**Effect of growth retardants, cytokinins and auxins
on the multiplication and rooting in vitro of
Alstroemeria x hybrida „Juanita”**

**MALGORZATA PODWYSZYŃSKA, ELEONORA GABRYSZEWSKA,
ANDRZEJ PRZYBYŁA**

Research Institute of Pomology and Floriculture, ul. Pomologiczna 18, 96-100 Skierniewice, Poland

(Received: October 1, 1998)

Abstract

Rhizome cultures of „Juanita” Polish cultivar of *Alstroemeria x hybrida* were used to enhance an effectiveness of micropropagation method of new cultivars and selections.

The effect of cytokinins (BAP, kinetin and 2iP), auxins (IAA, IBA and NAA), growth retardants (paclobutrazol and flurprimidol) alone or in combination were studied in relation to rhizome branching, aerial shoot production and rooting of rhizome. The greatest number of aerial shoots as well as the shortest shoots were observed at the highest BAP concentration (6 mg l⁻¹). However, the rhizomes had the poorest rooting ability. BAP at low concentrations combined with kinetin or 2iP also strongly stimulated aerial shoot formation and rhizome branching. Unfortunately, those shoots were of poor quality. Application of BAP at low concentration with paclobutrazol (0,1–0,5 mg l⁻¹) or flurprimidol (0,01–0,1 mg l⁻¹) in presence of 1 mg l⁻¹ NAA resulted in high number of aerial shoots (5–6), reduction of their length and higher rooting ability of the rhizomes. Growth retardants applied with NAA strongly stimulated formation of the roots but suppressed their elongation.

Abbreviations: BAP – 6-benzylaminopurine; kinetin – 6-furfurylaminopurine; 2iP – 6-[γ , γ -dimethylallylamino]purine; IAA – indole-3-acetic acid; IBA – indole-3-butyric acid; NAA – naphthaleneacetic acid; paclobutrazol (ICI PP-333) – (2-RS,3-RS)-1-(4-chlorophenyl)-4,4-dimethyl-2(1,2,3-triazol-1-yl)-pentan-3-ol; flurprimidol (Dowelanco) – α -(1-methylethyl)- α -[4-trifluoromethoxy]phenyl]-5-pyridinemethanol.

INTRODUCTION

Alstroemeria belongs to the first group of ten most valuable cut flowers. Considering many good features of alstroemeria such as high productivity, wide color range, low energy requirement for cultivation and a long vase-life, this plant merits to become more popular. Traditional propagation method by division of rhizome is too slow for large scale production, therefore micropropagation through rhizome multiplication has been developed (Gabryszevska and Hempel, 1985;

Gabryszewska, 1995, 1996; Bond and Alderson, 1993ab). However, in case of some Polish cultivars, multiplication rate and rhizome rooting obtained on standard media containing BAP and NAA (Gabryszewska and Hempel, 1985) or on medium modified by addition of 2,5 mg l⁻¹ kinetin (Podwyszyńska, 1997) were unsatisfactory. Therefore, on the authority of many authors who applied growth retardants for in vitro cultures, paclobutrazol and flurprimidol were incorporated in study to stimulate bud formation and to improve quality of rhizomes appropriate for direct transplanting into greenhouse. Chin (1982) was the first who reported that growth retardant ancymidol promoted shoot and root formation of asparagus in vitro culture. Further study showed that ancymidol and paclobutrazol combined with cytokinins induced strong shoot proliferation (Ziv, 1990). In spite, Bond and Alderson (1993b) found that paclobutrazol did not influence alstroemeria growth, whereas changed explant morphology.

The objective of this study was to improve efficiency in multiplication and rooting stages through obtaining branched and easy-to-root rhizomes containing numerous, short aerial shoots.

MATERIALS AND METHODS

Polish cut flower cultivar of *Alstroemeria x hybrida* – „Juanita” was used for this study. It was bred with gamma rays in 1984 and registered in 1990 (Przybyła 1992a,b, 1996). This is mutant of sterile hybrid with orange red flowers, stem height of 90 cm and good keeping quality. „Juanita” starts flowering in March giving production in greenhouse 190 flower stems m⁻² in the 1st year, 2nd year – 220 and in plastic tunnel – 230. Year-round blooming is possible under good light condition. In vitro rhizome cultures were initiated from apical and axillary rhizome tips as described by Gabryszewska (1995). Then, these rhizomes were cultured on the basic medium described below and supplemented with 2,5 mg l⁻¹ BAP, 2,5 mg l⁻¹ kinetin and 1 mg l⁻¹ NAA (Podwyszyńska, 1997). This composition of plant growth regulators was used as the control for examination of the BAP and 2iP effect (Table 1B). In all experiments rhizome cultures were grown on the following basic medium: Murashige and Skoog (1962) macro- and microelements, 40 g l⁻¹ sucrose, 40 mg l⁻¹ adenine, 100 mg l⁻¹ myoinositol, 100 mg l⁻¹ glutamine, 10 mg l⁻¹ glycine, 5 mg l⁻¹ nicotinic acid, 10 mg l⁻¹ thiamine, 1 mg l⁻¹ pyridoxine, 0,2% Gelrite. The pH was adjusted to 5,6 prior to autoclaving at 120°C for 15 min. Rhizome cultures were incubated in growth chamber at 25°C under 16 h photoperiod provided by warm-white fluorescent lamps at 55 mmol m⁻²s⁻¹ photosynthetic photon flux density. For the experiments the uniform rhizome segments were used with apical rhizome tip and two aerial (upright growing) shoots which were cut off above the 1st internode. These rhizome segments derived from the rhizome cultures established on BM medium containing 2,5 mg l⁻¹ BAP and 2 mg l⁻¹ NAA. They were cultured in jars (330 ml) containing 40 ml of a medium, at 5-week interval.

For the experiments, concentrations of cytokinins and auxins were chosen on the basis of the previous study (Gabryszewska and Hempel, 1985). Effect

of cytokinins (BAP, kinetin and 2iP) and growth retardants (paclobutrazol – ICI and flurprimidol – Dowelanco) was studied in relation to rhizome branching, aerial shoot production and growth, and rooting ability of rhizome. All growth regulators were added into the media before autoclaving. Data were collected 5 weeks later: number of aerial shoots per rhizome, aerial shoot length, number of roots per rhizome and rhizome multiplication rate defined as the number of separable rhizome segments (explants with one apical rhizome tip and 1 – 3 aerial shoots cut off under 1st internode) obtained after division of the primary rhizome.

Effect of different auxins (NAA, IBA, IAA) alone or combined with growth retardants was studied at the rooting stage under in vitro conditions and subsequently, after transfer of the rhizomes into a greenhouse. Rooting in vitro was evaluated 4 weeks later by determining number of rooted rhizomes per 4 rhizomes in the vessel, number of roots per rhizome, root length (mm) and number of aerial shoots per rhizome.

All treatments contained 5 replications, each consisting of 4 or 5 rhizome segments randomly selected. Final data were the means of 2 experiments. The data were subjected to analysis of variance and means were compared by Duncan's multiple range test at the significance level $P < 0,05$.

RESULTS AND DISCUSSION

Results of the first experiment showed that the greatest number of aerial shoots as well as the shortest shoots and the highest multiplication rate were observed at the highest BAP concentration – 6 mg l^{-1} (Table 1A). However, these rhizomes had the poorest rooting ability. Similar results were obtained in previous study (Gabryszewska and Hempel, 1985); it was also found that 2iP did not induce rhizome branching or aerial shoot production but increased shoot length. However, in current study, 2iP combined with 3 mg l^{-1} BAP strongly stimulated rhizome branching and aerial shoot formation (Table 1B). The greatest aerial shoot number – 6 and high multiplication rate – 3,6 were noted at 2 mg l^{-1} 2iP. Unfortunately, these shoots were of poor-quality (long and thin). Similar synergistic effect of cytokinins was observed by Van der Kriecken et al. (1990) in tobacco in vitro cultures. The effect of BAP on flower bud formation was significantly enhanced by 2iP at concentration which was by itself physiologically inactive. Authors found that 2iP competitively inhibits BAP inactivation as evidenced by the observation that 2iP causes a significant increase in the endogenous concentration of free BAP (physiologically active) at the expense of the glucoside BAP (inactive).

Table 1
Effect of cytokinins and flurprimidol in presence of 1 mg l^{-1} NAA on growth and rooting of alstroemeria „Juanita” multiplied in vitro

Growth regulators concentration (mg l^{-1})	No. of aerial shoots per rhizome	Aerial shoot length (mm)	Multiplication rate of rhizome	No. of roots per rhizome
A*				
BAP 1,5	3,7 a	55,5 c	2,5 a	0,8 b
BAP 3	4,3 a	36,9 b	2,8 ab	0,1 a
BAP 6	5,8 b	25,8 a	3,3 b	0,0 a
B*				
BAP 2,5 + kinetin 2,5	4,9 ab	31,0 a	2,3 a	0,0 a
BAP 3,0	4,3 a	37,0 a	2,6 ab	0,1 ab
BAP 3,0 + 2iP 1,0	5,2 ab	58,1 c	3,3 ab	0,7 b
BAP 3,0 + 2iP 2,0	6,0 b	49,5 b	3,6 b	0,2 ab
BAP 3,0 + 2iP 4,0	4,5 a	49,2 b	3,7 b	0,3 ab
C*				
Flur 1,0	2,7 a	6,6 a	1,5 a	5,0 d
BAP 1,5	3,3 ab	55,1 d	2,5 ab	0,8 a
BAP 3,0	4,3 bc	37,0 c	2,8 b	0,0 a
BAP 1,5 + Flur 0,1	5,9 d	20,6 b	4,3 c	1,6 ab
BAP 1,5 + Flur 0,5	5,6 cd	10,7 a	4,6 c	3,2 bc
BAP 1,5 + Flur 1,0	6,2 d	9,9 a	4,6 c	3,7 cd

*Effects of growth regulators A, B and C were analyzed in separate experiments. Means in each column followed with the same letter do not differ at significance level $P < 0,05$; Duncan's multiple range test.

Table 2
Effect of auxins on in vitro aerial shoot production and rhizome rooting of alstroemeria „Juanita”.

Auxin* concentration (mg l^{-1})	No. of rooted rhizomes/4	No. of roots per rhizome	Root length (mm)	No. of aerial shoots per rhizome	Aerial shoot length (mm)
A*					
NAA 1	3,9 a	5,1 a	11,1 b	2,7 a	42,6 b
2	3,6 a	8,3 a	9,2 ab	2,2 a	42,2 b
4	3,4 a	6,6 a	6,3 a	2,7 a	21,7 a
B*					
IBA 5	2,3 a	3,7 ab	6,0 a	2,3 c	68,5 a
IBA 5+ IAA 0,25	1,6 a	1,6 a	13,4 b	1,7 ab	81,5 a
IBA 10	2,7 a	6,4 b	3,4 a	1,4 a	94,4 a
IBA 10+IAA 0,25	1,7 a	1,5 a	9,8 ab	1,9 bc	101,1 a

*Effects of auxins A and B were analyzed separately. Means in each column followed with the same letter do not differ at significance level $P < 0,05$; Duncan's multiple range test.

Study concerning auxin effect showed that NAA strongly stimulated root formation and did not cause excessive shoot elongation (Table 2). The well rooted rhizomes with 2–3 aerial shoots of high quality were found in presence of 1 mg l⁻¹ NAA. In presence of NAA roots were short and thick. IBA stimulatory effect was much weaker; low number of rooted rhizomes was noted and aerial shoots were long and thin. These results confirm data of previous study (Gabryszewska and Hempel, 1985). These authors also noted that IAA had very little effect on rhizogenesis. On the other hand, there are some reports showing an additive effect of two auxins combination on root formation (Podwyszyńska, 1992; Khosh-Khui and Sink, 1982). Unfortunately, in the current study, an addition of IAA to IBA-medium inhibited root formation but, instead, stimulated aerial shoot and root elongation, decreasing shoot quality. Therefore, NAA at 1 mg l⁻¹ was chosen as the best auxin treatment and used in most experiments mentioned below. Moreover, rhizomes treated with NAA characterized with the best growth after transplanting to a greenhouse and nearly 100% of plants survived. Unfortunately, such rhizomes contained an unsatisfying number of aerial shoots (2–3). Addition of growth retardants to media containing auxins did not enhance aerial shoot number. Combination of 1 mg l⁻¹ NAA with 0,1 mg l⁻¹ paclobutrazol or 0,05 mg l⁻¹ flurprimidol significantly increased root number, dramatically reduced length of roots and shoots and caused their marked thickening (Fig. 2). These growth retardants combined with IBA and IAA used at concentrations shown in table 2B had similar effect but did not influence root thickness (data not presented).

Phenomenon of the fast shoot elongation and poor rooting ability, observed in *alstroemeria* in vitro, indicated that these could be due to high endogenous gibberellins level which are known to inhibit root formation and stimulate shoot elongation (Khunachak et al., 1987). These authors found that growth retardants, through inhibition of gibberellins synthesis, greatly enhanced the growth of vigorous shoots and roots of asparagus shoot cultures. Burkhart and Meyer (1991) reported that gibberellins inhibited rooting in some woody species. The authors also noted that growth retardants promoted in vitro rooting of white pine shoots; flurprimidol was less effective than ancymidol. In our study flurprimidol applied in presence of NAA did not stimulate aerial shoot production and rhizome branching but strongly induced root formation (Table 1C). These results correspond with data provided by Werbrouck and Debergh (1997) who indicated that another growth retardant – paclobutrazol did not show bud inductive effect on cytokinin free medium, while in presence of BAP boosted the shoot inducing effect of this cytokinin in *Spathiphyllum sp.* multiplied in vitro. In current study, the combination of BAP at low concentration (1,5 – 3 mg l⁻¹) with flurprimidol (0,1 – 1 mg l⁻¹) resulted in an increased multiplication rate, high number of aerial shoots (5–6), increased thickness of shoots and roots, reduction of their length and improved rooting ability of rhizomes (Table 1C). However, some aerial shoots, developed in presence of flurprimidol at above mentioned concentrations, were fasciated; moreover this chemical retarded further plant growth in greenhouse (data not presented). Therefore, in our later experiment, BAP was combined with flurprimidol as well as with paclobutrazol, however, at lower concen-

trations of $0,005 - 0,1 \text{ mg l}^{-1}$ (Fig. 1). Results showed that flurprimidol affected the rhizome growth more distinctly comparing to paclobutrazol. The highest number of aerial shoots as well as the shortest shoots and the highest rooting ability were found at $0,05 - 0,1 \text{ mg l}^{-1}$ flurprimidol. Paclobutrazol applied at the highest concentration ($0,1 \text{ mg l}^{-1}$) reduced only shoot length. The differences among growth retardants may be explained by their various ability to inhibit gibberellin biosynthesis. Flurprimidol is a pyrimidine derivate and paclobutrazol is triazole. They reduced gibberellin level by inhibiting kaurene oxidation in the GA pathway (Rademacher, 1990). These inhibitors may act on different sites and with different efficiency (Khunachak et al., 1987; Coolbaugh et al., 1978). Thus, in the further study paclobutrazol was applied at higher concentration (Table 3). Combination of $0,5 \text{ mg l}^{-1}$ paclobutrazol with $1,5 \text{ mg l}^{-1}$ BAP or $0,1 \text{ mg l}^{-1}$ paclobutrazol with 3 mg l^{-1} BAP resulted in well branched rhizomes containing numerous, short aerial shoots of good quality (5–6) and approximately 1 root (Table 3, Fig. 3). Observation in greenhouse showed that rhizomes acclimatized and continued growth within 100%, regardless of growth regulators combination. Plants derived from medium supplemented with $1,5 \text{ mg l}^{-1}$ BAP, $0,5 \text{ mg l}^{-1}$ paclobutrazol and 1 mg l^{-1} NAA grew the best. They formed new roots within 2–3 weeks and new shoots within 2–4 weeks (data not presented).

On the basis of the current study efficiency of alstroemeria micropropagation method was enhanced. The method permits propagation in vitro of new Polish cultivars of cut and pot alstroemeria.

Table 3
Effect of BAP and paclobutrazol in presence of 1 mg l^{-1} NAA on growth and rooting of alstroemeria 'Juanita' multiplied in vitro

Cytokinin concentration (mg l^{-1})	No. of aerial shoots per rhizome	Aerial shoot length (mm)	Multiplication rate of rhizome	No. of roots per rhizome
BAP 1,5	3,3 a	55,1 d	2,5 a	0,8 a
BAP 1,5 + Pac 0,1	4,2 ab	37,0 c	2,9 a	0,8 a
BAP 1,5 + Pac 0,5	5,2 bc	12,3 a	4,3 b	1,0 a
BAP 3,0	4,2 ab	37,2 c	2,8 a	0,0 a
BAP 3,0 + Pac 0,1	6,0 c	27,2 b	4,5 b	0,7 a

Means in each column followed with the same letter do not differ at significance level $P < 0,05$; Duncan's multiple range test

Fig. 1. Effect of paclobutrazol and flurprimidol in presence of 1.5 mg l^{-1} BAP and 1 mg l^{-1} NAA on growth and rooting of *alstroemeria* 'Juanita' multiplied in vitro.
Mean separation by Duncan's multiple range test at significance level $P < 0.05$.

Fig.2. Effect of paclobutrazol and flurprimidol in presence of 1 mg l^{-1} NAA on rhizome development and rooting of *alstroemeria* 'Juanita' in vitro; (C) control medium containing only NAA; (Fl) + 0.05 mg l^{-1} flurprimidol; (Pac) + 0.1 mg l^{-1} paclobutrazol.

Fig 3. Effect of BAP and paclobutrazol in presence of 1 mg l^{-1} NAA on aerial shoot growth and rhizome rooting ability of *Alstroemeria* „Juanita” multiplied in vitro (mg l^{-1}): 1) BAP 1.5; 2) BAP 1.5 + paclobutrazol 0.1; 3) BAP 1.5 + paclobutrazol 0.5; 4) BAP 3 + paclobutrazol 0.1.

Acknowledgements

The authors wish to thank ICI Chemicals for kindly supplying paclobutrazol, Mrs. Jadwiga Białek for her excellent technical assistance.

REFERENCES

- Bond S., Alderson P.G., 1993a. The effect of explant density, temperature and light on rhizome growth in vitro of *Alstroemeria*. J. Hort. Sci. 68: 855–859.
- Bond S., Alderson P.G., 1993b. The influence of apical dominance on the in vitro multiplication of the rhizome of *Alstroemeria*. J. Hort. Sci. 68: 905–910.
- Burkhart L.F. and Meyer M.M., 1991. The gibberellin synthesis inhibitors, ancymidol and flurprimidol, promote in vitro rooting of white pine microshoots. Plant Cell Rep. 10: 475–476.
- Chin C.K., 1982. Promotion of shoot and root formation in asparagus in vitro by ancymidol. Hort. Sci. 17: 590–591.
- Coolbaugh R.C., Hirano S.S. and West C.A., 1978. Studies on the specificity and site of action of α -cyclopropyl- α -(p-methoxyphenyl)-5-pyrimidine methylalcohol (ancymidol), a plant growth regulator. Plant Physiol. 62: 571–576.
- Gabryszewska E., 1995. Plant regeneration of *Alstroemeria* in vitro. Acta Agrobotanica 48: 95–104.
- Gabryszewska E., 1996. The influence of temperature, daylength and sucrose concentration on the growth and development of *Alstroemeria* x *hybrida* „Zebra” in vitro. Acta Agrobotanica 49: 131–140.
- Gabryszewska E., Hempel M., 1985. The influence of cytokinins and auxins on *Alstroemeria* in tissue culture. Acta Hort. 167: 295–300.
- Khosh-Khui M., Sink K.C., 1982. Rooting-enhancement of *Rosa hybrida* for tissue culture propagation. Scientia Hort. 17: 371–376.
- Khunachak A., Chin C.K., Le T. and Giafagna T., 1987. Promotion of asparagus shoot and root growth by growth retardants. Plant Cell Tiss. Org. Cult. 11: 97–110.
- Murashige T., Skoog F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473–497.
- Podwyszyńska M., 1992. In vitro propagation of *Aglaonema* sp. Folia Hort. IV: 105–114.
- Podwyszyńska M., 1997. Micropropagation of *Calathea ornata* Koern. Biologia Plantarum 39: 179–186.
- Przybyła A., 1992a. Mutation breeding of *Alstroemeria* in Poland. Acta Hort. 325: 561–565.
- Przybyła A., 1992b. Polish cultivars of *Alstroemeria*. Acta Hort. 325: 567–570.

- Przybyła A., 1996. Badania nad indukowaniem mutacji u *Alstroemeria* L. oraz ocena wartości użytkowej otrzymanywanych mutantów. Zesz. Nauk. Inst. Sad. Kwiac. Monografie i Rozprawy, 1–37.
- Rademacher W., 1991. Inhibitors of gibberellin biosynthesis: applications in agriculture and horticulture. In: Gibberellins (ed. Takahashi N., Phinney B.O., MacMillan J.) Berlin, Germany; Springer Verlag (1991): 296–310.
- Van der Krieken W.M., Croes A.F., Smulders M.J.M. and Wullems G.J., 1990. Cytokinins and flowers bud formation in vitro in tobacco, role of the metabolites. Plant Physiol. 92: 565–569.
- Werbrouck S.P.O., Debergh P.C., 1997. Possible role of gibberellins in the interaction between cytokinins and pesticides. Acta Hort. 447: 59–62.
- Ziv M., 1990. The effect of growth retardant on shoot proliferation and morphogenesis in liquid cultured gladiolus plants. Acta Hort. 280: 207–214.

Wpływ retardantów wzrostu, cytokinin i auksyn na mnożenie i ukorzenianie in vitro *Alstroemeria x hybrida* „Juanita”

Streszczenie

Do doświadczeń nad zwiększeniem efektywności mikrorozmnażania nowych odmian i linii hodowlanych *Alstroemeria x hybrida* wykorzystano kultury kłączy polskiej odmiany „Juanita”.

Wpływ cytokinin (BAP, kinetyna i 2iP), auksyn (IAA, IBA i NAA) dodawanych do pożywki oddzielnie lub łączenie z retardantami wzrostu (paklobutrazol – ICI i flurprimidol – Dowelanco) badano w odniesieniu do rozgałęziania się kłączy, formowania pędów nadziemnych i ukorzeniania. Najkrótsze i najliczniejsze pędy nadziemne obserwowano przy najwyższym stężeniu BAP (6 mg l⁻¹). Jednakże kłącze charakteryzowało się najniższą zdolnością do ukorzeniania. BAP w niższych stężeniach stosowana łączenie z 2iP lub kinetyną także silnie stymulowała rozgałęzianie kłączy i wyrastanie pędów nadziemnych. Jednakże ich jakość była niska (długie i cienkie). Zastosowania BAP w niskim stężeniu łącznie z paklobutrazolem (0,1–0,5 mg l⁻¹) lub flurprimidolem (0,01–0,1 mg l⁻¹) w obecności 1 mg l⁻¹ NAA pozwoliło uzyskać kłącza o dużej zdolności do ukorzeniania z licznymi pędami nadziemnymi. Retardanty wzrostu stosowane z NAA silnie stymulowały ukorzenianie kłączy lecz jednocześnie hamowały wzrost wydłużeniowy korzeni.