Artificial elevation of glutathione affects symptom development in ZYMV-infected Cucurbita pepo L. plants

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Received May 17, 2006; accepted October 20, 2006; published online December 4, 2006
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Summary

Styrian oil pumpkin seedlings (Cucurbita pepo L. subsp. pepo var. styriaca GREB.) were treated for 48 h with 1 mM OTC (L-2-oxothiazolidine-4-carboxylic acid) in order to artificially increase cellular glutathione content. They were inoculated with zucchini yellow mosaic virus (ZYMV) 10 days later. The effects of OTC treatment and ZYMV infection on glutathione levels were examined at the subcellular level by immunogold labeling of glutathione using a transmission electron microscope (TEM). These effects were further tested at the whole-tissue level by high performance liquid chromatography (HPLC). Such tests were carried out a) on roots, cotyledons and the first true leaves immediately after OTC treatment in order to analyze to which extent OTC increases glutathione levels in different cell compartments as well as in the whole organ; and b) in older and younger leaves and in roots three weeks after ZYMV inoculation in order to study how possible effects of OTC on symptom development would correlate with glutathione levels at the subcellular level and in the whole organ.

Immunocytological and biochemical investigations revealed that, 48 h after OTC treatment, glutathione content had increased in all investigated organs, up to 144% in peroxisomes of cotyledons. Three weeks after ZYMV inoculation, glutathione labeling density had significantly increased within intact cells of infected leaves, up to 124% in the cytosol of younger leaves. Roots showed decreased amounts of glutathione in the TEM. Biochemical studies revealed that OTC treatment resulted in 41 and 51% higher glutathione content in older and younger ZYMV-infected leaves, respectively, in comparison to untreated and ZYMV-infected plants. Evaluation of symptom development at this point revealed that all untreated ZYMV-infected plants had symptoms, whereas only 42% of OTC-treated ZYMV-infected plants showed signs of symptoms. Quantification of ZYMV particles revealed that all organs of OTC-treated and ZYMV-infected plants contained significantly decreased amounts of ZYMV particles over a period of five weeks when compared to the same organs of untreated ZYMV-infected plants. We can conclude that OTC treatment and subsequently elevated glutathione contents within Styrian oil pumpkin plants led to a strong decrease in virus content, which was accompanied by a suppression of ZYMV-induced symptoms as well as...
reduced and delayed symptom development within plants exhibiting symptoms.

**Abbreviations**

GSH  reduced glutathione  
GSSG  oxidized glutathione  
HPLC  high performance liquid chromatography  
OTC  L-2-oxothiazolidine-4-carboxylic acid  
TEM  transmission electron microscope  
TMV  tobacco mosaic virus  
ZYMV  zucchini yellow mosaic virus

**Introduction**

Plants react to pathogen attack with changes in levels of antioxidants and related enzymes due to pathogen-induced oxidative stress and activation of defense genes. Ascorbate and glutathione (and its redox state) are thought to play key roles in a plant’s ability to develop successful defense strategies against pathogens [2, 5, 15, 25]. Glutathione seems to play an important role in the development of pathogen resistance as elevated levels of reduced glutathione (GSH) and thiols have been observed in leaves of resistant barley [6, 34, 36, 37] and oat plants [35, 36], although not in susceptible lines during powdery mildew attack. In susceptible lines, unchanged [35–37] or even decreased levels of GSH have been found [34, 36]. Decreased levels of glutathione have also been found during Botrytis cinerea infection in leaves and various cell compartments (mitochondria, chloroplasts and peroxisomes) of systemically infected tomato plants [23, 24] as well as in leaves of *Avena sativa* infected with the fungal pathogen *Drechslera* [11].

Elevated glutathione levels have also been found to be involved in the development of resistance during plant-virus interactions. In resistant tobacco plants inoculated with tobacco mosaic virus (TMV), an elevation of GSH was observed in both infected lower and uninfected upper leaves. Increased GSH levels in the uninfected upper leaves occurred two weeks after the inoculation of the lower leaves and appeared to be accompanied by the development of systemic acquired resistance [9]. Furthermore, the same group has found that, besides GSH, the majority of enzymatic and non-enzymatic antioxidants were more active in resistant tobacco plants than they were in the non-resistant species after inoculation with TMV [19]. In cucumber (*Cucumis sativus*) and Styrian oil pumpkin (*Cucurbita pepo* L. subsp. *pepo* var. *styriaca* GREB.) plants, systemically infected with zucchini yellow mosaic virus (ZYMV), an increased activity of antioxidative enzymes, like ascorbate peroxidases and superoxide dismutases, has been reported [31]. The author hypothesized that the ascorbate-glutathione cycle might play an important role in the detoxification of H$_2$O$_2$ during virus infection.

In previous work, we demonstrated that, during ZYMV-infection, elevated glutathione levels only occurred among single, intact cells of infected *Cucurbita pepo* plants, but not in sections of whole leaves where glutathione levels remained unchanged [41]. Since the investigated leaves showed strong symptoms of ZYMV disease, the amount of glutathione accumulating within whole leaves was clearly not sufficient to prevent the development of symptoms. Therefore, the aim of the present study was to test whether an artificial elevation of the glutathione level is able to induce any signs of resistance or tolerance (e.g. suppression, reduction or delay of symptom development) in susceptible lines of *Cucurbita pepo* L. plants during ZYMV infection.

In this experiment, glutathione was artificially elevated by treating seedlings with increased amounts of L-2-oxothiazolidine-4-carboxylic acid (OTC). Treatment of OTC has been known to enhance glutathione levels by either reducing its degradation due to the inhibition of the metabolism of 5-oxo-L-proline by OTC (which instead of 5-oxo-L-proline is used as a substrate by 5-oxo-L-prolinase) or by serving as an intracellular delivery system for cysteine (which is the rate-limiting substrate during glutathione synthesis in plants [21, 30], via S-cysteine, the product of hydrolysis of OTC by 5-oxo-L-proline is used as a substrate by 5-oxo-L-prolinase) or by serving as an intracellular delivery system for cysteine (which is the rate-limiting substrate during glutathione synthesis in plants [21, 30], via S-cysteine, the product of hydrolysis of OTC by 5-oxo-L-proline is used as a substrate by 5-oxo-L-prolinase). OTC has been proven to artificially increase glutathione levels in spinach plants [16], tobacco plants [13], pea leaves [14], and poplar leaves [20]. In TMV-infected tobacco leaf discs, OTC-treatment strongly increased glutathione levels. Subsequently, it suppressed the development of necrotic lesions and