The influence of temperature, daylength and sucrose concentration on the growth and development of *Alstroemeria* 'Zebra' *in vitro*

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A b s t r a c t

The influence of temperature, daylength and sucrose concentration on the growth and development of *Alstroemeria* 'Zebra' *in vitro* was investigated. Only slightly more lateral rhizomes were formed at 25°C in comparison with 17°C on the medium with BA. Presence of BA in the medium strongly increased number of upright growing shoots and more shoots were formed in temperature 25°C than in temperature 17°C. Rhizome multiplication and formation of upright growing shoots were not significantly influenced by daylength. Sucrose was required for the formation of lateral rhizomes, upright growing shoots and roots. The highest number of lateral rhizomes was observed on the medium containing 60 and 80 g l⁻¹ sucrose and BA. Presence of BA in the medium with different sucrose concentrations markedly influenced the formation of upright growing shoots; the highest number of shoots was found on the explants cultured on the media with 20 and 30 g l⁻¹ sucrose. Low and high concentrations of sucrose inhibited upright growing shoots formation and their elongation. Rhizogenesis was not observed on rhizomes growing on the medium without sucrose and with 10 g l⁻¹ sucrose, either in the presence or absence of NAA. The number of roots per plantlet on the medium with NAA increased with increasing sucrose levels. The highest sucrose concentrations slightly inhibited roots formation.

INTRODUCTION

Alstroemeria is a rhizomatous ornamental plant which is gaining popularity as a cutflower. In the last years there has been some interest in using *Alstroemeria* as a pot plant. The increased interest in *Alstroemeria* is due particularly to its low energy requirement for cultivation and a long vase-life of its flowers.

Traditionally, plants are propagated through division of rhizomes of greenhouse grow plants. However, traditional division of rhizomes is too slow for large scale production and therefore micropropagation methods, especially rhizome multiplication, have been developed (H u s s e y et al., 1979; G a b r y s z e w s k a and

Hempel, 1985; Lin and Monette, 1987; Pierik et al., 1988; Buitendijk et al., 1992; Pedersen and Brandt, 1992; Bridgen and Chunshe ng Lu, 1994). Micropropagation does not always give satisfactory results because some cultivars have rather low multiplication rate.

In natural conditions, the growth and development of rhizome, upright growing shoots and flowers are affected by environmental conditions: temperature and light (Vonk Noordegraaff, 1981). Low temperature (17°C) and short day stimulated the growth and branching of rhizome in greenhouse conditions, while high temperature (25°C) and short day influenced the growth and development of upright growing shoots (Vonk Noordegraaff, 1981).

Growth and development of plants *in vitro* is controlled by the interaction among exogenous and endogenous hormones, mineral nutrition and environmental factors (light, temperature). Also, sucrose level is an important factor in the regulation of differentiation and growth of plants *in vitro*. The effects of sucrose concentration on organ formation and growth were found in *Lilium* (Takayama and Misawa, 1979, 1980, 1982) and *Calathea* (Podwyszyńska, 1995, in press).

The aim of this investigations is to determine whether there is an influence of daylength, temperature and sucrose concentration on the growth and development of *Alstroemeria* 'Zebra' *in vitro*.

MATERIAL AND METHODS

Rhizome cultures of *Alstroemeria x hybrida* 'Zebra' were grown initially on proliferation medium containing MS salt mixture (Murashige and Skoog, 1962), 0.4 mg l⁻¹ thiamine, 100 mg l⁻¹ inositol, 8 mg l⁻¹ BA, 3 % sucrose and 0.8 % commercial agar. The pH of the medium was adjusted to 5.7 before autoclaving for 17 min at 1 MPa. Cultures were incubated in a growth chamber at 23°C-25°C under 16h light/8h dark cycles. The light was provided by white fluorescent lamps at 40 μmol m⁻² s⁻¹.

The experiments were carried out with rhizomes segments having two upright growing shoots (decapitated at isolation to length of 0.5 cm) and two axillary buds and rhizome apical meristem. They were then placed, 5 explants per Erlenmayer flask, on MS media containing 4 mg l⁻¹ BA or 4 mg l⁻¹ NAA and on the media without growth regulators (control). The sucrose concentration in the medium was 30 g l⁻¹ (Experiment 1).

Experiment 1

The effects of temperature (17°C and 25°C), daylength (8h, 12h, 16h) and darkness on the growth and development of rhizome *in vitro* were examined.

Experiment 2

The influence of sucrose concentration (10, 20, 30, 40, 50, 60, 70, 80, 90 g l⁻¹), different sucrose concentration + BA and different sucrose concentration + NAA on

the growth and development of rhizome, upright growing shoots and roots were investigated.

Thirty explants were used per each treatment and the experiment was conducted twice. After 8 weeks of culture growth, the number of lateral rhizomes and upright growing shoots and length (mm) of main rhizomes, lateral rhizomes, upright growing shoots, number of roots and length (mm) of roots were determined. The results of experiment were calculated statistically and subjected to analyses of variance. Duncan's test was used to estimate the difference between means at a 5 % level of significance.

RESULTS AND DISCUSSION

The main rhizome branching was observed occasionally on the medium without cytokinin. The axillary buds of rhizomes were stimulated by the BA – 4 mg/l⁻¹ in the medium to develop secondary rhizome branching (Tab. 1). Only slightly more lateral rhizomes were formed at 25°C in comparison with 17°C on the medium with BA. Presence of BA in the medium strongly increased number of upright growing shoots and more shoots were formed in temperature 25°C than in temperature 17°C (Tab. 2).

Table 1

The influence of temperature, daylength and darkness on the growth and development of rhizome of *Alstroemeria* 'Zebra' on the medium with 4 mg/l⁻¹ BA or on the medium without growth regulators

Day length	Cytokinin	Temperature	Number of lateral rhizomes	Length of main rhizome (mm)	Length of lateral rhizome (mm)
8h	-BA	17°C	0.0 a	7.8 a	0.3 a
		25°C	0.1 a	8.9 ab	0.9 ab
	+BA	17°C	2.4 bc	12.5 cde	5.5 cd
		25°C	2.6 cd	12.4 cde	6.3 d
12h	-BA	17°C	0.2 a	9.5 ab	0.8 ab
		25°C	0.4 a	8.3 ab	2.2 bc
	+BA	17°C	2.4 bc	14.4 e	7.3 d
		25°C	2.6 bcd	12.6 de	6.7 d
16h	-BA	17°C	0.1 a	10.8 cd	0.4 a
		25°C	0.1 a	9.9 abc	0.8 ab
	+BA	17°C	2.6 bcd	13.9 e	7.4 d
		25°C	3.1 d	14.1 e	6.3 d
Darkness	-BA	17°C	0.1 a	9.2 ab	0.5 ab
		25°C	0.2 a	8.1 ab	1.3 ab
	+BA	17°C	2.0 b	10.6 bcd	5.3 cd
		25°C	2.8 cd	10.8 bcd	4.6 cd

Table 2

The influence of temperature, daylength and darkness on the growth and development of upright growing shoots of *Alstroemeria* 'Zebra' on the medium with 4 mg l⁻¹ BA or on the medium without growth regulators

Day length	Cytokinin	Temperature	Number of upright growing shoots	Length of upright growing shoots (mm)
8h	-BA	17°C	3.0 a	78.0 c
		25°C	2.4 a	81.8 c
	+BA	17°C	8.2 c	14.3 a
		25°C	11.7 e	12.3 a
12h	-BA	17°C	3.2 a	80.9 c
		25°C	3.2 a	64.0 b
	+BA	17°C	8.2 c	17.6 a
		25°C	10.6 de	16.0 a
16h	-BA	17°C	3.0 a	79.2 c
		25°C	3.0 a	79.1 c
	+BA	17°C	9.2 cd	19.1 a
		25°C	10.6 de	17.1 a
Darkness	-BA	17°C	2.9 a	89.4 c
		25°C	3.1 a	88.6 c
	+BA	17°C	6.7 b	13.0 a
		25°C	9.2 cd	14.7 a

The results are not consistent with the observation of P i e r i k et al. (1988) who found that rhizome multiplication rate was not affected by temperature in the range 15-21°C, only significant inhibition of the rhizome multiplication occurred at 24°C in comparison with 15, 18 and 21°C. L i n and M o n e t t e (1987) found that the quality of the cultures was superior when initiation was maintained at 8°C rather than 22°C. The elongation of shoots was suppressed by BA (Tab. 2); in contrast to this, cytokinin stimulated the growth of main and lateral rhizomes (Tab. 1). P i e r i k et al. (1988) stated that BA strongly stimulated the rhizome multiplication rate and number of upright growing shoots, and decreased the length of the upright growing shoots.

Despite the fact that *Alstroemeria* is sensitive for growth and flowering to daylength (V o n k N o o r d e g r a f f, 1981), it was demonstrated that rhizome multiplication and differentiation of upright growing shoots *in vitro* were not significantly influenced by daylength (Tab. 1, 2).

The number of lateral rhizomes was lowest on the medium without sucrose or on the medium containing 10 gl⁻¹ sucrose and without BA (Tab. 3, Fig. 1 A). Presence of BA – 4 mg l⁻¹ in the medium without sucrose increased only marginally the number of lateral rhizomes. Application of BA in the medium with sucrose

stimulated the branching of rhizomes; approximate 2.1-2.9 lateral rhizomes were found (Tab. 3, Fig. 1 B). The highest number of lateral rhizomes was observed on the medium containing 60 and 80 gl^{-1} sucrose and BA. On the control medium (without sucrose), the number of upright growing shoots was higher on the explants cultured in the presence of BA in comparison with the explants growing on the medium without BA (Tab. 4, Fig. 1 A, B). The formation of upright growing shoots strongly increased with the addition of different sucrose concentrations; approximately 3.1-4.0 upright growing shoots were obtained. Presence of BA – 4 mg l^{-1} in the medium with different sucrose concentrations markedly influenced the formation of upright growing shoots; the highest number of shoots was found on the explants cultured on the medium containing sucrose in concentrations 20 and 30 gl^{-1} , whereas the lowest number of upright growing shoots was obtained on the explants growing on the medium with 10 and 90 gl^{-1} of sucrose. Elongation of upright growing shoots was very weak on the sucrose-free medium, either in the presence or absence of BA (Tab. 4, Fig. 1 A, B). Different sucrose levels variously influenced the growth of upright growing shoots; the longest shoots were formed on the rhizome cultured on the medium with 20 and 30 gl^{-1} of sucrose. At the higher concentrations of sucrose (70-90 gl^{-1}) in the medium the shoots growth was suppressed. Addition of BA to the media with different sucrose concentrations strongly inhibited elongation of upright growing shoots. NAA – 4 mg l^{-1} in the sucrose-free and sucrose-enriched medium had weak promotory effect on the formation of upright growing shoots (Tab. 5, Fig. 1 A, C). The length of upright growing shoots cultured on the media with NAA and different sucrose levels decreased in comparison with the length of shoots growing on the NAA-free media with various sucrose levels. P i e r i k et al. (1988) demonstrated that a higher rhizome multiplication rate of *Alstroemeria* was obtained with sucrose than with glucose. The sucrose concentration markedly influenced the rhizome branching, 30-40 gl^{-1} being optimal. T a k a y a m a and M i s a w a (1979) showed that sucrose is required for the production of bulblets of *Lilium speciosum*.

Table 3

The influence of sucrose and BA – 4 mg l^{-1} on the growth and development of rhizome of *Alstroemeria* 'Zebra'

Concentrations of sucrose (gl^{-1})	Number of lateral rhizomes	
	sucrose	sucrose + BA
0	0.3a	0.8a
10	0.4a	2.4bc
20	0.5ab	2.8bc
30	0.6ab	2.4bc
40	0.7ab	2.7bc
50	0.5ab	2.4bc
60	0.7ab	2.9c
70	0.6ab	2.7bc
80	0.8ab	2.9c
90	1.0ab	2.1b

A — The growth of rhizome, upright growing shoots and roots on the media containing sucrose in the following concentrations: 1 – control (no sucrose), 2 – 10 g l^{-1} , 3 – 20 g l^{-1} , 4 – 30 g l^{-1} , 5 – 40 g l^{-1} , 6 – 50 g l^{-1} , 7 – 60 g l^{-1} , 8 – 70 g l^{-1} , 9 – 80 g l^{-1} , 10 – 90 g l^{-1}

B — The growth of rhizomes on the media containing BA – 4 mg l^{-1} and sucrose in the following concentrations: 1 – control (no sucrose), 2 – 10 g l^{-1} , 3 – 20 g l^{-1} , 4 – 30 g l^{-1} , 5 – 40 g l^{-1} , 6 – 50 g l^{-1} , 7 – 60 g l^{-1} , 8 – 70 g l^{-1} , 9 – 80 g l^{-1} , 10 – 90 g l^{-1}

C — The growth of rhizome and roots on the media containing NAA – 4 mg l^{-1} and sucrose in the following concentrations: 1 – control (no sucrose), 2 – 10 g l^{-1} , 3 – 20 g l^{-1} , 4 – 30 g l^{-1} , 5 – 40 g l^{-1} , 6 – 50 g l^{-1} , 7 – 60 g l^{-1} , 8 – 70 g l^{-1} , 9 – 80 g l^{-1} , 10 – 90 g l^{-1}

Fig. 1. The growth and development of rhizome, upright growing shoots and roots of *Alstroemeria* 'Zebra' on the media containing different concentrations of sucrose and growth regulators

Table 4

The influence of sucrose and BA – 4 mg l⁻¹ on the growth and development of upright growing shoots of *Alstroemeria* 'Zebra'

Concentrations of sucrose (g l ⁻¹)	Number of upright growing shoots		Length of upright growing shoots (mm)	
	sucrose	sucrose + BA	sucrose	sucrose + BA
0	1.9 a	2.4 a	6.8 a	6.8 a
10	3.1 ab	5.5 b	55.4 cd	9.9 bc
20	3.1 ab	8.0 c	57.1 d	13.9 ef
30	3.6 b	8.0 c	62.2 d	14.3 ef
40	4.0 b	7.5 bc	46.9 bcd	14.6 f
50	3.8 b	7.0 bc	52.4 cd	12.5 de
60	3.5 b	7.3 bc	40.6 bcd	11.0 cd
70	3.2 b	6.5 bc	37.4 bcd	10.8 bcd
80	3.4 b	6.3 bc	25.2 abc	8.9 b
90	3.9 b	5.5 b	19.4 ab	9.6 bc

Table 5

The influence of sucrose and NAA – 4 mg l⁻¹ on the growth and development of upright growing shoots of *Alstroemeria* 'Zebra'

Concentrations of sucrose (g l ⁻¹)	Number of upright growing shoots		Length of upright growing shoots (mm)	
	sucrose	sucrose + NAA	sucrose	sucrose + NAA
0	1.9 a	3.0 ab	6.8 a	16.2 a
10	3.1 ab	3.3 ab	55.4 cd	25.4 a
20	3.1 ab	3.4 ab	57.1 d	32.5 a
30	3.6 b	2.7 a	62.2 d	32.3 a
40	4.0 b	3.1 ab	46.9 bcd	28.9 a
50	3.8 b	3.0 ab	52.4 cd	29.6 a
60	3.5 b	2.8 ab	40.6 bcd	21.4 a
70	3.2 b	3.2 ab	37.4 bcd	18.5 a
80	3.4 b	3.8 b	25.2 abc	12.9 a
90	3.9 b	3.8 b	19.4 ab	15.1 a

Differentiation was not observed when sucrose was omitted from the medium. On the other hand, high sucrose concentrations inhibited the kinetin-induced bulb scale differentiation of *Lilium speciosum* cv. Uchida (Takayama and Misawa, 1982). Also, Bach and Pawłowska (1992) found for *Hyacinthus orientalis* that with higher levels of sucrose the mean number of shoots and bulblets decreased. When no sugar was included neither shoot or bulblets organogenesis could be observed. Podwyszyńska (1996, in press) showed that raised concentration of sucrose up to 45 g l⁻¹ promoted shoot growth and branching of *Calathea ornata* and improved their quality.

Rhizogenesis was not observed on rhizomes growing on the medium without sucrose and with 10 gl^{-1} of sucrose, either in the presence or absence of NAA (Tab. 6, Fig. 1 A, C). The rhizomes cultured on the media containing 20 to 90 gl^{-1} of sucrose produced 0.3 to 1.7 roots. NAA greatly enhanced rooting on the media with sucrose; the number of roots per plantlet increased with increasing sucrose levels. The highest number of roots on rhizome explants was found at 70 gl^{-1} of sucrose in the medium. The high sucrose concentration (80, 90 gl^{-1}) slightly inhibited root formation. Root elongation depended on the various sucrose levels; the longest roots were formed on the rhizomes cultured on the media with sucrose in concentrations 40-90 gl^{-1} . Presence of NAA in the medium inhibited root elongation in all sucrose levels. The optimum level of sucrose for root and rhizome laterals formation was higher than for upright growing shoots formation. These results were consistent with the results reported by T a k a y a m a and M i s a w a (1979). They found that optimum level of sucrose for lily bulblet formation was lower than for root formation. Levels of sucrose probably affect root growth through regulation of the hormonal level as suggested by B u t h e r and S t r e e t (1960).

Other effects of sucrose on organ formation probably are mediated through changes in osmotic potential (T r a n T h a n h V a n, 1977; B o r k o w s k a and S z c z e r b a, 1991). It was suggested (T r a n T h a n h V a n, 1977) that under the same auxin/cytokinin ratio, organogenesis varies according to the quantity and quality of carbohydrates, the action of which seems to be both of metabolic and osmotic nature.

T a b l e 6

The influence of sucrose and NAA – 4 mg l^{-1} on the growth and development of roots of *Alstroemeria* 'Zebra'

Concentrations of sucrose (gl^{-1})	Number of upright growing shoots		Length of upright growing shoots (mm)	
	sucrose	sucrose + NAA	sucrose	sucrose + NAA
0	0.0 a	0.0 a	0.0 a	0.0 a
10	0.0 a	0.0 a	0.0 a	0.0 a
20	0.3 a	0.5 a	2.0 ab	2.7 b
30	0.5 a	4.1 a	3.9 ab	3.0 b
40	1.4 a	6.2 a	9.3 b	4.9 b
50	1.3 a	6.2 a	8.7 b	3.8 b
60	1.7 a	9.6 a	7.4 b	3.8 b
70	1.4 a	10.9 a	8.1 b	3.7 b
80	1.0 a	9.3 a	5.9 b	2.2 b
90	1.1 a	6.6 a	5.7 b	2.6 b

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Wpływ temperatury, długości dnia i różnych stężeń sacharozy na wzrost i rozwój *Alstroemeria* 'Zebra' *in vitro*

Streszczenie

Badano wpływ temperatury, długości dnia oraz różnych stężeń sacharozy na wzrost i rozwój kłącza, pędów nadziemnych i korzeni *Alstroemeria* x *hybrida* 'Zebra'. Fragmenty kłącza rosnące na pożywce z BA tworzyły nieznacznie więcej bocznych rozgałęzień w temperaturze 25°C niż w temperaturze 17°C. Obecność BA w pożywce silnie stymulowała powstawanie pędów nadziemnych; więcej pędów tworzyło się na fragmentach kłącza rosnących w temperaturze 25°C w porównaniu z temperaturą 17°C. Długość dnia nie miała wpływu na różnicowanie się bocznych rozgałęzień kłącza i pędów nadziemnych. Sacharoza była czynnikiem niezbędnym w procesie różnicowania i wzrostu kłącza, pędów nadziemnych i korzeni alstremerii.

Najwięcej bocznych rozgałęzień kłącza stwierdzono na pożywce zawierającej BA i sacharozę – 60 i 80 g l⁻¹. Wpływ BA na powstawanie pędów nadziemnych i ich wzrost zależał od stężenia sacharozy w pożywce; najwięcej pędów nadziemnych tworzyło się na fragmentach kłącza rosnących na pożywce zawierającej 20 i 30 g l⁻¹ sacharozy. Niskie i wysokie stężenia sacharozy hamowały proces różnicowania pędów, a także ich wzrost wydłużeniowy. Rizogenezy nie obserwowano na fragmentach kłącza rosnących na pożywce bez sacharozy i z sacharozą w stężeniu 10 g l⁻¹, zarówno w obecności NAA, jak i bez auksyny. Liczba korzeni tworzących się na fragmentach kłącza rosnących na pożywce z NAA wzrastała wraz ze wzrostem stężenia sacharozy w pożywce; jednakże najwyższe stężenia sacharozy nieznacznie hamowały powstawanie korzeni.