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Antagonistic Changes between Abscisic Acid and Gibberellins in Citrus Fruits Subjected to a Series of Different Water Conditions

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ABSTRACT

The relationship between absicisic acid (ABA) and gibberellin (GA) changes in developing fruitlets from both Clementina (*Citrus clementina*, Hort ex Tan) and Okitsu (*Citrus unshiu*, (Mak) Marc.) trees subjected to changing water conditions was investigated. The treatments consisted of a series of water stress, rainfall, and re-irrigation periods. To confirm the effectiveness of the imposed water changes, leaf water potential and soil moisture were measured. The data indicated that there were antagonistic changes between ABA and GA_{20}, because in both species ABA increased and GA_{20} decreased during water stress, whereas re-hydration via either rainfall or irrigation reduced ABA but increased GA20. In addition, the data indicated that during water stress GA1 also decreased, whereas GA_8 did not change. After re-hydration, however, levels of GA_{20} products, in general were rather dependent upon the hormonal levels induced in the previous water status. In conclusion, the results showed the occurrence of antagonistic changes between the levels of ABA and GA_{20} in developing citrus fruitlets subjected to changing water conditions. The data might suggest that gibberellin 20-oxidase is regulated by water stress in citrus fruits.

Key words: Abscisic acid; Clementine mandarin; Gibberellin; Irrigation; Okitsu satsuma; Rainfall; Water potential

INTRODUCTION

In the Mediterranean area, citrus fruit growth is limited by water deficiency (Bain 1958; Goldshmidt and Monselise 1977). High temperatures, together with dry environments, cause tree dehydration, and in this situation, growth is generally prevented and abscission is stimulated. In citrus, both growth attenuation and abscission activation have been associated with increases in abscisic acid (ABA) content (Takahashi and others 1975; Talon and others 1990; Sagee and Erner 1991; Zacarias and

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others 1995). Furthermore, exogenous ABA increased fruit abscission in defoliated citrus plants (Gómez-Cadenas and others 2000). On the other hand, evidence for ABA changes and movement in the responses of citrus to water stress is also significant, as in many other plants (Sauter and others 2001). For example, in citrus, ABA accumulated in water-stressed roots and leaves, and the resulting high levels decreased to basal levels soon after re-watering. Re-hydration also restored xylem movement (Tudela and Primo-Millo 1992) and increased the ABA content of xylem fluid (Gómez-Cadenas and others 1996). This observation suggested that ABA was actively transported through the xylem after re-hydration. Furthermore, in previous work on citrus hormonal regulation, we proposed that ABA is primarily a signal sensitive to stress that appears to operate as a mediator between the adverse environment and the responses against stress (Gómez-Cadenas and others 1998; 2000).

Gibberellins (GAs) are also other important regulators of citrus growth. GAs are thought to be hormonal limiting factors controlling fruit growth (Talon and others 1990). The support for this idea comes from several studies reporting that exogenous GA_3 considerably improves fruit set. The endogenous GAs found in citrus fruits are mainly members of the 13-hydroxylation pathway (Talon and others 1992) leading to $GA_{1,}$ which is the bioactive GA regulating developmental processes (Zeevaart and others 1993). In general, GA_1 is elevated in developing fruitlets with a high probability of setting and lower in non-growing or abscinding fruits (Talon and others 1990; Ben-Cheikh and others 1997).

Taken together, the above observations indicate that it can be anticipated that ABA and GA levels may show antagonistic changes in the control of citrus fruit growth, suggesting the occurrence of a coordinated mechanism. A well-documented example of the antagonism between ABA/GA can be found in the regulation of seed germination in monocots. In maize (White and others 2000; White and Rivin 2000), the mutual antagonism of ABA and GAs during seed development appears to govern the choice between germination and maturation. The same authors suggested that the changing hormonal balance provides temporal control over the process, and this idea also appears to support similar works in rice (Yang and others 2001) and barley (Ho and others 2003).

In general, the hormonal changes that take place during citrus fruit development under normal growth conditions are very subtle and therefore very difficult to observe (Gómez-Cadenas and others 2000; Talon and others 1992). However, the responses to environmental stresses such as water deficiency may offer a way to confirm the prediction that ABA and GA levels show antagonistic behavior, the main goal of this work. In this regard, it is well known that there is a strong association between ABA and drought, although as far as we know, GAs have never been formally implicated in the responses to water stress and their role in this regard is therefore totally unknown. In this work, to elucidate the relationship between ABA and GAs in response to water availability, we have determined the changes that occur in developing citrus fruits subjected to changing water conditions. The study has been performed under field conditions and in parallel in two different citrus species to gain knowledge of the natural processes regulating citrus fruit growth and abscission.

MATERIALS AND METHODS

Plant Material

Adult trees *Citrus clementina*, Hort ex Tan, cv. ''Clementina de Nules'' (12 years old) and *Citrus unshiu*, (Mak) Marc, cv. Okitsu (8 years old) mandarins grafted on Carrizo citrange (*Citrus sinensis*, Osb. × *Poncirus trifoliata*, Raf) rootstocks were used in the studies described here. Both cultivars, like many other citrus species, are sensitive to water deficits. All plants were grown in experimental fields at the Instituto Valenciano de Investigaciones Agrarias (Moncada, Valencia, Spain).

Water Availability Treatments

To study the hormonal changes associated with water availability during fruit development, the normal irrigation schedule (regular hydration) in both species was altered as follows: In Clementina, irrigation was arrested from anthesis (day 0) until 76 days after anthesis (DAA). Re-hydration was subsequently restored. Rainfall occurred at days 31 ($30.0\ \mathrm{l\ m^{-2}}$), 38 ($3.3\ \mathrm{l\ m^{-2}}$), 42 ($12.3\ \mathrm{l\ m^{-2}}$), 56 ($5.5\ \mathrm{l\ m^{-2}}$), and 66 ($6.1\ \mathrm{l\ m^{-2}}$). Therefore, in Clementina the irregular hydration treatment consisted of a succession of water stress (WS, days 0–30), rainfall (RF, days 31–76), and re-hydration (RH, days 77–) periods. This irregular hydration treatment will be referred to as WS + RF + RH. Similarly, in Okitsu irrigation was arrested from days 0 to 87 DAA, and rain was concentrated on days 6 ($9.3\ \mathrm{l\ m^{-2}}$), 9 ($20.1\ \mathrm{l\ m^{-2}}$), 22 ($11.5\ \mathrm{l\ m^{-2}}$), and 34 ($21.2\ \mathrm{l\ m^{-2}}$). Therefore, in Okitsu the irregular hydration treatment consisted of a succession of water stress (days 0–5), rainfall (days 6–34), water stress (days 35–87), and re-hydration (days 88–). This irregular hydration treatment will be referred as WS + RF + WS+ RH.

Developing fruitlets were sampled periodically during the different phases of growth from anthesis to ripening. After growth measurements, samples were lyophilized, ground, and stored at −20 °C until analyses. Fruit drop was recorded on populations of approximately 200 tagged fruitlets each week, and final yield was registered.

Leaf Water Potential and Soil Moisture

In Clementina, leaf water potential measurements were performed periodically during the periods of water stress, rainfall, and re-hydration. Measurements were made in a pressure chamber (model 3000, Soil Moisture Equipment, Santa Barbara, CA) (Scholander and others 1965).

Soil moisture was also measured regularly in Clementina during the period of irrigation deficiency (0–70 DAA). Moisture measurements were carried out at 47 cm depth according to the Time Domain Reflectrometry (TDR) method (Trace system, 6051 × 1 model; Soil Moisture Equipment Crop). This method is based on the determination of the apparent dielectric constant (Ka) of soil that is related to moisture (Topp and Davies 1985).

ABA Extraction and Analysis

The ABA extraction and analysis were based on the procedures described by (Whitford and Croker 1991 and Else and others 1996), but with some modifications. Samples of lyophilized citrus fruitlets, generally 50 mg dry weight (DW), were homogenized in 5 ml of methanol and stirred overnight at 4 °C. To verify recoveries during extraction and purification, $[^3H]$ABA (about 1000 Bq) was added to the homogenized samples. The methanol extracts were evaporated to dryness under reduced pressure, and residues were loaded onto a QAE Sephadex A-25 (Pharmacia) anion-exchange column (5 ml volume). After C_{18} cartridge (500 mg) (Altech Associates) purification, residues were methylated, dried, partitioned, and dissolved for GC-MS-MS analyses. Aliquots of 3.0 μl were injected into a fused silica WCOT BPX5 capillary column (25 m × 0.22 mm × 0.25 μm film thickness) (Scientific Glass Engineering). For quantitative analyses, appropriate amounts of $[^2H_3]$ ABA as internal standard were added to the samples at the time of extraction. The first MS was adjusted to select the pair of precursor ions, m/z 190/193 (Met-ABA/$[^2H_3]$Met-ABA), and the second MS rendered the product ions, m/z 162/165, that were used for quantification.

Gibberellin Extraction and Analysis

The procedure for gibberellin (GA) analysis has been previously reported in detail (Ben-Cheikh and others 1997). Determination of GA involved sequential purification through adsorption, polyvinylpolypyrrolidone, and ion exchange chromatography. Further purification was obtained with C_{18} Sep-pack cartridges and reverse-phase high performance liquid chromatography (HPLC). Fractions of HPLC containing the GA of interest were derivatized and separately analyzed in a Fisons 8000 gas chromatograph coupled to a quadrupole MD 800 Fisons mass spectrometer. The samples (2 μl) were injected in splitless mode; the helium (He) inlet pressure was 85 Kpa, and the injector, interface, and MS source temperatures were 250°, 250°, and 200 °C, respectively. Ion electron impact masses at 70 eV were acquired in selected ion monitoring (SIM) mode. For quantification, different amounts of internal standards were used, and ions were monitored (dwell times = 80 ms) as follows: for GA_{29}/$[^2H_2]GA_{29}$-methyltrimethylsilyl (MeTMSi), m/z 508 and 506; GA_{20}/$[^2H_2]GA_{20}$-MeTMSi, m/z 420 and 418; GA_1/$[^2H_2]GA_1$-MeTMSi, m/z 508 and 506; and GA_8/$[^2H_2]GA_8$-MeTMSi, m/z 596 and 594.

Statistical Analyses and Reproducibility of the Determinations

In both species, three independent blocks of eight trees each per treatment ([a] regular irrigation and [b] irregular irrigation) were established for fruit growth and hormonal determinations. Thus, fruit growth, yield, and abscission measurements were the average of at least nine independent determinations. In the three blocks, independent extractions of ABA and GA were performed in fruits harvested from anthesis to after the re-hydration period (77 DAA in Clementina and 88 DAA in Okitsu).

For GA quantitation, different amounts of $[17-^2H_2]GA_{20}$ (99.6% enrichment), $[17-^2H_2]GA_{29}$ (99.8%), $[17-^2H_2]GA_1$ (99.6%), and $[17-^2H_2]GA_8$ (99.7%) were added to the methanol extracts as internal standards (Mehouachi and others 2000).

In Clementina, leaf water potential measurements were performed on at least three mature leaves from three representative trees per treatment. Soil moisture values were also the average of three independent measurements per treatment, repeated twice.

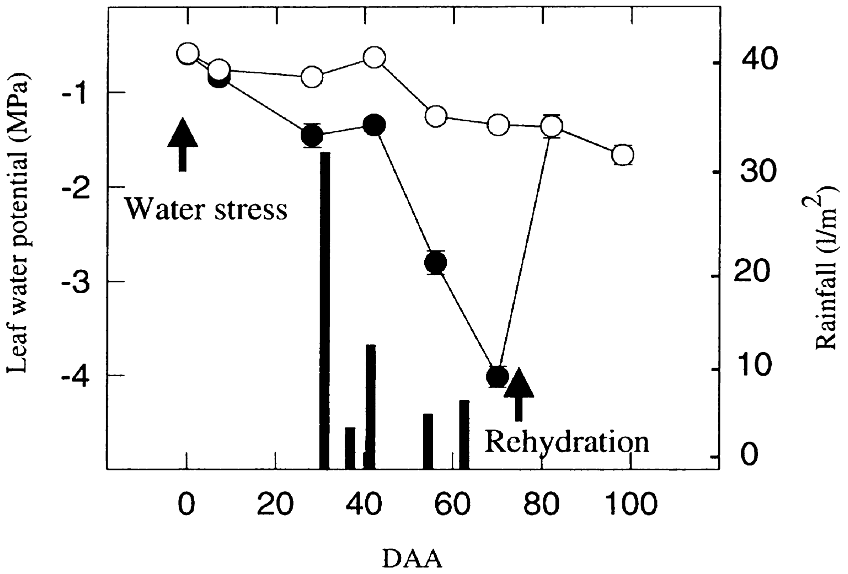

Figure 1. Leaf water potential in Clementina trees. Rainfall occurred from day 31 to day 76 with the intensity described in *Materials and Methods*. Plants were either well watered (regular hydration, [○]) or subjected to a series of different water conditions (irregular hydration, [●]). The irregular water supply treatment consisted of the succession of water stress (WS, days 0–30), rainfall (RF, days 31–76), and rehydration (RH, 77–100) periods. Day 0 was the day that anthesis occurred. Data are means ± SE, and each value was determined in at least three different plants with three replicates per plant. DAA: days after anthesis.

Data were generally compared using the least significant difference (LSD) test ($p \leq 0.05$). Statistical analyses were performed with StatGraphics Plus for Windows, V.2.1 (Statistical Graphics Corp., Rockville, MD).

RESULTS AND DISCUSSION

Changes in Leaf Water Potential and Soil Moisture

In the present work, we proposed to investigate under field conditions the ABA and GAs changes that occur in developing citrus fruits of both Clementina and Okitsu. We subjected the trees to changing water conditions to elucidate the relationship between these hormones in response to water availability. Both species are seedless mandarins and have been the subject of previous hormonal studies related to fruit-set and growth (Talon and others 1990; Zacarias and others 1995).

To confirm the effectiveness of the water changes, leaf water potential and soil moisture were first determined in Clementina mandarin. Water status of plants was determined by measuring leaf water potential during a succession of water stress (WS, days 0–30), rainfall (RF, days 31–76), and rehydration (RH, days 77–100) periods. During WS, leaf water potential was reduced from –0.60 to –1.45 MPa, and at 42 DAA it slightly recovered because of the rain. Thereafter, this parameter reached a minimum value (–4.0 MPa) 70 days after treatment (2.7-fold lower than levels found in control plants), although it was still raining, suggesting that the water contribution was not enough to restore normal leaf water potential. In contrast, re-hydration increased it to control values in a few days (−1.36 MPa) (Figure 1). Soil moisture was measured during a water stress (WS, days 0–30) + rainfall (RF, days 31–76) period during 70 DAA. The percentage of moisture in well-watered soil varied from 20% to 24%. Moisture was progressively reduced (13%) during WS after the suppression of irrigation. Thereafter, during the rainfall period, moisture decline was arrested and moisture percentage remained constant (Figure 2). These observations indicated that the treatment of irregular hydration reduced the water availability of the plants.

Changes in Fruit Growth and Abscission

Fruit development and abscission are additional parameters that help to evaluate the effectiveness of the water conditions imposed (Table 1). In both species, the irregular hydration treatment significantly reduced the number of fruits, although in Okitsu, the remaining fruits were bigger and heavier than in normally irrigated trees. The increase of fruit abscission by water stress in citrus has been demonstrated in other studies (Hilgeman 1977; Kriedemann and Barrs 1981; Barbera and others 1988), and the involvement of ABA on fruit abscission has also been shown (Gómez-Cadenas and others 2000).

Changes in Fruit Abscisic Acid

Abscisic acid amounts were determined in developing fruitlets of both Clementina and Okitsu

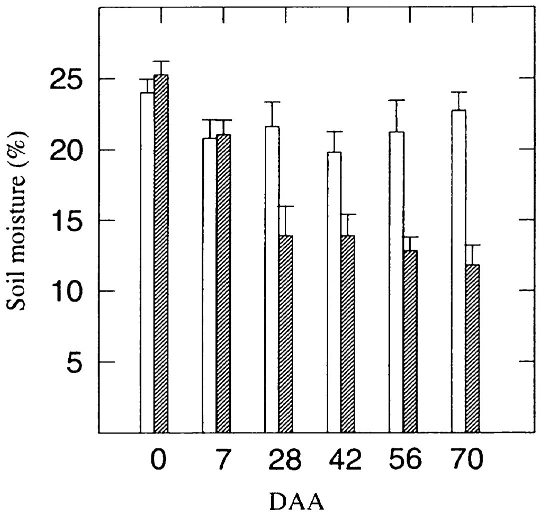

Figure 2. Moisture (%) in irrigated (regular hydration [☐]) and non-irrigated soils (irregular hydration [▨]) of Clementina orchards. Rainfall occurred from day 31 to day 70 with the intensity described in *Materials and Methods*. In non-irrigated soils the changing water conditions consisted of the succession of water stress (WS, days 0–30) and rainfall (RF, days 31–76) periods. Day 0 was the day that anthesis occurred. Data are means ± SE of three determinations repeated twice. DAA: days after anthesis.

mandarins cultivated under changing water conditions. In Clementina, endogenous ABA levels were determined in fruits subjected to a succession of water stress (days 0–30), rainfall (days 31–76), and re-hydration (days 77–) (WS + RF + RH) (Figure 3). Although the statistical analyses indicated that there were no differences between dates in control well-watered plants, the treatment of water stress applied at anthesis induced an increase of ABA levels in fruits that reached a maximum at 28 DAA. At this time, the accumulation of ABA (649 ng g^{-1} DW) produced by water stress was about 350% higher than at day 0. In contrast, the period of rain registered later (42 DAA), reduced ABA levels (21%). Two days after re-watering (day 77), more ABA reduction (69%) was observed. Finally, ABA levels increased thereafter to reach similar amounts in control and treated plants (251 and 221 ng g^{-1} DW, respectively) once normal irrigation was definitively established.

In Okitsu, the treatment consisted of a succession of water stress (days 0–5), rainfall (days 6–34), water stress (days 35–87), and re-hydration (days 88–) (WS + RF + WS + RH). Abscisic acid levels were relatively high (612 ng g^{-1} DW) at the beginning of the stress treatment but decreased (29%) after the rainfall period. Again, the second period of WS increased (166%), whereas subsequent re-hydration reduced (36%) fruit ABA amounts (Figure 4).

Therefore, the data indicated that, in developing fruitlets of Clementina and in Okitsu, ABA increased during water stress, whereas hydration through both rainfall and irrigation reduced these amounts. Increases of ABA content in response to water stress have been reported in several studies in many plants, including mandarins (Gómez-Cadenas and others 1996). In this study, for example, it was shown that severe and sudden water stress induced massive ABA increases (8- to 24-fold) in vegetative organs. This apparent discrepancy with the slight ABA rises (2–3-fold) found in the present work might suggest that ABA synthesis under water stress is differentially regulated in vegetative and reproductive organs. In addition, it has been reported that the ABA response is in general much more prominent under severe stress conditions (Weill and others 1979). It is worth mentioning that the apparent discrepancies between the evolution of the leaf water potential and soil moisture determinations and ABA levels appear to reflect the fact that these two parameters do not exactly reveal the water status of the fruit and therefore are not good markers of fruit water status. However, the determinations of these two parameters clearly indicated that the treatments were effective.

Changes in Fruit Gibberellins

For proper comparison, GA levels in developing fruitlets were studied in the same samples used for ABA determinations. The GAs analyzed were GA_{20}, GA_1, GA_{29}, and GA_8. These C-13 hydroxylated GAs are endogenous in citrus and are metabolically related in precursor-product ($GA_{20} \rightarrow GA_1 \rightarrow GA_8$; $GA_{20} \rightarrow GA_{29}$) relationships (Talon and others 1992; Ben-Cheikh and others 1997), GA_1 being the bioactive GA regulating developmental processes (Zeevaart and others 1993).

In Clementina, GA_{20} amounts in fruits at day 21 were higher in controls than in water-stressed plants (Figure 5). The release of water stress by rainfall increased GA_{20} levels. Thus, determinations performed at 42 (3.3 ng g^{-1} DW) and 76 DAA (6.6 ng g^{-1} DW) in WS+RF plants showed no statistical differences from regularly watered plants. Two days after re-watering (day 77), GA_{20} levels were the highest (9.8 ng g^{-1} DW), and thereafter a strong decrease (0.1 ng g^{-1} DW) occurred. In general, GA_1 contents were the lowest and showed much attenuated changes. Its levels, therefore, were fairly similar or uniform between fruits of both treatments. Gibberellin A_1 was lower during water stress

Table 1. Final Number, Diameter and Fresh Weight of Clementina and Okitsu Fruits from Well-watered Plants (Regular Hydration) and from Plants Subjected to a Series of Different Water Conditions (Irregular Hydration). In Clementina, the irregular water supply treatment consisted of the succession of water stress (WS, days 0–30), rainfall (RF, days 31–76), and re-hydration (RH, days 77–) periods. In Okitsu, this treatment consisted of the succession of water stress (days 0–5), rain fall (days 6–34), water stress (days 35–87), and re-hydration (days 88–) periods. Day 0 was the day when anthesis occurred. For additional details, see *Materials and Methods*. For each species, data within each column followed by dissimilar letters differ significantly at $p \leq 0.05$.

Treatments	Fruit number / tree	Fruit diameter (mm)	Fruit fresh weight (g)
Clementina			
Regular hydration	353^a	59.6^a	99.2^a
Irregular hydration	266^b	56.6^b	93.5^b
Okitsu			
Regular hydration	116^a	62.6^b	83.3^b
Irregular hydration	96^b	64.9^a	102.1^a

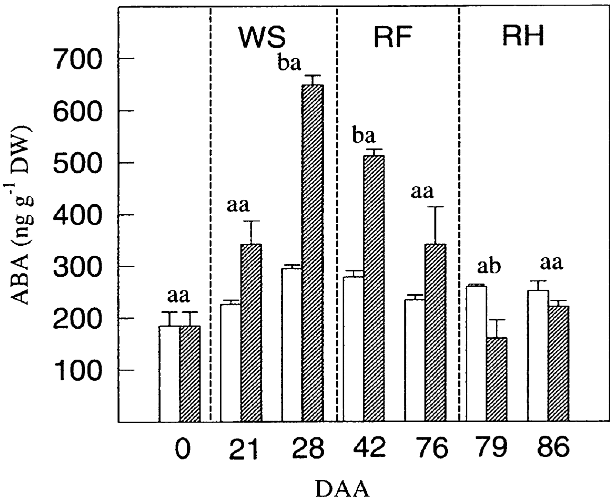

Figure 3. Abscisic acid (ABA) levels in developing Clementina fruitlets from well-watered plants (regular hydration [□]) and from plants subjected to a series of different water conditions (irregular hydration [▨]). The irregular water supply treatment consisted of a succession of water stress (WS, days 0–30), rainfall (RF, days 31–76), and re-hydration (RH, days 77–) periods. Day 0 was the day that anthesis occurred. For additional details on the treatment see *Materials and Methods*. Data were compared for each date ($n \geq 9$) using the Least Significant Difference (LSD) test. Dissimilar letters above columns differ significantly at $p \leq 0.05$. Error bars show SE. DAA: days after anthesis.

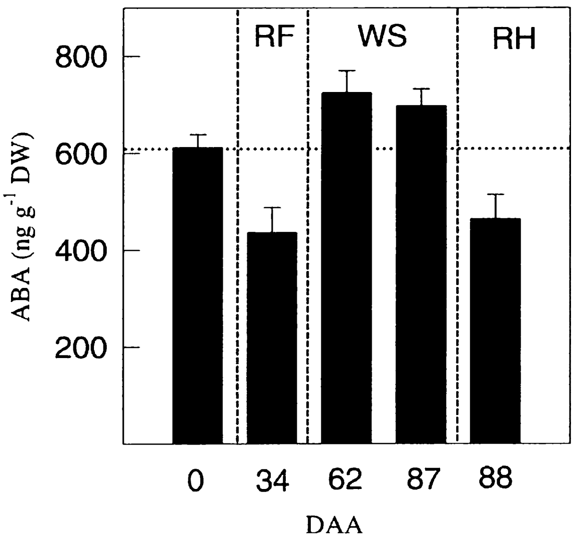

Figure 4. Abscisic acid (ABA) levels in developing Okitsu fruitlets from well-watered plants (regular hydration [.....]) and from plants subjected to a series of different water conditions (irregular hydration [■]). In the regular irrigation treatment, data are means of ABA values determined throughout the whole experimental period. The irregular water supply treatment consisted of a succession of water stress (WS, days 0–5), rainfall (RF, days 6–34), and water stress (WS, days 35–87) + re-hydration (RH, days 88–) periods. Day 0 was the day that anthesis occurred. For additional details on the treatment see *Materials and Methods*. Data are means ($n \geq 9$), and error bars show SE. DAA: days after anthesis.

at 21 DAA (1.4 ng g^{-1} DW). During the following period of RF, there was an initial reduction of GA_1 levels (42 DAA, 0.7 ng g^{-1} DW) and subsequent increases (76 DAA, 1.3 ng g^{-1} DW). On this day, GA_1 levels were higher in the control treatment (1.8 ng g^{-1} DW). After re-hydration, GA_1 levels were similar to those found in control plants. Levels of GA_{29} were high (14 and 15.3 ng g^{-1} DW, in regularly and irregularly watered fruits, respectively, 21 DAA); in general, those changes were opposite to those showed by GA_{20}, according to the precursor-product connection of these 2 GAs. In contrast to GA_{20}, for example, the transition from WS to RF reduced GA_{29} (6.2 ng g^{-1} DW) with respect to

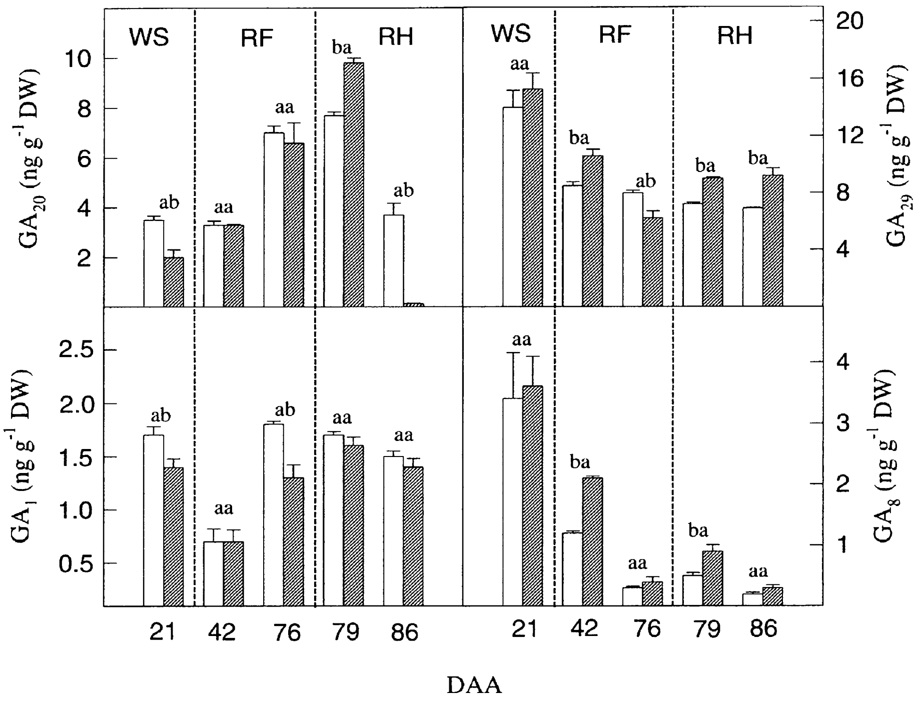

Figure 5. Gibberellin (GA) levels in developing Clementina fruitlets from well-watered plants (regular hydration [☐]) and from plants subjected to a series of different water conditions (irregular hydration [▨]). The irregular water supply treatment consisted of the succession of water stress (WS, days 0–30), rainfall (RF, days 31–76), and re-hydration (RH, days 77–) periods. Day 0 was the day that anthesis occurred. For additional details on the treatment see *Materials and Methods*. Data were compared for each date ($n \geq 9$) using the Least Significant Difference (LSD) test. Dissimilar letters above columns differ significantly at $p \leq 0.05$. Error bars show SE. DAA: days after anthesis.

controls (8 ng g^{-1} DW) at 76 DAA. However, re-hydration increased its levels (9 ng g^{-1} DW, 79 DAA) in parallel to GA_{20}, although GA_{29} did not show the "rebound effect" observed a few days later in the GA_{20} pattern. Regarding GA_8, the 2 β-hydroxylated product of GA_1, levels of this catabolite were reduced during the transition from WS (3.6 ng g^{-1} DW, 21 DAA) to RF (0.4 ng g^{-1} DW, 76 DAA). GA_8 levels increased slightly soon after re-hydration and were lower thereafter.

In Okitsu, the GA changes in general confirmed the tendencies observed in Clementina (Figure 6). For GA_{20}, the shift from prolonged WS (0.5 ng g^{-1} DW) to RH (1.4 ng g^{-1} DW) was accompanied by increases in its levels, whereas the transition from RF (3.9 ng g^{-1} DW) to WS (0.8 ng g^{-1} DW) resulted in lower amounts. Levels of GA_1 were low and pretty much constant and stable under moderate WS (1.5 ng g^{-1} DW, 62 DAA), RF (1.5 ng g^{-1} DW) and RH (1.8 ng g^{-1} DW). Small reductions in GA_1 and GA_{29} were noticed after prolonged WS (0.8 and 1.9 ng g^{-1} DW, respectively, 87 DAA). No other significant changes were found for GA_{29}. The levels of GA_8 were low shortly after anthesis and almost undetectable later on. Therefore, the data showed that in both species the water conditions imposed induced similar tendencies in the patterns of GA change. Thus, hydration via rainfall and irrigation increased, whereas WS reduced, GA_{20}. Gibberellin A_1 levels were low and changes were not pronounced. Levels were very similar during moderate WS, RF, and irrigation. However, minor and higher reductions, respectively, occurred during the transitions from WS to RF and during prolonged water stress. In general, levels of GA_{29} were lower under long WS. Higher GA_8 levels were found in both species at the beginning of the study, although amounts decreased thereafter to very low or to undetectable levels.

Antagonism between ABA and GA

The above results obtained in developing fruits of two different citrus species indicated the occurrence of a certain antagonism between ABA and GA changes during water stress and re-hydration periods. The antagonistic changes are conspicuous between ABA and GA_{20}, because the data in each cultivar showed that ABA increased and GA_{20} decreased during water stress, whereas hydration either through rainfall or irrigation reduced ABA levels but increased those of GA_{20}. The GA_{20} changes might be conditioning the levels of the other GAs studied because GA_{20} is the direct precursor of GA_1 and GA_{29}, whereas GA_1 is the precursor of GA_8. Thus, the data clearly indicated that during prolonged water stress GA_1 also decreased. In contrast, absolute levels of all GA_{20} products after re-hydration, for example, appeared to be generally related to the magnitude of the alteration of hormonal levels induced by the severity of the previous water status. One suggestion that can be deduced

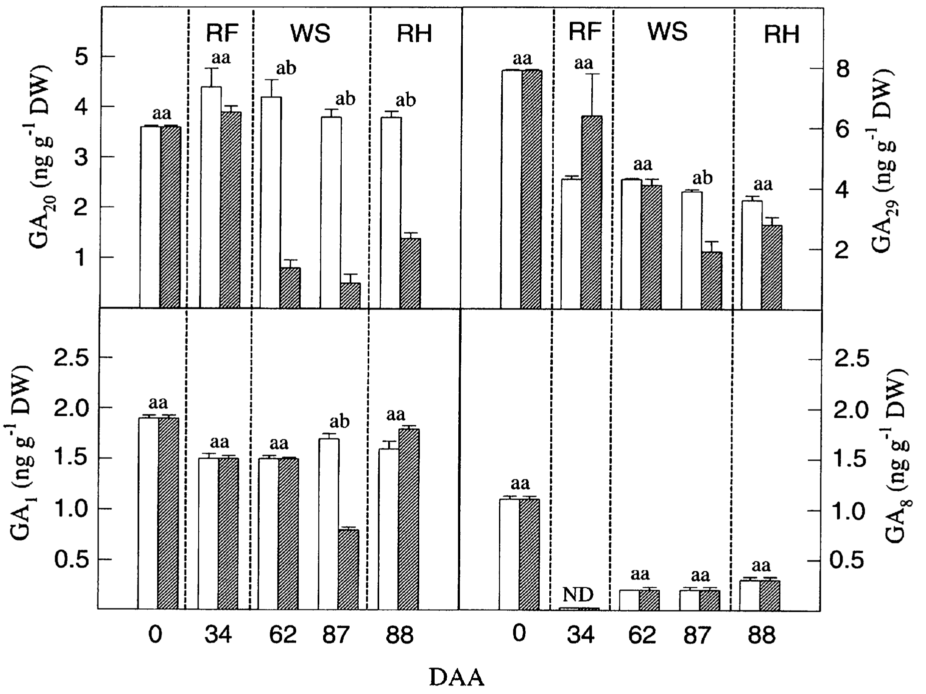

Figure 6. Gibberellin (GA) levels in developing Okitsu fruitlets from well-watered plants (regular hydration [□]) and from plants subjected to a series of different water conditions (irregular hydration [▨]). The irregular water supply treatment consisted of a succession of water stress (WS, days 0–5), rainfall (RF, days 6–34), and water stress (WS, days 35–87) + re-hydration (RH, days 88–) periods. Day 0 was the day that anthesis occurred. For additional details on the treatment see *Materials and Methods.* Data were compared for each date ($n \geq 9$) using the Least Significant Difference (LSD) test. Dissimilar letters above columns differ significantly at $p \leq 0.05$. Error bars show SE. DAA: days after anthesis; ND: not detected.

from these observations is that the changes induced by different water conditions on these other gibberellins are attenuated or "buffered" through their main effects upon GA_{20}, which could thus act as the main regulator of the bioactive GA_1. These tendencies were certainly apparent in both species, although the hormonal basal levels found in each were dissimilar (Figures 3, 4, 5, and 6). The differences in GA_1 content, the bioactive GA, was in previous work related to the higher capabilities of this cultivar to develop fruit. The high GA_8 amounts found in Clementina were similarly associated with elevated 2 β-hydroxylation activity that may explain the higher amounts of GA_{29} observed in this report.

The idea of opposite effects between ABA and GAs is not new; it has been suggested many times that ABA and GAs act antagonistically in the control of seed development in monocots. Thus, it was hypothesized that ABA antagonized a positive signal for precocious germination in maize (White and others 2000). This antagonism is generally visualized as crosstalk between GA and ABA signaling pathways, as in the cereal aleurone system (Ho and others 2003), although it is also accepted that the changing hormonal balance may provide temporal control over germination (White and Rivin 2000; Yang and others 2001). However, the work presented here offers new insights into the problem of ABA/GA antagonism because it expands this concept to dicots and to other developmental processes such as fruit development. It should be noted that work in citrus has previously been published reporting correlations between ABA/GA ratios and the fruit-setting ability of fruits (Sagee and Erner 1991; Zacarias and others 1995). In addition, this work suggests that gibberellin 20-oxidase, the enzyme that controls GA_{20} levels, might be a target of water stress in the GA biosynthetic pathway. We have previously shown in citrus that for GA_{20}, temperature-induced changes correlated with GA20-oxidase expression (Vidal and others 2003) and that in long-day plants, GA_{20} levels are photoperiodically controlled through the regulation of GA_{20} oxidase (Talon and Zeevaart 1992).

In conclusion, the results suggest the occurrence of antagonistic changes between the levels of ABA and GA_{20} in developing citrus fruitlets subjected to changing water conditions.

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