Effects of Paclobutrazol and Flurprimidol on Water Stress Amelioration in Potted Red Firespike

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SUMMARY. This study evaluated the effects of paclobutrazol (PBZ) and flurprimidol on the morphological and physiological characteristics of potted red firespike (*Odontonema strictum*) under drought stress. PBZ and flurprimidol were applied as a soil drench at 0.24 mg/pot. Untreated plants acted as a control. The plants were exposed to drought stress 2 weeks after plant growth regulator (PGR) application. Another group of plants treated with PGRs was watered regularly. A reduction in plant height, plant diameter, and growth index (GI) was observed in plants treated with PGRs and drought stress 5 weeks after beginning the study. Drought stress reduced plant height by 45% compared with control. Flurprimidol under drought stress decreased plant diameter and GI by 36% and 76%, respectively, compared with the control. The least leaf area and plant dry weight were observed in plants drenched with flurprimidol and grown under drought stress. Drought stress also delayed flowering and the number of plants flowering. Plants treated with PBZ had the highest photosynthesis rate, 54% more than untreated plants under drought stress alone. The lowest stomatal conductance (g_s) was measured in plants under drought stress alone or drought plus PBZ. Application of PBZ-enhanced red firespike drought tolerance reducing adverse effects of water stress on photosynthesis during the experiment.

Water stress is one of the major abiotic stresses negatively affecting plant production and quality (Rabert et al., 2014). Plant responses to water stress involve either adaptive changes in metabolism and overall plant structure or deleterious effects such as reduced net photosynthesis and yield or leaf abscission (Durner, 2013; Vu et al., 2001). The effect of water stress on the decrease in photosynthetic activity has been primarily attributed to stomata closure and reduced transpiration (Centritto et al., 2003; Franks and Farquhar, 2001). The most obvious result of a decrease in net photosynthesis is growth reduction (i.e., plant dry weight and cell size) with greater sensitivity in cell enlargement (Hsiao,

1973) and consequently, reduced branching, plant size, and leaf area (Durner, 2013).

The gibberellin synthesis inhibiting PGRs are regularly used to restrict cell elongation in plants. In addition to a reduction of plant size, PGRs are applied to improve compactness and increase other functional aspects, such as resistance against both abiotic and biotic stresses (Navarro et al., 2007). Several studies reported the effectiveness of these chemicals in reducing the unfavorable effects of water stress (Banón et al., 2001; Berova and Zlatev, 2003). Artega (1995) reported treatment with gibberellin inhibitors resulted in increased net photosynthesis in addition to drought resistance. Other studies have shown plants treated with PGRs used less water compared with untreated plants even

when the PGRs did not affect plant growth (Atkinson and Crisp, 1983; Conover, 1994; Poole and Conover, 1992; Ranney et al., 1989).

Paclobutrazol, a widely used growth retardant, is reported to have antitranspirant effects with a clear impact on some physiological and biochemical aspects of the water relations in plants (Asare-Boamah et al., 1986; George and Nissen, 1992). Many studies have focused on the protective effects of PBZ against drought stress and increasing plant survival rates after transplanting (Asare-Boamah et al., 1986; Fletcher et al., 2000; Marshall et al., 2000). Previous studies have also reported reduced transpiration rates and increased drought tolerance in some tree seedlings after using triazoles (Sailerova and Zwiazek, 1997; Shen and Zeng 1993). Eliasson et al. (1994) reported a reduction in water loss and increased survival of in vitro plants of black cherry (*Prunus serotina*) after adding PBZ and uniconazole to the culture media. The influence of PBZ on increased drought tolerance was due to a reduction in transpiration, shorter plant height, diminished biomass and leaf area, and an increase in stomatal resistance (Fernández et al., 2006). Flurprimidol is a triazole similar to PBZ and uniconazole in both chemical structure and mode of action. The effect of flurprimidol on improving adverse effect of drought stress in plants has not been studied.

Although PGR research has been conducted on firespike (Rezazadeh et al., 2015), no studies have been published examining the effects of PGRs on drought tolerance of firespike growth and flowering. The objectives of this study are to examine the physiological and morphological responses of firespike to water stress after being treated with PBZ and flurprimidol and determine whether these PGRs increase firespike drought tolerance.

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
2.54	inch(es)	cm	0.3937
6.4516	inch ²	cm ²	0.1550
16.3871	inch ³	cm ³	0.0610
28.3495	oz	g	0.0353
28,350	oz	mg	3.5274×10^{-5}
1	ppm	mg·L ⁻¹	1
(°F - 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

Material and methods

Firespike is a flowering perennial in the Acanthaceae, native to the tropical and subtropical regions of Central America (Daniel, 1995). It has potential for use as a flowering potted plant because of the attractive red spikes, dark green foliage, and natural bloom time (Rezazadeh et al., 2015). Firespike is reported to be a short-day plant (Garofalo, 2002); therefore, vegetative stock plants of red firespike were maintained under long days (natural daylength plus 4-h night interruption lighting with 60-W incandescent lights from 2200 to 0200 HR) to prevent floral initiation occurring before starting the experimental treatments. The experiment was started in Sept. 2014. Medial, single-node cuttings of red firespike were rooted. Four weeks later, the rooted cuttings were transplanted to 6-inch-diameter pots containing a commercial potting substrate (Sunshine Mix 1; Sun Gro Horticulture, Bellevue, WA) and grown under natural daylength (12-h daylight) in a double-layer polyethylene-covered greenhouse. Plants were pinched 2 weeks after transplanting leaving two to three nodes. One plant was grown per container. Greenhouse air temperature set points were 64/74 °F (night/day). The plants were fertigated using 200 ppm nitrogen from 20N-4.4P-16.6K liquid fertilizer (Peters Peat-Lite 20-10-20; Scotts, Marysville, OH). Soil drench application of PBZ and flurprimidol at 0.24 mg/pot were applied when the axillary shoots were 3 to 5 cm long (≈ 14 d after pinching). Treated plants and the untreated control were grown for 2 weeks for PGR uptake and watered by hand as needed to keep the substrate moist. Two weeks after PGR treatment, the plants in the drought treatments were subjected to water stress by watering every 7 d until completion of the study. There were three PGR treatments including a no PGR control, 0.24-mg/pot PBZ drench, 0.24-mg/pot flurprimidol drench, and two watering treatments: plants watered daily and plants watered every 7 d, the drought treatment.

Eight replications were used in a complete randomized design. Five weeks after the start of the drought

treatment, physiological and morphological parameters of the plants were measured. Plant height (from the soil level to the top point of the plant) was measured weekly and at termination of the study. Plant diameter (the average of the widths measured at the widest point of the plant and 90° to the widest point), total leaf area (LI-3100 area meter; LI-COR, Lincoln, NE), and plant dry weight were recorded. A plant GI was calculated as $GI = \pi \times (\text{average width}/2)^2 \times \text{height}$. Days to flower and number of replications flowering were recorded. Days to flowering were calculated from date of sticking the cuttings to the date when the first open flower was observed. Data collection was ended when $\approx 85\%$ of all plants flowered. g_s and net photosynthesis were measured on fully expanded leaves with a portable photosynthesis system (LI-6400XT; LI-COR). The temperature and photosynthetic photon flux was set based on ambient temperature and light. Data were analyzed using the general linear models procedure of SAS (version 9.3; SAS Institute, Cary, NC) to determine the effect of PGRs on drought tolerance and physiological and morphological response of plants. Means were separated using Fisher's protected least significant difference test at $P \leq 0.05$.

Results and discussion

Plant height was affected by both PGRs and drought stress throughout the experiment (Table 1). The drought-stressed plants after 5 weeks of treatment were 45% shorter (17 cm shorter) than control plants. There were no differences between the plant heights in plants treated with PBZ either watered regularly or under drought stress (35 and 31 cm, respectively). However, plants treated with PBZ under drought stress were 11 cm taller than untreated plants under drought, indicating PBZ increased drought tolerance in the plants and reduced the adverse effects of drought stress on plant height (Table 1). Flurprimidol at the same concentration was not as effective as PBZ on increasing drought tolerance with flurprimidol + drought treated plants no taller than the drought control.

Plant diameter in plants treated with flurprimidol alone or under

drought stress was 32% or 36% less than control plants (Table 1). Drought treatment alone decreased plant diameter by 21% compared with control plants. Drought stress and PGRs reduced GI. Plants under drought only had 65% lower GI than the control (Table 1). Plants treated with flurprimidol grown under drought stress had 76% less GI compared with control plants (Table 1). Drought treatment and flurprimidol + drought had the lowest plant dry weight at 32% and 29% less than the control (Table 1). Drought stress + PBZ drench reduced dry weight by 22% compared with well-watered control plants. Flurprimidol and PBZ treatments combined with drought stress had a 56% and 46% reduction in the leaf area, respectively, compared with control plants (Table 1). Plants treated with PGRs only had greater leaf area than those treated with both drought and PGRs. Greater leaf area was also observed by Ruter and Martin (1994) in two container-grown landscape plants treated with PBZ.

Based on these results, growth characteristics of firespike, such as plant height, leaf area, GI, and plant dry weight, decreased when drought and PGRs treatments were combined. Reduction in plant height, leaf area, and plant dry weight in plants treated with PBZ under stress has also been reported by Fernández et al. (2006). These morphological modifications, such as the reduction of the leaf area, plant dry weight, and plant height, in drought-treated firespike can be explained as the induction of drought tolerance mechanisms to reduce the transpiration rate and water consumption (Alarcón et al., 1999; Gao et al., 1988; Sánchez-Blanco et al., 2004).

Days to flower and number of plants flowering were affected by drought stress. Number of days to flower increased 16 d in plants under drought only, compared with control. Plants treated with PBZ and flurprimidol plus drought stress flowered 4 and 5 d later than those treated with PGRs without drought. Only two plants ($n = 8$) grown under drought stress flowered. Drought stress also reduced the number of flowers by 33% in plants treated with flurprimidol compared with the control plants. There were no differences

Table 1. Effects of drought treatments and 0.24 mg/pot paclobutrazol (PBZ) or 0.24 mg/pot flurprimidol on firespike final plant height, plant diameter, leaf area, flowering, net photosynthesis, and stomatal conductance (g_s). Drought was induced by watering at 7-d intervals.

Treatments	Plant ht (cm) ^a	Plant diam (cm) ^a	Growth index (GI) (cm ³) ^b	Plant dry wt (g) ^c	Leaf area (cm ²)	Flowering replications (no.)	Time to flowering (d)	Net photosynthesis ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	g_s ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)
Control	37 a ^x	34 a	34,187 a	32 a	3,656 a	6 a	103 f	9.9 b	0.14 b
Drought	20 c	27 bc	11,811 cd	22 d	1,964 c	2 c	119 a	6.1 c	0.04 c
PBZ	35 ab	30 b	24,461 b	28 b	2,203 b	6 a	111 d	13.1 a	0.15 b
Flurprimidol	30 b	23 d	12,112 cd	26 c	2,016 bc	6 a	109 e	12.8 a	0.17 ab
PBZ + drought	31 ab	26 c	17,043 c	25 c	1,967 c	6 a	115 b	12.4 a	0.08 c
Flurprimidol + drought	21 c	22 d	8,052 d	22 d	1,962 c	4 b	114 c	9.2 b	0.21 a

^a1 cm = 0.3937 inch, 1 g = 0.0353 oz, 1 cm² = 0.1550 inch².

^bGI = $\pi \times (\text{average width}/2)^2 \times \text{height}$; 1 cm³ = 0.0610 inch³.

^cMeans within a column followed by the same letter are not significantly different according to the least significant difference means comparison test at $P = 0.05$.

in number of flowers between the other treatments (Table 1).

There was a decline in g_s in water-stressed plants with PBZ compared with untreated control plants. g_s reduction in PBZ treated plants under drought stress indicated better stomatal regulation (Fernández et al., 2006), which may be explained as a mechanism for reducing transpiration rate and water loss. Unstressed plants treated with PGRs and the control plants did not differ in g_s (Table 1). g_s in PBZ + drought treatments dropped to levels similar to the control drought-stressed plants, whereas flurprimidol + drought plants had a similar g_s to the unstressed control plants. Fernández et al. (2006) reported stomatal density was increased in PBZ treatments under drought stress.

Net photosynthesis in plants treated with PBZ or flurprimidol under drought was 51% and 34%, respectively, higher than in those under drought only. Paclobutrazol, flurprimidol, and PBZ + drought all had a higher net photosynthesis than the drought-stressed and unstressed control. A reduction in net photosynthesis has been primarily attributed to the closure of stomata controlling the transpiration rate (Centritto et al., 2003; Franks and Farquhar, 2001). In our study, the greatest net photosynthesis and lowest g_s was in plants treated with PBZ under drought stress suggesting PBZ can efficiently regulate stomata performance and enhance drought resistance. These results indicate PBZ was able to prevent reduction in net photosynthesis under water stress conditions. Triazole compounds have been reported to affect stomatal pore size, thickness of upper and lower epidermis, and the number of stomata (Kishorekumar et al., 2006) all of which may affect transpiration rate and g_s .

PGR treatments reduced wilting symptoms in drought-stressed plants and improved desiccation resistance especially between irrigation intervals (observational data not shown). The triazole PGRs reduced transpiration, increased the relative water content, membrane stability, and significantly increased yield in wheat grown under water stress (Sairam et al., 1995). Rabert et al. (2014) suggests the enhanced drought tolerance in triazole-treated plants is due to maintaining a balance between formation and

detoxification of activated oxygen species, resulting in an improved response of plants to drought-induced oxidative stress. Sankar et al. (2007) showed the adverse effects of water stress can be minimized with the application of PBZ, which increased the antioxidant levels and activities of scavenging enzymes.

This study showed PBZ at 0.24 mg/pot induced drought-stress resistance in red firespike plants. More specifically, plants showed more resistance to wilting between irrigation intervals. The ability of these compounds to increase drought resistance may be explained by the reduced leaf area and plant height in treated plants. Paclobutrazol helped to regulate g_s efficiency and influence net photosynthesis, which contributed to significantly drought tolerance in treated plants. Paclobutrazol is useful in controlling plant size and increasing drought tolerance in firespike.

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