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Plant regeneration of *Alstroemeria in vitro*

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Abstract

The regenerative ability of explants from various organs of *Alstroemeria* plants was investigated. Rhizome apical and axillary tips cultured on the Murashige and Skoog medium with BA – 2 mgl^{-1} and NAA – 0.5 mgl^{-1} were the best among the tissue tested as initial explants. Five weeks after isolation the rhizome with 1-4 upright growing shoots were obtained. The types of rhizome explants influenced development and growth of lateral rhizomes and upright growing shoots. There were no significant differences in number of roots formed on various kind of rhizome explants. Rooting was strongly influenced by NAA. Subapical segments of vegetative stem, segments of flower pedicels and parts of ovary did not regenerate rhizome or roots but occasionally callus was formed on the medium with kinetin – 2 mgl^{-1} and NAA – 2 mgl^{-1}. Segments excised from vegetative stem sporadically developed roots on the medium with NAA or IBA in concentrations 3 and 9 mgl^{-1}.

INTRODUCTION

With classic propagation methods, the commercial introduction of new *Alstroemeria* cultivars would take many years. For that reason a rapid *in vitro* method of propagation could be of considerable value for breeding (Buitdendijk et al., 1992; Bridgen and Chunsheng Lu, 1994) and introduction of new cultivars to the horticulture production.

Ziv et al. (1973) studied the influence of kinetin and naphtaleneacetic acid (NAA) on regeneration of plantlets from subapical segments of inflorescence stems from "Ligtu" hybrids. Also, Ornstrup et al. (1992) found that apical parts of the vegetative stem gave the best shoot regeneration on the media with BA and NAA. Most of the studies for in vitro culture of *Alstroemeria* are directed towards multiplication of the rhizome, but multiplications rates are low. Hussey et al. (1979)

stated that rhizomes may be obtained *in vitro* directly from rhizome tips on the media with 6-benzyladenine (BA). Also, plantlets of *Alstroemeria* were regenerated from rhizome tips cultured in vitro by Gabryszewska and Hempel (1984), Lin and Monette (1987) and Pierik et al. (1988), Buitendijk et al. (1992).

The purpose of this paper is to compare the capability of *in vitro* regeneration of various explants of *Alstroemeria* plants.

MATERIAL AND METHODS

Explants from above-ground organs

The experiments were performed with the following explants of *Alstroemeria* cv. Regina:

- subapical segments of vegetative stem,
- segments of flower pedicels,
- parts of ovary.

The subapical segments of vegetative stem, 5 cm long, were taken from the plants in the vegetative stage. All leaves were removed from the stem. The segments of stem, 0.3 cm long, were placed on the medium in natural position (the basal end down).

In the experiment, with the influence of auxins on regeneration, segments of stem were sliced longitudinally in half and placed with the unwounded surface on the medium. The flower pedicels were cut in pieces 1.0 cm long and put on the medium in natural position. The ovaries were separated by cutting from flower buds in two stages: young flower buds, flower buds before blooming. After isolation the ovaries were cut longitudinally in half and placed with the unwounded surface on the medium.

The explants were surface sterilized in 1.5 % chloramine T solution (with a few drops of Tween 40) for 10-20 minutes with continuous stirring and then rinsed 3 times (3 x 10 min.) in sterile distilled water.

The basic nutrient medium consisted of the Murashige and Skoog (1962) macro- and micro-salts (except Fe) at full strength, NaFeEDTA – 40.3 mgl^{-1}, sucrose – 20 gl^{-1} and agar – 8 gl^{-1}. The pH of the medium was adjusted to 5.7 before adding the agar.

In the experiment concerning the influence of auxins on regeneration of explants from vegetative stem, following auxins were used: indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), naphtaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2.4-D) in concentrations: 0.1, 0.3, 1.0, 3.0, 10.0 mgl^{-1}.

In the other experiments the explants were grown on the medium with mixture of kinetin – 2 mgl^{-1} with NAA – 2 mgl^{-1}. The explants were placed one per tube containing 10 ml of medium. Each combination contained 20-40 explants. Explants were cultivated in growth chamber at 23°-25°C under 16 h light/8 h dark cycles. The light was provided by white fluorescent lamps at 40 $\mu molm^{-2}s^{-1}$.

After 8 weeks of cultivation, following parameters were determined:
- number of contaminated explants,
- number of survived explants,
- number of dead explants,
- number of explants with callus growth on the cut surface,
- number of explants with regenerted roots.

Explants from under-ground organs

The experiments were done with parts of rhizome of *Alstroemeria* cv. King Cardinal, Red Sunset, Regina, Mona Lisa and Zebra. The explants were taken from vigorously growing plants. Two kinds of explants were used: apical and axillary rhizome tips. Surface sterilization of rhizome pieces was as follows: few seconds in 70 % ethanol solution, 20 min. in 1.5 % chloramine T (with a few drops of Tween 40) and then rinsed 3 times (3 x 10 min.) in sterile distilled water. Apical and axillary rhizome tips with pieces of rhizome tissue were isolated and dipped for 5 seconds in 0.75 % solution of chloramine T. Afterwards, the explants were placed on solid media. Rhizome explants were grown on culture medium as follows: Murashige and Skoog (1962) macro- and micro-salts (except Fe) at full strength, NaFeEDTA – 40.3 mgl^{-1}, myo-inositol – 100 mgl^{-1}, thiamine – 0.4 mgl^{-1}, BA – 8 mg^{-1}, NAA – 0.5 mgl^{-1}, sucrose – 30 gl^{-1} and agar – 8 gl^{-1}.

Each explant was placed one per Erlenmayer flask containing 10 ml of medium. In the experiments from 5 to 37 apical rhizome tips and from 4 to 72 axillary rhizome tips were isolated. Cultures were incubated in growth chamber at 23°-25°C under 16h light/8 h dark cycles. The light was provided by white fluorescent lamps at 40 $\mu molm^{-2}s^{-1}$.

After 5 weeks of cultivation of the explants, following parameters were determined:
- number of contaminated explants,
- number of dead explants,
- number of explants that had regenerated the callus,
- number of explants that had regenerated the rhizome.

Different types of rhizome explants

The experiments were carried out on rhizome explants cv. Zebra obtained by multiplication *in vitro*. The following explants were used:
- a piece of a rhizome with 2 upright growing shoots and with an apical rhizome tip (Fig. 1 A),
- a piece of a rhizome with 2 upright growing shoots and without an apical rhizome tip (Fig. 1 B),
- a piece of a rhizome without 2 upright growing shoots which were cut off above the 1st internode and with an apical rhizome tip (Fig. 1 C),
- a piece of a rhizome without 2 upright growing shoots which were cut off above the 1st internode and without an apical rhizome tip (Fig. 1 D).

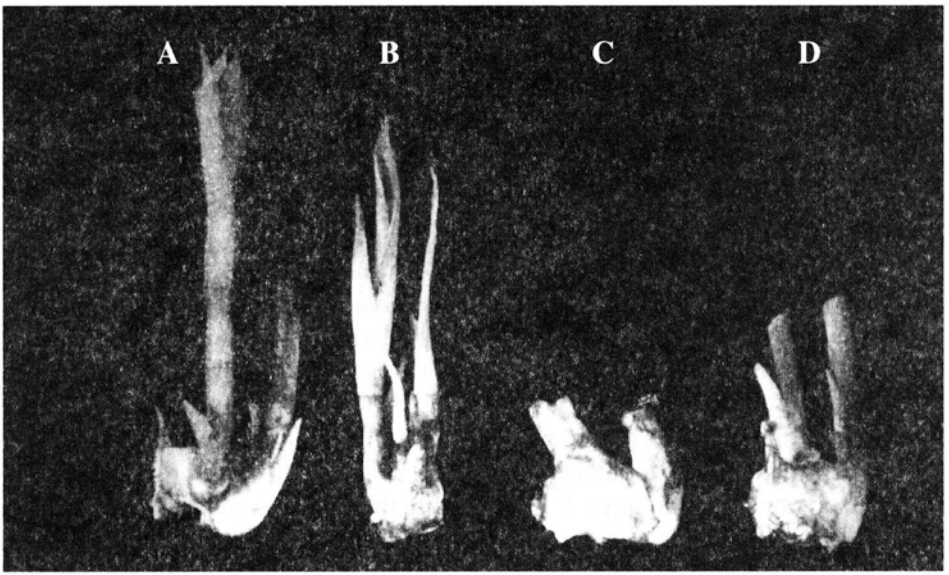

Fig. 1. The different types of rhizome explants of *Alstroemeria* cv. Zebra

A – piece of a rhizome with 2 upright growing shoots and with an apical rhizome tip, B – piece of a rhizome with 2 upright growing shoots and without an apical rhizome tip, C – piece of a rhizome without 2 upright growing shoots which were cut off above 1st internode and with an apical rhizome tip, D – piece of a rhizome without 2 upright growing shoots which were cut off above 1st internode and without an apical rhizome tip

Rhizome explants were placed on the M u r a s h i g e and S k o o g (1962) medium as previously described with BA – 4 mgl^{-1} or NAA – 4 mgl^{-1}. Rhizome explants growing on the media without growth regulators were taken as controls. The experiment was repeated two times with 25 replications in each treatment.

After 5 weeks of culturing, the following parameters were determined:
– number of branches on the rhizome,
– number and length (mm) of upright growing shoots,
– number and length (mm) of roots.

The data of the experiment were subjected to the variance analysis, and Duncan t-test was used for the mean separation at 5 % level of significance.

RESULTS

Regeneration on the explants from above-ground organs

The explants from above-ground organs were contaminated approximately in 22 %. About 42 % non-contaminated explants were dead. Subapical segments of vegetative stem, parts of flower pedicels and ovary gave similar results on the media containing kinetin – 2 mgl^{-1} and NAA – 2 mgl^{-1} (Tab. 1). The callus development

was observed on the cut surface of all types of explants. No further growth of callus or regeneration of organs was observed upon subculturing the explants on the fresh medium with the same concentration of growth regulators. Then callus turned brown and died. Differentiation of organs or growth of callus was not observed on stem explants growing on the control media without auxins (Tab. 2). Eight weeks after isolation all explants were dead. Likewise, the browning and necrosis of explants were found on the media containing various concentrations of auxins (NAA, IBA, 2.4-D); approximately 92 % of isolated stem explants died. The rest of explants survived and developed callus on the cut surface. The best callus growth or root initiation was observed on the stem explants growing on the media with NAA or IBA in concentrations 3 and 9 mgl^{-1} (Tab. 2).

Regeneration on the explants from under-ground organs

Approximately 58 % of the rhizome explants were contaminated. Apical and axillary tips were the best as initial explants. Five weeks after isolation rhizomes with 1-4 upright growing shoots were obtained on the media with BA – 8 mgl^{-1} and NAA – 0.5 mgl^{-1} (Tab. 3; Fig. 2 A, B). Regeneration and growth of rhizome and upright growing shoots were better for apical than axillary rhizome tips (Tab. 3). About 85 % isolated apical tips survived and regenerated new rhizomes while for axillary rhizome tips the ratio was 78 % (Tab.3). Adding BA resulted in outgrowth of axillary rhizomes of branched structures which may be divided into single or double rhizome segments and subcultured. Differences in reactions of cultivars to BA concentrations were observed. Cultivars King Cardinal, Zebra and Red Sunset had more dynamic growth of rhizome and produced more upright growing shoots than Regina and Mona Lisa. Some deformations of rhizome, upright growing shoots and leaves were found in cv. Zebra on the media with BA – 8 mgl^{-1} and NAA – 0.5 mgl^{-1}.

Regeneration of rhizome lateral branches, upright growing shoots and roots on different type of rhizome explants

The number of lateral rhizome branches and number of upright growing shoots on the medium without growth regulators depended on the kind of rhizome explants. The largest number (1.5) of lateral rhizome branches was found on the rhizome explants without 2 upright growing shoots which were cut off above the 1st internode and without rhizome tip (Tab. 4). The best regeneration of upright growing shoots (3.8) was observed on the rhizome explants with 2 upright growing shoots and with a rhizome tip. Rooting occurred sporadically on the control medium. Table 4 shows the effect of addition of BA or NAA on organogenesis from rhizome explants. BA strongly influenced the rhizome lateral branches multiplication and formation of upright growing shoots. Approximate 1.6-2.3 lateral branches were obtained in all kinds of rhizome explants on the medium with 4 mgl^{-1} BA. The largest number of upright growing shoots (6.7-7.7) was found on the rhizome explants with rhizome tip (Tab. 4).

Table 1

Comparison of the regeneration ability of explants from above-ground organs of *Alstroemeria* cv. Regina
Kinetin – 2 mgl^{-1} and NAA – 2 mgl^{-1}

Type of explant	Number of dead explants	Total number of explants	Number of contaminated explants	Number of survived explants that formed callus
Parts of ovary from young flower buds	10	3	4	3
Parts of ovary from flower buds before blooming	10	2	1	7
Segments of flower pedicels	10	4	3	3
Subapical segments of vegetative stem	40	13	12	15

Table 2

The effect of various concentrations of auxins (IAA, NAA, 2.4-D, IBA) on regeneration of subapical segments of vegetative stem from *Alstroemeria* cv. Regina. Each combination consists of 20 explants

Auxins (mgl^{-1})		Number of contaminated explants	Number of dead explants	Number of survived explants (no regeneration)	Number of explants that formed callus	Number of explants that formed roots
Control		2	18	0	0	0
IAA	0.1	1	17	0	0	0
	0.3	1	17	0	2 (+)	0
	1.0	7	9	1	3 (+)	0
	3.0	1	19	0	0	0
	9.0	2	15	0	3 (+)	0
NAA	0.1	2	17	0	1 (+)	0
	0.3	5	14	0	1 (+)	0
	1.0	1	16	0	3 (+)	0
	3.0	3	15	0	2 (+++)	1
	9.0	0	17	0	3 (+++)	3
2.4-D	0.1	5	13	0	2 (+)	0
	0.3	3	15	0	2 (+)	0
	1.0	3	15	0	2 (+)	0
	3.0	2	18	0	0	0
	9.0	1	19	0	0	0
IBA	0.1	2	18	0	0	0
	0.3	4	13	2	1 (+)	0
	1.0	3	15	0	2 (+)	0
	3.0	3	16	0	1 (++)	1
	9.0	1	17	0	2 (+++)	1

(+), (++), (+++) extent of callus formation

Table 3

Comparison of the regeneration ability of explants from underground organs of *Alstroemeria* cv. King Cardinal, Red Sunset, Regina, Mona Lisa and Zebra
BA – 8 mgl^{-1} and NAA – 0.5 mgl^{-1}

Cultivar	Type of explant*	Total number of explants	Number of contaminated explants	Number of dead explants	Number of explants that formed rhizome
King Cardinal	A	84	34	2	48
	B	153	95	20	38
Red Sunset	A	22	10	0	12
	B	27	12	0	15
Regina	A	5	0	0	5
	B	4	1	0	3
Mona Lisa	A	6	1	0	5
	B	6	0	0	6
Zebra	A	5	3	1	1

* See explanations:
A – apical rhizome tip, B – axillary rhizome tip.

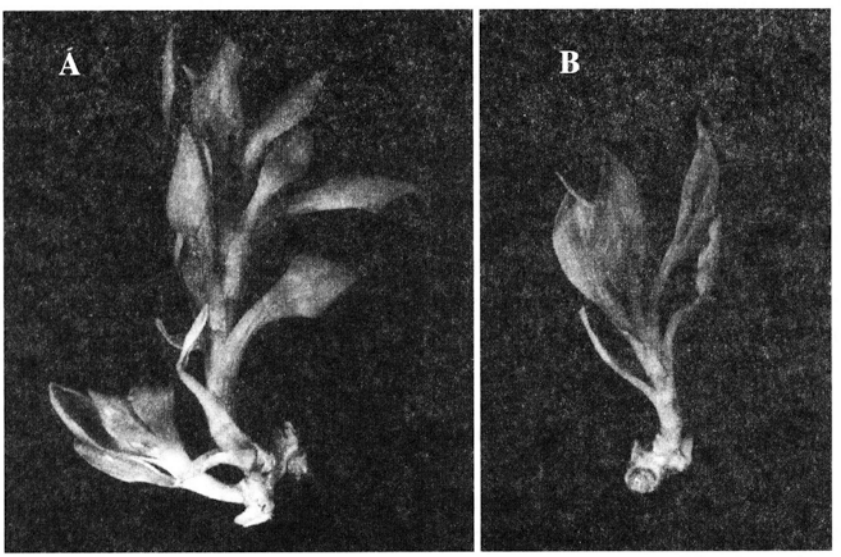

Fig. 2. The growth of an apical rhizome tip (A) and an axillary rhizome bud (B) of *Alstroemeria* cv. King Cardinal on the medium with BA – 8 mgl^{-1} and NAA – 0.5 mgl^{-1}

Table 4

The influence of types of rhizome explants and growth regulators (BA, NAA) on organogenesis of *Alstroemeria* cv. Zebra
BA – 4 mgl^{-1}, NAA – 4 mgl^{-1}

Type of rhizome explant	Growth regulator	Number of lateral rhizome branches	Number of upright growing shoots	Length of upright growing shoot (mm)	Number of roots	Length of root (mm)
A	Control	0.4 a	3.8 c	34.8 cde	0.2 a	5.6 a
	BA	2.3 d	7.7 e	15.8 a	0.0 a	0.0 a
	NAA	0.4 a	3.8 c	35.0 cde	11.4 b	5.5 abc
B	Control	0.6 a	2.9 bc	41.8 e	0.5 a	13.9 abc
	BA	1.6 bc	5.5 d	20.5 ab	0.0 a	0.0 a
	NAA	0.0 a	2.4 ab	43.3 e	12.0 b	6.5 c
C	Control	0.5 a	2.8 bc	37.2 de	0.2 a	13.2 ab
	BA	1.9 bcd	6.7 e	14.8 a	0.0 a	0.0 a
	NAA	0.3 a	2.8 b	26.9 bc	12.6 b	4.8 abc
D	Control	1.5 b	2.7 ab	31.8 cd	0.2 a	32.5 a
	BA	2.0 cd	5.4 d	14.2 a	0.0 a	0.0 a
	NAA	0.2 a	1.7 a	38.2 de	9.8 b	7.2 c

* See explanations:
A – piece of a rhizome with 2 upright growing shoots and with an apical rhizome tip,
B – piece of a rhizome with 2 upright growing shoots and without an apical rhizome tip,
C – piece of a rhizome without 2 upright growing shoots which were cut off above 1st internode and with an apical rhizome tip,
D – piece of a rhizome without 2 upright growing shoots which were cut off above 1st internode and without an apical rhizome tip.

Shoots growth in length on BA medium was inhibited, and roots formation was not observed. Addition of NAA – 4 mgl^{-1} had no promoting effect on regeneration of lateral rhizome branches and on formation of upright growing shoots. Rooting was strongly influenced by NAA. There were no significant differences in number of roots (9.8-12.6) formed on various kinds of rhizome explants. Elongation growth of roots was inhibited by NAA.

DISCUSSION

The data presented in this paper show that explants from above-ground organs (subapical segments of vegetative stem, segments of flower pedicels, parts of ovary) cultured on the Murashige and Skoog medium with kinetin – 2 mgl^{-1} and NAA – 2 mgl^{-1} did not regenerate rhizome or roots but occasionally callus was formed.

Segments excised from vegetative stem sporadically developed roots on the medium with NAA or IBA in concentration 3 and 9 mgl^{-1}. Lin and Monette (1987) stated that subapical segments from vegetative stems or inflorescenence stems and flower pedicels were dead in 6 weeks after culture initiation on the medium with IAA and cytokinins. On the other hand, Ornstrup et al. (1994) found that apical parts of vegetative stems gave best shoot regeneration on the medium containing BA and NAA. Also, buds and roots regeneration directly from the stem tissue on the medium with kinetin and NAA was obtained by Ziv et al. (1973).

Rhizome apical or axillary tips cultured on the medium with BA – 8 mgl^{-1} and NAA – 0.5 mgl^{-1} were the best among the tissues tested as initial explants. A high regeneration ability of rhizome tips was stated by Hussey et al. (1979), Lin and Monette (1987) and Pierik et al. (1988). Because the rhizome tips grow below the surface of the soil, they are quite difficult to disinfect, and this is one of the major problems of micropropagation of *Alstroemeria*. Pederson and Brandt (1992) developed a procedure for disinfection of rhizome tips, based on trimming of scale leaves 3 times, alternating with short (1-10 min.) immersions in disinfectants. Using disinfection in three steps, they obtained 80 % uninfected, growing explants; no significant difference could be detected between Korsolin (active components 8 % glutaraldehyde and 3.8 % formaldehyde) and NaOCl as disinfectant. In our experiment, application of ethanol and two steps disinfection in chloramine T, gave 42 % uninfected and growing rhizome explants.

An axillary rhizome initial is present at the base of each upright growing shoot but most are suppressed by the dominance of rhizome terminal meristem. Removing of the terminal meristem or addition of cytokinins influenced the outgrowth of an axillary rhizome, producing a branched structure (Hussey et al., 1979; Lin and Monette, 1987; Pierik et al., 1988). Presented results indicate that removing of apical rhizome tip and upright growing shoots on rhizome explants released of axillary buds from apical dominance of rhizome better than cutting only apical rhizome tip or upright growing shoots. Removing of apical rhizome tip and upright growing shoots is probably connected with elimination of endogenous auxins which are responsible for inhibition of axillary rhizome initial. Application of exogenous cytokinins (BA) strongly stimulated the outgrowth of an axillary rhizome in all types of rhizome explants.

Influence of BA on release of axillary shoots from apical dominance in monocotyledonous species was investigated by Hussey (1976). Apical dominance of species from family Iridaceae (*Gladiolus, Sparaxis, Iris, Schizostylis*) was released by very low concentration of BA. High concentrations of BA influenced the second branching of shoots. A strong apical dominance of the species from families: *Liliaceae* and *Amaryllidaceae* (*Hyacinthus, Lilium, Fritillaria, Narcissus, Hippeastrum*) was released and growth of axillary buds was obtained by application of high BA concentrations.

The auxin (NAA) promoted the rooting on rhizome explants but differences in number of roots were not observed between the different types of rhizome explants.

Lin and Monette (1987) reported that more roots were produced on rhizome explants containing a rhizome apical meristem than on rhizome sections lacking such a meristem.

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Regeneracja *Alstroemeria in vitro*

Streszczenie

Badano zdolności regeneracyjne eksplantatów izolowanych z różnych organów alstremerii. Największe zdolności regeneracyjne wykazywały pąki wierzchołkowe i kątowe kłącza rosnące na pożywce Murashige i Skooga zawierającej BA – 2 mgl^{-1} i NAA – 0,5 mgl^{-1}. Po 5 tygodniach wzrostu eksplantatów inicjalnych uzyskiwano kłącze zawierające od 1-4 pędów nadziemnych. Rodzaj eksplantatu kłączowego, stosowanego podczas mnożenia, wpływał na powstawanie i rozwój bocznych rozgałęzień kłącza oraz pędów nadziemnych. NAA silnie stymulował tworzenie się korzeni, jednakże nie stwierdzono różnic w liczbie korzeni w zależności od rodzaju eksplantatu kłączowego. Na fragmentach pędów nadziemnych i szypułek kwiatostanowych oraz na eksplantatach izolowanych z zalążni na pożywce zawierającej kinetynę – 2 mgl^{-1} i NAA – 2 mgl^{-1} nie obserwowano regeneracji kłącza i korzeni, natomiast tworzył się kalus. Fragmenty izolowanych pędów nadziemnych sporadycznie wytwarzały korzenie na pożywce zawierającej NAA lub IBA w stężniach 3 i 9 mgl^{-1}.