Influence of salicylic and jasmonic acids on the antioxidant systems of tomato (*Solanum lycopersicum* cv. Superchief) plants under biotic stresses

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Abstract

Changes in the activity of antioxidant enzymes, proline, and total soluble carbohydrates (TSC) were evaluated in tomato (*Solanum lycopercicum* cv. Superchief) plants in response to salicylic acid (SA) and jasmonic acid (JA) and inoculation with CMV (Cucumber mosaic virus) on days 0, 1, 2, 4, 6, 8, and 15 after virus inoculation (dpi). Results showed that proline content increased in all treatments except for control while TSC content decreased in all treatments except the control. TSC content decreased by 50% in CMV treated plants, but the reduction was very slow in hormone treatments. SA decreased the catalase (CAT) activity until 6 dpi and then increased CAT activity to 15 dpi while other treatments except the control increased CAT activity over time. The highest activity of peroxidase (POD) was observed in SA+JA treatment and the highest activity of superoxide dismutase (SOD) and phenylalanine ammonia lyase (PAL) was related to SA+JA+CMV treatment. These results show that combination of SA with JA can control the CMV in tomato plants cv. Superchief through the inhibition of TSC reduction which is an indicator of normal photosynthesis capacity.

Keywords: *Cucumber mosaic virus* (CMV); tomato; proline; oxidative stress

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Introduction

When plants are subjected to biotic and abiotic stresses, reactive oxygen species (ROS) like superoxide, hydrogen peroxide (H_2O_2), and hydroxyl radicals are generated (Dat et al., 2000). In order to avoid the harmful effects of ROS, plants have evolved an effective scavenging system composed of enzymatic antioxidants such as superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POD, EC 1.11.1.7), catalase (CAT, EC 1.11.1.6), and H_2O_2 generating and degrading enzymes (Hajiboland and Hasani, 2007). Salicylic acid (SA) is a phenolic derivative distributed in a wide range of plant species. Many researchers monitored the role of SA in defense and pathogen resistance. SA could contribute to maintaining cellular redox homeostasis through the regulation of antioxidant enzyme activities and induction of

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the alternative respiratory pathway (Moore et al., 2002).

Jasmonic acid (JA) and its methyl ester, methyl jasmonate (Me-JA), are naturally occurring plant growth regulators, which play pervasive roles in several physiological and biochemical processes in plants. It was reported that exogenous application of JA induces different physiological responses to various abiotic stresses (Ding et al., 2001). Also, it has been found that JA induced the expression of plant-defense genes in response to different pathogen attacks (Fayza and Sabrey, 2006).

Zhu et al. (2014) reported that SA and JA are essential for systemic resistance against *Tobacco mosaic virus* (TMV) in *Nicotiana benthamiana* and foliar application of JA followed by SA triggered the strongest systemic resistance against TMV. SA treatment on tomato plants infected with *Potato virus X* (PVX) enhanced the plant resistance via changing in the physiological parameters, activation of secondary metabolism, and expression of some antioxidant and pathogenesis related protein (PRs) genes (Falcioni et al., 2014). It seems that plant response to hormones under infection with viruses differs in various conditions and it is related to virus, plant, internal, and external factors. When working with CMV results maybe different due to these reasons. So, more studies are required to confirm the real effects of SA and JA application on the control of CMV in tomato plants.

Tomato (*Solanum lycopercicum*) plants are one of the most important vegetable crops all over the world which is exposed to many biotic stresses like pathogens and infections (Herlihy et al., 2003). Cucumber mosaic virus (CMV) from the genus *Cucumovirus* in the family Bromoviridae is a widespread virus which infects 1287 plant species (Zitikaite and Urbanaviciene, 2010). It is transmitted by aphids through sap and affects vegetables and is one of the most important viruses (Yang et al., 2016).

The objectives of this study were to determine the effect of CMV on tomato plant (cv. Superchief) antioxidants systems, photosynthetic status, proline and total carbohydrate content, and the influence of SA and JA on the amelioration of these traits against virus infection.

Materials and Methods

Plant material and treatments

In order to investigate the effect of salicylic and jasmonic acids on the response of tomato (*Solanum lycopersicum* cv. Superchief) to the oxidative stress derived by CMV, a factorial experiment was conducted based on a completely randomized design with three replications. Tomato seeds were sterilized using 5% sodium hypochlorite and planted in sterilized clay loamy soil in paper pots. Four weeks after planting, seedlings were transplanted to the controlled insect-proof greenhouse with 25/18 °C day/night temperature and 16/8 hours day/night photoperiod. Treatments were *cucumber mosaic virus* (CMV), salicylic acid (SA), jasmonic acid (JA), and their combinations as described below:

1) Foliar application with distilled water as control treatment
2) Inoculation with CMV
3) Foliar application with 1 mM SA
4) Foliar application with 0.5 mM JA
5) Foliar application with 1 mM SA and 0.5 mM JA
6) Foliar application with 1 mM SA and CMV inoculation
7) Foliar application with 0.5 mM JA and CMV inoculation
8) Foliar application with 1 mM SA and 0.5 mM JA and CMV inoculation

SA and JA at concentrations of 1 and 0.5 mM, respectively were sprayed on plants 24 hours before inoculation with CMV. CMV which was propagated on cucurbit plants was mechanically inoculated on tomato plants. A 0.1 g of cucurbit plant tissue was smashed in 1 ml inoculation buffer and the obtained extract was used for inoculation on the tomato leaves by gently rubbing with Carborundum (silicon carbite). Samples were taken after 0, 1, 2, 4, 6, 8 and 15 days post inoculation (dpi) and were immediately transported to a liquid nitrogen tank and kept frosted in -70 °C until measurements.

Measurement of proline content

The proline content was determined according to Bates et al. (1973). Leaf samples (0.1 g) were extracted in 10 ml of 3% sulfosalicylic acid

overnight and centrifuged at 1500 rpm for 10 min. The supernatant (2 ml) was mixed with 2 ml of ninhydrin solution and 2 ml glacial acetic acid for 1 h at 100 °C in water bath. Then, the reaction was stopped in an ice bath and the mixture was extracted with 4 ml toluene. The absorbance was read at 520 nm and the proline content was expressed as mM g^{-1} on a fresh weight basis.

Measurement of total soluble carbohydrates

TSC was determined using anthrone sulfuric acid method described by Scott and Melvin (1956). Briefly, 1 g of dried sample was homogenized with 80% ethanol for 15 min on a water bath. The extract was filtered and oven dried at 60 °C and added to 10 ml of 1.5 N sulfuric and heated at 100 °C for 6 h. TSC content was calculated as mg g^{-1} dry weight.

Enzyme extraction and activity measurement

Tomato plant leaf samples (1 g) were homogenized with 5 ml of potassium phosphate buffer (50 mM, pH 7.5) in a mortar. The buffer consisted of 1 mM EDTA, 1 mM dithiotreitol, and 2% polyvinyl pyrolidone (PVP). This homogenate was centrifuged at 15000 g for 25 min and supernatants were used for antioxidant enzymes assay (Kar and Mishra 1976).

Superoxide dismutase (SOD) assay

SOD (EC 1.15.1.1) activity was determined based on the procedure proposed by Giannopolitis and Ries (1977). Twenty µl of the supernatant was added to 50 mM potassium phosphate buffer (pH 8), 9.9 mM L-Methionine, 57 mM NBT (nitro blue tetrazolium), and 0.025 % triton X-100. The reaction started with the addition of 10 µl riboflavin under a fluorescent lamp for 10 min. The absorbance was measured for both blank and control at 560 nm.

Catalase (CAT) assay

Measurement of CAT (EC 1.11.1.6) activity was performed according to Aebi (1984). Twenty µl of the enzyme supernatant was added to 1.5 ml of reaction mixture containing 30 µl water, 50 µl of 1 M Tris-HCl buffer (pH 8.0), 5 mM EDTA, and 900 µl of 10 mM H_2O_2. The absorbance was recorded at 240 nm for 60 sec.

Peroxidase (POD) assay

POD (EC 1.11.1.7) activity was measured according to Kato and Shimizu (1987). Three ml reaction mixture containing 1.5 ml 0.1 m potassium phosphate buffer (pH 7), 600 µl of 10 mM guaiacol, and 800 µl of 4 mM H_2O_2 was added to 100 µl of the enzyme extract and the absorbance was recorded at 470 nm.

Phenylalanine ammonia lyase (PAL) activity

PAL activity (EC 4.3.1.5) was measured according to the method described by Assis et al. (2001). Extraction solution containing 50 mM sodium borate, 1 g PVP, 5 mM MEP and 2 mM EDTA was added to 200 mg leaf samples and then centrifuged at 20000 g for 20 min at 4 °C. The solution was finally filtrated to get crude extract. Then, 250 µl of crude extract was added to a reaction mixture containing 500 µl of 30 mM L-phenylalanine and 750 µl of 30 mM sodium borate buffer (pH 8.7). After 10 min the substrate was added and the reaction was stopped with 0.1 ml 6 N HCl. PAL activity was determined for 90 min at 30 °C by the production of cinnamate at 290 nm.

Results

Proline and total soluble carbohydrate

Proline content increased in all treatments except for the control from 0 to 15 dpi. The highest proline content (4.63 mM g^{-1} FW) was observed in SA + JA + CMV at 15 dpi which was not different with SA + CMV treatment at 15 dpi (Table 1). In contrast to proline, TSC content decreased over time in all treatments except for the control. In CMV inoculated plants TSC content decreased nearly by 50%, but the reduction speed was slower in hormone treatments compared with CMV (Table 2). The highest TSC content was related to the control at 15 dpi (4.78 mg g^{-1} DW).

Antioxidant enzymes activity

The highest CAT activity was observed in SA + JA + CMV treatment at 15 dpi (394 U min^{-1} g^{-1} FW) that was not different from JA treatment at the same time (Table 1). In the control and treatments consisting SA (SA and SA + JA) activity of CAT decreased until 6 dpi and then increased to 15 dpi, but in JA and CMV treatments the CAT activity increased over time. The highest POD activity (452 U min^{-1} g^{-1} FW) was related to SA + JA at 15 dpi which was not different from JA + CMV and SA + JA + CMV at 8 dpi and JA at 15 dpi (Table 1). The highest SOD activity (192 U mg^{-1} protein) was related to SA-treated plants at 15 dpi (Table 1) that was not different from SA + JA, SA + CMV and SA + JA + CMV at 15 dpi, and SA + JA + CMV at 8 dpi.

PAL activity

PAL activity increased in all treatments over time (Table 1) except for CMV and SA + JA + CMV which showed a different trend. The highest PAL activity was observed in SA + JA and SA + CMV treatments at 15 dpi (173 and 169 U mg^{-1} protein, respectively).

Discussion

Proline and total soluble carbohydrate (TSC) content

Many reports are present which indicate a significant increase in proline content under environmental stresses (Pazarlar et al., 2013; Berber and Onlu, 2012). Accumulation of proline activates a hypersensitive response to the biotic infections (Radwan et al., 2007) and scavenges the ROS (Fabro et al., 2004; Chen and Dikman, 2005). SA and JA also can induce proline accumulation in healthy and infected plants (Gholi-Tolouie et al., 2017).

In this experiment TSC content increased only in healthy plants over time and the other treatments showed sever reduction in TSC under virus infection and hormone treatments (Table 1). The reduction of TSC was not significant when SA applied was in combination with JA. Khalil et al. (2014) reported that TSC content decreased in tomato leaves, but increased in roots when infected by TYLCV (*Tomato yellow leaf curl bigeminivirus*). Reduction of TSC content in infected or hormone-treated plants was attributed to the decrease in photosynthesis capacity or increase in the respiration rate (Radwan et al., 2007; Hemida, 2005).

Antioxidant enzymes activity

Oxidative stress occurs when high levels of ROS are produced in plants and the enzymatic and non-enzymatic defense mechanisms are present for scavenging free radicals (Sedghi et al., 2012). Yang et al. (2016) demonstrated that ROS have been previously considered as very harmful molecules at high levels, but recently they are believed to be secondary messengers in stress transduction. H_2O_2 is the first candidate for stress signaling at low levels (Deng et al., 2016). It is confirmed that SA application decreases the CAT activity at the early days of infection of plants to pathogens causing an H_2O_2 burst and subsequent activation of defense systems including CAT activity on the days following the infection (Yarullina et al., 2011). It has been reported that the content of ROS in the JA-treated plants was the highest, but the damage was the least. This is an evidence for the role of ROS as secondary messengers which is mediated by JA during the virus resistance (Yang et al., 2016). Liao et al. (2012) demonstrated that in the tomato plants infected with TMV, both CAT and POD activities increased and the highest activities were observed when SA was used. Infection of *Momordica charantia* plants with CMV increased SOD and CAT activities and JA application improved plant resistance to CMV (Yang et al., 2016).

PAL activity

PAL is necessary for the synthesis of phenolic compounds such as flavonoids, phenyl propanoids, and lignin in plants (Ali et al., 2007). PAL activity in tomato leaves increase by SA application under PVX infection (Falcioni et al.,

Table 1
Comparison of means for the effect of hormone treatment and CMV infection in studied traits of tomato leaves

Treatment	Time (day)	Proline (mmol g^{-1} FW)	TSC (mg glucose g^{-1} DW)	CAT (unit min^{-1} g^{-1} FW)	POD (unit min^{-1} g^{-1} FW)	SOD (unit min^{-1} g^{-1} FW)	PAL (unit mg^{-1} protein)
Control	0	1.12	3.17	212	211.3	75.33	62.66
	1	1.02	3.56	208	203.3	65.33	71.33
	2	0.95	3.89	195	204	52.66	74
	4	0.92	4.15	176	192.3	59.66	72.33
	6	0.93	4.35	155	175	63.33	84.66
	8	0.95	4.51	124	165.3	73	83
	15	1.02	4.78	108	154.3	74.33	94.66
CMV	0	1.14	3.20	210	213.3	76.33	65.66
	1	1.28	3.12	217	217.3	69.66	71.66
	2	1.39	2.91	243	214	84.33	85.33
	4	1.61	2.57	268	285.6	95	91.66
	6	1.84	2.13	281	303.3	104.33	92.33
	8	1.96	1.96	302	383	124	93
	15	2.05	1.71	314	248.3	133.33	85.66
SA	0	1.13	3.15	213	222	72	65.33
	1	1.36	3.05	201	229	66	64.66
	2	1.74	2.87	187	233	94.66	71.66
	4	1.92	2.71	163	284	119.66	85.66
	6	2.28	2.48	142	334.6	142.33	104.66
	8	2.81	2.31	189	379.3	160.66	134.33
	15	3.15	2.22	225	409.3	192	163
JA	0	1.30	3.16	215	207.6	73	64
	1	1.32	3.02	221	245	74.66	59.33
	2	1.62	2.84	236	262.3	83.33	65.66
	4	1.89	2.59	284	304.6	105.66	83
	6	2.28	2.41	315	364.3	130.33	97
	8	2.7	2.27	368	391.3	159.66	125.33
	15	3.1	2.01	394	433	173.66	143.66
SA+JA	0	1.15	3.14	210	217.3	74	66
	1	1.62	3.05	215	243	83.66	64.66
	2	1.95	2.76	204	249.3	93.66	74.66
	4	2.24	2.60	200	308.6	116.66	90
	6	2.61	2.51	189	378.3	150.33	116.33
	8	2.94	2.45	256	410	164	144.33
	15	3.27	2.36	294	452	183	173
SA+CMV	0	1.14	3.16	214	218.6	64.66	65.66
	1	1.84	3.01	221	239.3	83.33	68
	2	2.17	2.85	217	268.3	101	73.33
	4	2.56	2.63	225	310.3	127.66	94.66
	6	2.94	2.41	271	363	156.33	123.3
	8	3.67	2.24	302	392	167	144
	15	4.57	2.11	334	374.3	183.33	169
JA+CMV	0	1.11	3.17	217	221	71	62.33
	1	1.67	2.97	226	229	80.33	66
	2	1.86	2.61	241	287.3	94.66	70.66
	4	2.04	2.43	276	321.3	116.33	87.33
	6	2.64	2.21	297	363.3	144.33	128.66
	8	3.17	2.08	349	433.3	170.66	152.66
	15	3.82	1.91	381	381.3	175.66	143.66
SA+JA+CMV	0	1.15	3.15	215	208	75.33	62.66
	1	1.87	3.02	230	237.6	71	57.33
	2	2.46	2.96	225	273.6	86	75.33
	4	2.94	2.76	268	326.6	120.33	93.66
	6	3.61	2.64	291	382	161.33	132.33
	8	4.12	2.53	317	436.3	188.66	139.66
	15	4.63	2.37	394	369	190.33	164
LSD		0.27	0.86	11.3	22.6	9.1	5.3

Means in each column are not greater than LSD have no significant difference at p<1%. TSC: total soluble carbohydrates.
TSC: total soluble carbohydrates; CAT: catalase activity; POD: peroxidase activity; SOD: superoxide dismutase activity; PAL: phenylalanine ammonia lyase activity.

2014). SA and JA activate PAL to produce phenolics and establish the systemic resistance network (Asghari and Hasanlooe, 2015). In tomato cv. Falat, increase in the activity of PAL was observed in response to CMV and application of SA and JA (Gholi-Tolouie et al., 2017). Li and Zou (2017) reported that SA, calcium ion or their combination

enhanced the PAL activity in tomato against *Botrytis cinerea*.

In conclusion, application of SA + JA can improve the resistance of tomato cv. Superchief to CMV infection and compensate the carbohydrate loss in the leaves caused by CMV.

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