

# The Improvement of Growth, Water Relations, and Oxidative Reduction of Pistachio Rootstock in Drought Stress Conditions with Beta-Aminobutyric Acid Pretreatment

Poboljšanje rasta, odnosa vode te smanjenje oksidativnih oštećenja podloga pistacija u uvjetima sušnoga stresa predtretmanom beta-aminobutričnom kiselinom

**Karimi, S., Shokri, M.**

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**Fakultet agrobiotehničkih znanosti Osijek, Poljoprivredni institut Osijek**

Faculty of Agrobiotechnical Sciences Osijek, Agricultural Institute Osijek

# THE IMPROVEMENT OF GROWTH, WATER RELATIONS, AND OXIDATIVE REDUCTION OF PISTACHIO ROOTSTOCK IN DROUGHT STRESS CONDITIONS WITH BETA-AMINOBUTYRIC ACID PRETREATMENT

Karimi, S., Shokri, M.

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## SUMMARY

*Environmental stresses are inevitable during the plant growth. The  $\beta$ -aminobutyric acid (BABA) induces systemic resistance in plants. The efficiency of BABA pretreatments (0.5 and 10 mM) in inducing drought tolerance in the 'Akbari' pistachio rootstock in controlled environmental conditions was investigated. The BABA solutions were sprayed three times every two days on pistachio seedlings at the 15-leaf stage. Subsequently, the irrigation of plants was withheld for 25 days, and the effects of BABA pretreatments on the growth, water relations, and oxidative damage indices in plants were investigated. The BABA pretreatments prevented the reduction of shoot dry and fresh mass under a drought stress by inhibiting the leaf abscission and maintaining the relative water content. Owing to the leaf abscission, leaf water potential and turgidity of drought-stressed plants were similar to the control plants. However, the measurement of osmotic potential indicated that the BABA pretreatments improved turgor potential in leaves of the drought-stressed plants by inducing osmotic regulation. A declined membrane stability index and chlorophylls, as well as the malondialdehyde accumulation in line with the hydrogen peroxide accumulation in the leaves, indicated the development of oxidative damage under drought. Still, the BABA pretreatment restricted the severity of oxidative damages. In this regard, a 5 mM BABA pretreatment was the most effective one in the anti-drought plant protection.*

**Keywords:** malondialdehyde; osmoregulation; oxidative damage; *Pistacia vera L.*; water relation

## INTRODUCTION

Global warming and climate change have become serious threats to agricultural production in the third millennium. These phenomena have caused widespread droughts and intensified salinization of lands in arid and semi-arid regions. The continuation of this trend will lead to a sharp decline in access to water for agriculture in these areas. Since the normal plant-cell metabolism directly or indirectly depends on water availability (Farooq et al., 2009), drought and water shortage are the most important factors limiting the growth and yield of plant crops. A decreased osmotic and water potential, along with the disappearance of turgidity of

tissues, stomata closure, and a reduced growth are the specific symptoms of drought stress (Gleason et al., 2017). Severe drought stress reduces photosynthesis, disrupts physiological processes, inhibits plant growth, and eventually leads to plant death (Farooq et al., 2009). Under drought stress conditions, the concentration of mineral elements in the soil solution increases, which is accompanied by an increase in the osmotic pressure of the soil solution and may lead to a decreased metabolic activity of the roots, which limits the absorption

(1) Assoc. Prof. Soheil Karimi (skarimi@ut.ac.ir), Maryam Shokri, M. Sc. – student – University of Tehran, College of Aburairhan, Imam Reza Blvd., Pkadasht, 3391653755 Tehran, Iran.

of elements and reduces the concentration of nutrient elements in leaves (Isaakidis et al., 2004).

The application of new technologies to improve the water distribution and the identification and utilization of drought-tolerant species and genotypes are valuable approaches to the development of agriculture in dry lands (Karimi et al., 2017a). However, even the tolerant plants manifest a high sensitivity to drought stress during some critical periods of growth and need special care and support. In this regard, seed and plant preconditioning techniques have demonstrated a high efficiency in preparing plants to deal with drought during critical growth stages. The preconditioning treatments consist of the use of physical or chemical treatments that enhance the expression of genes related to the stress tolerance in plants (Funnekotter et al., 2016). As a result, the preconditioned plants are able to respond to sudden changes in environmental conditions in a faster and stronger way (Karimi et al., 2017b). The  $\beta$ -aminobutyric acid (BABA) is a non-protein amino acid that acts as a signal associated with environmental and biological stresses (Tavallali et al., 2008). Studies have circumstantiated that the application of this substance can increase the expression of many genes associated with the plant responses to stress (Vijayakumari et al., 2016). In this regard, the BABA prepares the plants for unfavorable conditions by increasing the transcription of abscisic acid and ethylene genes (Zimmerli et al., 2008; Schwarzenbacher et al., 2020). Increasing the accumulation of abscisic acid following the application of BABA increases the plant's tolerance to salinity and drought stress by accelerating stomatal closure and reducing water consumption (Karimi et al., 2017b). Moreover, an increased activity of antioxidative enzymes has been reported following the application of BABA (Du et al., 2012; Baccelli and Mauch-Mani, 2016).

Assuming that the induction of such responses may induce drought tolerance, the effects of BABA application on drought tolerance of 'Akbari' pistachio (*Pistacia vera* L.) seedlings in the present study were evaluated under controlled environmental conditions. Pistachios are cultivated in hot and dry regions of the world, and therefore the pistachio orchards are always threatened by drought and water shortage. In the early stages of growth, when the young transplants have a limited root system, drought can impose more destructive effects on this plant and cause a stunted growth. Consequently, introducing efficient preconditioning methods to induce drought tolerance in the young pistachio seedlings is important.

## MATERIAL AND METHODS

Germinated seeds of the 'Akbari' pistachio were planted in pots containing perlite and coco peat (ratio 2:1 w:w). Each pot contained the same amount of the culture medium (1400 g). The plants were irrigated with distilled water until a two-leaf stage. Subsequently, the

plants were irrigated with the half-strength Hoagland's nutrient solution (pH = 7.1) every other day. Uniform seedlings at the fifteen-leaf stage were selected for the experiments 120 days after planting.

To evaluate the effects of BABA preconditioning on drought tolerance of the plants, four treatments were applied: 1) Control treatment (irrigated plants), 2) Drought stress treatment, 3) 5 mM BABA pretreatment + drought stress, and 4) 10 mM BABA pretreatment + drought stress. In the control treatment, the plants were irrigated to the field capacity (FC) level during the two-day intervals. During each irrigation, the pot weight was increased to the FC level. In treatments 3 and 4, during one week before imposing drought stress, 10 ml of the respective BABA solutions were sprayed on the plants three times during a two-day interval. Then, the plants were subjected to the drought stress. Drought stress was imposed by withholding the plant irrigation. In this regard, the pots were irrigated to the FC level, and their irrigation was stopped for 25 days thereafter. In treatment 4, the plants were subjected to the drought stress treatment without any BABA pretreatments. To restrict the rate of pot dehydration, the growing medium surface was covered with a polyethylene sheet.

At the end of the drought period, leaf number, plant height, shoot biomass, leaf area and plant water content were measured. Leaf greenness was assessed using a leaf chlorophyll meter device (Soil Plant Analysis Development, SPAD-502; Minolta Camera Co., Japan). Leaf water potential ( $\Psi_L$ ) was measured by a pressure bomb device (Soil Moisture Equipment Corp., USA) at midday. Leaf osmotic potential ( $\Psi_S$ ) was measured using a cryoscopic osmometer (Osmomat 030, Gonotec, Germany). Turgor potential ( $\Psi_p$ ) was calculated by subtracting the osmotic potential from the leaf water potential. Relative water content (RWC) was measured in the fully developed young leaves. Fresh mass (FM) of 15 uniform leaf disks were measured. Later, the leaf discs were rehydrated in distilled water in the darkness, and their turgid mass (TM) was recorded. Having the samples dried at the temperature of 75 °C for 72 h, the samples' dry mass (DM) was recorded. The RWC was calculated applying the following equation (Eq. 1):

$$\text{Eq. 1. RWC} = 100 \times (\text{FM} - \text{DM}) / (\text{TM} - \text{DM})$$

Membrane stability index (MSI) was measured in the leaves according to the method discussed by Arora et al. (1998). Fifteen leaf disks from the expanded young leaves were floated in 10 ml of deionized water and heated to 40 °C for 30 min. The samples' electrical conductivity was measured ( $\text{EC}_1$ ) and the samples were heated in boiling water for 10 min. Subsequent to the measurement of samples' electrical conductivity for the second time ( $\text{EC}_2$ ), the MSI was calculated applying Eq. 2:

$$\text{Eq. 2. MSI} = 1 - (\text{EC}_1/\text{EC}_2) \times 100$$

The concentration of chlorophylls was measured in 20 leaf disks after extraction with the eighty-percent acetone. After centrifugation, the absorbance of supernatants was measured at 663.2 and 646.8 nm, and the concentrations of pigments were calculated using the formula developed by Lichtenthaler (1987).

The leaves' hydrogen peroxide ( $H_2O_2$ ) content was determined according to the method developed by Velikova et al. (2000). After extraction with the trichloroacetic acid (TCA) and potassium phosphate buffer, the samples were centrifuged. KI was added to the samples, and their absorbance was measured at 390 nm using a spectrophotometer (PerkinElmer, Lambda 25, USA). Malondialdehyde (MDA) concentration in the leaves was determined according to the method described by Heath and Packer (1968). The samples were extracted with the TCA, and the supernatants were mixed with thiobarbituric acid solution in the twenty-percent TCA after centrifuging. Subsequent to the heating in boiling water bath for 30 mins., the samples' absorbance was measured at 532 and 600 nm. After withdrawing the nonspecific absorbance at 600 nm, MDA was calculated applying an extinction coefficient of  $155 \text{ mM cm}^{-1}$ .

The experiments related to this study were performed in a greenhouse with the day/night temperatures of 26/22 °C and relative humidity of 20%. The experiment was performed in a completely randomized design with seven replicates. Statistical analyses were performed using the SPSS v. 21. Duncan's multiple range test (DMRT) at a probability level of  $P \leq 0.05$ , which was applied for the sake of a mean comparison.

## RESULTS AND DISCUSSION

Drought stress significantly reduced plant growth indices. Plant height, shoot biomass, and fresh mass decreased under the drought stress compared to the control treatment (Table 1). The BABA pretreatments mitigated the growth of the drought-stressed plants to the control plants level. In most of the measured growth indices, no significant difference was observed between the 5- and 10-mM BABA treatments (Table 1). Growth inhibition is the first obvious response of plants to drought conditions (Gupta et al., 2020). Pistachio growth is reduced under the drought stress (Tajabadi Pour, 2004; Khoyardi et al., 2016). It is known that water deficiency by decreasing the cell turgidity affects the cell growth and development, especially in the stems and leaves (Yadollahi et al., 2011). Therefore, the early noticeable effects of dehydration are the formation of smaller leaves and a reduced plant height (Gupta et al., 2020). Besides, a part of the growth limitation under the drought stress is effectuated due to a declined carbon assimilation. Thus, the drought stress can directly affect the biochemical processes related to photosynthesis and can indirectly reduce the carbon dioxide entry into the leaf (Karimi et al., 2015). A water content reduction leads to the concentration of chlorophyll in leaf tissue, which can describe a marginal increase in leaf greenness of the drought-stressed plants. The BABA pretreatment resulted in the maintenance of leaves and the plant growth under drought (Table 1). However, in this regard, 5 mM BABA pretreatment was more effective than the 10 mM one. These improvements were primarily observed due to the plant's increased ability to preserve water in the leaf tissue and secondly due to the improvement in plant health status.

**Table 1. The effects of BABA pretreatments on shoot growth and leaf greenness (SPAD values) of pistachio rootstock under drought stress**

Tablica 1. Utjecaj predtretmana BABA na rast izbojka i indeks sadržaja klorofila (SPAD) podloga pistacije u uvjetima sušnog stresa.

Treatments / <i>Tretmani</i>	Plant height (cm) / <i>Visina biljke (cm)</i>	Shoot dry mass (mg) / <i>Suha masa izbojka (mg)</i>	Shoot fresh mass (g) / <i>Svježa masa izbojka (g)</i>	Leaf greenness (SPAD value) / <i>Indeks sadržaja klorofila (SPAD)</i>
Irrigated / <i>Navodnjavano</i>	34.2 <sup>ab</sup>	846.7 <sup>a</sup>	3.43 <sup>a</sup>	41.7 <sup>a</sup>
Drought stress (D) / <i>Sušni stres</i>	29.0 <sup>c</sup>	752.6 <sup>b</sup>	2.57 <sup>b</sup>	46.4 <sup>a</sup>
BABA 5mM + D	36.2 <sup>a</sup>	874.9 <sup>a</sup>	3.35 <sup>a</sup>	42.4 <sup>a</sup>
BABA 10mM + D	33.1 <sup>b</sup>	863.6 <sup>a</sup>	3.21 <sup>a</sup>	41.6 <sup>a</sup>
<i>ANOVA</i>				
Treatments / <i>Tretmani</i>	11812.6*	22293.7**	981828.9**	12.93 <sup>ns</sup>
Error / <i>Pogriješka</i>	1839.6	3228.4	31118.5	10.13

† The differences among the treatments were analyzed by the ANOVA; \*\*, \*, and <sup>ns</sup> indicate that the treatment effects were significant at  $P \leq 0.01$  and  $P \leq 0.05$  or non-significant, respectively. The means ( $n = 7$ ) followed by the same letters are not significantly different according to the DMRT at  $P \leq 0.05$ . / *Razlike između tretmana analizirane su ANOVOM; \*\*, \* i <sup>ns</sup> indiciraju da su učinci tretmana bili značajni pri  $P \leq 0,01$  i  $P \leq 0,05$ , odnosno beznačajni. Srednje vrijednosti ( $n = 7$ ) iz kojih slijede ista slova nisu značajno različite prema DMRT-u pri  $P \leq 0,05$ .*

A decrease in shoot fresh mass occurred primarily due to the leaf abscission, which was accompanied by a significant reduction in plant leaf area under the drought stress (Table 2). A decreased leaf area is a drought adaptation mechanism that assists the plant to survive under the drought stress by reducing the transpiration surface (Mirfattahi et al., 2017). Since the average area of each leaf remained unchanged under the drought stress (Table 2), it was concluded that the level of evapotranspiration of pistachios is limited by the leaf abscis-

sion during a short-term drought stress. Thus, the rate of the plant's leaf area decline indicates the severity of drought stress inflicted to the plant. In the plants treated with the 5 mM BABA, leaf abscission was not observed under the drought stress, but in the plants treated with 10 mM BABA the plant leaf area decreased; however, this effect was less pronounced than in the untreated plants. These results indicate a higher efficiency of 5 mM BABA treatment in the improvement of pistachio seedlings' drought tolerance.

**Table 2. The effects of BABA pretreatments on leaf growth indices in pistachio rootstock under drought stress**

Tablica 2. Učinci preddretmana BABA na pokazatelje rasta listova podloga pistacije pod sušnim stresom.

Treatments / <i>Tretmani</i>	Leaf no.(per plant) / <i>Br. listova (po biljci)</i>	Leaf no.(changes) / <i>Br. listova (promjene)</i>	Mean leaf area (cm <sup>2</sup> ) / <i>Srednja površina lista (cm<sup>2</sup>)</i>	Leaf area (cm <sup>2</sup> ) / <i>Površina lista (cm<sup>2</sup>)</i>
Irrigated / <i>Navodnjavano</i>	19.1 <sup>a</sup>	+5.7 <sup>a</sup>	3.23 <sup>a</sup>	68.6 <sup>a</sup>
Drought stress (D) / <i>Sušni stres</i>	16.0 <sup>b</sup>	-9.6 <sup>c</sup>	3.01 <sup>a</sup>	49.8 <sup>b</sup>
BABA 5mM + D	20.3 <sup>a</sup>	+2.4 <sup>ab</sup>	3.47 <sup>a</sup>	70.4 <sup>a</sup>
BABA 10mM + D	18.8 <sup>a</sup>	+0.9 <sup>b</sup>	3.39 <sup>a</sup>	64.5 <sup>a</sup>
<i>ANOVA</i>				
Treatments / <i>Tretmani</i>	3594.2*	6805.4**	256.5 <sup>ns</sup>	2561.2*
Error / <i>Pogrješka</i>	954.9	1581.3	190.6	753.4

† The differences among the treatments were analyzed by the ANOVA; \*\*, \*, and <sup>ns</sup> indicate that the treatment effects were significant at  $P \leq 0.01$  and  $P \leq 0.05$  or non-significant, respectively. The means ( $n = 7$ ) followed by the same letters are not significantly different according to the DMRT at  $P \leq 0.05$ . / *Razlike između tretmana analizirane su ANOVOM; \*\*, \* i <sup>ns</sup> indiciraju da su učinci tretmana bili značajni pri  $P \leq 0,01$  i  $P \leq 0,05$ , odnosno beznačajni. Srednje vrijednosti ( $n = 7$ ) iza kojih slijede ista slova nisu značajno različite prema DMRT-u pri  $P \leq 0,05$ .*

In addition to the leaf abscission, a part of the shoot fresh mass loss of was effectuated due to a significant reduction in the plant's water content (Table 3). In the drought stress treatment, leaf RWC was significantly lower than that in the other treatment (Table 3). Therefore, it can be concluded that the BABA pretreatment prevented a loss in the fresh mass by improving the plant's water level. Previous research has proven that BABA enhances the stomata control over water loss under a drought stress (Du et al., 2012; Lievens et

al., 2017; Schwarzenbacher et al., 2020) while inducing the abscisic acid accumulation in leaves.

Investigating water potential components indicated that the BABA pretreatments or drought stress did not have significant effects on the  $\Psi_L$  and  $\Psi_P$  in the leaf tissue (Table 3).  $\Psi_S$  in the BABA-preconditioned plants was significantly lower than that in other treatments. The highest  $\Psi_S$  was observed in the control and drought stress treatments (Table 3).

**Table 3. The effects of BABA pretreatments on leaf water potential ( $\Psi_L$ ), osmotic potential ( $\Psi_S$ ), turgor potential ( $\Psi_P$ ) and relative water content (RWC) of pistachio rootstock under drought stress**

Tablica 3. Utjecaj preddretmana BABA na vodni potencijal lista ( $\Psi_L$ ), osmotski potencijal ( $\Psi_S$ ), potencijal elastičnosti ( $\Psi_P$ ) i relativan sadržaj vode (RWC) podloga pistacije pod sušnim stresom.

Treatments / <i>Tretmani</i>	$\Psi_L$ MPa	$\Psi_S$ MPa	$\Psi_P$ MPa	RWC (%)
Irrigated / <i>Navodnjavano</i>	-0.955 <sup>a</sup>	-1.909 <sup>a</sup>	0.954 <sup>a</sup>	85.6 <sup>a</sup>
Drought stress (D)	-0.940 <sup>a</sup>	-1.920 <sup>a</sup>	0.980 <sup>a</sup>	75.1 <sup>b</sup>
BABA 5mM + D	-1.020 <sup>a</sup>	-2.198 <sup>b</sup>	1.178 <sup>a</sup>	85.6 <sup>a</sup>
BABA 10mM + D	-0.990 <sup>a</sup>	-2.116 <sup>b</sup>	1.126 <sup>a</sup>	86.8 <sup>a</sup>
<i>ANOVA</i>				
Treatments / <i>Tretmani</i>	48.39 <sup>ns</sup>	1.030*	0.042 <sup>ns</sup>	630.96*
Error / <i>Pogrješka</i>	87.26	0.421	0.026	83.55

† The differences among the treatments were analyzed by the ANOVA; \*\*, \*, and <sup>ns</sup> indicate that the treatment effects were significant at  $P \leq 0.01$  and  $P \leq 0.05$  or non-significant, respectively. The means ( $n = 7$ ) followed by the same letters are not significantly different according to the DMRT at  $P \leq 0.05$ . / *Razlike između tretmana analizirane su ANOVOM; \*\*, \* i <sup>ns</sup> indiciraju da su učinci tretmana bili značajni pri  $P \leq 0,01$  i  $P \leq 0,05$ , odnosno beznačajni. Srednje vrijednosti ( $n = 7$ ) iza kojih slijede ista slova nisu značajno različite prema DMRT-u pri  $P \leq 0,05$ .*

The preservation of tissue water content is essential for the maintenance of protoplasm hydration and continued metabolic activity under drought (Zivcak et al., 2016). In this study, water availability to the plants was investigated by the measurement of water potential components and RWC in the leaves. Leaf water potential is usually used for evaluating the water availability to plant (Farooq et al., 2009). By reducing water availability to pistachio seedlings, Tajabadi Pour et al. (2004) observed a significant decrease in  $\Psi_L$  and  $\Psi_S$ . However, in the present study, no significant changes were observed in the  $\Psi_L$ ,  $\Psi_S$ , or  $\Psi_P$  in the leaves of drought-stressed plants. This was due to the leaf abscission of the drought-stressed plants, which assisted them to preserve water by restricting the leaf area. On the other hand, the preservation of RWC,  $\Psi_L$ , and  $\Psi_P$  in the preconditioned plants was due to the  $\Psi_S$  decline in the leaves. These results indicated that an active osmoregulation in the preconditioned plants assisted water conservation under a drought stress. Osmoregulation is a critical mechanism for the maintenance of water level in the leaves of drought-tolerant plants (Karimi et al., 2012; Yang et al., 2015). Previous studies have also indicated an enhanced level of osmoregulation, increased expression of the genes related to the biosynthesis of abscisic acid, and an accelerated stomatal closure for the maintenance of water content in the BABA-treated plants (Du et al., 2012; Karimi et al., 2017b).

The ability to preserve water content under a water stress reflects a greater integrity of the plasma membrane and its ability to withstand degradation and mechanical damage due to dehydration (Yu et al., 2017). In this regard, at the end of the experimental period, the highest membrane stability index was observed in the 5 mM BABA treatment, which was not significantly

different from the control one. However, drought stress significantly affected the plasma membrane integrity (Table 4). The acceleration of leaf aging and abscission under the drought stress are the consequences of damage to the plasma membrane, proteins, nucleic acids, and photosynthetic pigments (Gechev et al., 2006). These damages are the consequences of stomatal closure for water conservation, which impairs a normal cellular metabolism (Walley et al., 2013), resulting in the accumulation of reactive oxygen species in the cells and an oxidative damage inflicted to the cell's critical components (Farooq et al., 2009). In the present study, significant reductions in chlorophyll concentration and membrane stability under a drought stress (Table 4) indicated the occurrence of oxidative damage to the leaves. These injuries occurred simultaneously to an increased level of  $H_2O_2$  and the accumulation of MDA in the tissue (Table 4). The accumulation of MDA, as a byproduct of lipid peroxidation, indicates the severity of oxidative damage (Karimi et al., 2017b). The BABA pretreatment significantly reduced the tissue damage indices (Table 4) and improved the chlorophyll concentration in the leaves of drought-stressed plants (Table 4). In such plants, the level of  $H_2O_2$  was maintained at the control level, and no significant change in the MDA content was observed (Table 4). These observations suggested that the BABA regulated the antioxidant defense network of the stressed plants, especially at a concentration of 5 mM. Accordingly, Du et al. (2012) demonstrated the effects of BABA on an increase in antioxidant activity in the wheat leaves. The studies have confirmed that the BABA improves the oxidative stress tolerance by activating the genes involved in the cell defense system (Zimmerli et al., 2008).

**Table 4. The effects of BABA pretreatments on the membrane stability index (MSI) and chlorophyll, malondialdehyde (MDA), and hydrogen peroxide ( $H_2O_2$ ) content in the leaves of pistachio rootstock under drought stress.**

Tablica 4. Utjecaj preddretmana BABA na pokazatelje stabilnosti membrane (MSI) te sadržaj klorofila, malondialdehida (MA) i vodikova peroksida ( $H_2O_2$ ) podloga pistacije pod sušnim stresom.

Treatments / <i>Tretmani</i>	MSI (%)	Chlorophyll / <i>Klorofil</i> (mmol m <sup>-2</sup> )	MDA (nmol g <sup>-1</sup> )	H <sub>2</sub> O <sub>2</sub> (μmol g <sup>-1</sup> )
Irrigated / <i>Navodnjavano</i>	68.8 <sup>ab</sup>	409.7 <sup>a</sup>	642.4 <sup>b</sup>	1.51 <sup>b</sup>
Drought stress (D) / <i>Sušni stres</i>	60.4 <sup>c</sup>	360.7 <sup>c</sup>	853.2 <sup>a</sup>	2.93 <sup>a</sup>
BABA 5mM + D	70.4 <sup>a</sup>	403.9 <sup>a</sup>	555.8 <sup>b</sup>	1.22 <sup>b</sup>
BABA 10mM + D	66.8 <sup>b</sup>	385.2 <sup>b</sup>	620.5 <sup>b</sup>	1.75 <sup>b</sup>
ANOVA				
Treatments / <i>Tretmani</i>	3791.5**	1189.7**	128.72**	29.66*
Error / <i>Pogriješka</i>	477.5	177.6	21.40	9.16

† The differences among the treatments were analyzed by the ANOVA; \*\* and \* indicate that the treatment effects were significant at  $P \leq 0.01$  and  $P \leq 0.05$ , respectively. The means ( $n = 7$ ) followed by the same letters are not significantly different according to the DMRT at  $P \leq 0.05$ . / *Razlike između tretmana analizirane su ANOVOM; \*\* i \* indiciraju da su učinci tretmana bili značajni pri  $P \leq 0,01$  odnosno pri  $P \leq 0,05$ . Srednje vrijednosti ( $n = 7$ ) iza kojih slijede ista slova nisu značajno različite prema DMRT-u pri  $P \leq 0,05$ .*

## CONCLUSION

Leaf abscission in pistachio was observed under the drought stress, which was in line with the development of oxidative damage in leaves. Plant growth and biomass significantly decreased due to a drought-induced leaf abscission. However, leaf abscission improved water availability to the remaining leaves of the drought-stressed plants while limiting the transpiration surface. The BABA pretreatments restricted oxidative damage to the leaves and prevented leaf abscission under the drought stress. The preservation of plasma membrane integrity and chlorophyll were in line with the restriction of H<sub>2</sub>O<sub>2</sub> accumulation in the leaves of the BABA-preconditioned plants. Preservation of leaf water content and turgidity was due to active osmoregulation after BABA pretreatments. Therefore, it was concluded that BABA preconditioning induces drought tolerance in the pistachio rootstock by engaging multiple tolerance mechanisms. Based on the results, foliar application of 5 mM BABA was suggested for the preconditioning of the young pistachio plants.

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## POBOLJŠANJE RASTA, ODNOSA VODE TE SMANJENJE OKSIDATIVNIH OŠTEĆENJA PODLOGA PISTACIJA U UVJETIMA SUŠNOGA STRESA PREDTRETMANOM BETA-AMINOBUTRIČNOM KISELINOM

### SAŽETAK

**Tijekom rasta biljaka okolišni stresovi su neizbježni. U biljaka  $\beta$ -aminobutrična kiselina (BABA) daje otpornost na stresne čimbenike. U ovom istraživanju analizirana je djelotvornost predtretmana BABA (0,5 i 10 mM) pri kontroliranim uvjetima suše kod podloga pistacija Ackbari. Otopine BABA prskane su na sadnice pistacije tripot svaka dva dana tijekom faze od petnaest listova. Potom je navodnjavanje biljaka uskraćeno 25 dana, a istraživani su učinci predtretmana BABA na rast, povezanost s vodom i pokazatelje oksidativnoga oštećenja biljaka. Predtretmani BABA spriječili su smanjenje suhe i svježe mase izboja izloženih sušnomu stresu zaustavljajući odbacivanje listova i održavajući relativan sadržaj vode. Zbog odbacivanja listova, vodni potencijal lista i elastičnost biljaka izloženih suši bili su slični kontrolnim biljkama. No, mjerenje osmotskoga potencijala pokazalo je da su predtretmani BABA poboljšali potencijal elastičnosti listova biljaka izloženih suši izazivajući osmotsku regulaciju. Smanjen pokazatelj stabilnosti membrane i klorofila, kao i nakupljanje malondialdehida u skladu s nakupljanjem vodikova peroksida u listovima, ukazalo je na razvoj oksidativnoga oštećenja u sušnim uvjetima. Ipak, predtretman BABA ograničio je težinu oksidativnih oštećenja. S time u vezi, predtretman s 5 mM BABA bio je najučinkovitiji u svladavanju sušnog stresa.**

**Ključne riječi:** malondialdehid, osmoregulacija, oksidativno oštećenje, *Pistacia vera* L., vodni režim biljaka