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Growth and Biochemical Response of Ocimum Basilicum l. Plant Under Critical Drought Environmental

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***Abstract*---**Study curried out to evolution response growth and biochemical of Ocimum basilicum L. under drought conditions. Plant grown under treatment conditions (24 hours with - 54°C, 21°C, 55-70% RH) and plant were transferred to growth chamber for 6 weeks. The results showed that the rate of increase in chlorophyll a and b was significant with an increase rate of 1.7 and 0.6 compared to control after three days of treatment. The results also showed that H2O2 content was increased and reached to 1.3, 2.8 and 3.5X compared to the control after three, six and nine days of treatment respectively. One the other hand the levels of the enzyme APX activity, Lipid peroxidation and protein which was conducted on the tissue of basil leaves, increase compared with control after three days of treatment.

***Keywords*---**biochemical response, Ocimum Basilicum, critical drought, drought conditions.

Introduction

Basil belongs to the genus Ocimum, belong to the family of Lamiaceae, and is generally known as basil. Basil in general and its leaves in general are soft and the size of its leaves ranges between 3 to 11 cm and the width of the leaves is about 1 to 6 cm, as they usually begin with a branch from the center of the stem to the outside, and white flowers grow on them at the top of the plant and reach a height from the ground level approximately from 30 to 130 cm. The basil plant has been famous for its wide variety since ancient times and is also distinguished by its aromatic scent as well as in the aromatic industries and the oil industry, as

well as in cooking uses, and ornamental plants, tropical in Asia and America, Central and South America Basil extracts were allocated in traditional medicines, in addition to its use with insecticides, fungicides, and antimicrobials, and the commercial types traded in the market belong to the type (Ocimum) basilicum. Also basil plays an important role through vitamin D, which the body needs for proper growth and development, and vitamin D is considered one of the important vitamins that the body has because it has a role in the manufacture of important proteins.

Plants exposure to fluctuated environmental conditions increases the level of the production of reactive oxygen species (ROS) such as, singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$). The toxic effect of these species in plants is avoided in the plant cells and their organelles by detoxification processes which include an anti-oxidant components of enzymatic and non-enzymatic (Apel and Hirt, 2004; Mittler, 2002; Scandalios, 2005). The favored responses in gene expression are for specific antioxidant enzymes. In addition, their differences in subcellular localization and biochemical properties of and the presence of non-enzymatic mechanisms (Vranova *et al.*, 2002). Non-enzymatic antioxidants are include ascorbate (AsA) and glutathione (GSH) necessary for the defense of plant against oxidative stress (Foyer and Noctor, 2005; Mittler, 2002). Also contain flavonoids, phenolic compounds, alkaloids, tocopherol and carotenoids (Gratão *et al.*, 2005). Antioxidant enzymes such as ascorbat peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX) and peroxiredoxin (PrxR) are present in all subcellular compartments (Scandalios, 2005). The main hydrogen peroxide detoxification system in plant chloroplasts is the ascorbate-glutathione cycle (Asada, 1992). The APX act as specific electron donor to reduce H_2O_2 to water. The importance of APX plays a role in ROS scavenging in cytosol, mitochondria and peroxisomes in addition to chloroplasts (Shigeoka *et al.*, 2008). APXs activity generally increases along with other enzymes activities, such as CAT, SOD, and GSH reeducates in response to environmental stress (Shigeoka *et al.*, 2002).drought, at different time points of exposure according to the type of abiotic stress). Aim of current study to ovulation response of *Ocimum basilicum* L. plant under critical drought environmental.

Methodology

Plant materials and growth conditions

Basil (Ocimum) basilicum grown under controlled conditions (24 hours with - 54°C, 21°C, 55-70% RH) on 2:1:1 beetamose, perlite and vermiculite. Trays filled with plant pots were transferred to the growth chamber under controlled conditions and after 6 weeks, the plants were exposed to one of the following abiotic stresses (drought, at different time points of exposure according to the type of abiotic stress) or maintained in the growth chamber conditions without any changes.

Drought

Drought treatment, plants were irrigated with tap water three times a week for a period of six weeks of growth in controlled conditions, and then the plants

introduced to a water deficit continued into three levels (3 days, 6 days or 9 days) with holding water. At the end of each specific drought exposure, plant sample leaves collected directly in liquid nitrogen plant growth chamber and stored at -80°C until further analysis. Drought treatment was applied as described by (Egert *et al.*, 2013). Controlled samples were continued irrigated three times a week for further (3 days, 6 days or 9 days) after the plant reached to a period of six weeks of growth in controlled conditions.

Quantification of chlorophylls

Chlorophyll measurement, 20mg tissue samples were ground in liquid nitrogen and homogenized in 1 ml of 80% acetone. The sample suspension was mixed by vortex and followed by incubation for 1h in darkness. The samples centrifuged at 13000 rpm for 15 min and the supernatants were collected in new tubes to use for measurement. Chlorophyll content in aqueous acetone was quantified spectrophotometrically by measuring the absorbance of the extract at 646.6 nm and 663.6 nm. The total chlorophyll content (chlorophyll *a* and *b*) in each sample was calculated according to (Porra,. 2005).

Total chlorophyll [µg/mg] = (17.76(A646.6) - 7.34 (A663.6)) / fresh weight
Chl *a* [µg/mg] = (12.25(A663.6) - 2.55(A646.6)) / fresh weight
Chl *b* [µg/mg] = (20.31(A646.6) - 4.91 (A663.6)) / fresh weight

Measurement of H_2O_2

Leaf hydrogen peroxide content of treated and control samples were assayed as described by (Christou *et al.* 2013). Frozen leaf material (100mg) was homogenized on ice with 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 *g* for 15 min at 4 °C. the supernatant was transferred to new tube and 0.5 ml of the supernatant was added to 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M KI and mixed gently. The absorbance of assay mixture was read at 390 nm and the content of H_2O_2 was calculated based on a standard curve of known concentrations of H_2O_2.

Lipid peroxidation

The level of lipid peroxidation was analyzed with the thiobarbituric acid tast, which determines malondialdehyde (MDA) as a product of lipid peroxidation (Hodges *et al.*, 1999). Briefly, 50 mg of aerial tissue was homogenized in 1 mL of 80% (v/v) ethanol on ice. Following centrifugation at 16,000g for 20 min at 4°C, the supernatant transferred to new tube and 0.5 mL of supernatant was mixed with 0.5 mL of 20% (w/v) trichloroacetic acid containing 0.65% (w/v) thiobarbituric acid. The mixture was incubated at 95°C for 30 min and then immediately cooled in an ice bath. After centrifugation at 10,000g for 10 min, the absorbance of the supernatant was measured at 532 nm, subtracting the value for nonspecific absorption at 600 nm. The MDA concentration was calculated from the extinction coefficient 155 mM^{-1} cm^{-1} (Hodges *et al.*, 1999).

Protein quantification

Protein extracts from the treated and control samples were determined baled on Bradford method (1976). Briefly, 50 mg of aerial tissue was homogenized in 1 mL of 100 mM HEPS buffer (pH 7.6) on ice. Following centrifugation at 13,000 rpm for 10 min at 4°C, the supernatant transferred to new tube and 0.2 mL of supernatant was mixed with 1ml Bradford reagent and the absorbance of the supernatant was measured at 595 nm and the content of protein was calculated based on a standard curve of known concentrations of BSA (Bradford, 1976).

Ascorbate peroxidase specific activity

Ascorbate peroxidase activity was determined as described by Baier et al. (2000). Briefly, 50mg frozen plant material was extracted in 125µl extraction buffer (100 mM HEPES-NaOH pH 7.6). After 5 min centrifugation at 13,000 rpm at 4oC, the supernatant was transferred to a fresh reaction tube. 50µl crude extract was added to 1 ml assay buffer (50 mM HEPES-NaOH pH7.6) and 50µl 5mM ascorbate. The reaction was started after addition of 100 µl 3 mM H_2O_2. The absorbance of the supernatant was measured spectrophotometricaly was carried out at 290 nm. Activity of ascorbate peroxidases were standardized on the protein contents.

Results and Discussions

Effects on chlorophyll A and chlorophyll d content

The results showed that the rate of increase in chlorophyll A was significant with an increase rate of 1.7 compared to control after three days of treatment. Whereas, the results showed that chlorophyll b increased with an average increase of 0.6 in chlorophyll b, compared to control after three days of treatment. In both measurements a and b, production rates were significant compared to the control group. After six days of continuing treatment, the results showed that the rate of chlorophyll production increased and reached 1.9 times compared to the control after six days of treatment. While the rate of chlorophyll production showed increases of

1.5 times compared to the control after six days of treatment. In both measurements, the production rates were significant compared to the contro. After nine days of treatment, the results showed that the rate of chlorophyll a production changed significantly since the rate was increased and reached 2.5 compared to the control. While the production rate of chlorophyll b showed an increase of 2.2 times compared to the control and the increase was significant. These results indicate that all treatments in drought showed significant effects. The change in production level was compared between three, six and nine days. The results showed that the rate of chlorophyll A production was significantly changed between three control and three treatment days It did not change significantly between three and six days ineffective. While it was significant between three and nine days of treatment and reached an increase of 2.5 times in the level of production. The rate of anthocyanin production also showed an

increase of 2.6 times compared to between six and nine days, which is significant. As shown in Figure 1.

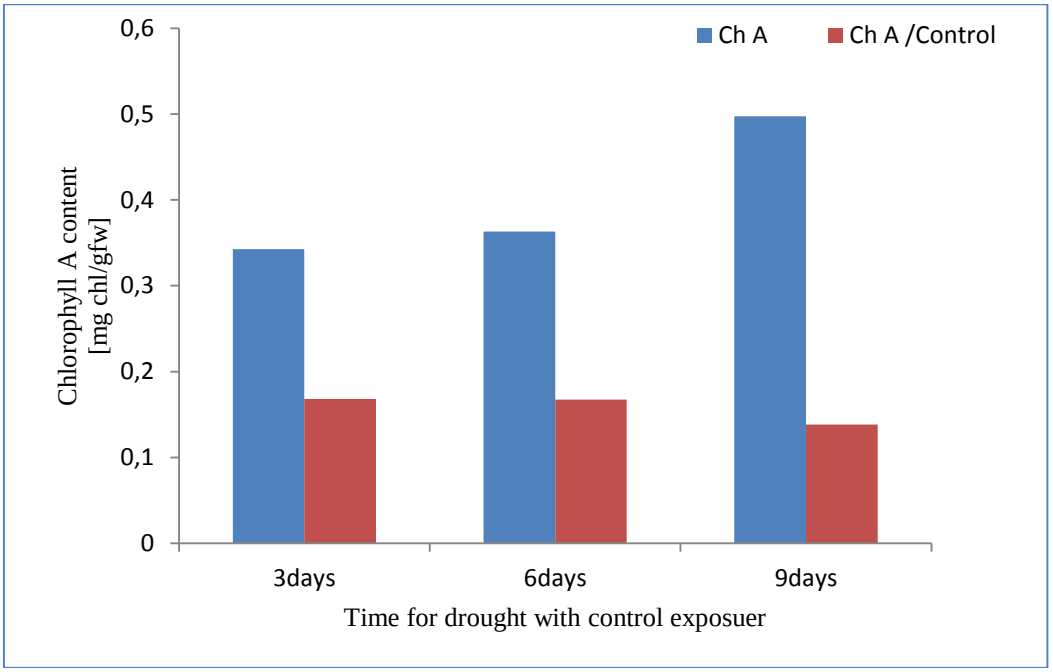

Figure 1. Effect of drought with different time points in comparison to control on chlorophyll a production of *Ocimum basilicum* leaves

There is a significant change in chlorophyll-b production rates between three days of treatment and three days of control, and the change was significant between three and six days. It was also significant between three and nine days of treatment and reached a 1.6 times increase in the production level. The rate of chlorophyll production showed increases of up to 1.1X in the period between six and nine days, which is significant. As shown in (Fig. 2).

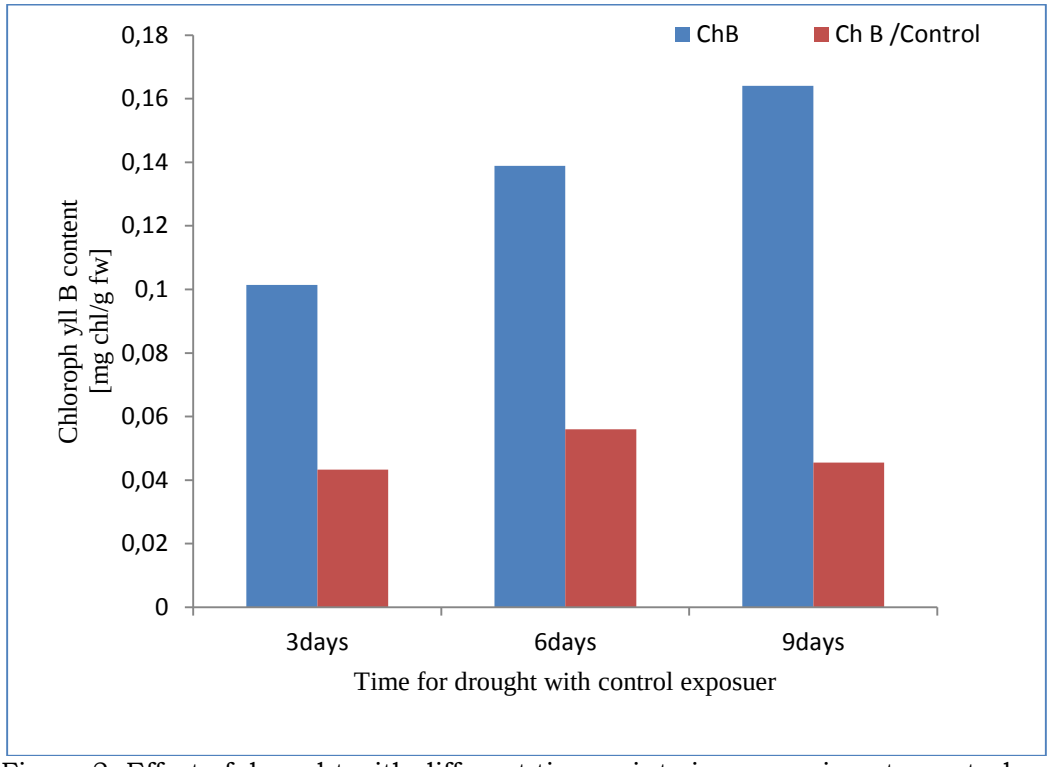

Figure 2. Effect of drought with different time points in comparison to control on chlorophyll b production of *Ocimum basilicum* leaves

Determination of chlorophylls content as an indirect method for estimation of bioproductivity and to understand of the photosynthetic regime of plants. Chlorophylls content are considered as physiological parameter, chlorophyll content under drought stress has already been reported (Fan et al., 2011). This might be caused by oxidation of chloroplast lipids and changes in the structure of pigments and proteins (Marcińska et al., 2013).

Effects on H_2O_2 content

The results showed that H_2O_2 content was increased and reached to 1.3X, 2.8X and 3.5X compared to the control after three, six and nine days of treatment respectively. This induction was significant at three, six and nine days The change in production level was compared between the three, six and nine days. The results showed that H_2O_2 production rate was significant changed of treatment and reached 2.1X increase in production level between three and six days. While it was significant between three and nine days of treatment and reached 2.6X increase in production level. In addition, the H_2O_2 production rate showed increases reached to 1.2X in compared between six and nine days which was significance. As shown in the figure 3. The accumulation of H2O2 in the plant known as the oxidative blast is one of the early events of the plant defense response to abiotic and biotic stresses)Jajic et al., 2015).Drought leads to an increase in H_2O_2 accumulation in a linear manner with stress intensity because chloroplasts (Hossain et al., 2015; Oelze, M. L. et al.2012), mitochondria and

peroxisomes are the sites as well as the primary target for production of reactive oxygen species under drought stress. (Farooq et al., 2009; Sharma and Dubey, 2005).

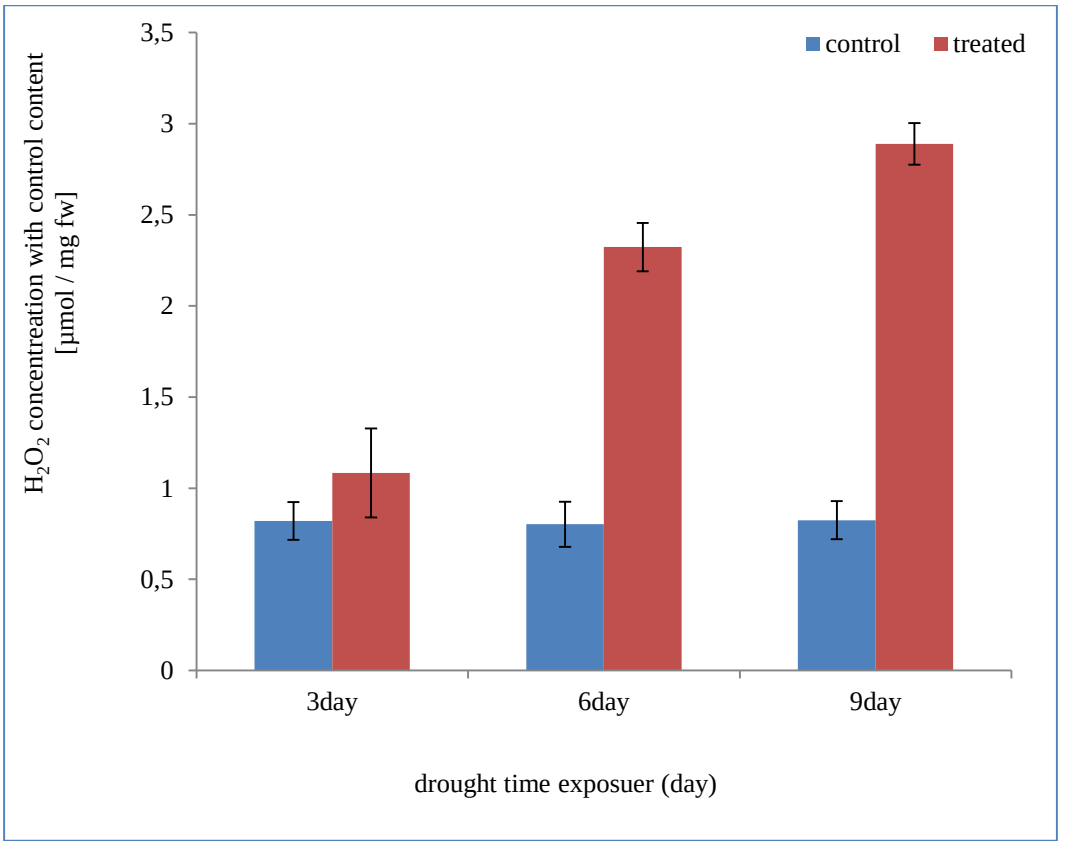

Figure 3. Effect of drought with different time points in comparison to control on H_2O_2 content of *Ocimum basilicum* leaves

Effects on lipid peroxidation level

The results showed that lipid peroxidation level was slightly non-significant increases with time dependent manner and reached to 1.3X, 1.6X and 2X compared to the control after three, six and nine days of treatment respectively, as shown in the The change in production level was compared between the three, six and nine days. The results showed that lipid production rate was not significant changed of treatment and reached 1.4X increase in production level between three and six days. While it was significant between three and nine days of treatment and reached 1.7X increase in production level. Also the lipid production rate showed increases reached to 1.2X in compared between six and nine days which was significance. Drought induces the generation of reactive oxygen species (ROS) causing oxidative stress in plants (Farooq et al., 2009).While the ROS can directly attack membrane lipids and increase lipid peroxidation (Alkhsabah et al.,2018). Overproduction of ROS increases the content of malondialdehyde (MDA) that considered an indicator of oxidative damage (Moller

et al., 2007). As our laboratory results show correlation between H_2O_2 accumulation in response to drought severity and the increases of the content of MDA for the same treatment as shown in figure 4 and 5.

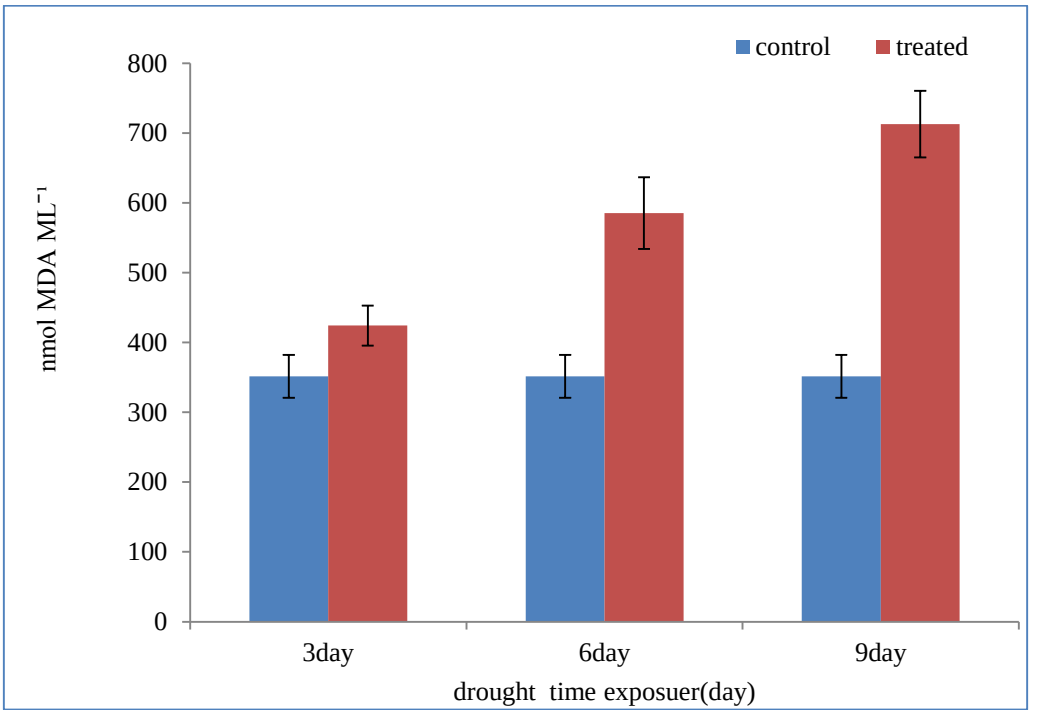

Figure 4. Effect of drought with different time points in comparison to control on lipid peroxidation level of *Ocimum basilicum* leaves

Effects on protein content

The results showed that protein production rate increase and reached 1.5X compared to the control after three days of treatment. After six days of treatment the results showed that protein production rate was significant and reached 1.6X compared to the control. After nine days of treatment the results showed that protein production rate was significant changed since the rate increases and reached 1.7X compared to the control. The change in production level was compared between the three, six and nine days. The results showed that protein production rate was not significant changed of treatment and reached 1.07X increase in production level between three and six days. While it was not significant between three and nine days of treatment and reached 1.66X increase in production level. Also the protein production rate showed increases reached to 1.04X in compared between six and nine days which was not significance. As shown in the figure 4.

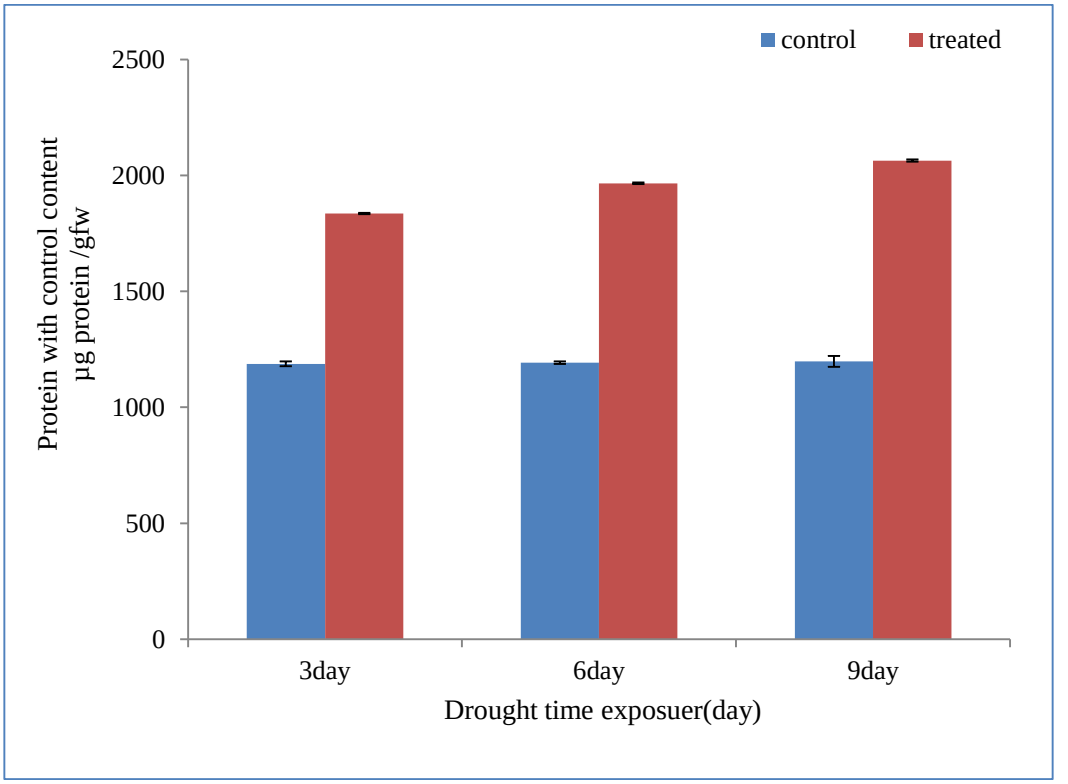

Figure 5. Effect of drought with different time points in comparison to control on protein content of *Ocimum basilicum* leaves.

While the significant change in protein content may be in response to the severity of abiotic stress to enhance plant drought tolerance. This occurs through the high abundance of proteins involved in energy production, amino acid synthesis, protein and destination synthesis, and the antioxidant defense system (Alkhsabah et al., 2018; Alsharafa., et al 2017;Merewitz et al., 2011; Narita et al., 2004).Reactive oxygen species cause lipid peroxidation, and consequently membrane injuries, protein degradation and enzyme inactivation (Sairam et al., 2005). Oxidative stress may cause protein oxidation, with a loss of enzyme activity (Berlett and Stadtman, 1997). In addition the production of reactive oxygen species is linear with the severity of drought stress, which leads to enhanced peroxidation of membrane lipids and degradation of nucleic acids, and both structural and functional proteins (Farooq et al., 2009).

Effects on APX content

The results of the experiment used to detect the levels of the enzyme APX activity, which was conducted on the tissue of basil leaves, showed that they were all significant by the treatment. When the results showed that total APX activity increase and reached 2.8X compared to the control after three days of treatment. After six days of treatment, the results showed that total APX activity was significant changed since the rate increases and reached 2.9X compared to the control. After nine days of treatment the results showed that total APX activity

rate was significant changed since the rate increases and reached 3.3X compared to the control. In addition, the change in production rate was compared between the three, six and nine days. The results showed that APX production rate was not significant changed of treatment and reached 1.03X increase in production rate between three and six days. While it was significant between three and nine days of treatment and reached 1.2X increase in production rate. In addition, the APX production rate showed increases reached to 1.14X in compared between six and nine days which was significance. As shown in the figure 6. While Various environmental stimuli, such as drought, salt stress, high light levels, high and low temperatures and abscisic acid, modulate the expres sion of APX-encoding genes (Alsharafa., et al 2017; Caverzan et al., 2012).APX is considering one of ROS-scavenging enzymes (Apel and Hirt, 2004).To reduce the effect of oxidative stress, which cooperate to maintain the integrity of photosynthetic membranes under oxidative stress, directly search for ROS or may act by production of non-enzymatic antioxidants (Apel and Hirt, 2004).While APX plays a major role in the adaptation of plants to stresses. (Koussevitzky et al., 2008).Therefore APX plays a major role in the acclimatization of plants to stresses (Koussevitzky *et al.*, 2008).

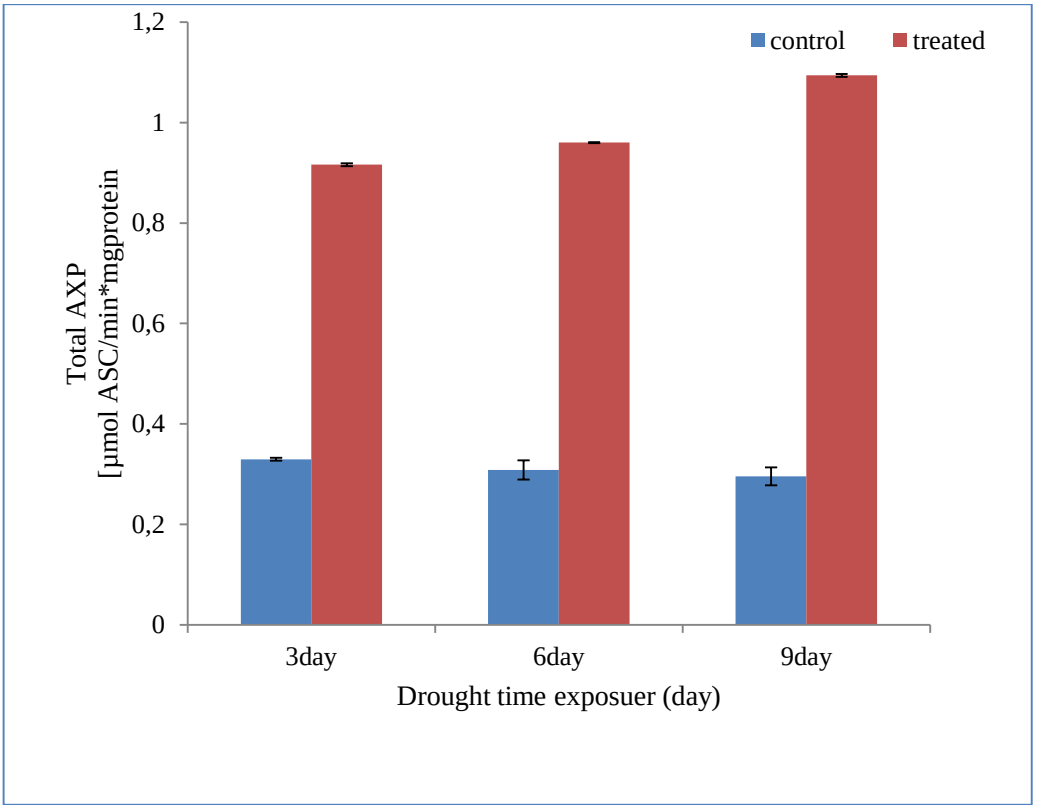

Figure 6. Effect of drought with different time points in comparison to control on APX activity of *Ocimum basilicum* leaves. Data are mean values ± SD of n=3. The significance of difference was calculated using Student's *t* test with $P \leq 0.05$.

Conclusion

The drought condition leaded to physiology response of *Ocimum basilicum* L. The growth and biochemical properties can be effected by critical environmental as a reaction or method of defense against stress.

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