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RESEARCH PAPER

Induction of direct organogenesis from aerial explants of scented alstroemeria genotypes

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Abstract

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Alstroemeria is an important pot plant and cut flower in the ornamental plant market, and its propagation mainly occurs by rhizome division. However, this method is inefficient and time-consuming and may contribute to the spread of viruses. This study focused on developing in vitro propagation protocols using aerial explants of *Alstroemeria caryophyllaea* and *A. 'Sweet Laura'*, two scented genotypes. Aerial explants of alstroemeria were regenerated by testing three types of aerial explants and four concentrations of cytokinins. Both *A. caryophyllaea* and *A. 'Sweet Laura'* showed the highest induction rate from shoots, with values of 35 and 63.3%, respectively. For both genotypes, the optimum BAP concentration for direct organogenesis from buds was 1.0 mg L⁻¹. Finally, a successful protocol for in vitro propagation of *A. caryophyllaea* and *A. 'Sweet Laura'* through direct organogenesis from aerial explants was generated.

Keywords: *Alstroemeria caryophyllaea*, *Alstroemeria 'Sweet Laura'*, growth regulators, tissue culture.

Abbreviations: BAP - 6-benzylaminopurine; MS - Murashige and Skoog (1962) medium.

Introduction

Alstroemeria is a rhizomatous monocot native to South America belonging to the *Alstroemeriaceae* family (Bayer, 1987; Muñoz and Moreira, 2003). Many varieties have been developed through breeding techniques, such as mutagenesis (Przybyla, 2000; Aros *et al.*, 2012a), polyploidization (Lu and Bridgen, 1997; Takayuki, 1999) and mainly interspecific hybridization (Burchi *et al.*, 1997). Currently, this species represents an important pot plant and cut flower in the ornamental plant

market due to its attractive flowers and long vase life (Hoshino, 2008). *Alstroemeria* is commonly vegetatively propagated by rhizome division. However, this system of propagation is inefficient and time-consuming (Lin *et al.*, 1997) and may contribute to the spread of viruses affecting yield during cultivation (Van Zaayen, 1995).

In vitro micropropagation is a frequently used alternative to propagate alstroemeria hybrids (Khaleghi *et al.*, 2008) and native species (Aros *et al.*, 2017), and several studies have validated the efficiency of this technique (Chiari and Bridgen 2000; Khaleghi *et al.*, 2008). Most studies assessing in vitro propagation of alstroemeria have

been performed using sections of rhizome as the explant (Yousef *et al.* 2007; Pumisitapon *et al.*, 2011; Aros *et al.*, 2017), but a low multiplication rate was often observed (Lin *et al.*, 1998). In vitro propagation of alstroemeria through immature (Van Schaik *et al.*, 1996) and mature embryos (Hutchinson *et al.*, 1994) has also been reported, showing a high multiplication rate, particularly when an embryogenic callus was induced (Akutsu and Sato, 2002; Kim *et al.*, 2006). However, this method is not useful for clonal propagation of commercial varieties. More recently, studies have been conducted on micropropagation from aerial explants, such as floral apices (Pedraza-Santos *et al.*, 2006), nodes with axil tissue (Kim *et al.*, 2006) and leaf base (Nasri *et al.*, 2013). Organs such as leaves and stems for use as explants show several advantages for in vitro propagation because they provide more material for propagation than rhizomes, and aerial explants potentially present less contamination than underground explants (Lin *et al.*, 1998).

In addition to the type of explant, several other factors are involved in in vitro propagation. The effect and interaction of growth regulators in particular on the in vitro propagation of alstroemeria have been widely studied (Lin *et al.*, 1997; Cruz *et al.*, 2003; Hamidoghli *et al.*, 2007), and these studies mainly focused on the interaction between auxin and cytokinin concentrations to improve shoot regeneration from the explants (Pedraza-Santos, 2006; Ongaro and Leyser, 2008). Moreover, the effect of photoperiod and temperature during in vitro propagation of alstroemeria has also been reported (Cruz *et al.*, 2003; Pedraza-Santos *et al.*, 2006).

Each genotype has its own multiplication rate when cultured and propagated in vitro (Lu and Bridgen, 1996; Chiari and Bridgen, 2000; Hoshino, 2008). Furthermore, this character is typically considered a selection criterion in breeding programs when a new variety is released. Therefore, specific in vitro propagation protocols are needed for each genotype studied. Considering that floral scent

has not been targeted as an important trait in alstroemeria breeding programs (Aros *et al.*, 2012b; Aros *et al.*, 2015), little is known about the propagation of fragrant genotypes. This study aimed at developing an efficient protocol of in vitro propagation for two scented alstroemeria genotypes: the Brazilian species *Alstroemeria caryophyllaea* and *Alstroemeria* ‘Sweet Laura’, which was obtained by crossing *Alstroemeria aurea* x *A. caryophyllaea* (Bridgen *et al.* 2009). The effects of aerial explants from different organs and the concentration of cytokinins (6-benzylaminopurine; BAP) in the culture media were evaluated to optimize the micropropagation of these two genotypes.

Materials And Methods

Plant material

Aerial explants were collected from selected lines of *Alstroemeria caryophyllaea* (Alstroemeria Breeding Program at the University of Chile) and *Alstroemeria* ‘Sweet Laura’ (Alstroemeria Breeding Program at Cornell University), which were both grown under greenhouse conditions at the Faculty of Agricultural Sciences, University of Chile. Three types of aerial explants were evaluated: leaves (obtained from the apical zone of the plant), young shoots (1 cm long without extended leaves) and stem sections (1 cm long containing an axillary bud). Explants were disinfected by immersion in 0.9% NaOCl (15 min), followed by rinsing (3 min) three times using distilled sterilized water.

Explant induction

Twenty-four treatments resulting from the combination of three explants (shoot, leaf, stem), four concentrations of cytokinin (0.0, 0.5, 1.0 and 2.0 mg L⁻¹ of BAP) and two genotypes (*Alstroemeria caryophyllaea* and *A.* ‘Sweet Laura’) were assessed in a completely randomized factorial design.

Fifteen replicates per treatment were performed, and the experimental unit consisted of one explant cultured in a Petri dish (disposable, 60 x 15 mm) containing 20 ml of MS medium (Murashige and Skoog, 1962), 30 g L⁻¹ sucrose, and 7 g L⁻¹ agar, with the pH adjusted to 5.8. The medium was supplemented with 0.1 mg L⁻¹ indolebutyric acid (IBA) in combination with the different concentrations of BAP as previously described. In vitro explants were grown under controlled conditions of a photoperiod of 16/8 hours light/dark (regime provided by lamps with density photosynthetic photon flux of 900 mol photons m⁻²s⁻¹) and 20 °C. After 4 weeks, induced explants (explants showing a 1 mm growth) were transferred to the regeneration medium. The percentage of explants exhibiting oxidation, contamination and callus development was also recorded.

Explant regeneration

Induced explants were transferred to test tubes (15 x 100 mm) containing 10 ml of liquid medium with MS and 30 g L⁻¹ sucrose supplemented with 2 mg L⁻¹ BAP, and the pH was adjusted to 5.8. The explants were grown under the same controlled conditions described previously. After 8 weeks, the number of regenerated shoots per explant, the length and width (cm) of regenerated shoots, the number of regenerated leaves per explant, and the length and width (cm) of regenerated leaves were evaluated in regenerated explants.

Histological study

To determine the meristematic zone from which organogenesis was induced, transverse histological sections were obtained from samples of induced leaf petiole of *Alstroemeria* 'Sweet Laura'. Samples were obtained at days 0 and 7 from in vitro cultivation based on the methodology described by Prat *et al.* (2008). Samples were fixed in a solution of FAA (4% formalin, 70% acetic acid, and 70% alcohol at a ratio of 5:5:90

v/v), dehydrated in ethanol (70, 80 and 90% for 30 minutes each time), and finally infiltrated in JB4 solution (Polyscience, Inc., Warrington, PA). Using an ultramicrotome (model 1516, Leitz, Germany), sections 10 microns thick were generated, mounted on a sealed slide with Canada balsam, and stained with toluidine blue for observation under light microscopy.

Statistical analysis

Data of induced, regenerated, necrotic, contaminated explants and callus development were analyzed using the Bliss transformation (arcsen√%). Logarithmic transformation of the data was applied, including evaluation of regenerated shoots. An analysis of variance was performed using Infostat software. Means were compared using Tukey's HSD (honest significant difference) test for multiple pairwise comparisons with a significance level of 0.05.

Results

Explant induction

Induction was observed in *Alstroemeria caryophyllaea* shoots and leaves and *A.* 'Sweet Laura' shoots, leaves and stems. For both genotypes, shoot explants showed the highest induction rate compared with leaf and stem, with values of 63% and 35% for *A.* 'Sweet Laura' and *A. caryophyllaea*, respectively. A higher induction rate of shoots and stems was observed in *A.* 'Sweet Laura', whereas no significant differences were noted between induced leaves observed in *A. caryophyllaea* (15%) and *A.* 'Sweet Laura' (18.3%) (Table 1).

Regarding the interaction between the cytokinin concentration and genotype, no significant differences were observed between genotypes except those explants cultured with 1 mg L⁻¹ BAP, which showed a significantly higher induction rate with *A.* 'Sweet Laura' explants (64.4%) than with *A.*

Table 1. Induced explants observed after 4 weeks considering the interaction between genotype and type of explant (n=60).

Genotype	Induced explants (%)					
	shoot		leaf		stem	
<i>A. caryophyllaea</i>	35.0	aB	15.0	bA	0.0	bB
<i>A. 'Sweet Laura'</i>	63.3	aA	18.3	bA	20.0	bA

*Different capital letters in the column indicate significant differences according to Tukey's test ($P \leq 0.05$).

**Different small letters in the row indicate significant differences according to Tukey's test ($P \leq 0.05$).

caryophyllaea (33.3%). In addition, 1 mg L⁻¹ BAP showed the highest explant induction, particularly in *A. 'Sweet Laura'*, where significant differences were noted compared with the other concentrations. The lowest induction value obtained was in explants cultured without BAP supplementation in both *A. caryophyllaea* (4.4%) and *A. 'Sweet Laura'* (11.1%); however, significant differences were not observed with the remaining BAP concentrations applied, particularly 1 mg L⁻¹ (Table 2).

Table 2. Induced explants observed after 4 weeks considering the interaction between genotype and BAP concentration (n=60).

Genotype	Induced explants (%)							
	0 mg L ⁻¹ BAP	0.5 mg L ⁻¹ BAP	1.0 mg L ⁻¹ BAP	2.0 mg L ⁻¹ BAP				
<i>A. caryophyllaea</i>	4.4	bA	8.9	bA	33.3	aB	20.0	abA
<i>A. 'Sweet Laura'</i>	11.1	cA	37.8	bA	64.4	aA	22.2	bcA

*Different capital letters in the column indicate significant differences according to Tukey's test ($P \leq 0.05$).

**Different small letters in the row indicate significant differences according to Tukey's test ($P \leq 0.05$).

Explant induction was concentrated during the second week for both genotypes and all the explants evaluated, particularly those growing in the medium supplemented with 1 mg L⁻¹ BAP, where the highest number of induced explants was observed. Only shoots and stems were induced in the first week, whereas only shoots of *A. caryophyllaea* grown with 2.0 mg L⁻¹ BAP showed induction in the fourth week (Figures 1 and 2). *A. caryophyllaea* shoots were induced with

all the BAP concentrations evaluated, whereas leaves were only induced with 1.0 and 2.0 mg L⁻¹ BAP. *A. 'Sweet Laura'* shoots were also induced with all BAP concentrations, but the absence of this growth regulator in the culture media did not produce induction in either stems or leaves.

Explant regeneration

Direct organogenesis was observed after explant induction. Elongation of shoots and subsequent leaf development were observed during shoot regeneration. On the other hand, leaves developed new shoots from the base of the petiole, and stems showed elongation and the development of new leaves from the axillary bud.

Interactions between factors (genotype, explant and BAP concentration) were not significant. Thus, the effect of each factor on the regeneration rate was independently analyzed. The genotype

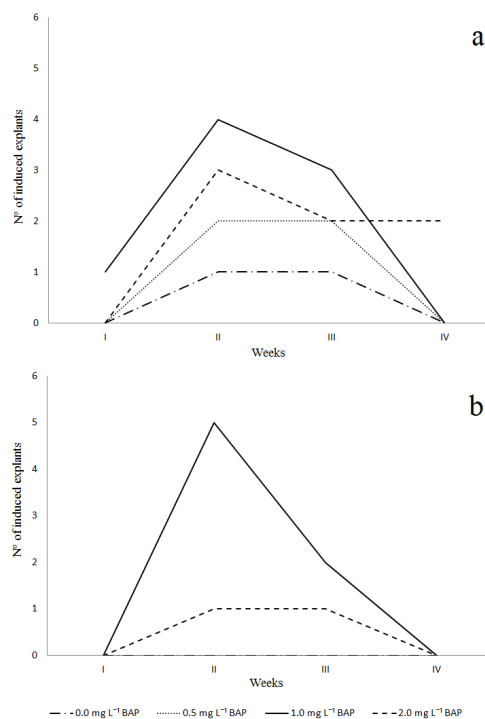


Figure 1. Induction of shoots (a) and leaves (b) of *A. caryophyllaea* in vitro cultured under different BAP concentrations for 4 weeks.

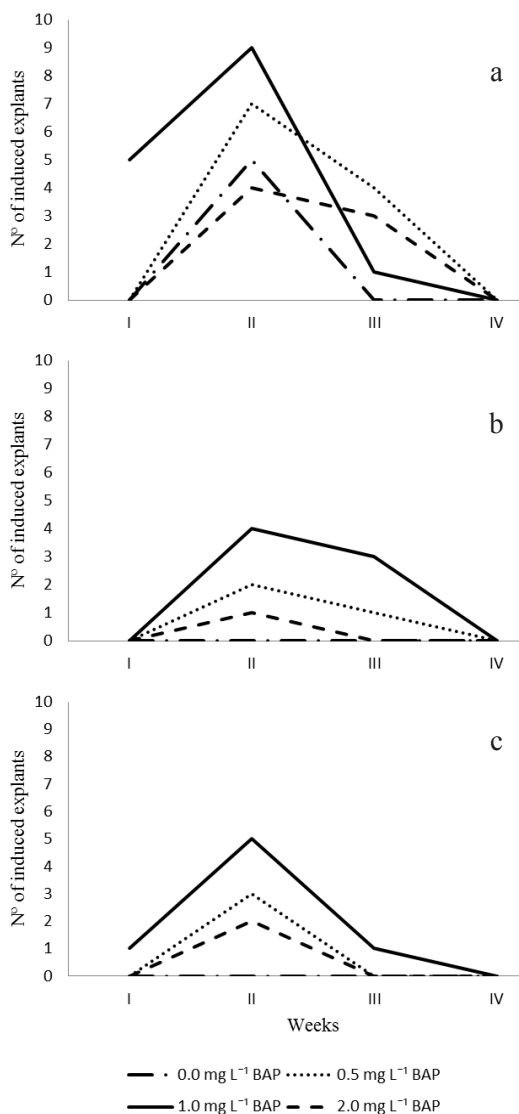


Figure 2. Induction of shoots (a), leaves (b) and stem (c) of *A. 'Sweet Laura'* in vitro cultured under different BAP concentrations for 4 weeks.

A. 'Sweet Laura' showed higher explant regeneration (21.7%) than *A. caryophyllaea* (8.3%). Regarding the explants, the shoot showed the highest regeneration (30%), and this result was significantly different from that of leaves and stems, with 10% and 5% regeneration, respectively. As observed during induction, explants previously cultured with 1.0 mg L⁻¹ BAP showed significantly higher regeneration (30%) than the other BAP concentrations evaluated (Table 3).

Table 3. Effect of genotype (n = 180), explants (n = 120) and BAP concentration (n = 90) on the regeneration observed from aerial explants of *A. caryophyllaea* and *A. 'Sweet Laura'*.

Treatment		Regeneration (%)	
Genotype	<i>A. 'Sweet Laura'</i>	21.7	a
	<i>A. caryophyllaea</i>	8.3	b
Explant	Shoot	30.0	a
	Leaf	10.0	b
	Stem	5.0	b
BAP concentration (mg L ⁻¹)	0.0	4.4	b
	0.5	12.2	b
	1.0	30.0	a
	2.0	13.3	b

*Different letters in indicate significant differences within each factor (genotype, explant and BAP concentration) according to Tukey's test ($P \leq 0.05$).

Regarding the growth of regenerated explants, the genotype showed an interaction with the type of explant with respect to the shoot length and width and leaf length. Thus, shoots presented the longest regeneration particularly in *A. 'Sweet Laura'*, where the shoot length (3.59 cm) showed significant differences compared with shoots regenerated from leaves (0.29 cm) and stems (1.01 cm). Regenerated shoots were also wider when using the shoot as the explant (0.18 cm) from *A. 'Sweet Laura'*. Maximum leaf length was observed in explants obtained from shoots of *A. caryophyllaea* (0.65 cm) and *A. 'Sweet Laura'* (1.62 cm) (Table 4).

For all the growth parameters evaluated, only shoots and leaves regenerated from shoots showed significant differences when comparing both genotypes. Thus, shoots and leaves showed higher growth in *A. 'Sweet Laura'* explants (Table 4).

Concerning the number of leaves produced per shoot, interactions between factors (genotype, explant and BAP concentration) were not significant, so the factors were independently analyzed. *A. 'Sweet Laura'* showed more leaves per shoot (1.81) than *A. caryophyllaea* (0.88), while shoot explants produced the highest number of leaves per shoot (3.24). This result was significantly different compared with the number of leaves from

Table 4. Genotype and explant effect on the growth of shoots and leaves of *A. caryophyllaea* and *A.* ‘Sweet Laura’ regenerated from aerial explants.

Genotype	Explant	Shoot		Leaf			
		Length (cm)	Width (cm)	Length (cm)	Width (cm)		
<i>A. caryophyllaea</i>	Shoot	1.23	aB	0.08	aB	0.65	aB
	Leaf	0.85	abA	0.02	abA	0.23	aA
	Stem	0.00	bA	0.00	bA	0.00	aA
<i>A.</i> ‘Sweet Laura’	Shoot	3.59	aA	0.18	aA	1.62	aA
	Leaf	0.29	bA	0.03	bA	0.29	bA
	Stem	1.01	bA	0.03	bA	0.73	bA

*Different capital letters indicate significant differences between genotypes for each explant according to Tukey’s test ($P \leq 0.05$).

**Different small letters indicate significant differences among explants for each genotype according to Tukey’s test ($P \leq 0.05$).

the leaf (0.17) and stem (0.63) explants. Finally, 1.0 mg L⁻¹ BAP significantly increased the number of leaves per shoot, revealing significant differences compared with the remaining concentrations evaluated (Table 5).

Histology

Transverse histological sections obtained from induced leaf petiole of *A.* ‘Sweet Laura’ showed that explants cultured in the induction medium (supplemented with 1 mg L⁻¹ BAP) at day 0 presented vascular bundles scattered in parenchyma composed of cells larger than those observed in the epidermis. Small cells with evidence of meristematic development were not observed (Figure 3A). After 7 days, the epidermal layer of the leaf petiole showed a transition to slight bumps on the surface due to the onset of meristem development, which subsequently served as the origin of new shoots by direct organogenesis (Figure 3B).

Discussion

Direct organogenesis from aerial explants

Direct organogenesis occurred in aerial explants of *A.* ‘Sweet Laura’ and *A. caryophyllaea*. Callus development was not observed, which is positive

Table 5. Effect of genotype (n = 180), explants (n = 120) and BAP concentration (n = 90) on the number of leaves produced per shoot regenerated from aerial explants of *A. caryophyllaea* and *A.* ‘Sweet Laura’.

Treatment		N° leaves per shoot	
Genotype	<i>A.</i> ‘Sweet Laura’	1.81	a
	<i>A. caryophyllaea</i>	0.88	b
Explant	Shoot	3.24	a
	Leaf	0.17	b
	Stem	0.63	b
BAP concentration (mg L ⁻¹)	0.0	0.58	b
	0.5	1.52	b
	1.0	4.13	a
	2.0	1.83	b

*Different letters in indicate significant differences within each factor (genotype, explant and BAP concentration) according to Tukey’s test ($P \leq 0.05$).

for the clonal propagation of cultivars because the risk of genetic variability induction by somaclonal regeneration is reduced. This result is consistent with results obtained by Lin *et al.* (1997; 1998) and Nasri *et al.* (2013) in *astroemeria*, where callus production was not observed. Nevertheless, other studies suggested that shoots and leaves cultured in media supplemented with TDZ and IBA (Cruz *et al.*, 2003) or BAP and KIN (Pedraza-Santos *et al.*, 2006) tend to produce callus development

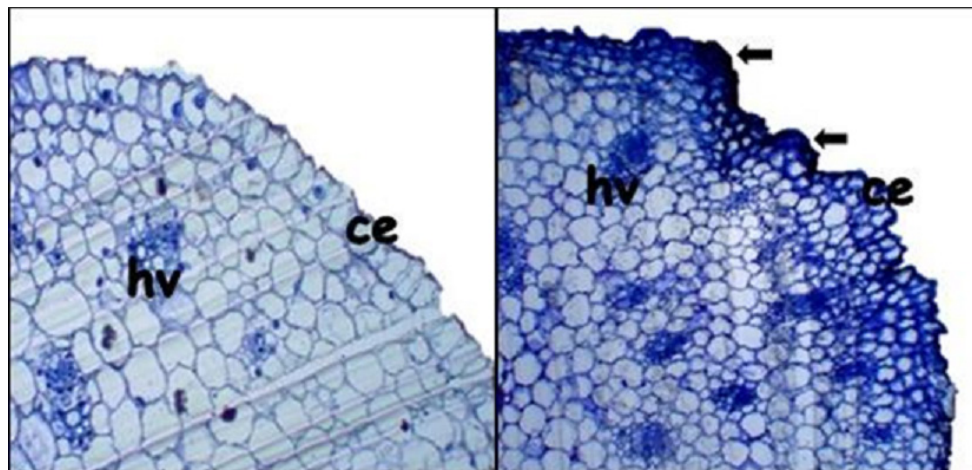


Figure 3. Transverse histological sections obtained from induced leaf petiole of *A.* 'Sweet Laura' at day 0 (a) and after 7 days of in vitro culture on induction medium.

*Arrows indicate bumps due to the starting of meristem development.

* ce = epidermal layer; hv = vascular bundles.

with the potential to induce somatic embryogenesis. Lin *et al.* (2000) observed the generation of a compact callus from nodal stem segments, while Pedraza-Santos *et al.* (2006) suggested that leaves and stems are explants with the potential to generate an embryogenic callus.

Direct organogenesis was observed in leaves by Lin *et al.* (1997) and Nasri *et al.* (2013) and in immature inflorescences by Pedraza-Santos *et al.* (2006). Regarding regeneration from leaves, Lin *et al.* (1997) reported similar results to those obtained in this study, observing petiole elongation and widening of the nodal zone. In addition, Lin *et al.* (1998) and Nasri *et al.* (2013) reported induction from the area between the base of the leaf and the stem. Moreover, Lin *et al.* (1997) suggested that the length of the petiole adhered to the leaf could affect the regeneration rate and the number of shoots induced.

Induction of new shoots from aerial explants

Only stem sections of *A.* 'Sweet Laura' were induced, whereas no induction from this explant was observed in *A. caryophyllaea*. Pedraza-Santos *et al.* (2006) failed to generate direct organogenesis

from stems of *Alstroemeria* cv. 'Yellow King', and induction occurred only in inflorescences (40%). However, Lin *et al.* (1997) observed 36% shoot induction in leaves of *Alstroemeria* 'VV024'. In this study, only 15 and 18.3% induction was observed from leaves of *A. caryophyllaea* and *A.* 'Sweet Laura', respectively. However, induction from shoots reached up to 63.3% in *A.* 'Sweet Laura' (Table 1). Hence, clear effects of the genotype and the type of explant were noted when alstroemeria was propagated in vitro.

Considering the effect of growth regulators in this study, 1 mg L⁻¹ BAP produced the highest induction rate regardless of the genotype and explant type used. Nasri *et al.* (2013) obtained similar results with explants of an *Alstroemeria ligtu* hybrid using supplementation with 1 mg L⁻¹ BAP and 0.1 mg L⁻¹ of AIB. By contrast, Lin *et al.* (1997) observed the highest explant induction of *Alstroemeria* 'VV024' with 2.2 mg L⁻¹ TDZ. Thus, cytokinins seem to be crucial for the induction of new shoots from aerial explants of alstroemeria. Nevertheless, treatments without BAP supplementation led to induction in both *A. caryophyllaea* (4.4%) and *A.* 'Sweet Laura' (11.1%) (Table 2). According to related literature (George *et al.*, 2008; Ongaro and Leyser, 2008),

those explants that result in induction despite being grown in cytokinin-free media may reflect the effect caused by endogenous hormones.

For both genotypes, the induction rate responded positively to increasing concentrations of BAP until peaking at 1 mg L⁻¹ BAP. Afterward, the induction rate decreased with supplementation of 2.0 mg L⁻¹ BAP (Table 2). Nasri *et al.* (2013) agreed with these results, also reporting a peak of shoot induction from alstroemeria aerial explants when using 1 mg L⁻¹ BAP. According to Hutchinson *et al.* (2010), high levels of growth regulators inhibit optimal morphogenic responses in alstroemeria explants, possibly due to a negative feedback mechanism. Moreover, to increase in vitro explant induction, Pedraza-Santos *et al.* (2006) suggested the use of liquid medium since they observed the induction of a greater number of adventitious buds. Increased diffusion of ions and oxygen and thus increased absorption of nutrients by the explant, unlike gelled culture medium, could explain the effects of liquid medium.

Regeneration of induced explants

As previously mentioned, direct regeneration was observed after explant induction. All the explants that achieved regeneration produced only one shoot. These results differ from the results obtained by Nasri *et al.* (2013), who observed the development of adventitious buds reaching regeneration of up to 2.33 shoots per induced explant after 8 weeks. Furthermore, Pedraza-Santos *et al.* (2006) achieved 2.7 buds per explant after 4 weeks by supplementing the culture media with KIN (2.5 mg L⁻¹) and BAP (1.5 mg L⁻¹) and growing the explants in complete darkness and 8 °C for 15 days. On the other hand, Lin *et al.* (1998) observed higher organogenesis from young leaves excised closer to the apex (82.5%) and a greatest number of shoots per explant (4.5).

Considering the results of this study and those obtained by other authors (Hoshino, 2008; Pum-
isutapon *et al.*, 2011; Kyte *et al.*, 2013), the best regeneration results of alstroemeria explants were

obtained by supplementing the culture media with cytokinins. Furthermore, Seyyedyousefi *et al.* (2013) suggested that low concentrations of auxins (ANA) are also required for the development and growth of leaf primordia during the regeneration of alstroemeria explants.

Explants cultured with 1.0 mg L⁻¹ BAP showed more growth and development of regenerated plants, for example in the number of leaves per stem (Table 5) and the shoot length (data not shown). Growth regulator supplementation during in vitro culture, such as TDZ (2.2 mg L⁻¹), BAP (1 mg L⁻¹) and NAA (0.1 mg L⁻¹), significantly increased the length of regenerated shoots obtained from *A. aurantiaca* 'Rosita' and the number of leaves (Hutchinson *et al.*, 2010).

Cytokinins cause shoot induction by stimulating cell division and reducing apical dominance which, according to Seyyedyousefi *et al.* (2013), explains why higher BAP concentrations decrease in shoot length. Nasri *et al.* (2013) observed 7.3 and 10.7 leaves per explant from an *Alstroemeria ligtu* hybrid after 4 and 8 weeks, respectively, in medium supplemented with 1 mg L⁻¹ BAP and 0.1 mg L⁻¹ AIB. These results are higher than those obtained in the present study, where 3.59 was the maximum number of leaves per explant (Table 4). However, it is also important to consider the effect of the genotype during regeneration.

Effect of oxidation on explant regeneration

For some explants, oxidation was observed, likely related to regeneration, as an increase in oxidation seems to reduce regeneration. Thus, 100% of oxidation observed in stems was related to poor regeneration (5%). However, shoots showed only 2% oxidation and the highest regeneration rate (30%) (Figure 4). Furthermore, considering that significant differences were observed among the explants in terms of oxidation rate, different susceptibilities of the explants to oxidation could be suggested.

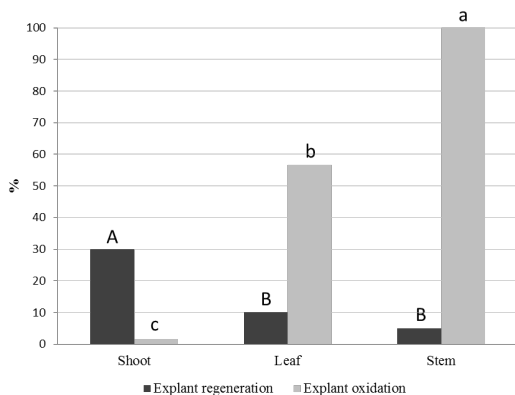


Figure 4. In vitro regeneration and oxidation of aerial explants of *A. 'Sweet Laura'* and *A. caryophyllaea*.

*Different capital letters indicate significant differences among explants for % of regeneration according to Tukey's test ($P \leq 0.05$).

**Different small letters indicate significant differences among explants for % of oxidation according to Tukey's test ($P \leq 0.05$).

The effect of oxidation on explant regeneration could be explained by energy accumulation when light absorption in leaf tissue exceeds the photosynthetic capacity of this organ, which subsequently produces reactive oxygen species and triggers photooxidative deterioration (Apel and Hirt, 2004). Lin *et al.* (1998) also observed browning in explants without

regeneration, while no oxidation was observed by Pedraza-Santos *et al.* (2006) when growing in vitro explants in darkness.

Considering the promising results of this research, particularly for regeneration using shoots as explants, this protocol could be implemented for in vitro propagation of *A. caryophyllaea* and *A. 'Sweet Laura'* to avoid the limitations of in vivo propagation. Moreover, further studies should be conducted to adjust this protocol to other cultivars of alstroemeria.

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Resumen

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Alstroemeria es una especie importante en el mercado de plantas ornamentales y flores de corte y su propagación es principalmente realizada por división de rizomas. Sin embargo, este método es ineficiente, altamente demandante de tiempo y puede contribuir a la dispersión de virus. Este estudio tuvo como objetivo desarrollar un protocolo de propagación in vitro usando explantes aéreos de *Alstroemeria caryophyllaea* y *A. cv. 'Sweet Laura'*, dos genotipos aromáticos. Los explantes aéreos de alstroemeria fueron regenerados probando tres tipos de explantes y cuatro concentraciones de citoquininas. Tanto *A. caryophyllaea* como *A. cv. 'Sweet Laura'* mostraron la mayor tasa de inducción desde brotes, con valores de 35 y 63,3%, respectivamente. Para ambos genotipos la concentración óptima de BAP para desarrollar organogénesis directa desde brotes fue de 1.0 mg L⁻¹. Finalmente, se generó un protocolo exitoso para la propagación in vitro de *A. caryophyllaea* y *A. cv. 'Sweet Laura'* a partir de explantes aéreos.

Palabras clave: *Alstroemeria caryophyllaea*, *Alstroemeria cv. 'Sweet Laura'*, cultivo de tejidos, reguladores de crecimiento.

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