



Antioxidant Glutathione Impacts on Faba Bean Plants Grown Under Drought Stress Conditions

Salwa A. Orabi¹, Amany A. Ramadan¹ and Manal F. Mohamed²

¹Botany Dept., National Research Centre (NRC), 33 El-Buhouth St., 12622 Dokki, Giza, Egypt.

²Field crop Dept., National Research Centre (NRC), 33 El-Buhouth St., 12622 Dokki, Giza, Egypt.

Received: 12 August 2021

Accepted: 20 Sept. 2021

Published: 20 Oct. 2021

ABSTRACT

Drought as abiotic stress create the reactive oxygen species (ROS) responsible for drastically hinder of plants growth and productivity via alternations in their physiological and biochemical processes. So, the objective of this study was to examine the possibility of using the antioxidants like glutathione as foliar spray on *Vicia faba* plants to eliminate the adverse effect of drought stress. A pot experiment was conducted at the wire house of the National Research Centre, Dokki, Giza, Egypt; to study the effect of foliar treatment with glutathione (100 and 200 ppm) under water stress (different irrigation intervals 4, 8 and 12 days; skipping irrigation) to elucidate drought influence on growth, some physiological and biochemical attributes of faba bean (*Vicia faba* L.) plants. Data showed that increasing water stress duration induced reduction in all growth parameters (Plant height, number of leaves and shoot dry weights/plant) compared with those of the untreated plants irrigated every 4 days. Drought stress led to increases in the enzymatic (APX, GR and PPO), non-enzymatic components (Total phenols, proline, GSH and DPPH) and lipid peroxidation levels. Foliar treatments of 100 and 200 ppm of glutathione led to an increment in growth parameters and yield components on stressed and unstressed plants. Also, increase the enhancement of leaves organic solutes (Phenols, proline, glutathione and total antioxidants). In the same time, the antioxidant enzymes increased and lipid peroxidation decreased due to glutathione treatments compared to corresponding control. Moreover, there are a strong positive correlation coefficient between No. of seed/plant, seed yield/plant and the morphological parameters (Shoot length, No. of leaves and shoot dry weight). Meanwhile, strong negative correlation coefficient with MDA was detected. It could be concluded that glutathione increased *Vicia faba* plants tolerance to water deficiency stress.

Keywords: *Vicia faba*, Glutathione, antioxidant enzyme activity, DPPH, Correlation coefficient

1. Introduction

Faba bean (*Vicia faba* L.) is one of the most popular legume food with high yield capacity and high protein content (Up to 30%) which contains most of the necessary amino acids for both human and animal nutrition, but low concentrations of sulfur amino acids (Gaber *et al.*, 2000).

Egyptian new reclaimed land is characterized as arid and semi-arid regions with poor soil nutrients and unfavorable environmental conditions (drought or high temperature) Furlan *et al.* (2016) demonstrated that drought (abiotic stress factor) with global warming phenomenon considered an important environmental stress for plant growth, since it ultimately causes yield reduction of commercial crops, e.g. Rice, wheat, maize and faba bean. Moreover, the production of ROS considered the key process in plant physiological response to drought which considered responsible for advanced oxidative damage when its accumulation reaches the confident edge level, thus inhibited growth and ultimate cell death (Molassiotis *et al.*, 2016). Moreover, severe hindering for plants growth and productivity induced due to ROS accumulation with fluctuations in plants bio-process (Abdallah *et al.*, 2019). Water stress is known to increase the amount of secondary metabolites accumulated in plants which is known as a defense mechanism of plants that make plants capable of altering their cellular

Corresponding Author: Salwa A. Orabi, Botany Dept., National Research Centre (NRC), 33 El-Buhouth St., 12622 Dokki, Giza, Egypt. E-mail: dr.salwaorabi@yahoo.com

metabolism to invoke various defense mechanisms and adaptation under water stress conditions (El-Tayeb, 2006). This mechanism involves the synthesis and accumulation of small osmolytes (Compatible solutes), such as proline, glycine betaine, sugars and some inorganic ions that help the cells to maintain their dehydrated state and the structural integrity of the membranes (Chaves *et al.*, 2003) that enhances plants resistance to drought and cellular dehydration (Ramanjulu and Bartels, 2002). A decline in the growth and development of plants as a result of drought stress may be attributed to reduction in the growth-promoting hormones, cell elongation, cell expansion and mitosis during cell division (farooq *et al.*, 2009). Global, climatic change causes the drought stress (Shehab *et al.*, 2010). Therefore, there is a need to solve this trouble by using antioxidants and or osmoprotectants. Also, dehydration of tissue inhibits the photochemical activities and decreases the activities of enzymes in the Calvin cycle (Monakhova and Chernyadev, 2002). Worldwide, the harmful effect of drought as one of the major causes of crop yield loss estimated on average by more than 50% by reducing total biomass (Wang and Ballington, 2007).

Glutathione is an antioxidant in plants, animals, fungi, some bacteria and archaea; and capable of preventing damage to important cellular components induced by ROS, e.g. free radicals, peroxides, lipid peroxides and heavy metals (Noctor and Foyer, 1998). It is one of plants antioxidant defense system essential metabolites (non-protein thiols in plant cells) accumulated in low molecular weight and exists in both oxidized (GSSG) and reduced (GSH) forms which is related to regulation of cell differentiation, death and senescence, pathogen resistance and enzymatic activity (Ogawa, 2005). The increased accumulation of GSH contributes to osmotic adjustment, thereby improves salt tolerance in plants (Foyer and Noctor, 2005). Also, GSH detoxifies the methylglyoxal by enhancing the glyoxalase enzymes activities of glyoxalase system for enhanced salinity tolerance (Singla-Pareek *et al.*, 2003). Srivalli *et al.*, (2008) reported that GSH participates in the maintenance of cells redox homeostasis and plays a vital antioxidant role in cell defense and protection against various stresses. GSH acts as a free radical scavenger, since it is a part of the ascorbate–glutathione cycle, essential for scavenging of hydrogen peroxide (H₂O₂) (Orabi, 2004). While, it plays diverse roles, as signaling molecule maintain redox balance of cell, in seedling growth modulation (Hussain *et al.*, 2008), stabilization of membrane via reducing Na⁺ influx and oxidative stress by reducing lipid peroxidation. Therefore, antioxidants prevent the damage of important cellular constituents resulted by ROS and participate in overcoming partially the unfavorable conditions and alleviate their reduced effects on yield quantity and quality (Kumar *et al.*, 2013).

This experiment was carried out to study the effect of the possible promotive and protective effect of the antioxidant glutathione (GSH) on faba bean plants on growth, some physiological parameters and yield under different irrigation intervals.

2. Materials and Methods

A pot experiment in the wire-house at the National Research Centre, Dokki, Cairo, Egypt was carried out to study the effect of foliar spray of glutathione (GSH) on faba bean (*Vicia faba*, L.) plant in different concentrations on growth, some biochemical measurements and yield under different irrigation intervals (4, 8 and 12 days).

2.1. Experiment management:

During the winter season of 2017/2018. Seeds of *Vicia faba* plants, var. Giza 429, provided from the Agricultural Research Center, Giza, Egypt directly sown on the 15th November in earthenware pots of 40 Cm diameter filled with 15 Kg of clay loam soil. The mechanical and chemical analyses of the soil were determined according to the standard method described by Klute (1986) and results are shown in Table (1).

All pots received a recommended doses of N, P and K fertilizers, namely 6 g calcium super phosphate (15.5% P₂O₅), 1.5 g potassium sulphate (48% K₂O) and 1.5 g ammonium nitrate (33.5% N), which was added immediately before sowing. Plants were then thinned to two plants/pot at 10 days after sowing. Plants were regularly irrigated with tap water for 20 days; then the different irrigation intervals were established every 4 (IR₁), 8 (IR₂) and 12 (IR₃) days. All pots were weighted on a beam balance before and during the irrigation, to determine the degree of depletion in the soil moisture content

and the calculating amount of water was added. The general principal stated by Boutraa and Sanders, 2001 was used for the water treatments application.

Table 1: Physical, hydro-physical and chemical properties of the soil.

Sand (%)	Silt (%)	Clay (%)			Textural class				
25.80	36.00	38.00			Clay loam				
F.C. (%)	W.P. (%)	A.W. (%)	H.C. (cm/h)	B.D. (g/cm ³)					
31.01	16.20	14.81	1.19	1.10					
pH	EC (dSm ⁻¹)	Soluble cations (mole L ⁻¹)				Soluble anions (mole L ⁻¹)			
7.70	0.60	Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	Cl ⁻	CO ₃ ⁻	HCO ₃ ⁻	SO ₄ ⁻
		1.11	0.88	2.20	1.48	0.75	2.14	1.14	1.65

The experiment included 9 treatments which included all combinations between three irrigations intervals (IR1, IR2, and IR3) and three exogenous glutathione treatments (0, 100 and 200 mg/L). Each of these glutathione treatments was sprayed always early in the morning twice; the first after 40 days from sowing and the second time two weeks later during the plant's life.

2.2. Growth measurements

Samples were taken after 70 days from sowing to determine the morphological measurements and biochemical analysis. One plant/pot has been left for yield determination. The morphological measurements were plant height (cm), number of leaves/plant and dry weight of shoot (g/plant).

2.3. Yield measurements

When signs of full maturity stage showed, measurements for yield (No. of seeds/plant, seed yield and seed index) were recorded.

2.4. Biochemical analysis

Some biochemical aspects were determined including phenolic content which was measured as described by Danil and George (1972). Proline content was extracted and calculated according to Bates *et al.* (1973). The antioxidant activity (DPPH radical scavenging) was determined using the method of Liyana-Pathiranan and Shahidi (2005). Lipid Peroxidation was determined by measuring Malondialdehyde (MDA) content as described by Dhindsa *et al.* (1982). Reduced glutathione (GSH) was measured with Ellman's reagent according to Silber *et al.*, (1992).

The activity of the enzymes was assayed differently based on their types. The ascorbate peroxidase APX-specific activity (EC 1.11.1.11) was determined according to the methodology of Nakano and Asda (1981). Glutathione reductase activity was assayed according to Zanetti, (1979). Polyphenol oxidase (PPO) activity (EC 1.10.3.1) was determined according to Cho and Ahn, (1999).

2.5. Statistical analysis

A split plot design of 3 x 3 (9 treatments) with six replicates each was applied for this experimentation to test three irrigations intervals (IR₁, IR₂, and IR₃) and three exogenous glutathione treatments (0, 100 and 200 mg/L). Different irrigations intervals were assigned at random in the main plots, while sub-plots were devoted to the different exogenous glutathione treatments. The data were statistically analyzed on complete randomized design system according to Snedecor and Cochran (1990). Means were compared by using least significant difference (LSD) at 5% levels of probability. Simple correlation coefficients among studied parameters computed according to Steel and Torrie (1981).

3. Results and Discussion

3.1. Changes in growth parameters

Data in Table (2) showed the influence of the different concentrations of glutathione on some growth indicators of *Vicia faba* plants grown under drought stress in forms of different irrigation intervals (4, 8 and 12 days). The two levels of irrigation (IR₂ and IR₃) in the soil induced significant

($P < 0.05$) reduction in some morphological parameters (Plant height, number of leaves and shoot dry weight/plant) compared to plants grown under IR₁.

Table 2: Effect of glutathione treatments on some growth parameters of faba bean plants grown under water regime condition.

Irrigation (IR)	Glutathione (G) Conc. (mg/L)	Shoot length (cm)	No. of leaves	Shoot dry wt. (g)
IR ₁	0	66.67	11.00	3.48
	100	73.17	12.33	4.25
	200	75.00	13.33	4.80
IR ₂	0	62.00	9.67	2.76
	100	66.00	11.33	3.10
	200	68.33	12.00	3.65
IR ₃	0	59.83	8.33	2.08
	100	62.67	9.67	2.58
	200	64.33	10.83	2.80
LSD (0.05) for IR x G		NS	NS	NS
Means of irrigation effects (IR)	IR ₁	71.61	12.22	4.18
	IR ₂	65.44	11.00	3.17
	IR ₃	62.27	9.61	2.49
Means of glutathione effects (G)	0	62.83	9.67	2.77
	100	67.28	11.11	3.53
	200	69.22	12.05	3.73
LSD (0.05) for IR and G		1.84	0.88	0.51

NS: Not significant

These results came in line with Kassab *et al.* (2012) who detected that the two levels of water stress (50 & 75%) resulted in substantial decreases in the vegetative growth traits (plant height, root length, number of green leaves, root diameter, root fresh weight, foliage weight, total fresh weight, root dry weight, foliage dry weight and total dry weight) as compared to 100% water irrigation. These results agree also with El-Sayed *et al.* (2020) who found that the two levels of WHC (60 and 40%) in the soil induced significant reduction in some morphological parameters of peanut plants (Plant fresh and dry weights of both shoot and root and root length) compared to plants grown under 80% WHC.

The reduction in the growth parameters under drought stress may be attributed to the losses of tissue water which inhibited cell division and enlargement (Siddique *et al.*, 1999), or due to the decrease in the activity of meristematic tissues responsible for elongation (El-Sebai *et al.*, 2016). Moreover, total fresh and dry weights decreased due to the low levels of drought which might have resulted from a reduction in chlorophyll content and consequently, photosynthetic efficiency (Pimratch *et al.*, 2015). Drought stress can promote ROS production which induced the degradation of proteins and membranes, decreasing photosynthesis and consequently plant growth (El Bassiouny *et al.*, 2018). These obtained results are in agreement with these obtained by, Ahmed *et al.*, (2010) on sorghum, Orabi and El-Noemani, (2015) on faba bean, Orabi *et al.*, (2018) on canola and Abdallah *et al.*, (2019) on wheat plants.

Concerning the effect of tested material (glutathione) on growth criteria of *Vicia faba* plants, the obtained data showed that 100 and 200 mg/L spraying treatments significantly ($P < 0.05$) increased the measured growth parameters compared to their corresponding control. Sanchez-Fernandez *et al.*, (1997) mentioned that this increased growth by glutathione can be attributed to the improved cell division. The obtained results of promotive effect of glutathione on *Vicia faba* plants growth criteria are similar to those obtained by Orabi (2004) who detected that glutathione at 50,100,200, and 400 mg/L led to marked increments in all morphological traits (plant height, number of leaves, fresh and dry weights of leaves, fresh and dry weights of stem and leaf area) in all growth stages (vegetative, flowering and fruiting) of stressed cucumber plant. Dawood *et al.* (2020) found that glutathione at 100, 200 and 300 mg/L caused significant increases in shoot length, No. of leaves, fresh and dry weights/plant of three wheat cultivars (Egypt-2, Shandaweel-1 and Gemmeiza-11). The obtained positive results for GSH treatments are also in agreement with those reported by Orabi *et al.* (2017) on *Zea mays*, Sadak *et al.* (2017) on chickpea and Rehman *et al.* (2021) on wheat under saline conditions.

3.2. Changes in enzymatic antioxidant activities

The data in Table (3) indicated that water stress (IR₂ and IR₃) significantly (P<0.05) increased antioxidant enzymes in forms of ascorbate peroxidase (APX), glutathione reductase (GR), polyphenol oxidase (PPO) of tested plants. These results came on line with Ahmed *et al.* (2010) who investigated that limited irrigation of sorghum plant significantly increased the antioxidant enzyme (SOD, CAT) activities. In addition, Kassab *et al.* (2012) pointed out that the increase of the water regime levels (from 75 up to 50%) led to gradual increase in Guaiacol peroxidase (POX) and catalase (CAT) enzyme activities of fodder beat plant. Orabi and El-Noemani (2015) found also that increasing drought stress led to increased antioxidant enzymes (APX & GR) of faba bean plant. Moreover, Orabi *et al.*, (2018) detected that widening irrigation intervals from 4 to 8 to 12 days significantly increased the antioxidant enzyme activities (APX, CAT & PPO) in canola leaves. Also, Abdallah *et al.*, (2019) on wheat found that the change in the peroxidase (POX) activity in leaves of wheat exposed to water stress (60% and 40% WHC) were significantly increased than that of the control and 80% of WHC.

Foliar spraying of faba bean plants with glutathione (100 and 200 mg/L) showed highly significant increase (P<0.05) in various antioxidant enzymes activity compared to corresponding control plants. The highest level of glutathione treatments recorded the highest (P<0.05) values of antioxidant enzymes in plants either unstressed or drought stressed ones. The magnitude of these enzymes' enhancement increased with increasing drought level and glutathione treatments improved stress tolerance by increasing APX, GR and PPO activities with corresponding drought level as compared to the control. In this concern, Orabi (2004) investigated that plants treated with glutathione at 200mg/L resulted in remarkable increments in all antioxidant enzymes activity (SOD, CAT, POX, APX & GR) in stressed cucumber plants. Also, Orabi *et al.*, (2017) found obvious increases of (APX & GR) in *Zea mays* stressed plants treated with glutathione at 100 and 200 mg/L under raw sea water irrigation. In addition, sadak *et al.*, (2017) reported that foliar spraying of chickpea plants with glutathione (50, 100 and 150 mg/L) under saline stress and unstressed plants resulted in marked increases of ascorbate peroxidase (APX), glutathione reductase (GR), peroxidase (POX) and superoxide dismutase (SOD).

Table 3: Effect of glutathione treatments on antioxidant enzyme activities and lipid peroxidation contents in faba bean plants grown under water regime condition.

Irrigation (IR)	Glutathione (G) conc. (mg/L)	Antioxidant enzymes			MDA (µmol/g FW)
		APX (µmol/g FW)	GR (nmol/g FW)	PPO (µmol/g FW)	
IR ₁	0	0.89	361.73	16.11	7.84
	100	1.44	395.23	17.92	6.95
	200	1.79	468.91	20.16	6.06
IR ₂	0	1.12	388.53	19.09	11.10
	100	2.38	489.01	21.44	8.88
	200	2.49	542.60	22.72	8.19
IR ₃	0	1.30	422.02	24.32	12.30
	100	2.91	535.90	26.35	9.47
	200	3.18	569.40	26.88	8.58
LSD (0.05) for IR x G		0.48	NS	NS	0.99
Irrigation effects (IR)	IR ₁	1.37	408.62	18.06	6.95
	IR ₂	1.99	473.38	21.08	9.39
	IR ₃	2.46	509.11	25.85	10.12
Glutathione effects (G)	0	1.10	390.76	19.84	10.41
	100	2.24	473.38	21.90	8.43
	200	2.48	526.97	23.25	7.61
LSD (0.05) for IR and G		0.27	63.62	1.40	0.58

NS: Not significant

The high quantity of ascorbic acid substrate accumulates during abiotic stress results in the induction of high APX activity (Alves *et al.*, 2013). This high levels of APX defined as crucial for the maintenance of the antioxidant system that protects plant organs against damage due to abiotic stress (Shigeoka *et al.*, 2002). Glutathione reductase (GR), with a consequent oxidation of NADPH, is

responsible for converting glutathione from its oxidized form glutathione-disulfide to the reduced form glutathione (Diaz-Vivancos *et al.*, 2015). Thus, glutathione become antioxidant for –SH groups in some enzymes either via oxidizing some complexes or through the compensation of –SH groups using the glutathione-disulfide interchange response (Gill *et al.*, 2013); and become partly responsible for preserving a high ratio of glutathione / glutathione-disulfide in the cells (Duhan *et al.*, 2017). Thus, the APX emphasized to play a vital role in controlling the cell indigenous ROS levels or depollution of H₂O₂ by using two molecules of ascorbic acid to reduce it to water. More, the role of GSH and GR in H₂O₂ scavenging has been well established in the Halliwell-Asada pathway (Noctor and Foyer 1998). The increased level of GR activity results in the accumulation of GSH and boost plant stress tolerance. APX is a primary enzyme of the ASA–GSH cycle that suppresses the accumulation of H₂O₂ in most cellular compartments by catalyzing the conversion of H₂O₂ to water. APX has a high affinity for H₂O₂ than CAT and POX and it may have a more crucial role in the management of ROS stress or may be responsible for the fine modulation of ROS signaling.

3.3. Lipid peroxidation (MDA)

Data in Table (3) showed that with increasing the drought stress level (IR₂ and IR₃) Malondialdehyde content (MDA) of faba bean plants increased as compared with (IR₁). The high level of MDA concentration in plant leaves indicated that it is affected by drought stress exhibited by lipid peroxidation, which resulted in membrane damage. As the result of drought stress the ROS, in general, via dehydrogenation of unsaturated fatty acids cause strong lipid peroxidation that generate radicals attacks another molecules resulting in a waterfall of reactions destructive lipid structure. Lipid peroxidation decreases membrane fluidity, increase leakiness of the membrane and damage membrane proteins, enzymes and ion channels (Orabi *et al.*, 2010). The increase of MDA content indicates that the bulk oxidative lipid metabolism in leaves was enhanced by drought, suggesting a relationship between drought and oxidative stress (Bosch *et al.*, 2001). Ahmed *et al.*, (2010) pointed out that MDA of the two studied sorghum cultivars (pioneer, F840 and Giza-113) was increased with leakage of electrolytes under the effect of water shortage (skipping one irrigation). Hussein *et al.*, (2009) and Kassab *et al.*, (2012) on cotton and fodder beat respectively, detected Malondialdehyde (MDA) accumulation under drought stress which led to leakage of essential electrolytes. Orabi and El-Neomani (2015) reported that prolonged water stress resulted in increments in ROS (O₂⁻ & H₂O₂) lipid peroxidation and electrolyte leakage of faba bean plant. Abdallah *et al.*, (2019) revealed that wheat plant exposed to 60% and 40% of WHC showed a gradual and significant increase in malondialdehyde (MDA) compared to a control plant at 80% of WHC. Duhan (2016) stated that MDA content significantly increased during waterlogging, salinity and their combined stresses, representing undesirable influence on membrane integrity and finally membrane corrosion. These changes affect ion interchange capability of plasma membrane as well as some activities in the physiological membrane effectiveness.

Concerning the effect of glutathione treatments on MDA content, it is found that foliar application decreased significantly (P<0.05) the content of MDA in *Vicia faba* plant then decreased membrane damage as compared with corresponding control. The obtained results coincided with Orabi (2004) who detected that foliar spray with glutathione decreased lipid peroxidation and consequently membranes electrolyte leakage of cucumber plants. These results agree also with Sadak *et al.*, (2017) who found that foliar application of glutathione lowered H₂O₂ production, and reduced MDA content of chickpea plant. This might be achieved via glutathione-mediated direct ROS scavenging, antioxidative mechanism involved in eliminating ROS or stabilizing of membrane via its effect on antioxidant enzymes and/or increased contents of endogenous glutathione (Salama and Al-Mutawa, 2009).

3.4. Changes in antioxidant compound contents

Drought stress caused significant increases (P<0.05) in proline, total phenolic compounds, glutathione and total antioxidants or DPPH scavenging activity contents in leaves of faba bean plants as compared to control plants (Table 4). These results are in harmony with those obtained by Ezzo *et al.*, (2018) on moringa plants and Hellal *et al.*, (2019) on barley plants who showed that free amino acids, e.g. proline and TSS increased in water stressed plants. These results are also in agreement with those of El-Tayeb (2006) on faba bean and El-Tayeb and Ahmed (2010) on wheat cultivars.

Proline accumulation under drought stress has been reported and suggested to be a biochemical marker for increased stress tolerance in plant species under stress conditions. Many functions have been postulated for proline, as free amino acids, accumulation in plant tissues could be involved in the osmotic adjustment of plants (Gzik, 1999). It also could also be a protective agent of enzymes and membranes (Bandurska, 1993). When plant subjected to drought stress, plants maintain their water content by accumulation of compatible organic solutes which act as osmo-protectants (e.g. proline) in their cytoplasm (Harinasut *et al.*, 2000), since it was found to functions as hydroxyl radical scavenger (Hoque *et al.*, 2007). Proline also has a vital role to increase the capacity for O₂⁻ or H₂O₂ scavenging (Orabi and El-Neomani, 2015)

Concerning the effect of glutathione on proline content of *Vicia faba*, data in table (4) showed that glutathione foliar application increased the improvement of proline significantly (P<0.05) as compared with corresponding control. The tolerance of *Vicia faba* to drought stress might be due to modulated amino acid metabolism. Proline found also to play a vital role in osmotic adjustment, stabilization and protection of enzymes, proteins and membranes from the harmful effects of drought stress (Ashraf and Foolad, 2007). This is in alliance with our results where glutathione treatments induced more accumulation of proline and free amino acids in faba bean plants. The increased level of proline in faba bean plants might be due to enhanced biosynthesis key enzymes of proline in a way or another (Amini and Ehasanpour, 2005). Nahar *et al.* (2015) mentioned that under water stress, GSH treatment increased the endogenous ASA and GSH levels in mung bean seedlings. Also, Sadak *et al.* (2017) reported that the application of glutathione resulted in maximum elevation of phenol and free amino acids as well as proline contents of chickpea plant under the stress conditions. Orabi *et al.* (2017) found that application of tested maize plant with glutathione 100 and 200 ppm increased the amino acid (Proline), all the amino acids composition and the content of antioxidants (phenol, glutathione and ascorbic acid) compared to corresponding control under salinity stress. The elevated antioxidant levels can protect the photosynthetic apparatus from oxidative damage (Diao *et al.*, 2014). Increasing endogenous amino acids and hence protein composition is one of the characteristic features of GSH-induced impacts in salt stressed plants (Akladius and Abbas, 2013). Glutathione itself is a small protein composed of three amino acids linked together and may have a role in enhancing amino acids pool in plants (Robins and Davies, 1981). The reduction of the radical DPPH from purple colour to a yellow colour (diphenyl picryl hydrazine) is due to hydrogen donating ability of the antioxidants (Abd El Motty and Orabi, 2013), the extent of colour change or DPPH scavenging activity in table (4) showed the ability of untreated or specially glutathione treated plants to overcome or mitigate the harmful effects of oxidative stress under drought.

Table 4: Effect of glutathione treatments on antioxidants contents in faba bean plants grown under water regime condition.

Irrigation (IR)	Glutathione (G) conc. (mg/L)	Phenols (mg/g FW)	Proline (µmol/g FW)	GSH (µmol/g)	DPPH (%)
IR ₁	0	1.80	11.20	4.48	74.06
	100	2.14	11.93	5.04	78.68
	200	2.21	13.09	5.38	82.23
IR ₂	0	2.24	12.28	4.95	79.12
	100	2.70	15.10	6.84	84.74
	200	2.93	16.80	7.10	87.92
IR ₃	0	2.60	14.05	6.10	82.74
	100	2.90	17.49	7.62	88.10
	200	3.08	19.13	7.80	88.84
LSD (0.05) for IR x G		0.12	1.79	1.82	NS
Irrigation effects (IR)	IR ₁	2.05	12.07	4.97	78.32
	IR ₂	2.62	14.73	6.30	83.92
	IR ₃	2.86	16.89	7.17	86.56
Glutathione effects (G)	0	2.21	12.51	5.18	78.64
	100	2.58	14.84	6.51	83.84
	200	2.74	16.34	6.76	86.33
LSD (0.05) for IR and G		0.10	1.04	1.44	2.94

NS: Not significant

3.5. Yield parameters

Data presented in Table (5) showed that drought stress decreased significantly ($P < 0.05$) yield parameters. On the other hand, foliar treatment of different concentrations of glutathione (100 and 200 ppm) significantly ($P < 0.05$) increased faba bean yield and its components (No. of seeds/plant, seed yield/plant (g) and seed index). The maximum increases were observed with the high conc. of glutathione. Drought stress results coincided with Kassab *et al.* (2012) who detected that fodder beat grown under limited water (75 & 50%) resulted in substantial decreases in yield traits (plant height, root length, root diameter, root weight/plant (g) , foliage yield/plant (g), total yield/plant (g), root yield/fed.(ton), foliage yield/fed.(ton) and biological yield/fed.(ton)). The obtained results confirmed also with those of Abdallah *et al.*, (2019) who reported the same trend on wheat plants exposed to 40 and 60% of water stress. i.e there are a negative relationship between yield and prolonging the period between irrigation, the highest decrement was in seed weight/ plant followed by seeds No/ plant then 100 seeds weight in plants received irrigation every 12 days in comparison with plants irrigated every 4 days intervals (Orabi *et al.*, 2018).

Exogenous glutathione treatments (50, 100, 200 & 400 mg/L) for Cucumber plants grown under cold stress or suitable normal conditions have maintained good protection, improved growth, development and yield with Orabi (2004). Zaki *et al.*, (2019) found that treatment with glutathione with cyano bacteria (CB) bio-fertilizer gave the best results of Soy bean yield traits (pods number, 100-seed weight & dry seed weight) in comparison with the individual treatments or control. Dawood *et al.* (2020) found that foliar treatment with different concentrations of glutathione and/or selenium significantly increased wheat grain yield and most of its components (shoot height, spike length, number of spikelets/spike, biological yield/teller, grain yield/teller, straw yield/teller, grains index, biological yield (ton/fed) and grain yield (ton/fed)) of three wheat cultivars. The obtained results showed that all applied treatments had a promoting effect on the grain yield and its components (Table 5). The empowering effect of glutathione could be explained by Buwalda *et al.* (1990) who indicated that its effect as a reservoir of reduced sulfur as the amino acid cysteine which is a component of the antioxidant glutathione.

Table 5: Effect of glutathione treatments on some yield parameters in faba bean plants grown under water regime condition.

Irrigation (IR)	Glutathione (G) conc. (mg/L)	No. of seeds	Seed yield / plant (g)	Seed index
IR ₁	0	11.67	8.81	0.75
	100	13.67	11.00	0.80
	200	14.67	12.20	0.83
IR ₂	0	9.34	7.31	0.78
	100	11.33	9.30	0.82
	200	12.33	10.10	0.83
IR ₃	0	7.33	5.73	0.78
	100	8.67	7.03	0.81
	200	9.33	7.47	0.83
LSD (0.05) for IR x G		NS	0.80	NS
Irrigation effects (IR)	IR ₁	13.34	10.67	0.79
	IR ₂	11.00	8.90	0.81
	IR ₃	8.44	6.74	0.81
Glutathione effects (G)	0	9.45	7.28	0.78
	100	11.22	9.11	0.81
	200	12.11	9.92	0.83
LSD (0.05) for IR and G		0.88	0.46	0.04

NS: Not significant

Moreover, Glutathione enhances the chlorophyll biosynthesis or decreases its degradation and integrated into primary metabolism, and it can affect the functioning of the signal transduction pathway by modulating cellular redox state (Khattab, 2007) and thus return in increasing crop yield.

The improvement in chlorophyll fluorescence traits might also enhance the re-translocation of stem reserves towards developing grains associated with sequenced applied moringa leaf extract (MLE)

and/or GSH improved hormonal homeostasis and signaling (Cheng *et al.*, 2015). Another possible reason can be enhanced endogenous cytokinin (zeatin) levels to delay premature leaf senescence with foliar-applied MLE in sequence with MLE or GSH (Tetley and Thimann, 1974). Delaying senescence have been reported as one of the potential measurable traits to improve osmotic and tissue tolerance in wheat (Mujeeb-kazi *et al.*, 2019).

3.5. Correlation coefficients among tested parameters in faba bean plants

It is observed from Table (6) that there are significant high positive correlation coefficients among the antioxidant substances (Phenol, proline and DPPH) and the antioxidant enzymes (PPO, APX and GR) as well as the glutathione content (from 0.73 to 0.95 at $P < 0.01$). Moreover, the correlations between the antioxidant enzymes to each other and the glutathione content observed also to be strong positive correlation ranged from 0.71 to 0.91 ($P < 0.01$).

Regarding yield parameters (seed yield /p and No. of seeds/p; Table 5), it is showed that there is a strong positive correlation ranged from 0.85 to 0.95 ($P < 0.01$) among these parameters and the morphological criteria (shoot length, shoot dry weight and No. of leaves; Table 2).

Table 6: Correlation coefficients among tested parameters of faba bean plants sprayed with glutathione under water regime condition.

	Shoot length	Shoot dry wt.	No. of leaves	PPO	GSH	APX	GR	MDA	Phenols	Proline	DPPH	Seed yield/P	No. of seeds
Shoot length													
Shoot dry wt.	0.878**												
No. of leaves	0.86**	0.82**											
PPO	-0.45*	-0.40	-0.38										
GSH	-0.30	-0.24	-0.14	0.85**									
APX	-0.08	-0.04	0.04	0.77**	0.91**								
GR	-0.23	-0.12	0.002	0.73**	0.82**	0.71**							
MDA	-0.89**	-0.82	0.87**	0.36	0.16	-0.11	0.06						
Phenols	-0.35	-0.34	0.20	0.88**	0.95**	0.84**	0.80**	0.26					
Proline	-0.29	-0.27	0.14	0.87**	0.93**	0.90**	0.83**	0.12	0.92**				
DPPH	-0.16	-0.19	0.07	0.84**	0.89**	0.82**	0.73**	0.09	0.91**	0.87**			
Seed yield/p	0.95**	0.88**	0.90**	0.48**	-0.28	0.07	-0.16	-0.87**	-0.34	-0.29	0.16		
No. of seeds	0.92**	0.85**	0.87**	0.55**	-0.38*	0.07	-0.18	-0.86**	-0.42	-0.36	-0.25	0.45**	
Seed index	0.08**	0.06	0.20	0.49**	0.48**	0.49**	0.48	-0.02	0.51**	0.55**	0.52**	0.13	0.07

It is worth mentioning that seed yield and No. of seeds/p significantly ($P < 0.01$) negatively correlated with MDA, being -0.87 and -0.86, respectively. This explain the reduction in MDA with GSH spraying at 100 and 200 ppm (Table 3). Moreover, these two characters also positively correlated ($P < 0.01$) by about 0.48 and 0.55 with antioxidant enzyme (polyphenol oxidase, PPO).

It could be concluded that glutathione treatments could overcome oxidative damage and improve antioxidant defense mechanisms (enzymatic and non-enzymatic compounds), growth and yield under stress. It has succeeded to help plants to resist drought stress induced from prolonging periods between irrigation of faba bean plants.

References

- Abd El-Motty, E.Z. and S.A. Orabi, 2013. The Beneficial Effects of Using Zinc, Yeast and Selenium on Yield, Fruit Quality and Antioxidant Defense Systems in Navel Orange Trees Grown under Newly Reclaimed Sandy Soil. *J. App. Sci. Res.*, 9(10): 6487-6497.
- Abdallah, M.M.Sh., H.M.S. El-Bassiouny, and M.A. AbouSeeda, 2019. Potential role of kaolin or potassium sulfate as anti-transpirant on improving physiological, biochemical aspects and yield of wheat plants under different watering regimes. *Bulletin of the National Research Centre*, 43: 134.
- Ahmed, A. G., M.A. Bekheta, and S.A. Orabi, 2010. Influence of arginine on growth and productivity of two sorghum cultivars grown under water shortage. *International J. of Acadmic Res.*, 1:7280.
- Akladios, S.A., and S.M. Abbas, 2013. Alleviation of sea water stress on tomato plants by foliar application of aspartic acid and glutathione. *Bangladesh Journal of Botany*, 42(1): 31–43.
- Alves, M.S., S.P. Dadalto, A.B. Gonçalves, G.B. De Souza, V.A. Barros, and L.G. Fietto, 2013. Plant bZIP transcription factors responsive to pathogens: a review. *International Journal of Molecular Sciences*, 14: 7815–7828.

- Amini, F. and A.A. Ehsanpour, 2005. Soluble proteins, proline, carbohydrates and Na^{and}/K^{and} changes in two tomato (*Lycopersicon esculentum*, Mill) cultivars under *In vitro* salt stress. Amer. J. Biochem. Biotechnol., 1: 212-216.
- Ashraf, M. and M.R. Foolad, 2007. Roles of glycine betaine and proline in improving plant abiotic stress tolerance. Environ. Exp. Bot., 59: 206–216.
- Bandurska, H., 1993. *In vivo* and *In vitro* effect of proline on nitrate reductase activity under osmotic stress in barley. Acta Physiol. Plant., 15: 83-88.
- Bates L.S., R.P. Waldan and L.D. Teare, 1973. Rapid determination of free proline under water stress studies. Plant Soil, 39: 205–207.
- Bosch, M.S., T. Jubany and L. Mari Alegre, 2001. Drought – induced senescence is characterized by a loss of antioxidant defenses in chloroplasts. Plant cell Environ., 24: 1319-1327.
- Boutraa, A. and F.E. Sanders, 2001. Influence of water stress on grain yield and vegetative growth of two cultivars of bean (*Phaseolus vulgaris*, L.). Journal of Agronomy and Crop Science, 186: 229-237.
- Buwalda, F., I. Stulen, L.J. De Kok, and P.J.C. Kuiper, 1990. Cysteine, γ -glutamylcysteine and glutathione contents of spinach leave as affected by darkness and application of excess sulfur. II. Glutathione accumulation in detached leaves exposed to H₂S in the absence of light is stimulated by the supply of glycine to the petiole. Physiol. Plant., 80: 196–204.
- Chaves, M.M., J.P. Maroco, and J.S. Pereira, 2003. Understanding plant responses to drought from genes to the whole plant. Funct. Plant. Biol., 30: 239-264.
- Cheng, M.C., K. Ko, W.L. Chang, W.C. Kuo, G.H. Chen, and T.P. Lin, 2015. Increased glutathione contributes to stress tolerance and global translational changes in Arabidopsis. Plant J., 83: 926–939.
- Cho, Y.K. and H.K. Ahn, 1999. Purification and characterization of polyphenol oxidase from potato: II. Inhibition and catalytic mechanism. J. Food Biochem., 23: 593–605.
- Danil, A.D. and C.M. George, 1972. Peach seed dormancy in relation to endogenous inhibitors and applied growth substances. J. of Amer. Society for Hort. Sci., 17: 621-624.
- Dawood, Mona G., Sadak, Mervat Sh., A.B. Bakry, and H.H. Kheder, 2020. Effect of glutathione and/or selenium levels on growth, yield and some biochemical constituents of some wheat cultivars grown under sandy soil conditions. Bulletin of the National Research Centre, 44: 158.
- Dhindsa, R.S., P. Plumb-Dhindsa, and D.M. Reid, 1982. Leaf Senescence and lipid peroxidation: effects of some phytohormones and scavengers of free radicals and singlet oxygen. Physiol. Plant, 56: 453–457.
- Diao, M., L. Ma, J. Wang, J. Cui, A. Fu, and H. Liu, 2014. Selenium promotes the growth and photosynthesis of tomato seedlings under salt stress by enhancing chloroplast antioxidant defense system. Journal of Plant Growth Regulation, 33(3): 671–682.
- Diaz Vivancos, P., A. Simone, G. de Kiddle, and C.H. Foyer, 2015. Glutathione-linking cell proliferation to oxidative stress. Free Radical Biology and Medicine, 89: 1154–1164.
- Duhan, S., 2016. Effect of waterlogging, salinity and their interaction on growth, oxidative and carbohydrate metabolism in pigeonpea (*Cajanus cajan*, L. Mill sp.) genotypes. Ph.D. Thesis, CCS Haryana Agricultural University, Hisar, India.
- Duhan, S., A. Kumari, and S. Sheokand, 2017. Effect of water logging and salinity on anti-oxidative system in pigeonpea plant leaves at different stages of development. Research on Crops, 18(3): 559–568.
- El Bassiouny, H.M.S., A.A. Abd El-Monem, M.M.S. Abdallah, and K.M. Soliman, 2018. Role of arbuscular mycorrhiza, α -tocopherol and nicotinamide on the nitrogen containing compounds and adaptation of sunflower plant to water stress. Biosci. Res., 15(3): 2068–2088.
- El-Sayed, S., Ramadan, Amany A. and F. Hellal, 2020. Drought stress mitigation by application of Algae extract on peanut grown under sandy soil conditions. Asian J. of Plant Sci., 19(3): 230-239.
- El-Sebai, T.N., M.M.S. Abdallah, H.M.S. El-Bassiouny, and F.M. Ibrahim, 2016. Amelioration of the adverse effects of salinity stress by using compost, *Nigella sativa* extract or ascorbic acid in quinoa plants. Int. J. Pharm. Tech. Res., 9(6): 127–144.
- El-Tayeb, M.A., 2006. Differential response of two *Vicia faba* cultivars to drought: growth, pigments, lipid peroxidation, organic solutes, catalase and peroxidase activity. Acta Agron. Hung., 54: 25-37.

- El-Tayeb, M.A. and N.L. Ahmed, 2010. Response of wheat cultivars to drought and salicylic acid. *American-Eurasian Journal of Agronomy*, 3(1): 01-07.
- Ezzo, M.I., E.M. Abd Elhamid, M.Sh. Sadak, and M.A. Aboelfetoh, 2018. Improving drought tolerance of moringa plants by using trehalose foliar treatments. *Bioscience Research*, 15(4): 4203-4214.
- Farooq, M., A. Wahid, N. Kobayashi, D. Fujita, and S.M.A. Basra, 2009. Plant drought stress: Effects, mechanisms and management *Agron. Sustain. Dev.*, 29: 153–188.
- Foyer, C.H. and G. Noctor, 2005. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *Plant Cell*, 17: 1866–1875.
- Furlan, A.L., E. Bianucci and S. Castro 2016. Signaling role of ROS in modulating drought stress tolerance in drought *Stress Tolerance in Plants*, Vol. 1: Physiology and Biochemistry, L.S. Tran, Ed., Springer, Cham, Switzerland.
- Gaber, A.M., H.A.M. Mostafa, and A. Ramadan, Amany, 2000. Effect of gamma irradiation of faba beans (*Vicia Faba*) plant on its chemical composition, Favism causative agent and hormonal levels. *Egypt. J. Physiol. Sci.*, 24: 1–16.
- Gill, S.S., N.A. Anjum, M. Hasanuzzaman, R. Gill, D.K. Trivedi, I. Ahmad, E. Pereira, and N. Tuteja, 2013. Glutathione and glutathione reductase: a boon in disguise for plant abiotic stress defense operations. *Plant Physiology and Biochemistry*, 70: 204–212.
- Gzik, A., 1999. Accumulation of proline and pattern of α -amino acids in sugar beet plants in response to osmotic, water and salt stress. *Environ. Exp. Bot.*, 36(1): 29-38.
- Harinasut, P., S. Srisunak, S. Pitukchaisopol, and R. Charoensataporn, 2000. Mechanisms of adaptations to increasing salinity of mulberry: Proline content and ascorbate peroxidase activity in leaves of multiple shoots. *Science Asia.*, 26: 207-211.
- Hellal, F., M. Abdel-Hady, I. Khattab, S. El-Sayed, and A. Chedly, 2019. Yield characterization of Mediterranean barley under drought stress condition. *AIMS Agriculture and Food*, 4(3): 518–533.
- Hoque, M.D.A., E. Okuma, M.N.A. Banu, Y. Nakamura, Y. Shimoishi, and Y. Murata, 2007. Exogenous proline mitigates the detrimental effects of salt stress more than exogenous betaine by increasing antioxidant enzyme activities. *J. Plant Physiol.*, 164: 553-561.
- Hussain, T.M., M. Hazara, Z. Sultan, B.K. Saleh, and G.R. Gopal, 2008. Recent advances in salt stress biology: a review. *Biotechnol. Mol. Biol. Rev.*, 3: 8–13.
- Hussein, M.M., M.A. Bakheta and S.A. Orabi, 2009. Influence of abscisic acid and benzyl adenine on some growth characters, endogenous hormones and some chemical constituents of cotton plants grown under drought stress. *Egyptian Journal of Agronomy*, 31: 253-270.
- Kassab, O.M., S.A. Orabi, and A.A. Abo Ellil, 2012. Physiological response to potassium application in fodder beet plant grown under water stress. *Australian J. of Basic and Applied Sciences*, 6(13):566-574.
- Khattab, H., 2007. Role of glutathione and polyadenylic acid on the oxidative defense systems of two different cultivars of canola seedlings grown under saline conditions. *Aust. J. Basic Appl. Sci.*, 1(3): 323–334.
- Klute, A., 1986. "Method of Soil Analysis", 2nd ed. Part 1. Physical and Mineralogical Methods. Part 2. Chemical and Microbiological Methods Properties. Modifon, Wisconsin, U.S.A.
- Kumar, V., M. Lemos, M. Sharma, and V. Shriram, 2013. Antioxidant and DNA damage protecting activities of *Eulophia nuda* Lindl. *Free Rad. Antioxidants*, 3(2): 55–60.
- Liyana-Pathiranan, C.M. and F. Shahidi, 2005. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. *J. of Agric. and Food Chem.*, 53: 2433-2440.
- Molassiotis, A., D. Job, V. Ziogas, and G. Tanou, (2016). Citrus plants: a model system for unlocking the secrets of NO and ROS inspired priming against salinity and drought. *Frontiers in Plant Science*, 7: 553.
- Monakhova, O.F. and I.I. Chernyadev, 2002. Protective role of Karoline-4 in wheat plants exposed to soil drought. *Appl. Biochem. Microbiol.*, 38:373-380.
- Mujeeb-kazi, A., R. Munns, A. Rasheed, F.C. Ogonnaya, N. Ali, P. Hollington, I. Dundas, N. Saeed, R. Wang, P. Rengasamy, M.S. Saddiq, J.L.D.D. Le'on, M. Ashraf, and S. Rajaram, 2019. Breeding strategies for structuring salinity tolerance in wheat. *Adv. Agron.*, 155: 121–187.

- Nahar, K., M. Hasanuzzaman, M.M. Alam, and M. Fujita, 2015. Glutathione-induced drought stress tolerance in mung bean: Coordinated roles of the antioxidant defence and methylglyoxal detoxification systems. *Plants*, 7, plv069.
- Nakano, Y. and K. Asada, 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.*, 22: 867-880.
- Noctor, G. and C.H. Foyer, 1998. Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology*, 49 (1): 249–279.
- Ogawa, K., 2005. Glutathione-associated regulation of plant growth and stress responses. *Antioxid. Redox Sign.*, 7: 973–981.
- Orabi, S.A. and A.A. El-Noemani, 2015. Role of proline in improving drought tolerance of faba bean plants via antioxidant responses to enhanced generation of superoxide anion radical and hydrogen peroxide. *American-Eurasian Journal of Sustainable Agriculture*, 9(1): 31-42.
- Orabi, S.A., 2004. Physiological impacts of cold injury on cucumber (*Cucumis stivus* L.) plant Ph.D. Thesis, Fac. of Sci., Cairo, Univ., Egypt.
- Orabi, S.A., T.A.E. El-Shahawy, and F.A. Sharara, 2017. The compensatory effect of glutathione on alleviating salinity-induced modulations in growth and biochemical traits in maize irrigated with diluted seawater. *Agricultural Engineering International: CIGR Journal*, Special issue: 80–90.
- Orabi, S.A., M.M. Hussein., S.S. Zaki, and F.A. Sharara, 2018. Influence of hydrogen peroxide on growth, yield and biochemical constituents of canola plants grown under different irrigation interval. *Curr. Sci. Int.*, 7(3):407-418.
- Orabi, S.A., S.R. Salman and M.A.F. Shalaby, 2010. Increasing resistance to oxidative damage in cucumber (*Cucumis sativus*, L.) plants by exogenous application of salicylic acid and paclobutrazol. *World J. of Agric. Science*, 6(3): 252-295.
- Pimratch, S., S. Butsat, and T. Kesmala, 2015. Application of blue-green algae and mineral fertilizers to direct seeding lowland rice. *Science Asia*, 41: 305–314.
- Ramanjulu, S. and D. Bartels, 2002. Drought and desiccation-induced modulation of gene expression in plants. *Plant Cell Environ.*, 25: 141-151.
- Rehman, H.U.R., H.F. Alharby, A.A. Bamagoos, M.T. Abdelhamid, and M.M. Rady, 2021. Sequenced application of glutathione as an antioxidant with an organic biostimulant improves physiological and metabolic adaptation to salinity in wheat. *Plant Physiology and Biochemistry*, 158: 43–52.
- Robins, R.J. and D.D. Davies, 1981. The role of glutathione in amino-acid absorption. Lack of correlation between glutathione turnover and amino-acid absorption by the yeast *Candida utilis*. *Biochemical Journal*, 194(1): 63–70.
- Sadak, Mervat Sh., Abd Elhamid, Ebtihal M. and Ahmed, Marwa M.R.M., 2017. Glutathione Induced Antioxidant Protection against Salinity Stress in Chickpea (*Cicer arietinum*, L.) Plant. *Egypt. J. Bot.*, 57(2): 293-302.
- Salama, K.H.A. and M.M. Al-Mutawa, 2009. Glutathione-triggered mitigation in salt-induced alterations in plasmalemma of onion epidermal cells. *Int. J. Agric. Biol.*, 1(1): 639–642.
- Sanchez-Fernandez, R., M. Fricker, L.B. Corben, N.S. White, N. Sheard, C. Leaver, M.V. Montagu, D. Inze, and M.J. May, 1997. Cell proliferation and hair tip growth in the *Arabidopsis* root are under mechanistically different forms of redox control. *Proc. Natl. Acad. Sci. USA*, 94: 2745–2750.
- Shehab, G.G., O.A. Ahmed, and H.S. El-Beltagi, 2010. Effects of various chemical agents for alleviation of drought stress in rice plants (*Oryza sativa* L.). *Not. Bot. HortiAgrobot. Cluj Napoca*, 38: 139–148.
- Shigeoka, S., T. Ishikawa, M. Tamoi, Y. Miyagawa, T. Takeda, Y. Yabuta, and K. Yoshimura, 2002. Regulation and function of ascorbate peroxidase isoenzymes. *Journal of Experimental Botany*, 53: 1305–1319.
- Siddique, M., R.B. Hamid, and M.A. Islam, 1999. Drought stress effect on photosynthetic rate and leaf gas exchange of wheat. *Bot. Bull. Academia Sinica*, 40: 141–145.
- Silber, R., M. Farber, E. Papopoulos, L.D. Nervla Liebes, and M. Bruch, 1992. Glutathione depletion in chronic lymphocytic leukemia B-lymphocytes. *Blood*, 80: 2038-2040.
- Singla-Pareek, S.L., M.K. Reddy, and S.K. Sopory, 2003. Genetic engineering of the glyoxalase pathway in tobacco leads to enhanced salinity tolerance. *Proc. Natl. Acad. Sci. Unit. States Am.*, 100: 14672–14677.

- Snedecor, G.W. and W.G. Cochran, 1990. "Statistical Methods". 8th Ed. Iowa State Univ. Press Ames, Iowa, U.S.A. 609.
- Srivalli, S. and R. Khanna-Chopra, 2008. Role of glutathione in abiotic stress tolerance. In: Khan N.A., Singh S., Umar S. (eds) Sulfur Assimilation and Abiotic Stress in Plants. Springer, Berlin, 207–225.
- Steel, R.G.D. and J.H. Torrie, 1981. Principles and procedures of statistics. A biometrical approach, 2nd Ed. McGraw-Hill Company, 633-639.
- Tetley, R.M. and K.V. Thimann, 1974. The metabolism of oat leaves during senescence: I. Respiration, carbohydrate metabolism, and the action of cytokinins. *Plant Physiol.*, 54: 294–303.
- Wang, S.Y. and J.R. Ballington, 2007. Free radical scavenging capacity and antioxidant enzyme activity in deerberry (*Vaccinium stamineum*, L.). *Science Direct*, 40: 1352-1361.
- Zaki, S.S., E.E. Belal, and M.M. Rady, 2019. Cyanobacteria and Glutathione Applications Improve Productivity, Nutrient Contents, and Antioxidant Systems of Salt-Stressed Soybean Plant *International Letters of Natural Sciences*, 76: 72 -85.
- Zanetti, G., 1979. Rabbit liver glutathione reductase, purification and properties. *Arch. Biochem. Physiol.*, 198: 241-246.