Research Journal of Medical Sciences 4 (4): 252-254, 2010
ISSN: 1815-9346

Requirement of Organic Factors for the Growth of *Datura stramonium in vitro* Tissues Cultured

[1]Ali Parsaeimehr and [2]Omid Alizadeh
[1]Department of Plant Biotechnology, National Academy of Science, Yerevan, Republic of Armenia
[2]Islamic Azad University, Firoozabad Branch, Iran

Abstract: Addition of a wide variety of organic extracts to culture media often results in favorable tissue responses in this aim we conducted an experiment on *Dutura stramonium* because of its essential role in medicine industry and its Alkaloids, two organic factor one thiamine-HCl and the other myo-inositol applied in this expirement, we wants to find out that the organic factors are essential in a tissue culture or not according to this aim the hypocotyls used as explants from the seeds as 3 cm length of Dutura stramonium by callus induction 100 mg L^{-1} of myo-inositol, 0.5 mg L^{-1} of thiamine-HCl used for organic factor. Three pieces of callus 30±0.1 g were transfer to a 100 mL Erlenmeyer flask with 50 mL of medium and incubated for 1 month in the dark at 25°C and the fresh weight of callus in each flask was determined after harvesting and comparing together for obtaining the results. Results were presented as the average of 15 replications for each treatment, the absence of thiamine-HCl or myo-inositol caused the decrease amounth in the growth of the *Datura stramonium* callus which been cultured on the complete medium with thiamine-HCl and myo-inositol for 8 months. Well according to this result its suggested that thiamine-HCl and myo-inositol are thought to be necessary for the growth of the callus. But the callus that had been cultured on the medium without thiamine-HCl or myoinositol for one month before the test could grow even on the medium without thiamine-HCl or myo-inositol at almost the same rate as the growth on the complete medium, From this fact the results obtained in considered to be due to a degeneration of the ability to biosynthesize thiamine-HCl and myo-inositol which occurs in the callus during its long term subculture on the medium with thiamine-HCl and myo-inositol. These degenerated abilities are restored by culturing the callus on a medium without thiamine-HCl and/or myo-inositol. The callus which lost the ability cannot grow without thiamine and requires it. But when the callus continued to becultured without thiamine, it must biosynthesize thiamine to continue to live. The ability may be restored and the callus begins to synthesize it. Once it is again supplied sufficiently by biosynthesis, the callus no longer requires added thiamine and continues to grow even without thiamine in the culture medium.

Key words: *Datura stramonium*, tissues cultured, organic factor, HCL, theamine, Iran

INTRODUCTION

The role of organic factors in tissue culture is so complicated in some of plant tissue cultures they are vital and in the others they are not essential, *Dutura stramounium* from solanaceae is one of the importance plants which use in industrial drug because of its enrichments alkaloids substances, its alkaloids have to many variable effects on eyes, nervous system, heart and blood circulation so by the base of these information's its clear that callus culture for the aim of suspension and producing the secondary metabolites in bioreactors and extracting substance like Atropine, Heuciamin and Scopolamine, organic factors are playing a valid roles. Plant tissue and cell culture media are generally made up of some or all of the following components: macronutrients, micronutrients, vitamins, amino acids or other nitrogen supplements, sugar(s), other undefined organic supplements, solidifying agents or support systems and growth regulators. Several media formulations are commonly used for the majority of all cell and tissue culture work, well organic factors in the *in vitro* tissue cultured except sugar as a source of energy or agar as a supporter for tissue culture or 2,4-D, NAA as an auxin or BA as a cytokine which are essential for plant tissue culture but several organic factors have been considered necessary for tissue cultures. It is known by experts that a plant is able to biosynthesize the organic substances necessary for its vitality. This fact suggests that even cultured cells may not require any organic constituent except sugar as an energy source. Actually, however, in the media so far available for tissue culture,

Corresponding Author: Ali Parsaeimehr, Department of Plant Biotechnology, National Academy of Science, Yerevan, Republic of Armenia

various organic constituents have been added such as vitamins, amino acids, nucleic acids, hormones and/or natural extracts such as yeast extract, coconut milk etc., besides an energy source and inorganic nutrients (Butenko, 1964; Murashige and Skoog, 1962; Shoo, 1972; White, 1963; Yeoman, 1973).

Addition of a wide variety of organic extracts to culture media often results in favorable tissue responses. Supplements that have been tested include protein hydrolysates, coconut milk, yeast extracts, malt extracts, ground banana, orange juice and tomato juice. However, undefined organic supplements should only be used as a last resort and only coconut milk and protein hydrolysates are used to any extent today (Demeyer and Dejaegere, 1988).

Protein (casein) hydrolysates are generally added to culture media at a concentration of 0.05-0.1% while coconut milk is commonly used at 5-20% (/v) (Demeyer and Dejaegere, 1993). Even in the medium by Murashige and Skoog (1962) thiamine-HCl and myo-inositol are used as organic constituents.

From the point of the original properties of the plant cells themselves, the thiamine-HCl and myo-inositol are not supposed to be required for tissue culture. In this aim we used these nutrients in the study in this experiment for the *Datura stramonium* tissue culture.

MATERIALS AND METHODS

Seed disinfection: Seeds of *Datura stramonium* used and their surface sterilized in 0/1% $HgCl_2$ and 1/0% SDS for 3 min and placed on half-strength MS (Murashige and skoog, 1962) medium with 0/8% agar for germination the incubation condition for *in vitro* culture should adjust on 25±1°C and 16 h photoperiod of approximately 28 $\mu Em^{-2}sec^{-1}$, the hypocotyls used as explants from the seeds as 3 cm length.

Callus induction, subcultures and organic factors: The complete medium for the present experiments consists of the mineral salts of Linsmaier and Skoog (1965), 100 mg L^{-1} of myo-inositol, 0.5 mg L^{-1} of thiamine-HCl, 2mg 2,4-D, 3.0~o sucrose and 1.0~o agar applied for this experiment. Subculture on the complete medium in the dark at 25°C for 5 months by transplanting tissue every month.

Three pieces of callus 30±0.1 g were transfer to a 100 mL Erlenmeyer flask with 50 mL of medium and incubated for 1 month in the dark at 25°C and the fresh weight of callus in each flask was determined after harvesting and comparing together for obtaining the results.

RESULTS AND DISCUSSION

Results were presented as the average of 15 replications for each treatment. As it shown in Table 1 the absence of thiamine-HCl or myo-inositol caused the decrease amount in the growth of the *Datura stramonium* callus which been cultured on the complete medium with thiamine-HCl and myo-inositol for 8 months. Well according to this result it's suggested that thiamine-HCl and myo-inositol are thought to be necessary for the growth of the callus.

But the callus that had been cultured on the medium without thiamine-HCl or myoinositol for 1 month before the test could grow even on the medium without thiamine-HCl or myo-inositol at almost the same rate as the growth on the complete medium (Table 1 and 2). These results suggest that the *Datura stramonium* callus originally. Requires neither thiamine-HCl nor myo-inositol for growth (Butenko, 1964).

So in this aim, consequently the callus was cultured on a medium without either thiamine-HCl or myo-inositol for a term of about 5 months and growth was then compared to the one on the complete medium. As shown in Table 3 the growth of this callus on the medium without thiamine-HCl and myo-inositol showed 89.5% of that on the complete medium well according to this result we can suggest that no organic factor is required for the growth of *Datura stramonium* callus except sugar as an energy source, agar as a tissue supporter and 2, 4-D as an auxin. These three are thought to be essential for tissue culture for *Datura stramonium.* From this fact, the results shown in Table 4 are considered to be due to a

Table 1: Effect of myo-inositol absence on the growth of Dutra's callus

Medium used for test	Fresh weight of callus (g)	Percentage
Complete	3.45	100.0
Myo-inositol	3.35	97.1

Table 2: Effect of thiamin-HCl absence on the growth of Dutras callus

Medium used for test	Fresh weight of callus (g)	Percentage
Complete	3.84	100.00
Thiamin-Hcl	3.96	103.12

Table 3: Effect of absence of both thiamine-HCl and myo-inositol on the growth of Dutra's callus

Medium used for test	Fresh weight of callus (g)	Percentage
Complete	3.64	100.0
Thiamin-Hcl and myo-inositol	3.26	89.5

Table 4: The Effect of absence of thiamin-HCl or myo-inositol on the growth of Dutra's callus

Medium used for test	Fresh weight of callus (g)	Percentage
Complete	3.40	100.00
Thiamine-Hcl	0.82	24.11
Myo-inositol	0.78	22.94

degeneration of the ability to biosynthesize thiamine-HCl and myo-inositol which occurs in the callus during its long term subculture on the medium with thiamine-HCl and myo-inositol. These degenerated abilities are restored by culturing the callus on a medium without thiamine-HCl and/or myo-inositol. The callus becomes able to grow without thiamine-HCl and/or myo-inositol as observed in fallowing tabels.

The results described clearly show that organic nutrient requirements for growth of callus are closely related to the culture histories of the callus. This correlation may be understood by an interpretation based on the changing ability to biosynthesize organic nutrients during culture of the callus. For an example, in the case of the thiamine nutrient when the callus is cultured on the medium with thiamine, the callus needs not biosynthesize thiamine.

Then during the long term culture with thiamine, the ability to biosynthesize thiamine may become lower and in some case completely degenerate. The callus which lost the ability cannot grow without thiamine and requires it. But when the callus continued to be cultured without thiamine, it must biosynthesize thiamine to continue to live. The ability may be restored and the callus begins to synthesize it. Once it is again supplied sufficiently by biosynthesis, the callus no longer requires added thiamine and continues to grow even without thiamine in the culture medium (Nishi, 1974).

CONCLUSION

According to the results, it seems that by ascending the period time of callus vitality, the calli alleviate its requirements to the organic factors for instance thiamine nutrient in a tissue culture the callus is cultured on the medium with thiamine, the callus don't need to biosynthesize thiamine. Then during the long term culture with thiamine, the ability to biosynthesize thiamine. The callus which lost the ability cannot grow without thiamine and requires it but after sub culturing the callus of *Datura stramonium* can approve to synthesis the organic factors which we applies in this experiment.

REFERENCES

Butenko, R.G., 1964. Nutrition of Tissue Culture. In: Plant Tissue Culture and Plant Morphogenesis, Artman, M. (Eds.). Israel Program for Scientific Translations, Jerusalem, Israel, pp: 23-53.

Demeyer, K. and R. Dejaegere, 1988. Influence of the mineral nutrition on yield and alkaloid content in *Datura stramonium*. Medelingen van de Faculteit, 53: 1723-1725.

Demeyer, K. and R. Dejaegere, 1993. Influence of nitrogen on the alkaloid content of *Datura stramonium*. Acta Hortic., 331: 35-38.

Linsmaier, E.M. and F. Skoog, 1965. Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant, 18: 100-127.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth bioassays with tobacco tissue culture. Physiol. Plant, 15: 473-497.

Nishi, T., 1974. Requirement of organic factors for the growth of *Ephedra* tissues cultured *in vitro*. J. Plant Res., 87: 337-340.

Shoo, K., 1972. Constituents of Medium for Plant Tissue Culture. In: Plant Tissue Culture, Takeuchi, M., I. Ishihara and T. Furuya (Eds.). Asakura-Shoten Co. Ltd., Tokyo, Japan, pp: 50-85.

White, P.R., 1963. The Cultivation of Animal and Plant Cells. Ronald Press Co., New York, pp: 228.

Yeoman, M.M., 1973. Tissue (Callus) Cultures Techniques. In: Plant Tissue and Cell Culture, Street, H.E. (Ed.). Blackwell Scientific Publications, Oxford, pp: 31-58.