



Original Contribution

STUDY OF GENOTYPE EFFECT, DIFFERENT MEDIA AND TIME OF EXPLANTING ON CALLUS INDUCTION IN *CEREUS PERUVIANUS* MILL.(CACTACEAE)

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ABSTRACT

Cereus peruvianus callus have a lot of alkaloids compounds and medicine extracted, which is important in medicine, medical and agricultural industries. The experiments were performed using Complete Randomized Design (CRD) and results were analyzed using one way ANOVA. MS medium supplemented with different concentrations of (2,4-D), kinetin and NAA were used. The study was carried out on Increasing callus weight in two different seasons of *Cereus peruvianus* var. *monstrosus* and *Cereus peruvianus* var. *tortuosus*. Results showed the callus induction of *Cereus peruvianus* var. *tortuosus* in MS supplemented with 4 mg/L of 2,4-D in summer and 4 mg/L of NAA in spring was highest, and in MS medium supplemented with (6mg/l) of kinetin in spring and summer was the lowest for *Cereus peruvianus* var. *monstrosus*. There were significant differences between two varieties of callus weight. Also there were significant differences between media treatment in summer and spring.

Key word: callus induction, *cereus monstrosus*, *cereus tortuosus*, regeneration

INTRODUCTION

Cereus peruvianus is an ornamental- medicinal plant from Cactaceae family, native to Brazil and Argentina, adapted to subtropical region (1). Most varieties esp. *cereus peruvianus* var. *monstrosus* and *Cereus peruvianus* var. *tortuosus* are used as medicinal plant. This plant is effective to treat patients with prostate, breast cancer, eye, kidney and heart disease and neuro-degenerative disorders. It also has a rejuvenating effect (2). Its nectar contains unsaturated fatty acids, alkaloids, and acetate used in agriculture and pharmaceuticals (3, 4). The most important fatty acids are Oleic acid $C_{18:1}$, Linoleic acid $C_{18:2}$, Pantadecanoic acid $C_{15:2}$, Myristic acid $C_{14:2}$, Palmitolenic acid $C_{16:1}$, Heptadecanoic acid $C_{17:2}$ and Decanoic acid $C_{10:2}$, since alkaloids of cereus callus in

comparison with mature plant is 1.7:1 (5). The best medium for callus induction was Ms-medium including 4 mg/l 2,4-D, 4-6 mg/l kinetin, % 15 coconut nectar (6). According to the study of some cacti regeneration like *Apontia* the best medium for callus induction is 2m 2,4-D, 2mg/l NAA (7). In *Notocactus* micropropagation in a media of 1.1 mg/l 2,4-D and 1 mg/l BA (8). We had the best callus induction. For *Ariocarpus* embryo generation 1 mg/l NAA and 2-3 mg/l BA was the best medium (9) and intergeneration of *Carifanta* from root the most callus induction was on a medium of 2 mg/l 2,4-D and 1 mg/l kinetin (10). When *Torbino Carpus* cactus was cultured in *in-vitro* culture by adding 1-3 mg/l BA to Ms medium, callus growth increased (11). Culturing *cereus* or extracting alkaloids have not experienced in Iran. The present study gives consolidated information about Genotip effect, different media and time of explanting on callus induction in *Cereus peruvianus*.

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MATERIALS AND METHODS

The experiments were repeated two times during the years of 2007 and 2008 at Tissue Culture Research Laboratory, Agriculture Biotechnology Research Institute Central Region of Iran. Seedlings (two-year-old) with a height of 15-20 cm originated from the Cactus International greenhouse were used as a source of plant materials. The stem explants from *Cereus peruvianus* var. *monstrosus* and *Cereus peruvianus* var. *tortuosus* were surface-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. stems were cut into smaller segments and were cultured on Ms-medium enriched with sucrose %3, 8 g/l phytoagar, growth regulators, pH=0.8 (**Table 1**). They were placed in a dark growth chamber at $25\pm 1^{\circ}\text{C}$ for 45 days. After 3 weeks culturing explants were subculture on a fresh medium. Within 45 days of incubation, callus weights were evacuated. This test was carried out in spring and summer by using factorial complete randomized design with 3 replications, 5 explants in each replication. Analyzing data was performed by using SAS and comparing averages was done by Duncan test and a probability value of %5.

Table 1. Combination of medium using experiment

PGR(mg/l)	No	PGR(mg/l)	No
Ms+4 mg/L NAA +2 mg/L kinetin	5	Ms+4 mg/L 2, 4-D	1
Ms+4 mg/L NAA +4 mg/L kinetin	6	Ms+4 mg/L 2, 4-D+2 mg/L kinetin	2
Ms+4 mg/L 2, 4-D+6 mg/L kinetin	7	Ms+4 mg/L 2, 4-D+4 mg/L kinetin	3
		Ms+4 mg/L NAA	4

RESULTS

Based on culture, variety and season, (a probability value of %1) and for culture treatment, and variety ($P\leq\%5$) was considered significant. (**Table 2**), the average increasing of callus weight in different seasons showed a significant difference. It was 2.64 in spring (**fig. 1, 2**) and 2.56 in summer (**fig. 3, 4**) (**Table 3**). Also it was 2.91 in var. *tortuosus* (**fig 2, 4**) and 2.34 in var. *Monstrosus* (**Fig. 1, 3**). The most effective media treatment was 4, including Ms+4mg/l NAA and media treatment 7, with Ms+4mg/l NAA+4mg/l Kinetin was the lowest, In callus induction, NAA was more effective than 2,4-D and Kinetin. Two weeks after culturing, callus induction was started. Kinetin reduced callus induction and by increasing this hormone, friable calluses converted into compact ones esp.in *Cereus peruvianus* that the most calluses were friable. Friable calluses were light yellow and compact ones were greenish yellow.

DISCUSSION

The most effective factors on callus induction are hormone concentration, genetic and time of getting explants. Also composition and amount of auxine is important. All in all, 1-2 mg/l 2,4-D is necessary for callus induction but about

Cactaceae we need more (8), In this study, callus induction and friability decreased when cytokinin increased. We had the lowest compact callus induction in media treatment 4, MS-medium supplemented with (4mg/l 2,4-D+6mg/l kinetin). These finding were not in agreement with the result of Diolivera (1994) and Machad (1996) in micropropagation of *cereus peruvianus*. They found that on this media callus growth was higher and friable in comparison to the media containing lower kinetin (5-12). The results were in contract with Goldamer about *Ario carpus* micropropagation. He reached that the highest callus induction by adding 3 mg/l Banzil Adenin and 1 mg/l NAA (9). In this study, the result showed that callus induction in spring was more than summer. Ekiz *et al* (1997) and Wernicke *et al* (1997) found that wheat callus induction in spring was more than other seasons and most plants are the same (13- 14). Bosila *et al* (2003) reported that absorbed light by mature plant, (because of its effect on auxin), subculture, genotype and explants had a great effect on callus size and amount of callus (15). Increase of callus weight for all treatments in spring and increase of callus induction could be affected by genotype, plant cells and hormones. Plant vegetative activity

and increase of auxin was an important cause of callus induction in spring. Auxin had a great effect on callus induction. The callus induction in NAA treatment was more compared with 2,4-D. Chailakhyan et al (1975). Reported that plant changes caused by growth and increase of daylight affected florigene and decomposition started. Environmental changes (hormones)

displayed the strongest effect. The concentration of hormones in a medium like kinetin or vitamins and glucose were important causes in callus induction. Considering the effect of temperature on auxin production, it will increase in summer and physiological changes of shoot regeneration increases kinetin that in composition with NAA, is the best callus induction media treatment(2).

Table 2. The result schedule of variances of induction callus and comparison average the medium effect on increasing of fresh weight of *Cereus peruvianus* using DMRT (in summer season and spring).

increasing of fresh weight callus Mean square	Degree of Freedom	Variable sources
0/31**	1	season
6/92*	1	var
3/86*	6	Media treatment
0/02**	6	Treatment hormone * var
0/73**	1	season * var
0/64**	6	Media treatment * season
0/89*	6	Media treatment * season * var
----	38	error bar
8/7		cv

*showeds that significantly different at 5% level ** showed that significantly different at 1% level and ns showed that not

Table 3. Increasing of fresh weight callus in cereus genotype in summer season and spring

increasing of fresh weight callus		Increasing in <i>Cereus</i> Genotypes callus increasing of fresh weight	
summer	spring	var monstrosus	var tortuosus
2/56 b	2/64 a	2/34 b	2/91 a



Fig. 1. The callus of *C. peruvianus* var monstrosus in spring season



Fig. 2. The callus of *C. peruvianus* var tortuosus in spring season

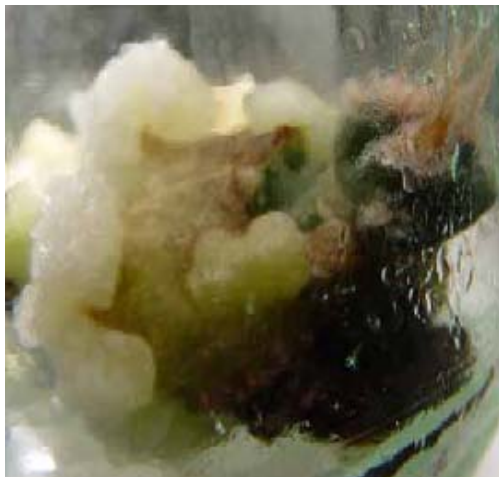


Fig. 3. The callus of *C. peruvianus* var *monstrosus* in summer season



Fig. 4. The callus of *C. peruvianus* var *tortuosus* in summer season

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