

MITIGATION OF PEG-INDUCES DROUGHT STRESS IN WHEAT (*Triticum durum*) BY EXOGENOUS APPLICATION OF PROLINE

Hayoun Houda, Ykhlef Nadia, Ryma Bouldjedj, Nadir Belbekri

Genetic, Biochemistry and Plant Biotechnology Laboratory, Faculty of Natural Sciences, Constantine 1 University,
Constantine 25000, Algeria

*Corresponding author. E-mail: hayoun.houda@yahoo.fr

ABSTRACT

Drought is considered one of the most important environmental stresses that has serious limitations on production of most crop plants, including wheat. Proline is an amino acid closely associated with plant responses to environmental constraints. This research aims to study the response of four varieties of durum wheat (*Triticum durum*) subjected to water stress stimulated by polyethylene glycol (20% PEG-6000) which is equivalent to osmotic potential of -1.2 Mpa. A total of 4 wheat genotypes were grown hydroponically and four treatments were imposed. Wheat plants exposed to drought stress showed reduced growth, which was correlated with reduced relative water content, chlorophyll content and stomatal conductance as evidenced by principle component analysis (PCA). It also revealed that electrolyte leakage and malondialdehyde (MDA) accumulations were closely related with the declined growth and wheat plants development under drought stress. Importantly, application of 6 mM of exogenous proline improved the growth responses of wheat plants to drought stress. Proline strengthened numerous physio-biochemical processes coupled with decreasing in MDA levels which indicates successful adaptation of wheat plants to drought stress. The results of PCA strongly supported that application of proline in stressful conditions ameliorated the responses of wheat mostly by enhancing physiological and biochemical activities. Eventually, it can be concluded that proline supplementation is one of the useful approaches to alleviate the adverse effects of water stress on wheat crop.

Keywords: polyethylene glycol, drought tolerance, *Triticum durum*, exogenous proline.

INTRODUCTION

Cereals such as wheat, rice, and corn are the most important sources of daily calories and protein among the world's key staple crops. Wheat was the first crop to be domesticated, and it is now the world's most widely consumed food (Abhinandan et al., 2018). Drought is one of the most significant factors restricting crop production around the world because it reduces: plant water content, cell turgor, and affects also transpiration rates (Bahador and Tadayon, 2020). The effects of drought on the wheat plant are variable, depending on the plant's phenological stage, as well as the duration, intensity, and frequency of drought (Sarto et al., 2017). The main consequences of drought in crop plants is slowing down plant growth and development as a result of inhibiting photosynthesis due to the decreased CO₂ availability resulted from stomatal closure (Flexas et al., 2006; Chaves et al., 2009). Water stress inhibits plant growth

by disturbing various biochemical and physiological processes including chlorophyll biosynthesis, membrane degradation and proteins structure destabilization (Kosar et al., 2015). Moreover, leaf water potential and relative water content (RWC) also decrease when plants are subjected to water stress (Arzani and Ashraf, 2016). Additionally, certain potential indicators such as electrolyte leakage, lipid peroxidation (MDA) and reactive oxygen species (ROS) were dramatically high under drought conditions (Abdelaal et al., 2020).

Maintenance of osmotic balance to turgor pressure offset loss is one of principal plants' response to drought. To counteract lower water potential, plant cells make osmotic adjustments by increasing the accumulation of osmolyte solutes (Blum, 2017). Proline is one of the most frequently accumulated osmolyte, playing an essential role in osmoregulation to mitigate the injurious impact of stresses including drought (Dar et

al., 2020), known for their contribution to stress tolerance in *different* ways. As proline acts as the molecular chaperons it has the ability to maintain protein integrity, prevents their aggregation and boosts the activity of several enzymes. (Szabados and Savouré, 2010; Hayat et al., 2012). *Exogenous applications of osmoprotectants* such as proline has attracted researchers due to their high important roles in-plant's defense system against the injurious impact of stress (Hayat et al., 2012; Hossain et al., 2019). According to Abdelaal et al. (2020), 10 mM proline application on water stressed barley plant increased plant dry weights and relative water content as well as chlorophyll concentration. Semida et al. (2020) noticed in onion exposed to drought stress that proline foliar application enhanced growth characteristics, sugar content and leaf water content (RWC). Farooq et al. (2017) reported that the use of proline as an osmotic protector against water stress caused the accumulation of high content of chlorophyll, proline and glycine betaine in the wheat plant. Based on the ideas mentioned above, this study aimed to assess the impact of exogenous proline application on morpho-physiological and biochemical responses of four durum wheat varieties (*Triticum Durum*) subjected to water stress stimulated by PEG-6000 *under controlled conditions*. The study focused on the evaluation of physiological parameters (RWC, stomatal conductance and photosynthetic parameters such as chlorophyll contents, osmolyte accumulation (proline, soluble sugar) and oxidative stress markers (lipid peroxidation, membrane stability).

MATERIAL AND METHODS

Plant material and growth conditions

Seeds of four wheat genotypes (*Triticum durum.*), Waha (W), Bidi 17 (B17), Wahbi (WB) and Ain Lahma (AH) were obtained from Technical Institute of Field Crops (ITGC), Constantine. The experiment was conducted at the laboratory of Plant Genetics, Biochemistry and Biotechnology, University of Mentouri Brothers, Constantine I, Algeria. The seeds of wheat varieties were initially

sterilized with sodium hypochlorite solution and then washed with distilled water, then placed in the petri dishes on a wet Whatman paper under optimal conditions of germination in the dark at 25°C temperature. After germination, the seedlings of all genotypes were transplanted to containers (15x25 cm) of hydroponic system containing nutrient solution (Broughton and Dilworth, 1971) at a volume of 300 ml per container. The experiment was carried out in conditioned growth chamber (16 h light/8 h dark photoperiod and at a temperature of 25°C). The seedlings were then cultured in four different nutrient solutions designed as:

Group 1: plants were grown in nutriment solution BD and was kept as control (without PEG and exogenous proline treatment) (C).

Group 2: plants were grown in nutriment solution BD containing 6 mM proline (C+P).

Group 3: plants were grown in nutriment solution BD containing 20% PEG-6000 (S).

Group 4: plants were grown in nutriment solution BD containing 20% PEG-6000 + 6 mM proline (S+P).

The proline and PEG concentration were based on preliminary experiments and four replicates per genotype per treatment were performed. Broughton and Dilworth's nutrient solution containing (0 or 6 mM proline) was replaced every week to replenish nutrients. However, the first application of treatments (20% PEG-6000) was applied after a week of growth, and the second application was applied in the third week. After one month of growth, the leaves of control and stressed seedlings have been harvested (in the third stage for the wheat).

Assessment of physiological and biochemical parameters

1. Growth measurement

After one month of growth, four plants were selected for each treatment to estimate *linear measurements mean value* such shoot and root length measurements.

2. Determination of Relative Water Content (RWC) and Stomatal conductance

Relative water content was measured on leaf sections obtained from the third fully

developed leaves. According to Barrs (1968), the leaves were quickly sealed and fresh weights were determined immediately after excision. After placing them in distilled water for 24h, turgid weight were estimated. Leaf dry weights were measured after drying leaf samples in oven for 24 h at 80°C. Finally, relative water content was calculated according to the following formula:

$$\text{RWC (\%)} = \frac{(\text{fresh weight} - \text{dry weight}) \times 100}{(\text{turgid weight} - \text{dry weight})}$$

Stomatal conductance was measured using a porometer (AP4 Delta-T Devices, Cambridge, UK) on the abaxial leaf surface.

3. Determination of chlorophyll content

Chlorophyll contents were measured according to Lichtenthaler and Wellburn (1983) protocol. 0.1 g of fresh leaf tissue was crushed in 2 ml of acetone (80%). The light absorption of leaf extract solution was determined at 645, 663 and 470 nm on a spectrophotomete. The pigment concentrations were calculated according to the following equations:

Chlorophyll a:

$$\text{Chl a} = 12.21 \times \text{DO (663)} - 2.81 \times \text{DO (645)}$$

Chlorophyll b:

$$\text{Chl b} = 20.13 \times \text{DO (645)} - 5.03 \times \text{DO (663)}$$

$$\text{Total chlorophyll} = \text{Chl a} + \text{Chl b.}$$

4. Determination of total soluble sugars

The soluble sugar content was measured according to Dubois et al. (1956) method. An amount of approximately 0.1 g of Fresh materials were extracted in 80% ethanol for 48 h. After alcohol evaporation, the residue was homogenized with 20 mL of distilled water. Afterward, 1 ml of the extract was treated with 1ml of 5% phenol and 5mL of concentrated sulfuric acid. After 20 min of incubation at 30°C, absorbance was measured at 485 nm. Soluble sugar content were determined using glucose standard.

5. Proline content determination

Free proline content in-leaves was measured using Troll and Lindsley method

(1955). 0.1 g of fresh leaves of control and treated plants were extract in 2 mL of 40% methanol for 60 min at 85°C. 1 mL of the extract was added into the test tube containing ninhydrin, acetic acid and orthophosphoric. The reaction mixture was heated in a boiling water bath at 100°C for 60 min. After cooling the mixture on ice, 5 mL of toluene was added and thoroughly mixed. Finally, the toluene phase was separated and the absorbance of the pink red upper phase was recorded at 520 nm using a spectrophotometer against a toluene blank.

6. Determination of total protein content

Quantification of total soluble protein was carried out using the method described by Bradford (1976). Thus, a quantity of 0.2 g of fresh plant tissue was grinded with 0.6 mL of Tris-Hcl buffer (pH 8.1). The mixture was centrifuged 20 min at 4°C. Then, 10 µl of the obtained extract was added to 5 µl of Bradford solution and 290 µl of extraction buffer. Thereafter, the absorbance was measured at 595 nm. The protein content was determined by spectrophotometer using bovine serum albumin as standard.

7. Estimation of Lipid Peroxidation and Electrolyte Leakage (EL)

Lipid peroxidation was estimated by measuring the level of malondialdehydes (MDA) as an indicator of membrane stability. Heath and Paker method (1968) was followed. Fresh samples of 0.5 g leaves were ground and homogenized in 0.1% trichloroacetic acid (TCA), and centrifuged at 12,000 rpm for 15 min. To an aliquot (0.5 mL) of the supernatant, solution of 0.5% thiobarbituric acid (TBA) in 20% TCA that was added. The blend was heated at 95°C for 30 min and directly cooled in an ice bath. Centrifugation was carried out at 12,000 rpm for 15 min and absorbance was measured at 532 nm by a spectrophotometer. The non-specific value at 600 nm absorption was subtracted. The total MDA contents were calculated using the extinction coefficient at $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

Electrolyte leakage was quantified as a way to estimate the degree of membrane

integrity. For the determination of electrolyte leakage (EL), the procedure of Bajji et al. (2002) was followed. Fresh samples of leaves were washed and sliced into tiny fragments, then placed in a test tube filled with 10 mL distilled water and incubated for 24 h at room temperature. Preliminary EC1 was measured, samples were boiled at 100°C for 60 min once more and the second EC2 was estimated. Total EL was calculated using the following relation: $EL (\%) = (EC1/EC2) \times 100$.

8. Statistical Analysis

All experimental data reported as averages of four replicates and analyzed by two-ways analysis of variance (ANOVA). The data were expressed as the mean \pm standard error. Significant differences between means were determined using the Newman and Keuls test, at $p \leq 0.05$. A statistical software package was also used to do principal components analysis (PCA) (SPSS V.26).

RESULTS AND DISCUSSION

Linear leaf and root growth

The effects of water stress and exogenous proline on the linear growth of durum wheat varieties were evaluated after one month of growth, by measuring the shoot and root length. Under PEG treatment a significant decrease in shoot and root length were reported ($p \leq 0.05$, Table 1) for all studied genotypes. This decline reached -32% in leaves of Wahbi and -26% in roots of Waha respectively, compared with controls plants. The addition of proline in the nutrient solution was found to be effective in improving seedling growth under control and water stress conditions. Under PEG treatment, an increase in growth estimated at +22.07% in leaves of Wahbi and +16.98% in roots of Waha in comparison to the stressed plants without proline.

Table 1. Shoot length (cm) and root length (cm) of durum wheat subjected to different PEG and exogenous proline treatments

Genotype	Shoot length (cm)				Root length (cm)			
	Treatment				Treatment			
	C	S	C+P	S+P	C	S	C+P	S+P
B17	18,55 \pm 0,65 ^a	14,67 \pm 0,78 ^c	19,43 \pm 0,59 ^a	18,14 \pm 0,17 ^b	15,70 \pm 2,34 ^a	13,15 \pm 3,99 ^c	16,18 \pm 0,23 ^a	15,43 \pm 0,68 ^a
WAHA	15,92 \pm 2,37 ^b	11,48 \pm 2,35 ^c	17,15 \pm 1,45 ^b	16,32 \pm 0,45 ^b	14,45 \pm 2,37 ^b	9,77 \pm 0,46 ^c	13,90 \pm 0,74 ^b	11,43 \pm 0,43 ^c
WAHBI	13,03 \pm 1,81 ^b	8,85 \pm 0,54 ^d	14,20 \pm 2,28 ^b	12,01 \pm 1,61 ^b	13,73 \pm 0,73 ^b	10,45 \pm 1,38 ^c	13,83 \pm 0,82 ^b	12,23 \pm 2,07 ^b
A.LAHMA	13,95 \pm 0,81 ^b	9,75 \pm 0,70 ^d	14,73 \pm 0,30 ^b	14,03 \pm 0,63 ^b	13,71 \pm 0,57 ^b	12,53 \pm 2,06 ^c	16,20 \pm 0,35 ^a	13,32 \pm 1,18 ^b

Values represent the means of four replicates \pm SD. C (0 PEG + 0 proline), C+P (0 PEG + 6 mM proline), S (20% PEG + 0 proline), S+P (20%PEG + 6 mM proline). Different letters in columns show significant differences ($p \leq 0.05$) according to Student-Newman-Keuls Test.

Relative water content (RWC%) and stomatal conductance

The percentage of relative water contents (RWC) differed among various treatments (Figure 1). The highest percentage of RWC was observed in control treatment for all studied genotypes. However, a significant reduction in RWC was found in response to PEG- imposed water stress compared to the

control. The percentage of decreasing is more important in Wahbi genotype with -41.79%. The addition of exogenous proline did not significantly affect the response of control plants. Nonetheless, under stressed condition, the application of exogenous proline improved the relative leaves water content for all studied genotypes. The RWC value was equal to untreated control.

HAYOUN HOUDA ET AL.: MITIGATION OF PEG-INDUCES DROUGHT STRESS IN WHEAT (*Triticum durum*) BY EXOGENOUS APPLICATION OF PROLINE

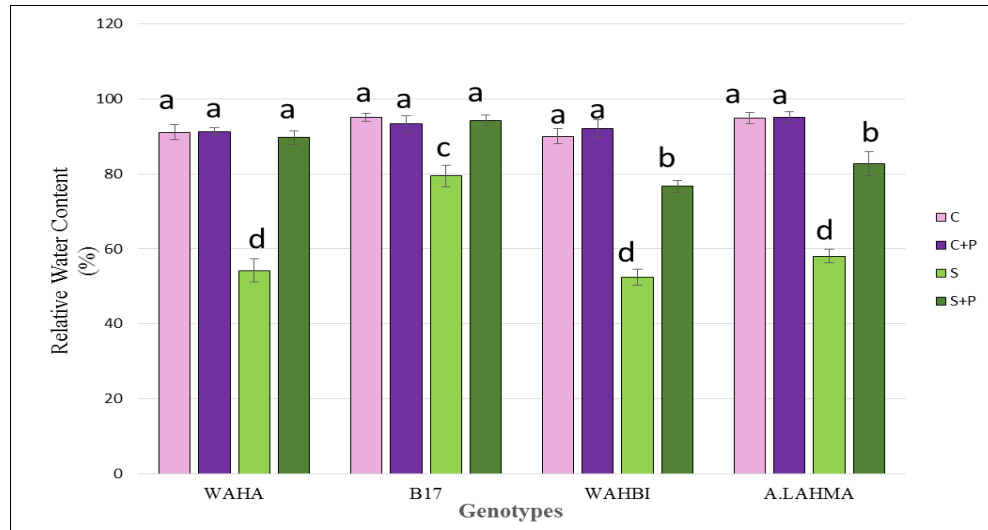


Figure 1. Effect of exogenous proline on relative water content in different genotypes of wheat under control and PEG-induced water stress.

Values are the mean \pm SD of four replicates. Different letters show significant differences ($p \leq 0.05$).

Stomatal conductance was significantly influenced by imposed stresses in all studied genotypes (Figure 2). The reduction in stomatal conductance was -75.94% in Waha genotype leaves compared to control plants. The presence of proline under

stressed condition induce a significant increase in stomatal conductance. The percentage of increasing is important in W genotype with 266.18% in comparison of the stressed without proline.

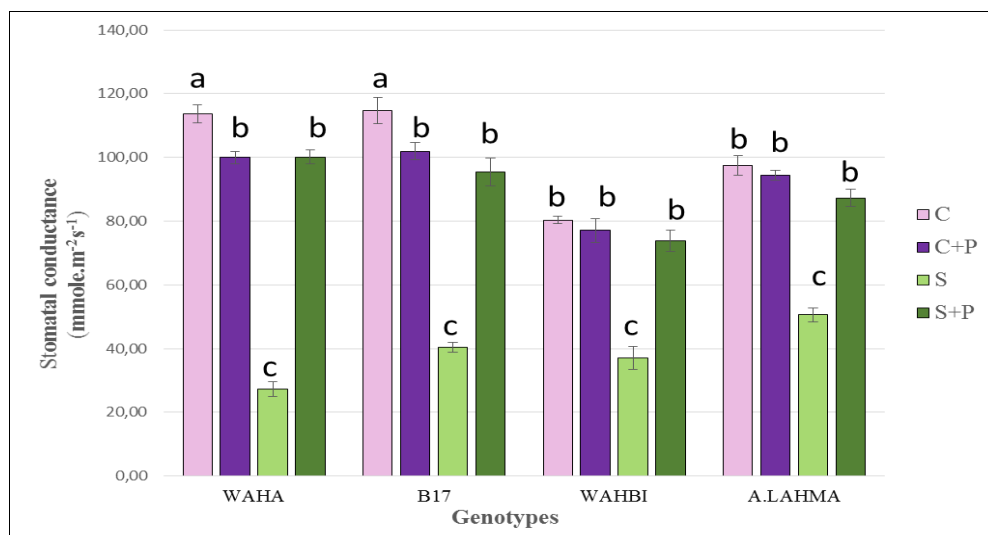


Figure 2. Effect of exogenous proline on stomatal conductance in different genotypes of wheat under control and PEG-induced water stress.

Values are the mean \pm SD of four replicates. Different letters show significant differences ($p \leq 0.05$).

Chlorophyll Content

The results obtained show a significant difference in the variation of chlorophyll content between control and stressed plants (Figure 3). Chlorophyll content decline under

water stress with a reduction of -16.18% in Waha genotype. Proline addition in the nutrient solution reduced the adverse effects of water stress on chlorophyll content.

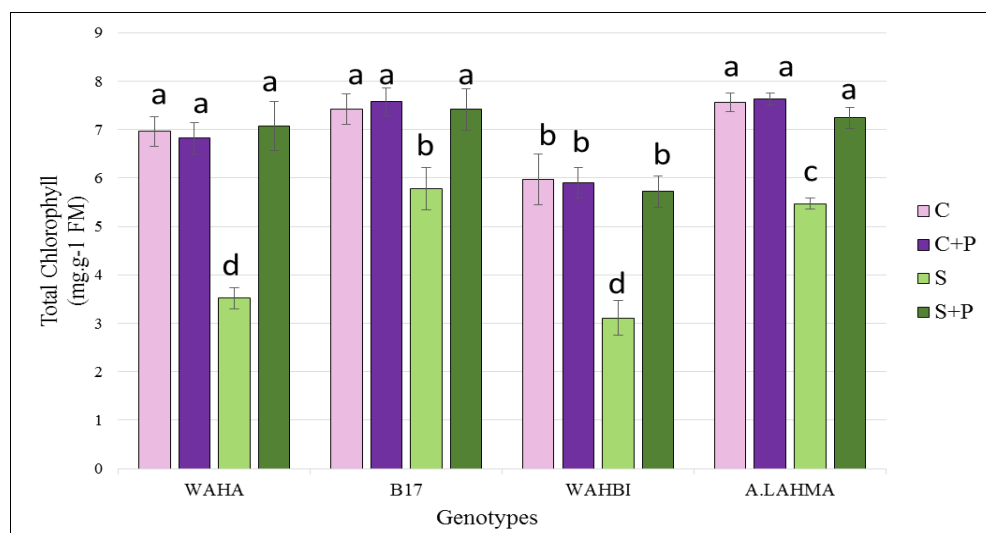


Figure 3. Effect of exogenous proline on chlorophyll content in different genotypes of wheat under control and PEG-induced water stress.

Values are the mean \pm SD of four replicates. Different letters show significant differences ($p \leq 0.05$).

Lipid Peroxidation and Electrolyte Leakage (EL)

Data presented in (Table 2) showed a high production of malondialdehyde (MDA) during PEG-imposed water stress for all studied genotypes. The recorded values were between 71.85 and 41.86 ($\text{nMol.g}^{-1}\text{FW}$) in Waha and A.lahma leaves varieties, respectively. A considerable increase (358.02%) in MDA

content was observed in Waha genotype compared to the control. Also the presence of PEG generated a significant increase in electrolyte leakage (+144.57%) in Waha variety. Under stressful conditions a significant decrease in membrane alteration and MDA production was recorded following the addition of proline to the nutrient solution.

Table 2. Malondialdehyde and Electrolyte leakage of durum wheat subjected to different PEG and exogenous proline treatments

Genotype	MDA ($\text{nMol.g}^{-1}\text{FW}$)				EL (%)			
	Treatment				Treatment			
	C	S	C+P	S+P	C	S	C+P	S+P
B17	20,79 \pm 1,20 ^d	61,49 \pm 2,07 ^b	17,35 \pm 1,08 ^d	33,27 \pm 2,25 ^d	7,30 \pm 0,74 ^c	15,82 \pm 1,45 ^a	7,34 \pm 0,56 ^c	6,77 \pm 0,73 ^c
WAHA	15,68 \pm 0,54 ^d	71,85 \pm 3,10 ^a	15,08 \pm 0,80 ^d	16,64 \pm 1,74 ^d	6,70 \pm 0,67 ^c	16,40 \pm 0,87 ^a	6,12 \pm 0,83 ^c	8,12 \pm 0,52 ^c
WAHBI	17,74 \pm 0,91 ^d	59,41 \pm 3,68 ^b	18,05 \pm 2,30 ^d	19,92 \pm 1,23 ^d	7,93 \pm 0,83 ^c	14,45 \pm 0,88 ^a	8,17 \pm 0,60 ^c	6,77 \pm 0,71 ^c
A.LAHMA	21,73 \pm 1,93 ^d	41,86 \pm 1,38 ^b	23,70 \pm 2,43 ^d	20,91 \pm 1,52 ^d	7,84 \pm 0,97 ^c	11,53 \pm 0,87 ^b	8,21 \pm 1,08 ^c	11,13 \pm 0,77 ^b

Values represent the means of four replicates \pm SD. C (0 PEG + 0 proline), C+P (0 PEG + 6 mM proline), S (20% PEG + 0 proline), S+P (20%PEG + 6 mM proline). Different letters in columns show significant differences ($p \leq 0.05$) according to Student-Newman-Keuls Test.

Endogenous Proline content

Proline content variations in wheat leaves are presented in (Figure 4). It showed a marked accumulation in stressed plants compared to the control. The important increase was registered in stressed plants for

Wahbi genotype with 369.27%. The effect of exogenous proline under stress conditions resulted in a significant increase in endogenous proline content. The highest accumulation of proline was recorded in A.lahma variety with 91.17% increase in leaves.

HAYOUN HOUDA ET AL.: MITIGATION OF PEG-INDUCES DROUGHT STRESS IN WHEAT (*Triticum durum*) BY EXOGENOUS APPLICATION OF PROLINE

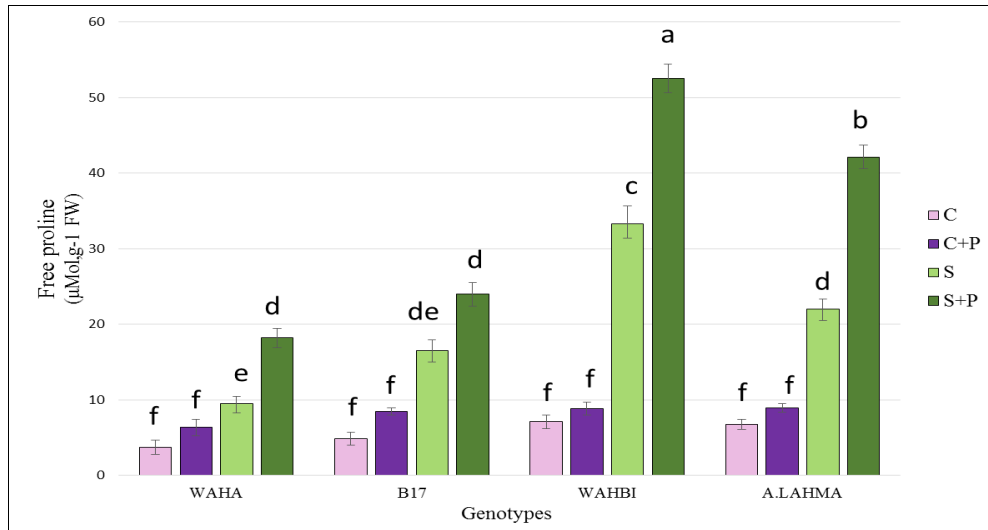


Figure 4. Effect of exogenous proline on free proline content in different genotypes of wheat under control and PEG-induced water stress.

Values are the mean \pm SD of four replicates. Different letters show significant differences ($p \leq 0.05$).

Soluble sugar accumulation

The soluble sugar content increases significantly under PEG-induced water stress conditions as shown in (Figure 5). The important percentage (+393.38%) of increasing

is observed in Wahbi genotype compared to the control. Under PEG-induced water stress conditions, the presence of proline, induced a variation in the accumulation of soluble sugars compared to stressed plants.

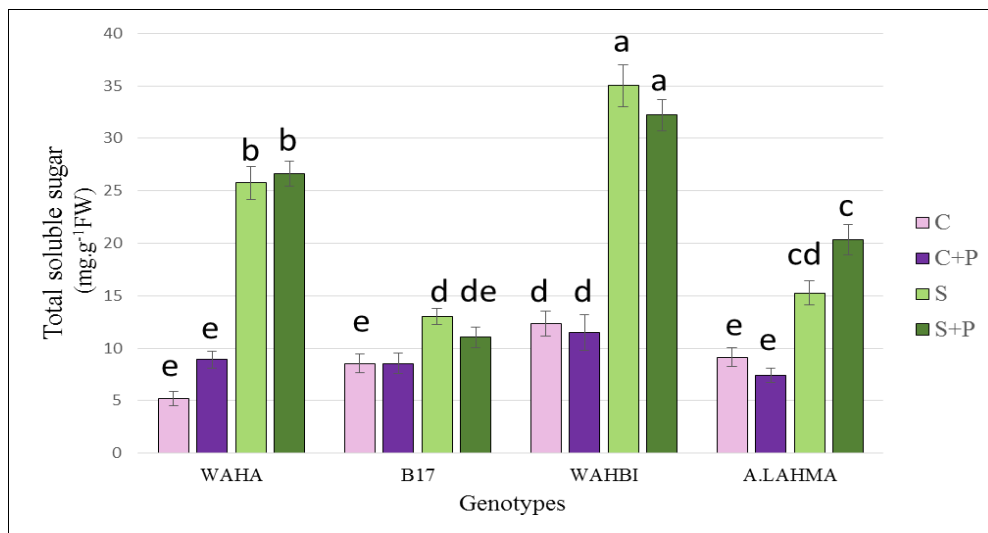


Figure 5: Effect of exogenous proline on total soluble sugar content in different genotypes of wheat under control and PEG-induced water stress.

Values are the mean \pm SD of four replicates. Different letters show significant differences ($p \leq 0.05$).

Total soluble protein content

PEG treatment reduced soluble protein content in leaves, estimated at - 94.43% in Wahbi cultivars (Figure 6). In contrast, the application of 6 mM proline increased

protein content under stress condition. The highest value (4.06 mg.g⁻¹ DW) was recorded in cultivar B17 which correspond to a 186.44% increase compared to stressed plants without proline.

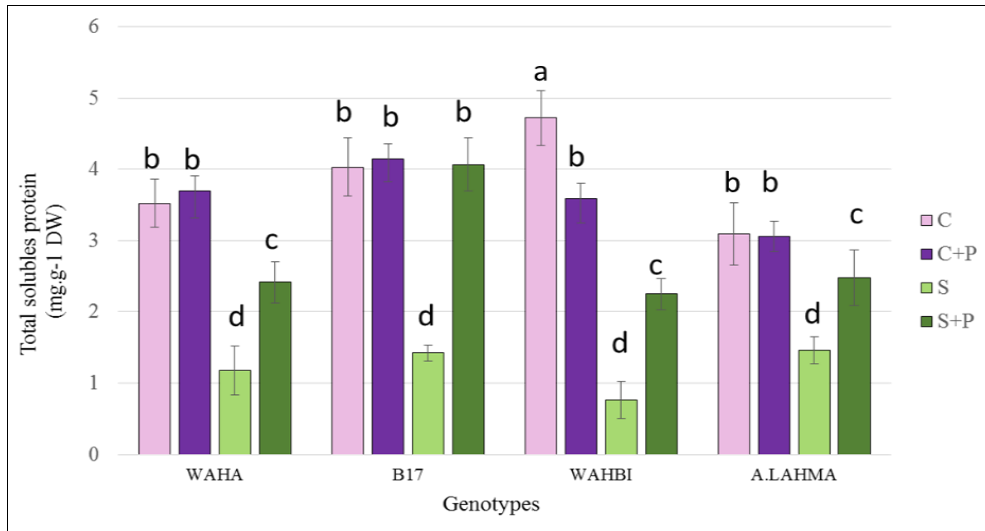


Figure 6. Effect of exogenous proline on total soluble protein content in different genotypes of wheat under control and PEG-induced water stress.

Values are the mean \pm SD of four replicates. Different letters show significant differences ($p \leq 0.05$).

Principal Component Analysis (PCA)

A principal component analysis was carried out to evaluate the response of the durum wheat genotypes to the different imposed treatments and to determine the correlations between the different studied parameters (morpho-physiological and biochemical). The correlations of the variables measured with the first two axes (axis F1 and axis F2) are shown in (Figure 7); they

explained 70.50 and 14.17%, respectively, i.e., 84.68% of complete database.

Axis F1 was positively defined by RWC, chlorophyll content, stomatal conductance and proteins content while it was negatively correlated with oxidative status parameters (EL and MDA). Axis F2 is defined by proline content and total soluble sugars, parameters involved in osmo-adjustment, showed a strong positive correlation.

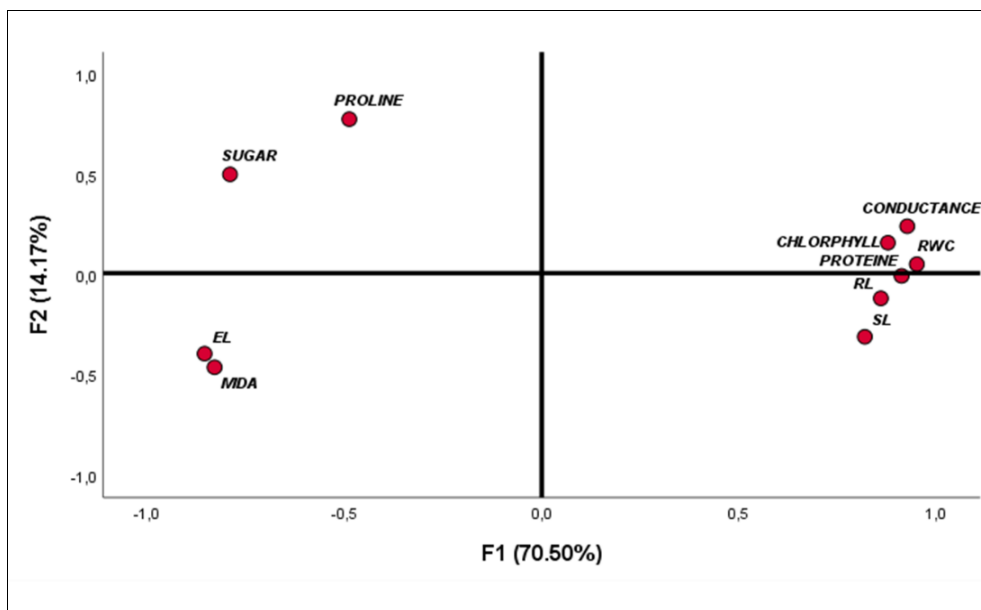


Figure 7: Correlation diagram between the studied parameters under different experimental conditions. The measured parameters included shoot length (SL), root length (RL), relative water content (RWC), stomatal conductance, chlorophyll content, malondialdehyde (MDA), electrolyte leakage (EL), proline, sugar and protein content.

HAYOUN HOUDA ET AL.: MITIGATION OF PEG-INDUCES DROUGHT STRESS
IN WHEAT (*Triticum durum*) BY EXOGENOUS APPLICATION OF PROLINE

The graphical representation of four varieties under different treatment (Figure 8) indicates that under control condition (C) and with exogenous proline (C+P) all studied varieties are superposed and form a single group (G1) on the positive side of the F1 and had a close association with most morphological and physiological parameters, while the stressed plants without exogenous proline are showed on the negative side of the same axis and had an intimate association with MDA and EL (G2). These results confirmed that water stress causes the disturbance of

physiological functions which is accompanied by cellular homeostasis disruption induces lipid peroxidation with the accumulation of malondialdehyde (MDA).

Interestingly, application of exogenous proline during PEG- imposed water stress is beneficial for plants (G3) to maintain their development and functions. These results imply that exogenous proline, was an effective compound reducing the harmful effects of water stress and the improvement of plant responses to this stress.

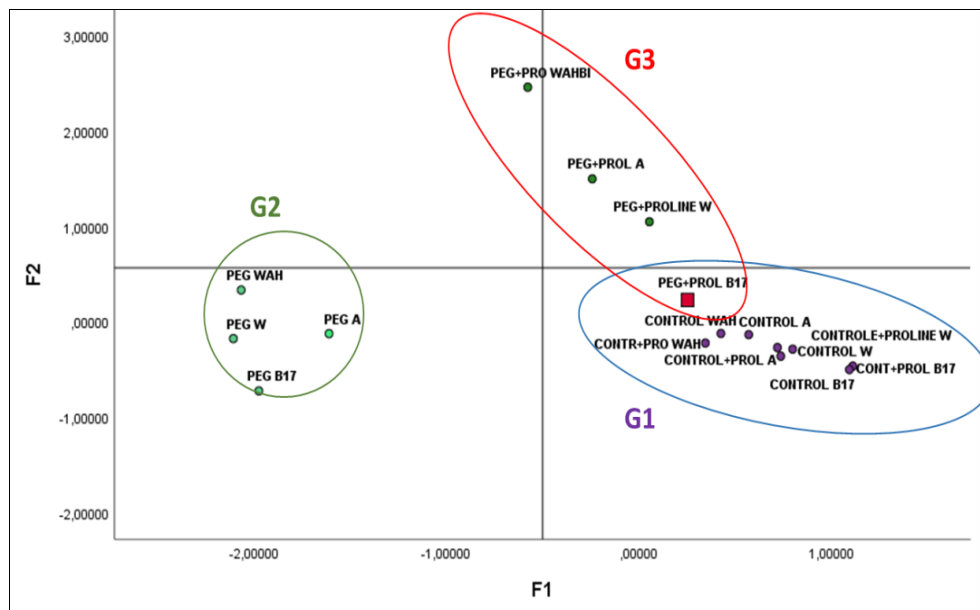


Figure 8: Graphing diagram of wheat based on studied parameters under different experimental conditions

Water stress is one of the main factors limiting plants' growth and decreasing the production of vegetable crops. It causes several physiological and biochemical changes including cell membrane degradation, a decrease of tissue water content; and alters photosynthetic pigments. These effects contribute negatively on plant growth, development and, consequently, reduce crop productivity (Semida et al., 2020). In this research, *shoot and root length were negatively affected by the stress induced by PEG-6000*. In fact, the capacity of plants to absorb water generally; declines in osmotic-stressed plants, reduces the relative turgidity and causes protoplasm dehydration due to turgor loss that results in reduced cell

proliferation and cell division which eventually reduced the growth plant (Kumari et al., 2014). Nevertheless, exogenous proline (6 mM) application mitigated the inhibition of plant growth for all studied varieties, these effects may be correlated with enhanced absorption of essential nutrient, especially Mg^{+2} , k^{+} and Ca^{+2} which stimulates plants' growth (Kavi Kishor et al., 2015). Similar results have been obtained in many species, such as onion (Semida et al., 2020).

Photosynthetic pigment levels and relative water content are considered important physiological indicators to assess the tolerance levels of various crops to different abiotic stresses, including water stress (Hussain et al., 2021). In our investigation,

treatment with PEG-6000 significantly reduced these two parameters for the genotypes studied compared to their controls. Khalilzadeh et al. (2016) also reported that water stress significantly reduced the wheat photosynthetic pigment content, which could be due to increased ROS levels, which led to lipid peroxidation and consequently to chlorophyll degradation. Moreover, the presence of exogenous proline under stress conditions attenuated the decrease in chlorophyll content and contributed to maintain high TRE compared to untreated stressed plants. This would probably be due to active osmoregulation, following the establishment of water stress tolerance mechanism, namely osmotic adjustment. Pervaiz et al. (2019) showed the beneficial effect of foliar application of proline that modulated the physiological and biochemical processes in wheat cultivars. Similar results were obtained by Altuntaş et al. (2020) on corn.

In this study, PEG-6000 negatively affected the stomatal conductance of all tested genotypes. During water deficit, the closure of the stomata allows the plant to maintain a favorable water balance. Exogenous proline promotes the uptake of K^+ ions by guard cells, which allows the regulation of turgor pressure and consequently the increase of stomatal conductance.

The products of membrane lipid peroxidation, in particular MDA considered as an indicator of oxidative stress in plants (Ma et al., 2015). Osmotic stress, affect cell membrane frequently associated with the increasing membrane permeability and losing of membrane integrity. In our survey, a significant increase has been reported for both MDA content and the rate of EL in leaves of plants exposed to PEG. This results are caused by excessive production of reactive oxygen species (ROS) in wheat leaves (Hanif et al., 2021). However, in the presence of exogenous proline a significant decreasing in MDA content and the rate of EL were observed. Proline helps in free radicals scavenging, therefore preventing plants from negative effects of these ROS

during drought stress. Concordant results were observed in sugar beet under water stress (Alkahtani et al., 2021).

Facing the harmful effects of water stress, plants proceed to an osmotic adjustment action through the synthesis of osmolytes such as sugars and proline. They maintain the membrane structure and stabilization, as they also intervene in the elimination of reactive oxygen species (ROS) induced by stress (Ghosh et al., 2021). In this study, the effect of PEG-6000 resulted in a significant accumulation of proline and sugars in wheat leaves. The accumulation of proline may be due to stimulation of its biosynthesis or inhibition of its degradation and slowed protein oxidation rate (Hayat et al., 2021). According to Hayat et al. (2012) sugars could act as osmolytes and contribute to the protection of enzymes as well as the integrity of the plasma membrane in response to osmotic stress. On the other hand, exogenous proline significantly amplifies the accumulation of endogenous proline under stress conditions and produces antioxidant enzymes (Bhaskara et al., 2015). These results confirm those obtained by Hayat et al. (2021) on pigeon pea seedlings and Noreen et al. (2018) on soft wheat cultivars.

PEG-6000 caused a significant drop in total protein content. In general, water stress alters the expression, accumulation and synthesis of proteins and thus causes protein denaturation. Nonetheless, an increase in protein content has been observed in the presence of proline under stress conditions, this is mainly due to the role of proline in protecting the structure and stabilizing proteins (Ashraf and Foolad, 2007).

CONCLUSIONS

This study was planned to examine the physiological and biochemical processes that occur in durum wheat plants in response to PEG- imposed water stress. The growth characters, physiological and biochemical parameters of wheat were considerably influenced by PEG treatment. Nevertheless, our results showed that application of exogenous proline at a 6 mM level can

HAYOUN HOUDA ET AL.: MITIGATION OF PEG-INDUCES DROUGHT STRESS
IN WHEAT (*Triticum durum*) BY EXOGENOUS APPLICATION OF PROLINE

reduce the negative effects of water stress in durum wheat plants by improving growth performance, which might be related to enhance total chlorophyll, relative water content and improved cell turgor.

Proline treatment also alleviated the membrane damage and reduced MDA level. These results indicate the capacity of proline to improve the tolerance of wheat plants confronted to water constraint. Hence, our findings provide an important prospect for proline use in the amelioration of vegetable production under water stress.

ACKNOWLEDGEMENTS

We thank Dr. Abdelkader Benbelkacem, Research Director of the National Institute of Agronomic Research of Algeria - Constantine. We also thank Mrs. Chafika Zahraoui.

REFERENCES

- Abdelaal, K.A.A., Rashed, S.H., Ragab, A., Hossain, A., EL Sabagh, A., 2020. *Yield and quality of two sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Döll) cultivars are influenced by foliar application of salicylic acid, irrigation timing, and planting density*. Acta Agriculturae Slovenica, 115(2): 273-282.
- Abhinandan, K., Skori, L., Stanic, M, Hickerson, N.M.N., Jamshed, M., Samuel, M.A., 2018. *Abiotic stress signaling in wheat - An inclusive overview of hormonal interactions during abiotic stress responses in wheat*. Frontiers in Plant Science, 9(6): 1-25.
- Alkahtani, M.D., Hafez, Y.M., Attia, K., Rashwan, E., Husnain, L.A., AlGwaiz, H.I., Abdelaal, K.A., 2021. *Evaluation of silicon and proline application on the oxidative machinery in drought-stressed sugar beet*. Antioxidants, 10: 398.
- Altuntaş, C., Demiralay, M., Sezgin Muslu, A., Terzi, R., 2020. *Proline-stimulated signaling primarily targets the chlorophyll degradation pathway and photosynthesis associated processes to cope with short-term water deficit in maize*. Photosynth, 144: 35-48.
- Arzani, A., and Ashraf, M., 2016. *Smart engineering of genetic resources for enhanced salinity tolerance in crop plants*. Critical Reviews in Plant Sciences, 35(3): 146-189.
- Bahador, M., and Tadayon, M.R., 2020. *Investigating of zeolite role in modifying the effect of drought stress in hemp: antioxidant enzymes and oil content*. Industrial Crops and Product, 144: 1-10.
- Bajji, M., Kinet, J.M., Lutts, S., 2002. *The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat*. Plant Growth Regulation, 36(1): 61-70.
- Barrs, H.D., 1968. *Determination of water deficits in plant tissues*. Water Deficits and Plant Growth: 235-368.
- Bhaskara, G.B., Yang, T.H., Verslues, P.E., 2015. *Dynamic proline metabolism: importance and regulation in water limited environments*. Frontiers in Plant Science, 6: 484.
- Blum, A., 2017. *Osmotic adjustment is a prime drought stress adaptive engine in support of plant production*. Plant Cell Environment, 40(1): 4-10.
- Bradford, M.M., 1976. *A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding*. Analytical Biochemistry, 72: 248-254.
- Broughton, W.J., and Dilworth, M.J., 1971. *Control of leghaemoglobin synthesis in snake beans*. Biochemical Journal, 125(4): 1075-1080.
- Chaves, M.M., Flexas, J., Pinheiro, C., 2009. *Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell*. Annals of Botany, 103: 551-560.
- Dar, M.I., Naikoo, M.I., Rehman, F., Naushin, F., Khan, F.A., 2020. *Proline accumulation in plants: Roles in stress tolerance and plant development*. Osmolytes and Plants Acclimation to Changing Environment: Emerging Omics Technologies, Springer, 1: 155-166.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.T., Smith, F., 1956. *Colorimetric method for determination of sugars and related substances*. Analytical Chemistry, 28(3): 350-356.
- Farooq, M., Nawaz, A., Chaudhry, M., Indrasti, R., Rehman, A., 2017. *Improving resistance against terminal drought in bread wheat by exogenous application of proline and gamma-aminobutyric acid*. Journal of Agronomy and Crop Science, 203(6): 464-472.
- Flexas, J., Bota, J., Galmés, J., Medrano, H., Ribas-Carbo, M., 2006. *Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress*. Physiologia Plantarum, 127: 343-352.
- Ghosh, U.K., Islam, M.N., Siddiqui, M.N., Khan, M.A.R., 2021. *Understanding the roles of osmolytes for acclimatizing plants to changing environment: a review of potential mechanism*. Plant Signal Behav. 16(8): 1913306.
- Hanif, S., Saleem, M., Sarwar, M., Irshad, M., Shakoor, A., Wahid, M., 2021. *Biochemically triggered heat and drought stress tolerance in rice by proline application*. Journal of Plant Growth, 40: 305-312.
- Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J., Ahmad, A., 2012. *Role of proline under*

- changing environments: A review.* Plant Signaling. Behavior, 7(11): 1456-1466.
- Hayat, K., Khan, J., Khan, A., Ullah, S., Ali, S., Fu, Y., 2021. *Ameliorative effects of exogenous Proline on photosynthetic attributes, nutrients uptake, and oxidative stresses under cadmium in Pigeon pea (Cajanus Cajan L.).* Plants, 10(4): 796.
- Heath, R.L., and Packer, L., 1968. *Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation.* Archives of Biochemistry and Biophysics, 125(1): 189-198.
- Hossain, M.A., Kumar, V., Burritt, D.J., Fujita, M., Mäkelä, P., 2019. *Osmoprotectant-mediated abiotic stress tolerance in plants.* Proline Metabolism and Its Functions in Development and Stress Tolerance, Springer: 41-72.
- Hussain, R., Ayyub, C.M., Shaheen, M.R., Rashid, S., Nafees, M., Ali, S., Butt, M., Ali, M., Maqsood, A., Fiaz, S., 2021. *Regulation of osmotic balance and increased antioxidant activities under heat stress in Abelmoschus esculentus L. Triggered by Exogenous Proline Application,* Agronomy, 11(4): 685.
- Kavi Kishor, P.B., Hima Kumari, P., Sunita, M., Sreenivasulu, N., 2015. *Role of proline in cell wall synthesis and plant development and its implications in plant ontogeny.* Frontier. Plant Science, 544 (6): 1-17.
- Khalilzadeh, R., Sharifi, R.S., Jalilian, J., 2016. *Antioxidant status and physiological responses of wheat (Triticum aestivum L.) to cycocel application and bio fertilizers under water limitation condition.* Journal of Plant Interaction, 11: 130-137.
- Kosar, F., Akram, N.A., Ashraf, M., 2015. *Exogenously-applied 5-aminolevulinic acid modulates some key physiological characteristics and antioxidative defense system in spring wheat (Triticum aestivum L.) seedlings under water stress.* South African Journal of Botany, 96: 71-77.
- Kumari, A., Sairam, R.K., Singh, S.K., Krishna, G.K., 2014. *Early growth response: an indicator of subsequent growth and yield of wheat genotypes grown under simulated water stress condition.* Indian Journal Plant Physiology, 19: 94-100.
- Lichtenthaler, H.K., and Wellburn, A.R., 1983. *Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents.* Biochemical. Society Transactions, 11: 591-592.
- Ma, J., Du, G., Li, X., Zhang, C., Guo, J., 2015. *A major locus controlling malondialdehyde content under water stress is associated with Fusarium crown rot resistance in wheat.* Mol. Genet. Genomics, 290: 1955-1962.
- Noreen, S., Akhter, M.S., Yaamin, T., Arfan, M., 2018. *The ameliorative effects of exogenously applied proline on physiological and biochemical parameters of wheat (Triticum aestivum L.) crop under copper stress condition.* Journal of Plant Interactions, 13(1): 221-230.
- Pervaiz, A., Iqbal, A., Khalid, A., Manzoor, A., Noreen, S., Ayaz, A., Zafar, Z.U., Ashraf, M., 2019. *Proline induced modulation in physiological responses in wheat plants.* Journal of Agriculture and Environmental Science, 8: 112-119.
- Sarto, M.V.M., Sarto, J.R.W., Rampim, L., Rosset, J.S., Bassegio, D., da Costa, P.F., Inagaki, A.M., 2017. *Wheat phenology and yield under drought: a review.* Australian Journal of Crop Science, 8: 941-946.
- Semida, W.M., Abdelkhalika, A., Mohamed, O.A., Rady, M.O.A., Abd El-Mageed, T.A., 2020. *Exogenously applied proline enhances growth and productivity of drought stressed onion by improving photosynthetic efficiency, water use efficiency and up-regulating osmoprotectants.* Scientia Horticulturae, 272: 109580.
- Szabados, L., and Savouré, A., 2010. *Proline: a multifunctional amino acid.* Trends Plant Science, 15(2): 89-97.
- Troll, W., and Lindsley, J.A., 1955. *Photometric method for the determination of proline.* Journal of Biological Chemistry, 215: 655-660.