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Action of abscisic and gibberellic acids on senescence of cut gladiolus flowers

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ABSTRACT: The gladiolus flower is classified as insensitive to ethylene. Thus, the signals that initiate senescence are poorly understood. This study evaluated the role of abscisic and gibberellic acids on postharvest senescence of 3 cultivars of cut gladiolus flowers (*Gladiolus grandiflora* Hort.). Stalks were harvested and placed in test tube containing 100 mL distilled water or an aqueous solution of abscisic acid (100 and 150 µM ABA), gibberellic acid (100 µM GA_3), and fluridone (1 mM) for 24 h. Subsequently, flower stalks were placed in a test tube with distilled water, and the following variables were determined: stem longevity, fresh weight change, water uptake rate and transpiration rate during the vase life, as well as membrane stability index and lipid peroxidation in 5 stages of flower development. In another experiment, the florets were removed by cutting down the pedicel. After removal, florets were placed in a beaker containing 10 mL distilled water or the following solutions: 100 or 150 µM ABA; 100 µM GA_3; 1 mM fluridone; and 100 µM ABA + 100 µM GA_3, followed by immersion in distilled water after 24 h in each treatment. Fresh weight change and transpiration rate of florets were assessed every 24 h. Abscisic acid is involved in the induction of senescence-related events in gladiolus flowers, such as high loss of membrane stability and abnormal flower opening. GA_3 regulates the action of ABA in the maintenance of cell membrane and opening of gladiolus flowers.

Key words: *Gladiolus grandiflora* Hort., petal senescence, hormone signaling, fluridone.

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INTRODUCTION

The gladiolus flower is valued by consumers for its lush beauty florets and by producers for its relative ease of production and good economic return. The flower longevity of gladiolus is 5 to 7 days on average, depending on the lifetime of the individual florets and the opening rate of the remaining buds (Serek et al. 1994; Kumar et al. 2014). Gladiolus is insensitive to ethylene and its endogenous physiological level does not influence flower senescence (Serek et al. 1994; Arora et al. 2006).

The action of plant hormones on the regulation of flowers senescence insensitive to ethylene is still poorly understood. Knowing the role of the senescence-signaling hormone is important in the choice of methods for preserving the ornamental quality of cut flowers (van Doorn and Woltering 2008). Recent studies observed an increase in endogenous concentration of abscisic acid (ABA) during senescence of ethylene-insensitive flowers (Wei et al. 2003; Hunter et al. 2004). According to Panavas et al. (1998), exogenous ABA promotes the loss of membrane stability and increased content of endogenous ABA prior to the increase in hydrolytic enzymes activity at the opening of the lily flowers. Zhong and Ciafre (2011) reported that the application of ABA accelerated the senescence of lily petals, suggesting a direct role of ABA in the early steps of senescence in petals regardless of the endogenous levels of ethylene.

The importance of ABA on floral senescence might be determined if its synthesis or action in a particular tissue could be regulated. Fluridone has been used in several studies aiming to inhibit ABA synthesis, and its efficiency was proven in most cases (Hunter et al. 2004; Zhong and Ciafre 2011). For the inhibition of ABA action, some studies reported the use of gibberellin (GA_3) on pulsing or vase solution. Kumar et al. (2014), in an experiment with *Gladiolus* sp. cv. Snow Princess and Dhanwantari, found that, even with high levels of ABA in the petals, membrane stability and absorption of vase solution were not affected when GA_3 solution was present. Also in gladiolus, Kumar and Gupta (2014) observed increased water uptake, delayed opening of basal flower, more florets open for stems, and prolonged longevity when flowers were dipped into or sprayed with 100 ppm gibberellic acid. Saeed et al. (2014) reported that GA_3 at the concentration of 25 $mg \cdot L^{-1}$, in vase solution, contributed to increase the fresh weight and longevity of cut gladiolus stems.

This experiment aimed to evaluate the role of abscisic and gibberellic acids on postharvest floral senescence of 3 cultivars of *Gladiolus grandiflora* Hort.

MATERIAL AND METHODS

Location and raw materials

Stems of gladiolus (*Gladiolus grandiflora* Hort.) cultivars Amsterdam, Red Beauty, and Veronica were used in the study. The stems were collected on the field of São Francisco Farm, located in Viçosa, Minas Gerais, Brazil (lat 20°45'S, long 42°51'W), at 651 m altitude.

Experiment description

After cleaning, standardization (50 cm length), and weighting, stems were placed into test tubes containing 100 mL distilled water or an aqueous solution of 100 µM ABA, 150 µM ABA, 100 µM GA_3, and 1 mM fluridone (Kumar et al. 2014). Thereafter, stems were placed into test tubes containing distilled water. The tubes were sealed with cotton wool and aluminum foil to prevent loss of solution by evaporation. The test tubes were maintained in the laboratory at room temperature of 22 ± 2 °C, relative humidity of 60 ± 20%, and light intensity of 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

All experiments were performed with 5 replicates and each experimental unit had a minimum of 3 flower stems.

Stem longevity

The longevity of the stems was determined as the time (number of days) between the beginning of the treatment and the onset of wilt in at least 50% of the flowers.

Fresh weight change

Stems were weighed every day in order to assess the fresh weight change, considering the initial weight of 100%.

Water uptake rate

The water uptake rate was determined according to the methodology described by van Doorn and Vaslier (2002). The stems were placed in individual tubes initially weighed containing 100 mL deionized water. Every day, the tubes were weighed with and without stems. To undo the effects of evaporation, the upper end of the tubes was wrapped with 4 layers of PVC film. The water uptake rate of each solution was obtained as the volume of solution absorbed in $mg \cdot g^{-1}$ FW, calculated by the following formula: $V = (S_i - S_f)/ST_f$, →

where V represents the volume of solution absorbed; S_i is the initial solution weight; S_f is the final solution weight; ST_f is the final stem weight.

Transpiration rate

The transpiration rate was estimated according to the method described by van Doorn and Vaslier (2002), in $mg \cdot g^{-1}$ FW, by subtracting the loss of fresh matter of stalks from the absorbed volume solution, by the following formula: $T = Vs - (ST_f - ST_i)$, where T is the transpiration rate ($mg \cdot g^{-1}$ FW); Vs is the volume of solution absorbed (mg); ST_f is the final stem fresh weight (g); ST_i is the initial stem fresh weight (g).

Membrane stability index

The membrane stability index was determined according to the method of Hassan and Ali (2014), with slight modifications. In each treatment, 5 discs (with 10 mm diameter) of flower tissue were placed in an Erlenmeyer flask with 20 mL deionized water. The discs were removed from the third basal flower of stems, at 5 flower opening development stages: (I) fully closed; (II) partially open; (III) fully open; (IV) partially senescent (indicating wilting of petal margins); and (V) senescent (Serek et al. 1994). After 6 h at room temperature, the initial conductivity (C1) was measured with a conductivity meter (Digimed, MD-31 model). The Erlenmeyer flask was heated to 90 °C for 2 h. Total conductivity (C2) was re-measured after cooling. Membrane stability was calculated as follows: $\% MSI = [1 - (C1/C2)] \times 100$.

Lipid peroxidation

Lipid peroxidation was determined by quantifying the malondialdehyde (MDA) content using the thiobarbituric acid test (TBA) (Cakmak and Horst 1991). Frozen tissue (0.2 g) was homogenized in 1 mL 1% (w/v) trichloroacetic acid (TCA) using a mortar and a pestle. The homogenate was centrifuged at 12,000 x g for 15 min. Then, 1.5 mL 20% TCA containing 0.5% (w/v) TBA was mixed with 0.5 mL of supernatant. The mixture was heated in boiling water for 20 min, cooled to room temperature, and centrifuged at 3,000 x g for 4 min. The absorbance of the supernatant fraction was measured at 532 nm in a spectrophotometer and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The amount of MDA was calculated from the extinction coefficient of 155 $mM^{-1} \cdot cm^{-1}$ and expressed as $nmol \cdot g^{-1}$ FW (Heath and Packer 1968). The samples were removed from the third basal flower of stems, at 5 flower opening development stages: (I) fully closed; (II) partially open; (III) fully open; (IV) partially senescent (indicating wilting of petal margins); and (V) senescent (Serek et al. 1994).

Study of isolated florets

In another experiment, florets were removed by making a cut below the pedicel with a blade. After removal, buds were placed in a beaker containing 10 mL of each solution, constituting the following treatments: distilled water, 100 µM ABA, 150 µM ABA, 100 µM GA_3, 100 µM ABA + 100 µM GA_3, and 1 mM fluridone, followed by 100 mL of distilled water after conditioning for 24 h.

Fresh weight change and transpiration rate were assessed every 24 h, in accordance with the methodology used in the previous experiment.

Statistics

Analysis of variance (ANOVA) was run at 1% significance level for 2 factors (cultivar and treatment) on the following variables: fresh weight (after 24 h), water uptake, and longevity. Tukey's test was applied at 5% probability for membrane stability index and descriptive statistics based on mean values and standard error for the other variables.

RESULTS AND DISCUSSION

No significant interaction was found between cultivars and treatments for any variable or for the isolated effect of cultivars. Significant effects of treatments were detected on fresh weight change, water uptake, and longevity (Table 1). →

Table 1. Summary of data relating to analysis of variance for fresh weight, water uptake, and longevity of 3 cultivars of gladiolus subjected to treatments with ABA, GA_3, and fluridone.

	Fresh weight (after 24 h)	Water uptake ($mg \cdot g^{-1}$ FW)	Longevity (days)
Cultivar	0.0476^{ns}	0.1662^{ns}	0.7755^{ns}
Treatments	5.0576^{**}	50.9904^{**}	10.1429^{**}
Cultivar × Treatment	0.4205^{ns}	0.6583^{ns}	0.9286^{ns}
CV (%)	**5.36**	**12.22**	**14.48**

**Significant at 1% probability by F-test; ^{ns}Non-significant.

Stem longevity

Treatments with ABA decreased the longevity of gladiolus stems. However, 100 µM GA_3 contributed to maintain the quality for a longer period (Figure 1).

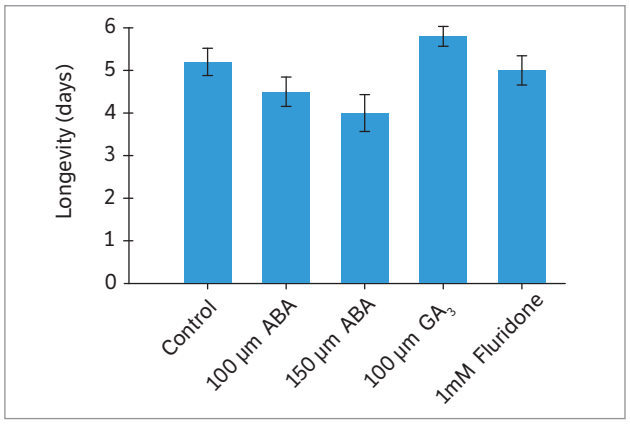

Figure 1. Longevity of cut gladiolus flowers subjected to treatments with ABA, GA_3, and fluridone. The columns represent the average values of cultivars Amsterdam, Red Beauty, and Veronica in each treatment. Vertical bars represent the standard error of the mean.

The longevity of stems treated with 100 and 150 µM of ABA was 4.4 and 4 days, which decreased by 13.46 and 23.07%, respectively, compared with the control. However, in the stems subjected to solution of 100 µM GA_3, the average was 5.8 days, which corresponded to a percentage increase of 11.53% in longevity, when compared with stems in water (control). The use of 1 mM fluridone did not influence the longevity of the stems, which was 5 days, compared with the 5.2 days of control flowers.

Singh et al. (2008) and Faraji et al. (2011) also found increased gladiolus vase life when treated with GA_3. According to Hunter et al. (2004), the senescence rate can be changed with a variation in the relative concentrations of GA_3 and ABA in the flower tissue. The GA_3 at 25 $mg \cdot L^{-1}$ in vase solution contributed to the increased longevity of gladiolus flowers (Saeed et al. 2014).

Fresh weight change

Stems subjected to 150 and 100 µM ABA initially had the highest fresh weight gain percentages up to the second day, with 12.06 and 7.55%, respectively, compared with control (Figure 2). This difference can be understood because ABA induces earlier floret opening, increasing fresh weight (Kumar et al. 2014). Yet, it was found that flower opening was irregular and senescence was accelerated beginning in the second day, when there was a decrease of 13 and 8% in fresh weight until the fourth day, in the stems treated with 150 and 100 µM ABA, respectively. The reduction in fresh weight is attributed to decreased water uptake and increased respiration induced by the senescence process (Ezhilmathi et al. 2007; Sairam et al. 2011).

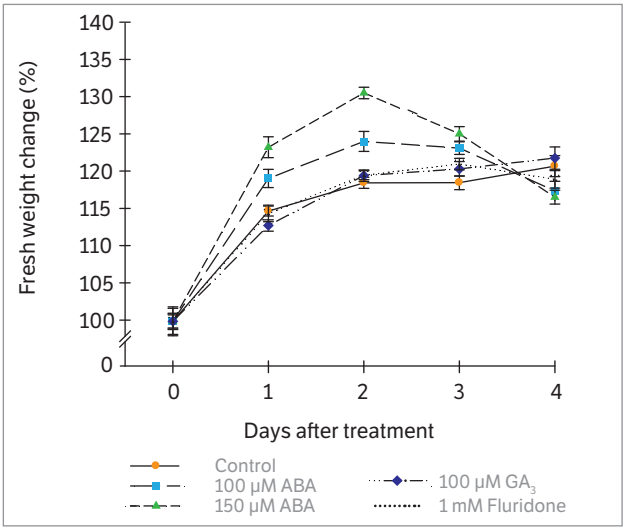

Figure 2. Fresh weight change of cut gladiolus flowers subjected to treatment with ABA, GA_3, and fluridone. Lines represent mean values of cultivars Amsterdam, Red Beauty, and Veronica in each treatment. Vertical bars represent the standard error of the mean.

On the other hand, the use of 100 µM GA_3 provided an upward trend in fresh weight until the fourth day, which revealed the onset of senescence. Similar results were found by Kumar et al. (2014), who applied GA_3 at the same concentrations in vase solution to inhibit the activity of ABA in gladiolus flowers. In this study, there was also a similar behavior between the control and fluridone treatments.

Water uptake and transpiration rate

The water uptake rate was reduced in the stems treated with ABA (Figure 3a). Control and stems treated with 100 µM GA_3 had the highest rates of water uptake over time. Compared with the control, in stems treated with 100 µM ABA, the cumulative water uptake decreased from 1,086 to 629 $mg \cdot g^{-1}$ FW, whereas, at the concentration of 150 µM, water uptake was 678 $mg \cdot g^{-1}$ FW. Stems in both treatments had 44.65 and 34.97% lower water uptake rate than control stems. In a study conducted by Kumar et al. (2014), the authors also →

found a decrease in water uptake rate of the conditioning solution with ABA in gladiolus stems. Kumar and Gupta (2014) found increased water uptake with immersion and foliar spray of 100 ppm GA_3 in gladiolus stems.

The response shown by water uptake rate is supported by the transpiration rate (Figure 3b). The stems treated with ABA had lower transpiration rates than those found in the other treatments, while the stems of control and treatment with 100 µM of GA_3 had the highest rates.

It is known that GA_3 contributes to the maintenance and preservation of cell membrane stability, inhibiting mainly membrane proteases (Saeed et al. 2014). Ezhilmathi et al. (2007), in an experiment with the antioxidant 5-sulfosalicylic, found an increase in water uptake and decrease in water transpiration rate, attributed to elevated cell membrane stability and low level of reactive oxygen species.

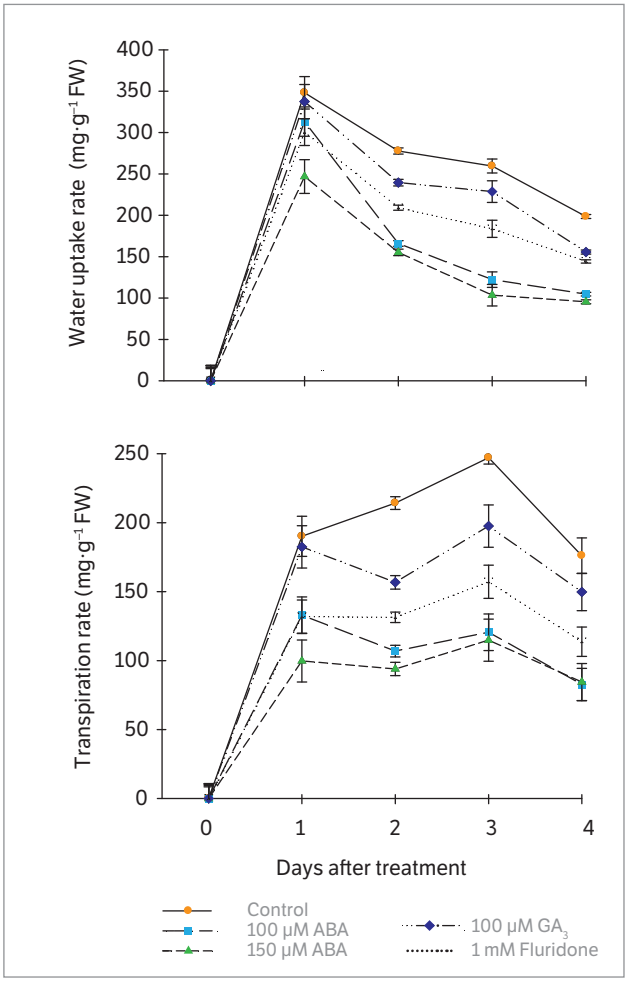

Figure 3. (a) Water uptake and (b) transpiration rate of cut gladiolus flowers subjected to treatment with ABA, GA_3, and fluridone. Lines represent mean values of cultivars Amsterdam, Red Beauty, and Veronica in each treatment. Vertical bars represent the standard error of the mean.

Membrane stability index

The electrolyte leakage is used as a parameter for evaluation of tissue damage such as loss of membrane selective permeability (Gerailoo and Ghasemnezhad 2011). There was a significant interaction between flower development stage and treatments for membrane stability index (Table 2). The membrane stability indices of petals differed significantly between the control and the treatments, as well as between the development stages. There was no significant difference between the initial and final stages of development; in other words, stages I and V (data not shown).

Treatments with ABA significantly reduced membrane stability index of flower from stage III, reaching the end of stage IV with 68.16 and 63.76%, respectively (Table 2). In the treatments with 100 µM GA_3 and 1 mM fluridone, however, no significant difference on membrane stability index was shown between stages, but values were significantly higher than in the treatments with 100 and 150 µM of ABA and similar to those obtained with control treatment.

The low level of membrane stability leads to greater leakage of solutes caused by the varying permeability of cell membranes (Zhong and Ciafre 2011; Kumar et al. 2014). In this study, the stems treated with GA_3 showed higher membrane stability rates. Hunter et al. (2004) and Kumar et al. (2014) also obtained similar results when using the potted GA_3 solution. According to Saeed et al. (2014), GA_3 contributes to the maintenance of vase life quality of gladiolus flowers →

Table 2. Membrane stability index of gladiolus flowers subjected to treatment with ABA, GA_3, and fluridone.

Stages	Membrane stability index (%)				
	Control	ABA (100 µM)	ABA (150 µM)	GA_3 (100 µM)	Fluridone (1 mM)
II	85.75 aA	75.66 aA	79.48 aB	77.69 aB	74. 99 aB
III	77.97 bA	70.00 bBC	67.32 bC	77.30 aA	73.75 aAB
IV	77.72 bA	68.16 bBC	63.76bC	73.93 aA	72.23 aAB

Means followed by the same lower case letter in the column and capital letter in the line do not differ statistically by Tukey's test at 5% probability.

by benefiting membrane stability. However, the optimal concentration should be studied beforehand; otherwise, the result may be negative. In their work, Saeed et al. (2014) found that the lowest concentration of GA_3 used (25 mg·L^{-1}) significantly contributed to maintain cell membrane stability.

The loss of membrane integrity is the final stage of the irreversible senescence associated with lipid peroxidation of membranes (Shahri and Tahir 2011).

Malondialdehyde content

Lipid peroxidation and membrane stability were inversely proportional and closely associated with flower senescence. Lipid peroxidation (MDA content) was increased during the 5 stages of flower development in all treatments and control (Figure 4). The flowers treated with 150 µM ABA had the highest MDA content from the second stage of development (II) when compared with the other treatments and the control, indicating that these flowers underwent greater cell membrane damage. The difference was more evident in the senescence stage (III), in which there was a difference of 14.68 and 9.99 nmol·g^{-1} FW compared with the stems treated with GA_3 and fluridone, respectively. These results are similar to those reported by Hatamzadeh et al. (2012), in gladiolus flowers, who also observed an increase in MDA content until the last stage of senescence.

Lipid peroxidation is mediated by reactive oxygen species caused by loss of membrane stability (Arora and Singh 2006). According to Zhong and Ciafre (2011), iris petals treated with ABA had an increase in the total activity of proteases that act on membrane proteins. Additionally, ABA also regulates ion leakage, anthocyanin degradation, phospholipid degradation, RNA degradation, and RNase activity (Zhong and Ciafre 2011). Thus, the induced ABA increased membrane permeability in gladiolus flowers due to the oxidation of lipids present in the plant cell membrane.

Flowers treated with GA_3 had the lowest MDA content in the 5 developmental stages (Figure 4). Gibberellic acid was also reported to delay the protease activity and degradation of chlorophyll (Eason et al. 2002; Silva 2003). According to Wood and Paleg (1974), GA_3 is known to restrict the oxidation of polyunsaturated fatty acids, which can lead to a reduction of lipid peroxidation. The extension of vase life of *Polyanthus tuberosus* flowers has been attributed to GA_3, which prevents the degradation of the protein by promoting protein synthesis and limiting the protease activity (Su et al. 2001; Arora and Singh 2004; Kant and Arora 2012).

Fresh weight change of the florets

When the florets were studied separately, there was a difference in the percentage of fresh weight variation as a function of time and type of treatment (Figure 5). As noted in the previous experiment, the treatment with 150 µM ABA initially had the highest fresh weight gain, which occurred →

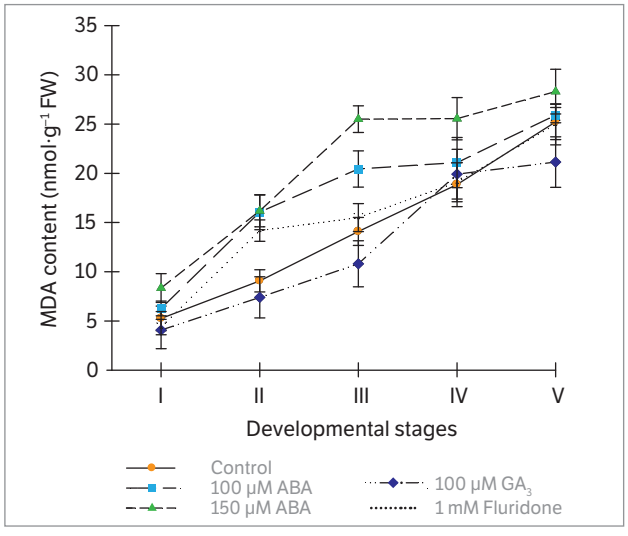

Figure 4. Malondialdehyde content of cut gladiolus flowers subjected to treatment with ABA, GA_3, and fluridone. Lines represent mean values of cultivars Amsterdam, Red Beauty, and Veronica in each treatment. Vertical bars represent the standard error of the mean.

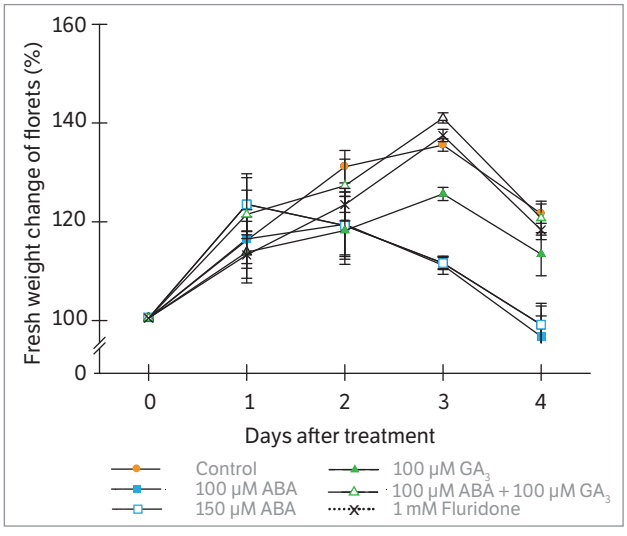

Figure 5. Fresh weight change of gladiolus florets subjected to treatment with ABA, GA_3, and fluridone. Lines represent mean values of cultivars Amsterdam, Red Beauty, and Veronica in each treatment. Vertical bars represent the standard error of the mean.

only on the first day, with the rapid induction of flower opening. After the first day, the treatments with 100 and 150 µM of ABA had a sharper decline, reaching the fourth day with a difference of 19.73 and 24.37%, respectively. The florets subjected to pulsing with 100 µM ABA + 100 µM GA_3 obtained the highest peak (third day) of weight gain in relation to others, especially the florets treated with ABA at 100 and 150 µM, reaching a percentage difference of 23%.

The florets treated with 100 µM GA_3 showed similar effects to those treated with 100 µM of ABA in vase solution during the first 2 days. According to the results found by Serek et al. (1994) with gladiolus florets, the fresh weight change that accompanies opening and senescence is explained by marked differences of respiration patterns in both stages. In the present experiment, the control of floral opening may explain this difference between treatments with ABA and GA_3 in relation to fresh weight, suggesting that the ABA/GA_3 ratio must control the flower development (Figure 6). The control and the treatment with 1 mM fluridone had the same behavior, as also observed in the previous experiment.

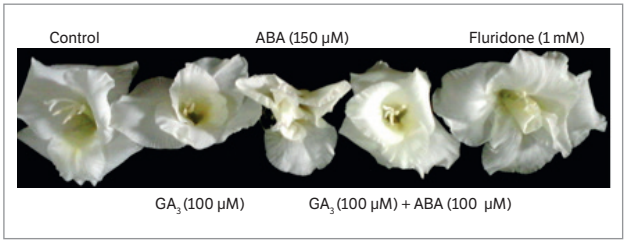

Figure 6. General appearance of gladiolus florets subjected to treatment with ABA, GA_3, and fluridone.

Transpiration rate of the florets

The florets treated with ABA in both concentrations had the highest transpiration peaks (Figure 7). Florets treated with 150 µM ABA had a peak on the first day after the treatment, whereas in those treated with 100 µM ABA, the peak was delayed to the third day.

The florets subjected to 100 µM GA_3, 1 mM fluridone, and 100 µM ABA + 100 µM GA_3 showed similar behavior in the transpiration rate until the second day, which may have been caused by the stabilization of fresh weight and subsequent increase in the rate of water uptake, due to the induction of flower opening. The lack of fluctuations in control was due to the stabilization of opening and fresh weight, which remained constant from the start. These results corroborate Ezhilmathi et al. (2007), who found that increased membrane permeability or ion leakage reduces the water retention capacity by the cellular osmotic potential, which, in this experiment, was attributed to the action of ABA.

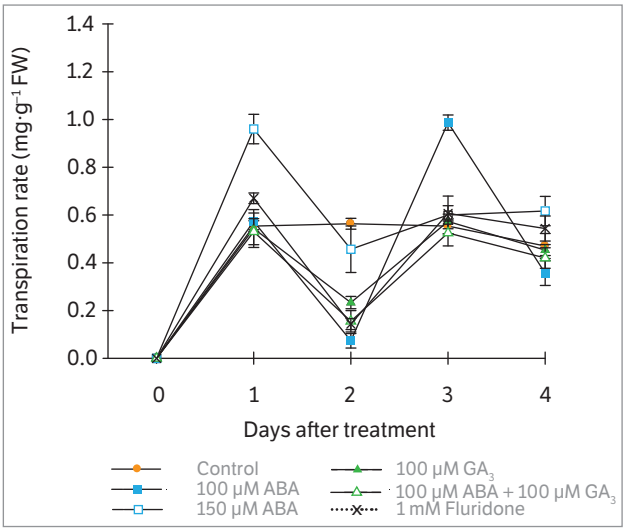

Figure 7. Transpiration rate of gladiolus florets subjected to treatment with ABA, GA_3, and fluridone. Lines represent mean values of cultivars Amsterdam, Red Beauty, and Veronica in each treatment. Vertical bars represent the standard error of the mean.

CONCLUSION

Abscisic acid is involved in the induction of senescence-related events in gladiolus flowers, such as high loss of membrane stability and abnormal flower opening. Gibberellic acid regulates the action of ABA in the maintenance of cell membrane and opening of gladiolus flowers. The hormone action on individual gladiolus florets needs more attention and, thus, these data can pave the way for further studies in this direction.

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