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Role of Tryptophan or Prozac (5-hydroxytryptamine) on some Osmolytes and Antioxidant defense system of Sunflower cultivars grown in Saline soil

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Abstract : A field experiment was conducted during two successive seasons in different saline soil levels (EC 1.56, 4.68and 7.83 ds/m) on two sunflower (*Helianthus annuus L.*) cultivars (Hysun 336 and Euroflor). Seeds were soaked prior to sowing in saline soil with tryptophan or Prozac (5-hydroxytryptamine) at different concentrations (0.0, 2.5 and 5.0 mg/l), to improve tolerance. Generally salinity stress increased total soluble sugars, proline, free amino acids and total phenol contents in both sunflower cultivars. On the other hand, the antioxidant enzymes activities catalase, peroxidase, polyphenol oxidase and Phenylalanine ammonialyase decreased with increasing salinity level. The macroelements (N, P, K and Mg) and some microelements (Fe, Mn, Zn and Cu) contents were decreased while; the sodium content was gradually increased with increasing salinity levels of both cultivars. Pretreatment of sunflower seeds with different concentrations of tryptophan or prozac could improve the adverse effects of salinity stress by increasing the solute and antioxidant enzyme. Moreover, in shoots of Hysun 336 was a higher osmolytes concentration contributing to osmotic adjustment and the higher antioxidant enzymes activity than those of Euroflor under salinity stress.
Key words: Antioxidant enzymes, Osmolytes, Prozac, Salinity, Sunflower, Tryptophan.

Introduction

Soil salinity becomes a serious problem in both agricultural and natural soils. Saline soils are limiting factors to agriculture in arid and semi- arid regions, crop growth and production[1]. Plants sowing in saline soil primarily exposed to osmotic stress and secondarily ion toxicity stress. Specific ion effects may cause direct toxicity or alternatively and/or may affect plant nutritional balances[2]. Plants have protective mechanisms to recognize and respond rapidly to the adverse environmental cues[3]. One of these metabolic adaptations is the stimulation of osmoprtectants synthesis as free amino acids, proline and soluble sugars. These compatible osmolytes not only act as osmoregulators but they may also protect the structure of different biomolecules and membranes [4] or act as free-radical scavengers that protect DNA from damaging effects of ROS [5]. In this regard, [6] reported that phenolic compounds plays an important role in scavenging free radicals and protects plants against the damaging effects of increased ROS levels due to water stress. Moreover, plants have developed different adaptive mechanisms, to reduce oxidative damage resulting from water deficiency, via the biosynthesis of a cascade of antioxidants. Antioxidant defense system enhancement is an important strategy to scavenge ROS by antioxidant enzyme and with non-enzymatic antioxidants[7].

The use of amino acids as a precursor of plant growth promoters is one approach to minimize the effect of salinity on plant growth and productivity. A common precursor of plant hormone auxin is L-Tryptophan,

which affects the physiological processes of plants after uptake directly or indirectly after transforming into auxins (IAA)[8]. [9]reported that, L-Tryptophan was very effective in increasing salt tolerance through increasing K^+, N, Ca^{2+} +, Mg^{2+} and P and reduced Na^+ and Cl^- content in wheat plant. Moreover, the tryptophan pathway plays a defensive role in plants[10]. Prozac (5-hydroxytryptamine), melatonin (Nacetyl- 5-methoxytryptamine) are another tryptophan-derived compound, have been recorded in several medicinal plant species[11].[12] suggested that tryptophan-derived compound have a role in plant defense against stress. In addition, the plant hormone, indole-3-acetic acid (IAA) is created from biogenic amine such as serotonin, prozac and melatonin[13]. Plants adaptation to environmental changes by using biogenic monoamines such as serotonin, tryptamine, prozac and tyramine as a function of mitogenic factor. In plants, serotonin and tyramine are conjugated to form phenolic compounds via thioester linkages during the synthesis of hydroxycinnamic acid amides[14]. It has been propose that tryptophan-derived compound (Prozac) plays a hormonal role in plant defense against stress[15].

Sunflower is one of an essential oil seed crops all over the world, and it is also an important crop in Mediterranean areas where it can tolerate salinity up to EC equals to1.7 ds/m[16]. Sunflower (*Helianthus annuus* L.), a new World plant, has been developed into a valuable source of edible oil and meal. Sunflower could be successful to increase the domestic production by selecting the proper cultivars which are suitable to different soil and climatic conditions. It is valued for it anti-cholesterol properties [17].

The target was studying the influence of soaking the seeds of sunflower in tryptophan or prozac and sowing in different levels of saline soils. This study includes how far they regulate the plant osmolytes, antioxidant defense system, and macro and microelement contents under different levels of saline soils of both sunflower cultivars.

Materials and methods

Experimental conditions*:*

Two field experiments were conducted at the Agricultural station of Agricultural Faculty, Fayoum University, Fayoum Governorate, Egypt. During two successive seasons, three experimental sites were chosen with physical and chemical analysis as shown in (Table 1). Soil analysis was carried out according to [18, 19].

Table (1): Physical and chemical analysis of cultivated soils with different soil salinity levels of 1.56, 4.68 and 7.83 dS m^{-1} at the experimental farm of the Faculty of Agriculture, Fayoum University, Egypt.

Properties	Site 1 (1000 mg/l) salt	Site 2 (3000 mg/l)salt	Site 3 (5000 mg/l) salt
Mechanical analysis			
Sand%, coarse	3.15	3.75	2.85
Fine	63.85	65.25	47.15
Silt%	19.75	20.25	20.50
Clay%	13.25	10.75	29.50
Soil texture	Sand loamy	Sand loamy	Sand clay loamy
Chemical analysis			
pH (1:2.5)	7.36	7.64	7.81
EC (dsm^{-1})	1.56	4.68	7.83
Organic matter%	1.42	1.38	1.25
CaCO3	9.34	8.56	8.05
Total N%	0.09	0.07	0.06
Available nutrients (mg/Kg soil)			
P	5.16	7.02	8.36
K	201.24	198.1	181.15
Fe	7.03	5.94	5.37
Mn	1.52	1.04	0.98
Zn	0.88	0.79	0.76
Cu	0.67	0.59	0.63

At soil preparation fertilizers supplemented with full dose 200 kg/fed of calcium superphosphate (15.5% P_2O_5), 200 kg/fed ammonium nitrate (33.5% N) and 50 Kg/fed potassium sulphate (48% K_2O) were incorporated into the top 15 cm of the soil. Normal agricultural practices common in the area were followed. Seeds of the two cultivars of sunflower (Hysun 336 and Euroflor) were carried from Agricultural Research Centre Assuit branch, Egypt. Chemical compounds (tryptophan or prozac) were supplied from SIGMA – ALDRICH Company. Soaking of seeds of the two cultivars was for 12 h in different concentrations of tryptophan or prozac (0.0, 2.5 and 5.0 mg/l). Seeds of the two cultivars were separately sown in the mentioned three experimental sites at two successive seasons. The seeds of the two cultivars were sown in split split plot design with four replications in rows 4-meter long, 0.60-meter apart and 6 ridges with total area (14.4 m^2). Hill spacing was 10 cm within the row. Seeds were sown at 3-5 seeds in each hill. The sites of each experiment put as main plot, tryptophan or prozac as subplot and concentrations of both compounds as sub sub plot. Irrigation took place immediately after sowing, then everyone week's intervals according to agronomic practices in the district. Thinning was carried out at 15 days after sowing to secure two plants per hill on both sides of the ridge.

Plant sampling:

Four plant samples/plot were harvested 50 days after sowing for chemical analysis. Determination of total soluble sugars, total amino acids, macro (N, P, K, Na, Mg and Ca) and microelements (Fe, Mn, Zn and Cu) in the dry tissues were determined. Total phenols & proline contents and some enzyme activities (CAT, POX, PPO and PAL) also determined in the fresh tissues.

Chemical analysis:

Total soluble sugars (TSS):

Total soluble carbohydrates (TSS) were extracted by overnight submersion of dry tissue in 10 ml of 80% (v/v) ethanol at 25°C with periodic shaking, and centrifuged at 600g. The supernatant was evaporated till completely dried then dissolved in a known volume of distilled water to be ready for determination of soluble carbohydrates [20] .TSS were analyzed by reacting of 0.1 ml of ethanolic extract with 3.0 ml freshly prepared anthrone (150 mg anthrone + 100 ml 72% H_2SO_4) in boiling water bath for ten minutes and reading the cooled samples at 625 nm using Spekol Spectrocololourimeter VEB Carl Zeiss **[21].**

Total phenol:

A known weight of the fresh samples of shoots were taken and extracted with 85% cold methanol (v/v) for three times at 0^OC. The combined extracts were collected, dried under vacuum and made up to a known volume with distilled water. Then 0.5 ml of the extraction was added to 0.5 ml Folin, shaken allowed to stand for 3 min. Then one ml of saturated sodium carbonate was added to each tube followed by distilled water shaken and allowed to stand for 60min. The optical density was determined at wave length of 725 nm using spectrophotometer as described by **[22].**

Proline:

Proline was assayed according to the method described by **[23]** 2ml of proline extract, 2ml of acid ninhydrin and 2ml of glacial acetic acid were added and incubated for 1 h in a boiling water bath followed by an ice bath. The absorbance was measured at 520 nm using Spekol Spectrocololourimeter VEB Carl Zeiss. A standard curve was obtained using a known concentration of authentic proline.

Free amino acids:

Free amino acid content was extracted according to the method described by **[24].** Free amino acid was determined with the ninhydrin reagent method [25]. 1 ml acetate buffer (pH 5.4) and 1 ml chromogenic agent were added to 1 ml free amino acid extraction. The mixture was heated in boiling water bath for 15 min. after cooled in tap water, 3 ml ethanol (60% v/v) was added. The absorbance at 570 nm was then monitored using Spekol Spectrocololourimeter VEB Carl Zeiss.

Assay of enzymes activities:

Enzyme extractions were collected following the method described by [26]. Leaf tissues were homogenized in ice-cold phosphate buffer (50 mM, pH 7.8), followed by centrifugation at 8,000 rpm and 4°C for 15 min. The supernatant was used immediately to determine the activities of enzymes.

Polyphenol oxidase:

(PPO, EC 1.10.3.1) activity was determined using a spectrophotometric method based on an initial rate of increase in absorbance at 410 nm [27]. Phosphate buffer solution pH 7 (0.1 M, 1.95 ml), 1 ml of 0.1 M pyrogallol as a substrate and 50 µl of the enzyme extract were pipetted into a test tube and mixed thoroughly. Then the mixture was rapidly transferred to a 1-cm path length cuvette. The absorbance at 410 nm was recorded continuously at 25°C for 5 min.

Peroxidase:

(POX, EC 1.11.1.7) activity was assayed by the method of [28]. The reaction mixture used for estimating the peroxidase enzyme (POX) contained 2 ml of 0.1 M phosphate buffer (pH 6.8), 1 ml of 0.01 M pyrogallol, one ml of 0.005 M H_2O_2 and 0.5 ml of the enzyme extract. The solution was incubated for 5 min at 25°C after which the reaction was terminated by adding 1 ml of 2.5 N H_2SO_4. The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a reagent blank prepared by adding the extract after the addition of 2.5 N H_2SO_4 at the zero time.

Catalase:

(CAT, EC 1.11.1.6) activity was determined spectrophotometrically by following the decrease in absorbance at 240 nm [26]. The mixture (3 ml) contained 1.9 ml phosphate buffer (50 mM, pH7.0), 100 µl enzyme extract, and 1 ml 0.3% H_2O_2. The reaction was initiated by adding enzyme extract. One unit of CAT activity was defined as the 0.01 deduction in absorbance at 240 nm per minute.

Phenylalanine ammonialyase:

(PAL, EC 4.3.1.5) activity was determined as the rate of conversion of Lphenylalanine to trans-cinnamic acid at 290nm [29]. Sample containing 0.4ml of enzyme extract was incubated with 0.5ml of 0.1M borate buffer, pH-8.8 and 0.5ml of 12mM L-phenyl alanine in the same buffer for 30 min at 300C. The amount of trans-cinnamic acid synthesized was calculated using its extinction coefficient of 9630 M-1 cm-1. Enzyme activity was expressed as synthesis of trans-cinnamic acid (in nmol quantities) min-1 g-1 fresh weight. The enzyme activities were calculated by [30].

Mineral ions:

Macro and microelement contents of were determined according to the method described by [31]. N and P were determined using Spekol Spectrocololourimeter VEB Carl Zeiss. While, estimation of Ca, K and Na contents were done by the use of flame photometer. Also, Mg, Fe, Mn, Zn and Cu contents were estimated using atomic absorption spectrophotometer.

Statistical analysis:

The data were statistically analyzed on split split plot design according to [32]. Means were compared by least significant difference (LSD) at 5% levels of probability.

Results:

Total soluble sugars:

Salt stress induced the increased of total soluble sugar contents in two sunflower cultivars with increasing soil salinity from EC 1.56 to 4.68 to 7.83 ds/m (Table 2). The percentages of increase were 8 % & 26% in Hysun and by 19% & 38% in Euroflor cultivars under soil salinity 4.68 & 7.83 ds/m, respectively.

Data in Table2. showed that, Presoaking application of both sunflower cultivar seeds at different concentrations of tryptophan or prozac (2.5 and 5.0 mg/l) at different soil salinity levels caused significant increases in total soluble sugars contents as compared with the corresponding salinity level. It is noticed that, Hysun 336 is more pronounced accumulation of total soluble sugar in the first level of saline soil (EC 1.56 ds/m) than Euroflor but in the second and third levels (4.68 and 7.83) the cultivar Euroflor is more pronounced accumulation of total soluble sugar. The Table 2 clearly shows that the effect of tryptophan or prozac (5.0 mg/l) were the most effective treatments respectively, since it increased of total soluble sugar by (10% &8%) , (23% &21%) and (24% & 23%) in Hysun 336 and (9% &7%) , (22% &21%) and (26% & 24%) in Euroflor in EC 1.56 to 4.68 to 7.83 ds/m respectively.

Proline and total amino acids:

Data recorded in Table (2) showed that, proline and free amino acid gradual increased of Hysun 336 and Euroflor cultivars with increasing soil salinity from (1.56 to 4.68 &7.83 ds/m). The amounts of increases were observed (4% &20%) and (!7% &37%) in proline and (3% &16%) and (16% &37%) in amino acid at Hysun 336 and Euroflor cultivars respectively .

Data in Table (4). As compared with the corresponding salinity level, application of tryptophan or prozac at different concentrations (2.5and 5 mg/l) caused significant increases in proline and free amino acids contents. It is noticed that, Hysun 336 is more pronounced accumulation of total proline and free amino acid in the first level of saline soil (EC 1.56 ds/m) than Euroflor but in the second and third levels (4.68 and 7.83) the cultivar Euroflor is more pronounced accumulation of proline and free amino acid as compared with the corresponding treatment.

Total Phenol:

Data presented in Table (2) showed the total phenol content significantly increased gradually with the increase of salinity level in both sunflower cultivars. The EC of soil increasing salinity from 1.56 to 4.68 &7.83 ds/m increased the phenol contents 14% and 27% in Hysun and 15% and 29% in Euroflor cultivars respectively.

Application of tryptophan or prozac (2.5 & 5.0 mg/l) induced slightly increases on total phenol content of Euroflor and Hysun 336 cultivars as compared with the corresponding salinity levels Table 2. It is noticed that, the phenol contents in Euroflor cultivar more than of Hysun 336 cultivars in the control plants and all treatments used.

Table (2): Effect of tryptophane (Tryp) or prozac on osmolytes of sunflower cultivars Hysun 336 (Cult 1) and Euroflor (Cult 2) under different saline soil (S) levels.

Treatment			Total soluble sugar		proline		amino acid		Total phenol	
			mg/g dry weight						mg/g fresh weight	
(S)EC (dsm^{-1})	Material (mg/l)		Cult 1	Cult 2	Cult 1	Cult 2	Cult 1	Cult 2	Cult 1	Cult 2
1.56	0.0		19.43	18.40	2.040	1.903	4.837	4.377	0.933	0.960
	Tryp	2.5	20.70	19.46	2.197	1.973	5.363	4.920	0.963	1.027
		5.0	21.46	20.06	2.277	2.047	5.533	5.107	1.003	1.047
	Prozac	2.5	20.03	18.90	2.147	1.967	5.260	4.737	0.997	1.017
		5.0	21.03	19.83	2.233	2.013	5.370	4.877	1.003	1.037
4.68	0.0		21.00	21.90	2.117	2.217	4.960	5.097	1.067	1.103
	Tryp	2.5	25.10	26.10	2.550	2.657	5.477	5.753	1.103	1.130
		5.0	25.93	26.66	2.647	2.740	5.650	5.907	1.133	1.160
	Prozac	2.5	24.26	25.20	2.380	2.563	5.377	5.630	1.100	1.117
		5.0	25.50	26.46	2.537	2.563	5.487	5.640	1.127	1.153
7.83	0.0		24.40	25.46	2.447	2.607	5.623	5.990	1.183	1.240
	Tryp	2.5	29.60	30.90	3.100	3.220	6.337	6.743	1.210	1.287
		5.0	30.36	32.20	3.160	3.283	6.467	6.917	1.227	1.323
	Prozac	2.5	29.50	30.73	2.980	3.143	6.257	6.657	1.227	1.310
		5.0	30.00	31.53	3.087	3.227	6.380	6.793	1.263	1.347
LSD at 5%			0.99		0.103		0.241		0.052	

Mineral Contents:

Data presented in Table (3) the nitrogen, phosphorous, calcium and magnesium contents of both sunflower cultivars Hysun 336 and Euroflor increased with increase of the soil salinity level (EC 1.56; 4.68; and 7.83 dsm^{-1}). As compared with the corresponding salinity level pretreatment of sunflower seeds with different concentrations of tryptophan or Prozac (2.5 and 5 mg/l) increased significantly of N, P, and Mg under all salinity levels in both cultivars. On the other hand, Ca contents observed non significant variation between treatments of both sunflower cultivars with tryptophan or prozac as compared with the corresponding salinity level. Results in Table 4 showed that the effect of different salinity levels in response to K+, Na+ and K+/Na+ ratio of both sunflower cultivars. Sodium content significantly increased with increasing salinity level in both cultivars. On the other hand, K+ content and K+/Na+ ratio in both cultivars gradual decreased significantly with Na+ content was increased. In the meantime, Hysun 336 cultivar showed higher significant values of K+/Na+ ratio as compared with Euroflor cultivar. Soaking sunflower seeds in either tryptophan or prozac showed significant decrease in Na+ content in both cultivars at EC 4.68 and 7.83 ds/m levels of salinity. Pretreatment of seeds with either tryptophan or prozac (2.5 and 5.0 mg/l) induced significant increases in K+ and K+/Na+ ratio in both cultivars as compared with the corresponding salinity level. The effect of salinity on microelement contents of sunflower cultivars, data Table 5 revealed that, increasing salinity level caused gradual decrease in Fe, Mn, Zn and Cu contents in both cultivars. In the mean time, soaking both cultivars of sunflower seeds in tryptophan or prozac caused slight increase in the microelement as compared with the corresponding salinity level. Meanwhile, the higher concentration of either tryptophan or prozac (5.0 mg/l) was more effective than 2.5 mg/l in improving Fe Mn, Zn and Cu content by using under all salinity level.

Table (3): Effect of tryptophan (Tryp) or prozac on macroelement contents of sunflower cultivars Hysun 336 (Cult 1) and Euroflor (Cult 2) under different saline soil (S) levels.

Treatment			N		P		Ca		Mg	
(S) EC (dsm^{-1})	Material (mg/l)		mg/100g dry weight							
			Cult 1	Cult 2	Cult 1	Cult 2	Cult 1	Cult 2	Cult 1	Cult 2
1.56	0.0		1677	1650	1770	1680	2393	2136	325	314
	Tryp	2.5	1757	1740	2123	1976	2393	2140	369	354
		5.0	1937	1890	2333	2256	2400	2143	389	377
	Prozac	2.5	1747	1717	2121	1942	2390	2203	374	362
		5.0	1870	1867	2234	2176	2403	2143	392	377
4.68	0.0		1623	1570	1632	1478	1900	1770	314	300
	Tryp	2.5	1680	1627	1921	1656	1903	1773	350	336
		5.0	1817	1763	2241	1933	1910	1777	361	344
	Prozac	2.5	1667	1610	1832	1519	1900	1773	352	338
		5.0	1747	1697	2001	1733	1910	1780	362	348
7.83	0.0		1547	1503	1332	1244	1623	1547	292	272
	Tryp	2.5	1633	1557	1661	1471	1627	1550	325	303
		5.0	1763	1643	1854	1665	1630	1553	339	316
	Prozac	2.5	1617	1543	1544	1356	1630	1550	327	303
		5.0	1680	1617	1765	1534	1633	1557	337	318
LSD at 5%			47.45		56.28		47.38		12.61	

Table (4): Effect of tryptophan (Tryp) or prozac on macroelement contents of sunflower cultivars Hysun 336 (Cult 1) and Euroflor (Cult 2) under different saline soil (S) levels.

Treatment			K		Na		K/Na	
(S) EC (dsm^{-1})	Material (mg/l)		mg/100g dry wt					
			Cult 1	Cult 2	Cult 1	Cult 2	Cult 1	Cult 2
1.56	0.0		1193	1150	235	253	5.07	4.55
	Tryp	2.5	1363	1297	231	251	5.90	5.16
		5.0	1440	1370	226	246	6.37	5.57
	Prozac	2.5	1347	1283	231	250	5.83	5.13
		5 .0	1403	1347	229	247	6.13	5.45
4.68	0.0		1137	1063	437	470	2.60	2.26
	Tryp	2.5	1290	1203	390	401	3.31	3.00
		5.0	1360	1267	360	376	3.78	3.37
	Prozac	2.5	1263	1180	393	403	3.21	2.93
		5 .0	1337	1237	353	380	3.79	3.26
7.83	0.0		1053	1023	711	728	1.48	1.41
	Tryp	2.5	1187	1153	612	630	1.94	1.83
		5.0	1243	1203	573	594	2.17	2.03
	Prozac	2.5	1173	1143	634	650	1.76	1.75
		5.0	1220	1187	581	601	2.10	1.98
LSD at 5%			43.98		8.92		0.06	

Table (5): Effect of tryptophan (Tryp) or prozac on microelement contents of sunflower cultivars Hysun 336 (Cult 1) and Euroflor (Cult 2) under different saline soil (S)levels.

Treatmemt			Fe		Mn		Zn		Cu	
(S) EC (dsm^{-1})	Material (mg/l)		mg/100g dry weight							
			Cult 1	Cult 2	Cult 1	Cult 2	Cult 1	Cult 2	Cult 1	Cult 2
1.56	0.0		51.80	49.6 7	37.20	33.53	26.00	24.20	18.30	17.53
	Tryp	2.5	53.70	51.4 7	38.97	35.37	28.17	25.97	20.50	19.33
		5 .0	56.6 7	54.2 0	42.00	38.27	28.33	26.1.3	20.87	19.60
	Prozac	2.5	52.83	50.60	38.20	34.47	28.03	25.83	20.83	19.53
		5 .0	55.37	52.73	41.83	38.03	28.27	25.97	21.03	19.76
4.68	0.0		47.40	45.70	31.57	29.83	21.17	19.57	16.13	14.13
	Tryp	2.5	49.13	47.23	32.80	31.30	22.77	21.20	18.10	15.77
		5 .0	51.67	49.47	35.47	33.87	22.63	21.23	18.33	15.83
	Prozac	2.5	48.27	46.50	32.20	30.83	22.57	20.90	18.23	15.80
		5 .0	50.10	48.23	35.07	33.53	22.53	21.00	18.37	15.97
7.83	0.0		38.07	33.10	23.23	22.37	15.87	14.40	11.47	10.53
	Tryp	2.5	39.47	34.30	23.70	23.10	17.23	15.87	12.97	11.77
		5 .0	41.73	36.37	26.30	25.50	17.27	15.93	13.10	11.67
	Prozac	2.5	38.87	33.70	23.57	22.83	17.07	15.77	13.07	11.73
		5 .0	40.83	35.37	26.00	25.17	17.17	15.90	13.13	11.80
LSD at 5%			1.329		1.082		1.392		0.922	

Enzyme Activities:

In response to salinity stress either alone or in combination with each of the tryptophan or prozac is illustrated (Table 6). The changes in the activities of the various enzymes observed that, CAT, POX, PPO and PAL decreased significantly with increased soil salinity in both cultivars. The magnitude of reduction was increased in Euroflor more than Hysun336. Application of tryptophan or prozac led to increases in CAT, POX, PPO and PAL activities as compared with corresponding salinity level. The most pronounced effect was recorded in response to 5.0 mg/l tryptophan or prozac in CAT, POX PPO and PAL in both cultivars respectively. In general, prozacat 5mg/l was the most pronounced treatment effect in increasing the all enzyme activities.

Table (6): Effect of tryptophane (Tryp) or prozac on enzyme activities (ug/g fresh weight/hour) of sunflower cultivars Hysun 336 (Cult 1) and Euroflor (Cult 2) under different of saline soil (S) levels.

Treatment			CAT		POX		PPO		PAL	
(S) EC (dsm^{-1})	Material (mg/l)		Cult 1	Cult 2	Cult 1	Cult 2	Cult 1	Cult 2	Cult 1	Cult 2
1.56	0.0		190	163	153	140	123	117	227	203
	Tryp	2.5	217	200	163	153	163	150	253	227
		5.0	233	213	210	177	183	173	283	253
	Prozac	2.5	227	193	167	167	170	163	273	237
		5.0	243	223	210	190	187	183	297	257
4.68	0.0		157	143	127	117	107	93	203	187
	Tryp	2.5	207	190	147	137	143	133	233	210
		5.0	223	207	180	157	167	150	260	233
	Prozac	2.5	210	183	170	163	153	143	243	220
		5.0	227	197	190	177	177	167	253	237
7.83	0.0		127	113	107	87	73	57	177	163
	Tryp	2.5	167	153	127	113	127	110	203	87
		5.0	193	170	153	137	140	127	227	207
	Prozac	2.5	173	157	160	150	133	83	213	197
		5.0	197	183	177	163	157	140	223	223
LSD at 5%			1.4		1.5		2.4		2.7	

Discussion:

Total soluble sugars:

Our results demonstrated that the increasing salinity levels up to 7.83 EC dsm^{-1} increased total soluble sugars in Hysun 336 and Euroflor plants Table 2. Similar results have been found by number of plant species [33, 34.]. [35] recorded that the main solutes involved in osmotic adjustment in some plants are the organic acids when the plant was under salinity stres. Prozac or tryptophan pretreatments under different salinity levels induced significant increases in TSS contents of sunflower shoots. In this respect it could be concluded that, prozac or tryptophan play a hormonal role and alleviated the inhibitory effect of salinity stress by marked increase in TSS contents via osmotic adjustment of plant cell [36]. In this connection, application of tryptophan or prozac generally stimulated the increase of total soluble sugars in both sunflower cultivars under salinity stress, via increasing endogenous levels of certain phytohormones [15].

Proline and Free amino acids:

Salt stress induced the increases in proline and free amino acid in of Euroflor and Hysun 336 cultivars with increasing soil salinity (Table 2). Accumulation of proline and amino acids cause the osmotic adjustment in sunflower plants under salinity stress. Similar results have been reached by [34] and [37] on sunflower and wheat

plants, respectively. Cytoplasmic enzymes can be protect by proline [38] concomitantly with scavenging hydroxyl radicals [39]. Thus, it could be suggested that salt tolerance was promoted through activation of proline synthesis and hydrolysis of protein into free amino acids to act as osmoprotectants in the different organs of sunflower plant. The inhibitory effect of salt stress on the both cultivars of sunflower was alleviated by tryptophan and 5-hydroxy tryptophan (anti-stress) treatments through increasing proline contants and/or enhancing the biosynthesis of other amino acids and their incorporation into protein [12]. L-Tryptophan is an amazing amino acid because it may act as an osmolyte, modulates stomatal opening and ion transport regulator [40]. Moreover, a higher proline and amino acid accumulation contributing to osmotic adjustment was observed in shoots of salinity stress Hysun 336 than those of Euroflor.

Total Phenols:

Total phenols may play a valued role in the regulation of plant metabolic processes and overall plant growth [41]. Data represented in (Table 2) show significant increases in total phenol contents with the increase in salinity levels. These results are in harmony with [42]and [34] on flax plant on sunflower plant respectively. Moreover, many antioxidant enzymes used phenols as a substrate; so, it mitigates the drought stress damages [43]. In this connection, the other roles of phenol in plant are to protect cells from potential oxidative damage and increase stability of cell membrane [44]. Also, [45] recorded an accumulation of phenolic compounds in response to abiotic stress. In general application of tryptophan and 5-hydroxytryptamine in both sunflower cultivars under the different salinity levels caused significant changes in phenol contents as compared with those of the corresponding salinity level. In the meantime, tryptamine, prozac and tryamine are involved in adaptation to environmental changes in plants.

Mineral Contents:

Salinity stress caused a decrease in nitrogen, phosphorous, calcium, magnesium and potassium contents paralleled to gradually increase in sodium content as the soil salinity increased of two sunflower cultivars Tables 3 and 4. Salinity exerted a more pronounced effect on the nutrient content particularly for Euroflor than for Hysun 336. In this connection, [46] on wheat reported that sensitive cultivars had significant great leaf Na+ and Cl- concentration and lower K+/Na+ ratio and K+ versus Na+ selectivity than all salt tolerant lines. Moreover, [47] found that K^+, K^+/Na^+ ratio and Ca^{2+} decreased in response to the treatments with the different concentrations of sea water on *Vicia faba* plant. Also, [48] and [49] indicated that the photosynthesis and the biochemical processes directly inhibit through ion toxicity and lead to water stress by increasing accumulation of sodium (Na+) and (Cl-) ions. Moreover, [50] reported that, strains of salt –tolerance plant were associated with both high capacity for osmotic adjustment and a great ability to take up ions followed by translocates them to leaves.

In addition, under salt stress conditions the uptake of Ca^{2+} and Mg^{2+} decreased may be due to either the cruel effect of Na^+ and K^+ on these cations or decreased transport of Ca^{2+} and Mg2+ ions. In this connection, [9] found that application of tryptophan induced reduction in Na+ and Cl^- contents that was parallel with the increase in K^+, Ca^{2+}, Mg^{2+} and P contents in the wheat shoots.

Table 5 found that, Fe, Mn, Zn and Cu contents in both cultivars caused gradual decrease with increasing salinity level. In the meantime, application of tryptophan or prozac in both cultivars of sunflower caused slight increase in the microelement as compared with the corresponding salinity level. In this connection, [51] suggested that, high NaCl may affect iron absorption and lead to Fe deficiency or toxicity. NaCl caused to decrease N, K^+, Ca^{+2}, Cu and Fe in the shoot tissue [52]. However, the excess and deficiency of Fe, Zn, Cu and Mn may leads to disturbance of ionic homeostasis which have various roles in the metabolism of plant tissues and are vital for the regularity of physiological processes [53]. In this regard, [54] concluded that, Fe, Mn, Cu, and Zn as transition metals mainly have unpaired electrons and they are good catalysts of oxygen reduction. Additional cationic micronutrients (Fe^{++}, Mn^{++}, Zn^{++}) play essential roles as cofactors and activators of enzymes. [55] and [9] report that in different crop species application of the tryptophan increased mineral contents.

Enzyme Activities:

Antioxidative enzymes are a key element in the defense mechanisms. Activities of antioxidant enzymes of plants under stress show many changes[35]. Superoxide dismutase, catalase, and peroxidase are enzymes that responsible for ROS-scavenging. These enzymes are involved in reducing of H_2O_2 from cells under salinity

stress [35]. Data in Table 6 showed that the activities of CAT, POX, PPO and PAL enzymes decreased significantly with increased soil salinity in both cultivars.. The reduction in enzyme activities indicated that these enzymes were unable to completely neutralize H_2O_2 resulted from the oxidative salt stress [56]. Application of tryptophan or 5-hydroxy tryptophan (Prozac) marked increases in CAT, PPO, POX and PAL activities under the different levels of soil salinity in sunflower plants as compared with the controls. Therefore, treatment with tryptophan or 5-hydroxy tryptophan counteract the adverse effect of salinity on metabolic activities via decreasing the ROS and thereby increasing resistance to salt stress. The same results were obtained by [33] on wheat. Moreover, its noticed that the increase of the microelements (Cu, Mn, Fe, Zn) in sunflower cultivars concomitantly with improvement in antioxidant enzyme activities Tables (5&6) might elucidate the SOD, catalases the reaction of dismutation of superoxide radicals: $2O_2^{\bullet-} + 2H+ \rightarrow H_2O_2 + O_2$. SOD is a metaloprotein with metals Mn, Cu, Zn, and Fe as co-factor [57], Mn-SOD was found in the matrix mitochondrium, Cu-Zn-SOD is found in cytosol and chloroplasts. Also, Fe-SOD, was detected in some plants [58]. Catalase catalases the reaction: $2\ H_2O_2 \rightarrow 2H_2O + O_2$. Finally, we concluded more relationships between activity of antioxidant enzymes and Mn, Fe, Zn, and Cu level in the sunflower cultivars.

The changes in the activity of PAL indicated that, PAL decreased significantly with increased soil salinity in both cultivars. There is a positive relationship between total phenolic compounds and PAL activity in sunflower plants. On the other hand, [42] indicated that PAL activity was stimulated under salt stress and it is involved in the biosynthesis of phenolic compounds. Thus, in the present results, soaking sunflower seeds in tryptophan and 5-hydroxytryptamine could improve the tolerance ability against salinity stress by increasing PAL activity which involved in the biosynthesis of phenolic compounds. Moreover, many antioxidant enzymes used phenols as a substrate; so, it mitigates the drought stress damages [43].

Concolusion:

Tryptophan or 5-hydroxy tryptophan (Prozac) counter act the adverse effect of salinity e of both sunflower cultivars (Euroflor and Hysun 336) via increasing formation of osmoprotectant compounds as total soluble sugars, proline and free amino acids. In addition to enhancing antioxidant enzyme activities. Moreover, in shoots of Hysun 336 was a higher osmolytes concentration contributing to osmotic adjustment and the higher antioxidant enzymes activity than those of Euroflor under salinity stress.

References

1. Bray EA, Bailey-Serres J, Weretilnyk E (2000). Responses to a biotic stresses in biochemistry and molecular biology of plants. American Society of Plant Physiologists1158–1249.
2. Silva C, Martinez V, Carvajal M (2008). Osmotic versus toxic effects of NaCl on pepper plants. Biologia Plantarum 52 (1), 72–79.
3. Demirevska K, Simova-Stoilova L, Fedina I, Georgieva K, Kunert K (2010). Response of oryzacystatin I transformed tobacco plants to drought, heat and light stress. J Agron Crop Sci. 196: 90–99.
4. Hare PD, Cress, WA, Van Staden J (1998). Plant, Cell & Environment 21, 535–553.
5. Ashraf M, Foolad MR (2007). Roles of glycine betaine and proline in improving lant abiotic stress tolerance. Environ Exp Bot. 59:206–216.
6. Petridis A, Therios I, Samouris G, Tananaki C (2012). Salinity induced changes in phenolic compounds in leaves and roots of four olive cultivars (Olea europaea L.) and their relationship to antioxidant activity. Environ. Exp. Bot. 79, 37–43.
7. Hasanuzzaman M, Hossain MA, Teixeira da Silva JA, Fujita M (2012). Plant response and tolerance to abiotic oxidative stress: Antioxidant defense is a key factor. In: Venkateswarlu B, Shanker SC, Maheswari M (eds) Crop Stress and its Management: Perspectives and Strategies. pp. 261–315. Springer, New York.
8. Khalid A, Arshad M, Zahir ZA (2006). Phytohormones: microbial production and applications. p. 207-220. In: Biological Approaches to Sustainable Soil Systems. (Ed.): N. Uphoff, A.S. Ball, E. Fernandes, H. Herren, O. Husson, M. Laing, C. Palm, J. Pretty, P. Sanchez, N. Sanginga and J. Thies. Taylor & Francis/CRC, Boca Raton, Florida
9. El-Bassiouny HMS (2005). Physiological responses of wheat to salinity alleviation by nicotinamide and tryptophan. Inter. J. of Agric & Biol., 7(4):653–659.

10. Hussein MM, Faham SY , Alva AK (2014). Role of Foliar Application of Nicotinic Acid and Tryptophan on Onion Plants Response to Salinity Stress Journal of Agricultural Science, 6, 8, 41-51.
11. Murch SJ, Campbell SSB, Saxena PK (2001). The role of serotonin and melatonin in plant morphogenesis: regulation of auxin-induced root organogenesis in in vitro-cultured explants of St. John's wort (*Hypericum perforatum* L.). In Vitro Cell Dev Biol Plant 37: 786–793.
12. Lookadoo SE, Pollard AJ (1991). Chemical contents of stinging trichomes of Cnidoscolus texanus. Journal of Chemical Ecology 17:1909–1916.
13. Arnao MB, Herna´ ndez-Ruiz J (2006). The physiological function of melatonin in plants. Plant Signaling and Behavior 1, 89–95.
14. Dalin L, Kang K, Choi JY, Ishihara A, Back K, Seong-Gene Lee SG (2008). HPLC analysis of serotonin, tryptamine, tyramine, and the hydroxycinnamic acid amides of serotonin and tyramine in food vegetables J Med Food 11:385-389.
15. Abdel-Monem AA, El-Bassiouny HMS, Rady, MM, Gaballah MS (2010). The role of tryptophan and prozac (5-hydroxy tryptophan) on the growth, some biochemical aspects and yield of two sunflower cultivars grown in saline soil. International Journal of Academic Research 2, 4. 254-262.
16. Caterina R D, Giuliani MM, Rotunno T, Caro AD, Flagella Z (2007). Influence of salt stress on seed yield and oil quality of two sunflower hybrids. Annals of Appl. Biol., 151(2): 145- 154.
17. Khatoon A , Qureshi MS, Hussain MK (2006). Effect of Salinity on Some Yield Parameters of Sunflower (*Helianthus annuus L.*). Inter. J. Agric & Biol., 2(4): 382-384.
18. Black, CA, DD Evans, White JL, Ensminger LE, Clark FF (1965). Methods of soil analysis. The American Soc. Agro., Inc. New York.
19. Jackson ML, (1973). Soil chemical analysis. Constable Co. Ltd., London.
20. Homme PM, Gonzalez B, Billard J (1992). Carbohydrate content, frutane and sucrose enzyme activities in roots, stubble and leaves of rye grass (Lolium perenne L.) as affected by sources/link modification after cutting. J. Plant Physiol., 140: 282-291.
21. Yemm EW, Willis AJ (1954). The respiration of barley plants. IX. The metabolism of roots during assimilation of nitrogen. New Phytolo., 55: 229-234.
22. Danil AD, George CM (1972). Peach seed dormancy in relation to endogenous inhibitors and applied growth substances. J. Amer. Soc. Hort. Sci., 17: 621-624.
23. Bates LS, Waldren RP, Teare IDT (1973). Rapid determination of free proline for water stress studies. Plant Soil. 39: 205-207.
24. Vartainan N, Hervochon P, Marcolte L, Larher F (1992). Proline accumulation during drought rhizogenesis in Brassica napus var. Oleifera. Plant Physiol., 140: 623-628.
25. Yemm EW, Cocking EC (1955). The determination of amino acids with ninhydrin. Analyst., 80: 209-213.
26. Chen JX, Wang XF (2006). Plant physiology experimental guide. Higher Education Press, Beijing, pp: 24–25, 55–56.
27. Soliva RC, Elez P, Sebastián M, Martín O (2001). Evaluation of browning effect on avocado purée preserved by combined methods. Innovative Food Science and Emerging Technologies, 1: 261-268.
28. Kumar KB, Khan PA (1982). Peroxidase and polyphenol oxidase in excised ragi (Eleusine coracana cv. PR 202) leaves during senescence. Indian J Exp Bot., 20: 412–416.
29. Dickerson DP, Pascholati SF, Hagerman AE, Butler LG, Nicholson RL (1984). Phenylalanine ammonia lyase and hydroxyl cinnamate CoA ligase in maize mesocotyls with Helminthosporium madis or Helminthosporium carbonium. Physiol. Plant Pathol., 25: 111-123.
30. Kong FX, Hu W, Chao WL, Sang WL, Wang LS (1999). Physiological responses of Mexicana to oxidative stress of SO2. Environ. and Exp. Bot., 42: 201-209.
31. Chapman HO, Pratt PE (1978). Methods of Analysis for Soils, Plants and Water. Univ. of California Agric. Sci. Priced Publication, 4034: 50.
32. Snedecor G W, Cochran WG (1980). Statistical methods. 7th edition, Iowa State University Press, Ames, Iowa.
33. Hassanein RA, Bassuony FM, Baraka DM, Khalil RR (2009). Physiological Effects of Nicotinamide and Ascrobic Acid on Zea mays Plant Grown Under Salinity Stress. I-Changes in Growth, Some Relevant Metabolic Activities and Oxidative Defense Systems. Res. J. of Agri. and Biol. Sci., 5(1): 72-81.

34. Rady MM, Sadak MSh, El-Bassiouny HMS, Abd El-Monem AA (2011). Aleviation the adverse effects of salinity stress in sunflower cultivars using nicotinamide and α-tocopherol Aust. J. Basic & Appl. Sci., 5(10): 342-355
35. Abdallah MMS , Abdelgawad ZA , El-Bassiouny HMS (2016). Alleviation of the adverse effects of salinity stress using trehalose in two rice varieties. South African Journal of Botany, 103: 275–282.
36. Park S, Kang K, Lee K, Choi D, Kim YS, Back K, (2009). Induction of serotonin biosynthesis is uncoupled from the coordinated induction of tryptophan biosynthesis in pepper fruits (Capsicum annuum) upon pathogen infection. Planta , 230 (6): 1197-1206.
37. El Bassiouny HMS, Abd Allah MMS, Rady MM, Gaballah MS, El-Sebai TN (2015). Role of Blue-Green Algae, Glutathione and Salicylic Acid on The Oxidative Defense Systems of Wheat Plant Grown in saline soil International Journal of Pharm Tech Research,8(10),pp 18-31.
38. Nikolopoulos D, Manetase Y (1991). Compatible solutes and in vitro stability of Salsola soda enzymes: Proline incopatiility. Phytochem., 30: 411-413.
39. Hoque MdA, Okuma E, Banu MNA, Nakamura Y, Shimoishi Y, Murata Y (2007). Exogenous proline mitigates the determintal effects of salt stress more than exogenous betaine by increasing antioxidant enzyme activities. J. Plant Physiol., 164: 553-561.
40. Rai. V. K (2002). Role of amino acid in plant responses to stresses. Biol. Plantarum J., 45: 481-487.
41. Abdallah MMS, El-Bassiouny HMS, Bakry AB, Sadak MSh (2015). Effect of Arbuscular Mycorrhiza and Glutamic Acid on Growth, Yield, Some Chemical Composition and Nutritional Quality of Wheat Plant Grown in Newly Reclaimed Sandy Soil. RJPBCS 6: 1038- 1054.
42. El Hariri1 DM, Sadak M Sh, El-Bassiouny HMS (2010). Response of flax cultivars to ascorbic acid and α – tocopherol under salinity stress conditions. International Journal Of Academic Research 2: 201- 210
43. Bakry BA, El-Hariri DM, Sadak MSh, El-Bassiouny HMS (2012). Drought Stress Mitigation By Foliar Application Of Salicylic Acid In Two Linseed Varieties Grown Under Newly Reclaimed Sandy Soil. Journal of Applied Sciences Research, 8(7): 3503-3514.
44. Burguieres E, McCxue P, Kwon Y, Shelty K (2006). Effect of vitamin C and folic acid on seed vigour response and phenolic-antioxidant activity. Biores. Technol. 95: 1393-1404.
45. Rivero R M, Ruiz J M, Garcia PC, Lopez – Lefebre L R, Sanchy E, Romero L (2001). Resistance to cold and heat stress : accumulation of phenolic compounds in tomato and water melon plants. Plant Sci., 160: 315-321.
46. El- Bassiouny HMS, Bekheta M A (2001). Role of putrescine on growth, regulation of stomatal aperture, ionic contents and yield by two wheat cultivars under salinity stress. Egyptian J. Physiol. Sci, 2-3: 235-258.
47. Shukry WM, El-Bassiouny HMS (2002). Gibberellic acid effects on protein pattern, hydrolytic enzyme activities and ionic uptake during germination of Vicia faba in sea water Acta Botanica Hungarica 44 (1-2), 145- 146
48. Zaho GQ, Mab BL, Ren CZ (2007). Growth gas, exchanges chlorophyll fluorescence and ion content of naked oat in response to salinity. Crop Sci., 47: 123-131.
49. Kiarostami Kh., Mohseni R, Saboora A (2010). Biochemical changes of Rosmarinus officinalis under salt stress. J. of Stress Physiol. & Biochem., 6: 114-122.
50. Rahnama A, Poustini K, Tavakkol-Afshari R, Ahmadi A, Alizadeh H (2011). Growth properties and Ion distribution in different tissues of bread wheat genotypes (Triticum aestivum L.) differing in salt tolerance. J. Agron. Crop Sci., 197: 21–30.
51. Yousfi S, Wissal M, Mahmoudi H, Abdelly C, Gharsalli M (2007). Effect of salt on physiological responses of barley to iron deficiency. Plant Phys. Biochm., 45: 309-314.
52. Turan MA, Katkat V, Taban S (2007). Variations in proline, chlorophyll and mineral elements contents of wheat plants grown under salinity stress. J. Agron., 6: 137-141.
53. Kabata-Pendias A, Mukherjee AB (2007). Trace Elements from Soil to Human. Springer- Verlag, Berlin-Heidelberg-New York, 550 pp.
54. Welch RM Graham RD, (2004). Breeding for micronutrients in staple food crops from a human nutrition prospective. J. Exp. Bot. 55, 353–364.
55. Wyszkowska J (1999). Modification of faba bean chemical composition caused by precursors of plant growth regulators and soil microorganism I. Effect of L–tryptophan and beta–indolyl acetic acid. Biuletyn–Naukowy, 5: 55–63.
56. Shalata A, Neumann P (2001). Exogenous ascorbic acid (vitamin C) increases resistance to salt stress and reduces lipid peroxidation. J. of Experimental Bot. 52(364): 2207-2211.

57. Bowler C, Van Montagu M, Inze D (1992). Superoxide dismutase and stress tolerance. Ann. Rev. Plant Physiol. Plant Mol. Biol. 43: 83-116.
58. Stroiński A (1999). Some physiological and biochemical aspects of plant resistance to cadmium effect. I. Antioxidative system. Acta Physiologiae Plantarum 21 (2): 999:175-188.

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