Mitigating the Harmful Effects of Water Deficiency Stress on White Lupine (*Lupinus albus* L.) Plants by Using Algae Extract and Hydrogen Peroxide.

Asmaa M. Mogazy¹; Engy A. Seleem² and G. F. Mohamed³*

¹Biology and Geology Department, Faculty of Education, Ain-Shams University, Cairo, Egypt.
²Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Giza, Egypt.
³Department of Agricultural Botany, Faculty of Agriculture, Fayoum University, Fayoum, Egypt.

**ABSTRACT**

Water deficit is a major growth limitation factor affecting plant growth and productivity. To counteract the harmful effects of drought stress, lupine plants were sprayed with two concentrations of algae extract (1 g L⁻¹ and 2 g L⁻¹) and hydrogen peroxide solution (0.5% and 1%) under low irrigation level. Morphological, physiological and anatomical studies were performed. It was found that all treatments enhanced plant height, root length, number of leaves, average leaf area, shoot and root fresh and dry weights. A significant increment in chlorophyll a and b, carotenoids and total pigments contents, as well as a significant decrease in electrolyte leakage was noted in treated plants compared with untreated under water deficit. Increment in levels of osmoprotectants such as proline, total free amino acids, glycine betaine and total soluble sugars contents was highly significant in case of 2 g L⁻¹ algae extract treatment compared with untreated plants group. Anatomical studies of root, stem and leaf of treated and untreated plants revealed that 2 g L⁻¹ algae extract treatment followed with 0.5% H₂O₂ induced significant increments in the thickness of the studied plant organs compared with the untreated plants under water deficit conditions. In general, the concentrations of 2 g L⁻¹ algae extract and 0.5% H₂O₂ were the most effective doses regarding all the studied characteristics.

**Keywords:** Algae extract, anatomy, drought, hydrogen peroxide, lupine, osmoprotectants.

**INTRODUCTION**

By 2030, many countries will suffer water shortages in addition to poor water quality which is considered as a global problem for food markets (Pieters et al., 2004). According to Kasim et al., 2013, by 2050, drought is expected to affect more than 50% of arable lands. Shortage of water could be induced naturally in response to climate change or artificially due to anthropological activities (Al-Riffai et al., 2019). Water deficiency is an abiotic stress with a drastic effect on agricultural productivity. Plant responses to water stress include decreases in plant growth and development, plant pigments content, CO₂ uptake, in addition to accumulation of reactive oxygen species (ROS) (Winnicki et al., 2019).

Consequently, induction of crop tolerance to water shortage is a major concern of many recent agricultural studies (Cantale et al. 2018).

Lupine (*Lupinus albus* L.) is a leguminous plant that is rich in high good quality protein percentage (44%), and seed dry matters like oil and dietary fiber, lupine cultivation reached to 661 thousand hectares in 2013 with Australia, Russian Federation and Ukraine as the world’s highest production areas (El-Harty et al. 2016). In Egypt, *Lupinus* is cultivated in different soil types because its agricultural requirements are very low. The annual production of lupine in Egypt is approximately 20488 kg/ha ranking the ninth on the world at 2018 (FAO 2020). Harmful effects of water shortage on lupine plants include flowers falling, decrease of pods number, and low productivity and seed chemical composition changes, e.g., increasing alkaloid content (Hane 2017).

Algae extract was reported as a useful biostimulant for plant growth, it is rich with auxins and cytokinins which are plant growth regulators (Marhoon and Abbas, 2015). *Chlorella* spp., *Spirulina* spp. and *Amphora* spp. microalgae are important commercially to produce algal products such as β-carotene (Pro-vitamin A), amino acids, lutein and phycocyanin (Badr et al. 2017). *Amphora coffeaeformis* is a marine and fresh water diatom which produces biochemical components like protein, carbohydrates and lipids (Rajaram et al., 2018). In addition, *Amphora coffeaeformis* extract is rich with high levels of plant pigments like chlorophyll, carotenoids as β-carotene, macro and micro-elements especially phosphorous, iron and zinc and antioxidants components (El-Sayed et al. 2018). Youssef and Ali (1998) stated that algal extracts (*Anabena oryzae*, *Nostoc calcicola* and *Spirulina* spp.) improved some biometric characteristics of cowpea plants (shoots fresh and dry weights and roots and length of shoots). According to Aung (2011), growth rate and leaf area increased by using *Spirulina* suspension treatments on mung bean.

Hydrogen peroxide (H₂O₂) is a stable Reactive Oxygen Species (ROS) which is naturally produced during cell metabolism especially under stress (Ismail et al. 2015).

It has great role in signaling several physiological processes like photosynthesis, respiration, transpiration and
translocation (Kilic and Kahraman 2016). Furthermore, Wang and Song (2008) explained that abscisic acid increases under drought stress leading to stomatal closure, and according to the relationship between H₂O₂ and abscisic acid, the latter increases the former in plant cell inducing stomatal closure under stress. Spraying H₂O₂ on soybean plants helps in maintaining leaf water content and overcoming drought stress problems (Ishibashi et al. 2011), induces adaptation to osmotic stress in maize (Terzi et al. 2014), enhances barely seed germination and seedling growth under salinity (Kilic and Kahraman 2016).

The aim of this study is to introduce new approaches to reduce global loss in lupine plant production by enhancing plant performance under water shortage conditions, investigate the physiological effect of algae extract and H₂O₂ treatments on lupine plants under water deficit conditions and to study the morphological and anatomical changes induced by both treatments in lupine plants.

MATERIALS AND METHODS

Two pot experiments were conducted at Faculty of Education, Ain-shams University, Cairo, Egypt in two growing seasons 2017/2018 and 2018/2019.

Plant material

Lupine seeds were obtained from Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. Plastic pots (25 cm in diameter) were filled with clay soil mixed with sandy soil (4:1:v:v). Homogenate seeds were selected, sterilized by 0.1% HgCl₂ (w/v) and sown at a rate of six seeds per pot on 23rd of October 2017 and 19th October 2018. The pots were kept in appropriate place receiving direct sun light, then seedlings were thinned to three per pot six days after emergence.

Experimental procedures

Seedlings were watered twice per week for two weeks. Drought stress was induced after 30 days from seed sowing at the emergence of first 2-3 true leaves.

The experiments were arranged in complete randomized block design with three replicates, three pots for each replicate (9 pots for each treatment). The treatments was divided as follows: control; normal irrigation (3-day interval), drought stressed plants; plants irrigated at 8-day interval, drought stressed treated plants; plants irrigated 8-day interval and sprayed with: a- H₂O₂ at the concentrations of 0.5 and 1%, b- Algae extract (Amphora coffeaeformis) at the concentrations of 1 and 2 g L⁻¹. The aforementioned substances were first applied 55 days from sowing date and the foliar spray was repeated after two weeks from the first one.

Algae extract

Algae extract (Amphora coffeaeformis) was acquired from Algal Biotechnology Unit, National Research Centre (NRC), Giza, Egypt. It was isolated from the local irrigation water of Egypt. Preparation of Algae extracts was described by El-Sayed et al. (2018).

Data recorded

Morphological characters

After 85 days from sowing, random samples of 15 plants per treatment (5 plants per replicate) were taken at flowering stage (55 days from drought treatment) The following morphological parameters were recorded in both seasons: plant height, root length, leaf number/plant, average leaf area (cm²) was measured with an area meter (Planix Digital Planimeter, Model 2107). Also, shoot and root fresh and dry weights/plant (g).

Physiological characters

1- Determination of electrolyte leakage (EL)

Electrolyte leakage was determined according to Lutt et al. (1996) using five leaf disc from the youngest fully expanded leaf. Electrical conductivity was determined in two values; (EC₁) and (EC₂) at room temperature and 121°C, respectively and the (EL) value was calculated as (EC₁/EC₂) and expressed in percentage.

b- Determination of photosynthetic pigments

Photosynthetic pigments were extracted using chilled acetone solution. Three wave lengths were used 645, 663, and 480 nm, the absorbance of the supernatant was recorded against 80% acetone as blank. The chlorophyll contents were estimated according to Arnon (1949), while the carotenoid content was determined according to the formula given by Kirk and Allen (1965).

c- Determination of osmolyte compounds

The proline content in fresh plants was estimated according to the method of Bates et al. (1973). Absorbance was determined at 520 nm.

Sugars were determined according to Dubois et al. (1956) and then absorbance was measured at 485 nm.

Total free amino acids were estimated following the method of Yemm and Cocking (1955). The absorbance was measured at 570 nm.

Glycine betaine was extracted from air dried plants using deionized water according to Grieve and Grattan (1983). The absorbance was measured at 365 nm.

Anatomical studies

Anatomical specimens were taken for each of: control, drought stressed, drought stressed treated plants from the middle portions of root, stem and leaflet blade of compound leaf on the middle internode of the stem. The selected specimens were chosen from plants aged 55 days from drought stress treatment.

The specimens, were killed and fixed in F.A.A. solution (50 ml 95% ethyl alcohol + 10 ml formalin + 5 ml glacial acetic acid + 35 ml distilled water) for 48 hours.

Thereafter, samples were washed in 50% ethyl alcohol, dehydrated and cleared in tertiary butyl alcohol series, embedded in paraffin wax of 54-56 C m.p. Cross sections, 20 thick, were cut by a rotary microtome, adhesived by Haupt’s adhesive and stained with the crystal violet-erythrosin combination (Nassar and El-Sahhar, 1998). Slides were photographed and read to determine histological aspects and measurements of different characters were expressed in microns (µ).

Statistical analysis

Data of each season were statistically analyzed according to Snedecor and Cochran (1994), New Least Significance Differences (New L.S.D.) at 5% level of probability were calculated to determine the mean comparisons among treatments.

RESULTS AND DISCUSSION

Morphological characters

Table (1) revealed that plants exposed to water deficit conditions showed a prominent significant decrease in all morphological characters estimated, except root length, which increased more than the control plants in both seasons.

Foliar application with algae extract (1 and 2 g L⁻¹) enhanced significantly all morphological characters in both seasons under drought conditions compared with the untreated plants, with exception of root length in the first and second seasons, and number of leaves/plant and root
fresh weight/plant in the first season which increased insignificantly at 1 g L⁻¹ extract. Moreover, all vegetative traits in both seasons increased significantly at the concentration 0.5% of hydrogen peroxide, while 1% of hydrogen peroxide caused significant increase only in plant height and leaf area in both seasons in comparison with the untreated plants under the same stress conditions. Among all these treatments, algae extract at 2 g L⁻¹ gave the highest significant values for all the morphological studied characters under drought stress conditions, however these values are still less than those of the control plants.

Water deficiency has adverse effects on plant growth and physiological processes; cell structure and enlargement is affected due to loss of turgor and inhibition of different metabolic processes which in turn affects plant growth rate (Zlatev and Lidon, 2012). Inhibition of shoot growth was recorded as an adaptive response helping plants to cope with drought stress by diverting essential solutes to face stressful conditions such as osmotic adjustment (Neumann, 2008).

The results of this study are in agreement with Emam et al. (2010) reported that Plant height, leaves number and area and dry weight of stem branches of legumes were reduced under soil water deficit. Ouzzoumoudj et al. (2014) which recorded a significant decrease in plant height of broad bean under drought conditions. Moreover, Prabha and Negi (2014) on capsicum plants and Siddiqui et al. (2015) on faba bean, stated that drought stress affected plant height, root and shoot fresh and dry weights and leaf area.

On the other hand, an enhancement was detected in morphological characters in response to both treatments with algae extract and H₂O₂. Algae extracts -specifically A. coffeeiformis- are characterized by the presence of many useful phyto-constituents including photosynthetic pigments such as chlorophyll and carotenoids (β-carotene and fucoxanthin) which enhance different biological activities of the cell (El-Sayed et al., 2018). Moreover, algae extract is rich with auxins and cytokinins which enhance cell division and enlargement leading to increase in plant vegetative characteristics (Marhoon and Abbas, 2015). The application of algae extract to tomato plants under drought stress enhanced plants fresh weights (Petrozza et al., 2014). According to Kasim et al. (2015), seaweed extract enhanced root depth, shoot height and leaf area of wheat plants under drought stress. Likewise, Abu Seif et al. (2016) reported that foliar application of snap bean with fresh water algae (Spirulina) improved plant length, leaf area per plant and plant dry weight (g).

Application of Spirulina algae extract increased significantly vegetative growth and yield/feed in sugar beet plants (Enan, et al., 2016).

Recently, an increasing concern was given to the importance of H₂O₂ in counteracting the adverse effects of plant stress. Despite being an ROS, H₂O₂ plays an important role in many physiological and biochemical processes of plant such as: seed germination, root system development, flowering and senescence (Niu and Liao, 2016). Ishibashi et al. (2011) on soybean showed that exogenous application of H₂O₂ enhanced the up-regulation of stress responsive genes and improved drought stress tolerance., Sun et al., (2016) on cucumber stated that the biomass of plants significantly increased by H₂O₂ application under drought stress. Orabi et al., (2018) stated that H₂O₂ improved growth and yield characters of canola plant with specific emphasis on dry matter of shoots and root, seed yield/plod and seed number/plant.

Table 1. Effect of algae extract and hydrogen peroxide on vegetative growth characters of lupine plants under water deficit conditions during two seasons of 2017/2018 and 2018/2019.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Vegetative growth characters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st season</td>
</tr>
<tr>
<td></td>
<td>Plant height (cm)</td>
</tr>
<tr>
<td>Control</td>
<td>45.9</td>
</tr>
<tr>
<td>Drought</td>
<td>30.7</td>
</tr>
<tr>
<td>Drought+Algae 1g L⁻¹</td>
<td>37.6</td>
</tr>
<tr>
<td>Drought+Algae 2 g L⁻¹</td>
<td>42.5</td>
</tr>
<tr>
<td>Drought+H₂O₂ 0.5%</td>
<td>38.1</td>
</tr>
<tr>
<td>Drought+H₂O₂ 1%</td>
<td>35.2</td>
</tr>
<tr>
<td>New L.S.D. (0.05)</td>
<td>4.21</td>
</tr>
</tbody>
</table>

Physiological characters

Membrane stability

An increase in electrical conductivity indicates loss of membrane integrity which increases the leakage of ions; the damage of great range of plant tissues could be tested by determining the ability of plasma membranes to control the rate of ion movement in/out of cells (Masoumi et al., 2010). Data in Table (2) showed threefold increments in the percentage of electrical conductivity (EL) in plants that experienced drought compared with control plants which received sufficient amount of water. While all treatments showed a significant decrease in the EL percentage compared with drought suffering untreated plants, both treatments algae extract (2 g L⁻¹) and hydrogen peroxide (0.5%) showed the least values compared with other treatments. Prabha and Negi (2014) showed that membrane stability was affected in drought stressed plants due to loss of membrane integrity which resulted in elevated leakiness of ions and in turn an increase in electrical conductivity. A noticeable electrolyte leakage was reported as a result of prolonged drought stress (Orabi and El-Noemani, 2015).

According to Sun et al. (2016), H₂O₂ treatment decreased electrolyte leakage and increased the capacity for osmotic adjustment regaining cell turgor in drought stressed plants.

Esmaielpour et al. (2020) indicated an increase in electrolyte leakage in basil plants in response to drought conditions followed by enhancement in growth and a noticeable decrease in electrolyte leakage after the application of seaweed extract.
Table 2. Effect of algae extract and H$_2$O$_2$ treatments on electrolyte leakage, chlorophyll a, b, carotenoids and total pigments contents in lupine plants under water deficit conditions.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>EL %</th>
<th>Chl a mg/g F. wt.</th>
<th>Chl b mg/g F. wt.</th>
<th>Carotenoids mg/g F. wt.</th>
<th>Total pigments mg/g F. wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.6</td>
<td>0.783</td>
<td>0.548</td>
<td>0.490</td>
<td>1.821</td>
</tr>
<tr>
<td>Drought</td>
<td>65.9</td>
<td>0.387</td>
<td>0.194</td>
<td>0.221</td>
<td>0.802</td>
</tr>
<tr>
<td>Drought +Algae 1 g L$^{-1}$</td>
<td>36.1</td>
<td>0.622</td>
<td>0.361</td>
<td>0.516</td>
<td>1.31</td>
</tr>
<tr>
<td>Drought +Algae 2 g L$^{-1}$</td>
<td>25.6</td>
<td>0.743</td>
<td>0.511</td>
<td>0.441</td>
<td>1.695</td>
</tr>
<tr>
<td>Drought +H$_2$O$_2$ 0.5%</td>
<td>27.2</td>
<td>0.686</td>
<td>0.498</td>
<td>0.385</td>
<td>1.564</td>
</tr>
<tr>
<td>Drought +H$_2$O$_2$ 1%</td>
<td>39.8</td>
<td>0.574</td>
<td>0.305</td>
<td>0.250</td>
<td>1.129</td>
</tr>
<tr>
<td>New L.S.D. (0.05)</td>
<td>3.18</td>
<td>0.010</td>
<td>0.011</td>
<td>0.007</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Photosynthetic pigments

Results in table (2) showed changes in pigments contents in lupine plant shoots under water deficit conditions in treated and untreated stressed plants compared with well irrigated control plants. Generally, a significant increment in chlorophyll a, b, carotenoids and total pigments contents was recorded in response to treatments compared with untreated plants under drought stress. Algae extract (2 g L$^{-1}$) treatment showed almost 2-fold increment in chlorophyll a, carotenoids and total pigments contents compared with untreated plants. Also, hydrogen peroxide 0.5% treatment showed a significant enhancement in the same contents. On the other hand, both treatments 1 g L$^{-1}$ algae extract and 1% H$_2$O$_2$ recorded less effect on photosynthetic pigments contents.

Photosynthesis is a major plant metabolic process which is crucial for crop production, so it is directly affected by water deficit conditions (Farooq et al., 2009).

Statistical analysis in treatment activity of photosynthetic enzymes were recorded as plant response mechanisms under stressful environments which in turn result in a clear reduction in photosynthetic activity (Nyachiro et al., 2001). The photosynthetic rate is adversely affected by the formation of reactive oxygen species (ROS) as well as increased activity of chlorophyllase and ethylene production (Farooq et al., 2009, Escalante-Magaña et al., 2019).

Plant pigments contents were significantly reduced in bean (Beinsan et al., 2003), Carthamus tinctorius (Siddiqi et al., 2009), wheat cultivars seedlings (Aly and Latif, 2011), and Indian mustard (Mostafaei et al., 2018) under water stress conditions. The aforementioned results supports the findings of this study as plant pigments content showed a significant decrease under water deficit conditions.

In accordance with the results of this study, algae extract treatment enhanced plant pigments content in snap bean (Abu Seif et al., 2016), sugarc beet plants (Eran et al., 2016). This effect could be attributed to the activity of antioxidants (Lee et al., 2008, El-Sayed et al., 2018) and growth promoter components in algae extract (El-Eslamboly et al., 2019). In addition, Sun et al., (2016) and Guler and Pehlivan (2016) claimed that pigments contents increased due to H$_2$O$_2$ treatment which alleviated the drought stress in cucumber and soybean plants, respectively. Moreover, Habib et al., (2020) recorded an increment in drought stressed wheat plant pigments contents in response to H$_2$O$_2$ treatment.

Osmolyte compounds

Plants tend to accumulate osmolytes such as soluble sugars, free amino acids, proline (Pro) and glycine betaine (GB) to adapt to stresses (Hoekstra et al., 2001). The present results showed that algae extract at 2 g L$^{-1}$ and H$_2$O$_2$ at 0.5% treatments induced an increment in studied osmolytes contents in plants subjected to drought in comparison with untreated ones under the same conditions.

Algae extract treatment 2 g L$^{-1}$ induced a significant increment in glycine betaine content as shown in Table (3) compared with drought stressed untreated plants. At the same time, 0.5% H$_2$O$_2$ treatment showed noticeable increment in the same contents. Moreover, total soluble sugars, total free amino acids and proline contents increased in response to 1 g L$^{-1}$ algae extract while, 1% H$_2$O$_2$ treatments gave the least value compared with untreated drought affected plants. Osmolytes present in the cytoplasm of plants play an important role in reducing cell osmotic potential and maintaining cell turgor (Pathan et al., 2004) without decreasing actual water content (Serraj and Sinclair, 2002). Proline (Pro) accumulation could be a plant mechanism to avoid water stress as it is one of the most important osmolytes associated with plants drought stress, in addition, it contributes to stabilizing sub-cellular structures (e.g. proteins and membranes) and scavenging free radicals (Hayat et al., 2012, Huang et al., 2014).

Accumulation of Glycine betaine (GB) was recorded in water stressed plants which assumed a protective role in stressed tissues (Park et al., 2006). Glycine betaine could maintain stable structures and activities of enzymes and protein complexes which reflect the integrity of membranes against the damaging effects of water stress (Sakamoto and Murata 2002). Moreover, under drought stress conditions, sugar accumulation was found to prevent the oxidation of cell membrane, maintain the turgidity of leaves and prevent dehydration of membranes and proteins, reduces the rate of photosynthesis and maintains the leaf water content and osmotic adjustment of plants (Samia et al., 2016). The present study showed a significant increase in (Pro) and (GB) contents in response to water stress. Findings of Silvente et al. (2012) on soybean and Lum et al. (2014) on rice support results of this study.

Table 3. Effect of algae extract and H$_2$O$_2$ treatments on proline, total soluble sugars, total free amino acids and glycine betaine contents in lupine plants under water deficit conditions.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Proline (mg/g FW)</th>
<th>Total s. sugars (mg/g FW)</th>
<th>Total free amino acids (mg/g FW)</th>
<th>Glycine betaine (mg/g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.701</td>
<td>24.78</td>
<td>6.09</td>
<td>1.13</td>
</tr>
<tr>
<td>Drought</td>
<td>1.035</td>
<td>32.33</td>
<td>17.01</td>
<td>2.98</td>
</tr>
<tr>
<td>Drought +Algae 1 g L$^{-1}$</td>
<td>1.982</td>
<td>42.6</td>
<td>25.34</td>
<td>3.45</td>
</tr>
<tr>
<td>Drought +Algae 2 g L$^{-1}$</td>
<td>2.103</td>
<td>49.50</td>
<td>32.65</td>
<td>4.41</td>
</tr>
<tr>
<td>Drought +H$_2$O$_2$ 0.5%</td>
<td>1.740</td>
<td>37.15</td>
<td>22.70</td>
<td>3.06</td>
</tr>
<tr>
<td>Drought +H$_2$O$_2$ 1%</td>
<td>0.985</td>
<td>28.09</td>
<td>13.81</td>
<td>3.41</td>
</tr>
<tr>
<td>New L.S.D. (0.05)</td>
<td>0.031</td>
<td>0.648</td>
<td>0.653</td>
<td>0.226</td>
</tr>
</tbody>
</table>

924
Results of the current study noted further accumulation in osmolytes content in response to algae extract and H₂O₂ treatments. According to Nair et al., (2012), algae extract increased proline and total soluble sugars contents contributing to abiotic stress tolerance in plants. In addition, application of algae extract increased sugar content in leaves and root of sugar beet plants (Enan et al., 2016). Sun et al., (2016) on cucumber showed an increase in soluble sugar and proline contents in drought stressed plants treated with H₂O₂. In addition, a positive correlation between H₂O₂ treatment and biosynthesis of proline under water stress has been reported in wheat plant (Habib et al., 2020).

Anatomical studies:

For anatomical studies, plants treated with algae extract at 2 g L⁻¹ and H₂O₂ at 0.5% concentrations under drought stress conditions (remarkable positive responses), in addition to untreated drought stressed and control plants (normal irrigation) were chosen.

1- The Root

The microscopical measurements (µ) and counts of root transverse sections of Lupine plants treated by 2 g L⁻¹ algae extract and 0.5% H₂O₂ grown under drought stress, the untreated drought stressed as well as the control plants are presented in Table (4) and Fig. (1).

Table 4. Measurements in microns (µ) of some anatomical features of root transverse sections of lupine plants treated with algae extract and hydrogen peroxide under water deficit conditions.

<table>
<thead>
<tr>
<th>Measurements (µ)</th>
<th>Control</th>
<th>Drought</th>
<th>Drought + Algae 2 g L⁻¹</th>
<th>±% to Drought</th>
<th>Drought + H₂O₂ 0.5%</th>
<th>±% to Drought</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root diameter</td>
<td>2585</td>
<td>1685</td>
<td>2170</td>
<td>+28.7</td>
<td>1880</td>
<td>+11.5</td>
</tr>
<tr>
<td>Cortex thickness</td>
<td>490</td>
<td>420</td>
<td>425</td>
<td>+1.2</td>
<td>360</td>
<td>-14.2</td>
</tr>
<tr>
<td>Vascular cylinder diameter</td>
<td>1525</td>
<td>830</td>
<td>1325</td>
<td>+59.6</td>
<td>1120</td>
<td>+34.9</td>
</tr>
<tr>
<td>Xylem thickness</td>
<td>432</td>
<td>225</td>
<td>357</td>
<td>+58.6</td>
<td>325</td>
<td>+44.4</td>
</tr>
<tr>
<td>Phloem thickness</td>
<td>75</td>
<td>60</td>
<td>75</td>
<td>+25</td>
<td>70</td>
<td>+16.6</td>
</tr>
<tr>
<td>Vessel diameter</td>
<td>44</td>
<td>31</td>
<td>42</td>
<td>+35.4</td>
<td>40</td>
<td>-29</td>
</tr>
<tr>
<td>Pith diameter</td>
<td>520</td>
<td>260</td>
<td>451</td>
<td>+73.4</td>
<td>330</td>
<td>+26.9</td>
</tr>
</tbody>
</table>

Concerning the drought effect, water deficit caused reduction in all anatomical characters compared with well irrigated control plants. On the other hand, plants which received 2 g L⁻¹ of algae extract under drought stress conditions exhibited the most remarkable thickness of root overcoming the untreated plants under the same drought stress. This increment could be attributed to the increase in cortex, vascular cylinder diameter and pith diameter. The increment in vascular cylinder diameter is attributed to the increase of secondary xylem and phloem thickness. Moreover, plants treated with H₂O₂ under drought stress showed an increase in root diameter exceeding the untreated plants at the same conditions by 11.5%.

However, the root diameter was less than that exhibited by plants treated with algae extract at 2 g L⁻¹. This increment is attributed to the increase in vascular cylinder, secondary xylem and phloem thickness, in addition to pith diameter. On the other hand, the cortex thickness showed a reduction of 14.3% less than the untreated stressed plants. The plants treated with algae extract at 2 g L⁻¹ showed a prominent increase in root diameter more than those treated by H₂O₂ at 0.5%. This increment is related to the increase in cortex thickness, vascular cylinder diameter, xylem tissue and pith diameter (Table 4 and Fig. 1).

Fig. 1. Transverse sections through median portion of the tap root of lupine plants sprayed with algae extract, and hydrogen peroxide under water deficit conditions, and the well watered control plants.

A- Control Plants;
B- Stressed Plants;
C- Stressed plants treated with 2 g L⁻¹ algae extract;
D- Stressed plants treated with 0.5% of hydrogen peroxide.

2- The Stem:

Microscopical counts of histological characters in transverse sections through the median intermode of lupine plants main stem are given in Table (5) and Fig. (2) for plants affected with drought stress, those treated with 2 g L⁻¹ algae extract and 0.5% H₂O₂ at the same stress level, and the well watered control plants. It is noted that drought stress severely affected the whole stem diameter which decreased...
by 37.2% less than the well irrigated control plants. This deficiency is completely reflected on all histological characters of the stem. On the other hand, plants treated with algae extract at 2 g L⁻¹ concentration under low water conditions gave the best results in which the whole stem diameter increased by 30.1% more than the drought stressed. This increment in stem diameter was linked to the remarkable increase in thickness of both stem wall as well as hollow pith diameter more than the drought stressed. It is worthy to mention that the plants treated by algae extract at 2 g L⁻¹ were thicker compared to those treated with H₂O₂ at 0.5% concentration under same stress conditions. Stem diameter of the former plants increased by 15.5% than the latter ones, and this increment could be attributed mainly to the increase of stem wall thickness, cortex thickness, xylem tissue, parenchymatous pith thickness and hollow pith diameter by 10.9, 6.2 and 23.3%, respectively.

### Table 5. Measurements in microns (µ) of some anatomical features of stem transverse sections of lupine plant treated with algae extract and hydrogen peroxide under water deficit conditions.

<table>
<thead>
<tr>
<th>Measurements (µ)</th>
<th>Control</th>
<th>Drought</th>
<th>Drought + Algae 2 g L⁻¹</th>
<th>±% to Drought</th>
<th>Drought + H₂O₂ 0.5%</th>
<th>±% to Drought</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem diameter</td>
<td>4650</td>
<td>2920</td>
<td>3800</td>
<td>+30.1</td>
<td>3290</td>
<td>+12.6</td>
</tr>
<tr>
<td>Stem wall thickness</td>
<td>1368</td>
<td>970</td>
<td>1171</td>
<td>+20.7</td>
<td>1055</td>
<td>+8.7</td>
</tr>
<tr>
<td>Epidermis Thickness</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>0.0</td>
<td>30</td>
<td>0.0</td>
</tr>
<tr>
<td>Cortex thickness</td>
<td>284</td>
<td>190</td>
<td>240</td>
<td>+26.3</td>
<td>220</td>
<td>+15.7</td>
</tr>
<tr>
<td>Fiber cap thickness</td>
<td>66</td>
<td>60</td>
<td>63</td>
<td>+5.0</td>
<td>63</td>
<td>+5.0</td>
</tr>
<tr>
<td>Phloem thickness</td>
<td>68</td>
<td>55</td>
<td>65</td>
<td>+14.5</td>
<td>60</td>
<td>+9.0</td>
</tr>
<tr>
<td>Xylem thickness</td>
<td>354</td>
<td>210</td>
<td>288</td>
<td>+37.1</td>
<td>238</td>
<td>+13.3</td>
</tr>
<tr>
<td>Vessel diameter</td>
<td>47</td>
<td>32</td>
<td>38</td>
<td>+18.7</td>
<td>36</td>
<td>+12.5</td>
</tr>
<tr>
<td>Parenchymatous pith thickness</td>
<td>548</td>
<td>425</td>
<td>475</td>
<td>+11.7</td>
<td>447</td>
<td>+5.1</td>
</tr>
<tr>
<td>Hollow pith diameter</td>
<td>1914</td>
<td>975</td>
<td>1449</td>
<td>+48.6</td>
<td>1175</td>
<td>+20.5</td>
</tr>
</tbody>
</table>

As a result of drought stress, the midrib as well as the lamina thickness was decreased by 31.7 and 32.4% less than the well irrigated control plants. Subsequently, the palisade and spongy tissues, and the dimension of midvein bundle were negatively affected compared with control plants. The reduction in midrib and the lamina thickness in drought stressed plant leaves could be attributed to the malformation in palisade and spongy layers as a result of exposing plants drought (Abd Elbar et al., 2019).
Moreover, Abd Elbar et al. (2019) explained that the rate of cell division of mesophyll decreases significantly by drought stress which indicates a decrease in leaf elongation.

Relative to drought stressed plants; it is obvious that spraying plants with algae extract at 2 g L⁻¹ enhanced all the anatomical characters of the leaflet. Midrib and lamina thickness increased by 30.3 and 31.3%, respectively. This thicker leaflet could be attributed to the increase in palisade and spongy tissues, and the dimension of midvein bundle (length and width).

Plants treated with H₂O₂ at 0.5% still occupy the second rank after algae extract in the development of anatomical structure of leaf. Data also revealed that the plants received algae extract at 2 g L⁻¹ showed 10.6 and 6.4% increments in midvein and lamina thickness over the plants treated by 0.5% H₂O₂ under the same drought conditions. This increment was reflected on all internal tissues; palisade, spongy, width of midvein bundle by 3.3, 4, and 10.7% higher than plants treated with 0.5% H₂O₂. The present results are almost in harmony with those obtained by (Boghdady et al., 2016) on chickpea, where, using 2 g L⁻¹ seaweed extract increased the stem diameter due to an increment in the thickness of cortex as well as phloem and xylem tissues. This concentration of seaweed also provided xylem vessels with wider cavities which in turn increased water conductive capacity due to increased area. In addition, the increased thickness of palisade and spongy tissues as well as vascular bundle of midvein lead to an increase in thickness of lamina and midvein of leaflet blades of chickpea. Moreover, Marhoon and Abbas (2015) stated that treatment of sweet pepper with seaweed extract at 6 g increased the thickness of cortex, and the diameter of vascular tissues significantly, in contrary, the thickness of pith area decreased compared with control. Salama et al. (2015) on basil plants and Salama et al. (2016) on stevia plants, stated that spraying seaweed extract at 1 and 1.5 g, respectively, caused an increase in stem diameter mainly resulting from the increment induced in thickness of cortex, phloem and xylem tissues and pith parenchyma area compared to the control. Likewise, seaweed extract induced increase in thickness of midvein and lamina due to the increase in the thickness of palisade and spongy tissues as well as in the dimensions of the midvein bundle, being in harmony with the present investigation.

The effect of spraying H₂O₂ on anatomical structure of lupine root, stem and leaves are extensively discussed in literature. According to Gil et al. (2009) the xylem vessel diameter and xylem/phloem ratio of roots and shoots tended to be greater for trees grown in soil treated with H₂O₂ than for controls, which resulted in increase in water conductivity and better enhancement of the aerial parts growth. Jafariyan and Zarea (2016) found that, soaking seeds of wheat plants in H₂O₂ at various concentrations (0, 25, 50, and 80%), enhanced the stomata density and increased the length and the anatomical features of leaf.

Table 6. Measurements in microns (µ) of certain anatomical features of leaflet blade transverse sections of lupine plant treated with algae extract and hydrogen peroxide under water deficit conditions

<table>
<thead>
<tr>
<th>Measurements (µ)</th>
<th>Control</th>
<th>Drought</th>
<th>Drought +Algae 2 g L⁻¹</th>
<th>±% to Drought</th>
<th>Drought +H₂O₂ 0.5%</th>
<th>±% to Drought</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid rib thick.</td>
<td>410</td>
<td>280</td>
<td>365</td>
<td>+30.3</td>
<td>330</td>
<td>+17.8</td>
</tr>
<tr>
<td>Lamina thick</td>
<td>222</td>
<td>150</td>
<td>197</td>
<td>+31.3</td>
<td>185</td>
<td>+23.3</td>
</tr>
<tr>
<td>Upper epidermis</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0.0</td>
<td>10</td>
<td>0.0</td>
</tr>
<tr>
<td>Lower epidermis</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0.0</td>
</tr>
<tr>
<td>Palisade tissue</td>
<td>110</td>
<td>70</td>
<td>93</td>
<td>+32.8</td>
<td>90</td>
<td>+28.5</td>
</tr>
<tr>
<td>Spongy tissue</td>
<td>90</td>
<td>60</td>
<td>78</td>
<td>+30.0</td>
<td>75</td>
<td>+25</td>
</tr>
<tr>
<td>Midvein bundle dimension</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>115</td>
<td>105</td>
<td>120</td>
<td>+14</td>
<td>115</td>
<td>+9.5</td>
</tr>
<tr>
<td>Width</td>
<td>160</td>
<td>130</td>
<td>155</td>
<td>+19.2</td>
<td>140</td>
<td>+7.6</td>
</tr>
</tbody>
</table>

Fig. 3. Transverse sections through leaflet mid-rib of lupine plants sprayed with algae extract, and Hydrogen peroxide under water deficit conditions, and the well watered control plants.

A- Control Plants.
B- Stressed Plants.
C- Stressed plants treated with 2 g L⁻¹ algae extract.
D- Stressed plants treated with 0.5% of hydrogen peroxide.
Asmaa M. Mogazy et al.

Fig. 4. Transverse sections through marginal portion of leaflet blade of lupine plant sprayed with algae extract, and hydrogen peroxide under water deficit conditions, and the well watered control plants.

A- Control Plants.
B- Stressed Plants
C- Stressed plants treated with 2 g L⁻¹ algae extract.
D- Stressed plants treated with 0.5% of hydrogen peroxide.

CONCLUSION

In summary, lupine plants exposed to drought stress exhibited a severe reduction in plant growth. On the other hand, the exogenous application of Amphora coffeaeformis extract (rich in protein, carbohydrates, lipids, pigments and plant growth regulators) alleviated the harmful effect of drought stress on lupine plants. Additionally, foliar application of H₂O₂ solution improved lupine plants tolerance to drought stress due to its role in some physiological processes. However, the algal Amphora coffeaeformis extract was more effective than H₂O₂ solution in helping plants to tolerate drought stress.

REFERENCES


Tخفيف الآثار الضارة الناتجة عن اجهاد نقص المياه على نباتات الترمس الأبيض باستخدام مستخلص الطحالب وفوق أكسيد الهيدروجين.

اسماء محمود مغازي1، انجي علاء سليم2 و جمال فرج محمد3

احترقت تجربتي اجريت في كلية التربية، جامعة عين شمس، في موسمين متتاليين 2018/2019 و2019/2019 وذلك بعد تحسين النمو والصفات في ظل ظروف نقص المياه باستخدام مستخلص الطحالب ”Amphora coffeaeformis” و مادة فوق أكسيد الهيدروجين. أوسميت النتائج أن معاملة البطاطس برش مستخلص الطحالب بتركيزات (0.5 و1 جم/لتر) و فوق أكسيد الهيدروجين بتركيزات (0.5 و 1 %) أدى إلى تحسين جميع الصفات المورفولوجية (طول النبات، عدد الأوراق، متوسط مساحة الورقة، الوزن الطات، و الجذور لكل من المجموع الخضري والجريتي) وذلك مقارنة بالنباتات الغير معالمة تحت نفس ظروف نقص المياه واعطت الجرعة 2 جم/لتر من مستخلص الطحالب وتركيز 0.5 % من فوق أكسيد الهيدروجين أعلى نسبة زيادة بالنسبة للتركيز الآخر. كما أدت معاملة نباتات الترمس بالمواد السابقة تذكرة أنها أدت إلى حدوث زيادة معنوية في محتوى النباتات من الصبغات (كليروفيل، كليروفيلا) والكروتنتين بالإضافة إلى المحتوى الكلي للثاني من الصبغات و محتوى النبات من البرولين، الإضافات الأمينية الحرة الكلوية Glycine betaine.

الدراسات التشريحية لكل من الجذر والساق والورقة أوضحت أن معاملة الفواكه غير العضوية تحت نفس ظروف نقص المياه، على العكس من الفواكه المعالمة بالماء. احتوت هذه الفواكه على لحوم الاماتية مع محتوى من الفواكه الساق تحت نفس ظروف نقص المياه "Electrolyte Leckage ‘EL’"

أوضح النتائج التي تم الحصول عليها في هذه الدراسة أن استخدام مستخلص الطحالب وفوق أكسيد الهيدروجين في نباتات الترمس الأبيض يؤدي إلى تحسين النمو والصحته، مما يتيح زيادة صلابة النباتات ضد اجهاد نقص المياه ومحتوى الفواكه الساق تحت نفس ظروف نقص المياه.