Theor Appl Genet (2000) 101:902–906 

**S. Micheletto · R. Boland · M. Huarte**

# Argentinian wild diploid *Solanum* species as sources of quantitative late blight resistance

Received: 30 August 1999 / Accepted: 30 December 1999

**Abstract** Greenhouse and field evaluations of quantitative resistance to late blight in genotypes of the wild Argentine diploid species *Solanum chacoense, Solanum commersonnii, Solanum microdontum* and *Solanum maglia* were performed with a complex race containing the virulence factors 1, 3, 4, 5, 7,10, 11 of *Phytophora infestans* and using the percentage of leaf area affected by late blight estimated at weekly intervals. From these readings the area under the disease progress curve (AUDPC) was calculated. Highly resistant and susceptible genotypes were identified. Resistance and variability were found in *S. chacoense,* which together with *S. commersonnii* showed a significantly higher level of resistance compared to the susceptible checks. Field evaluations of an $F_1$ progeny of 165 individuals, obtained from non-inbred diploid parents segregating for foliage quantitative resistance in *S. chacoense,* were also done by means of AUDPC. The polygenic nature of the resistance and some indication of the presence of both additive and interaction effects were evident.

**Key words** Potato late blight · Quantitative resistance · *Phytophthora infestans* · *Solanum*

## Introduction

Potatoes can be severely affected by late blight, a fungal disease caused by *Phytophthora infestans* (Mont.) De Bary, which can kill the foliage of the crop in a short time. Chemical crop protection is not only potentially hazardous and expensive, but is losing its efficacy as *P.infestans* has developed resistance to the leading fungicides. A valuable alternative control measure would be the use of resistant potato cultivars.

Genetic control of late blight in the foliage can be race-specific, controlled by the dominant alleles of *R* genes, but can also be effected through the action of general resistance which is considered to be more durable and based on polygenes (Umaerus and Umaerus 1994; Wastie 1991). In potato, 11 *R*-genes (*R*1–*R*11) have been found (Malcolmson and Black 1966; Shaw 1991) and four of them have been localized on the genetic map of potato (Leonards-Schippers et al. 1992; El-Kharbotly et al. 1994; Meksen et al. 1995). However, their immunity reaction can be easily overcome by the appearance of new virulent factors. Breeding efforts are directed to the increase of horizontal or quantitative resistance which is probably more durable than race-specific resistance. Quantitative resistance may also represent less pressure on the fungus to evolve into more virulent strains. The combined action of the two types of resistance may be desirable under practical breeding, although knowledge of the separate effects of each of them is of paramount importance for both scientific and practical purposes.

*Solanum tuberosum* (2n = 4*x* = 48) with two subspecies, *tuberosum* and *andigena*, is the most common cultivated potato. Quantitative resistance to late blight in the current breeding material of *S. tuberosum* is scarce. However, wild *Solanum* species are a known source of resistance to a wide range of pests and diseases. They constitute the most important part of this genus, with more than 228 species, and their ploidy ranges from diploid (2n = 2*x* = 24) to hexaploid (2n = 6*x* = 72). Another advantage of the use of the diploid species is that they facilitate the genetic analysis of polygenic characters (Rasmussen et al. 1998).

Genome analysis based on DNA polymorphism can reveal the genetic determinants of complex phenotypes and provide tools to maximize selection response. Molecular markers to assist the breeding for durable resistance to late blight can be useful as a substitute for disease screening and to select for disease-resistant QTLs. Molecular approaches have a great impact on under-

Communicated by G. Wenzel

S. Micheletto · M. Huarte (✉)
EEA INTA Balcarce, CC276, (7620) Balcarce, Argentina

S. Micheletto · R. Boland
Universidad Nacional del Sur, Dpto. Biología,
Bioqca. y Farmacia. San Juan 670,
(8000) Bahía Blanca, Argentina

standing resistance genes and also on molecular mechanisms of pathogenicity and virulence (Michelmore 1995). Additionally, the creation of an appropriate base populations is a prerequisite to make faster progress with the development of efficient markers.

This paper reports the results of greenhouse and field evaluations of quantitative resistance to late blight in genotypes of the wild Argentine diploid species *Solanum chacoense, Solanum commersonnii, Solanum microdontum* and *Solanum maglia*. Field evaluations of the $F_1$ progeny obtained from non-inbred diploid parents segregating for foliage quantitative resistance in *S. chacoense* is also presented.

## Materials and methods

### Fungal material

A complex race with the virulence factors 1, 3, 4, 5, 7,10 and 11 of *P. infestans* was provided by Marcela Van Damme (INTA-UNMDP). The isolates were cultured on tuber slices of the susceptible cultivar Bintje, at 100% HR under continuous low-intensity fluorescent illumination. The inoculum was prepared by rinsing the pieces of tubers with the sporulating fungus in sterilized water. The concentration of the suspension was determined by an haemocytometer at $4 \times 10^4$ sporangia/ml, both for greenhouse and field inoculations.

### Plant material

Genotypes of *S. microdontum*, *S. commersonii*, *S. chacoense* and *S. maglia* were inoculated with suspensions of the *P. infestans* complex and non-virulent ($R_0$) races, both in detached leaves and in the greenhouse, in order to identify genotypes with presence of *R* genes as has been reported in a previous paper (Micheletto et al. 1999). The material that showed compatibility with both races was assumed to be free of *R* genes and was used for this work. Details of the materials are given in Table 1.

### Greenhouse evaluations

Whole plants of 47 genotypes belonging to 16 accessions of *S. microdontum, S. commersonii, S. chacoense* and *S. maglia* free from *R* genes were inoculated with a suspension of the *P. infestans* complex race. Three to four 7-weeks-old plants were evaluated per genotype. High humidity and low temperatures were maintained inside the greenhouse. Seven days after inoculation, the intensity of foliage blight caused by *P. infestans* was measured by assessing the overall amount of necrotic tissue per plant on a Malcomson 1–9 scale (Cruickshank et al. 1982). All plants that obtained a score of 5–9 in the Malcomson scale were defined as resistant, while plants that obtained a lower score were considered susceptible.

### Field evaluations

The 1996/97 trial in the field consisted of two randomized blocks. Five plants per clone were planted in each plot. Forty seven genotypes of the wild species *S. chacoense, S. commersonii, S. maglia* and *S. microdontum* were also planted. Twenty eight of these genotypes were evaluated previously in the glasshouse. The plots were surrounded by the susceptible cultivars Bintje and Serrana INTA (infectors) to ensure a uniform natural infection. Disease progress was secured by artificial irrigation. Once the symptoms appeared, the percentage of leaf area affected by late blight was estimated at weekly intervals. From these readings the area under the disease progress curve (the AUDPC) was calculated according

**Table 1** Germplasm data from the INTA Balcarce Bank of the species *S. microdomtum* (*mcd*), *S.chacoense* (*chc*), *S.commersonii* (*cmm*) and *S. maglia* (*mgl*)

| Species | subspecies | Accession id. | Number of genotypes | Collection site | Elevation m.a.s.l[a] | Colection [a] form/eval.site |
|---|---|---|---|---|---|---|
| *mcd* | *gigantophyllum* | SCL 4611 | 2 | Catamarca Andalgalá | 1820 | No data/grh |
| *chc* | No data | ORHL 4822 | 3 | Salta R.de Lerma | 1600 | f/grh-fld |
| *chc* | No data | OKA 5611 | 1 | Salta R.de Lerma | 2150 | f/grh-fld |
| *chc* | No data | OKA 7309 | 4 | Corrientes San Cosme | 60 | f – t/grh |
| *chc* | No data | OKA 7497 | 2 | Salta Chicoana | 2700 | f/grh |
| *chc* | No data | CLALO 435 | 6 | Bs.Aires Pipinas | No data | No data/grh-fld |
| *cmm* | *commersonii* | OKA 5138 | 3 | Bs.Aires Pueyrredón | 30 | f/grh |
| *cmm* | *malmeanum* | OCL 7256 | 6 | Entre Ríos Gualeguaychú | 20 | f/grh-fld |
| *cmm* | *malmeanum* | OCL 7282 | 6 | Corrientes P.dlos Libres | 50 | No data/grh-fld |
| *cmm* | *malmeanum* | OCL 7291 | 3 | Entre Ríos Feliciano | 64 | f/grh |
| *cmm* | *malmeanum* | OCL 7292 | 5 | Entre Ríos Feliciano | 62 | f/grh |
| *cmm* | No data | OKA 7313 | 2 | Entre Ríos Paraná | 74 | f/grh |
| *mgl* | No data | CLM 865 | 1 | Mendoza | 1628 | t/grh-fld |
| *mgl* | No data | CLM 868 | 1 | Mendoza | 1770 | t/fld |
| *mgl* | No data | CLM 869 | 1 | Mendoza | 1780 | t/grh-fld |
| *mgl* | No data | CLM 870 | 1 | Mendoza | 1820 | t/grh-fld |
| *mgl* | No data | CLM 871 | 1 | Mendoza | 1789 | f/grh-fld |

[a] Height in meters above sea level
[b] Collection form: f = fruits; t = tubers; Evaluation site: grh = greenhouse; fld = field

to the method of Shaner and Finney (1977). An analysis of variance was run on the AUDPC values obtained.

The 1997/98 trial consisted of only one block, with similar plot conditions as in the previous year, in order to assess the year × genotype interaction. In this experiment, 35 of the genotypes indicated above, belonging to *S. chacoense*, *S. commersonii* and *S. maglia*, were evaluated. Records of the AUDPC were taken as above.

The parents and 165 genotypes of a *S. chacoense* segregating population obtained from a cross between a highly resistant genotype (OKA 5613.05) and a susceptible one (CLALO 435.08) were evaluated under field conditions during the 1997/98 season; 2–3 tubers per genotype were grown. Evaluations of late blight resistances were performed in the same way as indicated above and the AUDPC was calculated as an estimate of quantitative resistance.

## Results

### Screening for *P. infestans* quantitative resistance in four Argentinian wild species

All genotypes showed the disease after inoculations in the greenhouse. Of the 47 genotypes evaluated, 13 *S. commersonii*, ten *S. chacoense* and two *S. microdontum* showed high scores and were considered as resistant; 12 *S. commersonii*, six *S. chacoense*, three *S. maglia* and the susceptible control, Serrana, showed the lowest values and were considered susceptible. The results are presented in Table 2.

The results of the statistical analysis using the calculated variable AUDPC for the 1996/97 field evaluation showed high levels of variability among the evaluated species, as well as within each species (Table 3). Differences among genotypes within each accession were also important. In *S. commersonii*, accession OCL 7256 showed genotypes 1, 6 and 8 as susceptible while genotypes 2, 7 and 9 performed as resistant; in OKA 7292 genotypes 1, 2 and 4 were susceptible and genotypes 5 and 9 were resistant. In *S. chacoense*, CLALO 435 showed only one genotype as resistant while all the other five genotypes evaluated behaved as susceptible; a similar situation was observed in accession OKA 7302 where three resistant genotypes were found among the four tested. Therefore, accession specificity in relation to the proportion of resistant and susceptible genotypes is a relevant factor in the screening for horizontal resistance.

Resistance and variability were found in *S. chacoense*, and all three species showed a significantly higher level of resistance compared to the susceptible checks. *S. maglia* was not included in this analysis because of the low number of individuals assessed, and no variability was observed among them, all behaving as susceptible. The possibility that the individuals from *S. maglia* belong to a same genotype is currently being confirmed (A. Clausen, personal communication).

Table 4 shows the analysis of variance for 35 genotypes from *S. commersonii*, *S. chacoense* and *S. maglia* in two seasons of field evaluation for quantitative resistance. No genotype × year interaction was detected, and the behavior of them was consistent between years. The means for the two seasons are represented in Fig. 1, in which the lack of interaction is also evident.

From the results obtained with greenhouse and field evaluations, highly resistant and susceptible genotypes were identified.

**Table 2** Greenhouse screening for quantitative resistance to late blight in four wild *Solanum* species from Argentina. R: resistant; S: susceptible

| Species | Number of genotypes | | Frequency | |
|---|---|---|---|---|
| | R | S | R | S |
| *mgl* | 1 | 3 | 0.25 | 0.75 |
| *cmm* | 13 | 12 | 0.52 | 0.48 |
| *chc* | 10 | 6 | 0.63 | 0.38 |
| *mcd* | 2 | 0 | 1 | 0 |

**Table 3** Variability among and within species and the Duncan Multiple Range Test for AUDPC under field conditions in 1996/97

| Species | Mean square | *Pr > F* | Mean | Duncan grouping[a] |
|---|---|---|---|---|
| Serrana, Bintje[b] | | | 1977.49 | a |
| *cmm* | 37 171 | 0.0017 | 1513.98 | b |
| *chc* | 3 490 124 | 0.0001 | 723.98 | c |
| *mcd* | 1 507 517 | 0.0001 | 204.60 | d |

[a] Means with the same letter are not significantly different
[b] Susceptible controls

**Table 4** Analysis of variance for AUDPC in field evaluations in the 1996/97 and 1997/98 for 35 genotypes from three wild *Solanum* species from Argentina

| Source of variation | *df* | Mean square | *F* |
|---|---|---|---|
| Year | 1 | 2 835 882 | 7.589** |
| Species | 2 | 4 605 999 | 12.326** |
| Interaction (year × species) | 2 | 157 785 | 0.422 NS |
| Residual | 46 | 373 676 | |

### Field evaluation for resistance to late blight in a *S. chacoense* segregating population

The AUDPC distribution of the 165 individuals generated from the cross between the highly resistant genotype OKA 5613.05 and the susceptible one CLALO 435.08, both *S. chacoense* clones, is shown in Fig. 1. The distribution was normal with a slight deviation of frequencies towards resistance (W: Normal 0.900771, $P <$W 0.0001). The polygenic nature of the resistance and some indication of the presence of both additive and interaction effects seemed evident.

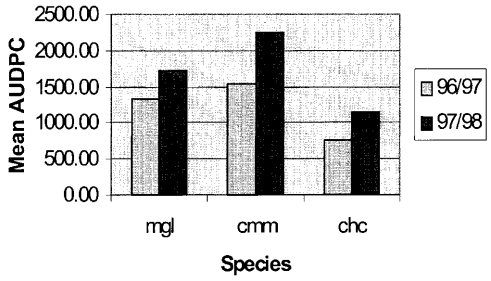


**Fig. 1** Means of AUDPC in two seasons for three wild species of *Solanum* from Argentina

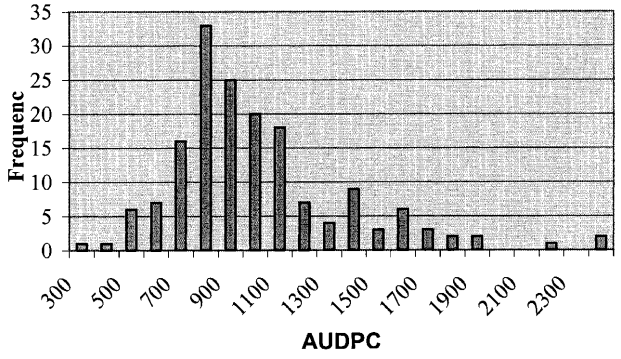


**Fig. 2** *S. chacoense* progeny field evaluation, 1997/1998

## Discussion

*S. chacoense* showed a high frequency of resistant genotypes, with low values of infection both in the greenhouse and the field. Although *S. microdontum* and *S. commersonii* also had resistant clones, the possibility that they bear *R* genes makes them not so interesting for quantitative resistance breeding since variation in quantitative resistance can be masked by segregation for qualitative resistance (Geiger and Heun 1989; Micheletto et al. 1999). The ranking of species in the greenhouse and in the field was similar, although the greenhouse evaluations are proposed only as a preliminary screening. Many methods of screening for resistance to late blight have been described based on detached leaves, whole plant or field tests. Tests appropriate for screening should be sufficiently controlled to give reproducible results and be capable of accurately identifying both highly susceptible and resistant genotypes, as was the whole plant test carried out under controlled conditions in a greenhouse (Stewart et al. 1983). It is also a prerequisite that the resistance identified in a glasshouse test is also reflected in field performance (Caligari et al. 1985), and more credit should probably be given to the field data because this test is more closely related to the conditions under which resistance will be expressed (Stewart et al. 1983).

The parameter used to calculate the resistance from field data (AUDPC) allowed the identification of valuable resistant and susceptible genotypes useful for the construction of segregating mapping populations, considering the lack of interaction between evaluation seasons and species. The AUDPC showed a high variability among and within species (Table 3) which confirms the value of these Argentinian species for resistance breeding.

Several approaches were previously used to determine whether genotypes of different (and also the same) accessions differ from each other with respect to their resistance genes. The selection of dissimilar parents from natural populations of wild potatoes species is likely to provide a large number of segregating markers, suitable for mapping and tagging agronomically important traits such as resistance to late blight. Well-assessed populations for mapping purposes are a current bottleneck. Therefore, focusing on developing mapping populations with quantitative resistance to late blight at the diploid level is fundamental in order to facilitate molecular data analysis. Molecular markers closely linked to quantitative traits can both ease and speed up the selection process of genotypes with durable resistance to late blight in plant breeding programs.

When the *S. chacoense* progeny was grown in the field under natural infection pressure in Balcarce, the plants showed a continuous distribution from high resistance to susceptibility. This observation indicated that it is likely that resistance to late blight is under the control of polygenes when the population has no *R* genes. This species has the advantage of bearing a low frequency of *R* genes, as was determined in both greenhouse and laboratory experiments (Micheletto et al. 1999). It also tuberizes easily in both the field and in the greenhouse. Thus, populations derived from this species segregating for quantitative resistance could have a high value for mapping this type of resistance.

The results of the phenotypic assessment of quantitative resistance should be verified in more locations and include the remaining individuals of the progeny in order to achieve more reproducibility of these data. In this way, the population will be valuable material to develop molecular markers linked with quantitative resistance to late blight.

**Acknowlegements** The authors thank Ms. Marcela Van Damme for helping in the preparation of the inoculum and The International Potato Center (CIP) for providing partial funding to carry out this research. These studies were conducted thanks to the guidance generated under the Global Initiative for Late Blight (GILB).

## References

Caligari PDS, Mackay GR, Stewart HE, Wastie RL (1985) Confirmatory evidence for the efficacy of a seedling progeny test for resistance to potato foliage blight [*Phytophthora infestans* (Mont.) de Bary]. Potato Res 28: 439–442

Cruickshank G, Stewart HE, Wastie RL (1982) An illustrated asessment key for foliage blight of potatoes. Potato Res 25: 213–214

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 *P. infestans* in progeny of dihaploid potato parents. Mol Gen Genet 242: 749–754
Geiger HH, Heun M (1989) Genetics of quantitative resistance to fungal disease. Annu Rev Phytopathol 27: 317–41
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
Malcolmson JF, Black W (1966) New *R* genes in *Solanum demissum* and their complementary races of *Phytophthora infestans* (Mont.) De Bary. Euphytica 15: 199–203
Meksen K, Leister D, Peleman J, Zabeau M, Salamini F, Gebhardt C (1995) A high-resolution map of the vicinity of the *R1* locus on chromosome V of potato based on RFLP and AFLP markers. Mol Gen Genet 249:74–81
Michelmore R (1995) Molecular approaches to manipulation of disease resistance genes. Annu Rev Phytopathol 15: 393–427
Micheletto S, Andreoni M, Huarte M (1999) Vertical resistance to late blight in wild potato species from Argentina. Euphytica 110/2: 133–138
Rasmussen JO, Nepper JP, Kirk HG, Tolstrup K, Rasmussen O (1998) Combination of resistance to potato late blight in foliage and tubers by intraspecific dihaploid protoplast fusion. Euphytica 102: 363–370
Shanner G, Finney RE (1997) The effect of nitrogen fertilization on the expression of slow mildewing resistance on Knox wheat. Phytopathology 67: 1051–1056
Shaw DS (1991) Genetics. In: Ingram DS, Williams PH (eds) *Phytophthora infestans*, the cause of late blight of potato. Advances in Plant Pathology, vol 7. Academic Press, pp 131–170
Stewart HE, Flavelle PH, McCalmont DC, Wastie RL (1983) Correlation between glasshouse and field tests for resistance to foliage blight caused by *Phytophthora infestans*. Potato Res 26:41–48
Umaerus V, Umaerus M (1994) Inheritance of resistance to late blight. In: Bradshaw JE., Mackay GR (eds) Potato genetics. CAB International, Wallingford, Oxford, UK, pp 365–401
Wastie RL (1991) Breeding for resistance. In: Ingram DS, Williams PH (eds) *Phytophthora infestans*, the cause of late blight of potato. Advances in Plant Pathology, vol 7. Academic Press, pp 193–223