Evaluation of Peroxydase Activity in Samples From Three Potato Varieties Inoculated With Potato Virus S and M Cultivated Under Drought Conditions

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Abstract. *Drought stress and virus infections are some of the major problems for potato production. Peroxidase (POD) activity could be an important indicator of potato growing process, reflecting the antioxidant capacity of plants, peroxidase being an enzyme with a strong contribution to the defense system of vegetables. This study presents the POD activity estimated in healthy plants and potato viruses S (PVS) and M (PVM) infected plants (from the Romanian varieties Cosiana, Azaria and Sevastia) cultivated in greenhouse, under salt and drought conditions (salt stress induced by plants irrigation with a solution NaCl 2g/L). The results show that the POD activity was influenced significantly by the PVS and PVM plants inoculation (especially in case of samples from resistant varieties to these viruses) and the antioxidant response of the potato plants was more intensive under drought conditions and under biotic stress (viruses inoculated).*

Key words: salt stress, POD activity, potato cultivars, PVM, PVS.

INTRODUCTION

Potato tubers (*Solanum tuberosum* L) are one of the most widespread sources of food and processing raw materials used in the food industry worldwide, due to their rich composition in valuable nutrients (Pang, 2019). However, this vegetable contains phenolic substances, substrates of an enzymatic browning reaction caused frequently by POD (peroxidase), affecting so the quality of the final products (Zhou et al., 2010; Zhu, 2017) in the periods of storage or in potato processing (Zhu and Hu, 2013), During their growth and development, potato plants are subjected to different types of biotic and abiotic stresses. The members of class III peroxidases (PRXs) are plant-specific enzymes and play important roles in defense processes, stress response in plants, growth and development of the plants, their functions in potato being poorly deciphered (Hiraga et al., 2001; Yang et al., 2020). Yang et al. (2020) noted the physiological roles and characteristics of potato plants by comprehensively analyzing the PRX gene family.

Potato plants are susceptible to water deficit (Heuer et al., 1998). Usually, under drought conditions, a strong increase in reactive oxygen species (ROS) was observed in plants from different species (Mano, 2002). For minimise the ROS damaging effects, the plants use enzymatic and no enzymatic antioxidants (Pellinen et al., 2002). Peroxidase (POD) is one of these enzymes having a strong activity in plants and an important function in the defense system of plants grown under stress conditions. Together with catalase and superoxide dismutase (Sun et al., 2018; Yang et al., 2013; Zhang et al., 2014), peroxidase improve the stress resistance of the plants, eliminates toxic elements, removes excess ROS (free radicals) in the plant, and produces some metabolites (Sun et al., 2018). POD is closely related to various important physiological activities of potato plants. POD can convert the toxic hydrogen peroxide for cells (H_2O_2) into H_2O with some series of activity changes, so it is often used to evaluate the plants stress tolerance (Sui et al., 2018; Song et al., 2019). POD is closely related to important physiological functions of plants especially under stress conditions induced by drought or by presence of the pathogens. Therefore, the activity of this enzyme can be used as an evaluation indicator of plants growth (Yang et al., 2013; Zhang et al., 2014).

Potato virus S (PVS) and potato virus M (PVM) are pathogens transmitted mechanically and by aphids in a nonpersistent manner (Cojocaru N., 1987; Loebenstein G., 2008). Similar with another potato viruses, the damage caused by these pathogens is both qualitative (commercial depreciation of tubers) and quantitative (reduction of production) and under favorable conditions, in case of sensitive potato varieties, the financial losses can be important, for consumption (potato tubers become unmarketable) and for seed potatoes (it could be downgraded) (Bădărău et al., 2012). Recently, Kumika and collaborators (2018) reported that potato pulp (product of starch industry), could be used for waste water (phenol-polluted) decontamination, due to its peroxidase content. They demonstrated that potato pulp may be used for the decontamination of 2,4-dichlorophenol (2,4-DCP) solutions in a peroxidase-catalyzed reaction (Kumika et al., 2015 and 2018). So, an alternative for using potato unmarketable or downgraded in this purpose is interesting.

There is limited information about the effect of potato viruses S and M infections, under salt stress on antioxidants plants response. Xu et al. (2008) reported in their papers that virus infection could improve drought tolerance (Wu, 1997). The objective of this preliminary study was to estimate the effect of interaction viruses S and M infection - water stress on the level of peroxidase activity in potato plants from three Romanian varieties cultivated under greenhouse conditions.

MATERIAL AND METHOD

In this study, three Romanian cultivars (Cosiana, Azaria and Sevastia) were analyzed, the first one being PVS and PVM medium resistant and the other two varieties resistant to these viruses. The health biological material (tubers) were obtained from the Breeding Department, National Institute of Research and Development for Potato and Sugar Beet Brasov. From each variety, 18 pots (with 1 eye pieces) in three repetitions were planted. Plants were grown in pots (18 x 14 cm) under greenhouse conditions. After 10 days from planting, 6 plants from each repetition have been mechanical inoculated, using a PVS source (secondary infection Carrera variety) and a PVM source (secondary infection Ostara cultivar). After the inoculation, ELISA tests have been made, in the aim to confirm the infection. Before and after inoculation, 3 leaves of lower, middle and upper part of plants were picked and analyzed for peroxidase activity. Drought stress were applied weekly for 3 plants non inoculated and for 3 infected PVS plants, respectively 3 infected PVM plants from each cultivar, potato plants being irrigated with a solution NaCl 2 g/L. The other plants were irrigated with water. After 75 vegetation days the lower, middle, and upper leaves of plants (health plants, PVS infected plants, PVM infected plants irrigated with water or with salt solution NaCl 2g/L) were picked and used for evaluate the peroxidase activity.

Determination of POD activities

The enzyme extraction was made by using 1g leaves grinded in 5 mL phosphoric acid buffer, this mix being after that centrifuged at 4000 r/min for 15 minutes at low temperature. For the enzyme reaction, the supernatant was taken and the volume being maintained to 25 mL (Li, 2000; Yang et al., 2020). For the reaction system there were used: 0.1 mL enzyme solution, 2.9 mL phosphoric acid buffer (0.05 mol/L), 1 mL H_2O_2 (2%) and 1 mL guaiacol (0.05 mol/L). After adding the enzyme, the absorbance at 470 nm was measured every minute, during four minutes, using a spectrophotometer DR2800 type (Hach Lange, Loveland, CO, USA) (Li, 2000; Yang et al., 2020). POD activity was calculated using the following formula:

$$\text{POD activity} = A_{470nm} \cdot V_T / (W \cdot V_s \cdot 0.01 \cdot t)$$

were V_T is the total volume of extract enzyme solution, W is fresh weight of sample, V_S is enzyme liquid volume for determination, and t is reaction time (Li, 2000; Yang et al., 2020).

Detection of PVS and PVM infection

A press with smooth roles was used for preparation leaf samples. The analysis was performed following the protocol described by Clark and Adams (1977) and using ELISA specific products from Bioreba (Shwitzerland). The rinsed microplates filled with substrate solution (p-nitro phenyl phosphate) were incubated 1 hour and the absorbance values determined at 405 nm (A_{405}) using a Tecan SunRise reader (Tecan, SunRiseTM, software MagellanTM, Männedorf, Switzerland). The samples having A_{405} values exceeding the cut-off (two times the average of healthy controls) were considered virus infected (Bădărău et al., 2012).

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) (Bădărău et al., 2012).

RESULTS AND DISCUSSION

Peroxidase (POD) are enzymes from class III peroxidases (PRXs), being plant-specific compounds with multiple functions in the plants growth and development. Many articles confirmed that PRX genes are widely involved in stress response in a lot of species (Wu et al., 2022).

In this study, the effect of some drought conditions on several antioxidants plants responses of healthy, PVS and PVM infected material was observed, by evaluation the changes of peroxidase (POD) enzyme activity under salt stress in case of three Romanian cultivars.

As shown in figure 1 and table 1 the varieties used were distinguished by different resistance to inoculation with the viruses studied. So, the most PVS and PVM resistant was the variety Sevastia, the percentage of infected plants observed after inoculation being the lowest (33.33%± 9.622 for PVS, respectively 27.78% ± 9.622 for PVM), compared with the other cultivars.

Table 1. Percent (%) of PVS and PVM infected material after inoculation

Variety	% PVS infected plants after inoculation	% PVM infected plants after inoculation
Cosiana	88.89 ± 9.622	83.33 ± 16.67
Azaria	61.11 ± 19.245	50.00 ± 9.622
Sevastia	33.33 ± 9.622	27.78 ± 9.622

Note: *Data represents the mean values (3 repetitions, 6 pots for each repetition) ± standard deviation
**ELISA test made after 4 weeks after inoculation (for identify PVS and PVM infected plants)

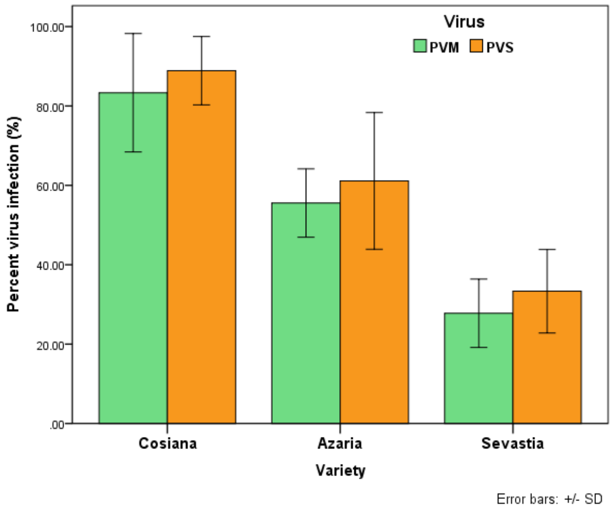

Figure 1. Percentage (%) of infected plants obtained after inoculation with PVS and PVM, in function of virus resistance level of varieties studied

The highest values of PVS and PVM infected plants (88.89% ± 9.622, respectively 83.33% ± 16.67) was determined in case of cultivar Cosiana, this one being very susceptible (in our experimental conditions) to both viruses. As can be seen in table 1, Azaria was the medium resistant variety, in this case the percent of infected plants was almost the same for PVS and PVM inoculated material (61.11% ± 19.245 for PVS, respectively 50.00% ± 9.622 for PVM).

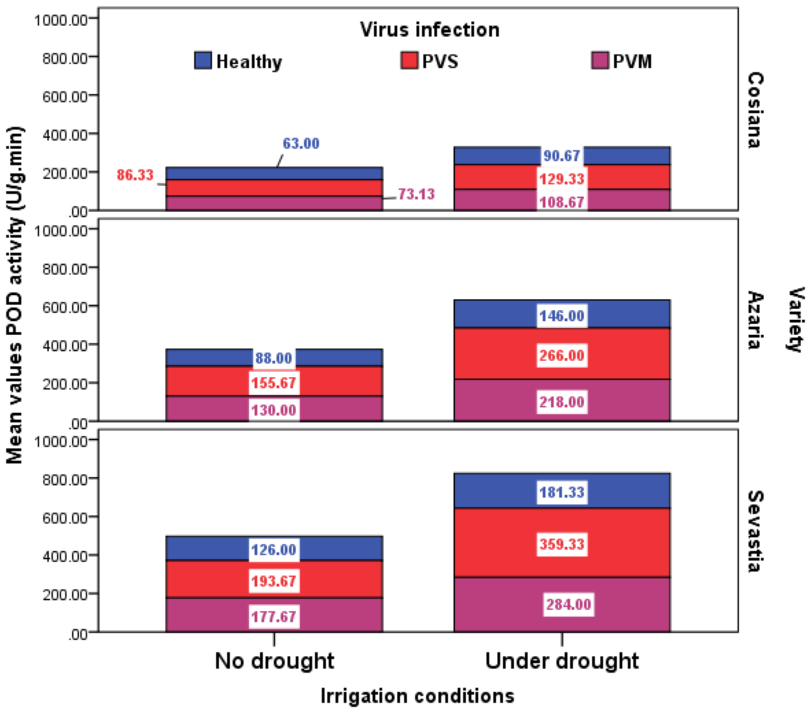

Figure 2. Changes of peroxidase activity of healthy and infected plants under salt stress

As can be seen in figure 2, the behavior of varieties under water stress conditions was similar for all cultivars, the POD activity increase under salt stress whatever the resistance for PVS or PVM inoculation. The most resistant variety Sevastia and the medium cultivar Azaria had distinguished by the higher values of POD activity in both healthy and infected plants, compared with the susceptible variety Cosiana (figure 2).

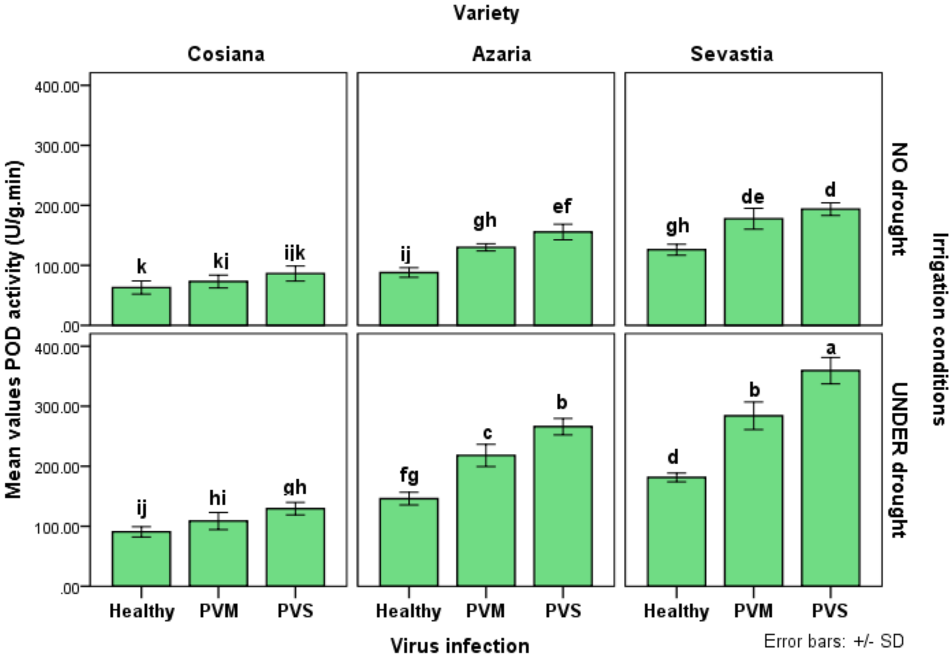

Figure 3. POD activity of healthy plants and potato virus S (PVS), potato virus M (PVM) infected plants, under drought conditions (irrigated with NaCl solution 2g/L) and not drought conditions (irrigated with water) twice weekly from 30-75 DAT. Watering was withheld at 75 vegetation days. Data are means ± SD of 3 experiments (n=3). Bars with different letters differ significantly by ANOVA and Duncan's test ($P<0.05$)

The mean values of POD activity (U/g·min) for varieties used in this study are presented in figure 3 and table 2. As shown in Figure 3 and Table 2, in cultivars Azaria and Sevastia (varieties medium resistant and resistant to PVS and PVM infection), the peroxidase activity was significantly increased in infected material compared to the healthy one under all irrigation conditions.

Under drought stress, POD activity increased significantly (P<0.05%) in infected PVS and PVM plants, the highest increase being observed in case of PVS infected material of variety Sevastia (359.669±22.081 U/g·min) (Figure 3, Table 2). As shown in table 2, for all cultivars, the peroxidase activity in case of PVM infected plants was significantly lower (p<0.05%) that those registered in case of PVS infected material.

Table 2. POD activity (U/g·min) of healthy plants and potato virus S (PVS), potato virus M (PVM) infected plants, under drought conditions (irrigated with NaCl solution 2g/L) and not drought conditions (irrigated with water). Changes of enzyme activity under salt stress

Conditions (irrigated conditions)	Material	Variety		
		Cosiana	Azaria	Sevastia
No drought (irrigated with water)	Healthy plants	63.110±11.015	88.432±7.539	126.533±10.874
	PVS infected	86.333±12.155	155.632±15.271	193.677±0.572
	PVM infected	73.314±10.073	131.318±9.704	177.683±17.637
Under drought (NaCl solution 2g/L)	Healthy plants	90.667±7.579	146.632±10.271	181.677±7.572
	PVS infected	**129.669±19.527**	**266.333±13.527**	**359.669±22.081**
	PVM infected	108.513±14.375	218.468±18.396	284.183±23.191

The behavior of plants from Cosiana cultivar (with low PVS and PVM resistance) was interesting. Compared to the other varieties, peroxidase activity was insignificantly higher in infected PVS and PVM plants compared to healthy plants under normal irrigation conditions. Only under water stress conditions, there was a significant increase in POD activity in Cosiana variety, but this increase was much smaller than that observed in the other two varieties used in this study (Figure 3, Table 2).

As shown in Figure 2 and 3, the POD activity of the healthy and virus infected plants was influenced by irrigation conditions and by variety (especially by the virus resistance of the material to inoculation).

The correlation coefficient Pearson revealed significantly values regarding the corelation between the POD activity and the irrigation conditions used in the experiments (Table 4). Also, the correlation coefficients Pearson presented in table 4 highlight that there is a significantly correlation ($p<0.01\%$) between POD activity and the percent of infected PVS and PVM plants after inoculation (respectively the variety resistance taking in to account the fact that in this study, we appreciated the level of material resistance using this percent).

Table 3. The correlation between POD activity, cultivars and virus infection

POD activitiy (U/g·min) Pearson Correlation (Significance threshold)	Irrigation condition (salt stress)	Variety	Type of virus	%PVS infected after inoculation	%PVM infected after inoculation
	0.489** (0.000)	0.656** (0.000)	0.289* (0.041)	-0.668** (0.000)	-0.632** (0.000)

**Correlationis significant for p<0.01.
N =54 (6 samples x 3 varieties x 3 repetitions)

The results of this study regarding the behavior of plants under drought conditions and under biotic stress are similar with those reported by another researchers (Sun et al., 2018; Zhang et al., 2014; Song et al., 2019). Among the plants tested, the virus infected biological material exhibited distinct symptoms and response to the stress, indicating a strong functional importance of the POD enzymes. Similar observations and phenomenon have been found and were reported in many species: maize (Sun et al., 2018), tomato (Zhang et al., 2018, Song et al., 2019, Wu et al., 2022), melon (Zhu et al., 2017) by suggesting that these enzymes play an essential role for the plants metabolism and for reducing the reactive oxygen species damaging effects. Maybe, enzymatic defense, such as POD enymes directly scavenge damaging free radicals (Bădărău et al., 2012). So, the data show a significant difference between the POD activity of the healthy and PVS, PVM infected plants from all the varieties tested (varieties with different resistance to these viruses), cultivated under drought conditions. However, it must be considered that the results presented in this paper arise from working with only a few of biological material and upper greenhouse growing conditions. Also, as was presented and in other researches (Bădărău et al., 2012) extended field trials would be made to confirm our research results.

CONCLUSION

Under drought conditions (salt stress), according to the data obtained in this study, the POD activity was influenced significantly by the PVS and PVM plants inoculation, especially in case of samples from resistant varieties. So, the infected material presented significantly increase of POD values, compared with the healthy material. The antioxidant response of the plants was more intensive under drought conditions and under biotic stress (virus inoculated).

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