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EFFECT OF ABSCISIC ACID ON THE GROWTH AND DEVELOPMENT OF LETTUCE (LACTUCA SATIVA 1.) UNDER VARIED IRRIGATION REGIMES

Mohamed Abdulla Al Muhairi

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جامعة الإمارات العربية المتحدة
United Arab Emirates University

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College of Food and Agriculture

Department of Aridland Agriculture

EFFECT OF ABSCISIC ACID ON THE GROWTH AND
DEVELOPMENT OF LETTUCE
(*LACTUCA SATIVA* L.) UNDER VARIED IRRIGATION REGIMES

Mohamed Abdulla Al Muhairi

This thesis is submitted in partial fulfillment of the requirements for the degree of
Master of Science in Horticulture

Under the Supervision of Dr. Abdul Jaleel Cheruth

December 2014

Declaration of Original Work

I, Mohamed Abdulla Al Muhairi, the undersigned, a graduate student at the United Arab Emirates University (UAEU) and the author of the thesis titled "Effect of abscisic acid on the growth and development of lettuce (*Lactuca sativa* L.) under varied irrigation regimes", hereby solemnly declare that this thesis is an original work done and prepared by me under the guidance of Dr. Abdul Jaleel Cheruth, in the College of Food and Agriculture at UAEU. This work has not previously formed the basis for the award of any degree, diploma or similar title at this or any other university. The materials borrowed from other sources and included in my thesis have been properly cited and acknowledged.

Student's Signature



Date

3rd Feb, 2015

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Abstract

Economically important vegetable crop lettuce (*Lactuca sativa* L.) of family Asteraceae was selected for the present investigation. It is being cultivated in UAE due to its commercial importance. In lettuce cultivation, major problem is the requirement of large quantity of irrigation water. The present study was aimed to reduce the water consumption of lettuce cultivation, for that, a varied irrigation regime was used with the application of abscisic acid (ABA). The parameters studied were growth, photosynthetic pigments, biochemical constituents, antioxidant potential and antioxidant enzymes activities in lettuce plants under drought stress and its response to ABA under stress. Drought stress decreased the morphological parameters like root, shoot length, total leaf number, fresh and dry weight in lettuce. The growth parameters increased in ABA treatments under drought stress. The pigment contents of the lettuce leaves also showed the same trend. But drought stress caused an increase in the biochemical constituents like proline and amino acid contents when compared with control. All these parameters also increased under individual ABA treatments and treatments under drought stress. ABA treatments to the unstressed plants caused an increase in these parameters. The non-enzymatic antioxidant molecules like ascorbate and α -tocopherol showed significant increase under drought condition in lettuce. ABA slightly reduced these contents. The antioxidant enzymes like superoxide dismutase, catalase and peroxidase showed significant increase under drought condition and ABA caused significant enhancement in these antioxidant enzymes under drought stress and also in unstressed conditions, thereby protecting the plants from the deleterious effects of drought stress. From the results of this investigation, it can be concluded that ABA at 10 $\mu\text{g/l}$ can be used as a potential tool to minimize the drought stress effects in lettuce cultivation.

Keywords: Lettuce, drought, morphology, pigments, antioxidant contents, antioxidant activity

Title and Abstract in Arabic

دراسة تأثير حمض الأبسيسيك على نمو و تطور نبات الخس تحت أنظمة ري مختلفة

المخلص

يعتبر محصول الخس (*Lactuca Sativa L.*) من محاصيل العائلة النجمية ذات القيمة الاقتصادية العالية ، و تتم زراعة هذا المحصول في المنطقة بشكل عام و في دولة الإمارات العربية المتحدة بشكل خاص لما فيها من عائد مربح، و يعد هذا المحصول من المحاصيل ذات الإحتياج المائي العالي، و يهدف البحث الحالي على توفير مياه دراسة مستويات مختلفة من مقننات مياه الري لمحصول الخس معرضا لمعاملات مختلفة من حمض الأبسيسيك (ABA) .

راسة على معايير متعددة منها معدلات النمو و أصباغ البناء الضوئي و مضادات الأكسدة و أنزيمات مضادات الأكسدة و المكونات الكيميائية-حيوية في النبات، تمت مقارنة هذه النتائج من محصول الخس تحت تأثير عاملين مختلفين: إجهاد الجفاف و تأثير حمض الأبسيسيك.

ر و طول الساق

جهد الجفاف مع تأثير حمض

الأبسيسيك عكست نتائج إيجابية في معايير معدلات النمو، و على نفس السياق ظهرت نتائج محتوى أصباغ البناء الضوئي في النبات منخفضة في معاملات إجهاد الجفاف و مرتفعة في معاملات إجهاد الجفاف مع تأثير حمض الأبسيسيك.

و أظهرت نتائج معاملات الجفاف ارتفاعا في تراكيز المكونات الكيميائية-حيوية مثل البرولين و الأحماض الامينية بالمقارنة مع المعاملات المعتمدة التي لم تعرض لإجهاد الجفاف، بالإضافة لذلك فإن جميع المعايير في التجربة أظهرت ارتفاعا نسبيا في معاملات إجهاد الجفاف مع تأثير حمض الأبسيسيك، و بالنسبة للمعاملات المعتمدة لإرتفاع شمل جميع معايير التجربة.

بالنسبة لمضادات الأكسدة اللا أنزيمية مثل أسكورات و ألفاتوكوفيرول فإنها أظهرت ارتفاعا ملحوظا في معاملات إجهاد الجفاف، و بالمقابل أظهرت معاملات إجهاد الجفاف مع تأثير حمض الأبسيسيك إنخفاضا طفيفا في هذه المعايير .

و أظهرت نتائج تراكيز مضادات الأكسدة الأنزيمية مثل فوق اكسيد الدسموتاز و الكاتالاز و البيروكسيداز ارتفاعا ملحوظا في معاملات إجهاد الجفاف، وكان تأثير حمض الأبسيسيك ملحوظا في تحسين مستوى تراكيز مضادات الأكسدة الأنزيمية في معاملات إجهاد الجفاف و كذلك المعاملات المعتمدة التي لم تعرض لإجهاد و في حماية النبات من التأثيرات الضارة الناتجة عن إجهاد الجفاف، و كنتيجة نهائية فإن هنالك إمكانية من استخدام حمض الأبسيسيك بتركيز 10 ميكروغرام/لتر لتخفيف الأعراض الناتجة من إجهاد الجفاف على محصول الخس.

الكلمات المفتاحية: الخس، الجفاف، مورفولوجي، أصباغ البناء الضوئي، محتوى مضادات-الأكسدة، نشاط مضادات-الأكسدة

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Above all, I bow before the Almighty for showering me with His blessings and enabling me to fulfill this endeavor.

Dedication

To my father

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Chapter 1: Introduction

Plants experience many different kinds of abiotic stress factors like higher concentration of salt, drought, temperature and heavy metals which largely influence plant development and crop productivity. They have become a major threat to food security due to constant changes of climate and deterioration of the environment (Suzuki et al., 2014). Among abiotic stress factors, drought has been the major environmental factor limiting the growth and productivity of agronomically important plants (Anjum et al., 2011). Plants have developed several biochemical as well as physiological and metabolic strategies in order to combat such abiotic stresses (Gupta et al., 2013). A noticeable feature of plant adaptation to abiotic stress factors is the activation of multiple responses involving complex gene interactions with many molecular pathways (Basu, 2012). Water deficit will be created when insufficient irrigation prevents a plant from normal growth and completion of life cycle (Zhu, 2002). In soil, insufficient moisture can be created due to shortage of rainfall (drought), coarse textured soils that retain little water in the root zone, or drying winds. Under water deficit conditions, plants suffer from cellular damage and this is typically accompanied by an increase in plant body temperature. Depending on the duration and extent of drought stress, a range of plant processes occurring at molecular, biochemical, cellular, and whole-plant levels may be altered (Chaitanya et al., 2003). This adaptation in plant response to water shortages can involve changes in the expression of gene-encoding proteins that contribute to drought adaptation. The proteins could be enzymes involved in the synthesis of hormones and changes in a plant's hormone levels, increasing inhibitors and reducing growth promoters (Wasternack and Hause, 2013).

In plants, drought stress affects growth and yield and its effect on yield is highly complex and involves processes as diverse as reproductive organs, gametogenesis, fertilization, embryogenesis, and seed development stress (Barnabas et al., 2008). Drought continues to have a significant impact in many parts of the world. (The latter still suffer from the drought the most). Drought is considered a major disaster occurring in almost all countries of the Arab region (Ragab and Hamdy, 2005). In the last several decades, the most productive agricultural regions were exposed to drought stress most years and in occasional years with severe drought. Commonly, drought stress synchronizes with extreme temperature, leading to even greater severity of drought stress (Barnabas et al., 2008).

The plant hormone, abscisic acid (ABA) is a stress hormone that is accumulated mainly under drought stress (Zhang et al., 2006). ABA is produced as roots begin dehydrating, initiates stomatal closure and the expression of stress-response genes and accumulates in drought-stressed plants. It is a key endogenous messenger in plants' responses to such stresses and understanding ABA signaling is essential for improving plant performance. A central response to water deficit is often increased synthesis of ABA, which in turn induces a range of developmental 'avoidance' and physiological or biochemical 'tolerance' mechanisms (Pastori and Foyer, 2002). ABA synthesis triggers major reprogramming of the transcriptome, stomatal closure and restraint on transpirational water loss (Raghavendra et al., 2010). But this adaptation for survival in many cases leads to a reduction in photosynthetic rate, grain filling and yield. There have been several studies on altering ABA levels by overexpression of its biosynthesis or catabolism genes using constitutive promoters. For example, transgenic over expression of NCED genes in tomato, Arabidopsis, bean and cowpea lead to enhanced ABA content in leaves or whole

plants and reduced transpiration (Iuchi et al., 2001). Drought stress induced ABA increase can cause pod abortion as shown by Liu et al. (2004). He found that ABA affected pod set directly via the processes within the ovary (i.e. cell division) or, indirectly, via influencing the availability of photosynthate sugar. Previously, Liu (2004) reported seed abortion in soybean under ABA influence. It is reported to gradually degrade upon removal of stress (Zhang et al., 2006). Zhang et al. (2011) reported that ABA pretreatment further increased the endogenous level in maize seedling. Pre-soaking seed treatment with ABA was reported to significantly enhance the antioxidant enzyme activity in maize seedlings subjected to water stress (Bano et al., 2012). Similarly, Nishiyama et al. (2011) found that the relative water content of ABA treated plants was higher under drought stress.

Lettuce (*Lactuca sativa*) belonging to the Asteraceae family is an important leafy vegetable known to contain many nutrients (Dan et al., 2014). Lettuce is one of the leaf-edible vegetables that should be free from water stress because it is extremely sensitive to drought due to a shallow root system (Kizil et al., 2012). Cultivated lettuce is an important salad crop which is grown throughout the world. Lettuce is also widely grown as a vegetable in home gardens. It is especially important as a commercial crop in Asia, North and Central America and Europe. China, U.S., Spain, Italy, India and Japan are among the world's largest producers. In UAE, the lettuce production is decreasing due to high amount of irrigation water needed for its cultivation, as ground water availability is limited in the region. Nutritionally, it rates low among other vegetable crops; 95% of the crop contains water with varying amounts of phosphorus, iron, sodium and potassium, depending on the morphological type. Lettuces leaves have higher levels of ascorbic acid, vitamin A and calcium (Martínez-Sánchez et al., 2011). Lettuce can be produced commercially wherever the

conditions suit: it is a cool season crop which requires good soil and an adequate water supply. Lettuce grows best in daytime temperatures of 18°C-25°C and a night temperature of 10°C-15°C. Lettuce typically accumulates a wide range of antioxidants, including enzymatic antioxidants (peroxidases, superoxide dismutase, and catalase) and nonenzymatic antioxidants (phytochemicals).

For the past several years, several scales of physiology have been applied to study responses to water deficit stress and methods to overcome drought stress in different plants (Reddy et al., 2004; Manivannan et al., 2007; Sankar et al., 2007). However, little information has been gained about the physiological basis in response to ABA treatments under drought stress and their optimum levels in lettuce. It seems necessary to do research related to the correlation between plant growth regulators and drought stress tolerance. The lettuce plant is susceptible to drought stress. There are many methods to overcome drought stress in vegetable plants. One among the methods is the application of hormones to overcome the stress effects. Moreover, very little is known about drought stress amelioration in lettuce with the application of hormones like ABA.

The objectives of the present study were to understand the effect of ABA in drought stress amelioration in lettuce plants through its effects on the plant's:

- Growth and yield
- Photosynthetic pigments
- Biochemical constituents
- Antioxidant potential
- Antioxidant enzymes

under drought stress conditions.

Chapter 2: Literature Review

2.1 Drought stress and its effects on plants Water is essential for all living organisms and it plays a very significant role in building plant metabolism. Among the ecological factors, water is the most important in determining plant growth and development; drought is one of the major stress factors in inhibiting the yields of crops. Water typically makes up 80 – 95% of the mass of growing plant tissues. As water is essential for plant production, the optimum use of available water must be made for higher yields. Any inappropriate environmental factor for living organisms is termed “stress” (Sade et al., 2013). Water deficit stress occurs when the availability of ground water is reduced and climatic conditions cause loss of water by evapotranspiration (Shao et al., 2007). Water availability and quality can be a limiting factor in plant growth (Ceylan et al., 2013). The impact of water shortage reduces growth, vigour and increases wilting and nutrient deficiencies.

All environmental stresses like drought, air pollution, temperature, salinity, heavy metals, pesticides and soil pH are major factors limiting plant growth and yield because, they affect almost all plant functions (Hernandez et al., 2001; Yue et al., 2011). Among all environmental stresses, drought is one of the most adverse factors to plant growth and yield (Yaqoob et al., 2012) and is a major abiotic factor that limits optimum plant productivity (Aazami et al., 2010). Usually, drought stress affects plant growth, and the extent of which the plants are affected depend on the stage of growth and the intensity of stress (Clavel et al., 2005). There should be basic knowledge about the biochemical and molecular responses of plants to drought as this is essential for understanding plant resistance mechanisms to water limited conditions (Reddy et al., 2004; Shao et al., 2005; Dashevskaya et al., 2013).

Drought is one of the most important environmental stresses which affect many physiological aspects of plants and causes huge damage to agriculture productivity every year (Xoconstle et al., 2010; Alizadeh et al., 2011). Rahdari and Hoseini (2012) reported that the membrane lipids and proteins will be disturbed under drought stress together with a decrease in the enzyme activity and transport capacity. In all agricultural regions, yields of rain-fed crops are periodically reduced by drought (Guan et al., 2010; Ghanbari et al., 2011; de Souza et al., 2013).

Producing plants that have the ability to withstand abiotic stress conditions is one of the priorities in plant science research (Fletcher et al., 2010). Stress tolerant plants adapt to these stresses through various morphological, physiological and molecular mechanisms (Drame, 2007).

2.2 Plant growth regulators and stress amelioration

Plant growth regulators can be defined as either natural or synthetic compounds that modify the plant growth and development pattern by exerting profound influence on many physiological processes and thereby increasing the productivity of crops (Kakimoto, 2003). There are a large number of synthetic organic chemicals possessing growth regulating properties and new ones are being added to the list periodically (Al-Khassawneh et al., 2006). The response induced by them varies with the plant materials and methods of application (Werner et al., 2001).

Fundamental processes of plant growth and development are mainly controlled by the plant hormones gibberellin (GA) and abscisic acid (ABA) (Xie et al., 2006). The response of strawberry to exogenous GA₃ is similar to that caused by certain natural environmental factors such as long days (LDs) and chilling (Tehranifar and Battey, 1996).

2.2.1. Abscisic acid (ABA) induced stress amelioration

ABA is a sesquiterpene (C-15) that contains three isoprene (C-5) units and is a product of the isoprenoid pathway as other hormones, including GA₃ (Dewick, 2002). In higher plants, ABA is derived from the cleavage product of the C-40 isoprenoid, all trans-violoxanthin (Seo and Koshiba, 2002). A direct pathway from the C-15 isoprenoid, farnesyl pyrophosphate has been described in the fungal species *Cercospora*; however, this pathway does not appear to function in higher plants (Nambara and Marion-Poll, 2005). Violoxanthin is cleaved to form one C-15 compound 9'-Cis-neoxanthin, which is then cleaved to form xanthoxin which is converted to ABA, and all these reactions are catalyzed by dioxygenase (Nambara and Marion-Poll, 2005).

ABA has long been considered an inhibitor due to its role in abscission, dormancy and the reduction of shoot elongation; however, evidence has accumulated to support a promoter role such as in inducing protein synthesis in seeds and, to some degree, in the defense against insect attacks (Davies and Zhang, 1991). ABA has major roles in many aspects of plant development including the regulation of stomatal closure and the initiation of adaptive responses to various environmental conditions like water deficit stress (Mauch-Mani and Mauch, 2005). ABA has been identified as a messenger in stress perception response pathways (Hirayama and Shinozaki, 2007) such as drought (Zhu, 2002) high temperature, low temperature and salinity stress (Popova et al., 1988). ABA is also involved in many physiological processes, such as photosynthesis regulation and stomatal movements (Kuromori et al., 2014). ABA may protect photosynthetic apparatus against photo damage by enhanced xanthophylls cycle (Zhu et al., 2011).

Regulation developmental processes by ABA, which induce tolerance to different stresses, and mediate the photosynthesis and respiration in leaves and also the inhibition of lateral root development, was reported by Giraudat et al. (1994) and also by Zhou and Leul (1998). ABA has a definite role in seed maturation and germination. ABA promotes stomatal closure by rapidly altering ion fluxes in guard cells. Other ABA actions involve gene expression regulation which provides stress tolerance (Fujita et al., 2011).

Previous reports indicate that one mode of ABA action may be related to its role in the reactive oxygen scavenging mechanism triggered in plant cells. It has been documented that ABA can cause increased generation of O_2^- (Jiang and Zhang, 2001) and H_2O_2 (Guan et al., 2000; Pei et al., 2000; Murata et al., 2001; Zhang et al., 2001). The activities of antioxidant enzymes such as superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase are enhanced in plant tissue under water stress by ABA treatment (Bellaire et al., 2000). ABA is involved in the sugar mediated regulation of gene expression where the glucose specific accumulation of ABA is essential for hexokinase mediated glucose responses (Arenas-Huertero et al., 2000, Finkelstein and Lynch, 2000).

Among the regulated physiological responses, ABA plays a central role in stress amelioration (Qin and Zeevaart et al., 2002). Previous studies demonstrated ABA involvement in modulation of expressions at the gene level of adaptive responses for plants in adverse environmental conditions (Zhang et al., 2006). ABA is also involved in the development of seeds and the synthesis of storage proteins and lipids in normal as well as stressed conditions (Sharp et al., 2000), leaf senescence and defense against pathogens (Zhao et al., 2001). In addition, ABA acts as a mediator in controlling adaptive plant responses to environmental stresses (Schroeder et al., 2001). In several instances, it

has been implicated in signal transduction at the single-cell level and ABA induces closure of stomata (Trejo et al., 1995).

ABA plays important roles in stomatal regulation and the stability of photosynthetic apparatus (Yin et al., 2005). ABA is a sesquiterpenoid generally synthesized from Xanthophylls (Taylor et al., 2000; Seo and Koshiba, 2002). ABA treatment enhanced the accumulation of anthocyanin, phenolics and ethylene production (Jiang and Joyce, 2003). ABA has been postulated to regulate the formation of xanthophylls in an inducible manner (Parry, 1993).

There are many free radical scavenging molecules occurring in plants, such as flavanoids, anthocyanins, carotenoids, dietary glutathiones, vitamins and endogenous metabolites with antioxidant activities (Larson, 1988; Ghasemzadeh and Ghasemzadeh, 2011; Agati et al., 2012). These antioxidants have free radical scavenging properties with singlet and triplet oxygen quenching, peroxide decomposing and enzyme inhibiting actions (Larson, 1988). Electron acceptors such as molecular oxygen, react easily with free radicals to become radicals themselves also referred to as reactive oxygen species (ROS). The ROS include superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\circ}OH$) (Cabisco et al., 2010).

In stress conditions, antioxidant acts as a main defense against free radical caused toxicity by protecting the damage caused by free radicals. The exposure of plants to exogenous and endogenous factors generates a wide range of reactive oxygen species (ROS) resulting in homeostatic imbalance (Valavanidis et al., 2013). In the plant cell, a complex antioxidative response system (ARS) consisting of non-enzymatic and enzymatic components, protects cellular constituents from oxidative damage by scavenging reactive oxygen species. The primary components of this defense system

include carotenoids, ascorbate, glutathione and tocopherol as well as antioxidant enzymes such as superoxide dismutase (SOD), catalase and the enzymes of the ascorbate – glutathione cycle (eg. Ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase) (Asada, 1999; Joseph and Jini, 2011).

Increasing evidence indicates that one mode of ABA action may be related to its role in the oxidative stress in plants cells. The plant hormone abscisic acid as a stress signal increased as a result of water stress and played an important role in the regulation of plant responses from the whole plant level (Davies and Zhang, 1991) to the cellular level (Shinozaki and Yamaguchi-Shinozaki, 1997). Hung and Kao (2003) reported that ABA increases the activities of SOD, APX, glutathione reductase (GR) and CAT. ABA also increases the activities of antioxidant enzymes such as superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase in plant tissue under water stress (Bellaire et al., 2000).

Chapter 3: Materials and Methods

A greenhouse experiment was conducted during the 2012 growing season to evaluate the morphological, physiological, yield response of lettuce under deficit irrigation with or without exogenous ABA application.

The greenhouse experiment was carried out in Al-Foah Experimental Station (27°N and 22°S latitude and 51°W and 57°E longitude) of the College of Food and Agriculture, UAEU in Al Ain city, 160 km East of Abu Dhabi the capital city of United Arab Emirates. The experimental period was from 10th of February 2012 to the 25th of May 2012.

The greenhouse environment was controlled for temperature and relative humidity. Accordingly, during the experimental periods, the temperature of the greenhouse was maintained at 24±2°C. The greenhouse allows the entrance of the natural light and hence artificial light was not applied. The methodologies adopted are described below.

3.1 Cultivation methods and experimental design

Lactuca sativa var. longifolia seeds were obtained from Lortolano company, Italy, and it was kindly provided by agricultural inputs commercial supplier “Shat Alarab”. Seed sowing was carried out manually on the 10th of February 2012 in polystyrene trays with 84 cells and filled with different media (peatmoss: perlite 2:1). In this experiment, the trays were kept in the greenhouse and irrigated as needed with tap water until germination. After emergence (15 days after sowing), the seedlings were thinned to retain three seedlings in each cell. Seedlings were under natural light conditions; ventilation was provided automatically when the air temperature exceeded 28°C by cooling system.

Plastic pots of 40 cm diameter and 45 cm height size were used for the study. The pots were filled with 10 kg of soil mixture containing red soil; sand and commercial potting soil at 1:1:1 ratio. Three seedling of lettuce were transplanted into each pot. Within a week, a second thinning was applied to retain the healthiest seedling for the experiment.

3.2 Drought stress induction in pot culture

The experiment was carried out with several drought levels and Abscisic Acid (ABA) concentrations applied exogenously. The drought factors were expressed at different irrigation intervals and fixed water quantity (200ml). The irrigation intervals were 24 hours (Control), 48 hours, 72 hours and 96 hours and water was applied manually per each pot. Moreover, lettuce samples were subjected to different concentrations of ABA. The ABA effect was studied on four different concentrations: not sprayed (control), 5 microgram/L, 10 microgram/L, 15 microgram/L and ABA concentrations were applied manually using a 500 ml sprayer for each plant.

The experiment had four levels for both variables. Consequently, the research covered 16 treatments including with the control units. In total, the experimental units were 48 replicated in three sets arranged in completely randomized block design (CRBD).

The experiment covered 16 treatments including control. The plants were allowed to grow up to 15 days on daily irrigation after transplanting. The samples were subjected to treatment from Day 15 after the transplanting. Samples were subjected to the ABA application on Days 7, 14, 21, 28 and 35 from drought induction (Sansberro et al., 2004). On Day 75, plants were uprooted gently, washed carefully and packed in labeled plastic bags. All samples were sent to the laboratory

to analyze growth parameters, pigments and biochemical contents, enzymatic and non-enzymatic antioxidants.

The chemicals used were obtained from Sigma, and are analytical grade chemicals and reagents.

3.3 Morphological parameters

3.3.1. Shoot and Root length

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

3.3.2. Number of leaves

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

3.3.3. Fresh weight and dry weight

After washing the plants in the 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 hot air oven for 24 hours. After drying, the weight was measured and the values were expressed in grams.

3.4 Pigment analysis

3.4.1. Chlorophyll and carotenoid

Chlorophyll and carotenoid were extracted from the leaves and estimated by the method of Arnon (1949).

Extraction

Five hundred milligrams of fresh leaf material was ground with 10 ml of 80 per cent acetone at 4°C and centrifuged at 2500 rpm for 10 minutes at 4°C. This

procedure was repeated until the residue became colourless. The extract was transferred to a graduated tube and made up to 10 ml with 80 per cent acetone and assayed immediately.

Estimation

Three milliliter aliquots of the extract were transferred to a cuvette and the absorbance was read at 645, 663 and 480 nm with a spectrophotometer (U-2001-Hitachi) against 80 per cent acetone as blank. Chlorophyll content was calculated using the formula of Arnon.

$$\text{Total chlorophyll (mg/ml)} = (0.0202) \times (A.645) + (0.00802) \times (A.663)$$

$$\text{Chlorophyll 'a' (mg/ml)} = (0.0127) \times (A.663) - (0.00269) \times (A.645)$$

$$\text{Chlorophyll 'b' (mg/ml)} = (0.0229) \times (A.645) - (0.00468) \times (A.663)$$

and expressed in milligram per gram fresh weight.

Carotenoid content was estimated using the formula of Kirk and Allen (1965) and expressed in milligrams per gram fresh weight.

$$\text{Carotenoid} = A.480 + (0.114 \times A.663 - 0.638 \times A.645)$$

3.4.2. Anthocyanin

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

In a pestle and mortar, five hundred mg of fresh tissue taken from the third leaf and from the periphery of the tuber tissue (0.5 cm from the epidermis and 1 cm from head of the root tuber) was ground in liquid nitrogen and extracted with 20 ml of 50 percent acetic acid overnight. The homogenate was centrifuged at 19,000 g for 15 minutes. The resultant supernatant was made up to 20 ml and 80 ml of McIlvaine's buffer (pH 3.0). The absorption measured at 530 nm in spectrophotometer. The anthocyanin contents were expressed in colour value $cv = 0.1 \times A_{530}/g \text{ fw}$.

3.5 Biochemical analysis

3.5.1. Proline

Proline content was estimated following the method of Bates et al. (1973).

Extraction

Five hundred mg of plant material was taken in a pestle and mortar and homogenized with 10 ml of 3 per cent aqueous sulfosalicylic acid. Then the homogenate was filtered through whatman No. 2 filter paper. The residue was re-extracted two times with 3 per cent sulfosalicylic acid and pooled. The filtrates were made upto 20 ml with 3 per cent sulfosalicylic acid and used for the estimation of proline.

Estimation

Two ml of extract was taken in a test tube and 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid were added to it. The mixture was incubated for one hour at 100°C in a water bath. The tubes were transferred to an ice bath to terminate the reaction. Then, to each test tube, 4 ml of toluene was added and mixed vigorously using a test tube stirred for 10-20 seconds. The toluene containing the chromophore was separated from the aqueous phase with the help of separating funnel and the absorbance was measured at 520 nm in a spectrophotometer using an appropriate blank. The proline content was determined from a standard curve prepared with proline and the results were expressed in milligram per gram dry weight.

3.5.2. Amino acids

Extraction and estimation of the amino acid content was followed by the method of Moore and Stein (1948).

Extraction

0.5 gram of plant material was taken in a pestle and mortar and homogenized with 10 ml of 80 per cent boiling ethanol. The extract was centrifuged at 800 rpm for 15 minutes and the supernatant was made upto 10 ml with 80 per cent ethanol and used for the estimation of free amino acids.

Estimation

One ml of ethanol extract was taken in a 25 ml test tube and neutralized with 0.1 N sodium hydroxide using methyl red indicator, to which, 1 ml ninhydrin reagent was added. The contents were boiled in a boiling water bath for 20 minutes; then 5 ml of diluting reagent was added, cooled and diluted to 25 ml with distilled water. The absorbance was read at 570 nm in a spectrophotometer. The standard graph was prepared by using glycine. The amino acid content was calculated using the standard graph. The results were expressed in milligrams per gram dry weight.

3.6 Antioxidants

3.6.1. Ascorbic acid

Ascorbic acid content was assayed as described by Omaye et al. (1979).

Extraction

One gram of fresh material was ground in a pestle and mortar with 5 ml of 10 per cent TCA; the extract was centrifuged at 3500 rpm for 20 minutes. The pellet was re-extracted twice with 10 percent TCA and supernatant was made to 10 ml and used for estimation.

Estimation

To 0.5 ml of extract, 1 ml of DTC reagent (2,4-Dinitrophenyl hydrazine-Thiourea-CuSO₄ reagent) was added and mixed thoroughly. The tubes were incubated at 37°C for 3 hours and to this 0.75 ml of ice cold 65 per cent H₂SO₄ was added. The

tubes were then allowed to stand at 30°C for 30 minutes. The resulting colour was read at 520 nm in spectrophotometer (U-2001-Hitachi). The ascorbic acid content was determined using a standard curve prepared with ascorbic acid and the results were expressed in milligrams per gram dry weight.

3.6.2. α -Tocopherol

α -Tocopherol activity was assayed as described by Baker et al. (1980).

Extraction

Five hundred milligrams of fresh tissue was homogenized with 10 ml of a mixture of petroleum ether and ethanol (2:1.6 v/v) and the extract was centrifuged at 10,000 rpm for 20 minutes and the supernatant was used for estimation of α -tocopherol.

Estimation

To one ml of extract, 0.2 ml of 2 per cent 2,2-dipyridyl in ethanol was added and mixed thoroughly and kept in dark for 5 minutes. The resulting red colour was diluted with 4 ml of distilled water and mixed well. The resulting colour in the aqueous layer was measured at 520 nm. The α -tocopherol content was calculated using a standard graph made with known amount of α -tocopherol.

3.7 Antioxidant enzymes

3.7.1. Superoxide dismutase (SOD, EC: 1.15.1.1)

Crude enzyme extract was prepared, for the assay of Superoxide dismutase by the method of Hwang et al. (1999).

Extraction

One gram of fresh tissue was homogenized with 10 ml of ice-cold 50 mM sodium phosphate buffer containing 1 mM PMSF. The extract was filtered through a double-layered cheesecloth. The extract was centrifuged at 12,500 rpm for 20 minutes

at 4 °C. The supernatant was saved and made up to 10 ml with extraction buffer and used for estimation of the SOD enzyme activity. The enzyme protein was determined by the Bradford (1976) method.

Estimation

Superoxide dismutase activity was assayed as described by Beauchamp and Fridovich (1971). The reaction medium was prepared and to 3 ml reaction medium, 1 ml of enzyme extract was added. The reaction mixture contained 1.17×10^{-6} M riboflavin, 0.1 M methionine, 2×10^{-5} potassium cyanide and 5.6×10^{-5} M nitroblue tetrasodium salt (NBT), dissolved in 0.05 M sodium phosphate buffer (pH 7.8). The mixture was illuminated in glass test tubes by two sets of Philips 40 W fluorescent tubes. Illumination started to initiate the reaction at 30°C for one hour. Those without illumination were saved as blank and kept in dark. The absorbance was read at 560 nm in the spectrophotometer against blank. Superoxide dismutase activity was expressed in units. One unit is defined as the amount of change in the absorbance by 0.1 per hour per milligram protein under the assay condition (Cherry, 1963).

3.7.2. Catalase (CAT, EC: 1.11.1.6)

Catalase activity was assayed as described by Chandlee and Scandalios (1984).

Extraction

Five hundred milligrams of frozen material was homogenized in 5 ml of ice cold 50 mM sodium phosphate buffer (pH 7.5) containing in 1mM PMSF. The extract was centrifuged at 4°C for 20 minutes at 12,500 rpm. The supernatant was used for enzyme assay.

Assay

The activity of enzyme catalase was measured using the method of Chandlee and Scandalios (1984) with modification. The assay mixture contained 2.6 ml of 50 ml of 50 mM potassium phosphate buffer (pH 7.0) 0.4 ml, 15 mM H₂O₂ and 0.04 ml of enzyme extract. The decomposition of H₂O₂ was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in units 1 mM of H₂O₂ reduction per minute per mg protein.

3.7.3. Peroxidase (POX, EC 1.11.1.7)

Peroxidase was assayed by the method of Kumar and Khan (1982). The Assay mixture of Peroxidase contained 2 ml of 0.1 M phosphate buffer (pH 6.8), 1 ml of 0.01 M pyrogallol, 1 ml of 0.005 M H₂O₂ and 0.5 ml of enzyme extract. The solution was incubated for 5 min at 25°C, after which the reaction was terminated by adding 1 ml of 2.5 N H₂SO₄. The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a blank prepared by adding the extract after the addition of 2.5 N H₂SO₄ at zero time. The activity was expressed in unit mg⁻¹ protein. One unit is defined as the change in the absorbance by 0.1 min⁻¹ mg⁻¹ protein.

3.8 Statistical Analysis

Statistical analysis was performed using two way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean ± SE for three samples in each group. P values ≤ 0.05 were considered as significant.

Chapter 4: Results

4.1 Morphological parameters

4.1.1 Shoot and Root length

In lettuce, the shoot length showed significant decrease with different irrigation regimes. The ABA application increased the shoot length significantly, except for 96hrs in T3 ($15 \mu\text{g}^{-1}$ of ABA). The maximum increase of shoot length was observed in the 72hrs irrigation regime in combination with T2 ($10 \mu\text{g}^{-1}$ of ABA application) as shown in Table 1.

Table 1. Effect of varied irrigation regimes, abscisic acid (ABA) and their combination on shoot length (cm plant^{-1}) in lettuce plants on 75 days after planting.

ABA Application ($\mu\text{g l}^{-1}$)	Irrigation regimes			
	24hrs	48hrs	72hrs	96hrs
0 (T0)	32.7 \pm 1.71a	28.9 \pm 2.43a	26.3 \pm 1.85a	24.5 \pm 1.77a
5 (T1)	30.8 \pm 2.54b	29.7 \pm 1.97b	27.5 \pm 2.05b	26.8 \pm 1.95b
10 (T2)	29.9 \pm 2.32c	30.9 \pm 1.68b	30.4 \pm 2.21c	27.1 \pm 2.73c
15 (T3)	29.9 \pm 1.87c	28.6 \pm 2.41a	28.1 \pm 1.84d	25.7 \pm 1.46b

Values are mean \pm SE of three replicates. Values, that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT) along the columns (different concentrations of ABA treatments)

The root length was increased significantly by drought stress in lettuce plants in all irrigation regimes except 96hrs when compared to control. The maximum increase was observed in the 72hrs irrigation regime. The root length again increased under ABA treatments under drought stress. The extent of increase was more in $10 \mu\text{g l}^{-1}$ ABA (T2) treated plants when compared to control and drought stressed plants (Table 2).

Table 2. Effect of varied irrigation regimes, abscisic acid (ABA) and their combination on root length (cm plant⁻¹) in lettuce plants on 75 days after planting.

ABA Application ($\mu\text{g l}^{-1}$)	Irrigation regimes			
	24hrs	48hrs	72hrs	96hrs
0 (T0)	25.94 \pm 2.03a	27.43 \pm 1.58a	30.77 \pm 2.77a	23.85 \pm 2.50a
5 (T1)	25.32 \pm 1.82a	30.33 \pm 1.86b	32.25 \pm 1.99b	24.78 \pm 2.61b
10 (T2)	24.68 \pm 2.57b	33.84 \pm 2.51c	34.88 \pm 2.31c	23.54 \pm 2.48a
15 (T3)	24.75 \pm 2.68b	32.59 \pm 1.94c	32.88 \pm 2.58b	20.21 \pm 1.99c

Values are mean \pm SE of three replicates. Values, that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT) along the columns (different concentrations of ABA treatments)

4.1.2 Number of leaves

There was significant reduction in the number of leaves with different irrigation regimes in lettuce plants on 75 days after planting. The maximum decrease was noted in the 96hrs irrigation regime. And, the ABA application didn't helped to overcome the stress situation during the 96hrs irrigation regime. But a significant increase in leaf number was observed on 72hrs irrigation interval plants with 10 $\mu\text{g l}^{-1}$ ABA spray (Table 3).

Table 3. Effect of varied irrigation regimes, abscisic acid (ABA) and their combination on number of leaves (number plant⁻¹) in lettuce plants on 75 days after planting.

ABA Application ($\mu\text{g l}^{-1}$)	Irrigation regimes			
	24hrs	48hrs	72hrs	96hrs
0 (T0)	35 \pm 2.57a	29 \pm 1.88a	27 \pm 2.35a	22 \pm 2.16a
5 (T1)	36 \pm 2.11a	31 \pm 1.79b	28 \pm 2.40b	24 \pm 2.05b
10 (T2)	39 \pm 1.96b	33 \pm 2.74c	31 \pm 1.76c	24 \pm 2.33b
15 (T3)	33 \pm 1.53c	31 \pm 2.10b	27 \pm 1.95a	24 \pm 1.82b

Values are mean \pm SE of three replicates. Values, that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT) along the columns (different concentrations of ABA treatments)

4.1.3 Fresh weight and dry weight

The root fresh weight decreased with drought stress in lettuce. The fresh weight increased under ABA treatments under drought stress. The extent of increase by ABA application was more in the 72hr irrigation regimes when compared to other irrigation intervals. ABA treatment alone decreased the root fresh weight but it was

not significant in $5\mu\text{g/l}$ ABA concentration (Table 4). The dry weight of roots showed the same trend as in the case of fresh weight. The maximum dry weight recorded was in the 72hrs irrigation regimes with $10\mu\text{g/l}$ ABA application (Fig. 1). At a higher concentration of ABA the fresh and dry weights showed a decreasing trend. Similarly, in the 96hr irrigation regime, the fresh and dry weights of roots showed significant decrease when compared to the control.

Table 4. Effect of varied irrigation regimes, abscisic acid (ABA) and their combination on root fresh weight (g plant^{-1}) in lettuce plants on 75 days after planting.

ABA Application ($\mu\text{g/l}$)	Irrigation regimes			
	24hrs	48hrs	72hrs	96hrs
0 (T0)	$29.86\pm 2.04\text{a}$	$29.48\pm 2.73\text{a}$	$30.74\pm 1.99\text{a}$	$29.96\pm 2.35\text{a}$
5 (T1)	$29.57\pm 1.78\text{a}$	$28.25\pm 1.95\text{b}$	$28.28\pm 2.53\text{b}$	$27.77\pm 2.86\text{b}$
10 (T2)	$26.91\pm 1.53\text{b}$	$26.57\pm 3.1\text{c}$	$31.95\pm 1.64\text{c}$	$22.85\pm 1.57\text{c}$
15 (T3)	$26.53\pm 2.46\text{b}$	$25.1\pm 2.05\text{c}$	$27.34\pm 2.06\text{b}$	$22.28\pm 1.93\text{c}$

Values are mean \pm SE of three replicates. Values, that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT) along the columns (different concentrations of ABA treatments)

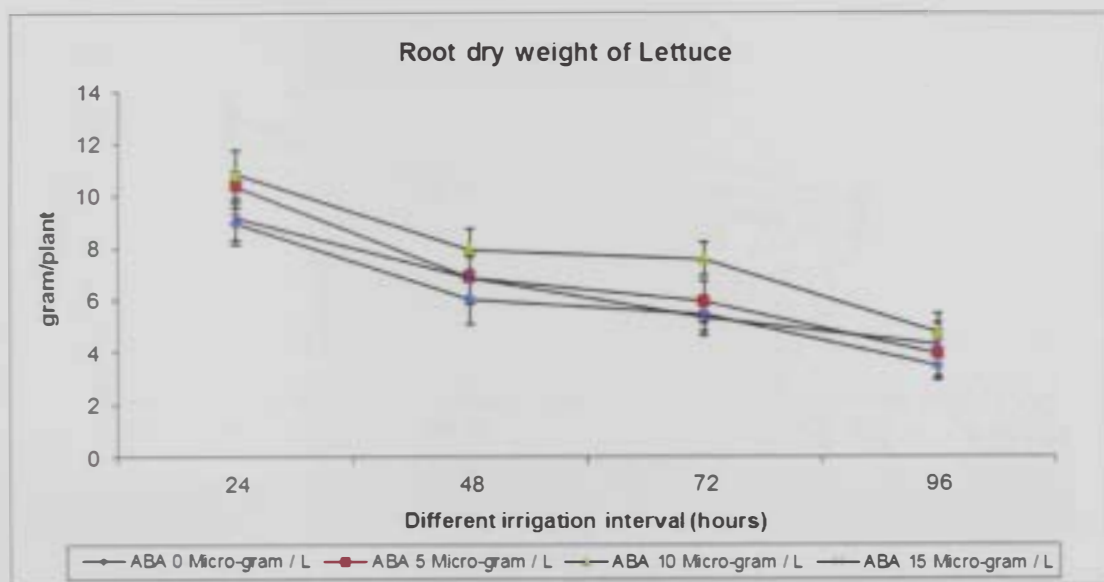


Fig. 1. Effect of varied irrigation regimes, abscisic acid (ABA) and their combination on root dry weight (g plant^{-1}) in lettuce plants on 75 days after planting.

Drought stress caused decreased fresh and dry weights in lettuce shoot. ABA treatment alone increased the fresh weight and this was mainly due to the increased

leaf number under ABA application. But there was no significant difference between the fresh weight increase in 10 and 15 ABA applications. The fresh and dry weights increased significantly under ABA treatments in all drought stressed plants. The extent of the increase was more in 72hrs with $10\mu\text{g l}^{-1}$ ABA application when compared to the control and drought stressed plants (Table 5 and Fig. 2).

Table 5. Effect of varied irrigation regimes, abscisic acid (ABA) and their combination on shoot fresh weight (g plant^{-1}) in lettuce plants on 75 days after planting.

ABA Application ($\mu\text{g l}^{-1}$)	Irrigation regimes			
	24hrs	48hrs	72hrs	96hrs
0 (T0)	108.64 \pm 9.67a	90.49 \pm 7.48a	88.46 \pm 6.32a	75.63 \pm 8.57a
5 (T1)	113.95 \pm 8.59b	92.56 \pm 8.86b	92.28 \pm 8.27b	89.29 \pm 7.92b
10 (T2)	128.22 \pm 7.84c	98.41 \pm 9.05c	101.03 \pm 9.58c	91.45 \pm 8.83c
15 (T3)	129.87 \pm 8.18c	96.38 \pm 8.36d	93.82 \pm 8.17b	86.90 \pm 9.94d

Values are mean \pm SE of three replicates. Values, that are not sharing a common superscript (a, b, c, d) differ significantly at $P \leq 0.05$ (DMRT) along the columns (different concentrations of ABA treatments)

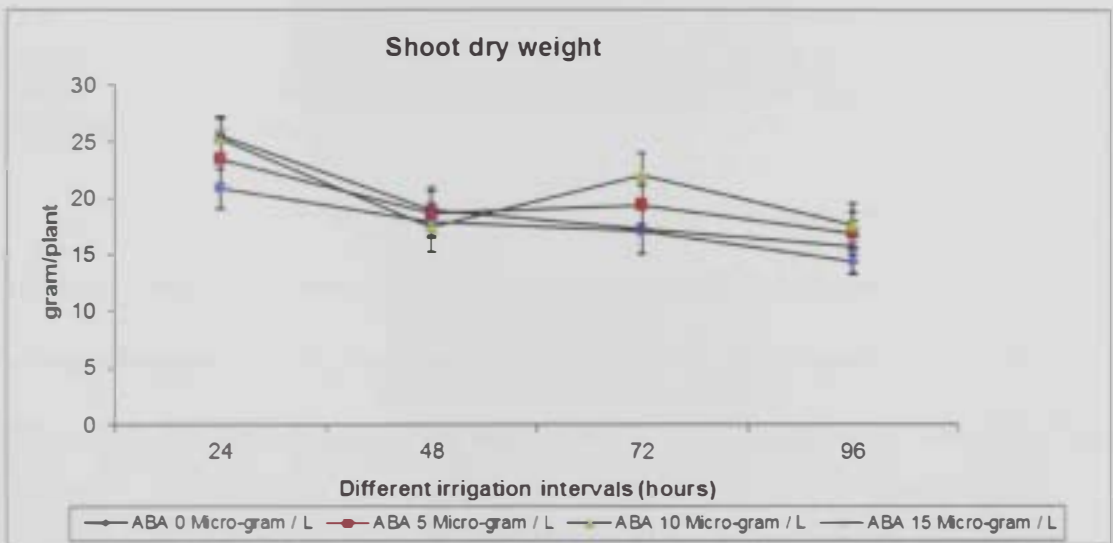


Fig. 2. Effect of varied irrigation regimes, abscisic acid (ABA) and their combination on shoot dry weight (g plant^{-1}) in lettuce plants on 75 days after planting.

4.2 Pigment analysis

4.2.1 Chlorophyll and carotenoid

The chlorophyll 'a' content (Table 6) of the lettuce leaves increased with ABA treatments when compared to the control and decreased with different irrigation regimes. Treatment with ABA increased the chlorophyll 'a' content. There was a significant increase in the 72hrs with ABA $10\mu\text{gl}^{-1}$ application. ABA in combination with drought increased the chlorophyll 'a' content and reduced the drought induced pigment inhibition in lettuce plants.

Table 6. Effect of varied irrigation regimes, abscisic acid (ABA) and their combination on chlorophyll 'a' (mg g^{-1} fresh weight) in lettuce plants on 75 days after planting.

ABA Application (μgl^{-1})	Irrigation regimes			
	24hrs	48hrs	72hrs	96hrs
0 (T0)	0.227 \pm 0.013a	0.204 \pm 0.017a	0.195 \pm 0.014a	0.181 \pm 0.018a
5 (T1)	0.233 \pm 0.022a	0.216 \pm 0.015b	0.205 \pm 0.016a	0.188 \pm 0.019a
10 (T2)	0.238 \pm 0.015a	0.212 \pm 0.018b	0.217 \pm 0.019b	0.195 \pm 0.015b
15 (T3)	0.219 \pm 0.018a	0.212 \pm 0.021b	0.201 \pm 0.013a	0.178 \pm 0.017c

Values are mean \pm SE of three replicates. Values, that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT) along the columns (different concentrations of ABA treatments).

The chlorophyll 'b' content of lettuce plant leaves also increased with ABA treatments. Drought induced a reduction in chlorophyll 'b' content in the leaves of lettuce when compared to well-watered control plants. ABA treatment increased the chlorophyll 'b' content and it was high in the 15 ABA application under the 72hr irrigation regime (Table 7).

Table 7. Effect of varied irrigation regimes, abscisic acid (ABA) and their combination on chlorophyll 'b' (mg g^{-1} fresh weight) in lettuce plants on 75 days after planting.

ABA Application ($\mu\text{g l}^{-1}$)	Irrigation regimes			
	24hrs	48hrs	72hrs	96hrs
0 (T0)	0.179 \pm 0.017a	0.162 \pm 0.014a	0.158 \pm 0.021a	0.141 \pm 0.016a
5 (T1)	0.182 \pm 0.009b	0.165 \pm 0.018a	0.165 \pm 0.017b	0.148 \pm 0.014a
10 (T2)	0.197 \pm 0.011b	0.172 \pm 0.016b	0.169 \pm 0.014b	0.156 \pm 0.019b
15 (T3)	0.185 \pm 0.016a	0.170 \pm 0.013b	0.171 \pm 0.018c	0.141 \pm 0.015a

Values are mean \pm SE of three replicates. Values, that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT) along the columns (different concentration) of ABA treatments)

The total chlorophyll content of the leaves of lettuce increased with ABA treatments. But drought stress caused a reduction in total chlorophyll content in leaves of lettuce plants. But the treatment with ABA increased total chlorophyll content to a large extent under drought stress (Table 8).

Table 8. Effect of varied irrigation regimes, abscisic acid (ABA) and their combination on total chlorophyll (mg g^{-1} fresh weight) in lettuce plants on 75 days after planting.

ABA Application ($\mu\text{g l}^{-1}$)	Irrigation regimes			
	24hrs	48hrs	72hrs	96hrs
0 (T0)	0.406 \pm 0.030a	0.366 \pm 0.031a	0.353 \pm 0.035a	0.322 \pm 0.034a
5 (T1)	0.415 \pm 0.031a	0.381 \pm 0.033a	0.37 \pm 0.033a	0.336 \pm 0.033a
10 (T2)	0.435 \pm 0.026b	0.392 \pm 0.034b	0.386 \pm 0.033b	0.351 \pm 0.034b
15 (T3)	0.404 \pm 0.034a	0.382 \pm 0.034a	0.372 \pm 0.031a	0.319 \pm 0.032a

Values are mean \pm SE of three replicates. Values, that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT) along the columns (different concentrations of ABA treatments).

The carotenoid content of the lettuce leaves increased with ABA treatments. Treatment with ABA increased the carotenoid content and it was significant in both 5 and 10 but there was no significant increase in 15 concentration ABA treatments over the control. Drought stress decreased the carotenoid contents. ABA in combination with drought increased the carotenoid content and reduced the drought induced pigment inhibition in lettuce plants (Fig. 3).

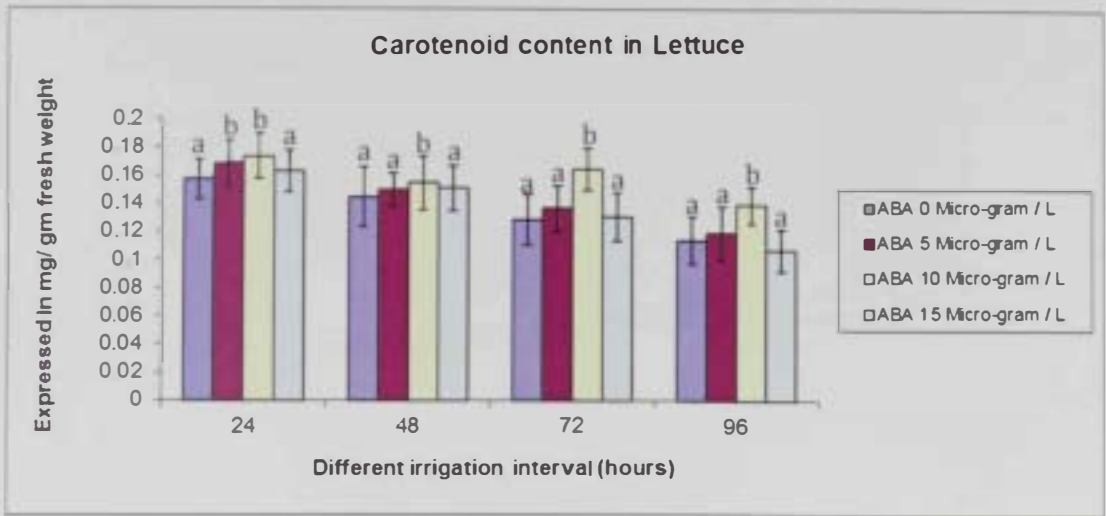


Fig. 3. Effect of varied irrigation regimes, Abscisic acid (ABA) and their combination on carotenoid contents (mg g^{-1} fresh weight) in lettuce plants on 75 days after planting.

4.2.2 Anthocyanin

Drought stress caused decreased anthocyanin content in lettuce leaves when compared with control on all samplings when compared to control. The anthocyanin content increased under individual ABA treatments and treatments under drought stress (Table 9).

Table 9. Effect of varied irrigation regimes, abscisic acid (ABA) and their combination on total anthocyanin (mg g^{-1} fresh weight) in lettuce plants on 75 days after planting.

ABA Application ($\mu\text{g l}^{-1}$)	Irrigation regimes			
	24hrs	48hrs	72hrs	96hrs
0 (T0)	0.316±0.025a	0.272±0.036a	0.264±0.026a	0.246±0.025a
5 (T1)	0.322±0.032a	0.283±0.027a	0.288±0.028a	0.265±0.018a
10 (T2)	0.338±0.021a	0.297±0.024b	0.314±0.031b	0.271±0.029b
15 (T3)	0.342±0.027b	0.305±0.019b	0.278±0.028a	0.256±0.022a

Values are mean \pm SE of three replicates. Values, that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT) along the columns (different concentrations of ABA treatments).

4.3 Biochemical analysis

4.3.1 Proline

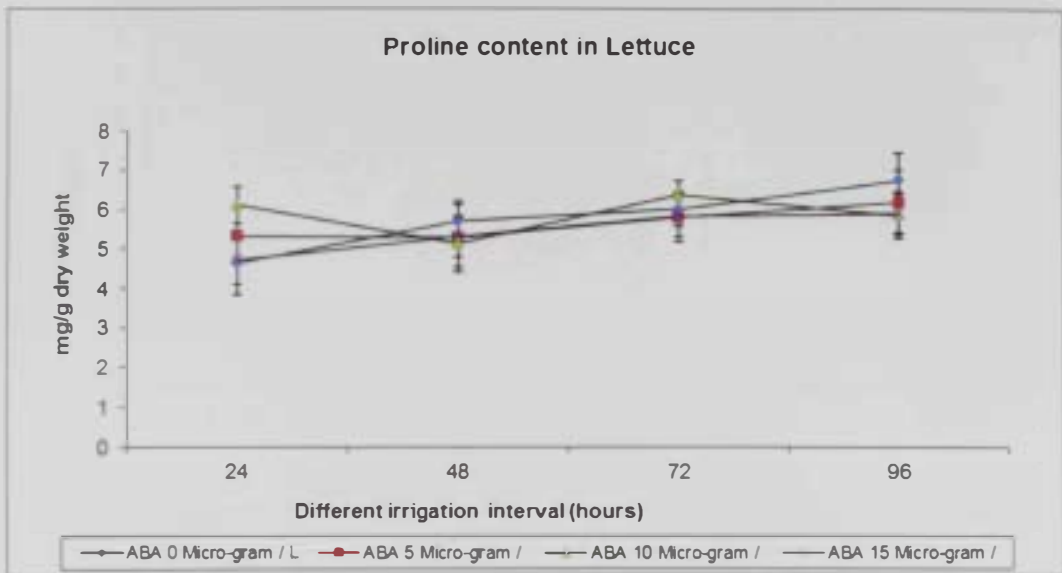


Fig. 4. Effect of varied irrigation regimes, abscisic acid (ABA) and their combination on proline contents (mg g^{-1} dry weight) in lettuce plants on 75 days after planting.

In lettuce, drought stress caused increased accumulation of proline content at all stages of growth. ABA also resulted in increased proline content in unstressed and stressed lettuce. ABA in combination with drought caused again enhancement in proline content when compared to stress and well-watered control plants. The 10 ABA treatment on the 72hr irrigation regime increased the proline content significantly when compared to all other treatments (Fig. 4).

4.3.2 Amino acids

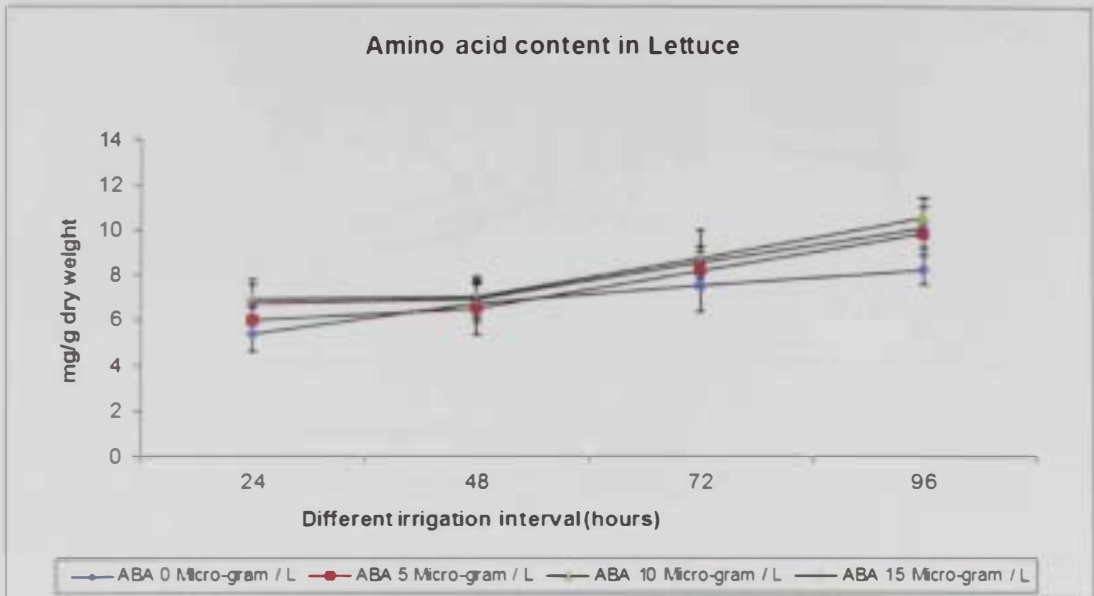


Fig. 5. Effect of varied irrigation regimes, abscisic acid (ABA) and their combination on amino acid contents (mg g^{-1} dry weight) in lettuce plants on 75 days after planting.

Water deficit stress increased the amino acid content in lettuce on all irrigation regimes. The amino acid content increased under individual ABA treatments and treatments under drought stress. The extent of increase was more in 15 ABA on the 96hr irrigation regime (Fig. 5).

4.4 Antioxidants

4.4.1 Ascorbic acid

In lettuce, ascorbic acid content was increased with drought stress when compared to control plants. ABA decreased the ascorbic acid content when compared to well watered and stressed plants (Fig. 6).

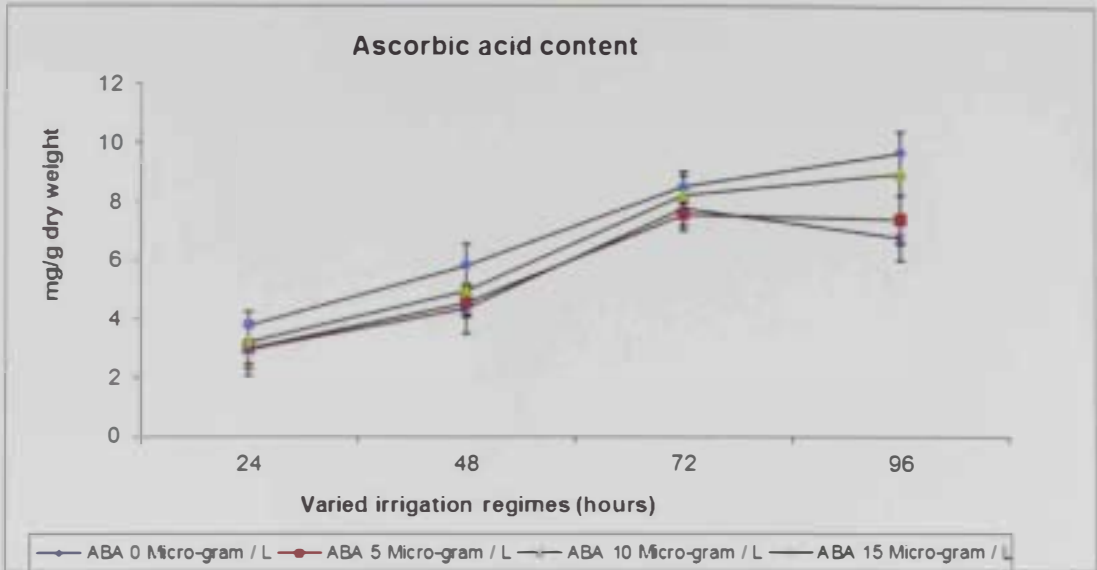


Fig. 6. Effect of varied irrigation regimes, abscisic acid (ABA) and their combination on ascorbic acid contents (mg g^{-1} dry weight) in lettuce plants on 75 days after planting.

4.4.2 α Tocopherol

The α -tocopherol of the drought stressed plant roots significantly increased when compared to control plants. ABA was an inhibitor of α -tocopherol individually and also under drought stress. ABA resulted a significant reduction in α -tocopherol content in lettuce when compared to the control and different irrigation regimes (Fig. 7).

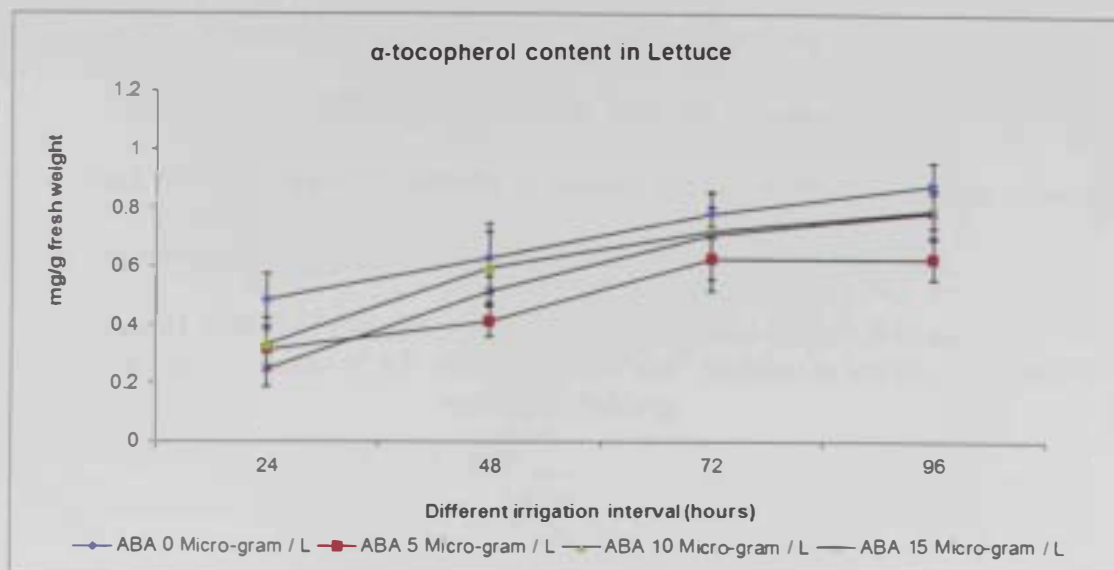


Fig. 7. Effect of varied irrigation regimes, abscisic acid (ABA) and their combination on α -tocopherol contents (mg g^{-1} fresh weight) in lettuce plants on 75 days after planting.

4.5 Antioxidant enzymes

4.5.1 Superoxide dismutase (SOD, EC: 1.15.1.1)

The activity of SOD increased by water deficit in lettuce. Only the 10 ABA treatment showed significant increase in SOD activity. Treatment with ABA resulted in an enhancement of SOD activity under drought stress. The maximum increase was in 10 ABA with the 72hr irrigation regime. More concentrations like 15 showed a decreased activity (Table 10).

Table 10. Effect of varied irrigation regimes, abscisic acid (ABA) and their combination on Superoxide dismutase (SOD) activity (unites mg^{-1} protein) in lettuce plants on 75 days after planting.

ABA Application ($\mu\text{g l}^{-1}$)	Irrigation regimes			
	24hrs	48hrs	72hrs	96hrs
0 (T0)	3.387 \pm 0.57a	4.638 \pm 0.73a	4.927 \pm 0.46a	5.859 \pm 0.77a
5 (T1)	3.424 \pm 0.42a	4.572 \pm 0.61a	4.881 \pm 0.52a	6.774 \pm 0.52a
10 (T2)	4.651 \pm 0.68b	5.517 \pm 0.47b	6.573 \pm 0.65b	7.551 \pm 0.48b
15 (T3)	3.707 \pm 0.44a	3.586 \pm 0.59a	5.685 \pm 0.55b	6.629 \pm 0.43a

Values are mean \pm SE of three replicates. Values, that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT) along the columns (different concentrations of ABA treatments).

4.5.2 Catalase (CAT, EC: 1.11.1.6)

The activity of CAT increased with different irrigation regimes. Treatment with ABA increased the CAT activity in control plants. On 96hrs irrigation regimes, there was no significant rise in CAT activity with ABA application (Table 11).

Table 11. Effect of varied irrigation regimes, abscisic acid (ABA) and their combination on catalase (CAT) activity (unites mg^{-1} protein) in lettuce plants on 75 days after planting.

ABA Application ($\mu\text{g l}^{-1}$)	Irrigation regimes			
	24hrs	48hrs	72hrs	96hrs
0 (T0)	4.194±0.48a	4.894±0.62a	5.336±0.44a	6.421±0.79a
5 (T1)	4.558±0.43a	4.727±0.55a	5.218±0.78a	6.213±0.86a
10 (T2)	5.234±0.61b	5.573±0.37b	6.988±0.52b	6.784±0.44a
15 (T3)	5.479±0.79b	4.609±0.43a	6.036±0.68b	6.793±0.50a

Values are mean \pm SE of three replicates. Values, that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT) along the columns (different concentrations of ABA treatments)

4.5.3 Peroxidase (POX, EC 1.11.1.7)

The peroxidase activity showed an increase in lettuce under drought conditions. Treatment with ABA, increased the peroxidase activity in all concentrations. ABA in combination with drought, increased the peroxidase activity on all irrigation regimes (Table 12). Higher concentration like 15 decreased the POX activity on control and all irrigation regimes.

Table 12. Effect of varied irrigation regimes, abscisic acid (ABA) and their combination on peroxidase (POX) activity (unites mg^{-1} protein) in lettuce plants on 75 days after planting.

ABA Application ($\mu\text{g l}^{-1}$)	Irrigation regimes			
	24hrs	48hrs	72hrs	96hrs
0 (T0)	1.782±0.17a	2.739±0.20a	3.216±0.16a	3.673±0.19a
5 (T1)	1.944±0.25a	2.576±0.26a	3.057±0.19a	3.562±0.23a
10 (T2)	2.273±0.16b	3.487±0.18b	4.254±0.21b	4.038±0.14b
15 (T3)	2.621±0.19c	2.494±0.14a	2.963±0.25a	3.365±0.18a

Values are mean \pm SE of three replicates. Values, that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT) along the columns (different concentrations of ABA treatments)

Chapter 5: Discussion

The present investigation was conducted to determine whether PGR, like ABA, increases lettuce drought tolerance and if such tolerance is correlated with changes in oxidative stress, osmoregulation and antioxidant potential. The results on growth, biochemical constituents, non-enzymatic antioxidants and antioxidant enzymes are discussed hereunder.

5.1 Morphological parameters

5.1.1 Shoot and Root length

The root length was increased by drought stress in lettuce plants when compared to the control on 75 DAS in all the drought intervals. The root length again increased under ABA treatments under drought stress. An increased root growth was reported by (Tahir et al., 2003) in mango tree under water stress. Water stress greatly suppresses cell expansion and cell growth due to the low turgor pressure (Shao et al., 2006).

Initially increased root growth was reduced in later stages due to severe water deficit stress. Declined cell enlargement and cell growth due to the low turgour pressure and also more leaf senescence under drought stress might cause the reduction in plant height (Liang et al., 2006). Past studies report that root to shoot ratio increases under water stress conditions to facilitate water absorption (Lambers et al., 1998) and is related to ABA content of roots and shoots (Sharp and Lenoble, 2002). The morphological and physiological responses to exogenous ABA application showed that ABA could play an important role to control drought tolerance in two *Populus* species (Yin et al., 2005).

Under drought stress and also ABA application, the shoot length of the lettuce plants decreased to a larger extent when compared to the control on all sampling days.

The plant height was reduced under drought stress in *Populus* species (Yin et al., 2005). Reduced plant height was reported in *Albizia* seedlings due to reduced stem length under drought stress (Sundaravalli et al., 2005). ABA induced growth inhibition was resulted from signal transduction at the single-cell level and thereby induces closure of stomata (Trejo et al., 1995).

5.1.2 Number of leaves

The number of leaves was reduced with the induction of drought stress in whole stages of lettuce plant growth. ABA in combination with drought increased the number of leaves slightly on 75 DAS under the 72 hour irrigation interval. Water deficit stress mostly reduced leaf growth and in turn the leaf area in many species of plants like *Ziziphus* sp (Zhang et al., 2004). The leaf growth was more sensitive to water stress in wheat, but it was not so in the case of maize (Nayyar and Gupta, 2006). The exogenous application of ABA will initiate stomatal closure and protect the plants from stress. Application of ABA at room temperature results in reduced leaf production in many plants (Swamy and Smith, 2001).

ABA role in mediating drought stress has been researched extensively. The stomatal movements and closure mechanisms are controlled by ABA thereby regulating water status in the plant through guard cell functions. ABA results in the induction of genes that encode enzymes and other proteins involved in cellular dehydration tolerance (Luan, 2002; Zhu, 2002).

5.1.3 Fresh weight and dry weight

Water deficit condition decreased the whole plant fresh weight to a larger extent in lettuce plants in all the water deficit intervals. 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 decreased under drought condition and this

might be the reason for suppression of cell expansion and cell growth due to the low turgor pressure. Regulated deficit irrigation and partial root drying caused a significant reduction in shoot biomass when compared to control in wheat plants (Shao et al., 2005).

Drought stress decreased the dry weight of lettuce plants in all the irrigation intervals when compared to the control plants. ABA treatments increased the dry weight considerably under drought stress. A decrease in plant biomass was reported in drought stressed wheat (Shao et al., 2007) and in *Asteriscus maritimus* (Rodriguez et al., 2005). Severe water stress may result in arrest of photosynthesis, disturbance of metabolism, and finally dying (Liang et al., 2006). Cell enlargement will be more inhibited by water stress more than cell division. Drought stress reduces plant growth reduction through involving various plants physiological and biochemical processes, like photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and hormones (Shao et al., 2007). ABA plays a critical role in regulating plant water status through guard cells and growth as well as by the 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).

5.2 Pigment concentration

5.2.1 Chlorophyll and carotenoid

There was a decrease in pigment content under drought stress in lettuce plants but treatment with ABA increased the chlorophyll 'a' content. The chlorophyll content in the wheat leaf decreased due to chemical desiccation treatments (Shao et al., 2006). A reduction in chlorophyll content has reported in drought stressed soybean plants by Zhang et al. (2006). The chlorophyll content decreased to a significant level at higher water deficits in maize and wheat plants (Nayyar and

Gupta, 2006). The carotenoid content of the lettuce leaves decreased with drought stress. Treatment with ABA increased the carotenoid content.

5.2.2 Anthocyanin

Drought stress caused a decrease in anthocyanin concentration in all the drought intervals in lettuce leaves. But the ABA spray increased the anthocyanin significantly when compared to control. Treatment with ABA increased anthocyanin accumulation in strawberry fruits (Jiang and Joyce, 2003). Effects of ABA treatment and night temperatures on anthocyanin composition in Pinot noir grapes were reported early by Mori et al. (2005).

5.3 Biochemical analysis

5.3.1 Proline

In lettuce, drought stress caused increased accumulation of proline content of the leaves at all water deficit irrigation intervals. ABA also resulted in increased proline content in lettuce in control and drought stressed plants. Water stress resulted in an increase in proline accumulation in sorghum (Yadav et al., 2005). Enhancement in proline content of stressed plants may be an adaptation to overcome the stress. Proline accumulated under stressed conditions supplies energy for growth and survival and thereby helps the plant to tolerate stress (Jaleel et al., 2007). Proline accumulation in plants might be a scavenger and acting as an osmolytes. ABA increased the proline content in *Phaseolus vulgaris* (Mackay et al., 1990).

5.3.2 Amino acids

Drought stress caused increased amino acid content when compared to the control in lettuce plants. The amino acid content increased under individual ABA treatments and treatments under drought stress. The amino acid content has been shown to increase under drought condition in sunflower (Manivannan et al., 2007).

Accumulated amino acids may occur in response to the change in the osmotic adjustment of their cellular contents (Shao et al., 2007). Amino acid accumulation plays a very important role in drought tolerance, probably through osmotic adjustment in different plant species, such as, *Radix astragali* (Tan et al., 2006).

5.4 Antioxidants

5.4.1 Ascorbic acid (AA)

The ascorbic acid content increased with drought in lettuce plants. ABA decreased the ascorbic acid content when compared to the control. Compared with the controls, plants under drought stress displayed increased AA levels throughout the experimental period, whereas ABA treatment lowered AA levels. Treatment in the ABA + drought group increased AA level lower than those of drought treatment alone. These results indicate that the enhancement of AA level is correlated with both exogenous ABA and drought stress. Similar results were reported by Guo et al. (2012) in pepper (*Capsicum annuum*) leaves subjected to chilling stress and exogenous ABA application. Ascorbate is one of the most extensively studied anti-oxidant and has been detected in the majority of plant cell types, organelles and apoplast (Smirnoff, 2000). Water stress resulted in significant increases in antioxidant AA concentration in turf grass (Zhang and Schmidt, 2000). A decrease in ascorbic acid was reported in ABA treatment in plants (Zhang et al., 2006).

5.4.2 α -Tocopherol

α -tocopherol of the drought stressed plants significantly increased when compared to control plants. ABA was an inhibitor of α -tocopherol individually and also under drought stress. ABA increased the α -tocopherol content, but it was significantly less than drought stressed and control plants. The active oxygen species formed at the membrane of wheat leaves under drought stress was efficiently removed

upon rehydration with an increase in the α -tocopherol and β -carotene (Bartoli et al., 1999). A similar result was observed in pea chilling stress (Simontacchi et al., 1993). Previous studies have demonstrated the important roles of AA in the tolerance of plants to environmental stresses. For instance, Li et al. (2011) showed that low temperature increases AA level in cucumber plants, through the enhanced recycling pathway, reduces the deleterious effects of environmental oxidative stress.

5.5 Antioxidant enzymes

5.5.1 Superoxide dismutase (SOD, EC: 1.15.1.1)

The activity of SOD increased in water deficit in lettuce plants. Treatment with ABA resulted in an enhancement of SOD activity under drought stress. The SOD activity increase under drought in *Phaseolus acutifolius* (Turkan et al., 2005). An increase in SOD activity was reported in *Carthamus tinctorius* plants under water deficit stress (Hojati et al., 2011). SOD activity increased under drought stressed higher plants (Reddy et al., 2004).

5.5.2 Catalase (CAT)

The activity of catalase increased in drought stressed plants when compared to control. ABA increased the catalase activity to a higher level than control. ABA increased the activities of antioxidant enzymes such as SOD, catalase, APX and glutathione reductase in plant tissue under drought freezing stress (Anderson et al., 1995; Bellaire et al., 2000; Yang et al., 2013).

5.5.3 Peroxidase (POX, EC 1.11.1.7)

The peroxidase activity showed an increase in the drought stressed plants. Treatment with ABA increased the peroxidase activity. *Radix astragali* plants under water deficit stress showed an enhancement in POX activity irrespective of different genotypes (Tan et al., 2006). Water deficit stress increased the POX activity in soybean plants (Zhang et al., 2006).

Chapter 6: Conclusion

The economically important vegetable crop lettuce (*Lactuca sativa* L.) of family Asteraceae was selected for the present investigation. 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 being cultivated in UAE due to its commercial importance. In lettuce cultivation, a major problem is the requirement of large quantity of irrigation water.

For the past several years, several techniques of physiology have been applied to overcome the water deficit and drought stress in field crops. However little information has been gained on the response to ABA treatments under drought stress and their ameliorative actions on lettuce. It seems necessary to study the correlation between plant growth regulators and drought stress tolerance. The present study was aimed to reduce the water consumption of lettuce cultivation, for that, a varied irrigation regime was used with the application of ABA.

The parameters studied were: growth, photosynthetic pigments, biochemical constituents, antioxidant potential and antioxidant enzymes activities in lettuce plants under drought stress and its response to ABA under stress. Drought stress decreased the morphological parameters like root, shoot length, total leaf number, fresh and dry weight in lettuce. The growth parameters increased in ABA treatments under drought stress. There was a slight increase in root length in early drought treatments, but later it decreased.

The chlorophyll and carotenoid contents of the lettuce leaves increased with age in the control and treated plants. Treatment with ABA increased the chlorophyll and carotenoid contents. ABA in combination with drought increased the chlorophyll

and carotenoid contents and reduced the drought induced pigment reduction in lettuce plants.

Drought stress caused an increase in the biochemical constituents like proline and amino acid contents when compared with control in lettuce. All these parameters also increased under individual ABA treatments and treatments under drought stress. ABA treatments to the unstressed plants caused an increase in these parameters.

The non-enzymatic antioxidant molecules like ascorbate and α -tocopherol showed significant increase under drought condition in lettuce. ABA slightly reduced the non-enzymatic antioxidant contents.

The antioxidant enzymes like superoxide dismutase, catalase and peroxidase showed significant increase under drought condition in lettuce. ABA caused significant enhancement in these antioxidant enzymes under drought stress and also in unstressed conditions.

From the results of this investigation, it can be concluded that ABA at 10 $\mu\text{g/l}$ can be used as a potential tool to minimize the drought stress effects on lettuce cultivation. A field level experiment and economic feasibility analysis are required to ascertain this conclusion, which will be done as future studies.

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