The Influence of Jasmonic Acid on the Amount and the Distribution of Cysteine Proteinase PLCP-2 in Healthy and PVY^{NTN} Infected Potato Plants (*Solanum tuberosum* L.)

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Abstract

The localization of cysteine proteinase PLCP-2 was investigated in potato plants (*Solanum tuberosum* L.) cultivar Désirée by electron microscopy. Healthy and PVY^{NTN} infected potato plants were grown *in vitro* on media with or without a supplement of jasmonic acid. We had already shown that PLCP-2 is present in leaves, stems, tips of shoots and tips of roots of healthy and PVY^{NTN} infected plants. It was detected in various cell types in protein bodies in vacuoles, in cytoplasm and in cell walls. There were significantly larger amounts of PLCP-2 in plants grown on medium with a supplement of jasmonic acid in both healthy and virus infected plants. More protein bodies in vacuoles were found in plants grown on medium with addition of jasmonic acid.

Keywords: *Potato virus Y*^{NTN}; potato (*Solanum tuberosum* L.); cysteine proteinase PLCP-2; jasmonic acid; immunolocalization; electron microscopy

INTRODUCTION

Proteases are ubiquitous enzymes that are essential in plant growth and development. They are responsible for the control of enzymatic pathways, the control of various cell regulators, the timing of the cell cycle, the supply of amino acids, the removal of abnormal or damaged proteins, the removal of mis-located proteins and programmed cell death (VIERSTRA 1993, 1996). The activity of proteases is regulated by proteinase inhibitors. PLCP-2 is potato leaf cysteine proteinase with molecular mass of about 22 kDa, isolated from potato leaves (POPOVIČ unpublished).

A broad range of effects of jasmonic acid was detected, such as the influence on organogenesis (RAVNIKAR *et al*. 1992; CAMLOH *et al*. 1996), the influence on biophysical properties of membranes (VILHAR *et al*. 1991), the reduction of total content of soluble proteins (VILHAR *et al*. 1995), the decrease of amount of chlorophylls and caretenoids (KOVAČ & RAVNIKAR 1998) and stimulation of secondary metabolite production (BAEBLER *et al*. 2002).

A model where pathogen attack or wounding induce the synthesis of jasmonic acid in plants and consequently the expression of proteinase inhibitors was suggested (FARMER & RYAN 1992; KOIWA *et al*. 1997). There is quite some evidence that pathogen infection and jasmonic acid influence the levels of proteinase inhibitors in plants (AGRAWAL *et al*. 2002; GRUDEN *et al*. 1997; KREFT *et al*. 1997; SHIN *et al*. 2001). There is some (AVROVA *et al*. 1999; POMPE-NOVAK *et al*. 2002), but significantly less information available dealing with cysteine proteinases. However, the influence of jasmonic acid on cysteine proteinases was, according to our knowledge, not described yet.

MATERIALS AND METHODS

Single-node cuttings of healthy and PVY^{NTN} secondary infected potato plants (*Solanum tuberosum* L. cultivar Désirée) were grown on modified Murashige-Skoog medium.

To investigate the role of jasmonic acid, 1 μM of jasmonic acid was added to the medium. Medium without jasmonic acid was taken as control. Plants were kept at 18 $\pm$ 2°C, with illumination at 70 μM per m^2/s and a photoperiod of 16 h.

After 5 weeks of cultivation, shoot tips, 1 mm^2 pieces of 7th leaf, 1 mm thick slices of stem between 7th and 8th internodes and root tips of the longest roots were taken. Pieces of plant tissue were fixed in a mixture of 2% paraformaldehyde and 0.1% glutaraldehyde in phosphate buffer, dehydrated in a series of ethanol solutions and embedded in LR White.

Ultrathin sections were incubated with polyclonal rabbit antibodies to cysteine proteinase PLCP-2 diluted 1:20 with 1% BSA in phosphate buffer, and with protein A – gold, diluted 1:200. Sections were stained with 3% uranyl acetate and Reynold's lead citrate. Samples were observed with a Philips CM100 transmission electron microscope at 80 kV.

All experiments were repeated two times.

RESULTS AND DISCUSSION

Our previous results have shown that PLCP-2 is present in protein bodies in vacuoles, in cytoplasm and in cell walls of shoot tips, leaves, stems and root

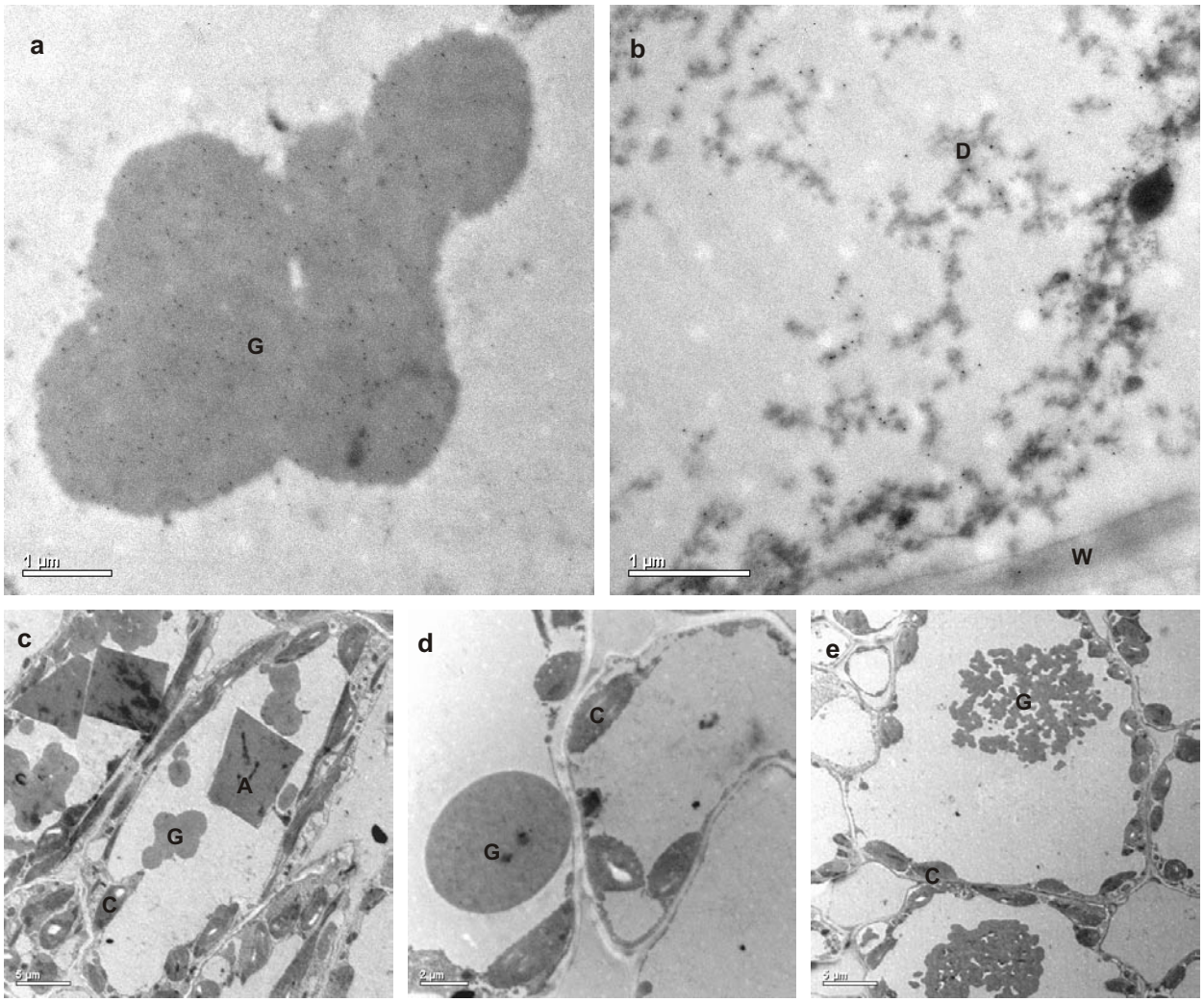

Figure 1a–e. Different types of inclusions in leaves of plants grown on medium with a supplement of jasmonic acid; G – globular protein body in vacuole; D – dispersed protein body in vacuole; A – angular inclusions in cytoplasm; W – cell wall; C – chloroplast

tips of healthy and PVY^{NTN} infected potato plants. The amount of PLCP-2 observed was low and no significant differences between localization of PLCP-2 in healthy and PVY^{NTN} infected plants were observed (POMPE-NOVAK *et al*. 2002).

There were significantly larger amounts of PLCP-2 in plants grown on medium with a supplement of jasmonic acid than in plants grown on control medium without a supplement of jasmonic acid. The majority of PLCP-2 was found in protein bodies in vacuoles. All globular (Figure 1a) and dispersed (Figure 1b) protein bodies in vacuoles were densely labeled with gold, while angular inclusions in cytoplasm and crystals in peroxisomes were not labeled. There were four different types of inclusions in leaves of jasmonic acid treated plants: globular protein bodies in vacuoles, dispersed protein bodies in vacuoles, angular inclusions in cytoplasm (Figure 1c) and crystals in peroxisomes. There were less globular protein bodies in vacuoles in stems, shoot tips and root tips than in leaves and there were no angular inclusions in the cytoplasm of cells of shoot and root tips. In plants grown on control medium, globular protein bodies in vacuoles were very rare and much smaller and there were no angular inclusions in the cytoplasm. In leaves of plants grown on the medium with the supplement of jasmonic acid, globular protein bodies in vacuoles measured up to 10 μm in diameter, angular inclusions in cytoplasm were about 6 μm in diameter and crystals in peroxisomes about 300 nm. Globular protein bodies were either condensed and spherical (Figure 1d) or a cluster of separate smaller spherical protein bodies (Figure 1e).

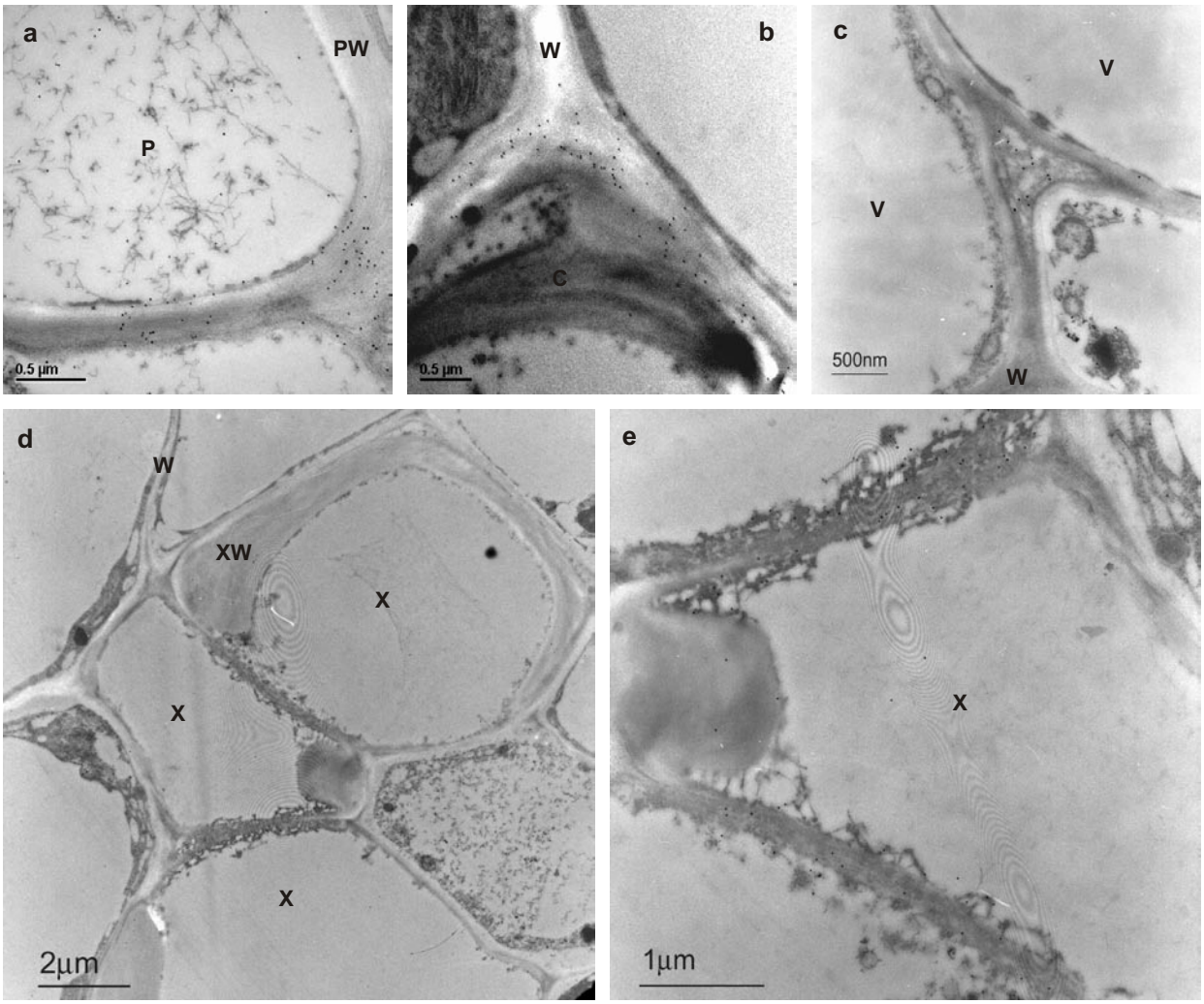

Figure 2. a – phloem cell in a leaf of a plant grown on medium with a supplement of jasmonic acid; b, c – parenhim cell in stem of a plant grown on medium with a supplement of jasmonic acid; d, e – xylem cells in stem of a plant grown on medium with a supplement of jasmonic acid; X – lumen of xylem; P – lumen of phloem; W – cell wall; WX – cell wall of xylem; WP – cell wall of phloem; C – chloroplast; V – vacuole

There were no significant differences in localization of PLCP-2 in xylem, in phloem (Figure 2a), in cell walls (Figure 2b) and in intracellular spaces (Figure 2c) between plants grown on the medium with the supplement of jasmonic and plants on the control medium.

PLCP-2 was found also on locations where degradation processes were going on. It was observed in cytoplasmatic residues of immature xylem, in cell walls of xylem cells that were in the process to join into single xylem vessel (Figures 2d and 2e) and all over the cells that seemed to be dying. Out of this, we can conclude that PLCP-2 has an essential role in degradation processes in potato plants.

No synergism of jasmonic acid treatment and virus infection was observed. Regarding PLCP-2 amount and location, no significant differences neither between healthy and PVY^{NTN} infected plants on the control medium were observed, nor between healthy and PVY^{NTN} infected plants on the medium with the supplement of jasmonic acid were found. There were, however, significantly larger amounts of PLCP-2 in plants grown on medium with the supplement of jasmonic acid than in plants grown on control medium. These results suggest that the answer of potato plants to PVY^{NTN} infection is not related to changes of proteinase PLCP-2 level.

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