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IMPACT OF ASCORBIC ACID ON GROWTH AND SOME PHYSIOLOGICAL ATTRIBUTES OF CUCUMBER (*CUCUMIS SATIVUS*) PLANTS UNDER WATER-DEFICIT CONDITIONS

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Abstract

Cucumber (*Cucumis sativus* L.) is a very popular vegetable, which is utilized all-over the world. In the current research, two cultivars of cucumber, Local and Hybrid were chosen to look at the effect of foliar-applied ascorbic acid on some key physio-biochemical attributes under varying water regimes. After one week of seed germination, the plants of both cucumber cultivars were subjected to 100% field capacity (control) and 60% field capacity (water deficit conditions. After one month of water stress treatment, ascorbic acid (AsA) at the rate of 0 (control), 50 and 100 mg L^{-1} was applied foliarly. After 15-day AsA application, the data were recorded which showed that drought stress significantly reduced the plant growth, chlorophyll contents, relative water contents (RWC), rate of photosynthesis (A), stomatal conductance, internal CO_2 concentration (C_i) and C_i/C_a, while drought caused an increase in relative membrane permeability (RMP), and proline and glycine betaine contents. AsA improved the shoot fresh and dry weights, chlorophyll *a*, RWC, C_i, C_i/C_a and proline contents. No change was observed in both cucumber cultivars under water-deficit and AsA treatments. Overall, growth improvement of cucumber plants under dry arid climate could be attributed to AsA-induced stimulation in the chlorophyll *a*, proline, RWC contents and C_i concentration.

Key words: *Cucumis sativus*, Water deficit stress, Gas exchange characteristics, Glycine betaine.

Introduction

The availability of optimum amount of water during whole life cycle of the plant is an essential element to obtain its better growth and yield production (Bartoli *et al.,* 1999; Nair *et al.,* 2008). So, the deficiency of water at any stage of plant growth and development can stimulate a number of plant processes including change in metabolism, physiology and morphology (Ashraf *et al.,* 2011). As an adaptive response to water stress, plant cells trigger the accumulation of different compounds such as proline, glycerol, sugars/carbohydrates, antioxidants, glycine betaine as well as secondary metabolites (Halimeh *et al.,* 2013; Kosar *et al.,* 2015; Waseem *et al.,* 2015). Of various drought-induced physiological changes occurring in plants, such as reduction in rate of photosynthesis/transpiration, relative water content (RWC) and stomatal regulation commonly occur (Chaves and Oliveira, 2004; Akram *et al.,* 2009; Tuna *et al*., 2010).

Scarcity of water causes oxidative stress due to over-accumulation of reactive oxygen species (ROS) in chloroplast followed by mitochondria (Ashraf, 2009). Over-production of ROS can cause death of cells (Imlay, 2003) due to interaction with vital membranes, proteins, DNA, RNA and nucleic acids. To detoxify the effect of ROS, plants produce some key ROS scavengers in the form of enzymatic or non-enzymatic antioxidants such as POD, CAT, SOD, tocopherol, ascorbic acid, glutathione and phenolics, etc. (Liu *et al*., 2009; Weng *et al.,* 2015). Ascorbic acid (AsA) is a non-enzymatic complex which enables plants to defend against stresses by reducing oxygen free radicals (Shafiq *et al.,* 2014). AsA is generally distributed in the cytosol of the plant and it acts as an antioxidant. Moreover, the plants with low ascorbate synthesis are quite sensitive to different environmental stresses which can adversely affect their growth and development (Alhagdow *et al.,* 2007; Ahmad *et al.,* 2013). Exogenous application of AsA has been very effective in improving plant growth and development by altering oxidative defense system, phytohormone signaling, cell expansion, ion transports and other related processes under stress or non-stress conditions (Pignocchi & Foyer, 2003; Darvishan *et al.,* 2013).

Cucumis sativus L. (cucumber), is a popular and well liked vegetable around the world. It is sensitive to drought and requires maximal amount of water during its growth (Ertek *et al.,* 2006; Wang *et al.,* 2010). The fruits of cucumber are sugary, refrigerant, haemostatic and boost. Therefore, usually it is used as a cooling agent and for treatment of skin problems (Akhtar *et al.,* 2011; Kaur *et al.,* 2014; Maheshwari *et al.,* 2014). A small number of bioactive compounds belonging to different chemical groups have been derived from this plant (Kaur *et al.,* 2014). Its yield and quality are affected adversely due to low soil water content (Mao *et al.,* 2003; Wang *et al.,* 2010). The purpose of the present study was to evaluate the efficiency of foliar applied AsA on growth and different physiological attributes particularly gas exchange characteristics, osmoprotectants and membrane permeability of cucumber plants subjected to water deficit conditions.

Materials and Methods

A pot experiment was conducted using two cultivars (Local and Hybrid) of cucumber (*Cucumis sativus* L.). Seeds of both cucumber cultivars were obtained from the Ayub Agricultural Research Institute, Pakistan. The experiment was set-up during January-March, 2015 in the research area of the GC Univ. Faisalabad, Pakistan. Seven kg soil was filled in each plastic pot, and initially 10 seeds were sown per pot. After one week of germination, five plants of uniform size per pot were maintained. After two weeks of germination, all pots were bifurcated, one for control (100% field capacity) and the other for water-deficit conditions (60% field capacity). Field capacity was calculated on the basis of saturation percentage. After fifteen days of maintenance of required levels of water stress treatment, ascorbic acid (AsA) was applied as a foliage spray at the rate of 50 and 100 mg/L in addition to no spay application. Fourteen days after foliar applied AsA, two plants were collected from each replicate, washed with water and recorded data for fresh weights of shoots. After air dry, all shoot samples were shifted to an oven adjusted at 72°C and remained therein till constant dry weight. The remaining plants were used for the determination of following attributes:

Chlorophyll contents: Fresh leaf material (0.5 g) was ground in 10 mL acetone (80%; v/v) and the absorbance recorded at 663 and 645 nm. Chlorophyll *a* and *b* were calculated following Arnon (1949).

Gas exchange attributes: Stomatal conductance, rate of photosynthesis, transpiration, and C_i were determined of all cucumber plants. All data were recorded at day-time with maximum sunshine (801-915 µmol m^{-2} s^{-1}) using a portable infra-red gas analyzer was used

Relative water contents (RWC): From each plant, third leaf was excised from the top and recorded fresh weight of all the leaves following Jones & Turner (1978). Immediately, the leaves were placed in a tub containing water for 3 hours and calculated the turgid weight. After this, all samples were kept in an oven for 48 hours and dry weight recorded.

Relative membrane permeability (RMP): A protocol proposed by Yang *et al.* (1996) was used to assess the RMP of water-stressed and non-stressed plants.

Leaf free proline: Following Bates *et al.* (1973), fresh leaf (0.5 g) was taken and ground finely in 10 mL 3% sulfosalicylic acid. Then, the extract was filtered and to 2 mL of filtrate 2 mL acid ninhydrin and 2 mL GAA were added. The mixture was heated in a water bath for 1 hour at 100°C, then cooled in ice bath and 4 mL of toluene were added. After vortexing the mixture, the absorbance of the upper layer was noted at 520 nm.

Glycine betaine (GB): We followed the method purposed by Grieve & Grattan (1983) to determine the leaf glycine betaine contents. Fresh leaf, 0.5 g, was taken and ground with 0.5% toluene (10 mL) and filtered. Then, one mL of H_2SO_4 (2 *N*) was added to one ml of the filtrate. Of which, 0.5 ml was mixed with 0.2 mL KI_3. The contents were vortexed and cooled, then five ml of 1, 2 dichloro ethane and 2.8 mL of ice cooled distilled water were added to it. After removing upper layer was discarded and the absorbance of the lower organic layer was read at 635 nm.

Statistical analyses: A three-way ANOVA of data for all the attributes was carried out to test the significance of different treatments on cucumber plants.

Results

Water-deficit conditions (60% field capacity) significantly (p≤0.001, 0.001) reduced the plant growth (shoot and root fresh and dry weights) of both (Local and Hybrid) cultivars of cucumber plants (Fig. 1). Foliar-applied ascorbic acid (AsA) was effective only in enhancing the shoot fresh and dry weights. Of both AsA levels, 50 mg L^{-1} was the most effective in improving shoot growth of cucumber plants grown under stress conditions. A non-significant response of cucumber roots was observed to exogenously applied AsA. Of both cucumber cultivars, Cv. Local was superior in growth performance under both water regimes.

A significant (p≤0.001) suppression was observed in the chlorophyll *a* and *b* contents due to arid conditions in cucumber leaves. Foliar-applied AsA enhanced chlorophyll *a* contents while no change was observed in chlorophyll *b* contents due to exogenously applied AsA (Fig. 1). The response of both cucumber cultivars was almost same to drought as well as AsA treatments.

Relative water contents (RWC) of cucumber leaves were affected adversely due to water deficiency (Fig. 1). Of both AsA levels, 100 mg L^{-1} was the most effective in improving RWC in cucumber leaves. A non-consistent difference was observed in RWC of both cucumber plants in response to AsA and drought stress treatments.

An increase (p≤0.05) was speculated in the relative membrane permeability (RMP) of both cucumber cultivars. However, no change was observed in the RMP due to foliar-applied AsA and water deficit conditions in cucumber plants (Fig. 1).

Imposition of drought stress significantly reduced the photosynthetic rate (A), sub-stomatal CO_2 concentration, stomatal conductance (g_s), and C_i/C_a, while transpiration rate (E) and water-use-efficiency (WUE) of both cucumber cultivars was remained unaffected (Fig. 2). Exogenously applied AsA was only effective in improving C_i and C_i/C_a of both cucumber cultivars. Difference between both cucumber cultivars was observed only in the photosynthetic rate, while the response of both cucumber cultivars in terms of all other gas exchange cultivars was almost unchanged (Fig. 2).

Proline accumulation was significantly increased in both cucumber cultivars due to drought stress. AsA improved proline accumulation in both cucumber cultivars. No significant difference was examined between the two cucumber cultivars in terms of proline accumulation under stress conditions (Fig. 2).

An increase was observed in the accumulation of GB contents in both cucumber cultivars due to water stress (Fig. 2). However, no change in GB contents was observed due to foliar applied AsA and water-deficit conditions in both cucumber cultivars.

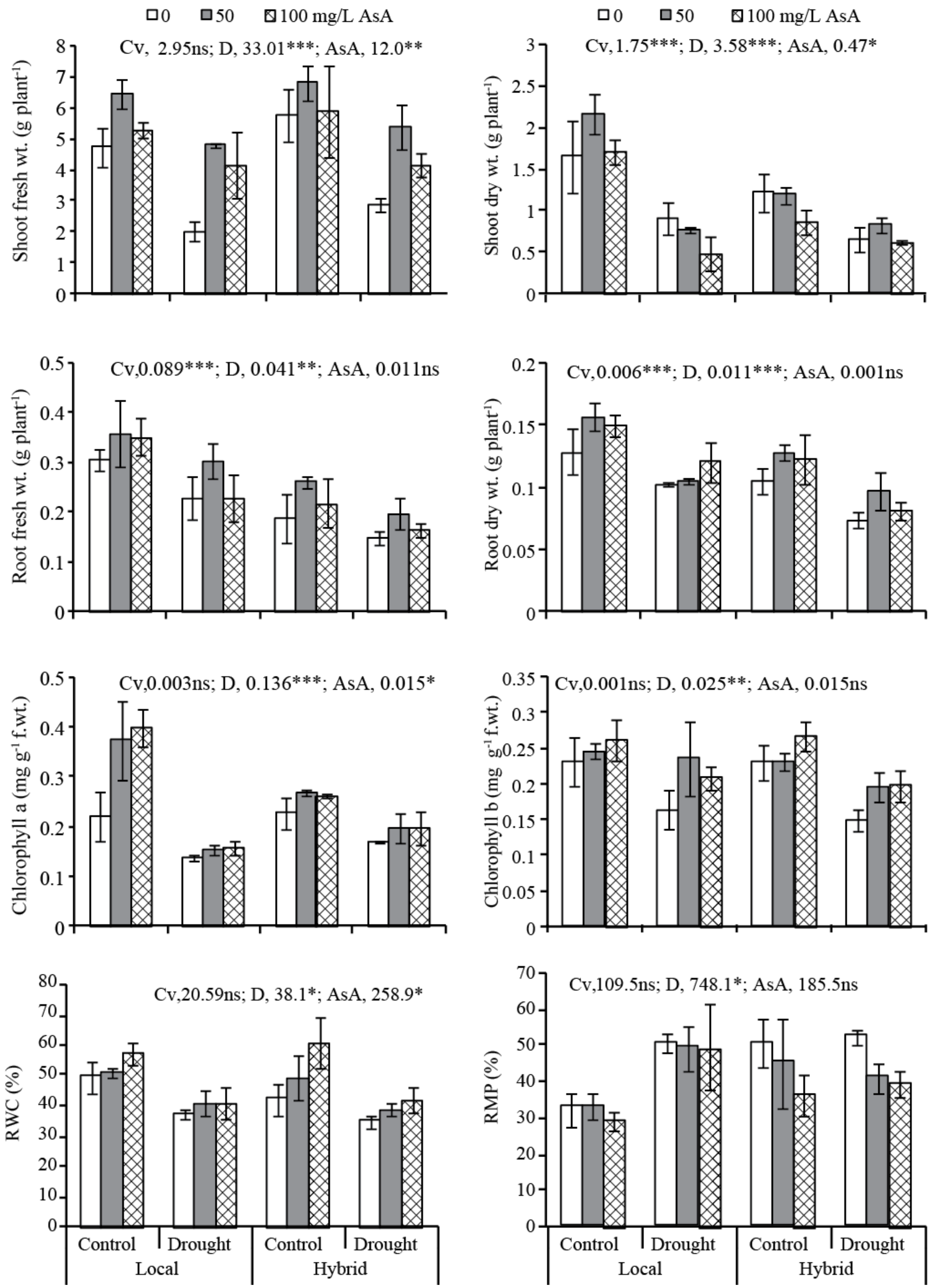

Fig. 1. Shoot and root fresh and dry weights, chlorophyll pigments, relative water content and relative membrane permeability of cucumber plants foliarly treated with ascorbic acid (AsA) grown under water deficit and non-stress conditions (Mean ± S.E.).
*, **, *** significant at 0.05, 0.01, 0.001 levels; ns, non significant; Cv, Cultivars and D, Drought.

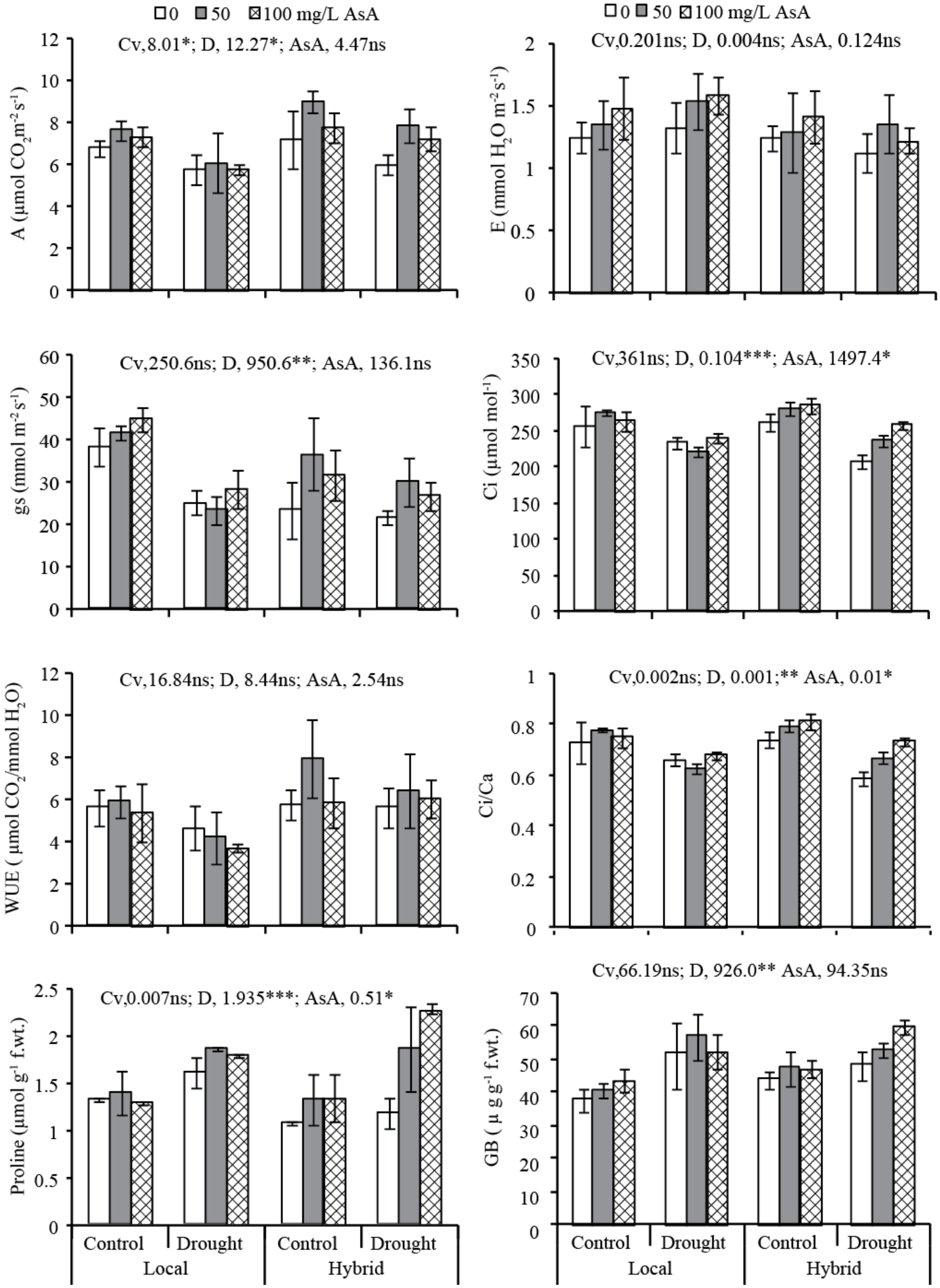

Fig. 2. Photosynthetic rate (*A*), transpiration (*E*), stomatal conductance (g_s), sub-stomatal CO_2 (C_i), water-use-efficiency (WUE), C_i/C_a, proline and glycine betaine contents of cucumber plants foliarly treated with ascorbic acid (AsA) grown under water deficit and non-stress conditions (Mean ± S.E.).

*, **, *** significant at 0.05, 0.01, 0.001 levels; ns, non-significant; Cv, Cultivars and D, Drought.

Discussion

It is well known that plant growth is severely restricted under water stress conditions due to a number of alterations in the physio-biochemical processes (Tas & Tas, 2007; Akram & Ashraf, 2011; Kosar *et al.,* 2015). In many economically important crops, a substantial reduction in growth and yield can be caused either by short- or long-term water stress (Javed *et al.,* 2011; Kamran *et al.,* 2009; Tesfamariam *et al.,* 2010). In the current study, water stress (60% field capacity) considerably inhibited the root and shoot fresh and dry weights of both (Local and Hybrid) cucumber cultivars. The adversaries of arid conditions on crop growth can be attributed to water-deficit induced disruption in chlorophyll biosynthesis, rate of transpiration or photosynthesis, stomatal regulations, essential nutrient uptake, signal transduction pathways, oxidative defense system, etc. (Arshad *et al.,* 2008; Sade *et al.,* 2011; Nagahatenna *et al.,* 2015). Of both cucumber cultivars, cv. Local was better in growth than cv. Hybrid under water limited environment.

Ascorbic acid as an antioxidant, is very effective in improving the plant biomass (Dolatabadian *et al.,* 2010; Ejaz *et al.,* 2012). In this work, varying levels (0, 50, and 100 mg L^{-1}) of foliar-applied AsA were effective in improving the plant growth of both cucumber cultivars. Previously, while working on okra (*Hibiscus esculentus*) plants, Amin *et al.* (2009) found that external treatment of AsA significantly improved the growth of okra plants and they attributed this growth improvement to AsA-induced stimulation in amino acids, protein contents and photosynthetic pigments under water stress conditions. Such type of beneficial results of AsA already deciphered by many researchers e.g. Malik & Ashraf (2012) in wheat, Dolatabadian *et al.* (2010) in corn, Shafiq *et al.* (2014) in canola under drought stress conditions.

One of the vital physiological processes, photosynthesis is mainly interlinked with chlorophyll biosynthesis (Ashraf & Harris, 2013). Chlorophyll contents usually decrease in plants on exposure to environmental stresses (Ashraf & Orooj, 2006; Ahmad *et al.,* 2007; Saleem *et al*., 2011). Due to inactivation of chlorophyll, rate of photosynthesis is also affected adversely (Amirjani & Mahdiyeh, 2013). Non-enzymatic antioxidant particularly ascorbic acid scavenges ROS and restricts chlorophyll degradation under stress conditions (Ashraf, 2009). In the present study, chlorophyll *a* and *b* contents decreased in both cucumber cultivars under dry conditions, and foliar-applied AsA was effective in improving chlorophyll *a* contents in both cucumber cultivars analogous to what has earlier been reported in drought-stressed plants of wheat (Azzedine *et al.,* 2011), cassia (Singh *et al.,* 2001), canola (Shafiq *et al.,* 2014), and maize (Dolatabadian *et al.,* 2009).

Gas exchange characteristics play a significant role in plant characteristics. Under water deficit conditions, alteration in photosynthesis and water-use-efficiency was observed and these processes are usually interrelated with stomatal regulation, internal CO_2 concentration and uptake of water at tissue or whole plant level under water deficit conditions (Kamran *et al.,* 2009). Foliar spray of AsA was found to be only effective in improving photosynthetic rate and C_i, so AsA-induced improvement in chlorophyll pigments can be interlinked with this change. During stress, activated oxygen compounds over-accumulate and production of antioxidants mitigates the adverse effects of water stress by scavenging H_2O_2, O_2^- and OH^- so they can be helpful in protecting the photosynthetic apparatus from oxidative damage (Ashraf, 2009; Ashraf & Harris, 2013). In the present study, RWC and WUE were found to be significantly reduced under water-deficit conditions. However, exogenous application of AsA was helpful in enhancing the RWC and water-use efficiency, so the AsA-induced improvement in these attributes can be associated with improvement in efficiency of AsA in water utilization and uptake which consequently can improve turgor potential and maintain water balance in cucumber plant tissues.

Both glycine betaine and proline contents significantly increased in cucumber plants under water-deficit conditions. However, foliar-applied AsA was effective only in enhancing the proline contents. The response of both cucumber cultivars was same in accumulation of proline or GB, so relatively better growth of cucumber cv. Local cannot be linked to these osmoprotectants. However, in accordance with our findings, a significant increase in GB and proline was already observed in a variety of plants under varying environmental cues (Zonouri *et al.,* 2014; Akram *et al.,* 2016). It is well established that high accumulation of osmoprotectants including trehalose, proline, GB and amino acids can be used as selection criteria for drought tolerance in different plant species (Ashraf & Foolad, 2007; Kamran *et al*., 2009).

In conclusion, drought stress significantly reduced plant growth, chlorophyll contents, relative water contents, rate of photosynthesis, stomatal conductance, C_i and C_i/C_a, while foliar applied AsA was useful in enhancing the plant growth, chlorophyll *a*, *A*, RWC, C_i, C_i/C_a and proline contents. Overall, growth improvement of cucumber plants under water stress treatments could be associated with AsA-induced improvement in chlorophyll pigments, proline, RWC contents and C_i concentration.

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