Running title: TESTING CULTIVAR RESISTANCE TO P. INFESTANS

In vitro evaluation of resistance of potato cultivars to *Phytophthora infestans*

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Summary

The resistance of ten potato cultivars (Agria, Ajax, Désirée Liseta, Kennebec, Majestic, Monalisa, Prima, Spunta and Tonda di Berlino) to *Phytophthora infestans* was analyzed in vitro using 8 fungal strains. An assay based on electrolyte leakage was used for screening leaves and tuber tissues with fungal culture filtrates. With almost all cultivars the resistance of leaves did not correlate with the resistance of tubers. Cv. Ajax appeared the least susceptible in both leaf and tuber tests, while the cv. Prima was the most susceptible in tuber tests.

Introduction

The oomycete *Phytophthora infestans* (Mont.) de Bary, the causal agent of potato late blight, is a serious threat for potato crops. Breeding potatoes for resistance to late blight was initiated soon after the severe epidemics of the last century in northern Europe. The recent and rapid worldwide changes of the fungus population (Fry et al., 1993; Goodwin et al., 1995) have resulted in a lack of potato cultivars with resistance against new races, and new serious outbreaks have been noted (Johnson et al., 1997). At present late blight can be controlled only by preventive spraying with fungicides.

Considering that the use of fungicides is expensive and an ecological hazard, new progress in potato late blight resistance is desirable.

In plant breeding the efficiency of selection depends on the speed and accuracy of the screening method. Breeders perform many trials in the field and greenhouse to select resistance, and normally they measure the development of lesions on single organs or on plants. This method is time and space-consuming, laborious and non-quantitative.

The electrolyte leakage assay (LEA) has been successfully used in other plant-pathogen systems for screening genetic resistance (Cristinzio & Saccardo, 1994; Cristinzio et al., 1994). It is quantitative, very simple and rapid to use. This paper reports experiments with LEA for a rapid screening of potato resistance to *P. infestans*.

Materials and methods

Isolates of Phytophthora infestans and plants

Eight isolates of *P. infestans* with different mating types, Ph430, 519, 520, 521 (A1), Ph 522 and 545 (A2), Ph523 and 548 (Self), collected from several Italian regions, were used for culture filtrate (CF) production. The fungi were grown for three weeks in a liquid medium (potato dextrose broth, Difco) on a rotary shaker (120 rev./min.) at 24 °C. Cultures were then filtered through filter paper and through a Nalgene filter (0,2 μm). The final supernatants were stored in aliquots at -20°C until required.

The following commercial potato cultivars were used: Agria, Ajax, Désirée, Kennebec, Liseta, Majestic, Monalisa, Prima, Spunta and Tonda di Berlino.

Electrolytes leakage assay (LEA)

LEA is based on the evaluation of electrolyte leakage from vegetables caused by the culture filtrate (CF) of the pathogen. Conductance of the solution was measured at 1h intervals using a conductivity meter with 20 electrodes (range 20μS-200mS/cm; K=1.0) connected to a computer with a specific program. Each value was the mean of at least five replicate

samples and was calculated as the difference from the reading at the beginning of the assay, corrected by subtracting that of the control.

For the tuber assay, five tubers from five plants per cultivar were used within three months of the harvest. For the leaf assay we collected at the pre-flowering stage the third and the fourth leaf from the top of plants, grown in pots under greenhouse conditions. Six tuber disks (6 mm diameter x 3 mm thickness) or six leaf disks (8 mm diameter) were placed in 20 ml of 15% CF at 25°C and magnetically stirred. Distilled water served as control.

Standardization of LEA

LEA was preliminarily standardized using four potato genotypes originating from the International Potato Center (CIP) Lima, Perù: one (702514 = r) without resistance genes, and the others (800987 = R2), (800995 = R10), (800996 = R11) with only one resistance gene. They were tested with two strains of *P. infestans* , Ph 442 (race 1.7) and Ph 524 (race 1.2.4.7.8.10.11), obtained from the Culture Collection of our Department. Using the leaflet inoculation method (Tooley et al., 1986) the first strain was aggressive only on the r-genotype and the latter strain on all the genotypes tested (Table 1a).

Data obtained by testing leaf tissues with the culture filtrates (CF) of the fungi were comparable with those from artificial inoculation (Table 1b). Mean of electrolyte leakage from the leaf tissues of the r-genotype caused by Ph 442 was statistically different from the others while that with Ph 524 was indistinguishable.

Results and Discussion

We did not observe statistically significant differences in our LEA measurements among *P.infestans* isolates on the 10 potato cultivars tested, with the exceptions of isolate 523 assayed on leaves and isolate 430 assayed on tubers (Table 2). We therefore assumed that LEA responses are uniform regardless of the isolates used in the assay. However, always more than one isolate was used in all our experiments.

All the cultivars tested in this work are susceptible to late blight, but the results obtained with LEA (Fig. 1A-1B) showed that Ajax was less sensitive than the others with the leaf tests, and among the less sensitives with the tuber tests, while Prima was much more sensitive in tuber tests.

There were statistical differences in susceptibility among the cultivars using either leaves or tuber tests in the analysis of the potato-*P. infestans* system with LEA. In accordance with results from other authors (Umaerus et al., 1983; Wastie, 1991) on the same cultivars, the susceptibility of the leaves and tubers were often different.

As a consequence of these results, we think that the above technique, performed with leaves and/or tubers incubated for a few hours in crude culture filtrates of some aggressive *P. infestans* isolates, may be useful for rapid screening in programmes of selection and breeding of potato for resistance to late blight. It may also be an alternative or a complement to the more traditional pathogenicity tests.

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Table 1a :

Virulence of two *Phytophthora infestans* strains inoculated on leaf tissue of four potato genotypes after 5 days incubation.

Genotype	Strain	
	Ph442 (race 1.7)	Ph524 (race 1.2.4.7.8.10.11)
702514 (r)	a*	a
800987 (R2)	n	a
800995 (R10)	n	a
800996 (R11)	n	a

* a = abundant sporulation; n = none

Table 1b :

Electrolyte leakage from leaf tissue of four potato genotypes caused by 15% culture filtrates of two *Phytophthora infestans* strains after 6 hours incubation. (Conductance values (μS) are based on the average of five replicates;
means in columns followed by same letter are not significantly different, according to Tukey-Kramer HSD, alfa = 0,05).

Genotype	Strain	
	Ph442 (race 1.7)	Ph524 (race 1.2.4.7.8.10.11)
702514 (r)	46 a	73 a
800987 (R2)	34 b	68 a
800995 (R10)	35 b	66 a
800996 (R11)	32 b	69 a

Table 2 :

Leakage of electrolytes from potato tissues after 6 hours caused by culture filtrates of 8 *Phytophthora infestans* isolates. Conductance values (microsiemens) are based on the average of five replicates from 10 cultivars (see text); means in columns followed by same letter are not significantly different, according to Tukey-Kramer HSD, alfa = 0,05.

Isolate N.	Leaves	Tubers
430	46 a	169 a
519	46 a	200 ab
520	48 a	220 b
521	50 ab	205 ab
522	48 a	224 b
523	56 b	222 b
545	44 a	227 b
548	41 a	217 b

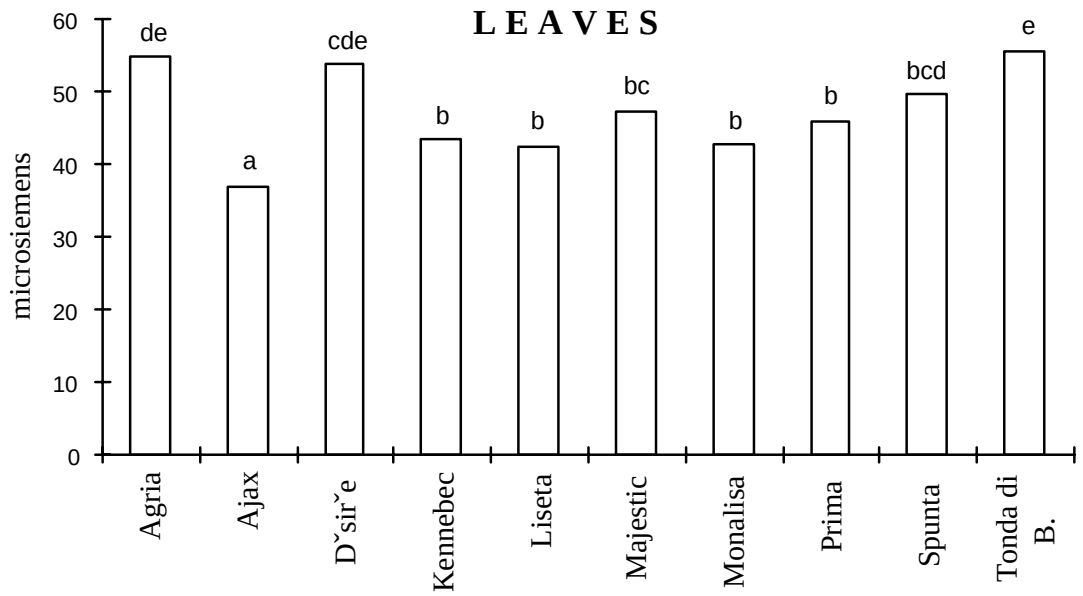
Fig.1A and 1B:

Electrolyte leakage after 6 hours from leaves and tubers of 10 potato cultivars, caused by culture filtrates of *Phytophthora infestans.*

Conductance values (microsiemens) are based on the average of five replicates from 8 strains.

Columns with same letter are not significantly different, according to Tukey-Kramer HSD (alfa=0,05).

Fig. 1A


LEAVES
microsiemens
60
50
40
30
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10
0
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a
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Agria
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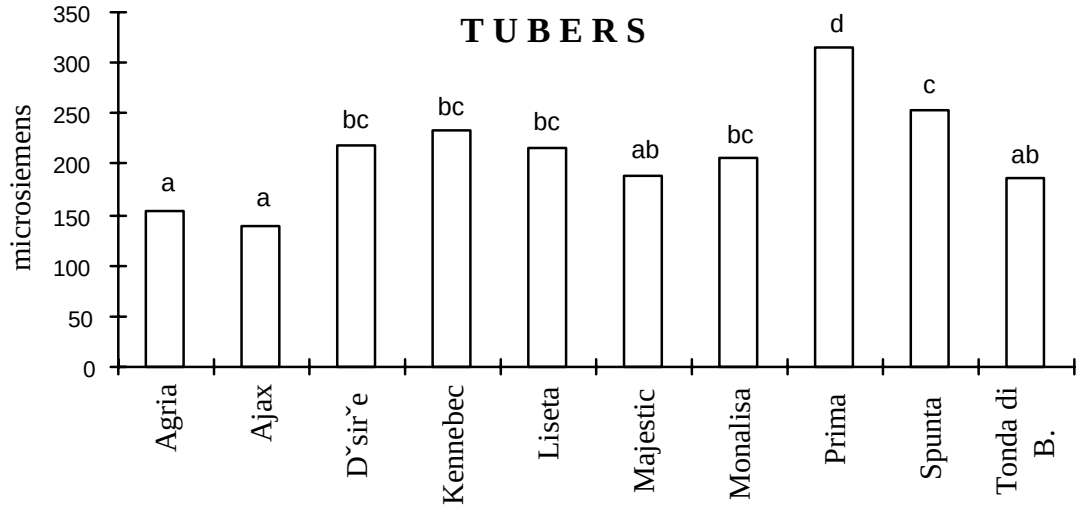
Kennebec
Liseta
Majestic
Monalisa
Prima
Spunta
Tonda di B.

1B


TUBERS
microsiemens
350
300
250
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150
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50
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a
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Tonda di B.