IN VITRO PRODUCTION OF CALLUS FROM *ZYGOPHYLLUM COCCINEUM* L.

EMAN, A. ALAM
Botany Department, National Research Centre, Dokki, Giza, Egypt
Corresponding author: Eman200980@hotmail.com

Abstract

Tissue culture technique was used to produce callus from the wild economic plant (*Zygophyllum coccineum* L.). MS medium supplemented with 2 mg/l 2, 4-D was the most suitable medium for callus induction using stem segments explants after 4 weeks. MS medium supplemented with 3 mg/l 2, 4-D was the most suitable medium for growth of this callus after 4 weeks.

[Eman, A. Alam. *IN VITRO* PRODUCTION OF CALLUS FROM *ZYGOPHYLLUM COCCINEUM* L.

Academia Arena 2011;3(1):6-9]. (ISSN 1553-992X). http://www.sciencepub.net.

Key words: ZYGOPHYLLACEAE, *IN VITRO* PRODUCTION OF CALLUS, *ZYGOPHYLLUM COCCINEUM* L.

Introduction

Genus *Zygophyllum* (Zygophyllaceae) includes many medicinal and economic plants which have diuretic, anthelimintic, antidiabetic, antibacterial and insecticidal effects (Elgamal *et al.,* 1995; El-Hefnawi, 1999; Nawairy *et al.,* 2002 and Assar and El-Sopky; 2003). Tissue culture technique was used to cultivate different genera of Zygophyllaceae for regeneration purposes such as *Zygophyllum xanthoxylon* (Bunge) (Sun, 2008), to produce more biologically active compounds such as ascorbic acid from callus of *Fagonia cretica* (Kapoor, 2002), alkaloids, saponins, flavonoids and phenolic compounds of antibacterial activity more than the intact plant (Eman *et al.,* 2010), diosgenin from callus of *Balanites aegyptiaca* (Gour and Kant, 2006) and beta -carboline and serotonin alkaloids and fatty acids from callus of *Peganum harmal*a (Piacetini *et al*., 2004).

Our work aims to produce callus from *Zygophyllum coccineum* using tissue culture technique because it is difficult to produce callus from it because it is succulent plant however the medicinal and economic importances of it.

Materials and Methods

Plant materials:

Samples of *Zygophyllum coccineum* L. were collected from Quatamia - Suez desert road (150 Km away from Suez City). Samples were authenticated by comparison with voucher specimens in the herbarium of Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt, where voucher specimens were deposited.

Methods:

1- Callus induction:

This experiment was carried out to study the effect of different concentrations of 2, 4-D on callus induction of *Zygophyllum coccineum* L. stem segments explants. Explants were surface sterilized by immersion in 70 % ethanol for 30-60 seconds, then soaked in 50% of sodium hypochlorite (commercial Clorox) for 15-20 minutes, then washed with sterile distilled water "3 times" (Hoda, 1994). Sterilized explants were aseptically transferred to sterilized MS medium (Murashige and Skoog, 1962); supplemented with 3% sucrose (Khafagi, 2000), 1% agar (Torres, 1989) and different concentrations of 2,4-D as follows.

Media	Hormones
1	1 mg/l 2,4-D
2	2 mg/l 2,4-D
3	3 mg/l 2,4-D

The callus cultures were incubated at 25± 2°C for four weeks. The percentage of calli induction was calculated every week during the incubation period.

2-Callus growth:

Callus of *Zygophyllum coccineum* L. stem segments explants can be maintained by subculturing on MS medium supplemented with different concentrations of 2, 4-D as previously mentioned in callus induction.

The callus cultures were incubated at 25± 2°C for four weeks. Weight of callus of hypocotyle explants was taken as an indicator of callus growth on each medium as follows:

+	≥ 1 g
++	≥ 2 g
+++	≥ 5 g

3- Statistical analysis:

Statistical analysis of all results was done using Fisher analysis of variance methodology. A least significant difference test was applied at 5% and 1% probability level to determine differences among treatment means (Steel and Torrie, 1984). The

MSTAT computerized package program was subjected to the regular statistical analysis of variance (Nissen *et al*., 1985). Each reading = mean of three replicates ± SD.

Results and Discussion

1- Callus induction:

Data in Table (1) revealed that, using stem segments explants of *Zygophyllum coccineum* L. to induce calli through the incubation period (four weeks) on the three used media, it was found that, there is a highly significant variation between the three used media for calli induction from stem segments explants. The three types of media can induce calli with special reference to medium number (2).

Table (1): Responses of stem segments explants of *Zygophyllum coccineum* L. to calli induction on three different media during four weeks.

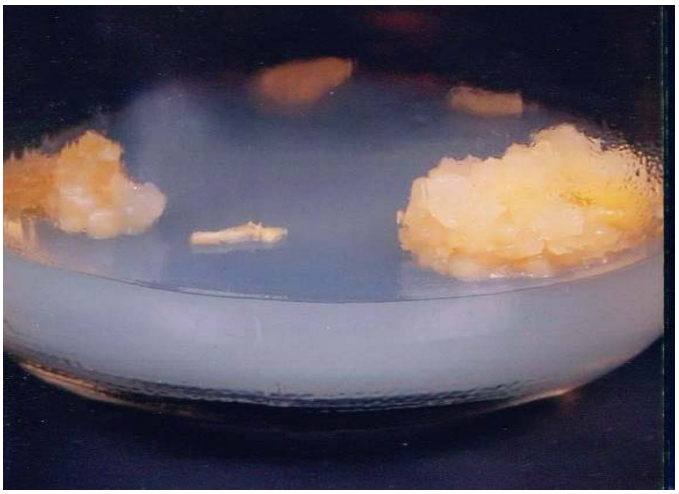
Media	1st week	2nd week	3rd week	4th week
1	0.22±0.10	0.44±0.09	0.55±0.10**	0.55±0.10**
2	**0.22±0.10**	**0.50±0.00**	**0.67±0.10****	**0.72±0.04****
3	0.14±0.10	0.28±0.25	0.28±0.25	0.28±0.25
L.S.D. (0.05)	**0.39**			
L.S.D. (0.01)	**0.40**			

2- Callus growth:

Data in Table (2) and Photos (1-2) revealed that, callus of *Zygophyllum coccineum* L. stem segments explants grew on media number **1,2** and **3**, with special reference to that on medium number **3**. These results agreed with Zhang and Kang, 2004; Khafagi *et al.,* 2004; Ibrahim and Khafagi, 2004; Mohan *et al*., 2004 and Gour and Kant, 2006 since they found that, *Nitraria tangutorum, Peganum harmala, Tribulus terrestris* and *Balanites aegyptiaca* respectively can induce calli using MS medium supplemented with 2,4-D.

Table (2): Qualitative estimation of callus growth of *Zygophyllum coccineum* L. stem segments after four weeks.

Media	Weight of callus (g)
1	+
2	++
3	**+++**



Photos (1-2

Photos (1-2): Callus growth of stem segments explants of *Zygophyllum coccineum* L. on medium number (3) after four weeks.

References

1. Elgamal, M.H.A.; Shaker, K.H.; Pollmann, K. and Seifert, K. . Triterpenoid saponins from *Zygophyllum species*. Phytochemistry1995; 40(4): 1233-1236.
2. El-Hefnawi, H. N. .Screening of some Sinai plants for their antimicrobial activity. Al-Azhar Journal of Microbiology1999;3:1-6.
3. Newairy, A.A.; Mansour, H.A.; Yousef, M.I. and Sheweita, S.A.. Alterations of lipid profile in plasma and liver of diabetic rats: effect of hypoglycaemic herbs. Journal of Environmental Science and Health art-B, Pesticides, Food Contaminants and Agricultural Wastes2002;37(5):475-484.
4. Assar, A.A. and El-Sobky, M.M.. Biological and histopathological studies of some plant extracts on larvae of *Culex pipiens* (*Diptera Culicidae*). Journal of the Egyptian Society of Parasitology2003; 33(1):189-200.
5. Sun .Rapid clonal propagation of Zygophyllum xanthoxylon (Bunge) Maxim., an endangered desert forage species.*In-Vitro*-Cellular-and-Developmental-Biology-Plant 2008;44(5):.396-400.
6. Kapoor, B.B.S.. Production of endogenous ascorbic acid from tissue cultures of *Fagonia cretica* L. . Journal of Phytological Research2002; 15(1): 111-112.
7. Eman, A. Alam; Gehan, H. Amin; Yassin, M . ElAyouty and Mohamed, S. Abdel-Hady. . Chemical Composition and Antibacterial Activity Studies on Callus of *Fagonia arabica* L.. Academia Arena 2010;2(12):91-106.
8. Gour, V.S. and Kant, T.. Optimized callus production in *Balanites aegyptiaca* (L.) Del. . Journal of Phytological Research 2006; 19(1):53-57.
9. Piacetini, M.P.; Piatti, E. and Faternale. Phospholipase C-dependent phosphoinositide breakdown induced by ELF-EMF in *Peganum harmala* callus . Biochemimie (Paris) 2004; 86(4-5):.343-349.
10. Hoda, A.M.. An attempt for producing callus from tomato plants resistant to salinity. M.Sc. Thesis1994, Vegetables and Horticulture Department, Faculty of Agriculture, Zagazig University, Egypt: 36.
11. Murashige, T. and Skoog, F.. A revised medium for rapid growth and bioassays with Tobacco tissue cultures. Physiology of Plants 1962; 15: 473-479.
12. Khafagi, I. . Management of growth and autotoxicity of *Cleome droserifolia* heterotrophic. Egyptian Journal of Botany 2000 ; 38(1-2):157-171.
13. Torres, K.. Tissue culture techniques in horticultural crops. Van nostrand reinhold, 1989, New York, Chapt., 2: 26-51.
14. Steel, R.G.D. and Torrie, J.H.: Principles and procedures of statistics 1984, Mc Graw Hill Book Co. Inc, New York , USA, 2nd ed.
15. Nissen, O.; Eisensmith, S.P.; Freed, R.; Everson, E.H.; Smail, V.; Weber, M.; Tohme, J.; Anderson, J.; Rorick, K.; Portice, G.; Rittersdorf, D.; Wolberg, P.; Bricker, B.; and Heath, T. : A microcomputer program for the design, management and analysis research experiments 1985. Version 4, Michigan State University and Agriculture University of Norway, USA.
16. Zhang, H.X. and Kang, X.Y.. Studies on tissue culture techniques of *Nitraria tangutorum*. Xibei Zhiwu Xuebao2004; 24(1):.56-64.
17. Khafagi, I.; Abdel-Kader, K.; Kord, M.; Dewedar, A. and Hamid, S.A.. *In vitro* eliciation of plant photosensitizers in tissue culture systems. Egyptian Journal of Physiological Science 2004; 25(1): 105.
18. Ibrahim, M. A. and Khafagi, I. K.. Effect of extremely low frequency magnetic field on seed germination. Egyptian Journal of Biophysics and Biomedical Engineering 2004 ; 2004(5): 41-57.
19. Mohan, J.S.; Kumar, V.V. and Aparna, V. . Somatic embryogenesis and plant

regeneration in *Tribulus terrestris* L. . Phytomorphology2004; 50 (3-4): 307-311.

11/16/2010