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BASIC AREAS - Article

Drought stress mitigation with humic acid in two *Cucumis melo* L. genotypes differ in their drought tolerance

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ABSTRACT: Different responses of two melon (*Cucumis melo* L.) genotypes (Şemame, drought and salt-tolerant and Ananas, drought and salt-sensitive) to drought stress with or without humic acid (HA) treatment were studied. The experiment was carried out under greenhouse conditions. The experimental design was two factorial randomized block with 4 replicates. HA treatment increased the shoot fresh and dry weights and leaf area of both genotypes under drought stress. HA stimulated accumulation of K and Ca ions, chlorophyll (SPAD value) and antioxidant enzyme activity (superoxide dismutase-SOD, catalase-CAT and glutathione reductase-GR) in both genotypes. This effect was more clear in the Şemame genotype than in Ananas. As a result, HA treatment has been proved to influence the ability of melon genotypes to cope with drought stress and to increase their tolerance.

Key words: melon, drought, plant breeding, lipid peroxidation, antioxidant enzymes.

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INTRODUCTION

Water deficit is generally known as drought and is expressed as the absence of the necessary water for normal plant growth and life cycle (Zhu 2002). Drought or water deficit considerably affects vegetable production in many parts of the world. It disturbs plant water relationships, reducing leaf size, root growth and root multiplication. Plants exhibit various physiological and biochemical responses to drought stress at cellular and whole organism levels. In leaves, closure of the stomata, membrane damage and changes in the activation of various enzymes occur (Zhang et al. 2013; Ariafar and Forouzande 2017; Hatami 2017; Kaya et al. 2018). Tolerance to water stress is a complex phenomenon involving a number of physiochemical processes at different stages of plant development. Various mechanisms have been developed by drought-tolerant plants to adapt to the stress. Examples of these mechanisms are: increased water uptake by developing large and deep root systems, reduction of water loss by accumulation of osmolites, prevention of membrane disintegration and enzyme activation, and increase in K and Ca ions uptake (Mahajan and Tuteja 2005; Lotfi et al. 2015; Kaya et al. 2018).

Melon (*Cucumis melo* L.) is an important horticultural product grown in arid and semiarid regions of the world. In general, it is known that melon is moderately resistant to drought, which causes various effects such as metabolic disturbances (Poudineh et al. 2015; Ariafar and Forouzandeh 2017). Application of humic acid (HA) to increase the resistance of drought tolerant melon genotypes is considered as a permanent method due to its anti-stress effects. Kulikova et al. (2005) reported that humic substances may work against environmental stresses. HA caused some changes in physical and chemical properties of the soil, such as water retention capacity, airing, pH and ion transportation (Lodhi et al. 2013). Humic substances are well known as stimulators of plant germination and growth (Dell'Amico et al. 1994). Arancon et al. (2006) reported that humic substances, which stimulate plant germination and growth, behave very similar to growth hormones. HA could promote plant growth by increasing the permeability of cell membrane, facilitate transport of essential elements within the roots and favor respiration (Cacco and Dell Agnolla 1984; Masciandaro et al. 2002). HA also positively affects the nutrient intake of plants and is particularly important for the transport and the availability of micronutrients (Sharif et al. 2002). HA, an important component of organic fertilizers and humic substances, can be used to improve plant growth by improving its leaves' water content, photosynthesis, antioxidant metabolism and enzymes activity, thus enhancing its tolerance (Fu Jiu et al. 1995; Al-Shareef et al. 2017). The aim of the present study was to assess the effects of HA treatment in two melon genotypes (Şemame, drought-tolerant, and Ananas, drought-sensitive) grown under drought stress conditions in terms of morphological, physiological and biochemical parameters.

MATERIALS AND METHODS

Plant Materials and Treatments

The study was carried out under controlled conditions in a greenhouse at Soil, Fertilizer and Water Resources Central Research Institute in Ankara (Turkey) from May 10th to the end of July 2017. The conditions in the greenhouse were as follows: relative humidity 50%-55%, and temperatures were 18/30 °C (day/night). Seeds of melon genotype were sown in pots filled with mixture of vermiculite:perlitle [1:1 (v/v)] (May 10th). At the 3-4 leaves stage (28 days-old), seedlings were transplanted into 7-L volume pots (22 cm deep and 25 cm diameter) containing medium-textured soil (soil texture: sand clay loam, pH; 7.75, EC; 1.28 dS·m^{-1}, organic matter: 0.54%, nitrogen: 0.18%, phosphate: 3.60%, potash: 0.86%), four seedling in each pot. At first, all pots were irrigated to field capacity. Pot weight was taken into account when determining the amount of irrigation water, and the pots were weighed on daily basis. Following planting of the seedlings, fertilizers containing 100 mg·kg^{-1} N, 25 mg·kg^{-1} P and 100 mg·kg^{-1} K were applied. One week after planting, the irrigation treatments started. The experiment was conducted in a randomized plots design with 2 factors and 4 replications. In the study, the first factor is the local Turkish melon genotypes (Şemame, drought and salt-tolerant, and Ananas, drought and salt-sensitive) (Kusvuran et al. 2011) and the second factor is the treatments (1. Nonstress control and HA: 100% of field capacity irrigation, 2. Drought stress: 50% field capacity irrigation and 3. Drought stress + HA: 50% field capacity irrigation + 2000 mg·L^{-1} HA). HA, contained 46% humic and fulvic acid.

HA was applied on the plants as liquid treatment with irrigation water within 3 days until reaching the final concentration of 2000 mg·L^{-1} (to obtain 2000 mg·L^{-1} of HA, →

500 mg·L^{-1} were added on the first two days and 1000 mg·L^{-1} of HA on the third day). Stress treatment started 35 days after seed sowing and plants were kept under these conditions for 42 days until harvest. Morphological measurements were made at harvest, shoot and roots were separated and weighed. Leaf samples were frozen in liquid nitrogen and stored at –80 °C for later physiological and biochemical analysis.

Morphological Evaluation

At the end of the study, random plants from each treatment and/or replicate were selected, then they were separated into shoots and roots. After the plants fresh weights were recorded, they were dried at 65 °C for 48 hours and the shoot and root dry weights were taken. The plants samples shoot and root lengths were measured using a digital ruler. For leaf area measurements, a leaf area meter of Licor LI-3000A was used.

Potassium (K) and Calcium (Ca) Ion Analysis:

K and Ca ions content of dried samples (1 g) digested in concentrated 0.1 N nitric acid (HNO_3) and perchloric acid ($HClO_4$) (4:1) were determined using coupled plasma atomic emission spectrometry (ICP-AES, Perkin Elmer Plasma 2000) (Kacar and İnal 2008).

Chlorophyll, Hydrogen Peroxide (H_2O_2) and Lipid Peroxidation Assay

SPAD index was measured by using chlorophyll meter SPAD 502 (Konica Minolta Sensing, Inc. Osaka/Japan). For each read, the average of 5 SPAD values measured at different points on a leaf was taken. The levels of hydrogen peroxide (H_2O_2) in melon leaves were measured according to Patterson et al. (1984). Lipid peroxidation was estimated by determining malondialdehyde (MDA) content in the leaves (Lutts et al. 1996) using an extinction coefficient of 155 mmol·L^{-1}.

Enzymatic Activities

For enzymes extraction, 0.5 g leaf samples were grinded in liquid nitrogen and milled with 5 mL extraction buffer (50.0 mmol·L^{-1} K-phosphate buffer, pH 7.6, and 0.1 mmol·L^{-1} Na_2-EDTA). The homogenate was centrifuged at 15000 rpm for 15 min and a supernatant was used for reading the different enzymes activity spectrophotometrically. The activity of superoxide dismutase (SOD), was assayed according to Cakmak and Marschner (1992). Accordingly, the reduction of nitro blue tetrazolium (NBT) induced by the superoxide radical at 560 nm is assumed. One unit of SOD activity was calculated as the amount of enzyme causing 50% inhibition of NBT reduction. Catalase activity was determined according to the method of Cakmak and Marschner (1992) and the disappearance of H_2O_2 at 240 nm was observed. The glutathione reductase (GR) activity was determined by the rate of decrease in absorbance of oxidized glutathione at 340 nm (Cakmak and Marschner 1992). One enzyme unit was defined as mmol·mL^{-1} oxidized glutathione per min.

Statistical Analysis

Analysis of variance (ANOVA) was performed to determine significant differences. The experimental design was a two-factor randomized block with 4 replicates. Means were seperated using Duncan Multiple Range Test at $p < 0.05$.

RESULTS AND DISCUSSION

The effect of "treatment and genotype" interaction on shoot fresh weight and leaf area ($p < 0.01$), shoot dry weight and root length ($p < 0.05$) was significant (Table 1). Fresh and dry weights of shoot-root, lengths of shoot-root and leaf area were decreased in both melon (*Cucumis melo* L.) genotypes as a response to drought stress (Table 2 and Fig. 1). Kron et al. (2008) reported that the decrease in transpiration rate under water stress significantly decreases plant length and dry matter content. The reduction was much higher in Ananas genotype than Şemame compared to the control plants. Şemame has been able to better preserve its relative water content, stomatal conductance, and leaf water potential under drought stress (Kıran et al. 2014). Furthermore, the ability of Şemame to absorb more water than Ananas may be related to its ability to stimulate the synthesis of these osmotic solutes (Gagneul et al. 2007). The accumulation and the roles of osmotic dissolutions have been revealed under drought stress. However, HA treatment has stimulating effects under drought condition, in terms of shoot fresh and dry weights of Ananas and in the stem length of Şemame. The stimulatory effect of HA was observed in both genotypes in terms of leaf area. In addition, humic substances have been →

shown to have stress-reducing effects on plants exposed to drought stress (Kulikova et al. 2005; Aydin et al. 2012). With its chelating properties, HA can increase nutrient uptake such as nitrogen and zinc, and support plant growth by reducing water loss (Zhang et al. 2013; Hatami 2017).

K and Ca are known for their important role in enhancing plants tolerance against drought stress (Nasri et al. 2008; Yuan-yuan et al. 2009). Water stress or drought prevents plants from uptaking nutrients from their growing media, which causes deficiency symptoms of such nutrients on plants (Abdalla and El-Khohiban 2007; Arjenaki et al. 2012). "Treatment and genotype" interaction was significant on K and Ca contents ($p < 0.05$ and $p < 0.01$, respectively) (Table 3). The results show that drought reduces the levels of K and Ca ions in the leaves of both genotypes (Table 2). Under drought stress, K and Ca ions contents decreased more in the Ananas genotype than in Şemame (about 4%, 9% and 17%, 15%, respectively) (Table 2). HA treatments resulted in an increase content of K and Ca and in both genotypes when compared to the control plants (Kaya et al. 2018).

In the two genotypes, SPAD chlorophyll level decreased under drought stress ($p < 0.05$) (Table 3), compared to the control chlorophyll content decreased by 15% in Şemame and 39% in Ananas melon genotype (Table 4). The reduction →

Table 1. ANOVA for morphological parameters related to the traits of melon genotypes treated with HA treatment under drought stress.

Source of variance	*df*	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	Shoot length	Root length	Leaf area
Treatment(T)	3	**	**	*	**	**	*	**
Genotype(G)	2	**	**	ns	**	**	ns	**
T x G	6	**	**	ns	ns	ns	*	**
Error	18	7.52	0.15	0.38	0.03	37.58	0.82	8.77
CV(%)		7.33	8.28	16.07	13.42	13.14	7.44	6.87

ns: nonsignificant, ** and *, significant in 1% and 5% area.

Table 2. The effect of "treatment x genotype" interaction on fresh and dry weights of the shoot, root length, leaf area, K and Ca contents.

Treatment	Genotype	Shoot fresh weight	Shoot dry weight	Root length	Leaf area	K	Ca
		($g \cdot plant^{-1}$)		cm	cm^2	(%)	
Control	Şemame	45.32 a	5.59 a	12.75 ab	56.74 a	1.24 b	1.55 a
	Ananas	39.12 b	4.85 b	13.25 a	54.57 a	1.11 b	1.09 c
DS	Şemame	40.51 b	5.22 ab	10.75 c	40.56 b	1.19 b	0.86 d
	Ananas	24.56 d	3.29 d	12.50 ab	28.25 c	0.92 c	0.92 d
DS + HA	Şemame	42.48 ab	5.31 ab	12.50 ab	41.23 b	1.40 a	1.39 b
	Ananas	32.45 c	4.08 c	11.67 bc	37.25 b	1.46 a	1.28 b

Within each column, means followed by the same letter do not differ significantly at $p < 0.05$. Drought stress (DS), humic acid (HA).

Table 3. ANOVA for some physiological and biochemical parameters related to the traits of melon genotypes treated with HA treatment under drought stress.

Source of variance	*df*	K	Ca	Chlf Spad	H_2O_2	MDA	SOD	CAT	GR
Treatment(T)	3	**	**	**	**	**	**	**	**
Genotype(G)	2	*	**	ns	**	**	**	ns	**
T x G	6	*	**	*	**	**	**	**	**
Error	18	0.01	0.01	3.92	0.01	0.02	5.34	38.93	0.03
CV(%)		8.67	7.37	8.81	11.35	5.22	8.56	4.56	4.81

ns: nonsignificant, ** and *, significant in 1% and 5% area. Chlorophyll (Chlf), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR).

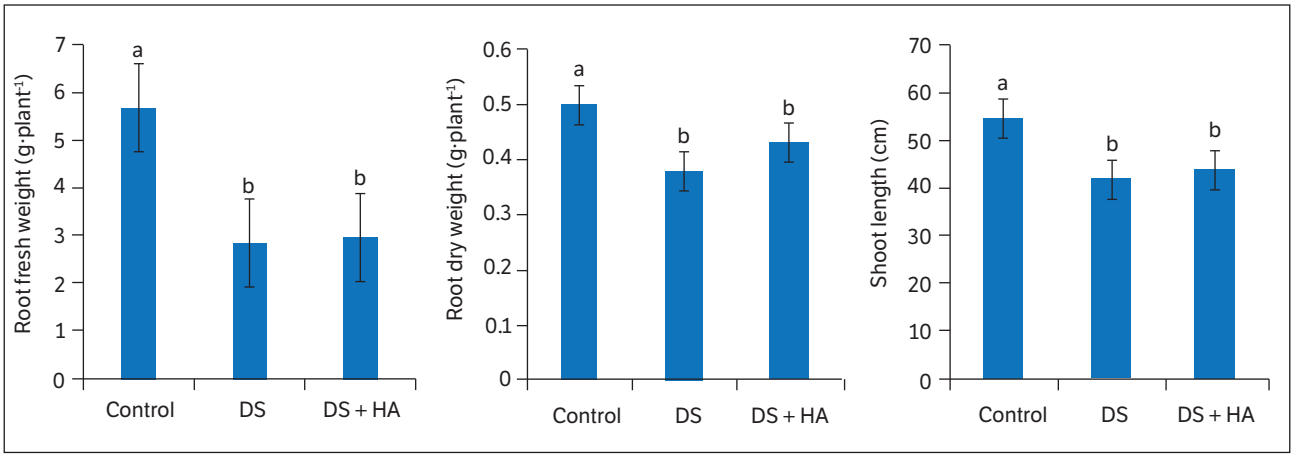

Figure 1. Effects of treatments on root fresh and dry weights and shoot length of genotypes. Means followed by the same letter do not differ significantly at $p < 0.05$. The data are means (± standard error) of four replication. Drought stress is shown as DS and humic acid as HA.

Table 4. The effect of "treatment x genotype" interaction on chlorophyll (Chlf), hydrogen peroxide content (H_2O_2) and malondialdehyde (MDA) contents, superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) activities.

Treatment	Genotype	Chlf	H_2O_2	MDA	SOD	CAT	GR
		Spad value	μmol·g^{-1} FW		U·min^{-1}·mg^{-1} FW		μmol·min^{-1}·mg^{-1} FW
Control	Şemame	24.00 ab	0.25 c	1.67 e	18.03 c	122.47 c	2.99 d
	Ananas	26.73 a	0.18 d	1.88 d	19 36 c	83.20 e	2.75 d
DS	Şemame	20.25 c	0.36 b	2.82 b	32.04 b	146.24 b	4.18 b
	Ananas	16.25 d	0.49 a	3.53 a	19.61 c	109.86 d	2.93 d
DS +HA	Şemame	24.23 ab	0.19 d	1.76 de	38.30 a	138.75 b	4.74 a
	Ananas	22.96 bc	0.27 c	2.24 c	34.63 b	221.29 a	3.76 c

Within each column, means followed by the same letter do not differ significantly at $p < 0.05$. Drought stress; DS, humic acid; HA.

in chlorophyll concentration in plants exposed to drought can be attributed to the increased activity of the chlorophyll-degrading enzyme (Reddy and Vora 1986). With HA, both genotypes developed chlorophyll pigment synthesis in plants exposed to drought stress. The influence of chlorophyll production increase due to HA was more obvious in Ananas (40%) compared to Şemame. Indeed, HA can chelate and improve uptake micronutrients such as Fe and Mn and make them readily available for the plant (Rupiasih et al. 2013; Meganid et al. 2015).

In our study, the effect of "treatment and genotype" interaction on H_2O_2 accumulation was found significant ($p < 0.01$) (Table 3). H_2O_2 was increased in both genotypes, in response to drought stress compared to the control plants (about Şemame, 44% and Ananas, 172%) (Table 4). In the literature, drought-tolerant genotypes have shown to have less membrane damage and H_2O_2 content than the sensitive ones (Sairam and Srivastava 2001; Kaya et al. 2018). HA treatment resulted in lower H_2O_2 accumulation in Şemame plants compared to untreated plants, but there was a higher level of accumulation in Ananas. This demonstrates lower reactive oxygen species (ROS) accumulation in the tolerant genotype, and therefore HA treatment is more effective.

The results in Table 3 show that MDA significantly increased as a result of drought stress in both genotypes ($p < 0.01$). When these increases were compared to the control plants, it was found to be 68% in Şemame and 87% in Ananas (Table 4). In HA treatments, plants exposed to drought stress showed a decrease in MDA content compared to plants not treated with HA and this result was more remarkable in the tolerant genotype Şemame. MDA is considered an indicator of lipid peroxidation assessment and damage to membranes (Lotfi et al. 2015). In the study, HA treatment limited the oxidation stress in melon genotypes under drought stress conditions. These effects can be attributed to the increase in antioxidant enzyme activities such as SOD, CAT and GR (Table 4). In addition, HA increases uptake of ions and cell permeability (Chen et al. 1990).

SOD, CAT and GR are the main enzymes detoxifies ROS (Gill and Tuteja 2010). In this study, antioxidant enzymes were significantly affected by drought stress (Table 3) ($p < 0.01$). The activity of SOD, CAT and GR enzymes was significantly increased (in Şemame respectively: 78%, 19% and 40%, in Ananas respectively: 1%, 32% and 7%) compared to the control plants (Table 4). Similar results have been reported by Lotfi et al. (2015), Hafez and Seleiman (2017) and Kaya et al. (2018). In addition to that, HA treatment significantly stimulated the activity of SOD, CAT and GR antioxidant enzymes in both genotypes subjected to drought stress. But the activities of SOD, CAT and GR were markedly stimulated only in Ananas (in Şemame respectively: 20%, 5% and 13%, in Ananas respectively: 76%, 101% and 28%) due to HA treatment (Table 4). It has been reported that higher SOD and GR activities of tolerant genotypes are associated with a more active ascorbate-glutathione cycles of these plants (Azooz 2004). In this study, the stimulation of SOD and CAT activity by the HA treatment in the sensitive genotype under stress may contribute to its tolerance to drought.

CONCLUSION

HA treatment has significant effect on the ability of melon genotypes to cope with drought stress and contributes to their tolerance. This contribution is more evident in the Şemame genotype. This seems to be particularly related to the ability to increase antioxidant enzyme activities to a high level.

AUTHORS' CONTRIBUTION

Conceptualization, Kıran S. and Ellialtıoğlu Ş. Ş.; Methodology, Kıran S. ; Investigation, Kıran S. U. and Baysal Furtana G.; Writing – Original Draft, Kıran S. and Baysal Furtana G.; Writing – Review and Editing, Talhouni M.; Resources, Baysal Furtana G. and Talhouni M.

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