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Influence of Salicylic Acid application on Oxidative and Molecular Responses and functional properties of *Capsicum annuum* L. cultivated in greenhouse conditions

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Abstract:
Growing techniques can affect the yield and nutritional quality of cultivated plant species. Owing to its high nutritional value, *Capsicum annuum* L. was used to investigate the effect of abiotic stress and elicitor on yield and fruit quality parameters under conditions of greenhouse. Elicitors are molecules that induce the activation of transduction cascades and hormonal pathways, which trigger induced resistance to environmental stress. Aim was evaluated the endogenous H_2O_2 production caused by the effect of different concentrations of salicylic acid (SA) in *C. annuum* in production after elicitation, and the production of secondary metabolites to relate their response in metabolic pathways. SA has induced an endogenous H_2O_2 and enzymatic activities related with plant defense as phenylalanine ammonia lyase (PAL) and catalase (CAT). Results showed that could be an indicator for determining application opportunity uses in agriculture for maintaining plant alert systems against a stress. The correlation of secondary metabolites and ability to remove free radicals of the sweet pepper was significant showed a correlation between the DPPH and ABTS compared with the control. The analysis revealed that significant increased activity of CAT and PAL and H_2O_2 endogenous were ($p<0.05$) perturbed in the stress-induced treatments: in some metabolic pathways as aminoacyl t-RNA biosynthesis.

Keywords: antioxidant capability, plant stress, elicitor, reactive oxygen species, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), PAL, CAT

Introduction

Increasing daily consumption of fruits and vegetables containing bioactive compounds has been a major public health focus for many years. Developing fresh produce containing greater concentrations of phytochemicals to have increasing effect on some health aspects can be an alternative approach to increase the exposure to the bioactive compounds [1,2]. *Capsucum annuum* are a good source of numerous antioxidant compounds, these contain more than 20 different carotenoids, abundant phenolic compounds (including flavonoids and condensable tannins) and vitamin C [3]. Also, there has been a renewed interest in studying and quantifying the bioactive compounds of fruits and vegetables due to their health-promoting properties [4].

Introduction

These compounds in food are important health-protecting factors and their presence can be modified due to the use of elicitors, stress abiotic or biotic; however, this practice could induce changes in fruit quality attributes [1]. *Capsucum annuum* are a good source of numerous antioxidant compounds, these contain more than 20 different carotenoids, abundant phenolic compounds (including flavonoids and condensable tannins) and vitamin C [3].

Plants are frequently exposed to different environmental stresses, which can be both biotic and/or abiotic. These stresses cause biochemical alterations as generation of hydrogen peroxide (H_2O_2) resulting in an early response of the plant defense mechanism, the oxidative burst, the generation of reactive oxygen species (ROS) [5,6].

Results and Discussion

The knowledge that phenols and flavonoids in higher plants produced in response to environmental factors, and assess the determination of total phenol focused on assessing elicitor and its concentration in seedling, so that a significant difference was observed between concentrations producing a greater amount of phenolic compounds in the concentration 10 mM SA for routine production and 0.1 mM for gallic acid with ($F = 176.47$, $P < 0.0001$) Figure 1.

Results and Discussion

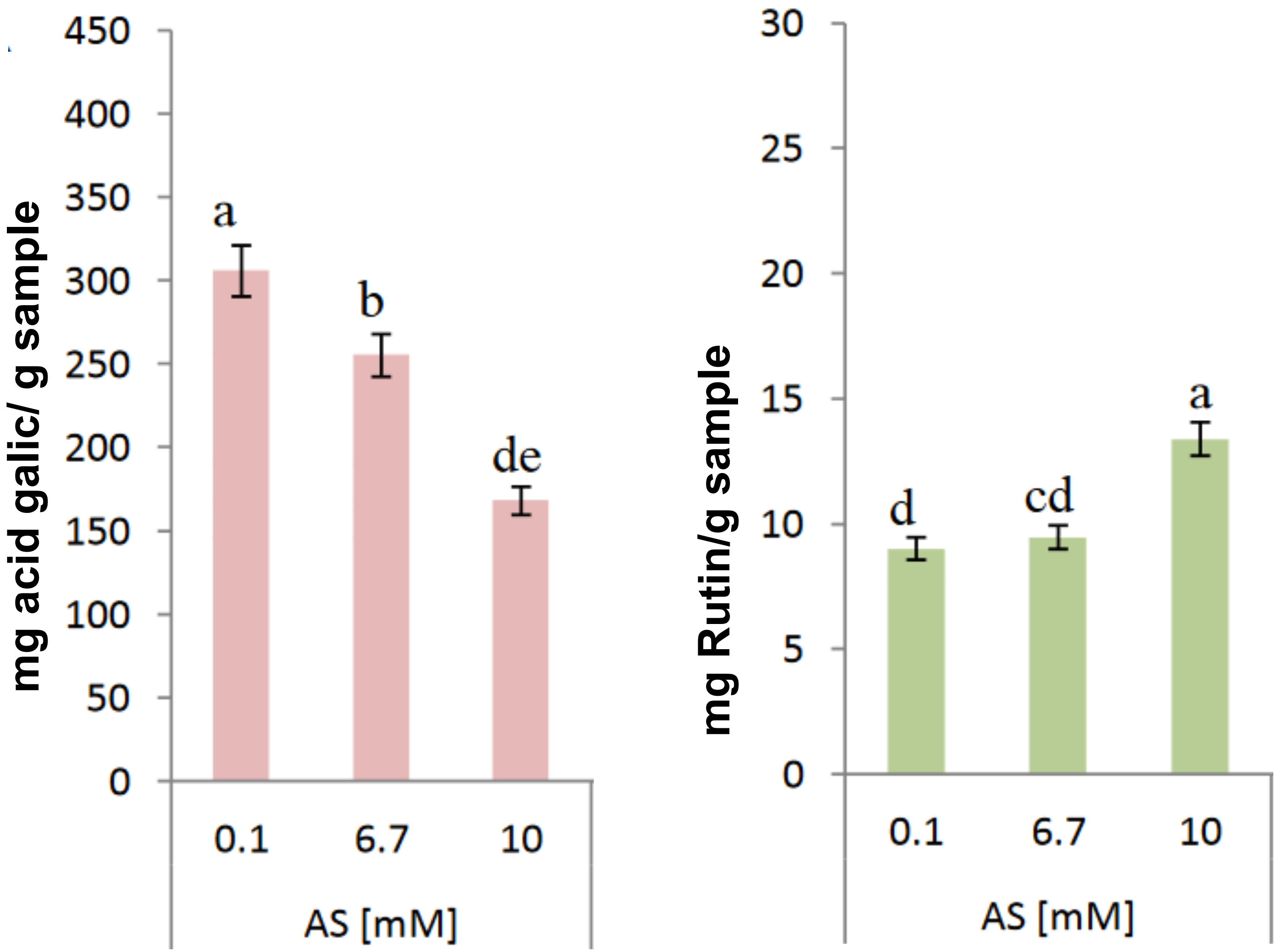


Figure 1. Phenolic Compunds in seedling of *Capsicum annuum*

Results and Discussion

Table 1. Flavonoid, tannins, and phenols contents in *Capsicum annuum.*

Treatment	Flavonoids (µg rutin/g sample)	±SD	Tannins (mg (+) cathechin/g sample)	±SD	Phenols (mg galic acid/g sample)	±SD
Control	237.577^{b}	54.999	2.726^{a}	0.689	0.016^{a,b}	0.003
SA 0.1 mM	211.332^{a,b}	32.675	2.709^{a}	0.417	0.020^{b,c}	0.011
SA 1 mM	275.124^{c}	59.381	2.669^{a}	0.667	0.016^{a}	0.002
SA 0.01 mM	220.510^{a,b}	40.898	2.835^{a}	0.400	0.014^{a}	0.003

Results and Discussion

(Table 1) The total phenolic content of *Capsicum annuum* was similar to those reported by Vinson et al., (7). The total content of phenols corresponding depending *Capsicum annuum*. This is shown by increasing the content of total phenolic above the control (treatment 1) which corresponds to 0.016 mg gallic acid equivalent/g which is significantly different. According Mejia-Teniente [5] the combination of elicitors at high concentrations the effect on oxidative stress in the plant and producing different secondary metabolites as well as total phenolic content. The highest concentration 275.124 µg rutin equivalent/g corresponding to treatment 1 mM SA 8 and the lowest concentration 220.510 µg rutin equivalent/g corresponding to Treatment 0.01 mM SA. *Capsicum annuumm* not presented significant differences between treatments in the amount of total tannins.

Results and Discussion

Table 2. ABTS and DPPH-free radical scavenging in *Capsicum annuum*

Treatment	ABTS (µmol eq. TROLOX/g sample)	±SD	DPPH (µmol eq. TROLOX/g sample)	±SD
Control	2.441^{a}	0.443	5.014^{a}	0.611
SA 0.1 mM	2.905^{b}	0.038	$5.382^{a,b}$	0.731
SA 1 mM	2.879^{b}	0.059	$6.246^{d,e}$	0.366
SA 0.01 mM	2.526^{a}	0.444	$5.704^{b,c,d}$	1.079

Results and discussion

The ABTS and DPPH radical scavenging capacity is shown in (Table 2). The radical capacities of *Capsicum annuum* were significantly different with the highest concentrations. This pattern was similar to that of the total content of phenolic compounds. Red sweet pepper with the highest concentrations were treatments 1 and 0.1 mM and not showed significant differences and lowest concentrations were treatment Control and 0.01 mM with 2.441 and 2.526 µmol trolox equivalents/g respectively. The absorption capacity for the DPPH radical in the *Capsicum annuum* highest concentrations 6.246 µmol trolox equivalent/g to treatment 0.01 mM and lowest concentrations 5.014 µmol trolox equivalent/g to treatment Control.

Results and Discussion

Table 3. Correlation between flavonoids, phenols, and tannins content with scavenging free radical capability (ABTS and DPPH) in *Capsicum annuum*.

Treatment	ABTS (µmoles eq. Trolox/g sample)	DPPH (µmoles eq. Trolox/g sample)	Flavonoids (µg rutin/g sample)	Phenols (mg galic acid/g sample)	Tanins (mg (+)catechin/g sample)	Regression equation	R^2	P<0.05
Control						-0.1371x + 14.69	0.64	0.000
						-2.0184x + 344.37	0.60	0.242
						-0.2925x + 17.769	0.99	0.011
SA 0.1 mM						0.5717x - 53.208	0.81	0.002
						0.0006x - 0.0155	0.91	0.014
						0.1832x - 7.9368	0.97	0.000
SA 1 mM						112.18x - 10625	0.99	0.000
						0.0189x - 1.8169	0.99	0.000
						-2.6022x + 255.52	0.99	0.000
						-12.551x + 1140	0.99	0.000
						-0.0021x + 0.161	0.99	0.000
						0.2911x - 17.392	0.99	0.000
SA 0.01 mM						-0.1052x + 11.839	0.61	0.000
						-0.1978x + 13.943	0.99	0.001

Results and Discussion

Furthermore, the total flavonoid content have been correlated with the ability to eliminate radicals by absorption of DPPH radical and ABTS radical was observed in treatment 1 mM with a significant correlation ($r = 0.99$, $P < 0.000$) and ($r = 0.99$, $P < 0.000$) respectively, therefore, indicates that the interaction of the elicitor and abiotic stress influences the production of flavonoids, Table 3 .

Results and Discussion

However, from the above discussion salicylic acid enhances the growth and productivity secondary metabolites of plants. Exogenous application of salicylic acid induces the resistance in plants, thereby provides a considerable protection against various biotic and abiotic stress [8,9]. The lower concentrations of salicylic acid have proved to be beneficial in enhancing the photosynthesis growth and various other physiological and biochemical characteristics of plants. However, at higher concentrations, SA itself may cause a high level of stress in plants and enhances the activities of antioxidants enzyme system.

Results and discussion

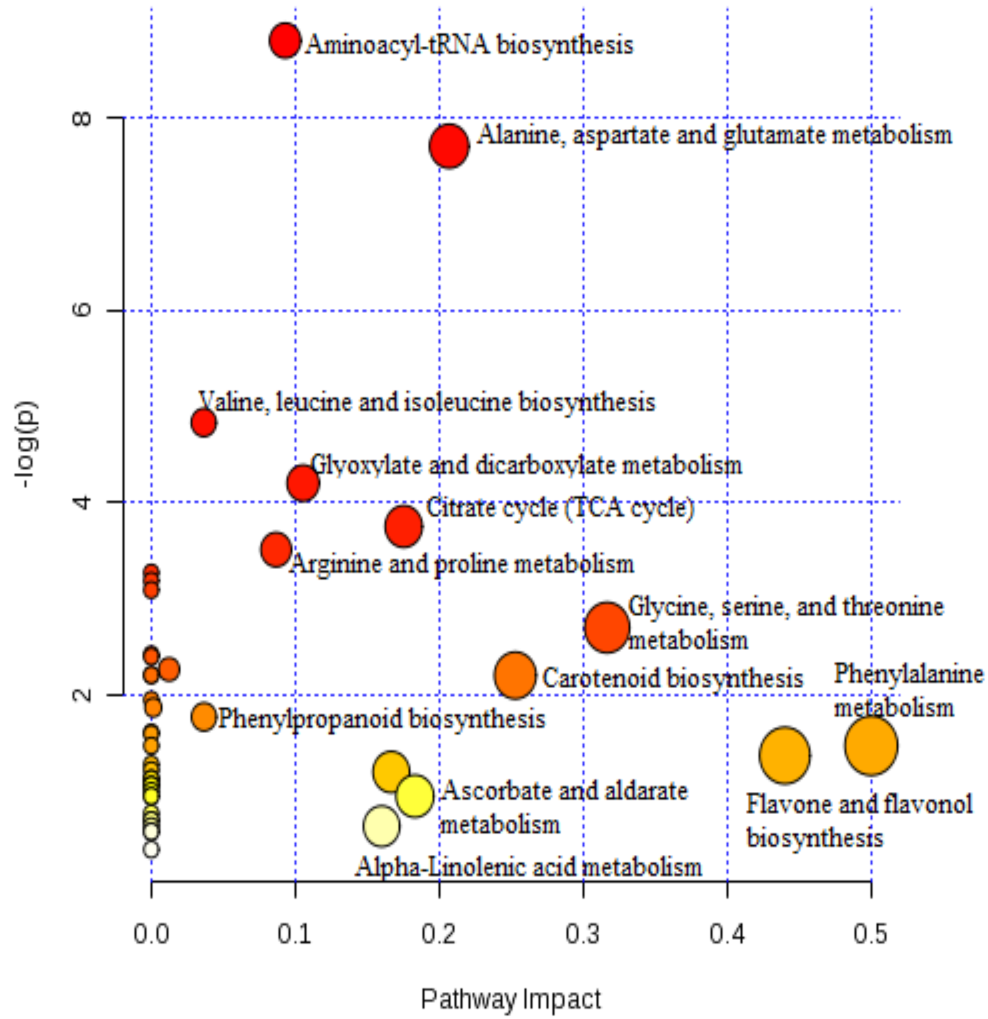
Table 4. Low molecular weight metabolites of sweet pepper *Capsicum annuum*.

Name of compound	Rt (min.)	Peak area (%)				Important iones in peaks: m/z
		Control	SA 0.1 mM	SA 1 mM	SA 0.01 mM	
Lactic acid	3.687	LLD	3.423	7.663	LLD	147-117-191-148
Succinic acid	7.557	1.751	LLD	3.284	2.753	247-147-148-172
Maleic acid	8.174	LLD	0.529	0.671	LLD	147-73-245-148
Malic acid	11.366	4.103	4.59	10.592	8.48	233-189-133-190
Ascorbic acid	19.43	2.745	10.005	8.855	82.472	73-147-332-205
Palmitic acid	20.678	7.131	9.961	8.458	1.811	313-145-117-93
Linoleic acid	23.363	4.56	6.556	9.204	1.203	262-97-147-190
Stearic acid	24.033	5.652	5.312	5.226	0.811	341-145-117-313
γ-Tocopherol	34.59	0.531	2.132	1.238	0.082	488-223-73-489-222
(+)-α-Tocopherol	35.78	LLD	12.41	8.75	0.335	502-237-73-503-236
Campesterol	336.78	3.037	5.971	4.196	0.195	343-73-129-43
Stigmasterol	37.122	1.031	1.438	1.209	0.049	484-255-129-313
β-Sitosterol	37.721	9.478	17.216	10.763	0.296	486-396-357-129

LLD: Lower Limit of Detection

Results and Discussion

Schematic of the metabolome following the metabolite pathway mapping of the impacted metabolites identified after effect stress-induced in *C. annuum* L. A color coded matrix represents intensity values of the metabolic pathway and effect of each metabolite, which has been log_2-transformed and mean-centered. (Color figure online). The analysis was performed using the MetaboAnalyst software.



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Results and Discussion

This study revealed that the metabolite levels were significantly altered in the treatment with the control. The differential metabolites induced by stress-induced radiation mainly included fatty acids, amino acids, organic acids, and phytosterols. Rahimi, et al. [10] investigated the effect of salicylic acid and Methyl jasmonate on growth, yield and essential oil quantity and quality of cumin (*Cuminum cyminum* L.), which main effect was an increase by the application of SA. This showed that SA significantly enhanced H_2O_2 production, phenylalanine ammonia-lyase (PAL) activity, and rosmarinic acid accumulation. The CAT cycle, an important pathway for the generation of energy, is the final pathway for the oxidation of carbohydrates, fatty acids, and amino acids. The disturbance of these three pathways in somatic tissue indicates disorders in the cell.

Conclusions

Results highlight that capsicum and elicitor can be considered a good source of antioxidant bioactive compounds, there is a synergistic interaction of biotic and abiotic stress applied to plant sweet pepper in their high concentrations, and shows a highest absorption capacity of the radical ABTS and DPPH, indicating synergistic interaction by particularly extractable polyphenols compounds (total phenolics, flavonoids and tannins).

Elicitors can promote important changes in secondary metabolites profile, aminoacids, and fatty acids which can have an impact in sensory traits affecting the quality of pepper as a result of their interactions with environment conditions.

Conclusions

In conclusion, the interaction of SA and CE showed a significant effect on the concentration of aminoacids, fatty acids, and phytosterols compared to the control treatment. The latter compounds are derived mainly from aminoacyl-tRNA biosynthesis; alanine, aspartate and glutamate metabolism.

Exogenous application of SA, significantly increased endogenous H_2O_2 as well as enzymatic activities related with plant defense as phenylalanine ammonia lyase and catalase.

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