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Original Contribution

EFFECT OF AREOLE AND CULTURE MEDIUM ON CALLUS INDUCTION AND REGENERATION *CEREUS PERUVIANUS* MILL. (CACTACEAE)

N. Karimi[1], M. R. Mofid[2*], M. Ebrahimi[3], R. Naderi[4]

[1]Islamic Azad University, Karaj branch, Karaj, Iran.
[2] Department of Biochemistry, School of Pharmacy and Isfahan Pharmaceutical Sciences Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
[3]Agricultural Biotechnology Research Institute, Central Region of Iran (ABRICI), Isfahan, Iran.
[4]Faculty of Horticultural Sciences and Plant Protection, University of Tehran , Tehran, 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*). Explants were cultured on MS (Murashige and Skoog, 1962) 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 . The frequency of Callus induction of *C. peruvianus* was highest in medium containing 4 mg/l 2,4- D or 4 mg/l NAA. Areol was not effective for callus induction possitivly and regeneration.

Key word: callus induction, *cereus peruvianus*, regeneration

INTRODUCTION

Cacti are dicotyledonous perennial plant with specialised features adapted for survival in arid and other climatic condition. *Cereus peruvianus* 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 active compounds, including plants provide many pharmacologically 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. The 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 the size of explants are effective factors on callus induction.

***Correspondence to:** *Mohammad reza Mofid, Dept. Biochemistry, Isfahan University Medical Sciences, Isfahan, Iran, Email:* mrezamofid@yahoo.de

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) 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. The **Table (3)** showed that different concentrations of the cytokinin (TDZ, kinetin and BAP) and auxin (NAA and 2,4-D) tested on regeneration of areol and produce frible callus. 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.

ethanol for one min and then they were treated with 1% (v/v) hypochlorite sodium

RESULTS

The explants derived shoot explant with aroel was used for shoot regeneration. The result showed **(Table 3)** that areol was not effective for callus induction and shoot regeneration *and callus induction* of *Cereus jamacaru was higher than Cereus hildmannianus*. **Fig. 1, 2**. The percentage of shoot regeneration was the highest (53.3 and 40) on medium containing 4 mg/L (w/v) of 2,4-D + 6 mg/l KIN+10 %coconut, and 4 mg/L (w/v) of 2,4-D +4 mg/l KIN+10% coconut respectively **Table 2**, **Fig. 3**. Results on the rootlets induction of the *in vitro* plantlet derived from shoot tip explants cultured in the MS basal medium supplemented with 4 mg/L2,4-D or NAA + different levels of KIN were summarized in **Table 3**, **Fig. 4**. The data obtained revealed that the highest root formation was obtained in MS medium supplemented with 4 mg/L 2,4-D+ 2 mg/L KIN, and 4 mg/L NAA + 2 mg/L KIN **(Table 4) Fig. 4 .**

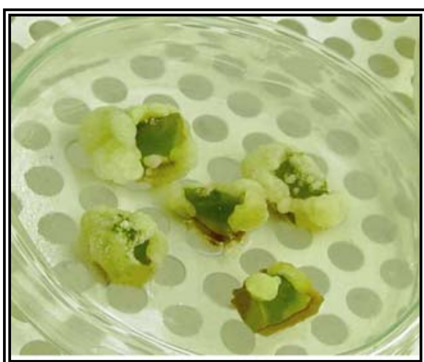

Figure 1. explant without areol (*c.jamacaru*)

Figure 2. explant with areol (*c.jamacaru*)

Figure 3. regeneration of aroel in *Cereus hildmannianus* and *Cereus jamacaru*.

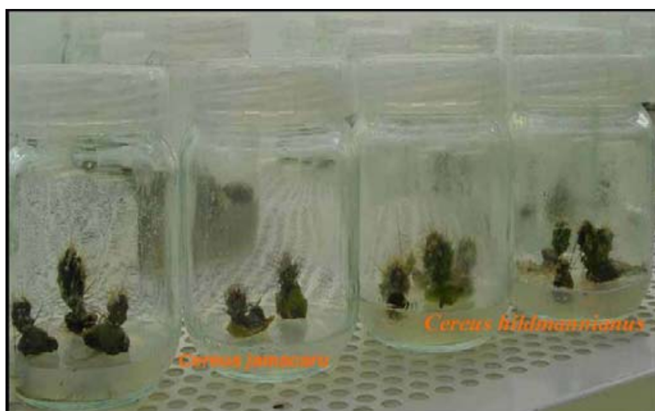

Figure 4. root regeneration in cereus

DISCUSSION

The result **(Table 1)** demonstrated that the areol have negative effect on callus induction. Similarly, for opuntia areol has been reported to reduce callus induction of leaf explants (4). The percentage of shoot regeneration was the highest on medium containing 4 mg/L (w/v) of 2,4-D+6 mg/l KIN+10 % coconut, and 4 mg/L (w/v) of 2,4-D +4 mg/l KIN+10% coconut respectively **(Table 4)**. Similarly, (Sandra Aparecida et al.,1994) (5) in micropropagation of Cereus peruvianus. The result revealed that 4 mg/L (w/v) of 2,4-D+2mg/L Kin and 4 mg/L (w/v) of NAA+2mg/L Kin were very good for formation of rootlets obtain from in vitro plantlets. These findings are in agreement with those reported by for (5) in micropropagation of *Cereus peruvianus*. In vitro induced habituation in tissue cultures, making explants independent from exogenous growth hormones for regenerative activities or on reducing explants regenerative ability (6) was not observed in the persent research.cactus,in general, is known to produce high levels of auxins (7), and this was evident in the ready rooting ability of the initial cultures in the present study. Accumulation of cytokinin like substances due to repeated culture in media containing cytokinin may have been one of the factors that contributed to balance out the high endogenous levels of auxins,and subsequently to improve regeneration in this study.further research to look into the changes in endogenous auxins and cytokinins in the culture is underway. Coconut water or milk is usually added to the culture medium of some species where the initial explants produce excessive amounts of phenolic substances which their oxidation products darken both the tissue and the medium (8). In cactus tissue cultures, the optimum levels of coconut milk for callus formation were 10% (9) For C. peruvianus species, 15% coconut water was initially necessary. However, coconut water seems unnecessary for maintenance during subsequent subcultures, because the 10% concentration was sufficient in the five initial subcultures, and in subsequent subcultures callus tissues continued to grow in the absence of coconut water. In N. profilera, callus formation from different sources was also dependent on the interaction of 2,4-D, coconut milk and kinetin, but coconut milk was required at all times for continued proliferation of callus on subculturing (5).

Table 1. *Effect of Areol on callus induction (CI) of C.jamacaru and C.hildmannianus*

2,4-D	*C.jamacaru* CI %		*C.hildmannianus* CI %	
	Without Areol	With Areol	Without Areol	With Areol
4	90±2.1 a	80±1.4 a	78±1.1 b	60±0.5 b

***Table 2.** Percentage ofplant regeneration and morphology of callus formed from c.hilmannianus and c.jamacaru stem explants on MS medium supplemented with different levels of Plant Growth Regulator*
*Explants with areol no produce plantlet.

No	PGR(mg/l)	Percentage of plantlet	
		c.hilmannianus (without aroel)	*c.jamacaru* (without aroel)
		plantlet	plantlet
1	coconut10+% kinetine lmg/4+2,4-Dmg/l4	A B 40	0
2	coconut10+ % kinetine l mg/6+2,4-Dmg/l4	A53/3	13/3
3	IAAmg/l1+ NAAmg/l1	D0	0
4	BAP mg/l1+ NAAmg/l0/05	D0	0
5	BAP mg/l0/7IBA+ mg/l0/5	D0	0
6	TDZ mg/l0/03NAA+ mg/l0/05	DC6/6	0
7	BAP mg/l1NAA+ mg/l0/1	D0	0
8	kinetinemg/ 0/5NAA+ mg/l0/2	D0	0
9	TDZ mg/l0/05NAA+ mg/l0/5	DC6/6	0
10	BAP mg/l2+ NAAmg/l0/05	D0	0
11	BAP mg/l1+ NAAmg/l0/2	D0	0
12	BAP mg/l0/1+ NAAmg/l0/1	B26/6	0
13	BAP mg/l2+ NAAmg/l0/1	D 0	0
14	BAP mg/l2+ GA_3 mg/l1	C13/3	0
15	BAP mg/l2+ NAAmg/l0/2	D0	0
16	TDZ mg/l0/07NAA+ mg/l0/05	D0	0
17	TDZ mg/l0/07NAA+ mg/l0/2	D0	0
18	TDZ mg/l1NAA+ mg/l0/1	B26/6	0
19	BAP mg/l2	D0	0
20	TDZ mg/l0/03NAA+ mg/l0/1	D0	0
21	TDZ mg/l0/05NAA+ mg/l0/1	D0	0
22	TDZ mg/l0/05NAA+ mg/l0/05	C13/3	0
23	TDZ mg/l0/03NAA+ mg/l0/1	0 D	0
24	BAP mg/l0/5IBA+ mg/l0/7	0 D	0
25	NAA mg/l0/05IBA+ mg/l0/03	0 D	0

***Table 3.** Percentage of root regeneration from c.hilmannianus and c.jamacaru stem explants on MS medium supplemented with different levels of Plant Growth Regulator.*

No	PGR(mg/l)	rooting percentage		
1	MS+ 4mg/l 2,4-D	*c.hilmannianus*	c.jamacaru	rooting percentage
2	MS+ 4mg/l 2,4-D+2mg/l kinetine	47/7 B	31/6 C	64/1AB
3	MS+1mg/l GA_3+2mg/l BAP	63/3 A	53/ 3 A	93/3A
4	MS+4mg/lNAA+2mg/l kinetine	0E	0 D	0E
5	MS+ 4mg/l2,4-D+6mg/l kinetine	33/3 BC	66/6 A	60AB
6	MS+ 0/1mg/l NAA+0/1mg/l BAP	13/3D	13/ 3 C	13/3D
7	MS+ 0/1mg/lNAA +2mg/l BAP	13/3E	26/6 C	0E
8	MS+ 4mg/l NAA	0 F	0 D	0E
9	MS + 4mg/l 2,4-D+4mg/l kinetine	39/9 BC	26/6 C	53/3AB
10	MS+ 4mg/l 2,4-D	29/9 C	26/6 C	33/3BC

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REFERENCES

1. Sawsan, S. Sayed, Abou-Dahab T.A., 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. Murashige,T., Skoog,F., 1962.A revised medium for rapid growth and bioassays with tobacco tissue, *physiol. Plant*.15:474-497.
4. Mondragon Jacobo, C ., 2001. cactus pear Domestication and Breeding, *plant Breeding Reviews*, 20 :151.
5. 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*. p.94-98.
6. Paek KY,Lee CH,Choi JK &Kwack .1984. Mass propagation of Nephrolepis exaltata by runner tip in vitro.J.Korean Soc.Hort.Sci.25:313-312
7. Hubstenberger JF,Clayton PW & Phillips GC .1992.Micropropagation of cacti (cactaceae).In:Bajaja YPS (ed) Biotechnology in Agriculture and Forestry.High-tech and Micropropagation IV 20 :.49-68 Springer,Berlin,Heidelberg,New York.
8. 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.
9. Steinhart, C.E ., 1962.Tissue culture of a *cactus. Science* ,137:545-546.
10. 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.
11. Drew, R.A. and Azimi, M., 2000. Micropropagation of Red Pitaya (Hylocereus undatus), Acta HortiCulture 575.
12. Garcıa-Saucedo. P , Valdez-Morales. M , Valverde. M , Cruz- Hernandez.M and Paredes-Lo pez. 2005. Plant regeneration of three Opuntia genotypes used as human food, *Plant Cell, Tissue* and *Organ Culture*, 80: 215–219.
13. Machado, M. F. and Prioli, A. J ., 1996. Micropropagation of Cereus peruvianus Mill. (Cactaceae) by areole activation, 32, 3:199-203.
14. Martín, R. , Mario, R., Goldammer K., Víctor., 2001. Micropropagation of *Turbinicarpus laui* glasset foster ,an endemic and endangered species, *In Vitro Cell. Dev. Biol. Plant* ,37 (400-404).
15. Molphe-Balch. E, Perez-Reyes, M. , Davila-Figueroa, C. and Villalobos-Amador.E., 2002.*In vitro* propagation of three species of columnar cacti from the sonoran desert.*Hortscience* , 37,4 :693-696.
16. Mohamed-Yasseen, Y., 2002. Micropropagation of pitaya (*Hylocereus* undatus britton et Rose) *In Vitro Cell. Dev. Biol. Plant* , 38:427–429.
17. O.A.Lameira and J. E. B. P .Pinto., 2006. *In vitro* propagation of *Cordia verbenacea* L. (*Boraginaceae*), Rev. Bras. Pl. Med., Botucatu, 8(102-104) n.esp.
18. Park, CH. and Walton, PD. 1989. Embryogenesis and plant regeneration from wild rye Elmus culture of Canada Canadensis L. *Plant Cell* , 8,5:289-291.
19. Papafotiou, M. and Balotis, G. and Louka .P ND Chronopoulos. 2001. *In vitro* plant regeneration of Mammillaria elongate normal and cristat formis,plant cell, *Tissue and Organ Culture*, 65:163-167 .
20. Sriskandarajah S., Al-Ramamneh E. and Serek M., 2004. Regeneration from phylloclade explants and callus cultures of schlumbergera and Rhipsalidopsis,plant cell,*Tissue and Organ Culture*, 78:75-81 .
21. Yasseen Mohamed, Yasseen, Shery, A. and Barringer, Yasseen M., 1995. Rapid propagation of tuna (Opuntia ficus-indica) and plant establishment in soil, *Biomedical* and *life sciences*, 42,1: 117-119.