J Plant Growth Regul (2011) 30:322–333
DOI 10.1007/s00344-011-9194-z

Interplay Between Abscisic Acid and Jasmonic Acid and its Role in Water-oxidative Stress in Wild-type, ABA-deficient, JA-deficient, and Ascorbate-deficient *Arabidopsis* Plants

Ricard Brossa · Marta López-Carbonell · Tana Jubany-Marí · Leonor Alegre

Received: 19 October 2010 / Accepted: 10 January 2011 / Published online: 5 February 2011
© Springer Science+Business Media, LLC 2011

Abstract The interplay between jasmonic acid (JA) and abscisic acid (ABA) in plant responses to water stress and in water-stress-enhanced oxidative stress was investigated in *Arabidopsis thaliana* plants subjected to water stress by water deprivation. For this purpose a drought assay was conducted using *Arabidopsis* mutants impaired in ABA (*aba2*), JA (*aos*), and ascorbate (*vtc1*) biosynthesis. Our results show an interaction between ABA and JA during their biosynthesis. Moreover, the coordinated action of ABA and JA protected wild-type, *aba2*, and *aos* plants from the effects of stress. However, this effect was not observed in the *vtc1* mutant, which showed a distinct decrease in the F_v/F_m ratio, concomitant with a marked fall in relative water content (RWC), despite high endogenous concentrations of JA and ABA. This finding indicates the relevance of ascorbate metabolism in plant acclimation to stress. Despite the interaction between the two phytohormones, drought-associated stomatal closure is regulated mainly by ABA and weakly by JA, whereas JA plays a role in the formation of antioxidants regulating ascorbate and glutathione metabolism. A time course analysis revealed the relevance of plant age and stress duration in the responses of the mutants compared to wild-type plants. Here we discuss the relationship between ABA, JA, ascorbate, and glutathione in plants under water stress.

Keywords Abscisic acid (ABA) · *Arabidopsis* mutants · Ascorbate · Gas exchange · Glutathione · Jasmonic acid (JA) · Oxidative markers · Phytohormones interplay · Water stress

Introduction

Drought is one of the most detrimental environmental conditions for plant survival and thus plants have evolved several mechanisms to cope with this condition. Water stress in plants can enhance oxidative stress (Apel and Hirt 2004), depending on both external parameters and endogenous plant factors such as the concentration of phytohormones and their interactions and the oxidative status of antioxidants.

The role of the phytohormone abscisic acid (ABA) in drought and its signal transduction to induce tolerance to stress has been well studied (Huang and others 2008). In addition, there is increasing evidence that jasmonic acid (JA) and its metabolically active derivatives (jasmonates) are also crucial signaling molecules involved in many plant responses to biotic and abiotic stresses (Creelman and Mullet 1997; Devoto and Turner 2003; Wasternack 2007; Balbi and Devoto 2008). However, the exact role of jasmonates in the response of plants to water stress is not known, but external applications of methyl JA (MeJA) have shown that jasmonates increase plant resistance to water stress induced by PEG (Li and others 1998) or by withholding water (Wang 1999). Furthermore, JA has been shown to increase in spear tips of *Asparagus officinalis* exposed to drought (Gapper and others 2002), and in *Carica papaya* seedlings and *Pinus pinaster* plants subjected to water stress (Mahouachi and others 2007; Pedranzani and others 2007). In addition, MeJA increases

R. Brossa · M. López-Carbonell · T. Jubany-Marí · L. Alegre (✉)
Departament de Biologia Vegetal, Facultat de Biologia, Universitat de Barcelona, Avda. Diagonal 645, 08028 Barcelona, Spain
e-mail: lalegre@ub.edu

in *Cistus albidus* subjected to drought (Jubany-Marí and others 2010). Moreover, jasmonates are involved in stomatal closure (Suhita and others 2004). Although JA and MeJA were previously thought to be key regulators of jasmonate responses, it has been demonstrated that it is the isoleucine conjugate of jasmonic acid (JA-Ile), the active form of JA, that acts in the signal transduction pathway (Staswick 2008; Pauwels and others 2010). However, no studies have been reported to date on the variations in endogenous concentrations of JA-Ile in plants subjected to water stress. Furthermore, a rapid increase in endogenous JA levels resulting from environmental stimuli leads to a concomitant increase in JA-Ile (Wasternack and Kombrink 2010), and it has not been ruled out that this could occur in conditions of water stress.

To date, the role of ABA and JA in plant responses to drought has been addressed independently for each hormone, thus overlooking the complex interactions between these two signaling pathways.

Physiological responses to drought include stomatal closure, decreased photosynthetic activity, and even generation of reactive oxygen species (ROS) causing plant death. Concomitant molecular reprogramming includes extensive changes in gene expression. ABA and JA are key signaling substances that control the expression of many genes (Bray 1997; Shinozaki and Yamaguchi-Shinozaki 1997, 2000; Riera and others 2005; Ahuja and others 2010). A recent study (Huang and others 2008) reported that many drought-regulated genes are annotated in response to other plant hormones (auxin, cytokinin, gibberellins, jasmonates, brassinosteroids), and among these, jasmonates appeared to be the most relevant of the non-ABA hormones affecting responsive genes to water stress.

Regarding the function of JA in plant defense against stress, it has been suggested that JA may interact with ABA synthesis under water stress conditions (Bandurska and others 2003; Adie and others 2007) and that ABA and JA could regulate stomatal closure (Acharya and Assmann 2009). Moreover, there is a marked relationship between JA and antioxidants. Ascorbate and glutathione are involved in plant metabolism and stress tolerance, and the balance between ascorbate and glutathione pools is crucial for the maintenance of redox homeostasis in plant cells (Noctor and Foyer 1998b). Furthermore, the levels of ascorbate and glutathione influence each other, as shown in the ascorbate-deficient *Arabidopsis* mutant *vtc1*, which has higher glutathione content compared to Col-0 (Fernández-García and others 2009). JA interacts strongly with redox processes (Foyer and Noctor 2009), and it has been shown that JA enhances synthesis of ascorbate and glutathione (Table 1). Moreover, it has been suggested that water-stress-induced JA is a signal that leads to the regulation of ascorbate and glutathione metabolism and that this is crucial for the acquisition of water stress tolerance (Shan and Liang 2010). In addition, it has been suggested that ABA accumulation in response to drought stress induces an increase in ascorbate, maintaining and even decreasing the ascorbate oxidative status in *C. albidus* plants (Jubany-Marí and others 2009).

Arabidopsis mutants impaired in JA, ABA, and ascorbate biosynthesis are useful tools for studying the interaction between ABA and JA in plant responses to stress, and their use contributes to knowledge of the relationship between ABA and JA interaction and the redox status of ascorbate and glutathione. Thus, in the present study we used two hormone-deficient mutants, namely, *aba2* and *aos*, and one ascorbate-deficient mutant *vtc1*.

Table 1 Summary of the effect of jasmonates on ascorbate and glutathione metabolism

Species	Ex./End. JA	Jasmonate	Conditions	Response	Reference
Arabidopsis thaliana	Ex. appli	JA and MeJA	Ozone	Increased AA Increased GSH	Sasaki-Sekimoto and others (2005)
A. thaliana	Ex. appli	MeJA	Suspension cells	Increased AA	Wolucka and others (2005)
A. tahliana	End. conc	MeJA	Wounding	Increased AA Increased GSH	Suza and others (2010)
A. thaliana	End. conc	JA	Water stress	Regulation of AA and GSH metabolism	In this study
Agropyron cristatum	End. conc	JA	Water stress (PEG)	Regulation of AA and GSH metabolism	Shan and Liang (2010)
Cistus albidus	End. conc	MeJA	Water stress	Increased AA	Jubany-Marí and others (2009)
Fragaria vesca	Ex. appli	MeJA	Water stress	Increased AA	Wang (1999)
Zea mays	Ex. appli	MeJA	Water stress	Increased AA	Li and others (1998)
Wolffia arrhiza	Ex. appli	JA	≠ [JA]	Regulation of AA and GSH. metabolism	Piotrowska and others (2010)

Ex. appli exogenous application of JA, *End. conc* endogenous concentration of JA, *AA* ascorbate, *GSH* glutathione

The *aos* mutant (Park and others 2002) is an *Arabidopsis* knockout mutant from a Col-6 (gl1) background, defective in the JA biosynthetic gene *CYP74A* (allene oxide synthase, AOS). Endogenous JA levels do not increase after wounding in this mutant and its phenotype shows severe male sterility.

The *aba2* mutant (Léon-Kloosterziel and others 1996) is from a Col-0 background and shows reduced seed dormancy and excessive water loss that leads to reduced rosette growth (Koorneef and others 1984). The *aba2* locus has been characterized biochemically and shows a lower capacity than the wild type to convert xanthoxin to ABA (Schwartz and others 1997). Thus, this mutant presents significantly lower ABA endogenous content and is therefore useful for the study of the physiological role of this hormone, including its interactions with antioxidants under drought and oxidative stress conditions.

The *vtc1* mutant, isolated from a Col-0 background for its sensitivity to ozone, is also sensitive to abiotic stresses such as UV-B light, sulfur dioxide, and freezing (Conklin and others 1996), and to water stress (Munné-Bosch and Alegre 2002). Despite the lack of ascorbate, this mutant does not suffer oxidative stress in nonstressful conditions (Veljobic-Jovanovic and others 2001). The *vtc1* mutant shows higher concentrations of ABA than wild-type *Arabidopsis* plants (Pastori and others 2003), and a relationship between ascorbate redox status and ABA has been proposed (López-Carbonell and others 2006).

The aim of the present study was to evaluate the role of the interplay between ABA and JA in plant resistance to drought and in the regulation of oxidative status of antioxidants. With this purpose, we performed simultaneous measurements of plant water status, ABA, JA, ascorbate, and glutathione redox status, and other oxidative stress markers such as the extent of lipid peroxidation and the F_v/F_m ratio. In addition, gas exchange was measured in the rosettes of *aos*, *aba2*, and *vtc1* mutants and then compared with wild-type *Arabidopsis* plants.

Material and Methods

Plant Material and Water-stress Treatment

Seeds of *Arabidopsis thaliana* Columbia (Col) ecotype wild-type (Col-0), jasmonic acid-deficient mutant (*aos*), abscisic acid-deficient mutant (*aba2*), and ascorbate-deficient mutant (*vtc1*) were surface sterilized and sown on MS agar medium under sterile conditions, then incubated for 4 days at 4°C.

At the stage of four leaves (2-week-old plants) the seedlings were transplanted to pots containing a mixture of peat/perlite/vermiculite (1:1:1 v/v/v) in a constant-environment chamber (8-h photoperiod, 90–110 µmol m^{-2} s^{-1}, air temperature between 22 and 24°C). After 5 weeks, two watering regimes were imposed for 12 days on wild-type and mutant plants: (1) Plants were watered to saturation with Hoagland's solution (Hoagland and Arnon 1950) twice a week (substrate at approximately 90% of relative water content [RWC]) throughout the experiment (well-watered [WW] plants), and (2) water was withheld from plants (water-stressed [WS] plants). The experimental period from germination was 7 weeks.

Plant water status, plant hormones, jasmonic (JA) and abscisic (ABA) acid, estimation of lipid peroxidation, malondialdehyde (MDA), maximum efficiency of photosystem II (F_v/F_m), ascorbate, ascorbate oxidative status, glutathione and glutathione oxidative status, and photosynthesis were analyzed in whole rosettes. At each sampling point we harvested ten rosettes per treatment and mutant. For biochemical analyses samples were immediately frozen in liquid nitrogen and stored at −80°C until use.

Plant Water-status Measurement

Plant water status was determined by measuring the relative water content (RWC %) (Turner 1981) as follows: RWC % = (FW − DW)/(TW − DW)*100, where FW is the fresh matter, TW is the turgid matter after rehydrating the leaves for 24 h at 4°C in darkness, and DW is the dry matter after oven-drying the leaves for 24 h at 80°C.

JA and ABA Analysis

Concentrations of JA and ABA were analyzed simultaneously by HPLC MS/MS using the methods of Segarra and others (2006) and López-Carbonell and others (2009) with slight modifications. Briefly, 100 mg of leaves were ground in liquid nitrogen with a mortar and pestle and extracted with 750 µl of methanol–water–acetic acid (90:9:1 v/v/v). Deuterium-labeled internal standards (40 ng of JA-d_5 and 40 ng of ABA-d_6) were added to each of the samples and replicates at the beginning of the extraction procedure. Extracts were vortexed for 5 min and incubated for 10 min at 4°C under ultrasonication (Vibra-Cell Ultrasonic Processor, Sonics & Materials Inc., Newtown, CT, USA) and subsequently centrifuged for 10 min at 10,000 rpm. The supernatants were collected and the pellets were re-extracted with 750 µl of the extraction solvent. Then they were pooled and filtered through a 0.22-µm polytetrafluoroethylene (PFTE) filter (Waters, Milford, MA, USA) and injected into the LC-MS/MS system.

Finally, 5 µl of each sample was injected into the LC system (Acquity UPLC, Waters) using a Waters X-Bridge C18 column (3.5 µm; 100 × 2.1 i.d.). Quantification used MS/MS on an API 3000™ triple quadrupole mass

spectrometer (AB Sciex, Danaher Corp, Washington, DC). Multiple reaction monitoring (MRM) acquisition was performed by monitoring the 209/59 transition for JA and 263/153 for ABA.

Leaf Gas Exchange and Fluorescence Measurements

Gas exchange was measured using an open IRGA LI-COR 6400 system (LI-COR Inc., Lincoln, NE, USA) equipped with an *Arabidopsis* chamber head. Photosynthetic measurements were performed under chamber light (100 μmol m^{-2} s^{-1}), air temperature (25°C), and 400 μmol mol^{-1} of CO_2. The measured gas exchange parameters net photosynthesis (A), transpiration rate (E), and stomatal conductance (G_s) were calculated according to equations developed by von Caemmerer and Farquhar (1981). Measures were taken once a week between 11 a.m. and 1 p.m.

Chlorophyll fluorescence measurements were made on attached leaves by means of the pulse-modulated fluorometer IMAGING-PAM (Walz, Effeltric, Germany). The fluorescence parameter measured was the maximum quantum efficiency of PSII photochemistry (F_v/F_m) (Genty and others 1989)

Estimation of Lipid Peroxidation

The extent of lipid peroxidation was estimated by measuring the amount of MDA in leaves following the method described by Hodges and others (1999). This method takes into account the possible influence of interfering compounds in the thiobarbituric acid-reactive substances (TBARS) assay.

Analyses of Reduced and Oxidized Forms of Ascorbate and Glutathione

For ascorbate and glutathione determination, 100 mg of leaves were ground in liquid nitrogen with a mortar and pestle, followed by the addition of 750 μl of extraction buffer (300 mM TCA; 2.5 mM EDTA). This mixture was then clarified with centrifugation at 10,000 rpm. Re-extraction with the same conditions was performed and the two supernatants were mixed. Finally, extracts were measured following Noctor and Foyer (1998a), where total glutathione was obtained by the glutathione reductase (GR) recycling assay, initially described by Tietze (1969); whereas for oxidized glutathione, determination samples were previously incubated with 2-vinylpyridine as described by Griffith (1980).

Reduced ascorbate (AA) and dehydroascorbate (DHA) were oxidized using ascorbate oxidase (AO) and dithiothreitol (DTT), respectively, as described in Foyer and others (1983). Ascorbate oxidase specifically oxidizes all reduced ascorbate in the sample; thus, the decrease in O.D. at 265 nm is related to AA concentration. In contrast, when the sample is incubated with DTT, DHA is reduced to AA and the increase in O.D. is proportional to the initial DHA concentration. The ascorbate oxidative status was estimated as DHA/(DHA + AA) and glutathione oxidative status as GSSG/(GSSG + GSH).

Statistical Analyses

Data were analyzed using PASW for Windows ver. 17.2 (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was used to compare mean values at a range of sampling times for RWC, plant hormones, ascorbate and glutathione content and oxidative status, gas exchange measurements, and fluorescence parameters. The post hoc Duncan's test was applied. A significance level of 95% ($p < 0.05$) is indicated in the figure captions. At each sampling time, significantly different means are marked with different letters.

Results

Time Course of Endogenous Concentrations of Jasmonic Acid and Abscisic Acid

Well-watered (WW) Col-0 and *aos* and *vtc1* mutants maintained a relative water content (RWC) of between 92 and 86%, whereas the *aba2* mutant showed a significantly lower RWC (ca. 70%) throughout the experiment. Six days after the onset of water stress, RWC started to decrease in water-stressed (WS) plants, and by the end of the experiment we observed significantly lower values of RWC in WS compared to WW plants, 15% lower in Col-0, 27% in *aba*, 39% in *aos*, and 51% in *vtc1* mutants. Particularly noteworthy was the sharp decrease in RWC (45%) observed in the *vtc1* mutant from days 9 to 12 of water stress (Fig. 1).

The endogenous concentration of JA was almost constant (ca. 0.2 nmol g^{-1} FW) in Col-0 and *aba2* plants; however, at the beginning of the experiment this hormone was 50% lower in the *aos* mutant than in Col0, *aba2*, and *vtc1* plants. Water stress induced an increase in endogenous concentration of JA, which reached values of 1.25 nmol g^{-1} FW in Col-0, *aba2*, and *vtc1* plants after 12 days of withholding water. However, although the increase in JA was progressive from the beginning of the experiment in Col-0 and *vtc1*, in the *aba2* mutant JA concentrations started to increase later, at 6 days of water stress (Fig. 1).

The ABA concentration in WW plants followed a similar trend to that of JA, increasing with age in the WW *vtc1* mutant. ABA content in the *aba2* mutant was 65% lower

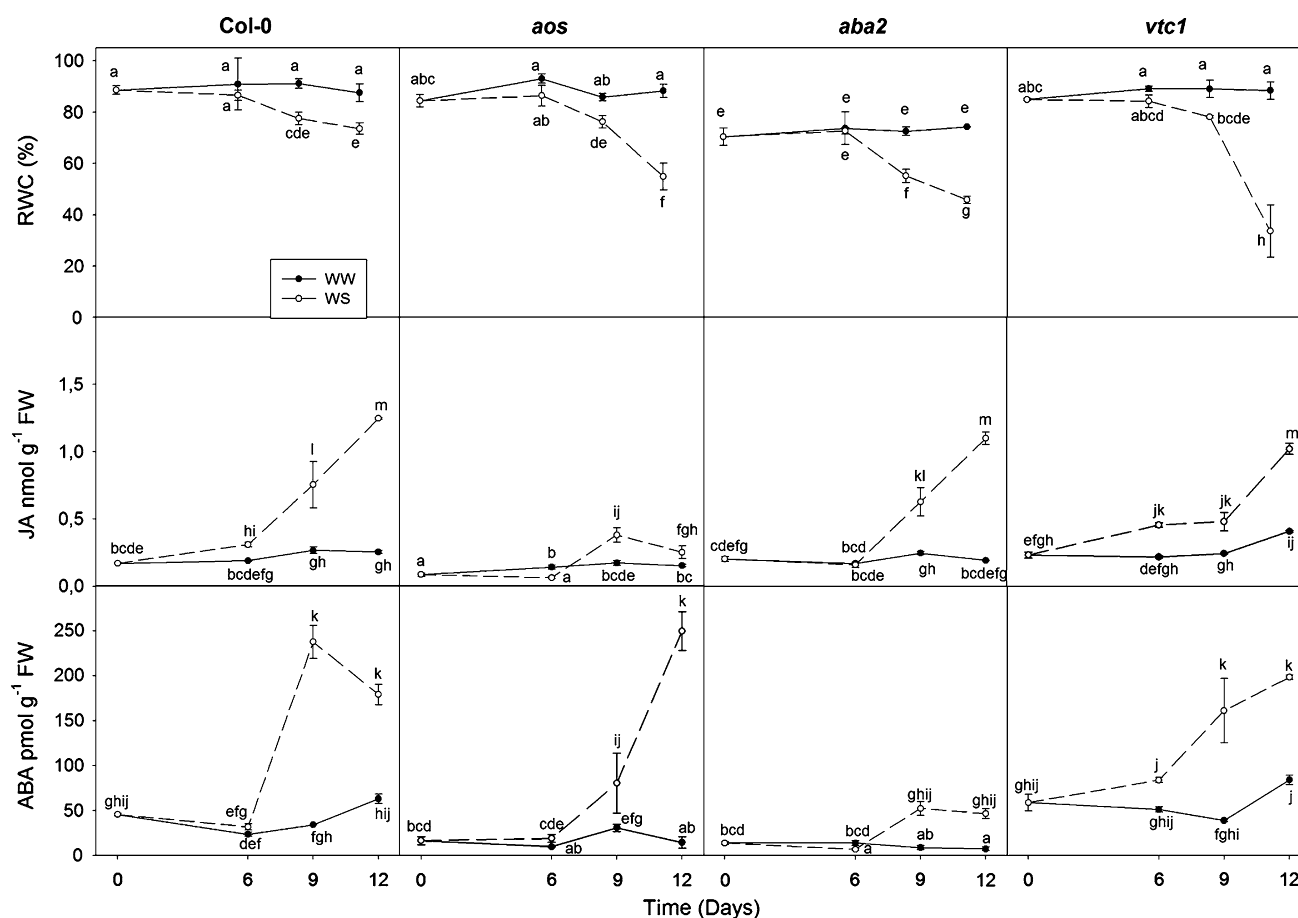

Fig. 1 Time course of relative water content (RWC %) and endogenous concentration of jasmonic acid (JA, nmol g^{-1} FW) and abscisic acid (ABA, pmol g^{-1} FW) in the rosettes of well-watered (*filled circle*, WW) and water-stressed (*open circle*, WS) *Arabidopsis* wild-type (Col-0) plants and JA-deficient (*aos*), ABA-deficient (*aba2*), and AA-deficient (*vtc1*) mutants. Water stress was induced by withholding water for 12 days. For each sampling time, significantly different means ($p < 0.05$) are marked with *different letters*. Data are means ± SE. At least three replicates of ten rosettes were used in each sampling point

than in the wild-type plants and in *aos* and *vtc1* mutants. In WS Col-0 plants, ABA reached a peak of 238 pmol g^{-1} FW after 9 days of water stress and tended to decrease thereafter. A progressive increase in ABA concomitant with water stress was observed in *aos* and *vtc1* mutants. The WS *aba2* mutant showed only a slight increase in this parameter.

Jasmonic Acid and Abscisic Acid Effects on the Regulation of Gas Exchange

Given the involvement of both ABA and JA in the regulation of stomatal movements, we compared gas exchange between mutants and wild-type *Arabidopsis* plants.

The time course of net photosynthesis (A), stomatal conductance (G_s), and transpiration rate (E) was determined from the beginning of the experiment until day 10, when significant differences in RWC and in the endogenous concentrations of JA and ABA were observed between WW and WS plants (Fig. 2).

WW Col-0 plants showed values of A of approximately 2.5 µmol m^{-2} s^{-1}, whereas A increased with rosette age in *aos* plants, reaching values approximately 2.5-fold higher than in the wild type. The *vtc1* mutant showed the lowest values of A throughout the experiment, about 1.4 µmol m^{-2} s^{-1} (Fig. 2). In the *aba2* mutant, water stress reduced the net photosynthesis rate to near or below the compensation point for CO_2.

The stomatal conductance rate (Fig. 2) in WW *aos*, *aba2*, and Col-0 plants increased with plant age during the water stress treatment. The *vtc1* mutant had higher G_s values than did Col-0 and *aos* plants, but the highest G_s values were observed in *aba2*, which were around fourfold higher than in wild type, *aos*, and *vtc1* plants. Water stress decreased G_s in Col-0, *vtc1*, and *aba2* (51, 88, and 25%, respectively) from the beginning of the experiment. The

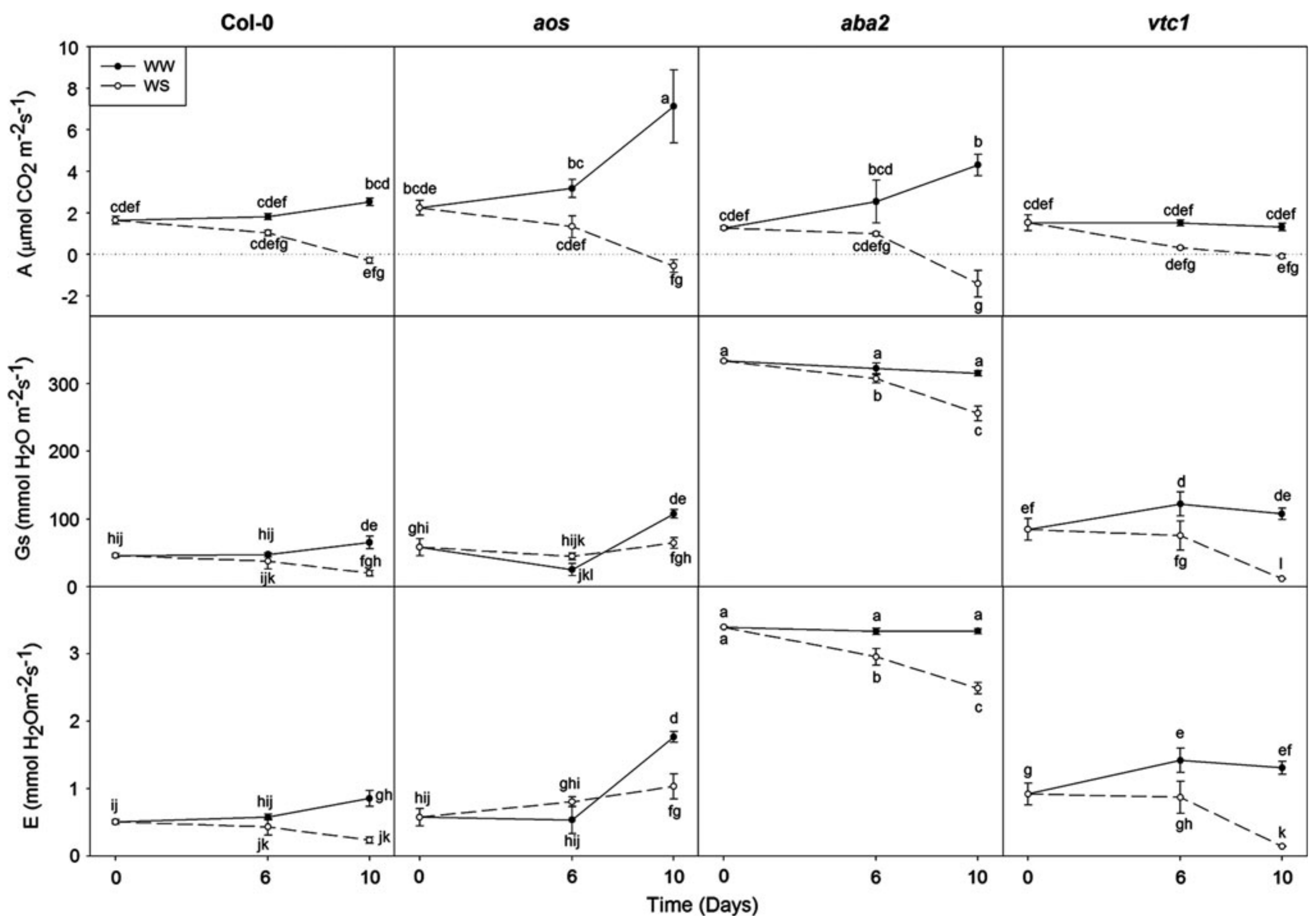

Fig. 2 Time course of net assimilation rate (A, μmol m^{-2} s^{-1}), stomatal conductance (G_s, mmol m^{-2} s^{-1}), and transpiration rate (E, mmol m^{-2} s^{-1}) in the rosettes of well-watered (*filled circle*, WW) and mildly water-stressed (*open circle*, WS) *Arabidopsis* wild-type (Col-0) plants and JA-deficient (*aos*), ABA-deficient (*aba2*), and AA-deficient (*vtc1*) mutants. Water stress was induced by withholding water for 12 days. For each sampling time, significantly different means ($p < 0.05$) are marked with *different letters*. Data are means ± SE. At least three replicates of ten rosettes were used in each sampling point

WS *aba2* mutant had a G_s about threefold higher than that of WS wild-type, *aos*, and *vtc1* plants.

The transpiration rate E (Fig. 2) followed a similar trend as that of G_s in WW and WS plants, and again the WW and WS *aba2* mutant showed the highest transpiration rate (ca. 3.3 and 2.5 μmol m^{-2} s^{-1}, respectively). The largest differences between WW and WS plants (90%) were observed in the *vtc1* mutant.

Jasmonic Acid and Abscisic Acid Effects on the Regulation of Oxidative Markers

The maximum efficiency of PSII photochemistry (F_v/F_m ratio; Fig. 3) was almost constant in WW plants and tended to decrease after 9 days of stress in Col-0, *aos*, and *aba2*. However, the *vtc1* mutant showed a clear decrease in this parameter from 0.81 to 0.13 at day 12 of stress. The damage to photosynthetic electron transport appeared to be irreversible in *vtc1* plants.

MDA was used as an estimation of lipid peroxidation. MDA levels tended to increase by the end of the experiment in WW Col-0, *aba2*, and *vtc1* plants (Fig. 3). This trend was not observed in the *aos* mutant. The lowest concentration of MDA was observed in WS *aos* plants.

The *aos* Mutant Showed the Lowest Variations in Total Ascorbate and Glutathione

Total ascorbate increased in Col-0 and in *aba2*, whereas concentrations were almost constant in *aos* and *vtc1*. In Col-0 plants, the total amount of ascorbate was influenced by both AA and DHA, which reached their maximum levels on day 9 of the experiment, although AA decreased on day 12. In the *aba2* mutant, the maximum level of AA was also observed at day 9, but total ascorbate remained constant until day 12 due to an increase in DHA and a decrease in AA. *aos* plants always maintained much lower AA and DHA levels did than Col-0 and *aba2* plants,

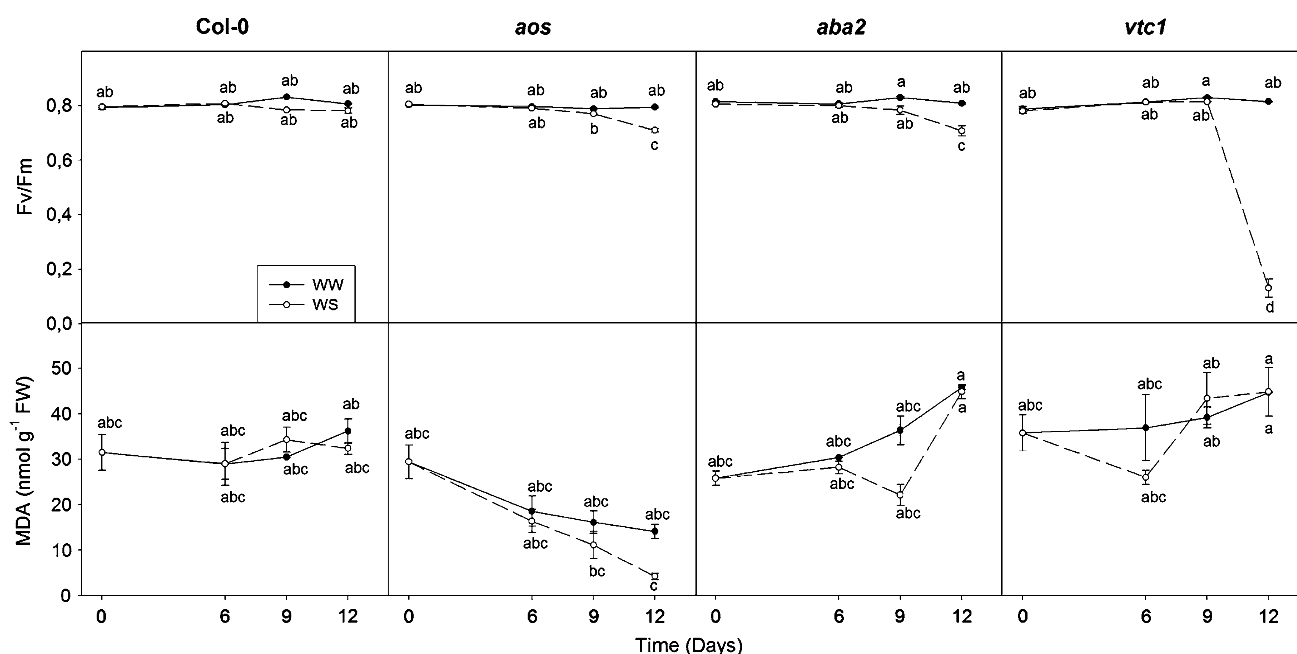

Fig. 3 Variations in the F_v/F_m ratio and MDA (nmol g^{-1} FW) in leaves of well-watered (*filled circle*, WW) and mildly water-stressed (*open circle*, WS) *Arabidopsis* wild-type (Col-0) plants and JA-deficient (*aos*), ABA-deficient (*aba2*), and AA-deficient (*vtc1*) mutants. Water stress was induced by withholding water for 12 days. For each sampling time, significantly different means ($p < 0.05$) are marked with *different letters*. Data are means ± SE. At least three replicates of ten rosettes were used in each sampling point

although there was a tendency toward an increase in the levels of total AA, mainly due to DHA, which increased from day 9. As expected, AA and DHA levels in the *vtc1* mutant were the lowest, despite a slight increase in total ascorbate; this was especially the case for DHA levels at the end of the experiment. AA increased in WS Col-0 and *aba2* plants but was almost constant in *aos* and *vtc1* mutants under the same conditions, although lower in these latter mutants (68 and 42%, respectively) than in Col-0 and *aba2* mutants. Meanwhile, DHA levels in WW plants were consistently about 70–80% lower than those of AA, except in the case of *vtc1* mutants where DHA levels were only 50% lower. Water stress induced a progressive increase in DHA levels in *aba2*, *vtc1*, and *aos* plants after 12 days of stress but not in Col-0, which showed the highest concentrations of DHA after 9 days of stress (Fig. 4).

No significant differences in the endogenous concentration of glutathione in WW Col-0 plants were observed. However, this parameter decreased significantly in *aos*, *aba2*, and *vtc1* mutants at the end of the experiment. The endogenous concentration of GSSG in WW plants was consistently lower than that of GSH, except in the case of the *vtc1* mutant. Water stress induced oscillations in GSSG levels mainly in Col-0 and *aba2* plants, but no significant differences were detected in the GSSG content of *aos* or *vtc1* mutants (Fig. 4).

Ascorbate oxidative status increased from day 9 to the end of the experiment in Col-0 and *vtc1* plants. A slight but significant increase was observed in *aos* and no differences were detected in the WW *aba2* mutant during the experiment. Water stress induced an increase in ascorbate oxidative status and this increase was visible after 9 days of withholding water. A slight but significant increase in glutathione oxidative status was observed after 9 days of stress in Col-0, *aba2*, and *vtc1* plants, whereas glutathione oxidative status at the end of the experiment was 41, 50, and 64% higher in WS Col-0, *aba2*, and *aos* plants, respectively, than in WW plants. No differences in glutathione oxidative status were observed between WW and WS *vtc1* mutants (Fig. 5).

Discussion

Here we provide new evidence for the physiological significance of the interaction between JA and ABA and their antioxidants' oxidative status in plant responses to drought. Our observations on *Arabidopsis* mutants with impaired JA (*aos*) and ABA (*aba2*) biosynthesis as well as in a mutant with impaired ascorbate biosynthesis (*vtc1*) suggest that there is an interaction between phytohormones and their antioxidants' oxidative status in plant responses to drought.

The role of phytohormone interactions in the regulation of plant responses to biotic and abiotic stress has been studied (Fujita and others 2006), and it has been demonstrated that the interplay between ABA and JA influences

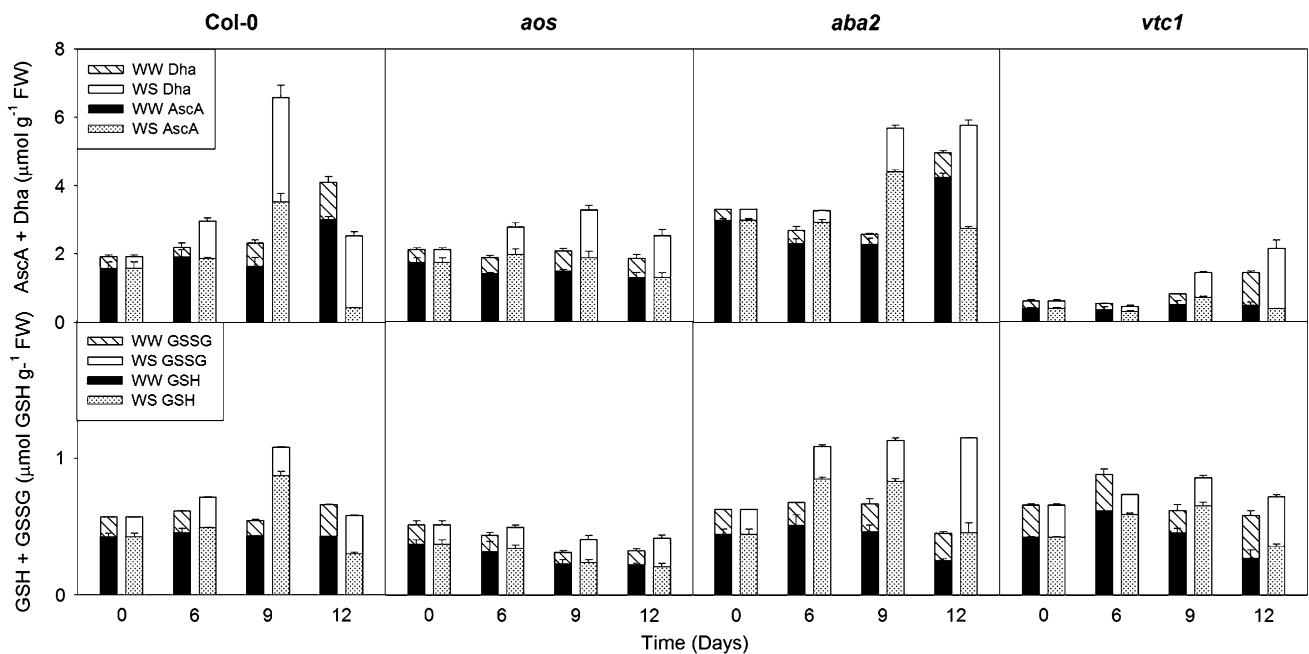

Fig. 4 Variations in total ascorbate (AA + DHA, µmol g^{-1} FW) and total glutathione (GSH + GSSG, µmol GSH g^{-1} FW) in the rosettes of well-watered (*filled circle*, WW) and water-stressed (*open circle*, WS) *Arabidopsis* wild-type (Col-0) plants and JA-deficient (*aos*), ABA-deficient (*aba2*), and AA-deficient (*vtc1*) mutants. Water stress was induced by withholding water for 12 days. Data are means ± SE. At least three replicates of ten rosettes were used in each sampling point

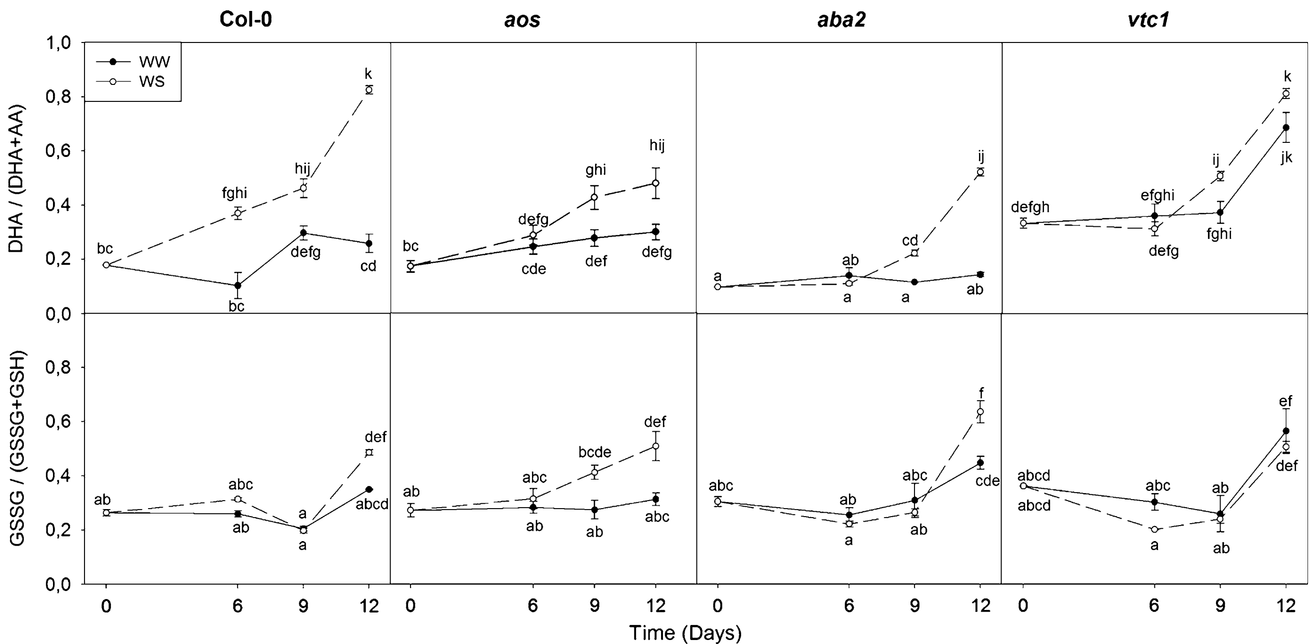

Fig. 5 Variations in oxidative state of ascorbate (DHA/AA + DHA) and glutathione (GSH/GSH + GSSG) in the rosettes of well-watered (*filled circle*, WW) and water-stressed (*open circle*, WS) *Arabidopsis* wild-type (Col-0) plants and JA-deficient (*aos*), ABA-deficient (*aba2*), and AA-deficient (*vtc-1*) mutants. Water stress was induced by withholding water for 12 days. For each sampling time, significantly different means ($p < 0.05$) are marked with *different letters*. Data are means ± SE. At least three replicates of ten rosettes were used in each sampling point

plant responses to biotic stress (Adie and others 2007) and has a plausible role in abiotic stress (Fujita and others 2006). Furthermore, it has been suggested that this interplay regulates plant responses to drought stress (Harb and others 2010). However, our understanding of the interplay between ABA and JA in the regulation of plant

responses to water stress is still incomplete. Thus, our results shed new light on this process and improve our knowledge of the role of hormones in the regulation of plant responses to stress.

ABA and JA Biosynthesis are Interlinked

Water stress induces a progressive increase in ABA (Zhu 2002; Nambara and Marion-Poll 2005) in conjunction with a significant decrease in RWC (Jubany-Marí and others 2009). Although we observed that the endogenous concentration of ABA increased and after 12 days reached the same values in WS Col-0 plants and *aos* mutants, the accumulation pattern of this hormone differed between these plants. At day 6 of water stress, the concentration of ABA in *aos* mutants was 75% lower than in wild-type plants. Moreover, the concentrations of JA in *aba2* after 9 days of stress were slightly lower than in Col-0 plants. Thus, on the basis of our simultaneous observations of ABA and JA in Col-0, *aos*, and *aba2* plants, we propose that JA influences ABA biosynthesis and, to a lower extent, ABA influences JA biosynthesis. Similarly, WS barley seedlings pretreated with JA showed a more than fourfold greater accumulation of ABA compared to WS barley seedlings not pretreated with JA (Bandurska and others 2003). Moreover, the role of JA in the regulation of ABA biosynthesis has been addressed (Kazan and Manners 2008). Furthermore, it has been suggested that ABA regulates JA biosynthesis in ABA-treated *Arabidopsis* plants (Adie and others 2007; Kazan and Manners 2008). However, to the best of our knowledge no studies to date have considered simultaneously endogenous variations in ABA and JA in *aos* and *aba2* mutants, and none have shown the influence of increasing levels of JA on ABA biosynthesis. Our data from the *vtc1* mutants also support the notion of a relationship between ascorbate metabolism and levels of endogenous jasmonates, as has been suggested previously (Suza and others 2010).

ABA content in WW *vtc1* mutants after 12 days of growth was higher than in Col-0 plants. These results are consistent with those reported by Pastori and others (2003), who showed that leaf ABA increased 1.6-fold in this mutant. This increase was higher as plants aged (López-Carbonell and others 2006). Simultaneously, JA levels followed the same pattern as ABA, again suggesting crosstalk between ABA and JA biosynthesis.

Stomatal Closure is Regulated Mainly by ABA and to a Lesser Extent by JA

The WW *aba2* mutant had the highest values of G_s and E, as has been described by Léon-Kloosterziel and others (1996), who reported that a lack of adequate amounts of ABA in the rosette of *aba2* leads to a higher transpiration rate and to excessive water loss. However, in contrast to these findings, we observed a slight but significant decrease in G_s and E in WS *aba2* plants in parallel with a slight increase in ABA and JA. The coordinated action of ABA and JA contributed to stomatal closure, although G_s and E were almost threefold higher in WS *aba2* compared to WW *aos*, *vtc1*, and wild-type plants. The high stomatal conductance and transpiration seen in WS *aba2* plants would explain the decrease observed in the rate of photosynthesis. It is particularly noteworthy that the greatest decrease in the CO_2 assimilation rate was observed in WS *aos* and *aba2* mutants. This indicates that in the *aos* mutant the decrease in CO_2 assimilation rate was more pronounced than the decrease in G_s and E. Thus, JA is required for the regulation of aspects of photosynthesis rather than stomatal conductance in WS plants (Reinbothe and others 1994). There were no significant differences in the CO_2 assimilation rate between WW *vtc1* and wild-type plants, an observation that is consistent with the study by Veljobic-Jovanovic and others (2001). During the first 10 days of stress, slight differences were observed in the RWC, which is consistent with a slight decrease in CO_2 assimilation rate, stomatal conductance, and transpiration.

Oxidative Markers

Remarkably, only the *vtc1* mutant showed a sharp decrease in F_v/F_m, which is an indicator of damage to the photosynthetic apparatus, from days 9 to 12 of water stress. This observation indicates that water stress triggers oxidative damage but only in conditions of prolonged stress. The decrease in F_v/F_m was concomitant with a sharp fall in RWC despite the high concentrations of JA and ABA observed by the end of experiment. These findings show that the levels of other compounds such as DHA are required for stomatal closure, or that other mechanisms such as xylem cavitation and consequent plant dehydration regulate oxidative stress in the *vtc1* mutant. Similar results have been reported in this mutant by Huang and others (2005), who observed that under salt stress conditions F_v/F_m had a much greater decrease in the *vtc1* mutant than in the wild type and that this difference increased as stress progressed. Thus, compared to *aos* and *aba2*, *vtc1* is the mutant that is most sensitive to prolonged stress. In addition, MDA tended to decrease progressively throughout the experiment in WW and WS *aos* plants. This observation indicates a relationship between JA and lipid peroxidation but not between ABA and lipid peroxidation, as MDA increased with plant age and also with stress in the *aba2* mutant. A possible relationship between MDA and jasmonate levels cannot be discounted (Jubany-Marí and others 2010). Furthermore, depending on the concentration, the

external application of JA stimulates the formation of lipid peroxides (Piotrowska and others 2010).

Ascorbate and Glutathione Formation Under Water Stress is Influenced Mainly by JA and to a Lesser Extent by ABA

The relationship between ABA and JA in regulating ascorbate and glutathione metabolism in plants under stress remains unknown (Shan and Liang 2010). In several plant species, ascorbate increases in response to exogenous JA, thereby suggesting that JA may regulate ascorbate metabolism in plants (Suza and others 2010; Sasaki-Sekimoto and others 2005). However, these reports did not provide information about ascorbate metabolism in mutants deficient in JA biosynthesis. In addition to the potential effects of JA signaling on ascorbate accumulation, conversely, the concentrations and redox status of ascorbate may also influence JA signaling. Our study using WW and WS *aos*, *aba2*, and *vtc1* mutants contributes to advancing our knowledge of the influence of endogenous JA on baseline ascorbate concentrations and also of the participation of JA in the regulation of ascorbate metabolism in response to water stress. Our results showed almost the same trends in the pattern of AA and total ascorbate in Col-0 and *aba2* plants, which increased in WW plants in an age-dependent manner. Similarly, AA and total ascorbate increased over the time course of the experiment in WS plants, in parallel with JA, reaching higher values than in WW plants.

The most conspicuous aspects of the *aos* mutant were the ascorbate and glutathione values, which did not increase in response to water stress, whereas a slight increase in JA was registered. Although initial ascorbate concentrations in the *aos* mutant were the same as those in Col-0 plants, the mutant did not experience any significant increase as water stress progressed. The small and non-significant differences observed in AA, DHA, and total ascorbate in the *aos* mutant showed that JA is required to regulate the variations in ascorbate metabolism in WS *Arabidopsis* plants. These findings confirm previous reports that have related ascorbate formation to jasmonates (Li and others 1998; Wang 1999; Sasaki-Sekimoto and others 2005; Wolucka and others 2005). Furthermore, the final value of ascorbate in WW Col-0 plants showed a slight increase, possibly induced by age. This increase was not detected in jasmonate-deficient plants.

No significant differences were observed in ascorbate metabolism in the *vtc1* mutant during the first 9 days of water stress, despite the high concentrations of JA. Only after 12 days of stress did we observe a sharp increase in DHA and a small decrease in AA. Thus, JA regulated ascorbate metabolism in Col-0 and *aba2* plants. JA increased the transcript levels and activities of ascorbate peroxidase (APX) and GR and the contents of AA and GSH in leaves under stress (Shan and Liang 2010). However, it did not do so in the *vtc1* mutant in which the induction of antioxidant genes by JA was abolished in part because of the impaired supply of GDP-Man substrate required by the action of JA. Moreover, according to Veljobic-Jovanovic and others (2001), there is little compensation for decreases in leaf ascorbate by increases in glutathione in the *vtc1* mutant.The results obtained with the *aos*, *aba2*, and *vtc1* mutants showed that JA is the major contributor to the regulation of ascorbate metabolism in *Arabidopsis* plants. Glutathione in WW and WS plants was higher in Col-0, *aba2*, and *vtc1* than in *aos* plants, thereby suggesting that JA participates in the control of GSH and total glutathione concentrations. These results are consistent with the role of JA in the regulation of glutathione metabolism in plants subjected to water stress (Shan and Liang 2010) but are not indicative of the proposed involvement of ABA in the regulation of glutathione metabolism.

In conclusion, our results suggest that water-stress-induced JA accumulation participates in the regulation of ascorbate and glutathione metabolism which in turn enhances antioxidant activity and protects *Arabidopsis* plants against oxidative damage induced by water stress. However, although jasmonates are key signalling components for the plant stress response, their effects are tightly regulated by ascorbate concentrations and metabolism. JA failed to regulate oxidative stress in the *vtc1* mutant under prolonged drought. These results contribute to our knowledge about the antioxidant metabolism in plants under water stress conditions.

We observed that water stress induced a sharp increase in ascorbate oxidative status in Col-0 and to a lesser extent in *aba2* and *aos* mutants. These findings provide evidence of a relationship between ABA, JA, and ascorbate oxidative status. Compared with *aba2*, *aos*, and Col-0 plants, the WW *vtc1* mutant showed the highest ascorbate oxidative status and this increased with plant age. No significant differences were observed between WW and WS plants. These results are consistent with the findings reported by Conklin and others (1996), who observed that ascorbate oxidative status does not change in *vtc1* plants under water stress conditions. Our data strongly indicate that changes in ascorbate content are the major factor regulating plant responses to oxidative stress. The redox status of glutathione followed the same pattern as shown by the ascorbate oxidative status, thereby also indicating the relationship with environmental cues and levels of ascorbate.

In conclusion, overall our results show that *aos*, *aba2*, and *vtc1* mutants are more sensitive to drought than wild-type *Arabidopsis* plants, and we provide evidence of the concerted interaction between JA and ABA and antioxidants in modulating of the responses of these plants to

water stress. Despite the interaction between JA and ABA, ABA is the key player in the regulation of stomatal movements, whereas JA plays a major role in the control mechanism regulating ascorbate and glutathione metabolism. However, ascorbate concentrations and metabolism are indispensable for JA/ABA crosstalk in the responses of *Arabidopsis* to drought.

Acknowledgments We thank the Serveis de Camps Experimentals and Serveis Científico-Tècnics (Universitat de Barcelona) for technical assistance. This work was supported by grant BFU2009-08865 from the Spanish Government. RB holds a FPI fellowship from the Spanish Government.

References

Acharya BR, Assmann SM (2009) Hormone interactions in stomatal function. Plant Mol Biol 69:451–462

Adie BA, Perez-Perez J, Perez-Perez MM, Godoy M, Sanchez-Serrano JJ, Schmelz EA, Solano R (2007) ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defences in *Arabidopsis*. Plant Cell 19:1665–1681

Ahuja I, de Vos RCH, Bones AM, Hall RD (2010) Plant molecular stress responses face climate change. Trends Plant Sci 15: 664–674

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–379

Balbi V, Devoto A (2008) Jasmonate signalling network in *Arabidiopsis thaliana*: crucial regulatory nodes and new physiological scenarios. New Phytol 177:301–318

Bandurska H, Stroinski A, Kubís J (2003) The effect of jasmonic acid on the accumulation of ABA proline and spermidine and its influence on membrane injury under water deficit in two barley genotypes. Acta Physiol Plant 25:279–285

Bray EA (1997) Plant responses to water deficit. Trends Plant Sci 2:48–54

Conklin PL, Williams EH, Last RL (1996) Environmental stress sensitivity of an ascorbic acid-deficient *Arabidopsis* mutant. Proc Natl Acad Sci USA 93:9970–9974

Creelman RA, Mullet JE (1997) Biosynthesis and action of jasmonates in plants. Annu Rev Plant Physiol 48:355–381

Devoto A, Turner JG (2003) Regulation of jasmonate-mediated plant response in *Arabidopsis*. Ann Bot 92:329–337

Fernández-García N, Martí MC, Jiménez A, Sevilla F (2009) Sub-cellular distribution of glutathione in an *Arabidopsis* mutant (*vtc1*) deficient in ascorbate. J Plant Physiol 166:2004–2012

Foyer C, Noctor G (2009) Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. Antioxid Redox Signal 11:862–905

Foyer C, Rowell J, Walker D (1983) Measurement of the ascorbate content of spinach leaf protoplasts and chloroplasts during illumination. Planta 157:239–244

Fujita M, Fujita Y, Noutoshi Y, Takahashi F, Narusaka Y, Yamaguchi-Shinozaki K, Shinozaki F (2006) Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. Curr Opin Plant Biol 9:436–442

Gapper NE, Norris GE, Clarke SE, Lill RE, Jameson PE (2002) Novel jasmonate amino acid conjugates in *Asparagus officinalis* during harvest-induced and natural foliar senescence. Physiol Plant 114:116–124

Genty B, Briantais JM, Barker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochem Biophys Acta 990:87–92

Griffith OW (1980) Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinilpyridine. Anal Biochem 106:207–212

Harb A, Krishnan A, Madana MR, Ambabaram MM, Pereira A (2010) Molecular and physiological analysis of drought stress in *Arabidopsis* reveals early responses leading to acclimation in plant growth. Plant Physiol 154(3):1254–1271

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. California Agricultural Experiment Station Circular 347, Berkeley, 32 pp

Hodges DM, DeLong JM, Forney CF, Prange RK (1999) Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanins and other interfering compounds. Planta 207:604–611

Huang C, Wenliang H, Guo X, Su P, Zhang L (2005) Increased sensitivity to salt stress in an ascorbate-deficient *Arabidopsis* mutant. J Exp Bot 56:3041–3049

Huang D, Wu W, Abrams SR, Cutler AJ (2008) The relationship of drought-related gene expression in *Arabidopsis thaliana* to hormonal and environmental factors. J Exp Bot 59:2991–3007

Jubany-Marí T, Munné-Bosch S, López-Carbonell M, Alegre L (2009) Hydrogen peroxide is involved in the acclimation of the Mediterranean shrub, *Cistus albidus* L., to summer drought. J Exp Bot 60:107–120

Jubany-Marí T, Prinsen E, Munné-Bosch S, Alegre L (2010) The timing of methyl jasmonate, hydrogen peroxide and ascorbate accumulation during water deficit and subsequent recovery in the Mediterranean shrub *Cistus albidus* L. Environ Exp Bot 69:47–55

Kazan K, Manners JM (2008) Jasmonate signaling: toward an integrated view. Plant Physiol 146:1459–1468

Koorneef M, Reuling G, Karssen CM (1984) The isolation and characterisation of abscisic acid-insensitive mutants of *Arabidopsis thaliana*. Physiol Plant 61:377–383

Léon-Kloosterziel KM, Alvarez-Gil M, Rujis GJ, Jacobsen SE, Olszewki NE, Schwartz SH, Zeevaart JAD, Koorneef M (1996) Isolation and characterization of abscisic acid-deficient *Arabidopsis* mutants at two new loci. Plant J 10:655–661

Li L, Van Staden J, Pager AK (1998) Effect of plant growth regulators on the antioxidant system in callus of two maize cultivars subjected to water stress. Plant Growth Regul 25:81–87

López-Carbonell M, Munné-Bosch S, Alegre L (2006) The ascorbate-deficient *vtc-1 Arabidopsis* mutant shows altered ABA accumulation in leaves and chloroplasts. J Plant Growth Regul 25:1–9

López-Carbonell M, Gabassa M, Jáuregui O (2009) Enhanced determination of abscisic acid (ABA) and abscisic acid glucose ester (ABA-GE) in *Cistus albidus* plants by liquid chromatoraphy-mass spectrometry in tandem mode. Plant Physiol Biochem 47:256–261

Mahouachi W, Arbona V, Gómez-Cadenas A (2007) Hormonal changes in papaya seedlings subjected to progressive water stress and re-watering. Plant Growth Regul 53:43–51

Munné-Bosch S, Alegre L (2002) Interplay between ascorbic acid and lipophilic antioxidants defences in chloroplasts of water stressed *Arabidopsis* plants. FEBS Lett 524:145–148

Nambara E, Marion-Poll A (2005) Abscisic acid biosynthesis and catabolism. Annu Rev Plant Biol 56:165–185

Noctor G, Foyer CH (1998a) Simultaneous measurement of foliar glutathione, γ-glutamylcysteine, and amino acids by high-

performance liquid chromatography: comparison with the two other assay methods for glutathione. Anal Biochem 264:98–110

Noctor G, Foyer CH (1998b) Ascorbate and glutathione: keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol 49:249–270

Park JH, Halitschke R, Kim HB, Baldwin IT, Feldman KH, Feyereisen R (2002) A knock-out mutation in allene oxide synthase results in male sterility and defective wound signal transduction in *Arabidopsis* due to a block in jasmonic acid biosynthesis. Plant J 31:1–12

Pastori GM, Kiddle G, Antoniw J, Bernard S, Veljovic-Jovanovic S, Verrier PJ, Noctor G, Foyer CH (2003) Leaf vitamin C contents modulate plant defense transcripts and regulate genes that control development through hormone signalling. Plant Cell 15: 939–951

Pauwels L, Barbero GF, Geerinck J, Tilleman S, Grunewld W, Cuéllar Pérez A, Chico JM, Vanden Bossche R, Sewell J, Gil E, García-Casado G, Witters E, Inzé D, Long JA, De Jaeger G, Solano R, Goossens A (2010) NINJA connects the co-represor TOPLESS to jasmonate signalling. Nature 464(7289):788–791

Pedranzani H, Sierra-de-Grado R, Viglioco A, Miersch O, Abdala G (2007) Cold and water stresses produce changes in endogenous jasmonates in two populations of *Pinus pinaster* Ait. Plant Growth Regul 52:111–116

Piotrowska A, Bajguz A, Czerpak R, Kot K (2010) Changes in the growth, chemical composition, and antioxidant activity in the aquatic plant *Wolffia arrhiza* (L.) Wimm. (Lemnaceae) exposed to jasmonic acid. J. Plant Growth Regul 29:53–62

Reinbothe S, Mollenhauer B, Reinbothe C (1994) The regulation of plant gene expression by jasmonates in response to environmental cues and pathogens. Plant Cell 6:1197–1209

Riera M, Valon C, Fenzi F, Giraudat J, Leung J (2005) The genetics of adaptive responses to drought stress: abscisic acid-dependent and abscisic acid-independent signalling components. Physiol Plant 123:11–119

Sasaki-Sekimoto Y, Taki N, Obayashi T, Aono M, Matsumoto F, Sakurai N, Suzuki H, Hirai MY, Noji M, Saito T, Masuda T, Takamiya K, Shibata D, Ohya H (2005) Coordinated activation of metabolic pathways for antioxidants and defence compounds by jasmonates and their roles in stress tolerance in *Arabidopsis*. Plant J 44:653–668

Schwartz SH, Léon-Kloosterziel KM, Koornneef M, Zeevaart JAD (1997) Biochemical characterization of the *aba2* and *aba3* mutants in *Arabidopsis thaliana*. Plant Physiol 114:161–166

Segarra G, Jáuregui O, Casanova E, Trillas I (2006) Simultaneous quantitative LC-ESI-MS/MS analyses of salicylic acid and jasmonic acid in crude extracts of *Cucumis sativus* under biotic stress. Phytochemistry 67:395–401

Shan C, Liang Z (2010) Jasmonic acid regulates ascorbate and glutathione metabolism in *Agropyron cristatum* leaves under water stress. Plant Sci 178:130–139

Shinozaki K, Yamaguchi-Shinozaki K (1997) Molecular responses to drought and cold stress. Curr Opin Biotechnol 7:161–167

Shinozaki K, Yamaguchi-Shinozaki K (2000) Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. Curr Opin Biotechnol 3:217–223

Staswick PE (2008) JAZing up jasmonate signalling. Trends Plant Sci 13:66–71

Suhita D, Agepati S, Raghavendra J, Kwack JM, Vavasseur A (2004) Cytoplasmic alkalinization precedes reactive oxygen species production during methyl jasmonate- and abscisic acid-induced stomatal closure. Plant Physiol 134:1536–1545

Suza WP, Avila CA, Carruthers K, Kulkarni S, Goggin FL (2010) Exploring the impact of wounding and jasmonates on ascorbate metabolism. Plant Physiol Biochem 48:337–350

Tietze F (1969) Enzymatic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem 27:502–522

Turner NC (1981) Techniques and experimental approaches for the measurement of plant water status. Plant Soil 58:339–366

Veljobic-Jovanovic SD, Pignocchi C, Noctor G, Foyer CH (2001) Low ascorbic acid in the *vtc-1* mutant of *Arabidopsis* is associated with decreased growth and intracellular redistribution of the antioxidants system. Plant Physiol 127:426–435

von Caemmerer S, Farquhar GD (1981) Some relations between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153:376–387

Wang SY (1999) Methyl jasmonate reduces water stress in strawberry. J Plant Growth Regul 18:127–134

Wasternack C (2007) Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. Ann Bot (Lond) 100:681–697

Wasternack C, Kombrink E (2010) Jasmonates: structural requirements for lipid-derived signals active in plant stress responses and development. ACS Chem Biol 5:63–77

Wolucka BA, Goosens A, Inzé D (2005) Methyl jasmonate stimulates the de novo biosynthesis of vitamin C in plant cell suspensions. J Exp Bot 56:2527–2538

Zhu JK (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Biol 53:247–273