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THE EFFECTS OF NEW TREATMENTS ON PVY INFECTED POTATO PLANTS UNDER DROUGHT CONDITIONS

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Abstract: *The effects of treatments with Satureja hortensis essential oils and exogenous H_2O_2 were evaluated in plants, testing positive after Potato virus Y (PVY) infection, under drought conditions. In vitro PVY infected and uninfected plants were transferred to a green-house, injected with a Satureja hortensis essential oils suspension and sprayed twice a week with H_2O_2 (1 mM, pH 5.6). Under drought conditions, minitubers produced by infected and treated plants had significantly more starch than the controls. The treatments had positive effects on infected minitubers, such as weight, reduction of number, starch content and sprouting. A signal role for Satureja hortensis essential oils and hydrogen peroxide in lessening symptoms is suggested.*

Key words: *Satureja hortensis essential oil, hydrogen peroxide, potato virus Y, drought stress.*

1. Introduction

Potato virus Y (PVY, *potyvirus* genus, family *Potyviridae*) is distributed worldwide. It causes losses in solanaceous crops such as potato (*Solanum tuberosum*), tobacco (*Nicotiana tabacum*) and tomato (*Lycopersicum esculentum)* crops [4]. In the case of potatoes, the virus not only leads to reduction by up to 90%, but also causes tuber necrosis in certain cultivars upon infection with the tuber necrosis strain of PVY (PVY^{NTN}) [4]. So, PVY is one of the world's most economically important viruses affecting potato crops. Thus, efforts to control PVY are essential when producing potatoes for market or seed [2], [4].

Satureja hortensis L. (summer savory - Family *Lamiaceae,* order *Lamiales*) is renowned for its aromatic and seasoning properties in food products but also for the antispastic and disinfecting properties. The essential oils are known for their antiseptic (antimicrobial, antifungal and antiviral) properties. It inhibits mould formation. This oil contains hydro-carbonated and oxygenated compounds like α and β pinene, α tujene, camphene, sabinene, myrcene, α phelandren, terpinene, limonene, cymene, 1,8 cineol, β phelandrene, linalol, caryophillene. The main compounds are carvacrol (about 35%) - which renders the characteristic smell - tymol and p-cymene [3]. They are also insect-repellent and antimicrobial, antiviral which could protect the plants. Maybe, one of these compounds or others could be involved in the process of signalling stress, in healthy and infected potato plants [1-3]. Plant cells have defensive responses to the pathogen attack

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associated with changes in the oxidative metabolism [5]. One of the consequences of stress is an increase in the cellular concentration of the reactive oxygen species (ROS), which are subsequently converted to hydrogen peroxide (H_2O_2). These ROS, particularly H_2O_2, play versatile roles in the normal plants' physiological processes and in resistance to stresses. H_2O_2 produced in excess is harmful, but lower concentrations are beneficial [5]. H_2O_2 is believed to play two distinct roles in pathogenesis. One involves the oxidative burst in the hypersensitive response, which restricts pathogen growth and the other activates plant defence responses, including induction of phytoalexins, second messengers or signalling intermediates, antioxidant enzymes and cell wall reinforcement.

Water stress is one of the most important environmental factors that limit the growth, yield and quality of potato crops. Potato plants are very susceptible to water deficit, which causes a severe reduction in leaf area, fresh weight and stolon development [6]. Plants under drought conditions 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 [6]. Similar mechanisms are triggered in plants during biotic and abiotic stress. A common response is the production of ROS including superoxide, singlet oxygen, hydroxyl and hydrogen peroxide oxygen. These ROS can be detrimental and promote deleterious effects in the most sensitive biological macromolecules [5], [6], leading to electrolyte leakage, changes in ion fluxes, lipid peroxydation, protein oxidation and imbalances in the oxidative systems at the subcelullar level. Under intense stress, different target molecules are damaged, resulting in cell death [5], [6]. To minimize ROS damaging effects, aerobic organisms developed both non-enzymatic and enzymatic antioxidants. Purely enzymatic defences, such as superoxide dismutase (SOD), catalase (CAT) and peroxidases (POXs) directly scavenge superoxyde radicals and H_2O_2, converting them into less reactive species. POXs have been associated with an ever-increasing number of physiological processes, especially detoxifying H_2O_2 [5]. Genetic and physiological evidence suggests that H_2O_2 acts as a signalling second messenger, mediating the acquisition of tolerance to both biotic and abiotic stresses and providing information about changes in the external environment [5], [6].

There is limited information about the occurrence of symptoms with the interaction between PVY and abiotic stress. Xu et al. (2008) showed in their papers that potato virus infections improve drought tolerance [8]. In this research we studied the effect of the virus - water stress contribution to the occurrence of symptoms in virus infected potato plants under essential oils treatments and H_2O_2 - mediated greenhouse conditions.

2. Material and Methods

Plant material. *Solanum tuberosum* L. microplants cv Roclas, testing virus free, were obtained from the Biotechnology Department (from the in vitro germoplasm collection of the Institute for Research and Development of Potato and Sugar Beet Braşov, Romania). Potato microplants were obtained from a previous selection under green house conditions, before inclusion in the in vitro collection. For obtaining positive material, a part of the plants have been mechanically inoculated, using a PVY secondary infected plant from Record variety. The infection of the material was confirmed by ELISA tests. Single node cuttings were *in vitro* propagated in test tubes in a Murashige and Skoog medium, at 20±1 °C under a 16 h photoperiod (fluorescent lights, 400-700 nm), in sterile

conditions. Forty PVY infected microplants and forty healthy microplants were transferred to pots (17x14 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 PVY was tested by ELISA.

ELISA test. A press with smooth roles was used for the preparation of leaf samples. The analysis was performed following essentially the protocol described by Clark and Adams (1977).

Stress and chemical treatments. All experiments were performed in triplicate. Microplants were transferred to pots and after 7, 14 and 21 days, all the plants (except for the controls) were injected with *Satureja hortensis* oil (1/100) 10 units for each plant. Seven days after 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 10 mL essential oils suspension (1/1000). The fertilization was done 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. Minitubers' number, weight and starch were recorded at 90 DAT as productivity estimation. The controls and the untreated plants were sprayed with distilled water. Six viruses infected (positive) and healthy (negative) plants were sprayed in randomized arrays for each chemical treatment, and each treatment was performed in three independent experiments.

Minitubers starch content. Starch content was determined by spectrophotometric assay by antrone reaction [7]. For each treatment, a composite 1 g sample of pith from three minitubers was used. Tissue was ground in a mortar with 10 mL 80% (v/v) ethanol [7]. The analyses were performed the day after harvesting.

Statistical analysis. Data were analyzed by ANOVA and Duncan's Multiple Range Test and the score was significant since $P < 0.05$ (IBM SPSS Statistics software). With the aim of illustrating the precision of the mean we used the confidence interval (CI).

3. Results and Discussions

The effects of treatments with *Satureja hortensis* essential oils and H_2O_2, were compared according to tuber harvest parameters (weight, starch, number, sprouting) of both healthy and virus infected (PVY) plants cv Roclas plants.

Weight. Minituber weight of PVY positive plants were significantly diminished compared to the control uninfected plants. The treatments significantly affected the minituber weight (Figure 1). PVY infected plants treated with essential oils and H_2O_2 had significantly ($P < 0.05$) increased minituber weight to similar values as the non-treated and unsprayed control. Minitubers produced by uninfected plants that were treated also significantly ($P < 0.05$) increased their weight in all treatments (Figure 1).

Minitubers produced under drought conditions by negative plants had significantly ($P < 0.05$) reduced weight by 36% compared to the uninfected plants subjected to irrigation.

Minitubers produced by treated and PVY inoculated plants under drought showed the highest weight of all the treatments under drought (Figure 1). Very interesting, the treatments with *S. hortensis* essential oils

and H_2O_2 significantly increased ($P < 0.05$) the minitubers' weight in infected plants compared to the uninfected plants treated with *S. hortensis* essential oils and H_2O_2.

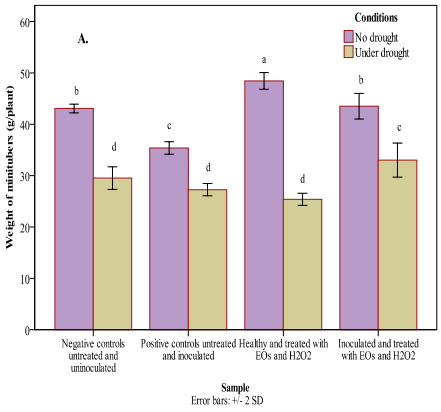

Fig. 1. *Tuber weight of healthy and PVY infected plants, under drought and non-drought conditions, following treatments with S. hortensis EOs and H_2O_2 (1 mM) or water (controls), twice weekly from 30-75 DAT. Data = means (n = 3). Bars with different letters differ significantly according to ANOVA and Duncan's test ($P < 0.05$)*

Number of minitubers. PVY inoculated and infected plants produced a higher number of minitubers but with less weight than the uninfected control plants. The treatments significantly reduced the minitubers' number compared to the uninfected control, to similar values as the uninfected control (Figure 2).

Similarly, under drought conditions, the infected control plants had significantly more minitubers than the healthy plants (negative controls). Interestingly, the treatments significantly reduced by 55% (compared to the infected controls) or to similar value (compared to the irrigated and uninfected controls) the number of minitubers in infected plants (Figure 2).

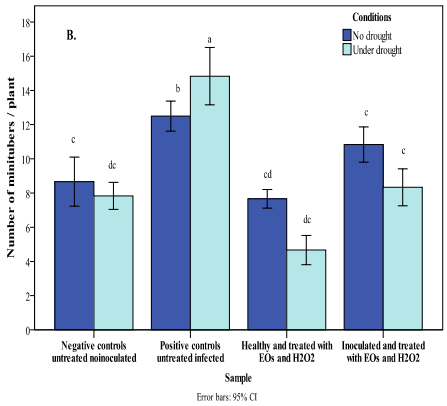

Fig. 2. *Minituber number of healthy and PVY infected plants following treatments with S. hortensis EOs and H_2O_2. Data = means (n = 3). Bars with different letters differ significantly according to ANOVA and Duncan's test ($P < 0.05$)*

In another research we discovered that the treatments with some essential oils, H_2O_2 and ascorbic acid significantly reduced minituber number compared to infected controls and significantly enhanced minituber weight [1], [2]. In the present research we observed these effects in all conditions: under drought and well-watered conditions.

Starch. PVY infection leads to a low starch content. The starch content in infected plants was significantly ($P < 0.05$) lower than in uninfected plants. However, the treatments with *S. hortensis* essential oils and H_2O_2 significantly ($P < 0.05$) enhanced the starch content in both infected (22% increase) and uninfected (16% increase) plants compared with their controls (Figure 3). Under drought stress, minitubers of uninfected and PVY inoculated plants significantly decreased in starch content by 33% and 39% respectively, compared to the irrigated uninfected controls.

The treatments applied on PVY infected plants induced significantly higher levels in the minitubers than those of the infected and untreated control plants (Figure 3).

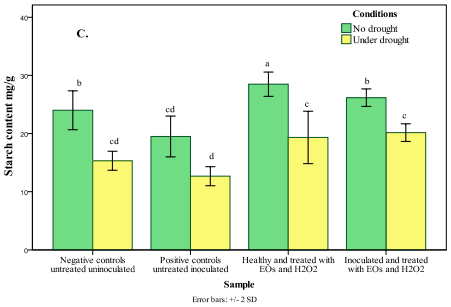

Fig. 3. *Starch content of healthy and PVY infected plants following treatments with S. hortensis EOs and H_2O_2. Data = means (n = 3). Bars with different letters differ significantly according to ANOVA and Duncan's test ($P < 0.05$)*

Sprouting. Minitubers from infected plants significantly reduced percentage of sprouting. Multiple sprouting (more than one sprout/minituber) was significantly ($P < 0.05\%$) enhanced by 12.4% by the treatments of PVY inoculated plants compared to the positive control plants (PVY inoculated and untreated) (Table 1). Sprouted tubers 140 days after harvest in potato healthy plants (virus free) and PVY inoculated (infected) and treated (injected with *S. hortensis* essential oils suspension and sprayed with 1 mM H_2O_2 from 30-75 DAT). Data are the means ± SD of three experiments. Means labelled with different letters differ significantly according to ANOVA and Duncan's test ($P < 0.05$).

No significant effect of the treatment on minitubers sprouting was observed on uninfected minitubers. The effect of essential oils and H_2O_2 treatments on sprouting was accentuated by drought stress. Infected minitubers had significantly (11.4%) reduced total sprouting compared to the healthy minitubers.

Essential oils and H_2O_2 significantly induced more sprouting in minitubers from inoculated plants, compared to the positive controls. The treatments with essential oils and H_2O_2 increased multiple sprouting in infected and uninfected minitubers compared to the controls (Table 1).

Effect of the treatments on tuber sprouting Table 1

Sample Tubers from:	Conditions	Number of tubers	Single sprouting [%]	Multiple sprouting [%]	No sprouting [%]	Total sprouting [%]
Negative Control	No drought	220-340	84.6 ± 1.4 (a)	15.4 ± 1.4 (c)	0.0 ± 0.0	100 (a)
Positive Control		340	77.4 ± 1.5 (bc)	16.4 ± 1.2 (c)	7.2 ± 0.8 (b)	93.8 (b)
Negative Control	Under drought	190-220	81.6 ± 2.1 (b)	17.0 ± 1.9 (b)	1.4 ± 0.2 (d)	98.6 (a)
Positive Control		320-410	68.9 ± 2.4 (cd)	18.4 ± 1.0 (b)	12.7 ± 2.3 (a)	87.3 (c)
Healthy treated plants	No drought	190-220	84.7 ± 2.1 (a)	15.3 ± 2.1 (c)	0.0 ± 0.0	100 (a)
Infected treated plants		220-240	68.3 ± 3.9 (cd)	27.9 ± 4.9 (a)	3.8 ± 2.0 (c)	96.2 (ab)
Healthy treated plants	Under drought	190-220	70.6 ± 2.7 (cd)	29.4 ± 2.7 (a)	0.0 ± 0.0	100 (a)
Infected treated plants		220-340	69.2 ± 4.8 (cd)	30.8 ± 4.8 (a)	0.0 ± 0.0	100 (a)

Minitubers' weight and starch levels were induced by the treatments under well watered conditions, but under drought stress both were enhanced only in infected plants. Previously we observed a significant enhancement of chlorophyll content by H_2O_2 treatment of PVY inoculated plants, resulting in the augmentation of minituber weight [1], [2].

4. Conclusions

The treatments with *Satureja hortensis* essential oils and H_2O_2 were favourable for the diminishing of stress-damage symptoms in infected plants. These treatments enhanced sprouting in tubers from PVY inoculated (infected) minitubers and *Satureja hortensis* essential oils and H_2O_2 - ameliorative damage effects were observed on potato plants growing under combined biotic (virus) and abiotic (drought) stress conditions.

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