Plant, Cell & Environment
Plant, Cell and Environment (2015) doi: 10.1111/pce.12536

Original Article

Jasmonoyl isoleucine accumulation is needed for abscisic acid build-up in roots of Arabidopsis under water stress conditions

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ABSTRACT

Phytohormones are central players in sensing and signalling numerous environmental conditions like drought. In this work, hormone profiling together with gene expression of key enzymes involved in abscisic acid (ABA) and jasmonate biosynthesis were studied in desiccating Arabidopsis roots. Jasmonic acid (JA) content transiently increased after stress imposition whereas progressive and concomitant ABA and Jasmonoyl Isoleucine (JA-Ile) accumulations were detected. Molecular data suggest that, at least, part of the hormonal regulation takes place at the biosynthetic level. These observations also point to a possible involvement of jasmonates on ABA biosynthesis under stress. To test this hypothesis, mutants impaired in jasmonate biosynthesis (*opr*3, *lox*6 and *jar1-1*) and in JA-dependent signalling (*coi*1) were employed. Results showed that the early JA accumulation leading to JA-Ile build up was necessary for an ABA increase in roots under two different water stress conditions. Signal transduction between water stress-induced JA-Ile accumulation and COI1 is necessary for a full induction of the ABA biosynthesis pathway and subsequent hormone accumulation in roots of Arabidopsis plants. The present work adds a level of interaction between jasmonates and ABA at the biosynthetic level.

Key-words: *coi1-16*; drought; hormone signal transduction; *jar1-1*; jasmonates.

INTRODUCTION

Plants are sessile organisms, which makes the adaptation to a hostile environment a key feature in their physiology. Among the environmental changes that have driven plant evolution, water availability is the most important factor affecting almost every aspect of plant physiology and metabolism (Arbona *et al.* 2010).

Phytohormones coordinate plant responses to stress, acting as bridges between the sensing process and the physiological responses (Brossa *et al.* 2011; Savchenko *et al.* 2014). Jasmonates are widely distributed among plant species and have been classically associated to the regulation of a multitude of developmental and stress response processes such as fruit ripening (Soto *et al.* 2012), production of viable pollen (Song *et al.* 2011), root growth (Raya-González *et al.* 2012), wounding (Koo & Howe 2009) and plant immunity (Browse 2009; Pieterse *et al.* 2012). Since the discovery of jasmonates, 12-oxo-phytodienoic acid (OPDA), jasmonic acid (JA) and methyl JA (MeJA) have been considered as the only bioactive molecules in the pathway. However, a deep investigation of the jasmonate-resistant (*jar1-1*) Arabidopsis mutant allowed the annotation of JAR1 as the enzyme responsible for jasmonoyl isoleucine (JA-Ile) biosynthesis (Staswick *et al.* 2002), which is the molecule that binds to COI1 and mediates jasmonate-dependent signalling (Fonseca *et al.* 2009). The initial binding of JA-Ile to COI leads to the recruitment of JAZ proteins to form a complex as JA co-receptors. At low JA-Ile levels, JAZ proteins act as transcriptional repressors of gene expression. However, after hormone levels increase and the reception complex is formed, JAZ are degraded in a SCF^{COI1}-dependent manner, and JA responses are activated (Sheard *et al.* 2010). Despite the central role that JA-Ile plays on jasmonate-dependent signalling, JA and OPDA have some specific activity in response to a number of environmental stress conditions (Böttcher & Pollmann 2009). Therefore, to gain knowledge in the putative activity of specific jasmonates, different mutants in the jasmonate biosynthesis pathway should be used. Fortunately, in Arabidopsis, a battery of different mutants can be found such as *lox6*, impaired in OPDA biosynthesis; *opr3* unable to convert OPDA to JA under wounding (Chehab *et al.* 2011), or the above mentioned *jar1-1*. It has recently shown that LOX6 contributes to the fast accumulation of JA and JA-Ile in wounded leaves (Chauvin *et al.* 2013).

Water stress triggers the accumulation of ABA, which is considered the key phytohormone regulating whole plant responses to this condition. ABA controls many adaptive responses such as activation of genes responsible for osmotic adjustment, root hydraulic conductivity, shoot and root growth, transpiration and organ abscission (Verslues & Bray 2006; Duan *et al.* 2013; Hong *et al.* 2013); moreover, ABA accumulation is the main responsible of maintaining primary root elongation under low water potentials by inhibiting

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ethylene production (Spollen *et al.* 2000). The ABA downstream signalling in response to water stress is well characterized but the initial switch connecting the primary sensation of water stress and the induction of ABA biosynthesis is still elusive. Jasmonate levels are rapidly and transiently increased by turgor reduction induced by water deficit (Creelman & Mullet 1997). Furthermore, jasmonate-mediated signalling in response to dehydration has been suggested (Arbona *et al.* 2010; De Ollas *et al.* 2013).

Despite the classical assignation of specific roles to each hormone, it is nowadays widely accepted that the expression of multiple genes and flux of various metabolic pathways must be coordinated to adjust the plant response to the severity of the stress, in a time- and tissue-specific manner. The concerted action of different signalling pathways allow a plastic hierarchy of cross-coordination that is just beginning to be understood (De Lucas & Brady 2013). ABA and JA signalling pathways can interact in several points and there is an overlap between ABA- and JA-induced gene expression and physiological processes (Fernández-Arbaizar *et al.* 2012). Furthermore, the inhibitory effect of ABA on Arabidopsis seed germination was enhanced when ABA was applied together with JA (Fernández-Arbaizar *et al.* 2012). At the molecular level, several lines of evidence point towards the interaction between JA and ABA signalling pathways. Recently, Lackman *et al.* (2011), described how MeJA can modulate *NtPYL4* transcript levels in tobacco plants and, on the opposite, ABA is required for JA biosynthesis under biotic stress pressure (Adie *et al.* 2007). Moreover, the induction of *MYC2* by ABA seems to rely on the JA-Ile receptor *COI1* according to Lorenzo *et al.* (2004). In rice, *OsbHLH148* (ortholog of Arabidopsis *MYC2*) interacts with *OsJAZs* in response to drought and its overexpression improves drought tolerance due to the increase in *OsDREB1* expression (Seo *et al.* 2011). These results allowed the proposal of a model for the *OsbHLH148*-related jasmonate signalling in drought stress in which ABA and JA act synergistically to confer stress tolerance. Results also suggested an influence of JA signalling on expression of ABA-dependent genes.

The putative interaction of jasmonates with ABA at the biosynthetic level in Arabidopsis plants subjected to abiotic stress is less understood. It was demonstrated that MeJA induced ABA accumulation in guard cells through the up-regulation of the 9-*cis*-epoxycarotenoid dioxygenase 3 (*NCED3*) gene (Hossain *et al.* 2011). In that work, both ABA and MeJA treatment promoted stomatal closure in wild-type plants following the same dose-dependent relationship and through the same secondary messengers. On the contrary, in *coi1-16* mutant plants, MeJA treatment did not elicit stomatal closure but ABA treatment did, whereas in *abi1*-2 plants, neither ABA nor MeJA elicited stomatal closure. However, it is not clear whether the JA-deficiency caused an impaired stomatal function. Actually, Brossa *et al.* (2011) compared different Arabidopsis genotypes under water stress conditions and no differences in stomatal conductance between Col-0 and a JA-deficient mutant (aos) were found under stress conditions.

These results are compatible with MeJA and ABA acting on signalling pathways converging in *ABI1* or also with JA influencing ABA signalling or, maybe, even inducing its biosynthesis (Hossain *et al.* 2011). Kim *et al.* (2009) suggested that in rice exposed to drought stress, MeJA could stimulate ABA biosynthesis. Contrasting results recently obtained by Savchenko *et al.* (2014), indicate that ABA accumulates in leaves of different Arabidopsis mutants impaired in jasmonate biosynthesis.

In the present work, hormonal interaction in response to water stress was investigated in roots and leaves of different Arabidopsis mutants. The hypothesis to test was that the rapid water stress-induced increase of jasmonate levels in Arabidopsis roots was necessary for a full induction of ABA biosynthesis and, hence, that jasmonate-dependent signalling was part of the water stress network.

MATERIALS AND METHODS

Plant material and stress treatments

Seeds were obtained from NASC (accession number, ecotype and reference of each line are summarized in Supporting Information Table S1). Most of the experiments were performed on 6-week-old *Arabidopsis thaliana* seedlings grown in hydroponic conditions. After stratification, seeds were suspended in sterile water and placed in a bottom-trimmed 1.5 mL tube with sterile sand using an automatic pipette. Microtubes were placed in plastic trays and in 5 L plastic boxes containing nutrient solution. Germinating seeds were covered with a plastic appliance to avoid dehydration. During the first week, plants were kept on tap water. Thereafter, water was replaced by a half-strength Murashige and Skoog (MS) medium for one more week and then kept on a full-strength MS medium for the rest of the growing period. A commercial fish tank pump was used to provide oxygenation to the medium. Plants were cultivated in a 16 h photoperiod, a light intensity of 150 μE m^{-2} s^{-1} and 60–80% relative humidity (RH).

For stress treatments, two different strategies were employed: the first one consisted in desiccating 6-week-old seedlings without any substrate in a plant growth chamber under controlled environmental conditions (Sanyo MLR-351H, Cardiff, UK). Seedlings were placed over a drying paper for different periods of time in the dark at constant temperature of 25 ± 2 °C. Well-watered plants were placed in 200 mL plastic pots with the same nutrient solution employed in the hydroponic growth system. Preliminary experiments indicated that plants placed on a water-saturated filter paper did not show any significant change in ABA or JA content for 300 min. The second experimental design consisted in transplanting 6-week-old plants to 200 mL plastic pots filled with fine-grain perlite as a substrate immediately prior to the experiment. Control plants were watered to saturation allowing water to leach from pots and stress plants kept in dry perlite. To exclude any putative artefact, roots were collected by manually turning the pot and gently grabbing the seedling by the rosette. The whole plant was immediately submerged in liquid nitrogen and

subsequently lyophilized. Once dry-frozen, remaining perlite pellets stuck to the roots were removed with a brush. Time lag between sampling and freezing was less than 30 s.

Relative water content

Relative water content (RWC) was calculated as:

$$RWC\,(\%) = [(FW - DW)/(TW - FW)] \times 100$$

Where FW is the fresh weight of leaves/roots, TW is the weight at full turgescence, achieved after soaking the leaves/roots in cold water (4 °C) for 24 h, and DW is the estimated weight after drying the leaves/roots for 4 h at 80 °C until a constant mass is achieved.

Hormone analyses

Hormone extraction and analysis were carried out essentially as described in (Durgbanshi *et al*. 2005) with slight modifications. Briefly, 0.4 g of frozen plant material was extracted in 5 mL of distilled water after spiking with 50 ng of [$^2H_6$]-abscisic acid, dihydrojasmonic acid, [$^2H_3$] N-[(-)-jasmonoyl]-Isoleucine and [$^2H_5$] *cis*-12-oxo-phytodienoic acid (Arbona *et al*. 2010). After centrifugation at 4000 *g* at 4 °C, supernatants were recovered and pH adjusted to 3.0 with a 30% acetic acid solution. The acidified water extract was partitioned twice against 3 mL of di-ethyl ether. The organic layer was recovered and evaporated under vacuum in a centrifuge concentrator (Speed Vac, Jouan, Saint Herblain Cedex, France). The dry residue was then resuspended in a 10% MeOH solution by gentle sonication. The resulting solution was filtered through regenerated cellulose 0.22 μm membrane syringe filters (Albet S.A., Barcelona, Spain) and directly injected into a UPLC system (Acquity SDS, Waters Corp., Milford, MA, USA). Separations were carried out on a C18 column (Nucleodur C18, 1.8 μm particle size, 50 × 2.1 mm, Macherey-Nagel, Düren, Germany) using a MeOH:H_2O (both supplemented with 0.1% acetic acid) gradient at a flow rate of 300 μL min^{-1} (See Supporting Information Table S2 for details of the stereoisomer forms detected). Hormones were quantified with a Quattro LC triple quadrupole mass spectrometer (TQD, Micromass, Manchester, UK) interfaced to the LC through an orthogonal Z-spray electrospray ion source. Three biological and two technical replicates were performed for each sample.

RNA extraction and cDNA synthesis

Total RNA was isolated using the Trizol method (TRI Reagent©, Sigma-Aldrich, Madrid, Spain) according to manufacturer's instructions. Total RNA was resuspended in DEPC-treated water, the RNA concentration was determined with a Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) and the integrity checked by gel electrophoresis. RNA was treated with DNase I (Promega, Madrid, Spain) to remove DNA contamination. Absence of genomic DNA was tested by PCR using intron-specific primers (Supporting Information Table S3) in RNase (Promega) treated samples. First-strand cDNA was synthesized from 1 μg total RNA using PrimeScript RT-PCR Kit from TaKaRa (Condalab, Madrid, Spain) following the manufacturer's protocol.

Real-time qRT-PCR analysis

Real-time qRT-PCR was carried out using a SmartCycler RealTime PCR system (Cepheid, Sunnyvale, CA, USA) and analysed using the manufacturer's software. The cDNAs were diluted 1:5 with nuclease-free water. Reactions were carried out using the SYBR Premix Ex Taq kit from TaKaRa (Condalab, Madrid, Spain). Specific primers for target and the endogenous reference genes were designed based on sequences from the TAIR genomic database with the NCBI Primer-BLAST software (Ye *et al*. 2012) listed in Supporting Information Table S3. Amplification fragments ranged from 150 to 200 bp, the optimal primer melting temperatures was set on 60 °C and primers were designed to span exon-exon junctions if possible. The conditions for each PCR were as follows: 95 °C for 15 min, followed by 45 cycles of 30 s at 94 °C, 30 s at 60 °C and 30 s at 72 °C. At the end of each experiment, a melting-curve analysis was performed using the instrument default parameters (60 s at 95 °C, then a ramps starting from 55 °C to 95 °C in 1 °C s^{-1} increments and finally, 30 s at 95 °C), which yielded one peak for each set of primers at a temperature between 77 °C and 82 °C, confirming the amplification of only a single product species during the runs.

Relative quantification was performed using the ΔΔCt method corrected with the corresponding primer efficiency obtained with serial dilution curves (Pfaffl *et al*. 2004). Three biological replicates were performed for each sample to obtain standard deviation.

Statistical analysis

Statistical analyses were performed using StatGraphics Plus (V. 2.1.) for Windows (Statistical Graphics Corp., Warrenton, VA, USA). Differences between treatments groups were compared by using the Fisher's least significant difference (LSD) test ($P \leq 0.05$).

RESULTS

Hormone profiles in roots of Arabidopsis under severe water stress conditions

A time-course experiment where hormone levels in roots of WT and *jar1-1* seedlings were quantified showed that ABA accumulation in response to severe dehydration was related to jasmonate biosynthesis. OPDA content transiently increased under water stress conditions (Fig. 1a). At the beginning of the experiment, root OPDA levels in WT plants were 0.148 ± 0.018 nmol g^{-1} but after 30 min of desiccation, they increased 3.2-fold. In later stages of desiccation, OPDA levels transiently increased again (0.293 ± 0.033 nmol g^{-1},

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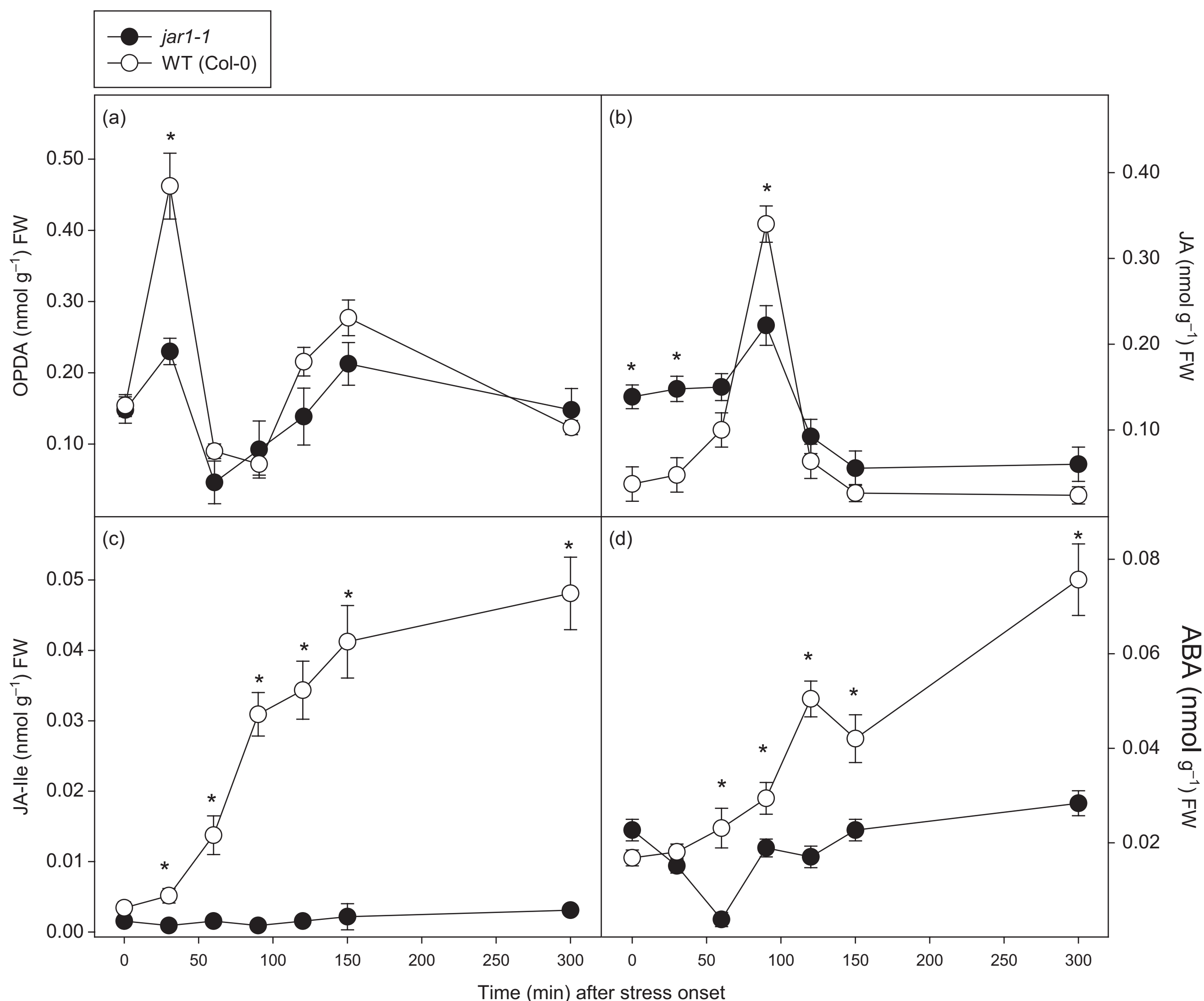

Figure 1. OPDA (a), JA (b), JA-Ile (c) and ABA (d) levels in roots of *Arabidopsis thaliana* WT,(Columbia-0, white circles) and *jar1-1* (black circles) under control conditions ($t = 0$) and dehydrated in air. Data are mean values ± standard deviation of three independent determinations. Asterisks denote statistical significance ($P \leq 0.05$) between control and stressed plants.

after 150 min). OPDA content in roots of well-watered *jar1-1* seedlings was similar to that found in WT. However, under water stress conditions, *jar1-1* plants did not show the initial transient accumulation of this metabolite. JA levels transiently increased after 90 min of stress imposition (8.8-fold higher than initial levels) to decrease to control levels thereafter (120 min). Roots of *jar1-1* plants showed a pattern of JA accumulation similar to WT seedlings although starting from higher initial values and reaching a lower transient increase (Fig. 1b). Levels of JA-Ile were also quantified due to its direct role in COI1-dependent responses in JAs signalling. After the stress onset, root JA-Ile content gradually raised to reach levels 6.6-fold higher than those found in roots of well-watered plants. Root JA-Ile levels in *jar1-1* plants remained at basal levels throughout the experiment (Fig. 1c). In WT plants, ABA accumulation was progressive throughout the experimental period to reach a 5.6-fold increase with respect to initial levels. However, in *jar1-1* plants under water stress conditions, root ABA content did not show any remarkable increase compared with the initial conditions (Fig. 1d). To sum up, desiccation significantly increased the content of jasmonates in Arabidopsis roots with each metabolite following a specific pattern. JA-Ile deficiency in *jar1-1* roots modified the accumulation pattern of both OPDA and JA, and also prevented ABA accumulation, a phytohormone biosynthetically not related to jasmonates.

Transcriptional control of drought-induced hormone increase

JAR1 transcripts in non-stressed *jar1-1* plants were detectable but with a very low abundance (80% lower than in WT seedlings; Fig. 2). In response to water stress, JAR1 expression in WT plants significantly increased throughout the

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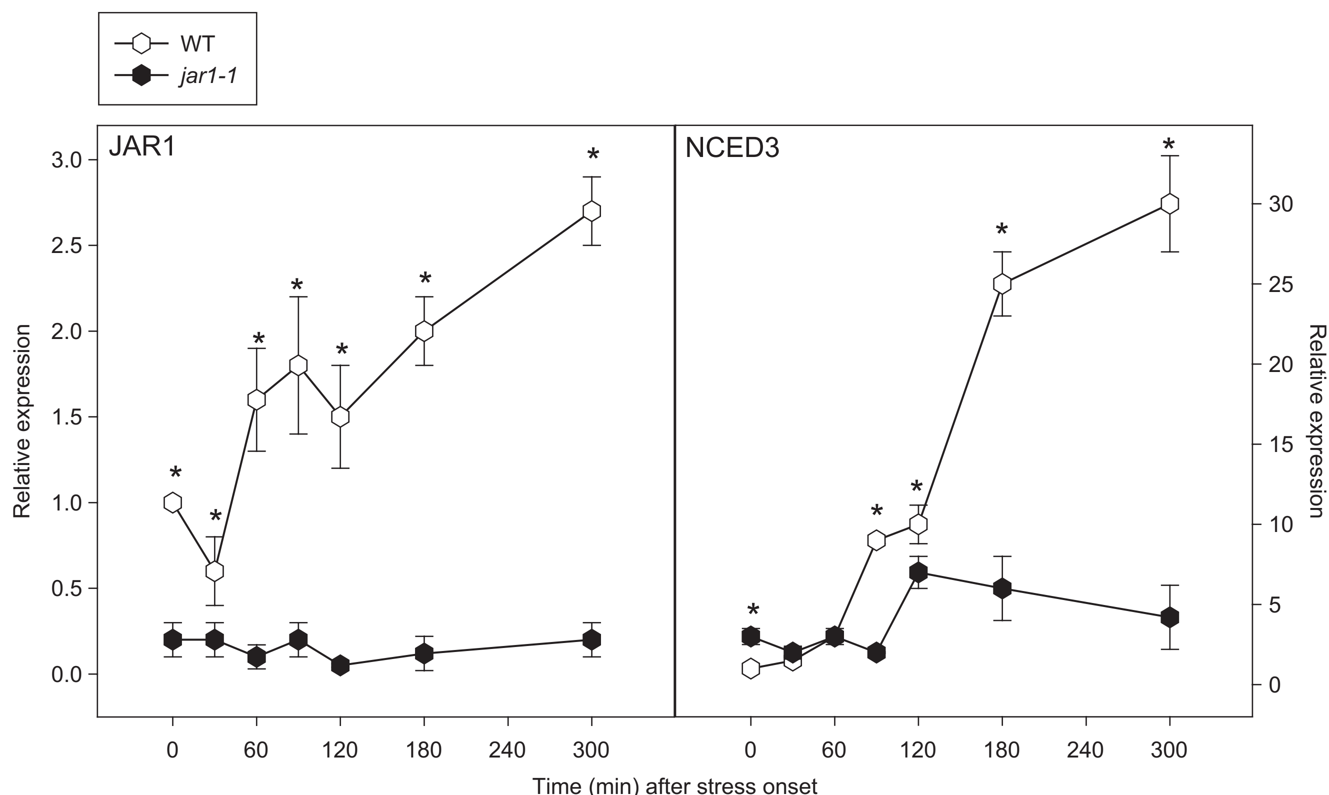

Figure 2. Relative expression of AtJAR1 (left) and AtNCED3 (right) in roots of *Arabidopsis thaliana* WT, (Columbia-0, white circles) and *jar1-1* (black circles) under control conditions ($t = 0$) and dehydrated in air. Data are mean values ± standard deviation of three independent determinations. Asterisks denote statistical significance ($P \leq 0.05$) between genotypes.

experiment. However, in *jar1-1* seedlings, expression of this gene remained at the same levels throughout the experiment, paralleling the minor quantities of JA-Ile found in those plants.

NCED3 expression in well-watered *jar1-1* plants was slightly higher than in WT seedlings. However, after 60 min of dehydration, a constant increase in the amount of *NCED*3 transcripts was detected in WT plants whereas only a slight transient rise was observed in *jar1-1* seedlings. At the end of the experiment *NCED3* expression in *jar1-1* plants was 85% lower than in WT. It should be noted that the expression of this gene is much higher than JAR1 even in well-watered plants.

Hormone profiles in roots of Arabidopsis under substrate dehydration

To exclude putative artefacts in the stress imposition system, a complementary water stress situation was assayed by transferring seedlings to a dry substrate. The progressive dehydration of seedlings in perlite did not allow to observe significant variations in OPDA levels between WT and *jar1-1* seedlings (Fig. 3a), only after 480 min, *jar1-1* seedlings had a significant lower content of this hormone. After 300 min of dehydration, JA accumulated in both genotypes (Fig. 3b), being levels in WT higher than in *jar1-1*; although with lower levels, the differential accumulation remained at 480 min of dehydration. Differences in hormone accumulation were more evident in the case of JA-Ile. In WT seedlings JA-Ile content increased (3.3-fold) after 300 min of stress imposition (Fig. 3c). On the contrary, stressed *jar1-1* seedlings had similar JA-Ile content than both WT and *jar1-1* under well-watered conditions. After 480 min of dehydration, JA-Ile levels were equivalent to those in well-watered plants for both genotypes. In potted plants, water stress induced a progressive accumulation of ABA in roots of WT seedlings to reach a 6.2-fold increase after 480 min of dehydration (Fig. 3d); similarly to what was observed in air-dried roots, *jar1-1* was unable to accumulate high amounts of ABA in roots and only a slight increase in the ABA content was observed after 480 min of dehydration. This system that allowed a more progressive desiccation resulted in a very similar (but not identical) accumulation of jasmonates and ABA. Importantly, despite the differences in the stress imposition, accumulation of ABA in *jar1-1* roots was still impaired.

Relative water content in plants under water stress conditions

To compare the extent of dehydration, RWC was measured in leaves and roots of different genotypes after 300 min of dehydration (Fig. 4). In leaves of plants transferred to dry

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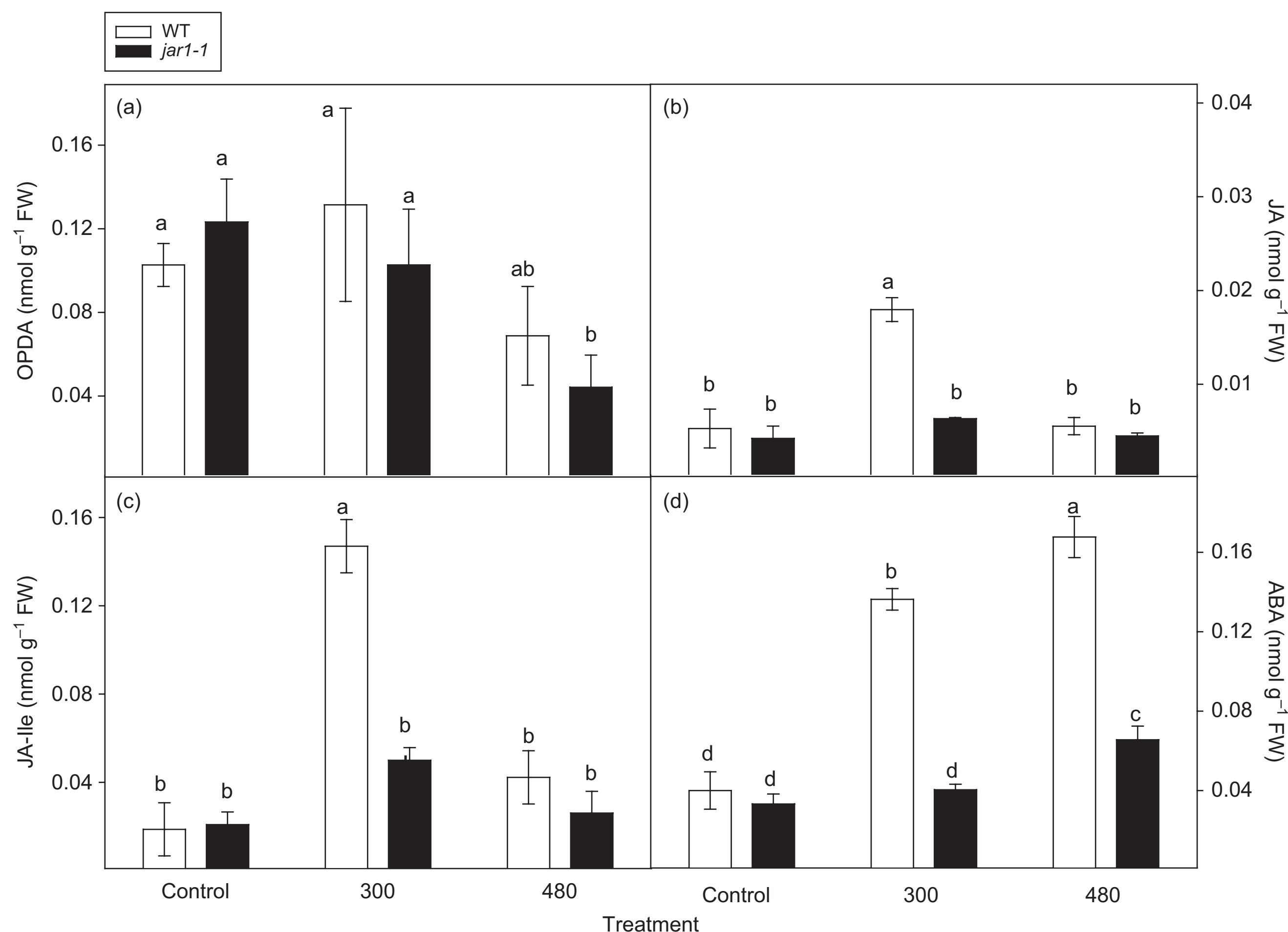

Figure 3. OPDA (a), JA (b), JA-Ile (c) and ABA (d) levels in roots of *Arabidopsis thaliana* WT (Columbia-0, white bars) and *jar1-1* (black bars) under control conditions and dehydrated in perlite. Data are mean values ± standard deviation of three independent determinations. Letters denote statistical significance ($P \leq 0.05$) between genotypes and groups.

perlite, all genotypes (WT, *opr3*, *lox6* and *jar1-1*) had a similar RWC. The simultaneous exposure of seedlings to desiccation with no substrate caused a similar degree of water loss in the aerial part of all genotypes. However, the different systems of dehydration (perlite or air) had a marked influence in root RWC. When seedlings of the different genotypes were transferred to dry perlite, root RWC decreased by a 19% on average without detecting differences among the different genotypes. Seedlings air-dried for 300 min suffered a reduction in RWC of 48% on average with little variations among genotypes. As shown in Supporting Information Fig. S1, plants transferred to a dry substrate suffered a more progressive dehydration and the genotypes studied in that experiment (WT and *jar1-1*) showed similar values of RWC throughout the experiment.

Hormone levels and gene expression in several mutants impaired in JA biosynthesis under water stress conditions

Hormone levels and gene expression in well-watered plants did not significantly vary at any of the data points analysed. No significant variations in OPDA levels in WT plants after the stress onset were found (Fig. 5a), which is coherent with data from Fig. 1 where it is shown that OPDA levels only increased after 30 min of water withdrawal. In control conditions, *opr3* plants, unable to accumulate JA, had root OPDA levels 2.9 times higher than WT plants. After the stress onset, OPDA content increased 1.6-fold respect to values found in well-watered plants, and remained at similar levels despite the extent of dehydration. On the contrary, *lox6* seedlings (impaired in OPDA biosynthesis) showed an opposite behaviour, with root OPDA content lower than WT throughout the experimental period (around 80% lower). In *jar1-1* seedlings, OPDA levels in well-watered conditions were similar to WT. However, upon stress imposition, root OPDA content in *jar1-1* plants did not increase.

In roots of WT seedlings, JA levels transiently increased 90 min after the stress onset (Fig. 5b). In *opr3* and *lox6* plants, basal JA content was slightly lower than in WT seedlings whereas in *jar1-1* plants it was higher. None of the mutant genotypes accumulated JA in their roots under water stress conditions. JA-Ile levels progressively increased in WT plants to reach a 15-fold increase (Fig. 5c) after 300 min of

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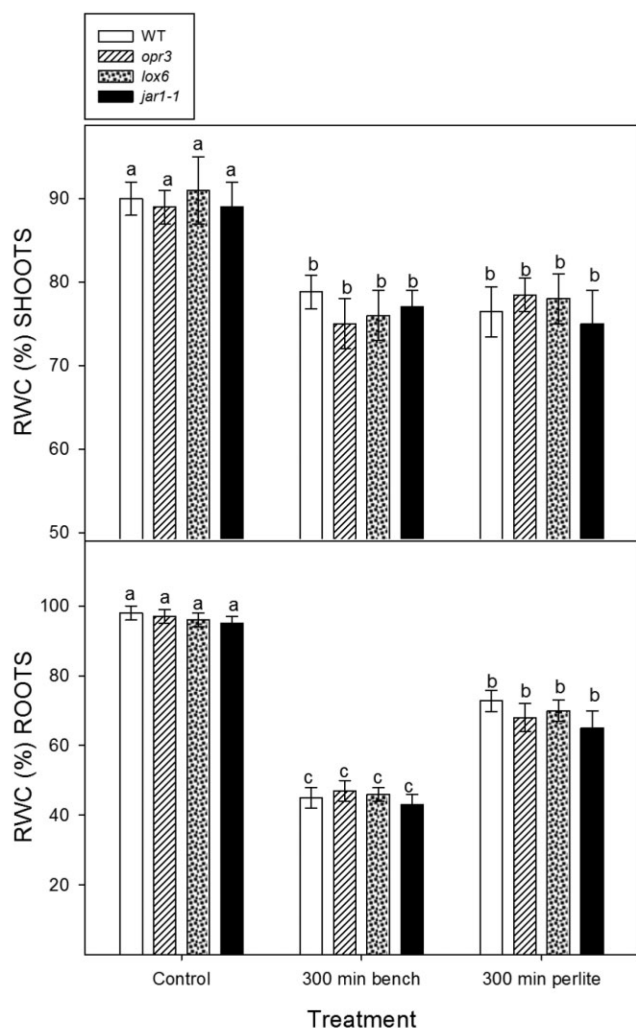

Figure 4. Relative water content in shoots and roots of *Arabidopsis thaliana* WT (Columbia-0, white bars), *opr3* (lined bars), *lox6* (dotted bars) and *jar1-1* (black bars) under control conditions ($t = 0$) and dehydrated in air or in dried perlite. Data are mean values ± standard deviation of three independent determinations. Letters denote statistical significance ($P \leq 0.05$) between genotypes and groups.

dehydration (49% loss of water). None of the jasmonate-deficient lines significantly accumulated JA-Ile in response to water stress; on the contrary, levels of this metabolite in *opr3* plants decreased a 43% throughout dehydration from relatively elevated initial levels. The *lox6* seedlings had a JA-Ile accumulation pattern similar to *opr3* plans although JA levels were slightly higher throughout the experiment. As expected, *jar1-1* plants had the lowest JA-Ile content of all tested lines and these low levels remained unaffected after stress imposition. Similarly to JA-Ile, ABA levels progressively increased in WT plants in response to desiccation (Fig. 5d). In the same way, ABA levels did not show any relevant rise in water-stressed seedlings of the lines deficient in JA-Ile or any of its precursors (OPDA and JA). Deficiency in each of the three main jasmonates has an impact on the pattern of accumulation of the other members of the biosynthetic pathway. Nevertheless, upon dehydration, the common output was the deficient accumulation of both JA-Ile and ABA.

ABI2 (a protein phosphatase 2C) and *ABI5* (member of the basic leucine zipper transcription factor family) were chosen to follow the ABA-dependent signalling because of their key function in the ABA signalsome. Figure 6 shows the relative amounts of *ABI2* and *ABI5* transcripts in roots of WT plants and jasmonate-deficient mutants. *ABI2* expression increased in WT plants (4.9-fold) in response to dehydration whereas it did not change in any of the mutants. Roots of stressed *opr3* and *lox6* seedlings had increased amounts of *ABI5* transcripts respect to controls; however, it should be noted that this up-regulation was less pronounced than in WT plants subjected to the same stress conditions. Interestingly, in *jar1-1* plants, *ABI5* expression did not respond to dehydration. In potted plants on dry perlite, variations in *ABI2* and *ABI5* expression in WT and *jar1-1* seedlings were comparable. *ABI2* and *ABI5* were up-regulated in roots of WT seedlings (6.2- and 2.9-fold increase, respectively) but it remained unchanged in *jar1-1* seedlings (Fig. 6a2,b2).

Expression of the water stress-responsive genes *RD29A* (responsive to ABA and to osmotic stress), *RD29B* (responsive to ABA), *RD22* (responsive to the MYC2-mediated ABA pathway) and *ERD1* (responsive to dehydration and cold) was analysed under both water stress systems (Fig. 7).

In air-dried plants, the amount of *RD29A* transcript increased 33-fold in WT seedlings after stress imposition. Conversely, *RD29A* expression was high in well-watered seedlings of *opr3* and *lox6* lines with respect to WT but it did not show any important variation under water stress conditions. *RD29A* expression in non-stressed *jar1-1* seedlings was not significantly different from WT; however, upon stress imposition, transcript abundance dropped to very low values, becoming almost undetectable. Similarly, *RD29A* was up-regulated in WT plants under substrate desiccation (24-fold), whereas in *jar1-1* seedlings, up-regulation was significantly lower (threefold, Fig. 7a2). *RD29B* expression increased in air-dried WT plants (11-fold). However, in jasmonate-deficient lines, the stress-induced increase of *RD29B* expression was much lower (only 2.5-fold in *opr3*) or even null (in *lox6* and *jar1-1*). Substrate desiccation (see Fig. 7b2) strongly up-regulated *RD29B* expression in WT, whereas only a minor increase in gene expression was detected in *jar1-1* seedlings. Similarly, *RD22* expression was up-regulated in response to severe stress in WT (1.5 fold) and *opr3* (3.2-fold) seedlings but it did not change in the rest of jasmonate-deficient lines. In plants transplanted to dry perlite *RD22* expression slightly increased in WT and remained unchanged in *jar1-1* (see Fig. 7c2). Finally, a clear increase in *ERD1* expression induced by stress was observed in roots of all assayed lines in both experimental systems. Therefore, despite particular differences in the transcriptional response to dehydration (both qualitative and quantitative) between lines in response to the different stress imposed, jasmonate-deficient lines did not up-regulate neither JA- (*RD22*) nor ABA- (*RD29A*,*B*) dependent genes. However, up-regulation of *ERD1* (belonging to a signalling

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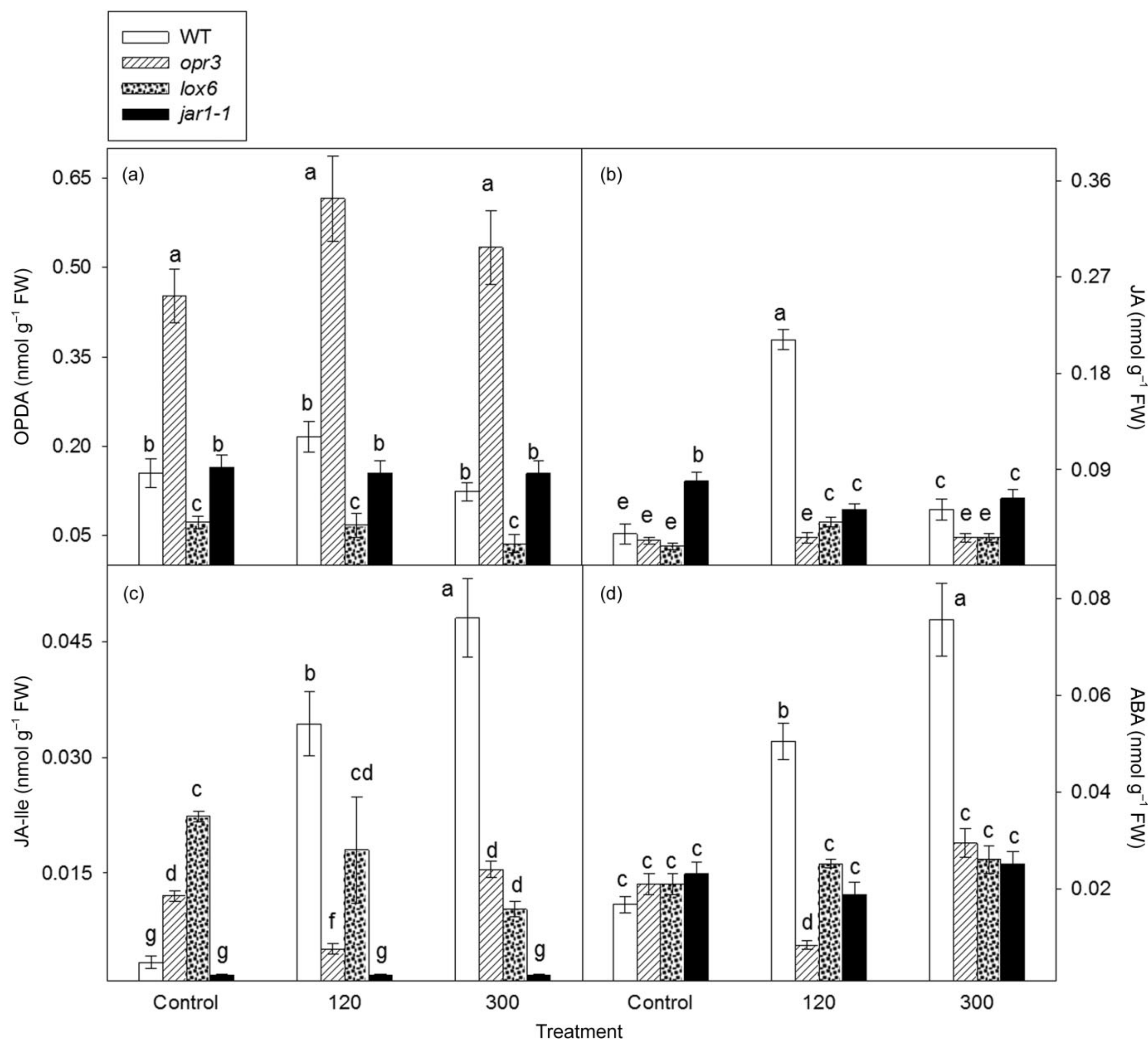

Figure 5. OPDA (a), JA (b), JA-Ile (c) and ABA (d) levels in roots of *Arabidopsis thaliana* WT (Columbia-0, white bars), *opr3* (lined bars), *lox6* (dotted bars) and *jar1-1* (black bars) under control conditions ($t = 0$) and dehydrated in air. Data are mean values ± standard deviation of three independent determinations. Letters denote statistical significance ($P \le 0.05$) between genotypes and groups.

pathway independent to both ABA and JA) remained unaltered or even magnified in JA-deficient lines.

Hormone levels in roots of ABA and JA deficient and insensitive mutants

Despite some differences in the basal levels (see Supporting Information Table S4), in response to water stress, all mutant genotypes (*coi1*-16, *abi2-1 and aba1-3*) showed important increases in the levels of JA-Ile. However, *jar1-1* roots did not modify JA-Ile levels under stress pressure. Stress-induced ABA accumulation was much lower in *aba1-3*, *jar1-1* and *coi1*-16 when compared with their respective WT lines. In the same conditions, *abi2-1* seedlings accumulated significantly more ABA than WT plants. It is worth noting that despite the opposite pattern of JA-Ile accumulation in roots of *jar1-1* and *coi1* plants, both mutants had lower levels of ABA in response to dehydration. On the other hand, both ABA-deficient or -insensitive mutants had higher levels of JA-Ile than the WT genotype.

Hormone profiles in leaves of Arabidopsis under severe water stress conditions

Levels of different jasmonates in leaves of stressed plants followed a pattern completely different from that observed in

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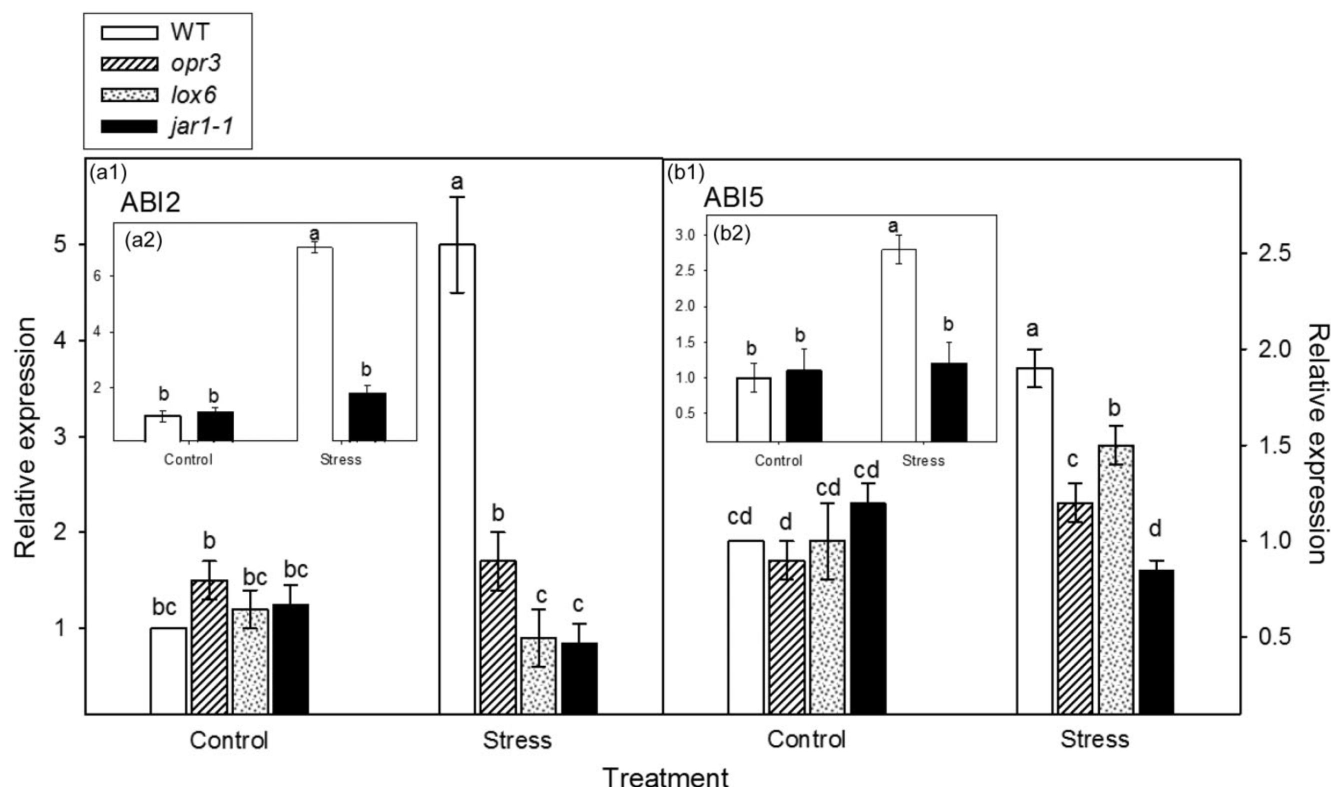

Figure 6. Relative expression of ABI2 (left) and ABI5 (right) in roots of *Arabidopsis thaliana* WT (Columbia-0, white bars), *opr3* (lined bars), *lox6* (dotted bars) and *jar1-1* (black bars) under control conditions and dehydrated in air (external figures a1–b1) or in perlite (insets a2-b2). Data are mean values ± standard deviation of three independent determinations. Letters denote statistical significance ($P \leq 0.05$) between genotypes and groups.

roots. OPDA, JA and JA-Ile contents in the aerial part decreased throughout the experimental period (Fig. 8). In WT seedlings, OPDA levels gradually decreased, reaching a minimum of 3.9 ± 0.9 nmol g^{-1} after 150 min of desiccation. In well-watered *jar1-1* seedlings, leaf OPDA levels were much lower than in WT plants, following a completely different pattern after stress imposition. Similarly, leaf JA content in WT plants gradually decreased (from 0.55 ± 0.07 nmol g^{-1}, at time 0 to 0.18 ± 0.02 nmol g^{-1} 150 min after the stress onset). In *jar1-1* seedlings, the initial leaf JA levels were similar to those in WT but, after stress imposition, JA content transiently increased (1.3-fold) to subsequently decrease as in WT plants throughout the rest of the experimental period. Leaf JA-Ile levels in WT seedlings rapidly decreased in response to desiccation. At time 0, JA-Ile content was 0.068 ± 0.013 nmol g^{-1}, and only 30 min after water withdrawal, it decreased to 0.035 ± 0.011 nmol g^{-1}. JA-Ile levels at the end of the experimental period were 89% lower than those in well-watered plants. As expected, in *jar1-1* seedlings, JA-Ile content was low throughout the experiment. Leaf ABA content progressively increased after the stress onset with very similar patterns in both genotypes (2.6- and 2.1-fold increase at the end of the experimental period in WT and *jar1-1* plants, respectively).

DISCUSSION

ABA accumulation in plants in response to stress conditions promotes stomatal closure to avoid water loss and activates the expression of multiple genes (Nakashima & Yamaguchi-Shinozaki 2013), which act as modulators of plant metabolism to cope with the adverse situation. Previous studies have identified *NCED3*, encoding for a 9-*cis*-epoxycarotenoid dioxygenase, as the limiting step in ABA biosynthesis under stress conditions (Nambara & Marion-Poll 2005). Nevertheless, the mechanism linking water deficit sensing and induction of *NCED3* expression is still unknown.

Most of the research involving ABA accumulation under stress conditions has focused on the aerial part (Roychoudhury *et al*. 2013). However, the root is the first organ to cope with water deficit and the long-distance hormonal signalling between roots and shoots under stress conditions is a key physiological process (Zhang & Davies 1989; Van der Weele *et al*. 2000; Puértolas *et al*. 2013). In a previous work, De Ollas *et al*. (2013) demonstrated that a transient JA accumulation is required for ABA biosynthesis induction in roots of water-stressed citrus plants. To better dissect this hormonal interaction, an experimental system was developed

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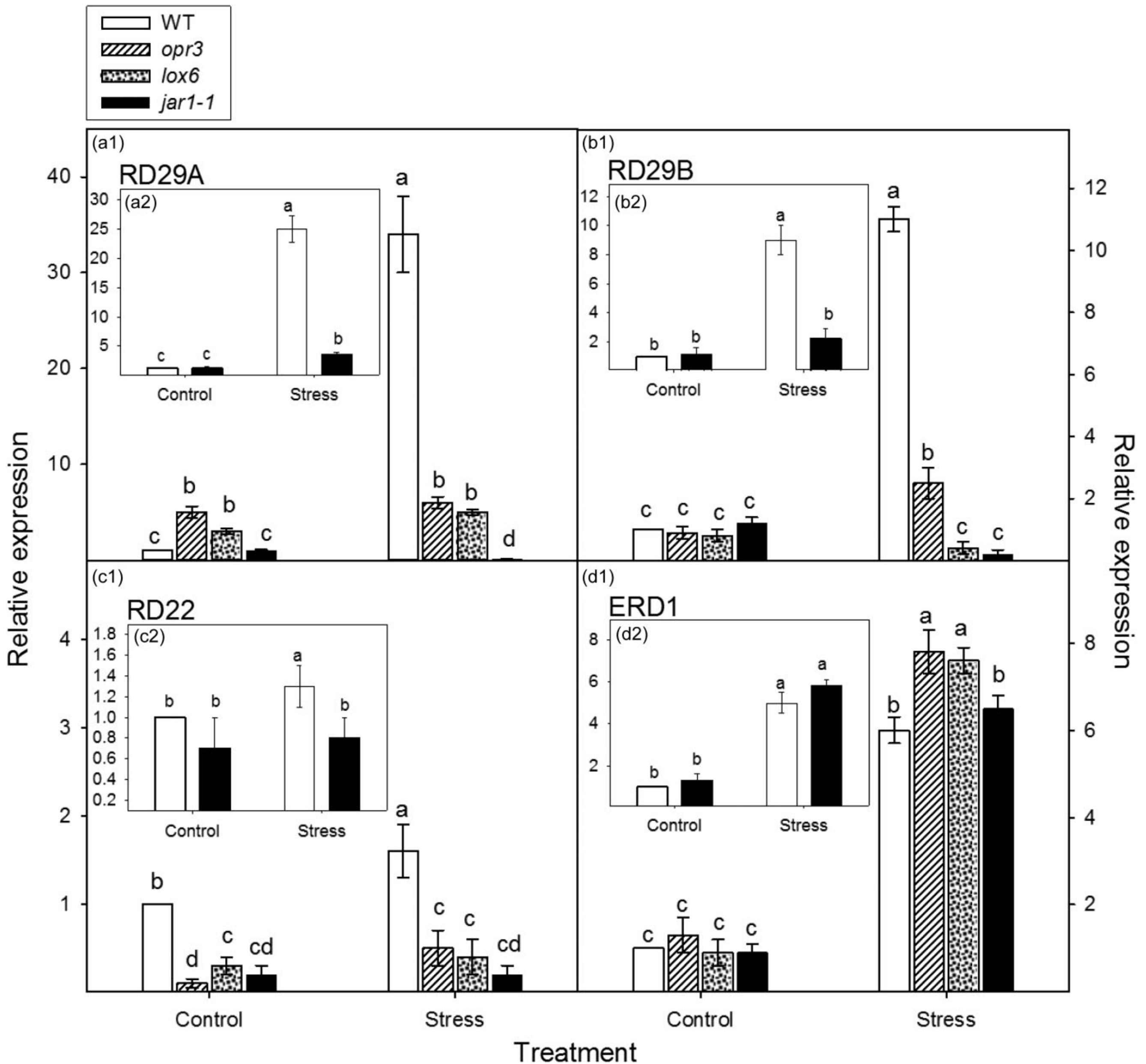

Figure 7. Relative expression of RD29A (a), RD29B (b), RD22(c) and ERD1 (d) in roots of *Arabidopsis thaliana* WT (Columbia-0, white bars), *opr3* (lined bars), *lox6* (dotted bars) and *jar1-1* (black bars) under control conditions and dehydrated in air (external figures A1-D1) or in perlite (insets figures A2-D2). Data are mean values ± standard deviation of three independent determinations. Letters denote statistical significance ($P \leq 0.05$) between genotypes and groups.

for dehydration of intact seedlings of the model plant *A. thaliana*. In addition, available mutant genotypes impaired in hormone perception and biosynthesis were included. Severe dehydration was employed to quantify fast variations in hormone levels in plants under stress. Furthermore, a method for root sampling that avoided mechanical damage to intact plants was implemented. Perlite desiccation was used complementary to air dehydration. RWC measurements (Fig. 3 and Supporting Information Fig. S1) indicated that the degree of desiccation in plants from the different genotypes was similar and allowed discarding any artefact in the experimental system.

In this work, JA-Ile and ABA concomitantly accumulated in roots of WT Arabidopsis plants subjected to the two different systems of dehydration following a pattern similar to that of citrus (De Ollas *et al*. 2013). Moreover, data show a transient accumulation of OPDA and JA after the stress imposition, supporting our previous model of JA involvement in plant responses to water stress. These observations suggest an interaction between both hormonal pathways in roots. In this sense, each line deficient in jasmonate biosynthesis (*opr3*, *lox6* and *jar1-1*) had a specific pattern of oxylipin accumulation under stress conditions. However, all lines were unable to accumulate JA-Ile after water

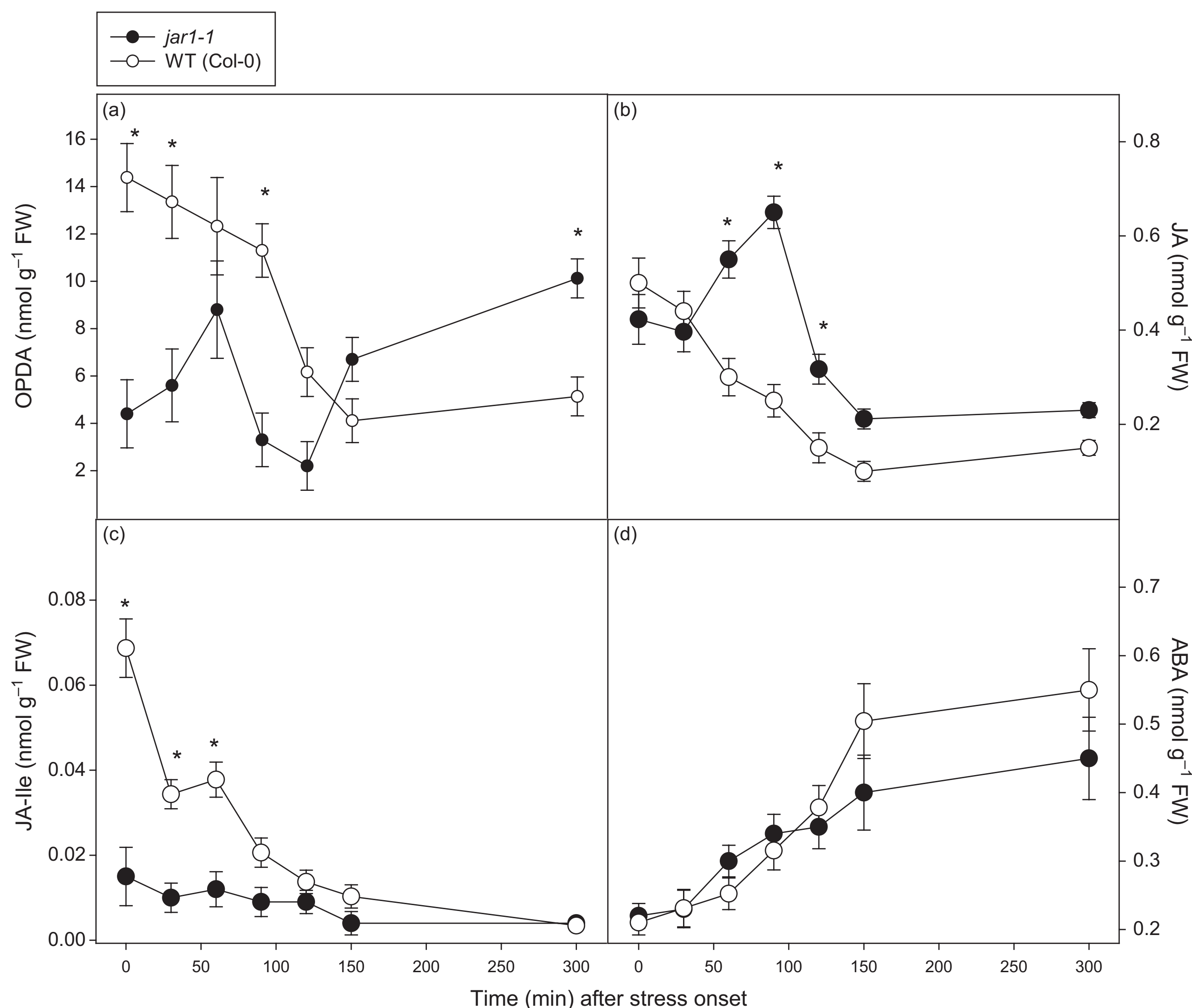

Figure 8. OPDA (a), JA (b), JA-Ile (c) and ABA (d) levels in leaves of *Arabidopsis thaliana* WT,(Columbia-0, white circles) and *jar1-1* (black circles) under control conditions ($t = 0$) and dehydrated in air. Data are mean values ± standard deviation of three independent determinations. Asterisks denote statistical significance ($P \leq 0.05$) between control and stressed plants.

withdrawal. This was expected in *jar1-1* seedlings because of their incapability to conjugate isoleucine to JA. In the case of *opr3* and *lox6* plants, the impairment in JA-Ile accumulation was associated to substrate deficiency because of impairments in oxophytodienoatereductase 3 and lipoxygenase 6 activities, respectively (Chehab *et al.* 2011; Chauvin *et al.* 2013). Interestingly, these jasmonate-deficient plants were also unable to accumulate ABA under two different water stress conditions, being ABA content in those lines significantly lower than in WT plants throughout the experiments. These data support the assumption that jasmonates and more specifically, JA-Ile accumulation, under water deficit is necessary for a fast ABA build up in roots of Arabidopsis plants. Data from these experiments (Figs 1, 3 & 5) pointed to JA-Ile as the main player in the jasmonate–ABA interaction under severe dehydration because of several facts: firstly, in *opr3* seedlings, OPDA content was significantly higher than in WT (or any other tested line) plants and, nevertheless, plants were unable to increase ABA levels. Secondly, JA levels in roots of *jar1-1* plants followed a pattern of accumulation similar to that in WT seedlings (although JA burst was lower). However, this significant increase in JA content did not have any effect on the induction of ABA biosynthesis. Thirdly, the best correlation between hormone deficiency and lack of ABA accumulation was found with JA-Ile, which is consistent with JA-Ile being the bioactive compound in *COI1*-dependent signalling (Fig. 1).

The high correlation between expression of genes coding for key enzymes of JA-Ile and ABA biosynthesis (*JAR1* and *NCED3*) and hormone contents in roots (Figs 1 & 2) indicates that, at least partially, the different ABA accumulation in WT and *jar1-1* plants derived from a differential regulation

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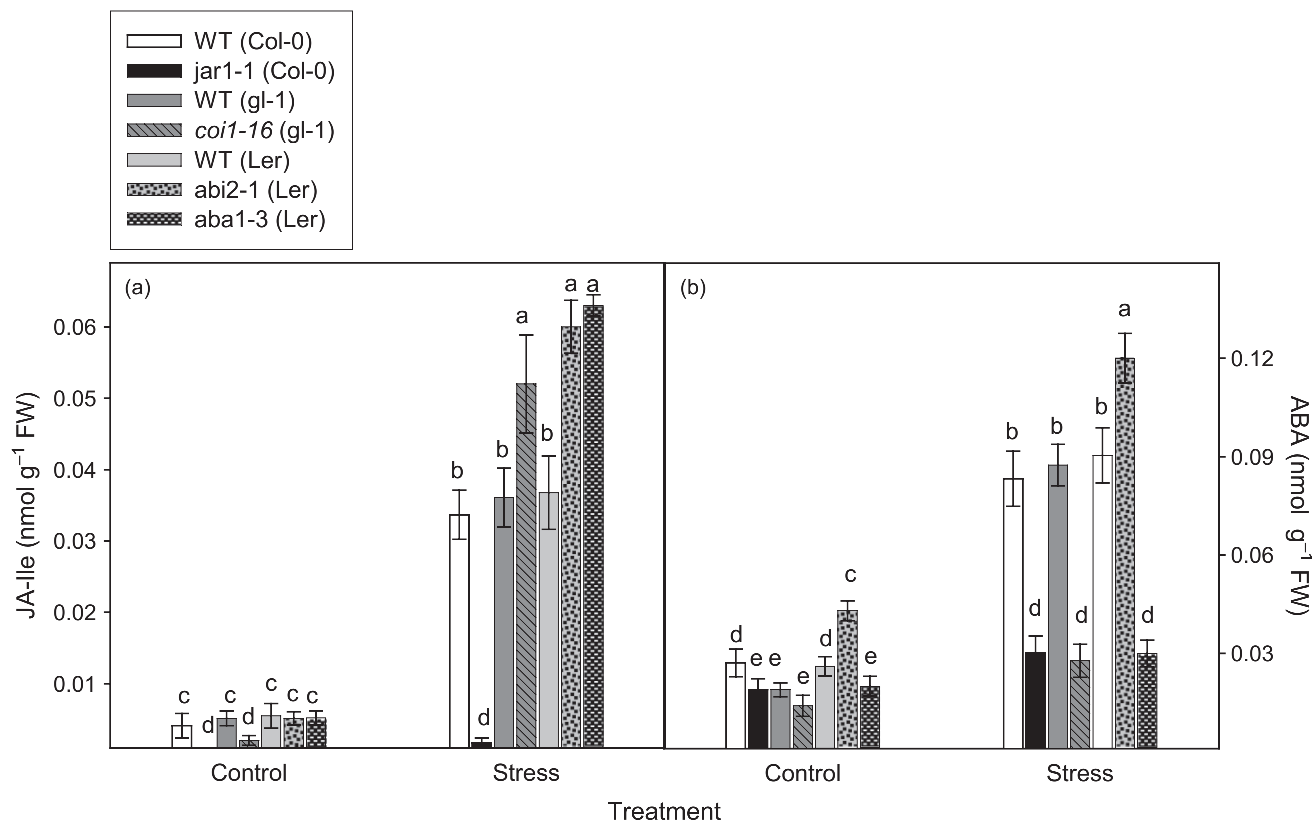

Figure 9. JA-Ile (a) and ABA (b) levels in roots of *Arabidopsis thaliana* WT Col-0 (white bars), *jar1-1* (Col-0, black bars), WT gl-1 (deep grey bars), *coi1-16* (gl-1, lined deep grey bars), WT Ler-1 (grey bars), *abi2-1* (Ler-1, dotted grey bars) and *aba1-3* (Ler-1, dashed grey bars), under control conditions and dehydrated in air. Data are mean values ± standard deviation of three independent determinations. Letters denote statistical significance ($P \leq 0.05$) between genotypes.

of ABA biosynthesis in roots. To assess the involvement of JA in ABA-dependent signalling, expression of genes coding for the protein phosphatase 2C, ABI2 and the basic leucine zipper transcription factor, ABI5, were analysed in roots of Arabidopsis seedlings under stress conditions. Expression of both genes in all jasmonate-deficient lines was down-regulated (Fig. 6), supporting that an intact JA biosynthesis pathway is necessary to accomplish a full induction of ABA biosynthesis and signalling in roots of Arabidopsis under stress conditions. The fact that plants with an intact ABA biosynthesis pathway were unable to accumulate ABA in early stages of water stress conditions to the same extent than WT plants points out to a missing signalling step, linking stress perception and ABA biosynthesis, probably through the regulation of *NCED3* expression.

Analysis of water stress-responsive genes was performed to assess the impact of jasmonate deficiency on ABA signalling in response to dehydration. Expression of both *RD29A* and *RD29B* genes was significantly lower in jasmonate-deficient seedlings respect to WT plants (Fig. 7). Down-regulation of *RD29B* was expected as ABA accumulation in jasmonate-deficient lines was low and *RD29B* promoter activation is dependent of the ABA-responsive AREB transcription factor (Roychoudhury *et al.* 2013). Nevertheless, since *RD29A* activation is only partially dependent on ABA signalling and full activation requires a stress-responsive *DREB* in addition to the AREB (Nakashima & Yamaguchi-Shinozaki 2013), a partial up-regulation was expected. The lack of any significant increase in expression suggested that the stress induction of the *DREB* was insufficient to activate transcription without an ABA-mediated induction of the *ABRE* element. Similarly, no important up-regulation of *RD22*, a *MYC2*-responsive gene, was detected in Arabidopsis plants impaired in JA signalling. On the other hand, induction of *ERD1* was similar in all tested lines, supporting the role of *ERD1* in an ABA-independent branch of stress responses (Tran *et al.* 2007) and confirming the lack of artefacts in the system.

Comparison of early JA-Ile and ABA accumulation patterns under water deficit in *jar1-1*, *coi1-16*, *abi2-1* and *aba1-3* offered deep insights into this hormonal interaction (Fig. 9). Firstly, JA-Ile content increased in *aba1-3* and *abi2-1* plants in a more pronounced way than in WT seedlings, indicating that JA-Ile accumulation in response to water stress is influenced by ABA accumulation and signalling but, in this case, with a slight negative influence. These data are not surprising as it is well-known that the alteration of the homeostasis of any hormone would influence the rest (Arbona *et al.* 2010). However, more work should be performed to better dissect this negative interaction. Secondly, roots of *coi1-16* seedlings

accumulated significantly more JA-Ile under the conditions assayed, suggesting that the lack of downstream signalling promotes an increased hormone accumulation trying to cope with the insensitivity (Paschold *et al*. 2008). Interestingly, roots of both *jar1-1* and *coi1-16* plants accumulated significantly less ABA than WT genotypes under dehydration, supporting the involvement of the jasmonate signal in the early ABA accumulation. In Grebner *et al*. (2013), the root-specific JA-deficient mutant *lox6* showed significantly lower survival rates after water withdrawal and recovery. Therefore, it seems than JA and ABA interplay in water stress conditions is able to affect plant tolerance.

Data on Fig. 8 support a root-specific role for the interaction studied. In leaves, no apparent correlation between JA-Ile and ABA profiles could be observed. Moreover, under the conditions of rapid desiccation studied in this work, jasmonate levels followed a decreasing pattern in WT plants. Savchenko *et al*. (2014) have recently found similar results and, at the end of a 5-day-drought period, ABA accumulated in different mutants impaired in oxylipin synthesis. Interestingly, in Brossa *et al*. (2011), it is suggested that, in *aos* plants, ABA accumulation was slower (75% lower after 9 days of water withdrawal) than in WT although the final levels were similar in both genotypes. Therefore, more work is still needed to understand the interaction among oxylipins and ABA in the aerial part of plants under water stress. It would be important to take in consideration the type of imposed stress (rapid desiccation could have different effects than a more progressive reduction of soil water potential), the pattern of hormone accumulation (and not only the end point), etc. Another important aspect to further study is how the putative signals travelling from root to shoot (and vice versa) affect this jasmonate–ABA interaction. For example, in our system, jasmonate accumulation in roots could be related to the depletion in shoots.

Results have been obtained using hormone-deficient and -insensitive mutants. Further research using hormone-overproducing mutants would reinforce the results obtained in Arabidopsis plants although previous work in citrus plants (De Ollas *et al*. 2013) showed that exogenous JA treatment increased ABA concentration in roots of plants under stress. However, it is important to point out that results obtained in situations where a hormone cue is absent and others where the same hormone is over-accumulated are not always compatible (Brossa *et al*. 2011; Hossain *et al*. 2011). To this respect, processes involved in hormone homeostasis and crosstalk among different signals are crucial in determining the physiological outcome.

To sum up, hormonal interaction in roots under different conditions is shown in this work. Signal transduction between water stress-induced JA-Ile accumulation and *COI1* is necessary for a complete early induction of the ABA biosynthesis pathway and subsequent hormone accumulation in roots of Arabidopsis plants. Although JA and ABA signal transduction pathways interact at several points (Lorenzo *et al*. 2004; Lackman *et al*. 2011), we have demonstrated that jasmonates, and specifically JA-Ile, modulate ABA biosynthesis pathway under water stress conditions.

ACKNOWLEDGMENTS

This work was supported by the Spanish Ministerio de Economía y Competitividad (MINECO) through grants AGL2010-22195-C03-01 and AGL2013-42038R to A.G.-C. Hormonal profiles were performed at Instrumental central facilities (SCIC) of Universitat Jaume I. The authors have no conflict of interest to declare.

REFERENCES

Adie B.A.T., Pérez-Pérez J., Pérez-Pérez M.M., Godoy M., Sánchez-Serrano J.J., Schmelz E.A. & Solano R. (2007) ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in Arabidopsis. *The Plant Cell* **19,** 1665–1681.

Arbona V., Argamasilla R. & Gómez-Cadenas A. (2010) Common and divergent physiological, hormonal and metabolic responses of *Arabidopsis thaliana* and *Thellungiella halophila* to water and salt stress. *Journal of Plant Physiology* **167,** 1342–1350.

Böttcher C. & Pollmann S. (2009) Plant oxylipins: plant responses to 12-oxo-phytodienoic acid are governed by its specific structural and functional properties. *FEBS Journal* **276,** 4693–4704.

Brossa R., López-Carbonell M., Jubany-Mari T. & Alegre L. (2011) 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. *Journal of Plant Growth Regulation* **30,** 322–333.

Browse J. (2009) Jasmonate passes muster: a receptor and targets for the defense hormone. *Annual Reviews in Plant Biology* **60,** 183–205.

Chauvin A., Caldelari D., Wolfender J.L. & Farmer E.E. (2013) Four 13-lipoxygenases contribute to rapid jasmonate synthesis in wounded *Arabidopsis thaliana leaves*: a role for lipoxygenase 6 in responses to long-distance wound signals. *New Phytologist* **197,** 566–575.

Chehab W.E., Kim S., Savchenko T., Kliebenstein D., Dehesh K. & Braam J. (2011) Intronic T-DNA insertion renders arabidopsis opr3 a conditional jasmonic acid-producing mutant. *Plant Physiology* **156,** 770–778.

Creelman R.A. & Mullet J.E. (1997) Biosynthesis and action of jasmonates in plants. *Annual Reviews in Plant Biology* **48,** 355–381.

De Lucas M. & Brady S.M. (2013) Gene regulatory networks in the Arabidopsis root. *Current Opinion in Plant Biology* **16,** 50–55.

De Ollas C., Hernando B., Arbona V. & Gómez-Cadenas A. (2013) Jasmonic acid transient accumulation is needed for abscisic acid increase in citrus roots under drought stress conditions. *Physiologia Plantarum* **147,** 296–306.

Duan L., Dietrich D., Ng C.H., Yeen Chan P.M., Bhalerao R., Bennett M.J. & Dinneny J.R. (2013) Endodermal ABA signalling promotes lateral root quiescence during salt stress in Arabidopsis seedlings. *The Plant Cell* **25,** 324–341.

Durgbanshi A., Arbona V., Pozo O., Miersch O., Sancho J.V. & Gómez-Cadenas A. (2005) Simultaneous determination of multiple phytohormones in plant extracts by liquid chromatography-electrospray tandem mass spectrometry. *Journal of Agricultural and Food Chemistry* **53,** 8437–8442.

Fernández-Arbaizar A., Regalado J.J. & Lorenzo O. (2012) Isolation and characterization of novel mutant loci suppressing the ABA hypersensitivity of the arabidopsis coronatine insensitive 1–16 (coi1-16) mutant during germination and seedling growth. *Plant Cell Physiology* **53,** 53–63.

Fonseca S., Chini A., Hamberg M., Adie B., Porzel A., Kramell R., . . . Solano R. (2009) (+)-7-iso-Jasmonoyl-L-isoleucine is the endogenous bioactive jasmonate. *Nature Chemical Biology* **5,** 344–350.

Grebner W., Stingl N.E., Oenel A., Mueller M.J. & Berger S. (2013) Lipoxygenase6-dependent oxylipin synthesis in roots is required for abiotic and biotic stress resistance of Arabidopsis. *Plant Physiology* **161,** 2159–2170.

Hong J.H., Seah S.W. & Xu J. (2013) The root of ABA action in environmental stress response. *Plant Cell Reports* **32,** 971–983.

Hossain M.A., Munemasa S., Uraji M., Nakamura Y., Mori I.C. & Murata Y. (2011) Involvement of endogenous abscisic acid in methyl jasmonate-induced stomatal closure in Arabidopsis. *Plant Physiology* **156,** 430–438.

Koo A.J.K. & Howe G.A. (2009) The wound hormone jasmonate. *Phytochemistry* **70,** 1571–1580.

Lackman P., González-Guzmán M., Tilleman S., Carqueijeiro I., Pérez A.C., Moses T., . . . Goossens A. (2011) Jasmonate signaling involves the abscisic acid receptor PYL4 to regulate metabolic reprogramming in Arabidopsis

and tobacco. *Proceedings of the National Academy of Sciences of the United States of America* **108,** 5891–5896.

Lorenzo O., Chico J.M., Sánchez-Serrano J.J. & Solano R. (2004) JASMONATE-INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in arabidopsis. *The Plant Cell* **16,** 1938–1950.

Kim E.H., Kim Y.S., Park S.H., Koo Y.J., Do Choi Y., Chung Y.Y., . . . Kim J.K. (2009) Methyl Jasmonate Reduces Grain Yield by Mediating Stress Signals to Alter Spikelet Development in Rice. *Plant Physiology* **149,** 1751–1760.

Nakashima K. & Yamaguchi-Shinozaki K. (2013) ABA signaling in stress-response and seed development. *Plant Cell Reports* **32,** 1–12.

Nambara E. & Marion-Poll A. (2005) Abscisic acid biosynthesis and catabolism. *Annual Reviews in Plant Biology* **56,** 165–185.

Paschold A., Bonaventure G., Kant M.R. & Baldwin I.T. (2008) Jasmonate perception regulates jasmonate biosynthesis and JA-Ile metabolism: the case of COI1 in *Nicotiana attenuata*. *Plant Cell Physiology* **49,** 1165–1175.

Pfaffl M.W., Tichopad A., Prgomet C. & Neuvians T.P. (2004) Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper Excel-based tool using pair-wise correlations. *Biotechnology Letters* **26,** 509–515.

Pieterse C.M.J., Van Der Does D., Zamioudis C., Leon-Reyes A. & Van Wees S.C.M. (2012) Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology* **28,** 489–521.

Puértolas J., Alcobendas R., Alarcón J.J. & Dodd I.C. (2013) Long-distance abscisic acid signaling under different vertical soil moisture gradients depends on bulk root water potential and average soil water content in the root zone. *Plant, Cell and Environment* **36,** 1465–1475.

Raya-González J., Pelagio-Flores R. & López-Bucio J. (2012) The jasmonate receptor COI1 plays a role in jasmonate-induced lateral root formation and lateral root positioning in *Arabidopsis thaliana*. *Journal of Plant Physiology* **169,** 1348–1358.

Roychoudhury A., Paul S. & Basu S. (2013) Cross-talk between abscisic acid-dependent and abscisic acid-independent pathways during abiotic stress. *Plant Cell Reports* **32,** 1–22.

Savchenko T., Kolla V.A., Wang C.Q., Nasafi Z., Hicks D.R., Phadungchob B., . . . Dehesh K. (2014) Functional convergence of oxylipin and abscisic acid pathways controls stomatal closure in response to drought. *Plant Physiology* **164,** 1151–1160.

Seo J., Joo J., Kim M., Kim Y., Nahm B.H., Song S.I., . . . Choi Y.D. (2011) OsbHLH148, a basic helix-loop-helix protein, interacts with OsJAZ proteins in a jasmonate signaling pathway leading to drought tolerance in rice. *The Plant Journal* **65,** 907–921.

Sheard L.B., Tan X., Mao H., Withers J., Ben-Nissan G., Hinds T.R., . . . Zheng N. (2010) Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor. *Nature* **468,** 400–405.

Song S., Qi T., Huang H., Ren Q., Wu D., Chang C., . . . Xie D. (2011) The jasmonate-ZIM domain proteins interact with the R2R3-MYB transcription factors MYB21 and MYB24 to affect jasmonate-regulated stamen development in Arabidopsis. *The Plant Cell* **23,** 1000–1013.

Soto A., Ruiz K.B., Ziosi V., Costa G. & Torrigiani P. (2012) Ethylene and auxin biosynthesis and signaling are impaired by methyl jasmonate leading to a transient slowing down of ripening in peach fruit. *Journal of Plant Physiology* **169,** 1858–1865.

Spollen W.G., Lenoble M.E., Samuels T.D., Bernstein N. & Sharp R.E. (2000) Abscisic acid accumulation maintains maize primary root elongation at low water potentials by restricting ethylene production. *Plant Physiology* **122,** 967–976.

Staswick P.E., Tiryaki I. & Rowe M.L. (2002) Jasmonate response locus JAR1 and several related Arabidopsis genes encode enzymes of the firefly luciferase superfamily that show activity on jasmonic, salicylic, and indole-3-acetic acids in an assay for adenylation. *The Plant Cell* **14,** 1405–1415.

Tran L.P., Nakashima K., Sakuma Y., Osakabe Y., Qin F., Simpson S.D., . . . Yamaguchi-Shinozaki K. (2007) Co-expression of the stress-inducible zinc finger homeodomain ZFHD1 and NAC transcription factors enhances expression of the ERD1 gene in Arabidopsis. *The Plant Journal* **49,** 46–63.

Van der Weele C.M., Spollen W.G., Sharp R.E. & Baskin T.I. (2000) Growth of *Arabidopsis thaliana* seedlings under water deficit studied by control of water potential in nutrient-agar media. *Journal of Experimental Botany* **51,** 1555–1562.

Verslues P.E. & Bray E.A. (2006) Role of abscisic acid (ABA) and *Arabidopsis thaliana* ABA-insensitive loci in low water potential-induced ABA and proline accumulation. *Journal of Experimental Botany* **57,** 201–212.

Ye J., Coulouris G., Zaretskaya I., Cutcutache I., Rozen S. & Madden T. (2012) Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics* **134**.

Zhang J. & Davies W.J. (1989) Abscisic acid produced in dehydrating roots may enable the plant to measure the water status of the soil. *Plant, Cell and Environment* **12,** 73–81.

Received 24 November 2014; received in revised form 2 March 2015; accepted for publication 9 March 2015

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Relative water content (%) in shoots and roots of *Arabidopsis thaliana* WT (Columbia-0, white bars), and *jar1* (black bars) under control conditions and dehydrated in perlite.

Table S1. Germ plasm accessions used in water stress experiments with their respective background, NASC identification and locus affected.

Table S2. Stereoisomer forms detected in the hormone quantification analysis.

Table S3. Primers designed for gene expression analysis of Arabidopsis seedlings by quantitative RT-PCR (qRT-PCR).

Table S4. Basal hormone levels in the different Arabidopsis accessions used in this study.

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