

Full Length Research Paper

Relationship between jasmonic acid accumulation and senescence in drought-stress

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Plants raised from shoot-tip cultures of apricot (DT: drought-tolerant genotype *Prunus armeniaca* L. cv. Ansu Maxim, and DS: drought-sensitive genotype *P. armeniaca* L. cv. Longwangmao) were grown in pots of peat-based compost. Water was withheld for 12 days. Gas chromatography–mass spectrometry analysis indicated that jasmonic acid (JA) levels in leaves of DT plants increased three-fold on day 12 after water was withheld, while there was little variation in leaf JA in DS plants for the whole drying period. There was no significant difference in the JA concentration in roots of the two apricot cultivars from 1 – 9 days. JA concentration in DT plants roots increased slightly by 12 days compared to JA concentration in DS. There was 100% abscission in DT plants at 13 days, earlier than 14 days for DS. Two months after rewatering, 12 of the 18 DT plants were alive, but all 18 DS plants were dead. Exogenous JA accelerated leaf senescence in both DT and DS plants by chlorophyll loss. After JA treatment there were increased malondialdehyde (MDA) levels in DT and DS plants. These results suggest that the transient JA accumulation from DT leaves may promote leaf senescence due to soil drying, thus avoiding excessive water loss, and aiding drought survival.

Key words: Apricot, drought stress, leaf, jasmonic acid, senescence.

INTRODUCTION

Jasmonic acid (JA) has been suggested to have a role in plant responses to water deficit because water stress induces expression of several genes that also respond to JA (Turner et al., 2002; Hashimoto et al., 2004). For example, expression of the genes encoding the soybean vegetative storage protein acid phosphatases (*VspA/VspB*) increased in plants when subjected to water deficit and in plants treated with JA (Mason and Mullet, 1990). In addition, *in vivo* soybean leaves that had been sufficiently

dehydrated to cause a 15% decrease in fresh weight, had ≈5-fold increased JA levels within 2 h, which declined to approximately control levels by 4 h (Creelman and Muller, 1995).

However, the JA concentration in apricot plants has not been studied. Several lines of evidence support the conclusion that JA has an important role in plant senescence. For example, methyl jasmonate and its precursor JA were first demonstrated to promote senescence in detached oat (*Avena sativa*) leaves (Ueda and Kato, 1980), and subsequently JA was shown to have a role in leaf senescence in *Arabidopsis* (He et al., 2002; Cao et al., 2006). It is not surprising that senescence was induced by water stress, but the relationship between JA induced in plant tissue by water deficit and senescence has not been studied. These data and others (Parthier, 1990) suggest that JA may have a role in leaf senescence

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Abbreviations: DT, Drought-tolerant genotype (*Prunus armeniaca* L. cv. Ansu Maxim); DS, drought-sensitive genotype (*P. armeniaca* L. cv. Longwangmao).

during water deficit. Therefore, the objective of this study was to test whether JA could promote leaf senescence and improve drought stress tolerance to apricot plants.

MATERIALS AND METHODS

Plant materials and growing conditions

Explants of apricot {drought-tolerant (DT): *Prunus armeniaca* L. cv. Ansu Maxim and drought-sensitive (DS): *P. armeniaca* L. cv. Longwangmao} were multiplied by shoot-tip culture and the resulting clones were rooted by auxin treatment, and then grown in peat-based compost. Plants were kept at 25/20°C for day/night in a greenhouse with supplementary lighting, maintaining a 16-h photoperiod with a minimum of 200 $\mu\text{mol m}^{-2}\text{s}^{-1}$ photosynthetically active radiation (PAR).

Apricot plants used in this study were grown in peat-based compost for 90 days. Each pot contained three plants of equal size, each with 18 – 20 leaves on the main stem. Plant heights were 28 – 30 cm. The experiment was performed in a growth cabinet with a 16-h photoperiod at 250 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PAR and air temperature 25/20°C for day/night. Humidity was not controlled. Drought was initiated by withholding water for 12 days. Rewatering treatments were performed during the night of day 12.

Relative water content of soil

FW represents fresh weight and DW represents dry weight. The relative water content of soil (RWC_{soil}) was calculated as $\text{RWC}_{\text{soil}} (\%) = [(\text{FW} - \text{DW}) \times 100 / \text{DW}]$. The soil samples were taken in steel cylinders (diameter: 3.5 cm and length: 14 cm) at sunset.

Abscission measurements

All leaves were tested for abscission by applying slight pressure by hand to each petiole near the blade. After this initial testing, the plants were watered to saturation and then water was withheld in a growth room. Abscission was determined for periods up to 14 days.

Jasmonate determinations

JA extraction and quantification were carried out according to a protocol modified from Gundlach et al. (1992). In brief, 100 ng of dihydro-JA (a gift from Chunjian Li, College of Resources and Environment, China Agricultural University) was added to samples as an internal standard. Extracted samples were analyzed by GC-MS [Trace2000 gas chromatograph linked to a Finnigan Voyager Thermo Quest mass spectrometer; column VF-5 (30 m \times 0.32 mm \times 0.25 μm); linear He flow at 36.3 cm s^{-1} ; column temperature step gradient 120°C for 2 min, 120 - 160°C at 10°C min^{-1} , 160 – 200°C at 4°C min^{-1} , 200 – 290°C at 30°C min^{-1} ; electron potential, 70 eV]. JA was quantified by selective ion monitoring (measuring ions $m/z = 224$ for JA methyl ester; $m/z = 226$ for methyl dihydrojasmonate). Mass spectra of the sample were identical to published spectra (data not shown; Gundlach et al., 1992; Creelman and Muller, 1995).

JA treatment

(\pm)-JA was from Sigma-Aldrich Inc. (Israel). To investigate the effect of different JA concentrations on the level of chlorophyll (Chl) in the DT leaves, the plants that were watered daily were sprayed with

different concentrations (25, 50, 75 and 100 μM) of (\pm)-JA. Leaf segments were sampled 3 days after JA treatment and their Chl contents were determined. Several groups of control DT leaves were neither dehydrated nor treated with JA and were frozen in liquid N_2 immediately.

The final experiment was to investigate the effects of JA on the levels of Chl and malondialdehyde (MDA) in the DT or DS leaves. The foliage of the DT or DS plants that were well watered was sprayed with 50 μM (\pm)-JA solution containing 0.02% Tween-20 (v/v) ('JA' treatment). On the day when water was withheld, the same volume of water containing 0.02% Tween-20 (v/v) was sprayed on the foliage of the DT or DS plants ('drought' treatment). The control was the well-watered treatment in which plants were also sprayed with the same volume of water containing 0.02% Tween-20 (v/v), but were kept well-watered throughout the experiment ('fully watered' treatment).

On the third and sixth day after spraying, leaves were harvested from the shoots of each plant. The leaves were immediately stored at -80°C until analysis. The Chl content of the leaf was measured spectrophotometrically (Ueda and Kato, 1980), and MDA content was measured as described by Franck et al. (1998).

Data analysis and statistics

Data were subjected to analysis of variance (ANOVA) procedures (SAS Institute, 9.1.3 ed, 2008). Appropriate standard errors of the means (S.E.) and L.S.D.s at $P = 0.05$ were calculated.

RESULTS

For both apricot cultivars the RWC_{soil} decreased from 95 - 24% over the 12 days in which water was withheld (Figure 1A). There was a strong decrease at 3 - 12 days and all apricot plants died after 14 days of water withholding, at which time RWC_{soil} reached 17%.

Survival of drought stress

Little natural abscission was observed for the well-watered apricot plants (Figure 1B); Obviously, higher abscission in both apricot cultivars were exhibited if water was withheld over the 14 days (data not shown); Higher abscission was observed in DT than DS plants after 3 days. For DT plants, 100% abscission was 13 days after water was withheld, but in DS this occurred later at 14 days.

Rewatering treatments were performed during the night of day 12 to investigate the drought resistance of the two cultivars. Plants with similar vigor were selected and equally well-watered prior to treatments (Figure 2A, D). There were severe water deficits after 12 days of withholding water. Leaves of DT plants lost water and wilted more severely (Figure 2B) than DS (Figure 2E). However, 12 of 18 DT plants were alive (Figure 2C) two months after rewatering, whereas all 18 DS plants were dead (Figure 2F). It seems highly likely that the way DT plants dealt with drought stress was to avoid having living tissues in a state of water deficit; thus when the drought was over, they could again grow and actively metabolize.

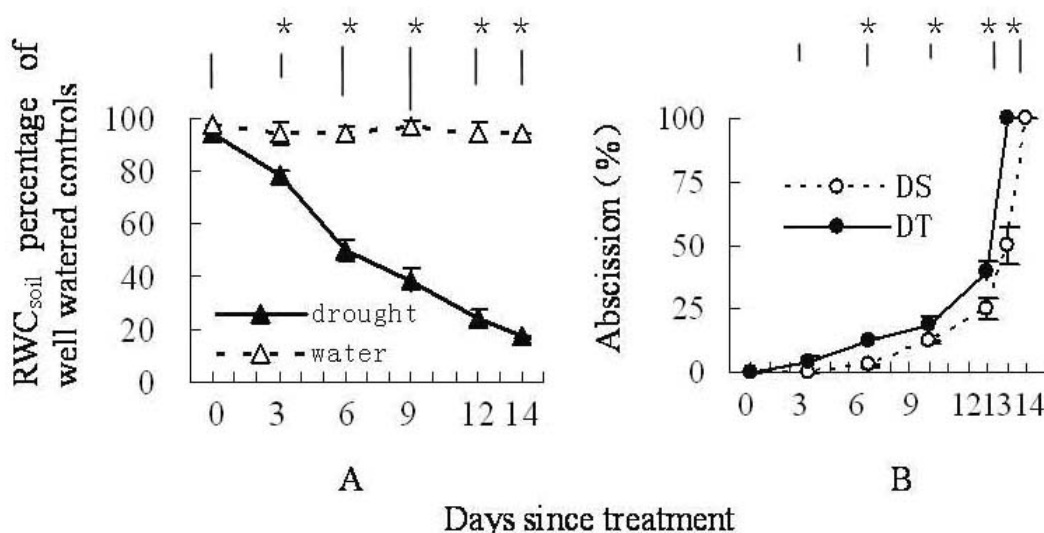


Figure 1. A) Relative water content of the soil (RWC_{soil}) during the drying period. B) Leaf abscission (%) resulting from drought stress in either DT or DS plants when water was withheld for 14 days. Each point is a Mean ± S.E. (n = 6). Vertical bars indicate L.S.D.s (P = 0.05) and asterisks denote significant difference between the two treatments at P < 0.05.

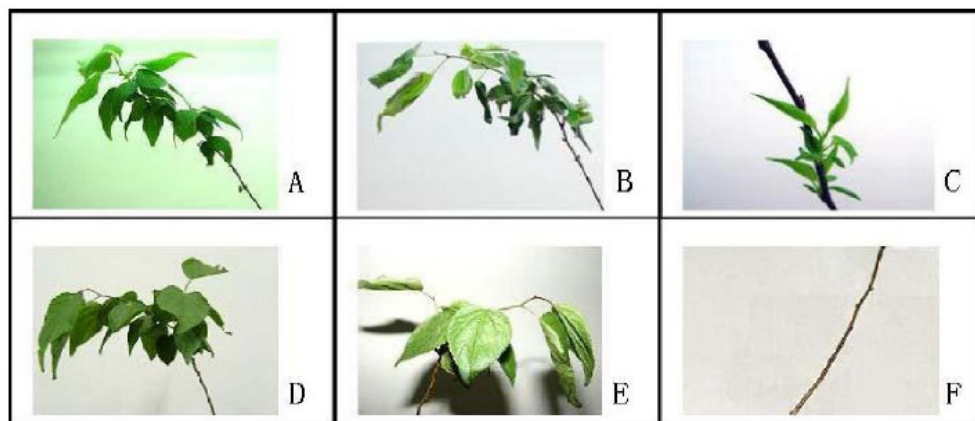


Figure 2. Survival of drought stress. DT (A, B and C) and DS plants (D, E and F) were grown in soil well-watered (A and D), dried for 12 days (B and E), and rewatered for 2 months (C and F).

Drought stress-induced JA accumulation

The JA concentration in apricot leaves and roots was analyzed by GC/MS selected ion monitoring (Figure 3). Since dihydro-JA was not detected naturally in leaves of the two apricot cultivars, it was added to samples as an internal standard. JA and dihydro-JA were not found in plant samples before they were methylated prior to GC/MS analysis. JA levels of leaves in well-watered treatment remained around 150 ng g⁻¹ DW in DT and 100 ng g⁻¹ DW in DS plants for the entire experiment. In both DS and DT plants, JA level did not change appreciably from 1 – 9 days. After the initiation of the soil drying treatment, the JA

concentration in DT reached about 580 ng g⁻¹ DW under drought stress. JA levels increased 3-fold by 12 days of water withholding and declined to approximately control levels one day after rewatering in DT plants.

JA levels of well-watered roots remained around 400 ng g⁻¹ DW in DT and 450 ng g⁻¹ DW in DS plants for the entire experiment. There was no significant difference in the root JA concentrations of the two apricot cultivars from 1 - 9 days. JA concentration in DT plants roots had slightly increased by 12 days compared to JA concentration in DS. In DT plant roots, the JA concentration increased seven-fold on day 13 (1 day after rewatering) compared to watered controls; this represented a lag time of 1 day after

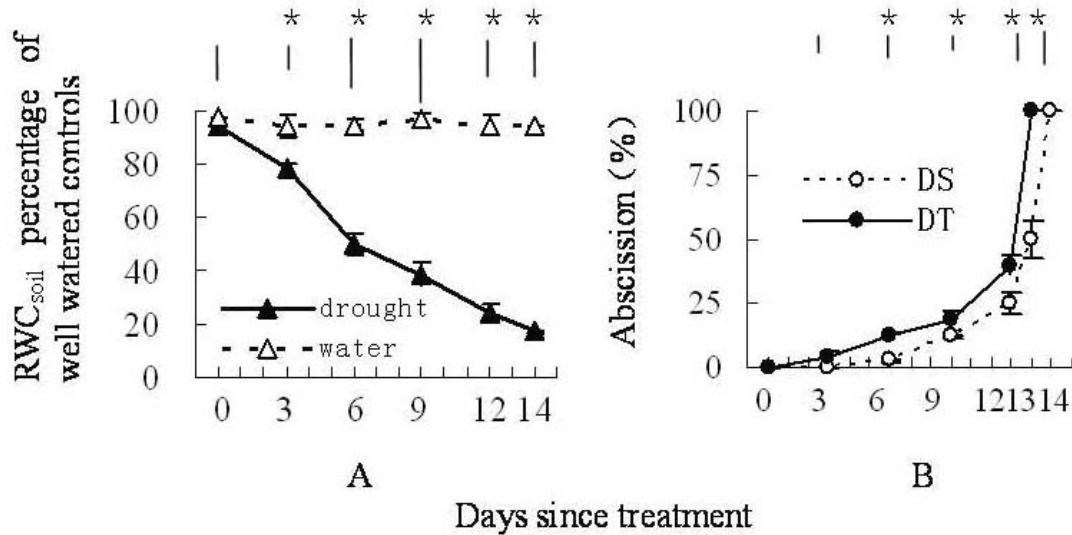


Figure 3. JA concentrations assayed either in leaves or roots of apricot plants. Water was withheld from plants for 12 days and then rewatered for 3 days. JA concentrations are the Means±S.E. of six samples assayed. Vertical bars indicate L.S.D.s (P = 0.05) and asterisks denote significant difference between the two apricot plants at P < 0.05.

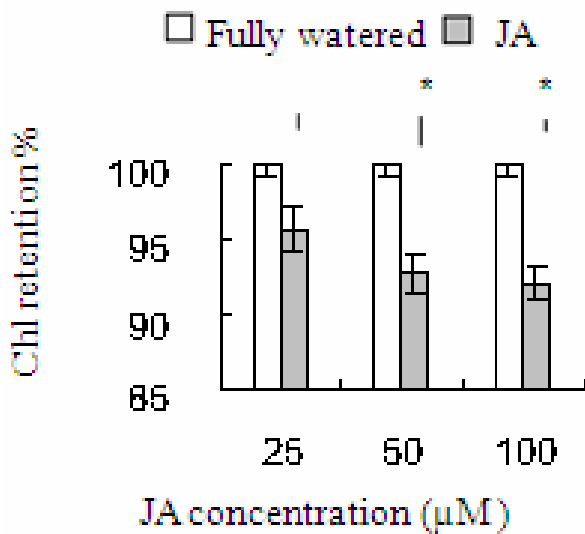


Figure 4. Effects of different concentrations of exogenously applied JA on the Chl loss of DT leaves at 3 d after treatment. Chl retentions are percentages of initial values. Each point is a mean ± S.E. (n=6). Vertical bars indicate L.S.D.s (P = 0.05) and asterisks denote significant difference between the two treatments at P < 0.05.

leaf JA accumulation reached a maximum.

Effect of JA on senescence

The exogenous application of JA induced the loss of leaf

Chl in a concentration-dependent style (Figure 4). On the third day following JA application when plants were not yet water stressed, the spraying of JA at 50 μM on the potted apricot plants showed a significant loss of leaf Chl in the JA-treated plants; decreased levels of Chl by about 15% compared with JA-untreated plants in DS or DT. There was no significant difference in Chl levels between two groups of JA-untreated plants ('fully watered' and 'drought' treatments) that were not yet drought-stressed after 3 days (Figure 5A). On the sixth day, when plants were subjected to moderate–severe water stress, the treatment induced a significant decline of Chl levels in leaf in the simple drought-treated plants, and there was an even lower level of leaf Chl in the JA-treated plants.

The application of 50 μM JA markedly enhanced the level of the dehydration-induced MDA accumulation (Figure 5B). On the third day, the level of MDA was lower in the leaves of well-watered plants and 'drought'-treated plants; however, in leaves of JA-treated plants, the MDA level significantly increased compared with JA-untreated plants. On the sixth day, the simple drought-treatment induced a rise in leaf MDA levels, and the JA treatment induced an even higher level of MDA.

DISCUSSION

Our data demonstrate that DT plants wilted and the leaves fell faster than DS plants under drought stress (Figures 1B, 2B, and 2E). DT plants behaved like *Leucaena leucocephala* which is known for drought tolerance. In summer, *L. leucocephala* will allow leaves to die in dry

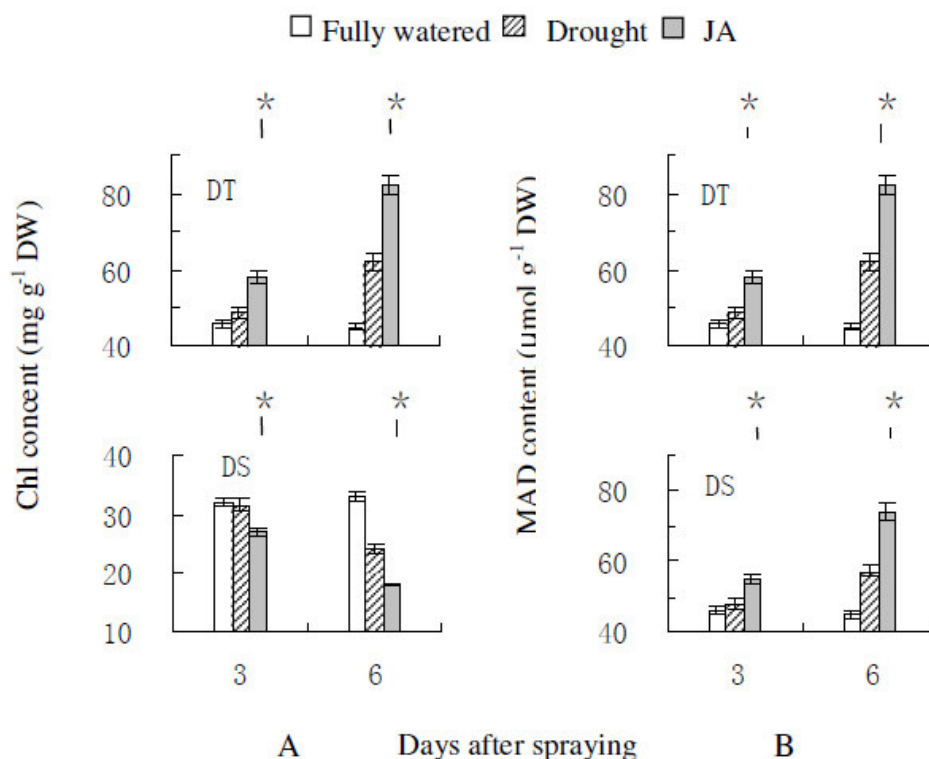


Figure 5. A) Concentration of Chl in leaves of DS and DT apricot plants after spraying with 50 μ M JA. B) Concentration of MDA in leaves of DS and DT apricot plants after spraying with 50 μ M JA. Each point is a Mean \pm S.E. (n = 6). Vertical bars indicate L.S.D.s (P = 0.05) and asterisks denote significant difference among the three treatments at P < 0.05.

periods and grow leaves in rainy periods. To survive drought stress, a plant must either extract more water from the soil, and/or effectively control the amount of water it loses through transpiration. DT plants and *L. leucocephala* adapt to drought stress using water-conserving mechanisms (which decrease water loss, reduce leaf growth, and accelerate leaf senescence). After a period of water deficit, plants have the ability to resume growth and restart their life cycle. It is not surprising that leaf abscission was induced by water stress. Although there was an association between leaf senescence and plant water deficits, the mechanism by which water stress induced abscission was not investigated.

Our GC-MS analysis showed sharp increases in leaf JA production rates in DT plants as severe water deficit developed. In preliminary experiments in which DT plants were rewatered after water was withheld for >13 days, all plants died. After 13 days drought treatment, RWC_{soil} reached 17%; DT plants could survive at RWC_{soil} >17% (Figure 1A). We also found that the difference between 12 and 13 days drought treatment was not only in leaves but also in roots of DT plants; at 12 days of drought treatment, the roots of DT plants were white and alive, but by 13 days of drought treatment they were brown and dead. When

water was withheld for 13 days, the DT leaves began to fall off. JA levels in DT leaves increased \approx 3-fold by 12 days (Figure 3) and disappeared on 13 days (data not shown) if water continued to be withheld. Thus JA levels did not remain high for a long time in DT leaves. In the present research, rewatering treatments were performed on day 12 of the drying treatment. It is interesting to note that two months after rewatering, the DT plants were alive and the DS were dead (Figure 2C, F). JA was not found in DS leaves on days 13 or 14 of the drying treatment. JA in DS leaves did not reach a maximum over the 14 days during which water was withheld (data not shown). Thus, to keep DT plants alive, rewatering was performed on day 12 of the drying treatment.

We also found that JA levels in the DT roots reached a maximum (one day after rewatering) after leaf JA maximum accumulation (12 days; Figure 3). Perhaps direct transport of drought-induced JA from leaves to roots can account for increased root JA pools after the drying treatments (Zhang and Baldwin, 1997). In higher plants, JA biosynthetic steps occur in fruit, seeds and leaves. Translocation of JA in the plant apparently takes place in the phloem, but both xylem and phloem translocation should be studied in more detail. However, in the present

study, why were JA levels in roots higher than that of leaves in DT plants for the entire experiment? Are increases in root JA levels in DT plants after rewatering dependent on *de novo* JA synthesis in roots or transportation of drought-induced JA from leaves to roots? These questions need further research.

It is known that JA regulates plant responses to both abiotic and biotic stress factors. JA may be death hormones (Engvild, 1989). A loss of Chl, the repression of genes related to photosynthesis at the transcriptional and translational levels (Reinbothe et al., 1994) and the degradation of Rubisco (Rakwal and Komatsu, 2000) are typical symptoms promoted by methyl jasmonate treatment in mature leaves. These symptoms resemble those of senescence. In the present study there was a significant loss of Chl and increased MDA after JA treatment in DT or DS (Figures 5A, 5B). There was a similar loss of Chl and increased MDA after JA treatment in the two woody species *Malus hupenensis* Pamp Rehd. (drought-tolerant species) and *Malus sieversii* (Ledeb) Roem. (drought-sensitive species) (data not shown). A senescence-promoting role might be associated with elevated JA levels in senescing leaves (Rao et al., 2000). It is known that senescence enables plants to withstand temporary or sustained water deficits (Levitt, 1980). Thus, a possible senescence-promoting role might be associated with JA induced by water deficit in plant tissue.

The senescence-promoting role might only be associated with JA under drought stress. ABA is generally recognized as a stress hormone that regulates expression of many drought responses (Liu et al., 2009). When plants wilt, ABA levels typically rise as a result of an increase in the rate of synthesis (Taylor, 1991). ABA has also been shown to promote leaf senescence (Ueda and Kato, 1980). ABA accumulation in higher plants in response to water deficit is thought to act as a signal for the initiation of processes involved in adaptation to drought and other environmental stresses (Bray, 1993). Our GC-MS analysis also showed sharp increases in leaf ABA production rates in DT plants as water deficit developed (data not shown). As we have pointed out, jasmonic acid responded to desiccation similarly to ABA. Further research is needed to determine what compound plays a pivotal role in promoting leaf senescence during water stress.

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