Benzyladenine and Gibberellic Acid Application Prevents Absciscic Acid-induced Leaf Chlorosis in Pansy and Viola

Nicole L. Waterland¹, John J. Finer, and Michelle L. Jones²

Department of Horticulture and Crop Science, The Ohio Agricultural Research and Development Center, The Ohio State University, 1680 Madison Avenue, Wooster, OH 44691

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Abstract. Drought stress during the shipping and retailing of floriculture crops can reduce postproduction shelf life and marketability. The plant hormone absciscic acid (ABA) mediates drought stress responses by closing stomata and reducing water loss. Applications of exogenous s-ABA effectively reduce water loss and allow a variety of species to survive temporary periods of drought stress. Unfortunately, s-ABA application can also lead to leaf chlorosis, which reduces the overall quality of economically important bedding plant species, including *Viola ×wittrockiana* (pansy). The goal of this research was to determine how to prevent s-ABA-induced leaf chlorosis in pansy and a closely related species, *Viola cornuta* (viola). All concentrations of both spray (250 or 500 mg·L⁻¹) and drench (125 or 250 mg·L⁻¹) s-ABA applications induced leaf yellowing. Young plants at the plug stage and 11-cm finished plants with one to two open flowers were further evaluated to determine if the developmental stage of the plants influenced s-ABA effectiveness or the development of negative side effects. Both plugs and finished pansies and violas developed leaf chlorosis after s-ABA applications, but symptoms were generally more severe in finished plants. The individual application of benzyladenine (BA), gibberellic acid (GA₄₊₇), or the ethylene perception inhibitor, 1-methylcyclopropane, before s-ABA application had no effect on the development of s-ABA-induced leaf chlorosis. However, applications of 5 or 10 mg·L⁻¹ BA and GA₄₊₇ as a mixture (BA + GA₄₊₇) before a drench or spray application of s-ABA prevented leaf chlorosis. The application of s-ABA and BA + GA₄₊₇ would allow floriculture crops to tolerate temporary periods of drought stress without any loss of postproduction quality.

Floriculture crops may encounter harsh environmental conditions during shipping and while on display in retail stores. These

poor postproduction environments can result in losses of 5% to 20% (Armitage, 1993; Healy, 2009). A major cause of postproduction decline is drought stress resulting from infrequent or inconsistent watering (Barrett and Campbell, 2006). Plants respond to water deficit by closing their stomata and decreasing transpirational water loss. This drought stress response is regulated by the plant hormone absciscic acid (ABA) (Malladi and Burns, 2007).

The exogenous application of ABA or ABA analogs delays drought stress-induced wilting in a variety of bedding and potted plants (Blanchard et al., 2007; Kim and van Iersel, 2008; Monteiro et al., 2001; Sharma et al., 2005, 2006; Waterland et al., 2010). Although ABA applications allow plants to survive temporary periods of drought stress, negative side effects, including leaf necrosis, chlorosis, or abscission, have been observed in some species (Blanchard et al., 2007; Kim and van Iersel, 2008; Waterland et al., 2010). We observed leaf chlorosis on drought-stressed *Tagetes patula* (marigolds), *Pelargonium ×hortorum* (seed geraniums), and pansies treated with drench or spray applications of s-ABA. Leaf chlorosis in pansies was

not directly induced by drought treatment, because symptoms were observed in both watered and drought-stressed plants that were treated with s-ABA (Waterland et al., 2010). It is unclear why s-ABA induced leaf chlorosis and senescence in pansies or how this side effect might be prevented.

ABA is the main plant hormone associated with drought stress, but most stress responses are the result of cross-talk between hormone signaling pathways (Huang et al., 2008). Although the application of a plant hormone may result in a specific response, these applications can also modify the synthesis and perception of other hormones. The resulting hormone modifications or the interactions between multiple hormones may cause the observed response. To understand a plant's response to an exogenous hormone application, it is therefore necessary to understand these potential hormone interactions. In most cases, drought stress results in an increase in the synthesis of ABA and ethylene and a decrease in the synthesis of auxin, gibberellins, and cytokinins (Nilsen and Orcutt, 1996). ABA applications enhance leaf senescence and abscission by increasing ethylene production and/or increasing ethylene sensitivity (Zacarias and Reid, 1990). Under severe and moderate drought stress, *Petunia ×hybrida* (petunia) show an increase in ethylene production as well as an increase in s-ABA, the biologically active form of ABA (Vardi and Mayak, 1989). Changes in the balance of ABA, ethylene, gibberellins, and cytokinins within the plant may be responsible for the development of leaf senescence (Blanchard et al., 2007; Waterland et al., 2010) and leaf abscission (Kim and van Iersel, 2008) after s-ABA applications.

The application of other plant hormones, including cytokinins and gibberellins, or the inhibition of ethylene perception may provide a means of preventing ABA-induced leaf chlorosis and abscission. Ethylene perception inhibitors such as 1-methylcyclopropane (1-MCP) have been used to prevent senescence and abscission in a variety of floriculture crops, including cut flowers and potted flowering plants (Blankenship and Dole, 2003). Cytokinins reduce leaf yellowing and senescence by delaying chlorophyll degradation (Gan and Amasino, 1995, 1997). Transgenic petunia and *Nicotiana tabacum* (tobacco) plants that overproduce cytokinins show delayed leaf senescence and reduced symptoms of leaf chlorosis after drought stress (Clark et al., 2004; Gan and Amasino, 1995). In some species, the application of gibberellic acids (specifically GA₄₊₇) reduces postproduction losses by preventing leaf senescence (Han, 1997; Ranwala et al., 2003; Ranwala and Miller, 1998). Even the use of cytokinins and gibberellins in combination is effective at reducing leaf chlorosis in a variety of floriculture crops (Funnell and Heins, 1998; Han, 2001; Kim and Miller, 2009; Ranwala et al., 2000). It is not known why senescence is prevented or delayed in some species by cytokinins and in others by gibberellins nor is it possible to predict which

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¹Current address: Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV 26506.

²To whom reprint requests should be addressed; e-mail jones.1968@osu.edu.

species will respond to a particular hormone or combination with delayed senescence. Plant responses to plant growth regulators (PGRs) are highly variable; therefore, all PGRs must be examined in different species, cultivars, and even at various developmental stages before useful recommendations can be developed (Gent and McAvoy, 2000).

The goal of this research was to determine how to prevent s-ABA-induced leaf chlorosis in pansy and viola. Although s-ABA applications allow pansies to survive temporary periods of drought stress, negative side effects like leaf chlorosis decrease overall crop quality (Waterland et al., 2010). Our specific objectives were 1) to determine if cultivar, developmental stage, method of application, and concentration influence the effectiveness of s-ABA and the occurrence of leaf chlorosis symptoms; 2) to identify an application method and/or concentration of s-ABA that would not induce leaf chlorosis; and 3) to determine whether s-ABA-induced leaf chlorosis can be prevented by the application of other PGRs, including cytokinins, gibberellins, or ethylene perception inhibitors.

Materials and Methods

Plant material

Viola xwittrockiana (pansy) 'Dynamite Purple' (Kieft-Pro-Seeds), 'Karma Rose Fire' (Goldsmith Seeds), or 'Matrix Blue Frost' (PanAmerican Seed) and *Viola cornuta* (viola) 'Penny Deep Blue' (Goldsmith Seeds), 'Skippy Lavender' (Kieft-Pro-Seeds), 'Sorbet Yellow Delight', or 'Sorbet Babyface Purple' (PanAmerican Seed) were used in the experiments as described subsequently. Plugs (young plants in the vegetative stage) were obtained from Green Circle Growers Inc. (Oberlin, OH) or Bob's Market (Mason, WV). Cell packs (1204s; 96.1 cm³/cell) of viola 'Sorbet Babyface Purple' and pansy 'Matrix Blue Frost' were obtained from Green Valley Growers Inc. (Ashland, OH) for all finished plant experiments (Expts. 1 and 2).

Expt. 1: s-abcisic acid treatment of 11-cm finished pansies and violas. Pansy 'Dynamite Purple', 'Karma Rose Fire', and 'Matrix Blue Frost' and viola 'Penny Deep Blue', 'Skippy Lavender', and 'Sorbet Babyface Purple' plugs or cell packs were transplanted to 11-cm pots with soilless greenhouse media (Pro-Mix-BX-Mycorise-Pro®; Premier Horticulture, Quebec, Canada). Plants were grown under natural irradiance with supplemental lighting provided as needed by high-pressure sodium and metal halide lamps (GLX/GLS e-systems GROW lights; PARSOURCE, Petaluma, CA). The average photosynthetic photon flux (PPF) was 330 μmol·m⁻²·s⁻¹ from 0700 to 1900 hr daily [mean daily light integral (DLI) = 18.4 mol·m⁻²·d⁻¹]. Mean greenhouse temperatures were 22.4/15.8 °C ± 2.0/1.6 day/night with daytime relative humidity of 51.6% ± 7.5%. Plants were irrigated daily with 200 mg N/L Peters Excel® Cal-Mag 15N-2.15P-12.5K (Scotts-Sierra Horticulture Products, Marysville, OH), which was reduced to 100 mg N/L 2 weeks before flowering. s-ABA

treatments were applied to finished pansies and violas when they reached a marketable stage of one to two open flowers per plant and the foliage and roots had reached the edge of the pot. Applications were on 26 Apr. 2008 for pansy 'Matrix Blue Frost' and viola 'Sorbet Babyface Purple' and 6 May 2008 for the other four cultivars. All plants were watered to container capacity 12 h before s-ABA (ConTego™; Valent BioSciences Corp., Libertyville, IL) application. s-ABA was applied according to manufacturer's recommendations as a drench

at 0, 125, or 250 mg·L⁻¹ (60 ml per container) or as a spray at 0, 250, or 500 mg·L⁻¹ (≈6.6 mL per plant) with the addition of 0.05% CapSil® (Aquatrols Corporation of America, Inc., Cherry Hill, NJ). Spray applications were applied with a Regulator Backpack® sprayer (H.D. Hudson Manufacturing Company, Chicago, IL). Half of the plants from each treatment were irrigated daily with 100 mg N/L. The other half (i.e., drought-stressed) had water withheld until the s-ABA-treated plants wilted and then they were

Fig. 1. Visual symptoms of wilting and daily wilt status ratings of 11-cm finished *Viola cornuta* (viola) and *Viola xwittrockiana* (pansy) after withholding water. Images are of viola 'Sorbet Babyface Purple' 5 d after the application of s-abcisic acid (s-ABA) and subsequent drought stress (A). The left image is of drench applications of 0, 125, and 250 mg·L⁻¹ s-ABA and the right image is of spray applications of 0, 250, and 500 mg·L⁻¹ s-ABA (A). Water was withheld until s-ABA-treated plants were wilted (wilt status rating of 3 or below), and wilt status ratings were determined daily (B). Day 0 observations were taken just before (1 to 2 h) s-ABA application and withholding of water. Wilt status ratings were from 1 to 5: 5 = completely turgid, 4 = soft to touch but still upright, 3 = starting to wilt and no longer upright, 2 = wilted with complete loss of turgor, and 1 = wilted to the point that leaves are dry and desiccated. Values are means ± SD of four replications (n = 4).

Table 1. Shelf life of drought-stressed 11-cm finished *Viola cornuta* (viola) and *Viola ×wittrockiana* (pansy) treated with s-ABA.²

Species and cultivar	Application method and s-ABA concn (mg·L ⁻¹)					
	Time until visually wilted (days)					
	Drench			Spray		
	0	125	250	0	250	500
<i>Viola cornuta</i>						
Penny Deep Blue	2.5 d	5.0 c	5.8 c	2.5 d	7.3 b	8.3 a
Skippy Lavender	2.3 e	3.3 d	5.0 c	2.3 e	6.5 b	7.3 a
Sorbet Babyface Purple	4.5 d	6.8 c	7.8 b	4.3 d	8.3 b	9.5 a
<i>Viola ×wittrockiana</i>						
Dynamite Purple	3.3 e	5.0 d	5.8 c	1.3 f	6.5 b	9.0 a
Karma Rose Fire	3.5 cd	4.0 c	5.5 b	2.5 d	8.3 a	9.0 a
Matrix Blue Frost	4.3 d	5.8 c	7.0 b	3.5 d	7.5 b	8.8 a

²Values are means of four replications (n = 4). Values in rows followed by different letters are statistically different at $P \leq 0.05$ (least significant difference test). Shelf life is the average number of days from Day 0 that it took each plant to show visible symptoms of wilt. This is equivalent to a rating of 3 (starting to wilt) or less on the wilt status rating scale of 1 to 5 in which 5 = completely turgid, 4 = soft to touch but still upright, 3 = starting to wilt and no longer upright, 2 = wilted with complete loss of turgor, and 1 = wilted to the point that leaves are dry and desiccated. Shelf life extension is the number of additional days from the time in which the control plants wilted until the s-ABA-treated plants wilted.

s-ABA = abscisic acid.

rewatered. Plants were held in the greenhouse under the environmental conditions described previously for all subsequent evaluations.

Expt. 2: Plant growth regulator applications to 11-cm finished pansies and violas. PGRs were applied to 11-cm finished pansy 'Matrix Blue Frost' and viola 'Sorbet Babyface Purple' before s-ABA application. Cell packs were transplanted and plants were grown in the greenhouse as described previously. Treatments for Expt. 2A began on 13 May 2008. Average PPF was 340 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from 0500 to 1900 HR daily (DLI = 17.6). Mean greenhouse temperatures were 22/15 °C $\pm$ 2.1/0.9 day/night with daytime relative humidity of 51.2 $\pm$ 5.6%. PGR treatments included spray applications ($\approx$ 4.4 mL per plant) of benzyladenine (BA) (MaxCel[®]; Valent BioSciences Corp.), GA₄₊₇ (ProVide[®]; Valent BioSciences Corp.), or BA + GA₄₊₇ (Promalin[®]/Fascination[®]; Valent BioSciences Corp.) were applied at the rates of 0, 1, 2, 5, or 10 mg·L⁻¹ (plus 0.05% CapSil[®]) 12 h before 0 or 250 mg·L⁻¹ s-ABA. Treatments also included 1-MCP (EthylBloc[®]; Floralife[®], Inc., Walterboro, SC), an ethylene perception inhibitor, at the manufacturer's recommended rate of 300 nL·L⁻¹ for 10 h before a drench application of 0 or 250 mg·L⁻¹ s-ABA. All plants in this experiment were irrigated daily with 100 mg N/L.

Based on the observations from the first PGR experiment outlined previously (Expt. 2A), a concentration of 5 mg·L⁻¹ BA + GA₄₊₇ was selected for the treatment of all six pansy and viola cultivars in Expt. 2B. Treatments began on 13 June 2008 under the greenhouse conditions described previously. Average PPF was 359 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from 0500 to 1900 HR daily (DLI = 19.8). Mean greenhouse temperatures were 24.3/18 °C $\pm$ 3.0/2.5 day/night with daytime relative humidity of 60.9 $\pm$ 8.7%. BA + GA₄₊₇ at 0 or 5 mg·L⁻¹ (plus 0.05% CapSil[®]) was applied 12 h before either a drench application of 0 or 250 mg·L⁻¹ s-ABA or a spray application of 0 or 500 mg·L⁻¹ s-ABA (plus 0.05% CapSil[®]). Half of the plants in this experiment were irrigated daily and the other half had water withheld for 5 d before being rewa-

Fig. 2. Leaf chlorosis ratings of finished 11-cm *Viola cornuta* (viola) and *Viola ×wittrockiana* (pansy) after a drench or spray application of s-ABA. Finished plants were irrigated daily with 100 mg N/L. Leaf chlorosis ratings for the whole plant were from 1 to 11: 11 = completely green with no sign of chlorosis, 10 = 10% or less leaf chlorosis, 9 = 11% to 20%, 8 = 21% to 30%, 7 = 31% to 40%, 6 = 41% to 50%, 5 = 51% to 60%, 4 = 61% to 70%, 3 = 71% to 80%, 2 = 81% to 90%, and 1 = 91% to 100% leaf chlorosis. Visual ratings were recorded daily with Day 0 observations taken just before (1 to 2 h) s-ABA application. Values are means $\pm$ SD of four replications (n = 4).

tered. Chlorophyll changes in the leaves were quantified with a chlorophyll content meter (SPAD-502; Konica Minolta Sensing, Inc., Osaka, Japan). The lowest basal leaf on each plant was measured at 5 d after s-ABA

application on both drought-stressed and watered plants.

Expt. 3: s-abscisic acid treatment of pansy and viola plugs. Viola 'Penny Deep Blue', viola 'Skippy Lavender', pansy 'Dynamite

Purple', and pansy 'Karma Rose Fire' plugs were received in 512-cell trays (5.4 cm³/cell) and viola 'Sorbet Yellow Delight' and pansy 'Matrix Blue Frost' were in 288-cell trays (7 cm³/cell). Plugs were randomly assigned to empty plug trays with equal spacing among all replicates and treatments. All plugs were grown and evaluated in the greenhouse under natural irradiance with supplemental lighting (as stated previously) and an average *PPF* of 250 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from 0700 to 1900 hr daily (DLI = 11.6). Mean greenhouse temperatures were 23.4/16.7 °C $\pm$ 1.2/3.7 day/night with a daytime relative humidity of 49.5% $\pm$ 8.5%. Five-week-old pansy and viola plugs were treated on 9 Apr. or 18 Apr. 2008 for initial determination of s-ABA effectiveness and leaf chlorosis. Plugs were sprenched (spray to drench, $\approx$ 43 mL per tray) with 0, 75, 125, 250, 500, or 1000 mg·L⁻¹ s-ABA (plus 0.05% CapSil®). Half of the plugs had water withheld for 2 d and were then rewatered. The other half were irrigated daily throughout the experiment with 100 mg N/L.

Expt. 4: Application of benzyladenine + gibberellic acid and s-ABA on pansy and viola plugs. Viola 'Penny Deep Blue', viola 'Sorbet Yellow Delight', pansy 'Dynamite Purple' and pansy 'Karma Rose Fire' plugs were received in 288-cell trays (7 cm³/cell). Replications and treatments were randomly assigned to empty plug trays as described for Expt. 3. Nine-week-old plugs were treated with BA + GA₄₊₇ before s-ABA application. Treatments began on 8 Jan. 2010 and plugs were grown and evaluated under the greenhouse conditions described previously. The average *PPF* during this experiment was 269 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from 0700 to 1800 hr daily (DLI = 11.6). Mean greenhouse temperatures were 21.9/15.6 °C $\pm$ 1.1/0.9 day/night with a daytime relative humidity of 29.0% $\pm$ 8.1%. Plugs were sprenched ($\approx$ 29 mL per tray) with 0 and 5 mg·L⁻¹ BA + GA₄₊₇ 12 h before an application of 0 or 1000 mg·L⁻¹ s-ABA (plus 0.05% CapSil®). All plugs were irrigated daily with 100 mg N/L.

Evaluations of wilt status and leaf chlorosis

Visual observations of wilt status and leaf chlorosis were taken daily for all plugs and finished plants. Day 0 was the day of s-ABA treatment. All observations on Day -1 or Day 0 were made just before (1 to 2 h) the application of PGRs. Wilt status ratings were from 1 to 5: 5 = completely turgid, 4 = soft to touch but still upright, 3 = starting to wilt and no longer upright, 2 = wilted with complete loss of turgor, and 1 = wilted to the point that leaves are dry and desiccated. The shelf life of drought-stressed plants was calculated as the number of days that it took plants to reach a wilt status rating of 3 or less, after which they were considered unmarketable. Leaf chlorosis ratings for the whole plant were from 1 to 11: 11 = completely green with no sign of chlorosis, 10 = 10% or less leaf chlorosis, 9 = 11% to 20%, 8 = 21% to 30%, 7 = 31% to 40%, 6 = 41% to 50%, 5

Fig. 3. *Viola cornuta* (viola) and *Viola xwittrockiana* (pansy) treated with other plant growth regulators (PGRs) to prevent s-ABA-induced leaf chlorosis. Violas and pansies were pretreated with benzyladenine (BA), gibberellic acid (GA₄₊₇), BA + GA₄₊₇, or 1-methylcyclopropene (1-MCP). BA, GA₄₊₇, and BA + GA₄₊₇ were applied at the rate of 5 or 10 mg·L⁻¹. Plants were treated with 300 nL·L⁻¹ 1-MCP for 10 h. Plants were treated 12 h later with drench applications of 0 or 250 mg·L⁻¹ s-ABA. Images are of viola 'Sorbet Babyface Purple' and are representative of these experiments. Photos were taken 2 d after s-ABA application. All plants were irrigated daily with 100 mg N/L.

Fig. 4. Chlorophyll content readings of *Viola cornuta* (viola) and *Viola xwittrockiana* (pansy) 5 d after s-ABA application in watered plants. Finished plants were irrigated daily with 100 mg N/L. Benzyladenine (BA) + gibberellic acid (GA₄₊₇) (0 or 5 mg·L⁻¹) was applied 12 h before using a drench (0 or 250 mg·L⁻¹) or spray (0 or 500 mg·L⁻¹) application of s-ABA. Data were analyzed using Proc GLM (generalized linear model) with means separated by letters. Values are means $\pm$ SD of four replications (n = 4).

= 51% to 60%, 4 = 61% to 70%, 3 = 71% to 80%, 2 = 81% to 90%, and 1 = 91% to 100% leaf chlorosis.

Statistical analysis

Reported values are the means $\pm$ SD of four replications, which is four plants in Expts. 1 and 2. For Expts. 3 and 4, six individual plugs were used as one replication. Data are the average of three replications for pansy 'Matrix Blue Frost' and viola 'Sorbet Yellow Delight' and four replications for all other pansies and violas in Expt. 3. Expt. 4 values are the average of four replications. All experiments were conducted in a completely randomized block design with Expts. 1 and 2 blocked by watering regimen, watered versus water withheld, and by replication. Expts. 3 and 4 were blocked by treatment. All values were analyzed using Proc GLM (generalized linear model) with least significant difference means separation ($P \leq 0.05$). Values were analyzed by SAS Version 9.1.3 (Statistical Analysis System; SAS Institute, Inc., Cary, NC).

Results

Expt. 1: Wilt status and shelf life extension in s-ABA-treated 11-cm finished pansies and violas. s-ABA treatments delayed wilting symptoms in drought stressed pansies and violas (Fig. 1; Table 1). Treated plants had increased shelf life compared with control plants (0 mg·L⁻¹ s-ABA), and shelf life extensions ranged from 0.5 to 7.7 d depending on the concentration of s-ABA, method of application, and cultivar (Table 1). The effectiveness of the s-ABA treatment increased at higher concentrations, and differences were observed between cultivars (Fig. 1B; Table 1). Plants treated with 125 mg·L⁻¹ s-ABA were the first to show symptoms of wilting when compared with the 250 or 500-mg·L⁻¹ treatments (Fig. 1B). Application of 500 mg·L⁻¹ s-ABA resulted in the greatest delay in wilting (Fig. 1B) and led to shelf life extensions of 5.0 to 7.7 d beyond those of the non-ABA-treated controls (Table 1). The longest shelf life extension was observed in pansy 'Dynamite Purple' and the shortest was in viola 'Sippy Lavender'. Three d after rewatering, all the pansies and violas treated with s-ABA recovered from drought stress, but 71% of control plants (0 mg·L⁻¹ s-ABA) died (data not shown).

Expt. 1: Leaf chlorosis of 11-cm finished pansies and violas treated with s-ABA. Although s-ABA was effective at delaying wilting, basal leaf chlorosis was induced in both finished pansies and violas (Figs. 2 and 3). The degree of leaf yellowing was similar in drought-stressed and nonstressed plants (data not shown); therefore, leaf chlorosis was further investigated in watered plants to directly evaluate the effect of s-ABA applications. Control plants (0 mg·L⁻¹ drench or spray) had little or no leaf chlorosis throughout the experiment (Fig. 2). ABA-induced leaf chlorosis was first observed on Day 1 in pansy 'Dynamite Purple' and viola 'Penny Deep Blue', Day 3 in viola 'Sorbet

Babyface Purple', and Day 2 for all other cultivars (Fig. 2). The severity of leaf chlorosis (as determined by the percentage of the plant that had yellow leaves) continued to increase until Day 5 or later depending on the cultivar. By Day 6, all cultivars treated with 500 mg·L⁻¹ s-ABA (except 'Sorbet Babyface Purple') had a rating of 7 or lower, indicating that 30% or more of the plant was yellow (Fig. 2).

Expt. 2A: Preventing s-ABA-induced leaf chlorosis with pre-application of other plant growth regulators. Pretreatment with the ethylene perception inhibitor, 1-MCP, did not reduce the development of leaf chlorosis in s-ABA-treated plants (Fig. 3). Spray applications of cytokinin (BA at 1, 2, 5, or 10 mg·L⁻¹) or gibberellins (GA₄₊₇ at 1, 2, 5, or 10 mg·L⁻¹) also did not reduce leaf

chlorosis (Fig. 3 and data not shown). In contrast, spray applications of BA + GA₄₊₇, at either 5 or 10 mg·L⁻¹, completely prevented s-ABA-induced leaf chlorosis based on visual observations (Fig. 3 and data not shown).

Expt. 2B: Preventing s-ABA-induced leaf chlorosis with pre-application of benzyladenine + gibberellic acid. SPAD meter readings indicated that s-ABA reduced the chlorophyll content in basal leaves by 25% to 85% depending on cultivar, concentration, and application method (Fig. 4). Spray applications of 500 mg·L⁻¹ s-ABA caused more leaf yellowing than drench applications of 250 mg·L⁻¹ in two of the six cultivars (viola 'Penny Deep Blue' and pansy 'Matrix Blue Frost') (Fig. 4). When plants were pretreated with 5 mg·L⁻¹ BA + GA₄₊₇,

Fig. 5. Wilt status ratings of *Viola cornuta* (viola) and *Viola x wittrockiana* (pansy) plugs after a spray application of s-ABA and 2 d of withholding water. Wilt status ratings were from 1 to 5: 5 = completely turgid, 4 = soft to touch but still upright, 3 = starting to wilt and no longer upright, 2 = wilted with complete loss of turgor, and 1 = wilted to the point that leaves are dry and desiccated (A). Visual ratings were taken daily with Day 0 being a completely turgid plug just before (1 to 2 h) s-ABA application. Values are the mean $\pm$ SD of three replications ($n = 3$) for viola 'Sorbet Yellow Delight' and pansy 'Matrix Blue Frost' and four replications ($n = 4$) for all other cultivars (B). Each replication consisted of six plugs.

A

B

Fig. 6. Leaf chlorosis ratings of *Viola cornuta* (viola) and *Viola ×wittrockiana* (pansy) plugs after a sprinch application of s-abscisic acid (s-ABA). Plugs were irrigated daily with 100 mg N/L. Leaf chlorosis ratings for the whole plant were from 1 to 11: 11 = completely green with no sign of chlorosis, 10 = 10% or less leaf chlorosis, 9 = 11% to 20%, 8 = 21% to 30%, 7 = 31% to 40%, 6 = 41% to 50%, 5 = 51% to 60%, 4 = 61% to 70%, 3 = 71% to 80%, 2 = 81% to 90%, and 1 = 91% to 100% leaf chlorosis (A). Visual ratings were recorded daily with Day 0 observations taken just before (1 to 2 h) s-ABA application (B). Values are the mean of three replications (n = 3) for viola 'Sorbet Yellow Delight' and pansy 'Matrix Blue Frost' or four replications (n = 4) ± SD for all other cultivars (B). Each replication contained six plugs.

Fig. 7. Visual observations and leaf chlorosis ratings of *Viola cornuta* (viola) and *Viola xwittrockiana* (pansy) plugs after applications of 0 or 5 mg·L⁻¹ benzyladenine (BA) + gibberellic acid (GA₄₊₇) and 0 or 1000 mg·L⁻¹ s-ABA. Images are of viola 'Sorbet Yellow Delight' 3 d after s-ABA application (A). Plugs were irrigated daily with 100 mg N/L. Leaf chlorosis ratings for the whole plant were from 1 to 11: 11 = completely green with no sign of chlorosis, 10 = 10% or less leaf chlorosis, 9 = 11% to 20%, 8 = 21% to 30%, 7 = 31% to 40%, 6 = 41% to 50%, 5 = 51% to 60%, 4 = 61% to 70%, 3 = 71% to 80%, 2 = 81% to 90%, and 1 = 91% to 100% leaf chlorosis (B). Visual ratings were taken daily. Day -1 was immediately before (1 to 2 h) BA and GA₄₊₇ applications (B). Values are the mean of four replications $\pm$ SD with each replication consisting of six plugs (n = 4) for all pansies and violas (B).

the chlorophyll content of leaves at 5 d after s-ABA application was the same as non-s-ABA-treated controls (Fig. 4).

Expt. 3: Wilt status of pansy and viola plugs treated with s-ABA. Sprinch applications of s-ABA delayed visible wilting in drought-stressed pansy and viola plugs. One d after withholding water, control plugs (0 mg·L⁻¹ s-ABA) were more wilted than all

s-ABA treatments (Fig. 5B). All control plugs were severely wilted and had reached a wilt status rating of 1 by Day 2. The 1000 mg·L⁻¹ s-ABA-treated plugs remained turgid longer than all other treatments. Plugs of pansy 'Matrix Blue Frost' and viola 'Sorbet Yellow Delight' treated with 1000 mg·L⁻¹ s-ABA did not show any wilting symptoms when water was withheld for 2 d. Concen-

trations of 75, 125, 250, and 500 mg·L⁻¹ of s-ABA were equally effective at preventing wilting, except in pansy 'Dynamite Purple' in which 500 mg·L⁻¹ was as effective as 1000 mg·L⁻¹ (Fig. 5B). Plugs were rewatered after they had been drought-stressed for 2 d. The survival rate of rewatered plugs was dependent on the concentration of s-ABA. With s-ABA sprinch applications of 75, 125, 250,

500, and 1000 mg·L⁻¹, pansy plugs had survival rates of 53.7%, 40.7%, 81.9%, 81.0%, and 95.8%, respectively, and viola plugs had survival rates of 49.1%, 56.5%, 75.0%, 75.0%, and 100%, respectively. The survival rate in all the control (0 mg·L⁻¹ s-ABA) plugs was 0% after rewatering (data not shown).

Expt. 3: Leaf chlorosis of pansy and viola plugs treated with s-abscisic acid. Watered plugs that were treated with s-ABA developed lower leaf chlorosis similar to what was observed on 11-cm finished plants (Fig. 6). In some instances, this leaf chlorosis was less severe at the plug stage. ABA-induced leaf chlorosis was observed on five of the six cultivars by Day 1 (Fig. 6B). On Day 3, viola 'Sorbet Yellow Delight' and pansy 'Matrix Blue Frost' had more severe leaf chlorosis at higher concentrations of s-ABA (500 and 1000 mg·L⁻¹) compared with the lower concentrations (75, 125, and 250 mg·L⁻¹) (Fig. 6B). Applications of s-ABA did not induce leaf yellowing in viola 'Skippy Lavender', whereas the most severe symptoms were observed in pansy 'Matrix Blue Frost' (Fig. 6B). Similar leaf chlorosis symptoms were observed in drought-stressed plugs after s-ABA application (data not shown).

Expt. 4: Preventing s-abscisic acid-induced leaf chlorosis with pre-applications of benzyladenine + gibberellic acid in pansy and viola plugs. Pansy and viola plugs sprayed with 1000 mg·L⁻¹ s-ABA and 0 mg·L⁻¹ BA + GA₄₊₇ developed leaf chlorosis 2 d after application (Fig. 7). Pretreatment with 5 mg·L⁻¹ BA + GA₄₊₇ prevented the development of leaf chlorosis on all plugs that received 1000 mg·L⁻¹ s-ABA (Fig. 7). All BA + GA₄₊₇-treated plugs were visually indistinguishable from the control plugs (0 mg·L⁻¹ s-ABA plus 0 mg·L⁻¹ BA + GA₄₊₇) (Fig. 7).

Discussion

All applications of s-ABA regardless of cultivar, developmental stage, method of application, or concentration were effective at delaying wilting symptoms in pansies and violas exposed to severe drought stress. However, we were unable to identify a combination of method of application and concentration of s-ABA that would effectively enhance drought tolerance in any of these plants without inducing leaf chlorosis. Leaf chlorosis in general was less severe in young plants (plugs) than in finished plants that were flowering. s-ABA-treated viola 'Skippy Lavender' developed little or no leaf chlorosis at the plug stage, but finished plants had 20% to 50% leaf chlorosis (leaf chlorosis rating of 8 to 6) depending on the s-ABA treatment. This indicates that responses to s-ABA will be highly dependent on both cultivar and developmental stage. Plant responses to PGRs are known to vary based on both of these factors (Gent and McAvoy, 2000).

A plant's response to hormones is controlled by both the hormone concentration and the exposure time. In pansies and violas,

higher concentrations of s-ABA resulted in more severe symptoms of leaf chlorosis. These exogenous applications can also increase or decrease the endogenous production of other plant hormones and alter tissue sensitivity to hormones. In some plants, exogenous ABA applications stimulate ethylene production (Sharp et al., 2000; Zhang et al., 2009). Ethylene is a gaseous plant hormone known to regulate leaf and flower senescence, and it has been associated with postproduction/postharvest leaf chlorosis and senescence in a variety of crops (Blankenship and Dole, 2003; Bleecker and Kende, 2000). Leaf senescence and abscission in ethylene-sensitive crops can be prevented by treatment with the ethylene perception inhibitor, 1-MCP (Blankenship and Dole, 2003; Celikel et al., 2002). In some species, including Easter lilies (*Lilium longiflorum*), leaf senescence is not associated with ethylene production and it is prevented by the application of BA and/or GA₄₊₇ (Franco and Han, 1997). In our experiment, 1-MCP did not prevent s-ABA-induced leaf chlorosis, indicating that ethylene may not be associated with leaf senescence in pansy and viola.

The application of PGRs that include cytokinins or gibberellins reduces or prevents leaf yellowing in a number of species. Cytokinins such as BA delay the development of leaf yellowing in a variety of floriculture crops, including lilies (*Lilium* sp.), tulips (*Tulipa gesneriana*), geraniums, gerbera daisy (*Gerbera jamesonii*), goldenrod (*Solidago canadensis*), zinnia (*Zinnia elegans*), and miniature roses (*Rosa ×hybrida*) (Franco and Han, 1997; Kim and Miller, 2009; Padhye and Runkle, 2008; Philosoph-Hadas et al., 1996; Pinto et al., 2005; Tjosvold et al., 1994). The application of only gibberellic acid, specifically GA₄₊₇, also effectively enhances postproduction quality by reducing the occurrence of leaf chlorosis and senescence. Basal leaf yellowing is reduced or prevented by GA₄₊₇ application in Easter and Oriental (*Lilium* sp.) lilies (Han, 1997; Ranwala and Miller, 2000; Ranwala et al., 2000, 2003). Neither BA nor GA₄₊₇ alone was effective at preventing, or even reducing, the s-ABA-induced leaf chlorosis that we observed in pansy and viola.

PGR applications that included a combination of both cytokinins and gibberellic acids (BA + GA₄₊₇) were the only treatment that effectively reduced s-ABA-induced leaf chlorosis. The application of PGRs that contain both BA and GA₄₊₇ also prevent postproduction cold-induced leaf chlorosis and senescence in many species of lilies, including potted Easter lilies, potted Oriental lilies (*Lilium* sp. 'Stargazer'), and cut Asiatic and Oriental lilies (*Lilium* sp.) (Funnell and Heins, 1998; Han, 1995, 1997, 2001; Ranwala and Miller, 1998, 2005; Ranwala et al., 2000, 2003; Whitman et al., 2001). Postproduction leaf yellowing in tulips is reduced after the application of BA and GA₄₊₇ (Kim and Miller, 2009). It remains unclear why BA + GA₄₊₇ are effective in combination but not individually in these species.

Application of BA + GA₄₊₇ before the application of s-ABA could be used by growers to prevent leaf chlorosis. One potential problem with using BA + GA₄₊₇ to reduce s-ABA-induced leaf chlorosis is increased internode elongation. The exogenous application of GA independently or in combination with cytokinins results in increased stem elongation in some species, including poinsettia (*Euphorbia pulcherrima*) and Easter lily (Blanchard and Runkle, 2008; Han, 2000; Ranwala et al., 2003; Ranwala and Miller, 1999; Whitman et al., 2001). In Easter lilies, undesirable stem elongation was minimized by determining the optimum concentration of GA₄₊₇ and using only foliar spray applications (Ranwala et al., 2003). At 21 d after treatment, we did not measure any differences in plant height resulting from BA + GA₄₊₇ applications (data not shown). Under our experimental conditions, the low concentrations of GA did not induce stem elongation in violas or pansies. Nineteen of 20 cultivars of tulips treated with BA + GA₄₊₇ also displayed no stem elongation (Kim and Miller, 2009). The pre-application of a PGR that contains low concentrations of BA + GA₄₊₇ provides a simple and practical way for growers to effectively use s-ABA to delay visual symptoms of wilting in drought-stressed crops without inducing leaf chlorosis.

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