Pak. J. Agri. Sci., Vol. 59(1), 43-53; 2022
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/22.923
http://www.pakjas.com.pk

Exogenous osmolytes supplementation improves the physiological characteristics, antioxidant enzymatic activity and lipid peroxidation alleviation in drought-stressed soybean

Irfan Iftikhar[1], Muhammad Anwar-ul-Haq[1,*], Javaid Akhtar[1] and Muhammad Maqsood[2]

[1]Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad-38040, Pakistan; [2]Department of Agronomy, University of Agriculture, Faisalabad-38040, Pakistan.
*Corresponding author's e-mail: haqgondal@gmail.com; haq.gondal@uaf.edu.pk

Soybean is considered among the crops that demand a plentiful water supply, particularly during the growth phase to achieve maximum production. The drought stress adversely reduces the productivity and yield of soybean as compared to other legumes crops. With the passage of time, the local varieties of soybean have declined their yield potential because they have no resistance against abiotic stresses. There is a need to explore the avenues that minimize the impact of drought on soybean plants. In this regard, a pot experiment was performed to assess the exogenously applied foliar supplementation of proline and glycine betaine osmolytes @10mM and 20mM on the various physical parameters, oxidative stress amelioration, and cessation of damaging lipid peroxidation that indicates the beneficial effects of osmolytes supplementation in drought-stressed soybean. The water stress was applied at 50% of field capacity sown with three different genotypes of soybean including Ajmeri, Rawal, and Faisal-Soy. This leads to the reduced morphological growth and physiological response in all three soybean genotypes in lieu of water stress conditions. The water stress also reduced the content of proline and glycine betaine and reduced the activity of antioxidant enzymes. It also enhanced the process of lipid peroxidation in all genotypes of soybean. The osmolyte treatments enhanced the resistance against drought stress conditions by improving the physiological response and antioxidant enzymatic activity. It also decreased the degree of lipid peroxidation evaluated through MDA and H_2O_2 contents. The most significant results were found in the Ajmeri genotype as compared to other genotypes at 20mM foliar application of proline. Therefore, it is suggested that the use of exogenous osmolytes especially proline is helpful in enhancing soybean production and alleviating the drought stress effects.
Keywords: Soybean, drought stress, osmolytes, proline, ajmeri.

INTRODUCTION

Since the population of the world is increasing rapidly, food security has become the most concerning issue of the world that leads the agricultural sector to start focusing on techniques that help to mitigate abiotic stresses (Gonulal, 2020; Sivakumar *et al.*, 2020). Water stress is the most devastating abiotic stress that reduces up to 50-60% of crop yield (Sadeghipour and Abbasi, 2012; Zulfiqar *et al.*, 2020; Basal and Szabo. 2020). The water scarcity that leads to drought stress, is a major concern in countries like Pakistan, especially since the last decade. In 2018, around 45% reduction in rainfall has been observed in Pakistan (FAO, 2019). Soybean belongs to the leguminous family, and it is considered one of the most substantial oilseed crops in Pakistan. It is consumed all over the world due to its nutritional value (Naamala *et al.*, 2016). It is known with different names in different regions of the world such as meat of field or meat without bones etc. It is comprised of 40-42% protein and 18-20% oil contents (Kumar *et al.*, 2019; Akram, 2019). The seeds of soybean contain different vitamins and antioxidants that reinforce their nutritional value (Meghvansi *et al.*, 2010; Silva *et al.*, 2011). It is equally significant for both animals along the human diet (Devi *et al.*, 2012). In reference to its dry-matter analysis, some comprised of 30% carbohydrates primarily including cellulose, hemicellulose, and polysaccharides (Zheng *et al.*, 2019). The soybean incorporates 80% of the total area of overall leguminous crops and 68% of the total production of leguminous crops (Sabagh *et al.*, 2019). According to International World Soybean Production-2017, the total production of more than 340 million metric tons (MMT) of soybean was recorded globally in 2016. The countries including the USA, Brazil, and Argentina are ranked as the top three territories based on their

Iftikhar, I., M. A.-ul-Haq, J. Akhtar and M. Maqsood. 2022. Exogenous osmolytes supplementation improves the physiological characteristics, antioxidant enzymatic activity and lipid peroxidation alleviation in drought-stressed soybean. Pakistan Journal of Agricultural Sciences. 59: 43-53.
[Received 26 July 2021; Accepted 21 Feb 2022; Published 18 Mar 2022]

production and cultivation of the soybean. The share of these countries in total production of soybean was documented 117.20 MMT, 114 MMT, and 57.80 MMT respectively according to World Agricultural Production USA 2017 (Asif *et al.,* 2021). It is processed and crushed into different soya products such as soymeal, soy-flour, soymilk, and many other products. The main consumption of soybean has been reported primarily in animal feed as compared to the human diet and it is estimated that only 3% proportion of total production is used in the human diet and the rest is used for animal feeds (Cardoso *et al.,* 2018).

Soybean was brought to Pakistan in the 1960s and it was commercially used in the 1970s. In the 1990s, a little research work was started on breeding and genetics of soybean but due to little concern and lack of resources, only a few varieties were developed which are still in use to date. With the passage of time, these varieties have declined their yield potential because they have no resistance against abiotic stresses while contrarily these stresses are increasing alarmingly every year in countries like Pakistan (Khurshid, 2017; Asad, 2020; Pushpavalli *et al.,* 2020). As reported by the United States Department of Agriculture (USDA), Pakistan has imported 2.5 MMT soybean in 2019-20. In 2018-19 Pakistan spent US $1.5 billion on the import of soybean. In Pakistan, the imports and consumption of soybean were increased by 455% and 478% respectively, in the year 2019-20 as compared to 2014-15 (Ahmed *et al.*, 2020).

The worldwide change in climate conditions has given birth to several abiotic stresses that affect the growth of plants and cause a major issue of food security worldwide. (Shao *et al.,* 2007; Cardoso *et al.,* 2018; Zheng *et al.,* 2019). Water scarcity is the main hotspot of abiotic stresses in the world that caused a substantial impact on the quality and quantity of the crops (Passioura, 2007). Drought stress caused a harmful impact on the agriculture sector and enhanced the issue of food security globally. The water shortage caused a negative impact on different growth stages of plants from germination to ripening (Shao and Butler, 2009). A series of changes were occurring in physicochemical parameters of the plant under drought stress conditions. The water deficiency decreased plant growth and productivity by more than 50% (Emami Bistgani *et al.,* 2017). Water scarcity is a big environmental challenge for Pakistan, and it is placed in the third position among the cruel water shortage countries (International Monetary Fund, 2018). As reported by the Pakistan Council of Research in Water Resources (PCRWR), the water consumption per annum was declined and Pakistan falls into vast water scarcity problematic countries. In the 1950s the water consumption was as much as 5000 m^3 per annum but it has currently decreased to 1000 m^3 per annum (Aziz *et al.,* 2018). Soybean demands an ample water supply during its growth process to attain high production (Waraich *et al.,* 2011).

The exogenously applied different phytohormones and fertigation played a significant role in the development index and rate of growth of plants over abiotic stress environment (Samarah *et al.,* 2009). The proline and glycine betaine has major contributions to the stability of the membrane by diminishing the process of lipid peroxidation. The osmolytes exhibited a leading role in the inhibited process of protein denaturing of plant cells and enhanced the transportation of ions through the membrane under drought stress conditions (Shinde *et al.,* 2019), but the role of foliar supplementation of osmolytes on soybean in drought stress conditions is still unknown consequently. The research was designed to sort out the resistant genotype of soybean among the locally available genotypes and assess the role of two different osmolytes namely proline and glycine betaine (GB) on the development and growth of soybean during drought stress conditions. The extreme abiotic conditions, like drought stress, also lead to the extravagant production and accumulation of reactive oxygen species that lead to oxidative stress. The reactive oxygen species are highly reactive species present in nature that can affect plant organelles, metabolites, and molecules by disturbing various metabolic pathways that ultimately lead to cell death. Over time, plants have evolved various defense mechanisms helpful for the generation of antioxidants required to ameliorate the deleterious ROS. The trial was carried out to evaluate the importance of osmolytes in the mitigation of the drastic effects of water stress particularly the oxidative stress that occurred by virtue of water shortage.

MATERIALS AND METHODS

The trial was performed at the Saline Agriculture Research Centre, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan. The three varieties of soybean namely Ajmeri, Rawal, and Faisal-Soy were selected based on genetic resistance against water scarcity among the available varieties in Pakistan. The seed of these soybean varieties was collected from Ayub Agriculture Research Institute (AARI), National Agriculture Research Centre (NARC), and all over Pakistan. The sandy clay loam soil for the pot experiment was obtained from the local field and after sieving the soil, the 12 kg soil was filled in all pots and five seeds of each genotype were sowed per pot. The drought stress was applied based on field capacity by using the gravimetric method. Subsequently, the impact of foliar supplementation of two standard osmolytes including proline and glycine betaine (GB) at 10mM and 20mM concentrations was estimated. The groups were made as T1: Control; T2: FC 50% (drought stress at 50% field capacity); T3: FC 50% + Proline 10mM; T4: FC 50% + Proline 20mM; T5: FC 50% + GB 10mM; T6: FC 50% + GB 20mM. The field capacity of the soil was maintained on regular basis by the gravimetric method (Wilcox, 1951).

Using complete randomized design (CRD), the treatment of osmolytes was started after 2-3 days of germination of the seedling. When plants were reached at the R4 leaf stage, the chlorophyll contents of plants were noted by using the average value of SPAD-502. Subsequently, the crop was harvested at the reproductive stage, and physical parameters including average shoot length, and average fresh and dry weight of shoots and roots were estimated in all experimental groups. The fully extended leaves were collected and subsequently, the membrane stability index (MSI) and relative water contents (RWC) were evaluated using (Turner, 1986) and (Sairam *et al.*, 2002) methods respectively. For MSI, The EC of samples was recorded after heating the samples at 40°C and 100°C. The EC of samples was recorded with the EC meter (WTW-330i). The concentration of proline and glycine betaine in the leaves was also estimated by (Bates *et al.*, 1973) and (Grieve and Grattan, 1983) methods respectively. The total protein was estimated by the Bradford (1976) technique and the antioxidants activity of catalase (CAT) and superoxide dismutase (SOD) was assessed by the methods of (Chance and Maehly, 1955) and (Giannopolitis and Ries, 1977) respectively. The activity of peroxidase (POD) and ascorbate peroxidase (APX) was measured by the protocol of (Bergmeyer and Bernt, 1974) and (Krivosheeva *et al.*, 1996) respectively. The lipid peroxidation parameters including the levels of malondialdehyde (MDA) and levels of H_2O_2 were measured using the protocols of (Zhang and Kirkham, 1996) and (Junglee *et al.*, 2014) respectively. All these parameters were measured by using a spectrophotometer (UV6000, R&M, USA). Results of all parameters were analyzed, and the level of significance was estimated using the software Statistix 8.1® (Torrie, 2014). The Pearson correlation among the different attributes was measured by utilizing the R-Studio.

RESULTS

Effect of exogenous application of osmolytes on morphological growth of soybean under drought stress: Water is a major constituent of different metabolic activities within the plant cell. The result of our experiment showed that

Table 1. Effect of exogenously applied different osmolytes on shoot length, fresh and dry weight.

Treatments	Shoot length (cm plant^{-1})			Shoot fresh weight (g plant^{-1})			Shoot dry weight (g plant^{-1})		
	Ajmeri	Rawal	Faisal-Soya	Ajmeri	Rawal	Faisal-Soya	Ajmeri	Rawal	Faisal-Soya
Control	75.3a	70.6b	74.0ab	31.33a	27.33bc	29.00b	10.87a	9.63c	10.14b
FC 50%	33.0j	29.0k	31.6jk	10.20klm	8.70m	9.13lm	2.06l	0.92n	1.57m
FC 50% + Proline 10mM	46.0fg	40.0hi	43.6gh	14.20hi	12.80ij	13.87hi	6.02gh	5.14ij	5.85gh
FC 50% + Proline 20mM	62.3c	55.6d	60.6c	25.63cd	23.57e	24.13de	8.43d	7.26e	8.00d
FC 50% + GB 10mM	41.0hi	34.0j	38.0i	12.47ij	10.63kl	11.60jk	5.58hi	4.12k	5.08j
FC 50% + GB 20mM	52.0de	48.0f	49.3ef	19.97f	15.27h	17.30g	6.81f	5.86gh	6.15g

a, b, ... mean values within a column, not bearing a common letters differ significantly ($P \leq 0.05$). FC= Field Capacity, GB= Glycine Betaine.

Table 2. Effect of exogenously applied different osmolytes on root fresh weight, root dry weight and relative chlorophyll contents (SPAD).

Treatments	Root fresh weight (g plant^{-1})			Root dry weight (g plant^{-1})			Relative chlorophyll (SPAD)		
	Ajmeri	Rawal	Faisal-Soya	Ajmeri	Rawal	Faisal-Soya	Ajmeri	Rawal	Faisal-Soya
Control	5.64a	4.83c	5.18b	2.06a	1.68c	1.90b	49.30a	41.57cd	45.37b
FC 50%	1.81l	1.05n	1.56m	0.69i	0.39k	0.48jk	32.57i	27.77j	29.57j
FC 50% + Proline 10mM	2.83gh	2.37jk	2.62hi	0.92gh	0.67i	0.84h	37.13efg	34.00hi	35.43gh
FC 50% + Proline 20mM	4.54d	3.63f	4.09e	1.67c	1.16e	1.37d	43.87bc	38.73ef	41.50cd
FC 50% + GB 10mM	2.46ijk	2.01l	2.25k	0.89gh	0.53j	0.73i	36.23fgh	32.57i	34.43hi
FC 50% + GB 20mM	3.02g	2.55ij	2.90g	1.06f	0.88h	0.98fg	39.23de	36.47fgh	37.60efg

a, b, ... mean values within a column, not bearing a common letters differ significantly ($P \leq 0.05$). FC= Field Capacity, GB= Glycine Betaine.

the growth of all genotypes was decreased drastically during the water stress as compared to control conditions. The results of morphological growth parameters were shown in Tables 1 and 2. The shoot length was decreased more than 50% in FC 50% group as compared to the control. In the case of shoot fresh and dry weights, they also got declined in FC 50% group as compared to control conditions. Regarding root fresh and dry weight, FC 50% group again exhibited the lowest values as expected. Among comparisons between different genotypes, the maximum fresh and dry weight of shoots and roots were recorded in the Ajmeri genotype. In reference to the relative effects of two different osmolytes proline and glycine betaine at different concentrations of 10mM and 20mM in mitigating the adverse consequences of water stress, the most significant results were found under the 20mM foliar application of proline. Moreover, among the comparison of different experimental genotypes, the shoot length along with the fresh and dry weight of shoots and roots were found to significantly improve in Ajmeri genotype with the least ameliorating effects on the Rawal genotype.

The photosynthetic efficiency is directly linked to the concentration of chlorophyll contents that is affected by the availability of water. The SPAD value indicates the chlorophyll matter in the leaves of the plant. The chlorophyll contents of all the genotypes were well expectedly found reduced under the drought stress condition as compared to the control. The foliar supplementation of 20mM proline gave the most significant results as compared to other treatments. The maximum chlorophyll contents were recorded in Ajmeri under the treatment of 20mM proline while the least benefit was found in the Rawal. The afore-mentioned results certainly indicate that the exogenously applied osmolytes enhance the overall morphological growth of soybean under drought stress conditions.

Role of osmolytes on water balance and membrane stability health of soybean under drought stress: The results of relative water contents (RWC) and membrane stability index

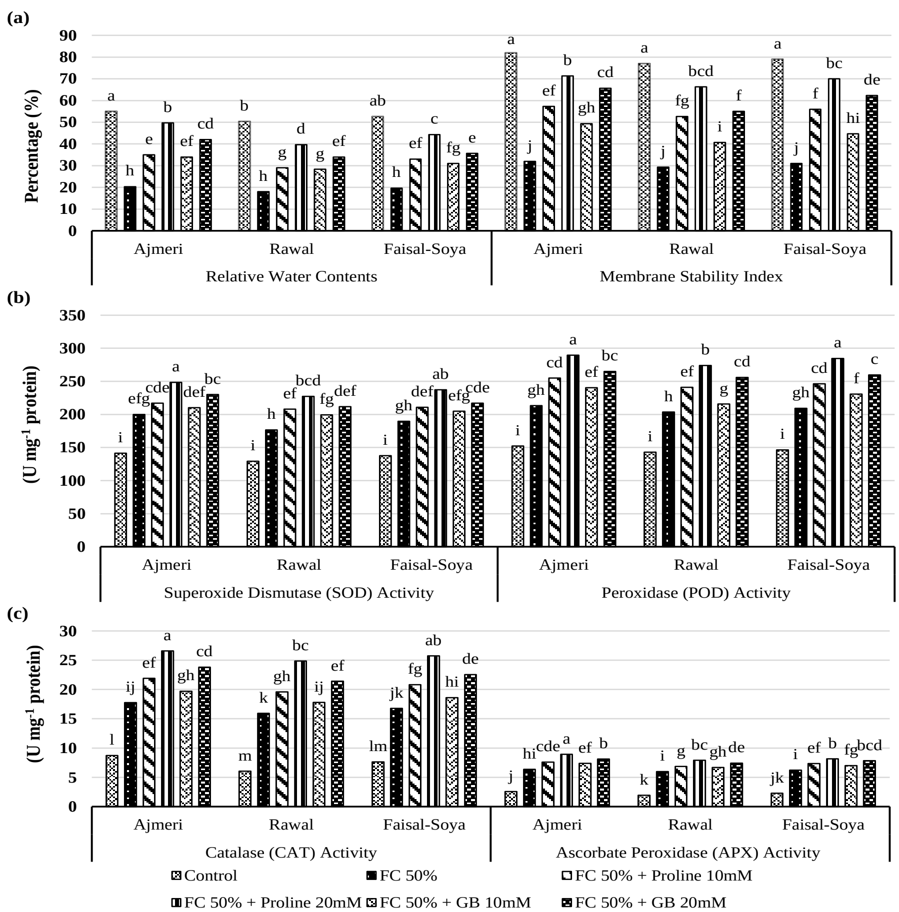

Figure 1. Effect of osmolytes on physiological parameters and antioxidant enzymatic activity [a) relative water contents and membrane stability index, b) superoxide dismutade and peroxidase, c) catalase and ascorbate peroxidase] of soybean under drought stress condition. a, b, ... mean values within a column, not bearing a common letters differ significantly (P≤0.05). FC= Field Capacity, GB= Glycine Betaine.

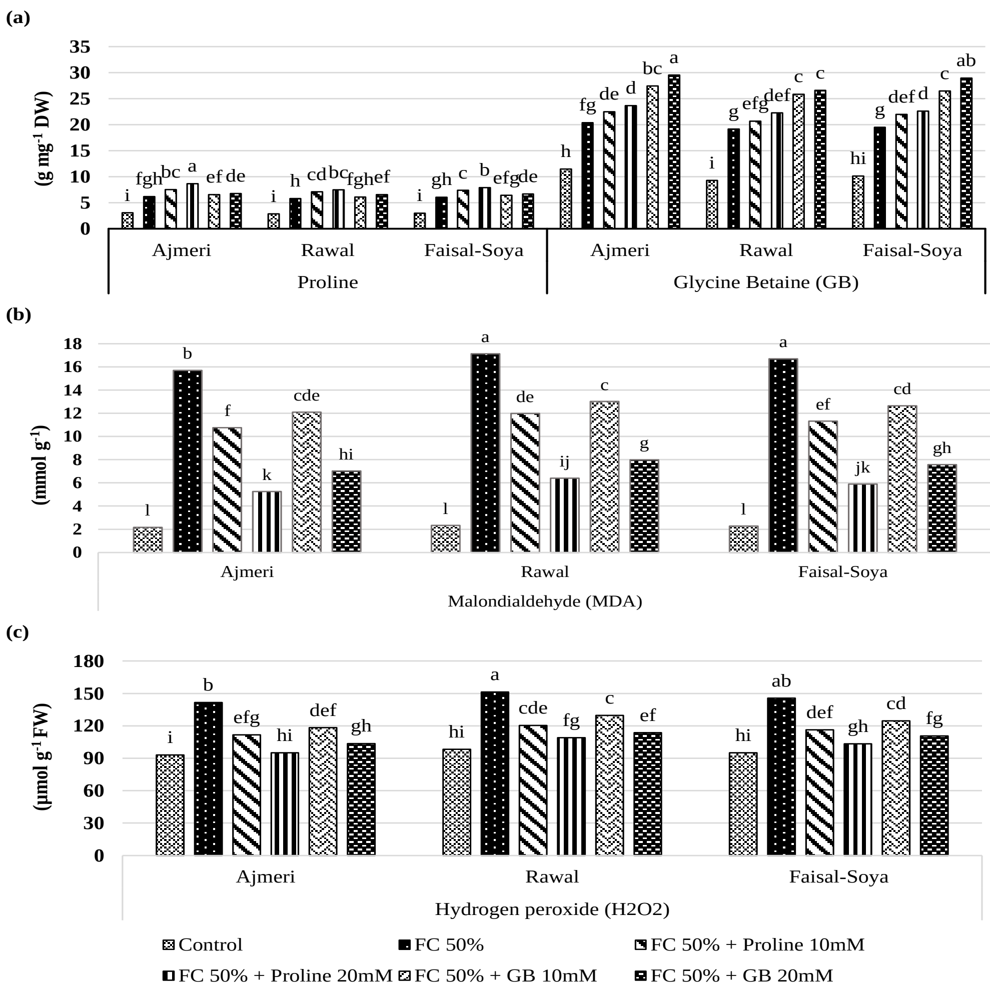

Figure 2. Effect of osmolytes on endogenous level of proline and glycine betaine and process of lipid peroxidation [a) proline and glycine betaine, b) Malondialdehyde (MDA), c) Hydrogen peroxide (H_2O_2)] of soybean under drought stress condition. a, b, ... mean values within a column, not bearing a common letters differ significantly ($P \leq 0.05$). FC= Field Capacity, GB= Glycine Betaine.

(MSI) as shown in Figure 1 (a) indicates that the water stress causes an adverse effect regarding both factors. Both the RWC and MSI attained a more than 50% reduction in FC 50% group in comparison to the control group. Regarding the comparison of the impact of both osmolytes viz. proline and glycine betaine at different concentrations of 10mM and 20mM, the most compelling effects were found under the 20mM foliar application of proline. Among the comparison between genotypes, the Ajmeri genotype exhibited the most appealing effects by the 20mM proline treatment hence refer that the Ajmeri genotype has more potential to alleviate the negative effects of drought stress under foliar supplementation of proline. The correlation between the different growth and physiological parameters were shown in Figure 3.

Impact of osmoprotectants on the activity of antioxidant enzymes under water stress conditions: The antioxidant enzymatic activity of SOD, POD, CAT, and APX enzymes in foliar tissues was well-expectedly found to increase in FC 50% group in comparison to the control group in lieu of water stress. The subsequent treatment of proline and glycine betaine osmolytes at concentrations of 10mM and 20mM under water deficit conditions further increased the enzyme activities especially in the group given the treatment of 20mM proline. The most promising results among different genotypes were found again in Ajmeri genotype as compared to Rawal and Faisal-Soya genotypes. The results are graphically presented in Figures 1 (b) and (c). Our findings suggest that the surge in the activity of antioxidant enzymes in treatment groups would help the plant in scavenging the reactive oxygen species (ROS) produced because of drought

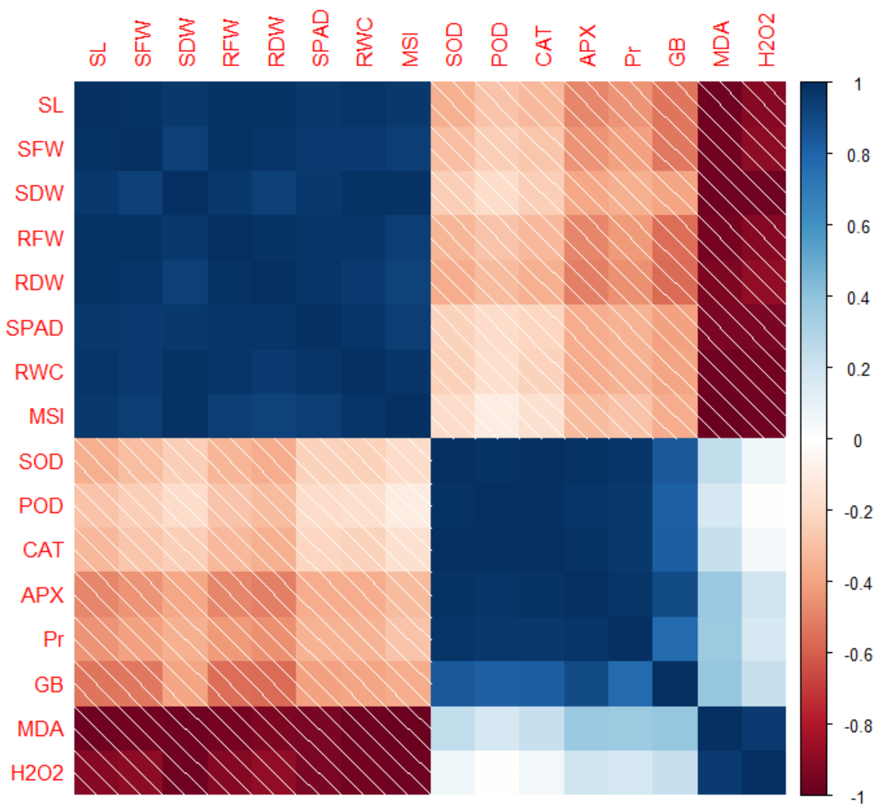

Figure 3. Correlation between different morphological, physiological, antioxidant enzymatic activity, osmoprotectant and rate of lipid peroxidation of soybean under drought stress conditions. **SL**= Shoot length, **SFW** = Shoot fresh weight, **SDW** = Shoot dry weight, **RFW** = Root fresh weight, **RDW**= Root dry weight, **SPAD** = Chlorophyll content, **RWC** = Relative water content, **MSI** = Membrane stability index, **SOD** = Superoxide dismutase, **POD** = Peroxidase, **CAT** = Catalase, **APX** = Ascorbate peroxidase, **Pr**. = Proline, **GB** = Glycine betaine, **MDA** = malondialdehyde, **H_2O_2** = Hydrogen peroxide

stress. The correlation of antioxidant enzymatic activity with other parameters was presented in Figure 3.

***Osmolytes contents of soybean under drought stress*: The osmolytes are very much crucial for the plant to deal with stress conditions. Through strengthening the plant's defense mechanism, it supports the plant in combating the abiotic stress environment. In this experiment, the level of osmolytes in the leaves of the plant increased significantly in FC 50% group in comparison to the control group. However, since the need for osmolytes gets more critical during the stress conditions, the typical rise in osmolytes level is unable to address the additional need for osmolytes for general physiological mechanisms. Interestingly, the exogenous supplementation of proline and glycine betaine osmolytes increased the plant osmolytes levels to an extent where they can satisfy the increased need for osmolytes to encounter the stress conditions more effectively. The results of the experiment were shown in Figure 2 (a). The maximum level of proline was found in the group treated with 20mM exogenous proline. While the maximum amount of glycine betaine was found in the group treated with 20mM exogenous glycine betaine. Among the comparison between genotypes, the Ajmeri genotype responded the best towards the exogenous supplementation of proline and glycine betaine osmolytes. The Pearson correlation was shown in Figure 3.

***Effect of exogenous application of osmolytes on lipid peroxidation of soybean under drought stress*:** Malondialdehyde (MDA), the main reactive aldehyde produced because of lipid peroxidation, is employed for the assessment of damage by the reactive oxygen species generated during drought stress. On the other hand, the level of hydrogen peroxide (H_2O_2) is also considered a characteristic feature to represent the impact of oxidative stress resulting from water stress conditions. In this research, the levels of both MDA and H_2O_2 levels were found anticipatedly improved under the water stress conditions as compared to the control. The graphical representation of results is shown in Figures 2 (b) and (c). The exogenously applied osmolytes decreased the lipid peroxidation exhibited by a decrease in the MDA contents and concentration of H_2O_2. The most significant results were recorded at 20mM foliar application of proline as compared to other treatments.

Among the genotypes' comparison, the Ajmeri genotype exhibited the best results in annulling the stress parameters with the help of exogenous treatment of osmolytes. In view of this, it is postulated that by enhancing the plant defense mechanism, exogenously applied osmolytes play a vital role in combating the lipid peroxidation caused by the stress conditions including drought stress. The rate of lipid peroxidation with other attributes were presented in Figure 3.

DISCUSSION

Drought stress decreases crop productivity that leads to economic damage for the farmers. (Sadeghipour and Abbasi, 2012). Insufficient water supply and/or lower rainfall in field areas affects both the vegetative and reproductive development of crops (Silva *et al.,* 2010). Soybean is considered among the crops that demand a plentiful water supply, particularly during the growth phase to achieve the maximum high productions. The drought stress adversely reduces the productivity and yield of soybean more severely as compared to other legumes crops (Lobato *et al.,* 2009; Masoumi *et al.,* 2010). Very few research is available in the literature archive regarding the evaluation of exogenous supplements that may help in the mitigation of drought stress in soybean. We evaluated the effect of exogenously applied foliar supplementation of proline and glycine betaine osmolytes on the various physical parameters, oxidative stress amelioration, and cessation of damaging lipid peroxidation that indicates the beneficial effects of osmolytes supplementation in drought-stressed soybean. The impact can equally be proposed for other stress-like conditions including high temperature and salinity (Ku *et al.*, 2013).

In our research experiment, the growth parameters including the shoot length, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight was evaluated to estimate the soybean overall growth. The results of our experiment suggested that the plant growth factors got reduced under the drought stress in comparison to control environments (Stolf-Moreira *et al.,* 2010). The drought stress decreased the water contents in leaves estimated through RWC and MSI in all genotypes. More water transpires from the stomata in response to photosynthetic activity as compared to available water to plants for their metabolic activity (Roldán *et al.,* 2008). The treatment of osmolytes significantly mitigated the adverse effects of water stress (Aslam *et al.,* 2011). The results can be associated with the phenomenon that exogenous osmolytes supplementation increased the osmotic potential of the plant in treatment groups as exhibited through RWC and MSI evaluation. The chlorophyll contents evaluated through SPAD also increased significantly in the treatment groups. Improvement in relative water contents and chlorophyll pigment by the supplementation of exogenous osmolytes rationalizes the increase in photosynthesis that resulted in improved growth of plants. The availability of more water for photosynthetic and other metabolic activities explains the increase in biomass produced by soybean genotypes under water stress conditions.

The generation of reactive oxygen species (ROS) is the primary consequence of water deficiency in plants that leads to the activation of defense systems in plants. Under water stress, various ROS including hydroxyl radical, hydrogen peroxide, and superoxide radical are synthesized and aggregated within the plant cells and result in oxidative stress to proteins, lipids, and nucleic acids along with various cell structures (Cruz De Carvalho, 2008). The combat this oxidative stress, the plant enhances the antioxidant enzymatic activity to mitigate the damaging effects of drought stress. It also activates several biochemical, molecular, and cellular processes that help the cell fight with oxidative stress burden (Samarah *et al.,* 2009). In the present study, all drought-stress groups demonstrated a slight improvement in the levels of antioxidant enzymes including SOD, POD, CAT, and APX. Nevertheless, this natural defense mechanism of increased antioxidant enzymes to combat oxidative stress is not enough against the continuously increasing levels of ROS. The foliar application of osmolytes was evaluated for potential assistance in antioxidant activity to alleviate the harmful effects of drought stress. Results exhibited a significant increase in the levels of all antioxidant enzymes in osmolytes treated groups.

The antioxidant enzyme SOD plays a vital part in the detoxification of superoxide radicals along with hydrogen peroxide produced during drought-stress conditions (Lakshmi Sahitya *et al.*, 2018). Various exogenously applied treatments have been reported to enhance the SOD activity and significant results have been reported in soybean, safflower, liquorice, and lupin (Pan *et al.,* 2006; Masoumi *et al.,* 2010; Mohammadi *et al.,* 2011). The result of our experiment demonstrated that the proline enhances the activity of SOD under drought stress conditions in comparison to FC 50% treatment. The antioxidant enzymes including POD and CAT are considered helpful in scavenging the H_2O_2 and O_2 into the less harmful molecules and protecting the plant from the toxic effect of drought stress (Mohsenzadeh *et al.*, 2006; Wang *et al.,* 2012; Abid *et al.*, 2016). We observed a significant increase in both POD and CAT levels in osmolytes treated groups indicating the supportive effect of foliar application of osmolytes in combating the fight with oxidative stress. Likewise, the antioxidant enzyme APX plays a vital role in the modification of H_2O_2 into less harmful entities by the glutathione-ascorbate cycle (Kurutas *et al.,* 2016). The foliar application of osmolytes exhibited an increase in the activity of APX suggesting the important role of osmolytes application during drought-stress conditions.

The reactive oxygen species produced in oxidative stress caused by drought stress may also lead to lipid peroxidation (Ullah *et al.*, 2018). Peroxidation of membrane lipids by ROS is an indication of membrane damage and leakage under drought stress conditions (Liu *et al.,* 2017). Primarily, the MDA and H_2O_2 concentration is measured to evaluate the extent of lipid peroxidation in plants. Malondialdehyde is the key secondary product of polyunsaturated fatty acid oxidation that offers the assessment of the damage resulted by the ROS produced during water stress (Wahid *et al.,* 2007; Farnese *et al.,* 2016). Likewise, the level of hydrogen peroxide also indicates the impact of oxidative stress. A rise in lipid peroxidation because of water stress has been also described by the other researchers (Roldan *et al.,* 2008; Uzhilday *et al.,* 2012; Pan *et al.,* 2006). In the present research, the levels of both MDA and H_2O_2 were found significantly improved under the water stress conditions as compared to the control and were found significantly improved in osmolytes supplemented groups. The foliar application of osmolytes increased the activity of antioxidant enzymes including super peroxidase, peroxidase, and catalases that bind the active site of ROS and save the plant from lipid peroxidation under drought stress conditions.

During the drought stress condition, compatible solute accumulation is the primary approach adopted by the plants for osmoprotection (Majumdar *et al.,* 2016). The mechanism of osmoprotection is dependent upon the close relationship of osmolytes with various components of the cell, resulting in the maintenance of turgor pressure through regulating the water contents inside the cells (Solanki and Sarangi, 2014). During drought stress, proline exhibits a significant role and acts as a signaling element to control the mitochondria performance and influence cell proliferation through activating various genes that are considered important for stress retrieval (Shinde *et al.,* 2016; Per *et al.,* 2017). Proline accumulation specifically helps in maintaining membrane integrity by declining the oxidation of lipids by means of defending cellular redox potential and scavenging free radicals (Osman, 2015; Rady *et al.,* 2016). Nevertheless, the level of osmolytes in the leaves improved significantly in the FC 50% group in comparison to the control group, the customary increase in osmolytes level is unable to address the additional need of osmolytes to combat oxidative stress conditions that occurred because of drought stress. Therefore, additional solute supplementation helps increase the endogenous levels of osmolytes that relieve the plant in ameliorating the toxic effects of water scarcity.

Many studies have proved that the different genotypes of the same crop have different enzymatic activity against drought stress (Wang *et al.,* 2012; Bennett *et al.,* 2004; Masoumi *et al.,* 2010). The three genotypes of soybean Ajmeri, Rawal, and Faisal-Soya were selected for our research experiment to evaluate the effect of osmolytes under drought stress conditions. Among all genotypes, the Ajmeri genotype responded the best towards foliar application of osmolytes. The activity of antioxidant enzymes was found more significant in Ajmeri genotype as compared to other genotypes. This higher activity of antioxidant enzymes decreased the lipid peroxidation process and enhanced the plant defense mechanism against stress conditions (Hojati *et al.,* 2011; Sharma *et al.,* 2012; Ibrahim *et al.,* 2016). Under drought stress conditions, the process of lipid peroxidation has a major contribution to diminishing the growth and physiological attributes of soybean with reference to substitution in the activity of antioxidant enzymatic and endogenous levels of osmolytes. The strong positive and negative correlation between the different parameters of growth, physiological, antioxidant enzymatic activity, the concentration of proline and glycine betaine, and rate of lipid peroxidation under drought stress is exhibited in Figure 3.

Conclusion: Drought stress is considered a threatening challenge to agricultural crops including soybean and since the threat is increasing alarmingly in countries like Pakistan, there is a desperate need for strategies to explore the avenues that minimize the drought effects. That being the case, we explored the role of foliar supplementation of osmolytes on soybean in drought stress conditions, and based on results, we suggest the use of exogenous osmolytes especially proline at 10mM concentration is helpful in enhancing soybean production and alleviating the drought stress effects.

Conflict of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' Contribution Statements: I. Iftikhar and M. Anwar-ul-Haq conducted the experiment and collected data. I. Iftikhar, M. Anwar-ul-Haq and J. Akhtar prepared the first draft of manuscript. I. Iftikhar, M. Anwar-ul-Haq, J.Akhtar and M. Maqsood planned the study and M. Anwar-ul-Haq and I. Iftikhar finalized the final draft of manuscript.

Acknowledgments: The financial assistantship provided by the Higher Education Commission (HEC) Islamabad, Pakistan, under the HEC Funded Project (NRPU-4624 and NRPU-13599) for this study, is highly acknowledged.

REFERENCES

Abid, G., M. M'hamdi, D. Mingeot, M. Aouida, I. Aroua, Y. Muhovski, K. Sassi, F. Souissi, K. Mannai and M. Jebara. 2017. Effect of drought stress on chlorophyll fluorescence, antioxidant enzyme activities and gene expression patterns in faba bean (*Vicia faba L.*). Archives of Agronomy and Soil Science. 63:536-552.

Ahmed, A., S. Aftab, S. Hussain, H.N. Cheema, W. Liu, F. Yang and W. Yang. 2020. Nutrient accumulation and distribution assessment in response to potassium application under maize–soybean intercropping system. Agronomy. 10:1-18.

Akram, Z. 2019. Future prospects of soybean in Pakistan. Biomed. Journal of Scientific and Technical Research. 15:15-17.

Asad, S. A., M. A. Wahid, S. Farina, R. Ali, and F. Muhammad. 2020. Soybean production in Pakistan: experiences, challenges and prospects. International Journal of Agriculture and Biology. 24:995-1005.

Asif, M., H. Abd-Ur-Rahman, A. Aziz, M. Adnan, A. Ali, M.E. Safdar, N. Akhtar, M.S. Hanif, M.A. Javed, M. Abdullah and F. Ur Rehman. 2021. Seed priming improves growth, yield and seed oil contents of soybean (*Glycine max L.)* cultivars under semi-arid conditions of Sargodha, Pakistan. Plant Cell Biotechnology and Molecular Biology. 22:27-37.

Aslam, R., N. Bostan, Nabgha e Amen, M. Maria and W. Safdar. 2011. A critical review on halophytes: salt tolerant plants. Journal of Medicinal Plants Research. 5:7108-7118.

Aziz, O., S. Hussain, M. Rizwan, M. Riaz, S. Bashir, L. Lin, S. Mehmood, M. Imran, R. Yaseen and G. Lu. 2018. Correction to: Increasing water productivity, nitrogen economy, and grain yield of rice by water saving irrigation and fertilizer-N management. Environmental Science and Pollution Research. 25:16616-16619.

Basal, O. and A. Szabo. 2020. Physiology, yield and quality of soybean as affected by drought stress. Asian Journal of Agriculture and Biology 8:247-252.

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

Bennett, J.O., O. Yu, L.G. Heatherly and H.B. Krishnan. 2004. Accumulation of genistein and daidzein, soybean isoflavones implicated in promoting human health, is significantly elevated by irrigation. Journal of Agricultural and Food Chemistry. 52:7574-7579.

Bergmeyer, H.U. and E. Bernt. 1974. UV-Assay with Pyruvate and NADH. In Methods of enzymatic analysis. 574-579.

Cardoso, C.C., B.M.O. Mendes and V.M.D. Pasa. 2018. Production and characterization of cold-flow quality biofuel from soybean oil using different alky and benzyl alcohols. Journal of Environmental Chemical Engineering. 6:2241-2247.

Chance, B., & A.C. Maehly. 1955. Assay of catalases and peroxidases. Methods in Enzymology. 764-775.

Cruz De Carvalho, M.H. 2008. Drought stress and reactive oxygen species: production, scavenging and signaling. Plant Signaling & Behavior. 3:156-165.

Devi, K.N., L.N.K. Singh, M. Sumarjit Singh, S. Basanta Singh and K. Khamba Singh. 2012. Influence of sulphur and boron fertilization on yield, quality, nutrient uptake and economics of soybean (*Glycine max*) under upland conditions. Journal of Agricultural Science. 4:1-10.

Emami Bistgani, Z., S.A. Siadat, A. Bakhshandeh, A. Ghasemi Pirbalouti and M. Hashemi. 2017. Interactive effects of drought stress and chitosan application on physiological characteristics and essential oil yield of *Thymus daenensis* Celak. The Crop Journal. 5:407-415.

Farnese, F.S., P.E. Menezes-Silva, G.S. Gusman and J.A. Oliveira. 2016. When bad guys become good ones: The key role of reactive oxygen species and nitric oxide in the plant responses to abiotic stress. Frontiers in Plant Science. 7:1-15.

Giannopolitis, C.N. and S.K. Ries. 1977. Superoxide dismutases: I. Occurrence in higher plants. Journal of Plant Physiology. 59:309-314.

Gonulal, E. 2020. Performance of sorghum× Sudan grass hybrid (*Sorghum bicolor* L. × *Sorhgum sudanense*) cultivars under water stress conditions of arid and semi-arid regions. Journal of Global Innovations in Agricultural and Social Science 8:78-82.

Grieve, C.M. and S.R. Grattan. 1983. Rapid assay for determination of water soluble quaternary ammonium compounds. Plant and Soil. 70:303-307.

Hojati, M., S.A.M. Modarres-Sanavy, M. Karimi and F. Ghanati. 2011. Responses of growth and antioxidant systems in *Carthamus tinctorius L*. under water deficit stress. Acta Physiologiae Plantarum. 33:105-112.

Ibrahim, E.A. 2016. Seed priming to alleviate salinity stress in germinating seeds. Journal of Plant Physiology. 192:38-46.

Junglee, S., L. Urban, H. Sallanon and F. Lopez-Lauri. 2014. Optimized assay for hydrogen peroxide determination in plant tissue using potassium iodide. American Journal of Analytical Chemistry. 05:730-736.

Khurshid, H. 2017. Miracle crop: the present and future of soybean production in Pakistan. MOJ Biology and Medicine. 2:Pp….????

Krivosheeva, A., D.L. Tao, C. Ottander, G. Wingsle, S.L. Dube' and G. Öquist. 1996. Cold acclimation and photoinhibition of photosynthesis in Scots pine. Planta. 200:296-305.

Ku, Y.-S., W.-K. Au-Yeung, Y.-L. Yung, M.-W. Li, C.-Q. Wen, X. Liu and H.-M. Lam. 2013. Drought stress and tolerance in soybean. A comprehensive survey of international soybean research—Genetics, physiology, agronomy and nitrogen relationships. 209-237.

Kumar, J., N. Sharma, G. Kaushal, S. Samurailatpam, D. Sahoo, A.K. Rai and S.P. Singh. 2019. Metagenomic insights into the taxonomic and functional features of kinema, a traditional fermented soybean product of sikkim himalaya. Frontiers in Microbiology. 10:1-17.

Kurutas, E.B. 2016. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Journal of Nutrition. 15:1-22.

Lakshmi Sahitya, U., M.S.R. Krishna, R. Sri Deepthi, G. Shiva Prasad and D. Peda Kasim. 2018. Seed antioxidants interplay with drought stress tolerance indices in chilli (*Capsicum annuum L*) seedlings. BioMed Research International. 2018.

Liu, Y., Q. Song, D. Li, X. Yang and D. Li. 2017. Multifunctional roles of plant dehydrins in response to environmental stresses. Frontiers in Plant Science. 8:2015-2018.

Majumdar, R., B. Barchi, S.A. Turlapati, M. Gagne, R. Minocha, S. Long and S.C. Minocha. 2016. Glutamate, ornithine, arginine, proline, and polyamine metabolic interactions: The pathway is regulated at the post-transcriptional level. Frontiers in Plant Science. 7:1-17.

Masoumi, H., M. Masoumi, F. Darvish, J. Daneshian, G. Nourmohammadi and D. Habibi. 2010. Change in several antioxidant enzymes activity and seed yield by water deficit stress in soybean (*Glycine max L.*) cultivars. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 38:86-94.

Meghvansi, M.K., K. Prasad and S.K. Mahna. 2010. Symbiotic potential, competitiveness and compatibility of indigenous Bradyrhizobium japonicum isolates to three soybean genotypes of two distinct agro-climatic regions of Rajasthan, India. Saudi Journal of Biological Sciences. 17:303-310.

Mohammadi, A., D. Habibi, M. Rohami and S. Mafakheri. 2011. Effect of drought stress on antioxidant enzymes activity of some chickpea cultivars. Journal of Agriculture and Environmental Sciences. 11:782-785.

Mohsenzadeh, S., M.A. Malboobi, K. Razavi and S. Farrahi-Aschtiani. 2006. Physiological and molecular responses of *Aeluropus lagopoides* (Poaceae) to water deficit. Environmental and Experimental Botany. 56:314-322.

Naamala, J., S.K. Jaiswal and F.D. Dakora. 2016. Microsymbiont diversity and phylogeny of native bradyrhizobia associated with soybean (*Glycine max L. Merr*.) nodulation in South African soils. Systematic and Applied Microbiology. 39:33-344.

Osman, H.S. 2015. Enhancing antioxidant–yield relationship of pea plant under drought at different growth stages by exogenously applied glycine betaine and proline. Annals of Agricultural Sciences. 60:389-402.

Pan, Y., L.J. Wu and Z.L. Yu. 2006. Effect of salt and drought stress on antioxidant enzymes activities and SOD isoenzymes of liquorice (*Glycyrrhiza uralensis Fisch*). Plant Growth Regulation. 49:157-165.

Passioura, J. 2007. The drought environment: Physical, biological and agricultural perspectives. Journal of Experimental Botany. 58:113-117.

Per, T.S., N.A. Khan, P.S. Reddy, A. Masood, M. Hasanuzzaman, M.I.R. Khan and N.A. Anjum. 2017. Approaches in modulating proline metabolism in plants for salt and drought stress tolerance: Phytohormones, mineral nutrients and transgenics. Plant Physiology and Biochemistry. 115:126-140.

Pushpavalli, R., J.D. Berger, N.C. Turner, K.H.M. Siddique, T.D. Colmer and V. Vadez. 2020. Cross-tolerance for drought, heat and salinity stresses in chickpea (*Cicer arietinum L.*). Journal of Agronomy and Crop Science. 206:405-419.

Rady, M.M. and K.A. Hemida. 2016. Sequenced application of ascorbate-proline-glutathione improves salt tolerance in maize seedlings. Ecotoxicology and Environmental Safety. 133:252-259.

Roldán, A., P. Díaz-Vivancos, J.A. Hernández, L. Carrasco and F. Caravaca. 2008. Superoxide dismutase and total peroxidase activities in relation to drought recovery performance of mycorrhizal shrub seedlings grown in an amended semiarid soil. Journal of Plant Physiology. 165:715-722.

Sabagh, A.E.L., A. Hossain, M.S. Islam, C. Barutçular, D. Ratnasekera, N. Kumar, R.S. Meena, H.S. Gharib, H. Saneoka and J.A.T. da Silva. 2019. Sustainable soybean production and abiotic stress management in saline environments. Australian Journal of Crop Science. 13:228-236.

Sadeghipour, O. and S. Abbasi. 2012. Soybean response to drought and seed inoculation. World Applied Sciences Journal. 17:55-60.

Sairam, R.K., K. Rao and G.C. Srivastava. 2002. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Science. 163:1037-1046.

Samarah, N.H., R.E. Mullen and I. Anderson. 2009. Soluble sugar contents, germination, and vigor of soybean seeds in response to drought stress. Journal of New Seeds. 10:63-73.

Shao, H. and E.C. Butler. 2009. The relative importance of abiotic and biotic transformation of carbon tetrachloride in anaerobic soils and sediments. Soil and Sediment Contamination. 18:455–469.

Shao, H.B., L.Y. Chu, G. Wu, J.H. Zhang, Z.H. Lu and Y.C. Hu. 2007. Changes of some anti-oxidative physiological indices under soil water deficits among 10 wheat (*Triticum aestivum L.*) genotypes at tillering stage. Colloids Surfaces B: Biointerfaces. 54:143-149.

Sharma, P., A.B. Jha, R.S. Dubey and M. Pessarakli. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany. 2012:1-26.

Shinde, H., A. Dudhate, D. Tsugama, S.K. Gupta, S. Liu and T. Takano. 2019. Pearl millet stress-responsive NAC transcription factor PgNAC21 enhances salinity stress tolerance in Arabidopsis. Plant Physiology and Biochemistry. 135:546-553.

Shinde, S., J.G. Villamor, W. Lin, S. Sharma and P.E. Verslues. 2016. Proline coordination with fatty acid synthesis and redox metabolism of chloroplast and mitochondria. Plant Physiology. 172:1074-1088.

Silva, E.N., R. V. Ribeiro, S.L. Ferreira-Silva, R.A. Viégas and J.A.G. Silveira. 2010. Comparative effects of salinity and water stress on photosynthesis, water relations and growth of *Jatropha curcas* plants. Journal of Arid Environments. 74:1130-1137.

Silva, G.F., F.L. Camargo and A.L.O. Ferreira. 2011. Application of response surface methodology for optimization of biodiesel production by transesterification of soybean oil with ethanol. Fuel Processing Technology. 92:407-413.

Sivakumar, J., J.E.P. Prashanth, N. Rajesh, S.M. Reddy and O.B. Pinjari. 2020. Principal component analysis approach for comprehensive screening of salt stress-tolerant tomato germplasm at the seedling stage. Journal of Biosciences. 45:1-11.

Solanki, J.K. and S.K. Sarangi. 2014. Effect of drought stress on proline accumulation in peanut genotypesInternational Journal of Advanced Research. 2:301-309.

Stolf-Moreira, R., M.E. Medri, N. Neumaier, N.G. Lemos, J.A. Pimenta, S. Tobita, R.L. Brogin, F.C. Marcelino-Guimarães, M.C.N. Oliveira, J.R.B. Farias, R. V. Abdelnoor and A.L. Nepomuceno. 2010. Soybean physiology and gene expression during drought. Genetics and Molecular Research. 9:1946-1956.

Torrie, H. 2014. Principles and Procedures of Statistics: A Biometrical Approach. by Robert G. D. Steel; James Review by: Gordon D. Booth Published by: American Statistical Association Stable. 76:753-754.

Turner, N.C. 1986. Adaptation to water deficits: a changing perspective. Australian Journal of Plant Physiology. 13:175-190.

Ullah, A., H. Manghwar, M. Shaban, A.H. Khan, A. Akbar, U. Ali, E. Ali and S. Fahad. 2018. Phytohormones enhanced drought tolerance in plants: a coping strategy. Environmental Science and Pollution Research. 25:33103-33118.

Uzilday, B., I. Turkan, A.H. Sekmen, R. Ozgur and H.C. Karakaya. 2012. Comparison of ROS formation and antioxidant enzymes in *Cleome gynandra* (C4) and *Cleome spinosa* (C3) under drought stress. Plant Science. 182:59-70.

Wahid, A., M. Perveen, S. Gelani and S.M.A. Basra. 2007. Pretreatment of seed with H2O2 improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. Journal of Plant Physiology. 164:2830-294.

Wang, S., D. Liang, C. Li, Y. Hao, F. Ma and H. Shu. 2012. Influence of drought stress on the cellular ultrastructure and antioxidant system in leaves of drought-tolerant and drought-sensitive apple rootstocks. Plant Physiology and Biochemistry. 51:81-89.

Waraich, E. A., R. Ahmad, & M. Y. Ashraf. 2011. Role of mineral nutrition in alleviation of drought stress in plants. Australian Journal of Crop Science. 5:764-777.

Wilcox, L.V. 1951. A method for calculating the saturation percentage from the weight of a known volume of saturated soil paste. Soil Science. 72:233-238

Zhang, J. and M.B. Kirkham. 1996. Lipid peroxidation in sorghum and sunflower seedlings as affected by ascorbic acid, benzoic acid, and propyl gallate. Journal of Plant Physiology. 149:489-493.

Zheng, Z., T. Lei, J. Wang, Y. Wei, X. Liu, F. Yu and J. Ji. 2019. Catalytic cracking of soybean oil for biofuel over γ-Al2O3/CaO composite catalyst. Journal of the Brazilian Chemical Society. 30:359-370.

Zulfiqar, F., N.A. Akram and M. Ashraf. 2020. Osmoprotection in plants under abiotic stresses: new insights into a classical phenomenon. Planta. 251:1.