Micropropagation of *Alstroemeria* Hybrids

Mark P. Bridgen[1], Joseph J. King, Charlotte Pedersen, Mark A. Smith, and Paul J. Winski

Department of Plant Science, U-67, 1376 Storrs Road, The University of Connecticut, Storrs, Connecticut 06269 U S A

Alstroemeria hybrids are grown for their cut flowers and, more recently, as potted and garden plants. Their beautiful, large inflorescences with colors of purple, lavender, red, pink, yellow, orange, white and bicolors have made them the tenth most popular cut flower at the Dutch flower auction. At the auction, their U.S. dollar value in 1990 was approximately $35,263,000 In addition to having beautiful colors, the flowers have long postharvest vase lives of 2-3 weeks. The plants prefer cool temperatures for growth, and once flowering has been initiated, an everblooming habit produces a high yield of flowers until flowering has ceased.

Alstroemeria is traditionally propagated asexually by the division of their rhizomes. The triploid nature of most commercial cut flower cultivars makes asexual propagation essential. However, this procedure is too slow and tedious for large, commercial propagators. Consequently, the dissemination of new cultivars is delayed and the cost of propagules is inflated Seed propagation from diploid and tetraploid *Alstroemeria* is possible and is used in the United States to produce mixed hybrids for large potted plants. However, due to the heterozygous nature of current cultivars, it is difficult to obtain consistent lines. Breeders are trying to develop homogeneous inbred seed lines and F1 hybrids, but at this point seeds are not commercially available.

Commercial propagators of *Alstroemeria* have resorted to rapid proliferation through micropropagation as a way to produce large numbers of clones. *In vitro* propagation can produce very large quantities of plants in a small space and is independent of the weather. In addition, pathogen free plants can be produced.

Research on the micropropagation of *Alstromeria* has been ongoing at the University of Connecticut since 1985 when our breeding program began. The following paper will summarize the information we have learned about propagation procedures from Stage I to Stage IV. Additional information on micropropagation of *Alstroemeria* which was learned from commercial and private laboratories and from published papers will also be presented. References will be given for work which was not completed in our laboratory.

STAGE 1—INITIATION OF ASEPTIC CULTURES

If seeds are used as the original explant source, it is very easy to start a culture. Simply place the seeds in 5% sodium hypochlorite for 10-15 minutes with constant stirring, rinse theseeds twice in sterile deionized water, and culture at 17-19°C. The

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[1]Author to whom communications should be addressed.

temperature is very important for quick germination in 10-14 days.

In situations where asexual clones are desirable, seed germination cannot be used. It is often very difficult to disinfect underground storage organs such as rhizomes, and *Alstromeria* is no exception A procedure has been outlined which will give 100% clean explants if proper techniques are followed

Terminal rhizome tips approximately 0.5-1.5 cm in length should be freshly harvested from actively growing plants. None of the shoots behind the growing point should be included with the growing tip Apical shoots should be used for the initial explant because they will have a faster multiplication rate than lateral shoots arising from the rhizome. Although some success culturing vegetative or floral shoots has been reported (Ziv et al., 1973), these are not the ideal tissues to use for micropropagation.

When harvesting rhizome tips, they should be placed in plastic bags or moist paper towels to prevent dessication. They should not be placed in water because of the potential uptake of pathogens by the vascular system may cause problems. Rhizome tips should have all visible media particles cleaned off under running tap, water with a fine bristled brush. The explants are then placed in 3% Korsolin (Ferrosan Co., 4600 Kose, Denmark) or 1% sodium hypochlorite for 10 min. Korsolin is a commercial greenhouse bench disinfectant which contains 3 8% formaldehyde and 8% glutaraldehyde. Explants are taken to the laminar flow hood after the initial sterilization step and placed in sterile distilled water before subsequent treatments. Explants further cleaned under a dissecting microscope and subsequently placed in 1% sodium hypochlorite for 5 min. followed by sterile water. The 2-4 outer leaves surrounding the explant should then be aseptically removed to expose the small growing point and rhizome. This piece is placed in 0.5% sodium hypochlorite for 1 minute and then sterile water until cultured. Most explants willproduce a whitish exudate at the point of incision; although this looks like a bacterium infection, it is not a contaminant

STAGE II - MULTIPLICATION

The components of the nutrient medium are critical for the proper development of *Alstromeria* rhizomes. When KH_2P0_4 levels were varied and all other Murashige and Skoog (MS) (1962) levels maintained the same, explants receiving KH_2PO_4 levels of 1.25-2.5 mM produced the most shoots and growing points and greatest fresh weight (Elliott, et al , 1992) Smith and Bridgen examined effects of variations in nitrogen, calcium, iron and magnesium concentrations on the growth and proliferation of rhizome explants *in vitro*. They observed significant linear responses to increased nitrogen up to 80 mM, but no differences to varying $NH_4:NO_3$ ratios. Low levels of $CaCl_2$ ranging from 0.09-0.3 mM were superior to high levels. When iron in the form of ferric EDTA was examined at levels up to 1 mM, treatments from 0.01-0.5 mM had the best response over time Magnesium sulfate levels up to 15 mM were tested and the highest level was superior for plant growth when Gel-Gro was used as a medium solidifier.

Experiments examining pH values of 5, 6, and 7 showed no differences; experiments examining sucrose levels from 1-5% demonstrated 3-4% sucrose is optimal (Pierik et al., 1988). In Smith and Bridgen's research (1992), rhizomes growing on media solidified with agar were compared to rhizomes on media solidified with Gel-Gro. In all cases, plantlets grew best when the medium was solidified with

Table 1. *Alstroemeria* medium

	Elements	Concentration (mM)
Macronutrients	NH_4NO_3	20.0
	KH_2PO_4	1.25
	KNO_3	19.0
	NH_4Cl	20.0
	$CaCl_2 \cdot 2H_2O$	0 3
	$MgSO_4 \cdot 7H_2O$	15 0
	NaFe-EDTA	0.1
Micronutrients	Murashige and Skoog levels	
Organics	Murashige and Skoog vitamins 30 g liter^{-1} sucrose	
Growth regulators (suggested ranges)		
Stage I and II:	4-22 µM Benzylaminopurine (1-5 mg liter^{-1}) (BAP)	
	0.054 µM Naphthaleneacetic acid (0.01 mg liter^{-1}) (NAA)	
Stage III.	0.54-2 7 µM NAA (0.1-0.5 mg liter^{-1}]	
Solidifying agent	1.2g liter^{-1} Gel Gro (w/v)	
Medium pH	5 6±0.1	

Gel-Gro. Although refinements of the *Alstroemeria* medium continue, a medium is presented in Table 1 which considers the data as well as important nutrient rates.

The type of cytokinin and its concentration are important factors for *in vitro* multiplication of *Alstroemeria*. Kinetin, 6-benzylaminopurine (BAP), and 6-(gamma, gamma) dimethylallylaminopurine (2iP) stimulate the branching of rhizomes and the latter two suppress the elongation of shoots as their concentration is increased (Gabryszewska and Hempel, 1985; Pierik et al 1988). BAP is most often recommended for *Alstroemeria* micropropagation in the range from 4.4-22 µM for increased numbers of rhizome branches and shorter lengths of erect shoots. Due to the variability in cultivar responses, there is no exact concentration of cytokinin to recommend; each laboratory should test their cultivars at the

recommended levels. Levels of cytokinin greater that 25 µM should be avoided due to the increased probability of mutations

The auxins, naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA) have been reported not to affect multiplication rates other than in an inhibitory role when applied at concentrations greater than 5 µM, (Pierik et al. 1988) However, earlier reports have shown stimulated growth when low levels of IBA or NAA are added to the multiplication medium (Gabryszewska and Hempel, 1985). Experience in our laboratory has shown advantages to adding a low level of NAA (Bridgen et al., 1990)

Experiments on the influence of temperature of rhizome multiplication have demonstrated that cultures growing at 18°C produce less of the black/brownish exudate than those growing at higher temperatures (Pierik et al , 1988). The exudate is common on some *Alstroemeria* cultivars, but is not a contaminate. Multiplication rates of rhizomes were not affected by temperatures in the range of 15-21°C. Temperatures as low as 8°C have been used successfully (Lin and Monette, 1987), but most commercial laboratories now grow *Alstroemeria* at 18±1°C

Irradiance levels from 1.5-9.7 W/m^2 were tested on *Alstroemeria* and showed no significant effect on multiplication rates; however, shoot length was decreased as irradiance increased (Pierik et al, 1988). Work done in our laboratory has shown high light as a disadvantage for multiplication. There is a negative correlation in the number of propagules produced as irradiance increases from 50 to 300µ mol s^{-1} m^{-2}. Also, when photoperiods between 8 and 16 hours were tested, no difference in the multiplication rate was noticed. Reports of a 1-2 week dark period at the beginning of the culture state it is either advantageous Lin and Monette, 1987) or of no harm to the plants (Pierik et al , 1988).

After cultures are established, the fastest multiplication will continue if explant divisions consist of a single growing point with a single shoot attached. The shoot should be decapitated at isolation to a length of 5 mm.

The length of the multiplication cycle and the number of cycles are also important factors for rhizome multiplication. A higher multiplication rate with less and shorter upright shoots was observed when plantlets were subcultured every 3 weeks for 5 cycles than when they were subcultured every 3.75, 5, or 7 5 weeks with 4, 3, or 2 cycles, respectively (Pierik et al , 1988).

Liquid media have been shown to have higher multiplication rates at lower cytokinin levels than semi-solid media, however vitrification is often a problem (Pierik et al , 1988) Commercial laboratories currently avoid liquid media for *Alstroemeria* due to vitrification problems.

STAGE III - ROOTING

Alhough the actual root development of *Alstroemeria* occurs during Stage IV, root initiation begins in Stage III. The initial explants will usually contain a growing bud with 2-3 additional, decapitated lateral shoots; this is slightly larger than explants for Stage II. Cytokinins should be left out of the Stage III medium. For optimal rooting, NAA should be added from 0.54-2.7 µM. IBA is adequate for root initiation, but it is less effective than NAA (Gabryszewska and Hempel, 1985).

Although no data are presented, Pierik et al. (1988) state that higher sucrose levels of 5% are better for rooting than the 3% used for stage II. They also state that

a photoperiod of 16 hours is superior to 8 hours and that a pH of 6 is better than pH values of 5 or 7

STAGE IV - ACCLIMATION

After 3-4 weeks on the Stage III medium, roots will be visible on the *Alstroemeria*. Plantlets can then be removed from the culture vessel and washed under warm tap water to remove the medium. Plugs or pots approximately 2.5 - 3 cm in size can be filled with pasteurized and pH-adjusted peat moss and used at this stage.

After removal from the culture vessels, plantlets can be acclimated in two ways They can be placed into a secondary growth room with high humidity and 12-16 hours of light or they can be placed into a shaded greenhouse. If the greenhouse is used, the plantlets should be tightly covered with plastic or placed in a fog or mist house to maintain high humidity After 4-6 weeks, plantlets may be removed from the secondary growth room, transplanted into larger pots and moved to the regular growing area. Fungicide should be applied regularly, as needed, to acclimating plantlets.

CONCLUSION

The micropropagation of *Alstroemeria* has been studied intensively during the past 6 years. This presentation reviews important aspects and procedures for success during Stages I-IV. However, this paper does not suggest one growth regulator formula for the successful culture of all cultivars. Micropropagators of *Alstroemeria* must realize that cultivars vary with their response to growth regulators and environmental conditions. Each laboratory should dedicate some resources to determine the optimum procedures for its specific cultivars.

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