

Gas Exchange, Chlorophyll and Growth Response of Three Orange Genotypes (*Citrus sinensis* [L.] Osbeck) to Abscisic Acid under Progressive Water Deficit

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ABSTRACT

A pot experiment was carried out to determine the changes in gas exchange, growth and chlorophyll contents of 'Washington Navel', 'Red Blood' and 'Shamouti' oranges (*Citrus sinensis* [L.] Osbeck) treated with 0.1 and 1 mM abscisic acid (ABA) under water deficit conditions in a partially controlled greenhouse for 150 days. The drought treatments were imposed by the depletion of 25, 50 and 75% of the available water (DAW). The results have shown the decline in growth of oranges grown under severe water deficit stress, with the reduction in leaf CO₂ assimilation rate (P_n), transpiration (T) and chlorophyll content. The growth and gas exchange were most impacted by imposing the irrigation at 75%DAW. Considerable genotypic variation in drought tolerance was observed. Based on gas exchange response, 'Washington Navel' was considered a drought sensitive cultivar, while 'Shamouti' was considered drought tolerant under the conditions of this study. 'Red Blood' was also affected but to a lesser extent. Exogenous application of ABA had no effect on growth, chlorophyll content and gas exchange parameters under well-watered conditions (25%DAW). Application of ABA at 1mM supported a considerably lower leaf P_n, T and chlorophyll, in addition to taller plants for 'Washington Navel', especially under severe drought stress.

Keywords: Abscisic acid, Orange, Water deficit, CO₂ assimilation rate, Transpiration, Chlorophyll.

INTRODUCTION

Water deficit is probably the most important stress factor determining plant growth and productivity. Agriculture in Jordan is restricted by a shortage of land receiving sufficient precipitation. More than 90% of the country's 9 million hectares of land receive less than 200 mm annual rainfall. Water shortage can cause plants to reduce their metabolic activity, causing a decrease in photosynthesis, carbon fixation, and ultimately, growth (Deputit and Caldwell, 1973; Miller and Shykoff, 1999).

Plant tolerance to drought results from both morphological adaptation and responses at the biochemical and physiological levels (Levitt, 1980; Save *et al.*, 1995; Batlang, 2006). At the cellular level, plant responses to water deficit may result from cell damage, whereas other responses may correspond to adaptive processes. Different mechanisms contribute to drought resistance in plants. These include the avoidance of water deficits by drought escape, water conservation and more efficient water uptake (Jones, 1983).

Stomatal control is the first, and perhaps the most important, step in plant responses to drought. Although decreased stomatal conductance reduces the rate of water loss to protect the plant from dehydration (Hanson and Hitz, 1982), it also decreases photosynthetic CO₂ assimilation (Cornic, 2000).

Citrus is one of the largest and most valuable

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evergreen fruit trees in many countries. It is a common fruit species in the Mediterranean basin. Most citrus species are grown under irrigated conditions because of the limited rainfall in the subtropical climate of Jordan. High temperatures jointly with dry environments induce tree dehydration that reduces growth (Agusti *et al.*, 2007). The ability of citrus species and cultivars to perform reasonably well in water stressed environments is an important trait for stability of production under drought stress conditions.

The response to water stress differs greatly among various species (García-Sánchez *et al.*, 2007). Within the *Citrus* genus, significant differences in their tolerance to water stress have been recognized by many investigators (Save *et al.*, 1995; García-Sánchez *et al.*, 2007). Several selection criteria have been proposed for selecting genotypes based on their performance in stress and non-stress environments. Differences among species in leaf morphology, leaf water potential, osmotic potential, photosynthesis and stomatal conductance are involved, to varying extents, in determining how a species responds to drought. The numerous studies indicate that citrus growth (Ortuno *et al.*, 2005) and flower-bud formation (Southwick and Davenport, 1986; Koshita and Takahara, 2004) can be drastically reduced as a result of water deficit. This growth reduction has been attributed to reduced mean net photosynthesis, stomatal conductance and leaf water potential (Save *et al.*, 1995; García-Sánchez *et al.*, 2007), to lower specific leaf area and to lower water use efficiency (García-Sánchez *et al.*, 2007).

Considerable variations in response to antitranspirants have been reported among species as well as in product efficacy. Metabolic antitranspirants have shown to be as or more effective in controlling water status than film-forming antitranspirants (like Vapor Gard and Clear Spray) and may have potential for protecting various plant species against water stress (Hummel, 1990). Application

of antitranspirants for citrus trees enhanced growth, improved water use efficiency (Hazarika and Parthasarathy, 2002), increased fruit set and yield (Shabaan *et al.*, 1998; Saleh and El-Ashry, 2006) and improved fruit quality (Saleh and El-Ashry, 2006).

The phytohormone abscisic acid (ABA) is thought to have played a major role in the induction of responses to water deficit in all plant species (Zeevaart and Creelman, 1988; Sagee and Erner, 1991). Available evidence suggests that the onset of drought or ABA treatment causes an increase in ABA concentration and ethylene production in citrus plants (Gomez-Cadenas *et al.*, 1996). ABA and its synthetic analogs have shown to increase net photosynthesis and reduce transpiration under drought and are thereby able to increase water use efficiency (Blake *et al.*, 1990).

The objectives of this study were to identify genotypic differences of three Orange (*Citrus sinensis* [L.] Osbeck) cultivars, namely 'Washington Navel', 'Red Blood' and 'Shamouti' to water deficit in the absence or presence of ABA, as an antitranspirant, by evaluating the effect of water stress and ABA on gas exchange properties, growth and chlorophyll content.

MATERIALS AND METHODS

Plant Material and Growth Conditions

A pot experiment to study the response of three orange cultivars to foliar abscisic acid (ABA) under water stress was conducted in a partially controlled glasshouse, in which temperature and ventilation can be controlled, located at the College of Agriculture, University of Mu'tah, Al-Karak, Jordan during the period May 16 – October 27, 2008. Three orange (*Citrus sinensis* [L.] Osbeck) cultivars, namely 'Washington Navel', 'Red Blood' and 'Shamouti', grafted on sour orange rootstock, were used. Uniform, nonbearing, two years old transplants were planted in May 16, 2008 using 10-liter plastic pots (27 cm in diameter, 25 cm in

depth) containing a sandy clay loam soil. Macro- and microelements were added at the 0.5-strength Hoagland nutrient concentration (Hoagland and Arnon, 1950) once every two weeks for one month before treatments application began. Chemical properties of the soil were determined according to the standard procedures of the United States Salinity Laboratory Staff (1954). These

properties are shown in Table 1. The data indicate that soil structure was sandy clay loamy, soil reaction was neutral, organic matter content was low, exchangeable cations and anions were slightly lower than the standard values used for diagnostic purposes and electrical conductivity was 0.7 dS/m. There was no salinity problem in the study soil at the beginning of the research.

Table 1. Chemical and physical properties of the soil used at the beginning of the experiment.

| Properties | |
|--------------------|-----------------|
| Texture | Sandy clay loam |
| pH | 7.3 |
| E.C. (dS/m) | 1.03 |
| Organic matter (%) | 0.48 |
| N(%) | 0.21 |
| P (ppm) | 171.1 |
| K (ppm) | 246.3 |
| Ca (ppm) | 454.3 |
| Mg (ppm) | 35.6 |
| Na (ppm) | 1.72 |
| Fe (ppm) | 8.2 |
| Mn (ppm) | 3.8 |
| Zn (ppm) | 1.3 |
| Cu (ppm) | 2.2 |

The soil surface was covered with a double layer of plastic mulch (70 µm in thickness) in order to prevent evaporation of water from pot surface. One transplant was planted in each pot. The transplants were trained to 3-4 shoots per plant one week after transplanting.

Drought Treatments

The plants were uniformly irrigated with potable water for two weeks before the initiation of drought treatments. Table 2 shows the average chemical composition of the irrigation water used throughout the experiment. After that, the irrigation treatments were started and the plants were subjected to four levels of water stress. The treatments were:

- a. Irrigation after depletion of 25% available water (DAW).
- b. Irrigation after depletion of 50% available water.
- c. Irrigation after depletion of 75% available water.

To calculate the amount of water for a given treatment, the following equation, as suggested by Israelsen and Hansen (1962), was adopted:

$$Q = AW \times D.wt.$$

Q: Quantity of added water (kg)

AW: Available water (Field capacity – Wilting point)%

D. wt. : Dry weight of soil / container (kg)

The AW was taken as the difference between root zone water storage at field capacity and permanent

wilting point. Field capacity and wilting point were determined by generating a pressure-volume curve. The plants were maintained under these conditions for five months. The 25% DAW was considered an unstressed control treatment.

ABA Treatments

The transplants were sprayed with deionized water in 0.5% methanol (control), 0.1 mM mixed isomer (Sigma) ABA in 0.5% methanol or 1 mM ABA one day before soil moisture treatments were initiated. Both ABA-treated and untreated plants were exposed to three moisture regimes.

Measurements

Measurements of gas exchange parameters [CO_2 assimilation rate and transpiration] were conducted during stress periods (just before irrigation) on the second intact leaf from the top of each plant using a leaf chamber analyzer (System Type LCA-4; Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands). Five measurements were made during the course of the experiment 2, 5, 8, 12 and 16 weeks after drought treatment initiation. The measurements were taken between 09:00 and 12:00 with the following specifications: leaf surface area= 3.25 cm^2 ; ambient CO_2 concentration= $352 \mu\text{mol mol}^{-1}$.

At the termination of the investigation, six randomly selected plants from each genotype and drought treatment were used to study the effect of ABA and drought on citrus cultivars. Growth measurements (plant height, trunk diameter, number of leaves per plant and leaf area) were conducted. The increase in plant height (at the terminal bud of the central leader) was determined between the dates May 15 and October 24, 2008. Trunk diameters 4 cm above the bud union were measured to the nearest 0.01 mm with a caliper oriented in the same direction for successive measurements.

Chlorophyll content was estimated according to Harborne (1973).

Experimental Design and Data Analysis

The experimental design was factorial in randomized complete block design (RCBD). Each treatment was replicated three times with two transplants per replicate. The data were subjected to analysis according to Snedecor and Cochran (1980). The analysis of variance (ANOVA) was used to determine significant differences. Means were compared by using Least Significant Difference (LSD) test at 5% level.

RESULTS

Gas Exchange Attributes

Prior to the initiation of drought treatments, photosynthesis (P_n) rates of control leaves were 9.14, 8.83 and $8.55 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-2}$, while for transpiration (T) rates were 2.80, 1.79 and $1.67 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ for the orange cultivars 'Washington Navel', 'Red Blood' and 'Shamouti', respectively (data not shown). Under well-water conditions (25%DAW), leaf P_n (Table 2) and T (Table 3) remained constant for all cultivars during the experimental period. ABA concentrations used had no significant influence on gas exchange parameters of all cultivars and gave practically no response. The imposition of moderate water deficit stress (50%DAW) significantly reduced gas exchange parameters of 'Washington Navel' and 'Red Blood' during the duration of the investigation (Tables 2 and 3). During the first month of water deficit, a slight reduction in both P_n and T was noticed; but after that, a progressive decrease in P_n and T has taken place in the previously mentioned cultivars. ABA application at both concentrations failed to maintain a better P_n of all cultivars than untreated ones. Transpiration started to decline below the initial control rate in only 1 mM ABA plants at 40 days of drought stress.

Table 2. Photosynthetic rate of 'Washington Navel', 'Red Blood' and 'Shamouti' oranges as influenced by abscisic acid under water deficit stress conditions.

| Days after treatment | Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-2}$) | | | | | | | | |
|-------------------------|--|-----------|-----------|------------|----------|----------|------------|----------|------------|
| | 0 mM ABA | | | 0.1 mM ABA | | | 1.0 mM ABA | | |
| | 25% DAW | 50% DAW | 75% DAW | 25% DAW | 50% DAW | 75% DAW | 25% DAW | 50% DAW | 75% DAW |
| Washington Navel | | | | | | | | | |
| 0 | 9.24 a | 9.40 a | 9.36 a | 8.943 a | 8.94 a | 8.42 a | 9.26 a | 8.66 a | 9.043 a |
| 20 | 9.53 a | 6 cd | 5.08 de | 8.84 ab | 6.69 bcd | 3.11 e | 8.96 ab | 4.54 de | 3.37 e |
| 40 | 9.3 a | 4.24 defg | 2.74 g | 7.89 ab | 3.63 fg | 2.97 g | 9.03 a | 2.72 g | 2.42 g |
| 60 | 8.98 ab | 5.83 cd | 4.35 def | 7.83 ab | 5.70 d | 3.9 efg | 8.59 ab | 4.16 def | 4.18 def |
| 80 | 9.34 a | 5.15 de | 4.31 de | 8.35 ab | 4.93 de | 4.93 de | 8.84 a | 4.91 de | 4.50 de |
| Red Blood | | | | | | | | | |
| 0 | 8.46 a | 9.39 a | 8.80 a | 8.943 a | 9.04 a | 9.35 a | 8.14 a | 8.64 a | 9.04 a |
| 20 | 8 abc | 9.44 a | 6.95 abcd | 8.84 ab | 8.73 ab | 7.84 abc | 7.77abc | 7.89 abc | 7.94 abc |
| 40 | 9.03 a | 6.63abcde | 3.84 gf | 7.89 ab | 8.26 ab | 4.05 efg | 8.017ab | 7.94 ab | 4.95 cdefg |
| 60 | 8.75 ab | 5.36 de | 3.35 fg | 7.83 ab | 5.42 de | 2.40 g | 7.89 ab | 4.16 def | 2.37 g |
| 80 | 7.58 abc | 6.10 cd | 3.31 e | 7.92 abc | 6.35 bcd | 3.36 e | 8.26 ab | 4.91 de | 3.40 e |
| Shamouti | | | | | | | | | |
| 0 | 8.43 a | 8.57 a | 8.56 a | 9.52 a | 8.43 a | 9.04 a | 8.48 a | 9.1 a | 10.04 a |
| 20 | 7.95 abc | 8.74 ab | 7.70 abc | 8.64 abc | 8.55 abc | 9.10 ab | 9.15 ab | 7.84 abc | 7.84 abc |
| 40 | 8.54 a | 7.90 ab | 6.80 abcd | 8.43 ab | 7.54 abc | 5.7 bcd | 8.54 a | 7.37abc | 4.7 defg |
| 60 | 9.14 a | 8.03 ab | 5.3 de | 9.04 ab | 7.32 bc | 3.7 efg | 9.25 a | 8.65 ab | 4.7 def |
| 80 | 8.43 a | 7.83 abc | 4.5 de | 9.09 a | 7.93 abc | 4.3 de | 8.23 ab | 8.12 ab | 3.40 e |

Means followed by different letters indicate significant differences at the 0.05 level according to the LSD.

Table 3. Transpiration rate of 'Washington Navel', 'Red Blood' and 'Shamouti' oranges as influenced by abscisic acid under water deficit stress conditions.

| Days after treatment | Transpiration rate (mmol H ₂ O m ⁻² s ⁻¹) | | | | | | | | |
|----------------------|---|------------|------------|------------|------------|----------|------------|------------|----------|
| | 0 mM ABA | | | 0.1 mM ABA | | | 1.0 mM ABA | | |
| | 25% DAW | 50% DAW | 75% DAW | 25% DAW | 50% DAW | 75% DAW | 25% DAW | 50% DAW | 75% DAW |
| | Washington Navel | | | | | | | | |
| 0 | 2.65 a | 2.77 a | 2.74 a | 2.96 a | 2.84 a | 2.84 a | 2.78 a | 2.58 a | 2.97 a |
| 20 | 2.67 a | 1.47 bcd | 1.48 bcd | 2.83 a | 1.36 bcd | 1.27 bcd | 2.85 a | 1.41bcd | 0.85 d |
| 40 | 2.72 a | 1.25 bcdef | 1.16 bcdef | 2.43 a | 1.11cdef | 0.19 hi | 2.77 a | 0.31 gh | 0.27 gh |
| 60 | 2.43 a | 0.99 def | 1.03 cdef | 2.71 a | 1.03 cdef | 0.19 hi | 2.64 a | 0.25 hi | 0.24 hi |
| 80 | 2.52 a | 1.10 def | 1.01 ef | 2.8 a | 1.02 ef | 0.24 h | 2.68 a | 0.33 h | 0.28 h |
| | Red Blood | | | | | | | | |
| 0 | 1.87 b | 1.65 b | 1.77 b | 1.79 b | 1.51 b | 1.79 b | 1.98 b | 1.64 b | 1.61 b |
| 20 | 1.45 bcd | 1.69 bc | 1.21 bcd | 1.84 b | 1.48 bcd | 1.40 bcd | 1.82 bcd | 1.46 bcd | 1.29 bcd |
| 40 | 1.56 bcd | 1.39 bcde | 1.16 bcdef | 1.72 b | 1.05 def | 0.79 fg | 1.60 bcd | 0.24 h | 0.27 h |
| 60 | 1.47 bcd | 1.08 cdef | 0.85 efg | 1.41bcd | 0.75 efg | 0.64 fgh | 1.69 b | 0.31 i | 0.38 ghi |
| 80 | 1.54 bc | 0.99 ef | 0.82 fg | 1.67 b | 0.86 fg | 0.82 fg | 1.7 b | 0.47 gh | 0.18 h |
| | Shamouti | | | | | | | | |
| 0 | 1.78 b | 1.51 b | 1.30 b | 1.62 b | 1.51 b | 1.42 b | 1.68 b | 1.50 b | 1.30 b |
| 20 | 1.86 b | 1.61 bcd | 1.02 cd | 1.39 bcd | 1.28 bcd | 1.02 cd | 1.72 bc | 1.34 bcd | 0.99 cd |
| 40 | 1.64 bc | 1.36 bcde | 0.84 ef | 1.48bcd | 1.25 bcdef | 0.88 ef | 1.57 bcd | 1.30 bcdef | 0.33 gh |
| 60 | 1.62 b | 1.62 b | 0.86 efg | 1.52 bc | 1.38 bcd | 0.78efg | 1.51 bc | 1.23 bcde | 0.18 hi |
| 80 | 1.68 b | 1.48 bcd | 0.82 fg | 1.56 bc | 1.39 bcde | 0.81 fg | 1.46 bcd | 1.14 cdef | 0.21 h |

Means followed by different letters indicate significant differences at the 0.05 level according to the LSD.

In the absence of ABA, irrigation after depletion of 75% available water (severe drought stress) was found to induce a general reduction in gas exchange parameters studied for all orange cultivars (Tables 2 and 3). The data revealed after 80 days of progressive drought, decreased rates of Pn equivalent to 53.9, 59.9 and 56.3% and of T equivalent to 64.8, 46.6 and 51.2%, for 'Washington Navel', 'Red Blood' and 'Shamouti' orange, respectively. Application of 1mM ABA decreased significantly T of all cultivars. Transpiration of 0.1mM ABA-treated plants of 'Red Blood' and 'Shamouti' was not affected. However, T of 'Washington Navel'

remained constant until 40 days of progressive drought, then it significantly reduced with 1mM ABA treatment.

Plant Growth

Under well-water conditions (25%DAW), the plants of all cultivars grew fairly well without any indication of growth restriction throughout the experiment. Application of ABA at both concentrations did not significantly affect growth of all genotypes (Fig. 1). When the irrigation was scheduled at DAW of 50%, there was an obvious variation in height of the studied cultivars by the termination of the experiment (Fig. 1). Compared to the unstressed controls, drought imposed

by 50% DAW caused a significant reduction in 'Washington Navel' height, whereas trunk diameter and number of leaves remained unaffected. Growth of 'Red

Blood' and 'Shamouti' was not significantly affected by this water level.

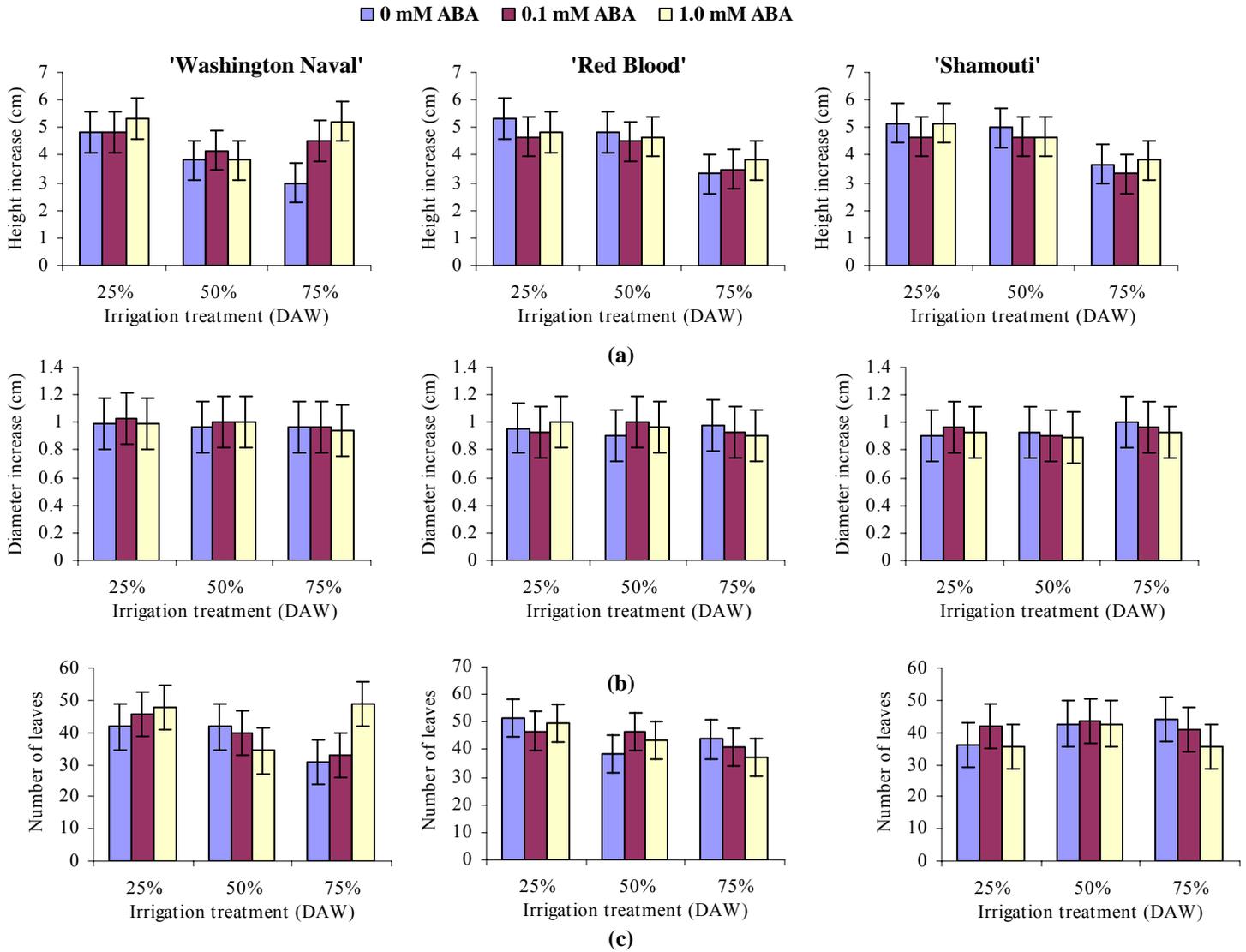


Figure 1. Height increase (a), diameter increase (b) and number of leaves (c) of 'Washington Navel', 'Red Blood' and 'Shamouti' oranges as influenced by abscisic acid concentration under water deficit stress conditions.

Exogenous ABA application at both concentrations had insignificant effects on height, trunk diameter and number of leaves for all cultivars. Furthermore, growth suppression was mainly observed when the depletion of available water increased to 75% which led to considerable reductions in plant heights of all cultivars. Number of leaves declined for all studied cultivars, but to a greater extent for 'Washington Navel' than for 'Red Blood' and 'Shamouti'. Trunk diameter and leaf area (data not shown) of all cultivars, on the other hand, showed no significant change with this water level. The low ABA concentration of 0.1 mM was sufficient to increase the height of 'Washington Navel' (sensitive genotype), while 'Red Blood' and 'Shamouti' (tolerant genotypes) did not respond even to the higher concentrations.

Chlorophyll Content

Under well-water conditions (25%DAW), leaf

chlorophyll contents remained at higher levels in all cultivars, and no genotype differences were observed (Fig. 2). ABA effect was, also, negligible. Similarly, irrigation based on 50%DAW led to insignificant effect on the concentration of the photosynthetic pigment of 'Washington Navel' and 'Shamouti'. The chlorophyll pigment contents in both untreated and 0.1mM ABA-treated plants were approximately the same. Substantial reductions in leaf chlorophyll contents of 'Washington Navel' and 'Red Blood' were found under irrigation with 75% DAW. Treatment with 1mM ABA was found to significantly maintain chlorophyll content of 'Red Blood'. However, chlorophyll contents of the other cultivars at both 1mM ABA concentrations did not improve over that of ABA-untreated plants.

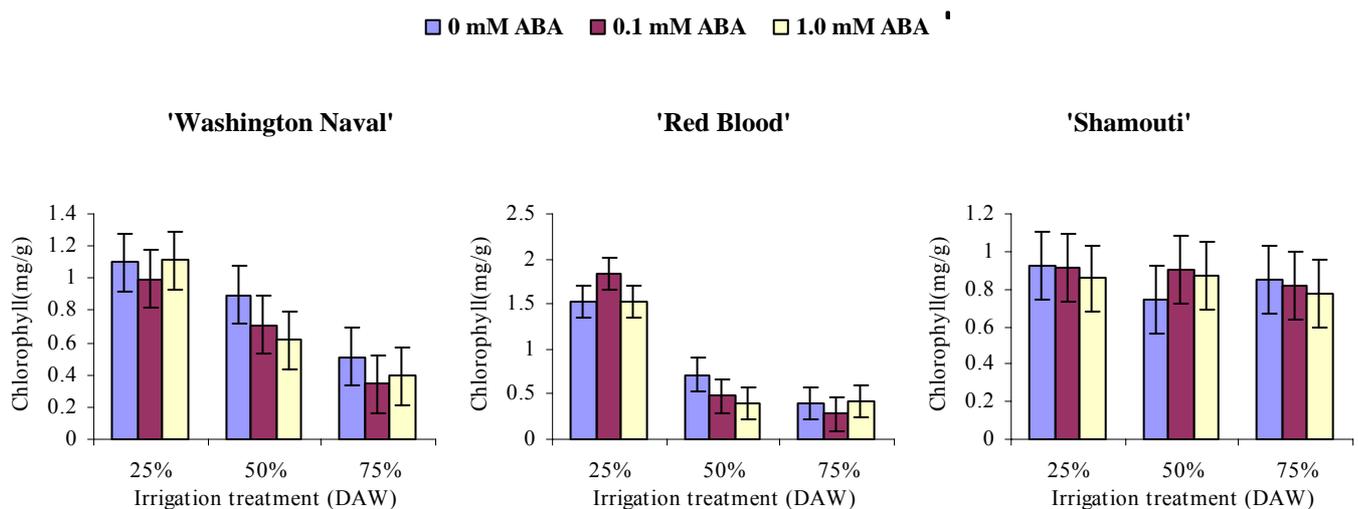


Figure 2. Total chlorophyll content of 'Washington Navel', 'Red Blood' and 'Shamouti' oranges as influenced by abscisic acid concentration under water deficit stress conditions.

DISCUSSION

Photosynthesis and transpiration decreased when the soil water moisture decreased. In the absence of ABA, a

considerable variation in Pn and T response to drought was detected among the tested cultivars with time. 'Shamouti' was relatively more tolerant to drought than

other cultivars, as indicated by less influence of Pn and T under prolonged water stress. The moderate drought stress (50%DAW) caused a progressive decline in Pn and T in both 'Washington Navel' and 'Red Blood'. 'Shamouti' exhibited lower insignificant Pn and T reduction in response to water deficit stress than the other cultivars during the period of the investigation, indicating that plants under mild stress were postponing tissue dehydration. Under severe drought stress (75% DAW), substantial reductions in Pn and T were found for most sampling dates. Drought has previously been reported to cause a decrease in citrus photosynthetic and transpiration rates (Joseph and Yelenosky, 1988; Arbona *et al.*, 2005; Ortuno *et al.*, 2005). The main effect of a soil water deficit on leaf carbon exchange rate is exerted through stomatal closure and reduction in plant growth. Moreover, the decrease in stomatal and mesophyll cells conductance results in lower internal carbon dioxide levels that significantly affect photosynthesis (Hanson and Hitz, 1982).

High ABA accumulation in water-stressed plants (Gomez-Cadenas *et al.*, 1996; Alves and Setter, 2000) and exogenous application of ABA lower both Pn and T by closing stomata (Blake *et al.*, 1990). The application of ABA could affect photosynthetic rates by prompting stomatal closure and preventing both photosystem degradation and water loss (Whitehand, 1998). Photosynthesis of all studied cultivars showed non-significant decline under moderate and severe drought stresses following ABA treatment at both concentrations. However, variable transpiration responsiveness to ABA has been observed among the different genotypes under water deficit. Treatment with 0.1 mM ABA did not cause a significant decline in A of all genotypes under moderate stress conditions compared to the untreated plants, but it caused a clear reduction in T of 'Washington Navel' under severe stress conditions.

Moreover, 1mM ABA treatment caused a significant reduction in T of 'Washington Navel' and 'Red Blood' under moderate stress conditions and a general decline in T of all genotypes under severe stress conditions. The current data indicate that the deleterious effects of severe drought stress on T of oranges can be reduced with a high concentration of ABA.

Water deficit has been shown to reduce vegetative growth for many species (Mahouachi *et al.*, 2006). It has profound effects on citrus growth in particular (Arbona *et al.*, 2005; Ortuno *et al.*, 2005). The three orange genotypes that were investigated in this study showed considerable differences in their response to different drought stress levels as well as to the different concentrations of ABA. Abscisic acid level had no influence on growth responses of all genotypes under non-stressed and moderate drought treatments. Orange plants of different genotypes were adversely affected by the low soil moisture level (75%DAW) imposed in the investigation.

In the current investigation, the decrease in leaf number could be considered as a principal cause of reduced growth through the reduction in assimilation supply and transpiration and may be proposed as a mechanism of water stress avoidance. Reduction in plant height and leaf number has been associated with the decline in cell division and enlargement and more leaf senescence (Shao *et al.*, 2008) and low turgor pressure (Cabuslay *et al.*, 2002). Similarly, the considerable reductions in 'Washington Navel' growth at 50% as well as 75% DAW seem to be related to the suppression of tissues' growth and indicated its sensitivity to water stress. On the contrary, negligible inhibition of 'Shamouti' growth under 50% and 75% DAW treatments might suggest that it is more tolerant to water deficit stress than the other cultivars.

ABA treatments partially alleviate the deleterious

effects of drought on orange growth. The low ABA concentration of 0.1 mM was sufficient to enhance shoot elongation at 75% DAW treatment in the drought-sensitive cultivar (Washington Navel), while in the less tolerant cultivar (Red Blood), a similar response occurred only at higher concentrations. 'Shamouti' growth was unaffected by various ABA concentrations. The influence of ABA on plant growth under stress conditions supports the findings of Arbona-Mengual *et al.* (2003) on citrus and Wang *et al.* (2003). The positive effect of ABA on the drought-sensitive cultivar could be related to reduced leaf water consumption (Schroeder *et al.*, 2001), increased osmotic adjustment (Guicherd *et al.*, 1997), cell turgor maintenance (Wang *et al.*, 2003) and the inhibition of ethylene evolution (Rajasekaran and Blake, 1999). Jiang and Huang (2002), however, found that ABA-enhanced drought tolerance was related to induction of dehydration protein synthesis. In citrus, it has been found that enhanced salt tolerance with ABA was attributed to its capability in reducing the number of chloride ions in the leaf tissues in addition to protecting the photosynthetic machinery, thus reducing defoliation (Arbona-Mengual, 2003).

There is good evidence that chloroplasts, site of ABA formation, are likely to be damaged by increasing water stress level (Agarwal *et al.*, 2005; Castrillo and Trujillo, 1994). In the absence of drought stress, there was no effect of ABA on chloroplast content. The observed decrease in chlorophyll content of both 'Washington Navel' and 'Red Blood' under 75%DAW treatment was

attributed to the inhibition of chlorophyll synthesis as well as to accelerated turnover of chlorophyll already present (Moreshat *et al.*, 1996). However, this decrease was significantly maintained and reduced (4.8%) in 'Red Blood' by the application of 1mM ABA. The current observation on the action of ABA on protecting the structure of chlorophyll pigment was similar to that found in ABA-treated wheat (Agarwal *et al.*, 2005) and coronatine-treated maize (Wang *et al.*, 2008).

In conclusion, the results of the current study have shown the decline in tolerance of oranges grown under severe water deficit stress, corresponded with reduction in gas exchange parameters and chlorophyll and growth inhibition. Considerable genotypic variation in drought tolerance was observed. 'Washington Navel' was considered a drought sensitive cultivar, while 'Shamouti' was considered drought tolerant. The biomass and gas exchange were most impacted by the imposition of irrigation at 75%DAW. Exogenous application of ABA at 1mM supported a considerably lower leaf CO₂ assimilation rate, transpiration and chlorophyll for 'Washington Navel' and 'Red Blood', especially when they were subjected to severe drought stress.

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(*Citrus sinensis* [L.] Osbeck)

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