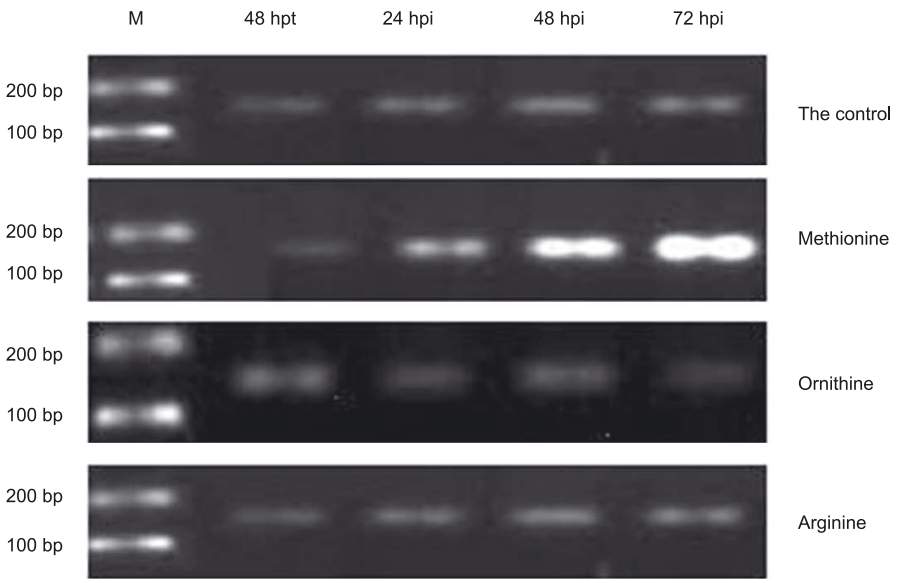

Fig. 3. Relative transcript amounts of the *PR-2* gene, *β*-1,3-glucanase, 48 hours post treatment (hpt) and 24, 48 and 72 hours post inoculation (hpi), in plants treated with L-methionine, L-arginine, L-ornithine and control plants. The increased expression of *PR-2* genes was observed 48 and 72 hpi in treated plants with L-methionine

Catalase activity assay

The results showed that catalase activity in plants pretreated with methionine, was significantly reduced 48 hpi. It seems that, in these conditions, the antioxidant activity of plant catalase was active (Fig. 4). The results of the analysis indicate that changes in the enzyme activity, in plants treated with L-methionine, L-arginine, and L-ornithine compared to the control, was not significant.

Peroxidase activity assay

The results of the analysis of peroxidase activity showed that pretreatment of plants with amino acid methionine increased the activity of this enzyme at 48 and 72 hpi. In contrast, L-arginine or L-ornithine treatments were not effective in increasing enzyme activity (Fig. 5).

Phenylalanine ammonia-lyase activity assay

Phenylalanine ammonia-lyase activity significantly increased at 48 and 72 hpi in plants treated with methionine compared to the control ($p < 0.05$). However there was not a significant difference between phenylalanine ammonia-lyase activity in plants treated with L-arginine or L-ornithine, compared to control (Fig. 6).

Discussion

Xcc is the causal agent of Asiatic citrus canker disease which causes economical damage to many citrus species and Rotaceous plants (Dewdney *et al*. 2012). No highly effective strategy has been presented to control the disease. One of the management strategies against pathogen attacks, is an activation of the plant's defense system

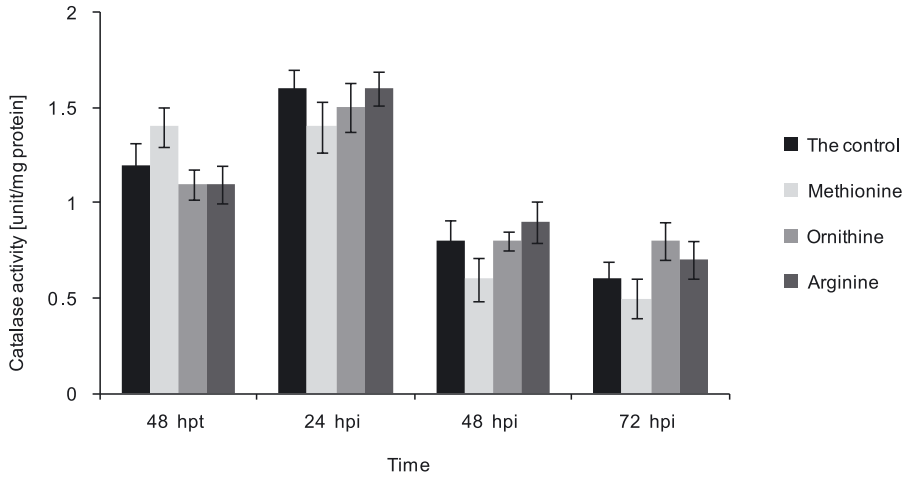

Fig. 4. Catalase enzyme activity inplants treated with: distilled water (the control), methionine, ornithine, and arginine, at 48 hours post treatment (hpt) and 24, 48, and 72 hours post inoculation (hpi)

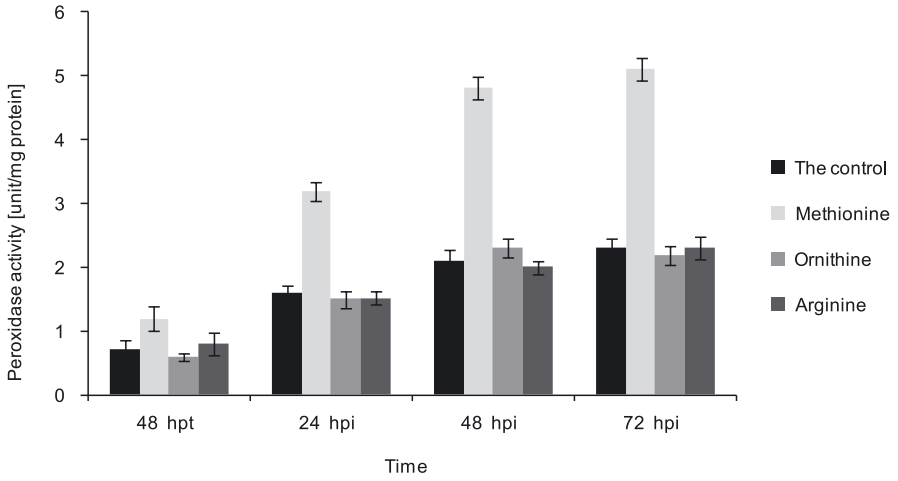

Fig. 5. Peroxidase enzyme activity in plants treated with: distilled water (the control), methionine, ornithine, and arginine, at 48 hours post treatment (hpt) and 24, 48, and 72 hours post inoculation (hpi)

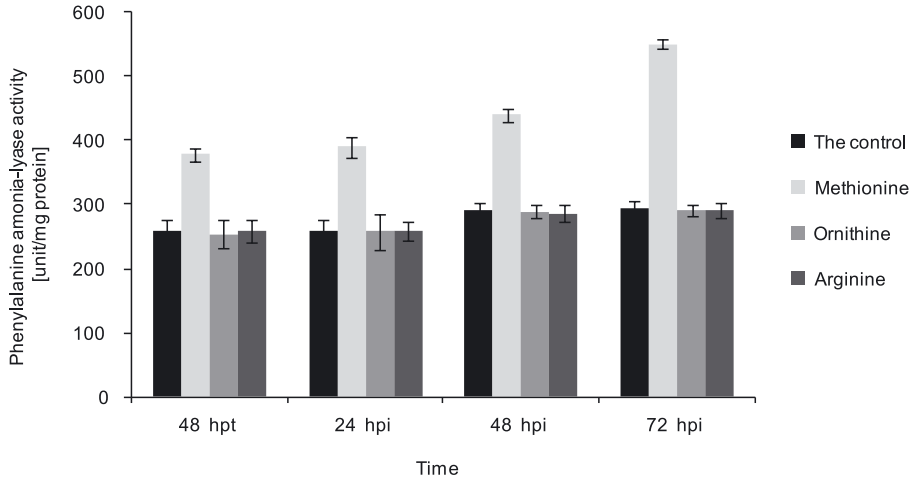

Fig. 6. Phenylalanine ammonia-lyase enzyme activity in plants treated with: distilled water (the control), methionine, ornithine, and arginine, at 48 hours post treatment (hpt) and 24, 48, and 72 hours post inoculation (hpi)

characterised by the accumulation of salicylic acid and the expression of *PR* genes (Hammerschmidt *et al*. 2001). Our study aimed at identifying agents that limit pathogen damage to the plant. For this purpose, we tested the effect of the amino acids L-arginine, L-ornithine, and L-methionine on induced resistance in lime plants against citrus canker disease. Our results indicate that among the tested treatments, L-methionine significantly reduced canker lesions in lime leaves. Analysis of the mRNA levels of *PR-2* gene by semi-quantitative RT-PCR, showed that *PR-2* gene (*β*-1,3-glucanase) enhanced expression in response to *Xcc* infection. The activity of *β*-1,3-glucanase was significantly induced 48 and 72 h after inoculation, but especially 72 h after inoculation. This result is supported by previous studies that reported *β*-1,3-glucanase accumulation in plants correlated with increased levels of induced resistance elicited by various elicitors (Graham and Leite 2007; Francis *et al*. 2009; Graham *et al*. 2011). Furthermore, an increase in peroxidase and phenylalanine ammonia-lyase enzyme activity was observed in plants treated with methionine compared to ornithine or arginine and the control treated plants.

Peroxidase controls the availability of H_2O_2 in the cell wall, which is a prerequisite for the cross-linking of phenolic groups in response to various external stresses, such as wounds, pathogen interactions and environmental constraints, through the formation of a physical barrier of lignin or suberin. High concentrations of phenolic compounds around wounds or pathogen-infected areas can restrict or weaken pathogen growth (Reimers and Leach 1991). Ballester *et al*. (2010) found that soluble peroxidase contributes to the beneficial effect of pathogen infection treatment by reducing disease incidence. Thus, the increase in peroxidase is one of the markers of induced resistance.

Phenylalanine ammonia-lyase is the first enzyme in the metabolic pathways of phenylpropanoids, which is responsible for the biosynthesis of p-coumaricacid derivatives, phytoalexin, and lignins that contribute to plant defense systems. Phenylalanine ammonia-lyase also participates in the biosynthesis of the defense hormone salicylic acid, which is required for both local and systemic acquired resistance in plants (Dixon and Paiva 1995). Induction of phenylalanine ammonia-lyase activities in-

creased significantly in plants treated with L-methionine. This result is in agreement with previous findings that phenylalanine ammonia-lyase is involved in increasing resistance and significantly increases in response to the stimulation of different resistance elicitors in citrus fruit (Droby *et al*. 2009; Ballester *et al*. 2010). The present study assessed the effect of the L-methionine on disease development. Enhanced disease resistance induced by L-methionine and riboflavin against powdery mildew infection has been demonstrated for melon, cantaloupe, squash, pea, and strawberry (Tzeng *et al.* 1996; Sarosh *et al*. 2005). The results from this study indicated that pretreatment of lime plants with L-methionine induced the levels of disease resistance against *Xcc*, and significantly reduced the severity of disease.

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