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The Effect of Exogenous Proline and Glycine Betaine on Phyto-biochemical Responses of Salt-stressed Basil Plants

Gehan Safwat[(1)#], Hemat S. Abdel Salam[(2)]

[(1)]Faculty of Biotechnology, October University for Modern Sciences and Arts (MSA), Cairo, Egypt; [(2)]Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agricultural Research Centre, Cairo, Egypt.

SALINITY is one of the most problematic issues for agriculture in terms of abiotic stresses, particularly in semi-arid regions. Important osmolytes which accumulate in the cell include proline and glycine betaine. In the present investigation, the effect of the aforementioned osmolytes were assessed on sweet basil (*Ocimum basilicum* L.), the roles of exogenous applications were estimated at 50 100 or 200mM) and salinity stress (50 ,75 or 100mM NaCl). Results showed that all concentrations significantly increased growth parameters including number of branches/plants, height, fresh weight, leaves, and chlorophyll content, all of which decreased with increased NaCl. 100- and 200-mM glycine betaine had the most beneficial effect in both seasons. Similarly, severe salt conditions had a significant increase in endogenous proline content of basil leaves. Foliar application of 100- and 200-mM glycine betaine had the most beneficial effect in both seasons. When SDS-PAGE analysis was conducted it showed 19 bands with molecular weights (MW) ranging from 14.47 to 175.43kDa. Moderate and high salinity stress treatments of irrigation water (75 and 100mM) blocked the synthesis of a 175.429kDa polypeptide that was not restored by all foliar application of proline and glycine betaine. Highest NaCl level (100mM) alone induced the synthesis of new polypeptide (63.41kDa molecular weight). Exogenous proline and glycine betaine improved physiological parameters and reduced oxidative damage. Results suggest increased tolerance to oxidative damage caused by salinity, and these protectants rendered better performance, by upregulating their antioxidant defence system.

Keywords: Abiotic stresses, Glycine betaine, *Ocimum basilicum* L. proline, Salinity, Sweet basil.

Introduction

The economic significance of Medicinal and aromatic plants, is undoubtful due to a continuous market demand for their products. Sweet basil (*Ocimum basilicum* L.) is one of the most in demand plants in this regard, it is an herbaceous plant belonging to *Lamiaceae* family, and has a widespread popularity for its uses in flavoring and medicinal applications. Essential oil from sweet basil is used considerably in multiple sectors including food, perfumery, and medical industries. The plant additionally encompasses a varied range of biological activities as well as antioxidant properties and contains several aromatic compounds (Lee et al., 2005). Several studies reported that the impact of salinity on basil plants reduce several growth variables (Fatemi & Aboutalebi, 2012; Bione et al., 2014; Heidari, 2012), reporting that, basil plants are negatively affected in growth variables under salt stress. Sweet basil is a perenial plant, native to Africa and tropical regions of Southeastern Asia, there are roughly 30 species, all of which possess distinct physical and chemical features (Telci et al., 2006). It contains medicinal value for its contents of antioxidants, beta-carotene, vitamins, and metals such as Mn, Cu, K, Mg (Marotti et al., 1996). Its properties include aiding digestion, lowering cholesterol and blood sugar

#Corresponding author email: gsafwat@msa.edu.eg
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as well as immune system support. Reports were also indicated for its value in treating of diseases such as diarrhea and vomiting in children, headaches, asthma, coughs, dental pains, and even kidney stones, (Sajjadi, 2006). Plants are exposed to multiple kinds stresses throughout their life cycles whether Biotic or Abiotic. These stresses can be from temperature, drought, heavy metal contamination, infection by pathogens, UV radiation, nutrient deficiency and salinity (Siripornadulsil et al., 2002). A major culprit of abiotic factors negatively influencing productivity all over the world is Salinity (Koca et al., 2007). According to Al-Karaki et al. (2001), as salt concentrations increase, the water absorbance potential of the plant decreases, which negatively influences major functions including osmotic balance, conductance of stomata, metabolism, nutrient absorbance hydraulic conductivity, intercellular CO_2 concentrations and net photosynthetic rate, all of which are debilitating factors, which decrease the plant's ability to grow and thrive. Salinity stresses are a major contributor of damaging agricultural yields globally, they reduce production, whether for sustenance or economic gain. Presently, there is an affirmation that that salinity affects 7% of the world's land area and 20% of irrigated lands (Parihar et al., 2014; Khan & Duke, 2001).

Plants attempt to synthesize some osmolytes endogenously such as proline, soluble sugars, polyols and amino acids and glycine betaine in response, as a mechanism to ameliorate the unfavorable effects caused by salinity (Rady et al., 2018). The production of osmolytes function to adjust and protect sub-cellular structures and revert the oxidative damage caused by ROS under high salt conditions (Hare et al., 1998). As previously mentioned, two significant organic osmolytes that alleviate damages and are produced in response to environmental stresses such as drought, salinity, extreme temperatures, UV radiation and heavy metals are Proline and Glycine betaine. Their explicit roles remain controversial in processes of plant osmo-tolerance remain controversial; however, the compounds are known to have positive effects on enzyme and membrane integrity along with adaptive functions in osmotic adjustments in plants grown under stress conditions (Muhammad & Majid, 2007). According to Umezawa et al. (2006) low levels of glycine betaine had been shown to safeguard macromolecules such as nucleic acids, proteins and lipids and act as a reservoir for carbon and nitrogen sources. Rhodes & Hanson (2003) added that it can also protect membrane functions at high concentrations of Na^+ and CL^-. Szabados & Savoure (2010) assessed proline and signified that it was an amino acid with the α-amino group present as a secondary amine, it is known as a proteinogenic amino acid and is essential for primary metabolism. Yan et al. (2000) reported protection from salt-induced oxidative stress by the exogenous application of proline, by increasing the antioxidative. Therefore, the main objective of the study was to test the effect of salinity on plant growth, nutritional acquisition and antioxidative system of salt-stressed basil plants through exogenous application of proline and glycine betaine.

Materials and Methods

This investigation was held within Horticulture Research Institute (HRI), Agricultural Research Center (ARC), Giza, Egypt, within their orchard and it was conducted across two seasons in 2019 and 2020. Sweet basil (*Ocimum basilicum* L.) seeds were acquired from the Research Center of Medicinal and Aromatic Plants, Giza, Egypt and were propagated in the nursery in the months of February 15th, 2019 and 2020. All agro-management procedures necessary for seedling production were abided by.

Experimental design

The adopted experimental design was randomized with the employment of three replicates across all experiments. Each replicate consisted of 5 pots; uniform thirty-day-old seedlings were transplanted into pots of 30cm diameter. Each pot was filled with a sandy clay mixture soil (1:1) and contained one seedling, irrigation was with tap water till mid-March. On March 15th Salinity treatments began for two months, with repeated irrigation at a rate of twice per week at 50, 75 or 100mM NaCl in addition to the control treatment (irrigation using tap water at 0.85mM NaCl). Leaching was done using tap water every 15 days for accumulated salts, up to the end of the experiment. Foliar spraying treatments were done once every week during the experiment period as follows:

No.	Treatment	Concentration
1	Control	Tap water (0.85mM NaCl)
2	Proline	50mM

3	Proline	100 mM
4	Proline	200 mM
5	Glycine betaine	50 mM
6	Glycine betaine	100 mM
7	Glycine betaine	200 mM

Vegetative growth parameters

Plant height (cm) and branch numbers and herb fresh weight (g) were measured in June 15th, then samples from the leaves of all samples were collected for chemical analysis and detection of constituents.

Chemical analysis

Total chlorophylls (chlorophyll a and chlorophyll b) and Total carotenoids were detected according to the methods of Holm (1954) and Wettstein (1957). Total phenols were detected and analyzed according to the methods described by Swain & Hillis (1959). Total flavonoids were detected and analyzed spectrophotometrically using the methods described by Zhuang et al. (1992). Free amino acids were detected and determined by Yemm & Cocking (1955) and finally proline was assayed according to Handa et al. (1983).

SDS-PAGE analysis of proteins

According to the methods of Laemmli (1970), proteins were separated electrophilically using Sodium Dodicyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE).

Statistical analysis of data

An adoption of a completely randomized trial was adopted for the study. The statistical analysis of the present data was carried out according to Snedecor & Cochran (1989). Results were averaged and then compared using the new L.S.D. values at 5% level (Gomez & Gomez, 1984).

Results

Growth paramters

Generally, all *Ocimum basilicum* growth characteristics decreased progressively with the increase of NaCl concentrations up to 100mM compared to herb fresh weight (g) of basil plants significantly increased with the exogenous application of all proline and glycine betaine concentrations compared to the control (tap water). The concentration of 200mM glycine betaine was found to be most effective one under severe salinity (100mM); improving plant height, branches number and fresh weight of basil plant and exceeded the control by 32.91 and 33.13%, 42.83 and 43.56% and 21.32 and 23.50% in the first and second seasons, respectively (Tables 1, 2).

Pigment content

Leaves' content of total chlorophyll steadily decreased with higher NaCl contents in irrigation water (Table 3). Foliar applications with all proline and glycine betaine treatments boosted total chlorophyll contents when compared to the control (tap water), highest total chlorophyll contents were achieved by spraying plants with proline at 200mM recording 1.601 and 1.763mg/g in the 1st and 2nd seasons, respectively. On the other hand, lowest total chlorophyll contents were observed when basil plants sprayed with tap water giving 1.205 and 1.373mg/g in the first and second seasons, respectively.

Contrariwise, increasing NaCl concentration in irrigation water positively affected leaves carotenoids content (Table 3). Foliar applications with all proline and glycine betaine treatments decreased carotenoids content when measured against the control (tap water), and the highest carotenoids content was found with control treatment under highest level of saline irrigation water (100mM NaCl) recording 0.895 and 0.868mg/g in the first and second seasons, respectively; while lowest carotenoids content under severe salinity were observed when basil plants sprayed with proline at 200mM giving 0.609 and 0.605mg/g in the first and second seasons, respectively.

Total phenols

Total phenols increased with higher NaCl concentrations up to 100mM compared to control during both seasons (Table 4). In regards to the results of spraying treatments, exogenous foliar applications improved total phenols in basil leaves under salinity conditions when compared with controls, glycine betaine at 100 and 200mM logged the highest values of 0.226, 0.228% and 0.239, 0.241% in the 1st and 2nd seasons, respectively.

Total flavonoids

Leaves' content of total flavonoids gradually increased with rising NaCl in irrigation water (Table 4). All proline and glycine betaine treatments enhanced the content of flavonoids in

leaves under conditions of salinity compared to the control. Foliar application of 100- and 200-mM glycine betaine had the most favourable result (0.091 and 0.086g/100g in the 1st season, and 0.099 and 0.100g/100g in the 2nd season). Application of both high Glycine betaine concentrations by foliar spraying had more stimulatory effects on total flavonoids level in higher salt stress when likened to the control plants at corresponding levels of salinity (Table 4).

TABLE 1. Impact proline and glycine betaine on plant heights and branches number of salt-stressed basil plants

		Plant heights (cm)					No. of branches/plants				
First season											
NaCl (mM) treatments		**Control**	**50**	**75**	**100**	**Mean**	**Control**	**50**	**75**	**100**	**Mean**
Control		57.963	50.040	46.067	40.663	48.683	8.470	6.510	5.577	4.467	6.256
Proline (50mM)		63.200	52.893	49.133	48.000	53.307	8.917	7.450	6.097	5.370	6.958
Proline (100mM)		64.737	56.460	50.137	51.947	55.820	9.717	7.933	6.643	5.763	7.514
Proline (200mM)		66.113	60.590	52.313	52.707	57.931	9.813	8.400	7.310	5.907	7.858
Glycine betaine (50mM)		65.487	55.113	49.453	49.263	54.829	8.877	7.080	6.493/;1	5.787	7.059
Glycine betaine (100mM)		66.723	59.127	53.093	50.027	57.243	9.017	7.770	7.177	6.157	7.530
Glycine betaine (200mM)		68.097	60.000	54.403	54.047	59.137	10.030	8.187	7.790	6.380	8.097
Mean		64.617	56.318	50.657	49.522		9.263	7.619	6.727	5.690	
New L.S.D.5%	Treatments	0.464					0.073				
	NaCl	0.351					0.055				
	interaction	0.928					0.146				
Second season											
NaCl (mM) treatments		**Control**	**50**	**75**	**100**	**Mean**	**Control**	**50**	**75**	**100**	**Mean**
Control		63.080	59.057	48.144	39.697	52.494	9.867	8.100	6.120	4.277	7.091
Proline (50mM)		66.183	65.353	50.854	47.619	57.502	10.280	9.643	6.703	5.233	7.965
Proline (100mM)		66.990	65.620	52.486	49.534	58.658	10.827	9.817	7.093	5.607	8.336
Proline (200mM)		67.180	65.476	55.215	50.908	59.695	10.920	10.103	7.723	5.853	8.650
Glycine betaine (50mM)		66.227	66.221	50.952	48.483	57.971	10.057	8.923	6.967	5.753	7.925
Glycine betaine (100mM)		67.913	67.923	56.910	50.312	60.765	10.487	9.547	7.503	5.900	8.359
Glycine betaine (200mM)		69.213	66.360	57.638	52.847	61.515	11.147	9.903	8.447	6.140	8.909
Mean		66.684	65.144	53.171	48.486		10.512	9.434	7.222	5.538	
New L.S.D. 5%	Treatments			0.328					0.061		
	NaCl			0.248					0.046		
	interaction			0.656					0.121		

TABLE 2. Impact of proline and glycine betaine on herb fresh weight of salt-stressed basil plants

	First season					Second season				
NaCl (mM) treatments	**Control**	**50**	**75**	**100**	**Mean**	**Control**	**50**	**75**	**100**	**Mean**
Control	214.34	164.90	131.80	123.67	158.68	218.30	168.43	132.27	120.97	159.99
Proline (50mM)	219.33	172.47	140.82	126.63	164.81	221.70	174.60	140.80	127.53	166.16
Proline (100mM)	220.77	179.83	149.53	139.40	172.38	223.80	182.27	149.90	139.00	173.74
Proline (200mM)	229.00	196.37	151.79	147.10	181.06	231.60	198.40	151.57	146.77	182.08
Glycine betaine (50mM)	214.90	171.33	142.63	140.63	167.37	217.73	172.53	142.83	139.90	168.25
Glycine betaine (100mM)	217.10	178.50	149.90	145.67	172.79	219.97	181.03	149.63	146.10	174.18
Glycine betaine (200mM)	220.77	184.00	154.40	150.03	177.30	224.03	185.67	155.40	149.40	178.63
Mean	219.46	178.20	145.84	139.02		222.45	180.42	146.06	138.52	
Treatments			1.14					1.04		
NewL.S.D.5% NaCl			0.86					0.78		
interaction			2.27					2.07		

TABLE 3. Influence of exogenous application of proline and glycine betaine on pigment content of salt-stressed basil plants

	Total chlorophyll (A+B) (mg/g)					Carotenoids (mg/g)				
	First season									
NaCl (mM) treatments	**Control**	**50**	**75**	**100**	**Mean**	**Control**	**50**	**75**	**100**	**Mean**
Control	1.704	1.174	1.013	0.927	1.205	0.486	0.665	0.710	0.895	0.689
Proline (50mM)	2.069	1.403	1.174	1.077	1.431	0.445	0.551	0.616	0.646	0.564
Proline (100mM)	2.071	1.433	1.213	1.152	1.467	0.468	0.517	0.592	0.624	0.550
Proline (200mM)	2.214	1.581	1.417	1.193	1.601	0.474	0.505	0.573	0.609	0.540
Glycine betaine (50mM)	2.065	1.247	1.050	0.959	1.330	0.478	0.553	0.632	0.802	0.616
Glycine betaine (100mM)	2.068	1.351	1.166	1.062	1.412	0.475	0.533	0.616	0.767	0.598
Glycine betaine (200mM)	1.995	1.316	1.173	1.060	1.386	0.480	0.525	0.594	0.719	0.579
Mean	2.027	1.358	1.172	1.062		0.472	0.550	0.619	0.723	
New L.S.D. Treatments			0.058					0.010		
5% NaCl			0.044					0.008		
interaction			0.115					0.019		
	Second season									
NaCl (mM) treatments	**Control**	**50**	**75**	**100**	**Mean**	**Control**	**50**	**75**	**100**	**Mean**
Control	1.960	1.335	1.199	0.998	1.373	0.439	0.681	0.720	0.868	0.677
Proline (50mM)	2.392	1.688	1.293	1.093	1.617	0.422	0.582	0.624	0.685	0.578
Proline (100mM)	2.381	1.763	1.325	1.166	1.659	0.473	0.515	0.607	0.634	0.557
Proline (200mM)	2.548	1.803	1.476	1.224	1.763	0.486	0.447	0.564	0.605	0.525
Glycine betaine (50mM)	2.341	1.304	1.174	0.988	1.452	0.466	0.592	0.644	0.792	0.624
Glycine betaine (100mM)	2.347	1.423	1.274	1.046	1.522	0.470	0.578	0.632	0.733	0.603
Glycine betaine (200mM)	2.374	1.488	1.244	1.074	1.545	0.460	0.561	0.595	0.708	0.581
Mean	2.335	1.544	1.283	1.084		0.459	0.565	0.627	0.718	
New L.S.D. Treatments			0.052					0.013		
5% NaCl			0.039					0.010		
interaction			0.095					0.024		

TABLE 4. Impact of proline and glycine betaine on total phenols and flavonoids content of salt-stressed basil plants

	Total phenols (%)					Total flavonoids (g/100g)				
First season										
NaCl (mM) treatments	**Control**	**50**	**75**	**100**	**Mean**	**Control**	**50**	**75**	**100**	**Mean**
Control	0.167	0.194	0.203	0.215	0.195	0.051	0.064	0.057	0.060	0.058
Proline (50 mM)	0.173	0.200	0.214	0.238	0.207	0.059	0.067	0.069	0.082	0.069
Proline (100 mM)	0.173	0.204	0.219	0.242	0.210	0.054	0.070	0.074	0.082	0.070
Proline (200 mM)	0.175	0.213	0.243	0.264	0.224	0.055	0.078	0.110	0.107	0.087
Glycine betaine (50mM)	0.175	0.211	0.224	0.260	0.218	0.064	0.083	0.077	0.106	0.082
Glycine betaine (100mM)	0.179	0.225	0.237	0.264	0.226	0.065	0.089	0.095	0.115	0.091
Glycine betaine (200mM)	0.175	0.226	0.243	0.268	0.228	0.052	0.095	0.097	0.099	0.086
Mean	0.174	0.211	0.226	0.250		0.057	0.078	0.083	0.093	
New L.S.D.5% Treatments			0.002					0.002		
NaCl			0.002					0.002		
interaction			0.005					0.005		
Second season										
NaCl (mM) treatments	**Control**	**50**	**75**	**100**	**Mean**	**Control**	**50**	**75**	**100**	**Mean**
Control	0.179	0.208	0.211	0.230	0.207	0.061	0.070	0.069	0.070	0.068
Proline (50 mM)	0.186	0.215	0.223	0.254	0.220	0.068	0.076	0.080	0.093	0.080
Proline (100 mM)	0.186	0.219	0.227	0.230	0.216	0.068	0.080	0.085	0.100	0.083
Proline (200 mM)	0.184	0.228	0.251	0.281	0.236	0.066	0.089	0.109	0.119	0.096
Glycine betaine (50mM)	0.186	0.226	0.233	0.277	0.230	0.068	0.087	0.090	0.115	0.090
Glycine betaine (100mM)	0.190	0.240	0.245	0.281	0.239	0.072	0.101	0.103	0.119	0.099
Glycine betaine (200mM)	0.186	0.242	0.251	0.285	0.241	0.068	0.102	0.109	0.123	0.100
Mean	0.185	0.225	0.235	0.263		0.067	0.087	0.092	0.106	
New L.S.D.5% Treatments			0.009					0.002		
NaCl			0.007					0.002		
interaction			0.017					0.005		

Free amino acids

Amino acids increased with higher NaCl concentrations up to 100mM compared to the control during both seasons (Table 5). The increase was more pronounced in plants under highest level of salinity (0.808 and 0.722g/100g) in 1st and 2nd seasons. When looking at the spraying treatments, all applications heightened free amino acids contents in basil leaves under conditions of salinity compared with the control except glycine betaine at 50 mM in the first season. A remarkable increase in free amino acids compositions in plants irrigated with 100 mM NaCl was achieved by foliar application of all proline concentrations (50, 100 and 200mM) recording the highest values at 0.949, 0.951 and 0.924g/100g in the 1st season and 0.828, 0.812 and 0.885g/100g in the 2nd season.

Proline

As NaCl concentrations increased, up to 100mM, during both seasons a gradual increase in proline amounts compared to the control (Table 5) was witnessed. Under severe salinity conditions, there was a substantial increase in the endogenous proline content for basil plants (0.233 and 0.268mg/g^{-1} in the 1st and 2nd seasons, respectively) was observed. As for the effect of spraying treatments, all exogenous foliar applications enhanced proline content of basil plants. Foliar application of 100- and 200-mM proline had the most beneficial effect (0.246 and 0.256mg/g^{-1} in the 1st season and 0.284 and 0.289mg/g^{-1} in the 2nd season. Lower proline content was observed when plants sprayed with tap water at 0.144 and 0.165mg/g^{-1} in the 1st and 2nd seasons, respectively.

TABLE 5. Influence of exogenous application of proline and glycine betaine on free amino acids and proline content of salt-stressed basil plants

		Free amino acids (g/100g)					Proline (mg/g^{-1})				
First season											
NaCl (mM) treatments		**Control**	**50**	**75**	**100**	**Mean**	**Control**	**50**	**75**	**100**	**Mean**
Control		0.501	0.574	0.668	0.713	0.614	0.113	0.187	0.150	0.126	0.144
Proline (50mM)		0.663	0.839	0.843	0.949	0.824	0.149	0.256	0.257	0.278	0.235
Proline (100mM)		0.756	0.805	0.821	0.951	0.833	0.215	0.249	0.252	0.267	0.246
Proline (200mM)		0.802	0.749	0.896	0.924	0.843	0.235	0.236	0.275	0.278	0.256
Glycine betaine (50mM)		0.612	0.573	0.529	0.613	0.582	0.138	0.196	0.186	0.205	0.181
Glycine betaine (100mM)		0.838	0.654	0.669	0.806	0.742	0.194	0.204	0.218	0.247	0.216
Glycine betaine (200mM)		0.870	0.686	0.654	0.703	0.728	0.202	0.227	0.221	0.228	0.219
Mean		0.720	0.697	0.726	0.808		0.178	0.222	0.223	0.233	
New L.S.D.5%	Treatments			0.031					0.007		
	NaCl			0.023					0.005		
	interaction			0.062					0.014		
Second season											
NaCl (mM) treatments		**Control**	**50**	**75**	**100**	**Mean**	**Control**	**50**	**75**	**100**	**Mean**
Control		0.430	0.528	0.518	0.520	0.499	0.128	0.214	0.171	0.147	0.165
Proline (50mM)		0.569	0.722	0.784	0.828	0.726	0.170	0.292	0.293	0.320	0.269
Proline (100mM)		0.821	0.701	0.752	0.812	0.772	0.245	0.283	0.287	0.321	0.284
Proline (200mM)		0.896	0.665	0.785	0.885	0.808	0.268	0.269	0.307	0.314	0.289
Glycine betaine (50mM)		0.526	0.554	0.579	0.600	0.565	0.157	0.224	0.213	0.234	0.207
Glycine betaine (100mM)		0.742	0.575	0.697	0.702	0.679	0.215	0.245	0.249	0.284	0.248
Glycine betaine (200mM)		0.770	0.639	0.644	0.710	0.691	0.223	0.253	0.245	0.257	0.244
Mean		0.679	0.627	0.680	0.722		0.201	0.254	0.252	0.268	
New L.S.D.5%	Treatments			0.022					0.008		
	NaCl			0.017					0.006		
	interaction			0.045					0.015		

SDS-PAGE protein electrophoresis

SDS-PAGE analysis (Fig. 1) revealed a total of 19 bands with molecular weights (MW) ranging from 14.47 to 175.43kDa. Moderate and high salinity stress treatments of irrigation water (75 and 100mM) blocked the synthesis of a 175.429kDa polypeptide that was not restored by all foliar application of proline and glycine betaine. Highest NaCl level (100mM) alone induced the synthesis of new polypeptide (63.41kDa molecular weight). Under severe salinity conditions; high glycine betaine concentrations (100 and 200mM) showed overly expressed protein bands of 34.645 and 36.016kDa, and higher proline concentrations (100 and 200mM) induced the synthesis of a new polypeptide (102.44kDa molecular weight). Highest NaCl concentrations with or without foliar application of both compounds at 100 and 200mM increased the expression of a 29.99kDa polypeptide. Finally, proline or glycine betaine treatments and the presence of various salt treatments stimulated changes of the protein patterns with diverse concentrations. The new pattern for proteins which is visible due to salinity stress and exogenous applications reflects the expression of basil plant salinity tolerance.

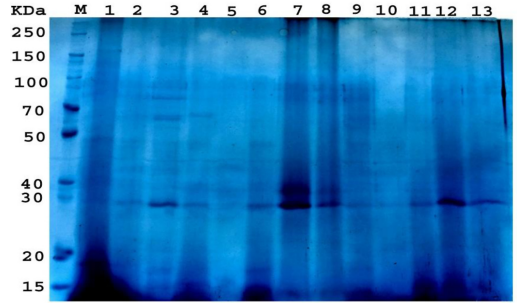

Fig. 1. Electrophoretic banding patterns of basil plant grown under salinity stress as affected by proline and glycine betaine foliar applications [(M) Molecular weight markers,

(1) Control, (2) 75mM NaCl, (3) 100mM NaCl, (4) 100mM NaCl + 50mM Glycine betaine, (5) 75mM NaCl + 100mM Glycine betaine, (6) 75mM NaCl + 200mM Glycine betaine, (7) 100mM NaCl + 100mM Glycine betaine, (8) 100mM NaCl + 200mM Glycine betaine, (9) 100mM NaCl + 50mM proline, (10) 75mM NaCl + 100mM proline, (11) 75mM NaCl + 200mM proline, (12) 100mM NaCl + 100mM proline, (13) 100mM NaCl + 200mM proline]

Discussion

As shown in the above tables, salinity stress inhibited growth of basil plants in all assessed parameters, including decreasing plant height, branches number/plant, fresh weight and total chlorophyll, there could be two possible justifications for this inhibition. The First could be attributed to the reduction of the plant's ability to absorb water due to the presence of salt in the soil. This reduction generally results in a lengthier growth rate, and that is the osmotic consequence for salinity. The second could be possible injuries occurring to the cells within transpiring leaves, due to excess amounts of salts penetrating the transpiration streams, and these injuries may cause further damage thus decreasing photosynthetic and growth potentials (Munns et al., 2006; Shehata & Nosir, 2019; Elhindi et al., 2017).

The retardant effect of salinity stress on the development and growth of other plant species was also determined; for example, its effects were reported on *Senna occidentalis* by Reda (2007) and on *fava beans* by Dawood et al. (2014), as well as on *Oryza sativa* by Hasanuzzaman et al. (2014). In the current study all growth parameters and total chlorophyll of basil plants significantly increased with the foliar application of proline and glycine betaine at varying concentrations exogenously compared to the control (tap water). Butt et al. (2016) recommended a similar approach when investigating chili peppers, the results of foliar application of proline under salt stress conditions stimulated the shoot and root length, plant fresh and dry mass and photosynthetic rate. Glycine betaine is a dipolar at the physiological pH, but electrically neutral in nature. Wani et al. (2013), stated that the essential role carried out by glycine betaine in plants exposed to salinity stress is the protection of plant tissues via osmotic adjustment (OA), photosynthetic apparatus protection and scavenging of ROS, and stabilization of proteins like Rubisco. Generally, the content of total phenols and flavonoids gradually increased with higher NaCl concentrations in irrigation water. All proline and glycine betaine treatments improved phenols and flavonoids content of leaves under salinity conditions compared with the control. This is in support of Rezazadeh et al. (2012)'s theory on the protective effects on artichokes by leaf polyphenols against the oxidative stress generated by salinity and secondary metabolites may play a role in salinity tolerance. When SDS-PAGE was conducted, it produced variations in the protein electrophoretic patterns under salinity stress. The appearance of a varied pattern reflected the foliar treatments of proline or glycine betaine as polypeptide bands. Salinity stress may be identified through proteins related to salt stress and in this study a specific polypeptide was synthesized when compared with control conditions, this result was in alignment with agreement with Bavei et al. (2011) and Sobhanian et al. (2016). In this study, the highest salinity stress (100mM) and higher proline concentrations (100 and 200mM) synthesized new polypeptides (63.41 and 102.44kDa molecular weights, respectively) as stress-specific proteins. These new proteins may play an important role in triggering a special system that helps the whole plant against salinity stress. These proteins may have an osmo-protective function (Dure, 1993) or protecting cellular structures (Close & Lammers, 1993). The most important mechanism involved in cell protection against salt stress is the induction of *de novo* synthetic protein groups (Kermode, 1997). These stress proteins may provide a stored form of nitrogen that may be re-utilized when the stress is over (Badr et al., 1998). Under severe salinity conditions of irrigation water, glycine betaine and proline concentrations (100 and 200mM) showed over expressed protein bands of 34.645 and 36.016kDa. These proteins may have a critical function in plant development under biotic and abiotic stresses (Amini et al., 2007). From the above results, it can be concluded that spraying the plant with proline under moderate salt stress to enhance the plant growth, salt photosynthetic pigments and proline content of sweet basil. The application on this medicinal plant offers favourable changes in an economically beneficial and eco-friendly manner. The physiological mechanism behind the resistance and enhancement is not well-known and may require further research. Counteracting the effects of salinity, and aiding recovery is a significant aim, that is heavily attempted within the

literature. The combination specified may provide benefit in offering an alternative economical and environmentally friendly solution.

Conclusions

Salinity is one of the main abiotic factors negatively influencing plant productivity. The aim of this investigation was to achieve the possibility of alleviating the harmful effects of irrigation water salinity on vegetative growth traits, nutritional acquisition and antioxidative system of salt-stressed basil plants through the exogenous application of proline and glycine betaine. Under severe salinity conditions, spraying the plant with glycine betaine and proline showed over expressed protein bands of 34.645 and 36.016kDa. These proteins may have a critical function in plant development and enhance the plant growth of sweet basil under biotic and abiotic stresses.

Conflict of interests: The authors confirm that there is no conflict of interest to disclose

Author contribution: Dr. Safwat: Writing the article and following up its publication, in addition to participating in selecting the research point, planning the experiments and analyzing the results of experiments. Dr. Abdel Salam: Selecting the research point, carrying out the experiment and conducting the practical experiments and helping in the revision of the article

Ethical approval: Not applicable.

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تأثير المعاملة بالبرولين و الجليسين بيتين على نبات الريحان أثناء الإجهاد الملحى

چيهان صفوت[1]، همت سميح عبد السلام[2]

[1]كلية التكنولوجيا الحيوية - جامعة اكتوبر للعلوم الحديثة والآداب- جيزة- مصر، [2]قسم النباتات الطبية والعطرية- معهد بحوث البساتين- مركز البحوث الزراعية- الجيزة- مصر.

تعد الملوحة احد اكبر المشاكل التى تواجه الزراعة خاصة فى المناطق الجافة وشبه الجافة. تهدف الدراسة الحالية إلى دراسة اثر المعاملة بالبرولين و الجليسين بيتين بتركيزات 50، 100 و200 ملليمولر على نبات الريحان (*Ocimum basilicum* L.) أثناء التعرض للاجهاد الملحى لكلوريد الصوديوم بتركيزات 50، 75 و 100 ملليمولر. أظهرت النتائج زيادة مؤشرات النمو كالوزن الرطب وطول النبات وعدد الأوراق والفروع وكذلك محتوى الكلوروفيل عند المعاملة بكلا من البرولين والجليسين بيتين في كلا من الموسم الاول والثاني كما أظهرت النتائج نقص القيم المسجلة للمؤشرات السابقة عند المعاملة بكلوريد الصوديوم. ادى استخدام البرولين والجليسين بيتين عند جميع التركيزات المستخدمه إلى زيادة المحتوي من الفينولات الكليه والفلافونويد في الأوراق لنبات الريحان مقارنة بالنبات الغير معامل . كان لاستخدام الجليسين بيتين بتركيزات 100 و200 ملليمولر أفضل الاثر على النمو كما ادى لزيادة محتوى الأوراق من البرولين. أظهر التفريد الكهربى (SDS-PAGE) للبروتينات 19 حزمة تراوحت اوزانها الجزيئية بين 14.47 و 175.43 كيلو دالتون. اظهرت المعاملة بكلوريد الصوديوم بتركيزات 75 و 100 ملليمولر توقف لانتاج عديد ببتيد ذو وزن جزيئى 175.429 كيلو دالتون. ادى التعرض لكلوريد الصوديوم بتركيز 100 ملليمولر فى غياب المعاملة بالبرولين و الجليسين بيتين إلى ظهور ببيتيد متعدد جديد ذو وزن جزيئى 63.41 كيلو دالتون. اوضحت الدراسة ايضا قدرة البرولين والجليسين بيتين على التحسين الفسيولوجى وتقليل الضرر التأكسدى الناتج من الاجهاد الملحى وأن هذه المركبات قادرة على الحماية ضد اضرار الملوحة عن طريق رفع القدرة الضد تأكسدية للنبات .