The Oxidative Processes Induced in Cell Suspensions of *Solanum* Species by Culture Filtrate of *Phytophthora infestans*

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Oxidative Response, *Phytophthora infestans*, Polygenic Resistance

Solanum genotypes that differ in the level of polygenic resistance to the oomycete plant pathogen *Phytophthora infestans* were studied for their oxidative response to culture filtrate (CF) of the pathogen. Reactive oxygen species (ROS) production, peroxidase activity and lipid peroxidation have been studied in the CF-treated cell suspensions derived from leaves of the resistant *S. nigrum* (nonhost) and *S. tuberosum* cv. Bzura as well as from the susceptible *S. tuberosum* cv. Tarpan and clone H-8105. In both the resistant and susceptible cells the CF induced similar processes, but these varied with respect to the kinetics and intensity. In all cells probably the membrane-bound NADPH oxidase, was responsible for the ROS production. This process was more intensive and prolonged in the susceptible cells than in the resistant ones. The CF treatment slightly affected peroxidase activity in all cells studied. Lipid peroxidation that occurred as a consequence of the ROS accumulation was pronounced mainly in the susceptible cells. We suggest that lack of stringent control of the oxidative processes and sensitivity to the pathogen toxins may be decisive for limited polygenic resistance in potato.

Introduction

One of the earliest plant responses to pathogens or their elicitors is a rapid synthesis and release of reactive oxygen species (ROS) termed "oxidative burst" (Wojtaszek, 1997; Murphy *et al.*, 1998). The ROS include: superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$). Usually, in any system producing $O_2^{\cdot -}$, a substantial amount of H_2O_2 is also generated by dismutation, spontaneous or catalysed by superoxide dismutase (SOD). The detection of hydroxyl radicals is often unsuccessful because of its short half life time and extreme reactivity. Current studies on the source of ROS in plant / pathogen interactions point to multiplicity of mechanisms in different systems of hosts and pathogenic agents. Among several possibilities, two enzymatic systems of ROS synthesis receive most attention, namely: 1) the membrane-bound NADPH oxidase analogous to the mammalian neutrophil one, 2) the pH-dependent production of H_2O_2 by cell-wall peroxidase (Bolwell and Wojtaszek, 1997; Murphy *et al.*, 1998).

The ROS produced in response to pathogen are assumed to perform several functions in diverse aspects of disease resistance. They have been shown to be implicated in direct killing of the pathogen or host cells (hypersensitive response, HR) and in an irreversible membrane damage via lipid peroxidation (Rogers *et al.*, 1988). On the other hand, lipid peroxides generated simultaneously may serve as precursors of intercellular signals (Ebel and Mithöfer, 1998) and / or as antimicrobial agents (Croft *et al.*, 1993). The ROS may be involved in oxidative cross-linking of cell wall proteins (Brisson *et al.*, 1994). They may also act as an intracellular signal regulating gene expression (Levine *et al.*, 1994; Wojtaszek, 1997).

Classically, the ROS production was described as a biphasic process. The first phase appears to be non-specific, being produced in response to numerous elicitors. The second phase is thought to

Abbreviations: CAT, catalase; CF, culture filtrate; DPI, diphenyleneiodonium; LDC, luminol-dependent chemiluminescence; MES, 2-(N-Morpholino)ethanesulfonic acid; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate; SOD, superoxide dismutase; TBA, 2-thiobarbituric acid; TBARS, 2-thio-barbituric acid reactive substances; TCA, trichloroacetic acid; TRIS, 2-amino-2-(hydroxymethyl)-1,3-propandiol.

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correlate with resistance of the plant to the pathogen and to be specific for an incompatible interaction resulting in hypersensitive cell death. However, Glazener *et al.* (1996) showed that occurrence of the second phase is not sufficient to trigger the HR. Vera-Estrella *et al.* (1993) reported that elicitors from *Cladosporium fulvum*: specific (intracellular fluid isolated from tomato leaves infected with the fungus) and non-specific (glycopeptide elicitor prepared from culture filtrate of the fungus) similarly induced the ROS response in tomato cell suspension. In potato (*Solanum tuberosum*) leaves inoculated with zoospores of oomycete plant pathogen *Phytophthora infestans*, the compatible race induced only the first phase, while the incompatible one initiated both phases of ROS production. The biphasic ROS response was correlated with HR after pathogen penetration (Chai and Doke, 1987). On the contrary, Freytag *et al.* (1994) observed HR in both incompatible and compatible interactions between *S. tbr* cv. Datura and *P. infestans* after successful penetration of the pathogen into plant tissue. Furthermore, Kamoun *et al.* (1999) reported that the HR appeared also in the interaction between potato cultivars displaying partial (field) resistance to *P. infestans*.

The reports mentioned above prompted us to examine the oxidative burst and the relevant processes in potato plants that are polygenically resistant or susceptible to *P. infestans*; we have also attempted to find whether these reactions might serve as biochemical markers of this type of general resistance. The plant production of ROS in response to pathogen varies depending on the nature of elicitor, plant species and age or developmental stage of the plant cell (Murphy *et al.*, 1998). Therefore, most studies on ROS generation are performed with cell suspensions treated with cell-free elicitors. This simplified system allows to deal with uniform cell type in a defined growth stage, with synchronous response to inducer; it also allows to focus on the plant side of the interaction. In the present studies we have used leaf-derived cell suspensions of potato, of the polygenically resistant cv. Bzura, and susceptible cv. Tarpan and clone H-8105. As a reference, we have also assayed the cells of *S. nigrum*, wild species, nonhost, completely resistant to *P. infestans*. The resistance of the genotypes used was evaluated in leaflet tests by IHAR, Młochów Research Center (Poland). The cell suspensions were treated with the culture filtrate of *P. infestans*, as non-specific elicitor. Here we compare the elicitor-induced ROS production, peroxidase activity and lipid peroxidation in these cells with differential polygenic resistance.

Materials and Methods

Cell suspensions

Cell suspensions from plants: *Solanum tuberosum* cv. Bzura, cv. Tarpan and clone H-8105, and *Solanum nigrum* established from leaf-derived calli, were cultured on MS medium (Murashige and Skoog, 1962) as described previously (Awan *et al.*, 1997).

The cell cultures in the log growth phase were collected and resuspended at the concentration of 0.05 g ml^{-1} in the assay medium consisting of 175 mM mannitol, 0.5 mM $CaCl_2$, 0.5 mM K_2SO_4, 5 mM 2-[N-Morpholino]ethanesulfonic acid (MES) and water adjusted to pH 5.3. The cells were preincubated for two hours on a rotary shaker under standard growth conditions (Awan *et al.*, 1997).

Preparation of culture filtrate from Phytophthora infestans

Two different isolates of *P. infestans*, complex races MP-306 with virulence factors 1,2,3,4,7,10,11 and MP- 322 with additional virulence factors 6 and 8, were kindly provided by IHAR, Młochów Research Center (Poland). The pathogen growth and preparation of the culture filtrate were described by Awan *et al.* (1997). Briefly, *P. infestans* was maintained on rye agar medium at 15 °C in the dark. CF was prepared from the pathogen (80 sporangia ml^{-1}) grown in liquid Henniger medium (Henniger, 1958). The cultures were incubated for six weeks at 20 °C, in the dark. Medium separated from the oomycete was dialysed for 48 h against water and lyophilised. The CF was quantified as μg glucose equivalents per ml.

Elicitor treatment of cells

The cells preincubated in the assay medium were treated with CF at the concentration of 14 μg glucose equivalents ml^{-1} cell suspension. In some experiments a whole series of CF concentrations were tested. The control cells were treated with

an equal amount of distilled water. Immediately following the treatment, cell suspensions were returned to standard growth conditions for a specific time period.

Chemiluminescence assay

ROS level was monitored by the luminol-dependent chemiluminescence (LDC) using a luminometer 1250 (Bio-Orbit, Finland) according to the original procedure of Glazener *et al.* (1991). The cell sample of 850 µl was transferred to the luminometer cuvette. Luminol in 300 mM MES buffer pH 7, was automatically added to the final concentration of 80 µM and the sample was vortexed. The level of chemiluminescence was measured after an initial delay of 30 s after addition of the luminol solution. The non-elicited, control cells were measured in parallel with the CF treated cells. To determine the effect of ROS scavengers, various amounts of catalase (EC 1.11.1.6 from bovine liver, Sigma) or superoxide dismutase (EC 1.15.1.1 from bovine erythrocytes, Sigma) were added to the elicited cell sample of 850 µl. After incubation for one minute, the luminol solution was automatically added to the final concentration of 80 µM. The probes were vortexed for 5 s and LDC was recorded. In some experiments, portions of diphenyleneiodonium (DPI, Calbiochem) were added to the cells 10 min prior to the CF treatment. LDC was recorded after an appropriate time of incubation.

Peroxidase activity

Peroxidase activities were determined according to Rychter and Lewak (1971). Portions of the elicited cells (from the batches used for LDC measurements) were collected by vacuum filtration. The cell-free residues were used for the measurement of activity of extracellular peroxidases. The cells were ground in liquid nitrogen and homogenised in 0.05 M 2-Amino-2-(hydroxymethyl)-1,3-propandiol (Tris)-HCl pH 7.2 containing 6 mM L-cysteine and 6 mM L-ascorbic acid. The extracts were centrifuged at 10000×*g* at 4 °C for 10 min (SORVALL RC 26 Plus). The reaction mixture containing 2 mM benzidine solution, 0.2 M acetate buffer pH 5.0 and 0.2 M H_2O_2 was incubated in water bath at 37 °C. The assay sample consisted of 1.2 ml of the reaction mixture and the enzyme extract to the final volume of 2 ml.

The changes in absorbance at 590 nm were recorded after lag time of 20–30 s. Enzyme activity was expressed as ΔA_{590}* min^{-1} *mg^{-1} of protein. Protein content was determined using the method of Bradford (1976).

Lipid peroxidation

Lipid peroxidation was measured as 2-thio-barbituric acid-reactive substances (TBARS) according to the modified method of Oteiza and Bechara (1993). The elicited cells (from the batches used for LDC measurements) were collected by vacuum filtration and washed with distilled water. The cells were ground in liquid nitrogen, homogenised in 5% TCA and then centrifuged at 10000×*g* at 4 °C, for 10 min. The reaction mixture containing the cell extract, 0.3% (w/v) SDS, 0.25% (w/v) TBA in 50 mM NaOH and 6% (v/v) HCl in the final volume of 1 ml was incubated at 80 °C for 40 min. TBARS were extracted with 1 ml of 1-butanol. After centrifugation at 2250×*g* for 10 min, the specific A_{532} of the organic phase and the non-specific A_{600} were measured. The results were expressed as $(A_{532}-A_{600})*g^{-1}$ of fresh weight.

Presentation of data

The presented data are the mean values ± SD from two independent experiments, each sample in duplicate. The significance of differences between mean values was determined by the Student's t-test.

Results and Discussion

Intensity and duration of ROS production

In all cell suspensions studied the culture filtrate (CF) of *P. infestans* of both isolates used: MP-322 or MP-306 (differing in virulence), induced accumulation of ROS, as shown by changes in LDC (Fig. 1). In the control, untreated cells no changes in LDC were observed during the whole period of exposure (results not shown). In all cell suspensions, despite the differences in the type and level of the resistance, the ROS accumulation started to increase 10 to 20 min after CF treatment but further process proceeded in a different manner. In

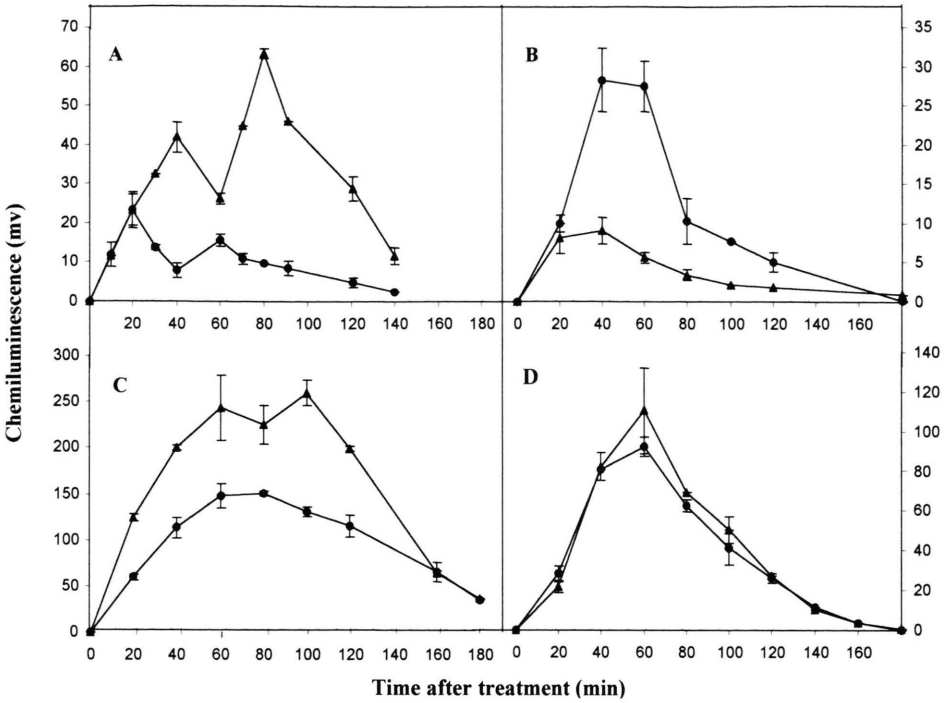

Fig. 1. Time course of ROS production by cell suspensions treated with CF (14 μg glucose equivalents per ml) of *P. infestans* isolates (•) MP-322, (▲) MP-306 measured using the luminol-dependent chemiluminescence (LDC) method. (A) *S. ngr*, (B) *S. tbr* cv. Bzura, (C) *S. tbr* cv. Tarpan, (D) *S. tbr* clone H-8105. The values are mean ± SD (n = 4).

the most resistant *S. ngr* (nonhost), two phases in the ROS generation could be distinguished: after a rapid initial increase, ROS accumulation decreased transiently, then rose again to a higher level, and afterwards lowered slowly to the control level. In contrast, in all *S. tbr* cell suspensions the ROS production took place in one phase only, but they differed in timing and intensity of the process. In the resistant Bzura cells the accumulation of ROS reached a maximum at about 40 min after CF treatment and then decreased promptly. In the susceptible clone H-8105 a high ROS production lasted up to 60 min, whereas in Tarpan the ROS accumulation increased gradually and remained at a high level for a prolonged time (about 80 min); afterwards, it decreased slowly to the initial level. Generally, in the resistant cells, *S. ngr* and *S. tbr* cv. Bzura, the ROS accumulation was lower than in the susceptible cells. The resistant cells only showed differences in the intensity and duration of the phases of ROS accumulation in response to both CF of MP-306 and MP-322, albeit the general pattern of the kinetics of the process remained the same. On the other hand, in *S. ngr* cells only, the ROS generation was biphasic. According to Świeżyński *et al.* (1993) differences in the responses of potato genotype to *P. infestans* isolates differing in virulence may indicate the presence of specific resistance. The author admitted that in potato breeding material, in our case cv. Bzura, unidentified specific resistance to *P. infestans* was likely. In *S. ngr* (nonhost) such differences might be due to genes responsible for the nonhost type of resistance of this genotype. Currently, distinction between resistance genes involved in the nonhost or field resistance and R genes remains unclear (Kamoun *et al.*, 1999). Differences in the oxidative burst observed at the level of cultured cells of the genotypes studied seem to reflect the differences in their relative resistance as evaluated in leaflet tests (see Introduction). Moreover, initially we have also observed that only *S. ngr* leaves exhibited HR in response to CF (MP-322) treatment, whereas the leaves of the *S. tbr* genotypes showed

some necrosis (cv. Bzura) or necrosis and chlorosis (cv. Tarpan and clone H-8105, data not shown). Our finding of two phases in the oxidative burst in *S. ngr* is in concert with Dorey s *et al.*, (1999) report, who also noted a biphasic ROS production in tobacco cell suspension treated with β-megaspermin, the elicitor causing HR in tobacco leaves, similar to that observed by us in CF-treated *S. ngr* leaves. The second phase is considered to relate to the recognition process between nonhost/ pathogen at the species level and cultivar/ race at the subspecies level (Baker *et al.*, 1993). In our case, in the resistant Bzura cells such interaction apparently did not occur.

Since the cells studied differed in the kinetics of the ROS production, it seemed reasonable to perform further comparative experiments using the cells being at a comparable stage of CF-induction (basing on the data in Fig. 1). Since in *S. ngr* cells the two phases of ROS production might be controlled by different mechanisms, these cells were investigated at the time points that corresponded with peaks of the phases.

The elicitor CF (MP-322) dose dependence of the ROS production was examined in cells that already reached a high level of the ROS. In *S. ngr* cells the highest dose of CF significantly inhibited ROS generation during the first phase, whereas this effect was not observed in the second phase (Fig. 2A). Such a differential response might be related to nonspecific and specific character of the respective phases. In the resistant *S. tbr* Bzura cells the increased doses of CF enhanced ROS production (Fig. 2B). In contrast, in the susceptible *S. tbr* cells of Tarpan and clone H-8105 the ROS production lowered after treatment with higher doses of CF (Fig. 2C,D). Keeping in mind that CF of *P. infestans* contained toxic substances that could influence cell reactions (Buiatti and Ingram, 1991; see ref. Awan *et al.*, 1997), it is conceivable that this reduction of ROS production in the susceptible cells was due to their sensitivity to a high concentration of the toxins and/or a propagation of oxidative damage. This is in agreement with our previous finding that viability of the Tarpan cells was significantly decreased by CF treatment, while

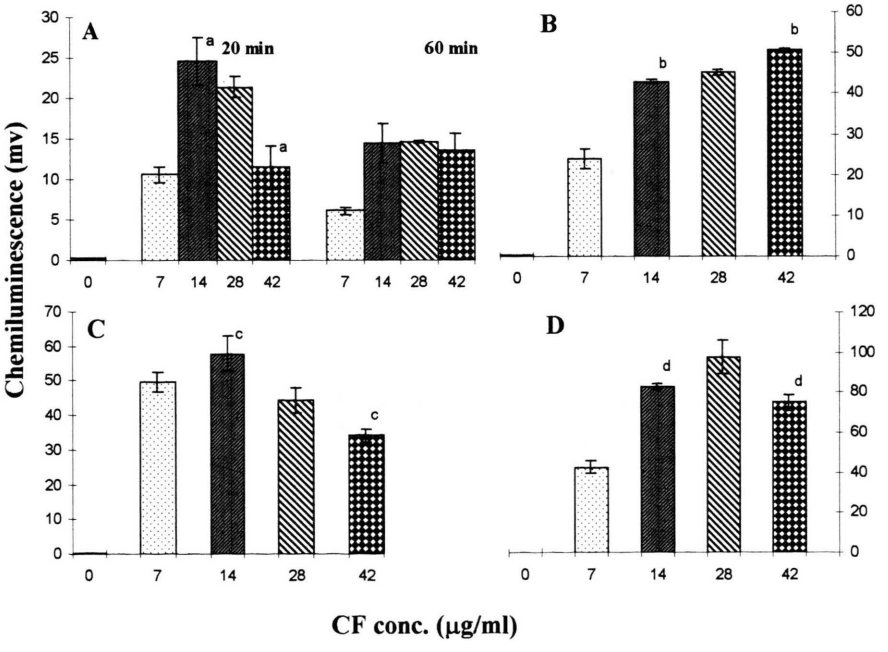

Fig. 2. Dose-dependent production of ROS by cell suspensions (A) *S. ngr*, (B) *S. thr* cv. Bzura, (C) *S. tbr* cv. Tarpan, (D) *S. tbr* clone H-8105 treated with CF MP-322 (7–42 µg glucose equivalents ml^{-1}). The ROS production was measured by the LDC method at 60 min after CF treatment or as indicated. The values are mean ± SD (n = 4). Letters indicate significant differences between mean values at $p < 0.05$.

the Bzura cells were only slightly affected (Awan *et al.*, 1997).

Effects of ROS scavengers

To assess whether there are any differences in the composition of ROS accumulated by the elicited cells we have used exogenous catalase (CAT) and superoxide dismutase (SOD). To exclude possible effects of other processes on the composition of ROS accumulated, which might take place during a prolonged CF treatment, the experiments with ROS scavengers were carried out with cells being just at the beginning of the stage of a high ROS accumulation. The *S. ngr* cells were consequently examined at the time intervals corresponding to the peaks of both phases.

In all types of cells studied, addition of CAT to the elicitor-treated cells caused a rapid decrease in the level of ROS accumulated (Fig. 3). It points to H_2O_2 as a predominant component of the ROS detected. This referred mainly to the Bzura and Tarpan cells, in which exogenous SOD had no effect on the level of detected ROS. In the *S. ngr* and H-8105 cells only addition of 100 units SOD caused an increase in the ROS accumulation. This fact may indicate the presence of a certain amount of $O_2^{\cdot -}$ in the ROS produced. Thus, these results suggest that all types of the CF – treated cells accumulated mostly H_2O_2; the latter may be directly generated or produced by a very rapid dismutation of the initially generated $O_2^{\cdot -}$, particularly in the case of *S. ngr* and H-8105 cells.

Identification of the systems of ROS production

According to the identified systems of ROS production the mammalian-type NADPH oxidase generates $O_2^{\cdot -}$, while pH-dependent cell-wall peroxidase may be responsible for H_2O_2 production (Bolwell *et al.*, 1999). At present, for discrimination between these two systems, diphenyleneio-

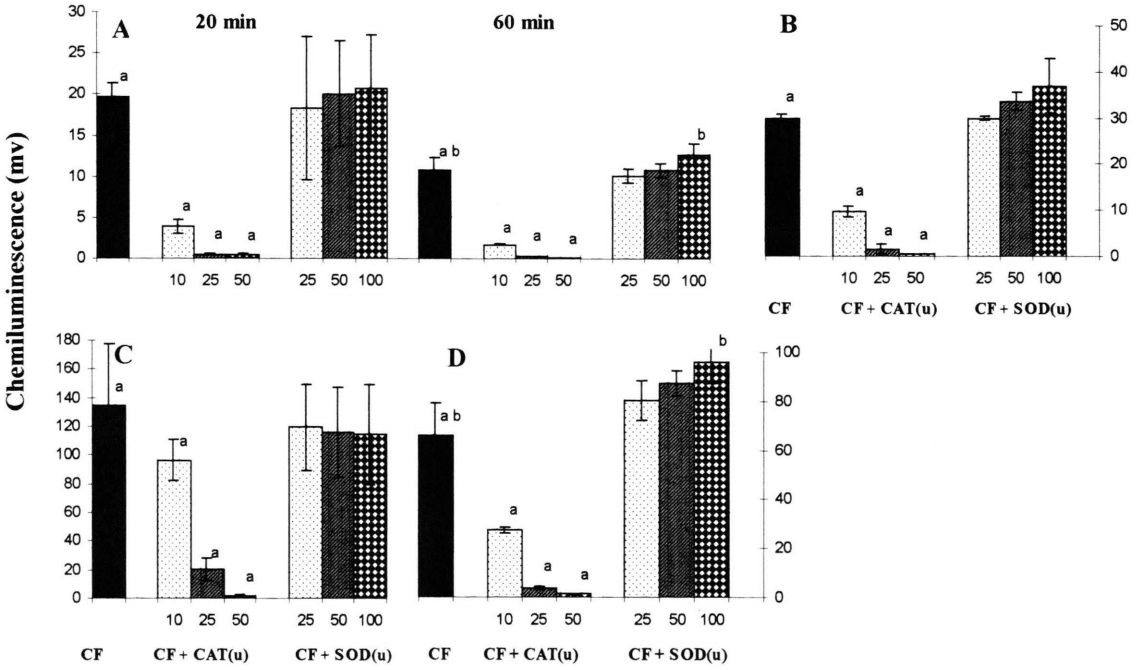

Fig. 3. Effects of CAT (10,25,50 units) and SOD (25,50,100 units) on ROS accumulation in (A) *S. ngr*, (B) *S. tbr* cv. Bzura, (C) *S. tbr* cv. Tarpan, (D) *S. tbr* clone H-8105 cells treated with CF MP-322 (14 μg glucose equivalents ml^{-1}). The ROS accumulation was measured by the LDC method at 40 min after CF treatment or as indicated. CAT or SOD were added to CF-treated cells 1 min prior to the measurement. The values are mean ± SD (n = 4). Letters indicate significant differences between mean values at $p < 0.05$. CAT: one unit will decompose 1.0 μmole of H_2O_2 per min at pH 7.0 at 25°C while the H_2O_2 concentration falls from 10.3 to 9.2 mM. SOD: one unit will inhibit the rate of reduction of cytochrome c by 50% in a coupled system with xanthine and xanthine oxidase at pH 7.4 at 25°C in a 3.0 ml reaction volume.

donium (DPI), a potent inhibitor of the mammalian NADPH oxidase, and pH effects are studied (Bolwell *et al.*, 1998; Piedras *et al.*, 1998). Despite many objections concerning the limited specificity of DPI, inhibition of the oxidative burst by its low concentrations (for cells 1–25 μM) may suggest participation of the NADPH oxidase, whereas the peroxidase system, is inhibited by up to 100–250 μM DPI and requires pH 7–7.2 (Wojtaszek, 1997; Bolwell and Wojtaszek, 1997; Frahry and Schopfer, 1998).

To identify the systems responsible for ROS production in all types of the cells used, we have investigated in parallel the ROS production and activities of cellular and extracellular peroxidases in the cells treated with CF or both DPI and CF. In this study, we examined the cells (*S. tbr* cv. Bzura and clone H-8105) being at the stage of a high level of ROS accumulation (chosen basing on the data in Fig. 1). Since in *S. ngr* and *S. tbr* cv. Tarpan cells the ROS accumulation was prolonged, it was conceivable that different systems might be responsible for ROS generation at the early and late stages of response. Therefore, we decided to examine these cells after a short and a prolonged time of CF treatment.

When DPI was added to the cells prior to CF treatment, the ROS production was inhibited significantly in comparison with those induced by CF alone (Fig. 4). The higher concentrations of DPI were used, the stronger inhibitory effects were observed. In both *S. ngr* and *S. tbr* cv. Tarpan cells, inhibition of CF-induced ROS production by DPI was more pronounced at the late stage of response than at the early stage (Fig. 4A,C).

The peroxidase activity was measured in the cell samples taken from the same batches, which were used in the assays with DPI. In all cell types studied, the activity of the cellular peroxidases almost did not change after CF or DPI and CF treatments (data not shown). In cells of clone H-8105 only CF treatment resulted in an increase in extracellular peroxidase activity by about 30%. However, in the presence of DPI the CF-induced peroxidase activity decreased to the level of control, untreated cells (101 $\Delta A^{*}min^{-1}{*}mg^{-1}protein$). In

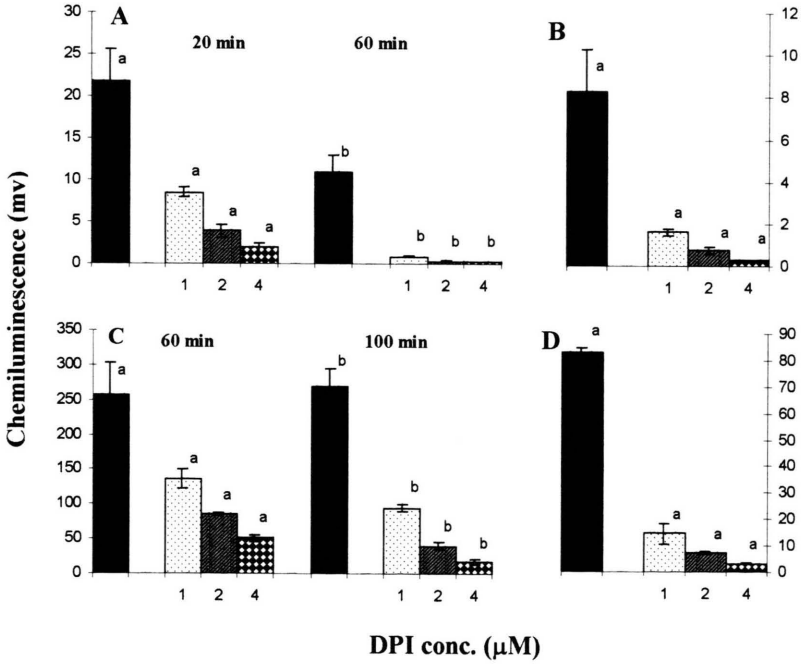

Fig. 4. Effect of DPI on ROS production by cell suspensions treated with CF (14 μg glucose equivalents ml^{-1}). (A) *S. ngr*, (B) *S. tbr* cv. Bzura, (C) *S. tbr* cv. Tarpan, (D) *S. tbr* clone H-8105. *S. ngr* treated with CF MP-322, *S. tbr* treated with CF MP-306 (solid bars). DPI was added to the cells 10 min prior to CF treatment. The ROS production was measured by the LDC method at 50 min after CF treatment or as indicated. The values are mean ± SD (n = 4). Letters indicate significant differences between mean values at $p < 0.05$.

other *S. tbr* cells studied, the treatments with CF or DPI and CF had no significant effect on the extracellular peroxidase activity. In *S. ngr* cells treated with CF alone the activity of the extracellular peroxidase was reduced in the first phase, but did not change significantly in the second one, when compared to the control, untreated cells (110 $\Delta A*min^{-1}*mg^{-1}$protein). This observation suggested that CF affected different isoenzymes in both phases. The presence of DPI in the elicited *S. ngr* cells did not affect the extracellular peroxidase activity in both phases. Thus, in all cells studied, the ROS production was substantially reduced in the presence of very low concentrations of DPI, whereas at the same time the peroxidase activity remained on a rather high level. Furthermore, the assays for the ROS production by the cells were conducted at a lower pH (5.3), similar to that of the cell growth medium, in order to eliminate a possible shock due to pH change. Moreover, we have also previously found that in the CF-treated Tarpan and Bzura cells the extracellular pH did not increase above 6 (Awan *et al.,* 1997). So, all above suggests that similar systems, most probably the mammalian-type NADPH oxidase, were responsible for the oxidative burst in all cell types studied, and $O_2^{\cdot -}$ generated was dismutated too rapidly to be detected. This is in agreement with the data obtained for potato by Doke *et al.* (1996).

Lipid peroxidation

To investigate a possible effect of ROS accumulation on membrane damage we have measured lipid peroxidation in the elicited cells, immediately after the oxidative burst and in those, where the ROS were scavenged by the added CAT. In the *S. ngr* cells elicitation did not lead to an increase in lipid peroxidation. In the *S. tbr* resistant Bzura treated with CF, lipid peroxidation increased by about 6%, whereas in the susceptible Tarpan and clone H-8105 up to 12%–15%, respectively. The addition of CAT to the cells prior to CF treatment resulted in a decrease in the level of lipid peroxidation near to that in the non-elicited controls (about $A_{532}-A_{600} = 0.77*g^{-1}FW$). This observation suggested that the presence of the ROS led to lipid peroxidation in elicited cells. An increase in lipid peroxidation following the elicitor-induced oxidative burst has also been reported by Rogers *et al.*, 1988; Vera-Estrella *et al.*, 1992; May *et al.*, 1996. Lipid peroxidation may also be enhanced by lipoxygenase activity (see Introduction). Since a substantial increase in the latter was usually observed later than the maximum of the oxidative burst, so in our case the involvement of lipoxygenase activity in lipid peroxidation should rather be excluded.

Concluding remarks

Two major types of resistance in potato to *P. infestans* are recognized: specific resistance determined by R genes and general, field resistance dependent on the combined action of many genes. The latter, being durable to this highly variable pathogen, appears to be an important value in the potato breeding programmes (Colon *et al.*, 1995). In our studies *S. tbr* genotypes used represent a relative high (cv. Bzura) and low (cv. Tarpan and clone H-8105) polygenic resistance to *P. infestans.* The *S. ngr* posses nonhost resistance, that usually is described as similar to the cultivar-race specific resistance (Hadwiger and Culley, 1993).

The obtained data showed that both polygenically resistant and susceptible potato cells are capable of initiating the same reactions in response to CF treatment, however, these reactions varied with respect to the timing and intensity. In all cells studied the oxidative burst is probably determined by similar mechanisms. An intensive and prolonged ROS accumulation in the susceptible Tarpan and clone H-8105 cells may result from: 1) an intensive ROS production, 2) a low efficiency of endogenous systems decomposing ROS, 3) sensitivity of the cells to toxic substances contained in CF. To elucidate the two first possibilities additional studies are needed. The third one is indirectly confirmed by our data on effects of increased doses of CF on the ROS production in the susceptible cells (Fig. 2) as well as our previous findings on the decreased viability and the high and prolonged increase in extracellular pH (an early event in plant/pathogen interaction) in CF treated Tarpan cells (Awan *et al.*, 1997). Furthermore, an increase in lipid peroxidation in the susceptible cells may indicate that in these cells antioxidant systems are not efficient enough to control the level of free radicals. In the resistant cells, the CF-induced ROS production seems to be strictly

regulated. Thus, we propose that lack of stringent control of the oxidative processes and sensitivity to the toxins may be distinctive features of potato susceptibility to *P. infestans*. This suggestion is in concert with the opinion of Buiatti and Ingram (1991) on the correlation between resistance to pathogen and tolerance to toxins.

Since model cell suspension system may not accurately reflect the complex interactions in the whole plant, studies with plant leaves are in progress, in order to confirm our present conclusions.

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