

Apical Dominance in *Alstroemeria* Cultured In Vitro

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Abstract

Apical dominance in *Alstroemeria* is studied to develop an improved propagation protocol for this crop. Four types of explants were prepared: an intact rhizome with two intact shoots (+R+2S), an intact rhizome with two decapitated shoots (+R-2S), a decapitated rhizome with two intact shoots (-R+2S), and a decapitated rhizome with two decapitated shoots (-R-2S). The explants were cultivated on solid MS medium with 9 μ M 6-benzylaminopurine (BAP). -R-2S explants showed the highest and +R+2S the lowest axillary-bud outgrowth. Outgrowth in -R+2S and +R-2S explants was intermediate. So, axillary buds are released by removal of the rhizome tip and by removal of the shoot tips. In both decapitated shoots and decapitated rhizomes, application of lanolin with 3-indolebutyric acid (IBA) to the cut end restored apical dominance. This indicates that both tips exert an effect via basipetally transported auxin.

INTRODUCTION

Alstroemeria plants consist of aerial shoots and a rhizome. Growth occurs sympodially: at each node, the apex of the horizontally growing rhizome changes direction of growth from horizontal to vertical, forms a shoot, and then stops growing. At the same time, the first axillary bud at the node grows out horizontally and continues as rhizome. Thus, the rhizome is composed of segments of axillary shoots. The second (next higher) axillary bud in a shoot may stay dormant or may develop into a lateral rhizome (Van Schaik, 1998). Micropropagation by forced outgrowth of the second axillary bud is the preferred method for *Alstroemeria* (Pedersen et al., 1996), but gives a low multiplication rate (ca. 1.2-1.8 per four weeks). To increase the multiplication rate, several factors have been studied (Pierik et al., 1988), but no significant improvements have been achieved. Elucidation of the mechanism(s) of apical dominance seems to be highly relevant to improve propagation but has received little attention.

Apical dominance is the control over the outgrowth of axillary buds exerted by the shoot apex. It has been found early that auxin produced at the shoot apex and transported basipetally has a major role in the inhibition of axillary bud outgrowth (review in Cline, 1994). Recently, evidence has been obtained for the occurrence of a carotenoid-like, inhibitory regulator (Mouchel and Leyser, 2007). Bond and Alderson (1993) report that removal of both aerial shoot and rhizome tips significantly increases rhizome branching. This suggests that both tips exert apical dominance over axillary buds, thus preventing branching in *Alstroemeria*. However, addition of the auxin transport inhibitor 2,3,5-triiodobenzoic acid (TIBA) in the culture media does not lead to an increased multiplication rate, suggesting that the mechanism of apical dominance in *Alstroemeria* is a complex phenomenon (Bond and Alderson, 1993). These data imply that a better understanding of how apical dominance controls axillary bud outgrowth is required for improvement of *Alstroemeria* propagation.

Aim of this work was to investigate the role of aerial shoot and rhizome tips and exogenous auxin application in the control of axillary bud outgrowth.

MATERIALS AND METHODS

Established cultures of two *Alstroemeria* cultivars '24098 2B' and 'Sara' were obtained from Könst Alstroemeria (Nieuwveen, NL) and Van Zanten Plants (Rijsenhout,

NL) respectively. In experiment 1, four different types of explants were prepared (Fig. 1). An intact rhizome with two intact shoots (+R+2S), an intact rhizome with two decapitated shoots (+R-2S), a decapitated rhizome with two intact shoots (-R+2S), and a decapitated rhizome with two decapitated shoots (-R-2S). In experiment 2, we applied IBA-lanolin (0, 0.1, 1.0, 10, and 100 mg/g) to all cut ends of -R-2S explants. In experiment 3, we applied IBA-lanolin (30 mg/g) to separate cut ends of -R-2S explants. All explants were cultivated in MS medium containing 9 μ M BAP + 40 g/L sucrose + 2 g/L gelrite (pH 5.8). The number of lateral rhizomes was measured after a four week period. It should be noted that the shoot apex is located 'above' the inhibited axillary bud, and the rhizome tip 'below'.

RESULTS AND DISCUSSION

In plants with a strong apical dominance, axillary buds hardly grow out unless they are decapitated (Cline, 1994). To investigate the role of aerial-shoot and rhizome tips in the inhibition of axillary-bud outgrowth, we analyzed bud outgrowth in explants from which aerial shoots and/or the rhizome tip had been removed. Bud outgrowth in both cultivars was similar. -R-2S explants showed the highest bud outgrowth indicated by the maximum number of lateral rhizomes. +R+2S explants had the lowest bud outgrowth and bud outgrowth of +R-2S and -R+2S was intermediate (Fig. 2). These data indicate that both aerial shoots and the rhizome tip inhibited axillary-bud outgrowth in *Alstroemeria*. This corresponds to the previous observation by Bond and Alderson (1993).

Since the apical tip is the primary site of biosynthesis of inhibitory auxin, and since in other species, lanoline paste with auxin may restore apical dominance after decapitation, we applied IBA-lanolin to the cut ends of shoots and the rhizome in -R-2S explants. Increasing concentrations of IBA-lanolin from 0 to 100 mg/g reduced bud outgrowth, compared to untreated control. The highest reduction was found at 100 mg/g IBA-lanolin. Lanolin itself (0 mg/g IBA-lanolin) showed a very small inhibitory effect (Fig. 3). These observations correspond to the observations in many herbaceous and woody plants (Cline, 1996, 2000) and in the classical auxin-replacement experiment in *Vicia faba* (Thimann and Skoog, 1933).

In the experiment shown in Figure 4, a paste with 30 mg IBA/g lanolin was applied to the cut ends of either shoots or rhizome or to both. IBA reinstated apical dominance at all positions. It should be noted that in all experiments, axillary-bud outgrowth at shoot 1 (S1) which is far from rhizome tip, was higher than at shoot 2 (S2) which is next to rhizome tip (Fig. 1 and data not shown). This may be due to axillary buds at different locations having an unequal potential growth rate (Miguel et al., 1998) or to distinct levels of apical dominance.

CONCLUSIONS

In *Alstroemeria*, decapitation of rhizome and shoot tips releases the inhibited axillary buds from the control exerted by apical dominance. Apical dominance can be restored by application of exogenous auxin to the cut ends of decapitated rhizome and/or shoots. Interestingly, the aerial-shoot tip is located above the inhibited axillary bud, but the rhizome tip is located below. Thus, direct inhibition by auxin transported downwards from the rhizome tip is difficult to conceive. It has been suggested that the extent of apical dominance is related to the capacity of a stem to transport auxin (Bennett et al., 2006).

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Literature Cited

Bennett, T., Sieberer, T., Willett, B., Booker, J., Luschnig, C. and Leyser, O. 2006. The *Arabidopsis MAX* pathway controls shoot branching by regulating auxin transport. *Curr. Biol.* 16:553-563.

- Bond, S. and Alderson, P.G. 1993. The influence of apical dominance on the in vitro multiplication of the rhizome of *Alstroemeria*. *J. Hort. Sci.* 68:905-910.
- Cline, M.G. 1994. The role of hormones in apical dominance. New approaches to an old problem in plant development. *Physiol. Plant.* 90:230-237.
- Cline, M.G. 1996. Exogenous auxin effects on lateral bud outgrowth in decapitated shoots. *Ann. Bot.* 78:255-266.
- Cline, M.G. 2000. Execution of the auxin replacement apical dominance experiment in temperate woody species. *Amer. J. Bot.* 87:182-190.
- Miguel, L.C., Longnecker, N.E., Ma, Q., Osborne, L. and Atkins, C.A. 1998. Branch development in *Lupinus angustifolius* L. I. Not all branches have the same potential growth rate. *J. Exp. Bot.* 49:547-553.
- Mouchel, C.F. and Leyser, O. 2007. Novel phytohormones involved in long-range signaling. *Curr. Opin. Plant Biol.* 10:473-476.
- Pedersen, C., Hansen, C.W., Brandt, K. and Kristiansen, K. 1996. *Alstroemeria* plantlets can be induced to flowering by cold treatment during in vitro culture. *Sci. Hort.* 66:217-228.
- Pierik, R.L.M., Van Voorst, A., Booy, G., Van Acker, C.A.M., Lelivelt, C.L.C. and De Wit, J.C. 1988. Vegetative propagation of *Alstroemeria* hybrids in vitro. *Acta Hort.* 226:81-89.
- Thimann, K.V. and Skoog, F. 1933. Studies on the growth hormones of plants. III. The inhibition action of growth substance on bud development. *Proc. Nat. Acad. Sci. USA* 19:714-716.
- Van Schaik, C.E. 1998. Regeneration and transformation of *Alstroemeria*. Ph.D. Thesis. Wageningen Agricultural University, Wageningen.

Figures

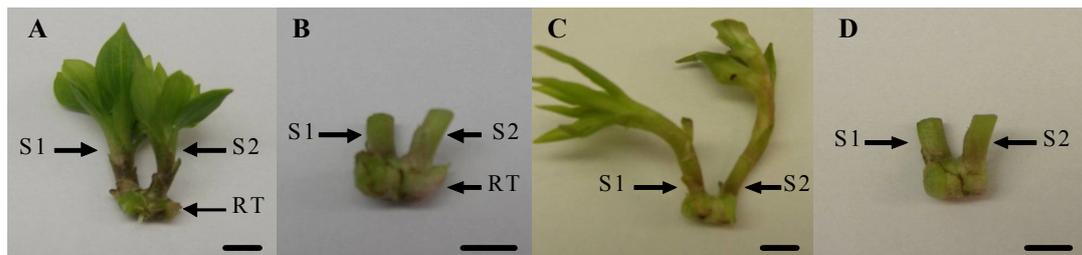


Fig. 1. The four types of explants used in the studies: +R+2S (A), +R-2S (B), -R+2S (1C), -R-2S (D). S1 and S2 are the aerial shoots; RT is the rhizome tip. All Bars are 5 mm.

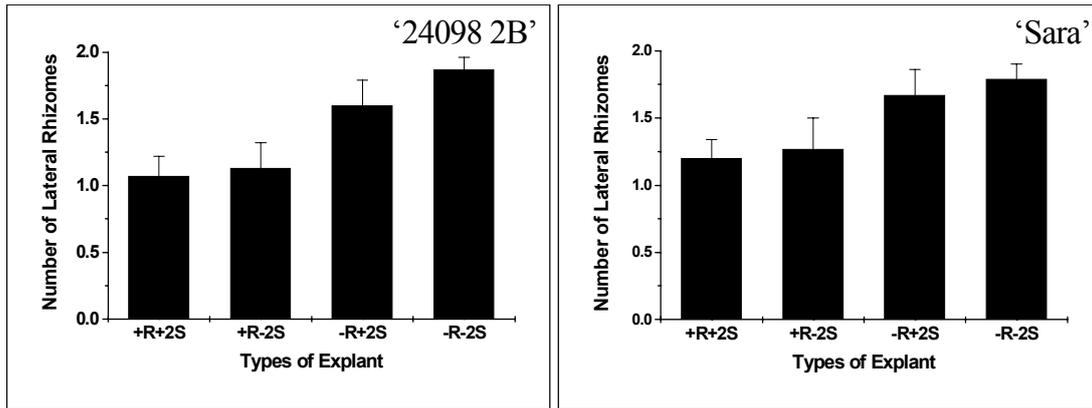


Fig. 2. Effect of decapitation of the aerial shoot or the rhizome tips on outgrowth of axillary buds into lateral rhizomes in cultivars '24098 2B' and 'Sara', n = 15, bars indicate SEM.

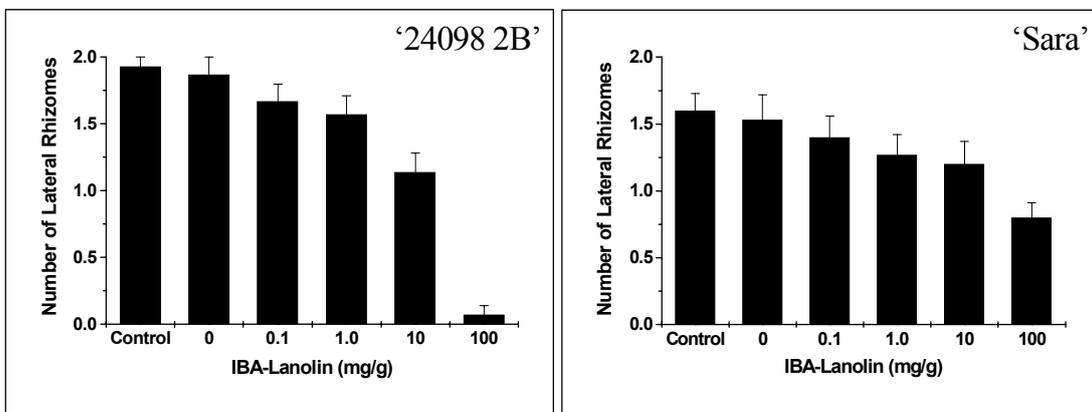


Fig. 3. Effect of application of IBA-lanolin (0-100 mg/g) on outgrowth of axillary buds into lateral rhizomes in -R-2S explants of cultivars '24098 2B' and 'Sara', n = 15, bars indicate SEM.

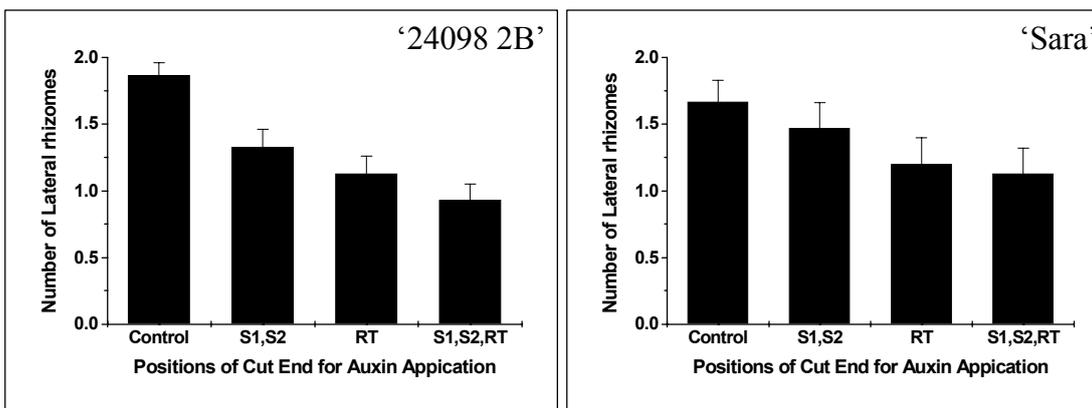


Fig. 4. Effect of application of IBA-lanolin (30 mg/g) on outgrowth of axillary buds into lateral rhizomes in -R-2S explants of cultivars 2B' and 'Sara', n = 15, bars indicate SEM.