Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(11):174-179

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Enhancement of Secondary Metabolite Production in *Abrus precatorius* Linn. via Genetic Transformation

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ABSTRACT

Although Glycyrrhiza glabra is the recognized source of glycyrrhizin, its production in tissue culture has not been successful by tissue culture. Abrus precatorius is an alternative source for glycyrrhizin and was thus taken up in tissue culture technique for glycyrrhizin production. The yield enhancement for the glycyrrhizin production was done by means of genetic transformation using Agrobacterium tumefaciens. When compared with untransformed callus resulted in 8.665 ± 0.144 g/l dry cell weight biomass and 0.427 ± 0.0016% glycyrrhizin, which was 2.4-fold times higher in productivity in comparison to control untransformed cultures.

Keywords: *Abrus precatorius*; Callus induction; Growth curve; Phytoharmones

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INTRODUCTION

Plants produce bio-chemicals that are of importance in the healthcare, food, flavour and cosmetics industries. Many pharmaceuticals are produced from the secondary metabolites of plant. Often the source plants are cultivated in tropical or subtropical, geographically remote, areas which are subjected to political instability, drought, disease and changing land use patterns and other environmental factors. In addition, the long cultivation period between planting and harvesting make selection of high-yielding strains difficult. These factors have generated considerable interest in the use of plant cell culture technologies for the production of pharmaceuticals and other plant derived bio-chemicals [1]. The plant cell culture technology is now sufficiently advanced to allow for large quantities of relatively homogeneous, undifferentiated cells to be produced. Plant cell and tissue culture systems are complementary and may provide competitive metabolites production systems when compared to whole plant extraction. Callus culture is formation of mass of cells in a solidified media, whereas cell suspension culture is cells suspended in liquid media. Large scale plant suspension cultures are expected to be suitable for industrial production of plant derived bio-chemicals [2,3].

Secondary metabolism is the synthesis and metabolism of endogenous compounds by specialized proteins. The results of these processes are secondary metabolic products. They are an expression of cell specialization which is either triggered by the process of cell differentiation of or represents an aspect process of plant development [4,5].

Agrobacterium mediated genetic transformation is the dominant technology used for the production of genetically modified transgenic plants. *Agrobacterium* genetically transforms its host by transferring a well-defined DNA segment from its tumor-inducing (Ti) plasmid to the host-cell genom. In nature, the transferred DNA (T-DNA) carries a set of oncogenes and opine-catabolism genes, the expression of which, in plant cell, lead to neoplastic growth of the transformed tissue and the production of opines, amino acid derivatives used almost exclusively by the bacteria as a nitrogen source [6,7].

Abrus precatorius is used as abortifacient, antidote, aphrodisiac, con-traceptive, anti-diuretic, emetic, and also in the treatment of convulsion, cough, diarrhea, gingivitis, gonorrhea, hook-worms, jaundice, ophthalmic disease, rheumatism, trachoma, vermifuge, etc. Abrus is possibly native of Indian, Africa and most of the tropics [8,9].
Previous workers have claimed that glycyrrhizin is the sweet principle of *A. precatorius* leaves. According to some literature the roots contain higher percentage of glycyrrhizin than leaves. Chemically, glycyrrhizin is a triterpeniod glycosidic saponin present in roots and rhizome of Liquorice plant *Glycyrrhiza glabra* and roots and leaves of *A. precatorius* (Wild liquorice or Indian liquorice). The crude dried aqueous extract 4-25% (also known as black liquorice) may contain glycyrrhizinic acid in the form of calcium, magnesium and potassium salt. Glycyrrhizin, which consists in the calcium and potassium salts of glycyrrhizic acid (Glycyrrhizinic acid) is converted upon hydrolysis into the aglycone glycyrrhetic acid (glycyrrhetinic acid) plus two molecules of glucouronic acid. The acid form is not particularly water-soluble, but its ammonium salt is soluble in water at pH greater than 4.5 [10-12].
Present investigations were undertaken with a view to enhance production of glycyrrhizin in *abrus precatorius* utilizing tissue culture techniques like genetic transformation. Glycyrrhizin is a phytoconstituent of importance for the pharmaceutical and food industry. Glycyrrhizin used as hapatoprotective, immunomodulator, Anti HIV, expectorant, flavoring agent/ sweetener, antitumor, anti-inflammatory agent etc. Thus it is of prime important plant constituent of medicinal values and our aim was to establish a protocol/ procedure for its enhanced production by tissue culture techniques [13,14].

MATERIALS AND METHODS

Collection of Seeds

Seeds of *Abrus precatorius* were collected from medicinal plant garden, Department of Pharmaceutical Sciences, Dr. H. S. Gour University Sagar, M. P. with identification no. Bot. / H / 5865.

Aseptic Germination of Seeds

The viable seeds were taken in a culture tube (slightly tilted position) and washed under running tap water. The seeds were surface sterilized by 70% ethanol for 1-2 min, then with sodium hypochlorite (4%) for 10-12 min and then rinsed 7-8 times with sterilized double distilled water. Now the sterile seeds were aseptically germinated at 25 ± 2°C at 12-12 h photoperiod at normal humidity conditions. The germination of seeds of *Abrus precatorius* started at 8^{th} day, 90% germination was observed at 12^{th} day, after 14^{th} day germinated seed had two distinct cotyledons. Finally the grown seedlings were aseptically transferred to sterile 250 ml conical flask for further growth [15,16].

Initiation and Maintenance of Callus Culture

Explants from aseptically germinated plantlets were aseptically transferred with sterile forcep to a solid (0.8% agar) MS medium with each with proper and combination of cytokinin and auxin hormones in a petri-dish and 250 ml conical flask. These were incubated at 25 ± 1°C on the solid MS medium in dark for initial seven days till callus induction started. After callus induction the culture plates were incubated under 12/12 hr light/dark cycle and at light regimens ranging from 1500-2500 lux provided by fluorescent tubes. Among the various plant growth regulators attempted the NAA (1 mg/l) + kinetin (1 mg/l) was the best optimized concentration for the induction and growth of calli of *Abrus precatorius* explants. After ten days compact, friable creamy whitish colored callus induced in leaf explant on NAA (1 mg/l) + kinetin (1 mg/l) with 65% callogenesis. Callus cultures were maintained by sub culturing regularly at interval of every three weeks on the fresh medium (Table 1). It was maintained at the same hormonal concentration, which was best for callus initiation of *Abrus precatorius* explant during subsequent sub-culturing [17,18]. A total of 20 explants were taken for each observation; each experiment performed in triplicate (n=3).

Table 1: Effect of hormonal concentration on *Abrus precatorius* leaf explants

Hormonal concentration	Days after which callus initiation starts	Callus characteristics	% callogenesis
2,4-D (1 mg/l)	14-16	Creamish coloured Callus	20%
NAA (1 mg/l)	-	-	0
Kn (1 mg/l)	-	-	0
NAA (1 mg/l) + Kn (1 mg/l)	12-Oct	Friable creamy whitish coloured	65%
NAA (1 mg/l) + BAP (2 mg/l)	13-15	Compact pale coloured	40%
2,4-D(1 mg/l)+ BAP (2 mg/l)	16-18	Compact pale coloured	25%

RESULTS AND DISCUSSION

Callus Growth Determination

Suspension cultures were initiated by aseptic transfer of friable fraction of callus equivalent to 1 to 1.5 g/l fresh cell weight into 250 ml Erlenmeyer flask containing 50 ml of liquid MS medium. The plant hormones added were NAA + Kn 1 mg/l each. Culture were incubated on a gyratory shaker at 110 ± 10 rpm at 25 ± 1°C under 12/12 h light/dark regime. The suspension culture was maintained in MS medium containing NAA (0.5 mg/l) and Kn (0.5 mg/l) without agar and subcultured at an interval of two weeks at 25 ± 1°C and 110 ± 10 rpm [19,20].

Determination of Glycyrrhizin

(a) Qualitative estimation was performed by TLC with solvent system Toluene: ethyl acetate: glacial acetic acid (12.5:7.5:0.5) [21]

(b) Quantitative estimation was performed by HPLC on Schimadzu (Integration: Hewlett Packard) HPLC with photodiode array detector, SPD-10 MA system, glycyrrhizin was separated on Phenomenex (250 × 4.6 mm) RP_{18} 5 µ column (Table 2). Methanol: water (70:30) Isocratic solvent was used for estimation of glycyrrhizin as its hydrolysis product 18-β-glycyrrhetinic acid [22-24]. Results were mean ± SD of triplicate samples (Figure 1).

Table 2: Glycyrrhetinic acid (GA) content in suspension culture of *Abrus precatorius*

S.No.	Culture age (days)	Culture Biomass ($gL^{-1}DW$)	%Glycyrrhetinic acid content
1	5	1.005 ± 0.31	0.0032 ± 0.0022
2	10	2.837 ± 0.27	0.0057 ± 0.0034
3	15	4.605 ± 0.61	0.089 ± 0.0012
4	20	5.456 ± 0.11	0.105 ± 0.0038
5	25	5.304 ± 0.40	0.097 ± 0.0024
6	30	4.322 ± 0.38	0.058 ± 0.0025

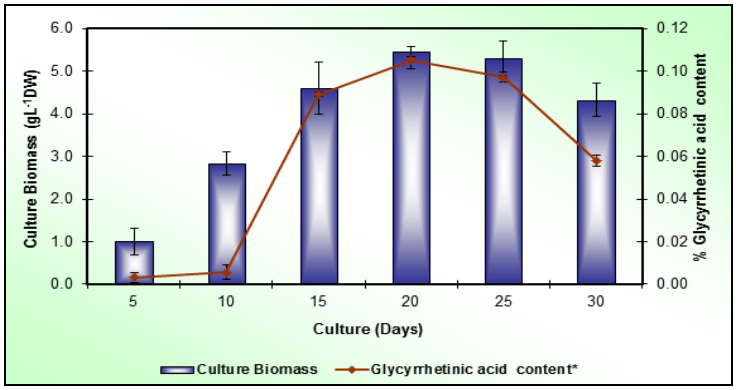

Figure 1: Glycyrrhetinic acid (GA) content in suspension culture of *Abrus precatorius*

Transformed Callus Culture

In the present investigation *Agrobacterium tumefaciens* NCIM 2943 was used to establish *Abrus precatorius* transformed cell culture. *Agrobacterium tumifaciens* (NCIM 2943) was obtained from National Collection of Industrial Microorganisms (NCIM) Pune. *Agrobacterium tumefaciens* NCIM 2943 was maintained in YEB media and subcultured at regular interval of 30 days (Tables 3-7). Culture of bacterial strain was maintained at 26°C. [25,26]

Optimization of Various Conditions for the Establishment of Transformed Callus Culture of *Abrus precatorius* Explants

Table 3: Effect of media on transformed callus culture

S. No.	Transformation experimental conditions	Characteristics	Days after Callusing starts	Intensity of formed callus	% *Trans-formation Efficiency
1	MS-Media	Unorganized friable callus	5-Apr	++++	80%
2	GB-5 Media	Soft Callus mass of Cream colour	7-Jun	++	65%

Table 4: Effect of pH on transformed callus culture

1	pH 4.5 ± 0.3	Tissue necrosis	--	---	----
2	pH 5.5 ± 0.3	Compact Callus mass	5	++	68%
3	pH 6.5 ± 0.3	Soft friable growth	4-5	++++	72.20%
4	pH 7.5 ± 0.3	Loose callus mass	6	+++	70%

Table 5: Effect of density of bacterial inoculum

1	0.50 (OD_{600})	Low growth	6-7	++	52.11%
2	1.00 (OD_{600})	Best callus growth	4-5	++++	61%
3	1.50 (OD_{600})	Good callus	5-6	+++	59%

Table 6: Effect of infection time of inoculum

1	15 min	Low callus growth	6	++	59%
2	30 min	Best callus growth	5	+++	55%
3	45 min	Good gall formation	4	+++	43.20%

Table 7: Effect of concentration of acetosyringone

1	0 µM	Whitish Callus formed	5-6	+++	67.2%%
2	50 µM	Good gall formation	4-5	++++	73.30%
3	100 µM	Profusely grown gall	4-5	++++	78%

Note: Each experiment was done in duplicate with ten explants per treatment. *Percentage Transformation Efficiency calculated on the basis of opine positive crown gall culture.

Confirmation of Transformed Cells

The transformation conformation was done by opine assay using Electrophoresis 64% transformation efficiency was found [27].

Initiation of Transformed Cell Suspension Cultures of *Abrus precatorius*

Suspension cultures were initiated by aseptic transfer of friable fraction of callus equivalent to 0.5 to 1.0 g fresh cell weight into 250 ml Erlenmeyer flask containing 50 ml of liquid Murashige and Skoog (MS) medium without any growth hormone. Cultures were incubated on a gyratory shaker at 110 rpm at 25 ± 1°C under 12/12 h light/ dark regime (Tables 8 and 9). The suspension culture was maintained in liquid MS medium without any plant growth hormone and subcultured at an interval of two weeks at 25 ± 1°C and at 110 r.p.m under 12/12 h light/ dark regime (Figure 2) [28,29].

Table 8: Growth parameters of transformed cell suspension culture of abrus precatorius

Culture Period (Days)	*Mean Fresh Weight (g)	*Mean Dry Weight (mg)	% Biomass Increase (FW)
5	0.118 ± 0.06	16.29 ± 2.14	290%
10	1.236 ± 0.41	29.56 ± 3.27	398%
15	3.005 ± 0.44	78.21 ± 3.21	487%
20	5.723 ± 0.64	108.00 ± 2.42	580%
25	4.995 ± 0.57	72.22 ± 1.00	560%
30	4.977 ± 0.20	52.33 ± 2.08	501%

*Results were mean ± SD of triplicate sample

Table 9: Glycyrrhetinic acid (GA) content in transformed suspension culture of abrus precatorius

Culture period (Days)	Culture biomass (gL^{-1} DW)*	Glycyrrhetinic acid content (% DW)*
0	-	-
5	1.770 ± 0.35	0.0041 ± 0.0025
10	3.772 ± 0.168	0.099 ± 0.0013
15	6.642 ± 0.102	0.286 ± 0.0042
20	8.665 ± 0.144	0.427 ± 0.0016
25	7.994 ± 0.22	0.402 ± 0.0030
30	5.148 ± 0.186	0.301 ± 0.0012

*Results were mean ± SD of triplicate samples.

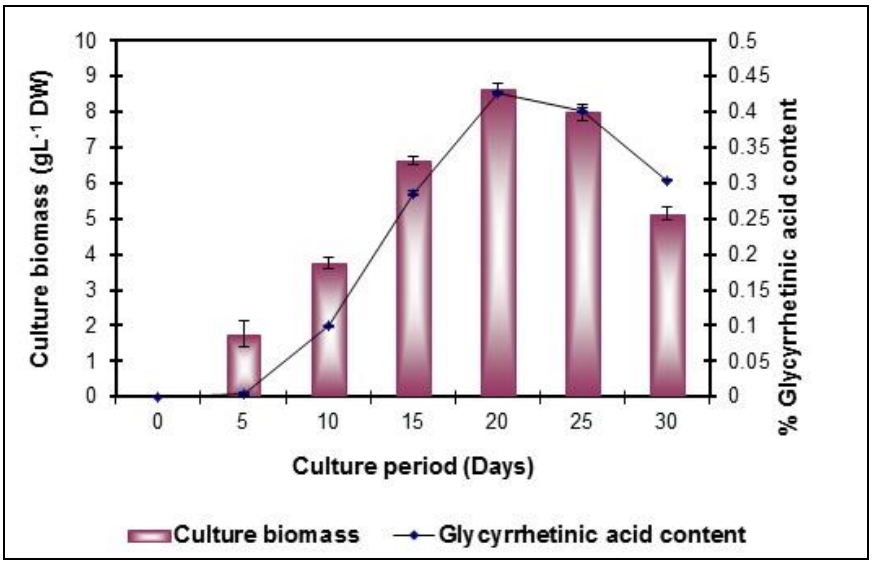

Figure 2: Glycyrrhetinic acid (GA) content in transformed suspension culture of *Abrus precatorius*

CONCLUSION

When compared untransformed callus culture with transformed callus culture, give increase in GA content. *A. tumefaciens* transformed cultures of *A. precatorius* were successfully induced from *in vitro* grown plants with 64% transformation efficiency. Complete growth production of *A. precatorius* cells in the suspension culture were established. Maximum biomass (8.665 ± 0.144 g/Ldry weight) and Glycyrrhetinic acid accumulation (0.427 ± 0.0016% DW) were obtained on the 20th day of cultivation. This study represents a successful approach for the production of Glycyrrhetinic acid content by transformed cell suspension cultures from *A. precatorius*. Significantly higher Glycyrrhetinic acid content accumulation was obtained in comparison to that reported earlier. This yield enhancement could generally be applied for the development of cell culture-based bioprocesses for mass-scale production of commercially important phytochemicals. Further research work in this direction is under progress.

REFERENCES

[1] T Gaspar; C Kevers; C Penel; H Greppin; DM Reid; TA Thorpe. *In Vitro Cellular Develop Biology-Plant.* **1996**, 32(4), 272-289.
[2] AM Aly; L Al-Alousi; Salem HA. *Aaps Pharmscitech*. **2005**, 1, 6(1), E74-82.
[3] S Agrawal; N Ghatak; R Arora; M Bhardwaj. *Pharmacol Res Commun*. **1970**, 2(2), 159-163.
[4] BA Akinloye; LA Adalumo; *Niger J Pharm.* **1981**, 12(2), 405.
[5] DWang, AM Wu. Carbohydrate microarrays for lectin characterization and glyco-epitope identification. Lectin: Analytical Technologies, **2007**, 167-192.
[6] C Arias; H Scragg; A Stafford; M Rodriguez. *Plant Cell Tissue Organ Culture.* **1993**, 34, 77-82.
[7] A Baldi; V Dixit. *Bioresource Technol*. **2008**, 99(11), 211-213.
[8] AP Basu. *Indian J Pharm*. **1993**, 35(6), 203.

[9] Y Blume; Y Alla; R Vladimir; B Oleg; B Galina; P Alexey P. *Cell Biology Int.* **2008**, 32(5), 566-570.
[10] W Chan; J Staba**.** *Lloydia*. **1965**, 28, 55.
[11] S Chauhan; B Singh; G Kimothi; S Agarwal. *Indian J Pharm Sci*. **1998**, 60(4), 251-253.
[12] W Chilton; E Hood. *Chilton M Phytochem.* **1985**, 24, 221-224.
[13] T Chong; M Abdullah; O Lai; F Nor; N Lajis. *Proc Biochem*. **2005**, 40, 3397-3405.
[14] V Desai; M Sirsi. *Indian J Pharm*. **1966**, 28, 164-165.
[15] D Dhingra; M Parle; S Kulkarni. *J Ethnopharmacol.* **2004**, 91, 361-365.
[16] F DiCosmo; M Misawa. *Trends Biotechnol*. **1985**, 3, 318-322.
[17] J Ebel; A Mithofer. *Planta*. **1998**, 206, 335-348.
[18] P Engstrom; P Zambryski; M Montagu; S Stachel. *J Molecular Biology*. **1987**, 197(4), 635-645.
[19] M Escobar; A Dandekar. *Trends Plant Sci*. **2003**, 8, 380-386.
[20] T Furuya; K Matsumoto; M Hikichi. *Tetrahedron Letters*. **1971**, 2567-2569.
[21] V Gaudin; T Vrain; L Jouanin. *Plant Physiol Biochem*. **1994**, 32, 11.
[22] S Gelvin. *Mol Biol Rev*. **2003**, 67, 16-37.
[23] E Gengaihi; M Karawya. *Herba Hungarica.* **1988**, 27(1), 27-34.
[24] H Hayashi; H Fufui; M Tabata. *Plant Cell Rep*. **1988**, 7, 508-511.
[25] J Killacky; MSF Ross; TD Turner. *Planta Medica*. **1976**, 30, 310-316.
[26] T Murashige; F Skoog. *Physiol Plant*. **1962**, 15, 473.
[27] VK Saxena; DN Sharma. Fitoterapia, **1999**, 70, 328-329.
[28] CS Shah; JG Bhatta. *Indian J Pharm*. **1973**, 35(3), 102-104.
[29] T Sharma; VK Dixit. *Ind J Pharm Sci.* **2006**, 68 (4), 448.