Trakia Journal of Sciences, Vol. 8, No. 1, pp 33-37, 2010

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

Original Contribution

EFFECT OF GENOTYPE, EXPLANT SIZE AND POSITION ON CALLUS INDUCTION IN *CEREUS PERUVIANUS* MILL. (CACTACEAE)

N. Karimi[1], M. R. Mofid[2*], M. Ebrahimi[3] and S. M. Khayyam Nekouei[3]

[1]Karaj Islamic Azad university, Iran,
[2] Dept. of Clinical Biochemistry - School of Pharmacy, Isfahan University of Medical Sciences, Iran.
[3]ABRICI, Agricultural Biotechnology Research Institute Iran (ABRII), Iran

ABSTRACT

Cereus peruvianus (Cactaceae) is an important medical plant. The study was carried out on callus induction of *Cereus jamacaru* f. monstrosus and *Cereus hildmannianus* fma monstrosa (*Cereus peruvianus*). Apical and lateral explants were cultured on Murashige and Skoog media with factorial combinations of the auxins indole-3-acetic acid (NAA), 2, 4-dichlorophenoxyacetic acid (2,4-D) and N-(2-furanyl-methyl)-1-purine-6 amine (kinetin) at the concentrations 1, 2, 3, 4 mg/l . Positive results were obtained from the apical and lateral explants in all conditions tested, but apical explants gave the highest callus induction in all growth regulator combinations tested. Explants' size doesn't have a significant impact.

Key words: callus induction, cereus peruvianus, size, position

INTRODUCTION

Cereus peruvianus (family *Cactaceae*) is a climbing cactus of tropical origin and has a widely branched crown. They are mostly treelike or shrubs like column cacti and rather attractive, they grow quickly and easily and can be used for many ornamental purposes (1). Alkaloids purified from plants provide many pharmacologically active compounds, including leading chemotherapy drugs. As is generally true of secondary metabolites, overall productivity is low, making commercial production expensive. Alternative production methods remain impractical, leaving the plant as the best source for these valuable chemicals. Result obtained from other studies revealed that *Cereus peruvianus* is one of the important sources of alkaloid and it is very effective to treat patients with prostate and breast cancer. Comparison of alkaloid production by *C. peruvianus* plants and by callus tissues indicated that alkaloid levels were almost twice as high in callus tissues as in shoots of C. peruvianus plants. The ratio of alkaloid concentration between mature plant and morphologically undifferentiated cells of callous tissue was 1:1.7 (2).Genotype, Composition of culture medium, Physiological state of the donor plant and explants and position of the explants on the plant, as well as size of explants are effective factors on callus induction.

*** Correspondence to**: *Mohammad reza Mofid, Clinical Biochemistry, Isfahan University Medical Sciences, Iran,Email: mohrezamofid@hotmail.com*

MATERIALS AND METHODS

The experiments of this study were carried out at Tissue Culture Research Laboratory, Agriculture Biotechnology Research Institute Central Region of Iran in order to investigate the behaviour of the consecutive *in vitro* micropropagation stages of *Cereus peruvianus* under the effect of different concentrations of cytokinin (kinetin) and auxin (NAA, 2,4-D), and different types of culture substrates for callus induction. The experiments were repeated two times during the years of 2006 and 2007. Seedlings (one-year-old) with a height of 15-20 cm originated from the Cactus International greenhouse (*Cereus hildmannianus* and *Cereus jamacaru*) were used as a source of plant materials. The stem explants were surfaces- sterilized with 96% (v/v) ethanol for one min and then they were treated with 1% (v/v) hypochlorite sodium with one or two drops of tween-20 for 20 min, followed by rinsing three times with sterile distilled water. The culture medium under trials consisted of the macro- and micro-elements and vitamins of MS-medium (3)

enriched with 30 g/l sucrose and 0.8% (w/v) phytoagar. Apical and lateral explants were used for this experiment. The explants were aseptically placed on full strength MS-medium supplemented with Naphthalene acetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D) and N-(2-furanyl-methyl)-1-purine-6 amine (kinetin) at the concentrations 1, 2, 3, 4 mg/l. All the used culture media were adjusted to pH 5.7±0.1 and autoclaved at 121°C and 1.2 kg/cm^2 for 20 minutes before using. The stem explants were placed vertically in 200 ml capacity glass jars containing 25 ml medium. The cultures were incubated at 25±1°C under dark condition. After 4-6 weeks culturing, the calli were recultured on fresh medium of the same components. The experiments were performed using Complete Randomized Design (CRD) and results were analyzed using one way ANOVA.

RESULTS

Data in **Table 1, 2** revealed that different concentrations of cytokinin (kinetin) and auxin (NAA and 2,4-D) tested in this trial had a significant effect on callus induction of explants. Data in **Table 3** showed that different size of explant (0/5 and 1 cm) hadn't significant effect on callus induction **Figure 2 (F)** . These results showed that the highest callus induction per explants was obtained after 45 days in MS-medium supplemented with 4 mg/l 2,4-D. The addition of KIN significantly reduced the caulogenesis efficiency in treatment containing 2,4-D which means KIN could not promote and proliferate the growth of callus tissue. Callus induction in *Cereus jamacaru* was better than *Cereus hildmannianus* **Figure1 (A)** and apical explants gave the highest callus induction in all growth regulator combinations compared to lateral explants **Figure 1 (B,C)**. The callus which induced from leaf explants on MS basal medium containing 4 mg/L (w/v) of 2,4-D was friable **Figure 2 (D)**. The addition of KIN up]to 4 mg/L (w/v) to the medium containing different concentrations of 2,4-D leads to the production of callus which is compact instead of friable **Figure 2 (E)**.

A ***B***

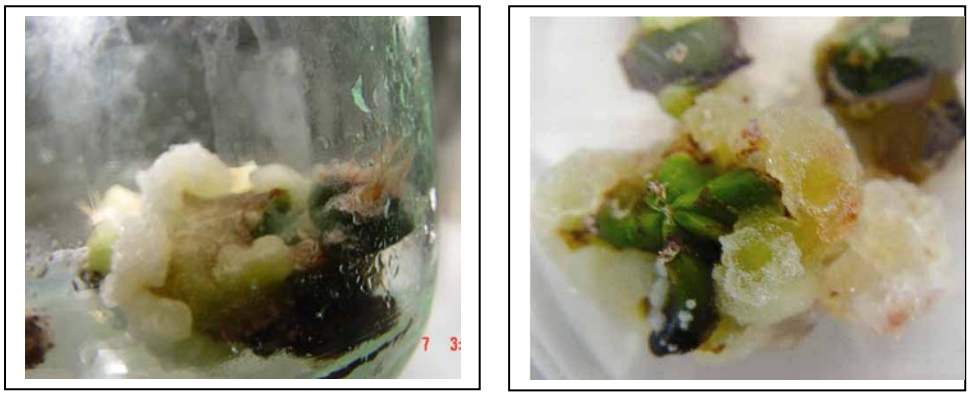

Fig.1 A. explant position in (*c hildmannianus*) B. explant position in (*c. .jamacaru*) (apical meristem)

C ***D*** ***E***

Fig. 2 C. compact callus D. frible callus E. effect explant size

Table 1: *Percentage of callus induction (CI) and morphology of callus formed from Cereus peruvianus stem explants on MS medium supplemented with different levels of 2,4-D and KIN supplied after 6 weeks of cultures at 25±1ºC under light condition.*

Phytohormones supplied in WPM basal medium (mg/L) 2,4-D KIN		CI %	CI %	Morphology of callus formed from stem explants	
		C.jamacaru	*C.hildmannianus*	*C.jamacaru*	*C.hildmannianus*
1	0	34±2.0 e	30±0.7 e	PY , friable	Friable
2	0	65±2.0 c	59±1.0 c	PY , friable	Friable
3	0	75±1.6 b	69±0.6 b	PY , friable	Friable
4	0	90±2.2 a	85±1.1 a	PY , friable	Friable
1	1	27±1.1 e	21±1.0 e	PY ,compact	Friable
2	1	53±1.4 d	50±1.2 d	PY ,compact	Friable
3	1	60±1.6 c	55±1.0 c	PY ,compact	Friable
4	1	72±2.3 b	65±1.2 b	PY ,compact	Friable
1	2	25±2.3 e	20±1.3 e	PY,compact	Friable
2	2	43±2.3 ed	44±1.3 d	PY,compact	Friable
3	2	52±1.3 d	50±1.1 d	PY ,compact	Friable
4	2	65±2.4 c	61±2.1 c	PY ,compact	Friable
1	3	15±1.5 f	10±1.0 f	PY ,compact	Compact
2	3	34±1.4 e	30±1.0 e	PY ,compact	Compact
3	3	42±2.3 ed	35±1.3 e	PY ,compact	Compact
4	3	50±2.6 d	45±1.6 d	PY ,compact	Compact

Value noted as mean ± standard deviation (n=10) Percentage of callus weight (CW) PY indicates pale yellow color, PG indicates pale green color.

DISCUSSION

The auxin concentration used for callus induction in this study was high compared to the ones reported for callus induction in other species (4). The treatment with 4 mg/l concentrations of 2,4-D was the most effective treatment for callus induction of C.peruvianus. **Table 1** showed that the addition of KIN reduced the caulogenesis efficiency in treatments containing 2,4-D. It means that the KIN didn't promote and proliferate the growth of callus tissue. Although auxin is capable to promote the growth of callus, according to Wernicke and Milkovits (1987)(5), high concentrations of 2,4-D were able to inhibit callusing of basal segments and it has affected as a herbicide. High concentration of 2,4-D may prevent cell division due to increase of internal auxin higher than critical threshold value (6). The explants size was found not affecting the success of culture but the explants size of 0.5 cm × 0.5 cm was resulted in higher callus induction compared to explants size of 1 cm × 1cm. These findings are in agreement with those reported by Dabekaussen et al. (2003) (7), Espinasse et al. (1989)(8) and Duong Tan Nhut et al. (2006) (9) in Effect of genotype, explants size, position, on shoot generation of Gerbera jamesonii by receptacle transverse thin cell layer culture. Results **Table 3** showed, that apical explants gave the highest callus induction in all growth regulator combinations compared to the lateral explants. Various research undertakings on this plant have also proved that apical merristem was very appropriate for callus induction (10). The calluces which induced from stem explants on MS basal medium containing different

concentrations of 2,4-D were friable. Similarly, (6) Barringtonia racemosa the addition of KIN up to 2 mg/L (w/v) to the medium containing different concentrations of 2,4-D has been reported to produce friable callus instead of compact callus.

Table 2: *Percentage of callus induction (CI) and morphology of callus formed from cereus previonus stem explants on MS medium supplemented with different levels of NAA and KIN supplied after 6 weeks of cultures at 25±2ºC under light condition*

Phytohormones supplied in MS basal medium (mg/l)		% CI	% CI	Morphology of callus formed from stem explants	
NAA	KIN	*C.jamacaru*	*C.hildmannianus*	*C.jamacaru*	*C.hildmannianus*
1	0	24±2.1 e	20±2.1 e	PG, friable	Friable
2	0	55±2.6 c	49±2.6 c	PG, friable	Friable
3	0	67±2.2 b	61±2.2 b	PG, friable	Friable
4	0	78±1.4 a	73±1.4 a	PG, friable	Friable
1	1	21±2 e	16±2 e	PG , friable	Friable
2	1	43±1.6 d	37±1.6 d	PG , friable	Friable
3	1	53±1.5 c	49±1.5 c	PG , friable	Friable
4	1	61±2 b	55±2 b	PG , friable	Friable
1	2	15±1.1 ef	10±1.1 ef	PG, compact	Friable
2	2	32±1.4 de	27±1.4 de	PG, compact	Friable
3	2	44±2.2 d	40±2.2 d	PG, compact	Friable
4	2	54±1.3 c	49±1.3 c	PG , compact	Friable
1	3	10±1.1 f	7±1.1 f	PG, compact	Compact
2	3	27±1.4 e	20±1.4 e	PG, compact	Compact
3	3	32±1.5 de	27±1.5 de	PG, compact	Compact
4	3	43±1.3 d	33±1.3 d	PG, compact	Compact

Value noted as mean ± standard deviation (n=10) Percentage of callus weight (CW) PG indicates pale green color, PG indicates pale green color.

Table 3: *Effect of explant size and position on callus induction (CI) of C.jamacaru and C.hildmannianus*

2,4-D	*C.jamacaru* CI %	*C.hildmannianus* CI %	Size(cm)	Position
4	90±2.1 a	78±1.1 b	1×1	Apical
4	92±1.9 a	86±0.9 a	0/5×0/5	Apical
4	60±1.2 b	50±0.9 d	1×1	Lateral
4	62 ±2.4 b	59±1.2 c	0/5×0/5	Lateral

ACKNOWLEDGEMENTS

The project was funded by the Tissue Culture Research Laboratory, Agricultural Biotechnology Research Institute Central Region of Iran (ABRICI). The authors would like to thank Dr. S. M. Khayyam Nekouei Responsible ABRICI and Dr.Behbahani for critical review of the manuscript.

REFERENCES

1. Sawsan, S., Sayed, T.A., Abou-Dahab and Youssef. E.M.A., 2005. *In vitro* propagation of cactus (*Cereus peruvianus* l.) Arab J. Biotech., 8. 1:169-176.
2. Oliveira, AJ. and Machado, MFPS. , 2003. Alkaloid production by callous tissue

cultures of *Cereus peruvianus* (cactaceae),.104, 2: 149-155.
3. Murashinge, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue, *Physiol. Plant*.15:474-497.
4. Smith, R.H., Burdick, P.J. Anthony, J. and Reilley, A.A ., 1991. *In vitro* propagation of *Coryphantha macromeris. Hortscience* 26:315.
5. Wernicke, W. and Milkovits, L ., 1984. Developmental gradients in wheat leaves-response of leaf segments in different genotypes cultured in vitro. J. *Plant Physiol*. 115: 49–58.
6. Behbahani.M, Abdul Manaf A., Muse, R. and Banu Mohd, N., 2007. Anti-oxidant and anti-inflammatory activities of leaves of *Barringtonia racemosa.*
7. Dabekaussen, M.A.A., Pierik, R.L.M., van der Laken J.D. and Hoek S.J ., 2003. Affecting areole activation in vitro in the cactus Sulcorebutia alba Rausch, *Horticulture scientica*, 46,3-4: 283-294.
8. Espinasse, A., Lay, C. and Volin, J., 1989. Effects of growth regulator concentrations and explant size on shoot organogenesis from callus derived from zygotic embryos of sunflower (*Helianthus annuus* L.), *Plant cell*, Tissue culture and organ culture, 17, 2-3 :171-181.
9. Duong, T.. , Truong, T., Nguyen T., Nguyen T., Nguyen, T., Nguyen Q. T. and Nguyen Hong Vu., 2006. Effect of genotype, explant size, position, and culture medium on shoot generation of *Gerbera jamesonii* by receptacle transverse thin cell layer culture, *Horticulture science*. 2 ,111:146-151.
10. Augusto, F., 2006. Fatty Acids Production and Callus Cultures of *C. peruvianus*., *Plant Sciences* , 1:368.
11. Dixon, R.A ., 1985. Isolation and maintenance of callus and cell suspension culture. In:Dixon,R.A.,ed. Plant cell culture,a practical approach.Oxford:IRI,Press Limited;1-20.
12. de Oliveira, S.A., Machado, M.F.P.S., Prioli, A.J. and Mangolin, C.A. 1995. *In vitro* propagation of *Cereus peruvianus* MILI. (Cactaceae), *In vitro* cell. Dev.Biol. 94-98.
13. White J. W., Allen, M. Easley, and Strock P., 1987. Effects of Growth Regulants on *Opuntia basilarus*, *Hort. Sciencce*. 13, 2: 181-182.
14. Malda, G., Backhaus, RA., Martin, C., 1999. Alterations in growth and crassulacean acid metabolism (CAM) activity of in vitro cultured cactus, *Plant Cell, Tissue and Organ Culture*. 58: 1–9.