J. Agric. Sci. Mansoura Univ., 34 (5): 5165 - 5175, 2009

EFFECT OF EXOGENOUS APPLIED ANTIOXIDANTS ON ENDOGENOUS ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANTS IN PEPPER PLANT GROWN UNDER SALINITY STRESS CONDITIONS

Sakr, M. T. and Reda S.A. Metwally
Bot.Dept.Faculty of Agric. Mansoura Uni. Egypt
sakmoheb@yahoo.com

ABSTRACT

Two pot experiments were performed at the Experimental Station Farm . Faculty of Agriculture Mansoura university during two successive summer seasons of 2007 and 2008. This work was conducted to study the role of some exogenous applied antioxidant on endogenous enzymatic and non-enzymatic antioxidants under different salinity stress levels of sweet pepper cv. California wonder.

The data show that all salinity levels and each of applied antioxidants(presoaking+foliar spray) as well as their interactions slightly increased the contents of different non-enzymatic antioxidants (phenols, Ascorbic and Glutathione) in both shoot and fruits of pepper plant during the two growth seasons. Moreover SWE combined with highest salinity level treatment was the most effective in this respect.

Moreover each of salinity stress levels (2000, 4000 or 6000 mg/l) increased Super oxide dismutase (SOD) and Ascorbic peroxidase (APX) activity in both shoot and fruits of pepper plants. In addition The data show that any of applied antioxidants (HA, SA, ASA, Tochopherol, Put., SWE) alone or combined with different salinity stress levels also increased the activity of SOD, APX enzymes in both shoot and fruits of pepper plant, during the two growth seasons.

INTRODUCTION

Non-enzymatic antioxidants contents:

Regarding ascorbic acid (ASA),the sequence of events in the plant tissue subjected to drought stress are: increased production of ROS and of oxidized target molecules; increases in the expression of genes for antioxidant functions; increases in the levels of antioxidative systems and antioxidants, and increased scavenging capacity for ROS, resulting in tolerance against the drought stress (Mano, 2002).

The non-enzymatic plant antioxidants can be classified into two major types: (1) AA-like scavengers, and (2) pigments such as carotenoids. ASA is an important antioxidant, which reacts not only with H2O2 but also with O2, OH and lipid hydroperoxidases. On the other hand, ASA has been implicated in several types of biological activities in plants: (1) as an enzyme co-factor, (2) as an antioxidant, and (3) as a donor/ acceptor in electron transport at the plasma membrane or in the chloroplasts, all of which are related to oxidative stress resistance (Conklin, 2001). APX uses ASA and oxidizes it to monodehydroascorbate (MDA). MDA may give rise to dehydroascorbate (DHA). Both MDA and DHA will then be reduced to regenerate the ascorbate pool. This type of scavenging is thought to occur near PSI, thereby

minimizing the risk of escape and reaction of ROS with each other (Foyer and Noctor, 2000). AA is water-soluble and also has an additional role in protecting or regenerating! oxidized carotenoids or tocopherols (Imai, *et al.* 1999). ASA is a major metabolite in chloroplasts of higher plants and represents about 10% of the soluble carbohydrate pool in leaves (Noctor and Foyer, 1998).

ASA regenerates tocopherol from tocopheroxyl radical providing membrane protection (Thomas, *et al.,* 1992).

As forPhenols,Navarro, *et al.,* (2005), found that pepper plants grown under three saline treatments (0, 15, and 30 mM NaCl) had some antioxidant activity fractions such as, lycopene, β-carotene, ascorbic acid, total phenolic compounds and reducing sugars were enhanced.

Alexieva, (2001), stated that in pea plants the increase in phenols was significantly induced by drought stress .

Hale, *et al.,* (2005), found that total phenolic glycoside concentration were increased due to drought stress.

Regarding,Glutathion,the antioxidant (GSH) defense must keep active oxygen understanding of the key roles of enzymes such as SOD, APX and catalase in antioxidant defense. While these individual enzymes have central roles in the antioxidant defense network, the explorationof the enzymes involved in the synthesis and metabolism of ascorbate and glutathione .

The enzymes ascorbate peroxidase, glutathione reductase, superoxide dismutase and monodehydroascrbate reductasem, among others, are involved in the regeneration of glutathione and ascorbate that are important in detoxification of ROS (Foyer and Mullineaux, 1994).

As for,Enzymatic antioxidants,plants posses antioxidant systems in the form of enzymes such as SOD, APX, GR, DIIAR, CAT and metabolites viz., ascorbic acid, glutathione, α-tocopherol, carotenoid, flavanoids etc. (Smirnoff ,1995). These antioxidant enzymes and metabolites are reported to increase under various environmental stresses (Yu and Rengel,1999) as well as comparatively higher activity has been reported in tolerant cultivars than the susceptible ones .It is possible that better salinity resistance maintain higher activity of antioxidant enzymes viz., SOD, GR and CAT resulting in lower H2O2 production, lipid peroxidation and higher membrane stability(Sairam, *et al.* ,2002).

MATERIALS AND METHODS

Two pot experiments were performed at the Experimental Station Farm , Faculty of Agriculture Mansoura university during two successive summer seasons of 2007 and 2008. This work was conducted to study the role of some antioxidant materials in alleviation the harmful effect of different salinity stress levels on sweet pepper cv. California wonder.

Seed of sweet pepper (*Capsicum annum* L.) cv. California wonder were sown on 15 th February in both 2007 and 2008 seasons. Seedling were transplanted at the age 45days (6.7 leaves) on the 1 st of April in pots (50 cm inner diameter) containing 8 kg of air dried loamy soil at the rate of two plant/

pots. Each pot was supplied limit amounts of 20.5 % N in form ammonium sulphate at the rate (2.5 kg), 15.5 % P_2O_5 in the form calcium superphosphate at the rate (1.5 kg) and 48 % K2O in the form potassium sulphate at the rate (1 kg) before planting and added 1.5 kg ammonium sulphate 30, 60 and 120 days after transplanting.

Four levels of artificial sea water used by dissolving known weight of natural salt crust, in tap water. The natural salt crust was brought directly from the salterns of Rashid, El- Beheira Governorate, Egypt where the Mediterranean sea water is evaporated, air dried, thoroughly crushed using porcelain mortar and pestle .The four salinity levels used:
1)- Tap water (320 mg/l). ,2)- 2000 mg/l. ,3)- 4000 mg/l. ,4)- 6000 mg/l..
The amount of salt for each salinity level was calculated, dissolved in the proper amount of tap water and used for experimental investigation.

Applied antioxidants were: 1)- Tap water.,2)- Humic acid (1000 mg/L).,3)- salicylic acid (250 mg/L)., 4)- Ascorbic acid (250 mg/L) .5)- Putrescince (1 mg/L).,6)- Seaweed extract (1000 mg/L).

The seeds were presoaking in any of applied antioxidants for 8 hours before sowing and the plants were foliar sprayed with any of each applied antioxidants at 30,60,80,120 and 150 after trans planting under salinity stress levels.Plant samples were collected after 80 days to determine the contents of antioxidants as well as enzymatic antioxidant activity.

Non-enzymatic antioxidant determination

Total ascorbate determination:

0.5 g of leaf was ground in 50 ml of 2% (w/v) metaphosphoric acid using mortar and pastel and centrifuged for 30 min at 13 000 rpm at 4°C. The ascorbate content (µ.mol / g FW) was measured in the supernatant at 25°C, .The absorbance of red color was measured at 520 nm A blank value for each sample was obtained by adding a few crystals of ascorbic acid, completely reducing the dye and rendering it colorless according to Omaye et al (1979).

Total glutathione determination

The level of total glutathione(GSH) was determined with Ellman's reagent according to *De Vos et al (1992).* 300 ul of sample buffer were mixed with 630 ul of 0.5 M K_2HPO_4 and 25 ul of mM 5, 5 -dithiobis (2-nitrobenzoic acid) (final pH 7). The Absorbance at 412 nm was read after 2 min. GSH was used as a standard.

Total phenols determination:

1g of dry defeated ground leaves were macerated in 5-10 ml 80% ethanol for at least 24 hours at 0°C, the alcohol was clarified, the remained residue was re-extracted with 5-10 ml 80% ethanol 3 times. At the end, the clarified extract was completed to 50 ml using 80% ethanol. The colorimetric method of Folin-Denis as described by Daniel and George (1972) was employed for the chemical determination of phenolic compounds .quantities were determined by reading the developed blue color at 725 nm. Using 0.5 ml 80% ethanol and reagents only as a blank.(Daniel and Georg,1972 and A.O.A.C.,1967).

2-Enzymatic antioxidant activity determination:

-Ascorbate peroxidase activity determination:

Ascorbate peroxidase (APX) was assayed spectrophotochemically according to Fielding (1978).The assay was carried out at 25°C in 1.0 cm light path cuvette and the reaction mixture was consisted of 1500 ul phosphate buffer, 20 µl EDTA, 1000 µl sodium ascorbate and enzyme extract (20 µl). After mixing the reaction was initiated by adding the 480 µl H_2O_2 and decreasing in optical density at 290 nm against blank (without extract) was continuously recorded every minute (for two minutes).

-Super oxide dismutase (SOD) activity determination:

Leaf samples were collected in a ice bucket and brought to the laboratory. Leaves were then washed with distilled water and surface moisture was wiped out. Leaf samples (0.5g) were homogenized in ice cold 0.1M phosphate buffer (pH 7.5) containing 0.5Mm EDTA with pre-chilled pestle and mortar. The homogenate was transferred to centrifuge tubes and was centrifuged at 4 ℃ in Beckman refrigerated centrifuge for 15 min at 15000 X g .The supernatant was transferred to 30 ml tubes and referred to enzyme extract .

SOD activity was estimated by recording the decrease in absorbance of superoxide-nitro blue tetrazolium complex by the enzyme . About 3ml of reaction mixture containing 0.1ml of 1.5M sodium carbonate, 0.2mL of 200mM methionine, 0.1ml of 2.25Mm Nitro-blue tetrazolium, 0.1ml of 3mM EDTA, 1.5ml of 100mM potassium phosphate buffer, 1ml distilled water and 0.05 ml of enzyme were taken in test tubes in duplicate from each enzyme sample. Two tubes without enzyme extract were taken as control. The reaction was started by adding 0.1ml riboflavin (60mM) and placing the tubes below a light source of two 15 w florescent lamps for 15 min. reaction was stopped by switching off the light and covering the tube with black cloth. Tubes without enzyme developed maximal color. A non- irradiated complete reaction mixture which did not develop color served as blank. Absorbance was recorded at 560nm and one unit of enzyme activity was taken as the quantity of enzyme which reduced the abaorbance reading of samples 50% of comparison with types lacking enzymes(Dhindsa *et al.*,1981)

The dates of all experiments were statistically analyzed as technique of the analysis of variance (ANOVA) according to Gomez and Gomez(1984). The treatment means were compared using the least significant differences (LSD).

RESULTS

Non-enzymatic antioxidants contents:

Total phenols, Ascorbic and Glutathione content:

The data in table(1,2,3) show that all salinity levels and each of applied antioxidants as well as their interactions slightly increase the contents of different non-enzymatic antioxidants (phenols, Ascorbic and Glutathione) in both shoot and fruits of pepper plant during the two growth seasons. Moreover SWE combined with highest salinity level treatment was the most effective in this respect.

Table (1): Effect of salinity stress levels and antioxidants (presoaking and foliar spray) as well as their interactions on total phenols (mg/100g F.w) in shoot and fruits of pepper plant during the two growing seasons (2007 & 2008).

Treatment / Antioxidant (mg/l)	Salinity levels (mg/L)									
	0.00	2000	4000	6000	Mean	0.00	2000	4000	6000	Mean
	Season 2007					Season 2008				
	Pepper shoot									
Tap water	46	50	67	73	59	44	49	65	76	58
HA (1000)	43	51	58	71	55	45	54	64	73	59
SA (250)	40	45	54	63	50	43	47	57	67	53
ASA (250)	49	54	68	83	63	48	56	71	78	63
Put. (1)	41	45	68	78	58	40	48	60	79	56
SWE (1000)	65	74	76	93	77	64	79	80	99	80
Mean	45	51	63	74		45	52	63	74	
LSD at 5%	Antioxidant: 2.71 Salinity: 3.73 Interaction:5.65					Antioxidant: 3.71 Salinity: 4.74 Interaction: 6.7				
	Pepper fruits									
Tap water	26	31	38	45	35	28	34	40	50	38
HA (1000)	28	30	39	43	35	30	34	42	46	38
SA (250)	27	30	44	51	38	28	33	45	54	40
ASA (250)	34	39	45	43	42	35	38	46	52	42
Put. (1)	28	35	40	50	38	30	34	44	51	39
SWE (1000)	36	42	56	63	49	38	45	57	64	51
Mean	29.	34	43	49		31	36	45	51	
LSD at 5%	Antioxidant: 3.71 Salinity: 4.73 Interaction: 6.65					Antioxidant: 2.71 Salinity: 3.74 Interaction: 5.7				

HA : humic acid, SA : Salicylic acid, ASA : Ascorbic acid, Put : Putrescine, SWE : seaweeds extract

Table (2): Effect of salinity stress levels and antioxidants (presoaking and foliar spray) as well as their interactions on total Ascorbic acid (mg/100g F.w) in shoot and fruits of pepper plant during the two growing seasons (2007 & 2008).

Treatment / Antioxidant (mg/l)	Salinity levels (mg/L)									
	0.00	2000	4000	6000	Mean	0.00	2000	4000	6000	Mean
	Season 2007					Season 2008				
	Pepper shoot									
Tap water	129	153	184	215	170	127	140	180	200	161
HA (1000)	154	177	275	294	225	175	186	280	283	231
SA (250)	164	203	280	290	234	160	189	277	280	226
ASA (250)	175	245	300	364	271	167	200	288	294	237
Put. (1)	180	200	300	329	252	175	200	288	315	244
SWE (1000)	174	196	299	322	247	160	186	287	317	237
Mean	163	194	261	289		162	183	260	275	
LSD at 5%	Antioxidant:9.71 Salinity 8.73 Interaction: 13.65					Antioxidant: 8.71 Salinity: 9.74 Interaction: 12.7				
	Pepper fruits									
Tap water	79	86	123	150	109	80	99	104	143	106
HA (1000)	107	164	171	174	154	110	152	155	168	146
SA (250)	138	154	171	181	161	117	149	171	180	154
ASA (250)	154	182	196	200	183	146	160	184	199	172
Put. (1)	166	170	192	199	181	150	165	181	186	170
SWE (1000)	141	165	183	189	169	158	170	192	199	179
Mean	137	157	176	184		134	153	169	178	
LSD at 5%	Antioxidant:7.71 Salinity 9.73 Interaction: 12.65					Antioxidant: 6.71 Salinity: 8.74 Interaction: 13.7				

HA : humic acid, SA : Salicylic acid, ASA : Ascorbic acid, Put : Putrescine, SWE : seaweeds extract

Table (3): Effect of salinity stress levels and antioxidants(presoaking and foliar spray) as well as their interactions on glutathione (u mol/g.F.w in shoot and fruits of pepper plant during the two growing seasons (2007 & 2008).

Treatment / Antioxidant (mg/l)	Salinity levels (mg/L) 0.00	2000	4000	6000	Mean	0.00	2000	4000	6000	Mean
	Season 2007					Season 2008				
	Pepper shoot									
Tap water	220	225	238	246	232	215	220	230	240	226
HA (1000)	222	227	230	240	229	216	225	230	245	229
SA (250)	227	232	235	242	234	220	230	230	245	231
ASA (250)	245	260	280	240	256	235	240	275	280	257
Put. (1)	225	231	250	257	240	215	225	240	250	232
SWE (1000)	259	270	280	295	276	240	260	270	290	265
Mean	230	237	251	254		222	230	243	255	
LSD at 5%	Antioxidant: 8.71 Salinity: 5.73 Interaction:11.65					Antioxidant: 7.71 4.8 Salinity: 6.74 Interaction: 11.7				
	Pepper fruits									
Tap water	226	231	244	252	238	221	220	236	246	230
HA (1000)	228	233	236	246	235	222	225	236	251	233
SA (250)	233	238	241	248	240	226	236	236	246	236
ASA (250)	251	266	286	296	274	241	246	281	286	263
Put. (1)	231	237	256	263	246	221	231	246	256	238
SWE (1000)	265	276	286	301	282	246	266	276	296	271
Mean	236	243	257	265		228	235	249	259	
LSD at 5%	Antioxidant: 7.71 Salinity:6.73 Interaction: 11.65					Antioxidant: 7.71 Salinity: 4.74 Interaction: 11.7				

HA : humic acid, SA : Salicylic acid, ASA : Ascorbic acid, Put : Putrescine, SWE : seaweeds extract

Enzymatic antioxidants activity:

Data presented in tables (4,5) show that each of salinity stress levels (2000, 4000 or 6000 mg/l) increased Super oxide dismutase (SOD) and Ascorbic peroxidase (APX) activity in both shoot and fruits of pepper plants.

Table (4): Effect of salinity stress levels and applied antioxidants as well as their interaction on Superoxide dismutase(SOD) activity (units/g frech weight) in shoot and fruits of pepper plant during the two growing seasons (2007 & 2008).

Treatment / Antioxidant (mg/l)	Salinity levels (mg/L) 0.00	2000	4000	6000	Mean	0.00	2000	4000	6000	Mean
	Season 2007					Season 2008				
	Pepper shoot									
Tap water	307	350	355	362	343	300	340	350	360	337
HA (1000)	310	335	347	358	337	325	340	350	360	343
SA (250)	312	320	325	328	321	315	320	325	330	322
ASA (250)	314	350	360	390	353	330	360	370	400	365
Put. (1)	313	318	325	330	321	320	325	330	335	327
SWE (1000)	٢١٥	360	380	390	361	330	370	390	410	375
Mean	310	332	341	351		316	336	345	356	
LSD at 5%	Antioxidant: n.s Salinity: 0.73 Interaction: 1.65					Antioxidant:5.71 4.8 Salinity : 8.74 Interaction: 11.7				
	Pepper fruits									
Tap water	315	358	363	370	351	308	348	358	368	345
HA (1000)	318	343	355	366	345	333	348	358	368	351
SA (250)	320	328	333	336	329	323	328	333	338	330
ASA (250)	322	358	368	398	361	383	368	378	408	384
Put. (1)	321	326	332	338	329	328	333	338	343	335
SWE (1000)	323	368	388	398	٢١٨	338	378	398	418	3830
Mean	318	340	349	359		329	344	353	364	
LSD at 5%	Antioxidant: 7.71 Salinity: 8.73 Interaction: 12.65					Antioxidant: 7.71 Salinity: 9.74 Interaction: 13.7				

HA : humic acid, SA : Salicylic acid, ASA : Ascorbic acid, Put : Putrescine, SWE : seaweeds extract

Table (5): Effect of salinity stress levels and applied antioxidants as well as their interaction on ascorbic peroxidase (APX) activity (units/g protein/min) in shoot and fruits of pepper plant during the two growing seasons (2007 & 2008).

Treatment / Antioxidant (mg/l)	Salinity levels (mg/L)									
	0.00	2000	4000	6000	Mean	0.00	2000	4000	6000	Mean
	Season 2007					Season 2008				
	Pepper shoot									
Tap water	125	133	137	141	134	120	125	130	140	128
HA (1000)	130	135	140	146	137	135	139	142	148	141
SA (250)	130	145	150	154	144	130	145	150	155	145
ASA (250)	135	155	160	170	155	139	160	165	170	158
Put. (1)	132	135	140	150	139	130	135	140	145	137
SWE (1000)	140	148	155	160	150	150	155	162	170	159
Mean	131	139	145	152		131	139	144	152	
LSD at 5%	Antioxidant: 1.71 Salinity:2.73 Interaction: 3.65					Antioxidant:1.71 4.8 Salinity: 2.74 Interaction: 3.7				
	Pepper fruits									
Tap water	131	139	143	147	140	126	131	136	146	134
HA (1000)	136	141	146	152	143	141	145	148	154	147
SA (250)	136	151	156	160	150	136	151	156	161	151
ASA (250)	141	161	166	176	161	145	166	171	176	164
Put. (1)	138	141	148	156	145	136	141	146	151	143
SWE (1000)	146	154	161	166	156	156	161	168	176	165
Mean	137	145	151	158		137	145	150	158	
LSD at 5%	Antioxidant: 4.71 Salinity: 5.73 Interaction: 7.65					Antioxidant:4.71 Salinity: 5.74 Interaction: 7.7				

HA : humic acid, SA : Salicylic acid, ASA : Ascorbic acid, Put : Putrescine, SWE : seaweeds extract

In addition The data show that any of applied antioxidants (HA, SA, ASA, Tochopherol, GSH, Citric , Put., SWE) alone or combined with different salinity stress levels also increased the activity of SOD, APX on enzymes in both shoot and fruits of pepper plant, during the two growth seasons.

DISCUSSION

Enzymatic antioxidant activity :

The metabolism of active oxygen, peroxidases in plants are also involved in the biosynthesis of cell wall including lignification and suberization. Considerable evidence shows that high peroxidase activity is correlated with the reduction of plant growth. This might be attributed to peroxidase catalysis of ferulic acid conversion to diferulic acid on polysaccharides, the feruloylation of hemicelluloses, or the insolubilization of hydroxyproline-rich glycoprotein causing cell wall stiffening. Morphologically, the most typical symptom of saline injury to a plant is retarded growth due to inhibition of cell elongation, resulting in a stunted plant.

To mitigate and repair damage initiated by AOS. Treated plants have developed a complex antioxidant system. The primary components of this

system include carotenoids, ascorbate, glutathione and tocopherols, and enzymes such as superoxidase dismutase(SOD), catalase(CAT), glutathione peroxidase(GPX), peroxidase(APX) and the enzymes involved in the ascorbate glutathione cycle. Ascorbate peroxidase, dehydroascorbate reductase, monodehydroascorbate reductase and glutathione reductase. Many components of these antioxidant defence system can be found in different subcelluler compartments.

Vaidyanathan, *et al.,* (2003). Plants posses antioxidant systems in the form of enzymes such as SOD, APX, GR, DIIAR, CAT and metabolites viz., ascorbic acid, glutathione, α-tocopherol, carotenoid, flavanoids etc. Smirnoff, (1995). These antioxidant enzymes and metabolites are reported to increase under various environmental stresses Yu and Rengel, (1999) as well as comparatively higher activity has been reported in tolerant cultivars than the susceptible ones , Sairam, *et al.,* (2002).

Non enzymatic antioxidants :

1- Ascorbic acid (AA) contents:

The non-enzymatic plant antioxidants can be classified into two major types: (1) AA-like scavengers, and (2) pigments such as carotenoids. ASA is an important antioxidant, which reacts not only with H_2O_2 but also with O_2, OH and lipid hydroperoxidases. On the other hand, ASA has been implicated in several types of biological activities in plants: (1) as an enzyme co-factor, (2) as an antioxidant, and (3) as a donor/ acceptor in electron transport at the plasma membrane or in the chloroplasts, all of which are related to oxidative stress resistance (Conklin, 2001). APX uses ASA and oxidizes it to monodehydroascorbate (MDA). MDA may give rise to dehydroascorbate (DHA). Both MDA and DHA will then be reduced to regenerate the ascorbate pool. This type of scavenging is thought to occur near PSI, thereby minimizing the risk of escape and reaction of ROS with each other (Foyer and Noctor, 2000). ASA is a major metabolite in chloroplasts of higher plants and represents about 10% of the soluble carbohydrate pool in leaves (Noctor and Foyer, 1998).

2- Total phenol contents:

Phenolics are diverse secondary metabolites (flavonoids, tannins. hydroxycinnamate esters and lignin) abundant in plant tissues (Grace and Logan, 2000). Polyphenols possess ideal structural chemistry for free radical scavenging activity, and they have been shown to be more effective antioxidants in vitro than tocopherols and ascorbate.

Another mechanism underlying the antioxidativc properties of phenolics is the ability of flavonoids to alter peroxidation kinetics by modification of the lipid packing order and to decrease fluidity of the membranes (Amor, *et al.,* 2000). These changes could sterically hinder diffusion of free radicals and restrict peroxidative reactions. Moreover, it has been shown recently that phenolic compounds can be involved in the hydrogen peroxide scavenging cascade in plant cells.

Phenolic compounds retard or inhibit lipid autoxidation by acting as radical scavengers (Namiki, 1990) and, consequently, are essential antioxidants that protect against propagation of the oxidative chain.

Total phenols in pepper tended increase gradually with increasing salinity levels in soil. This increase showed some tendency to adjust osmotically against salt stress. Moreover, stress condition leads to an increase in phenolic compounds (Amor, *et al.*, 2000). These phenolic compounds could be a cellular adaptive mechanism for scavenging oxygen free radicals during stress and this free radical scavenger and others such as ascorbate could be readily oxidized in the system of tissue representing subcelluler damages. In addition, Kurup, *et al.*, (1994), pointed out that stressed marigold plants contained higher concentrations of free phenolic compounds, due to enhanced biosynthesis and formation of lignin than controls.

Reduced Glutathion contents:

GSH can function as an antioxidant in many ways. (1)It can react chemically with singlet oxygen, superoxide and hydroxyl radicals and therefore function directly as a free radical scavenger Noctor and Foyer, 1998). (2) GSH may stabilise membrane structure by removing acyl peroxides formed by lipid peroxidation reactions (Price, *et al.*, 1990). (3)GSH is the reducing agent that recycles ascorbic acid from its oxidised to its reduced form by the enzyme dehydroascorbate reductase (Loewus, 1988). (4) GSH can also reduce dehydroascorbate by a non-enzymatic mechanism at pH > 7 and GSH concentrations greater than 1 mM. This may be a significant pathway in chloroplasts whose stromal pH in the light is about 8 and GSH concentrations may be as high as 5 mM (Foyer and Halliwell, 1976).

REFERENCES

A.O.A.C. (1967). Official Methods of Analysis of the association of official analytical chemists. Washington D.C., U.S.A.

Alexieva, V., Sergiev, I. Mapelli, S. and Karanov1Acad. M. Popov,A. (2001). The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat Plant, Cell & EnvironmentVolume 24 Issue 12 Page 1337.

Amor, Y.; Chevion, M and Levinc, A. (2000). Anoxia pretrealment protects soybean cells against H_2O_2-induced cell death: possible involvement of peroxidases and of alternative oxidase& Technol., 25: 511-521.

Conklin, P., (2001). Recent advances in the role and biosynthesis of ascorbic acid in plants. Plant Cell Environ; 24:383-94.

Daniel, H.D. and. George, C. M (1972). Peach seed dormancy in relation to indogenous inhibitors and applied growth substances. J. Amer. Soc. Hort Sci. 97:651-654

De Vos, C. H.; Vonk,; M. J .Vooijs, . R. and Hek, S. (1992). Glutathione depletion due to copper-induced phytochelation synthesis causes oxidative stress in *silene cucblus*. Plant physiology. 98: 859-858.

Dhindsa, R.S., Plumb-Dhindsa, P.and Throne, T.A. (1981). Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. J.Exp.Bot 32, pp. 93-101.

Fielding, J. (1978). A biochemical and cytochemical study of peroxidase activity in root of *Pisum sativum*. J. of Experimental Botany. 29: 969-981

Foyer, C.H. and Mullineaux, P. (1994). Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants. CRC Press. Boca Raton. FL. ISBN 0-8493-5443-9.

Foyer, C.H. and Noctor, G. (2000). Oxygen processing in photosynthesis: regulation Foyer and signaling. New Phytol.; 146: 359-388.

Grace. S. C. & Logan. B. A. (1996). Acclimation of folair antioxidant systems to growth irradiance in three broad-leaved evergreen species. - Plant Physiol. 112: 1631-1640.

Hale, K. H., Herms, D. A. Hansen, R. C. Clausen, T. P. and Arnold, D. (2005). Effects of Drought Stress and Nutrient Availability on Dry Matter Allocation, Phenolic Glycosides, and Rapid Induced Resistance of Poplar to Two Lymantriid Defoliators.J.Amer.Ecol. 5: 2601-2620.

Kurup, S. S.; Nalwadi, U.G. and Geibel, M. (1994). Phenolic biosynthesis in relation to moisture stress in marigold. Acta Hort., 381: 488-493.

Loewus, F.A., (1988). Ascorbic acid and its metabolic products. In: The Biochemistry of Plants, Vol. 14. Preiss, J. (ed) Academic Press, New York, p. 85-107.

Mano, J., (2002). Early events in environmental stress. Taylor Francis pub. 217-45 .

Nabati, D. A., R.E.Schmidt, and D.J. Parrish. (2005). Alleviation of salinity stress in Kentucky bluegrass by plant growth regulators and iron. Crop Science 34:198-202.

Namiki, M. (1990). Antioxidants/antimutagens in food. CRC Critical Reviews in Food Science and Nutrition, 29, 273–300.

Navarro, J. M.; P. Flores,; C. Garrido, and V. Martinez (2006). Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. Food Chemistry 96 (2006) 66–73.

Navarro, J. M.; P. Flores,; C. Garrido, and V. Martinez (2006). Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. Food Chemistry 96 (2006) 66–73.

Noctor, G. and Foyer, C.H. (1998). Ascorbate and glutathione: keeping active oxygen under control. Annual Review of Plant Physiology and Plant Molecular Biology 49: 249–279.

Omaye, S. T., Turnbm, J. D. and Saubermch, H. E. (1979). Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods in Enzymologyi. Vol. 62. Academic Press. New York. Pp 1-

Price, A.H. and G.A.F. Hendry. (1991). Iron-catalysed oxygen radicalformation and its possible contribution to drought damage in nine native grasses and three cereals. Plant, Cell and Environment 14:477-484.

Sairam, R.K. and Srivastava, G.C. (2002). Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress, Plant Sci 162 (2002), pp. 897–904.
Sies, H. (1991). Oxidative stress: Oxidant and Antioxidant. London: Academic Press.
Thomas, C.E., McLean, L.R. Parker ,R.A and Ohlweiler , D.F. (1992). Ascorbate and phenolic antioxidant interactions in prevention of liposomal oxidation. Lipids 27: 543–550.
Vaidyanathan, H., P. Sivakumar, R. Chakrabarty, and G. Thomas, (2003). Scavenging of reactive oxygen species in NaCl-stressed rice (Oryza sativa L.)—differential response in salt-tolerantand sensitive varieties. Plant Science 165 : 1411–1418.

تأثير المعاملة بمضادات الأكسدة على المحتوى الداخلى لمضادات الأكسدة الإنزيمية وغير الإنزيمية فى نبات الفلفل النامى تحت ظروف الاجهاد الملحى

محب طه صقر و رضا سيد أحمد متولى
قسم النبات الزراعى – كلية الزراعة – جامعة المنصورة

أجريت تجربتى أصص فى مزرعة محطة البحوث بكلية الزراعة –جامعة المنصورة فى الموسم الصيفى لعامى ٢٠٠٧& ٢٠٠٨ لدراسة دور بعض مضادات الأكسدة (معاملة خارجية) على المحتوى الداخلى لمضادات الأكسدة الإنزيمية و غي الإنزيمية فى نبات الفلفل صنف كاليفورنيا النامى تحت ظروف الإجهاد الملحى .

أظهرت النتائج أن كلاً من مستويات الاجهاد الملحى المختلفة وكذلك المعاملة نقعا ورشا بأى من مضادات الأكسدة وكذلك معاملات التداخل من مستويات الاجهاد الملحى و مضادات الأكسدة أدت الى زيادات واضحة فى المحتوى الداخلى من مضادات الأكسدة غير الانزيمية (الفينولات – الاسكوربيك – الجلوتاثيون) فى كل من المجموع الخضرى والثمار لنبات الفلفل خلال موسمى الزراعة.

ولوحظ أن المعاملة SWE (مستخلص أعشاب البحر) بالتداخل مع المستوى المرتفع من الاجهاد الملحى (٦٠٠٠ مج/لتر) كانت المعاملة الاكثر فاعلية فىهذا الشأن .

ولوحظ أن مستويات الاجهاد الملحى المختلفة (٢٠٠٠&٤٠٠٠& ٦٠٠٠مج /لتر) أدت الى زيادة النشاط الانزيمى لكلب من انزيمى SOD (سوبر أكسيد ديزميوتيز) & APX (اسكوربيت بيروكسيديز) فى كل من المجموع الخضرى وثمار نبات الفلفل

كما لوحظ أن المعاملة بأى من مضات الاكسدة (هيوميك – سالسيليك – توكوفيرول – بتروسين – مستخلص أعشاب البحر) منفردة أو بالتداخل مع أى من مستويات الاجهاد الملحى المختلفة الى زيادة النشاط الانزيمىل لانزيمى SOD) & APX فى كل من المجموع الخضرى وثمار الفلفل صنف كاليفورنيا خلال موسمى ٢٠٠٧& ٢٠٠٨.