Effect of Drought Stress on Some Growth Parameters and Several Biochemical Aspects in Two Pumpkin Species

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Abstract

For considering the effect of drought stress on some morphological and biochemical changes in two pumpkin species, *Cucurbita maxima* L. and *Cucurbita pepo* L. a kind of experiment was done by field culture in three water dispersal levels with field capacity, 2/3 field capacity, and 1/3 field capacity, based on the factorial design in random block form with four replications. The results indicated that increasing the stress level, leaves water potential under drought stress decreased in comparison to the control sample in both species. But with increasing drought stress, root length increased too. Also during drought stress, root soluble carbohydrates content, ascorbic acid content, dehydroascorbic acid, catalase, polyphenol oxidase, and peroxidase enzymes activity increased significantly in 5% level, according to the results with increasing the stress, soluble carbohydrates content decreased in leaf.

Keywords: antioxidant enzymes, drought stress, growth parameters, pumpkin

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Introduction

Plant growth and development are under influence of genetics characters and environmental conditions. Some factor like temperature, moisture, radiation, feed and gases can influence plant growth and development and cause reduction and increase in their function. For each environmental factor, there is one range of good conditions for plant growth. Outer from this extent, because of pressure of the mentioned factors, are called stress.

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One of the important environmental stresses is drought stress, which may come out under low raining, high temperature and wind speed conditions and decrease severely the production rate in places that are deal with this phenomenon (Bohnert & Jensen., 1996).

Iran with the average of the annual raining rate 260mm, become dry and semidry regions and half of the agricultural fields are existed in these regions (Chassemi et al., 1995). Plants in drought stress conditions respond to stress with morphological, physiological and metabolically changes in all of its organs. In cell level, plant respond to water depletion like cell injuries, but acclimation with stress can get the different

responses. Today great numbers of induced genes are known in different plant species under drought stress and a lot of investigations were done by scientists in molecular plant tolerance to drought stress (Balakrishman et al., 2005).

Drought not only decreases the plant growth and development but also cause changes in metabolism process, these changes can resist the plant in front of different environmental changes (Anjum et al. 2011). Shoot and root suitable response to low water potential, is a kind of acclimation towards stress conditions especially drought(Özenç, 2008). Some scientists believe that water reduction in plant decrease leaves number and size and decrease shoot length (Watkinson et al., 2006). Also drought stress reduces leaves growth but increase substances transport to root and root length in compare to control plants (Peleg and Blumwald, 2011). Also in other reports is narrated that plants root length increase in drought stress conditions (Niinemets, 2010). Accumulation of soluble carbohydrates was shown in different plant species, some scientists believe that polysaccharides damage especially starch increase monosaccharide (Kowitcharoen, et al. 2018). Drought stress increase starch polysaccharide decomposition and increase accumulation of soluble carbohydrates like fructose and sucrose, plant with increasing the amount of soluble carbohydrates in root help osmotic potential and absorb more water (Kowitcharoen et al., 2015). Completely soluble carbohydrates increase in shoot and root because of these reasons: 1. Carbohydrates dissolution damage like starch causes increase in soluble carbohydrate 2. soluble carbohydrate is synthesis from other ways except photosynthetic ways 3. Plant completely growth prevent or reduction (Kowitcharoen et al., 2017). Some scientists identify a kind of carbohydrates under drought stress that balance the drought effect on photosynthetic genes translation and transcription, like Rubisco enzyme genes coder during drought stress, these reports have shown a kind of control mechanism in which accumulate carbohydrates in some parts of plants in drought stress conditions (Daryanto, et al. 2017).

Different investigations were done about stress effects on antioxidant enzymes activity, for example increase of antioxidant enzymes especially peroxidase enzymes were expressed in the reports (Laxa et al. 2019). In another survey indicated that drought with increasing peroxidase activity, prevent reactive oxygen species disadvantages effects on cell membrane (Kobayashi et al. 2019). Some scientists said that polyphenol oxidase enzyme increase during drought stress, this increase is the main reason for defensive role of this enzyme against drought stress and eliminate oxygen radical under UV radiation (Tuladhar et. Al. 2021). Catalase enzymes decompose hydrogen peroxide in peroxisomes. This enzyme activity is sensitive to drought and high temperature stress, so enzyme activity changes usually depend on the amount of plant ability for decomposing hydrogen peroxide (Fekia et al. 2015). In plants antioxidant enzymes like catalase and ascorbic acid are biological defensive mechanism against reactive oxygen species (Cassia, et al. 2018). Also detoxify lipids peroxide and hydrogen peroxide (Alvarez-Robles et al,2020). Different investigations indicate that ascorbate and dehydroascorbate increase in stress leaves in compare to control (Debolt et al., 2007; Rosales et al., 2006). Some scientists believe that stress has negative effect on some morphological characteristics like leaf level, stomata numbers and the amount of evaporation. Shedding leaf in most of herbaceous and woody plants is related to the lack of water (Ouledali et al. 2019). This investigation indicates the drought stress on experimental species and related criteria with drought stress.

The main objective of this study is investigating the drought stress effect on some biochemical process in two pumpkins species to consider the defensive plant mechanisms against drought.

Materials and Methods

The grains of two pumpkin species: *Cucurbita maxima* L. and *Cucurbita pepo* L. were provided from Seed and Plant Improvement Institute, Karaj, Iran. The seeds were cultured in soil with leaf mold: sand: clay (1:1:2 from left to right respectively). The field capacity value of the soil, determined by pressure plate apparatus (Lindsay, 1988) was used to calculate 3 water application

regimes: (1) re watering to field capacity (FC); (2) watering with a volume nominally 1/3FC; (3) watering nominally 2/3 FC. Calculations after the experiment showed that the actual amount of watering for field capacity were: 2700 g plant $^{-1}$, 1/3 FC: 900 g plant^{-1} and 2/3 FC 1800 g plant^{-1}.

Plant Field Culture

After culturing a lot of numbers of healthy and sterile grains in reservoir form and stress function from germination stage, healthy plants were selected and transferred to the field soil in Ashtiyan central province that is the part of Iran central plateau. In this study among the Cucurbita family, two species were chosen *Cucurbita maxima* L. and *Cucurbita pepo* L. For investigating drought stress changes on growth, development, morphological and biochemical characteristics of two species, some samples from different parts of plant were selected and sent to the laboratory for analysis. Shoot and root plant samples after drying in 70 °C for 72 hours were put in the paper form packs to utilize for growth parameters and some chemical compounds determination assay.

Root Length Measurement

Root length was measured by millimeter ruler and for each treating groups, four replications were proposed, the replication average calculated for each sample.

Leaf Area Measurement

Millimeter graph paper method used for leaf area measurement. Here a leaf is taken and traced over graph paper, and the grids covered by the leaf are counted to give the area. This method can be both inaccurate and time consuming, and it is also impractical when many measurements are necessary.

Leaf Water Potential Measurement

Leaf water potential was measured by random sampling from leaf samples in the end of leaf growth stage by use of Pressure Chamber Device Sky Instrument England model (1900) (Boyer,1967).

Biochemical Measurement

Ascorbate and dehydroascorbate concentration assay

The amount of ascorbic acid was estimated using de Pinto et al. (1999) method. 0.5 g of leaf was homogenized in metaphosphoric acid 5% (w/v) and centrifuged for 15 minutes. 0.3 ml of solution was mixed with 0.75 ml KH_2PO_4 buffer and 0.3 ml distilled water and kept in room temperature for 10 minutes. Then, 0.6 ml TCA 10% (w/v), 0.6 ml orthophosphoric acid 44% (w/v), 0.6 ml dipyridil (C10H8N2) 4% (w/v) and 10 µl FeCl3 were added and shaken for 20 min in 40 °C.

The same method was used in order to measure dehydroascorbate content. 0.5 g of leaf was homogenized in metaphosphoric acid 5% (w/v) and centrifuged for 15 minutes. 0.3 ml of solution was mixed with 0.75 ml KH_2PO_4 buffer and 0.15 ml DDT and kept in room temperature for 10 minutes. Then, 0.15 ml NEM 5% (w/v) was added and again kept in room temperature for 10 minutes. Then, 0.6 ml TCA 10% (w/v), 0.6 ml orthophosphoric acid 44% (w/v), 0.6 ml dipyridil (C10H8N2) 4% (w/v) and 10 µl $FeCl_3$ were added and shaken for 20 min in 40 °C. The absorbance was measured spectrophotometrically at 525 nm wavelength for both of them. Ascorbate and dehydroascorbate concentrations were calculated based on mgg-1 FW.

Total Soluble carbohydrates assay

Total soluble and insoluble sugars of shoot and root tissues were determined by the phenol sulfuric acid method as described by Kochert et al. (1978). Briefly, 5 ml of ethanol (70%) was added to 0.05 g of dry shoot and root samples and maintained in refrigerator for one week. Then, the mixture was centrifuged at 10000 g for 15 min at room temperature and supernatant was used for determination of soluble sugars content, Glucose was used to prepare a standard curve and data was expressed as mg g^{-1} DW.

Extraction of Antioxidants

To extract antioxidant enzymes, 0.5 g of leaves were ground using a tissue grinder in 8 ml of cooled phosphate buffer (pH 7.0, containing 1% (w/v) polyvinylpyrrolidone) and 0.2 g quartz sand

in test tubes that were placed in an ice bath. The homogenate was centrifuged at 15000 × g for 20 min at 4 °C. The supernatant was used for assays of enzyme activity (catalase, peroxidase and polyphenoloxidase).

Catalase assay(CAT)

Activities of catalase (CAT) were measured using the method of Kar and Mishra (1976) with modification. The CAT reaction solution (3 ml) contained 50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2 and 0.1 ml enzyme extract. Reaction was initiated by adding enzyme extract. Changes in absorbance of the reaction solution at 240 nm were read every 20s.One-unit CAT activity was defined as an absorbance change of 0.01-unit min^{-1}.

Peroxidase assay(GPX)

Activities of peroxidase were measured using the method of Kar and Mishra (1976) with modification. For guaiacol peroxidase acivity assay the reaction mixture (3.0 ml) contained 0.1 M phosphate buffer (pH 6.80), guaiacol (30 mM), H_2O_2 (30 mM) and 0.3 ml enzyme extract. Changes in absorbance of reaction solution at 470 nm were determined every 20s. One unit GPX activity was defined as an absorbance change of 0.01-unit min^{-1}. The activity of each enzyme was expressed on a protein basis.

Polyphenol oxidase assay(PPO)

PPO activity was determined by the method of Liu et al (2005). The standard reaction mixture contained 1.5 ml of 40 mmol/l catechol and 2.3 ml of 0.1 mol/l phosphate buffer (pH 6.5) in a 10 ml test tube, and was placed in a 25 °C water bath for 5 minutes. Then, 0.2 ml of crude enzyme was added to the test tube and mixed thoroughly. Immediately, the increase in absorbance was measured at 420 nm with a UV-spectrophotometer (Mapada UV1600; Mapada, Shanghai, China). The reaction time for PPO was 2 min, and the activity was expressed in units – one unit = 0.001ΔA420/min/g fresh weight (FW).

Statistical Analysis

Data collected from were analyzed using SPSS software (version 15). One-way analysis of variance (ANOVA) and the statistical significance of the results were analyzed by using Duncan test. The charts were designed by using Excel software.

Results

In this investigation the increase of drought stress at 2/3and 1/3 field capacity showed that the root length boost significantly in compare to control, it means that the plant efforts for obtaining water in stress conditions. So in each three treatments there are significant differentiations between two species (Fig. I).

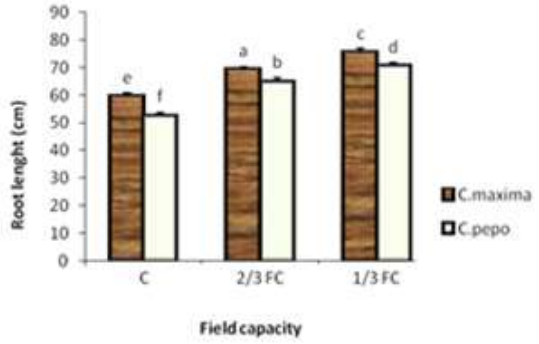

Fig. I. Effects of drought stress on root length base on Duncan test P<0.05.

In relation with leaf area the results showed the reduce effects of drought stress are studied on all of the plants leaf level (Fig. II). In this assay the amount of leaf water potential in three treatments had insignificant differentiations (Fig. III). Also the ascorbate and ascorbate peroxidase data assay indicated that water drought treatments increased significantly in drought conditions in compare to control plants in both species (Fig. IV, V).

The results showed that the antioxidant enzymes activity like peroxidase and polyphenoloxidase increase in water stress treatments (Fig. VI, VII). Catalase enzyme activity increased a little in mild stress but in the severe stress decreased in compare to control plants (Fig. VIII). The amount of carbohydrates solution decreased a little in leaf level (Fig.IX, X).

Discussion

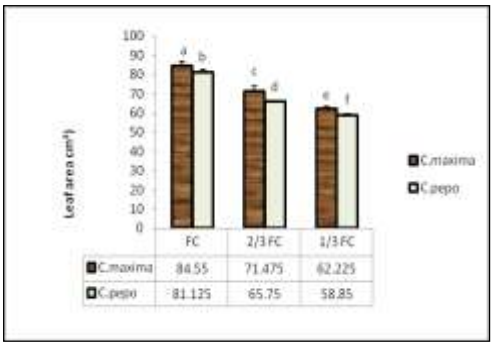

Fig. II. Effects of drought stress on leaf area base on Duncan tes

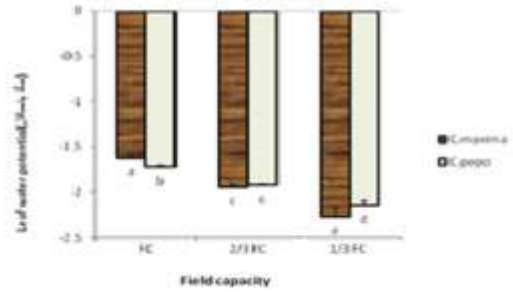

Fig. III. Effects of drought stress on leaf water potential base on Duncan test P<0.05.t P<0.05.

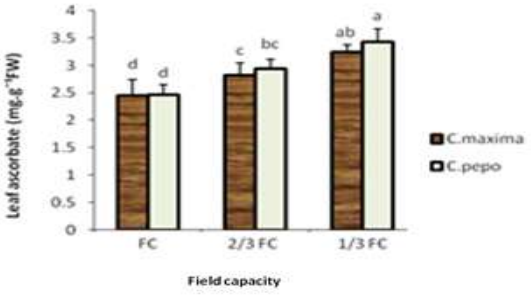

Fig. IV. Effects of drought stress on ascorbate amount base on Duncan test P<0.05.

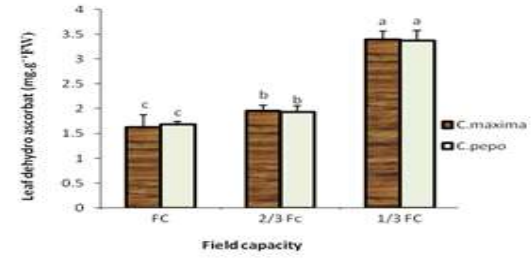

Fig. V. Effects of drought stress on dehydroascorbate amount base on Duncan test P<0.05.

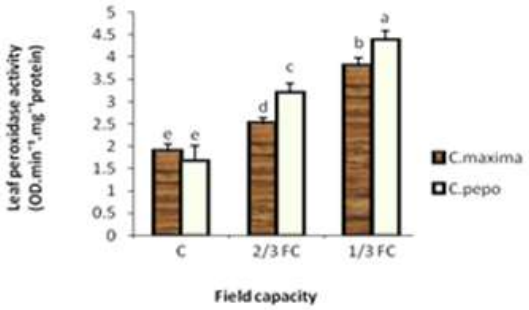

Fig. VI. Effects of drought stress on peroxidase enzyme activity base on Duncan test P<0.0.

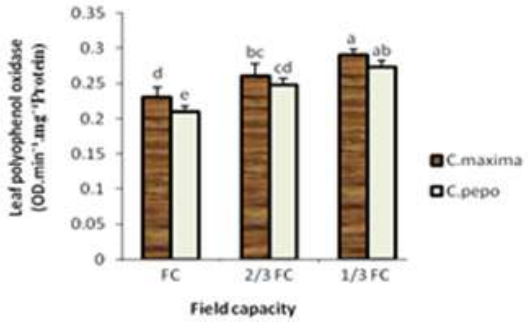

Fig. VII. Effects of drought stress on leaf polyphenoloxidase enzyme activity base on Duncan test P<0.05.

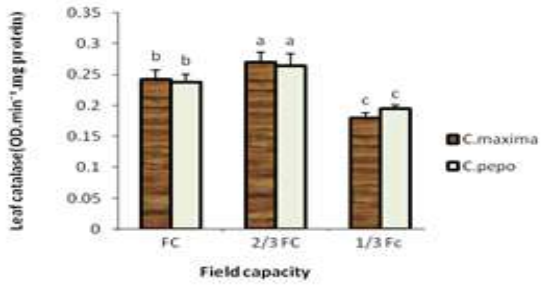

Fig. VIII. Effects of drought stress on catalase enzyme activity base on Duncan test P<0.05.

Water plays an important role in plant growth and development, so that water stress becomes one of the restriction factors that effects on biochemical and physiological process, and prevents agricultural plant's genetic potential.

Plants usually defense with different operations against environmental stresses (Kusaka et al., 2005). The obtaining results showed that direct relation between the

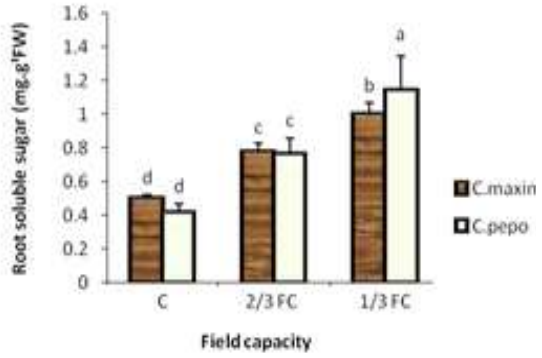

Fig. IX. Effects of drought stress on root soluble carbohydrates base on Duncan test P<0.05amount base on Duncan test P<0.05.

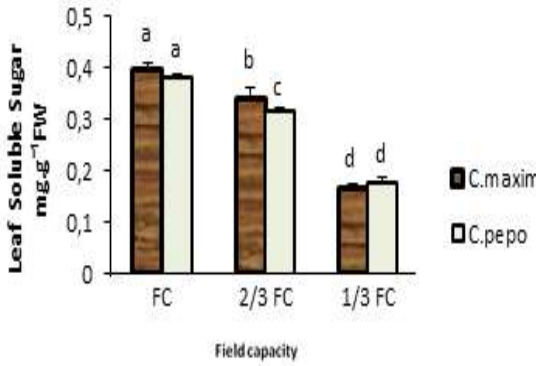

Fig. X. Effects of drought stress on leaf soluble carbohydrates base on Duncan test P<0.05.

increase of drought levels and reduction of growth parameters (Osama et al. 2019). In the present study drought stress decreased shoots growth and increase root length. This suitable root and shoot response to low water potential is a kind of adaptation to drought conditions (*Chaturvedi et al., 2019*). In this study the continuous growth of root length increase getting water from soil, also to approve this study (Liptay et al., 1998) these scientists had shown that *Zea mays* L. and tomato's roots length increase during drought stress, so plant able to absorb more water of deeper soil layers. So that in some plants exist a stronger root system for increasing plant ability to absorb water, that is introduced as basic mechanism to drought adaptation (Kusaka et al., 2005). Different changes like making deeper root for absorbing water, dying later than usual (maintain turgor), proper root system, leaf level reduction for diminishing the amount of evaporation, pore closure and osmotic adjustment are plants function in dealing with drought (Biswas et al. 2021). To approve this study, water reduction in potato plant was decreased the number and size of the leaves and shoot length (Watkinson et al., 2006). Drought stress decreased leaves growth but increase taking materials to root and root length in compare to control plants. The leaf level reduction and soil water potential reduction are corresponded by other plants like sunflower (Synerri et al., 1993) and tobacco (Pastori et Trippi, 1993).

Soluble sugars are one of the major osmotic compounds that accumulate in different parts of the plant (Babazadeh Darjazi et al. 2019). In the present study, in both pumpkin species and all the treatments, drought stress caused a significant increase in soluble carbohydrates in all the plant's leaves. Furthermore, drought adaptation needs some reactions to change some metabolites and increase some amino acids and soluble carbohydrates (Sunka et al., 2003). In this investigation, the soluble carbohydrates decreased in stressed leaves, but the amount of this parameter increased in the root. One of the reasons for carbohydrate reduction in plant leaves under drought stress is drought effects on thylakoids' membrane, the amount of chlorophyll a, b, and the amount of photosynthesis (Chang et al. 2019). El_Tayeb (2005) reported that on chenopods had expressed that drought stress increase starch polysaccharide decomposition and increase soluble carbohydrates' accumulation like fructose and sucrose in which maintain helping turgor in the plant. So during leaf low water potential, carbohydrates accumulation

can play its role in cell osmotic adaptation (Shanker & Sharma, 2005).

In this investigation, drought stress increased the amount of carbohydrate in root in stressed plants. The reports were expressed that plants by increase of root soluble carbohydrate help reduction in root osmotic potential, so absorb more water. The present study is corresponded clearly by other experiments on leaf soluble carbohydrates' reduction (Ripoll et al, 2014). So this result is clarified by leaf level, photosynthesis and synthesis of organic compounds reduction in stress conditions. Also, the results showed that root soluble carbohydrates increase with water stress that is derived from structural and insoluble carbohydrates' composition in low water conditions.

Peroxidase is one of the important enzymes in plant oxidative stress that can sweep hydrogen peroxide. Hydrogen peroxide is the major product of superoxide dismutase activity. Eliminates malondialdehyde peroxidase. Malondialdehyde promotes lipid membrane peroxidation and facilitates cell membrane breakdown (Farzamisepehr and Norouzi,2018). In this study drought stress increased peroxidase enzyme activity in all of the stress treatments. In other reports indicated that peroxidase activity in mature leaves increase three times in compare to control plants in drought conditions, but there wasn't any increase in immature leaves (Jiang and Huang, 2001). In some studies, indicated that different resistant numbers of wheat plant have high peroxidase activity in stress conditions. So the increase of peroxidase activity prevents reactive oxygen species disadvantage effects and adjusts antioxidant enzymes activity and balances respectively the amount of O_2, H_2O_2 (Lee and Shiu, 2005).

Polyphenol oxidase enzyme increased during drought stress and in some cases didn't change (Zhang and Sun, 2021). The results of this investigation showed the defensive role of this enzyme in front of oxidative stress corresponds with other reports. Polyphenol oxidase is a kind of enzymes that have mechanisms for eliminating oxygen radical under UV radiation, heat and drought stress conditions (Dawood et al., 2021). Phenolic compounds (poly phenols) are acted as obstructive antioxidants of composing free radicals by polyphenol oxidase and peroxidase enzymes. These enzymes cause phenolic compounds oxidation to intermediate compounds like quinons in oxygen presentation (Choudhuri, 2004). This enzyme activity reduction in present study prevents this compounds reduction in stressed plants. In plants biological defensive mechanisms are against reactive oxygen species like catalase, ascorbic acid and etc, also substances like tocopherol and beta carotene (Dawood et al., 2021).

Ascorbates extensively are acted as unit oxygen and hydroxyl radicals' eliminator so avoided from reproducing free radicals chained reactions. Ascorbic acid with ascorbate peroxidase plays a role in detoxifying lipid peroxidase and hydrogen peroxide (Kumar et al. 2019). In this investigation, the increase of ascorbate and dehydroascorbate was seen in the drought stress condition in all treatments. A lot of reports based on antioxidant compounds activity (Waśkiewicz et al.2014). A mount of ascorbate and dehydroascorbate increased in UV radiation and drought stress leaves. Also in long lasting treatment the time of drought stress in Sinapis seedlings the amount of ascorbate and dehydroascorbate increased in compare to control (Jaskulak et al., 2019). Ascorbate is the first important antioxidant that reacts straightly with hydrogen peroxide, hydrocyle radicals, supperoxide and unit oxygen, in many aspects control redox and antioxidant activity in plant cells and its role as a response agent to stress and cofactor

enzyme were the most important topic in many investigations related to the drought stress in recent years (Debolt et al., 2007). Also in another study ascorbate with its antioxidant characteristic can protect cell membrane from drought stress and free radicals damage (Orsavová et al., 2019). So the increase of ascorbate and dehydroascorbate during in this investigation are the parts of defensive mechanisms against drought stress damage.

Conclusion

In conclusion the pumpkin plant in drought stress condition used appropriate defensive mechanisms to decrease stress damages. Furthermore, many investigations would do to identify Cucurbita genus with remedial features and its suitable actions in dealing with drought stress. Also with identifying the stressed resistance items, best methods and proper watering can protect the plant in low water conditions from drought damages.

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