WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 2, 1215-1220. Research Article ISSN 2277– 7105

EFFECT OF SOME GROWTH HORMONES AND COLCHICINE ON BIOMASS AND GLYCYRRHETINIC ACID PRODUCTION IN CALLUS CULTURE OF *GLYCYRRHIZA GLABRA* LINN.

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Article Received on
06 Dec 2014,

Revised on 27 Dec 2014,
Accepted on 19 Jan 2015

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ABSTRACT

The study was aimed towards the production of medicinally important glycyrrhetinic acid from the callus culture of *Glycyrrhiza glabra* using various phytohormones and chemical mutagen colchicine. Supplementation of MS media with various combinations and concentrations of phytohormones enhanced glycyrrhetinic acid level in stem, leaf and root derived callus. When the calli derived from some selected medium were exposed to various concentrations of colchicines, the glycyrrhetinic acid content was further increased.

KEY WORDS: *Glycyrrhiza glabra*, callus culture, colchicine.

INTRODUCTION

Plants are the only economic source of a number of well established and important drugs. At a rough estimate, 25% of the modern drugs are directly or indirectly derived from plant products. The technology for the production of 18 β-acyl glycyrrhetinic acid from *Glycyrrhiza glabra* has been developed and the production is likely to be undertaken in near future.[1] *G. glabra* is used as an expectorant, antitussive, anti-inflammatory and antiallergic agent.[2 5] Revers [6] and Landarlan [7] reported antiulcerative property which is due to β - glycyrrhetinic acid and its glycoside, glycyrrhizin. These two compounds have also been found effective in rheumatoid arthritis, hepatitis and in Addison's disease.[8,9] The antimicrobial activity of *G. glabra* is due to the presence of flavones, licoflavone which has been found effective in preventing cytotoxicity against *E. coli*.[10,12] Hatano *et.al*.[13] isolated an anti HIV (Human Immunodeficiency Virus) phenolic constituent, licopyranocoumarin from the roots and stolon of *Glycyrrhiza* spp.

Earlier some work has been reported on the static and suspension culture of *G. glabra* for biomass production and only a few works has been reported on the regulatory aspect of glycyrrhetinic acid production using chemical mutagens.[14-16] Therefore, in the present investigation attempts have been made to study the effect of various phytohormones and a chemical mutagen, colchicine, on the glycyrrhetinic acid production in the callus culture of *G. glabra.*

MATERIALS AND METHODS

Various manipulations in Murashige and Skoogs medium (MS medium)[17] were made using different combinations and concentrations of Naphthalene acetic acid (NAA), Indole-3-acetic acid (IAA), 2,4-Dichlorophenoxy Acetic acid (2,4-D) and Benzyl aminopurine (BAP) and the media were sterilized (120°C. 15lb/sq.in. for 30 mins.).

After proper surface sterilization, nearly 5mm long stem, leaf and root explants of *G. glabra* were transferred separately on the culture slants and the culture tubes were stored in dark for one week to initiate the callus proliferation, after which the culture tubes were exposed to florescent light (1600-2000Lux, 16 hrs.) at 26± 1°C. After five weeks, the calli were withdrawn from the tubes and growth index (Final dry wt. – Initial dry wt. / Initial dry wt.) determined. The callus mass was dried at 60°C for 48 hrs and dried mass so obtained was estimated for glycyrrhetinic acid content. The media exhibited best results with stem, leaf and root explants were further used for studying the effect of colchicine on growth and glycyrrhetinic acid production.

About 30 days old stem derived callus (grown on MS medium containing 2.0 mg/l each of NAA and BAP), leaf derived callus (grown on MS medium containing 2.0 mg/l of NAA and 2.5 mg/l of BAP) and root derived callus (grown on MS medium containing 0.5 mg/l of IAA, 0.5 mg/l of 2,4-D and 0.1 mg/l of BAP) were transferred to fresh liquid medium with the same composition of phytohormones and shaken in a rotary shaker at 100 rpm for 10 days. The cells were filtered by a nylon fabric and treated with different concentrations of colchicine ranging from 0.02 to 0.25% for two hours with constant shaking in a rotary shaker at 100 rpm.

After these treatments the cells were recovered by nylon fabric and washed repeatedly with sterile distilled water. Later these cells were transferred to fresh static medium with the same

hormonal combination and allowed to grow into calli for five weeks. The calli were harvested separately and growth index (GI) and glycyrrhetinic acid (GA) content was calculated.

Estimation of Glycyrrhetinic acid content

Various methods for the estimation of glycyrrhetinic acid content in plants include HPLC [18], gravimetric[19], colorimetric[20], polarographic[21], paper chromatographic[22], TLC[23], GLC [24] and TLC densitometer.[25] The procedure for GA estimation prescribed in British Pharmacopoeia[26] was followed in the present investigation. Briefly, the estimation procedure involved hydrolysis of one gram callus powder with 1M HCl and 1,4-Dioxan for two hours. After filtering the filter was dried at 105°C and extracted with chloroform. The chloroform extract was evaporated to dryness and the residue was dissolved in 10 ml mixture containing equal volumes of chloroform and methanol. Measured quantity of this solution was applied on chromatoplates of silica gel G and placed in TLC chamber containing ethyl acetate, ammonia (1M) and absolute ethanol (60:27:13). The developed plates were dried and examined under UV light; the area corresponding to glycyrrhetinic acid was scrapped off, treated with 10 ml of absolute ethanol and filtered through sintered glass filter. The glycyrrhetinic acid content was determined specrophotometrically by measuring absorbance at 250nm.

RESULTS AND DISSCUSSION

In the first step of present experimentation various combinations of phytohormones were screened for biomass and glycyrrhetinic acid production. The media which produced better results with stem, leaf and root explants were selected further for studying the effect of colchicine. In second step, various concentrations of colchicine ranging from 0.02 to 0.25% were tried for the production of glycyrrhetinic acid content in stem, leaf and root derived callus culture of *G. glabra*. The observations show that various combinations of phytohormones in all used concentrations favored the callus growth in stem, leaf and root segments of *G. glabra* as compared to phytohormones free (control) medium. In stem and leaf explants, the best growth was obtained in the MS medium containing 2mg/l each of NAA and BAP that exhibited maximum GI of 5.86 (control: 1.84) in stem and 6.22 (control: 1.78) in leaf derived callus. In case of root explant, the highest GI of 4.38 was obtained with the medium containing 2mg/l 2,4-D and 0.1 mg/l BAP while control medium could not induce callusing even in five weeks time.

The observations show enhanced production of glycyrrhetinic acid when MS medium was supplemented with various combinations of phytohormones. In stem derived callus the best results, however, were obtained with MS medium containing 2mg/l each of NAA and BAP which produced maximum glycyrrhetinic acid of 2.14% (control 0.56%). in leaf derived callus maximum amount of glycyrrhetinic acid 2.04% (control: 0.48%) was obtained with the MS medium containing 2mg/l of NAA and 2.5mg/l of BAP while in case of root derived callus, maximum glycyrrhetinic acid of 3.90% was obtained with the medium containing 0.5mg/l of IAA, 0.5mg/l of 2,4-D and 0.1mg/l of BAP.

Various concentrations of colchicine ranging from 0.02 to 0.25% were tried for the production of Glycyrrhetinic acid from stem, leaf and root culture of *G. glabra*. it was observed that initial concentrations (i.e. 0.02 to 0.15%) of colchicine increased the glycyrrhetinic acid content in stem, leaf and root derived cultures. However, it was 0.1% colchicine which produced maximum of 2.86% glycyrrhetinic acid (control: 2.14%) in stem and 4.40% glycyrrhetinic acid (control: 3.90%) in root derived callus, while 0.05% colchicine produced a maximum of 2.84% glycyrrhetinic acid (control: 2.04%) in leaf derived callus culture of *G. glabra*.

Initial concentrations i.e. 0.02 to 0.10% of colchicine did not show any beneficial or detrimental effect on biomass production as the growth indices were slightly increased. Thereafter as the colchicine concentration increased the growth indices decreased.

G. glabra and its important bioactive constituents glycyrrhetinic acid and glycyrrhizin have been in great demand because of their high therapeutic value but due to nonconductive climatic conditions the drug does not grow well. Keeping this in mind, tissue culture technique was adopted for the production of medicinally important glycyrrhetinic acid using various phytohormones and a chemical mutagen colchicine from the callus culture of *G. glabra*.

As various physical and chemical mutagens produce multiplication, addition, alteration or aberration in the chromosomes, such mutants can be utilized to enhance enzyme activity and, in turn, the yield of metabolites is increased. This phenomenon has been tried in the present investigations for securing new genotype from the mutant plant culture of *G. glabra*.

TABLE I: Effect of Growth Hormones on Growth index (GI)* and Glycyrrhetinic acid (GA) content in Callus Culture of *G. glabra*. (CULTURE AGE- 5 WEEKS)

S. No.	Growth hormones (mg/l)	STEM		LEAF		ROOT	
		GI ±SD	GA ±SD	GI ±SD	GA ±SD	GI ±SD	GA ±SD
	2,4-D:BAP						
1.	1:0.1	4.12±0.12	1.08±0.06	4.84±0.12	1.12±0.05	3.46±0.12	2.34±0.07
2.	2:0.1	5.62±0.13	1.86±0.04	5.96±0.14	1.64±0.04	4.38±0.11	2.92±0.06
3.	3:0.1	5.18±0.13	1.22±0.07	3.68±0.11	0.98±0.03	3.52±0.10	2.66±0.07
4.	4:0.1	4.22±0.14	0.94±0.05	3.22±0.10	0.92±0.04	3.90±0.12	2.52±0.05
5.	2:0.2	3.68±0.16	0.90±0.05	4.62±0.14	1.22±0.04	3.48±0.14	3.22±0.06
6.	2:0.3	3.86±0.15	0.88±0.05	3.46±0.13	1.12±0.05	3.04±0.14	3.10±0.05
	NAA:BAP						
7.	2:1.0	3.86±0.11	1.66±0.05	4.48±0.10	1.52±0.04	3.02±0.12	2.32±0.04
8.	2:1.5	4.32±0.10	1.98±0.04	5.92±0.12	1.82±0.05	3.42±0.10	2.60±0.05
9.	2:2.0	5.86±0.12	2.14±0.06	6.22±0.13	1.96±0.04	3.11±0.13	2.96±0.07
10.	2:2.5	5.12±0.13	2.02±0.05	6.12±0.16	2.04±0.05	2.98±0.10	2.50±0.05
11.	1:1.0	3.66±0.14	1.52±0.04	3.98±0.13	1.46±0.07	2.88±0.13	1.90±0.04
12.	1:2.0	3.92±0.12	1.64±0.05	4.32±0.12	1.42±0.06	3.08±0.12	1.88±0.06
	IAA:2,4-D:BAP						
13.	1.0:1.0:0.1	4.50±0.13	1.18±0.03	5.02±0.11	1.12±0.04	3.20±0.12	3.02±0.06
14.	0.5:0.5:0.1	5.03±0.11	1.62±0.05	5.33±0.12	1.48±0.03	3.96±0.12	3.90±0.08
15.	0.5:1.0:0.1	4.68±0.14	1.40±0.04	4.92±0.11	1.23±0.04	3.83±0.12	3.12±0.04
16.	0.5:2.0:0.1	4.00±0.12	1.10±0.05	4.60±0.13	1.02±0.03	3.63±0.11	2.98±0.06
17.	Control	1.84±0.08	0.56±0.04	1.78±0.11	0.48±0.02	-	-

***Growth index (GI) = [(final dry wt. - initial dry wt.) / initial dry wt.]**

TABLE II: Effect of Colchicine on Growth index (GI)* and Glycyrrhetinic acid (GA) content in Callus Culture of *G. glabra*. (CULTURE AGE- 5 WEEKS)

S. No.	Colchicine (%)	STEM		LEAF		ROOT	
		GI ±SD	GA ±SD	GI ±SD	GA ±SD	GI ±SD	GA ±SD
1.	0.02	6.02±0.17	2.32±0.99	6.28±0.15	2.38±0.08	4.48±0.12	4.00±0.08
2.	0.05	5.95±0.17	2.52±0.06	5.92±0.15	2.84±0.07	4.12±0.11	4.18±0.05
3.	0.10	5.63±0.18	2.86±0.08	5.63±0.18	2.28±0.08	3.65±0.14	4.40±0.08
4.	0.15	4.88±0.13	2.40±0.07	4.92±0.13	1.88±0.06	2.84±0.16	4.12±0.07
5.	0.20	4.23±0.12	2.12±0.06	4.18±0.12	1.75±0.05	1.98±0.14	3.92±0.09
6.	0.25	3.48±0.17	1.95±0.05	2.55±0.17	1.52±0.06	1.54±0.14	3.54±0.10
7.	Control	5.86±0.12	2.14±0.06	6.12±0.16	2.04±0.05	3.96±0.12	3.90±0.08

***Growth index (GI) = [(final dry wt. - initial dry wt.) / initial dry wt.]**

ACKNOWLEDGEMENT

Authors are grateful to the Council of Scientific and Industrial Research, New Delhi for financial assistance.

REFERENCES

1. Handa, S.S. The Pharmacos, 1994; 31: 11.
2. Kinoshita T., Saitoh T. and Shibata S. Chem. Pharm. Bull. 1996; 24: 991.
3. Van Hulle C., Breakman P. and Vandewalle M., Planta Medica, 1971; 20: 276.
4. Saitoh T. and Shibata S., Tetrahedron, 1975; 312: 4461.
5. Kobayashi M., Noguchi H. and Sankawa D., Chem. Pharm. Bull. 1985; 33: 3811.
6. Revers F.E., Med. Tijdscher Geneesk (1946), 90, 135: (1948), 92, 2968: 1948; 92: 3567.
7. Landerlan, S.A., British Patent, 1, 447, 162, 25 Aug. 1976.
8. Wagner H., Hikino H. and Farnsworth N.R., Economic and Med. Plants Research. 1985; 1: 55.
9. Hayashi H., Fukui H. and Tabata M., Plant Cell Report, 1988; 1: 508.
10. Fukui H., Gota K. and Tabata M., Chem. Pharm. Bull. 1988; 36: 4174.
11. Sabaih M., Mansouri S., Ramezoman M. and Gholam H.A. Int.J.Crude Drug Res. 1987; 25: 72.
12. Kuo S., Shankel D.M., Telikepalli H. and Mitscher L.A. Mutat. Res. 1992; 282(2): 93.
13. Hatano T., Yashuhara T., Kukuda T., Noro T. and Okuda T., Chem. Pharm. Bull. 1989; 37(11): 3005.
14. Tailang M. and Kharya M.D., Indian Drugs, 1997; 33 (10): 507-10.
15. Tailang M. Kharya M.D. and Dixit V.K., I.J.Pharm. Sciences, 1997; 59(1): 26-29.
16. Tailang M. and Kharya M.D., I.J. of Med. And Aromatic Plant Sciences, 1998; 20(1): 36-41.
17. Murashige T. and Skoog F., Physiol. Plant, 1962; 15: 473.
18. Killacky J., Ross M.S.F. and Turner T.D. Planta Medica, 1978; 30: 310.
19. Dox A.W. and Plaisahse G.P., J. Am. Soc., 1961; 38: 2156.
20. Hiraga Y., Endo H., Takahashi K. and Shibata S., J. Chromatogr. 1984; 292: 451.
21. Cundiff R.H., Anal. Chem. (1964), 36, 1871.
22. Qurust H., Janson A.P. and Wostmann B.S.I., Res. Tran. Chim. 1955; 74: 1975.
23. Hada H. and Inagaki M., Yakugaku Zassi. 1970; 78: 795.
24. Kurono G. and Saski S., Yakugaku Zassi. 1970; 90: 497.
25. Gootjes J. and Nauta W., Pec. Trav. Chim. Pays Bas. Belg. 1964; 73: 886.
26. British Pharmacopoeia, 1988. Her Majesty Stationary Office, London, 1998; 2: 335.