Gen. Appl. Plant Physiology, 2008, Special Issue, 34 (3-4), 203-214

PHYTOHORMONE LEVELS OF DROUGHT-ACCLIMATED LAUREL SEEDLINGS IN SEMIARID CONDITIONS

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Summary. The effect of acclimation to drought in seedlings of Mediterranean evergreen sclerophyll *Laurus nobilis* L. (laurel) was studied in semiarid field conditions. The phytohormones abscisic acid (ABA), gibberellic acid (GA_3), zeatin (Z) and indole-3-acetic acid (IAA) contents, plant water potential, photochemical efficiency, and morphological characters were determined. Seedlings were acclimated to drought in nursery where half of the plants were subjected to water deficit (50 % of field capacity) during 5 months. The other half was irrigated regularly at field capacity and served as controls. Then seedlings were transplanted to semiarid field conditions around Urla (Aegean Region-Turkey) while exposed to drought stress. Measurements were done ten months after transplanting. Plant water potential reached -2.5 MPa in acclimated seedlings and -1.5 MPa in non-acclimated controls. GA_3 and Z contents of leaves were higher in acclimated seedlings as compared to control ones, while ABA was not changed. On the contrary, IAA content of the leaves of acclimated seedlings was reduced. The photochemical efficiency of PSII in acclimated seedlings was maintained at a higher level than in controls, while leaf area and plant height were

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reduced. Increased surviving capacity in acclimated seedlings was recorded. The data indicate that acclimation of seedlings improves their adaptation ability to drought. The particular strategy of laurel seedlings for better field performance can be associated with an efficient decrease in leaf water potential as well as alterations in photosynthesis, growth and phytohormone levels.

Key words**:** Acclimation, chlorophyll fluorescence, drought, *Laurus nobilis,* phytohormones, water potential.

INTRODUCTION

Drought is one of the major environmental constraints that adversely affect plant growth and yield worldwide (Boyer, 1982). In particular, the Mediterranean climate shows a strong seasonality in water availability and temperature. Irradiance and temperature are high during summer, but precipitations are minimal. Accordingly, the dry, hot and cloudless summer, with its high evaporative demand, is the most stressful period for the local flora (Mooney, 1981). Plants respond to water deficit and can adapt to drought conditions through various physiological and biochemical mechanisms. Phytohormones have critical roles in regulating these responses to stress. Especially abscisic acid (ABA) has been considered to be one of the main endogenous signals to trigger various acclimations when plants were exposed to drought (Zhu, 2002). However, there were contradictory reports on the variation of gibberellic acid (GA_3), zeatin (Z) and indole-3-acetic acid (IAA) contents under water stress (Pustovoitova et al., 2004; Yang et al., 2001; Xie et al., 2003).

Laurus nobilis L. (laurel) is a slow growing, natural evergreen member of Mediterranean region vegetation. As an important aromatic plant, fresh or dried laurel leaves are commonly used as food flavoring and herbal tea and its essential oil is used in cosmetics and for medicinal purposes. De Lillis (1991) reported that laurel is a drought tolerant species, but young seedlings are vulnerable to drought stress in early phases of the development. For the purpose of reforestation of damaged land in South and West Coasts of

Anatolia and increase in commercial laurel production, drought tolerance potential of young seedlings must be improved until they are established in the soil.

The purpose of this work was to study the effect of acclimation to drought on improving drought tolerance of laurel seedlings. The changes of phytohormone contents (abscisic acid, gibberelic acid, zeatin and indol-3-acetic acid), plant water potential, photochemical efficiency of PSII, morphological characters and plant survival ratio were measured. Non-acclimated (control) and acclimated seedlings subjected to drought stress in semiarid field conditions were investigated.

MATERIALS AND METHODS

Laurel (*Laurus nobilis* L.) seeds were germinated and grown in 1.0 l plastic pots filled with homogenized soil containing organic manure (5 %) and grown under natural photoperiod under semi-controlled conditions at the Ege Forestry Research Institute Nursery. One-month-old seedlings were used in the experiment. For acclimation to drought half of the plants were subjected to water deficit (50 % of field capacity) during 5 months. The other half was irrigated regularly at field capacity and served as controls (non-acclimated). Then seedlings were transplanted to reforestation area in Urla (Aegean Region-Turkey) in randomized complete block design with four replicates. The field has a Mediterranean type dry climate with annual rainfall of 400-600 mm on average, however, almost no rain in July and August. Measurements were done after ten months of transplanting (at the beginning of September), when the summer drought shows its maximum effects on seedlings in field conditions.

Leaf water potential was measured with Scholander pressure chamber using terminal shoots of seedlings. Midday photochemical efficiency of photosystem II (Fv/Fm) was measured with a plant efficiency analyzer (Hansatech, UK) in dark-adapted (30 min) samples. The leaf area was measured with a common desk-top scanner and software (Flaeche.exe) on the base of the method of O’Neal et al. (2002), using the fully expanded leaves. The seedlings height was measured before transplanting and at the beginning of September. The surviving ratio was expressed as a percent of alive seedlings remaining after drought stress period versus total number of transplanted seedlings. Extraction and purification of the four hormones (ABA, GA_3, Z and IAA) were carried out as previously described by Atici et al. (2005). The hormone content was determined by High Performance Liquid Chromatography (HPLC) using isocratic system. HPLC apparatus was equipped with Waters 6000A pump (Waters, Hicrom Ltd. Uk); ultraviolet detector (Unicam Analytical Systems, Cambridge, UK) and µBondapak C18 column (Waters, Hicrom) using acetonitrile (12.00 %; pH 4.98) as the mobile phase. The flow rate, pressure and wavelength were 2 ml min^{-1}, 13.8 MPa, and 265 nm, respectively. Under these conditions, the retention times of GA_3, Z, IAA and ABA were determined to be 2.85, 3.88, 7.17 and 22.21 minute for standards, respectively.

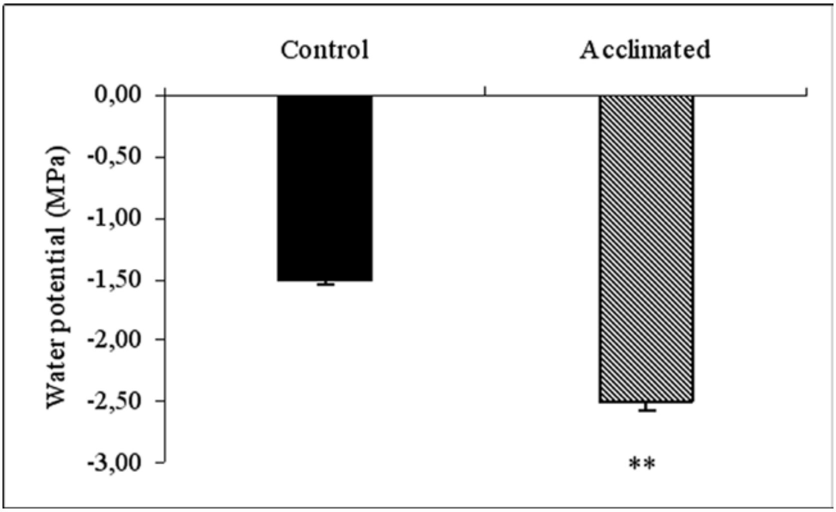

Fig. 1. Midday water potential of control and acclimated laurel plants in field. One-month-old healthy seedlings were subjected to water deficit treatments for five months. Half of the laurel seedlings in the nursery were irrigated regularly at field capacity and served as controls (non-acclimated). The other part was subjected to water deficit (50 % of field capacity) during 5 months for acclimation to drought. Then seedlings were transplanted to reforestation area and grown in field ten months. Measurements were taken at the end of summer drought. Results are means of four replicates and were compared by LSD test at the 0.01 level of confidence.

The significance of differences was tested by one way ANOVA using SPSS for Windows and mean values were compared by LSD test at the 0.01 level of confidence.

RESULTS

Ten months after transplanting of laurel seedlings to dry field conditions, leaf water potential reached -2.5 MPa in acclimated seedlings and -1.5 MPa in non-acclimated controls (Fig.1). Thus, the leaf water potential of the acclimated seedlings was significantly ($p<0.01$) lower than controls (167 %).

The photochemical efficiency of PSII of acclimated laurel seedlings (0.77) was about 138 % higher than of control seedlings (0.557) (Fig. 2).

Water stress in field conditions severely inhibited the growth of acclimated laurel seedlings as compared with the control. Plant height of acclimated seedlings was 41 % less than that of control (Fig. 3B). Similar to plant height, leaf area was inhibited under water stress to about 73 % in

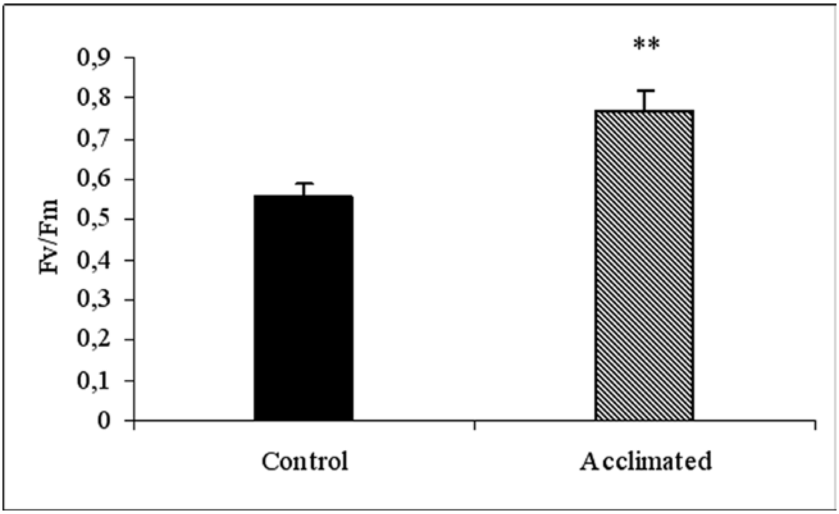

Fig. 2. Effects of drought on midday photochemical efficiency of photosystem II (Fv/Fm) of control and acclimated laurel plants in field. Other details are as shown in the legend to Figure 1.

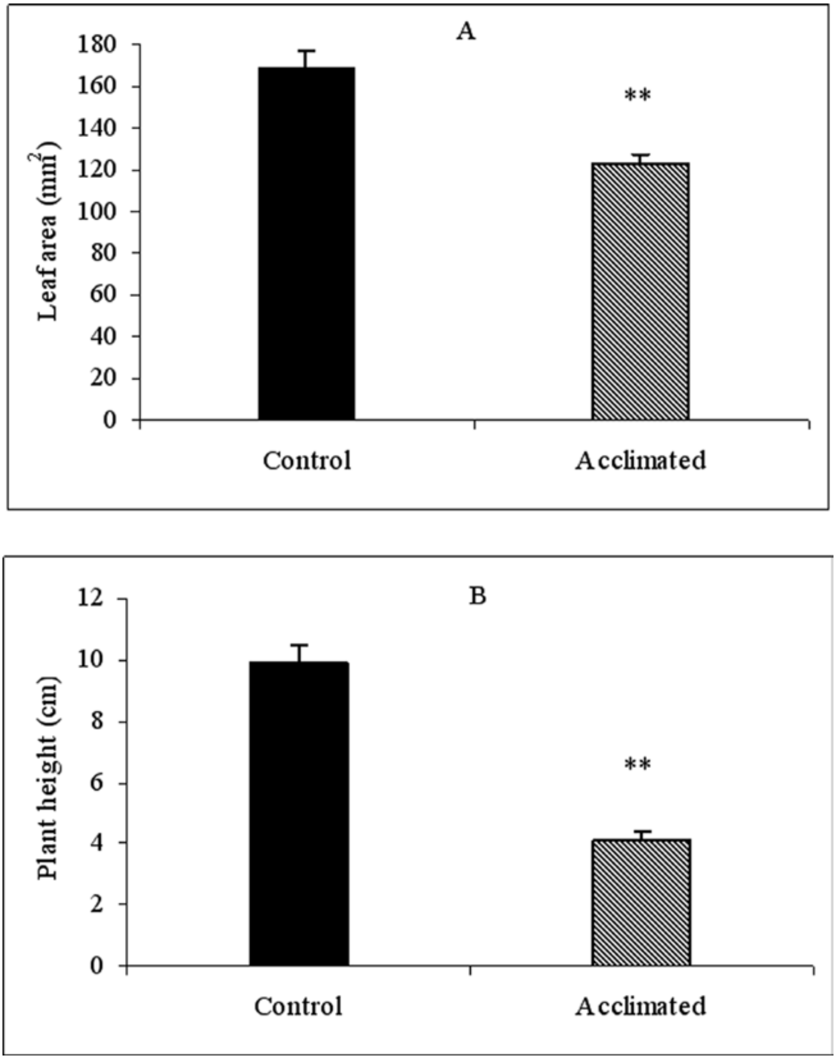

Fig. 3. Effects of drought on morphological characters, leaf area (A) and plant height (B) of control and acclimated laurel plants in field. Other details are as shown in the legend to Figure 1.

acclimated seedlings when compared with control (Fig. 3A).

Surviving ratio in acclimated seedlings was about 2.2 times higher (45.8 %) than in non-acclimated control seedlings (20.8 %) (Fig. 5.).

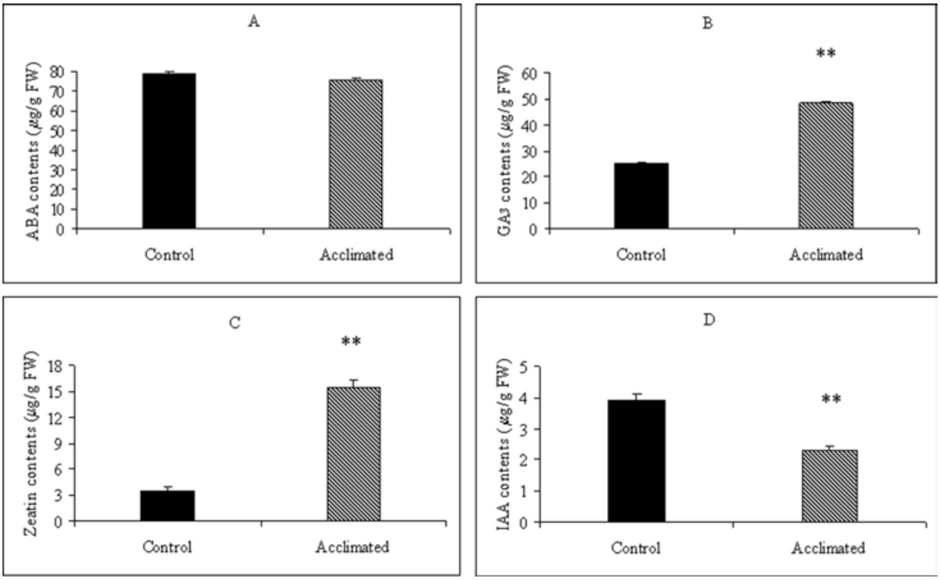

Fig. 4. Effects of drought on abscisic acid (ABA), gibberelic acid (GA3), zeatin (Z) and indol-3-acetic acid (IAA) contents in control and acclimated laurel plants in field. Other details are as shown in the legend to Figure 1.

The data on the content of four phytohormones (ABA, GA_3, Z and IAA) showed that at the end of drought period, there was no obvious difference in ABA content between the acclimated and control leaves (Fig. 4A). In contrast to ABA, GA_3 content of acclimated seedlings increased sharply (about 192 %, $p<0.01$) as compared to non-acclimated controls (Fig. 4B). In a similar manner, Z content in leaves of acclimated seedlings was found almost 4.43 times higher ($p<0.01$) than in controls (Fig. 4C). In contrast to other phytohormones, a significant decrease in IAA content (59 %, $p<0.01$) in acclimated seedlings as compared to controls was observed (Fig. 4D).

DISCUSSION

Laurel plants suffer from water shortage, especially at the seedling stage in field. In the present study we acclimated seedlings to drought in the nursery and then transplanted them to semiarid field conditions i.e. exposed

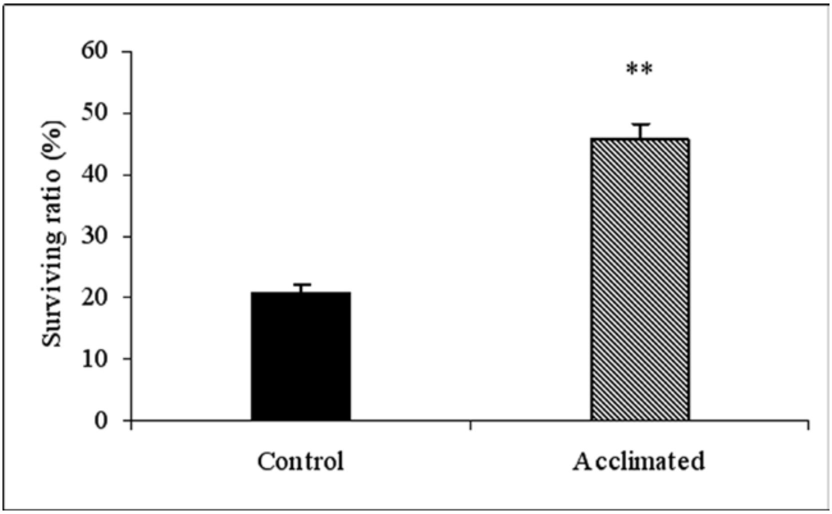

Fig. 5. Effects of drought on surviving ratio of control and acclimated laurel plants in field. Other details are as shown in the legend to Figure 1.

to drought stress. Efficient induction of physiological and morphological alterations in acclimated laurel seedlings was observed. Thus, reduction in leaf area and plant height in acclimated seedlings (Fig. 3A, 3B) can be regarded as an adaptive morphological response to drought (Taiz and Zeiger, 2002).According to Castillo (2008) drought tolerance is observed in plants with low water potential. In our study, the lower water potential in acclimated seedlings (Fig.1) may contribute to maintaining of a better photosynthetic performance (Fig. 2) and generally to improved adaptation ability to drought. Our results are in agreement with those of Zunzunegui et al. (2005) who reported that the tolerant woody species of Mediterranean climate were able to control water deficit, showing low values of leaf water potential and only slight decrease of photochemical efficiency.

Acclimation of laurel seedlings to drought had significant differential effects on ABA, GA_3, Z and IAA contents. Abscisic acid is a well known stress hormone that has multiple functions, including induction of genes involved in water stress protection and stomata closing (Seki et al., 2002). Our results showed that drought period did not cause different ABA accumulation in control and acclimated seedlings (Fig. 4A). Low leaf water potential (-1.5 MPa, referred to as severe stress) in control seedlings seems to be enough for inducing ABA accumulation. The same ABA level was attained at a lower water potential (-2.5 MPa) in acclimated seedlings which was consistent with previous studies (Pustovoitova et al., 2004). It is reported that ABA inhibits growth and leaf area expansion (Alves and Setter, 2000) in plants. Although both group of laurel seedlings had similar level of ABA, much lower plant height and leaf area of drought exposed acclimated seedlings provide evidence that different signals such as IAA may have a role besides ABA. It can be supposed that the decrease of IAA (Fig. 4D) in acclimated seedlings subjected to drought is related to reduced growth performance (Fig. 3A, 3B). Decline of IAA content under water stress is a well known phenomenon (Wang et al., 2008; Yang et al., 2001), this being in accordance with our results. Higher GA_3 and Z content in acclimated seedlings corroborated with their more negative water potential and the better maintained photochemical efficiency of PSII (Fig. 1, 2, 4B, 4C). All these adaptive responses reflected the increased surviving capacity of acclimated seedlings when exposed to drought stress in field conditions (Fig. 5.). In conclusion the data indicate that acclimation of laurel seedlings improve their adaptation ability to water deficit in field conditions. The particular strategy of laurel seedlings for better field performance can be associated with an efficient decrease in leaf water potential as well as alterations in photosynthesis, growth and phytohormone levels.

***Acknowledgements*:** This study was supported by Republic of Turkey Ministry of Environment and Forestry and Aegean Exporters' Associations Project (15. 6102).

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