Differential Responses of Potato Cell Suspensions to a Culture Filtrate of 
Phytophthora infestans

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General (polygenic) resistance of plant hosts to an attack by a range of pathogens is an 
important feature of plant defense responses against the infection. In search of biochemical 
markers defining this resistance, cell suspensions derived from leaves of potato (Solanum 
tuberosum L.) cvs. Tarpan and Bzura that are polygenically susceptible and resistant to Phy­ 
tophthora infestans, respectively, were inoculated with culture filtrate (CF) of the fungus. Cell 
suspension of Tarpan responded to CF treatment by a higher extracellular alkalinization and 
more significant reduction in their viability and growth than those of the Bzura cultivar. The 
stimulation of phenylalanine ammonia-lyase (PAL) activity, but not of β-1,3-glucanase, was 
more significant in CF treated Bzura cells than in Tarpan ones. The obtained results sug­ 
gest that sensitivity to the fungal toxins and variation of PAL activity may represent useful 
markers for the evaluation of polygenic resistance in potato.

Introduction

Interactions between plant hosts and their pathogens or pathogen cell free extracts or specific 
metabolites, known as elicitors, trigger several plant defense responses. Very early responses, 
considered to be involved in recognition and initial signalling events, include changes in plasma mem­ 
brane potential and ion fluxes (Atkinson et al., 1990; Baker et al., 1993; Kombrink and Somssich, 
1995). Subsequently, various biochemical pathways are activated, i.e. phenylpropanoid pathways 
(where phenylalanine ammonia-lyase (PAL) is the key enzyme), synthesis of antimicrobial sub­ 
stances, “phytoalexins” and accumulation of pathogenesis-related (PR) proteins comprising a 
wide range of different plant defense proteins, in­
cluding hydrolytic enzymes, β-1,3-glucanase and 
chitinase (Kombrink and Somssich, 1995).

Cell-free extracts as well as metabolites of plant pathogens, commonly known as “elicitors”, trigger 
the same biochemical responses in plants and cul­
tured cells as does infection by pathogens (Ebel 
and Cosio, 1994; Boller, 1995). In studies on de­
­fense responses in potato tissues, culture filtrate 
(CF) and mycelial homogenate (MH) of P. infes­
tans as well as arachidonic acid (AA) have been 
used as biotic elicitors (Friend, 1991). The CF con­
tains a number of fungal metabolites, including 
polysaccharides and glycoproteins (Stolle and 
Schuber, 1985; Davidse et al. 1986; Huet et al., 
1994). MH is a mixture of hyphal components, 
whereas AA is an unsaturated fatty acid present 
in the cell wall of P. infestans but absent in potato 
tissues (Bostock et al., 1981). In our early studies, 
detached leaflets of potato cvs. Tarpan and Bzura 
that are polygenically susceptible and resistant to 
P. infestans, respectively (Swiezynski et al., 1993), 
were treated with AA, CF and MH. The leaflets 
of Bzura cv. developed earlier and showed more necrosis than those of Tarpan after these treat­
ments (Awan et al., 1995). These results were in 
agreement with the observations that leaves of po­
tato cvs. with a high level of general resistance were highly sensitive to the components of CF 
from P. infestans (Davidse et al., 1986).

To investigate further the general potato resis­
tance to P. infestans, in the present study cell sus­
pension cultures of both cvs., Tarpan and Bzura, established from leaf-derived calli, were used. Cultured cell suspensions with uniform cell type, defined growth stage and synchronous response to stimulation are frequently used as models for the characterization of defense responses at biochemical and molecular levels (Kombrink and Somssich, 1995). We report here the kinetics and changes in extracellular pH, cell viability, cell growth and enzymatic activities in cell suspensions treated with CF of *P. infestans*. In preliminary studies, we had found that the response of cell suspensions from both cvs. to the treatments with AA and MH was either less or similar to that with CF (Maciejewska et al., 1995).

**Materials and Methods**

**Potato cell suspensions**

Cell suspension cultures of both cvs. Tarpan and Bzura, established from leaf-derived calli, were maintained on MS medium (Murashige and Skoog, 1962) supplemented with vitamins (Gamborg et al., 1968), glycine (2 mg l⁻¹), hydrolysed casein (500 mg l⁻¹), sucrose (3%) and phytohormones: 2,4-D (2 mg l⁻¹), NAA (2 mg l⁻¹) and BAP (0.1 mg l⁻¹). The cultures were kept on a rotary shaker (120 r min⁻¹) at 26 °C in the dark and subculturing in the fresh medium was done every 8th day. Cell cultures in the log growth phase, 7 – 8 days after subculturing, were used for all experiments, except the determination of cell growth, where cell suspensions were used just after subculturing.

**Fungal growth and preparation of culture filtrate**

*P. infestans* (MP 245, complex race) obtained from Potato Research Institute Mlochów (Poland) was maintained on rye agar medium supplemented with cholesterol (50 μg ml⁻¹) (Hohl, 1991) at 15 °C in the dark. After every three months, the fungus was passed through tuber slices of susceptible potato cv. Tarpan to maintain the virulence. Concentrated CF was prepared from fungi grown for six weeks in the liquid Henniger medium (Henniger, 1959). The medium was separated from the fungus by filtration, the filtrate dialyzed for 48 h against water at 4 °C, the dialysate lyophilized, the residue taken up in one-tenth of the initial volume and the resulting solution filter-sterilized. The CF was quantified as μg glucose equivalents per ml. Carbohydrate content was determined by colorimetric method using glucose as standard (Dubois et al., 1956).

**Extracellular pH**

Cells from the cultures were collected by vacuum filtration, washed with the fresh growth medium, sieved to remove the bigger aggregates, again filtered and resuspended (0.1 g fresh wt. ml⁻¹) in the fresh medium. Aliquots of 20 ml were taken in 100 ml Erlenmeyer flasks and preincubated for 2 h on a shaker (120 r min⁻¹) at 26 °C. These cell suspensions were inoculated with CF (10 μg glucose equivalents ml⁻¹). The controls were treated with distilled water. Extracellular pH was recorded by glass electrode fitted with digital monitor (Pharmacia).

**Cell viability**

Cells were prepared and treated with CF as described above. The viability of cell suspensions was determined by MTT [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (Aldrich) colorimetric assay (Sanchez et al., 1994). A 50 μl of MTT solution (5 mg ml⁻¹) was added to 500 μl of cell suspension. After 2 h incubation at 26 °C, the mixture was supplemented with 500 μl isopropanol containing 0.04 n HCl and centrifuged at 2000 r min⁻¹ for 10 min. The absorbance (test wavelength 570 nm – reference wavelength 630 nm) in control was taken as 100% viability and cell viability (%) was calculated from the ratio of the value for the treated cells to that in control.

**Cell growth**

After subculturing, 70 ml cell suspensions were taken in 300 ml Erlenmeyer flasks. Two days later, CF (5 μg glucose equivalents ml⁻¹) or sterile distilled water was added to the cell cultures. Cell growth was determined in 10 ml aliquots from zero to eight days after subculturing with two days intervals. For determination of dry weight, cells obtained by vacuum filtration were incubated at 60 °C until the constant weight was achieved.
Cell suspension treatment, extraction and enzyme assay

Aliquots (20 ml) of cell suspension in the log growth phase were treated with CF (10 μg glucose equivalents ml⁻¹); to the controls sterile distilled water was added. After specific time period of the treatment, cells were collected by vacuum filtration, washed with distilled water, freeze-dried in liquid nitrogen and stored at -80 °C.

PAL and β-1,3-glucanase assays

For PAL assay, frozen cells (0.5 g) were ground in liquid nitrogen and homogenized in 2 ml of 0.2 M borate (Na⁺) buffer, pH 8.8, containing 14 mM 2-mercaptoethanol. The extract was centrifuged at 14,000 x g for 20 min. PAL activity in the supernatant was determined using colorimetric method (Havir and Hanson, 1968). The reaction mixture of 4 ml containing the enzyme extract (200–300 μg proteins), 0.12 M borate buffer, pH 8.8 and 22 mM L-phenylalanine was incubated at 30 °C for 60 min. The content of trans-cinnamic acid was determined at 290 nm and calculated using molar coefficient (Havir and Hanson, 1968).

For β-1,3-glucanase assay, frozen cells (1.0 g) were ground in liquid nitrogen and homogenized in 3 ml of 0.5 M sodium acetate buffer, pH 5.2. The extract was centrifuged at 14,000 x g for 20 min and supernatant desalted on Sephadex G-25 column. β-1,3-glucanase activity in the pooled elution fractions containing protein was determined essentially as described by Kombrink and Hahlbrock (1986), except that reducing sugars were determined with 4-hydroxybenzoic acid hydrazide (Lever, 1972).

The activities of both enzymes were calculated in katal, i.e. mole product released per s, and expressed as relative to controls. Protein was determined by the method of Lowry et al. (1951).

Results and Discussion

Suspension cultured cells of potato cultivars possessing different levels of polygenic resistance varied in their responses to CF of P. infestans. The CF treatment caused an increase in extracellular pH of both cell suspensions as compared to the controls, where pH of Tarpan cell culture remained constant during the whole experiment, while in Bzura cell suspension it increased slightly after 1 h (Fig. 1). In CF treated Tarpan cell suspension, the increase in pH lasted for up to 6 h of the treatment, reaching at maximum about one unit. In Bzura cells, the pH increase was less pronounced and lasted up to 2 h (Fig. 1). There were no further changes in the response of both cell suspensions treated with twice higher concentration of CF (data not shown).

The viability of Tarpan cells was significantly reduced by CF treatment and only 33% cells exhibited viability at 6 h after treatment. In contrast, Bzura cells were slightly affected as 81% cells remained viable at the same time (Table I). The viability of both cells did not decrease further in response to twice higher concentration of CF (data not shown).

An increase in extracellular pH as well as cell death are attributed to recognition of incompatibility in pathogen/non-host and race/cultivar interactions (Atkinson et al., 1990; Baker et al., 1993; Baker and Mock, 1994). In this light, one might expect that the response of Bzura cells to CF treatment should be more pronounced than that of Tarpan ones. However, we should keep in mind that CF of P. infestans contains substances that are...
Table I. The effect of culture filtrate (CF) of *P. infestans* on the viability of cell suspensions of potato cvs. Bzura and Tarpan. Cells were prepared and treated with CF (10 μg glucose equivalents ml⁻¹) as described in the Materials and Methods. Cell viability was determined by MTT colorimetric method (Sanchez et al., 1994). The value of controls (treated with distilled water) was considered as 100% viability and cell viability (%) was calculated from the ratio of the value for the treated cells to that in controls. Results are means of three independent experiments each consisting of four replicates (± SE).

<table>
<thead>
<tr>
<th>Time [h]</th>
<th>Bzura</th>
<th>Tarpan</th>
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<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.5</td>
<td>100</td>
<td>71 ± 8</td>
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<tr>
<td>1</td>
<td>100</td>
<td>61 ± 2</td>
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<tr>
<td>4</td>
<td>90</td>
<td>40 ± 2</td>
</tr>
<tr>
<td>6</td>
<td>81 ± 6</td>
<td>33 ± 1</td>
</tr>
</tbody>
</table>

toxic to potato root cap cells (Galal et al., 1991), leaf tissues (Galal et al., 1991; Huet et al., 1994) as well as to mesophyll protoplasts and anther microspores (Möller et al., 1992). It has also been stated that plasma membrane is the main target for a number of fungal toxins (Kohmoto and Otani, 1991). Therefore, it is conceivable that the changes observed in Tarpan cells are mostly due to damages caused by the fungal toxins and their effects are predominant over possible defense reactions.

To verify further the results obtained for cells’ viability after CF treatment, we tested the effect of CF on the cell growth. The CF (5 μg glucose equivalent ml⁻¹) was added to both cell suspensions after two days of subculturing. Using such young cells allowed us to follow their response to CF treatment until reaching the log growth phase. The growth of Tarpan cells was completely blocked just after two days (Fig. 2a), whereas the growth of Bzura cells was not affected as compared to the control (Fig. 2b). A visible browning of Tarpan cells was noted within one day after treatment, while Bzura cells became brown 4–6 days after inoculation, when they reached the log phase of growth. Thus, the cells of both cultivars varied in this response in respect of their age.

Browning of cells is most frequently attributed to activation of phenylpropanoid pathways, including PAL activity (Fritzmeier et al., 1987), processes that are stimulated by different stress conditions, including pathogenesis (Dixon and Paiva, 1995). Accordingly, we have measured the activity of PAL in both Tarpan and Bzura cells (Fig. 3a). Although basic PAL activity was almost similar in both cultivars, after CF treatment stimulation of the enzyme activity was higher and more prolonged in resistant Bzura cells than that in susceptible Tarpan ones. The results confirm earlier studies on PAL role in defence response (Fritzmeier et al., 1987; Mozzetti et al., 1995).
In contrast to PAL activity, the constitutive level of β-1,3-glucanase activity as well as the degree of its stimulation after CF treatment were higher in Tarpan than those in Bzura cells (Fig. 3b). This was unexpected, since β-1,3-glucanase, among other PR proteins, is rapidly and markedly induced in potato leaves after CF treatment or infection by *P. infestans* (Schröeder *et al.*, 1992; Kombrink *et al.*, 1988). Whatever the reason for this discrepancy, it appears that the induction of β-1,3-glucanase activity following CF treatment is not the major marker of pathogen resistance in the Bzura (resistant) cultivar.

In summary, our results indicate that potato cultivar with lower level of polygenic resistance is more sensitive to CF containing the fungal toxins. It was also found that CF treatment induced defense reactions in cells of both resistant and susceptible cvs. In the cells of resistant cultivar, no toxic effects were noticeable and even if they were, the cells were able to cope with them and to activate defense strategies efficiently. Based on the obtained results, we propose that sensitivity to the fungal toxins and variation of PAL activity may be used as useful markers for the evaluation of polygenic resistance in potato.

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