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Comparison of defence responses to *Botrytis cinerea* infection in tomato plants propagated *in vitro* and grown *in vivo*

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Abstract

Defence reactions: O_2^- generation, superoxide dismutase, catalase, guaiacol peroxidase and ascorbate peroxidase activities after *B. cinerea* infection in tomato plants propagated *in vitro* and grown *in vivo* have been compared. Infection resulted in rapid O_2^- generation. Superoxide dismutase activity increase was slower than O_2^- response. In plants propagated *in vitro* catalase and guaiacol peroxidase activities after infection were induced less strongly than in plants grown *in vivo*. K_2HPO_4 pretreatment of plants grown *in vitro* enhanced significantly the activities of catalase and guaiacol peroxidase after infection. Slight restriction of *B. cinerea* infection development in *in vitro* propagated plants pretreated with K_2HPO4 was observed.

INTRODUCTION

The use of *in vitro* culture methods in propagating many plant species of economic importance has increased considerably during the last decade. However, *in vitro* propagated plantlets in the phase of acclimatization to greenhouse conditions are highly susceptible to pathogen attack. One can assumed that in the stage of acclimatization *in vitro* plantlets defence system is less strongly or not so rapidly mobilized than that of plants propagated by traditional methods. Therefore studies on the defence system of *in vitro* propagated plantlets have been undertaken and attempts have been made to induce their defence reactions.

It has been demonstrated that defence responses observed in infected plants can be also induced by elicitors (S c h n e i d e r and U l l r i c h, 1994). Elicitors include preparations of pathogenic microorganisms as well as abiotic compounds. Recently it has been found that some inorganic salt components of *in vitro* culture media, applied

at higher concentrations, may also act as elicitors (I r v i n g and K u ć, 1990). Induction of plant defence reactions in response to elicitor treatment may lead to increased resistance to subsequent infections.

In this work we compared defence reactions of *in vitro* propagated tomato plantlets and plants grown from seeds in a growth chamber and tried to induce defence responses of *in vitro* multiplied plantlets using K_2HPO_4 as an elicitor. We examined O_2^- generation and superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (PO) and ascorbate peroxidase (APX) activities as defence responses after *B. cinerea* infection.

MATERIALS AND METHODS

Tomato plants. Tomato plants (*Lycopersicon esculentum Mill.* cv. „Perkoz") were grown from seeds in soil, in a growth chamber at 23°C and with 16 h photoperiod or were propagated *in vitro* on MS medium supplemented with 1 mg/l BAP and 0.05 mg/l IAA. Plantlets from in vitro culture were rooted for 1 week on MS medium with 1 mg/l NAA. Then plantlets were transferred to perlite and grown in a growth chamber at 23°C and with 16 h photoperiod. To induce defence responses one part of in vitro multiplied plantlets was rooted on the same medium supplemented with 5 mM K_2HPO_4.

***Botrytis cinerea* infection.** Tomato plants grown from seeds were inoculated with fungal conidia at the age of 2 weeks and *in vitro* propagated plants, both K_2HPO_4 pretreated and non-treated, after transfer to perlite. *B. cinerea* conidial suspension contained 1 x 10^6 conidia in 1 ml. After inoculation plants were kept at 100% relative humidity. Leaves were examined for enzyme activity and O_2^- content 1, 2, 3 and 5 days after inoculation. Control plants were examined simultaneously.

Preparation of enzyme extracts. The leaves were homogenized (1 : 5 w/v) in 1 M NaCl in 50 mM phosphate buffer pH 7.0 containing 1% PVP, 1 mM EDTA. For assay of APX extracts were prepared in the same medium containing 1 mM sodium ascorbate. The homogenate was centrifuged and the supernatant was used as enzyme extract to assay SOD, CAT, PO and APX activities.

The detection of O_2^- was based on its ability to reduce nitro blue tetrazolium (NBT) and was performed according to D o k e (1983). Five fresh leaf discs (Ø 0.8 cm) were immersed in 3 ml 0.01 M potassium phosphate buffer pH 7.8 containing 0.05% NBT and 10 mM NaN_3 for 1 hour. Then the mixture was heated at 85°C for 15 minutes and cooled rapidly. The discs' activity to reduce NBT was expressed as increased absorbance per hour per disc.

Enzyme assays. The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of NBT using the method of Beauchamp and Fridovich as described by D h i n d s a et al. (1981). CAT activity was measured spectrophotometrically according to D h i n d s a et al. (1981) and that of guaiacol peroxidase as described by M a e h l y and C h a n c e (1954). APX was determined

with sodium ascorbate as a substrate according to N a k a n o and A s a d a (1981) but we used 265 nm instead of 290 nm (an absorbance coefficient of 13.7 $mM^{-1}cm^{-1}$). All results are the means of three separate experiments.

RESULTS AND DISCUSSION

In tomato plants, both grown from seeds and propagated *in vitro*, *B. cinerea* infection resulted in rapid O_2^- formation (Fig. 1). However, the highest increase in O_2^- formation, about 200% of control level, was detected earlier in plants multiplied *in vitro*. The rate of SOD activity increase was slower than O_2^- response (Fig. 2). SOD activity reached the highest level only 3 days after *B. cinerea* inoculation when O_2^- production decreased significantly. The fall back of O_2^- content after its early increase could be at least partly the effect of increasing SOD activity. CAT activity increased gradually during infection development and reached the highest level 5 days after inoculation, both in plants grown *in vivo* and those multiplied *in vitro* (Fig. 3). However, during the whole examined period CAT induction was about 20% weaker in *in vitro* propagated plantlets in comparison with plants grown from seeds. *B. cinerea* infection resulted in a significant increase in PO activity in plants grown *in vivo* (Fig. 4). The activity increase was visible 1 day after inoculation and persisted for 5 days. The highest activity, about 290% of control, was detected 5 days after inoculation. In plants propagated *in vitro* PO activity increase was detected later and 5 days after inoculation was about 1.5 times lower than that in infected plants grown *in vivo*. On the other hand the induction of APX was observed earlier and was slightly stronger in plants propagated *in vitro* e.g. 3 days after inoculation the APX activity in plants cultured *in vitro* and grown *in vivo* was 160% and 120% of control, respectively (Fig. 5).

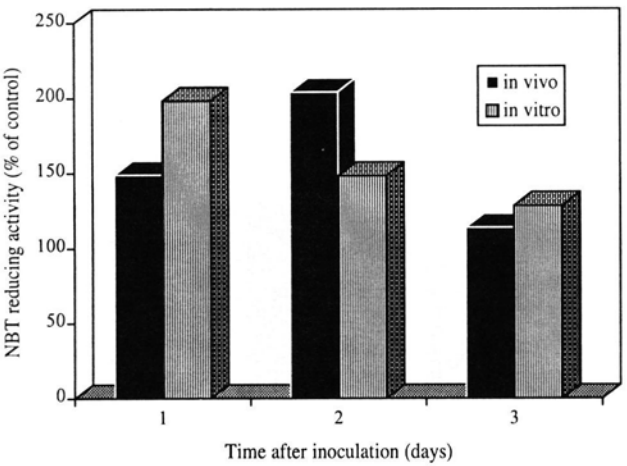

Fig. 1. Time course of O_2^- generation in *B. cinerea* infected tomato leaves

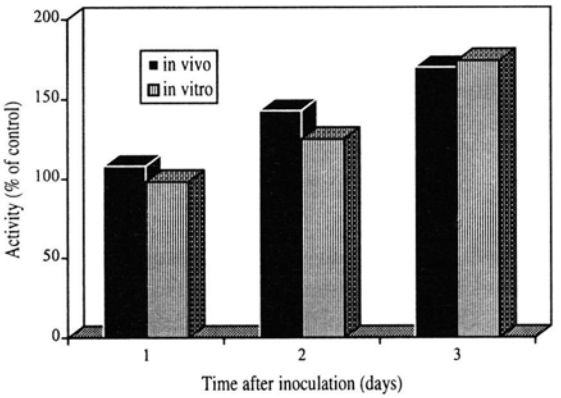

Fig. 2. Superoxide dismutase activity in tomato leaves after *B. cinerea* infection.

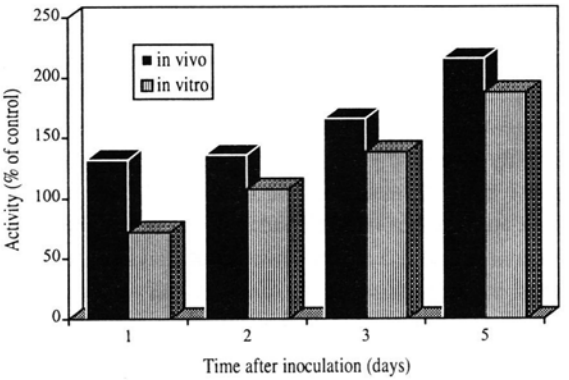

Fig. 3. Catalase activity in tomato leaves after *B. cinerea* infection.

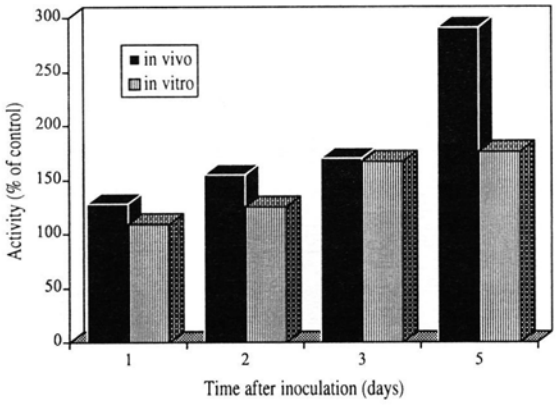

Fig. 4. Guaiacol peroxidase activity in tomato leaves after *B. cinerea* infection.

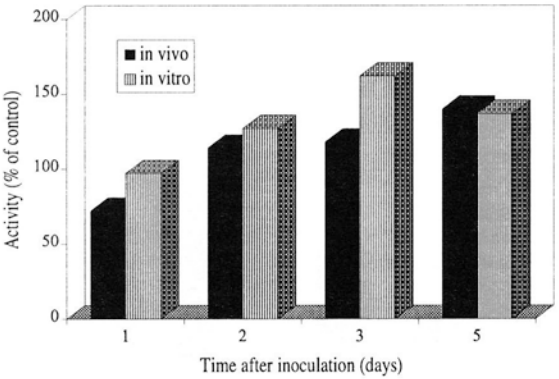

Fig. 5. Ascorbate peroxidase activity in tomato leaves after *B. cinerea* infection.

It has been reported that active oxygen species and antioxidant enzymes may contribute to the multicomponent mechanism of disease resistance. O_2^- generation may be directly involved in hypersensitive response (D o k e, 1983). The antioxidant mechanisms that may contribute to plant pathogenesis include SOD, CAT and APX (B a k e r and O r l a n d i, 1995). The cell wall bound peroxidases have been reported to oxidase NAD(P)H leading to the formation of O_2^- and H_2O_2, the latter is used in the formation of lignin (H a m m e r s c h m i d t, 1984). Our results seem to support the reports indicating that active oxygen species and antioxidant mechanisms: SOD, CAT and peroxidases may play a role in plant – pathogen interactions.

We observed that in *in vitro* propagated plantlets the induction of CAT and PO in response to infection was weaker than in plants grown from seeds. It seemed interesting to examine whether there is a correlation between the diminished activity of CAT and PO and the high susceptibility of *in vitro* propagated plantlets in the phase of acclimatization to *in vivo* conditions. We used K_2HPO_4 to elicit CAT and PO activity in *in vitro* cultured tomato plantlets. It has been reported that foliar sprays of oxalate and phosphate salts solutions protected cucumber plants against a broad range of fungi, bacteria and viruses (M u c h a r r o m a h and K u ć, 1991) and phosphate and bicarbonate induced protection against powdery mildew on rose plants (R e u v e n i et al., 1994). However, the mechanism of protection induced by these chemicals remains unknown. G o t t s t e i n and K u ć (1989) have suggested that protection is triggered by a process involving the sequestering of calcium from host tissues.

We observed that in K_2HPO_4 treated plantlets the activity of CAT did not change significantly (Fig. 6) whereas PO activity was elicited on an average 30% above the control level (Fig. 7). In plantlets pretreated with K_2HPO_4 and then infected the activity of CAT, 1 day after inoculation, increased about 40% above the level in the infected, non-treated ones. The activity of PO after infection in plantlets pretreated with K_2HPO_4 was induced stronger than after infection without pretreatment 100% and 40%, 1 and 3 days after inoculation, respectively. We observed that infection development in K_2HPO_4 pretreated plantlets was slightly restricted as compared with non-pretreated

ones. It seems that enhanced CAT and PO activities are not sufficient to increase resistance of *in vitro* propagated plantlets in the stage of acclimatization. It was reported that the lack of epicuticular wax on the leaves of *in vitro* propagated plantlets is also associated with low survivability during hardening off (T o r r e s, 1989).

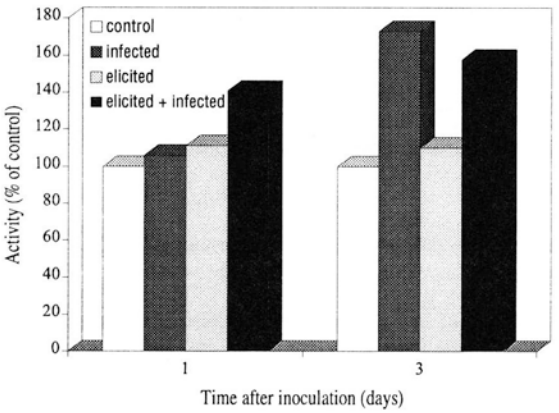

Fig. 6. Catalase activity in *in vitro* propagated tomato leaves after K_2HPO_4 pretreatment and *B. cinerea* infection.

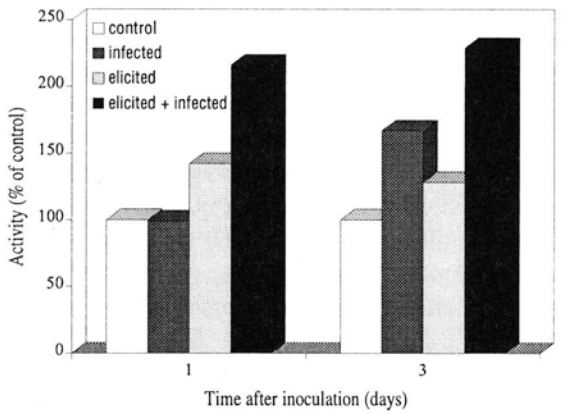

Fig. 7. Guaiacol peroxidase activity in *in vitro* propagated tomato leaves after K_2HPO_4 pretreatment and *B. cinerea* infection.

In conclusion our results indicated that the K_2HPO_4 pretreatment sensitizes plantlets from *in vitro* culture resulting in more rapid increase in CAT and PO activities after infection. It seems possible that enhanced activities of CAT and PO after inoculation, induced in phosphate pretreated plantlets, may contribute to the slight restriction of *B. cinerea* infection development observed.

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REFERENCES

Baker C.J., Orlandi E., 1995. Active oxygen in plant pathogenesis. Annu. Rev. Phytopathol. 33: 299-321.

Dhindsa R.S., Plumb-Dhindsa P., Thorpe T.A., 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. 32: 93-101.

Doke N., 1983. Involvement of superoxide anion generation in the hypersensitive response of potato tuber tissues to infection with an incompatible race of *Phytophthora infestans* and to the hyphal wall components. Physial. Plant Pathol. 23: 345-357.

Gottstein H.G., Kuć J., 1989. Induction of systemic resistance to anthracnose in cucumber by phosphates. Phytopathology 79: 176-179.

Hammerschmidt R., 1984. Rapid deposition of lignin in potato tuber tissue as a response to fungi non-pathogenic on potato. Physiol. Plant Pathol. 24: 33-42.

Irving H.R., Kuć J.A., 1990. Local and systemic induction of peroxidase, chitinase and resistance in cucumber plants by K_2HPO_4. Physiol. Mol. Plant Pathol. 37: 355-366.

Maehly A.C., Chance B., 1954. The assay of catalases and peroxidases. In: Glick D., (Ed.) Methods of biochemical analysis. 1: 357-425. Interscience Publishers Inc., New York.

Mucharromah E., Kuć J., 1991. Oxalate and phosphates induce systemic resistance against diseases caused by fungi, bacteria and viruses in cucumber. Crop Protection. 10: 265-270.

Nakano Y., Asada K., 1981. Hydrogen peroxide is scavenged by ascorbate – specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22: 867-880.

Reuveni R., Agapov V., Raviv M., 1994. Effect of foliar sprays of phosphates on powdery mildew (*Sphaerotheca pannosa*) of roses. J. Phytopathology. 142: 331-337.

Schneider S., Ullrich W.R., 1994. Differential induction of resistance and enhanced enzyme activities in cucumber and tobacco caused by treatment with various abiotic and biotic inducers. Physiol. Mol. Plant Pathol. 45: 291-304.

Torres K.C., 1989. Tissue culture techniques for horticular crops, New York, Avi Book: 57-63.

Porównanie reakcji obronnych po infekcji *Botrytis cinerea* u roślin pomidora otrzymanych techniką *in vitro* i rozmnażanych z nasion.

Streszczenie

W pracy porównywano reakcje obronne: generowanie O_2^-, aktywność dysmutazy ponadtlenkowej (SOD), katalazy (CAT), peroksydazy guajakolowej (PO) i peroksydazy askorbinianowej (APX) w roślinach pomidora otrzymanych techniką *in vitro* i rozmnażanych z nasion po inokulacji *B. cinerea*. Stwierdzono, że w wyniku infekcji następowało szybkie generowanie O_2^-. Wzrost aktywności SOD następował później niż generowanie O_2^-. W odpowiedzi na infekcję rośliny rozmnażane *in vitro* reagowały słabszą indukcją CAT i PO. Przedinfekcyjne traktowanie roślin rozmnażanych *in vitro* K_2HPO_4 powodowało znacznie silniejszą indukcję CAT i PO po infekcji. Obserwowano niewielkie ograniczenie rozwoju choroby w roślinach rozmnażanych *in vitro* pretraktowanych K_2HPO_4.