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THE INFLUENCE OF MEDIUM SOLIDIFYING AGENTS AND DOUBLE-PHASE MEDIUM ON THE GROWTH AND DEVELOPMENT OF *COSMOS ATROSANGUINEUS* (HOOK.) VOSS *IN VITRO*

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Abstract. Influence of different medium gelling agents (Agar-Agar Sigma, Lab-Agar™ Biocorp, Bacto-Agar Difco, Gelrite) and two types of medium (solidified by Agar-Agar and double-phase medium) on branching and growth of *Cosmos atrosanguineus* shoots was investigated. Shoot tips obtained from aseptical tissue cultures were grown for 6 weeks on Murashige and Skoog medium containing BA in concentration of 1 mg · dm^{-3}. It was found that induction of axillary shoots was the best on double-phase medium (addition of liquid medium after 4 weeks of shoot cultures in vitro). No significant differences were found in regeneration potential and elongation of shoots depending of the media solidified by studied agars or Gelrite.

Key words: *Cosmos atrosanguineus*, cosmos, agar brand, Gelrite, double-phase medium, branching of shoots

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Introduction

The most characteristic feature of *Cosmos atrosanguineus* (Hook.) Voss are chocolate scented flowers. Dark red-brown, sometimes almost black, velvety flowers on long, slender, reddish brown stems bloom from early summer to autumn. Chocolate cosmos is a tuberous-rooted, tender perennial native to Mexico. It may be overwintered indoors where not hardy. The best places for cultivation are borders or containers where the flowers can be appreciated up close. They also are good cut flowers. These plants do not produce seeds, so that they have to be propagated vegetatively, by division of tubers. Because this method of propagation is very slow, studies on in vitro propagation of *C. atrosanguineus* were undertaken.

It is well known that solidifying agents as well as double-phase medium have significant effect on the developmental processes of in vitro growing plants (Griffis *et al.* 1991; Marcelis-Van Acker & Scholten 1995; Scholten & Pierik 1998; Serrano-Martinez *et al.* 2012; Scherwinski-Pereira *et al.* 2012).

The aim of this experiment was to investigate the effect of agar brand, Gelrite and double-phase of medium on branching and growth *in vitro* of *C. atrosanguineus* shoots.

Material and methods

Shoot tips of *C. atrosanguineus* taken from aseptically grown shoot cultures were used in this experiment. They were cultivated on the basic Murashige & Skoog (MS) (1962) medium containing: mineral salts and thiamine – 0.4 mg · dm^{-3}, pyridoxine – 0.5 mg · dm^{-3}, nicotinic acid – 0.5 mg · dm^{-3}, glycine – 2 mg · dm^{-3}, myo-inositol – 100 mg · dm^{-3}, sucrose – 30 g · dm^{-3} and supplemented with cytokinin, benzyladenine (BA), in concentration of 1 mg · dm^{-3}. The different gelling agents (Agar-Agar Sigma – 6.5 g · dm^{-3}, Lab-Agar™ Biocorp – 6.5 g · dm^{-3}, Bacto-Agar Difco – 6.5 g · dm^{-3}, Gelrite – 2.0 g · dm^{-3}) and double-phase medium (addition of liquid medium to the Agar-Agar medium after 4 weeks of culture) were tested.

Shoot tips were placed into 250 ml Erlenmeyer flasks, with five shoots per flask. Each combination consisted of 4 flasks – 20 shoots. Each flask with 5 explants was a replication.

Table 1. The influence of gelling agent type on growth and development of *Cosmos atrosanguineus* main shoot after 6 weeks of *in vitro* culture.

Type of gelling agent	Length of main shoot (mm)	Number of leaves on main shoot	Fresh weight of main shoot (mg)
Agar-Agar Sigma	27.8 b*	9.1 a	66.2 c
Bacto-Agar Difco	29.9 a	8.4 ab	74.8 ab
Lab-Agar	29.0 ab	7.9 b	68.1 bc
Gelrite	30.4 a	8.6 ab	82.7 a
Mean	29.3	8.2	73.0

* values in vertical columns followed by the same letter do not differ significantly at p = 0.05.

Table 2. The influence gelling agent type on number and growth of *Cosmos atrosanguineus* axillary shoots after 6 weeks of *in vitro* culture.

Type of gelling agent	Number of axillary shoots	Length of axillary shoots (mm)	Fresh weight of axillary shoots/explant (mg)
Agar-Agar Sigma	4.7 a*	10.3 a	62.5 b
Bacto-Agar Difco	5.1 a	13.1 a	69.8 b
Lab-Agar	4.7 a	12.6 a	63.5 b
Gelrite	5.4 a	13.2 a	80.5 a
Mean	4.9	12.3	69.0

* values in vertical columns followed by the same letter do not differ significantly at p = 0.05.

The cultures were maintained at 22°C± 2°C with a photon flux of 35 $\mu M \cdot m^{-2} \cdot s^{-1}$ and a 16-h photoperiod. The following characters were evaluated after 6 weeks of culture: length and fresh weight of main shoot, number of leaves and axillary shoots on main shoot, length and fresh weight of axillary shoots.

The results of the experiment were analyzed statistically using a standard statistical procedure with one factorial design with use of Tukey test to estimate the differences between the means at a 5% level of significance.

Results and discussion

On the basis of statistical analysis it was proven that type of medium gelling agent has a significant effect on growth and development of the main shoot of *Cosmos atrosanguineus*. Shoots of the biggest length and fresh weight were obtained on the medium solidified with Gelrite (30.4 mm, 82.7 mg respectively). Significantly lower values were noted when Agar-Agar Sigma was used (27.8, 66.2 mg respectively) (Tab. 1). Similarly, number of regenerating axillary shoots, their length and fresh weight were the highest on the media solidified with Gelrite (Tab. 2). No differences in number and length of axillary shoots were observed regarding addition of Gelrite or agar to the medium. Many authors reported about advantageous effect of Gelrite used to solidify the media on growth and regeneration of plants in tissue cultures (Bailey *et al.* 1986; McRae & Van Staden 1990; Griffis *et al.* 1991; Kozak & Dąbski 1998).

Comparing growth of the main shoot on the media solidified with agar and on the double-phase media, significantly better elongation of shoots was noted on the double-phase media. The obtained shoots formed more leaves and characterized with higher fresh weight when they were cultivated on double-phase media when compared to shoots cultivated on the media solidified with Agar-Agar (Tab. 3). The number of regenerating axillary shoots was also significantly higher on the double-phase media. Their length was similar on both media settings but the fresh weight was over

Table 3. The influence of medium setting on growth and development of *Cosmos atrosanguineus* main shoots after 6 weeks of *in vitro* culture.

Medium condensation	Length of main shoot (mm)	Number of leaves on main shoot	Fresh weight of main shoot (mg)
Solidified by Agar-Agar	27.8b*	8.0 b	66.2 b
Double-phase medium	33.0 a	9.2 a	98.7 a
Mean	30.4	8.6	82.4

* values in vertical columns followed by the same letter do not differ significantly at p = 0.05.

Table 4. The influence of medium setting on number and growth of *Cosmos atrosanguineus* axillary shoots after 6 weeks of *in vitro* culture.

Medium condensation	Number of axillary shoots	Length of axillary shoots (mm)	Fresh weight of axillary shoots/explant (mg)
Solidified by Agar-Agar	4.7 b*	10.3 b	62.5 b
Double–phase medium	7.4 a	11.3 a	154.9 a
Mean	6.0	10.8	108.7

* values in vertical columns followed by the same letter do not differ significantly at p = 0.05.

two times higher on the double-phase media (Tab. 4). An advantageous effect of double-phase media on number and length of shoots was observed in case of *Rosa hybrida* L. cv. *'Sweet Promise'* (Maene & Deberth 1985), *Pyrus domestica* Medik. (Wang 1991), *Helianthemum marminorense* Alcaraz, Peinado & Mart. Parras (Serrano-Martinez *et al.* 2012), *Ananas comosus* (L.) Merr. (Scherwinski-Pereira *et al.* 2012).

Conclusions

1. Double-phase medium has the most favourable influence on the induction of axillary shoots of *Cosmos atrosanguineus* in tissue culture.
2. The type of gelling agent has no significant effect on the multiplication rate.
3. Elongation growth of axillary shoots is improved by medium solidified with Gelrite or Bacto-Agar Difco.

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