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Mutation Induced Bioproduction of Glycyrrhetinic Acid from Callus Culture of *Glycyrrhiza Glabra* Linn.

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Glycyrrhiza glabra callus cells grown on modified MS medium were exposed to various concentrations of Ethyl methane sulfonate (EMS). EMS in 0.2 to 0.3% concentration, favoured glycyrrhetinic acid production while suppressing the cell growth. Methyl methane sulfonate (MMS) in the same concentrations however, favours both the glycyrrhetinic acid production as well as cell growth.

GLYCOSIDES are one of the most extensively investigated class of compounds among the secondary metabolitites because of their therapeutic importance. Extensively expored Glycyrrhiza glabra is an important ingredient of many Chiness traditional formulations used as expectorant, antitussive, anti-inflammatory and antiallergic agent[1-4]. Recently anti-ulcerative property due to β-glycyrrhetinic acid and its glycosideglycyrrhizin has been reported[5]. Glycyrrhizin and β-glycyrrhetinic acid have also been found effective in rheumatoid arthritis, hepatitis and Addison's disease[6-7].

In the recent past, static and suspension cultures of this plant have been examined for biomass and product yields. However, no work has been reported on the regulation of glycyrrhetinic acid production using chemical mutagens. Therefore, in the present investigation, attempts have been made to study the effects of chemical mutagens on glycyrrhetinic acid content in *G. glabra*.

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MATERIALS AND METHODS

Ethyl methane sulfonate (EMS) and methyl methane sulfonate (MMS) were the chemical mutagens chosen and their stock solutions were prepared in phosphate buffer (pH 7.).

In the present studies, Murashage and Skoog's medium with some hormonal modifications was used. In preliminary studies various combinations and concentrations of naphthalene acetic acid (NAA), indole acetic acid (IAA) and benzyl amino purine (BAP) were tried for enhancing callus growth and glycyrrhetinic acid (GA) content in stem, leaf and root explants of *G. glabra*. The media which gave good callus growth as well as GA content in stem, leaf and root explants were selected for studying the effects of EMS and MMS.

About 30 days old stem derived friable callus (grown) on MS medium containing 2 mg/l each of NAA and BAP, leaf derived callus grown on MS medium containing NAA (2 mg/l) and BAP (2.5 mg/l) and root derived callus grown on MS medium containing IAA (0.5 mg/l), 2,4-D (0.5 mg/l) and BAP (0.1 mg/l) were transferred to fresh liquid medium with the same composition and shaken in a rotary shaker (100 rpm) for two weeks. The cells were filtered through a nylon fabric and they were treated with

Table I

Effect of EMS on growth and Glycyrrhetinic Acid Content in the Callus Culture of Glycyrrhiza glabra (Culture Age - 5 Weeks)

S. No.	EMS%	STEM		LEAF		ROOT	
		GI	%GA	GI	%GA	GI	%GA
1.	0.05	5.42	2.12	5.90	1.88	3.62	4.30
2.	0.10	5.16	2.28	5.84	1.92	3.28	4.38
3.	0.15	4.62	2.36	5.68	2.06	3.08	4.28
4.	0.20	4.12	2.44	5.22	2.28	2.76	4.00
5.	0.25	4.02	2.52	4.68	2.42	2.48	3.82
6.	0.30	3.76	2.86	4.26	2.30	2.20	3.58
7.	0.35	3.48	2.50	3.64	2.12	1.98	3.38
8.	0.40	3.12	2.36	3.12	2.10	1.62	3.06
9.	0.45	2.22	2.18	2.26	1.96	1.38	2.76
10.	0.50	1.76	2.10	1.68	1.82	1.20	2.12
11.	Control	5.86	2.34	6.12	2.04	3.96	4.28

GI = (Final dry wt. - Initial dry wt.)/ Initial dry wt.
Average of five readings.

different concentrations of EMS (0.05-0.5%) and MMS (0.05-0.4%) solutions for two hours with constant shaking in a rotary shaker.

After these treatments the cells were washed repeatedly with sterile distilled water. Later these cells were transferred on to static media and were allowed to grow into calli for five weeks. The calli were harvested separately and growth index (GI) was calculated in each case as shown in Table I and II.

Estimation of Glycyrrhetinic Acid

Several methods for the estimation of glycyrrhetinic acid in plants have been reported. Killacky and Turner[9] determined GA content in liquorice by HPLC. Many methods of GA estimation include separation of constituents by paper chromatography, TLC, GLC and estimation by gravimetric, colorimeteric, polarographic and TLC densitometer. The GA estimation method prescribed in the British parmacopoeia[10] was followed in the present studies.

Method for Estimation of GA

One gram callus powder was hydrolysed by 1 M HCl and 1,4-dioxan for two h. After filtering, the filter was dried at 105° and extracted with chloroform. The chloroform extract was evaporated to dryness and the residue was dissolved in 10 ml of mixture containing equal volumes of chloroform and methanol.

Measured quantity of this solution was applied on to chromatoplate of silica gel G and placed in chamber containing ethyl acetate, ammonia (1 M) and absolute ethanol (60:27:13). The developed plates were dried and examined in UV light, the area corresponding to glycyrrhetinic acid was

Table - II

Effect of MMS on growth and Glycyrrhetinic Acid Content in Callus Culture of Glycyrrhiza glabra (Culture Age - 5 Weeks)

S.No.	MMS %	STEM		LEAF		ROOT	
		GI*	%GA	GI	%GA	GI	%GA
1.	0.05	5.84	2.40	6.20	2.12	4.02	3.78
2.	0.10	5.92	2.52	6.62	2.18	4.20	3.92
3.	0.15	6.26	2.88	7.18	2.26	4.82	4.16
4.	0.20	6.88	2.44	7.82	2.34	4.12	4.50
5.	0.25	6.18	2.30	7.22	2.30	3.62	4.18
6.	0.30	5.38	2.22	6.52	2.16	3.38	4.00
7.	0.35	5.12	2.10	5.92	2.00	2.82	3.92
8.	0.40	4.20	1.96	5.18	1.88	2.28	3.80
9.	Control	5.86	2.34	6.12	2.04	3.96	4.28

* GI = (Final dry wt. - Initial dry wt.)/ Intial dry wt.
Average of five readings.

scrapped off, treated with 10 ml of absolute ethanol and filtered through sintered glass filter. The absorbance was measured at 250 nm and the GA content was determined spectrophotometrically.

RESULTS AND DISCUSSION

EMS Treatment

EMS in all applied concentration gradually decreased the growth indices in all three explants. However, GA content was enhanced in the stem, leaf and root derived callus producing maximum GA content of 2.86% (control-2.34%) with 0.3% EMS in stem callus, 2.42% (Control-2.04%) with 0.25% EMS in leaf callus and 4.38% (control-4.28%) with 0.1% EMS in root callus.

MMS Treatment

Compared to control increased growth indices in MMS treated tissues were observed. However, it was 0.2% MMS which gave the highest GI of 6.88 (control-5.86%) in stem and 7.82 (control- 6.12) in leaf derived callus. In root derived callus, highest GI of 4.82 (control-3.96) was observed with 0.15% MMS.

Glycyrrhetinic acid content was also increased in MMS treated callus as compared to control. In stem derived callus, highest GA content of 2.88 (control-2.34%) was observed with 0.15% MMS. In leaf derived callus, highest GA of 2.35% (control-2.04%) and in root derived callus highest GA of 4.50% (control-4.28%) were observed with 0.2% MMS treatment.

Requirement of specific climatic and soil conditions, scanty flowering and seed bearing factors restrict the large scale cultivation of *G. glabra*. In raising a new crop, about 20% of the valuable suckers comprising the drug morphology has to be sacrificed. High therapeutic value with its increased demand

have been the main driving force to take up this plant under tissue culture study for the regulation of glycyrrhetinic acid content using chemical mutagens.

The present investigations reveal that ethyl methane sulfonate favoured glycyrrhetinic acid production in leaf and stem derived callus in moderate concentrations of 0.25 and 0.3%. Methyl methane sulfonate however, favours glycyrrhetinic acid production and callus growth in minimum to moderate concentrations of 0.05 to 0.2% in stem, leaf and root derived callus. MMS, therefore, may be considered as better mutagenic agent as it enhances glycyrrhetinic acid production as well as biomass.

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