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SALICYLIC ACID STIMULATES FLOWERING IN MICROPOPAGATED GLOXINIA PLANTS

ÁCIDO SALICÍLICO ESTIMULA LA FLORACIÓN EN PLANTAS MICROPROPAGADAS DE GLOXINIA

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SUMMARY

Micropropagated gloxinia (*Sinningia speciosa* Benth.) seedlings transferred to greenhouse conditions, were treated with salicylic acid (SA) to test its effect on flowering. Concentrations of 1.0 to 0.0001 µM of SA were sprayed on the shoots on three occasions. Results showed that all SA concentrations tested increased the total number of flowers per plant by 25 to 37 %. Flower length increased 11 % by SA at 1.0 µM. All SA treated plants flowered 6 d earlier and had higher leaf area compared to control plants.

Index words: *Sinningia speciosa,* salicylic acid, flowering, plant growth regulator.

RESUMEN

Plántulas de gloxinia (*Sinningia speciosa* Benth.) en condiciones de invernadero fueron tratadas con ácido salicílico (AS) para evaluar su efecto en su expresión floral. Concentraciones de 1.0 a 0.0001 µM de AS fueron asperjadas en el dosel de las plántulas en tres ocasiones. Los resultados mostraron que todas las concentraciones de AS probadas, incrementaron de 25 a 37 % el número total de flores por planta. Además, con 1.0 µM de AS se aumentó la longitud de la flor en 11 %. Todos los tratamientos del AS acortaron en por lo menos 6 d la floración de las plantas y causaron una mayor área foliar, en comparación con el testigo.

Palabras clave: *Sinningia speciosa,* ácido salicílico, floración, regulador de crecimiento de plantas.

INTRODUCTION

Salicylic acid (SA) or orthohydroxybenzoic acid is an endogenous plant phenol recognized as a plant growth regulator that influences numerous physiological processes (Raskin, 1992). It has been reported that exogenous applications of SA to plants affect several of their physiological processes, such as stomatal closure (Larqué-Saavedra, 1978; 1979); control of ion absorption and transport (Harper and Balke, 1981); inhibition of ethylene synthesis (Huang *et al*., 1993; Leslie and Romani, 1986); induction of adventitious roots (Kling and Meyer, 1983); biomass accumulation in *Glycine max* and *Pinus patula* (Gutiérrez *et al.,* 1998; San-Miguel *et al.,* 2003); reduction of stress by salinity in *Triticum aestivum* (Shakirova *et al.,* 2003); and stimulation of growth and differentiation of transformed *Catharanthus* roots (Echevarría-Machado *et al.,* 2007).

Quiroz-Figueroa *et al*. (2001) have also reported that picomolar concentrations of SA applied to the culture medium increase cell growth and somatic embryogenesis in tissue cultures of *Coffea arabica*. Regarding the effect of SA on the flowering processes, Oota and Cleland (1975) and Cleland and Ben-Tal (1982) showed that the application of SA in the growth medium of *Lemna gibba* could substitute photoperiod effect on flowering promotion. Endogenous SA levels however, have not yet been proven to be responsible for the flowering effect, although it is known that flowering is affected by photoperiod and stress factors (Shimakawa *et al.,* 2012).

Flowering is a complex process regulated by genetic and environmental factors. In order to study this morpho-physiological event in the plant cycle, it is important to have a reliable bioassay system. In this respect, valuable information of the flowering process has been obtained in previous studies using *in vitro* plants as reported by Zhang (2007), or using homogeneous African violet plants (*Saintpaulia ionantha* Wendl.) obtained via micropropagation (Martin-Mex *et al.,* 2005). Since micropropagated plants are sensitive to external application of plant regulators, the present research used gloxinia (*Sinningia speciosa* Benth.) vitroplants to evaluate if the flowering is affected by SA (Larqué-Saavedra *et al*., 2007)

MATERIALS AND METHODS

Gloxinia cv. 'Ultra' vitroplants were obtained from leaf explants. For shoot regeneration, Murashige and Skoog (1962) culture medium (MS) was used in 100 mL of a hormonal balance of 8.87 µM of benzylaminopurine and 2.69

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μM of naftalenacetic acid . For rooting promotion, shoots were cultured in 50 mL magenta boxes containing MS medium at half concentration, 1.9 g L^{-1} of agar Phytagel®, iron and vitamins at full concentration, and no growth regulators. Incubation conditions were: 27 ± 2 °C temperature, 16-8 h of light-dark photoperiod, and 37 μmol m^{-2} s^{-1} light intensity.

After plant removal from the culture medium, their roots were washed to eliminate agar and then transplanted into plastic pots filled with Cosmopeat® and Agrolite® (3:1). Pots were covered with transparent plastic bags to maintain high levels of humidity and transferred to greenhouse conditions with minimum and maximum temperatures of 19/30 °C night/day, under light conditions of 650 ± mol m^{-2} s^{-1} and 13 h photoperiods. Plants were watered daily and fertilized weekly with a solution of 170 mg L^{-1} of soluble fertilizer (19N-19P-19K; Haifa Chemicals, Ltd.). After 18, 25 and 32 d the seedlings were sprayed with solutions of salicylic acid (Merck®) prepared at three concentrations (1.0, 0.01 and 0.0001 μM). Distilled water was applied as a control. Tween 20® was added to the solution as a surfactant. A completely random experimental design was used with 10 replicates per treatment.

Number of leaves and total leaf area (LI-3000A Leaf Area Meter; Li-COR, Inc.) were recorded 100 d after the first SA application; days to flowering were also recorded (when 50 % of the plants had opened their flowers). The total number of flowers was registered each week and the length and width of the flowers were measured in cm with a ruler. Data were analyzed using ANOVA (SAS, 2004).

RESULTS AND DISCUSSION

The *in vitro* cultivation of gloxinia produced vigorous plants with well-developed shoots suitable for the present research work. The pattern of gloxinia flower exposure (Figure 1) shows that all plants treated with SA flowered 24 d after the last application, while the control plants did not reach flowering until day 33. Thereafter, the rate of flowering was similar in all treatments, but by the end of this study (76 d after last application of SA) the control plants had exposed only eight flowers while the plants treated with SA had produced 10 to 11 flowers, without significant differences ($P \leq 0.05$) among treated plants. Therefore, the application of SA increased the number of flowers per plant by 25 to 37 %, compared to the control plants. Besides inducing an earlier flowering and a greater number of flowers per plant, the SA treatments also increased flower size, in particular with the 1.0 μM SA concentration, which produced flowers 17 % longer and 11 % wider (Figure 2).

Even though SA treatments did not have a significant effect on the number of leaves, all SA concentrations were able to increase the total leaf area per plant, most significantly when

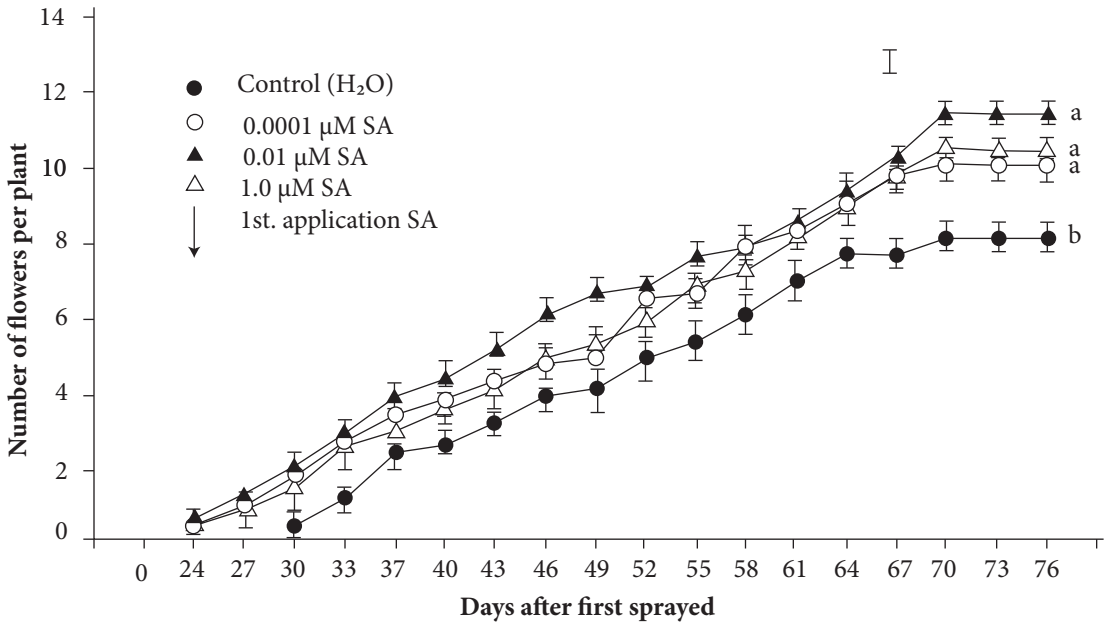

Figure 1. Pattern of flowering in gloxinia (*Sinningia speciosa*), vitroplants after shoot applications of salicylic acid. Vertical bars in each mean are the standard deviations (n = 10), and means with the same letter are not significantly different (Tukey, 0.05).

applied at 0.01 µM which caused an increase of 49 % compared to the control (Table 1).

These results clearly show that the application of SA accelerates flowering initiation in gloxinia in-vitro plants when they were acclimated to greenhouse conditions. Oota and Cleland observed similar results (1975) in *Lemna gibba*. It is interesting to note that the lowest concentration of SA tested here at picomolar level (0.0001 µM) was sufficient to affect gloxinia flowering, as described above; these results are similar to those observed by Martin-Mex *et al.* (2005) in the flowering process of micropropagated African violet plants treated in early stages with salicylic acid. Other morpho-physiological changes caused by SA at picomolar concentrations have been reported for embryogenesis of *Coffea* (Quiroz-Figueroa *et al.,* 2001) and root transformation of *Catharanthus* (Echevarría-Machado *et al.,* 2007).

It should be noted there is scarce information available on the response of micropropagated plants to growth regulators when treated during the acclimation period. At this stage, plant tissue may have fewer physical barriers to exogenous treatments, such as gases (carbon dioxide, ozone, etc.) or chemicals (growth regulators, herbicides, etc.) or even pathogens that might be present in the *ex vitro* environment. Plant hormones such as absicic acid (ABA) have been applied during this period to increase plant endurance for further development, (Pospíšilová *et al.,* 1998; 2007), thus suggesting that early application of hormones might be recommended.

The fact that SA treatments induced an earlier expression of flowers by 6 d is of particular interest, although this response cannot be explained with these data. Further work is needed in this area in order to propose a framework for the mechanisms involved in this process. However, it may be possible that salicylic acid stimulated flowering by inducing a greater uptake of nutrients, since it has been demonstrated that root systems grow larger as a result of SA (Gutiérrez *et al.* 1998; Echevarría-Machado *et al*., 2007). It is also possible that SA could have affected the specific expression of the CONSTANTS (Co) protein, which is said to be crucial for flowering induction.

CONCLUSIONS

The present research work showed that micropropagated gloxinia plants are sensitive to low concentrations

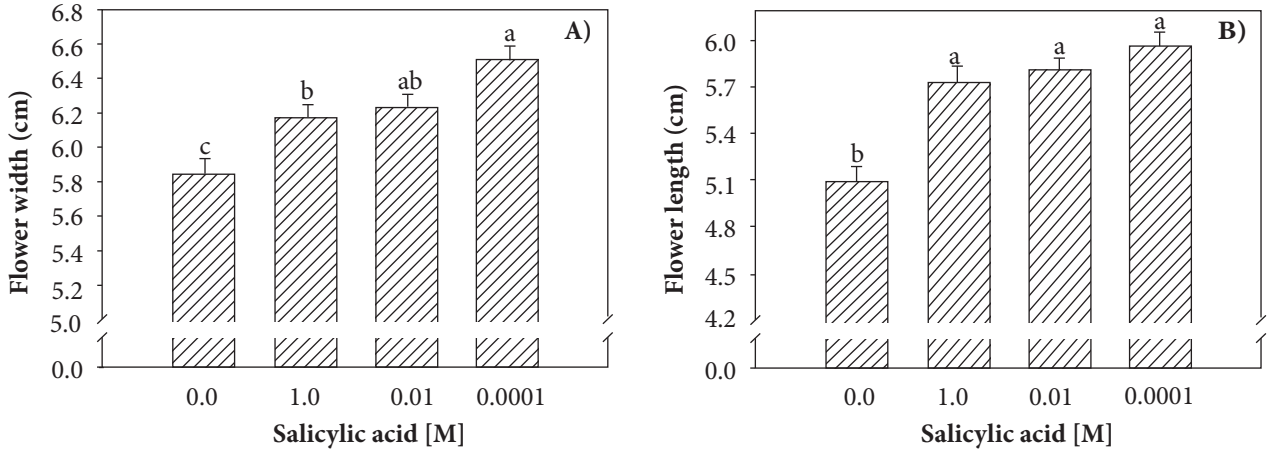

Figure 2. Effect of salicylic acid applications on the shoot of gloxinia (*Sinningia speciosa*) on flower size (A and B). Vertical lines on each bar represent the standard deviations, and means with the same letter are not statistically different (Tukey, 0.05).

Table 1. Effect of salicylic acid sprayed on plants of *Sinningia speciosa* cv. 'Ultra', on three morphological traits.

Treatment SA [µM]	Number of leaves per plant	Leaf area (cm^2) per plant	Days to flower
0	14 ± 0.4 a	456.4 ± 16 c	30 ± 3 b
0.0001	13 ± 0.6 a	496.2 ± 23 bc	24 ± 2 a
0.01	13 ± 0.4 a	680.9 ± 12 a	24 ± 3 a
1.0	13 ± 0.6 a	565.2 ± 16 b	24 ± 2 a

Values are the means, ± standard error (n = 10). Means with the same letter are not significantly different (Tukey, 0.05).

of salicylic acid, since concentrations as low as 0.0001 µM sprayed on the shoots of this ornamental plant increased the number of flowers per plant by 37 %, as well as augmenting both flower length and width. Finally, it was possible to confirm that salicylic acid induces early flowering in gloxinia vitroplants.

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