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Responses of the Ascorbate-Glutathione Cycle to Necrotic Virus Infections in Tobacco

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Summary

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Malondialdehyde (MDA) levels, and activities of ascorbate peroxidase (AP), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) were determined in *Nicotiana tabacum* L. *cv.* Xanthi-nc infected by tobacco mosaic virus (TMV) and in *Nicotiana benthamiana* L. infected with tobacco necrosis virus. TMV infections led to considerably increased MDA levels in *N. tabacum* leaf tissues incubated under light, but less noticeably in darkness. In virus infected leaves AP activities increased substantially, while DHAR activities decreased in both plant-virus interactions. In TMV infected *N. tabacum* leaves elevated GR activities were also observed. It is supposed that antioxidative reactions influence necrotization and thereby symptom expression in virus infected tobacco plants.

Introduction

Environmental stress factors and microbial infections can increase the formation of reduced, reactive derivatives of molecular oxygen in plant tissues. This oxidative stress can initiate lipid peroxidation processes leading to membrane destruction and cell death. It is supposed that these oxidative processes are responsible for the occurrence of rapid cell death (appearance of necrotic lesions) elicited by infections in incompatible host-pathogen interactions (hypersensitive responses). Hydrogen peroxide seems to play a central role in the oxidative burst

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elicited by pathogen infections in plants (BAKER & ORLANDI 1995). Several antioxidative systems are known to participate in plant defense reactions against oxidative stress. The ascorbate-glutathione cycle plays a principal role in the cellular antioxidative defense by removing excess hydrogen peroxide (FOYER & al. 1994, EL-ZAHABY & al. 1995). Fungal infections were shown to induce different components of the ascorbate-glutathione cycle and other antioxidative processes (GÖNNER & SCHLÖSSER 1993, EL-ZAHABY & al. 1995). The activity of glutathione S-transferase (which is able to catalyze the breakdown of lipid hydroperoxides that derives from lipid peroxidation processes) can be induced by fungal infections, fungal elicitors (MAUCH & DUDLER 1993), and viral infections (GULLNER & al. 1995a, b).

Only limited information is available on the role of antioxidants in plant response reactions to virus infections. In the present study the function of ascorbate-glutathione cycle was investigated in tobacco plants inoculated with viruses that cause local hypersensitive necrosis. *Nicotiana tabacum* L. *cv.* Xanthi-nc and *Nicotiana benthamiana* L. were inoculated with tobacco mosaic virus (TMV) and tobacco necrosis virus (TNV), respectively. Levels of malondialdehyde (MDA), and the activities of ascorbate peroxidase (AP, E.C. 1.11.1.11.), dehydroascorbate reductase (DHAR, E.C. 1.8.5.1.), and glutathione reductase (GR, E.C. 1.6.4.2) were determined in the inoculated and healthy control leaves.

Abbreviations: AP, ascorbate peroxidase; DHAR, dehydroascorbate reductase; GR, glutathione reductase; TMV, tobacco mosaic virus; TNV, tobacco necrosis virus.

Materials and Methods

Seeds of tobacco plants (*Nicotiana tabacum* L. *cv.* Xanthi-nc, and *Nicotiana benthamiana* L.) were sown in soil and grown under normal greenhouse conditions (18-23 °C; supplemental light: 160 mE m^{-2} s^{-1} for 8 h per d; relative humidity: 75-80%). The second and third true leaves of 2-month-old *N. tabacum* plants were inoculated with a suspension of the U_1 strain of TMV in sodium phosphate buffer (10 mM; pH 7.0) using Celite as an abrasive. For MDA determinations leaf discs were cut from control and virus inoculated leaves 1 h after inoculation and placed on polystyrene beads (diameter 5 mm) floating on tap water in Petri dishes. In additional experiments the second and third true leaves of 2-months-old *N. benthamiana* plants were inoculated by TNV suspensions. Mock inoculated plants were used as controls. Chemicals were purchased from Sigma (St. Louis, MO, U.S.A.).

For enzyme activity determinations leaves (0.5 g) were frozen in liquid nitrogen and suspended in 3 ml of cold 0.2 M TRIS/HCl buffer (pH 7.8) containing 3% soluble polyvinylpyrrolidone and 0.1 mM EDTA-Na_2. The homogenate was strained through muslin and centrifuged at 8000 g for 20 min at 4 °C. The supernatants were used as enzyme source. AP activity was determined spectrophotometrically at 290 nm according to NAKANO & ASADA 1981, except that in the assay mixture the concentrations of H_2O_2 and ascorbic acid were 0.5 and 0.25 mM, respectively. DHAR and GR activities were measured as previously described (KLAPHECK & al. 1990).

Malondialdehyde (MDA) levels were determined with 2-thiobarbituric acid reagent (POPHAM & NOVACKY 1991).

At least three independent parallel experiments were carried out in each case. The significant difference between mean values were evaluated by Student's t-test. Differences were considered to be significant at $P = 0.05$.

Results and Discussion

The visible necrotic lesions appeared on the infected leaves approx. 2 d after inoculation in both plant-virus interactions. Prior to symptom appearance gradually increasing MDA levels were observed in TMV-infected *N. tabacum* leaf discs incubated under continuous illumination, in accordance with earlier observations in intact plants (ÁDÁM & al. 1990). Less considerable accumulation of MDA was observed in disks incubated in darkness (Fig. 1). These results showed that lipid peroxidation was largely light-dependent and that photosynthetic electron transport processes are involved in the production of active oxygen species leading to increased lipid peroxidation. The hypersensitive reaction in *N. tabacum* L. *cv.* Xanthi-nc elicited by TMV was preceded by an oxidative burst (the increased production of superoxide anion), but not in systemically infected tobacco plants (DOKE & OHASHI 1988). Increased MDA levels were observed also in virus infected cowpea plants (BELEID EL-MOSHATY & al. 1993).

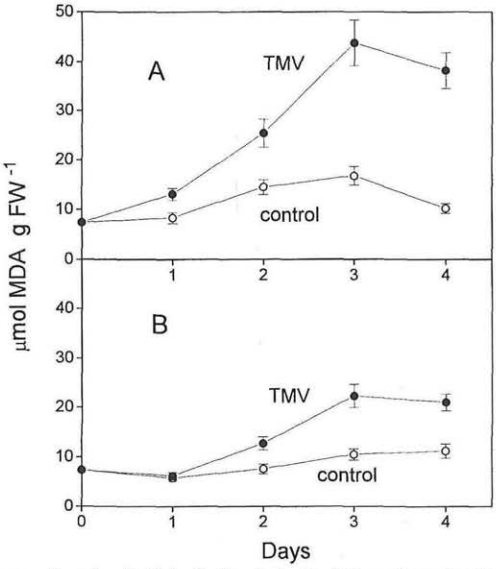

Fig. 1. Increase of malondialdehyde levels in leaf discs from healthy and tobacco mosaic virus (TMV) infected *N. tabacum* leaves. Leaf discs were incubated (A) under continuous light (150 µE m^{-2} s^{-1}), or (B) in darkness. Means of three independent experiments ± SD are shown.

Our studies proved that viral infections led to substantial changes also in the activities of enzymes of the H_2O_2 decomposing ascorbate-glutathione cycle. In TMV-infected *N. tabacum* leaves the AP activity increased up to 190% of control 4 d after

inoculation (Fig. 2). Similar induction of AP activity was found also in TNV infected *N. benthamiana* leaves (Fig. 2). To gain more information on AP the investigation of AP isoenzymes will be necessary. Since the chloroplastic AP isoforms lose activity rapidly upon extraction, ascorbate containing extraction medium has to be used for the determination of these isoforms (MITTLER & ZILINSKAS 1993).

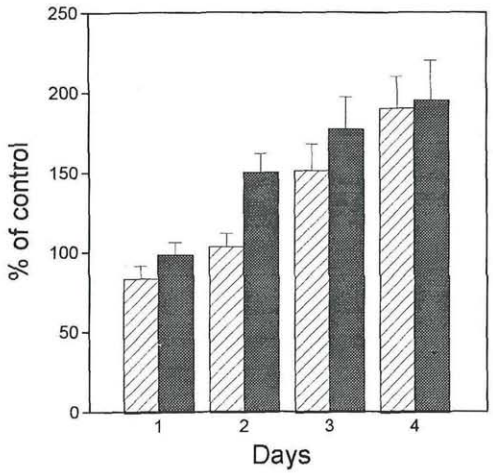

Fig. 2. Changes of ascorbate peroxidase (AP) activity in tobacco mosaic virus (TMV) infected *N. tabacum* and tobacco necrosis virus (TNV) infected *N. benthamiana* leaves. Means of three replicate experiments ± SD are shown. AP activities in control plants were 2.2 ± 0.17 and 1.31 ± 0.03 μmol ascorbate g FW^{-1} min^{-1} in *N. tabacum* and *N. benthamiana*, respectively (n = 4). Symbols: hatched columns: TMV - *N. tabacum* interaction; gray columns: TNV - *N. benthamiana* interaction.

In contrast to AP, decreased DHAR activities were observed in tobacco leaves 2 d after inoculation (66% and 63% of control after TMV and TNV infections, respectively). Activities of DHAR in uninfected healthy *N. tabacum* and *N. benthamiana* leaves were 0.11 ± 0.03 and 0.14 ± 0.02 μmol dehydroascorbate g FW^{-1} min^{-1}, respectively (n = 4).

The first detectable change of GR activity appeared 48 h after viral infection in TMV-infected *N. tabacum* leaves: the activity slightly decreased, down to 83% of control. However, after this transient drop the GR activity increased significantly and 4 d after inoculation it reached 152% of the control. The control GR activity was 0.13 ± 0.01 μmol NADPH g FW^{-1} min^{-1} (n = 4). Elevated AP (KUBO & al. 1995) and GR (EDWARDS & al. 1994) activities have been detected in plants exposed to various abiotic stress effects. In powdery mildew infected barley leaves elevated AP and unaltered GR activities were detected (EL-ZAHABY & al. 1995). It seems that the increased antioxidative defense ability of plants contributes to increased stress resistance.

Although several members of the ascorbate-glutathione cycle were activated by virus infections, decreasing DHAR activity may significantly hinder the recycling of dehydroascorbate and the normal function of this cycle. The other oxidized form of ascorbate, monodehydroascorbate, may also be produced after viral infection and can compensate for decreases in DHAR activities by decreasing the possibility of dehydroascorbate formation. Nevertheless, these redox changes can not prevent lipid peroxidation processes and the development of necrotic symptom expression. Further studies (the comparison of incompatible and compatible host-virus interactions) are necessary for a better understanding of the role of the ascorbate-glutathione cycle in plant resistance against viral infections associated with lipid peroxidation and host tissue necrosis.

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