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Effects of One Plant Activator on Peroxidase and Protease Enzymes in Two Tomato (*Solanum lycopersicum* L.) Varieties

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ABSTRACT

The aim of this research was to investigate the effect of one plant activator on peroxidase (EC 1.11.1.7) and protease (EC 4.3.1.1) activity in Solanum lycopersicum Riogrande and H2274 varieties. Plant activator was applied to 10 weeks old seedlings in recommended dose, two and four fold doses (2,4 and 8 ml/L) by spraying to the leaves under in vivo conditions. The leaves of the seedlings were harvested for analysis 24 and 48 h after plant activator applications. All of the experiments were replicated three times. Peroxidase activity changing in S. lycopersicum varieties while compared with control group. 48 h after 8 ml/L activator application in Rio Grande and H2274 varieties POX activity increased as 248% and 247.4% respectively. Protease activity changing in S. lycopersicum varieties was compared with the control group. 48 h after 4 ml/L activator application in RioGrande and H2274 varieties PRO activity increased as 90.6% and 83.7%, respectively. Plant activator application has changed the total peroxidase and total protease levels depending to the time and concentrations in different levels. Thus, the use of chemicals with low doses and activators can prevent different plant related diseases by increasing the efficiency of self defence mechanisms in plants by virtue of which our country can hold an important place in agriculture in future and also foreign sales will contribute to resolve the phyto-pathological problems of tomato.

Keywords: Plant activator, Peroxidase, Protease, *S. lycopersicum*, R*iogrande, H2274*

INTRODUCTION

Various enzymes are synthesized in plants which are also involved in the defence mechanism of which the peroxidases are utterly important. Peroxidases (EC 1.11.1.7) are the precursor enzymes that are synthesized in chloroplasts in a large majority of plants and also actively take part in the functioning of the defence mechanism [1]. Peroxidases are involved in defence reactions of plants against pathogens and every kind of stress factors [2]. Peroxidase (POX) cleaves water and oxygen by toxic H_2O_2 after metabolic events, so the POX reaction is measured by monitoring the formation of oxygen [3]. Proteases are involved in many aspects of plant physiology and development. On the other hand, they are necessary for protein turnover, degradation of damaged, misfolded and potentially harmful proteins and also provide free amino acids required for the synthesis of new proteins. Studies have described proteases are associated with several types of plant PCD, including involved in all aspects of the plant life cycle ranging from the mobilization of storage proteins during seed germination to the initiation of cell death and senescence programs [4-6]. Proteases also take part in oxidative stress, tracheary element development and the selective breakdown of regulatory proteins by the ubiquitin/proteasome pathway by which they are able to control the key aspects of plant growth, development, and defence [7,8]. In recent years, demand of conventional fungicides used against pathogen related diseases are gradually decreasing as producers and consumers becoming more health conscious and biological control and Integrated Pest Management (IPM) programs becoming more popular. Bioactivators used to increase natural disease resistance were one of the basics in biological control. The use of plant activators has increased in recent years with the developments in organic agricultural practices. Use of plant activators to reduce plant diseases is relatively new and very few activators are commercially available. Green

Miracle is a long chain fatty acid based new generation stress alleviator for improving the plant health. By virtue its reflective nature Green Miracle alleviates the plant from thermic stress by reducing the rate of transpiration through reflecting greater amount of light that fall on the plant.

MATERIALS AND METHOD

Plant materials

Two varieties of *S. lycopersicum* Mill. Riogrande and H2274 seeds were taken and treated with distilled water for 5 h before the sowing process. Then, seeds were sowed in plastic pots (10 × 30 cm) containing a mixture of 1:2 perlite-peat. Seedlings were grown under plant growth chamber conditions (light/dark of 16/8 h at 25 ± 20°C), relative humidity 60-70%. Ten weeks old seedlings were used for plant activator applications.

Plant activator

In this research Green Miracle plant activator were used for the experiments. This plant activator was taken from Konserve Microbial Agriculture and Animal Product Ltd. Company. Green Miracle is a long chain fatty acid based new generation stress alleviator for improving the plant health. To determine the effects of the plant activator, the criteria which can be defined as the total protease and peroxidase in leaves were studied.

Preparation of leaf extracts

Healthy leaves of Riogrande and H2274 varieties of tomato seedlings were harvested for the applications. For the preparation of crude leaf extract, 0.5 g of fresh leaf were homogenized in 5 ml of cold sodium phosphate buffer (0.05 M, pH 6.5) for 30 s and then centrifuged at 13,000 rpm for 20 min at 4°C.

Enzyme analyses

Peroxidase activity in leaf extracts was assayed spectrophotometrically. 1 ml of assay mixture containing 0.05 M sodium acetate buffer (pH 6.5), 0.2 ml of 0.1 M pyrogallol, 0.1 ml of 90 mM H_2O_2 and an aliquot of the crude leaf extract containing 10-40 µg proteins were mixed together immediately before the evaluation. The peroxidase enzyme activity was measured at 300 nm according to Kanner and Kinsella [9].

Protease activity in the crude leaf extracts was assayed spectrophotometrically. 0.5 ml enzyme solution (supernatant) was added to 2.5 ml casein solution and kept under 30°C without shaking in a water bath for incubation for 20 min. Again, 2.5 ml TCA (Tri-Carboxyl-Acid) solution was added to each test tube and kept under 30°C without shaking in a water bath for incubation for 30 min. After that, suspension filtered through a coarse filter paper tube with a clean glasstube. 0.5 ml of filtrate was taken to a clean glass tube and 2.5 ml 0.5 M Na_2CO_3 was added to each tube. Two-fold diluted 0.5 ml Folin-Ciocalteu reagent was added to each tube. The tubes were stored at room temperature without shaking for 30 min. The protease enzyme activity was measured at 660 nm in spectrophotometer [10].

RESULTS AND DISCUSSION

Plant activators have been used extensively in recent years in the fight against harmful organisms. Using the plant activator to pre-stimulation of plant defence systems provides protection against pests and disease by which it is possible to improve the efficiency. Thus, the plant activators can used to combat pests which can be antibacterial, fungicidal, insecticidal, etc. [11]. According to our research results, peroxidase activity changing in RioGrande and H2274 varieties were determined after plant activator application, while compared with the control group. Most effective application for POX changing was the 8 ml/L application after 48 h in RioGrande and H2274 varieties where the POX activity increased 248% and 247.4% respectively. Most effective total protease activity changing was 4 ml/L application after 48 hours in RioGrande and H2274 varieties as 90.6% and 83.7% respectively. Results of these assays and statistical data of SPSS represented in Tables 1 and 2.

Table 1: Statistical relationships between the groups after 24 and 48 h plant activator applications in *S. lycopersicum* RioGrande seedlings; ** Statistically meaningful groups gave ($p<0.05$)

Group	POX activity (mg/mL/min)	St. Dev.	Compared Groups	Protease activity (U/ml/min)	St. Dev.		Time (h)
Control	54	85.44	1.4**	0.1177	0.00153	1.2**	24
2 ml/L	54.29	49.771	2.4**	0.1577	0.0145	1.3**	24
4 ml/L	58.236	664.816	3.4**	0.1947	0.0205	2.3**	24
8 ml/L	112.9	618.304		0.132	0.015	3.4**	24
Group	**POX activity (mg/mL/min)**	**St. Dev.**	**Compared Groups**	**Protease activity (U/ml/min)**	**St. Dev.**		**Time (h)**
Control	533.333	76.376	1.4**	0.118	0.003	1.4**	48
2 ml/L	536.166	59.448	2.3**	0.1783	0.00751	2.3**	48
4 ml/L	617.466	33.871	2.4**	0.2253	0.0145	2.4**	48
8 ml/L	187.94	53.935	3.4**	0.1553	0.00651	3.4**	48

Table 2: Statistical relationships between the groups after 24 and 48 h plant activator applications in *S. lycopersicum* H2274 seedlings; ** Statistically meaningful groups gave ($p<0.05$)

Groups	POX activity (mg/mL/min)	St. Dev.	Compared Groups	Protease activity (U/ml/min)	St. Dev.		Time (h)
Control	547.467	338.711		0.135	0.1808	1.2**	24
2 ml/L	510.467	256.642		0.1967	0.013	1.3**	24
4 ml/L	568.667	382.797	3.4**	0.224	0.0165	2.3**	24
8 ml/L	1.203.333	450.925		0.1677	0.009	1.4** 3.4**	24
Groups	**POX activity (mg/mL/min)**	**St. Dev.**	**Compared Groups**	**Protease activity (U/ml/min)**	**St. Dev.**		**Time (h)**
Control	547.467	338.711	1.3**	0.1353	0.0155	1.2** 1.3**	48
2 ml/L	615.267	373.258	2.4**	0.2047	0.0105	2.3**	48
4 ml/L	627.167	312.903	3.4**	0.2477	0.0155	2.4** 3.4**	48
8 ml/L	1.902.433	487.828	1.4**	0.1747	0.0145	1.4**	48

Table 3: ANOVA test for protease activity in *S. lycopersicum* RioGrande (24 and 48 h)

ANOVA					
Mean value					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.009	3	0.003	13.414	0.015
Within Groups	0.001	4	0.000		
Total	0.010	7			

Table 4: ANOVA test for protease activity in *S. lycopersicum* H2274 (24 and 48 h)

ANOVA					
Mean value					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.011	3	0.004	420.854	0.002
Within Groups	0.000	4	0.000		
Total	0.011	7			

Table 5: ANOVA test for POX activty in *S. lycopersicum* Rio Grande (24 and 48 h)

ANOVA					
Mean value					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	13460171	3	4486724	6.359	0.053
Within Groups	2822110	4	705,527		
Total	16282280	7			

Table 6: ANOVA test for POX activty in *S. lycopersicum* H2274 (24 and 48 h)

ANOVA					
Mean value					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	14532873	3	4844291	7.702	0.039
Within Groups	2515731	4	628,933		
Total	17048603	7			

If the value is less than 0.05, the difference between the examined data or groups is statistically significant (95% confidence). Thus, there is a statistically significant difference shown in Tables 3-6. There is no difference by 0.053 in Table 5 but if the confidence level is taken as 90%, there will be a difference.

In our research, *L. esculentum* Mill Rio Grande and H2274 varieties POX activity increased 248% and 247.4% respectively. A similar study was carried out by Kiprak [12] on the change of POX activity in *Capsicum annuum*. Peroxidase activity changing in *Capsicum annuum* L. var. *grossum* seedlings was determined in different levels, after plant activators application, while compared with control group. 24 h after 1.2 ml/L dose of Crop-Set application to the *in vivo* growth seedlings of *Capsicum annuum* L. var *longum*, POX activity increased 180% and after 48 h 2.4 ml/L dose of Crop-Set application. Both of the plant activators significantly increased POX activity in both plant varieties according to the exposure time. In our work in vivo conditions related to POX enzyme production; the highest enzyme activity was observed at 48 h in the plant groups to which the plant activator was applied. In *S. lycopersicum* H2274 and *S. lycopersicum* RioGrande species, the most effective dose was found to be four times the dose of the recommended dose.

There are a variety of studies on the use of casein as substrate for proteolytic activities in different plants. Mahajan et al. [13] used casein as a substrate to screen twenty plants of the *Euphorbiaceae* family for proteolytic activities in leaves. Among these selected plants; *Pedilanthus tithymaloides* has the highest caseinolytic activity, followed by *Euphorbia tirucalli*, *Euphorbia nivulia* and *Euphorbia nerifolia* 9.0, 6.42, 4.56 and 4.38 U/mg proteins, respectively.

Our study has found that, the protease activity of tomatoes RioGrande and H2274 was found to be increased. In another research conducted by Veerasamy et al. [14] zeatin application also showed increased in protease activity. In our research; total protease activity increasing in RioGrande and H2274 varieties after 48 h with 4 ml/L plant activator applications was as 90.93% and 83.07%, respectively.

CONCLUSION

As a result, exports of agricultural products due to pesticide residues to minimize the role of the terms of the negative effects, plant activators is necessary to traditional chemical control of the manufacturer as a more preferred alternative methods. Plant activators, to per unit area more and high-quality product that delivers, drugs commonly used in agricultural production today is inevitable.

Future research in this field will be; an active role in the functioning of the defence mechanism and protease, a key enzyme in the metabolism of the protein, interact each other. Various inhibitors will be used to interaction between these two enzymes.

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