RESEARCH PAPER

Studies on *in vitro* propagation and biochemical analysis of *Trigonella foenum-graecum* L.

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Trigonella foenum - graecum L is a important medicinal plant which, is rare and extensively used in traditional system of medicine. It belongs to the family Fabaceae. The present study was mainly aimed to develop a protocol for the successful micro propagation and biochemical analysis of compounds present in the callus as well as in the *in vitro* plant. The explants slected for the present study includes, cotyloden, hypocotyls, shoot tip epicotyls. The shoot tips explants inoculation on MS medium with auxins and cytokinins alone and in combinations showed shoot initiation along a shoot initation with basal callus formation. The chlorophyll pigment content in the cullus with different morphology and the *in vitro* regenerated plant was assessed. Total chlorophyll value was estimated as 2.7277 mg/g. The total protein content in the vitro regeneration plant and morphological different callus were estimated by Lowry's. and acryl amide gel electrophoresis. The protein content of the yield grown plants was estimated as 0.789 mg/g fresh weight and that of callus was estimated as 0.421 mg/g fresh weight. The seeds of field-grown plant as well as green friable callus obtained in 2,4-D of field - grown showed maximum amount of protein content. Peroxidase enzyme activity of callus was also determined. Green friable callus obtained from a combination of 2,4-D showed maximum peroxidase activity. The presence of secondary metabolites *in vitro* plant as well as callus indicated that *in vitro* system is a possible source for the isolation of Diosgenin.

Key words **:** Phytochemical, Diosgenin, Trigonella, Peroxidase activity, *In vitro* propagation

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INTRODUCTION

In India, medicinal plants are used extensively in system on medicines like Ayurveda, Unani, Sidha and homeophathy. The country richly endowed with range of plants with medicinal value represent great nature resources. Plant biotechnology can bring many benefits to medicine, environment and Industry, it also has a wide range of possible application in food and forming, the biotechnology method of plant improvement, manipulation and selection at cellular level, plant biotechnology utilizes plant cells or tissues to improved variety of products ranging form to one other biotic or abiotic stress or possessing some unique features not possible by conventional breeding approaches. The production of secondary products is an application of tissue culture technology. Secondary products such as gums, resins, alkaloids, antibiotics, enzyme are potentially available form cell culture technology (Luckner and Nover, 1997). *Triognella foencum graecum* Linn belongs to the family Fabaceae. It is one of an endangered plant having lots of medicinal value. The present study aims at *in vitro* studies on the plant, analysis of primary metabolites and analysis of secondary metabolites using TLC.

RESEARCH METHODOLOGY

Media preparation:

Trigonella foenum-graecum Linn belongs to the family Fabaceae, A certified seed obtained from Tamil Nadu Agricultural University (TNAU) was used in the present study. MS medium (Murashige and Skoog, 1962) was used for the

present study stock solution of macro and micronutritents, ions, vitamins and hormones were prepared in double distilled water and stored in refrigeration at 4 ± 1^{0}C. Plant growth regulators were dissolved in few drops of sodium hydroxide or ethanol as the case may be and then made up to the desired volume (mg/w/v) by adding distilled water.

Establishment of sterile cultures:

Prior to inoculation the laminar air flow chamber and all instruments such as forceps, Surgical blades and Petridishes were sterilized.

Stomatal analysis:

Mature leaves from field grown and *in vitro* regenerated plants used for stomatal study. Upper and lower epidermal peels were taken and stained with saffrarnine. Stomatal frequency and size were calculated according to Salisbury's formula using micrometer.

Biochemical analysis:

Chlorophyll content of leaves from field grown plants as well as morphologically different cali obtained from MS medium supplemented with 2 mg/l 2, 4-D was determined chlorophyll estimation, by using the laboratory manual (Jeyaraman, 1992).

Protein estimation:

Total soluble protein content in leaf samples were estimated using Lowry's method (Lowry *et al.*, 1951).

Peroxidase activity:

Peroxides activity of callus maintained on Ms medium with 3mg/l BA was determined after 15 days of subculture.

Phytochemical analysis:

In vitro and the control plants were weighed and dried in a hot air oven at 60^{0}C and powered. The desired samples were weighed and petroleum either extract (40 to 60^0) was taken by means of a soxhlet extractor.

These extracts were used for TLC (Thin layer chromatography) for the detection and separation of steroidal components. Callus section were stained in Sudan black (3% Sudan black in absolute) for three minutes, washed in distilled water. These sections were then mounted in glycerin and observed liquids appeared brown in colour.

RESEARCH FINDINGS AND ANALYSIS

The cotyledon explants produced maximum amount of callus induction. The cotyledon explants produced maximum amount of callus than the hypocotyls explants (Table 1, 2) (Fig. 1 and 2).

Table 1 : Effect of auxin on callus induction from cotyledon, hypocotyl explant

Hormone 1 concentration mg/l		Morphology of callus		Fresh weight of callus*	
		Cotyledon	Hypocotyl	Cotyledon	Hypocotyl
2,4-D	0.5	Pale green friable	Pale green friable	1.277	0.984
	1	Pale green friable	Pale green friable	1.731	1.209
	2	Green friable	Green friable	1.213	0.972
NAA*	0.5	Pale green friable	Creamy friable	1.261	1.258
	1	Pale green friable	Creamy friable	0.827	0.852
	2	Pale green friable	Pale green friable	0.651	0.667

*mean value of 7samples * NAA – Napthaline acetic acid

Table 2: Effect of auxin and cytokinins in combination on the cotyledon and hypocotyl

Hormone concentration (mg/1)		Morphology of callus		Amount of callus	
		Cotyledon	Hypocotyl	Cotyledon	Hypocotyl
2.4D:BA	0.5:0.5	Pale green friable	Pale green friable	1.287	1.242
	0.5:1.0	Green friable	Green compact	1.259	1.223
	0.5:2.0	Green friable	Green compact	0.972	0.842
	0.5:3.0	Green friable	Green compact	0.721	0.734
2,4D:KIN	0.5:0.5	Green friable	Green friable	0.878	0.924
	0.5:1.0	Green friable	Green friable	1.004	0.927
	0.5:2.0	Pale green friable	Pale green friable	0.731	1.041
	0.5:3.0	Pale green friable	Pale green friable	1.221	1.216
NAA:KIN	1.0:0.5	Green friable	Green friable	0.727	0.812
	2:1.0	Pale green friable	Pale green friable	1.021	1.003
	3:1.5	Pale green friable	Pale green friable	0.944	0.874

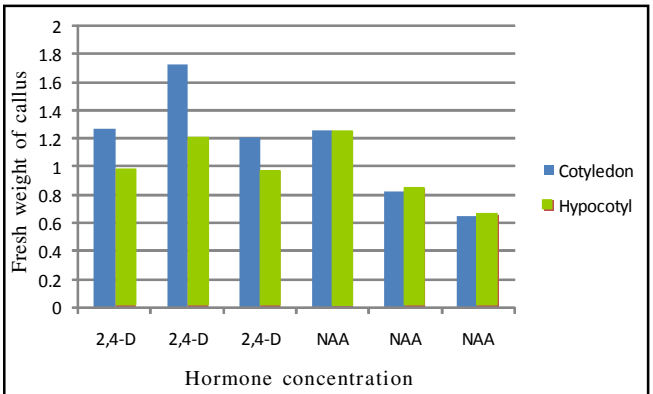

Fig. 1 : Effect of auxin on callus induction from cotyledon, hypocotyl explant

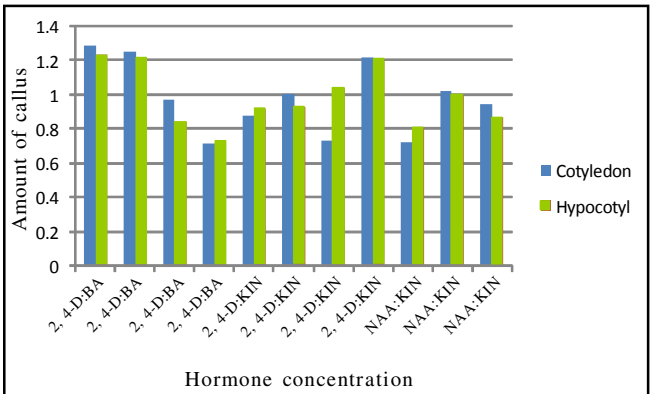

Fig. 2 : Effect of auxin on callus induction from cotyledon, hypocotyl explant

Effect of BA on shoot multiplication:

In the present study the shoot tip inoculated in MS medium supplemented with 0.5 mg/ 1 BA showed regeneration without callus formation produced a single shoot with maximum height of 6.1 cm. (Redwan and Kokate, 1980) (Table 3, 4).

Effect of KIN on shoot multiplication:

In the present study the shoot tip inoculation on MS medium supplemented with 0.5 mg /I KIN showed regeneration without any callus formation produced a single shoot with maximum height of 4 cm (Redwan and Kokate, 1980) (Table 3, 4).

Table 3 : Effect of BA or KIN alone on shoot tips explants

Hormone concentration mg/1		Number of shoots (after 30 days)	Length of shoots (after 30 days)
BA	0.5	2	3.6
	1	2	6.1
	2	1	4.5
Kin	0.5	4	1.5
	1	2	4
	2	1	3.8

Table 4 : Effect of auxins and cytokinin combination of shoot tip explant

Hormone concentration (mg/1)		Number shoots	Length of shoots	Basal callus	
				Morphology	Amount of callus
2,4-D:BA	0.5: 0.5	1	3.9	Green compact	++++
	0.5:1	1	1.3	Green compact	++
	0.5:2	1	2.5	Green compact	+++
2,4-D:Kin	0.5:0.5	1	1.5	Green friable	++
	0.5:1	2	2.1	Green friable	++
	0.5:2	1	1.8	Creamy friable	++

In the present study major photosynthesis pigments like chlorophyll a, b and total chlorophyll were analysed in the callus. The maximum amount of chlorophyll was in callus maintained on MS medium supplemented with 2, 4-D mg/I. Similar result was also reported by (Sharon and Bhaskare, 1998) (Table 5).

Table 5 : Chlorophyll estimation in callus and leaf sample

Hormone concentration mg/1	Morphology of the callus	Chlorophyll (a) g/g Fr.wt	Chlorophyll(b) g/g Fr.wt	Total Chlorophyll g/g Fr.wt
Field grown plant	–	2.2576	0.4756	2.7277
2,4-D 1 Mg/1	Brown friable	0.0343	0.1443	0.1746

The protein content of the field grown plants was estimated to be 0.789 mg/g fresh weight and that of callus was estimated as 0.421 mg/g fresh weight (Table 6).

Table 6 : Protein estimation of morphologically different calli and leaves from field grown plant

Hormone concentration mg/1	Morphology of the callus	Protein content mg/g
Field grown plant	–	0.789
2,4-D 1 Mg/1	Brown friable	0.421

The callus obtained from 2,4 D 2mg/l showed the maximum peroxidase activity. The control plants also showed an average amount of peroxidase activity (Table 7).

The present study revealed the high *in vitro* response of different explants in this species. The biochemical analysis

Table 7 : Peroxidase activity of morphologically different callus		
Hormone concentration mg/1	Morphology of the callus	Protein content mg/gm
Field grown plant	–	540
2,4-D 1 Mg/1	Brown friable	564

of primary metabolites showed similar amount in control as well is in invitro shoots. The results in the study indicated the high potential of this species for utilizing the plant tissue and cell culture technology for the extraction of valuable phyto chemical. Thus, *Trigonella foenum* culture can prove to be an efficient source for the pharmaceutical industry for the production of valuable life saving medicines. *Trigonella fornum-graecum* seed is a source of raw material for the steroid industry. (Kaul *et al*., 1969). In view of economic constrains candidates for commercial production via plant cell culture are limited to a few types of high value plant specific compounds (Balasimha and Tewari, 1978) This includes diosgenin derived hormone precursors. Further advances in our understanding of immunology and related areas should permit the development of new selective and sensitive bio assays to guide the isolation of bioactive natural products.

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