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Physiological Role of Glycinebetaine in Alleviating the Deleterious Effects of Drought Stress on Canola Plants (*Brassica napus* L.)

Mona G. Dawood and Mervat Sh. Sadak

Botany Department, National Research Centre, Dokki, Giza, Egypt

ABSTRACT

Pot experiments were conducted during two successive seasons (2011/2012 and 2012/2013) at wire house of the National Research Centre, Giza, Egypt to study the effect of soaking canola seeds with glycinebetaine (GB) (10mM, 15mM and 20mM) on growth, photosynthetic pigments, osomoprotectants, antioxidant enzymes, seed yield quality and quantity, in fever of antioxidant compounds and fatty acids composition in the yielded canola plants which subjected to moderate and severe drought stress (75% FC and 50% FC). Moderate and severe drought stress caused marked decreases in canola plant growth parameters (shoot height, root length, fresh and dry weights of shoot and root/plant) and significant decreases in photosynthetic pigments and IAA accompanied by significant increases in osomoprotectants (proline and total soluble sugars), MDA and H_2O_2 in tissues of canola leaf relative to control plants. Antioxidant enzymes activities (POX, PPO, SOD, CAT and APX) were significantly increased accompanied by significant decreases in NR due to drought stress. Drought stress at 75% FC and 50% FC decreased seed yield/plant, oil and carbohydrate, total phenolic content, tannins, flavonoids and antioxidant activity accompanied by significant increases in protein content of the yielded seeds. Drought stress increased total saturated fatty acids and decreased unsaturated fatty acids relative to control plants. On the other hand, GB treatments proved to be effective in enhancing growth parameters and photosynthetic pigments of drought stressed plants. Glycinebetaine treatments at different levels caused significant increases in IAA, proline, total soluble sugars and significant decreases in MDA, H_2O_2, antioxidant enzymes (POX, PPO, SOD, CAT, APX and NR) in canola plants irrigated with different levels of water relative to corresponding controls. All GB treatments caused significant increases in seed yield, oil, carbohydrate, protein, total phenolic content, tannins, and antioxidant activity of the yielded seeds and non-significant increases in flavonoids in the yielded canola seeds either in plants irrigated with 75% FC or 50% FC relative to corresponding controls. The increases in seed yield/plant due to 20 mM GB were 30.80% and 60.28% at 75% FC and 50% FC respectively relative to corresponding controls. The fatty acid profile of canola oils showed different responses to GB treatments either in unstressed plants or drought stressed plants. Oleic and linoleic acids were increased accompanied by decreases in linolenic and erucic acids under the interaction effect of GB treatments and drought stress (75% FC and 50% FC) and these results led to decreases in total saturated fatty acid and increases in unsaturated fatty acid relative to corresponding controls. Generally, 20 mM GB was the most pronounced and effective treatment in alleviating the deleterious effect of moderate or severe drought stress on canola plants.

Key words: *Brassica napus L.,* osmoprotectants, water deficit, seed quality, antioxidant, fatty acid composition.

Introduction

Canola (*Brassica napus* L.) is one of the most important sources of vegetable oil in the world, currently holds the third position among oilseed crops after palm oil and soybean (FAO, 2011). Canola seeds are characterized by low erucic acid and glucosinolate. Since, high levels of erucic acid and glucosinolate in rapeseed are considered toxic to human and animal. Safe limits for these compounds have been described as less than 2% of erucic acid in oil and less than 30 µmol g^{-1} of glucosinolate in oil free meals. Canola oil has the lowest saturated fatty acids content among vegetable oils and thus presents an increasing demand for diet-conscious consumers. The residue left after oil extraction is rich in proteins and can be used for animal feeding (Din *et al*., 2011).

Plants experience drought stress due to high rate of transpiration or due to low supply of water to roots. So, any degree of water imbalance at any growth stage adversely affects the crop growth and its development. The adverse effects of drought stress on plant biomass production are due to inhibition in cell expansion, alterations in plant metabolism and reduction in the activities of different metabolic enzymes such as enzymes in the Calvin cycle (Ashraf *et al*., 2013) as well as reduction in respiration, translocation, ion uptake, and levels of growth promoters (Praba *et al.,* 2009). Moreover, the drought stress affects on a number of biochemical and molecular processes, which results in stomatal closure, decrease in rate of transpiration, pigment content, photosynthesis and thereby partial or full inhibition in growth and development (Lawlor and Cornic, 2002).

Corresponding Author: Mona G. Dawood, Botany Department, National Research Centre, Dokki, Giza, Egypt
E-mail: monagergis@yahoo.com

In addition, drought stress leads to oxidative stress in plant cells and enhancement the production of reactive oxygen species (ROS) as superoxide radical, hydrogen peroxide, hydroxyl radical, and singlet oxygen. The production of ROS has harmful effects on different plant physiological and metabolic processes such as photosynthesis and antioxidant defense system leading to lipid peroxidation, chlorophyll destruction, biological macromolecule deterioration, membrane dismantling, ion leakage, and DNA-strand cleavage (Hossain *et al.,* 2013). Further, damage to fatty acids of membrane could produce small hydrocarbon fragments including malondialdehyde (MDA) that is considered as one important sign of membrane system injury. Salt and drought stress resulted in a marked increase in MDA and H_2O_2 levels in different plant species including rapeseed and mustard due to inadequate induction of antioxidant system (Hossain *et al.,* 2013).

To counter the deleterious effects of ROS, plants have developed the scavenging mechanism of ROS categorized as enzymatic and non-enzymatic antioxidants, acting as a defense mechanism to regulate the ROS levels. The enzymatic antioxidants include superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), glutathione peroxidase (GPX) and non-enzymatic antioxidants include ascorbate, glutathione and phenolic compounds (Hossain *et al*., 2013). The degree of damage by ROS depends on the balance between the production of ROS and its removal by antioxidant scavenging mechanism. Superoxide radicals that emerge as result of stress in the plant tissues are transformed into hydrogen peroxide (H_2O_2) and molecular O_2 by the SOD enzyme (Kusvuran *et al.,* 2013). The metabolism of H_2O_2 is dependent on various functionally interrelated antioxidant enzymes such as CAT and POX. Since, CAT and POX activities coordinate with SOD activity and playing a central protective role in O^{2-} and H_2O_2 scavenging process (Hoque *et al.*, 2007).The CAT enzyme plays a role in converting H_2O_2 into water and molecular oxygen (Kusvuran *et al.,*2013). Further, the H_2O_2 is also detoxified to H_2O in the ascorbate-glutathione cycle**.** APX uses ascorbate as electron donor in the first step of the ascorbate-glutathione cycle and is considered the most important plant peroxidase in H_2O_2 detoxification (Noctor and Foyer, 1998).

Non-enzymatic antioxidants are vital substances that possess the ability to scavenge the free radical induced oxidative stress. In plant tissues, many phenolic compounds are potential antioxidants: flavonoids, tannins and lignin precursors may work as ROS-scavenging compounds (Rice-Evans *et al.,* 1997). These antioxidants act as a cooperative network, employing a series of redox reactions. Moreover, it has been shown that phenolic compounds can be involved in the hydrogen peroxide scavenging cascade in plant cells (Ali and Alqurainy, 2006). Flavonoids are the plants secondary metabolic compounds, which act effectively as scavengers of oxidizing molecules including singlet oxygen and free radicals. They also play an important physiological role in plant stress tolerance (Ali *et al*., 2007)

Many plants tolerate stress by production of different types of organic solutes called osmoprotectants (or compatible solutes) which lower the osmotic potential and attract water molecules into the cell and ultimately maintain the cell turgor. These compatible solutes including soluble sugars, sugar alcohols, proline and glycinebetaine, *etc* are low molecular weight, highly soluble in water and non-toxic to plant even at higher cytosolic concentration. Generally, these compatible solutes protect plants from stress injury through different means, including protection of cytoplasm and chloroplasts from Na^+ damage and scavenging of reactive oxygen species (Smirnoff and Cumbes, 1989), stabilization of proteins and protecting membrane structure (Bohnert and Jensen, 1996), maintaining the osmotic balance, and general maintenance of physiological stability of plants under stressful conditions (Farooq *et al.,* 2009).

The application of osmoprotectants has been considered as a shotgun approach to increase plant drought tolerance. Glycinebetaine (GB) is a quaternary ammonium compound, an amino acid derivative and regarded as one of the most effective compatible solutes that protect plants from injury of abiotic stresses.

GB application improves growth, survival and tolerance of a wide variety of plants under various stress conditions (Ashraf and Foolad, 2007) by regulating a number of physiological and biochemical processes (Qureshi *et al*., 2013), maintaining turgor pressure (Agboma *et al*., 1997), enhancing net CO_2 assimilation rate (Lopez *et al.,* 2002), protecting the functional proteins and enzymes (e.g. Rubisco), and lipids of the photosynthetic apparatus and maintaining electron flow through thylakoid membranes (Allakhverdiev *et al*., 2003) and regulation of photosynthetic machinery and ion homeostasis (Raza *et al*., 2007). Further, GB induces defense response in crops against reactive oxygen species (ROS) produced due to biotic and abiotic stresses and plays a vital role in the process of osmotic adjustment in many crops under environmental stresses (Gadallah,1999). Moreover, it may be act as anti-transpirant which allowed the plant to access more water for a long period and facilitates photosynthesis as reported by Agboma *et al*. (1997). It is interesting to note that, the high levels of glycinebetaine do not exert adverse effects on protein structure, enzyme activities, membrane functions and metabolic processes occurring within the cell (Rhodes and Hanson, 1993). Shahbaz *et al*. (2011) mentioned that foliar-applied 50 mM GB was the most effective concentration in enhancing various growth attributes and grain yield as well as the levels of some key metabolites of wheat cultivars under drought stress conditions.

Naturally produced GB can easily be collected as a relatively inexpensive natural by-product from high-producing plants such as sugar beets (Rhodes and Hanson, 1993). This may make application of GB an economically feasible approach to counteract the adverse effects of environmental stresses on crop productivity. This work aimed to study the effect of soaking canola seeds with glycinebetaine at different concentrations on growth, photosynthetic pigments, osomoprotectant, antioxidant enzymes, seed yield quality and quantity, in fever of antioxidant compounds and activity as well as fatty acids composition in the yielded canola plants which subjected to various levels of drought stress.

Materials and Methods

Two pot experiments were conducted during two successive seasons (2011/2012 and 2012/2013) at wire house of the National Research Centre, Giza, Egypt.

Materials

Canola seeds (*Brassica napus* L.var. Pactol) were obtained from Oilseed Department, Agricultural Research Centre, Giza, Egypt. Glycinebetaine (GB) was purchased from Sigma Aldrich.

Methods

Healthy seeds were surface sterilized with 1 % (v/v) sodium hypochlorite for approximately 2 min, followed by washing thoroughly with distilled water. The seeds were divided into four groups, the first group was soaked with distilled water as control (GB_0), while the second, third and fourth groups were soaked with three different concentrations of glycinebetaine at 10mM, 15mM and 20mM named as GB_1, GB_2 and GB_3 respectively for 12 hours then allowed to dry at room temperature (25 °C) for about 1h.Regarding chemical fertilizers, Ca-superphosphate (15.5 % P_2O_5) was applied at a rate of 10g/ pot before sowing. Nitrogen fertilizer as ammonium sulfate (20.5 % N) was applied at the rate of 2g/ pot twice at 3 and 5 weeks old plants.

Each experiment was carried out in plastic pots (30 cm in diameter) filled with clay soil. The seeds were sown at a depth of 3cm in the middle of November during the two successive seasons. Each experiment comprised 12 treatments with 10 replicates in a complete randomized design.

At three leaves stage, thinning was done leaving 3 seedlings per pot. The plants were watered regularly to field capacity (FC) till the drought treatments were imposed. The plants were exposed to drought stress after 30 days from sowing. The canola plants were subjected to two levels of drought stress i.e. 75% FC and 50% FC named as D_1 and D_2 respectively while control plants (D_0) were irrigated with 95% FC (full field capacity).

Data recorded

During vegetative stage (after 60 days from sowing) plant samples were collected to determine shoot height, root length, fresh and dry weights of shoot and root/plant. The collected fresh leaves from treated and control plants were used for analyses of photosynthetic pigments, indole acetic acid, antioxidant enzymes (POX, PPO, SOD, CAT, APX and NR) as well as hydrogen peroxide (H_2O_2) and lipid peroxidation (MDA). Whereas, oven- dried leaves for 72 h at 70 ºC were ground to a powder and kept in a desiccators to determine proline and total soluble sugars.At harvest, plants were collected to determine seed yield /plant (g). The collected seeds were cleaned (to separate the overlapping parts) and ground for determination of oil. The yielded oils were used for determination and identification of fatty acids composition. The defatted meals were used for determination of total protein, carbohydrate, phenolic content, tannins, flavonoids and antioxidant activity.

Measurements

Photosynthetic pigments (Chlorophyll a, chlorophyll b and carotenoids) were estimated using the method of Moran (1982). Indole acetic acid was determined according to the method reported by Larsen *et al.* (1962). Proline was estimated according to Bates *et al.* (1973). Total soluble sugars were determined according to Smith *et al.* (1956). 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}.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}. For enzyme determination**:** The method used for extracting the enzyme was that of Mukherjee and Choudhuri (1983). Peroxidase (POX, EC 1.11.1.7) activity assayed according to the method described by Bergmeyer (1974). Polyphenol oxidase (PPO, EC 1.10.3.1) activity assayed using the method of Kar and Mishra (1976). Superoxide dismutase (SOD, EC 1.12.1.1) activity measured according to the method of Dhindsa *et al.* (1981). Catalase (CAT, EC 1.11.1.6) activity assayed according to the method of Chen *et al.* (2000). The activity of ascorbate peroxidase (APX, EC 1. 11. 1. 11) was assayed according to Chen and Asada (1992). The activity of nitrate reductase (NR, EC 1. 7. 1. 1) was measured according to Jaworski (1971). The NR activity was expressed as nano moles of nitrite produced per gram fresh weight per hour (nM NO_2 / g

FW h^{-}). Seed oil content was determined using Soxhlet apparatus and petroleum ether (40-60 ^{o}C) according to (AOAC, 1990). Methyl esters of fatty acids were prepared from an aliquot of total lipid according to Harborne (1984). Fatty acid composition was determined quantitatively by gas liquid chromatography of methyl ester using a "HEWLETT PACKARD HP 6890 series GC system" instrument equipped with a flame ionization detector (FID). The capillary column "HP-INNOWAX polyethylene glycol"; length (30 m); diameter (530 mm) and film thickness (1 µm). Two injections were made from each sample. The operating conditions were: initial temp. 120ºC; final temp. 240ºC and detector temp. 300ºC. The nitrogen, hydrogen and air flow rates were 30, 30 and 300 ml/min respectively. The defatted meals were used for further analyses. Total carbohydrates were determined according to Dubois *et al.*(1956). The protein content was determined by microkjeldahl method according to (AOAC, 1990). Total phenolic compounds were determined according to the method described by Zhang and Wang (2001). Tannins were determined using the modified vanillin hydrochloric acid (MV-HCl) as reported by Maxson and Rooney (1972). Total flavonoid contents were measured by the aluminum chloride colorimetric assay as described by Ordoñez *et al*. (2006). The free radical scavenging activity was determined according to Brand- Williams *et al*. (1995) using the 1.1-diphenyl-2-picrylhydrazil (DPPH) reagent.

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) for a randomized complete block design after testing for homogeneity of error variances according to the procedure outlined by Gomez and Gomez (1984). Statistically significant differences between means were compared at $P \leq 0.05$.

Results and Discussion

Growth parameters

Moderate and severe drought stress (75% FC and 50% FC) caused marked decreases in canola plant growth parameters (shoot height, root length, fresh and dry weights of shoot and root/plant) relative to control plants (D_0GB_0) (Table 1). Since, moderate and severe drought stress (D_1GB_0 and D_2GB_0) decreased shoot dry weight/plant by 14.56% and 42.19% and root dry weight/plant by 25.6% and 30.4% respectively relative to control plant (D_0GB_0). These results are in harmony with Abass and Mohamed (2011) who reported that both fresh and dry weights of shoots and roots of common bean decreased with increasing drought stress and these reductions may be due to the metabolic disorders induced by stress and generation of ROS. The decline in shoot height and root length in response to drought might be due to decrease in cell elongation, cell turgor, cell volume and eventually cell growth (Banon *et al.,* 2006). The inhibition in root growth may be attributed to reduced extensibility of the root tip tissue due to hardening of the expanding cell walls. Further, Hussain *et al*. (2012) mentioned that the water deficit in root zone caused increase in root respiration rate, imbalance in the utilization of carbon resources, decrease in the production of adenosine triple phosphate and an increase in the production of ROS.

Table 1. Effect of glycinebetaine (GB) on some growth parameters of canola plants grown under different levels of drought stress (D) (means of two successive seasons)

Treatments		Shoot system			Root system		
		Height (cm)	Fresh wt/plant (g)	Dry wt/plant (g)	Length (cm)	Fresh wt/plant (g)	Dry wt/plant (g)
D_0	GB_0	56.0	51.01	6.66	20.5	4.67	1.25
	GB_1	58.6	58.27	6.88	22.0	5.43	1.36
	GB_2	62.0	59.95	7.45	24.0	6.08	1.42
	GB_3	66.0	62.02	10.08	23.2	6.36	1.85
D_1	GB_0	49.0	32.70	5.69	17.5	2.29	0.93
	GB_1	51.0	36.63	5.85	19.0	2.82	0.97
	GB_2	55.5	42.60	5.97	20.0	3.53	0.99
	GB_3	60.8	48.36	6.86	21.0	4.36	1.38
D_2	GB_0	31.5	17.57	3.85	14.0	1.56	0.87
	GB_1	45.0	28.00	4.47	14.67	2.50	0.88
	GB_2	47.0	31.70	5.08	16.5	2.83	0.92
	GB_3	46.5	30.00	5.31	17.0	2.62	0.95
LSD 5%		7.13	6.13	2.13	1.01	0.17	0.11

D_0 (95% FC); D_1(75 % FC);D_2(50 % FC);GB_0(0 mM);GB_1(10 mM);GB_2(15mM);GB_3(20 mM).

On the other hand, GB treatments proved to be effective in enhancing shoot height, root length, fresh and dry weights of shoot and root under unstressed, moderate and severe drought stressed plants (Table 1). It was noted that 20 mM GB at unstressed, moderate and severe drought stress (D_0GB_3 , D_1GB_3 and D_2GB_3) caused increases in dry weight of shoot by 51.35%, 20.56% and 37.92% respectively and increases in dry

weight of root by 48.0%, 48.38% and 9.19% relative to corresponding controls (D_0GB_0, D_1GB_0 and D_2GB_0) respectively. The beneficial role of GB in promoting shoot and root biomass in wheat under drought stress has already been reported by Mahmood *et al.* (2009). In addition, Aldesuquy (2014) mentioned that exogenous application of GB could counteract the adverse effects of drought by improvement of growth vigor of root and shoot, leaf area, retention of pigments content, increasing the concentration of osmoprotectants, keeping out the polysaccharides concentration and/or stabilization of essential proteins.

Photosynthetic pigments

Moderate and severe drought stress (75% FC and 50% FC) caused significant decreases in all components of photosynthetic pigments relative to control plant (D_0GB_0) (Table 2). Abass and Mohamed (2011) reported that contents of photosynthetic pigments in leaves of common bean plants were significantly decreased with increasing the level of drought stress. Drought inhibits the photosynthesis process of plants may be due to oxidative stress by causing pigment photo-oxidation, damaging to photosynthetic apparatus and leading to decrease in photosynthetic carbon assimilation (Din *et al.,* 2011).

On the other hand, GB treatments at all concentrations caused marked increases in photosynthetic pigments in fresh leaf tissues of unstressed plants (95% FC) as well as in fresh leaf tissues of plants that exposed to moderate (75% FC) and severe (50% FC) drought stress relative to corresponding controls (Table 2). Glycinebetaine application was found to be effective in mitigating the harmful effects of water deficit conditions on photosynthetic capacity of plants possibly due to its role in preventing photoinhibition (Ma *et al.,* 2006), protection of Rubisco enzyme and lipids of the photosynthetic apparatus and maintaining electron flow through thylakoid membranes thereby maintaining photosynthetic efficiency (Allakhverdiev *et al*., 2003; Shahbaz *et al*., 2011).

Table 2. Effect of glycinebetaine (GB) on photosynthetic pigments, IAA, proline and total soluble sugars of canola leaves at different levels of drought stress (D) (means of two successive seasons)

Treatments		Photosynthetic pigments (mg/g fresh weight)			IAA (µg/ 100g fresh weight)	Proline (mg/100 g dry weight)	Total soluble sugars (mg/g dry weight)
		Chlorophyll a	Chlorophyll b	Carotenoid			
D_0	GB_0	1.93	0.64	0.58	18.45	30.46	19.18
	GB_1	2.56	0.74	0.59	31.45	36.26	19.75
	GB_2	2.53	0.95	0.69	56.16	49.42	22.35
	GB_3	2.89	0.96	0.77	73.90	61.17	31.75
D_1	GB_0	1.52	0.54	0.35	13.25	44.02	30.85
	GB_1	1.92	0.70	0.38	26.55	48.16	36.10
	GB_2	2.36	0.73	0.66	40.60	62.70	45.25
	GB_3	2.25	0.75	0.63	56.65	76.44	46.75
D_2	GB_0	1.18	0.48	0.22	9.35	58.71	48.58
	GB_1	1.27	0.69	0.24	22.39	60.19	50.25
	GB_2	1.55	0.71	0.37	38.55	64.19	50.85
	GB_3	1.69	0.72	0.49	42.75	79.39	57.60
LSD 5%		0.26	0.08	0.18	4.39	1.37	5.81

D_0 (95% FC); D_1(75 % FC);D_2 (50 % FC);GB_0(0 mM); GB_1(10 mM);GB_2(15mM);GB_3(20 mM).

IAA

Drought stress (moderate and severe) significantly decreased IAA concentration in fresh canola leaves by 28.18% and 49.32% respectively than that of control (D_0GB_0) (Table 2). These decreases may be due to increase the destruction of IAA by increasing the activity of IAA oxidase (Bano and Samina, 2010).

On contrast, Table 2 indicates that GB treatments significantly increased IAA concentrations in unstressed plants as well as in drought stressed plants by increasing GB concentrations relative to corresponding controls. 20 mM GB had the highest beneficial effect in ameliorating the harmful effect of drought stress on IAA either under moderate or severe drought stress relative to corresponding controls. Aldesuquy (2014) mentioned that exogenous spray with GB may increase the drought tolerance by acceleration of growth promoters (IAA, GA_3 and cytokinins) and at the same time reduced accumulation of inhibitor represented by ABA in flag leaves of wheat plants.

Compatible solutes (proline and total soluble sugars)

Under water stress, plants accumulate greater quantity of compatible organic solutes, which shield them from stress through stabilizing of membranes, tertiary structures of enzymes and proteins (Ashraf and Foolad, 2007). Table 2 shows that either moderate or severe drought stress caused significant increases in proline concentration of canola leaves relative to control plants (D_0GB_0). This finding is in consistent with Keyvan (2010) who found that under water stress, proline is accumulated in wheat cultivars and this accumulation is positively correlated with stress tolerance. These increases may be attributed to reduced proline

oxidase, proline catabolising enzymes as mentioned by Debnath (2008). In addition, proline has been considered as a carbon and nitrogen source for rapid recovery from stress and acting as stabilizer for membranes and some macromolecules and also as a free radical scavenger (Mousavi *et al*., 2009).

Moreover, Table 2 shows clearly that GB treatments significantly increased proline concentrations in canola leaves under moderate and severe drought stress as well as in unstressed plants relative to corresponding controls. The accumulation of proline under drought stress and GB treatment is consistent with the early findings of Hussain *et al.* (2009).

Regarding soluble sugars, it was found significant increases in soluble sugars due to moderate and severe drought stress relative to control plants (D_0GB_0) (Table 2). Soluble sugars are accumulated in plants of many species that are subjected to water stress. Mousavi *et al*. (2009) stated that water stress caused a remarkable increase in sugars content that might play a role in the osmotic adjustment. In addition, accumulations of soluble carbohydrates increase the resistance of plant to drought stress (Keyvan, 2010).

Table 2 shows clearly that all concentrations of GB increased soluble sugars in unstressed plants (95% FC) as well as in drought stressed plants (75% FC and 50% FC) and these increases were significant at the highest level of GB (20 mM) relative to corresponding controls. These results are similar to those obtained by Ibrahim (2004) who found that salinity stressed sorghum plants that treated with GB, accumulated more soluble sugars than the salinity stressed plants only.

Lipid peroxidation and H_2O_2

Table 3 shows that either moderate or severe drought stress caused significant increases in MDA and H_2O_2 in fresh leaf tissues relative to control plants. The malonyldialdehyde (MDA) is regarded as a marker for evaluation the degree of the lipid peroxidation or damage to plasmalemma and organelle membranes that associated with damages provoked by ROS due to environmental stresses (Ozkur *et al.,* 2009). Drought stress caused modification in the lipid matrix of the plasma membrane and changes in the physical organization of the membrane (Mirzaee *et al.,* 2013). In addition, it caused marked increases in MDA and H_2O_2 levels in different plant species including rapeseed and mustard may be due to inadequate induction of antioxidant system as mentioned by Hossain *et al.* (2013).

Meanwhile, GB treatments significantly decreased MDA and H_2O_2 in fresh leaf tissues of canola plants irrigated with 95% FC or 75% FC or even 50% FC relative to corresponding controls. The most pronounced treatment was 20 mM GB. The GB-treatment reduced the MDA contents which led to the cell membrane stability by reducing ROS (Farooq *et al*., 2010) and substantially ameliorates the impacts of drought on membrane integrity and stability in the maize plants (Anjum *et al*.,2012). This reduction in MDA contents could be attributed to the putative role of osmolytes in alleviating the deleterious effects of stress on the structure of cell membranes and activities of different enzymes as well as reducing the generation of highly destructive free radicals (Smirnoff and Cumbes, 1989; Ali, 2011).

Antioxidant enzymes

Antioxidant enzymes (POX, PPO, SOD, CAT and APX) were significantly increased accompanied by significant decreases in NR due to moderate and severe drought stress relative to control plants (Table 3). In this context, increased activities of CAT, POX and APX has been reported in plants grown under stress conditions (Hoque *et al*., 2007). Gharache *et al.* (2013) mentioned that increases in SOD and APX activities promote the removal of reactive oxygen species (ROS), thus conferring higher drought resistance to plants. Abedi and Pakniyat (2010) showed that SOD and POX activities were increased in some canola cultivars and linked with protection from oxidative damage due to drought stress. Superoxide is converted by the SOD enzyme into H_2O_2 (Noctor and Foyer, 1998) which must be scavenged to O_2 and water by the antioxidant enzymes such as CAT, POX and APX (Ozkur *et al*., 2009). Further, under drought stress, the activities of CAT and POX were increased and involved in elimination of H_2O_2 from stressed cells and presumed to limit cellular damage and enhance the plants oxidative capacity to defend stress (Nojavan and Khorshidi, 2006). High activity of CAT indicated drought tolerance in some of the canola cultivars (Omidi, 2010). APX scavenges peroxide by converting ascorbic acid to dehydroascorbate (Ozkur *et al*., 2009). The activities of certain enzymes especially nitrate reductase sharply decreased as water deficit increased (Hussain *et al.,* 2012).

Table 3 shows that all GB treatments significantly increased antioxidant enzymes (POX, PPO, SOD, CAT, APX and NR) in canola plants irrigated with different levels of water relative to corresponding controls. Since, GB treated plants can maintain higher antioxidative enzyme activities in a reasonable level to prevent damage caused by oxidative stress (M*a et al*., 2006). Similarly, Raz*a et al.* (2007) found that the adverse effects of salt stress on wheat plants can be ameliorated by exogenous application of GB as it acts on accelerating the activities of antioxidant enzymes and changing water relations and ion homeostasis. In another study Hoque *et al. (*2007) found that exogenous application of GB alleviated the salt induced reduction in the activities of antioxidant enzymes such as CAT and POX activities. GB protects membranes and proteins against the destabilizing effects of dehydration during abiotic stress and it has some ability to scavenge free radicals (Ashraf and Foolad, 2007).

Table 3. Effect of glycinebetaine (GB) on malondialdehyde, hydrogen peroxide and antioxidant enzymes of fresh canola leaves at different levels of drought stress (D) (means of two successive seasons)

Treatments		MDA	H_2O_2	POX	PPO	SOD	CAT	APX	NR
		µmol /g fresh wt		Unit /min /g fresh wt					(nM NO_2^- /g fresh wt / h)
D_0	GB_0	8.11	2.69	333	18.30	18.35	18.75	0.413	300
	GB_1	4.41	1.95	402	26.13	20.82	28.62	0.432	342
	GB_2	4.52	1.11	592	33.95	24.30	36.65	0.451	362
	GB_3	4.31	0.91	672	41.00	27.20	46.60	0.481	384
D_1	GB_0	14.11	11.74	474	24.1	23.45	38.90	0.482	280
	GB_1	11.90	7.83	558	35.45	24.70	43.80	0.512	292
	GB_2	10.77	7.44	693	39.10	30.40	58.35	0.542	311
	GB_3	9.36	6.49	720	45.53	35.50	60.60	0.560	335
D_2	GB_0	23.45	13.98	529	33.30	33.60	49.20	0.528	242
	GB_1	16.34	11.44	647	38.75	40.55	59.85	0.552	280
	GB_2	13.01	10.30	805	47.15	45.75	64.45	0.560	301
	GB_3	7.97	9.36	893	60.62	53.40	72.85	0.580	320
LSD 5%		0.27	0.27	17.29	2.13	2.65	2.87	0.023	10.25

D_0 (95% FC); D_1(75 % FC);D_2 (50 % FC);GB_0(0 mM);GB_1(10 mM);GB_2(15mM);GB_3(20 mM).

Wang *et al*. (2010) suggested that GB induces increase in osmotic adjustments for drought tolerance by improving antioxidative defense system including antioxidant enzymes in wheat crop. Our results are in line with those of Ma *et al*. (2006) who found that GB-treated wheat plants that exhibited increased SOD and APX activities showed higher photosynthetic activity and water stress tolerance. Furthermore, GB-treated rice plants exhibit increases in SOD, POX and CAT activities, indicating a more efficient quenching of ROS (Farooq *et al*., 2010).

Seed yield,oil,carbohydrate and protein contents.

It is worthy to mention that the yield and biochemical composition of a plant mainly depends on growth conditions, which is markedly affected by water availability. Table 4 indicates that drought stress at 75% FC and 50% FC significantly decreased seed yield/plant, oil and carbohydrate contents accompanied by significant increases in protein content relative to control plants (D_0GB_0). The decreases in seed yield /plant were 24.76% and 55.24% due to drought stress at 75% FC and 50% FC respectively relative to control plants. Drought stress reduced the crop yield due to reduction in photosynthetic pigments (Anjum *et al*., 2003) and diminished activities of calvin cycle enzymes (Ashraf *et al*., 2013). Ali *et al.* (2010) stated that changes in seed chemical composition could have been due to the reason that low water supply during the plant life affects many enzymes whose activity is reduced under water stress conditions and leading to changes in metabolic activities that result in altered translocation of assimilates to seeds. Ali and Alqurainy (2006) mentioned that the main cellular components susceptible to damage by free radicals are lipids (peroxidation of unsaturated fatty acids in membranes), proteins (denaturation), carbohydrates and nucleic acids.The reduction in the oil content under drought stress could be due to oxidation of some of the polyunsaturated fatty acids (Singh and Sinha, 2005). Carbohydrate changes are of particular importance because of their direct relationship with such physiological processes as photosynthesis, translocation, and respiration. Water stress decreased the pigments concentration in leaves which results in inhibition of photosynthetic activity, in turn it leads to less accumulation of carbohydrates in mature leaves and consequently may decrease the rate of transport of carbohydrates from leaves to the developing seeds. Regarding increments in protein content under drought stress, Ahmad *et al*. (2009) reported that seed protein content of sunflower was significantly higher in irrigation missing at flowering stage than in control, when crop was given normal irrigations.

Regarding GB effect, Table 4 shows that all GB treatments caused significant increases in seed yield, oil, carbohydrate, and protein contents in the yielded canola seeds either in plants irrigated with 75% FC or 50% FC relative to corresponding controls. It is clear that, the 20 mM GB was the most pronounced and effective treatment in alleviating the deleterious effect of moderate or severe drought stress. The increases in seed yield/plant due to 20 mM GB were 30.80% and 60.28% at 75% FC and 50% FC respectively relative to corresponding controls. In other words, these results indicate that 20 mM GB had effective role under severe drought stressed conditions more than moderate drought stressed conditions. Glycinebetaine (GB) promotes plant growth and yield under normal or stress conditions due to its osmoprotective effect on photosynthetic machinery and regulation of ion homeostasis (Raza *et al*., 2007) as well as improving CO_2 assimilation in plants under drought stress (Hussain *et al*., 2008) and because of its role in biosynthesis and transport of hormones like cytokinins that may have a role in the transport of photoassimilates (Taiz and Zeiger, 2006).

Table 4. Effect of glycinebetaine (GB) on seed yield, chemical composition and antioxidant activity of the yielded canola seeds at different levels of drought stress (D) (means of two successive seasons).

Treatments		Seed yield/plant (g)	Oil (%)	Carbohydrate (%)	Protein (%)	Phenolic content (mg/g)	Tannins (mg/100g)	Flavonoids (mg/g)	Antioxidant activity (%)
D_0	GB_0	3.15	43.92	19.50	18.66	15.95	0.82	0.37	31.0
	GB_1	3.32	44.47	20.65	22.45	19.05	0.97	0.44	32.0
	GB_2	3.35	45.68	21.45	22.49	19.30	1.20	0.42	34.0
	GB_3	4.07	45.01	23.92	22.98	22.30	2.34	0.55	37.0
D_1	GB_0	2.37	40.20	15.52	21.83	14.70	0.80	0.36	26.0
	GB_1	2.84	42.48	19.06	22.31	17.15	1.11	0.41	28.5
	GB_2	2.95	43.20	17.50	23.58	17.20	1.22	0.42	30.0
	GB_3	3.10	43.09	20.66	24.54	17.60	1.39	0.44	31.0
D_2	GB_0	1.41	32.99	13.13	22.67	8.45	0.39	0.32	20.5
	GB_1	1.48	35.08	14.77	23.87	14.25	0.58	0.33	22.5
	GB_2	1.64	36.45	17.66	26.11	15.15	0.94	0.35	23.5
	GB_3	2.26	38.05	17.84	27.29	17.90	1.11	0.47	24.8
LSD 5%		0.18	1.07	0.86	0.50	1.09	0.16	0.09	1.51

D_0 (95% FC); D_1 (75 % FC); D_2 (50 % FC); GB_0(0 mM); GB_1(10 mM); GB_2(15 mM);GB_3(20 mM).

Phenolic content, tannins, flavonoids and antioxidant activity

Moderate and severe drought stress (75% FC and 50% FC) caused marked decreases in total phenolic content, tannins, flavonoids and antioxidant activity of the yielded seeds (Table 4). These decreases were significant in total phenolic content and antioxidant activity. Total phenolic contents in oilseeds are very important for the oxidative stability of the polyunsaturated fatty acids of oils and indicative of antioxidant activity (Rice-Evans *et al.,* 1997; Ali *et al*., 2010; Ali *et al*., 2013). These decreases in total phenolic contents of canola seeds under drought stress are similar to those reported byAli *et al*. (2010); Ali and Ashraf (2011) who mentioned that water deficit significantly decreased the concentration of phenolic compounds in maize. Regarding flavonoid content, in contrast to our results of Table 4, Ali et al. (2010) indicated that water stress significantly increased the flavonoids contents in maize plants. Meanwhile, the decreases in antioxidant activity under drought stress are in agreement with Ali *et al*. (2013) who reported that the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity, decreased significantly in maize due to drought stress.

On the other hand, Table 4 shows that all GB treatments caused significant increases in total phenolic content, tannins, and antioxidant activity of the yielded seeds and caused non-significant increases in flavonoid contents either in plants irrigated with 95% FC or 75% FC or 50% FC relative to corresponding controls. 20 mM GB was the most pronounced and effective treatment. The increases in antioxidant activity due to 20 mM GB were 19.35 %, 19.23 % and 20.97% at 95% FC, 75% FC and 50% FC respectively relative to corresponding controls. Ali and Ashraf (2011) revealed that foliar-applied compatible solutes as glycinebetaine could enhance the levels of phenolic compound in maize under water deficit conditions. Ali *et al*. (2013) indicated that exogenous application of osmolyte as proline increased flavonoids in drought stressed maize plants. A positive correlation in seed oil antioxidant activity and different antioxidant compounds under the effect of water deficit condition and exogenous application of organic osmolytes had already been reported in some earlier studies in maize (Ali *et al*., 2010; Ali and Ashraf, 2011). In addition, GB had the capacity to scavenge free radicals which is more important than their role as a mere osmolyte (Ashraf and Foolad, 2007; Ali, 2011).

It has been reported that exogenously applied osmolytes increased the contents of antioxidant compounds under stress conditions. The increases in these metabolites due to osmolytes was found to be negatively associated with leaf MDA contents, thus showing the role of these osmolytes in plant oxidative defense mechanism by increasing the accumulation of these antioxidant secondary metabolites under stress conditions. Such effects of exogenously applied osmolytes in increasing the contents of these compounds may be due to the reason that these compatible solutes act as a regulatory or signaling molecule to activate multiple physiological and biochemical processes as well as plant adaptation processes under stress conditions (Ashraf and Foolad, 2007; Ali, 2011).

Fatty acid composition

The fatty acid profile of canola oils (Table 5) reveals that drought stress caused increases in saturated fatty acids (palmitic , stearic, arachidic) accompanied by decreases in behenic acid relative to control plants. Meanwhile, unsaturated fatty acids (oleic and linoleic) were decreased by drought stress accompanied by increases in linolenic, gadoleic and erucic acids. Generally, moderate and severe drought stress increased total saturated fatty acids and decreased unsaturated fatty acids relative to control plants. Environmental stresses such as drought have significant effects on seed oil fatty acid composition (Flagella *et al.,* 2002; Ali *et al.,* 2010; Ali *et al*., 2013).

Results in Table 5 are similar to those reported by Pham-Thi *et al.* (1986) who reported that water deficiency decreased the degree of fatty acids unsaturation which was attributed to the inhibition in the

biosynthesis of polyunsaturated fatty acids and suppression in the activities of desaturases. Mekki *et al.* (1999) also showed that drought stress increased the percentage of palmitic acid and reduced unsaturated fatty acids. Ensiye and Khorshid (2010) studied the response of safflower to irrigation regimes and reported that the oil content and oleic and linoleic acid percentage were significantly reduced by drought stress. Tohidi-Moghadam *et al*. (2011) mentioned that water deficit stress conditions decreased canola oil and linoleic acid contents, but increased stearic, α linolenic, and gadoleic acids contents. Ullah *et al*. (2012) stated that drought stress reduced the oil quality of canola by decreasing oleic acid content and increasing the erucic acid content. Ali *et al.* (2014) stated that maximum erucic acid content was observed in treatments of 60% irrigation and minimum erucic acid content was observed in plants treated with100% irrigation level.

The fatty acid profile of canola oils showed different responses to GB treatments in unstressed plants and drought stressed plants (Table 5). Since, well irrigated plants (95% FC) showed increases in stearic acid accompanied by decreases in behenic acids relative to control plants. Palmitic acid was increased by 10 mM GB and decreased by 15 and 20 mM GB. Hence, total saturated fatty acids was increased slightly by 10 mM GB and decreased by 15 and 20 mM GB. Further, oleic acid and erucic acids were decreased by GB treatments while linoleic, linolenic and gadoleic acids were increased. So, GB treatments caused slight changes in total unsaturated fatty acid in unstressed plants. Regarding interaction between drought stress and GB treatments, it was noted that moderate and severe drought stress caused decreases in palmitic and stearic acid and different responses in arachidic and behenic due to different GB treatments. Oleic and linoleic acids were increased accompanied by decreases in linolenic and erucic acids under the interaction effect of GB treatments and drought stress (75% FC and 50% FC) and these results led to decreases in total saturated fatty acid and increases in unsaturated fatty acid relative to corresponding controls.

Compatible solutes (GB or proline) improved the oil quantity and quality due to their protective effect on cellular structures during fatty oil biosynthesis and storage, which occurs in liposomes or oleosomes in seeds during seed filling stage (Taiz and Zeiger, 2006; Ali *et al*.,2013). Ali (2011) stated that exogenous GB improved the quality of oil by decreasing the un-saponifiable matter and increasing oil saponification and iodine values, the measure of oil unsaturation.

Table 5. Effect of glycinebetaine (GB) on fatty acid composition of the yielded canola oils at different levels of drought stress (D).

Treatments		Fatty acid composition (%)									TSFA	TUFA
		16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1		
D_0	GB_0	3.88	0.49	61.93	19.98	9.24	1.43	0.37	1.54	0.89	7.34	92.41
	GB_1	4.41	0.65	53.25	22.44	15.29	1.37	0.73	1.13	0.63	7.56	92.34
	GB_2	3.82	0.59	43.23	32.67	16.43	1.23	0.32	1.12	0.51	6.76	93.16
	GB_3	3.34	0.68	43.94	31.99	15.81	1.59	0.48	1.28	0.73	6.89	92.95
D_1	GB_0	4.54	0.77	56.87	17.79	15.15	1.67	0.77	0.98	1.31	7.96	91.89
	GB_1	4.32	0.73	68.60	16.56	5.69	1.75	0.52	0.66	1.0	7.46	92.37
	GB_2	3.43	0.74	63.39	19.72	7.63	2.18	0.84	1.03	0.96	7.38	92.54
	GB_3	3.44	0.95	63.69	20.42	8.14	----	1.40	0.68	1.13	5.07	94.78
D_2	GB_0	6.22	1.56	59.16	13.37	10.81	2.64	3.19	1.14	1.56	11.56	88.09
	GB_1	3.20	1.17	61.09	19.74	7.78	3.12	2.04	0.93	0.88	8.42	91.53
	GB_2	2.90	1.20	62.90	20.03	6.67	1.32	2.27	1.17	1.33	6.59	93.20
	GB_3	3.57	1.52	65.60	15.47	8.62	1.43	1.37	1.14	1.20	7.66	92.26

D_0 (95% FC); D_1 (75 % FC); D_2 (50 % FC); GB_0(0 mM); GB_1(10 mM); GB_2(15 mM);GB_3(20 mM).
16:0(palmitic);18:0(stearic);18:1(oleic);18:2(linoleic);18:3(linolenic);20:0(arachidic);20:1(gadoleic);22:0(behenic);22:1(erucic);
TSFA(total satutated fatty acids); TUFA(total unsaturated fatty acids).

References

Abass, S.M. and H.I. Mohamed, 2011. Alleviation of adverse effects of drought stress on common bean (*Phaseolus vulgaris* L.) by exogenous application of hydrogen peroxide. Bangladesh J. Bot., 41: 75-83.

Abedi, T. and H.Pakniyat, 2010. Antioxidant enzyme changes in response to drought stress in ten cultivars of oilseed rape (*Brassica napus* L.). Czech J. Genet. Plant Breed., 46: 27-34.

Agboma, P., T. Sinclair, K. Jokinen, P. Peltonen-Sainio, and E. Pehu, 1997. An evaluation of the effect of exogenous glycinebetaine on the growth and yield of soybean. : Timing of application watering regimes and cultivars. Field Crops Res., 54:51-64.

Ahmad, M., A. Haji, A. Bukhsh, A. U. Malik, M. Ishaque and S. H. Sadiq, 2009. Performance of sunflower response to exogenously applied salicylic acid under varying irrigation regimes. The J. of Animal and Plant Sci., 19: 130-134.

Aldesuquy, H. S.,2014. Glycinebetaine and salicylic acid induced modification in water relations and productivity of drought wheat plants. J. of Stress Physiology and Biochem., 10 : 55-73.

Ali, A. and F. Alqurainy, 2006. Activities of antioxidants in plants under environmental stress. In: MotohashiN (ed.), The lutein-prevention and treatment for diseases. Trans-world Research Network, India PP.187-256.

Ali, M., G. Khan and F. Akbar, 2014.The effect of different levels of irrigation and potassium (K) application on seed erucic content for different varieties of brassica under field conditions. Chem. and Materials Res., 6:97-100.

Ali, Q., 2011. Exogenous use of some potential organic osmolytes in enhancing drought tolerance in maize (*Zae mays* L.). A thesis submitted in partial fulfillment of the requirements for the degree of doctor of philosophy. Botany Dep., Fac. of Sci., Univ. of Agric., Faisalabad, Pakistan.pp.312.

Ali, Q., F. Anwar, M. Ashraf, N. Saari, and R. Perveen, 2013. Ameliorating effects of exogenously applied proline on seed composition, seed oil quality and oil antioxidant activity of maize (*Zea mays* L.) under drought stress. Int. J. Mol. Sci., 14: 818-835.

Ali, Q. and M. Ashraf, 2011. Exogenously applied glycinebetaine enhances seed and seed oil quality of maize (*Zea mays* L.) under water deficit conditions. Environ. Exp. Bot., 71:249-259.

Ali, Q., M. Ashraf, and F. Anwar, 2010. Seed composition and seed oil antioxidant activity of maize under water stress. J. Am. Oil Chem. Soc., 87:1179-1187.

Ali, Q., M. Ashraf, and H.R. Athar, 2007. Exogenously applied proline at different growth stages enhances growth of two maize cultivars grown under water deficit conditions. Pak. J. Bot., 39: 1133-1144.

Allakhverdiev, S. I., H. Hayashi, Y. Nishiyama, A. G. Ivanov, J. A. Aliev, V. V. Klimov, N. Murata and R. Carpentier, 2003. Glycinebetaine protects the D1/D2/Cytb559 complex of photosystem II against photo-induced and heat-induced inactivation. J. Plant Physiol., 160:41-49.

Anjum, F., M. Yaseen, E. Rasul, A. Wahid and S. Anjum, 2003. Water stress in barley. I. Effect on chemical composition and chlorophyll content. Pakistan J. Agric. Sci., 40: 45-49.

Anjum, S. A., M. F. Saleem, L. Wang, M. F. Bilal and A. Saeed, 2012. Protective role of glycinebetaine in maize against drought-induced lipid peroxidation by enhancing capacity of antioxidative system. Australian J. Crop Sci., 6:576-583.

A.O.A.C., 1990. Official Methods of Analysis. 20th edition. Association of Official Analytical Chemists, Arlington, Virginia, U.S.A. (No.920.39 for oil and 984.13 for protein).

Ashraf, M. and M.R. Foolad,(2007. Role of glycinebetaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot., 59:206-216.

Ashraf, M., M. Shahbaz and Q. Ali, 2013. Drought-induced modulation in growth and mineral nutrients in canola (*Brassica napus* L.). Pak. J. Bot., 45: 93-98.

Bano, A. and Y. Samina, 2010. Role of phytohormones under induced drought stress in wheat. Pak. J. Bot., 42: 2579-2587.

Banon, S.J., J. Ochoa, J.A. Franco, J.J. Alarcon, and M.J. Sanchez-Blanco, 2006. Hardening of oleander seedlings by deficit irrigation and low air humidity. Environ. Exp. Bot., 56: 36-43.

Bates, L.S., R.P. Waldan, and L.D. Teare, 1973. Rapid determination of free proline under water stress studies. Plant and Soil, 39: 205-207.

Bergmeyer, H.U., 1974. Methods of Enzymatic Analysis*"* I. Second edition. Academic Press. New York.

Bohnert, H. J. and R. J. Jensen, 1996. Strategies for engineering water-stress tolerance in plants.Trends Biotecnol., 14: 89-97.

Brand-Williams, W., M.E. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. Lebensmittel Wissenschaften und Technologi, 28: 25-30.

Chen, G. and K. Asada, 1992. Inactivation of ascorbate peroxidase by thoils requires hydrogen peroxide. Plant Cell Physiol., 33: 117-123.

Chen, Y., X. D. Cao, Y. Lu, and X. R. Wang, 2000. Effect of rare earth metal ions and their EDTA complexes on antioxidant enzymes of fish liver. Bull. Environ. Contam. Toxicol., 65:357-365.

Debnath, M., 2008. Responses of *Bacopa monnieri* to salinity and drought stress in vitro. J. Medicinal Plants Res., 11: 347-351.

Dhindsa R., P. Plumb-Dhindsa and T. Thorpe, 1981. Leaf senescence correlated permeability, lipid peroxidation and decreased levels of superoxide dismutase and catalase. J. Exp. Bot., 32:93-101.

Din, J., S.U. Kans, J. Ali, and A.R. Gurmani, 2011. Physiological and agronomic response of canola varieties to drought stress. The J. of Animal and Plant Sci., 21: 78-82.

Dubois, M., K.A. Guilles, J.K. Hamilton, P.A. Rebers, and F. Smith, 1956.Colorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350-356.

Ensiye, A. and R. Khorshid, 2010. Effect of irrigation regimes on oil content and composition of safflower (*Carthamus tinctorius* L.) cultivars. J. of the American Oil Chemists' Society. 1527-1528.

Farooq, M., A. Wahid, N. Kobayashi, D. Fujita, and S.M.A. Basra, 2009. Plant drought stress: effects, mechanisms and management. Agronomy for Sustainable Development, 29: 185-212.

Farooq, M., A. Wahid, D.J. Lee, S.A. Cheema and T. Aziz, 2010. Comparative time course action of the foliar applied glycinebetaine, salicylic acid, nitrous oxide, brassinosteroids and spermine in improving drought resistance of rice. J. Agron. Crop Sci., 196:336-345.

Flagella, Z., T. Rotunno, E. Tarantino, R. Caterina, and A. Caro, 2002. Changes in seed yield and oil fatty acid composition of high oleic sunflower (*Helianthus annuus* L.) hybrids in relation to the sowing date and the water regime. Eur. J. Agron., 17: 221-230.

Food and Agriculture Organization (FAO), 2011. Crop Production Statistics. http://www.Fao.org.

Gadallah, M.A.A., 1999. Effect of proline and glycinebetaine on *Vicia faba* responses to salt stress. Biol. Plant., 42: 249-257.

Gharache, A., A.S. Kalidari, M.M. Rahimi, and M. Khalatbari, 2013. Evaluation the effect of different ranges super absorbent on quality and physiological characteristics of canola (*Brassica napus* L.) cvs Zarfam under water deficit stress. Technical J. of Engineering and Applied Sci., 3-2:165-169.

Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agricultural Research. John Wiley & Sons Inc., Singapore, p.680.

Harborne, J.B.,1984. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis 2nd_Edition, London, N.Y., P.15.

Heath, R.L. and L. Packer, 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives in Biochem. and Biophysics,125**:**189-198.

Hoque, M.A., E. Okcma, M.N.A. Banu, Y. Nakamura, Y.Shimoishi, and Y. Murata, 2007. Exogenouus proline mitigates the detrimental effects of salt stress more than exogenous betaine by increasing antioxidant enzyme activities, J. of Plant Physiology 164: 553-561.

Hossain, M. A., M. G. Mostofa, and M. Fujita, 2013. Cross protection by cold-shock to salinity and drought stress-induced oxidative stress in mustard (*Brassica campestris* L.) seedlings. Molecular Plant Breeding, 4: 50-70.

Hussain, M., M. Farooq, K. Jabran, H. Rehman and M. Akram, 2008. Exogenous glycinebetaine application improves yield under water-limited conditions in hybrid sunflower. Arch. Agron. Soil Sci., 54: 557-567.

Hussain, M., M.A. Malik, M. Farooq, M.B. Khan, M. Akram, and M.F. Saleem, 2009. Exogenous glycinebetaine and salicylic acid application improves water relations, allometry and quality of hybrid sunflower under water deficit conditions. J. Agron. Crop Sci., 195:98-109.

Hussain, S., A. Ali, M. Ibrahim, M. F. Saleem, and M. A. Alias Haji A. Bukhsh, 2012. Exogenous application of abscisic acid for drought tolerance in sunflower (*Helianthus annuus* L.): A review. The J. of Animal and Plant Sci., 22: 806-826.

Ibrahim, A. H., 2004. Efficacy of exogenous glycinebetaine application on sorghum plants grown under salinity stress. Acta Bot. Hungarica, 46: 307-318.

Jana, S. and M. Choudhuri, 1981. Glycolate metabolism of three submerged aquatic angiosperms during aging J. Aquat. Bot., 12: 345-354.

Jaworski, E.G., 1971. Nitrate reductase assay in intact plant tissues. Biochem. Biophys. Res. Commun., 43:1274-1279.

Kar, M. and D. Mishra, 1976. Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence. Plant Physiol., 57: 315-319.

Keyvan, S., 2010. The effects of drought stress on yield, relative water content, proline, soluble carbohydrates and chlorophyll of bread wheat cultivars. J. Anim. Plant Sci., 8:1051-1060.

Kusvuran,S., S. Ellialtioglu, and Z. Polat,2013. Antioxidative enzyme activity, lipid peroxidation, and proline accumulation in the callus tissues of salt and drought tolerant and sensitive pumpkin genotypes under chilling stress. Hort. Environ. Biotechnol., 54:319-325.

Larsen, P.A., S. Harbo, Klungron and T.A. Ashein, 1962. On the biosynthesis of some indole compounds in *Acetobacter xylinum*. Physiol. Plant, 15: 552-565.

Lawlor,D.W. and G. Cornic, 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell Environ., 25: 275-294.

Lopez, C.M.L., H. Takahashi and S. Yamazaki, 2002. Plant water relations of kidney bean plants treated with NaCl and foliarly applied glycinebetaine. J. Agron. Crop Sci., 188: 73-80.

Ma, Q. Q., W. Wang, Y. H.Li, D. Q. Li, and Q. Zou, 2006. Alleviation of photoinhibition in drought-stressed wheat (*Triticum aestivum*) by foliar-applied glycinebetaine. J. Plant Physiol., 163: 165-175.

Mahmood, T., M. Ashraf, and M. Shahbaz, 2009. Does exogenous application of glycinebetaine as a pre-sowing seed treatment improve growth and regulate some key physiological attributes in wheat plants grown under water deficit conditions. Pak. J. Bot., 41:1291-1302.

Maxson, E.D. and L.W. Rooney, 1972. Two methods of tannin analysis for *sorghum bicolor* (L.) Moench. Crop Sci., 12: 253.

Mekki, B. B., M. A. EL-Kholy, E. M. Mohamad, 1999. Yield, oil and fatty acids contents as affected by water deficit and potassium fertilization in two sunflower cultivars. Egyptian J. of Agronomy, 21: 67-85.

Mirzaee, M., A. Moieni, and Ghanati, F. (2013). Effects of drought stress on the lipid peroxidation and antioxidant enzyme activities in two canola (*Brassica napus* L.) cultivars. J. Agron. Sci. Tech., 15: 593-602.

Moran, R., 1982. Formula for determination of chlorophyllous pigments extracted with N.N. dimethylformamide. Plant Physiol., 69:1371-1381.

Mousavi, E. A., K. M. Kalantari, and S. R. Jafari, 2009. Change of some osmolytes accumulation in water-stressed colza (*Brassica napus* L.) as affected by 24-epibrassinolide. Iranian J. of Sci. and Technology, Transaction A, 33: A1.1-11

Mukherjee, S.P. and M.A. Choudhuri, 1983. Implication of water stress–induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in vigna seedling. Physiol. Plant., 58: 166- 170.

Noctor, G. and C. H. Foyer, 1998. Ascorbate and glutathione: keeping active oxygen under control. Ann. Rev. Plant Physiol. Plant Mol. Biol., 49: 249-279.

Nojavan, A.M. and M.Khorshidi, 2006. An investigation of vanillin imposed oxidative stress in corn (*Zea mays* L.) and the activities of antioxidative enzymes, Pakistan J. of Biolo. Sci., 9:34-38.

Omidi, H., 2010. Changes of proline content and activity of antioxidative enzymes in two canola genotype under drought stress. Am. J. Plant Physiol., 5: 338-349.

Ordoñez, A.A.L., J.D. Gomez, M.A. Vattuone and M.I. lsla, 2006. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chem., 97: 452-458.

Ozkur, O., F. Ozdemir, M. Bor, and I. Turkan, 2009. Physiochemical and antioxidant responses of the perennial xerophyte *Capparis ovata* Desf. to drought. Environ. Exp. Bot., 66:487-492.

Pham-Thi, A.T., C. Borrel-Flood, J. Vieira da Sila,; Justin, A.M. and P. Mazliak, 1986. Effects of water stress on lipid metabolism in cotton leaves. *Photochem*., 24 : 723-727.

Praba, M.L., J.E. Cairns, R.C. Babu, and H.R. Lafitte, 2009. Identification of physiological traits underlying cultivar differences in drought tolerance in rice and wheat. J. Agron. Crop Sci., 195:30-46.

Qureshi, K.M., S. Chughtai, U.S. Qureshi, and N. A Abbasi, 2013. Impact of exogenous application of salt and growth regulators on growth and yield of strawberry. Pak. J. Bot., 45:1179-1186.

Raza, S. H., H. R. Athar, M. Ashraf and A. Hameed, 2007. Glycinebetaine-induced modulation of antioxidant enzymes activities and ion accumulation in two wheat cultivars differing in salt tolerance. Environ. Exp. Bot., 60:368-376.

Rhodes, D. and A. D. Hanson, , 1993. Quarternary ammonium and tertiary sulfonium compounds in higher plants. Annu. Rev. Plant Physiol. Plant Mol. Biol., 44: 357-384.

Rice-Evans, C. A., N. J. Miller and G. Paganga, 1997. Antioxidant properties of phenolic compounds. Trends in Plant Sci., 2: 152-159.

Shahbaz, M., Y. Masood, S. Parveen, and M. Ashraf, 2011. Is foliar applied glycinebetaine effective in mitigating the adverse effects of drought stress on wheat (*Triticum aestivum* L.)? J. Appl. Bot. Food Tech., 84: 192-199.

Singh, S. and S. Sinha, 2005. Accumulation of metals and its effects in *Brassica juncea* (L.) Czern. (cv. Rohini) grown on various amendments of tannery waste. Ecotoxicology and Environmental Safety, Orlando, 62: 118- 127.

Smirnoff, N. and Q. J. Cumbes, 1989. Hydroxyl radical scavenging activity of compatible solutes. Phytochem., 28: 1057-1060.

Smith, F., M.A. Gilles, J.K. Hamilton, and P.A. Godees, 1956. Colorimetric method for determination of sugar related substances. Anal. Chem., 28: 350.

Taize, L. and E. Zeiger, 2006. Plant Physiology, 4th ed.; Sinauer Associates, Inc.:Sunderland, MA,USA.

Tohidi-Moghadam, H.R., H. Zahedi, and F. Ghooshchi, 2011. Oil quality of canola cultivars in response to water stress and super absorbent polymer application. Pesq. Agropec. Trop., Goiânia, 41: 579-586.

Ullah, F., A. Bano, and A. Nosheen, 2012. Effects of plant growth regulators on growth and oil quality of canola (*Brassica napus* L.) under drought stress. Pak. J. Bot., 44: 1873-1880.

Wang, G.P., Z. Hui, F. Li, M.R. Zhao, J.Zhang, and W.Wang, 2010. Improvement of heat and drought on photosynthetic tolerance in wheat by accumulation of glycinebetaine. Plant Biotech. Rep., 4: 212-222.

Zhang, W. and S. Y. Wang, 2001. Antioxidant activity and phenolic compounds in selected herbs. J. of Agric. and Food Chem., 49: 5165-5170.