GLASNIK INSTITUTA ZA BOTANIKU I BOTANIČKE BAŠTE UNIVERZITETA U BEOGRADU
BULLETIN DE L'INSTITUT ET DU JARDIN BOTANIQUES DE L'UNIVERSITE DE BEOGRAD
Tom XXIX, 1995, Beograd

UDC 588.7:582.564(497.11)
Original scientific paper

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FACTORS AFFECTING *IN VITRO* ROOTING OF *CRYPTANTHUS BROMELIOIDES* (OTTO & DIETR.)

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Todorović, I. and Vinterhalter, D. (1995): *Factors affecting in vitro rooting of Cryptanthus bromelioides (Otto / Dietr.)*. – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 143 - 148.

Shoot cultures of a decorative clone of *Cryptanthus bromelioides* Otto & Dietr. were maintained *in vitro* on MS (Murashige and Skoog, 1962) medium supplemented with BAP 0.5-1.0 mgl^{-1} and 0.1-0.2 mgl^{-1} NAA. Characteristic of this species is poorly developed root system same as in other epiphytic bromeliads. On the standard rooting medium comprising 0.5 mgl^{-1} IBA root length was only 9.4 mm with 3.8 roots per explant. We therefore investigated the effect of factors and conditions which are know to affect and improve root initiation and elongation. Among various factors including auxins IBA and NAA, light, inorganic nutrition, activated charcoal, phloroglucinol, ancymidol, fusicoccin, riboflavine and ascorbic acid the most effective was activated charcoal which 2.5 times increased the root length.

Key words: *in vitro*, propagation, rooting, shoot cultures, *Cryptanthus bromelioides*, epiphyte.

Ključne reči: *in vitro*, razmnožavanje, ožiljavanje, kulture izdanaka, *Cryptanthus bromelioides*, epifit.

INTRODUCTION

Cryptanthus bromelioides member of the *Bromeliaceae* familiy is a small plant with leaves arranged in a rosette. Like other bromeliads *C. bromeliodes* is an epiphyte and its root system is poorly developed. The poor root growth of bromeliads can be also obsrved under conditions of *in vitro* culture. Thus for instance in *Aechmea fasciata* Baker., the mean root length after 5 weeks of rooting on hormone free medium was only 12.45 mm (Vinterhalter & Vinterhalter, 1994). However addition of 1% activated charcoal to the medium more than doubled the length of roots in *Aechmea*. We therefore investigated *in vitro* rhizogenesis of *C. bromelioides*, with the aim to enhance rooting parameteres supplementing the medium with various rooting cofactors.

There is a number of studies dedicated to *in vitro* propagation of bromeliad species. *Aechmea fasciata* was propagated by Zimmer and Peiper (1974, 1975), Jones and Murashige (1974) and Ziv et al., (1986); *Tillandsia, Guzmania* and *Vriesea* by Mekers (1977) and Mekers and Van Onsem (1983); *Cryptanthus* by Davidson and Donnan (1977) and Mathews and Rao (1982); *Quesnelia* (Hosoki and Asahira (1980) and *Ananas* Mathews and Rangan (1979, 1981), Zepeda and Sagava (1981).

MATERIAL AND METHODS

Shoot cultures used in this investigation were introduced and successfully propagated *in vitro* as *Ananas comosus* L. Plants 1-2 years old were examined and reclassified according to Saakov (1983) as a decorative clone of *Cryptantus bromelioides* Otto & Dietr. (*C. acaulis* Lindl).

Shoot cultures were maintained on MS (Murashige & Skoog, 1962) medium supplemented with 0.5-1.0 mgl^{-1} BA and 0.1-0.2 mgl^{-1} NAA. Shoot culture stock was subcultured at 6-8 week intervals. The duration of rooting treatments was 4 weeks. Each treatment contained 25-30 replicates and was repeated at least twice. Shoots excised for rooting treatments were 35-40 mm long. Root length was measured as the length of the longest root on the rooted plant. Shoot cultures were maintained in 100 ml Erlenmeyer flasks or 125 ml blood transfusion bottles. Rooting treatments were performed in Ø 20 x 100 mm test tubes. All culture vessels were closed with cotton wool plugs. Medium pH was adjusted to 5.8 prior to autoclaving which was performed for 20-25 minutes at 114-115^{0}C.

Conditions in the growth room were: photoperiod 16/8 hours light to darkness, provided by cool white fluorescent lamps, irradiance 5.0-7.2 Wm^{-2} and temperature 25 $\pm$ 2^{0}C.

Adaptation of rooted plants was performed in glasshouse, plants were transferred in peat based substrates (mixture of peat, sand and humus). During adaptation plants were weekly sprayed with fungicides (containing 0.3% Captan).

RESULTS

The first group of treatments was performed with the aim to investigate the effect of auxins on rooting. IAA and NAA were applied i concentration 0-2.0 mgl^{-1} and shoots left in continuous contact with the rooting medium, Tab. 1. Rooting with auxins was nearly 100% efficient. Roots were short, the maximum root length (11.4 mm) was registered on hormone-free and medium with lowest IBA concentration - 0.1 mgl^{-1}.

Root length decreased with the increase of auxin concentration. IBA concentrations under 0.5 mgl^{-1} provided slender roots with numerous hairs whilst at higher IBA concentrations roots were thick and hairs were absent. In treatments with NAA roots were even shorter than on IBA supplemented media. The longest root 5.3 mm was registered on media with 0.1 mgl^{-1} NAA.

Tab. 1. – Effect of NAA and IBA on root elongation and the number of roots per rooted plant

Auxin, mgl^{-1}	Root length. mm ± SEM		Roots per rooted culture ± SEM	
	IBA	NAA	IBA	NAA
0	11.36 ± 0.8	11.73 ± 0.7	2.83 ± 0.3	2.70 ± 0.2
0.1	11.37 ± 1.0	5.27 ± 0.5	3.44 ± 0.2	3.72 ± 0.3
0.2	9.43 ± 0.4	nm	3.13 ± 0.2	nm
0.5	9.33 ± 0.6	4.91 ± 0.4	3.80 ± 0.3	5.00 ± 0.4
1.0	8.60 ± 0.9	3.73 ± 0.4	4.86 ± 0.7	6.61 ± 0.7
2.0	4.46 ± 1.0	3.10 ±0.2	6.73 ± 1.0	5.31 ± 0.4

nm - not measured

The mean number of roots per explant increased with auxin concentration reaching maximum 6.7 on 2.0 mgl^{-1} IBA and 6.6 at 1.0 mgl^{-1} NAA.

Since IBA enabled better elongation than NAA, all furhter experiments were performed with IBA supplemented media. Concentration 0.5 mgl^{-1} IBA was considered to be optimal providing moderately high values for both root elongation and roots per rooted explant parameters.

In the next experiment concentration of MS inorganic salts in the medium was decreased, Table 2. Treatments contained IBA either at 0.1 or 0.5 mgl^{-1}.

Tab. 2. – Effect of inorganic nutrition on root elongation and the number of roots per rooted plant

MS inorganic salts, %	Root length, mm ± SEM		Roots per rooted explant ± SEM	
	IBA 0.1 mgl^{-1}	IBA 0.5 mgl^{-1}	IBA 0.1 mgl^{-1}	IBA 0.5 mgl^{-1}
100	8.3 ± 0.6	8.6 ± 0.7	2.7 ± 0.2	4.3 ± 0.2
50	10.0 ± 0.9	11.6 ± 0.9	3.2 ± 0.2	2.7 ± 0.3
20	11.2 ± 0.7	13.6 ± 0.9	3.3 ± 0.3	3.8 ± 0.4
10	10.5 ± 0.9	14.1 ± 0.7	2.8 ± 0.2	3.4 ± 0.4
0	9.5 ± 0.5	9.6 ± 0.7	2.7 ± 0.2	3.0 ± 0.3

On both media root length increased with decrease of concentration of inorganic salts. Maximum for lower IBA concentration was reached at 1/5 and for higher IBA concentration on media containing 10% MS inorganic salts. Interesting results were obtained for the number of roots per rooted culture. At lower IBA concentration maximum was at 1/5 MS inorganic salts same as the maximum for root elongation. On

the higher IBA concentration (0.5 mgl^{-1}) values decreased with the concentration of inorganic salts. Rooting percentage in these treatments varied from 96,6 do 100%. On salt-free media roots were dark and thin, thread-like.

Next group of treatments was performed with the aim to evaluate the light requirement for rotting of *C. bromelioides*. Both light and dark treatments contained 0.5 mgl^{-1} IBA and full strength MS inorganic salts (Tab. 3). In light the mean number of roots per explant was higher and roots were longer than in dark treatment. Plants in dark treatments were etiolated and their shoot were longer (56.1 mm) than in plants cultured in light (40.1 mm).

Tab. 3. – Effect of light on root elongation and the number of roots per rooted plant

Treatment	Root length, mm ± SEM	Roots per rooted explant ± SEM	Shoot length, mm ± SEM
light	8.68 ± 0.3	4.46 ± 0.2	40.65 ± 0.7
darkness	6.68 ± 0.3	3.58 ± 0.2	56.14 ± 1.2

Finally, a number of substances which are known as cofactors of rhizogenesis were investigated, all on medium supplemented with 0.5 mgl^{-1} IBA and full strength MS inorganic salts, Tab. 4.

Tab. 4. – Effect of rhizogenesis cofactors on root elongation and the number of roots per rooted plant

Cofactor	concentration	root length. mm ± SEM	roots per rooted culture ± SEM
control		9.33 ± 0.6	3.8 ± 0.3
charcoal (activated)	1%	24.64 ± 0.2	4.1 ± 0.2
ancymidol	0.8 mgl^{-1}	7.60 ± 0.4	8.9 ± 0.7
fusicocin	10^{-10}M	7.00 ± 0.8	9.8 ± 1.0
phloroglucinol	162 mgl^{-1}	3.83 ± 0.2	6.9 ± 0.4
ascorbic acid	1 mgl^{-1}	4.63 ± 0.5	8.0 ± 0.7
riboflavine	1 mgl^{-1}	10.86 ± 1.3	4.0 ± 0.3

The most potent cofactor was 1% activated charcoal which increased root length 2.5 times in comparison to the control. Mean number of roots was not much higher than in the control. In contrast to activated charcoal other cofactors increased the number of roots per rooted explants and decreased root elongation.

Ancymidol, fusicocin and ascorbic acid more than doubled the number of roots per rooted explant. Phloroglucinol also increased root production but strongly inhibited root elongation same as ascorbic acid. Ancymidol and fusicocin moderately decreased root length.

Riboflavin slightly increased root elongation and roots per rooted plant above the values registered in the control.

Adaptation of plants rooted on IBA supplemented medium was very good, usually over 90% efficient. Adaptation of plants rooted on NAA supplemented medium was less efficient. After some time plants attain the characteristic rosette growth habit.

DISCUSSION

Our investigation showed that the optimal relation between root length and number was obtained on media with 0.5 mgl^{-1} IBA and 1/10 strength MS inorganic salts. Among various rhizogenesis cofactors activated charcoal significantly increased root length. Ancymidol and fusicocin did not affect much root elongation but increased the number of roots per explant. Phloroglucinol and ascorbic acid were inhibitory to root elongation but increased the production of roots. Riboflavine showed no marked effect on rooting parameters. Adaptation was much better if plant were rooted on IBA than on NAA supplemented media.

The obtained results are in accordance with results obtained with *Aechmea fasciata* (Vinterhalter and Vinterhalter, 1994) in which 1% activated charcoal was found to significantly increase root elongation.

ACKNOWLEDGEMENTS

Results presented here were part of work which Ivana Todorović presented in her final examination (graduating work) at Faculty of Biology, Belgrade.

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Rezime

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ISTRAŽIVANJE FAKTORA KOJI UTIČU NA *IN VITRO* OŽILJAVANJE VRSTE *CRYPTANTHUS BROMELIOIDES* (OTTO& DIETR.)

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Kulture izdanaka dekorativnog klona *Cryptanthus bromelioides* (Otto / Dietr.) su održavani *in vitro* na MS (M u r a s h i g e and S k o o g , 1962) podlozi uz dodatak 0.5-1.0 mgl^{-1} BAP i 0.1-0.2 mgl^{-1} NAA. Karakteristika ove vrste je slabo razvijen korenov sistem slično kao i kod drugih epifitnih bromelija. Tako na standardnoj polozi za ožiljavanje koja sadrži 0.5 mgl^{-1} IBA dužina korena bila je svega 9.4 mm dok je broj korenova po eksplantatu bio 3.8. Zbog toga su istraživani faktori za koje je poznato da utiču na inicijaciju i izduživanje korena. Između različitih faktora uključujući tu auksine IBA i NAA, svetlost, mineralnu ishranu, aktivni ugalj, floroglucinol, ancimidol, fuzikokcin, riboflavin i askorbinsku kiselinu najveći efekat ispoljio je aktivni ugalj koji je u koncentraciji 1% povećavao izduživanje korena 2.5 puta.