INTRODUCTION

Plants are repetitively confronted with several environmental stresses, both abiotic and biotic; sometimes plants can be defied with different stresses concurrently or consecutively (Hatmi et al., 2015). Photosynthesis is strongly inhibited by drought stress. The stimulation of photorespiration along with over-reduction of photosynthetic electron transport results in increased production of ROS (Hernández et al., 2012). Drought stress is one of the most adverse factors affecting plant growth and productivity. Exploring biochemical and molecular responses of plants to drought stress is essential for better perception of plant resistance mechanisms against water-limited conditions (Ahmad et al., 2016).

It is well known that abscisic acid (ABA) is involved in the control of...
ABA effects on lettuce under different irrigation regimes

ABA has been stated to participate in stress-induced readjustment of plant metabolism (Fujita et al., 2011). ABA regulates the biosynthesis of phenylpropanoids, particularly flavonols and anthocyanins (Tossi et al., 2012; Perrone et al., 2012). ABA induces stomatal closure and other physiological and biochemical modifications that allow plants to tolerate drought stress (Wilkinson and Davies, 2010). ABA plays a significant role in numerous responses to drought stress, such as induction of stress proteins, stomata closure and accretion of various metabolites for protection of cells from water deficit stress (Umezawa et al., 2010). Many studies have shown that ABA can inhibit the inward K⁺ channel to inhibit stomatal opening (Chen et al., 2013).

The economically important vegetable crop lettuce (Lactuca sativa L.), selected for the present investigation belongs to the family Asteraceae. Lettuce is cultivated worldwide and is one of the most consumed green leafy vegetables in the raw form for its taste and high nutritive value. It is cultivated in UAE due to its commercial importance. The major problem during lettuce cultivation is the requirement for large quantity of irrigation water. Lettuce is the most popular leafy vegetable and is appreciated for the salads; it encompasses health-promoting phytochemicals including polyphenols, carotenoids and vitamin C (Llorach et al., 2008). For the past several years, numerous techniques have been applied to overcome water deficit in field crops. However, limited information is gained on the response to ABA treatment under drought stress and its ameliorative actions during cultivation of lettuce. It seems necessary to study the correlation between plant growth regulators and drought stress tolerance. The present study aimed to reduce the water consumption of lettuce. For that purpose, different irrigation regimes were applied together with ABA application.

MATERIALS AND METHODS

Pot culture experiments were conducted in completely randomized block design (CRBD) in a greenhouse at Al-Foah Experimental Station (270N and 220S latitude and 510W and 570E longitude) of the College of Food and Agriculture, UAEU in Al Ain city. The greenhouse environment was controlled for temperature and relative humidity. During the experimental period, the temperature in the greenhouse was maintained at 24±2°C. Plastic pots of 40 cm in diameter and 45 cm in height were used. Pots were filled with 10 kg of soil mixture containing red soil; sand and commercial potting soil at a ratio of 1:1:1. Lactuca sativa var. longifolia seeds were obtained from Lortolano company, Italy and kindly provided by the commercial supplier “Shat Alarab”.

Treatments

Irrigation regimes

Drought treatments (48h, 72h and 96h) were expressed as different irrigation intervals and fixed field capacity of water irrigation quantity (200ml) and various irrigation levels were kept as control.

ABA application

Lettuce plants were subjected to different concentrations of ABA (5 µg L⁻¹, 10 µg L⁻¹ and 15 µg L⁻¹). ABA treatment
was applied as foliar spraying. Plants were allowed to grow up to 15 days upon irrigation. Plants were subjected to ABA treatment 7, 14, 21, 28 and 35 days after drought induction (Sansberro et al., 2004). On day 75 after planting, plants were uprooted gently, washed carefully and packed in plastic bags for analysis.

**Morphological parameters**

Plant height was measured from the soil level to the tip of the shoot and expressed in cm. Plant root length was measured from the point of the first cotyledonary node to the tip of the longest root and expressed in cm.

Total number of leaves, which were fully developed, were counted and expressed as number of leaves per plant.

After washing the plants with tap water, fresh weight was determined by using an electronic balance (Model – XK3190-A7M) and the values were expressed in grams. After taking fresh weight, the plants were dried at 60°C in a hot air oven for 24 h. After drying, the weight was measured and the values were expressed in grams.

**Pigment analysis**

**Chlorophyll and carotenoid contents**

Chlorophylls and carotenoids were extracted in acetone and measured spectrophotometrically (U-2001-Hitachi) by the method of Arnon (1949).

Chlorophyll content was calculated using the formulae of Arnon:

\[
\text{Total chlorophyll (mg/ml)} = (0.0202) \times (A_{645}) + (0.00802) \times (A_{663})
\]

\[
\text{Chlorophyll a (mg/ml)} = (0.0127) \times (A_{663}) - (0.00269) \times (A_{645})
\]

\[
\text{Chlorophyll b (mg/ml)} = (0.0229) \times (A_{645}) - (0.00468) \times (A_{663})
\]

Carotenoid content was estimated using the formula of Kirk and Allen (1965):

\[
\text{Carotenoids} = A_{480} + (0.114 \times A_{663} - 0.638 \times A_{645})
\]

**Anthocyanin content**

Anthocyanin was extracted and estimated by the method of Kim et al. (2002).

Five hundred mg of fresh tissue taken from the third leaf and from the periphery of the tuber tissue were ground with a pestle and mortar in liquid nitrogen, and extracted in acetic acid (50%, v/v) overnight. The homogenate was centrifuged at 19,000×g for 15 min. The resultant supernatant was made up to 20ml and 80ml of McIlvaine’s buffer (pH 3.0). The absorption was measured at 530 nm. The anthocyanin content was calculated according to the formula:

\[
\text{cv} = 0.1 \times A_{530}/\text{g FW}
\]

**RESULTS AND DISCUSSION**

**Morphological parameters**

In lettuce, shoot length showed a significant decrease at different irrigation regimes (Fig. 1). ABA application increased shoot length significantly, except for 96 h in T3 (15 µg L⁻¹ ABA). The maximum increase of shoot length was observed at 72h irrigation regime in combination with T2 (10 µg L⁻¹ ABA).

Root length was significantly increased as a result of drought stress in lettuce plants at all irrigation regimes except for 96 h treatment when compared to control. The maximum increase was observed at 72 h irrigation regime. Root length increased due to ABA treatment under drought stress. The increase was highest in ABA-treated plants (T2-
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**Figure 1.** Effect of different irrigation regimes, ABA and their combination on shoot length of lettuce plants on day 75 after planting. Values are means ±SE of three replicates.

10 µg L⁻¹ ABA) when compared to control and drought-stressed plants (Fig. 2).

Root length was increased by drought stress in lettuce plants when compared to control on day 75 after planting in all drought treatments. Root length increased also in response to ABA treatment under drought stress. Increased root growth was reported by Tahir et al. (2003) in mango trees under water stress. Water stress greatly suppresses cell expansion and cell growth due to the low turgor pressure (Ahadiyat et al., 2014). The initially increased root growth was reduced at the later stages due to severe water deficit stress. Declined cell enlargement and cell growth due to the low turgor pressure and also accelerated leaf senescence under drought stress might cause a reduction in plant height (Liang et al., 2006). In early studies it was reported that root to shoot ratio increased under water stress conditions to facilitate water
absorption (Lambers et al., 1998) and this was related to ABA content in roots and shoots (Sharp and Lenoble, 2002). The morphological and physiological responses to exogenous ABA application suggest an important role of ABA in the control of drought tolerance in two Populus species (Yin et al., 2004).

Under drought stress in combination with ABA application, shoot length decreased to a larger extent when compared to control on all sampling days in lettuce plants. Plant height was reduced under drought stress in Populus species (Yin et al., 2004). Reduced plant height was reported in Albizzia seedlings due to reduced stem length under drought stress (Sundaravalli et al., 2005). ABA-induced growth inhibition results from signal transduction at the single-cell level and thereby ABA induces closure of stomata (Trejo et al., 1995).

There was a significant reduction in the number of leaves at different irrigation regimes in lettuce plants on day 75 after planting. A maximum decrease was noted at 96h irrigation regime. ABA application didn’t help to overcome the stress situation during 96h irrigation regime, but a significant increase in leaf number was observed in plants at 72 h irrigation regime in combination with 10 µg L⁻¹ABA (Fig. 3).

The number of leaves was reduced under drought stress at all stages of lettuce growth. ABA in combination with drought increased the number of leaves slightly on day 75 after planting under 72h irrigation interval. Water deficit mostly reduces leaf growth and in turns, the leaf area in many plant species like Ziziphus sp. (Zhang et al., 2004). Leaf growth was more sensitive to water stress in wheat, but not in maize (Nayyar and Gupta, 2006). The exogenous application of ABA induces stomatal closure and protects plants from stress. Application of ABA at room temperature results in reduced leaf production in many plants (Swamy and Smith, 2001).

The role of ABA in mediating drought stress has been extensively researched. The stomatal movements and closure mechanisms are controlled by ABA thereby regulating water status in the plant through guard cells functions. ABA induces the induction of genes that encode

**Figure 3.** Effect of different irrigation regimes, ABA and their combination on the number of leaves in lettuce plants on day 75 after planting. Values are means ±SE of three replicates.
enzymes and other proteins involved in cellular dehydration tolerance (Luan, 2002; Zhu, 2002).

Root fresh weight decreased under drought stress in lettuce. The fresh weight increased in response to ABA treatment under drought stress. The extent of increase was higher at the 72h irrigation regime when compared to the other irrigation intervals. ABA treatment alone decreased root fresh weight but it was not significant after treatment with 5 µg L\textsuperscript{-1} ABA concentration (Fig. 4). The dry weight of roots showed the same trend as fresh weight. The maximum dry weight was recorded at 72h irrigation regime in combination with 10 µg L\textsuperscript{-1} ABA concentration. Fresh and dry weights showed a decreasing trend with increasing ABA concentration. Similarly, at 96-h irrigation regime, the fresh and dry weight of roots showed a significant decrease when compared to control (Fig. 4, 5).

Drought stress decreased both fresh

![Figure 4](image1.png)

**Figure 4.** Effect of different irrigation regimes, ABA and their combination on root fresh weight of lettuce plants on day 75 after planting. Values are means ± SE of three replicates.

![Figure 5](image2.png)

**Figure 5.** Effect of different irrigation regimes, ABA and their combination on root dry weight in lettuce plants on day 75 after planting. Values are means ± SE of three replicates.
and dry weight of lettuce shoot. ABA treatment alone increased shoot fresh weight which was mainly due to the increased leaf number in response to ABA application. There was no significant difference between the fresh weight increase in 10 µg L⁻¹ and 15 µg L⁻¹ ABA treatments. Both fresh and dry weight increased significantly during ABA treatments in all drought stressed plants. The extent of increase was higher in 72h with 10 µg L⁻¹ ABA treatment when compared to control and drought stressed plants (Fig. 6, 7).

Water deficit decreased plant fresh weight to a larger extent in all water deficit treatments. Similar results were observed in higher plants like Pearl millet (Kusaka et al., 2005) and *Abelmoschus esculentum* (Bhatt and Srinivasa Rao, 2005). The fresh weight decrease under drought conditions might be the reason for suppression of

Figure 6. Effect of different irrigation regimes, ABA and their combination on shoot fresh weight of lettuce plants on day 75 day after planting. Values are means ±SE of three replicates.

Figure 7. Effect of different irrigation regimes, ABA and their combination on shoot dry weight of lettuce plants on day 75 after planting. Values are means ±SE of three replicates.
cell expansion and cell growth due to low turgor pressure. Regulated deficit irrigation and partial root drying caused a significant reduction in shoot biomass when compared to control in rice plants (Ahadiyat et al., 2014).

Drought stress decreased the dry weight of lettuce plants at all irrigation regimes when compared to control. ABA treatments increased the dry weight considerably under drought stress. A decrease in plant biomass together with lipid degradation was reported in drought stressed vigna (Scotti-Campos et al., 2013) and in Asteriscus maritimus (Rodriguez et al., 2005). Severe water stress may result in arrest of photosynthesis, disturbance of metabolism, and finally death (Liang et al., 2006). Cell enlargement is inhibited by water stress more strongly than cell division. Drought stress reduces plant growth through involving various physiological and biochemical processes, like lipid degradation and hormones (Scotti-Campos et al., 2013). ABA plays a crucial role in regulating plant water status through guard cells and growth as well as by induction of genes that encode enzymes and other proteins involved in cellular dehydration tolerance which might be the reason for increased dry weight under drought stress (Luan, 2002; Zhu, 2002).

**Pigment analysis**

**Chlorophyll and carotenoid contents**

Chlorophyll \(a\) content (Fig. 8) of the lettuce leaves increased during ABA treatments when compared to control and decreased at different irrigation regimes. Treatment with ABA increased chlorophyll \(a\) content. There was a significant increase at 72h irrigation regime after 10 µg L\(^{-1}\) ABA application. ABA in combination with drought increased chlorophyll \(a\) content and reduced the drought induced pigment inhibition in lettuce plants.

Our results showed that chlorophyll \(b\) content also increased upon ABA treatments. Drought induced a reduction in chlorophyll \(b\) content when compared to control. ABA treatment increased chlorophyll \(b\) content and it was high after ABA application at 15 µg L\(^{-1}\) at 72-h irrigation regime (Fig. 9).

Total chlorophyll content increased

**Figure 8.** Effect of different irrigation regimes, ABA and their combination on chlorophyll \(a\) content in lettuce plants on day 75 after planting. Values are means ±SE of three replicates.
Figure 9. Effect of different irrigation regimes, ABA and their combination on chlorophyll b content in lettuce plants on day 75 after planting. Values are means ±SE of three replicates.

upon ABA treatments. On the other hand, drought stress caused a reduction in total chlorophyll content. Treatment with ABA increased total chlorophyll content to a larger extent under drought stress (Fig. 10).

Carotenoid content increased upon ABA treatments. Treatment with ABA increased carotenoid content over the control and this was significant at both ABA concentrations - 5 µg L⁻¹ and 10 µg L⁻¹ but there was no significant increase after application of 15 µg L⁻¹. Drought stress decreased carotenoid content. ABA in combination with drought increased carotenoid content and reduced the drought-induced pigment inhibition in lettuce plants (Fig. 11).

There was a decrease in pigment content under drought stress in lettuce plants but treatment with ABA increased chlorophyll a content. The chlorophyll

Figure 10. Effect of different irrigation regimes, ABA and their combination on total chlorophyll content in lettuce plants on day 75 after planting. Values are means ±SE of three replicates.
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Figure 11. Effect of different irrigation regimes, ABA and their combination on carotenoid content in lettuce plants on day 75 after planting. Values are means ±SE of three replicates.

content in rice leaves decreased due to chemical desiccation treatments (Ahadiyat et al., 2014). A reduction in chlorophyll content was reported in drought-stressed soybean plants (Zhang et al., 2006). Chlorophyll content decreased to a significant level at higher water deficits in maize and wheat plants (Nayyar and Gupta, 2006).

Anthocyanin content

Drought stress decreased anthocyanin content in lettuce leaves when compared with control. Anthocyanin content increased under individual ABA treatments and treatments under drought stress (Fig. 12).

Drought stress caused a decrease in anthocyanin content in lettuce leaves upon the drought regimes studied. However, spraying with ABA increased anthocyanin content significantly when compared to control. Similarly, treatment with ABA increased anthocyanin accumulation in strawberry fruits (Jiang and Joyce, 2003).

Figure 12. Effect of different irrigation regimes, ABA and their combination on total anthocyanin content in lettuce plants on day 75 after planting. Values are means ±SE of three replicates.
CONCLUSION

In the present study, we analysed growth and photosynthetic pigments in lettuce plants at different irrigation regimes and the response to ABA application as foliar spray under drought stress. Drought stress decreased the morphological parameters like root and shoot length, total leaf number, fresh and dry weight. The growth parameters increased upon ABA treatments under drought stress. There was a slight increase in root length during early drought treatment, but further on it decreased. Both chlorophyll and carotenoid contents increased with age in control and treated plants. Treatment with ABA also increased chlorophyll and carotenoid contents. ABA in combination with drought stress increased chlorophyll and carotenoid contents and reduced the drought-induced pigment reduction in lettuce plants.

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