EFFECT OF *SATUREJA HORTENSIS* ESSENTIAL OILS TREATMENTS ON CATALASE AND PEROXIDASE ACTIVITY IN POTATO VIRUS S INFECTED POTATO PLANTS UNDER DROUGHT CONDITIONS (PRELIMINARY STUDIES)

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Abstract

Responses of Satureja hortensis essential oils and H_2O_2 treatments were estimated in plants testing positive being infected with Potato Virus S (PVS), under drought conditions. Infected and uninfected microplants transferred to a green-house were injected with a Satureja hortensis essential oils suspension and sprayed twice a week with H_2O_2. The presence of virus decreased significantly catalase activity (CAT). Under drought stress, peroxidase (POX) activity increased significantly in infected plants, but the treatments only enhanced POX activity in uninfected plants compared to the CAT activity in the same experimental condition. An interesting role of Satureja hortensis essential oils and H_2O_2 in lessening the potato PVS infected microplants behavior is suggested.

Key words:Satureja Hortensis essential oil, potato A virus, droughtstress

INTRODUCTION

Massive imports of potato in last years, the continuous "migration" of seed potatoes from one area to another, climate change, inadequate treatments for disease vector control (especially aphids), viral pressure, resistance of varieties are just some of the factors that may favor the spread of viruses (Bădărău et al. 2021). One of these virus is Potato Virus S (PVS), a pathogen transmitted mechanically and by *Myzus persicae* in a nonpersistent manner (Cojocaru N., 1987, Loebenstein G., 2008b). The damage caused by this pathogens agent is both quantitative (reduction of production) and qualitative (commercial depreciation of tubers). In case of cultivation of sensitive varieties under favorable conditions, financial losses can be important both for potato consumption (it can become unmarketable) as for seed potatoes (it will be downgraded). Thus, efforts to control this pathogen are essential when producing potatoes for market or seed (Bădărău et al. 2021).

Satureja hortensis L. (summer savory – Family *Lamiaceae,* order *Lamiales*) is known for its antiseptic (antimicrobial, antifungal and antiviral) properties. It inhibits mould formation. Thisoil contains hydro-carbonated and oxygenated compounds like α and β pinene, a tujene, camphene, sabinene, myrcene, a phelandren, terpinene, limonene, cymene, 1,8 cineol, βphelandrene, linalol, caryophillene. The main compounds are carvacrol (about 35%) – wich imprints the characteristic smell –tymol and p-cymene (Bedoux et al. 2010). They are also insect-repellent and antimicrobial, antiviral wich could protect the plants (Petersen et al. 2001).

Potato plants are very susceptible to water deficit (this could cause a severe reduction in leaf area, fresh weight and plant development (Heuer et al.1998). Under drought conditions, the plants show an increase in reactive oxygen species (ROS) which leads to expression of genes associated with antioxidant functions for scavenging ROS, resulting in tolerance to drought stress (Mano 2002). In the aim to minimise these ROS damaging effects, the plants involved non-enzymatic and enzymatic antioxidants. Enzymatic defense, such as superoxide dismutase (SOD), catalase (CAT) and peroxidases (POXs) directly scavenge superoxyde radicals. H_2O_2, convert this radicals to less reactive species (Romero-Romero et al.2009). In plant pathogen relationships, ROS are involved in induction of defense genes, antioxidant enzymes such as CAT and POX and accumulation of secondary metabolites (Pellinen et al.2002).

There are limited information about appearance of symptoms with interaction between potato virus S and abiotic stress. Xu et al. (2008) showed in their papers that potato virus infection improve drought tolerance(Wu 1997).The goal of this preliminary research, was to study the effect of the virus – water stress interaction on the level of catalase and peroxidase activity in virus infected potato plants under essential oils treatments– mediated under greenhouse conditions.

MATERIALS AND METHODS

Plant material. Potato plants (PVS positive material and negative controls from Castrum variety) were obtained from a previous selection under green house conditions. The infection of the material was confirmed by ELISA tests. Single node cuttings were *in vitro* propagated in test tubes on Murashige and Skoog (2005) medium, at 20±1°C under a 16 h photoperiod (fluorescent lights, 400–700 nm), in sterile conditions. Forty PVS infected microplants and forty healthy microplants were transplanted to pots (17 x 14 cm) containing peat-moss under greenhouse conditions 30 days after the single-node subculture step. These plants were maintained under greenhouse conditions for 90 days after transplanting (DAT) and each pot was allocated to an experimental unit, with ten plants per treatment. Before the treatments and after 45 DAT the presence of PVS was tested by ELISA.

ELISA test. A press with smooth roles was used for preparation leaf samples. The analysis was performed following essentially the protocol described by Clark and Adams (1977) (100 µl from each reactives solutions). Microplates were filled with substrate solution (p-nitro phenyl phosphate) incubated 1 hour and the absorbance values were estimated at 405 nm (A_{405}) on Tecan reader (Magellan software). The samples having A_{405} values exceeding the cut-off (two times the average of healthy controls) were considered virus infected.

Stress and chemical treatments. All experiments were performed in triplicate. Microplants were transplanted to pots and after 7, 14 and 21 days, all the plants (excepting the controls) were injected with *Satureja hortensis* oil suspension (1/100) 10 units each plant. From 7 days later from the first injection, the plants were sprayed twice weekly for the next 2 months with 10 mL per plant of 1 mM H_2O_2 at pH 5.6 and the earth of the pots with 10mL essential oils suspension (1/1000). The fertilization was made every 15 days and the plants were watered twice a week. Ten infected plants and ten negative plants for each treatment were sprayed with H_2O_2 in randomized arrays and subjected to drought conditions. Drought stress (suppressed water) or well watered conditions were applied from 75 DAT up to harvest. Controls and plants untreated were sprayed with distilled water. Six infected (positive) and healthy (negative) plants were sprayed in randomized arrays for each chemical treatment, and each treatment was performed in three independent experiments.

Determination of CAT and POX activities. These analysis were made at 75 and 90 DAT in order to compare these parameters before and during drought stress and how the treatments mediated these responses. The enzyme extraction was perfomed using 59 mM porassium phosphate buffer at pH 7,2, containing 5 mM DTT (dithiotreithol), 1mM EDTA and 1% (w/v) PVP (Anderson MD, 1995). The extract was centrifugated at 11627g for 15 minutes at 4ºC. The supernatant was used for CAT and POX activities.

CAT (EC 1.11.1.6) activity was determined according to Aebi (1984). The total reaction mixture (3 ml) contained 50mM potassium and sodium phosphate buffer (pH 7) and 20 μl enzyme extract. The reaction was initiated by the addition of 30 mM H_2O_2. The decomposition was followed directly by the decrease in absorbance at 240 nm every 20 s for 3 minutes at 26ºC.

POX (EC 1.11.1.7) activity was determined according to Mora-Herrera et al. (2007). The total reaction mixture (3 ml) contained 50mM potassium and sodium phosphate buffer (pH 7), 3.33 mM guaiacol and 4 mM H_2O_2 The reaction was initiated by addition of 20ml of the enzyme extract and progress measurated directly by the increasement in absorbance at 430 nm at 30 s intevals for 3 min at 25±1°C.

Statistical analysis. Data were analyzed by ANOVA and Duncan's Multiple Range Test and scored as significant if $P<0.05$ (IBM SPSS Statistics software).

RESULTS AND DISCUSSIONS

In this work the effect of treatments with *Satureja hortensis* essential oils and H_2O_2, were compared on antioxidant responses (CAT and POX activities) of both healthy and virus infected (PVS) potato plants (variety Castrum). The treatments with *Satureja hortensis* essential oils and H_2O_2 were favorable for diminution in stress-damage symptoms in infected plants.

Compared to unifected plants, CAT activity decreased in infected plants, whereas essential oils and H_2O_2 did not induce significant changes ($P<0.05\%$) (fig 1A). When drought stress was applied to plants, CAT activity significantly ($P<0.05\%$) decreased compared to well-irrigated plants. Under drought stress, infected plants injected and sprayed with essential oils suspension and sprayed with H_2O_2 had 40% increased CAT activity (fig 1A).

Under optimal irrigation conditions, significant differences occured in the POX activity affected by the PVS presence. POX activity was augmented when the treatments were made on uninfected and infected plants. (fig 1B). Under drought stress, POX activity increased significantly ($P<0.05\%$) in infected plants, but the treatments only enhanced POX activity in uninfected plants compared to the CAT activity in the same experimental condition (fig 1B).

We used a similar model like in other work (Bădărău et al.2012) - a model based on the *in vitro*-to-green house system for investigate the effect of the interaction between the virus- water stress on the appearance of symptoms in infected plants treated with *S. hortensis* essential oils and H_2O_2 in mediated greenhouse conditions.

As we have been reported (Bădărău et al. 2021) under green house conditions, the infected plants exhibited specific symptoms such as mosaic in the foliage, reduced plant weight, stem tickening, internod shortening and reduced minitubers production (sometimes if the strain is very virulent systemic shock reaction and / plant death). Known symptoms usually for this kind of virus were absent under drought stress in the conditions of our experiments. In a green house the environmental stress was likely more stable with gradual changes compared to the field conditions where environmental changes can abruptly occur.

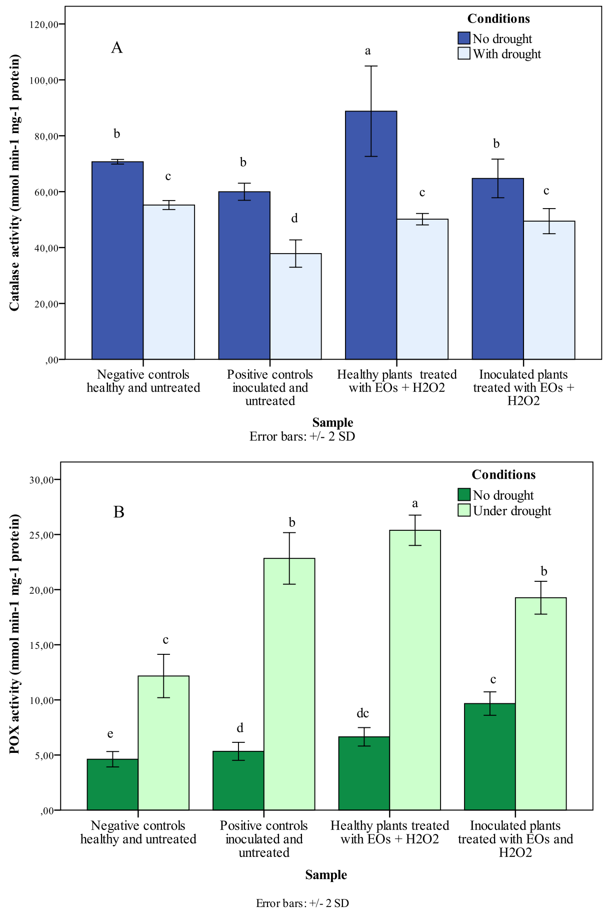

Figure 1. CAT activity (A) and POX activity (B) o fhealthy plants and potato virus S (PVS) infected plants,under drought conditions (■ dark colour of the bars) and not drought conditions (□ light colour of the bars), following treatments with *Satureja hortensis* (SH) essential oil and H_2O_2 (1mM) or water (controls), twice weekly from 30-75 DAT. Watering was withheld at 75 DAT. Data are means ± SD of 3 experiments (n=3). Bars with different letters differ significantly by ANOVA and Duncan's test (P<0.05).

The results obtined in this study confirmed that CAT inhibition is due to the virus. Essential oils and H_2O_2 application induced changes in CAT activity and H_2O_2 content, especially under drought stress. No significant differences in CAT activity were observed before drought stress. Treatment promoted inhibition of CAT activity during drought, but only in presence of the virus (fig 1A). Such enhancement could be important for the positive effects observed in minitubers from inoculated plants. Interestingly, by the beginning of the drought stress (75DAT), essential oils and H_2O_2 application resulted in a significantly higher CAT activity values in PVS inoculated plants compared to control plants, including the uninfected plants (fig 1A). Such differences suggest a signaling role of unknown compounds of essential oils and of H_2O_2 that could maybe induce positive effects in minitubers from inoculated plants, reducing number, starch content and sprouting, as there was mentioned in another study (Bădărău et al. 2012).

The presence of potato virus S (PVS) in potato plants induced augmentation of POX activity, as found to another pathogen and another plants (phytoplasma to apple trees, reported by Musseti et al. 2005). The drought effect on POX activity was amplified by essential oils and H_2O_2 application on uninfected material.

There are scarce information on the combined tolerance to biotic and abiotic stress. This research study demonstrates an ameliorative effect of the essential oils and H_2O_2 on the combined stresses. We presented the effect of water stress on the appearance of symptoms and the antioxidant response in virus infected potato plants under H_2O_2 –mediated greenhouse condition. As we have been reported for another virus (Bădărău et al. 2012), the treatments with essential oils suspension and low H_2O_2 concentration 1mM significantly reduced disease symptoms under drought stress for minituber production and starch accumulation, with repercussions in minituber size augmentation and induced multiple sprouting. The practical use of these treatments for overcoming damage in non-seed tubers, is a strong justification for continue investigation of the physiology and the effect of some compounds from *S. hortensis* essential oils and of H_2O_2.

CONCLUSIONS

The treatments with *Satureja hortensis* essential oils and H_2O_2 were favorable for diminution the stress-damage in infected plants under drought conditions, these aspects being highlight by a significant increase of catalase and peroxidase activity of treated material. So, under drought stress, infected plants injected and sprayed with essential oils suspension and sprayed with H_2O_2 had 40% increased CAT.

Further research is needed to determine the effects of treatments applied in this research in the aime to estimate the influence of cathalase and peroxidase activity on the tuber sprouting.

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