PROLINE AND ABA LEVELS IN TWO SUNFLOWER GENOTYPES SUBJECTED TO WATER STRESS

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Abstract. Abscisic acid (ABA) effects on proline content and water status were examined in two *Helianthus annuus* genotypes (cv. Nantio F1 and cv. Özdemirbey (TR3080)) exposed to drought and waterlogging stresses. Leaf expansion was inhibited by drought. ABA treatments decreased leaf area of both genotypes except when being waterlogged in Nantio F1. Relative water content (RWC) of the leaves decreased under drought stress. In ABA treated leaves, RWC had lower values than in non-treated leaves of Nantio F1. Dry weight (DW) decreased under drought stress. DW increased in ABA treated Nantio F1 genotype under drought stress and in the control plants, while DW of waterlogged plants decreased. Drought stress stimulated proline accumulation. ABA treatments caused an increase in the levels of proline content in respect to non-treated leaves of Özdemirbey. ABA contents were higher in plants under combination of drought-ABA and waterlogging-ABA treatment than in the control plants. The present data indicate that there are relations between proline content and water status. However, exogen ABA treatments change the water status and proline accumulation. Responses to exogen ABA treatment of both sunflower genotypes are different.

Keywords: proline, RWC, water status, ABA, *Helianthus annuus*

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INTRODUCTION

Low water potential caused by a soil water deficit is one of the major natural limitations of the productivity of natural and agricultural ecosystems, resulting in large economic losses in many regions. In the past, irrigation has been a key for an agricultural solution to this problem, but due to the increasing societal demands water supplies needed became at an increasingly high financial and environmental cost (Wu & Cosgrove, 2000).

Under severe water stress conditions caused by high salinty or drought, plants stop growing completely and accumulate solutes in cells in order to maintain the cell volume and turgor against dehidration. This phenomenon is known as osmotic adjustment. Osmotic adjustment has been observed in stems, leaves, roots and fruits (Nonami, 1998; Patakas et al., 2002).

Although all higher plants require free access to water, excess water in the root environment of land plants can be injurious or even lethal because it blocks the transfer of O_2 and other gases between the soil and the atmosphere. Under transient flooding, or irrigation followed by slow drainage, or in natural wetlands, plant roots can become O_2 deficient because of the slow transfer of dissolved O_2 in the water filled pore space of soil (Keles & Oncel, 2002).

Abscisic acid (ABA) is a plant hormone involved in the regulation of higher plants physiology, growth and development (Hurley & Rowarth, 1999; Neill & Burnett, 1999). It is involved in the mediating responses to the environmental stresses, notably to the stomatal closure and the induction of many stress – related gene products (Thompson et al., 2000). ABA levels in vegetative tissues can be elevated in response to various environmental stresses, most notably to drought. Therefore, ABA is referred to as a stress hormone (Taylor et al., 2000). ABA is synthesized in roots in response to stress (Shashidhar et al., 1996).

Dicot leaf growth is characterized by partly transient tip to base gradients of processes, structure and function. These gradients develop dynamically and interact with the dynamically developing stress conditions like drought. Under drought stress, growing leaves developed source function at smaller leaf size, before specific physiological adaptations to drought occurred (Schurr et al., 2000).

Cytokinins (CK) and Abscisic acid have been considered as signals in the root-to-shoot communications, co-ordinating the supply of water and nutrients by roots to the current growth requirements of shoots. Leaf growth is most inhibited under drought and salinity (Zdunek & Lips, 2001). Leaf growth repression under these conditions in most cases leads to a considerable accumulation of ABA (Davies et al., 2000; Sánchez-Blanco et al., 2002).

It is well known that proline accumulates in plants during the adaptation to various types of environmental stress, such as drought, salinity, high temperature, nutrient deficiency and exposure to heavy metals and high acidity (Öncel et al., 2000;

Ruiz et al., 2002). The principal role of proline probably is not to reduce the osmotic potential, but to protect enzymes against dehidration and salt accumulation (Thomas, 1990). Nieves et al. (2001) has pointed out that ABA treatment caused an increase in the levels of proline compared to the control.

Objectives of the present work are: (1) to determine the effects of ABA-drought and ABA-waterlogging combinations on proline accumulation and relative water content; and (2) to determine differences in the responses to exogen ABA treatment between *Helianthus annuus* genotypes.

MATERIALS AND METHODS

Plant material

Helianthus annuus L. cv. Nantio F1 and Özdemirbey seeds were obtained from the SAPEKSA tohumculuk Adana, Turkey. Seeds were soaked in aerated water for 1 d at 22^{o}C. Then they were transferred to plastic pots filled with sand: soil: manure mix (1/2/2). Plants were grown at 22°C and 65±5 % RH in a growth chamber under 480 μmol.m^{2}.s^{-1} light (day / night: 12 / 12 h).

Plants of each line were classified into three groups and exposed to water deficient, waterlogging and control conditions.

At the age of 3 weeks, they were exposed to water stress. Six pots were assigned at random to each treatment combination at every stress level. ABA (±) was obtained from Sigma Chemical Co. ABA solution (10^{-5} M) was applied three times at 1 d intervals by spraying the growing leaves. Controls were sprayed with distilled water. ABA treated plants, whether stressed or unstressed, were compared to the controls (sprayed with distilled water) at the same stress level. Plants were harvested on day 6 after the last ABA application. Plants were removed carefully from the pots and the roots were washed thoroughly with water and dried in paper towel.

Shoot growth and relative water contents (RWC)

Leaf area and shoot dry weight were measured after harvesting. Fresh weight (FW) 5 plants from each group was weighed and then dried in an oven at 60^{o}C for 1 night to obtain their dry weights (DW).

To determine plant RWC, nine leaves were weighed (fresh weight, FW) immediately after harvesting from the plant. The same tissues were then placed in a distilled water vial for 2 h at 25^{o}C and then their turgid weights (TW) were measured. The samples were then dried in an oven at 110°C for 24 h to obtain their dry weights (DW).

Relative water contents were calculated by the following formula:

$$RWC = \frac{(FW-DW)}{(TW-DW)} \times 100$$

Proline

Extraction and determination of proline was performed according to the method of Bates et al. (1973). Leaf samples (1 g) were extracted with 3 % sulphosalicylic acid. Extracts (2 ml) were held for 1h in boiling water by adding 2 ml ninhydrin and 2 ml glacial acetic acid, after which cold toluene (4 ml) was added. Proline content was measured by a spectrophotometer (Shimadzu UV 1601) at 520 nm and calculated as μmol g^{-1} DW against standard proline.

ABA analysis

ABA extraction was made according to Jeschke et al. (1997). Leaf samples were homogenized and extracted in 80 % methanol. Extracts were passed through a Sep-Pak C18-cartridge (Waters). Methanol was removed under reduced pressure and the aqueous residue was partitioned three times against ethyl acetate at pH 3.0. The ethyl acetate of the combined organic fractions was removed under reduced pressure. The residue was taken up in TBS- buffer (TRIS- buffered saline: 150 m mol L^{-1} $MgCl_2$ and 50 m mol L^{-1} TRIS : pH 7.8) and subjected to an immunologial ABA assay (ELISA) using ABA immunasay test kit (Phytodetek).

Statistical analysis

Results from the threefold application of all treatments were subjected to an analysis of variance using Minitab for Windows.

RESULTS

Leaf growth, RWC and DW

Drought treatment had a significant inhibitory effect on the growth of leaves of Nantio F1 and Özdemirbey (Fig.1). ABA treatment decreased leaf area in water-logging leaves of Özdemirbey (114,66 cm^2), and increased leaves area of Nantio F1 (118 cm^2). Stressed plants had a significantly lower ($P<0, 05$) leaf area than that of the control plants.

Drought treatment substantially inhibited leaves DW of Özdemirbey (359 mg $plant^{-1}$) compared to control plants (407 mg $plant^{-1}$) and Nantio F1 (414 mg $plant^{-1}$) (Fig. 1). ABA increased DW in the leaves of ABA treated plants of NantioF1 in the control (527 mg $plant^{-1}$) and under drought stress (477 mg $plant^{-1}$) ($P<0, 05$), while no significant differences were found in all treatments of Özdemirbey.

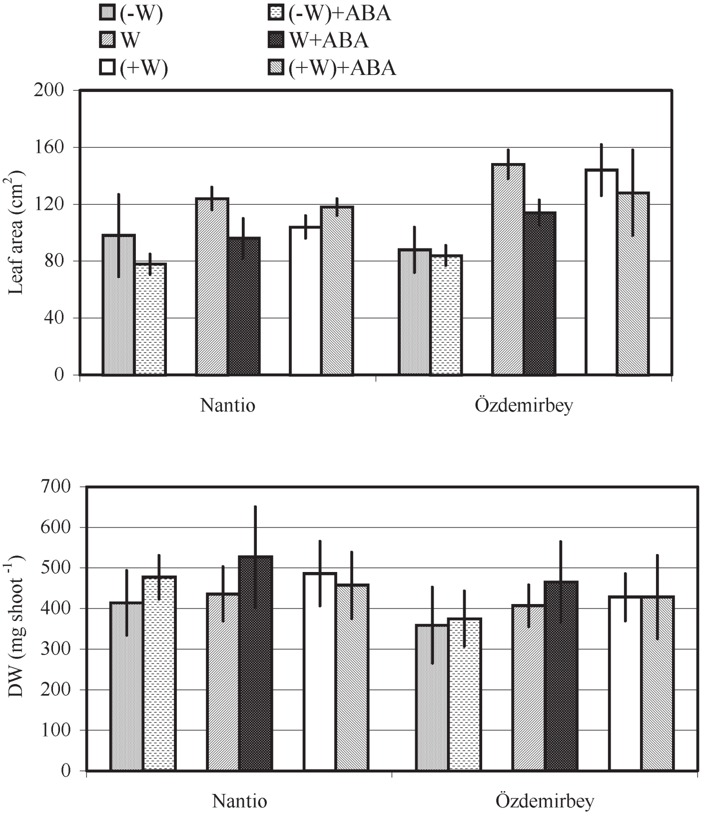
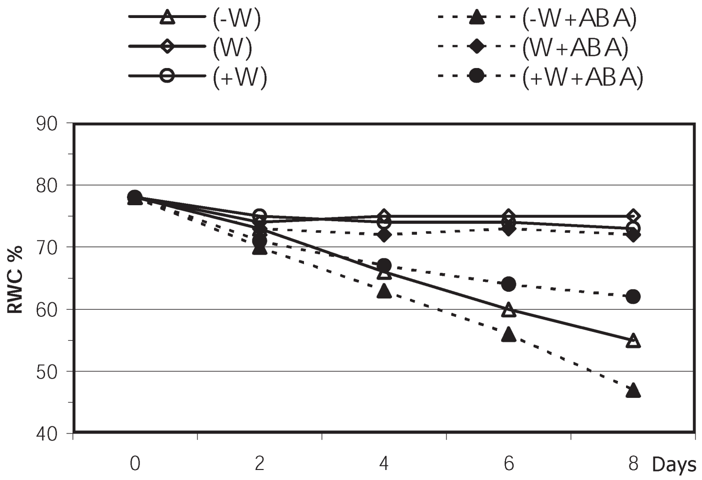


Fig. 1. Leaf area (water $P<0,05$) and DW (genotypes $P<0,05$) in sunflower genotypes under drought and waterlogging stresses. (-W) drought, (W) control, (+W) waterlogging.

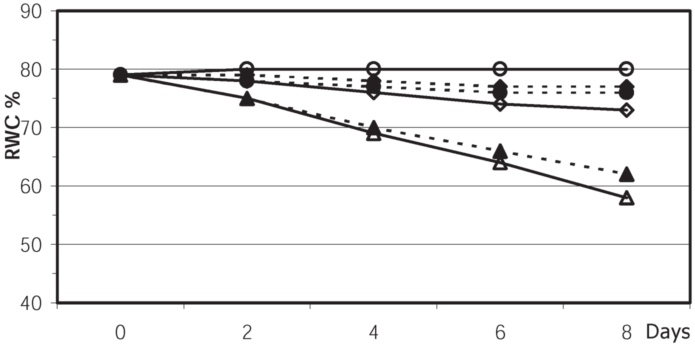
Drought stress had a significant inhibitory effect on RWC in leaves of both genotypes. During growth period, ABA inhibited RWC in drought stressed-Nantio F1 stronger than in Özdemirbey. RWC did not change in waterlogged-plants, but ABA treatment decreased RWC in these plants. Significant differences between NantioF1 and Özdemirbey under water treatments were found ($P<0,05$).

Free proline contents

In both genotypes, drought stress stimulated proline accumulation (Fig.3). However, proline was synthesized after 4 days in Nantio F1, while it was synthesized after 6 days in Ozdemirbey. Free proline decreased in ABA treated leaves of Nantio F1 (71,4 µmol g^{-1}), but increased in ABA treated leaves of Ozdemirbey (92 µmol g^{-1}). Significant differences in proline accumulation were revealed under stress conditions ($P<0,01$). Furthermore, significant differences in free proline content in drought



NantioF1



Özdemirbey

Fig. 2. RWC in sunflower genotypes under drought and waterlogging stresses (water $P<0,05$, genotypes $P<0,05$).

leaves during the growth period ($P<0,01$), and between Nantio F1 and Özdemirbey ($P<0,05$), were found.

ABA contents

ABA concentrations in the leaves of Özdemirbey (1010-1621 pmol mL^{-1}) were higher than the concentrations in the leaves of Nantio F1 (850-1579 pmol mL^{-1}) (Fig. 4). In the leaves of stress treated plants, ABA content was significantly higher ($P<0,05$). The ABA contents did not change significantly in ABA treated leaves.

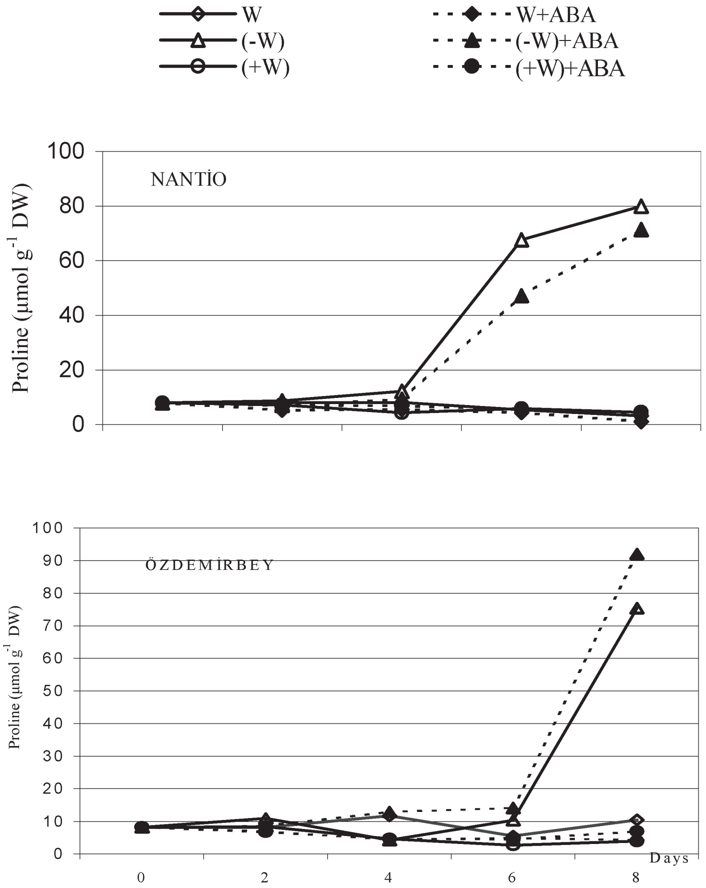


Fig. 3. Free proline contents in sunflower genotypes under drought and waterlogging stresses (water P<0,01, days P< 0,01, genotypes P<0,05).

DISCUSSION

Leaf growth is inhibited by drought. Meyer & Boyer (1981) indicated that water deficiency caused a decreased growth in higher plants. Similarly, Sánchez-Blanco et al. (2002) reported that the most important effects of drought stress were reduced leaf area and decreased growth rate. These results can be explained by the fact that drought had inhibitory effect on plant growth. We found that ABA treatments decreased leaves area of both genotypes except under waterlogging in Nantio F1. Bacon (1999) suggested that the plant growth regulator abscisic acid is the central controller of leaf expansion under drought. Davies et al. (2000) reported that leaf growth repression under drought conditions is in most cases linked to a considerable accumulation of ABA. RWC of the leaves decreased under drought stress. ABA treated leaves had

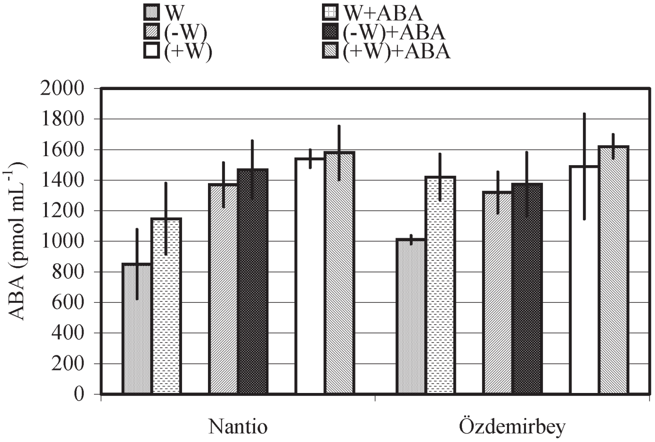


Fig. 4. ABA contents in sunflower genotypes under drought and waterlogging stresses (water P<0,05).

lower RWC than the non-treated leaves of Nantio F1, while no significant differences were found in Özdemirbey. Jing & Huang (2002) report that RWC decreases under drought stress. Similarly, Yürekli et al. (2001) found that RWC in the leaves of NaCl treated plants of *Licopersicum esculentum* was less than in the control plants.

The most important effect of drought stress is therefore the reduced growth rate. Our results show that DW is decreased by drought. These data are in accordance with the results reported by other authors (Sánchez-Blanco et al., 2002). Nantio F1 DW increased, while Özdemirbey DW did not change under waterlogging. In ABA treated Nantio F1, under drought and in the control plants DW increased, while DW of waterlogged plants decreased. However, there was no change in Özdemirbey. ABA can have a range of physiological and biochemical effects.

Drought stress stimulated proline accumulation. Proline concentration was low and almost constant in the leaves of the control and in the waterlogged plants during their development. The lower decrease of RWC in Özdemirbey compared to Nantio F1and the increase of proline levels at 6th days show that Özdemirbey could has higher drought tolerance than Nantio F1. Thomas (1990) point out that proline has been associated with drought resistance in barley. Similarly, Blum & Ebercon (1976) report that free proline accumulation in water-stressed leaves of grain sorghum is associated positively with “recovery resistance”, possibly by serving as a source of respiratory energy to the recovering plant. Schurr et al. (2000) suggested that free proline accumulated in water-stressed leaves of many crops. ABA treatment caused an increase in the levels of proline compared to non-treated leaves of Özdemirbey. These findings were supported by Nieves et al. (2001).

ABA concentration in the leaves of both stress treated plants is higher than that of the control plants. Hansen & Dörffling (1999) found that the ABA concentration in the xylem sap of drought-stressed sunflower plants was 10 times higher than in non-stressed plants. Similarly, it has been suggested that ABA rapidly increased in embryos (Thompson et al., 2000). We found ABA levels between 850-1600 pmol mL^{-1} in leaf extracts of both genotypes. Zhang et al. (2001) found that the maximum leaflet ABA concentration in stressed plants is 273 nmol kg^{-1} fresh weight. In our study, ABA concentrations in ABA treated plants had higher values than non-treated plants.

The present data indicate that there are close relations between proline accumulation and relative water content. However, exogen ABA treatments have decreased leaf expansion and increased shoot DW under drought stress. ABA has contrary effect on growth and RWC under waterlogging stress.

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