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Genetic Diversity within Wild Potato Species (*Solanum* spp.) Revealed by AFLP and SCAR Markers

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ABSTRACT

Exploitation of variability displayed by wild Solanum species for breeding the cultivated potato (S. tuberosum) requires phenotypic and genotypic characterization of germplasm resources. In the present work, a collection of 15 *wild Solanum species was investigated for resistance to pathotype Ro*2 *of the nematode Globodera rostochiensis. Most of the genotypes reduced reproduction of the nematode, compared to the control variety Spunta, a highly resistant genotype being an accession of S. tuberosum spp. andigena. The genetic variability of the Gro1 gene cluster, which confers resistance to some pathotypes of G. rostochiensis, was then studied in the Solanum species used in this study. For this purpose, SCAR markers for eight paralogues of Gro1 gene were developed. No species showed the same pattern of the resistant control genotype. Moreover, wide-genome variability was also assessed by using AFLP markers, which allowed species-specific markers to be identified for each genotype analyzed*

Keywords*: *Potato, Nematode Resistance, Globodera Rostochiensis, Gro1 Gene Cluster-SCAR Markers, AFLP Markers

1. Introduction

The genus *Solanum* contains more than 2000 species, distributed in very different habitats. Among these, more than 200 tuber-bearing species exist that could be particularly important for improving the cultivated potato, *Solanum tuberosum* L. Indeed, wild species are known to be important sources of plant pathogen resistance genes, as well as of many other interesting traits [1]. This has been underlined in subsection Potatoe of the *Solanum* genus, which includes several tuber-bearing wild species already used to improve the cultivated potato [2], particularly for resistance against the variety of pathogens that negatively affect potato production [3]. Moreover, in the last years, potato breeding deeply increased its efficiency by the aid of molecular markers [4,5]. Indeed, molecular fingerprinting of various potato wild species [6,7] and assisted-selection (MAS) [8] allow a better genetic resources managment and a more efficient gene transfer among *Solanum* species.

Among pathogens that affect potato production, the cyst nematodes *Globodera rostochiensis* and *G. pallida* cause severe damage to the cultivated potato and are found worldwide [9]. Resistance to *G. rostochiensis* has already been introgressed into *S. tuberosum* from some *Solanum* wild species, such as *S. andigena*, *S. vernei* and *S. spegazzinii* [10,11], and has been associated with single genes and quantitative trait loci (QTLs). As an example, the locus *H1* was introgressed from *S. andigena* and mapped on a distal position of chromosome V; it confers resistance to *G. rostochiensis* pathotypes Ro1 and Ro4 [12]. Another important source of broad spectrum resistance to potato cyst nematodes has been mapped on chromosome V (locus *Grp1*): it is a QTL and confers high resistance levels to *G. rostochiensis* pathotype Ro5 and to several populations of *Globodera pallida* [13]. This resistance was found in an interspecific hybrid resulting from a complex breeding scheme involving *S. tuberosum*, *S. vernei*, *S. vernei* ssp. *ballsii*, *S. olocense* and *S. tuberosum* ssp. *andigena*.

Finally, a source of resistance to *G. rostochiensis* pathotypes Ro1 and Ro5 derives from *S. spegazzinii*: it is due to the gene *Gro1* that was mapped on chromosome VII [14] and was then sequenced and characterized by means of positional cloning [15]. In particular, it was evidenced that the resistance gene, named *Gro1*-4, is part

of a complex cluster of paralogue genes, some of which seem to be true genes, and others pseudogenes. Therefore some of these paralogues could also confer resistance to other pathotypes of *G. rostochiensis* or to different pathogens, as already reported for the resistance gene *Mi*-1 in tomato [16]. This could be particularly interesting for finding sources of resistance to pathotype Ro2 of *G. rostochiensis*, which causes severe damage to cultivated potato in Italy.

Therefore our aim was to investigate a collection of *Solanum* wild species for: a) their response to *G. rostochiensis* pathotype Ro2, b) their genetic variability at a genome-wide level by AFLP markers, and c) their variability at the *Gro*1 gene cluster through the design of SCAR markers specific for different paralogues.

2. Materials and Methods

2.1. Plant Material

One accession from 15 *Solanum* wild species (listed in **Table 1**) was screened. Plant material was provided as true seed by the IR-1 Potato Introduction Project, Sturgeon Bay, WI. In addition to this material, a cultivar of *S. tuberosum* (cv. 'Spunta') and a diploid *S. spegazzinii* × *S. tuberosum* hybrid (P 40) were studied. The latter was kindly provided by Dr. Gebhardt (Max-Planck-Institut Koln, Germany) and is the resistant genotype used for RFLP mapping of locus *Gro1* and for *Gro1-4* cloning and sequencing [14,15].

Table 1. Accessions of *Solanum* wild species analyzed with their geographical origin: Plant Introduction number is indicated as well as the code used in the present work.

Species	Plant introduction number (P.I.)	Code	Geographical Origin
S. acaule	210029	ACL 1	Bolivia
S. boliviense	310974	BLV 1	Bolivia
S. bulbocastanum	243510	BLB 3	Mexico
S. canasense	265863	CAN 1	Peru
S. cardiophyllum	347759	CPH 2	Mexico
S. chacoense	133124	CHC 1	Uruguay
S. demissum	205625	DMS 1	Mexico
S. fendleri	458417	FEN 2	USA
S. hougasii	161726	HOU 1	Mexico
S. jamesii	275263	JAM 1	USA
S. multidissectum	8MLT-MI	MLT 1	Peru
S. phureja	IVP 35	IVP 35	Colombia
S. stoloniferum	275248	STO 1	Mexico
S. tarijense	265577	TAR 1	Bolivia
S. tuberosum ssp. andigena	205624	TBR1	Bolivia

Seeds for each accession were sterilized in 20% bleach for 10 min and were germinated *in vitro* on MS medium [17] in a growth chamber (24℃ and 16 h of light/day). 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℃. To produce plant material for this study, four week-old plants were transferred to styrofoam trays filled with sterile soil and acclimated in a growth chamber at 20℃. After two weeks, they were transferred to 5-cm-diameter plastic pots and grown in a temperature-controlled greenhouse (20–24℃).

2.2. Response to Globodera Rostochiensis

The 15 Solanum genotypes were tested for their response to pathotype Ro2 of Globodera rostochiensis. The symptoms revealed were compared with those of the susceptible cv. 'Spunta', used as control. The nematode population was reared on potato cv. Spunta in pots containing 2.8 dm3 of sandy soil (89% sand) in a greenhouse at 20 ± 2℃. To estimate the nematode population densities, three 200-g soil samples were processed with a Fenwick can. The cysts were separated from soil debris by means of flotation in alcohol [18], and then counted, crushed according to Bijloo's modified method [19] and their egg content determined. Five plants per genotype were transplanted into 5-cm diameter plastic pots containing organic potting soil and adapted to standard greenhouse conditions. Thirty days later, these plantlets were transplanted into 14-cm diameter clay pots containing 1000 cm^3 of steam-sterilized sandy soil (89% sand) infested with the nematode. At planting, the nematode population density was 20 eggs/g soil of pathotype Ro2. Pots were maintained in a greenhouse at 20 ± 2℃. Two months later, the plants were cut at ground level and the soil left to dry. Then the soil of each pot was mixed and a 200-g subsample processed as mentioned above to estimate the nematode population density. Reproduction rate was computed by measuring the eggs/g soil found at the end of the test against the eggs/g soil at the inoculum. All data were subjected to ANOVA in order to verify that response to the nematode was genotype dependent and after they were analyzed by Duncan's multiple range test [20].

2.3. AFLP Analysis

AFLP analysis was performed on plant material using the method described by Vos et al. [21] 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, five combinations of primers were used (*Eco*RI-ACT + *Mse*I-CTC; *Eco*RI-ACC +

*Mse*I-CAA; *Eco*RI-ACC + *Mse*I-CAT; *Eco*RI-ACC + *Mse*I-CTA; *Eco*RI-AAC + *Mse*I-CAG) with the *Eco*RI primer in each pair being labelled with FAM fluorochrome. AFLP fragments were separated by capillary electrophoresis on ABI Prism 3100 Avant Sequence Analyser (Applied Biosystems). AFLPs electropherograms were read and compared using Gene Mapper V3.7 software (Applied Biosystems). A panel was created for each primer combination and polymorphisms were scored as 1 (presence of fragment) or 0 (absence of fragment).

2.4. SCAR Analysis

For SCAR analysis, specific primers for each paralogue of the *Gro1* cluster (*Gro1-2*, *Gro1-3*, *Gro1-4*, *Gro1-5*, *Gro1-6*, *Gro1-8*, *Gro1-11*, *Gro1-14*) from P40 resistance allele [15] were designed using sequences available in GenBank (accession numbers AY196151-AY196158). For this purpose, sequences specific to each paralogue were identified by means of multiple-sequence alignment tools (CLUSTAL-W) [22] and pairwise alignment (Local BLAST-N) [23]. On these paralogue-specific sequences, primer pairs were constructed using E-Primer3 Software (http://emboss.sourceforge.net/) or manually. Primer specificity was verified by Local BLAST-N. *Gro*1-4 specific primers from Gebhardt et al. [5] were also used and are named 4RNA2.

PCR was performed in a total volume of 25 µl containing 0.2 mM dNTPs, 2 mM $MgCl_2$, 0.4 M of each primer and 1.25 U Taq DNA polymerase in the reaction buffer provided by the manufacturer (Invitrogen, Carlsbad, CA, USA). PCR conditions were as follows: 3 min at 94℃ followed by 35 cycles of 45 s at 92℃, 45 s at the primer pair specific annealing temperature, 1 min at 72℃ and finally 10 min at 72℃. Amplification patterns were compared and polymorphisms were scored as 1 (presence of fragment of expected size) or 0 (absence of expected fragment).

2.5. Cluster Analysis

Similarity between clones was calculated both on AFLP analysis and SCAR analysis data using the Jaccard coefficient: $J = a/(a + b + c)$, where a = number of bands present in x and y, b = number of bands present in x and absent in y, c = number of bands present in y and absent in x. The genetic similarities were graphically represented by an un rooted dendrogram constructed using the UPGMA clustering algorithm (Unweighted Pair Group Method). Genetic similarity calculations and dendrogram construction were performed using an NTSYS-pc package [24]. Bootstrap analysis were then performed using WinBoot Software with a bootsrapping value of 1000 [25].

3. Results

3.1. Response to *Globodera Rostochiensis*

ANOVA carried on the results of the resistance test gave significant F values for all considered parameters in tests with 15 and 84 degrees of freedom ($p < 0.01$). In particular F was 10.4 for eggs/g soil, 4.09 for eggs per cyst and 10.43 for the reproduction rate.

As shown by Duncan test results, in general, the nematode pathotype Ro2 reproduced significantly less on the accessions of the wild *Solanum* species than on the susceptible control cv. Spunta (**Table 2**). The number of eggs/g soil of pathotype Ro2 on the wild clones varied from 1/6 (group A, abc) to about 1/2 (group B, d) of that on cv. Spunta (63.9; group C, e). The only exception was clone MLT1 for which this value (67.9; group C, e) was similar to that of the susceptible control. Differences were also observed in the number of eggs per cyst and in the reproduction rate of the nematode. There were significantly fewer eggs per cysts than in the control for clones BLB3, CAN1, JAM1 and TBR1. For clones ACL1, BLB3, JAM1, STO1 and TBR1, the reproduction rates of pathotype Ro2 were < 1.

Table 2. Accessions of *Solanum* wild species analyzed with their geographical origin: Plant Introduction number is indicated as well as the code used in the present work.

Clone	Pathotype Ro2		
	Eggs/g soil (no.)	Eggs/cyst (no.)	Reproduction rate
ACL1	18.9 abc AB	129 bcde BC	0.9 abc AB
BLV1	21.1 abcd AB	145 cdef BCD	1.1 abcd AB
BLB3	18.5 abc AB	114 b AB	0.9 abc AB
CAN1	22.2 abcd AB	120 bc ABC	1.1 abcd AB
CPH2	25.2 abcd AB	152 def BCD	1.3 abcd AB
CHC1	32.3 cd B	131 bcde BC	1.6 cd B
DMS1	21.0 abcd AB	126 bcd BC	1.0 abcd AB
FEN2	26.0 bcd AB	152 bcde BCD	1.3 bcd AB
HOU1	33.0 d B	160 ef CD	1.6 d B
JAM1	18.3 abc AB	120 bc ABC	0.9 ab AB
MLT1	67.9 e C	174 f D	3.4 e C
IVP35	24.2 abcd AB	134 bcde BCD	1.2 abcd AB
STO1	17.6 ab AB	131 bcde BC	0.9 abc AB
TAR1	19.3 abcd AB	126 bcd BC	1.0 abcd AB
TBR1	11.5 a AB	80 a A	0.6 a A
Spunta	63.9 e C	154 def BCD	3.1 e C

3.2. AFLP Analysis

Using five primer pairs an average of 317 fragments per genotype were scored for a total of 1084 bins. The number of fragments scored for each genotype ranged from 148 for *S. cardiophyllum* to 470 for Spunta. The average number of selected bins per primer combination was 216, and ranged from 144 (*Eco*RI-ACC/*Mse*I-CAT) to 350 (*Eco*RI-ACT/*Mse*I-CTC) (data not shown). Most of the bins selected from each primer pair were polymorphic across the tested species (98.15%); only 20 were present in all the tested species. Among the polymorphic fragments, 88 were species-specific: the number of the species-specific fragments varied from 1 (for *S. tuberosum* subsp *andigena* and *S. fendleerii*) to 24 for *S. tarijense*. The most informative primer combinations identified from 26 to 33 species-specific fragments and allowed from 9 to 14 species to be discriminated (**Table 3**).

Dendrogram analysis grouped the tested genotypes into one main group (bootstrap values of 58%), with the species *S. tarijense, S. acaule S. bulbocastanum, S. jamesii, S. canasense* and *S. cardiophyllum* standing outside this cluster (**Figure 1**). The main group can be divided into two secondary branches, with a similarity coefficient between 28% and 39%. The similarity coefficient among species is never higher than 62% except for the two species S. *fendleerii* and *S. tuberosum* subs. *andigena* which group together with a similarity of about 79%.

3.3. SCAR Analysis

Each region of the *Gro*1-4 gene was compared to other *Gro*1 paralogue sequences available in GenBank by means of Local Blast. This analysis allowed the length of specific regions for each paralogue to be identified, as reported in **Table 4**. The regions which differed in length from the others were examined as paralogue-specific candidates, such as the region I intron for paralogue *Gro*1-5.

Where no evident difference in length was detectable, polymorphic sites (SNP or INDEL) were identified by CLUSTAL-W, as was the case of region III intron of paralogue *Gro*1-3 where various SNPs were found. This analysis allowed at least one paralogue specific region to be identified for each of the eight genes deriving from the *S. spegazzinii Gro*1 resistant allele. Where possible, coding regions were chosen for subsequent analysis. On each of these paralogue-specific regions a primer pair was designed with no annealing on different regions of *Gro*1 sequences.

The primers used for SCAR analysis are listed in **Table 5** and showed in **Figure 2**, including the primers for paralogue *Gro*1-4 from Gebhardt et al. [5].

Table 3. AFLP analysis: for each primer combination the number of species-specific fragments and of discriminated species are reported.

Primer combination	Species-specific fragment (no.)	Discriminated species (no.)
*Eco*RI-ACT/*Mse*I-CTC	26	9
*Eco*RI-ACC/*Mse*I-CAA	28	14
*Eco*RI-ACC/*Mse*I-CAT	0	-
*Eco*RI-ACC/*Mse*I-CTA	1	1
*Eco*RI-AAC/*Mse*I-CAG	33	12

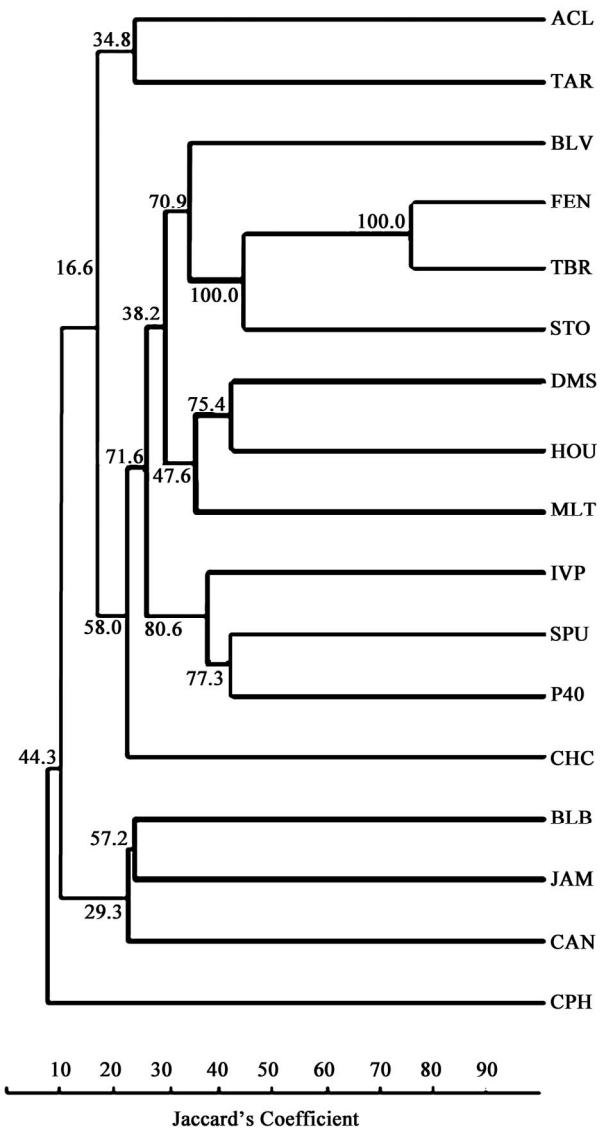

Figure 1. Unrooted dendogram built on the basis of UPGMA clustering of AFLP markers. The similarity on the x-axis is based on Jaccard's coefficient. Bootstrap values are reported at eachcluster node.

Table 4. Estimated length for each region of Gro1 paralogue sequences available in GenBank.

Accession N° (Gene)	Region length (bp)									Spliced RNA length	Unspliced RNA length
	5' UTR	**TIR**	**I intron**	**NBS**	**II intron**	**LRR**	**III intron**	**IV exon**	**3' UTR**		
AY 196151 (*Gro* 1-4)	93	496	5465	1095	76	1337	115	479	104	3604	9260
AY 196152 (*Gro* 1-5)	96	496	875	1095	76	1340	142	431	272	3730	4823
AY 196153 (*Gro* 1-2)	107	496	12092	1095	76	1337	142	479	272	3786	16096
AY 196154 (*Gro* 1-3)	78	512	946	1094	76	1337	144	514	n.d.	n.d.	n.d.
AY 196155 (*Gro* 1-6)	93	496	403	1094	76	1330	158	491	n.d.	n.d.	n.d.
AY 196156 (*Gro* 1-8)	n.d.	n.d.	n.d.	1095	76	1337	142	479	278	n.d.	n.d.
AY 196157 (*Gro* 1-11)	102	496	5199	1093	76	1284	142	479	272	3726	9143
AY 196158 (*Gro* 1-14)	n.d.	n.d.	n.d.	797	76	2266	82	509	n.d.	n.d.	n.d.

n.d.: the length of the region could not be estimated as no alignment was found with the corresponding region ends of Gro1-4.

Table 5. Primers used for each paralogue-specific SCAR marker. Melting temperature (Tm) used in PCR experiments is reported in column 4 as well as expected fragment size in column 5.

Paralogue	**Primer Code**	**Primer Sequence 5'-3'**	**Tm (℃)**	**Product length (bp)**
Gro 1-2	g1-2promF	atatagtgttagtgtgcttgg	56,0	299
	g1-2promR	cttatctcgcggtctaagtc		
Gro 1-3	g1-3IIIiF	cccgcatgaaaatataaatg	51,2	544
	g1-3IIIiR	ttgagattgtaaccgatatc		
Gro 1-4	4RNA2f*	tctttggagatactgattctca	54,7	602
	4RNA2r*	cgacctaaaatgaaaagcatct		
Gro 1-5	G1-5IiF	ctctatttttatttctgcgatgaac	56,4	127
	G1-5IiR	ggtatactccttttttcatctttac		
Gro 1-6	g1-6IVF	aatgtcgaatgatcccttca	54,2	202
	g1-6IVR	gagcaggcaataacttccaa		
Gro 1-8	g1-8TIRF	catgattacgaaatggactc	53,2	315
	g1-8TIRR	tttgatccagatgattgtcg		
Gro 1-11	g1-11p40promF	atgtaattccacaagtgagg	53,2	264
	g1-11p40promR	tttgcattagagcttcgtag		
Gro 1-14	g1-14nbsF	aataggcgtcagctcagtgc	57,4	190
	g1-14nbsR	tatgctcggccttaattgga		

Analysis was run on 15 *Solanum* wild species, on the cultivar 'Spunta' and the clone P40. All primer pairs were built to amplify only a fragment for the target paralogue and had no other amplification products in the positive control genotype P40. In some cases, faint amplified fragments of different size were attained, albeit not scored, because following sequencing, they did not exhibit sequence homology to any *Gro*1 paralogue. In other cases, clear amplified fragments of different size were attained and sequenced. They corresponded to *Gro*1 genes but exhibited INDEL mutations when compared to the target paralogue; consequently, a similarity value closer to other paralogues rather than to target one was obtained by BLAST analysis. Indeed, these mutations did not allow the specific paralogue of the cluster to be clearly identified (data not shown). Hence, these fragments were not scored either.

The PCR results are shown in **Figure 3**, where for each species the presence (value 1) or absence (value 0) of the expected amplified fragment is reported in tabular form. Some fragments were present in all or most of the wild species analysed and some proved to be only present in one or few species. In particular, *Gro*1-8 SCAR was the most common one, being present in all the 17 analysed genotypes, followed by *Gro*1-14 SCAR (present in 16 genotypes). By contrast, *Gro*1-4 SCAR was present only in clone P40, followed by *Gro*1-6 SCAR (present in 4 genotypes). The data were subjected to cluster analysis and the dendrogram shown in **Figure 3** was built as described in the methods. Cluster analysis highlighted two groups of identities. The first includes *S. canasense*, *S. hougasii* and *S. tuberosum* subsp. *andigena*, which all lacked the *Gro*1-4 and *Gro*1-6 SCARs. The second group comprised *S. boliviense* and *S. stoloniferum*, which both lack *Gro*1-3, *Gro*1-4, *Gro*1-6 and *Gro*1-11 SCARs. This clustering is not consistent with that produced by AFLP analysis.

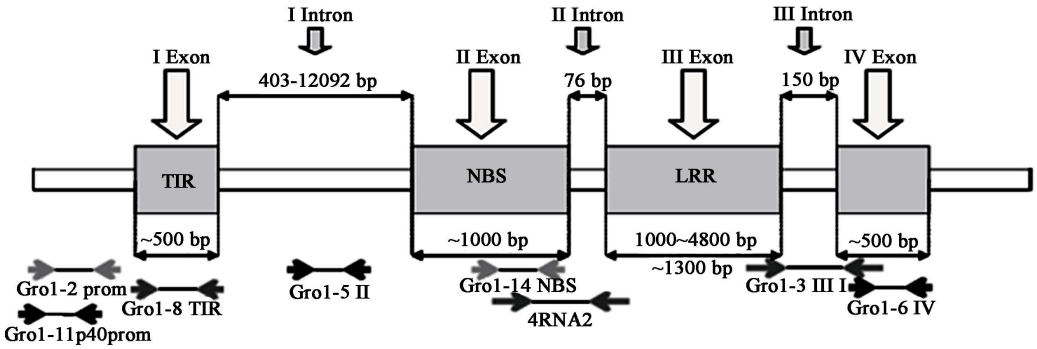

Figure 2. Exon/Intron organization of Gro 1 genes with the position of designed SCAR primers.

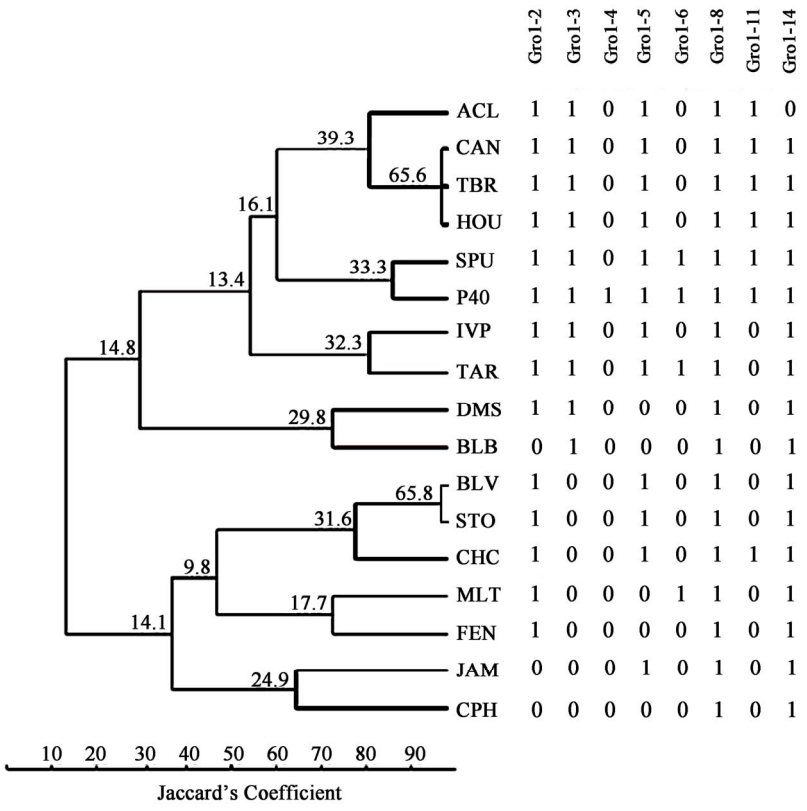

Figure 3. Unrooted dendogram built on the basis of UPGMA clustering of eight Gro 1 paraloguespecific SCAR markers. The similarity on the x-axis is based on Jaccard's coefficient. On theright-hand side of the figure the presence (1) or absence (0) of each SCAR marker is reported foreach genotype. Bootstrap values are reported at each cluster node.

4. Discussion

Characterization of variability among plant germplasm is a fundamental preliminary activity for plant breeding. While phenotypic variability has been characterized for centuries, the present-day challenge is to ascertain the relationship between genotypic and phenotypic variability in order to improve plant breeding programmes. With regard to phenotypic aspects, in the current study we observed resistance variability to *Globodera rostochiensis* pathotype Ro2 among 15 wild *Solanum* species. Interestingly, some *Solanum* species suppressed nematode reproduction, partially confirming the data of Hanneman and Bamberg [26]. In five of the 15 species tested, pathotype Ro2 had a reproduction rate < 1. The species *S. tuberosum* subsp. *andigena* is the most interesting because it suppressed nematode reproduction rates of pathotype Ro2 (0.6) and there were only 80 eggs per cyst of pathotype Ro2 compared to 153 in the control cv. Spunta. This wild species also exhibited a very low reproductio rate (0.3) in comparison with Spunta (10.3), when tested against pathotype Ro1 (data not shown). Therefore, this clone is promising for breeding programmes for resistance to pathotype Ro2 of *G. rostochiensis*. However, assessments of its response to other pathotypes of this cyst nematode and of *G. pallida* should also be made. Also, the species *S. bulbocastanum, S. jamesii* and *S. stoloniferum* should be further investigated, since they showed both a low reproduction rate and a reduced number of eggs per cyst with respect to the control cv. Spunta. Plant material in this work is also particularly suitable for an allelic characterization study and consequent phylogenetic elaborations since it consists of a pool of wild species of various geographical origins. All the material belongs to the *Solanum* genus, but different subgenera are represented and different polymorphism levels are detectable according to the various subgroup of material considered.

AFLP cluster analysis confirmed that the species considered are uniformly distributed on the genus tree as they showed almost uniform similarity coefficients, most of them lying between 30% and 50%. Eight of the 15 wild species had been previously characterized in more than one accession by AFLP analysis [27]. Although neither the clones analyzed in our work (different accession numbers) nor the restriction enzymes used were the same, the main structure of the cladogram found by Spooner et al. [27] was overall confirmed.

Besides genome-wide characterization of these species, locus-specific analysis of one resistance gene was also undertaken. In fact, the first step to improve the genetic background of potato cultivars through interspecific hybridization is to identify and characterize sources of resistance. In most cases, resistance depends on pathogen recognition by plant resistance factors and the specificity of the recognition is given from the interaction between R genes and Avr genes. These are usually involved in hypersensitivity response (HR) [28]. Due to their function, resistance genes typically undergo swift changes and continuously evolve, usually much faster than other gene classes. Their rapid evolution is mainly due to environmental factors: pathogens rapidly overcome acquired plant resistance, such that the plant evolutionary process accelerates to combat pathogen infection strategies [29]. The way in which resistance genes evolve and change has long been studied: it is widely stated that resistance genes are grouped into gene clusters containing several paralogue genes [30], as is the case of *I*2 [31], *Mi*-1 [32], *I*3 [33], *Gro*1 [15]. One of the most frequent gene cluster configurations is that of the gene *Xa*21 [34], where a functional gene is organized as a cluster with non-functional paralogues and truncated sequences. The *Gro*1 cluster could be similar, with the *Gro*1-4 functional gene linked to non-functional paralogues and gene fragments. This is consistent with the hypothesis that clusters could represent resistance gene storage and that frequent gene exchanges in the cluster lead to a new resistance strategy [35].

In order to characterize the 15 *Solanum* species at the *Gro*-1 locus, in the present study specific primers for each of the paralogues were constructed exploring the variability of different functional domains (TIR, NSB, LLR) and introns of the resistant allele *Gro*1-4, whose sequences are available in GenBank. Bioinformatic analysis of the P40 *Gro1* gene cluster by means of CLUSTAL-W alignment showed a very conserved region spanning NBS and LRR domains of the paralogues, but other regions of similarity could not be identified due to large insertions and repeated regions. In any case, the primers designed on the basis of these bioinformatic results allowed the presence/absence of each paralogue to be verified in each species analysed. As for the resistance gene *Gro*1-4, no genotype produced fragments like P40 specifically designed to amplify *Gro*1-4, not even those that exhibited resistance. This resistance, in fact, is probably due to genes other than *Gro* 1-4, as already reported in the literature [12,10]. Sequencing of the whole cluster *Gro*1 has been started in our laboratory in order to highlight the role of this cluster in nematode resistance of *Solanum tuberosum* subsp. *andigena* species.

The cluster analysis of SCAR results underlined the high similarity between *S. canasense*, *S. hougasii* and *S. tuberosum* subsp. *andigena* and between *S. boliviense* and S. *stoloniferum.* As for the first group, the species *S. canasense* showed a good level of resistance to pathotype Ro2, as well as *S. tuberosum* subsp. *andigena*. These two

species also shared the SCAR pattern of *Gro* 1 paralogues. Therefore, a sequence analysis of *Gro* 1 locus also for *S. canasense* is also desirable, since it could lead to the definition of which paralogue could be the putative resistance gene to pathotype Ro2. Inconsistency between the two unrooted dendrograms was expected since evolution of R genes is strongly driven by environment so that very different genomes can have very similar resistance traits and *vice-versa* [30].

In conclusion, molecular differences within 15 wild potato species were explored by generating AFLP fingerprints and SCAR profiles. Our study reveals a new set of markers that distinguish eight paralogues of the *Gro* 1 locus, potentially suitable for mapping, MAS and cloning purposes. These could represent a useful tool for genetic and breeding studies, if an association of these markers with the resistance trait can be confirmed [4]. For this purpose, the sequencing of the whole *Gro* 1 locus in the resistant species is necessary, as well as confirming of this resistance also in different environments.

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