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Effects of exogenous proline on the physiological characteristics of *Triticum aestivum* L. and *Lens culinaris* Medik. under drought stress

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ABSTRACT

Proline, which is an indicator of stress, is often considered as a good parameter for the testing of plants with good drought tolerance capacity. Thus, exogenous application of proline is a possible technique to avoid the deleterious effects of the drought on plant growth. The objectives of this study are to investigate the impact exogenous proline on the physiological behavior of two plant species, bread wheat, a monocot, and lentil, a dicot, under drought stress conditions. After several preliminary tests, optimal concentrations of exogenous proline were determined (6 mM for bread wheat and 2 mM for lentil) and both species were treated in normal and drought conditions. The results showed that water deficit affected both species leading to a reduction in growth, chlorophyll content and relative water content. Likewise, 15 % PEG-6000, which is equivalent to osmotic potential of -0.31MPa, caused a high accumulation of proline. In almost of cases we also noted a remarkable decrease in catalase (Cat), ascorbate peroxidase (APX) and gaiacol peroxidase (GPX) activities which was probably due to the oxidative stress caused by drought stress. The application of proline in stressful conditions reduced the deleterious effects caused by the stress on both species, due, particularly, to the accumulation of free endogenous proline and the increase of Cat, APX and GPX activities.

Key words: *Triticum aestivum* L.; *Lens culinaris* Medik.; exogenous proline; drought tolerance

IZVLEČEK

UČINKI DODAJANJA PROLINA NA FIZIOLOŠKE LASTNOSTI KRUŠNE PŠENICE (*Triticum aestivum* L.) IN NAVADNE LEČE(*Lens culinaris* Medik.) V RAZMERAH SUŠNEGA STRESA

Proline je kot indikator stresa pogosto uporabljen za testiranje odpornosti rastlin na sušo. Z dodajanjem prolina se je mogoče izogniti škodljivim učinkom suše na rast rastlin. Predmet te raziskave je bil preučiti vpliv dodajanja prolina v rastni medij na fiziologijo dveh rastlinskih vrst, krušne pšenice kot enokaličnice in navadne leče kot dvokaličnice, gojenih v razmerah sušnega stresa. Po predhodnih poskusih sta bili določeni optimalni koncentraciji prolina, 6 mM za krušno pšenico in 2 mM za navadno lečo. Obe vrsti sta bili obravnavani s prolinom v normalnih in v sušnih razmerah. Rezultati so pokazali, da je pomanjkanje vode prizadelo obe vrsti, kar je vodilo v zmanšano rast, manjšo vsebnost klorofila in zmanjšano relativno vsebnost vode. Podobno je obravnavanje s 15 % PEG-6000, kar je enakovredno osmotskemu potencialu -0.31MPa, povzročilo veliko kopičenje prolina v obeh vrstah. Opažen je bil tudi znaten upad v aktivnosti katalaze (Cat) in askorbat perkosidaze (APX), kar je bilo verjetno posledica oksidacijskega stresa, povzročenega s sušo. Uporaba prolina je pri obeh vrstah zmanjšala škodljive učinke sušnega stresa z akumulacijo endogenega prolina, kar je tudi zmanšalo aktivnosti Cat, APX in GPX.

Ključne besede: *Triticum aestivum* L.; *Lens culinaris* Medik.; eksogeni prolin; odpornost na sušo

1 INTRODUCTION

Stress is defined as a set of disorders that adversely affect the plants development (Farooq et al., 2009). Abiotic stresses shall include drought, cold, salinity, etc. These stresses would lead to reducing field crop yield by 70 % via morphological and physiological alterations. As a consequence, a better understanding of the tolerance mechanisms for these stresses is in itself a major economic challenge (Passioura, 2007). The tolerance of plants to drought stress is a complex phenomenon. When plants are exposed to drought

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stress, they synthesize the osmoregulatory compounds which play the role of a regulator of osmotic pressure and a stabilizer of enzymes and membranes (Farooq et al., 2009). Consequently, the synthesized compounds play a major role to overcome the negative impact of drought stress. The proline is considered to be as one of the most widely distributed and accumulated osmolyte under various drought stress conditions. It is a proteinogenic amino acid and its cyclic form provides an exceptional conformational rigidity (Szabados & Savouré, 2009; Lehmann et al., 2010). Free proline is involved in the osmotic potential adjustment in a variety of plants that are subjected to hyper osmotic conditions (Verbruggen & Hermans, 2008). Additionally, it has been proven that the proline functions as a chaperone molecule that preserves the integrity of proteins, prevents their aggregation (Rajendrakumar et al., 1994), maintains membrane integrity (Ashraf & Foolad, 2007) and acts as an antioxidant which eliminates free radicals (Sharma & Dietz, 2006; Liang et al., 2013). The involvement of proline in plant tolerance to stress has attracted researchers since it has an efficient and influential role to maintain good plant production under stress conditions. For example, overproduction of proline would play a role of cryoprotectant under chilling conditions (Gleeson et al., 2004). Proline applied exogenously ameliorated photosynthetic pigments content, stomatal conductance and CO_2 assimilation under drought and salinity conditions (Ben Hassine et al., 2008; Ben Ahmed et al., 2010). The results carried by Gleeson et al. (2004) showed that over-accumulation proline may have a role in protection of forest species from environmental stresses. However, a high concentration of proline can inhibit growth and causes other deleterious effects on cellular metabolism, according to Mani et al. (2002). Ashraf & Foolad (2007) suggest that the protective effect of proline depends on several parameters: the type of plant species, developmental stage, and the optimal concentration. On the basis of these parameters, we intended to investigate the consequences of the exogenous proline on the growth and the physiology for wheat and lentil subjected to drought stress. Since, osmotic stress can result in oxidative stress, we proposed to study antioxidant enzymes activities of catalase (Cat), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) which are the key enzymes of the adaptive response by maintaining the redox balance during oxidative stress. *Triticum aestivum* L. and *Lens culinaris* Medik have been choosen for this study due to their economic importance and because these two species belong to different plant classes (monocot and dicot).

2 MATERIALS AND METHODS

2.1 Plant material

The present study was conducted on two species, bread wheat (***Triticum aestivum*** 'Arz') and lentil (***Lens culinaris*****'**Dahra'). The seeds were obtained from Technical Institute of Field Crops (Algeria). These species have been chosen due to their great economic importance and to their belonging to different plant classes (monocot and dicot). Wheat var. Arz, harvested in the region of Tlemcen (West Algeria), is adapted to semi-arid region. Lentil var. Dahra, harvested in the region of Tiaret (West Algeria), presents an adaptation to drought and high temperatures. After germination, the seedlings of both species were transposed in potting soil-filled pots (65 g). The characteristics of the soil are: medium structure, pH 6, electrical conductivity (EC) of 450 $\mu S.cm^{-1}$, N 270 of mg l^{-1}, P_2O_5 of 150 mg l^{-1}, and K_2O of 300 mg l^{-1}. The pots were kept under ambient conditions (photoperiod 16 h and day/night temperatures 24/18 °C).

2.2 Treatments imposed on bread wheat and lentil seedlings

Treatments consisted in watering with 6 mM proline solution for bread wheat and 2 mM for lentil (C+P), with 15 % PEG-6000 solution (S) or 15 % PEG-6000 solution plus 6 mM proline solution for bread wheat and 2 mM for lentil (S+P). Control was watered with distilled water (C). The first application of treatments (50 ml) was applied after a week of growth, and the second application was applied in the third week. The leaves of control and stressed seedlings have been sampled after one month of growth (in the third stage for the bread wheat and in the seventh stage for the lentil leaves).

2.3 Growth measurement

For each treatment, growth measurement was taken on one month old plant. Five plants taken for each treatment, were used to calculate the mean of each parameter. The measurements taken were: height of shoot, fresh mass of leaves and roots, dry mass of leaves and roots (by oven drying at 80 °C for 48 h).

2.4 Determination of relative water content (RWC)

Leaves' turgor was estimated by the determination of RWC according to Barrs (1968). The leaf discs mass of lentil and leaf segments of wheat were measured immediately after sampling (fresh mass) and then, after

24 h of incubation in distilled water (mass at full turgor). Subsequently, the leaf discs were dried in oven at 80 °C for 24 h to obtain their dry mass. Relative water content was calculated from the following formula:

RWC = (fresh mass - dry mass) X 100/ (fresh mass of full turgor - dry mass)

2.5 Cell membrane integrity evaluation

Cell membrane integrity was estimated by measuring the relative leakage of electrolytes from foliar discs of the lentil and foliar segments of the wheat. The leakage of electrolytes was measured according to the method described by Bajji et al. (2002). Electrical conductivity was measured before (E1) and after the sample was boiled at 100 °C for 1 h (E2). The percentage of electrolytes leakage was calculated according to the following relation: Electrolyte leakage (%) = (E1/E2) X 100

2.6 Determination of photosynthetic pigments content

The contents of chlorophylls (a,b) and carotenoids were determined by extraction in 80 % acetone. Measured absorption values were used for chlorophyll and carotenoids contents calculation according to Lichtenthaler (1987):

Chl a ($\mu g\ ml^{-1}$) = 12,25 × OD_{663} - 2,79 × OD_{647}
Chl b ($\mu g\ ml^{-1}$) = 21,5 × OD_{647} - 5,10 × OD_{663}
Chl a+ Chl b ($\mu g\ ml^{-1}$) = 7,15 × OD_{663} + 18,71 × OD_{647}
Carotenoids ($\mu g\ ml^{-1}$) = (1000 * $D.O_{470}$ - 1,82) × (Chl a - 85,02)/198

2.7 Proline assay

The proline amount in the leaves was determined according to the method describded by Troll & Lindsley (1955) and modified by Magné & Larher (1992). Sample of 50 mg dry mass of leaves was homogenized with 1 ml of distilled water at 90 °C during 30 minutes. After centrifugation at 12000 rpm for 10 min, 500 µl aliquot of the supernatant was mixed with 1 ml of the reagent mixture (60 ml glacial acetic acid, 40 ml distilled water and 1 g ninhydrin) and heated in sealed test tubes at 95 °C for 30 minutes. After cooling down, 3 ml toluene was added to each sample. Proline content was read on a spectrophotometer at 520 nm and expressed as $mg.g^{-1}$ dry mass.

2.8 Total soluble protein quantification

An amount of 100 mg of samples were grinded with 1ml of 100 mM Tris-HCl buffer (pH 8.1) containing 10 % sucrose, 10 mM Na-EDTA and 0.05 % β-mercaptoéthanol. After centrifugation at 15000 rpm for 20 minutes, the supernatant was used to estimate soluble protein contents and antioxidant enzyme activity. Protein concentration was determined by measuring the absorbance at 595 nm. The protein content of each sample was determined by spectrophotometer according to the method of Bradford (1976) using Bovine Serum Albumin (BSA) as a protein standard.

2.8 Oxidative stress assessment

2.8.1 Measurement of lipid peroxidation

Lipid peroxydation was determined in terms of malondialdehyde (MDA) content by measuring the concentration of MDA, based on the method described by Alia et al. (1995). The leaf samples (100 mg) were weighed and homogenized in 2 ml of 0.1 % trichloroacetic acid (TCA) solution. The mixture was centrifuged at 12000 rpm for 20 minutes at 4 °C. Then, 0.5 ml of the supernatant was mixed with 0.5 ml of 0.5 % thiobarbituric acid (TBA) in 20 % TCA. The reaction mixture was heated in water bath at 95 °C for 30 minutes. cooled to room temperature and then centrifuged at 1000 rpm for 10 minutes. The absorbance of supernatant at 532 nm was determined and nonspecific absorbance of supernatant at 600 nm was subtracted from it. The MDA content was calculated by using the extinction coefficient of $\varepsilon = 155\ mM^{-1}cm^{-1}$ and expressed as nmol of MDA g^{-1} fresh mass.

2.8.2 Catalase activity

Catalase (Cat) activity was assayed as described by Dorey et al. (1998). The reaction mixture in a total volume of 2 ml contained 25 mM sodium phosphate buffer (pH 7.0) and 10 mM H_2O_2. The reaction was initiated by the addition of 100 µl of the protein exact containing enzyme, and Cat activity was assayed by monitoring the disappearance of H_2O_2 at 240 nm for 1 minute ($\varepsilon_{240} = 36\ mM^{-1}\ cm^{-1}$).

2.8.3 APX activity

Ascorbate peroxidase activity activity was determined according to Nakano and Asada (1981). The reaction mixture in a total volume of 1.5 ml contained 50 mM sodium phosphate buffer pH 7.0, 0.1 mM Na_4EDTA, 0.5 mM ascorbate, 0.1 mM H_2O_2, and 100 µl of the enzyme extract. H_2O_2-dependant oxidation of ascorbate was followed by decrease in the absorbance at 290 nm for 1 minute ($\varepsilon_{290} = 2.8\ mM^{-1}cm^{-1}$).

2.8.4 Guaiacol peroxidase (GPX)

Guaiacol peroxidase (GPX) activity was estimated according to MacAdam et al. (1992). For this assay, 100 mg of fresh leaves were homogenized in 1 ml of 50 mM (KH_2PO_4/K_2HPO_4) buffer (pH 6.5). The homogenate was centrifuged at 12000 g for 20 minutes at 4 °C. The supernatant was used for enzyme activity and protein content assays. All steps during enzyme extract

preparation were carried out at 4 °C. The reaction mixture contained 100 µl enzyme extract, 18 mM guaiacol and 50 µl H_2O_2. The enzyme activity was calculated using absorption coefficient for tetraguaiacol (26.6 mM^{-1} cm^{-1}) at 470 nm and was expressed as µmoles tetratgaicol.min^{-1}.mg^{-1} protein.

2.9 Statistical analysis

The results were depicted in histogram and table forms, representing the averages of the measured five values as well as their standard error (mean ± standard error). The results were evaluated statistically using the Student t-test at $P \leq 0.05$.

3 RESULTS

3.1 Shoot height

Osmotic stress led to an important reduction in shoot heigth, especially for wheat with decreases from 20.3±0.33 to 13.8±0.30 cm (-32.26 %). However, under normal conditions, the exogenous proline provoked a positive effect on wheat seedlings heigth (+28.33 %) but not significant effect on lentil seedlings. Under stressful conditions, applied proline increased highly bread shoot heigth (+48.87 %) versus a low increase for lentil (+8.46 %) (Figure 1).

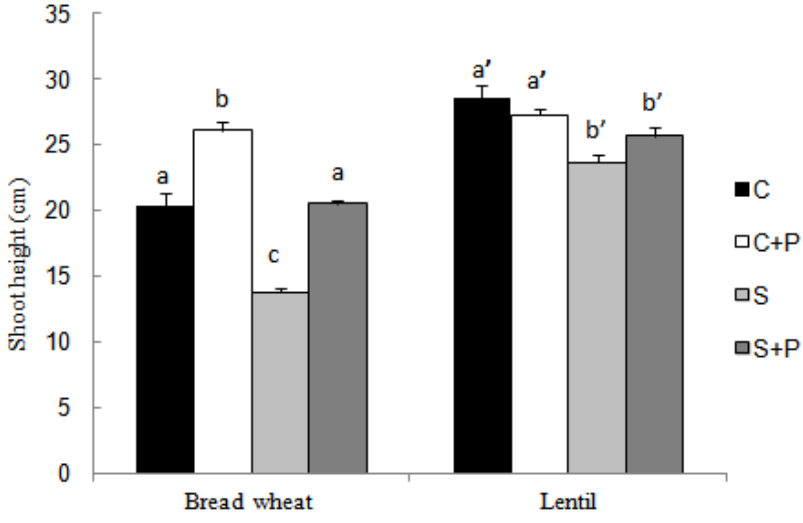

Figure 1: Effect of exogenous proline on shoot height of seedlings of *Triticum aestivum* and *Lens culinaris* under normal and drought stress conditions. Error bars represent the standard errors of the means and in some cases, the error bars are too small to be visible. For each species, different letters show significant differences ($P \leq 0.05$).

3.2 Fresh and dry leaves and roots mass

Osmotic stress induced a significant reduction ($P \leq 0.05$) in fresh and dry mass of leaves and roots of bread wheat and lentil seedlings. Under PEG exposure, exogenous proline induced a significant increase in fresh mass of wheat compared to stressed seedlings without exogenous proline. Thes increases reached +24.29 % and +24.71 % for leaves of bread wheat and lentil respectively, and +78.72 % and +47.12 % for roots of wheat and lentil respectively (Table 1).

Table 1: Effect of exogenous proline on fresh and dry mass of leaves and roots of bread wheat and lentil seedlings under normal and drought stress conditions

Treatments	Fresh mass of leaves (mg) Bread Wheat	Lentil	Dry mass of leaves (mg) Bread wheat	Lentil
C	290.32±33.62 **a**	534±13.13 **a'**	28.57±3.36 **a**	59.85±1.60 **a'**
C+P	319.47±6.32 **a**	461.93±12.31**b'**	37.15±3.5 **b**	51.37±1.08 **a'**
S	152.40±16.55 **b**	267.40±14.54 **c'**	20.22±2.88 **c**	34.03±1.91**b'**
S+P	267.35±14.42 **a**	332.37±8.17 **d'**	36.02±4.86 **b**	42.43±2.17 **c'**
	Fresh mass of roots (mg) Bread Wheat	Lentil	Dry mass of roots (mg) Bread wheat	Lentil
C	183.67±9.42 **a**	325.48±17.25 **a'**	16.3±1.85 **a**	20.37±2.28 **a'**
C+P	177.35±12.76 **a**	310.07±10.57 **a'**	17.35±4.65 **a**	20.5±20 **a'**
S	84.92±40.44 **b**	133.93±4.62 **b'**	10.77±1.17 **b**	10.93±2.47 **b'**
S+P	199.125±4.53**a**	239.35±20.97 **c'**	17.25±1.05 **a**	16.07±2.38 **a'**

Data represent mean ± standard error (SE). For each species and each organ, means in the same column, followed by ifferent letters are statistically different ($p < 0.05$).

3.3 Relative water content

Water stress induced a reduction of the relative water content (RWC) in leaves of wheat and lentil by 17.51 % and 17.45 %, respectively in comparison to the control plants. Under stressed conditions, the application of exogenous proline improved the relative water content of the leaves of both species. In the case of bread wheat, the RWC value was equal to untreated control (Figure 2).

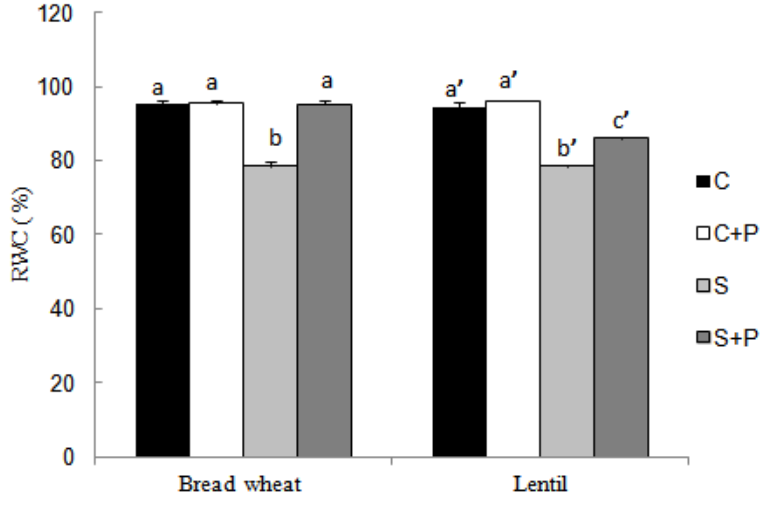

Figure 2: Effect of exogenous proline on relative water content of seedlings leaves of *Triticum aestivum* and *Lens culinaris* under under normal and drought stress conditions. Error bars represent the standard errors of the means. For each species, different letters show significant differences ($P \leq 0.05$).

3.4 Cell membrane integrity

Cell membrane integrity was assessed by electrolyte leakage. PEG treatment caused an important increase of electrolyte leakage in the leaves of wheat and lentil (52.03 % and 30.48 %, respectively) compared to the control plants. Under stress conditions, this electrolyte leakage has been reduced in the presence of exogenous proline in comparison to the stressed seedlings in the same rate for both species but with a lower absolute value for bread wheat (Figure 3).

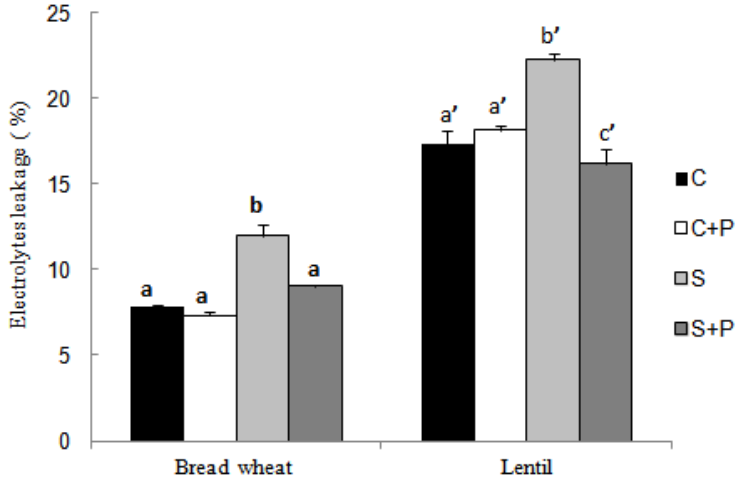

Figure 3: Effect of exogenous proline on electrolytes leakage of seedlings leaves of *Triticum aestivum* and *Lens culinaris* under under normal and drought stress conditions. Error bars represent the standard errors of the means. For each species, different letters show significant differences ($P \leq 0.05$).

3.5 Chlorophyll content

The total chlorophyll and carotenoids contents were significantly ($P \leq 0.05$) decreased under drought stress in both species. Under stress conditions, the addition of proline resulted in a significant increase ($P \leq 0.05$) in leaf chlorophyll contents of the wheat seedlings (+43.87 %) and carotenoids (+54.42 %) in comparison to the stressed without proline. In contrast, for the lentil seedlings, no positive effect has been observed. Under normal conditions and with exogenous proline, Chl a:Chl b ratio did not vary significantly for both species ($P \leq 0.05$). However, under water stress conditions, proline application on bread wheat increased this ratio but it had no effect on lentil (Table 2).

Table 2: Effect of exogenous proline on chlorophylls and carotenoids contents of leaves of bread wheat and lentil seedlings under normal and drought stress conditions

Treatments	Chlorophyll (a+b) (mg.g^{-1} fresh mass)		Chlorophyll a/ b		Carotenoids (mg.g^{-1} fresh mass)	
	Bread wheat	Lentil	Bread wheat	lentil	Bread wheat	lentil
C	0.061±0.004 **a**	0.23±0.04 **a'**	1.98±0.06 **a**	2.1±0.06 **a'**	2.31±0.18 **a**	0.042±0.005 **a'**
C+P	0.054±0.002 **a**	0.26±0.03 **a'**	1.95±0.07 **a**	2.23±0.01 **a'**	1.84±0.17 **a**	0.045±0.001 **a'**
S	0.038±0.001 **b**	0.18±0.044 **b'**	1.12±0.20 **b**	2.17±0.03 **a'**	1.21±0.10 **c**	0.033±0.007 **b'**
S+P	0.055±0.002 **a**	0.18±0.08 **b'**	1.93±0.40 **a**	2.16±0.11 **a'**	1.86±0.10**a**	0.034±0.001 **b'**

Data represent mean ± standard error (SE). For each species and each organ, means in the same column, followed by different letters are statistically different ($p < 0.05$).

3.6 Free proline content

The accumulation of proline was significantly higher in stressed plants (**S**) for both species in comparison to the control (+133.33 % for wheat and +114.58 % for lentil). In the absence of stress, application of proline induced significant differences ($P \leq 0.05$) in proline content with +44.44 % for wheat and +41.66 % for lentil. The presented results revealed that the highest level of proline content in leaves was recorded by supplying proline under both conditions (Figure 4).

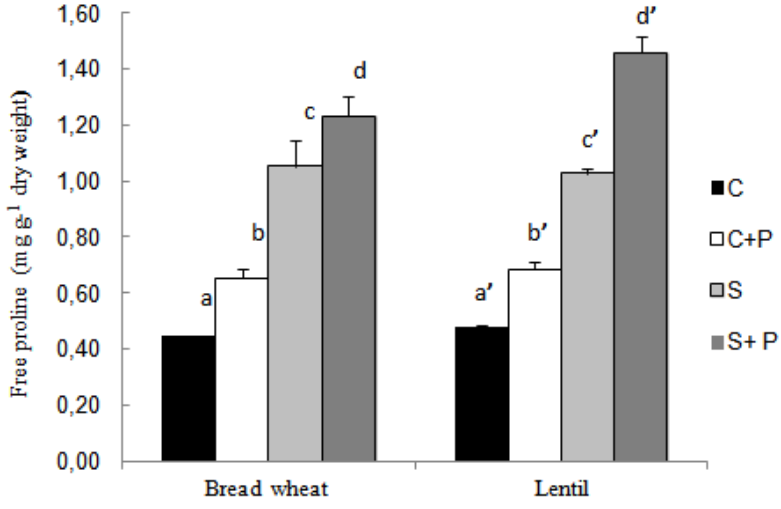

Figure 4: Effect of exogenous proline on free proline content of seedlings leaves of *Triticum aestivum* and *Lens culinaris* under under normal and drought stress conditions. Error bars represent the standard errors of the means. For each species, different letters show significant differences ($P \leq 0.05$).

3.7 Total soluble protein content

Osmotic stress induced a significant reduction ($P \leq 0.05$) of the total soluble protein content of lentil seedlings leaves (-48.48 % compared to the control). In contrast, for the wheat seedlings, no effect has been observed. Under PEG treatment, exogenous proline induced a significant increase in protein content of wheat (+27.08 %) and in lentil seedlings (+54.01 %) compared to stressed seedlings (S) (Figure 5).

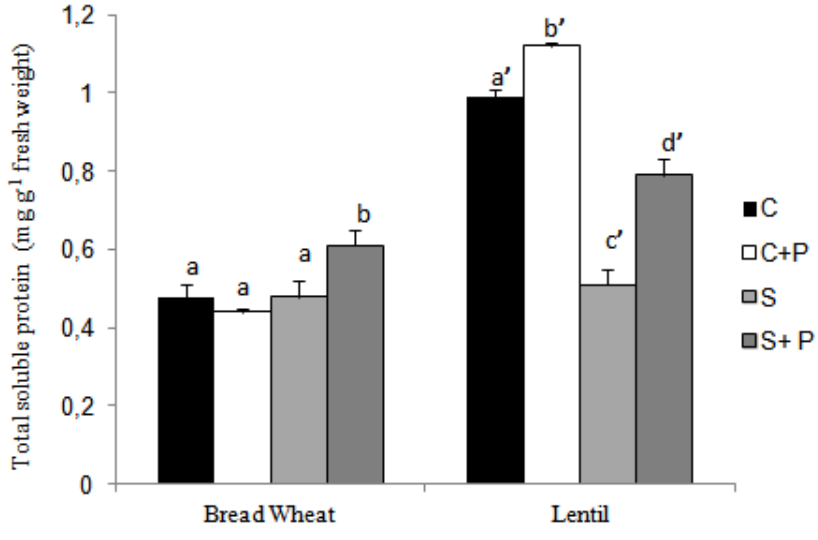

Figure 5: Effect of exogenous proline on total soluble protein content of seedlings leaves of *Triticum aestivum* and *Lens culinaris* under under normal and drought stress conditions. Error bars represent the standard errors of the means. For each species, different letters show significant differences ($P \leq 0.05$).

3.8 MDA content

PEG resulted in increased accumulation of MDA content by +89.26 % and +95.45 % in wheat and lentil seedlings, respectively, compared to control seedlings (Figure 6). Proline in combination with PEG reduced MDA content in bread (-87.72 %) and lentil (-53.48 %) compared to stressed without proline (S).

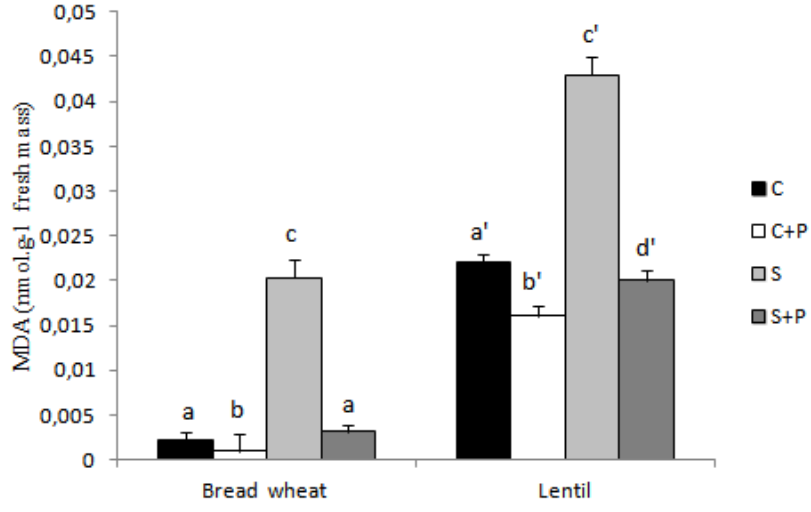

Figure 6: Effect of exogenous proline on the MDA content of seedlings of *Triticum aestivum* and *Lens culinaris* under under normal and drought stress conditions. Error bars represent the standard errors of the means. For each species, different letters show significant differences ($P \leq 0.05$).

3.9 Catalase activity

Under stressful conditions, there was no significant change in the catalase activity in lentil leaves, but this activity decreased significantly ($P \leq 0.05$) in the case of wheat (-45.35 %) compared to the control plants. Under these stressful conditions, the addition of proline amplified the catalase activity especially in wheat seedlings. The catalase activity increased for +197.02 % in the case of wheat and for +85.98 % in lentil compared to the stressed seedlings (S). Under unstressed conditions, the exogenous proline resulted in a significant decrease in catalase activity in the leaves of both species (-37.13 % for wheat and -30.44 % for lentil) in comparison to control seedlings (C) (Figure 7).

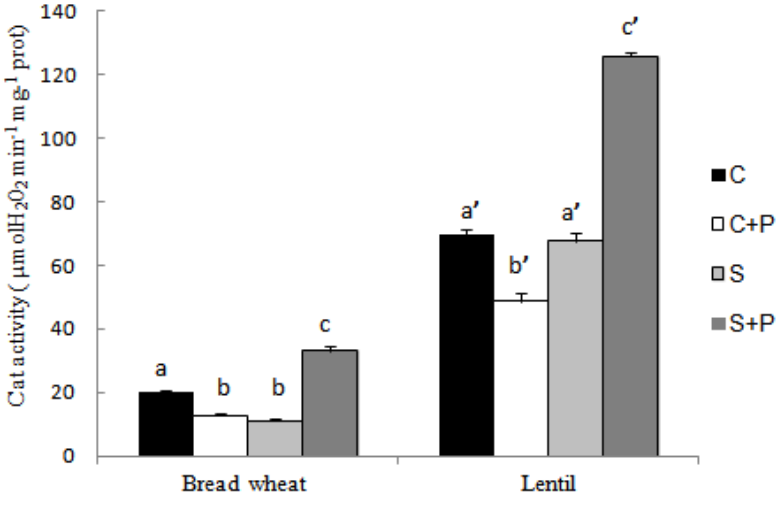

Figure 7: Effect of exogenous proline on the catalase activity of seedlings of *Triticum aestivum* and *Lens culinaris* under under normal and drought stress conditions. Error bars represent the standard errors of the means. For each species, different letters show significant differences ($P \leq 0.05$).

3.10 Ascorbate peroxidase activity (APX)

PEG caused a significant ($P \leq 0.05$) decrease in APX activity in the leaves of wheat and lentil (-67.50 % and -40.74 %, respectively) compared to the control (Figure 8). Also, the addition of proline under stress conditions increased APX activity in lentil (+56.25 %) in comparison to the stressed (S). However, exogenous proline reduced APX activity in wheat (-11.50 %).

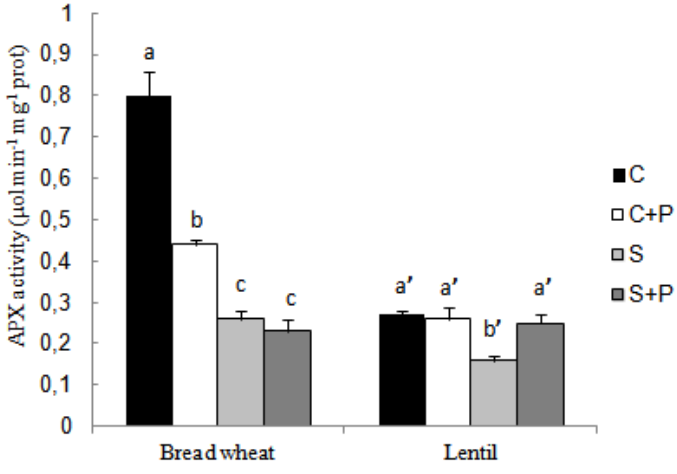

Figure 8: Effect of exogenous proline on the APX activity of seedlings of *Triticum aestivum* and *Lens culinaris* under under normal and drought stress conditions. Error bars represent the standard errors of the means. For each species, different show significant differences ($P \leq 0.05$).

3.11 Guaiacol peroxidase (GPX)

Drought stress caused a significant reduction ($P \leq 0.05$) in activity of GPX for both species**.** We noted -46.15 % for bread wheat and -20 % for lentil in comparison to the control plants.

Under normal and drought stress conditions, supply of proline significantly increased activity, especially for bread wheat (Figure 9).

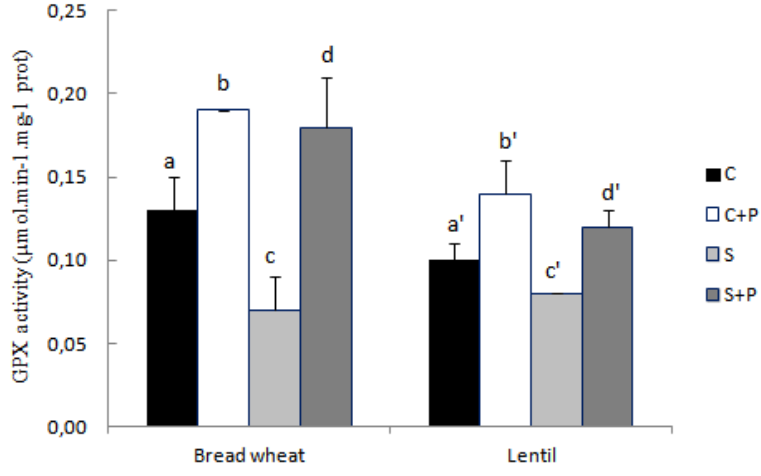

Figure 9: Effect of exogenous proline on the GPX activity of seedlings of *Triticum aestivum* and *Lens culinaris* under under normal and drought stress conditions. Error bars represent the standard errors of the means. For each species, different show significant differences ($P \leq 0.05$).

4 DISCUSSION

4.1 Effect of drought stress

In the presence of polyethylene glycol (PEG-6000), the results showed a decrease in growth of wheat and lentil seedlings. This can be explained by a increase of the osmotic pressure in the medium which prevented the absorption of water by the root system. Osmotic stress decreased the relative water content in both studied species, indicating that drought stress induced a decrease of turgor in wheat and lentil leaves.

A decrease in chlorophylls and carotenoids contents were also recorded. This consequently led to a reduction in growth resulting in a decrease in cell turgor and photosynthesis (Akcay et al., 2011). Exposure to drought stress leads to a significant effect in chlorophyll a and b contents (Ranjbarfordoei et al., 2000). The reduction in chlorophylls content under drought stress may be because of inhibition of chlorophyll synthesis or an acceleration of its degradation or membrane deterioration (Smirnof, 1995). Osmotic stress induced by PEG-6000 can lead to lipid peroxidation and, consequently, chlorophyll destruction also, with decreasing chlorophylls and carotenoids contents.

The effect of increased osmotic pressure caused by drought stress resulted in a significant increase in endogenous proline level in both species. It has been reported that the proline is an important compatible solute accumulated in higher plants under conditions of abiotic stress (Delauney & Verma, 1993). Proline plays a crucial role in osmoregulation and osmotolerance (Szabados & Savouré, 2009). It is considered as biomarker of stress. An increase in free proline content during dought stress conditions due to PEG has also been shown in different crops as maize (Meeta et al., 2013) and bread wheat (Ji et al., 2014). Handa et al. (1986) suggested that the level of proline accumulation depends not only on the plant osmotic potential or loss of turgor, but also on its level of stress adaptation of the plant. During stress, the expression of *P5CS* (pyrroline-5-carboxylate synthetase), but not of *P5CR* (pyrroline-5-carboxylate reductase) gene, is well correlated with proline content (Savouré et al., 1995).

Osmotic stress did not affect leaf total soluble protein content of wheat seedlings, but it reduced this content in the lentil. Changes in protein expression, accumulation, and synthesis have been observed in many plant species as a result of plant exposure to drought stress (Akhzari & Pessarakli, 2015; Li et al., 2018).The reduction in protein content in plants under drought stress is due to proteins synthesis inhibition, acceleration of the proteolysis process or reduction in the amino acids content (Dubey & Rani, 1990). Nitrate reductase, implied in protein synthesis, is the most altered enzyme by drought (Sepehr et al., 2012).

Drought stress led to oxidative stress illustrated for both species by enhanced electrolyte leakage. Electrolyte leakage is considered as an indicator of loss in membrane integrity as a result of lipid peroxidation (Bajji et al., 2002). Similar results were obtained by Bandurska et al. (2001). They reported that a high PEG concentration increased the membrane permeability of barley varieties. As consequence of this peroxidation, both species exhibited an obvious increase of MDA content which was directly related to the oxidative stress induced by PEG. However, plants possess a battery of antioxidant mechanisms, both enzymatic and non-enzymatic, by which ROS are removed from the cell (Noctor & Foyer, 1998).

Among non-enzymatic systems, proline provides a protective role in drought induced oxidative stress by reducing H_2O_2 levels and by increasing the antioxidant defense system (Molla et al., 2014). Catalase is the most responsive of enzymes mechanisms to H_2O_2 (Gondim et al., 2012). However, our results showed that this enzyme activity did not vary for lentil and has been significantly decreased in wheat seedlings. This decrease was reported by Alam et al. (2014). Contradictory results, where the catalase activity is increased, have been reported by Yuan et al. (2014) and Antonić et al. (2016). In fact, the behavior of antioxidant enzyme not only depends on the severity and duration of the stress treatment, but they also depends on the species and age of the plant (Carvalho, 2008). Our results showed that activities of APX and GPX decreased in response to osmotic stress. Indeed, Lokhande et al. (2010) and Jisha & Puthur (2015) observed a decrease in APX and GPX activities due to application of PEG.

4.2 Application of exogenous proline

Exogenous application of proline mitigated stress-induced inhibitory effects on the growth of both species. In the present study, proline supplementation increased free proline content in wheat and lentil leaves. However, this amino-acid was more accumulated in lentil leaves. Similar responses were observed in *Lepidium sativum* (Khalil & El-Noemani, 2012) and in rice (Hasanuzzaman et al., 2014; Samota et al., 2017). These facts allow us to suppose that exogenous proline was absorbed by the roots, transported and distributed to the leaves. Indeed, Bar-Nun and Poljakoff-Mayber (1977), showed that radioactive exogenous proline was incorporated in roots of *Pisum sativum* L. and *Tamarix tetragyna* Ehrenb.. Schobert et al. (1988) have reported

the same observation for castor bean roots. Thereafter, Rentsch et al. (1996) have showed the existence of proline transporters in plant. Moreover, Okuma et al. (2000) have demonstrated by non accumulation of glutamate that proline accumulation in *Nicotiana tabacum* L. cultured cells was due to exogenous proline. Free proline accumulation in both species due to stimulation by drought stress was, thus, increased by exogenous supplementation. The positive effects of proline on plant growth would be explained by its role as a nutrient, as well as its role as an osmoprotectant and its implication in osmoadjustment (Dawood et al., 2014). Our results showed that exogenous proline increased relative water content in both species despite stressful conditions allowing the plant to stabilize leaf water balance. Additionally, proline reduces transpiration via its regulatory effect of the opening/closing of the stomata (Yancey, 2005). Futhermore, proline supply showed that degradation in glutamate was increased (Mani et al., 2002). These results suggest that proline can be a good source of energy during stress too, and that the second step of the oxidation pathway is not rate limiting.

Exogenous proline increased the chlorophyll content, especially in bread wheat seedlings. These results are in accord with Rasheed et al. (2014). These authors noted that the addition of 20 mM proline under oxidative stress causes an increase in the chllorophylls and carotenoids contents in leaves of bread wheat. Shahid et al. (2014) showed that exogenous proline improved the growth by increasing photosynthesis. The effect of exogenous proline on chlorophyll contents may also have been due to stabilizing photosynthetic reactions (Abdelhamid et al., 2013).

Exogenous proline increased protein leaves content, especially in lentil seedlings. Similar results have been reported by Demir & Kocacaliskan (2002). These authors noted that the addition of 10 mM exogenous proline under salt stress causes an increase in the protein content in the bean leaf. Khedr et al. (2003) showed that 10 mM exogenous proline improves the salt-tolerance of *Pancratium maritimum* L. by protecting the protein turnover machinery against stress-damage and up-regulating stress protective proteins. Proline has been shown to act as a chemical protein chaperone and to prevent protein aggregation and thermodenaturation (Ignatova & Gierasch, 2006).

The prevention of oxidative stress-induced damages was resulted in a lesser membrane peroxidation thanks to enhanced catalase activity, especially for bread wheat. The same response was observed by Rady & Hemida (2016**)** in maize seedlings and Singh et al. (2016) in *Solanum melongena* L.

PEG had a strong effect on MDA production in both species. On the other side, significant reduction in MDA content was observed when proline was applied to wheat and lentil seedling. These results suggested positive correlation between decreased production MDA and increased in APX and Cat activities under stressful conditions.

Our study showed that under stressed conditions, the addition of proline enhanced APX activity in leaves of lentil. An increase in APX activity has been observed in sugarcane by application of 20 mM proline (Medeiros et al., 2015). In contrast, Kibria et al. (2016) showed that exogenous application of 25 and 50 mM proline, reduced APX activity in *Triticum aestivum*. Additionally, exogenous proline enhanced the activity of GPX. An increase in GPX activity was due to proline pretreatment.

5 CONCLUSION

The main aim of this study was to show the major role of exogenous proline in improving the plant stress tolerance. We conclude that for bread wheat and lentil, the addition of exogenous proline minimized the damages caused by water stress simulated by PEG-6000. Our results showed that the contribution of exogenous proline improved the growth of the aerial part, reduced membrane damages, and improved cell turgor. Additionally, the exogenous proline enhanced the activity of an antioxidant enzymes (Cat, APX and GPX), and increased the level of endogenous proline content. Interestingly, tolerance of bread wheat (a monocot) to water stress was more improved compared to lentil (a dicot). All these results suggest that proline is absorbed by the roots of the studied plants.Thus, under water stress, the presence of compatible solutes such as proline is a promising approach in the amelioration of vegetable production. However further investigations are needed to determine the most effective concentrations and number of applications as well as the most responsive growth stage(s) of each species. Furthermore, it remains to be understood the mechanisms of action of exogenous proline.

6 ABREVIATIONS

C:Control; C+P: Control+Proline; PEG-6000: Polyethelene glycol 6000; S: Stressed; S+P: Stressed+Proline**.**

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