

## General Resistance to Late Blight of *Solanum tuberosum* Plants Regenerated from Callus Resistant to Culture Filtrates of *Phytophthora infestans*

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**Summary.** Resistance of potato leaflets to culture filtrates of *Phytophthora infestans* is correlated with lower growth of the congenial parasite but not with lower sporulation.

**Key words:** Culture filtrate – Callus resistance – *Phytophthora infestans* – Potato – Regeneration

### Introduction

Wheeler and Luke (1955) first used phytotoxins in resistance breeding. Correlation of resistance to a parasite and resistance to its toxins is a necessary prerequisite for such a use of phytotoxins. Later on Kuo et al. (1970), Byther and Steiner (1972) and Matern et al. (1978) proved this correlation with some fungi. Strobel (1973) even demonstrated the molecular mechanism of susceptibility or resistance to *Helminthosporium sacchari*. He showed that this mechanism depends on the binding of the toxin Helminthosporoside to the plasmalemma. In contrast Payne and Yoder (1978) couldn't find a correlation between resistance to *Helminthosporium maydis* and resistance to its toxins.

Phytotoxins are useful tool for selection techniques in callus cultures. First Carlson (1973) reported a selection of callus for breeding purposes. Gengenbach et al. (1977) got plants resistant to *Helminthosporium maydis* by selection of callus.

We regenerated potato plants from callus, which is resistant to the culture filtrate of *Phytophthora infestans* (Behnke 1979). In the following paper we report on the resistance of the culture filtrate resistant plants.

### Material and Methods

34 plants of *Solanum tuberosum* were obtained by regeneration from 10 different calli. These calli were selected for resistance to

the culture filtrate of *Phytophthora infestans*. 15 control plants regenerated from 8 unselected calli. All calli originated from leaves of four different dihaploid clones of *Solanum tuberosum*. Leaves of plants regenerated from resistant calli exhibited more resistance to the culture filtrate than leaves of control plants (Behnke 1979). Dr. Erjevält (Svalöv) kindly supplied us the pathotype 1.2.3.4.5.7 of *Phytophthora infestans* and Dr. Schöber (Biologische Bundesanstalt, Braunschweig) the pathotype 1.2.3.4. These two pathotypes and a third one, isolated by ourselves, were cultivated on tubers of the cultivars 'Grata' and 'Agora' at 16°C. Before being inoculated the tubers were sterilised in 0,1% HgCl<sub>2</sub> for one hour. Suspensions of Sporangia were established simply by rinsing off cut surfaces of tubers with well growing mycelia. In order to get a concentration of about 250 sporengia / 25 µl we counted the number of sporengia of an aliquot of each suspension under the microscope. The suspension was diluted until the desired concentration was reached. Three times we picked up ten leaflets of the upper half of 49 plants for the infection test. These leaflets were placed (lower surface up) in a plastic box (28 cm × 46 cm), whose bottom was covered with humid quartz sand. 25 µl of a sporengia suspension was applied to each leaflet with a Hamilton syringe. In order to get a water saturated atmosphere we set four petri dishes (diameter 5 cm) filled with tap water inside the box and covered it with a glass sheet. After maintaining the box for four days at 16°C, we measured the main diameter of each lesion (Umaerus 1976). Five days after applying the sporengia suspension to the leaflets, we determined the number of sporengia which the fungus had newly produced per lesion. We put the respective leaflets of each plant in 20 ml tap water. After shaking carefully we counted the sporengia of 25 µl portions of each suspension under the microscope (three replicates).

### Results and Discussion

Four days after inoculation the average lesion size of control leaves measured 23.1 cm while the lesions of leaves from toxin resistant plants showed a diameter of 17.3 cm (Table 1). So the parasite grows 25% less on *Phytophthora*-toxin-resistant leaves than on control leaves. This difference is highly significant with a probability of  $p = 0,999$  (t-test). Only three of the investigated 34 plants presented a lesion size on their leaves, which differed not signifi-

cantly from lesion sizes of control plants: PM 3/6, PM 3/20 and PM 25/33, originating from the culture filtrate resistant calli PM 3 and PM 25. Leaves of PM 3/20 and PM 25/33 were also not culture filtrate resistant while leaves of PM 3/6 were.

The numbers of sporangia varied greatly. There was no significant difference between the control leaves and the leaves of culture filtrate resistant plants.

Three factors influence general resistance to *Phytophthora infestans*: infection efficiency, growth of the fungus

and sporulation of the parasite (Umaerus and Lihnell 1976). Because lower growth of the fungus is correlated with resistance to its culture filtrate, selection of callus may be an appropriate technique for breeding plants with general resistance to *Phytophthora infestans*. Perhaps the callus selection technique can be combined with other methods of breeding resistant clones e.g. the method of Umaerus (1969), who selected plants with a lower infection efficiency.

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### Literature

- Behnke, M. (1979): Selection of potato callus for resistance to culture filtrates of *Phytophthora infestans* and regeneration of resistant plants. *Theor. Appl. Genet.* **55**, 69-71
- Byther, R.S.; Steiner, G.W. (1972): Use of helminthosporoside to select sugarcane seedlings resistant to eye spot disease. *Phytopathology* **62**, 466-476
- Carlson, P.S. (1973): Methionine sulfoximine-resistant mutants of tobacco. *Science* **180**, 1366-1368
- Gengenbach, B.G.; Green, C.E.; Donovan, C.M. (1977): Inheritance of selected pathotoxin resistance in maize plants regenerated from cell cultures. *Proc. Natl. Acad. Sci. (Wash.)* **74**, 5113-5117
- Kuo, M.S.; Yoder, O.C.; Scheffer, R.P. (1970): Comparative specificity of the toxin of *Helminthosporium carbonum* and *Helminthosporium victoriae*. *Phytopathology* **60**, 365-368
- Matern, U.; Strobel, G.; Shepard, J. (1978): Reaction to phyto-toxin in a potato population derived from mesophyll protoplasts. *Proc. Nat. Acad. Sci. (Wash.)* **75**, 4935-4939
- Payne, G.A.; Yoder, O.C. (1978): Effect of the nuclear genome of corn on sensitivity to *Helminthosporium maydis* race T-toxin and on susceptibility to *Helminthosporium maydis* race T. *Phytopathology* **68**, 331-337
- Strobel, G. (1973): Biochemical basis of the resistance of sugarcane to eye spot disease. *Proc. Natl. Acad. Sci. (Wash.)* **70**, 1693-1696
- Umaerus, V. (1969): Studies on field resistance to *Phytophthora infestans*. 2. A method of screening young potato seedlings for field resistance to *Phytophthora infestans*. *Z. Pflanzenzücht.* **61**, 167-194
- Umaerus, V.; Lihnell, D. (1976): A laboratory method for measuring the degree of attack by *Phytophthora infestans*. *Potato Res.* **19**, 91-107
- Wheeler, H.E.; Luke, H.H. (1955): Mass screening for disease resistant mutants in oats. *Science* **122**, 1229

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**Table 1.** Diameter of lesions 4th day after inoculation of leaflets with *Phytophthora infestans*

Leaflets of plants from toxin resistant callus		Leaflets of control plants	
Plant	Diameter	Plant	Diameter
PM 2/1	14.8	HH 140/2	20.6
PM 2/5	16.9	HH 140/4	23.3
PM 2/8	14.4	HH 140/7	19.8
PM 2/12	19.0	HH 140/8	21.9
PM 3/6	23.4	HH 140/11	22.1
PM 3/7	15.6	HH 140/12	23.9
PM 3/20	22.9		
PM 3/22	18.3		
PM 4/10	15.4		
PM 4/12	19.5		
PM 4/14	15.9		
PM 5/2	16.6		
PM 5/4	14.8		
PM 5/5	15.8		
PM 26/26	13.6		
PM 27/2	15.6		
PM 27/6	15.1		
PM 30/1	15.8		
PM 30/2	19.3		
PM 30/4	17.0		
PM 30/5	19.4		
PM 25/6	18.6	HH 258/1	24.0
PM 25/12	18.9	HH 258/2	26.0
PM 25/14	18.7	HH 258/5	25.8
PM 25/15	15.0	HH 258/6	24.3
PM 25/16	16.8	HH 258/8	25.1
PM 25/30	16.3		
PM 25/33	21.5		
PM 25/41	18.4		
PM 25/44	16.4		
PM 41/4	14.1	HH 578/1	23.6
PM 41/6	18.1	HH 578/3	22.0
PM 41/9	19.8		
PM 31/1	11.0	H 282/2	21.3
		H 282/3	21.4
17.3 ± 7.4		23.1 ± 7.6	

P < 0,001