

## Short communication. Introgression of late blight (*Phytophthora infestans* L.) resistance from tuber-bearing *Solanum* wild species into cultivated potato

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### Abstract

We have analyzed resistance to *Phytophthora infestans* in five progenies derived from crosses between wild tuber-bearing potatoes (*Solanum okadae*, *Solanum canacense*, *Solanum bukasovii*, *Solanum jamessii* and *Solanum raphanifolium*) and cultivated diploid potato species (*Solanum phureja*, *Solanum goniocalyx* and a dihaploid of *Solanum tuberosum*). Different levels of resistance to *P. infestans*, from 11 to 81%, were found in leaves and tubers in the evaluated progenies. This variation indicates a variable number of major genes (R genes), minor genes and/or defeated R-genes, which have been transferred from the parental genotypes. Correlation analyses between leaf and tuber infection levels were low and not significant.

**Additional key words:** area under disease progress curve; breeding; potato.

### Resumen

#### Comunicación corta. Introgresión de la resistencia a mildiu (*Phytophthora infestans* L.) en la patata cultivada a partir de especies *Solanum* silvestres tuberíferas

Se ha analizado la resistencia a *Phytophthora infestans* en progenies derivadas de cruzamientos entre especies silvestres tuberíferas de *Solanum okadae*, *Solanum canacense*, *Solanum bukasovii*, *Solanum jamessii* y *Solanum raphanifolium* y especies cultivadas diploides de *Solanum phureja*, *Solanum goniocalyx* y un dihaploide de *Solanum tuberosum*. Se han encontrado diferentes niveles de resistencia, entre el 11 y el 81%, tanto en hojas como en tubérculos, en las cinco progenies evaluadas. Esta variación indica un número variable de genes mayores (genes R), genes menores y/o genes R superados, los cuales han sido transferidos de los genotipos parentales. El análisis de correlación de los niveles de infección entre hoja y tubérculo fueron bajos y no significativos.

**Palabras clave adicionales:** AUDPC; área bajo la curva de progreso de la enfermedad; mejora; patata.

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*Solanum* wild species have been used for potato breeding as important sources for improving resistance to diseases and environmental stresses and for introducing other agronomic characteristics of interest (Ruiz de Galarreta *et al.*, 1998; Spooner *et al.*, 2004). Some species from North and Central America have contributed to resistance against important potato diseases as for example against late blight caused by

the oomycete *Phytophthora infestans* (Song *et al.*, 2003). In the early twentieth century the wild tuber-bearing potato species *Solanum demissum* was identified as resistance source to *P. infestans* and introgression of resistance genes began through crosses. Since 1920, many scientific expeditions to Central and South America, representing centres of origin and biodiversity of potato, allowed to collect and describe over 200

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Abbreviations used: AUDPC (area under the disease-progress curve), *buk* (*S. bukasovii*), *can* (*S. canacense*), CV (coefficient of variation), EBN (endosperm balance number), *gon* (*S. goniocalyx*), *jam* (*S. jamessii*), *oka* (*S. okadae*), *phu* (*S. phureja*), *rap* (*S. raphanifolium*), *tbr* (*S. tuberosum*).

wild species and eight cultivated species (Hawkes, 1990). Several breeders found that the use of wild species as progenitors is tedious, difficult and it takes a long time to get useful pre-breeding material. This may be due to the fact that European and North American breeders work mainly with *S. tuberosum* ssp *tuberosum* and their haploids. The fertility and genetic viability of this species is very narrow and this problem becomes even more critical when crossing with wild species (García *et al.*, 2007). One exception has been the work of Peloquin *et al.* (1989), who obtained dihaploids of *S. tuberosum* with *S. phureja*. Also Glendining (1975) generated «neotuberosum» cultivars from crosses between *S. tuberosum* ssp. *tuberosum* and *S. tuberosum* ssp. *andigena* and several generations of selection for adaptation to the long days in the northern hemisphere. In contrary, South American breeders have the great advantage of high flexibility of ploidy present in different cultivated, so called «native» potato species which do not belong to *S. tuberosum* (Gabriel *et al.*, 1995, 2007; Estrada, 2000). *S. canasense* (*can*) is a wild species widespread in Peru and Bolivia. This species has been described as a valuable resistance source for *Streptomyces scabies* (Hosaka *et al.*, 2000), for the cyst nematode *Globodera pallida* (Castelli *et al.*, 2006) and for insects. *S. jamesii* is distributed between Mexico and USA and has certain frost tolerance. *S. raphanifolium* (*rap*) is mainly distributed in Peru and is considered as a natural hybrid between *S. canasense* and *S. megistacrolobum* (Spooner and Hijmans, 2001). Pérez *et al.* (2002) reported high resistance to *P. infestans* in *S. raphanifolium* and the presence of qualitative as well as quantitative resistance.

The hybridisation potential of potato depends on the ploidy level and the endosperm balance number (EBN). *S. tuberosum* has a ploidy EBN = 4x (4EBN), and its hybridization potential is highest with species 4x (4EBN) and 6x (4EBN) and hardly hybridises with species 4x (2EBN) and 2x (2EBN) (Spooner and Hijmans, 2001). On the other hand, crosses with wild species involve also the introgression of many unwanted characters and require multiple cycles of backcrosses and selection to remove these characteristics. Some native cultivated potato species from the Andes have high genetic variability, show resistances to abiotic stresses such as frost and drought, to biotic stresses caused by fungi, bacteria and viruses and contain also useful genes for quality traits.

The objective of this work was to evaluate the resistance to *P. infestans* in leaves and tubers of five potato

progenies derived from crosses between *Solanum* wild species and cultivated native potato species.

The wild species *S. okadae* (*oka*), *S. canasense*, *S. bukasovii* (*buk*), *S. jamesii* (*jam*) and *S. raphanifolium* were used as parental resistance sources against *P. infestans*. Controlled crosses were performed with *S. phureja* (*phu*), also partially resistant, with *S. gonniocalyx* (*gon*) and with a diploid of *S. tuberosum* (H88.31/34) accession and several progenies were obtained. A total of 367 leaflets and 294 tubers from progenies *oka*498063.6 × *phu*81 (C), *can*310956.8 × *phu*81 (D), *buk*210042.5 × *phu*81 (E), *jam*27521.48 × *gon*703354 (G) and H88 (H88.31/34, *tbr*) × *rap*636 (N) were evaluated for resistance to *P. infestans*. Resistance evaluations were performed in greenhouse and laboratory. Leaflets of young progeny genotypes were inoculated with spores of a complex local isolate. Four leaflets of each genotype were distributed in Petri dishes of 10 cm diameter in a completely randomised design. Two leaflets were placed in each Petri dish, using a total of 742 Petri dishes, including controls. Infection levels were evaluated 5, 6 and 7 days after inoculation. The percentages of necrotic or sporulating areas were assessed and the AUDPC (area under the disease-progress curve) values were calculated based on the sporulation intensities according to Gabriel *et al.* (2007). AUDPC (Bonierbale *et al.*, 2008) reflects the advance of the disease in time, so that families can be statistically compared. The relative AUDPC (AUDPCrel) is calculated by dividing AUDPC by total peak area and then standardized using the square root normal factor  $\sqrt{x+1}$ , to fit to a normal distribution.

Potato tubers were inoculated with the same isolate as used for leaf infections, following the methodology of Flier *et al.* (2001). Four tubers per genotype were placed in wet boxes which were distributed in a completely randomized design. The percentage of the infected surface of each tuber was determined 8, 11 and 14 days after inoculation. Analyses of variances and comparison of means for infection levels in progenies were performed using Proc GLM of SAS Software (SAS, 2000).

Distributions of infection levels were never adjusted to normal distributions ( $p < 0.5$ ), so that data were transformed as described above. All populations showed an asymmetric distribution for AUDPCrel towards right or left and a negative excess kurtosis ( $< 3$ ). AUDPCrel values in leaves of all families showed a coefficient of variation (CV) of 7.42% and the combined ANOVA was highly significant ( $p < 0.01$ ) for progenies.

**Table 1.** Average relative AUDPC values for *Phytophthora infestans* infections in leaves and severity of tuber infection (%) in five progenies. Least square means with the same letter are statistically not different ( $p < 0.05$ ) according to tests of equality of means with Proc GLM (SAS, 2000)

Progeny <sup>1</sup>	Male parent	Female parent	AUDPCrel <sup>2</sup>	Severity (%)
G	<i>jam</i> 27521.48	<i>gon</i> 703354	0.18a	10.77b
D	<i>can</i> 310956.8	<i>phu</i> 81	0.19ab	6.78a
C	<i>oka</i> 498063.6	<i>phu</i> 81	0.20ab	7.34a
E	<i>buk</i> 210042.5	<i>phu</i> 81	0.22b	12.38b
N	<i>H88.31/34</i>	<i>rap</i> 636	0.33c	11.28b

<sup>1</sup> G, *jam*27521.48 × *gon*703354; D, *can*310956.8 × *phu*81; C, *oka*498063.6 × *phu*81; E, *buk*210042.5 × *phu*81; N, *H88-31/34* × *rap*636. <sup>2</sup> The relative AUDPC (area under the disease-progress curve) is calculated by dividing AUDPC by total peak area and then standardized using the square root normal factor  $\sqrt{x+1}$  to fit to a normal distribution.

Table 1 shows the differences between least square means for average leaf and tuber resistance in the families. The highest average resistance level in terms of AUDPCrel in leaves was observed in progeny G (*jam* × *gon*), but was not different from those of progenies D and C. The significant lowest level was detected in progeny N (*H88* × *rap*).

Separate analyses of variances were performed for each progeny. In all families the ANOVA was significant for progeny genotypes. The ANOVA of progeny *oka* × *phu* (C) for example showed highly significant differences ( $p < 0.01$ ) between genotypes, with a CV of 2.44% and a coefficient of coincidence ( $R^2$ ) of 84%. These parameters indicate an appropriate adjustment to the model used for the analysis.

Progeny genotypes were classified corresponding to relative AUDPC classes into resistant (<0.21), moderate resistant ( $\geq 0.21$  and <0.35) and susceptible genotypes ( $\geq 0.35$ ), according to Gabriel *et al.* (2007). Figure 1 shows the relative frequency distributions of progeny genotypes within AUDPC classes in the five progenies. Large variation of resistance patterns between families can be observed. In progenies D, G and E we can see an excess of resistant genotypes ranging from 68 to 81% and, which is shifted towards more moderate resistant genotypes in progeny C (*oka* × *phu*). In this progeny 44.0% of the genotypes were resistant, 46% moderately resistant and 10% susceptible genotypes. In contrary, in progeny *H88* × *rap* (N) only 40% of the genotypes were resistant, 11% moderately resistant and 49.0% susceptible (Fig. 1).

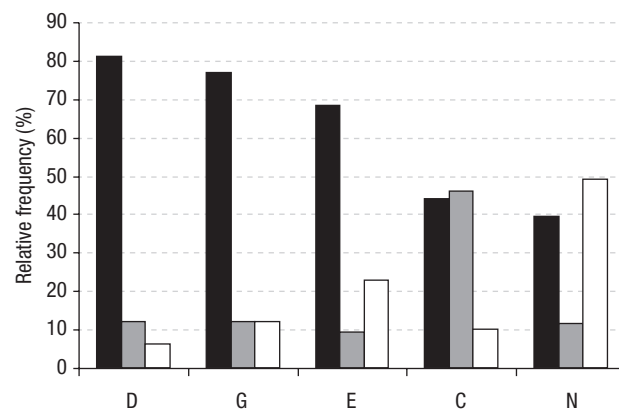
Tuber infection values were also transformed as described above in all families, since infection values did not adjust to normal distributions ( $p < 0.05$ ). The CV values oscillated between 22.2 and 47.9% in the families.

Analysis of variance for tuber infection showed also highly significant differences ( $p < 0.05$ ) between progenies.

Least square mean differences (Table 1) indicated that average tuber infection levels were lowest in progenies D and C (tuber infection severities of 6.78 and 7.34%, respectively) and not significantly different ( $p < 0.05$ ). Tuber infection severities in progenies G, N and E were significantly higher ranging between 10.77 and 12.38%, but without statistical differences between them.

Correlation coefficients between leaf and tuber resistance levels in the five progenies were low (Table 2), ranging from -0.069 to 0.328, and were never significant ( $p < 0.05$ ).

We used in our study new resistance sources to *P. infestans* in form of diploid wild species *jam*, *can*, *oka*, *buk* and *rap*. *S. okadae* is a wild tuber bearing species, which is distributed between Bolivia and Argentina (Coca and Montealegre, 2006; Patiño *et al.*, 2007; Saffarano *et al.*, 2008). In the present study genotypes



**Figure 1.** Frequency distributions of resistant (black), moderate resistant (grey) and susceptible genotypes (white) in classes of relative AUDPC values for each evaluated family.

**Table 2.** Correlation coefficients between resistance to *Phytophthora infestans* in leaves and tubers in five potato progenies (C, D, E, G, N).

	AUDPCrel				
	C	D	E	G	N
Severity of tuber infections (%)	-0.069 <sup>1</sup>	-0.226	0.328	-0.257	0.071
	0.742 <sup>2</sup>	0.325	0.298	0.273	0.598

<sup>1</sup> Pearson correlation coefficient. <sup>2</sup>  $Prob > |R|$  under  $H_0: Rho = 0$ .

from our progeny C showed good levels of leaf resistance. Also Barquero *et al.* (2005) found in somatic and sexual hybrids involving the wild species *S. bulbocastanum*, *S. circaeifolium* and *S. okadae*, high levels of resistance to *P. infestans*. However, evaluations of tuber resistance have not been reported previously for *S. okadae*. Hawkes (1990) reported also resistance to *P. infestans* in *S. canasense*.

The wild species *S. bukasovii* (progeny E) is distributed mainly between Bolivia and Argentina (García *et al.*, 2007) and has been described as resistant to *P. infestans* by Hawkes (1990). The predominance of genotypes with high resistance to *P. infestans* in foliage as well as in tubers in progeny G may suggest the presence of R genes.

Our results are in agreement with the observed resistances in these wild species and show that it is possible to transfer resistances from these species to cultivated potato through crosses. The variation of resistance levels observed in the progenies, indicate a variable number of major genes (R genes), minor genes and/or defeated R-genes, which have been transferred from the parental genotypes. Missing correlations between leaf and tuber resistance suggest that different resistance factors and mechanisms exist for these resistance types, which are probably controlled by different genes. Also for breeding separate selections for *P. infestans* resistance in leaves and tubers are required.

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