Influence of Garlic Extract On Enzymatic and Non Enzymatic Antioxidants in Soybean Plants (*Glycine Max*) Grown under Drought Stress

Heba Ibrahim Mohamed* and Samia Ageeb Akladious

Biological and Geological Sciences, Faculty of Education, Ain Shams University
El Makres St. Roxy, Cairo, Egypt (ARE) Post code: 1575
hebaibrahim79@yahoo.com

Abstract: Drought is an important environmental constraint limiting the productivity of many crops worldwide. Experiments were conducted to investigate the effects of foliar spray of natural extract (garlic extract) on drought stress in two cultivars of soybean (*Glycine max*) plants. Drought stress caused significant decrease in growth parameters (shoot and root length, area of leaves, fresh and dry weight of shoots and roots) and photosynthetic pigments (chl a, chl b, carotenoids and total pigments). On the other hand, drought stress caused significant increase in non enzymatic antioxidants (ascorbic acid, tocopherol and reduced glutathione), enzymatic antioxidants (glutathione reductase, superoxide dismutase and ascrobate peroxidase), oxidative damage (H_2O_2 content and lipid peroxidation) and osmolytes compounds (proline, total soluble sugars and total phenols) in shoots of the two cultivars of soybean plants (Giza 22 and 111). Moreover, foliar spray with two concentrations of garlic extract (400 and 600 ppm) enhanced all the above parameters that of the control plants and drought stressed plants. Electrophoretic studies of peroxidase and polyphenol oxidase isoenzymes showed wide variations in their intensities and densities among all treatments. It seems that garlic extract was able to enhance the tolerance of the studied plant to drought stress.

[Mohamed HI, Akladious SA. **Influence of Garlic Extract On Enzymatic and Non Enzymatic Antioxidants in Soybean Plants (*Glycine Max*) Grown under Drought Stress**. *Life Sci J* 2014;11(3s):46-58]. (ISSN:1097-8135). http://www.lifesciencesite.com. 9

Keywords: drought stress, soybean, garlic extract, non enzymatic antioxidants, enzymatic antioxidants, osmolytes compounds.

1. Introduction

Drought is the most severe abiotic stress factor limiting plant growth and crop production (Moussa, 2011; Rohbakhsh, 2013). Drought stress induces several physiological, biochemical and molecular responses in several crop plants, which would help them to adapt to such limiting environmental conditions (Arora *et al.,* 2002). It inhibits the photosynthesis of plants, causes changes of chloro-phyll contents and damages the photosynthetic apparatus (Escuredo *et al.,* 1998). It also inhibits the photochemical activities and decreases the activities of enzymes in the Calvin cycle (Monakhova and Chernyadev, 2002). It reduces respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Praba *et al.,* 2009). It breaks down the balance between the productions of reactive oxygen species (ROS) and the antioxidant defense system causing the accumulation of ROS which induces oxidative stress to protein, membrane lipids and disruption of DNA strands (El Tayeb, 2006).

Among the common responses in plants to abiotic stresses is the production of different types of organic solutes which include small molecules called osmoprotectant and antioxidant such as proline (Szabados and Savouré, 2010; Mohamed and Abdel-Hamid, 2013) and soluble sugar (Shao *et al.,* 2005) during stress. Water stress also increases the levels of soluble sugars (compatible solutes) as glucose, sucrose, sorbitol, galactose, raffinose, stachyose and soluble proteins while it reduces the amounts of polysaccharides, starch and total sugars (El Tayeb, 2006). Those compounds protect plants against stresses by cellular adjustment through the protection of membranes integrity and enzymes stability (Farooq *et al.,* 2009). When plants are subjected to various abiotic stresses, some reactive oxygen species such as superoxide ($O2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\cdot}OH$) and singlet oxygen ($^1O^2$) are produced (Li and Staden, 1998). These ROS may initiate destructive oxidative processes such as lipid peroxidation, chlorophyll bleaching, protein oxidation and damage to nucleic acids (Scandalios 1993). However, antioxidant enzymes such as superoxide dismutase (SOD), glutathione reductase (GR), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POD) and low-molecular antioxidants such as ascorbic acid, glutathione, α-tocopherol, flavonoids and carotenoids play a key role in scavenging those activated species (Sgherri *et al.,* 2000; El-Beltagi and Mohamed, 2013).

Soybean (*Glycine max* L. Merr) is an important legume crop, known for its high quality protein (40–

42%), oil content (18–22%) and beneficiary secondary metabolites such as isoflavones, phenolic compounds and saponins (Sakthivelu *et al.,* 2008).

Garlic, a bulb producing crop in the family *alliaceae*, has a very strong pungnant odour and it is known to contain an essential oil (sulphur) compounds. Garlic contains 0.1-0.36% of a volatile oil these volatile compounds are generally considered to be responsible for most of the pharmacological properties of garlic. Garlic contains at least 33 sulfur compounds like aliin, allicin, ajoene, allylpropl, diallyl, trisulfide, sallylcysteine, vinyldithiines, S-allylmercaptocystein, and others. Beside these sulfur compounds garlic contains 17 amino acids and their glycosides, arginine and others. Minerals such as selenium and enzymes like allinase, peroxidases, myrosinase, and others. The fresh extracts of *Allium sativum* can be used to improve the vegetative growth of many plants such as squash (Shafshak *et al.,* 2004). In addition, Morsy *et al.* (2009) found that garlic or onion extracts significantly improved all plant growth characteristics of cucumber plant, *i.e*. number of leaves/plant, number of flowers/plant, shoot and root length and fresh and dry weight of shoot and root system compared with non sprayed plants.

Garlic extract can be used to alleviate biotic and abiotic stresses. In this concern, El-Gamal and Hammad (2003) reported that garlic and yeast extracts are useful in counteracting the harmful effects exerted by cadmium on tomato plants. Also, Hammad (2008) studied the effect of foliar spray of garlic extract on growth, physiological aspects, anatomical structure as well as yield components of pea plants under water stress. He found that, the application of natural extracts caused significant increases in most tested parameters compared with control plants. The interactive effect of drought stress and the usage of natural substances resulted in significant increases in growth parameters, photosynthetic pigments, N, P and K content in leaves, enzymes activity and proline concentration compared with untreated plants. In addition, Abdo *et al.* (2012) used garlic cloves (30 ml/L.) for minimizing the harmful effects of environmental pollution caused by cadmium on vegetative and reproductive growth as well as on leaf anatomy and physiological behaviour of soybean. Moreover, Abbas and Akladious (2013) and Ali *et al.* (2013) reported that the usage of natural products can protect plants against abiotic and biotic stress by regulating many physiological processes.

The aim of the present study was to assess the effect of drought stress on physiological and biochemical attributes of two cultivars of soybean plants and to alleviate the effect of drought stress by using of natural extract (garlic).

2. Material and Methods

Plant material:

Soybean seeds of two cultivars (cv. Giza 22 and Giza 111) were used in this study. Seeds were obtained from the Crop Research Section, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

Garlic extract:

Garlic extract was prepared according to EL-Desouky *et al.* (1998) method. Well crushed garlic bulbils were subjected to ethanol 70% solvent and the extraction was left for 6 hr in dark and then filtered. The residue was re-extracted by new volume of ethanol. The filtered extract was evaporated to dryness under vacuum in rotary evaporator at 40ºC. The dried extraction was kept in deep freezer until use. Two concentrations were applied as foliar application (400 ppm and 600 ppm). Main contents of garlic extract have been analyzed by Arid Land Agricultural Research Unit Fac. of Agric. Ain Shams Univ. in Table (1).

Table 1. Some chemical constituents of garlic bulbils

According to Arid Land Agricultural Research Unit. Components	**Concentration** ppm
GA_3	16.33
IAA	Trace amount
ABA	Trace amount
Ca	0.0014
Mg	0.0012
SO_4	0.00018
Zn	66.5
Mn	94.4

Plant cultivation and drought treatments:

Matured seeds were surface sterilized with 70 % ethanol for 2 min, followed by 10 min in 5 % sodium hypochlorite (v/v) and rinsed with sterile water for four to six times and then grown in 30 cm diameter pots containing equal amounts of homogeneous soil. The soil characteristics were as follows: sandy loam in texture, sand 80%; silt 15.5%; clay 4.5%; pH, 7.8; EC 0.4 dS m^{-1} and organic matter 0.45%. This experiment was conducted under environmental conditions (day length 12–14 h, temperature 28–30°C and humidity 65%). The seeds were sown at 2-3 cm depth in each pot on 28th May and when emergence was complete (~7days) the seedling density was reduced to 10 seedlings / pot. After 7 weeks (flowering stage) from sowing, the plants were divided into four groups (5 pots/ group) and treated as follows:

- Plants of the 1^{rt} group were left without any treatments to serve as control (Normal or well watering irrigation (W_2D).

- Plants of the 2nd group subjected to drought stress and watered every 6 days (W_6D).
- Plants of the 3rd group were subjected to drought stress (W_6D) and sprayed by 400 ppm of garlic extract.
- Plants of the 4th group were subjected to drought stress (W_6D) and sprayed by 600 ppm of garlic extract.

The foliar spraying with garlic extract was carried out twice, at the age of 7 and 10 weeks. When the developed plants reached 14 weeks, 5 plants were carefully uprooted from the soil of each treatment where samples were analyzed for certain measures.

Determination of photosynthetic pigments:

Chlorophyll a, Chlorophyll b and Carotenoids were determined in soybean leaves. The spectrophotometric method recommended by Vernon and Seely (1966) was used. The pigment contents were calculated as mg g^{-1} fresh weight of leaves.

Assay of non enzymatic antioxidant:

Ascorbic acid contents: Content of ascorbic acid was estimated according to Mukherjee and Choudhuri (1983). The absorbance was recorded at 530 nm.

Tocopherols contents: The absorbance of α-tocopherol was recorded at 520 nm against ethanol as a blank (Philip *et al.,* 1954). The content of α-tocopherol in the extracts was calculated from the regression equation of the standard curve.

Glutathione contents: The measurement of total non protein SH group was carried out following the method of Cakmak and Marschner (1992).

Assay of enzymatic antioxidant:

The shoots were grinded in sodium phosphate buffer at pH 6.5 for GR, SOD and APX. The supernatant was used to measure the activity of the following enzymes:

Glutathione reductase (GR; **EC 1.6.4.2)** activity was determined based on the decrease in absorbance at 340 nm due to the oxidation of NADPH to NADP according to the method of Foyer and Halliwell (1976).

Superoxide dismutase (**SOD; EC 1.15.1.1)** activity was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium chloride (NBT). Absorbance was read at 560 nm according to Beauchamp and Fridovich (1971).

Ascorbate peroxidase (APX; EC 1.11.1.11) activity was estimated according to the method of Nakano and Asada (1981). Enzyme activity was determined by the decrease in absorbance of ascorbate at 290 nm.

Assay of oxidative damage:

H_2O_2 content: The H_2O_2 level was colorimetrically measured as described by Jana and Choudhuri (1981). The intensity of yellow color of supernatant was measure at 410 nm. H_2O_2 level was calculated using the extinction coefficient 0.28 μmol^{-1} cm^{-1}.

Lipid peroxidation: Lipid peroxidation was determined by estimating the malondialdehyde content following the method of Heath and Packer (1968). The absorbance of the resulting supernatant was recorded at 532 nm and 600 nm. The absorbance coefficient of malondialdehyde was calculated by using the extinction coefficient of 155mM^{-1} cm^{-1}.

Assay of osmolyte compounds:

Proline content was estimated by the method of Bates *et al.* (1973). **Soluble sugars** were determined based on the method of phenol sulfuric acid as described by Dubois *et al.* (1956). Pure glucose was used as standard. **Soluble phenols** were determined in accordance with Dihazi *et al.* (2003). The absorbance of the developed blue colour was read at 725 nm. Tannic acid was used as standard and the amount of soluble phenols was expressed as mg tannic acid g^{-1} dry weight.

Electrophoretic analysis of isozymes

Isozymes peroxidase and polyphenol oxidase were analyzed on 10 % polyacrylamide slab gels. Detection of peroxidase was carried out by the method described by Larsen and Benson (1970) and polyphenol oxidase was done by the method Sato and Hasegawa (1976). Isoenzyme banding patterns were recorded according to their relative front (Rf) values. The Rf value is the mobility of each isoenzyme band that traveled from the origin divided by the distance traveled by the front tracking dye (Powers *et al.,* 1989).

Statistical analysis:

All data were subjected to statistical analysis and means were compared by Duncan's multiple range test using Mstat C commuter package.

3. Results

Changes in growth parameters

Shoot and root length:

Results in table (2) showed that drought stress caused significant decrease in shoot and root lengths in soybean plants cv. Giza 111 but caused non significant effect in cultivar Giza 22 as compared with well watered plants. In addition, spraying the two cultivars of soybean plants with garlic extract caused significant increases in shoot and root lengths as compared with drought stressed plants and well watered plants.

Area of leaves (cm^2):

Non significant change in the area of leaves was observed in soybean plants cv. Giza 22 and 111 as a result of exposing the plants to drought stress (Table 2). On the other hand, spraying the plants with 400 and 600 ppm of garlic extract caused significant increase in leaves area as compared with either drought stressed plants or well watered plants.

Changes in photosynthetic pigments

Drought stress caused significant decrease in chlorophyll a, b and total photosynthetic pigments in leaves of soybean plants cv. Giza 22 and 111 as compared with control plants but had no effect on carotenoid contents in the two cultivars (Table 3). In addition, spraying soybean plants cv. Giza 22 and 111 with garlic extract (400 and 600 ppm) caused stimulation of the total photosynthetic pigments contents in leaves of the two cultivars as compared with control plants and drought stressed plants.

Table 2. Effect of foliar spraying of garlic extract on morphological characters of *Glycine max* (cv. Giza 22 and cv. Giza 111) plants grown under drought stress conditions. Means ± SD (*n=3*) of measurements on each three plants. Different letters indicate a significant difference at $P \leq 0.05$ according to Duncan's multiple range test.

Culti-vars	Treatment	Shoot length (cm)	Root length (cm)	Area of leaves (cm^2)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)
Giza 22	Control	39.33±1.53^{d}	31.67±0.58^{de}	163±56.3^{c}	5.41±1.10^{d}	1.45±0.40^{cd}	1.66±0.23^{d}	0.35±0.05^{bc}
	Drought	34.33±1.53^{d}	26.50±2.18^{e}	128±29.7^{c}	4.64±0.39^{d}	1.46±0.11^{cd}	0.81±0.09^{d}	0.24±0.04^{d}
	Drought+ 400 ppm	64.67±4.16^{bc}	38.67±2.31^{bc}	208±118.0 ^{b}	8.97±0.84^{bc}	2.40±0.38^{b}	2.28±0.25^{bc}	0.41±0.06^{ab}
	Drought+ 600 ppm	73.00±3.61^{b}	43.67±3.06^{ab}	240±21.03^{b}	11.83±0.92^{a}	3.00±0.23^{a}	2.35±0.16^{bc}	0.45±0.05^{a}
Giza 111	Control	56.33±9.29^{c}	33.33±1.53^{cd}	163±80.0^{c}	7.52±0.78^{c}	1.75±0.10^{c}	1.91±0.25^{cd}	0.36±0.07^{bc}
	Drought	32.67±9.29^{d}	26.33±2.89^{e}	153±29.3^{c}	5.13±0.84^{d}	1.32±0.22^{d}	1.11±0.16^{e}	0.28±0.05^{cd}
	Drought+ 400 ppm	77.33±13.80^{a} ^{b}	43.50±1.00^{ab}	2112±64.0^{b}	10.03±1.53^{ab}	2.30±0.24^{b}	2.51±0.33^{b}	0.43±0.06^{ab}
	Drought 600 ppm	87.73±5.77^{a}	48.00±7.55^{a}	279±23.7^{a}	11.49±1.27^{a}	2.90±0.33^{a}	3.05±0.55^{a}	0.46±0.02^{a}
L.S.D at 5%		**13.95**	**6.17**	**34.7**	**1.85**	**0.403**	**0.480**	**0.078**

Table 3. Effect of foliar spraying of garlic extract on photosynthetic pigments in leaves of *Glycine max* (cv. Giza 22 and cv. Giza 111) plants grown under drought stress conditions. Means ± SD (*n=3*) of measurements on each three plants. Different letters indicate a significant difference at $P \leq 0.05$ according to Duncan's multiple range tests.

Cultivars	Treatment	Chl a mg g^{-1} FW	Chl b mg g^{-1} FW	Car mg g^{-1} FW	Total pigments mg g^{-1} FW
Giza 22	Control	7.13±0.08^{c}	3.50±0.17^{d}	2.03±0.08^{d}	12.66±0.07^{f}
	Drought	6.66±0.12^{d}	2.53±0.12^{f}	2.64±0.03^{cd}	11.84±0.16^{g}
	Drought + 400 ppm	7.05±0.11^{c}	5.77±0.50^{a}	1.97±0.23^{d}	14.79±0.34^{d}
	Drought + 600 ppm	10.22±0.16^{a}	4.01±0.08^{c}	4.45±0.02^{ab}	18.68±0.19^{a}
Giza 111	Control	7.14±0.05^{c}	2.97±0.18^{e}	3.78±0.05^{ab}	13.89±0.13^{e}
	Drought	5.49±0.11^{e}	2.12±0.24^{g}	2.98±0.09^{bc}	10.59±0.06^{h}
	Drought + 400 ppm	8.67±0.16^{b}	3.03±0.13^{e}	4.02±0.07^{a}	15.73±0.14^{c}
	Drought + 600 ppm	10.10±0.13^{a}	4.85±0.21^{b}	3.12±0.05^{bc}	18.07±0.11^{b}
L.S.D at 5%		**0.200**	**0.363**	**0.743**	**0.248**

Changes in non enzymatic antioxidants activities

It is evident that drought stress caused significant increases in ascorbic acid, α-tocopherol and glutathione contents in shoots of soybean plants cv. Giza 22 and 111 as compared with control plants (Table 4). In addition, spraying plants with garlic extract showed significant increase ascorbic acid, α-tocopherol and GSH contents of shoots of the two cultivars above that of the corresponding controls.

Changes in enzymatic antioxidants

Drought stress resulted in considerable increase in the activity of GR, SOD and APX in shoots of soybean cv. Giza 22 and 111 as compared with control plants (Table 5). Application of garlic extract caused accumulation in GR activity in shoots of soybean cv. Giza 22, SOD activity and APX activity in the shoot of the two cultivars as compared with control plants.

Table 4. Effect of foliar spraying of garlic extract on non enzymatic antioxidants in *Glycine max* (cv. Giza 22 and cv. Giza 111) plants grown under drought stress conditions. Means ± SD (*n=3*) of measurements on each three plants. Different letters indicate a significant difference at $P \leq 0.05$ according to Duncan's multiple range tests.

Cultivars	Treatment	Ascorbic acid µg g^{-1} FW	α-tocopherol µg g^{-1} FW	Reduced glutathione (GSH) µg g^{-1} FW
Giza 22	Control	6.05 ± 0.24^{e}	27.24 ± 7.15^{d}	6.08 ± 0.18^{e}
	Drought	7.55 ± 0.51^{de}	33.72 ± 2.48^{c}	12.38 ± 0.33^{b}
	Drought + 400 ppm	9.82 ± 0.73^{bc}	44.13 ± 1.63^{b}	7.14 ± 0.21^{d}
	Drought + 600 ppm	10.22 ± 0.76^{bc}	45.82 ± 3.39^{b}	8.24 ± 0.82^{c}
Giza 111	Control	7.21 ± 1.39^{e}	41.01 ± 2.97^{b}	6.94 ± 0.90^{de}
	Drought	8.78 ± 0.52^{cd}	52.47 ± 4.35^{a}	15.75 ± 1.34^{a}
	Drought + 400 ppm	11.19 ± 0.88^{b}	56.89 ± 1.97^{a}	$7.75\pm0.09c^{d}$
	Drought + 600 ppm	14.52 ± 0.77^{a}	56.10 ± 0.86^{a}	8.60 ± 0.03^{c}
L.S.D at 5%		**1.46**	**6.16**	**1.006**

Table 5. Effect of foliar spraying of garlic extract on enzymatic antioxidants in *Glycine max* (cv. Giza 22 and cv. Giza 111) plants grown under drought stress conditions. Means ± SD (*n=3*) of measurements on each three plants. Different letters indicate a significant difference at $P \leq 0.05$ according to Duncan's multiple range tests.

Cultivars	Treatment	Glutathione reductase (GR) (unit min^{-1} g^{-1} FW)	Superoxide dismutase (SOD) (unit min^{-1} g^{-1} FW)	Ascorbate peroxidase (APX) (unit min^{-1} g^{-1} FW)
Giza 22	Control	1.01 ± 0.03^{e}	4.63 ± 0.24^{d}	0.203 ± 0.03^{e}
	Drought	2.06 ± 0.06^{b}	8.80 ± 0.52^{b}	0.537 ± 0.01^{b}
	Drought + 400 ppm	1.19 ± 0.04^{d}	6.80 ± 0.24^{c}	0.341 ± 0.02^{d}
	Drought + 600 ppm	1.37 ± 0.14^{c}	8.94 ± 0.90^{b}	0.443 ± 0.01^{c}
Giza 111	Control	1.16 ± 1.16^{de}	7.99 ± 0.85^{b}	0.229 ± 0.00^{e}
	Drought	2.63 ± 0.22^{a}	10.73 ± 0.47^{a}	0.865 ± 0.04^{a}
	Drought + 400 ppm	1.29 ± 0.02^{cd}	6.63 ± 0.63^{c}	0.369 ± 0.01^{d}
	Drought + 600 ppm	1.43 ± 0.01^{c}	7.08 ± 0.13^{c}	0.492 ± 0.01^{bc}
L.S.D at 5%		**0.166**	**0.912**	**0.055**

Changes in oxidative damage

H_2O_2 content:

As shown in Table 6, drought stress significantly increased the accumulation of H_2O_2 in shoots of soybean cv. Giza 22 and 111 as compared with control plants. The accumulation of H_2O_2 was higher in cultivar Giza 22 than Giza 111. However, foliar spray with garlic extract significantly reduced H_2O_2 level in shoots of soybean cv. Giza 22 and 111 when compared to drought stress plants and control plants.

Lipid Peroxidation:

Lipid peroxidation level in shoots of soybean plants cv. Giza 22 and 111 was assessed by MDA content. At drought stress, MDA content increased in cv. Giza 22 and111 as compared with the control plants (Table 6). In contrast, foliar spray with garlic extract 400 ppm and 600 ppm reduced the MDA levels by 82% and 67% in cv. Giza 22 and 89% and 75 in cv. Giza 111 as compared with drought plants.

Changes in osmolytes compounds

Proline (PRO) content:

The proline content in shoots of soybean plants cv. Giza 22 and 111 significantly increased under drought stress (Table 7). In addition, the proline content significantly increased in shoots of soybean plants cv. Giza 22 and 111 when the plants sprayed with the two concentrations of garlic extract (400 and 600 ppm) as compared with drought stressed plants and control plants.

Soluble sugars content:

Total soluble sugars content in shoots of soybean plants cv. Giza 111 significantly increased in plants exposed to drought stress and in plants sprayed with 400 and 600 ppm of garlic extract as compared with control plants (Table 7). On the other hand, in cultivar Giza 22, non significant change was observed in total soluble sugars content when the plants exposed to either drought stress or sprayed with 400 ppm of garlic extract whereas the same content showed significant increase when the plants sprayed with 600 ppm of garlic extract as compared with control plants.

Table 6. Effect of foliar spraying of garlic extract on oxidative damage (hygrogen peroxide and lipid peroxidation) in *Glycine max* (cv. Giza 22 and cv. Giza 111) plants grown under drought stress conditions. Means ± SD (*n=3*) of measurements on each three plants. Different letters indicate a significant difference at $P \leq 0.05$ according to Duncan's multiple range tests.

Cultivars	Treatment	H_2O_2 (µmole g^{-1} FW)	Lipid peroxidation (nmol MDA g^{-1} FW)
Giza 22	Control	0.838±0.03^{b}	31.68±9.35^{c}
	Drought	0.972±0.06^{a}	70.32±0.90^{a}
	Drought + 400 ppm	0.702±0.03^{c}	57.81±0.77^{ab}
	Drought + 600 ppm	0.663±0.14^{c}	47.35±2.71^{bc}
Giza 111	Control	0.546±0.03^{d}	35.44±2.95^{c}
	Drought	0.715±0.04^{c}	61.29±14.58^{ab}
	Drought + 400 ppm	0.400±0.03^{e}	54.45±6.97^{b}
	Drought + 600 ppm	0.346±0.04^{e}	45.76±12.0^{bc}
L.S.D at 5%		**0.111**	**14.64**

Total phenol content:

The obtained results revealed that total phenols content was significantly accumulated in shoots of soybean growing under drought stress condition (Table 7). In addition, foliar spray with garlic extract caused the accumulation of total phenol content in shoots of the two cultivars of soybean plants as compared with control plants. The accumulation of phenols was higher in cultivar Giza 111 than Giza 22.

Table 7. Effect of foliar spraying of garlic extract on osmolyte compounds (proline, soluble sugars and total phenol) in *Glycine max* (cv. Giza 22 and cv. Giza 111) plants grown under drought stress conditions. Means ± SD (*n=3*) of measurements on each three plants. Different letters indicate a significant difference at $P \leq 0.05$ according to Duncan's multiple range tests.

Cultivars	Treatment	Proline (µg g^{-1} DW)	Soluble sugars (mg g^{-1} DW)	Total phenol (mg tannic acid g^{-1} FW)
Giza 22	Control	8.7±0.10^{e}	62.87±5.63^{b}	1.65±0.38^{e}
	Drought	25.5±0.11^{cd}	73.47±3.45^{ab}	3.33±0.22^{cd}
	Drought + 400 ppm	32.9±0.22^{bc}	75.33±5.75^{ab}	3.55±0.84^{cd}
	Drought + 600 ppm	35.2±0.36^{abc}	86.80±7.66^{a}	3.71±0.95^{cd}
Giza 111	Control	20.4±0.91^{de}	66.27±9.60^{b}	2.62±0.59^{de}
	Drought	36.9±0.10a^{abc}	87.07±5.60^{a}	4.22±1.29^{c}
	Drought + 400 ppm	43.5±1.31^{ab}	83.33±13.58^{a}	5.75±0.14^{b}
	Drought + 600 ppm	46.4±0.86^{a}	87.73±4.05^{a}	7.81±0.69^{a}
L.S.D at 5%		**1.213**	**13.95**	**1.36**

Isoenzymes:

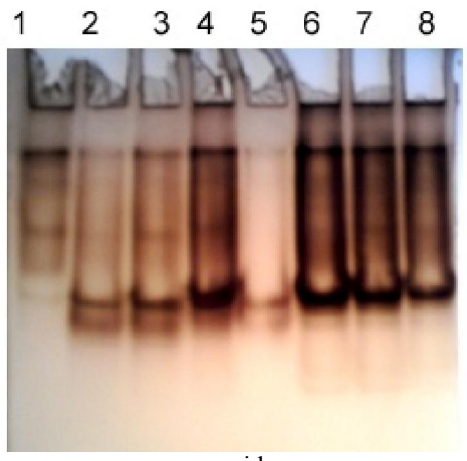
Peroxidase isoenzyme

Expression of the peroxidase isoenzyme was detected in soybean leaves treated with drought alone or in combination with garlic extract (400 and 600 ppm) using 10% native PAGE (Table 8 and Fig. 1). The results showed that eight bands were exhibited with different densities and intensities among the profiles of all treatments. The band which has Rf 0.11 was present in all treatments (common bands) of soybean leaves cv. Giza 111 and Giza 22. The band which has Rf 0.33 was common bands in soybean cv. Giza 22. The other bands were present in some treatments and absent in the others (polymorphic bands). The activity of peroxidase increased in drought treated plants and drought treated plants sprayed with two concentrations of garlic extract.

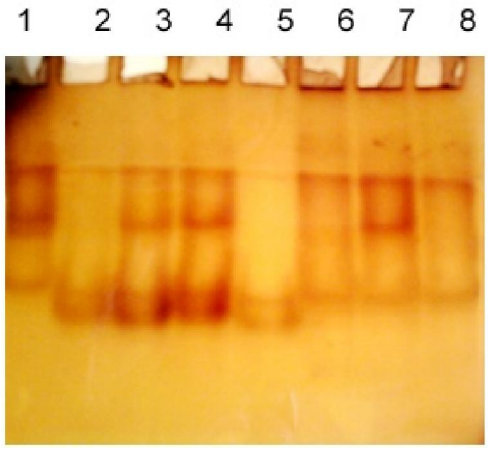
The induction of new isoenzymes and the change in the isoenzyme profile is considered to play an important role in the cellular defense against oxidative stress. Drought treatments induced different changes in POD isoenzyme patterns. The activity of POD increased in extracts of drought treated leaves of soybean cv. Giza 111 and Giza 22.

Table 8. Effect of foliar spraying of garlic extract on the intensity (%) of different isoenzymes profiles in *Glycine max* (cv. Giza 22 and cv. Giza 111) plants grown under drought stress conditions.

peroxidase								
Rf	**Giza 22**				**Giza 111**			
	Control	Drought	Drought + 400 ppm	Drought + 600 ppm	Control	Drought	Drought + 400 ppm	Drought + 600 ppm
0.11	15.5	6.8	6.6	8.7	8.2	11.7	9.7	8.7
0.19	6.3	7.5	4.9			4.8	8.4	5.1
0.33	5.1	8.4	6.3	13.5			7.1	10.7
0.36						8.4		
0.43	3.3							
0.51	3.3			20.0			16.9	14.6
0.53		10.9	14.5		14.2	20.0		
0.59		13.4	7.7	4.2	9.2	1.7	0.96	
0.69						1.2	0.94	0.55
0.73						1.1	0.90	0.50
NO. of bands	**5**	**5**	**5**	**4**	**3**	**7**	**7**	**6**
Polyphenol oxidase								
0.18	7.6	7.8	6.5	7.8				
0.21					7.5	9.4		
0.24							9.2	8.1
0.39	9.4	8.3		10.4		6.9		
0.42			8.8				13.8	6.5
0.51								5.5
0.65	7.8							
0.71			12.4	12.2	10.1	8.5	10.4	9.6
0.79		13.4	11.1	18.8	9.61			
0.18	7.6	7.6	6.5	7.8				
NO. of bands	**4**	**4**	**5**	**5**	**3**	**3**	**3**	**4**



peroxidase



polyphenol oxidase

Figure 1. Electrophoretic patterns of peroxidase and polyphenol oxidase isoenzyme of soybean leaves in response to foliar spray of garlic extract under drought stress.

1- Control (Giza 22)
2- drought
3- drought + 400 ppm garlic extract
4- drought + 600 ppm garlic extract
5- Control (Giza 111)
6- drought
7- drought + 400 ppm garlic extract
8- drought + 600 ppm garlic extract

Polyphenol oxidase isoenzymes

Polyphenol oxidase electrophoretic patterns are illustrated in table 8 and Fig. 1. Four bands with different intensities and densities were observed among the profiles of all treatments. One band was presented in all treatments (monomorphic bands) at Rf 0.18 in leaves of soybean cv. Giza 22 and at Rf 0.71 18 in leaves of soybean cv. Giza111. The other bands were presented in some treatments and absent in the others (polymorphic bands). The activity of polyphenol oxidase increased in drought treated plants sprayed with the two concentrations of garlic extract as compared with untreated plants.

4. Discussions

Drought stress caused significant decrease in plant growth. These results are in harmony with Abass and Mohamed (2011) who reported that the plant growth parameters of common bean (shoot and root length, fresh and dry weights of shoots and roots) decreased significantly with increasing drought stress as compared with control plants. Such decline in shoot and root length in response to drought might be due to either decrease in cell elongation, cell turgor, cell volume and eventually cell growth (Banon *et al.,* 2006), and/or due to blocking up of xylem and phloem vessels thus hindering any translocation through (Lavisolo and Schuber, 1998). Treatment with garlic extract caused increment in growth parameters and leaf area. These results are in harmony with El-Ghinbihi and Hassan (2007) who found that drought stress treatments exhibited significant reduction in vegetative growth characters represented by root length, plant height, number of branches and number of leaves, leaf area as well as dry weight of roots, stems and leaves. Foliar spray of pepper plants with natural extracts (yeast, garlic, eucalyptus) or ascorbic acid significantly enhanced all growth characters. In addition, Hanafy *et al.* (2012) mentioned that spraying of *Schefflera arboricola* plants with garlic extract increased leaf area.

Drought stress caused significant change in the fresh and dry weight of shoots and roots of cv. Giza 22 and cv. Giza 111 as compared with control plants. Similar results are obtained by Abass and Mohamed (2011) who reported that both fresh and dry weights of shoots and roots of common bean decreased with increasing drought stress. Also, Shitole and Dhumal (2012) found that drought stress caused reduction in fresh and dry weights of senna seedlings. Reduction in fresh and dry weights of shoots and roots of soybean plants under drought stress may be due to the metabolic disorders induced by stress and generation of ROS. There was inhibition of root growth which may be attributed to reduced extensibility of the root tip tissue due to hardening of the expanding cell walls.

Application of garlic extract increased fresh and dry weight of soybean plants. These results were in accordance with El-Ghinbihi and Hassan (2007) who found that foliar spray of pepper plants with natural extracts (yeast, garlic, eucalyptus) or ascorbic acid significantly increased dry weight of roots, stems and leaves under drought stress. Also, Zaki *et al.* (2008) found that, spraying of sweet pepper plants with garlic extract increased fresh and dry weight of plants.

Abass and Mohamed (2011) who reported that photosynthetic pigments contents in leaves of common bean plants were highly significantly decreased with increasing the level of drought stress. The reduction in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation. The decrease in the photosynthetic activity under drought stress may be due to stomatal or non-stomatal mechanisms. Stomata closure is one of the first responses to drought stress which result in declined rate of photosynthesis. The drought induced reduction in the chlorophyll content could be attributed to loss of chloroplast membranes, excessive swelling, distortion of the lamellae vesiculation and the appearance of lipid droplets. In addition, spraying soybean plants cv. Giza 22 and 111 with garlic extract (400 and 600 ppm) caused stimulation of the total photosynthetic pigments contents in leaves of the two cultivars as compared with control plants and drought stressed plants. These results are in line with El-Ghinbihi and Hassan (2007) who found that drought stress caused reduction in photosynthetic pigments (chl. a, chl. b, chl. a+b and carotenoids) of pepper plants but foliar spray of pepper plants with natural extracts (yeast, garlic, eucalyptus) or ascorbic acid significantly increased photosynthetic pigments under drought stress. Also, Hammad (2008) reported that, the interactive effect of drought stress and the usage of natural substances (garlic extract) increased photosynthetic pigments content in leaves of pea plants. Such promotional effects of garlic extracts upon the formation of chlorophyll might be due to the active role of such agents in the pathway of synthesis of α-amino levulinic acid, the precursor of chlorophyll biosynthesis.

Drought stress caused significant increases in ascorbic acid, α-tocopherol and glutathione contents in shoots of soybean plants cv. Giza 22 and 111 as compared with control plants. These results are in

harmony with those obtained by Abdul Jaleel *et al.* (2008) who found that the alba variety of *Catharanthus roseus* showed high levels of ascorbic acid content when compared to the rosea variety under drought stress. Also, Abdul Jaleel (2009b) stated that, α -tocopherol of the drought stressed *Withania somnifera* plant roots significantly increased when compared to control plants. In addition, Hasanuzzaman and Fujita (2011) found that the GSH content in rapeseed seedlings increased with increasing drought stress. The increase in GSH level in shoots of drought stressed soybean plants might be due to the increased GSH synthesis, decreased GSH degradation, increase in the transport of GSH and also involved as a substrate for GPX, which reduces H_2O_2 and organic peroxides, and therefore protects cell proteins and cell membranes against oxidation. Also, the increased GSH content might be due to the significant increase in GR activity as well as higher GSH biosynthesis. Ascorbic acid is one of the strongest non-enzymatic antioxidants that provide better protection against drought stress, regulation of cell elongation, protecting proteins and lipids and protecting cells against oxidative stress (Hasanuzzaman and Fujita, 2011). The increase in α -tocopherol content which observed under drought stress may be due to increase the activation of the expression genes responsible for the synthesis of tocopherols in plants. Additionally, GSH plays an indirect role in protecting membranes by maintaining α-tocopherol and zeaxanthin in a reduced state. GSH prevents the denaturation of proteins caused by the oxidation of protein thiol groups under drought stress.

In addition, spraying plants with garlic extract showed significant increase ascorbic acid, α-tocopherol and GSH contents of shoots of the two cultivars above that of the corresponding controls. Garlic extract contain GA_3. Growth regulators like GA_3 treatments increased the ascorbic acid content, α-tocopherol and reduced glutathione content in *Catharanthus roseus* (Abdul Jaleel *et al.,* 2009a).

Drought stress resulted in considerable increase in the activity of GR, SOD and APX in shoots of soybean cv. Giza 22 and 111 as compared with control plants . Similar results obtained by Hasanuzzaman and Fujita (2011) who reported that GR activity showed significant increase in rapeseed seedlings under mild drought stress, but it remained unchanged with severe drought stress. Also, the increase in SOD activity in shoots of drought stressed soybean might be due to the activation of preexisting SOD or due to synthesis of new SOD under drought conditions. Similar results showed that superoxide dismutse activity increased under drought stress in higher plants (Wang *et al.,* 2005; Turkan *et al.,* 2005; Abdul Jaleel *et al.,* 2008). In addition, APX activity increased under drought stress in many higher plants such as *Phaseolus acutifolius* **(**Turkan *et al.,* 2005) and *Catharanthus roseus* (Abdul Jaleel *et al.,* 2008).

Application of garlic extract caused accumulation in GR activity in shoots of soybean cv. Giza 22, SOD activity and APX activity in the shoot of the two cultivars as compared with control plants. These results are similar to that obtained by El-Ghinbihi and Hassan (2007) who found that drought stress caused reduction of the enzymatic activities (peroxidase and phenoloxidase) of pepper plants but foliar spraying of pepper plants with natural extracts (yeast, garlic, eucalyptus) or ascorbic acid significantly increased enzymatic activities under drought stress. Also, Hammad (2008) reported that the interactive effect of drought stress and the usage of natural substances (garlic extract) significantly increased enzymatic activities (peroxidase and phenoloxidase) in leaves of pea plants.

H_2O_2 and MDA content is accumulated in shoots of soybean plants under drought stress. These results are in accordance with Hasanuzzaman and Fujita (2011) who found that drought stress significantly increased H_2O_2 and MDA in the rapeseed seedlings. In addition, Saruhan *et al.* (2012) reported that drought stress increased MDA content in two maiza genotypes. However, foliar spray with garlic extract significantly reduced H_2O_2 and MDA level in shoots of soybean cv. Giza 22 and 111 when compared to drought stress plants and control plants. These results suggest that H_2O_2 may play a secondary role in the drought stress signaling network by inducing defense pathways in the early phase of drought. Therefore, it may be suggested that the increased level of H_2O_2 observed by other authors in the drought treated plants is due to oxidative damages, but eventually may also have a signal function (Zlatev and Lidon, 2012). Garlic extract contain calcium which had the function of preventing cell membrane injury and leakage as well as stabilizing cell membrane structure under adverse environmental conditions. Application of external calcium resulted in lower MDA content in liquorice cells compared with the contents in media without external calcium under water stress conditions (Li *et al.,* 2003). Lipid peroxidation was the first type of oxidative damage. Its overall effects were to decrease membrane fluidity; increase the leakiness of the membrane and damage membrane proteins, enzymes and ion channels (Shehab *et al.,* 2010).

The proline and soluble sugars content in shoots of soybean plants cv. Giza 22 and 111 significantly increased under drought stress. These results are in accordance with Abass and Mohamed (2011) who reported that the drought condition caused significant

increase in the proline and soluble sugars content in shoot of common bean plants.

In addition, the proline and soluble sugars content significantly increased in shoots of soybean plants when the plants sprayed with the two concentrations of garlic extract (400 and 600 ppm) as compared with drought stressed plants and control plants. These results are in accordance with El-Ghinbihi and Hassan (2007) who found that drought stress caused significant increase in proline content in leaves of pepper plants but foliar spray of pepper plants with natural extracts (yeast, garlic, eucalyptus) or ascorbic acid decreased the accumulation of proline under drought stress. Also, Hammad (2008) reported that the interactive effect of drought stress and the usage of natural substances (garlic extract) increased proline content in leaves of pea plants. The accumulation of PRO may be through an increase in its synthesis constantly with inhibition of its catabolism and may be a mechanism for stress tolerance. Also, Zaki *et al.* (2008) who found that spraying of sweet pepper plants with garlic extract showed non significant effect in total sugars contents in fruit of sweet pepper plants. The accumulation of soluble sugars compounds protects the cell under stress by balancing the osmotic strength of the cytosol with that of the vacuole and the external environment. The compounds also interact with cellular macromolecules as enzymes and stabilize their structure (El-Tayeb, 2006).

In this respect, the protection of soybean plants against drought stress by an exogenous application of garlic extract is believed to be caused indirectly as a result of its effect on proline accumulation which play a protective role as scavenges of ROS, resulted in improved adaptation ability and growth of plants under drought conditions (Türkan and Demiral, 2009). Also, Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress (Szabados and Savoure´, 2010).

Total phenols content was significantly accumulated in shoots of soybean growing under drought stress condition. These increases might be due to the increase in their biosynthesis. These results are in accordance with Azhar *et al.* (2011) who found that the total phenolic contents in desiajwain (*Trachyspermum ammi* L.) increased significantly with increasing drought stress levels. Drought stress reduces growth, so the carbon fixed during photosynthesis could be used to form secondary metabolites (phenolics) (Hale *et al.,* 2005). In addition, foliar spray with garlic extract caused the accumulation of total phenol content in shoots of the two cultivars of soybean plants as compared with control plants. The accumulation of phenols was higher in cultivar Giza 111 than Giza 22.

The activity of peroxidase increased in drought treated plants and drought treated plants sprayed with two concentrations of garlic extract. These results are in accordance with Abedi and Pakniyat (2010) who found that five POD distinct isoenzymes were presented in oilseed rape plants. The drought-stressed leaves were highly capable of increasing the number and intensity of POD isoforms. This could be considered as a response to drought induced oxidative damage, suggesting the enzymatic removal of H_2O_2 by POD.

The induction of new isoenzymes and the change in the isoenzyme profile is considered to play an important role in the cellular defense against oxidative stress. Drought treatments induced different changes in POD isoenzyme patterns. The activity of POD increased in extracts of drought treated leaves of soybean cv. Giza 111 and Giza 22. POD plays a role in decreasing the accumulation of H_2O_2 content, eliminating MDA (malondialdehyde) resisting cell peroxidation of membrane lipids and maintaining the cell membrane integrity.The activity of polyphenol oxidase increased in drought treated plants sprayed with the two concentrations of garlic extract as compared with untreated plants. These results are in accordance with Hammad (2008) who reported that the interactive effect of drought stress and the usage of natural substances (garlic extract) increased significantly enzymatic activities (peroxidase and phenoloxidase) in leaves of pea plants.

Conclusion

The present data suggest that garlic extract could trigger the activation of antioxidants in plants, which persists in the plants to alleviate the oxidative damage, leading to improvements in physiological attributes for the plants growth under drought conditions. Garlic extract can be used to alleviate the adverse effect of drought stress. The cultivar Giza 22 is more resistant to drought than Giza 111.

Corresponding Author:
Dr. Heba Ibrahim
Department of Biological and Geological Science
Ain Shams University
E-mail: hebaibrahim79@yahoo.com

References

1. Moussa HR. Low dose of gamma irradiation enhanced drought tolerance in soybean. Acta Agro Hung 2011; 59: 1–12.
2. Rohbakhsh H. Alleviating adverse effects of water stress on growth and yield of forage

sorghum by potassium application. Advances Environ Biol 2013; 7(1): 40-46.
3. Arora A, Sairam RK, Srivastava GC. Oxidative stress and antioxidative systems in plants. Curr Sci 2002; 82: 1227-1238.
4. Escuredo IP, Arrese-Igor C, Becana M. Oxidative damage in pea plants exposed to water deficit or paraquat. Plant Physiol 1998; 116: 173-181.
5. Monakhova OF, Chernyadev I.I. Protective role of kartolin-4 in wheat plants exposed to soil drought. Appl Biochem Microbiol 2002; 38: 373-380.
6. Praba ML, Cairns JE, Babu RC, Lafitte HR. Identification of physiological traits underlying cultivar differences in drought tolerance in rice and wheat. J Agron Crop Sci 2009; 195: 30-46.
7. El-Tayeb MA. Differential response of two Vicia faba cultivars to drought: growth, pigments, lipid peroxidation, organic solutes, catalase and peroxidase activity. Acta Agron Hung 2006; 54: 25-37.
8. Szabados L, Savouré A. Proline: a multifunctional amino acid. Trends Plant Sci 2010; 15: 89–97.
9. Mohamed HI, Abdel-Hamid AME. Molecular and biochemical studies for heat tolerance on four cotton genotypes. Romanian Biotechnol Letters 2013; 18(6): 7223-7231.
10. Shao HB, Liang ZS, Shao MA. Changes of anti-oxidative enzymes and MDA content under soil water deficits among 10 wheat (Triticum aestivum L.) genotypes at maturation stage. Colloids Surf. B: Biointerfaces 2005; 45: 7–13.
11. Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA. Plant drought stress: effects, mechanisms and management. Agronomy for Sustainable Development 2009; 29: 185–212.
12. Li L, Staden JV. Effects of plant growth regulators on the antioxidant system in callus of two maize cultivars subjected to water stress. Plant Growth Regul 1998; 24: 55–66.
13. Scandalios JG. Oxygen stress and superoxide dismutase. Plant Physiol 1993; 101: 7–12.
14. Sgherri CLM, Maffei M, Navari-Izzo F. Antioxidative enzymes in wheat subjected to increasing water deficit and rewatering. J Plant Physiol 2000; 157: 273–279.
15. El-Beltagi HS, Mohamed HI. Alleviation of cadmium toxicity in *Pisum sativum* L. seedlings by calcium chloride. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 2013; 41(1): 157-168.
16. Sakthivelu G, Akitha Devi MK, Giridhar P, Rajasekaran T, Ravishankar GA, Nedev T, Kosturkova G. Drought induced alterations in growth, osmotic potential and in vitro regeneration of soybean cultivars. General Appl Plant Physiol 2008; 34: 103-112.
17. Shafshak NS, Aggour ARA, Ali SMM. Effect of fertilization system on some squash cultivars production. Fourth Science Conference of Agricultural Science 2004; pp. 410-426. University of Assiut.
18. Morsy SM, Drgham EA, Mohamed GM. Effect of garlic and onion extracts or their intercropping on suppressing damping-off and powdery mildew diseases and growth characteristics of cucumber. Egyptian J Phytopathol 2009; 37: 35-46.
19. El-Gamal SM, Hammad SAR. Counteracting the deleterious effects of lead and cadmium on tomato plants by using yeast, garlic and eucalyptus extracts. Menofia J Agri Res 2003; 28: 737-755.
20. Hammad SA. Physiological and anatomical studies on drought tolerance of pea plants by application of some natural extracts. Annals Agric Sci (Cairo) 2008; 53: 285-305.
21. Abdo FA, Nassar DMA, Gomaa EF, Nassar RMA. Minimizing the harmful effects of cadmium on vegetative growth, leaf anatomy, yield and physiological characteristics of soybean plant [*Glycine max* (l.) merrill] by foliar spray with active yeast extract or with garlic cloves extract. Res J Agri Biol Sci 2012; 8: 24-35.
22. Abbas SM., Akladious SA. Application of carrot root extract induced salinity tolerance in cowpea (Vigna sinensis L.) seedlings. Pakistan J Bot 2013; 45: 795-806.
23. Aly AA, Mohamed HI, Mansour MTM, Omar MR. Suppression of powdery mildew on flax by foliar application of essential oils. J phytopathol 2.13; 6: 376-381.
24. El-Desouky SA, Wanas ALA, Khedr ZMA. Utilization of some natural plant extracts (garlic and yeast) as seed soaked materials of squash (Cucurbita pepo L.) Annals Agri Sci Moshtohor 1998; 36: 839-879.
25. Vernon LP, Seely GR. (1966). The chlorophylls. Academic Press. New York.
26. Mukherjee SP, Choudhuri MA. Implications of water stress induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. Plant Physiol 1983; 58: 166-170.
27. Philip B, Bernard L, William H. Vitamins and Deficiency Diseases, In, Practical Physiological Chemistry, McGraw-Hill company, INC: New York, Toronto, London, 1954; 1272–1274.

28. Cakmak I, Marschner H. Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. Plant Physiol 1992; 98: 1222-1227.
29. Foyer CH, Halliwell B. The presence of glutathione and glutathione reductase in chloroplasts: A proposed role in ascorbic acid metabolism. Planta 1976; 133, 21-25.
30. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochem 1971; 44: 276–287.
31. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplast. Plant Cell Physiol 1981; 22: 867-880.
32. Jana S, Choudhuri MA. Glycolate metabolism of three submerged aquatic angiosperm during aging. Aquatic Bot 1981; 12: 345-354.
33. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Bioph 1986; 125: 189-198.
34. Bates LS, Waldren RP, Teare I.D. Rapid determination of free proline for water-stress studies. Plant Soil 1973; 39: 205-207.
35. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Analytical Chem 1956; 28: 350–356.
36. Dihazi AD, Jaitt F, Zouine J, Hassni ME, Hardami I.E. Effect of salicylic acid on phenolic compounds related to data palm resistance to *Fusarium oxysporum* sp. Albedimis. Phytopathologia Mediterranea 2003; 423: 9-16.
37. Larsen AL, Benson WC. Variety specific variants of oxidative enzymes from soybean seeds. Crop Sci 1970; 10: 493-495.
38. Sato M, Hasegawa M. The latency of spinach chloroplast phenolase. Phytochemistry 1976; 15: 61.
39. Powers HR, Lin D, Hubbes M. Interspecific and intraspecific differentiation within the genus Cronartium by isozyme and protein pattern analysis. Plant Dis 1989; 73: 691-694.
40. Abass SM, Mohamed HI. Alleviation of adverse effects of drought stress on common bean (*Phaseolus vulgaris* L.) by exogenous application of hydrogen peroxide. Bangladesh J Bot 2011; 41: 75-83.
41. Banon SJ, Ochoa J, Franco JA, Alarcon JJ, Sanchez-Blanco MJ. Hardening of oleander seedlings by deficit irrigation and low air humidity. Environ Exp Bot 2006; 56: 36-43.
42. Lavisolo C, Schuber A. Effects of water stress on vessel size xylem hydraulic conductivity in *Vitis vinifera* L. J Exp Bot 1998; 49(321): 693-700.
43. El-Ghinbihi FH, Hassan MI. Effect of some natural extracts and ascorbic acid as foliar spray on growth, leaf water contents, chemical composition and yield of pepper plants grown under water stress conditions. Menofia J Agri Res 2007; 32: 683-710.
44. Hanafy MS, Saadawy FM, Milad SMN, Ali RM. Effect of some natural extracts on growth and chemical constituents of *Schefflera arboricola* plants. J Hort Sci Ornamental Plants 2012; 4: 26-33.
45. Shitole SM, Dhumal KN. Effect of water stress by polyethylene glycol 6000 and sodium chloride on seed germination and seedling growth of *Cassia angustifolia*. International J Pharmaceutical Sci Res 2012; 3: 528-531.
46. Zaki ME, Shafshak NS, Gabal MR, Shams AS. Effects of N-fertilizer source, biofertilizer and foliar spray with amino acids or garlic extract on growth, yield and fruit quality of sweet pepper plant. Annals Agri Sci Moshtohor 2008; 46: 533-544.
47. Abdul Jaleel CA, Gopi R, Manivannan P, Gomathinayagam M, Sridharan R, Panneerselvam R. Antioxidant potential and indole alkaloid profile variations with water deficits along different parts of two varieties of Catharanthus roseus. Colloids Surfaces B: Biointerfaces 2008; 62: 312–318.
48. Abdul Jaleel CA, Manivannan P, Wahid A, Farooq M, Somasundaram R, Panneerselvam R. Drought stress in plants: a review on morphological characteristics and pigments composition. International J Agric Biol 2009b; 11: 100–105.
49. Hasanuzzaman M, Fujita M. Selenium pretreatment upregulates the antioxidant defense and methylglyoxal detoxification system and confers enhanced tolerance to drought stress in rapeseed seedlings. Biol Trace Element Res 2011; 143: 1758–1776.
50. Abdul Jaleel CA, Gopi R, Panneerselvam R. Alterations in non-enzymatic antioxidant components of Catharanthus roseus exposed to paclobutrazol, gibberellic acid and Pseudomonas fluorescens. Plant Omics J 2009a; 2: 30-40.
51. Wang FZ, Wang QB, Kwon SY, Kwak SS, Su WA. Enhanced drought tolerance of transgenic rice plants expressing a pea manganese superoxide dismutase. J Plant Physiol 2005; 162: 465–472.

52. Turkan I, Bor M, Ozdemir F, Koca H. Differential responses of lipid peroxidation and antioxidants in the leaves of drought tolerant *P. actifolius* Gray and drought sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. Plant Sci 2005; 168: 223–231.
53. Saruhan N, Saglam A, Kadioglu A. Salicylic acid pretreatment induces drought tolerance and delays leaf rolling by inducing antioxidant systems in maize genotypes. Acta Physiol Plant 2012; 34: 97–106.
54. Zlatev Z, Lidon FC. An overview on drought induced changes in plant growth, water relations and photosynthesis. Emirates J Food Agri 2012; 24: 57-72.
55. Li M, Wang GX, Lin JS. Application of external calcium in improving the PEG-induced water stress tolerance in liquorice cells. Botanical Bulletin of Academia Sinica 2003; 44: 275-284.
56. Shehab GG, Ahmed OK, El-Beltagi HS. Effects of various chemical agents for alleviation of drought stress in rice plants (Oryza sativa L.). Notulae Botanicae Horti Agrobotanici Cluj 2010; 38: 139–148
57. Türkan I, Demiral T. Recent developments in understanding salinity tolerance. Environ Exp Bot 2009; 67: 2-9.
58. Azhar N, Hussain B, Ashraf MY, Abbasi K.Y. Water stress mediated changes in growth, physiology and secondary metabolites of desi ajwain (*Trachyspermum ammi* l.). Pakistan J Bot 2011; 43: 15-19.
59. Hale BK, Herms DA, Hansen RC, Clausen TP, Arnold D. Effects of drought stress and nutrient availability on dry matter allocation, phenolic glycosides and rapid induced resistance of poplar to two lymantriid defoliators. J Chemical Ecol 2005; 31: 2601-2620.
60. Abedi T, Pakniyat H. Antioxidant enzyme changes in response to drought stress in ten cultivars of Oilseed Rape (*Brassica napus* L.). Czech J Genetics Plant Breed 2010; 46: 27-34.

1/29/2014