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# Correlations between protoplast yield, tissue browning and late blight resistance in potato cultivars and wild *Solanum* spp.

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## Abstract

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A modified method for protoplast isolation from potato leaves was applied to 4 wild *Solanum* species and 21 *S. tuberosum* cultivars including 14 R-gene differentials. Protoplast yields were negatively correlated with field resistance to *Phytophthora infestans*. The yield was generally high ($1–2 \times 10^6$/g fresh weight) from species and cv. with low field resistance (1 exception) and low from those with high field resistance. The differentials with none or a single R-gene exhibited good yields (1 exception) whereas differentials with 2 R-genes yielded virtually no protoplasts. Tissues which released only few protoplasts leaked more phenolic material into the preincubation medium than did those releasing many protoplasts. The results implicate cell wall properties as factors of resistance and indicate similarities between the phenotypic expression of vertical and horizontal resistance.

## Introduction

Although the phytoalexin theory evolved from studies with the late blight fungus *Phytophthora infestans* on its potato host (Müller and Börger 1940) it is at present undecided whether or not phytoalexins play a major role in the reaction of resistant cultivars (Schöber 1980, Kuć et al. 1979), and other mechanisms such as lignification of host cell walls (Friend 1973) or papilla formation (Hohl et al. 1980) have been studied for their potential as factors of disease resistance in potatoes.

During a comparative investigation on protoplast release from a variety of potato cv. and wild *Solanum* species we noticed marked differences in yields among the various sources. In addition, the solutions in which the tissue samples were preincubated overnight stained brown to varying degrees. In this paper we present the data which indicate that there is a marked positive correlation between protoplast yield and degree of susceptibility for the late blight fungus *P. infestans* and of tissue browning.

## Materials and methods

The potato cv. were obtained from the following sources: Bintje and Eba (virus free) from Dr. F. A. Winiger at the "Eidgenössische Forschungsanstalt für landwirtschaftlichen Pflanzenbau, Zürich-Reckenholz, Switzerland", 12 late blight differentials from Dr. L. J. Turkensteen, Research Institute für Plant Protection, Wageningen, Holland. The wild *Solanum* species were made available to us by Ir. L. J. M. van Soest of the Gene-Bank at the FAL in Braunschweig, FRG. The potato cultivars were grown in a field plot, the *Solanum* spp. in the green house (average temperature appr. 20 °C, rel. humidity 60%).

Protoplasts were isolated from the first fully expanded leaflets with a modification of the methods of Shepard and Totten (1977) and of Upadhya (1975). To release protoplasts leaflets were surface sterilized and preincubated in 0.8 mM $CaCl_2$ (24 h, 4 °C) and subsequently incubated in 0.5% (w/v) Cellulase Onozuka R-10, 0.1% Macerozyme R-10 in 10 mM 2-N-morpholino-ethane sulfonic acid (MES, Sigma Chemical Corp.), 0.2 M sucrose, 0.4 mM $CaCl_2$, 2% (w/v) PVP-10 (polyvinyl pyrrolidone, MW 10,000, Sigma Chemical Corp.), 0.175 M KCl, 22.5 mM $MgCl_2$ for 2 h at 28 °C. Protoplasts were purified by flotation on 16% (w/v) Ficoll 400 and resuspended in medium A (Shepard and Totten 1977). Their viability was scored using methylene blue. During preincubation the medium turned brown with a UV absorbance shoulder at 265. The $E_{265}$ was determined for 12 cultivars.

## Results

In preliminary experiments not reported here the modified method described above was developed which gave consistent yields of protoplasts from the cv. Bintje and Eba. Even though Eba was grown under a variety of conditions in the field and in the greenhouse its protoplast yield was always well below that of Bintje. Yields also varied with the season. From July to October the yield for e.g. Bintje dropped from 2 to $0.4 \times 10^6$ protoplasts/g of fresh weight.

The same isolation method was applied to the 21 cultivars and 4 wild type species listed in table 1. To account for seasonal differences yields were always related to corresponding yields from Bintje with the tacit assumption that seasonal differences were similar in all plants tested. The results of protoplast yields and of browning of the preincubation medium are summarized in table 1.

The results indicate that either high field resistance or the presence of two but not one R-gene leads to a marked reduction in protoplast yield, usually well below 50% of the control (Bintje). The correlation is remarkable but with notable exceptions among the cultivated forms: In Rode Eersteling yields are low inspite of its low field resistance and the same applies to the R10-differential. Of interest are the cv. King Edward and Pentland Beauty. They are listed with low field resistance (9 and 7 respectively) in the 1979 edition of the "Index of European Potato Varieties" but with high field resistance in the 1976 edition. Their protoplast yields correspond to those of cv. with high field resistance.

Low protoplast yield is also correlated with enhanced browning of the incubation medium. Here again Rode Eersteling among the twelve cv. studied is exceptional in that its yield was low without a corresponding enhancement of the browning reaction. The differential R7 on the other hand yielded intermediate amounts of protoplasts despite a strong browning reaction.

Tab. 1. Protoplast yields and degree of tissue browning in potato cultivars and wild *Solanum* species carrying different types of resistance against *Phytophthora infestans.*

| Cultivar/species | R-gene(s) present | Field resistance (leaves)[1] | Protoplast yield (% of Bintje)[2] | Tissue browning (Bintje = 1.0)[3] |
|---|---|---|---|---|
| Differentials: | | | | |
| r (= Bintje) | none | low (7) | 100 | 1.0 |
| R1 | 1 | low* | 94 | nd |
| R2 | 2 | low* | 146 | nd |
| R3 | 3 | low* | 96 | nd |
| R4 | 4 | low* | 94 | nd |
| R5 | 5 | low* | 140 | 2.7 |
| R7 | 7 | low* | 54 | 5.5 |
| R10 | 10 | low* | 10 | 6.1 |
| R1, R2 | 1, 2 | low* | 10 | 7.2 |
| R1, R3 | 1, 3 | low* | 10 | 8.5 |
| R1, R4 | 1, 4 | low* | 10 | nd |
| R2, R3 | 2, 3 | low* | 23 | nd |
| R2, R4 | 2, 4 | low* | 21 | 5.8 |
| cultivated forms: | | | | |
| Bintje | none | low (7) | 100 | 1.0 |
| Holde | none | low (7) | 157 | 0.7 |
| Rode Eersteling | none | low (8) | 18 | 1.4 |
| King Edward | none | low/high (9, 2)[4] | 10 | nd |
| Pentland Beauty | 3 | low/high (7, 3)[5] | 32 | nd |
| Dekama | 3 | high (2) | 8 | 6.4 |
| Eba | 3 | high (3) | 47 | 1.8 |
| Alpha | none | high (3) | 13 | 20.0 |
| *S. tub.* ssp. *andigena* | none | low (9) | 93 | nd |
| Wild *Solanum* spp.: | | | | |
| *S. berthaultii* 18548 | ? | high (3) | 2 | nd |
| *S. berthaultii* 10063 | ? | high (1) | 2 | nd |
| *S. bulbocastanum* 8001 | ? | high (1) | 2 | nd |
| *S. hjertingii* 8091 | ? | high (1) | 15 | nd |

[1] 9 denoting lowest, 1 highest resistance (values taken from Index of European Potato Varieties, 1976, 1979)
[2] average yields appr. $1.2 \times 10^6$ protoplasts/g fresh wt.
[3] measured as $E_{265}$ of incubation medium (for Bintje $E_{265} = 0.36$)
[4] 9 according to Index 1979, 2 according to Index 1976
[5] 7 according to Index 1979, 3 according to Index 1976
* not accurately known but definitely very low

## Discussion

The results demonstrate that in general cv. with either low field resistance against *Phytophthora infestans* or with no more than one R-gene yield high numbers of protoplasts and develop low levels of browning. Two points are worth noting, (1) the pres-

ence of two but not one R-genes affects the protoplast yield in the same way as high field resistance and, (2) tissue browning, protoplast release and resistance appear to be interrelated phenomena.

The biochemical and genetic basis of field resistance and R-gene resistance are not well understood (Nelson 1978, VanderPlank 1982). Thus potential similarities and dissimilarities between the two types of resistance cannot be assessed at present. The results presented here show that even though none of the R-genes 1 to 4 affect protoplast yields substantially alone, any combination in pairs will lower these yields and thus mimics high field resistance for at least this character. In other words two R-genes combined will produce a phenotypic effect that is also evoked by high field resistance in the absence of any R-gene, i.e. by a different gene or gene combination. These similarities between the effects of R-genes and those of field resistance genes deserve further analysis.

Browning of the incubation medium and low protoplast yields are most easily related to resistance via the host cell wall. Browning of the incubation medium indicates production and release of phenolic compounds. Low protoplast yields indicate a low wall digestion rate. This links late blight resistance to increased browning and to increased wall resistance against enzymic degradation. The latter two might be interrelated via phenolics incorporated into the host cell walls after infection (Friend 1976, Wilson and Coffey 1980, Hächler and Hohl 1984) which possibly renders them more resistant to enzymic attacks by the pathogen. The possibility that innate cell wall differences in relation to enzymic degradation are present before infection cannot be excluded at present.

Alternatively one has to consider the possibility that the wall-degrading enzymes are directly inhibited by the phenolics. Yet, the results with the R-5 differential does not support this explanation since in this case high amounts of secreted phenolics do not appear to strongly inhibit the degrading enzymes.

In a recent paper McLauchlin (1983) also observed a positive correlation between field resistance against late blight and production of phenolics in three potato cultivars. Schöber (1971) described a positive correlation in potato tubers between field resistance and phenolase activity following wounding or infection. The long held view (e.g. Friend 1973) that phenolics are involved in resistance against late blight by changing host cell wall properties is supported by our results. Information on the type of linkage between the phenolics as the wall polymers is needed to understand this relationship and the biochemical basis for resistance in this system.

The view that the host cell wall is an important site of resistance is further strengthend by our observation that protoplasts of the resistant cv. Eba do not resist penetration by an avirulent race of the pathogen and do not produce wall-like papillae (Hohl et al. 1983) as is typical of infected tissue of this cultivar (Hächler and Hohl 1982).

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