

Characterizing Concentration Effects of Exogenous Abscisic Acid on Gas Exchange, Water Relations, and Growth of Muskmelon Seedlings during Water Stress and Rehydration

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ABSTRACT. Excess transpiration relative to water uptake often causes water stress in transplanted vegetable seedlings. Abscisic acid (ABA) can limit transpirational water loss by inducing stomatal closure and inhibiting leaf expansion. We examined the concentration effect of exogenous ABA on growth and physiology of muskmelon (*Cucumis melo* L.) seedlings during water stress and rehydration. Plants were treated with seven concentrations of ABA (0, 0.24, 0.47, 0.95, 1.89, 3.78, and 7.57 mM) and subjected to 4-day water withholding. Application of ABA improved the maintenance of leaf water potential and relative water content, while reducing electrolyte leakage. These effects were linear or exponential to ABA concentration and maximized at 7.57 mM. Gas-exchange measurements provided evidence that such stress control is attributed to ABA-induced stomatal closure. First, net CO₂ assimilation rate and stomatal conductance initially decreased with increasing ABA concentration by up to 95% and 70%, respectively. A follow-up study (≤ 1.89 mM ABA) confirmed this result with or without water stress and further revealed a close positive correlation between intercellular CO₂ concentration and net CO₂ assimilation rate 1 day after treatment ($r^2 > 0.83$). In contrast, ABA did not affect leaf elongation, indicating that stress alleviation was not mediated by leaf area adjustment. After 18 days of post-stress daily irrigation, dry matter accumulation showed a quadratic concentration-response, increasing up to 1.89 mM by 38% and 44% in shoot and roots, respectively, followed by 16% to 18% decreases at >1.89 mM ABA. These results suggest that excess levels of ABA delay post-stress growth, despite the positive effect on the maintenance of water status and membrane integrity. Another negative side effect was chlorosis, which accelerated linearly with increasing ABA concentration, although it was reversible upon re-watering. The optimal application rate of ABA should minimize these negative effects, while keeping plant water stress to an acceptable level.

Vegetable seedlings often suffer transient water stress after transplanting. This so-called transplant shock is caused by the imbalance between water uptake and transpiration. In newly transplanted seedlings, water uptake is reduced because of root injury during transplanting (Kramer, 1983) and disturbed root–soil contact (Burdett, 1990). In contrast to roots, shoots are relatively undamaged, maintaining high transpiration capacity. Moreover, upon transplanting, plants are exposed to direct sunlight, wind, and temperature extremes, which increase crop evapotranspiration. Successful field establishment depends on how quickly plants can recover water uptake capacity to support transpiration demand for normal growth.

Water stress increases accumulation of ABA in leaves (Davies and Jones, 1991). It is well documented that ABA acts as a stress signal, which triggers adaptive changes in physiology and morphology of plants (Taiz and Zeiger, 2002). For example, ABA synthesized in roots or mesophyll is transported to guard cells where it promotes stomatal closure by inducing net efflux of potassium ions and thus reducing turgor pressure (Fan et al., 2004; Li et al., 2006; Schroeder et al., 2001). It is

also known that ABA is involved in inhibition of leaf growth (Van Volkenburgh, 1999). Several studies reported that restricted leaf expansion was correlated with ABA increases in xylem sap (Ismail et al., 2002; Salah and Tardieu, 1997) or leaves (Alves and Setter, 2000; He and Cramer, 1996; Van Volkenburgh and Davies, 1983). In a study using ABA-deficient mutants, Bacon et al. (1998) demonstrated that ABA is required to mediate pH-regulated cell expansion in dehydrated barley (*Hordeum vulgare* L.). Whereas stomatal closure has an immediate effect in reducing transpirational water loss, restricted leaf expansion minimizes plant water use by limiting increases in transpirational area.

In addition to these functions in leaves, ABA plays an important regulatory role in root systems. Root growth is usually less inhibited than shoot growth under water deficit conditions (Creelman et al., 1990; Sharp et al., 2004; van der Wee et al., 2000; Watts et al., 1981). In maize (*Zea mays* L.) seedlings, Saab et al. (1990) proposed that endogenous ABA, which accumulates in root tips at low water potential, is required for the maintenance of primary root elongation. Their approach was to inhibit ABA accumulation using fluridone, an inhibitor of the carotenoid (ABA precursor) biosynthesis pathway, or using a mutant with deficient carotenoid synthesis. Inhibition of ABA accumulation by either method resulted in severe reductions in root elongation at low water potential. This finding was further confirmed in a subsequent study that showed full recovery of root elongation when ABA in the elongation zone

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was restored to normal levels with exogenous ABA (Sharp, 1994).

The overall effect of ABA can be summarized as an increase in root-to-shoot ratio, which, along with the regulation of stomatal closure, helps plants cope with water stress (Taiz and Zeiger, 2002). Thus, ABA application may reduce transplant shock in vegetable transplants. Berkowitz and Rabin (1988) found that bell pepper (*Capsicum annuum* L.) seedlings dipped entirely in 1 mM ABA solution had higher stomatal resistance and leaf water potential than untreated seedlings after transplanting. When irrigation was withheld for 15 h after transplanting to impose water stress, the improved water status by ABA resulted in increased field survival and yield. Similar results have been reported by Goreta et al. (2007). In their study, bell pepper seedlings were sprayed with ABA at 2000 mg·L⁻¹ (7.6 mM) and subjected to two cycles of 4-d water withholding in a greenhouse. They suggested that reductions in stomatal conductance (g_s) by ABA enabled the maintenance of leaf water potential and prevented increases in electrolyte leakage and leaf abscission. On the other hand, Latimer (1992) reported that root-drench application of ABA at 660 mg·L⁻¹ (2.5 mM) did not affect either transplant growth or field establishment of tomato (*Solanum lycopersicum* L.) seedlings under optimal irrigation. In maize seedlings, foliar application of 100 μM ABA increased root-to-shoot ratio but stimulated leaf chlorophyll degradation under water deficit conditions (Hejrnák and Kykalová, 2009).

The beneficial effects of exogenously applied ABA are not consistently evident in previous greenhouse and field studies. Most of these studies used a single concentration or narrow concentration range of ABA, which may not represent the optimal rate for the tested crop to promote desired responses. In fact, the magnitude of drought-induced increases in endogenous ABA varies among crop species, indicating a crop specific sensitivity to ABA (Davies and Jones, 1991). Furthermore, high-dose applications of ABA tend to have negative side effects such as leaf chlorosis and abscission (Kim and van Iersel, 2011; Waterland et al., 2010b). Therefore, exogenous ABA must be tested over a wide range of concentrations to accurately evaluate its potential as a stress control agent. The objective of this study was to characterize concentration effects of exogenous ABA on alleviating water stress and stimulating post-stress growth of muskmelon seedlings.

Materials and Methods

ABSCISIC ACID SOLUTIONS. The formulation of ABA used in this study was VBC-30025 (Valent BioSciences, Libertyville, IL) containing 90% of (+)-cis, trans-ABA. A stock solution was prepared according to the manufacturer's protocol using pre-weighed ABA and ethanol. Test solutions were prepared by diluting the stock solution with deionized water.

PLANT MATERIAL. Muskmelon 'Caravelle' seeds were sown in a polystyrene tray with 128 inverted pyramid cells each containing 30 mL of peat-lite mix (Speedling Peat-lite; Speedling, Sun City, FL). Seedlings were grown in a commercial nursery greenhouse (Speedling, Alamo, TX) for 40 to 45 d and then transferred to a greenhouse at the Texas A&M AgriLife Research and Extension Center in Uvalde, TX (lat. 29°1' N, long. 99°5' W), where experiments were conducted in Oct. 2006 and May 2007. During seedling growth in the commercial nursery, average daily air temperature ranged from 17 to 30 °C and 6 to 27 °C in 2006 and 2007, respectively.

GROWTH CONDITIONS AND TREATMENTS. In the first experiment (Expt. 1), 42-d-old seedlings were transplanted in a plastic tray (10.5 × 13 cm) with six cells each containing 60 mL of peat-lite mix. After transplanting, seedlings were fertilized with water-soluble fertilizer (20N-4.4P-16.6K) at 200 mg N/L and watered daily thereafter. When seedlings were 45 d old, ABA solutions prepared at 0, 0.24, 0.47, 0.95, 1.89, 3.78, and 7.57 mM (0, 62.5, 125, 250, 500, 1000, and 2000 mg·L⁻¹) were sprayed evenly over the seedlings using a handheld sprayer between 1100 and 1200 HR. Approximately 1 mL of ABA solution was applied per plant, which wetted the leaves thoroughly with little dripping. After spraying, seedlings were exposed to transient water stress by withholding water for 4 d. Irrigation was resumed when wilting occurred on all untreated (0 mM ABA) plants and performed daily thereafter. Seedlings were fertilized 11 d after ABA treatment (DAT) using the same rate as the first application and grown to 22 DAT. Day and night temperatures in the greenhouse were 20 to 32 °C and 15 to 22 °C, respectively, with a 11-h photoperiod. Maximum photosynthetic photon flux (PPF) at the canopy level was ≈1500 μmol·m⁻²·s⁻¹.

In the second experiment (Expt. 2), 51-d-old seedlings were transplanted individually in 10-cm square plastic pots (9.5 cm depth) containing 500 mL of peat-lite mix. After transplanting, seedlings were fertilized with water-soluble fertilizer (20N-4.4P-16.6K) at 200 mg N/L and watered every 2 d thereafter. When seedlings were 55 d old, ABA solutions prepared at 0, 0.47, and 1.89 mM (0, 125, and 500 mg·L⁻¹) were sprayed evenly over the seedlings using a CO₂-pressured backpack sprayer (Model T; Bellspray, Opelousas, LA) between 1100 and 1200 HR. The CO₂ backpack sprayer was equipped with a six-nozzle handheld boom and flat-fan nozzle tips (TP8002VS; TeeJet Technologies, Wheaton, IL) spaced 43 cm apart. Treatments were performed at 276 kPa to apply ≈1 mL of ABA solution per plant, which wetted the leaves thoroughly with little dripping. Irrigated control plants were watered every 2 d throughout the experiment, whereas transiently dehydrated plants were subjected to 6-d water withholding and watered every 2 d thereafter. Irrigation was resumed when wilting was visible on the untreated (0 mM ABA) plants. Seedlings were fertilized at 12 DAT using the same rate as the first application and grown to 15 DAT. Day and night temperatures in the greenhouse were 25 to 32 °C and 15 to 26 °C, respectively, with a 14-h photoperiod. Maximum PPF at the canopy level was ≈1500 μmol·m⁻²·s⁻¹.

In both experiments, plants were watered between 0800 and 0900 HR by subirrigation until the growing medium was fully saturated. All fertilizer applications were performed by drenching into the growing medium through irrigation.

GAS EXCHANGE. All gas-exchange measurements were made on an intact, unshaded, youngest expanded leaf, mostly the third leaf from the apex, between 1200 and 1400 HR. Two leaves per replication, each from a different plant, were used.

In Expt. 1, net CO₂ assimilation rate (A) and g_s were measured using a closed-flow infrared gas analyzer (LI-6200; LI-COR, Lincoln, NE) at 0, 1, 2, 3, 4, 10, and 22 DAT. The instrument was equipped with a 0.25-L uncontrolled environment chamber, which was customized to use a constant leaf area of 2.5 cm². Air flow rate was adjusted between 200 and 400 μmol·s⁻¹ for each measurement to maintain constant relative humidity in the chamber. Ambient CO₂ concentration and canopy-level PPF during the measurements ranged from 390 to 410 μmol·mol⁻¹ and 1000 to 1500 μmol·m⁻²·s⁻¹, respectively.

In Expt. 2, the same variables as in Expt. 1 and intercellular CO₂ concentration (C_i) were measured using an open-flow infrared gas analyzer (LI-6400; LI-COR) at 0, 1, 2, 3, 5, 6, and 15 DAT. The instrument was equipped with a 2 × 3-cm leaf chamber and a red plus blue light-emitting diode light source (6400-02B; LI-COR). During measurements, photosynthetically active radiation, reference CO₂ concentration, air flow rate, and block temperature were maintained constant at 1500 μmol·m⁻²·s⁻¹, 400 μmol·mol⁻¹, 500 μmol·s⁻¹, and 25 °C, respectively. Relative humidity in the sample chamber ranged between 50% and 70%.

LEAF CHLOROPHYLL INDEX. Immediately after gas-exchange measurement, chlorophyll index was measured on the same leaves using a chlorophyll meter (SPAD-502; Konica Minolta Sensing, Tokyo, Japan) in both experiments. Two readings were taken per leaf, ≈1 cm from the leaf margin and between major leaf veins.

PLANT WATER STATUS (EXPT. 1). Water potential (ψ) and relative water content (RWC) were measured on leaves of about the same age and size as those used for gas exchange measurements between 1200 and 1400 HR at 0, 2, 3, and 22 DAT. Leaf xylem pressure potential was measured as an estimate of leaf ψ using a pressure chamber (Model 3005; Soilmoisture Equipment, Santa Barbara, CA) as described by Taiz and Zeiger (2002). Another set of leaves was sampled, and four 1-cm diameter discs were cut from each leaf with a cork borer avoiding major leaf veins. Fresh weight (FW) of two leaf discs was recorded to determine RWC. The other two were used to determine electrolyte leakage. The samples were floated on deionized water in a petri dish and hydrated in the dark. After 4 h, the turgid weight (TW) was recorded, and the samples were subsequently dried to a constant weight at 85 °C to determine the dry weight (DW). Relative water content expressed as a percentage was calculated as follows:

$$\text{RWC} = [(\text{FW} - \text{DW}) / (\text{TW} - \text{DW})] \times 100$$

ELECTROLYTE LEAKAGE (EXPT. 1). Electrolyte leakage was determined by a modified procedure of Blum and Ebercon (1981) to assess the degree of cell membrane damage. Two leaf discs per plant as described for RWC were rinsed with deionized water and placed in a capped 60-mL test tube filled with 10 mL of deionized water. The test tubes were incubated for 24 h at 25 °C on a rotary shaker set at 100 rpm, and electrical conductivity in the incubated solution (EC1) was measured using a conductivity electrode (sympHony SP40C; VWR International, Radnor, PA). The test tubes were then autoclaved for 15 min at 120 °C and 103 kPa, and EC in the autoclaved solution (EC2) was measured on equilibration at 25 °C. Electrolyte leakage expressed as a percentage was calculated as follows:

$$\text{electrolyte leakage} = (\text{EC1} / \text{EC2}) \times 100$$

PLANT GROWTH (EXPT. 1). Leaves of approximately the same age and size as those used for other measurements were selected for leaf length measurements. Leaf length was measured from the lamina tip to the petiole attachment point non-destructively on the same leaves at 0 and 3 DAT. Relative leaf elongation rate (RLER) was calculated as follows:

$$\text{RLER} = d(\ln L) / dt$$

where d(lnL) is the difference in the natural logarithm of leaf length between two measurements and dt is the difference in time between two measurements.

At 22 DAT, two plants per replication were cut at the surface of growing medium and dried at 65 °C for 48 h to determine shoot DW. Roots were washed to remove from the growing medium and dried at 65 °C for 48 h to determine root DW.

STATISTICAL DESIGN AND ANALYSIS. In Expt. 1, treatments were seven concentrations of exogenous ABA. There were three replicates (trays) and six subsamples (plants) for each treatment arranged in a randomized complete block design.

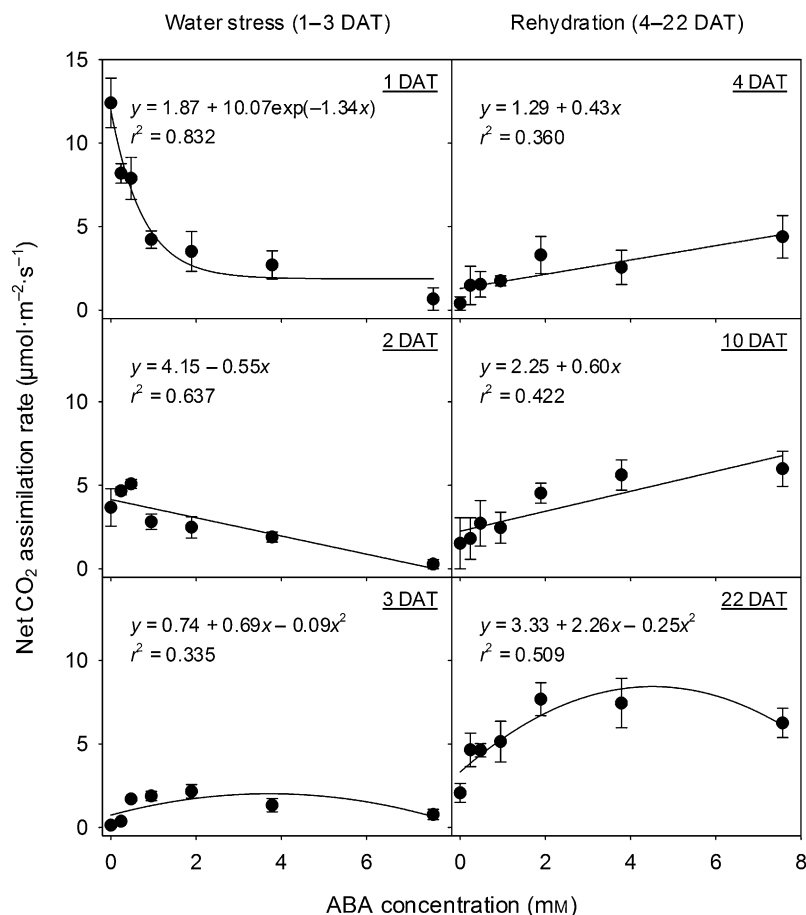


Fig. 1. Net CO₂ assimilation rate of muskmelon seedlings as a function of exogenous abscisic acid (ABA) concentration during water stress and rehydration (Expt. 1). Plants were sprayed with seven concentrations of ABA solution (0, 0.24, 0.47, 0.95, 1.89, 3.78, and 7.57 mM) at 1 mL per plant and subjected to water withholding. Irrigation was resumed 4 d after ABA treatment (DAT) and performed daily thereafter. Pre-treatment (0 DAT) mean ± SE was 10.4 ± 0.7 μmol·m⁻²·s⁻¹. Data are means ± SE (n = 3). Solid lines show fits to the following models: exponential decay (1 DAT), linear (2, 4, and 10 DAT), and quadratic (3 and 22 DAT).

Two plants per replication were used for each measurement. To characterize the dose-responses of dependent variables to ABA, we fitted each data set to the following four models: linear Eq. [1], quadratic Eq. [2], exponential decay Eq. [3], and exponential rise to an asymptote Eq. [4].

$$y = a + bx \quad [1]$$

$$y = a + bx + cx^2 \quad [2]$$

$$y = a + b \exp(-kx) \quad [3]$$

$$y = a + b[1 - \exp(-kx)] \quad [4]$$

where y is the predicted value of a dependent variable at ABA concentration x , and k is the rate constant. In Eqs. [1] and [2], a is the y intercept, b is the linear coefficient, and c is the quadratic coefficient. In Eq. [3], a is the lower asymptote, and b is the maximum decrease in y . The sum of a and b represents y for the control (0 mM ABA). In Eq. [4], a represents y for the control, and b is the maximum increase in y . The sum of a and b is the upper asymptote.

The model parameters were estimated using the NLMIXED procedure in SAS (Version 9.2; SAS Institute, Cary, NC). The model with the smallest value of Akaike's information criterion was selected as the best model for each data set. Models were considered non-significant when the following model parameters were not significantly different from zero ($P > 0.05$): b in Eq. [1], c in Eq. 2, and b or k in Eq. [3] and Eq. [4]. All models were fitted using all individual replicates ($n = 3$), although only mean values are shown in the figures below.

In Expt. 2, treatments were factorial combinations of two water stress levels (with or

without water withholding) and three ABA concentrations. There were four replicates and two subsamples for each treatment arranged in a split plot design with water stress as the main plot and ABA concentration as the subplot. All

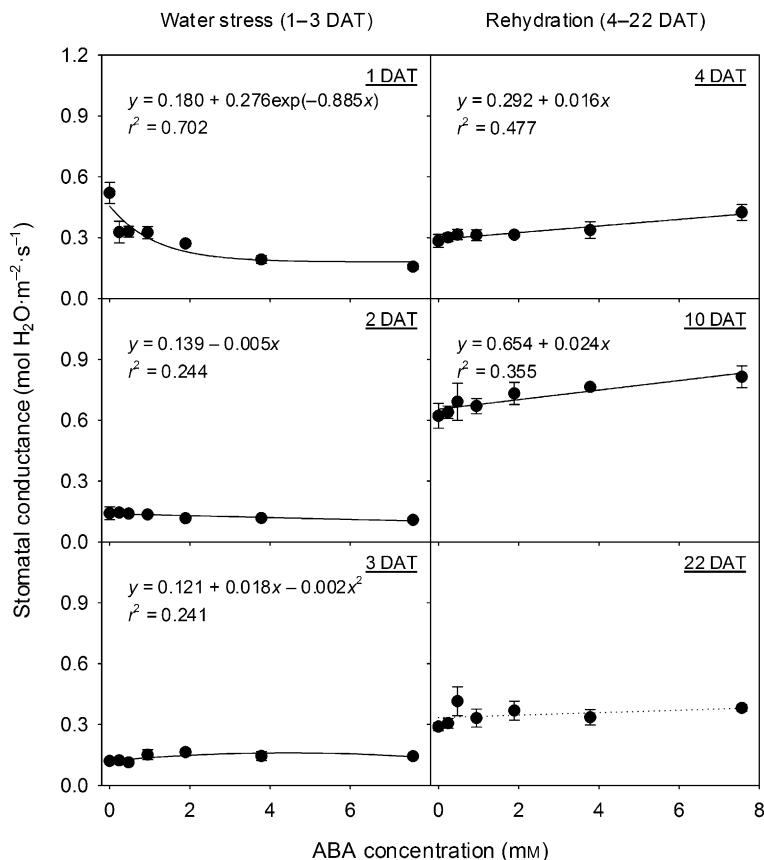


Fig. 2. Stomatal conductance of muskmelon seedlings as a function of exogenous abscisic acid (ABA) concentration during water stress and rehydration (Expt. 1). Plants were sprayed with seven concentrations of ABA solution (0, 0.24, 0.47, 0.95, 1.89, 3.78, and 7.57 mM) at 1 mL per plant and subjected to water withholding. Irrigation was resumed 4 d after ABA treatment (DAT) and performed daily thereafter. Pre-treatment (0 DAT) mean \pm SE was 1.2 ± 0.1 mol H₂O/m²/s. Data are means \pm SE ($n = 3$). Solid lines show fits to the following models: exponential decay (1 DAT), linear (2, 4, and 10 DAT), and quadratic (3 DAT). A dotted line shows a non-significant ($P > 0.05$) linear trend.

Table 1. Effects of transient water stress and abscisic acid (ABA) spray concentration on net CO₂ assimilation rate of muskmelon seedlings (Expt. 2).

Water stress ^c	ABA concn ^y (mM)	Net CO ₂ assimilation rate (μmol·m ⁻² ·s ⁻¹)							
		Time after ABA treatment (d)							
		0	1	2	3	5	6	15	
- Stress	0.00	16.9	16.8 ab ^x	16.4	16.3 ab	13.2 a	12.5 ab	10.8 ab	
	0.47	15.9	12.8 abc	13.3	15.6 abc	12.8 a	9.6 abc	9.8 b	
	1.89	17.0	8.5 c	12.8	18.5 a	14.5 a	11.3 abc	10.4 ab	
+ Stress	0.00	17.5	17.1 a	12.4	10.8 c	5.9 b	6.9 c	10.1 ab	
	0.47	17.4	11.2 bc	14.1	10.9 c	8.0 b	8.9 bc	11.8 ab	
	1.89	16.6	9.4 bc	12.1	12.0 bc	12.2 a	12.9 a	12.9 a	
Source of variation (P value)									
Water stress		0.879	0.941	0.203	0.000	0.010	0.213	0.034	
ABA concn		0.947	0.000	0.277	0.171	0.000	0.006	0.188	
Water stress \times ABA concn		0.853	0.375	0.158	0.738	0.014	0.002	0.071	

^zIrrigated control plants (- Stress) were watered every 2 d throughout the experiment, whereas transiently dehydrated plants (+ Stress) were subjected to 6-d water withholding and watered every 2 d thereafter. Measurements at 6 d after ABA treatment were made 4 h after re-watering.

^yPlants were sprayed with three concentrations of ABA solution (0, 0.47, and 1.89 mM) at 1 mL per plant.

^xMean separation in columns by the Tukey-Kramer test at $P \leq 0.05$.

Table 2. Effects of transient water stress and abscisic acid (ABA) spray concentration on stomatal conductance of muskmelon seedlings (Expt. 2).

Water stress ^z	ABA concn ^y (mM)	Stomatal conductance (mol H ₂ O/m ² /s)							
		Time after ABA treatment (d)							
		0	1	2	3	5	6	15	
– Stress	0.00	0.363	0.462 ab ^x	0.315	0.374 ab	0.294 a	0.257 ab	0.701	
	0.47	0.397	0.219 abc	0.233	0.414 a	0.259 ab	0.183 ab	0.636	
	1.89	0.346	0.102 c	0.179	0.412 a	0.268 a	0.201 ab	0.606	
		----- Water withholding -----			----- Rehydration -----				
+ Stress	0.00	0.338	0.468 a	0.190	0.158 b	0.071 c	0.125 b	0.471	
	0.47	0.375	0.160 bc	0.227	0.149 b	0.094 bc	0.148 ab	0.627	
	1.89	0.305	0.113 bc	0.144	0.168 b	0.171 abc	0.256 a	0.596	
Source of variation (<i>P</i> value)									
Water stress			0.838	0.892	0.138	0.000	0.023	0.402	0.559
ABA concn			0.700	0.006	0.121	0.894	0.287	0.081	0.825
Water stress × ABA concn			0.989	0.895	0.379	0.896	0.119	0.011	0.279

^zIrrigated control plants (– Stress) were watered every 2 d throughout the experiment, whereas transiently dehydrated plants (+ Stress) were subjected to 6-d water withholding and watered every 2 d thereafter. Measurements at 6 d after ABA treatment were made 4 h after re-watering.

^yPlants were sprayed with three concentrations of ABA solution (0, 0.47, and 1.89 mM) at 1 mL per plant.

^xMean separation in columns by the Tukey–Kramer test at *P* ≤ 0.05.

data analyses were, unless otherwise noted, run using the MIXED procedure with the Kenward–Rogers method (DDFM=KR) in SAS. When heteroscedasticity was indicated by a likelihood ratio test (*P* ≤ 0.05), the MIXED procedure was run with the GROUP option in the REPEATED statement. We tested the significance of main and interaction effects using the restricted maximum likelihood method (METHOD=REML), in which water stress, ABA concentration, and the interaction were fixed factors and replication and replication × water stress interaction were random factors. We also compared least squares means using the Tukey–Kramer test (ADJUST=TUKEY in the LSMEANS statement).

To assess stomatal and non-stomatal limitations to photosynthesis, we tested a linear correlation between *C*_i and *A* using the REG procedure in SAS. We assumed that *A* is proportional to *C*_i under stomatal limitation (Lawlor, 2002). The *r*² values were calculated separately for the irrigated control and transiently dehydrated plants at each measurement time. The correlation was considered non-significant when the slope was not significantly different from zero (*P* > 0.05).

Results

GAS EXCHANGE. Gas exchange showed a reversible inhibition in response to water stress and exogenous ABA in Expt. 1 (Figs. 1 and 2). Pre-treatment *A* (Fig. 1) and *g*_s (Fig. 2) were 10.4 μmol·m⁻²·s⁻¹ and 1.2 mol H₂O/m²/s, respectively. In the untreated control, *A* and

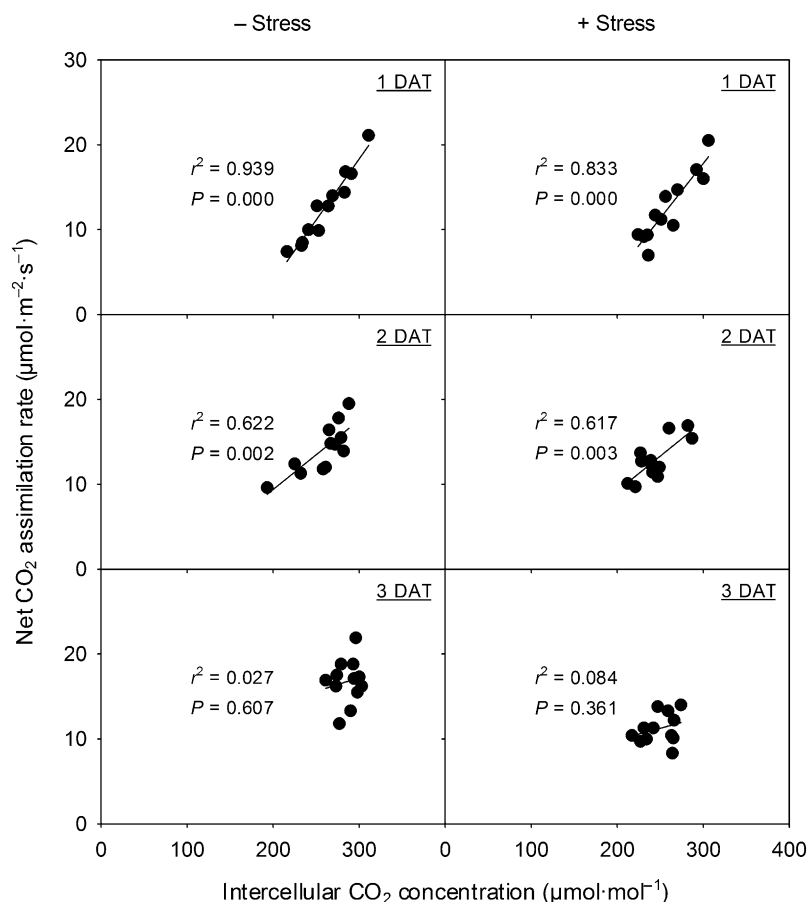


Fig. 3. Temporal declines in the linear correlation between intercellular CO₂ concentration and net CO₂ assimilation rate of muskmelon seedlings (Expt. 2). Plants were sprayed with three concentrations of abscisic acid solution (0, 0.47, and 1.89 mM) at 1 mL per plant. Irrigated control plants (– Stress) were watered every 2 d throughout the experiment, whereas transiently dehydrated plants (+ Stress) were subjected to 6-d water withholding and watered every 2 d thereafter. The correlation was weak (*r*² < 0.2) and non-significant (*P* > 0.05) from 3 to 15 d after ABA treatment (DAT). Data points are individual replicates (*n* = 4).

g_s started to decrease at 2 and 1 DAT, respectively, and were reduced by more than 90% at 3 DAT. After re-watering, A recovered slowly to 21% of the pre-stress level, whereas g_s recovered to half the pre-stress level at 10 DAT and decreased thereafter. In most measurements, A and g_s showed similar concentration-dependent responses to ABA. At 1 DAT, A and g_s decreased with increasing ABA concentration by up to 95% and 70%, respectively. This dose-response was described by an exponential decay with a steep decrease up to 0.95 and 3.78 mM ABA for A and g_s , respectively, followed by a gradual decrease. At 2 DAT, A and g_s continued to decrease with increasing ABA concentration, but the slope became linear and less steep. Thereafter, both A and g_s increased in response to ABA. Their dose-responses were quadratic at 3 DAT with an increase up to 1.89 mM ABA followed by a decrease, and then they became a linear increase shortly after re-watering at 4 and 10 DAT. At 22 DAT, the dose-response of A was quadratic with a peak recovery to 73% of the pre-stress level at 1.89 mM, whereas that of g_s did not fit any tested regression models.

In Expt. 2, A was significantly affected by water stress, ABA concentration, or the interaction except at 2 DAT (Table 1). At 1 DAT, A decreased by nearly half with increasing ABA concentration. In the irrigated control (– Stress), A in the untreated (0 mM ABA) plants remained at the pre-stress level until 3 DAT, whereas that in the ABA-treated plants gradually recovered to the pre-stress level by 3 DAT. From 3 to 15 DAT, A steadily decreased by 34% to 44% with no significant difference among the ABA treatments. Contrasting results were observed when water stress was imposed. In the untreated (0 mM ABA) plants, A decreased steadily from 1 to 5 DAT, being almost one-third of the pre-stress level at 5 DAT. The ABA-treated plants recovered A only from 1 to 2 DAT. From 2 to 5 DAT, A decreased to almost half the pre-stress level at 0.47 mM ABA, whereas it remained constant above two-thirds of the pre-stress level at 1.89 mM ABA. As a result, A showed a 2-fold increase with increasing ABA concentration at 5 DAT. This increase became gradually not significant after re-watering because recovery of A was inversely proportional to ABA concentration. At 15 DAT, A averaged 11% higher in the stress treatment than the irrigated control ($P = 0.034$). In all measurements except 15 DAT, g_s responded to water stress and ABA similarly to, but to a greater extent than A (Table 2). In Expt. 2, C_i was also measured and regressed against A (Fig. 3). Regardless of water stress, they showed strong positive correlations ($r^2 > 0.83$) at 1 DAT, but r^2 values gradually declined thereafter. From 3 to 15 DAT, the correlations were weak and non-significant.

LEAF CHLOROSIS. Leaf chlorosis, as indicated by reductions in chlorophyll index, was induced by both water stress and exogenous ABA in Expt. 1 (Fig. 4). In the untreated control,

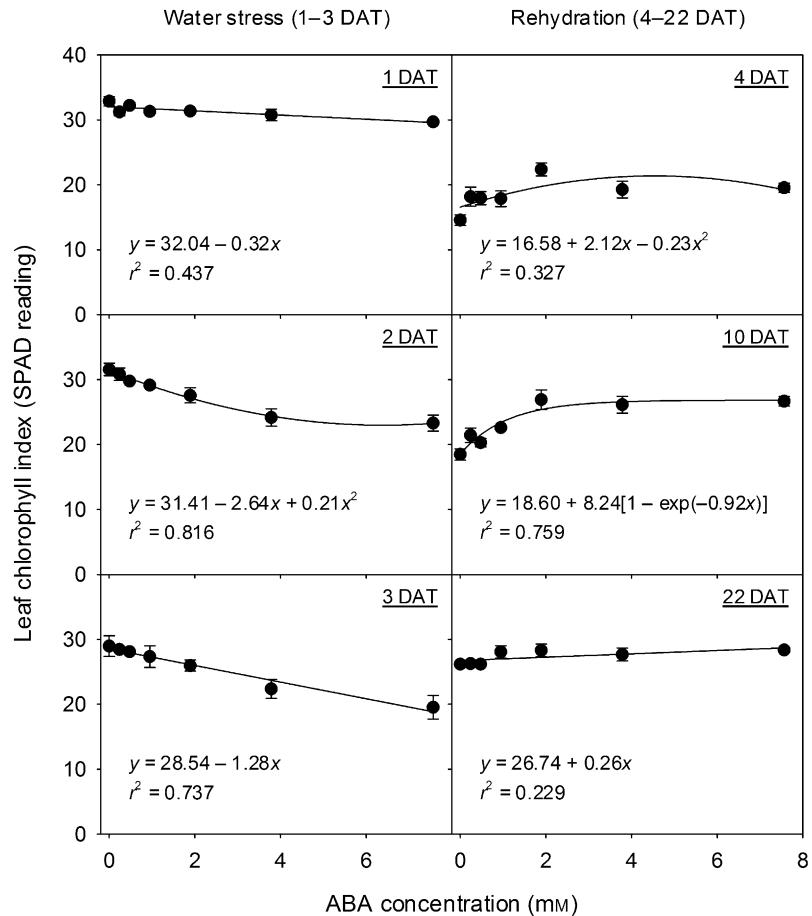


Fig. 4. Leaf chlorophyll index of muskmelon seedlings as a function of exogenous abscisic acid (ABA) concentration during water stress and rehydration (Expt. 1). Plants were sprayed with seven concentrations of ABA solution (0, 0.24, 0.47, 0.95, 1.89, 3.78, and 7.57 mM) at 1 mL per plant and subjected to water withholding. Irrigation was resumed 4 d after ABA treatment (DAT) and performed daily thereafter. Pre-treatment (0 DAT) mean \pm SE was 31.5 ± 0.3 . Data are means \pm SE ($n = 3$). Solid lines show fits to the following models: linear (1, 3, and 22 DAT), quadratic (2 and 4 DAT), and exponential rise to an asymptote (10 DAT).

chlorophyll index decreased only by 9% from 0 to 3 DAT (31.5 to 29.0) but decreased by more than half at 4 DAT (14.6), showing visible yellowing (Fig. 5). Thereafter chlorophyll index increased, especially after fertilization at 11 DAT. Leaf chlorosis was mostly corrected by 22 DAT with chlorophyll index recovering to 82% of the pre-stress level. In general, the dose-response of chlorophyll index to ABA was described as a linear decrease during the water stress period. The ABA-induced chlorosis progressed gradually with the maximum chlorophyll loss by ABA increasing from 10% at 1 DAT to 33% at 3 DAT. After re-watering, however, chlorophyll index increased in response to ABA. The dose-response was quadratic at 4 DAT with an increase up to 1.89 mM ABA followed by a slight decrease, but, at 10 DAT, it became an exponential rise reaching a plateau at 1.89 mM ABA. The maximum increase in chlorophyll index by ABA was 54% and 46% at 4 and 10 DAT, respectively. At 22 DAT, the dose-response exhibited a very gradual linear increase.

Similar effects of exogenous ABA on chlorophyll index were observed in Expt. 2 (data not shown). Transient chlorosis was induced by exogenous ABA, regardless of water stress.

PLANT WATER STATUS. Leaf ψ averaged -0.12 MPa across all treatments at 0 DAT (Fig. 6A). In the untreated control, leaf ψ

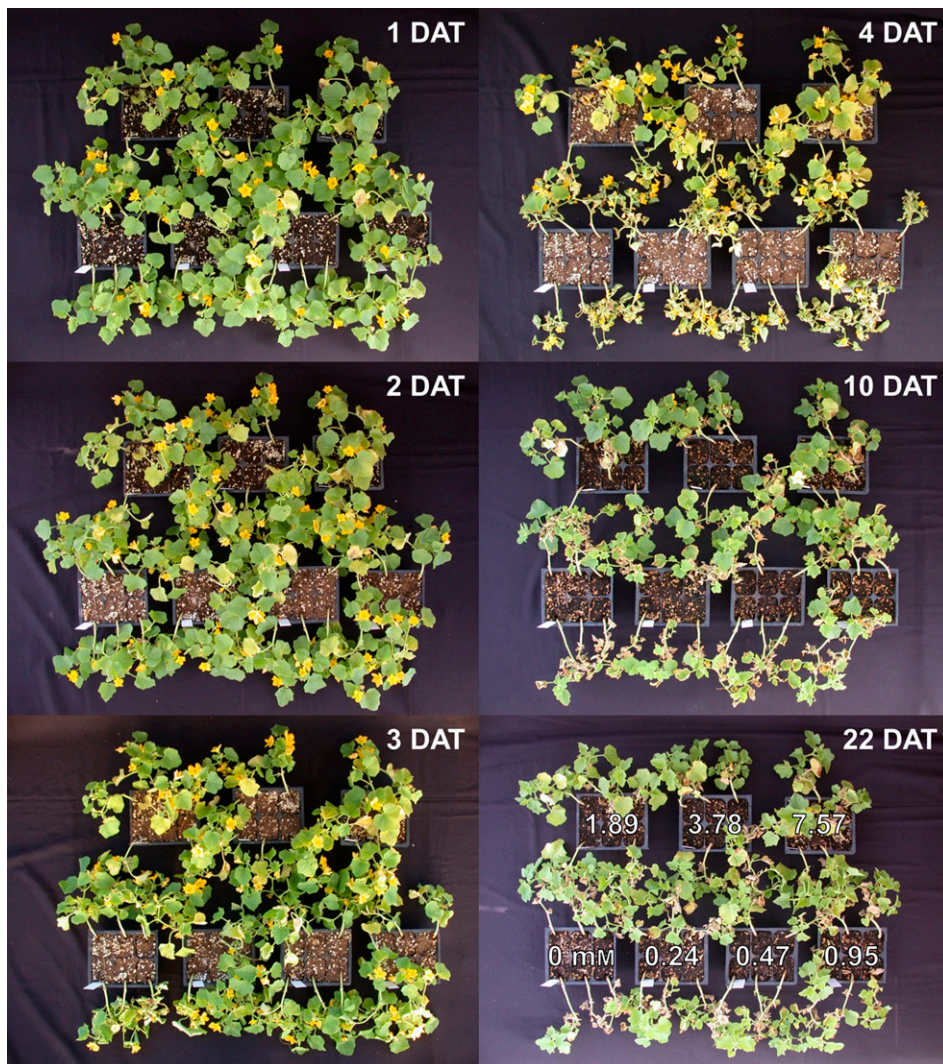


Fig. 5. Chlorosis and wilting in muskmelon seedlings (Expt. 1). Plants were sprayed with seven concentrations of abscisic acid (ABA) solution (from left to right and bottom to top in each image: 0, 0.24, 0.47, 0.95, 1.89, 3.78, and 7.57 mM) at 1 mL per plant and subjected to water withholding [0 to 3 d after ABA treatment (DAT)]. Irrigation was resumed 4 DAT and performed daily thereafter. The image at 4 DAT was taken immediately before re-watering.

decreased from the pre-stress value by 5-fold (-0.62 MPa) at 2 DAT and by 14-fold (-1.76 MPa) at 3 DAT. The magnitude of these reductions was lowered exponentially with increasing ABA concentration; leaf ψ increased sharply up to 0.95 mM ABA and then increased gradually to a plateau. Relatively high leaf ψ (>-0.6 MPa) was maintained at ≥ 0.95 mM ABA throughout the water stress period. At 22 DAT, leaf ψ was similar to the pre-stress level in all treatments.

Similar trends were observed for RWC (Fig. 6B). In the untreated control, RWC decreased from 93.6% at 0 DAT to 73.7% and 53.0% at 2 and 3 DAT, respectively. The magnitude of these reductions was lowered with increasing ABA concentration in a linear manner. Thus, RWC was more indicative of mild stress than leaf ψ . Increasing ABA concentration maintained RWC as high as 92.7% and 89.8% at 2 and 3 DAT, respectively. Similarly to leaf ψ , RWC was restored by re-watering to the pre-stress level in all treatments.

maintenance of leaf ψ and RWC (Fig. 6A–B), thus minimizing dehydration-induced damage to membranes (Fig. 7). These effects were linear or exponential to ABA concentration and were maximized at 7.57 mM. As a general rule, water stress can be classified as mild, moderate, and severe when RWC reductions are $<10\%$, 10% to 20%, and $>20\%$, respectively (Hsiao, 1973). According to these criteria, water stress at 2 DAT was moderate with ≤ 0.24 mM ABA and mild with ≥ 0.47 mM ABA, whereas water stress at 3 DAT was classified as severe with ≤ 1.89 mM ABA, moderate with 3.78 mM ABA, and mild with 7.57 mM ABA. Such a stress gradient also was evident by the severity of wilting (Fig. 5).

The alleviation of water stress may be associated with ABA-induced acclimation to water-limiting conditions. Stomatal closure is considered one of the first lines of defense against immediate dehydration (Chaves et al., 2002), and its regulation is known to be mediated by ABA. In this study, A and g_s initially decreased with increasing ABA concentration by

ELECTROLYTE LEAKAGE. Electrolyte leakage averaged 36.1% at 0 DAT and remained nearly constant (35.1% to 41.0%) until 2 DAT in all treatments (Fig. 7). In the untreated control, electrolyte leakage increased more than 2-fold from 2 to 3 DAT (36.1% to 84.1%), indicating that cell membrane damage was caused by severe leaf dehydration. The magnitude of cell membrane damage decreased sharply with increasing ABA concentration up to 1.89 mM and then plateaued near the pre-stress level (38.2% to 41.0%). At 22 DAT, electrolyte leakage was reduced to the pre-stress level in all treatments.

PLANT GROWTH. Leaf length (Fig. 8A) and relative leaf elongation rate (Fig. 8B) were unaffected by exogenous ABA during the water stress period. At the end of the rehydration period, shoot DW showed a quadratic ABA dose-response with an increase up to 1.89 mM ABA followed by a slight decrease (Fig. 9A). A similar but non-significant trend was found in root DW (Fig. 9B). The maximum dry matter increase by ABA application was 38% and 44% in shoot and roots, respectively. Root-to-shoot ratio ranged from 0.09 to 0.10 and did not fit any tested regression models (Fig. 9C).

Discussion

ABSCISIC ACID REDUCES WATER STRESS BY PROMOTING STOMATAL CLOSURE BUT NOT BY INHIBITING LEAF GROWTH. Foliar sprays of ABA applied before withholding water to muskmelon seedlings improved

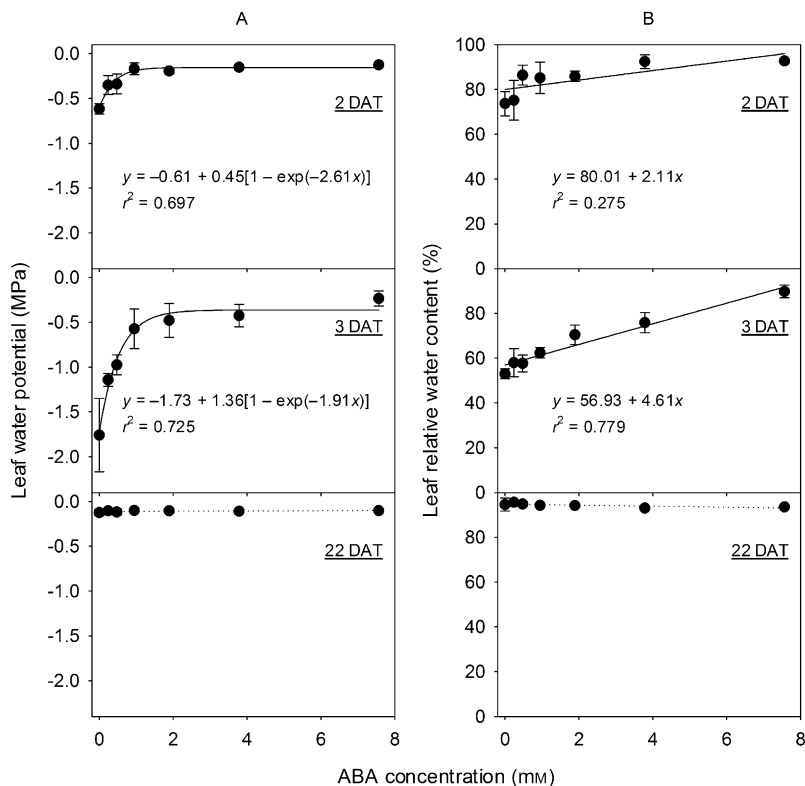


Fig. 6. Leaf water status of muskmelon seedlings as a function of exogenous abscisic acid (ABA) concentration during water stress and rehydration (Expt. 1): (A) water potential and (B) relative water content. Plants were sprayed with seven concentrations of ABA solution (0, 0.24, 0.47, 0.95, 1.89, 3.78, and 7.57 mM) at 1 mL per plant and subjected to water withholding. Irrigation was resumed 4 d after ABA treatment (DAT) and performed daily thereafter. Pre-treatment (0 DAT) means \pm SE were -0.12 ± 0.01 MPa (A) and $93.6\% \pm 0.2\%$ (B). Data are means \pm SE ($n = 3$). Solid lines show fits to the following models: exponential rise to an asymptote (A) and linear (B). Dotted lines show non-significant ($P > 0.05$) linear trends.

up to 95% (Fig. 1) and 78% (Table 2), respectively, suggesting that ABA-induced stomatal closure allowed rapid and dramatic water conservation at the expense of CO_2 supply to photosynthesis.

In addition to stomatal closure, ABA is involved in the inhibition of leaf expansion (Alves and Setter, 2000; Bacon et al., 1998; He and Cramer, 1996), which can reduce plant water use by limiting increases in transpirational area. However, leaf elongation during the water stress period was unaffected by exogenous ABA (Fig. 8). Because leaf length is an accurate indicator of leaf area in muskmelon (Panta and NeSmith, 1995), this result suggests that ABA-induced acclimation to water stress may not be mediated by leaf area adjustment in muskmelon seedlings. Cell expansion is a turgor-driven process and is extremely sensitive to dehydration (Taiz and Zeiger, 2002). Because plant water loss was inversely proportional to ABA concentration (Fig. 6A–B), turgor reduction may have limited cell expansion more severely at lower ABA concentrations, thereby potentially masking the effect of ABA on leaf expansion. However, in a previous study using pepper seedlings subjected to two cycles of 4-d water withholding, the maintenance of plant water status by exogenous ABA was associated with reductions in both g_s and leaf area (Goreta et al., 2007). These contrasting results indicate that ABA may regulate differential acclimation strategies depending on plant species.

DOSE-RESPONSE OF GAS EXCHANGE TO ABCISIC ACID DURING WATER STRESS AND RECOVERY. The initial dose-response of gas exchange to ABA was described by an exponential decay with a steep decrease up to 0.95 and 3.78 mM ABA for A and g_s , respectively, followed by a gradual decrease (Figs. 1 and 2). This change in slope gradient suggests that gas exchange became less responsive to increases in ABA above those high concentrations. The mechanism of ABA-induced stomatal closure is hydroactive, which depends on metabolic processes in guard cells. First, ABA binds to ABA protein receptors (Umezawa, 2011) and induces cytosolic Ca^{2+} elevations through extracellular influx and release from vacuoles (Schroeder et al., 2001). The elevated cytosolic Ca^{2+} level activates anion channels to promote anion release from guard cells, which in turn mediates the opening of outward K^+ channels (Schroeder et al., 2001). This net efflux of anions and K^+ is accompanied by osmotically mediated water movement out of the guard cells, leading to decreased guard cell turgor and stomatal closure. Therefore, ABA receptors, ion channels, and ions themselves (e.g., Ca^{2+} , Cl^- , K^+ , and organic anions) may be limiting factors for ABA signal transduction and thus effectiveness of exogenous ABA in inducing stomatal closure.

Under prolonged water stress, the dose-response of gas exchange shifted to a quadratic function with A and g_s increasing up to 1.89 mM ABA and decreasing with higher

ABA concentrations (Figs. 1 and 2). As noted previously, water loss and cell membrane damage progressed more severely at lower ABA concentrations (Figs. 6 and 7). Such impaired water relations can strongly inhibit enzymatic activities and cellular metabolism involved in photosynthetic processes (Lawlor, 2002). Moreover, rapid dehydration of leaf tissue can cause hydropassive stomatal closure independently of ABA. That is, when evaporative water loss from guard cells is faster than water movement from adjacent epidermal cells, guard cell turgor decreases, forcing stomata to close (Taiz and Zeiger, 2002). Similarly, stomata will remain closed if plant tissue is too dehydrated to permit guard cell turgor. Therefore, the quadratic response of A and g_s can be explained that metabolic impairment and hydropassive stomatal closure were the major limiting factors below 1.89 mM ABA, whereas above this concentration hydroactive stomatal closure by exogenous ABA was more inhibiting to gas exchange.

After re-watering, gas exchange generally increased linearly with increasing ABA concentration (Figs. 1 and 2). It is likely that ABA permitted maintenance of tissue hydration and membrane integrity (Figs. 6 and 7) and minimized metabolic impairment (Lawlor, 2002), thereby enabling fast recovery of gas exchange with rehydration. Additionally, inhibition of gas exchange by exogenous ABA is reversible by re-watering with no negative impact on subsequent recovery. This conclusion was also confirmed under well-watered conditions, where

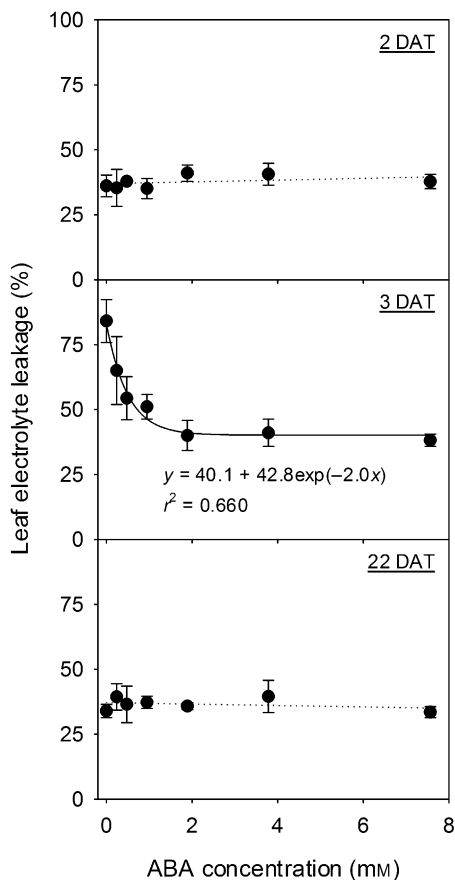


Fig. 7. Leaf electrolyte leakage of muskmelon seedlings as a function of exogenous abscisic acid (ABA) concentration during water stress and re-hydration (Expt. 1). Plants were sprayed with seven concentrations of ABA solution (0, 0.24, 0.47, 0.95, 1.89, 3.78, and 7.57 mM) at 1 mL per plant and subjected to water withholding. Irrigation was resumed 4 d after ABA treatment (DAT) and performed daily thereafter. Pre-treatment (0 DAT) mean \pm SE was $36.1\% \pm 4.0\%$. Data are means \pm SE ($n = 3$). A solid line shows an exponential decay fit. Dotted lines show non-significant ($P > 0.05$) linear trends.

complete recovery of gas exchange occurred within 3 d of ABA treatment (Tables 1 and 2). The transient effect of ABA is probably due to oxidation or conjugation that rapidly inactivates ABA in plant tissue (Davies and Jones, 1991). In contrast, ABA analogs (synthetic chemical structures) are known to have long-term consequences because of their high chemical stability (Abrams et al., 1997). Thus, to control short-term water stress (e.g., transplant shock, but not prolonged drought), the easily degradable natural ABA may be more suitable than its analogs.

STOMATAL AND NON-STOMATAL LIMITATIONS TO PHOTOSYNTHESIS. Although stomatal closure is an efficient strategy to conserve water, restricted entry of CO_2 lowers C_i and consequently limits A (Lawlor, 2002). This stomatal limitation to photosynthesis was demonstrated by high r^2 values (>0.83) for the positive correlation between C_i and A at 1 DAT (Fig. 3). In the absence of water stress, the subsequent decline in r^2 value was due to the recovery in both C_i and A , indicating stomatal re-opening resulting from degradation of exogenous ABA. In contrast, during water withholding, the decline in r^2 value occurred without the recovery in C_i and A , suggesting that non-stomatal factors became more important with progressive water stress.

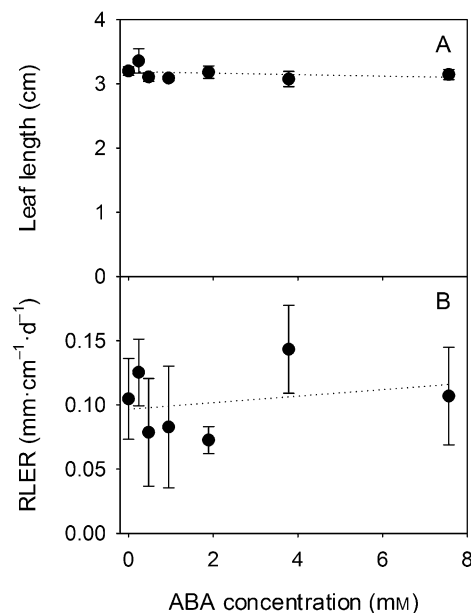


Fig. 8. Leaf elongation of muskmelon seedlings as a function of exogenous abscisic acid (ABA) concentration during water stress (Expt. 1): (A) leaf length measured 3 d after ABA treatment (DAT) and (B) relative leaf elongation rate (RLER) between 0 and 3 DAT. Plants were sprayed with seven concentrations of ABA solution (0, 0.24, 0.47, 0.95, 1.89, 3.78, and 7.57 mM) at 1 mL per plant and subjected to 4-d water withholding. Data are means \pm SE ($n = 3$). Dotted lines show non-significant ($P > 0.05$) linear trends.

Non-stomatal limitations may have been associated with impaired enzymatic activities and cellular metabolism, which are known to inhibit photosynthetic processes independently of CO_2 supply (Lawlor, 2002).

ABSCISIC ACID INDUCES TRANSIENT CHLOROSIS BUT REDUCES WATER STRESS-INDUCED CHLOROSIS. Leaf chlorosis is reported as a negative side effect of exogenous ABA in various crops (Blanchard et al., 2007; Hejnák and Kykalová, 2009; van Iersel et al., 2009; Waterland et al., 2010a). In this study, gradual but only transient leaf chlorosis was induced by exogenous ABA in muskmelon seedlings (Fig. 4). Notably, this chlorosis was accelerated linearly with increasing ABA concentration. Severe symptoms displayed uniform chlorosis across the entire lamina (Fig. 5) with up to 33% of chlorophyll loss by ABA (Fig. 4). The magnitude of chlorosis was comparable to that reported by Waterland et al. (2010b), who found 25% to 85% of chlorophyll loss in pansy (*Viola \times wittrockiana* Gams.) and viola (*Viola cornuta* L.) drenched with 0.95 mM ABA or sprayed with 1.89 mM ABA. The ABA-induced chlorosis can be attributed to the senescing effects of ABA, resulting from the gene expression of hydrolytic enzymes involved in chlorophyll breakdown (Weaver et al., 1998) or the stimulation of ethylene production (Gepstein and Thimann, 1981). A lack of nutrients, particularly N and Mg, is another factor promoting leaf chlorosis (Marschner, 1995). Because their uptake depends mainly on transpiration-driven mass flow (Havlin et al., 1999), reduced transpiration caused by ABA application may have limited N and Mg supply for chlorophyll formation and thus contributed to leaf chlorosis.

Leaf chlorosis was induced also by water stress mainly from 3 to 4 DAT, during which chlorophyll degradation was inversely proportional to ABA concentration (Fig. 4). As a result,

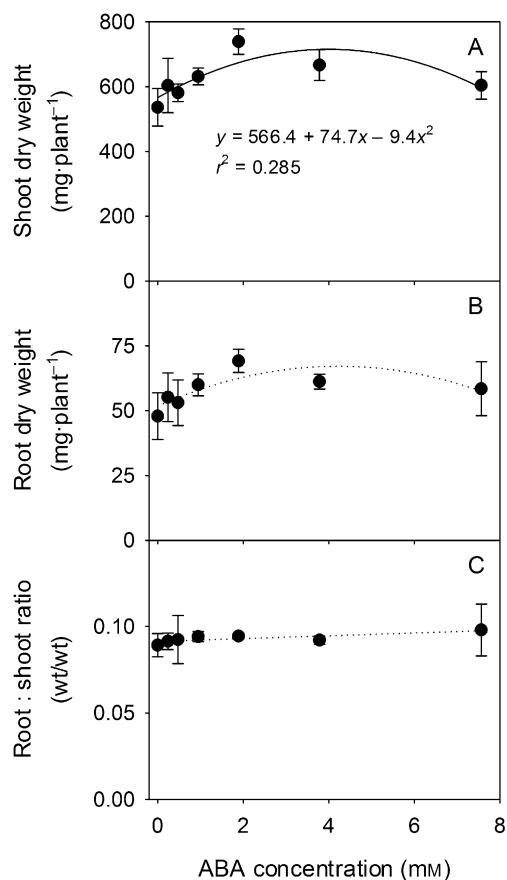


Fig. 9. Dry matter accumulation and partitioning of muskmelon seedlings as a function of exogenous abscisic acid (ABA) concentration after recovery from water stress [22 d after ABA treatment (DAT)] (Expt. 1): (A) shoot dry weight, (B) root dry weight, and (C) root-to-shoot ratio. Plants were sprayed with seven concentrations of ABA solution (0, 0.24, 0.47, 0.95, 1.89, 3.78, and 7.57 mM) at 1 mL per plant and subjected to water withholding. Irrigation was resumed 4 DAT and performed daily thereafter. Data are means \pm SE ($n = 3$). A solid line shows a quadratic fit. Dotted lines show non-significant ($P > 0.05$) quadratic (B) and linear (C) trends.

chlorosis became most severe in the untreated control, whereas it was minimized at ≥ 1.89 mM ABA. It seems that water stress induces chlorosis only during the severe stress period. It is also important to note that this process can develop very rapidly once initiated. The alleviation of chlorosis by ABA application was thus likely derived from maintenance of plant water status that minimized chlorophyll loss by dehydration. This effect remained significant during the post-stress recovery of chlorophyll. These results suggest not only that ABA-induced chlorosis is reversible by re-watering, but also that ABA can reduce leaf chlorosis depending on the degree of water stress.

EFFECTS OF ABSICISIC ACID ON POST-STRESS GROWTH. Although tissue dehydration and electrolyte leakage were minimized at the highest ABA concentration (Figs. 6 and 7), post-stress dry matter accumulation increased only up to 1.89 mM and decreased slightly with higher ABA concentrations (Fig. 9A–B). This observation raises the question of how excess levels of ABA negatively affected post-stress growth, despite the positive effect on the maintenance of water status and membrane integrity. One explanation could be the excessive inhibition of photosynthesis by ABA before re-watering, which may have

limited the supply of assimilates for dry matter production (Amthor, 2007).

Under water deficit conditions, ABA accumulation in leaves can suppress shoot growth (Taiz and Zeiger, 2002), whereas that in root tips is required for the maintenance of primary root elongation (Sharp et al., 2004; Spollen et al., 2000). However, exogenous ABA did not affect root dry matter partitioning in muskmelon seedlings (Fig. 9C). The lack of preferential root growth in response to ABA application may be due to the small rooting volume in our trays that restricted the capacity for root elongation (Nishizawa and Saito, 1998).

The effectiveness of ABA application in promoting post-stress growth appears to be determined by the balance between water stress control and inhibition of photosynthesis. Therefore, the expected degree of water stress and sensitivity of the targeted crop to exogenous ABA must be considered to determine the optimal application rate.

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