Genet Resour Crop Evol (2007) 54:1797–1806
DOI 10.1007/s10722-006-9201-6

RESEARCH ARTICLE

Resistance traits and AFLP characterization of diploid primitive tuber-bearing potatoes

Riccardo Aversano · Maria Raffaella Ercolano · Luigi Frusciante · Luigi Monti · James M. Bradeen · Gennaro Cristinzio · Astolfo Zoina · Nicola Greco · Salvatore Vitale · Domenico Carputo

Received: 16 June 2006 / Accepted: 13 December 2006 / Published online: 14 March 2007

Abstract Worldwide, a variety of pathogens negatively affect potato production, resulting in an estimated 22% annual yield reduction. Wild Solanum species represent a unique gene pool where all the traits necessary to improve the cultivated potato can be found. Therefore, breeding efforts for improved disease resistance and research aimed at characterizing wild germplasm have been extensively made. In this paper, sources of resistance to *Phytophthora infestans*, *Erwinia carotovora* subsp. *carotovora*, *Fusarium solani* and *Globodera* spp. have been investigated in several clones of two *Solanum* species originating from Central Mexico (*S. bulbocastanum* and *S. cardiophyllum*). Interestingly, we found sources of combined resistance to late blight and bacterial soft rot. This is an important finding considering that the development of resistant potato varieties has been hindered by the scarcity of resistant germplasm. In addition, we explored molecular differences within and between the two species generating AFLP fingerprints. By means of six primer pair combinations, we found 13 and 16 putative species-specific AFLP markers for *S. bulbocastanum* and *S. cardiophyllum*, respectively, and a bounty of markers useful for mapping, MAS, and cloning purposes. The phenotypic and molecular information associated to *S. bulbocastanum* and *S. cardiophyllum* for designing strategies of assisted selection are discussed.

Keywords Germplasm · Molecular markers · *Solanum bulbocastanum* · *S. cardiophyllum*

R. Aversano · M. R. Ercolano · L. Frusciante · L. Monti · D. Carputo (✉)
Department of Soil, Plant and Environmental Sciences, University of Naples ''Federico II'', Via Università 100, Portici 80055, Italy
e-mail: carputo@cds.unina.it

J. M. Bradeen
Department of Plant Pathology, University of Minnesota, 495 Borlaug Hall/1991 Upper Buford Circle, St. Paul, MN 55108, USA

G. Cristinzio · A. Zoina
Istituto di Patologia Vegetale, University of Naples ''Federico II'', Via Università 100, Portici 80055, Italy

N. Greco
Istituto per la Protezione delle Piante, Sezione di Bari, C.N.R., Via G. Amendola 165/A, Bari 70126, Italy

S. Vitale
C.R.A. – Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, Roma 00156, Italy

Introduction

The genus *Solanum* contains about 2,300 species distributed in temperate to tropical habitats, with the greatest diversity in Central and South America (Barroso et al. 1986). Among them,

particularly important for the genetics and breeding of tetraploid ($2n = 4x = 48$) cultivated potato (*S. tuberosum* L.) are the approximately 200 tuber-bearing species. These belong to the subsection Potatoe, and exist as ploidy series ranging from the diploid ($2n = 2x = 24$) to the hexaploid ($2n = 6x = 72$) level. Tuber-bearing *Solanum* species have a wide geographic distribution, ranging from the southern part of the United States to southern Chile. Consequently, they show a very large range of ecological adaptation. For example, some species, such as *S. acaule* Bitter, grow in the Andean region up to 4500 m, where frost events are very common. Other wild potatoes (e.g., *S. berthaultii* Hawkes and *S. tarijense* Hawkes) are adapted to the dry semi-arid conditions of Mexico. Some wild *Solanum* species also have developed strong resistances to a wide range of insects and diseases, including some of the worst pests of cultivated potato such as early and late blights, viruses, potato beetle, and soft rot. By contrast, cultivated potatoes have evolved under a limited range of non-extreme environmental conditions, and are often susceptible to biotic as well as abiotic stress conditions (Hawkes 1990).

Due to the evolutionary diversity of wild species and to the comparatively narrow genetic basis of *S. tuberosum* varieties, tuber-bearing Solanums provide an excellent and unique genetic diversity for potato breeding purposes. The possibility to introgress genes of interest from resistant species is favoured by the easy manipulation of whole sets of chromosomes through the use of *S. tuberosum* haploids ($2n = 2x = 24$) and $2n$ gametes (Carputo and Barone 2005). When sexual barriers hinder sexual hybridization, somatic fusion and genetic engineering may represent valid alternatives to conventional breeding approaches (Carputo et al. 2005; Orczyk et al. 2003). Among them, Mexican *S. bulbocastanum* and *S. cardiophyllum* may represent a useful source of noteworthy genes. Resistance to a number of pests and diseases has been reported by Watanabe et al. (1999), Bamberg et al. (1994), and Chen et al. (2003). Due to post-zygotic incompatibility isolation mechanisms, these species have not been used extensively in potato breeding. Interspecific bridge crosses between *S. bulbocastanum* and *S. tuberosum* have also been reported by Hermsen and Ramanna (1973). Somatic hybrids between potato and *S. bulbocastanum* were developed by Helgeson et al. (1993), and backcrossed progenies consistently displayed late blight resistance introgressed from the wild parent. *RB* gene, conferring resistance to *Phytophtora infestans*, was cloned from *S. bulbocastanum* and introgressed into *S. tuberosum* by means of *Agrobacterium*-mediated transformation (Song et al. 2003; van der Vossen et al. 2003, 2005). Chen et al. (2004) produced diploid and triploid hybrids between *S. pinnatisectum* and *S. cardiophyllum* with high resistance to late blight and colorado potato beatle.

To increase the efficiency of plant breeding programs, molecular markers may be of particular potential for understanding genetic relationships within potato germplasm, allowing researchers to establish a broad genetic base for breeding purposes (Bisognin and Douches 2002). Molecular fingerprints can be used to map traits of interest and to develop molecular linkage maps for useful wild potato species (Tanskley and McCouch 1997). One particularly attractive application of linkage maps is the identification of molecular markers for assisted selection (MAS) in breeding programs. MAS enables breeders to precisely introgress small genome sectors from wild or exotic accessions while reducing the incorporation of genes of undesirable effect.

In this paper, we report evaluation of genotypes of *S. bulbocastanum* and *S. cardiophyllum* for resistance to significant pathogens and pests. In addition, we explore their molecular fingerprints, demonstrating substantial interspecific polymorphism and documenting the potential for development of markers for MAS.

Materials and methods

Plant material

Fifteen clones belonging to two accessions, each of diploid Solanum species were screened. The wild species included two accessions (PI275190 and PI275188) of *S. bulbocastanum* Dunal subsp. *bulbocastanum* (respectively coded blb1 and blb2) and two accessions (PI 283062, PI 347759)

of *S. cardiophyllum* Lindl. subsp. *cardiophyllum* (cph1 and cph2). They were provided as true seed by the IR-1 Potato Introduction Project, Sturgeon Bay, WI. Seeds for each accession were sterilized in 20% bleach for 10 min and were germinated in vitro on MS medium (Murashige and Skoog 1962), in a growth chamber (24°C and 16 h of light/day). Random seedlings from each PI were chosen and named. All studied genotypes were maintained as micropropagated plants on MS medium with 1% sucrose and 0.8% agar, and incubated at 4000 lux, 16 h light, and 24°C. To produce plant material for this study, four week-old-plants were transferred into styrofoam trays filled with sterile soil and acclimated to *ex vivo* conditions in a growth chamber at 20°C. After two weeks, they were transferred into 5-cm-diameter plastic pots and grown in a temperature-controlled greenhouse (20–24°C).

Screening for *Phytophthora infestans* (Mont.) de Bary resistance

Inoculum was prepared with six isolates of *P. infestans* (three strains of A1 mating type and three of A2 mating type) collected from several southern Italy sites, and isolated from susceptible potato cultivars ('Marina' and 'Aiax'). To produce sporangia, the isolates were cultured in the dark for 12 days at 21°C, on V8p substrate (Cristinzio and Testa 1997). Sterile distilled water was then added onto the agar surface, and mycelium was stirred using a sterilized glass or rubber and filtered through two layers of cheesecloth. The suspension was diluted with distilled water to a final concentration of 20,000 sporangia ml^{-1} using a hemacytometer at 100× magnification. Four-week-old micro-propagated plants were planted in styrofoam trays in sterile soil and were arranged in a randomized complete block design. For each clone, 7–10 replications were used. When plants were 15–20 cm high, they were inoculated with the sporangia suspension. Disease severity values were estimated as the percentage of leaves with late blight symptoms at 7 and 14 days after inoculation. Severity values were scored using a scale of 0–5, where 0 = no disease, 1 = 3–24%, 2 = 25–49%, 3 = 50–74%, 4 = 75–94% and 5 = 95–100% infection. Based on an arbitrary scale, genotypes with infection index = 0–3% after 14 days were classified as ''resistant'', those with a value between 4 and 10% ''moderately resistant'' and others as ''susceptible''.

Screening for soft rot (*Erwinia carotovora* subsp. *carotovora*) resistance

Erwinia carotovora subsp. *carotovora* strain Ecc 009 obtained from the International Potato Center, Lima, Peru, was used as inoculum. Bacterial suspensions of 1×10^8 cfu/ml were used for all the inoculation tests and were prepared according to the procedures described by Sirianni (1998) with some modifications. Uniform and undamaged tubers were washed in running tap water and then surface sterilized in a 0.5% sodium hypochlorite solution for 20 min, rinsed with sterile water and then allowed to dry on sterile filter paper. Under a laminar flow cabinet, 3–5 holes (0.4 mm wide and 10 mm deep) were drilled into each tuber and, at the same time, inoculated with a 6 dental barbed broach previously dipped into the bacterial suspension. In each tuber, one hole was inoculated with sterile distilled water as a control while each of the other holes were inoculated with the bacterial suspension (about 5×10^5 bacteria cells for point inoculation). Ten tubers of each clone were inoculated with the bacterium, placed into a dew chamber, and incubated at 24°C for 72 h. Following incubation, tubers were sliced vertically through the infection points and the width of the rotted areas was measured. Based on the width of the rotted area, an arbitrary scale was used to classify clones as ''resistant'' (diameter of rotted area 0.4–2 mm) or ''susceptible'' (>2 mm).

Screening for *Fusarium solani* resistance

Monoconidial isolates of *F. solani* 1427 mc ISPaVe were isolated from potato cultivars ('Primura' and 'Monalisa') grown in northern Italy regions. The fungus was cultured on Potato Dextrose Agar (PDA) (Oxoid) for 15 days at 24°C. Conidia were washed from the plate with sterile distilled water and counted in a hemacytometer, and the suspension diluted in sterile distilled water to 106 conidia/ml. Each 5-week-old

plant was wounded at the stem base and inoculated with 5 ml of conidia suspension. All treated plants were grown in a greenhouse at 20 ± 3°C. Wilt symptoms developed between 14 and 21 days and were recorded with a 0–5 disease index scale described by Thanassoulopoulos and Kitsos (1985). Twelve plants of each accession were inoculated with fungal suspension and three plants were inoculated with distilled water.

Screening for *Globodera rostochiensis* and *G. pallida* resistance

The genotypes were tested against a southern Italy population of *Globodera rostochiensis* pathotype Ro2 and one of *G. pallida* pathotype Pa3 (Kort et al. 1977) and phenotypic responses were compared with those of susceptible cv. 'Spunta'. To prepare the inoculum the nematodes infested soil was processed with a Fenwick can. Cysts were separated from the soil debris by means of flotation in alcohol (Seinhorst 1974), counted, and crushed according to Bijloo's modified method (Seinhorst and Den Ouden 1966). Finally their egg content was determined. For the screening test the clones of wild species obtained from in vitro culture were transplanted in 5-cm-diameter plastic pots containing organic potting soil and adapted to standard greenhouse conditions. Thirty days later, these plantlets were transplanted again into 14 cm diameter clay pots containing 1000 cm^3 of steam sterilized sandy soil (89% sand) infested with 20 eggs/cm^3 of *G. rostochiensis* or *G. pallida*. The plots were maintained in a greenhouse at 20 ± 3°C. Plants were uprooted after 40 days, roots were weighed, gently washed in tap water, and cut in 0.5 cm long pieces. The nematodes were then extracted from the roots using the centrifugation method of Coolen (1979), counted, and classified into developmental stage, with each stage expressed as a percentage of the total nematode specimens per root. Clones whose roots did not allow the nematode to reach the adult female stage or in which the proportion of nematode females and cysts was either < 10% or < 70% of that of the susceptible cv. 'Spunta' were scored respectively as ''resistant'' and ''moderately resistant''. All the other clones were scored as ''susceptible''

AFLP (Amplified Fragment Length Polymorphism) analysis

AFLP analysis was performed on 15 genotypes from *S. bulbocastanum* and *S. cardiophyllum* and four varieties of *S. tuberosum* ('Blondy', 'Spunta', 'Russet Burbank' and 'Kathadin') using the method described by Vos et al. (1995) and the commercially available AFLP kit and protocol (Gibco-BRL AFLP analysis System I, Life Technologies, Gaithersburg, MD), which employs *Eco*RI and *Mse*I as restriction enzymes. For selective amplification, six combinations of primers were used (E-AGG + M-CTG; E-AGC M-CTA; E-AGC + M-CTG; E-ACA + M-CAG; E-ACT + M-CTT; E-ACA + M-CAT) with the E primer in each pair being radiolabeled with P-33. AFLP fragments were separated by electrophoresis on 6% denaturing polyacrylamide gels and visualized by exposing X-ray films to the dried gel for at least 24 h.

AFLPs fingerprints were compared and polymorphisms were scored as 1 (presence of fragments) or 0 (absence of fragment). Genetic similarity between clones was calculated as Jaccard's similarity coefficients: $\text{Jaccard}_{yx} = a/(a + b + c)$, where a = number of bands present in x and y, b = number of bands present in x and absent in y, and c = number of bands present in y and absent in x. The genetic similarities were graphically represented by a dendrogram constructed using the UPGMA (unweighted pair-group method, arithmetic average) clustering algorithm. Genetic similarity calculations and dendrogram construction were performed using NTSYS-pc package (Rohlf 1989).

Results

Screening for host resistance

Results of screening diploid wild species and *S. tuberosum* controls for late blight resistance are reported in Table 1. Mean disease severity values ranged from 0% (10 genotypes) to 42.5% (1 genotypes). Out of nine blb clones tested, six were highly resistant, whereas three were moderately resistant, with an infection value >4.0%. The

Table 1 Results from screening tests for resistance to *P. infestans*, *F. solani*, *G. rostochiensis*, *G. pallida* and *E. carotovora* subsp. *carotovora* of 15 genotypes of two diploid *Solanum* species: *S. bulbocastanum* (blb) and *S. cardiophyllum* (cph). *S. tuberosum* cultivar 'Spunta' is included as control

Genotypes	*P. infestans*		*F. solani*		*G. rostochiensis*		*G. pallida*		*E. carotovora*	
	% infected leaves	Reaction[a]	Disease index	Reaction[a]	Females and cysts no.	Reaction[a]	Female and cysts no.	Reaction[a]	Ø Lesion (mm)	Reaction[a]
S. bulbocastanum										
Blb 1A	0.0 a*	R	4.1 d*	S	16.6 a*	MR	8.5 a*	S	0.8 h*	R
Blb 1B	0.0 a	R	3.0 c	S	26.0 ab	MR	15.4 ab	S	1.0 h	R
Blb 1C	0.0 a	R	3.1 c	S	12.0 a	MR	24.2 abcd	S	5.2 b	S
Blb 1D	0.0 a	R	2.7 bc	S	33.4 bc	S	24.3 abcd	S	3.9 c	S
Blb 1E	4.4 ab	MR	1.5 a	MR	15.6 a	MR	17.4 abc	S	1.7 gh	R
Blb 2A	4.2 ab	MR	2.7 bc	S	14.5 a	MR	24.3 abcd	S	2.5 cfg	S
Blb 2B	0.0 a	R	2.5 b	S	23.0 ab	MR	28.8 bcd	S	6.7 a	S
Blb 2C	0.0 a	R	3.0 c	S	13.6 a	MR	14.0 ab	S	3.4 cde	S
Blb 2D	4.0 ab	MR	4.9 ef	S	17.2 a	MR	13.3 ab	S	2.0 fg	S
S. cardiophyllum										
Cph 1C	0.0 a	R	4.0 ef	S	18.2 ab	MR	15.5 abc	S	2.3 fg	S
Cph 2A	42.5 d	S	4.7 ef	S	22.2 ab	MR	36.5 d	S	–	–
Cph 2B	21.6 c	S	4.1 d	S	18.0 ab	MR	31.9 cd	S	2.8 def	S
Cph 2C	17.7 bc	S	4.9 ef	S	15.8 a	MR	22.6 abcd	S	0.9 h	R
Cph 2D	0.0 a	R	–	–	15.6 a	MR	34.4 d	S	2.5 efg	S
Cph 2E	15.2 bc	S	5.0 f	S	13.0 a	MR	25.7 bcd	S	3.8 cd	S
S. tuberosum										
Spunta	100.0 e	S	4.5 de	S	44.4 c	S	27.3 bcd	S	3.4 cde	S

* Data in each column sharing the same letter are not significantly different ($P = 0.05$) by the Duncan test.

[a] R: resistant; S: susceptible; MR: moderately resistant

mean foliar disease severity values of *S. cardiophyllum* clones ranged from 15.2% (cph 2E) to 42.5% (cph 2A). Only two clones (cph 1C and cph 2D) proved to be highly resistant. These clones did not show symptoms of late blight after inoculation. On the other hand, cph 2A was the most susceptible among the evaluated clones.

Most of the clones tested were highly susceptible to tuber soft rot (Table 1) and only four were classified as resistant (blb 1A, blb 1B, blb 1E, cph 2C). The mean width of the rotted area ranged from 0.8 mm (blb 1A) to 6.7 mm (blb 2B), both recorded within the clones of *S. bulbocastanum*. By contrast, *S. cardiophyllum* clones all showed a similar ranking in disease susceptibility when inoculated with Ecc. Only cph 2C was resistant, with a rotted area diameter of 0.9 mm. As for *Fusarium solani* resistance, out of 14 genotypes tested, only blb 1E showed a tolerant phenotype (disease index = 1.5) (Table 1). For all the other clones, disease index ranged from 2.5 (blb 2D) to 5.0 (cph 2E)

Table 1 also reports results on the reaction to nematode infection. Although the nine clones of *S. bulbocastanum* cannot be considered resistant to *G. rostochiensis*, nevertheless in their roots the development of this nematode was delayed, suggesting partial resistance. Indeed, the percentage of females and cysts was significantly lower than in the susceptible cv. 'Spunta'. The partial resistance of clones analyzed was also confirmed by the analysis of second stage juveniles (infective stages) (not reported). These stages were significantly higher in *S. bulbocastanum* clones compared to the susceptible cv. 'Spunta'. Partial resistance was also observed in the six clones of *S. cardiophyllum*. In the roots of all of these clones adult females and cysts were significantly less than in the cv. 'Spunta'. Therefore, most of the clones of *S. bulbocastanum* and all clones of *S. cardiophyllum* can be considered as moderately resistant to *G. rostochiensis*. As for *G. pallida*, the development of this nematode in the most blb and all cph clones was, in general, similar to that

of the control cv. 'Spunta'. Only blb 1A of *S. bulbocastanum* showed a significantly lower percentage of nematode females and cysts (8.5%) compared to cv. 'Spunta' (27.3%).

AFLP analysis

A total of 520 polymorphic bands were scored using six primer pairs. *S. bulbocastanum* and *S. cardiophyllum* genotypes could be clearly distinguished by their AFLP profiles, consistent with the results of Lara-Cabrera and Spooner (2004). The average number of fragments amplified per primer combination was 86.7, and ranged from 72 fragments (E-ACA/M-CAG) to 100 fragments (E-AGC/M-CTA) (data not shown). Most of fragments obtained from each primer pair were polymorphic across the tested species. The polymorphism frequencies within the *S. bulbocastanum* and *S. cardiophyllum* clones were respectively 70.3% and 75.5%. In particular, the total number of polymorphic bands was 204 for *S. bulbocastanum*, and 194 for *S. cardiophyllum*. Non-polymorphic fragments were recovered as well. Eight-five and sixty-one bands were found only in *S. bulbocastanum* and *S. cardiophyllum* genotypes, respectively. Among them, several species-specific bands present in all individuals of one species and absent in the other (13 in b/b and 16 in cph) were identified (Table 2).

Dendrogram analysis divided the tested genotypes into three main groups (Fig. 1). The first group was comprised only of *S. bulbocastanum* genotypes; the second one included all *S. cardiophyllum* genotypes. The *S. tuberosum* controls formed the third group. In order to obtain an estimate of the degree of differentiation among the groups, the average genetic similarities among the 15 wild genotypes and the four *S. tuberosum* were calculated. As expected, the highest average similarity values were shown by the genotypes belonging to the same species. Pairwise similarity varied from 0.49 to 0.98, with an average of 0.69 (data not shown). In the cph cluster, cph2A and cph2B genotypes showed the highest similarity (0.97), cph2D and cph1C the least similar (0.74). In general, all the *S. bulbocastanum* genotypes were similar (0.82–0.91) among them.

Discussion

To improve the genetic background of potato cultivars through interspecific hybridization, the first step is the identification of sources of resistance. In this work we focused on the

Table 2 Analysis of banding patterns generated by AFLP assay for nine *S. bulbocastanum* and six *S. cardiophyllum* genotypes using six primer pair combinations

Primer pair	Total number of bands, no.	Polymorphic loci, no.	Bands specific to each species, no.	Specific bands universal to all individuals, no. (%)
S. bulbocastanum				
E:AGG M:CTG	50	42	11	2 (4.0)
E ACT M:CTT	38	21	5	2 (5.3)
E:ACA M:CAT	59	37	17	1 (1.7)
E:AGC M:CTG	51	42	18	2 (3.9)
E:AGC M:CTA	49	37	17	3 (6.1)
E:ACA M:CAG	43	25	17	3 (7.0)
Total	290	204	85	13 (4.5)
S. cardiophyllum				
E:AGG M:CTG	48	40	12	1 (2.1)
E ACT M:CTT	50	32	17	5 (10.0)
E:ACA M:CAT	41	29	5	3 (7.3)
E:AGC M:CTG	36	31	9	1 (2.8)
E:AGC M:CTA	41	27	11	3 (7.3)
E:ACA M:CAG	41	35	7	3 (7.3)
Total	257	194	61	16 (6.2)

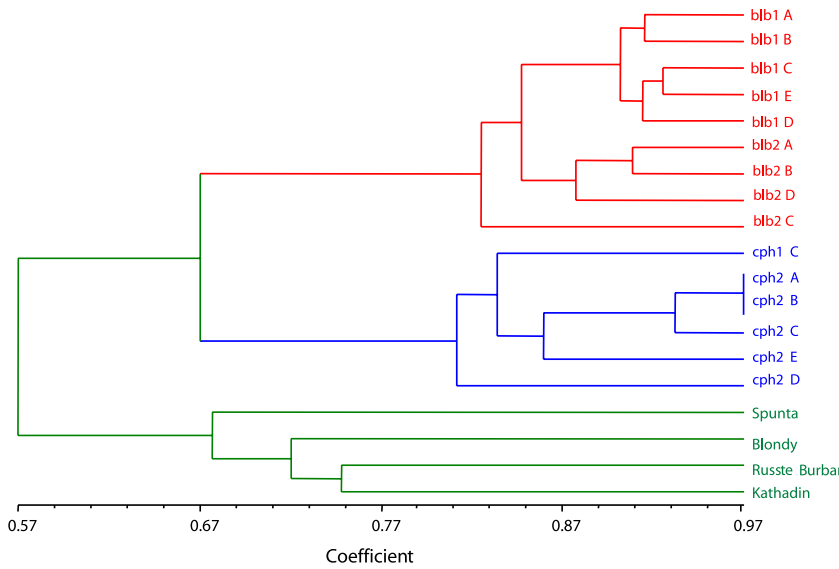

Fig. 1 UPGMA dendrogram of *S. bulbocastanum* (blb) and *S. cardiophyllum* (cph) genotypes and four *S. tuberosum* cultivated varieties, constructed on the basis of the genetic distance estimated by six AFLP primer pair combinations. The similarity on the x-axis is based on the Jaccard's coefficient

characterization of diploid potato germplasm with noteworthy resistances. Among all pathogens tested, *P. infestans* ranks beyond doubt as the world's most destructive crop disease (Garelik 2002). Indeed, breeding for resistance to light blight in potatoes was the first attempt at scientifically-based resistance breeding (Umaerus and Umaerus 1994). In the current study, nine genotypes resistant to late blight were identified, partially confirming data by Hanneman and Bamberg (1986). Interestingly, between and within species and even between genotypes of the same accession, we observed resistance variability for each target trait. For example, between clones of accession cph2 we found high variability in the percentage of leaves infected by *P. infestans*, resulting in the identification of one resistant and four susceptible clones from this single population. By contrast, our accessions of *S. bulbocastanum* showed a uniform low percentage of infected leaves, resulting in a high number of resistant clones. These observations are consistent with the well known high heterozygosity due to the potato outcrossing nature as well as to the allelic and genetic diversity of single resistance loci (Caicedo et al. 1999; Stahl et al. 1999; Mauricio et al. 2003; Rose et al. 2004) and R gene clusters (Van der Hoorn et al. 2001; Caicedo and Schaal 2004; Kuang et al. 2004; Smith et al. 2004; Xiao et al. 2004).

The sources of resistance we found in *S. cardiophyllum* and *S. bulbocastanum* suggest a co-evolution between these species and the oomycete fungus. The two potato species have widely overlapping distribution ranges in central Mexico, where *P. infestans* genetic recombination occurs and where high *P. infestans* genotypic diversity has been noted (Grunwald and Flier 2005). Presumably, the continuous intense infection pressure encountered in this region led to the high levels of resistance in *S. bulbocastanum* and *S. cardiophyllum*. Interestingly, we also found sources of combined resistance to late blight and bacterial soft rot (blb1A and blb1B). This is an important finding, considering the significant impact of these pathogens worldwide. Resistances successfully introgressed into commercial varieties are generally monogenic. Examples are the resistances to potato cyst nematode (*Globodera rostochiensis*), wart (*Synchytrium endobioticum*) and potato virus X. Unfortunately, often major genes and durability do not go together very well, because pathogens may overcome the resistance mechanisms. Breeders should look for major genes from other sources, that can lead to durable resistance. Some of the sources may well be the *S. bulbocastanum*, *S. cardiophyllum* clones we have identified. The pyramided genes should be combined with a genetic background of minor genes, to further reduce the risk of resistance breakdown.

Due to the presence of post-zygotic barriers, the diploid species examined in this study cannot be directly crossed with *S. tuberosum* haploids ($2n = 2x = 24$). Alternative strategies can be protoplast fusion, embryo rescue and bridge ploidies (Orczyk et al. 2003; Carputo and Barone 2005).

Once a researcher has successfully transferred the genes of interest from the donor species to cultivated potato, significant breeding challenges remain. Multiple generations of crossing and selection may be needed to achieve a clone with acceptable phenotypic attributes and each generation must be screened for retention of the newly introgressed disease resistance gene or genes. Phenotypically screening large numbers of genotypes for disease resistance can be a time-consuming and costly endeavor. The advent of DNA-based markers that are phenotypically neutral and the possibility to easily generate large-scale marker data sets has provided an indispensable tool for mapping and isolating useful genes and for MAS, reducing the need of large-scale disease resistance screenings. In potato, markers have been widely used to localize genetic factors controlling qualitative and quantitative expression of resistance both to late blight (Leonards-Schippers et al. 1992, 1994; El-Kharbotly et al. 1994, 1996; Naess et al. 2000), and to other pathogens and pests (reviewed in Gebhardt and Valkonen 2001).

In the present study, we explored molecular differences within and between two disease resistant wild potato species by generating AFLP fingerprints. Our study reveals a bounty of markers useful for mapping, MAS, and cloning purposes. Of special interest to potato improvement, we readily identified species-specific AFLP markers unique to one wild potato species and absent in cultivated potato. In the current study, by a six AFLP-primer pairs analysis, we found 13 and 16 putative species-specific AFLP markers for *S. bulbocastanum* and *S. cardiophyllum*, respectively. Even though we have not found any association between these AFLP markers and the resistance traits, we think they may represent useful tools for genetic and breeding studies. Indeed, they could be exploited for future wild genome-tracking and recombination analysis. In addition, they can be employed for accessing and studying genes from wild potato species (Naess et al. 2000). Finally, as highlighted by Barone (2004), our *S. bulbocastanum* and *S. cardiophyllum* species-specific markers may be used in negative assisted selection, i.e., choosing interspecific hybrids combining resistances with low wild genome content. This is an important prerequisite for designing strategies of assisted selection aimed to enhance the efficiency of the plant breeding by selecting against unfavorable alleles, and potentially, shortening the development time of resistant varieties.

Acknowledgments Contribution no. 127 from Department of Soil, Plant and Environmental Science. This research was carried out within the project ''Caratterizzazione ed utilizzazione di specie selvatiche diploidi di patata originarie del Sud America'' founded by Italian Ministry of Agriculture (MiPAF).

References

Bamberg JB, Martin MW, Schartner JJ (1994) Elite selections of tuber-bearing *Solanum* species germplasm. Inter-Regional Potato Introduction Station, NRSP–6, pp 56

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

Barroso GM, Peixoto AL, Costa CG, Ichaso CLF, Guimarães EF, Lima HC (1986) Sistemática de Angiospermas do Brasil, vol 3. Editora da Universidade Federal de Viçosa, Viçosa, Brazil

Bisognin DA, Douches DS (2002) Genetic diversity in diploid and tetraploid late blight resistant potato germplasm. Hort Sci 37(1):178–183

Caicedo AL, Schaal BA (2004) Heterogeneous evolutionary processes affect R gene diversity in natural populations of *Solanum pimpinellifolium*. Proc Natl Acad Sci USA 101:17444–17449

Caicedo AL, Schaal BA, Kunkel BN (1999) Diversity and molecular evolution of the *Rps2* resistance gene in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 96:302–306

Carputo D, Aversano R, Frusciante L (2005) Breeding potato for quality traits. Acta Hort 684:55–64

Carputo D, Barone A (2005) Ploidy level manipulations in potato through sexual hybridization. Ann Appl Biol 146:71–79

Chen Q, Kawchuk ML, Lynch DR, Goettel MS, Fujimoto DK (2003) Identification of late blight, Colorado potato beetle, and black leg resistance in three Mexican and two South American wild 2x (1EBN) *Solanum* species. Am J Potato Res 80:9–19

Chen Q, Lynch D, Platt HW, Li HY, Shi Y, Li HJ, Deasley D, Rakosy-Tican L, Theme R (2004) Interspecific

crossability and cytogenetic analysis of sexual progenies of Mexican wild diploid 1EBN species *Solanum pinnatisectum* and *S. cardiophyllum*. Am J Potato Res 81:159–169

Cristinzio G, Testa A (1997) Occurrence of the A2 mating type and self isolates of *Phytophthora infestans* in Italy. J Plant Pathol 79:121–123

El-Kharbotly A, Leonards-Schippers C, Huigen DJ, Jacobsen E, Pereira A, Stiekema WJ, Salamini F, Gebhardt C (1994) Segregation analysis and RFLP mapping of the *R1* and *R3* alleles conferring race-specific resistance to *Phytophthora infestans* in progeny of dihaploid potato parents. Mol Gen Genet 242:749–754

El-Kharbotly A, Palomino-Sánchez C, Salamini F, Jacobsen E, Gebhardt C (1996) *R6* and *R7* alleles of potato conferring race-specific resistance to *Phytophthora infestans* (Mont.) de Bary identified genetic loci clustering with the *R3* locus on chromosome XI. Theor Appl Genet 92:880–884

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

Gebhardt C, Valkonen JPT (2001) Organization of genes controlling disease resistance in the potato genome. Annu Rev Phytopath 39:79–102

Grunwald NJ, Flier WG (2005) The biology of *Phytophthora infestans* at its center of origin. Annu Rev Phytopath 43:171–190

Hanneman RE Jr, Bamberg JB (1986) Inventory of tuber-bearing *Solanum* species. Bulletin, 533 of Research Division of the College of Agriculture and Life Sciences. University of Wisconsin, Madison USA, pp 216

Hawkes J (1990) The potato: evolution, biodiversity and genetic resources. Belhaven Press, Oxford

Helgeson JP, Haberlach GT, Ehlenfeldt MK, Hunt G, Pohlman JD, Austin S (1993) Fertile somatic hybrids of potato and wild *Solanum* species: potential for use in breeding programs. Am Potato J 70:437–452

Hermsen JGT, Ramanna MS (1973) Double-bridge hybrids of *Solanum bulbocastanum* and cultivars of *Solanum tuberosum*. Euphytica 22:457–466

Kort J, Ross H, Rumpenhorst HJ, Stone AR (1977) An International scheme for identifying and classifying pathotypes of potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. Nematologica 23:333–339

Kuang H, Woo SS, Meyers BC, Nevo E, Michelmore RW (2004) Multiple genetic processes result in heterogeneous rates of evolution within the major cluster disease resistance genes in lettuce. Plant cell 16:2870–2894

Lara-Labrera SI, Spooner DM (2004) Taxonomy of North and Central American diploid wild potato (*Solanum* sect. *Petota*) species: AFLP data. Plant Syst Evol 248:129–142

Leonards-Schippers C, Gieffers W, Salamini F, Gebhardt C (1992) The *R1* gene conferring race-specific resistance to *Phytophthora infestans* in potato is located on potato chromosome V. Mol Gen Genet 233:278–283

Leonards-Schippers C, Gieffers W, Schäfer-Pregl R, Ritter E, Knapp SJ, Salamini F, Gebhardt C (1994) Quantitative resistance to *Phytophthora infestans* in potato: a case study for QTL mapping in an allogamous plant species. Genetics 137:67–77

Mauricio R, Stahl EA, Korves T, Tian D, Kreitman M, Bergelson J (2003) Natural selection for polymorphism in the disease resistance gene *Rps2* of *Arabidopsis thaliana*. Genetics 163:735–746

Murashige T, Skoog F (1962) A revised medium from rapid growth and bioassays with tobacco tissue cultures. Physiol Plantarum 15:251–258

Naess SK, Bradeen JM, Wielgus SM, Haberlach GT, McGrath JM, Helgeson JP (2000) Resistance to late blight in *Solanum bulbocastanum* is mapped to chromosome 8. Theor Appl Genet 101:697–704

Orczyk W, Przetakiewicz J, Nadoloska-Orczyk A (2003) Somatic hybrids of *Solanum tuberosum*—application to genetics and breeding. Plant Cell Tissue Organ Cult 74:1–13

Rohlf FJ (1989) NTSYSpc. Numerical Taxonomy and Multivariate Analysis System, vol 2.0, Exeter Software, Setauket, New York, USA

Rose LE, Bittner-Eddy PD, Langley CH, Holub EB, Michelmore RW, Beynon JL (2004) The maintenance of extreme amino acid diversity at the disease resistance gene, *RPP13*, in *Arabidopsis thaliana*. Genetics 166:1517–1527

Seinhorst JW (1974) Separation of *Heterodera* cysts from organic debris using ethanol. Nematologica 20:367–369

Seinhorst JW, Ouden H den (1966) An improvement of Bijloo's method for determining the egg content of Heterodera cysts. Nematologica 12:170–171

Sirianni P (1998) Superamento di barriere di incompatibilita' interspecifica attraverso la manipolazione della ploidia e dell' 'Endosperm Balance Number' per l'introgressione di geni utili in *Solanum tuberosum* L. ($2n = 4x = 48$) MSc Thesis, University of Naples ''Federico II'', Italy

Smith SM, Pryor AJ, Hulbert SH (2004) Allelic and haplotypic diversity at the *Rp1* rust resistance locus of maize. Genetics 167:1939–1947

Song J, Bradeen JM, Naess SK, Raasch JA, Wielgus SM, Haberlach GT, Liu J, Kuang H, Austin-Phillips S, Buell CR, Helgeson JP, Jiang J (2003) Gene *RB* cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. Proc Natl Acad Sci USA 100:9128–9133

Stahl EA, Dwyer G, Mauricio R, Kreitman M, Bergelson J (1999) Dynamics of disease resistance polymorphism at the *Rpm1* locus of *Arabidopsis*. Nature 400:667–671

Tanksley SD, McCouch SR (1997) Seed bank and molecular maps: unlocking genetic potential from the wild. Science 277:1063–1066

Thanassoulopoulos CC, Kitsos GT (1985) Studies on *Fusarium* wilt of potatoes. 1. Plant wilt and tuber infection in naturally infected fields. Potato Res 28:507–514

Umaerus V, Umaerus M (1994) Inheritance of resistance to late blight. In: Bradshow JE, Mackay GR (eds) Potato Genetics. CAB International, Wallingford, UK

van der Hoorn RAL, Kruijt M, Roth R, Brandwagt BF, Joosten MHAJ, De Wit PJGM (2001) Intragenic recombination generated two distinct *Cf* genes that mediate AVR9 recognition in the natural population of *Lycopersicon pimpinellifolium*. Proc Natl Acad Sci USA 98:10439–10498

van der Vossen E, Gros J, Sikkema A, Muskens M, Wouters D, Wolters P, Pereira A, Allefs S (2005) The Rpi-blb2 gene from *Solanum bulbocastanum* is an Mi–1 gene homolog conferring broad-spectrum late blight resistance in potato. Plant J 44:208–222

van der Vossen E, Sikkema A, Hekkert BteL, Gros J, Stevens P, Muskens M, Wouters D, Pereira A, Stiekema W, Allefs S (2003) An ancient R gene from the wild potato species *Solanum bulbocastanum* confers broad-spectrum resistance to *Phytophthora infestans* in cultivated potato and tomato. Plant J 36:867–882

Vos P, Hogers R, Bleker M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acid Res 23:4407–4414

Watanabe J, Orrillo M, Watanabe KN (1999) Evaluation of in vitro chromosome-doubled regenerates with resistance to potato tuber moth [*Phthorimaea operculella* (Zeller)]. Plant Biotechnol 16:225–230

Xiao S, Emerson B, Ratanasut K, Patrick E, O'Neill C, Bancroft I, Turner JG (2004) Origin and maintenance of a broad-spectrum disease resistance locus in *Arabidopsis*. Mol Bio Evol 21:1661–1672