in Biotron greenhouses at the University of Wisconsin-Madison. The selected lines were grown in triplicate and placed in a mist chamber 8 h before inoculation. The mist chamber held a 24-hour relative humidity of 100%, an 8-hour light period, a daytime temperature between 17 and 19°C and a nighttime temperature at 13 to 15°C. The lines were challenged with sporangial suspensions of *P. infestans* isolate US930287 (US-8 genotype, A-2 mating type) obtained from Dr. William Fry, Cornell University, Ithaca, NY. Measurements of the foliage blight were interpreted and scored according to the Malcolmson scale (Cruickshank et al., 1982). The scale was based on percentage of foliage infected and scores were as follows: 9, no visible infection; 8, <10% infection; 7, 11 to 25%; 6, 26 to 40%; 5, 41 to 60%; 4, 61 to 70%; 3, 71 to 80%; 2, 81 to 90%; 1, >90%; 0, 100% infection. Blight scores were recorded 4, 7, and 10 d after inoculation. An average score for the resistance of each clone was determined using the three replicates. Those plants with a score of 7 or higher were considered to be resistant.

RESULTS

Development of a PCR-Based Marker for *RB*

Plant disease resistance genes are often organized in tandem arrays (Michelmore and Meyers, 1998). Gene *RB* is located in the middle of an array of four resistance gene analogs (RGAs), *RGA1*/*rga1*, *RGA2*/*rga2* (*RB*/*rb*), *RGA3*/*rga3*, and *RGA4*/*rga4* in *S. bulbocastanum* clone PT29 (Song et al., 2003). The four resistance gene analogs share 69 to 79% sequence similarity. The *RB* and *rb* sequences exhibited 99.8% nucleotide identity. Since the breeding progenies derived from the potato × *S. bulbocastanum* somatic hybrids may contain any or all of the four *RGA*/*rga* sequences, the PCR marker for the *RB* gene must be specific to *RB* and distinguishable from *rb* as well as other *RGA*/*rga* sequences.

Six primer sets were designed within the *RB* gene. Most primers are located within the leucine-rich repeat (LRR) domain of the second exon (Fig. 1A), which is the most variable region within the *RB* locus compared to *rb* and the other three RGAs. Each primer set was designed to span less than 1 kb of DNA. Plasmid clones containing *RB*, *rb*, and the three other RGAs were used for initial testing of the primer sets. We found that only one of the six primer sets (Primers 1 and 1′, Fig. 1A) amplifies *RB* specifically. The second primer set (primers 2 and 2′) amplifies both *RB* and *rb*. The remaining four primer sets amplify *RB* and *rb*, as well as three other RGAs.

Primer 1 reads in the sense orientation of the *RB* allele and is located in a region of significant divergence in relation to the other RGAs (Fig. 1B). Primer 1 spans the coding region for the 15th LRR that is missing

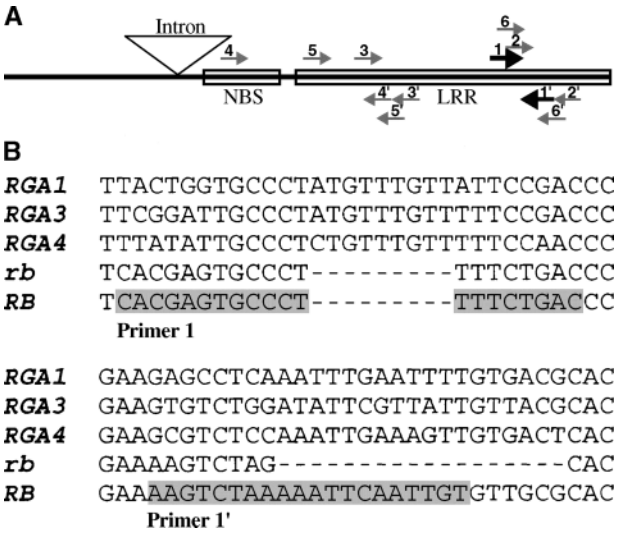

Fig. 1. Development of an *RB*-specific PCR marker. (A) Locations of six pairs of primers within the *RB* gene. Most of the primers are located within the leucine-rich repeat (LRR) domain in the second exon. (B) Alignment of the primer set no. 1 sequences in the corresponding regions of *RB*, *rb* and three RGAs. The exact primer sequences are highlighted in gray.

the final leucine compared to the consensus sequence. Primer 1′ reads in the antisense orientation of the *RB* allele and spans the coding region for the 18th complete LRR. Primer 1′ contains 12 bp that are not present within *rb* (Fig. 1B). This primer set no. 1 was used to amplify DNA from various lines that are known to either have or not have the *RB* gene. An expected 213-bp band was observed in all of the lines containing the *RB* gene (Fig. 2).

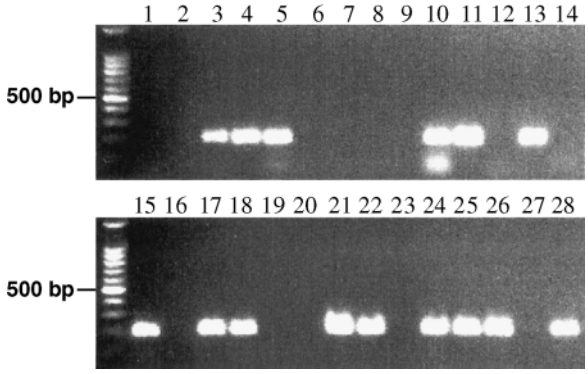

Fig. 2. Detection of the *RB* gene using the *RB*-specific PCR marker in plasmids or plants (transgenic Katahdin lines or backcrossed progenies derived from potato × *Solanum bulbocastanum* somatic hybrids) that are known to either have or not have the *RB* gene. Lane 1, potato cultivar Katahdin; Lane 6, negative control without template DNA; Lane 22, *S. bulbocastanum* clone PT29; Lanes 2, 7, 12, 25, 27, plasmids containing *RGA1*, *RGA4*, *rb*, *RB*, *RGA3*, respectively; Lane 3, 4, 11, 13, 17, 18, 21, 26, late blight–resistant transgenic Katahdin lines SP922, SP968, SP925, SP960, SP1006, SP1464, SP898, SP920, respectively; Lane 8, 9, 16, 19, 23, late blight–susceptible transgenic Katahdin lines SP937, SP941, SP991, SP947, SP953, respectively; Lane 5, 10, 15, 24, late blight–resistant BC1 clones J101A32, J101A45, J101A7, J101K6, respectively; Lane 14, 20, late blight–susceptible BC1 clones J101A39 and J101A20, respectively; Lane 28, late blight–resistant BC2 clone J101K6A22. A 213-bp band is amplified in all lanes that contain the *RB* gene.

Marker-Assisted Selection of *RB*

The potato breeding lines that potentially contain the *RB* gene were selected from progenies of crosses using a selected BC1/BC2 line derived from the potato × *S. bulbocastanum* somatic hybrids as the female parent and a potato cultivar or an elite breeding line as the male parent. The 4H lines (second field generation) and 8H lines (third field generation) (Table 1) had previously been field selected for two and three rounds, respectively, based on agronomic characteristics but not for late blight resistance. Field selections on agronomic performance include tuber appearance (tuber shape, eye depth, stolons), tuber number, size and uniformity, and reasonable early maturity. The WTS lines (Table 1) had previously been selected for their late blight resistance using detached-leaf assays. The late blight resistance of six WTS lines was also evaluated in an inoculated field test in Michigan (Douches et al., 2004). The field test showed that these six WTS lines are highly resistant to late blight.

A total of 110 breeding lines were tested for the presence of the *RB* PCR marker. The *RB* gene was detected in 45 (41%) of the lines. However, this number is inflated because the 52 WTS lines were selected for late blight resistance based on detached leaf assays performed earlier (Horia Groza, unpublished data, 2002–2003). Only 50% of the WTS lines contain the *RB* gene (Table 1), suggesting that the detached-leaf assays were error-prone. The *RB* gene was present among ~33% of 4H lines and ~32% of the 8H lines (Table 1), indicating that the extra round of field selection among the 8H lines did not affect the frequency of the *RB* gene. Both BC1 and BC2 lines derived from the potato × *S. bulbocastanum* hybrids were used as parents to generate the breeding lines. The *RB* gene is detected among 41% of the lines when BC2 lines were used as the female parent, slightly higher than the 25% when BC1 lines were used as the female parent (Table 1).

Confirmation of Late Blight Resistance in Marker-Positive Potato Lines

Seven marker-positive and seven marker-negative breeding lines as well as resistant and susceptible controls were chosen for late blight evaluation in the Biotron greenhouses. The relative humidity in the Biotron greenhouses can be maintained consistently at or above 90%

Table 1. Transmission of the *RB* gene in different breeding populations.

	Total progeny	Progeny with marker
WTS lines†	52	26 (50%)
4H lines	27	9 (33%)
8H lines	31	10 (32%)
BC1 clones as female‡	36	9 (25%)
BC2 clones as female‡	22	9 (41%)

† Five WTS lines showed strong late blight resistance in a field test (Douches et al., 2004). All these five lines were positive for the PCR marker. Line WTS1212–6 tested by Douches et al. (2004) was not included in the marker analysis.

‡ Four BC1 clones (J101K6, J101K27, J103K7, J103K8) and three BC2 clones (J101K6A19, J101K6A21, J101K6A22) were used as female parents in crosses.

Table 2. Greenhouse late blight evaluation of 14 randomly selected breeding lines.†

Lines	Average blight score 10 d after inoculation	Standard deviation	Presence of marker‡
WTS1218–3§	4.0	0	N
WTS1217–2§	7.7	0.57	Y
WTS1212–2¶	6.0	1.73	N
WTS1217–4¶	7.7	0.57	Y
WTS1271–1#	4.7	1.16	N
WTS1257–1§	7.0	1.53	Y
WTS1258–4¶	5.6	1.15	N
WTS1269–3¶	7.7	0.57	Y
4H5124–1#	8.3	1.16	Y
4H5123–2††	2.7	0.57	N
4H5133–6¶	4.0	2.0	N
4H5124–2¶	7.7	0.57	Y
8H4712–3#	8.0	0	Y
8H4710–2#	4.0	0	N
S. bulbocastanum (Clone PT29)§,¶,#,††	8.7	0.57	control
Russet Burbank§	1.8	1.7	control
Katahdin¶	1.2	0.41	control
Superior¶	1.5	0.84	control
Dark Red Norland#	1.0	0	control
Dark Red Norland#	0.7	0.58	control

† The evaluation was conducted in four separate experiments: 60 000, 73 000, 81 000, and 86 100 sporangia mL^{-1}.
‡ Y, PCR marker positive; N, PCR marker negative.
§ 73 000 sporangia mL^{-1}.
¶ 86 100 sporangia mL^{-1}.
# 81 000 sporangia mL^{-1}.
†† 60 000 sporangia mL^{-1}.

(Naess et al., 2000), which is critical to late blight resistance evaluation. This facility has been used to evaluate the late blight resistance of various germplasm developed from the potato × *S. bulbocastanum* somatic hybrids in our laboratory. The average score of late blight infection on the seven *RB* marker–positive plants was 7.7 (Table 2), representing ~14% foliage infection. These data indicate that the *RB* marker–positive plants are highly resistant to late blight (Fig. 3). The seven marker negative had an average blight score of 4.4 (Table 2), which corresponds to ~65% foliage infection. Thus, the PCR marker cosegregates with the resistant phenotype under greenhouse conditions (Fig. 3). Six WTS lines have also been tested in inoculated fields (Douches et al., 2004) and the resistance or susceptibility of these lines have also matched with the presence or absence of the PCR marker.

DISCUSSION

Potato breeding for late blight resistance began shortly after the beginning of the 19th century. Salaman and Wilson performed crosses between potato and *S. demissum* in the early 1900s, which were first used for resistance breeding in Scotland (Wastie, 1991). The first potato cultivar used in the U.S. with *S. demissum* genes was Empire, released in 1945 (Ross, 1986). Due to the breakdown of the R genes introduced from *S. demissum*, breeding efforts have been shifted to focus on developing cultivars with general or horizontal resistance. These efforts have yielded only moderately resistant or tolerant varieties. Such resistances are often not sufficient to protect the crop during heavy epidemics.

The short-lived R genes from *S. demissum* have prompted potato breeders and geneticists to look for resistance genes in other wild *Solanum* species (Colon and Budding, 1988; Douches et al., 2001; van Soest et al., 1984). High-level resistances have been found in several diploid Mexican species, including *S. bulbocastanum* and *S. pinnatisectum* Dunal (Chen et al., 2003; Helgeson et al., 1998; Kuhl et al., 2001). These species may have adapted to coexist with highly complex and dynamic *P. infestans* populations (Niederhauser and Millis, 1953; Niederhauser et al., 1954). Genetic mapping studies indicate that the resistance in both *S. bulbocastanum* and *S. pinnatisectum* may be conferred by a single gene or a few dominant genes (Kuhl et al., 2001; Naess et al., 2000). The *RB* gene cloned from *S. bulbocastanum* showed a broad-spectrum resistance against various known *P. infestans* strains. This result suggests that gene *RB* may have different molecular mechanisms for fighting off the late blight pathogen when compared to the R genes from *S. demissum*. Thus, the *RB* gene and possibly similar genes in the diploid Mexican *Solanum* species may provide more durable and effective late blight resistance for potato breeding.

Although crosses between potato and the Mexican diploid species are extremely difficult (Ramon and Hanneman, 2002), somatic hybrids between potato and these species can be readily developed by protoplast fusions (Helgeson et al., 1998; Menke et al., 1996; Oberwalder et al., 1998; Thieme et al., 1997; Ward et al., 1994). Such somatic hybrids provide excellent germplasm to introgress the late blight resistance from the Mexican diploid species into potato. The somatic hybrids between potato and *S. bulbocastanum* are partially fertile and numerous backcrossed progenies have been developed (Helgeson et al., 1998; Naess et al., 2001). The strong late blight resistances of several backcrossed progenies have been confirmed in numerous field tests, including in Toluca, Mexico (Helgeson et al., 1998). These backcrossed progenies have been used as parents by many potato breeding programs in the United States and Canada.

Molecular markers have been developed for selection of several important disease resistance traits in potato. Restriction fragment length polymorphism (RFLP) and

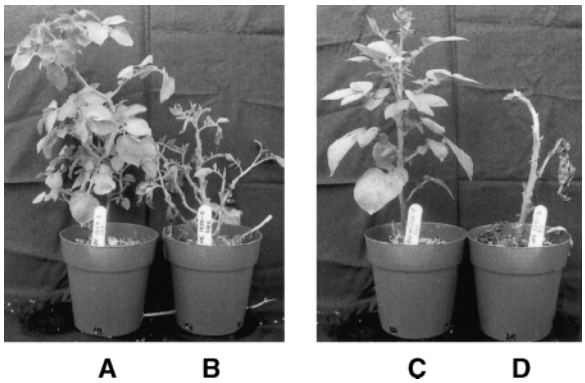

Fig. 3. Greenhouse evaluation of late blight resistance of breeding lines derived from potato × *Solanum bulbocastanum* somatic hybrids. The plants were challenged with *Phytophthora infestans* isolate US930287. Photos were taken 10 d after inoculation. A, a resistant line WTS1269–3; B, a susceptible line WTS1258–4; C, a resistant line 4H5124–2; D, a susceptible line 4H5133–6.

sequence-characterized amplified region (SCAR) have been identified for use in identifying potatoes containing the Ry_{adg} gene conferring resistance to *Potato virus Y* (Hamalainen et al., 1997; Kasai et al., 2000). PCR-based markers were developed for resistance to the potato root cyst nematode [*Globodera rostochiensis* (Woll.) Skarbilovich] (Niewohner et al., 1995). Significant efforts for genetic mapping and marker development have been made on quantitative resistance to *P. infestans* (Meyer et al., 1998; Oberhagemann et al., 1999; Trognitz et al., 2002). Although it has become routine to develop DNA markers closely linked with specific disease resistance traits, the efficiency of selection based on such markers varies, depending on whether the markers are user-friendly and how tightly linked they are to the target resistances. An increasing number of potato traits have been targeted for genetic mapping (Barone, 2004). These efforts will eventually result in more markers for marker-assisted selections.

The *RB* gene can be readily deployed into currently grown potato varieties by genetic transformation (Song et al., 2003). However, as of yet, transgenic vegetable crops have not been widely accepted. In general, consumers are still concerned that genetically modified (GM) foods contain risks based on relatively new science whose benefits and drawbacks have not been fully explored or explained (Finucane and Holup, 2005). Because of this public perception, releasing and field testing of GM fruits and vegetables in the United States has steadily declined since 1998 (www.nbiap.vt.edu/cfdocs/fieldtests1.cfm; verified 31 Oct. 2005). Thus, although the *RB* gene was cloned from a wild potato relative and it is now possible to develop transgenic potato cultivars that include only potato DNA without exotic DNA sequences from other organisms (Rommens et al., 2004), the fate of GM potato cultivars in the near future is uncertain. Until the public concerns are alleviated and scientific progress has advanced to provide the evidence needed to alleviate those concerns, it is necessary to introgress wild *Solanum* disease resistance genes, including the *RB* gene, using traditional breeding approaches. The methodology used to introgress and select for gene *RB* using a marker-based approach is an effective way to achieve this goal.

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REFERENCES

Agrios, G.N. 1997. Plant pathology. 4th ed. Academic Press, San Diego, CA.

Barone, A. 2004. Molecular marker-assisted selection for potato breeding. Am. J. Potato Res. 81:111–117.

Black, W., C. Mastenbroek, W.R. Wills, and L.C. Petersen. 1953. A proposal for an international nomenclature of races of *Phytophthora infestans* and of genes controlling immunity of *Solanum demissum* derivatives. Euphytica 2:173–179.

Chen, Q., L.M. Kawchuk, D.R. Lynch, M.S. Goettel, and D.K. Fujimoto. 2003. Identification of late blight, Colorado potato beetle, and blackleg resistance in three Mexican and two south American wild 2x (1EBN) *Solanum* species. Am. J. Potato Res. 80:9–19.

Colon, L.T., and D.J. Budding. 1988. Resistance to late blight (*Phytophthora infestans*) in 10 wild *Solanum* species. Euphytica 37:77–86.

Cruickshank, G., H.E. Stewart, and R.L. Wastie. 1982. An illustrated assessment key for foliage blight of potatoes. Potato Res. 25:213–214.

Douches, D., J. Coombs, K. Felcher, and W. Kirk. 2004. Foliar reaction to *Phytophthora infestans* in inoculated potato field trials in Michigan. Am. J. Potato Res. 81:443–448.

Douches, D.S., J.B. Bamberg, W. Kirk, K. Jastrzebski, B.A. Niemira, J. Coombs, D.A. Bisognin, and K.J. Felcher. 2001. Evaluation of wild *Solanum* species for resistance to the US-8 genotype of *Phytophthora infestans* utilizing a fine-screening technique. Am. J. Potato Res. 78:159–165.

Duncan, J.M. 1999. *Phytophthora*—An abiding threat to our crops. Microbiol. Today 26:114–116.

Finucane, M.L., and J.L. Holup. 2005. Psychosocial and cultural factors affecting the perceived risk of genetically modified food: An overview of the literature. Soc. Sci. Med. 60:1603–1612.

Flor, H.H. 1971. Current status of the gene-for-gene concept. Annu. Rev. Phytopathol. 9:275–296.

Fry, W.E., and S.B. Goodwin. 1997. Resurgence of the Irish potato famine fungus. Bioscience 47:363–371.

Garelik, G. 2002. Taking the bite out of potato blight. Science (Washington, DC) 298:1702–1704.

Hamalainen, J.H., K.N. Watanabe, J.P.T. Valkonen, A. Arihara, R.L. Plaisted, E. Pehu, L. Miller, and S.A. Slack. 1997. Mapping and marker-assisted selection for a gene for extreme resistance to potato virus Y. Theor. Appl. Genet. 94:192–197.

Hammond-Kosack, K.E., and J.D. Jones. 1996. Resistance gene-dependent plant defense responses. Plant Cell 8:1773–1791.

Helgeson, J.P., J.D. Pohlman, S. Austin, G.T. Haberlach, S.M. Wielgus, D. Ronis, L. Zambolim, P. Tooley, J.M. McGrath, R.V. James, and W.R. Stevenson. 1998. Somatic hybrids between *Solanum bulbocastanum* and potato: A new source of resistance to late blight. Theor. Appl. Genet. 96:738–742.

Johnson, R. 1984. A critical analysis of durable resistance. Annu. Rev. Phytopathol. 22:309–330.

Kasai, K., Y. Morikawa, V.A. Sorri, J.P.T. Valkonen, C. Gebhardt, and K.N. Watanabe. 2000. Development of SCAR markers to the PVY resistance gene Ry_{adg} based on a common feature of plant disease resistance genes. Genome 42:1–8.

Keen, N.T. 2000. A century of plant pathology: A retrospective view on understanding host-parasite interactions. Annu. Rev. Phytopathol. 38:31–48.

Kuhl, J.C., R.E. Hanneman, and M.J. Havey. 2001. Characterization and mapping of *Rpi1*, a late-blight resistance locus from diploid (1EBN) Mexican *Solanum pinnatisectum*. Mol. Genet. Genomics 265:977–985.

Leister, R.T. 2000. A resistance gene product of the nucleotide binding site-leucine rich repeats class can form a complex with bacterial avirulence proteins *in vivo*. Plant J. 22:345–354.

Lozoya-Saldana, H., C. Belmar-Diaz, J.M. Bradeen, and J.P. Helgeson. 2005. Characterization of *Phytophthora infestans* isolates infecting transgenic and somatic hybrid potatoes resistant to the pathogen in the Toluca Valley, Mexico. Am. J. Potato Res. 82:79.

Malcolmson, J.F., and W. Black. 1966. New R genes in *Solanum demissum* and their complementary races of *Phytophthora infestans*. Euphytica 15:199–203.

Menke, U., L. SchildeRentschler, B. Ruoss, C. Zanke, V. Hemleben, and H. Ninnemann. 1996. Somatic hybrids between the cultivated potato *Solanum tuberosum* L and the 1EBN wild species *Solanum pinnatisectum* Dun: Morphological and molecular characterization. Theor. Appl. Genet. 92:617–626.

Meyer, R.C., D. Milbourne, C.A. Hackett, J.E. Bradshaw, J.W. McNichol, and R. Waugh. 1998. Linkage analysis in tetraploid potato and association of markers with quantitative resistance to late blight (*Phytophthora infestans*). Mol. Gen. Genet. 259:150–160.

Michelmore, R.W., and B.C. Meyers. 1998. Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Res. 8:1113–1130.

Naess, S.K., J.M. Bradeen, S.M. Wielgus, G.T. Haberlach, J.M. McGrath, and J.P. Helgeson. 2000. Resistance to late blight in *Solanum bulbocastanum* is mapped to chromosome 8. Theor. Appl. Genet. 101:697–704.

Naess, S.K., J.M. Bradeen, S.M. Wielgus, G.T. Haberlach, J.M. McGrath, and J.P. Helgeson. 2001. Analysis of the introgression of *Solanum bulbocastanum* DNA into potato breeding lines. Mol. Genet. Genomics 265:694–704.

Niederhauser, J.S., J. Cervantes, and L. Servin. 1954. Late blight in Mexico and its implications. Phytopathology 44:406–408.

Niederhauser, J.S., and W.R. Millis. 1953. Resistance of *Solanum* species to *Phytophthora infestans* in Mexico. Phytopathology 43: 456–457.

Niewohner, J., F. Salamini, and C. Gebhardt. 1995. Development of PCR assays diagnostic for RFLP marker alleles closely linked to alleles *Gro1* and *H1*, conferring resistance to the root cyst-nematode *Globodera rostochiensis* in potato. Mol. Breed. 1:65–78.

Oberhagemann, P., C. Chatot-Balandras, R. Schäfer-Pregl, D. Wegener, C. Palomino, F. Salamini, E. Bonnel, and C. Gebhardt. 1999. A genetic analysis of quantitative resistance to late blight in potato: Towards marker-assisted selection. Mol. Breed. 5:399–415.

Oberwalder, B., L. Schilde-Rentschler, B. Ruoss, S. Wittemann, and H. Ninnemann. 1998. Asymmetric protoplast fusions between wild species and breeding lines of potato—Effect of recipients and genome stability. Theor. Appl. Genet. 97:1347–1354.

Ojiambo, P.S., J.O. Nyanapah, C. Lung'aho, J.K. Karinga, and H.M. Kidanemariam. 2000. Comparing different epidemiological models in field evaluations of selected genotypes from *Solanum tuberosum* CIP population A for resistance to *Phytopthora infestans* (Mont.) De Bary in Kenya. Euphytica 111:211–218.

Ramon, M., and R.E. Hanneman. 2002. Introgression of resistance to late blight (*Phytophthora infestans*) from *Solanum pinnatisectum* into *S. tuberosum* using embryo rescue and double pollination. Euphytica 127:421–435.

Rommens, C.M., J.M. Humara, J. Ye, H. Yan, C. Richael, L. Zhang, R. Perry, and K. Swords. 2004. Crop improvement through modification of the plant's own genome. Plant Physiol. 135:421–431.

Ross, H. 1986. Advances in plant breeding: Supplements to Journal of Plant Breeding. Vol. 13. p. 82–86. *In* W. Horn and G. Röbbelen (ed.) Potato breeding: Problems and perspectives. Verlag Paul Parey, Berlin.

Song, J., J.M. Bradeen, S.K. Naess, J.A. Raasch, S.M. Wielgus, G.T. Haberlach, J. Liu, H. Kuang, S. Austin-Phillips, C.R. Buell, J.P. Helgeson, and J. Jiang. 2003. Gene *RB* cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. Proc. Natl. Acad. Sci. USA 100:9128–9133.

Thieme, R., U. Darsow, T. Gavrilenko, D. Dorokhov, and H. Tiemann. 1997. Production of somatic hybrids between *S. tuberosum* L. and late blight resistant Mexican wild potato species. Euphytica 97:189–200.

Trognitz, F., P. Manosalva, R. Gysin, D. Nino-Liu, R. Simon, M.D. Herrera, B. Trognitz, M. Ghislain, and R. Nelson. 2002. Plant defense genes associated with quantitative resistance to potato late blight in *Solanum phureja* × dihaploid *S. tuberosum* hybrids. Mol. Plant Microbe Interact. 15:587–597.

van Soest, L.J.M., B. Schober, and M.F. Tazelaar. 1984. Resistance to *Phytophthora infestans* in tuber-bearing species of *Solanum* and its geographic distribution. Potato Res. 27:393–411.

Ward, A.C., J.S.-J. Phelpstead, A.E. Gleadle, N.W. Blackhall, S. Cooper-Bland, A. Kumar, W. Powell, J.B. Power, and M.R. Davey. 1994. Interspecific somatic hybrids between dihaploid *Solanum tuberosum* L. and the wild species *S. pinnatisectum* Dun. J. Exp. Bot. 45:1433–1440.

Wastie, R.L. 1991. *Phytophthora infestans*, the cause of late blight of potato—Breeding for resistance. Academic Press, San Diego, CA.