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Influence of Plant Growth Regulators and UV Supplementation on the Production of Secondary Metabolites in Tissue Cultures of *Glycine max*

Reena Panchlaniya1, Anurag Titov2
1Reearch Scholar, Department of Botony, Govt. Madhav Science PG College, Ujjain, India
2Professor, Department of Botony, Govt. Madhav Science PG College, Ujjain, India

ABSTRACT: The SMs have a lot of economic importance in the plant defense, plant breeding, pollination, ecological effects and others. Tissue cultures of *Glycine max* leaves explants were established in different culture media (added required hormones alone or in combination (NAA, 2.0 to 4.0 mg/l) and 2,4-D (2.0 to 4.0 mg/l)). Cultures were placed under filters which allowed only UV-B radiation to pass (continuously for 7 days (2 Hours in a day). In this study has been detected in UV-B treated callus extract as compared to the non-treated callus extract. Among all groups the best response were found in non- treated set of T10 groups of callus in MS media supplemented with, NAA (4 mg/L), 2,4-D (4 mg/L) and coconut water (10 ml/L). The UV treated set of T9 groups of callus in MS media supplemented with NAA (4 mg/L), 2,4-D (2 mg/L) and CW (10 ml/L) was found to be the best responsive so both sets (Treated and non-treated callus) were taken for phytochemical studies through TLC and HPLC. UV-B radiation (for short period) enhanced the production of SMs in Glycine max callus.

Keywords: Secondary metabolites (SMs), *Glycine max* (GM), PTC, UV-B light, Plant growth regulators (PGRs)

Introduction: Plant secondary metabolites can be defined as compounds that have no recognized role in the maintenance of fundamental life processes in the plants that synthesize them, but they play an important role in the interaction between plant and its environment. The production of these compounds is often low (less than 1% dry weight) and depends greatly on the physiological and developmental stage of the plant (Namdeo A. G., 2007). Levels of secondary metabolites are both environmentally induced as well as genetically controlled. The Secondary metabolites are also called as plant defensive compounds since these have been evolved to deter pathogens or herbivores such as insects and mammals (Makkar H. P. S. *et al*., 2009).

Classification Of Secondary Metabolites

Secondary metabolites could be classified into several categories according to various features like their chemical structure, solubility in different solvents, or the pathway of their biosynthesis (Katerova Z. *et al*., 2012). Classification of plant secondary metabolites based on various categoriesis shown in the Fig.1:

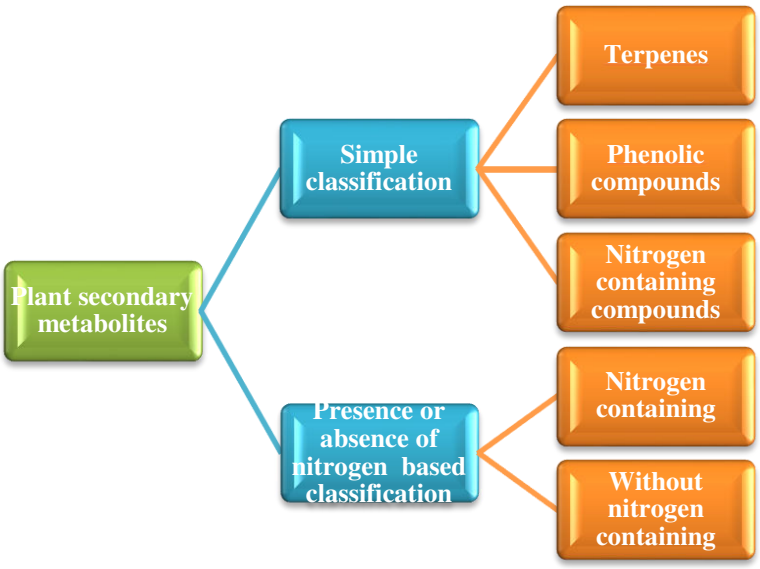


Fig. 1: Classification of plant secondary metabolites based on various categories

A simple classification of secondary metabolites includes tree main groups:

I. **Terpenes**, such as plant volatiles, cardiac glycosides, carotenoids and sterols.
II. **Phenolics,** such as phenolic acids, coumarins, lignans, stilbenes, flavonoids, tannins and lignin and
III. **Nitrogen containing compounds**, such as alkaloids and glucosinolates.

Another important classification is related to the presence or absence of nitrogen in their chemical structure. Thus secondary metabolites form two major groups:

I. **Nitrogen containing**– Alkaloids, non-protein amino acids, amines, cyanogenic, glycosides, and glucosinolates and
II. **Without Nitrogen containing**– Terpenes (mono-, sesqui-, di-, tri-, tetra-terpenes, steroids, saponins), phenolics (phenolic acids and phenylpropanoids), polyketides and polyacetylenes (Katerova *et al*., 2012).

PLANTS PROFILE

Scientific classification

Scientific classification

Kingdom : Plantae
Phylum : Magnoliophyta
Class : Magnoliopsida
Order : Fabales
Family : Fabaceae
Subfamily : Faboideae
Genus : *Glycine*
Species : *Glycine max*
Binomial name : *Glycine max*(L.) Merr.
Comman name : Soybean

Soybean is a major crop that has been extensively worked out for improvement in agronomic traits. Research on the physiological and biochemical mechanism of stress tolerance mainly biotic stress and secondary metabolite profiles are certainly an area of importance. The approach of tissue culture for analyzing the influence of stress on secondary metabolites. Exposure to any environmental stresses (like, UV radiation) in plants leads to the overproduction of secondary metabolites. These metabolite accumulations showed to the differential expression of key genes of respective metabolite biosynthetic pathway (Sudha, G. & G. A. Ravishankar, 2003).

Production of secondary metabolites is mainly achieved through the field-cultivation of plants. However, cultivation of plants of particular biotopes outside their natural ecosystem is a hard task as these fail to grow properly and are unable to withstand pathogen attacks. Therefore, this led researchers to consider plant cell, tissue or organ culture as an alternative way of production corresponding secondary metabolites. The technique of plant cell culture technology facilitates rapid production of secondary metabolites achieved by optimizing the cultural conditions, selecting high-production strains and employing transformation and immobilization techniques to enhance the production of theses metabolites (Kaur H., 2012).

Materials and Methods: Different experimental methods were executed during present study suggested by Saikia, M. *et al.*,(2013), Keskin, N. and Kunter, B. (2008), Dr. S.R.M. Mathew, R., & Sankarp, P. D. (2013), Predieri, S. *et al.,* (1993), Mendham, J. *et al.*, (2002), Dodds J.H. and Roberts L.W. (1985).In short the steps used in methodology process are as follows-

i. **Plant Tissue culture (PTC)**
ii. **Thin Layer Chromatography (TLC)**
iii. **High performance layer chromatography (HPLC)**

- **Basic principle of PTC:** The aseptic culture of plant protoplasts, cells, tissues or organs is done under controlled condition which leads to cell multiplication or regeneration of organs or whole plant.
- **TLC Principle:** TLC is a chromatographic technique, which is used for the separation of mixture of compounds. The Separation of compounds is based on the differential adsorption as well as partitioning of analysis between the liquid stationary phase and mobile solvent phase.
- **Basic Principle of HPLC:** HPLC is a chromatographic technique used in analytical chemistry and recent analytical biochemistry to separate a mixture of compounds for the purpose to identify, quantify and purify the individual specific components of the complex mixture. Separation is based on the tendency of analyte's polar (hydrophilic) or non polar (hydrophobic) nature between two liquid phases.

The steps used in PTC are as follows-Procedure: In the present study leaves of *Glycine max* and *Withania somnifera* were used for culture experiments.

- **Sterilization-** Surface sterilization procedures for Explants, laminar air flow, glassware, hands were carefully done. Explants were transferred to the laminar air flow for further process.
- **Preparation of Culture medium-** The best-suited media is MS media as suggested by Sharma H., (2008).
- **Composition of medium-** Media were prepared by mixing all the ingredients in the recommended composition then added required hormones alone or in combination (2,4-dichlorophenoxyacetic acid (2,4-D, 2.0 to 4.0 mg/l) and NAA (2.0 to 4.0 mg/l)). The pH was adjusted to be within 5.6–5.8 by using 1 mol/L HCl and NaOH after the addition of plant hormones then media were autoclaved at 121^0C temperature under 15 lbs pressure for 15 min. Ten groups of culture media were prepared by mixing supplements in the following composition (table 1):

Table 1: Ten Groups of culture media

S.No.	Groups	Chemical composition & Concentration
1.	T1	MS +2mg/L 2,4-D
2.	T2	MS +4mg/L 2,4-D
3.	T3	MS +2mg/L NAA+2mg/L 2,4-D
4.	T4	MS +2mg/L NAA+4mg/L 2,4-D
5.	T5	MS +4mg/L NAA+2mg/L 2,4-D
6.	T6	MS +4mg/L NAA+4mg/L 2,4-D
7.	T7	MS +2mg/L NAA+2mg/L 2,4-D+10ml/L Coconut water
8.	T8	MS +2mg/L NAA+4mg/L 2,4-D+10ml/L Coconut water
9.	T9	MS +4mg/L NAA+2mg/L 2,4-D+10ml/L Coconut water
10.	T10	MS +4mg/L NAA+4mg/L 2,4-D+10ml/L Coconut water

- **Preparation and Inoculation of Explants-** The sterilized leaf explants of *Glycine max* further cut aseptically into small pieces and inoculated on different culture tubes on induction media. Twenty five replicates of inoculated explants were prepared for each group. Culture tubes were kept in the light (50 µmol m^{-2} s^{-1}) with a 16/8 h light/ dark photoperiod at 25±2 °C for incubation.
- **Callus initiation and maintenance-** Culture tubes were carefully observed and the changes like colour, texture, any sign of contamination, swelling and initiation of results were recorded every day.

After a few days the cultured explants become slightly rough in texture, the surface may glistening reflected light and cracks developed. This was the sign of the beginning of callus formation.

- **Exposures to UV radiation- The culture flask were** transferred to a growth chamber where the UV radiation source consisted of 1.2 m, 40 Watt UV-B-300nm lamps. Cultures tubes were placed under filters which allowed only UV-B radiation to pass. UV-B radiation was provided for 2 hr/day for 7 days . All cultures also received white light from fluorescents lamps (25 µmol m^{-2} s^{-1}).

- **Measurements (Physiological Responses of Callus)**
  The data for Callus Induction rate/Induction Frequency, Growth Index (GI), morphology, Fresh weight and Dry weight were recorded.

- **Callus induction rate (%)-** The percentage of callus induction rate or Frequency of callus generation was calculated by the following formula:

$$\textbf{Callus induction rate}\ (\%) = \frac{\textbf{Numbur of explants formed callus}}{\textbf{Total numbure of explants cultured}} \times 100$$

- **Callus Size, Nature & Morphology -** The size of the callus was measured by digital Vernier Caliper without any spoiling/disturbing and recorded in mm. The nature and morphology was also noted down.

- **Callus Growth index -** The fresh weights and dry weights of callus groups were recorded and calculated Growth index by the following formula:

$$\textbf{GI}\ (\%) = \frac{\textbf{Initial weight (W0)} - \textbf{Final weight (W1)}}{\textbf{Initial weight (W0)}} \times \textbf{100}$$

W0- After 8 weeks of culturing, the callus was removed from culture medium, washed completely free of agar and the fresh weight in gram was recorded.

W1- Freshly weighed callus was placed on Whatman No. 01 filter paper and dried at 80ºC for 24 h in oven then the Dry weight was recorded.

The steps used in TLC are as follows:

- **Sample preparation and extraction-** Eight weeks old callus was transferred to 10 ml separate volumetric flask and dissolved in methanol. The solution was grinded for 20 min and filtered through Whatman filter paper no.41 and the filtrate was used for further investigation (Panday A. and Tripathi, S. 2014).

- **Developing solvent system or Mobile phase-** Mobile phase consists of chloroform: Ethyl acetate in the 60:40 ratio in SS-I and chloroform: Acetone: Formic acid in the 75:16.5:8.5 ratio in SS-II, both mobile phase were used for the separation of the phenolic compounds (flavonoid tannin) (Kathiresan, Prabhu *et al.*, 2011 and Mohammed,S. S. A., *et al.,* 2003).

- **Stationary phase-** Stationary phase was done using TLC plate coated with silica gel, which are commercially available 60 F254 (Merck silica gel 60 F254 plate).

- **Procedure and Rf value calculate** - A TLC plate coated with silica gel G was taken and gently draw a straight line across the TLC plate approximately 1 cm from the bottom. Methanolic callus extract was loaded on the TLC plate (silica gel-G) as a single spot at the center of the TLC plates and put in TLC chamber, covered with a lid. The sample was run until the solvent front reaches the top-end border of the plates. The developed plate was taken out from the chamber and the solvent front was marked and allowed to air dry at room temperature for few minutes. The TLC plates were observed under UV light. The spots were visualized in the UV inspection cabinet (254 nm to 365 nm) and the distance of separated spots were marked and separated distance was measured. The Rf value calculated by following formulas:

$$\text{Rf value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by solvent}}$$

- **High Performance Layer Chromatography (HPLC):** HPLC equipment – HPLC estimation of SMs was performed on Water modular HPLC system, equipped with UV detector. For estimation, Thermo C18 (Dimension 250 x 4.6) RP column, pump 715, software Data Ace, Injector and the mobile phase with the mixture of Acetonitrile: Methanol (50:50) at a flow rate of 1.0 ml/min and the column temperature at 25°C was used. The wavelength of UV detector was set at 254 nm. The HPLC was run and sample detected at 254 nm. The sample injection volume was 20µl and total 20 minutes run time.

- **Preparation of standard stock solution -** 10 mg of tannic acid was weighed accurately and transferred to separate 10ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000 ppm.

- **Preparation of working standard solution (mobile phase)-** From stock solutions of tannic acid 1 ml was taken and diluted up to 10 ml with this solution, 1.0, 1.5ml solution was transferred to 10 ml volumetric flasks and make up the volume up to 100 ml with methanol, gives standard drug solution of 10 µg/ ml concentration.The methanolic extract of *Glycine max* leaf explants callus was prepared and subjected to HPLC with Acetonitrile: Methanol (50: 50) as mobile phase and the peak for SMs was obtained in retention time.

Results and Discussion: In this study, an attempt was made to establish tissue culture from leaves of *Glycine max* and to investigate the biosynthesis potentiality of the cultured tissue to produce secondary metabolites. Therefore, the study surveyed the occurrence of these compounds in 8 weeks old callus and the results are presented in this chapter under the following headings-

a) *In vitro* studies of *Glycine max*- Callus initiation occurred in explants after 4-7 days of inoculation

b) **Nature and Morphology effect on callus:** Nature and Morphological changes of *Glycine max non-treated callus* groups exhibit Greenish yellow and friable callus. After UV-B treatment the callus colour was changed to Yellowish brown compact and the callus remain friable.

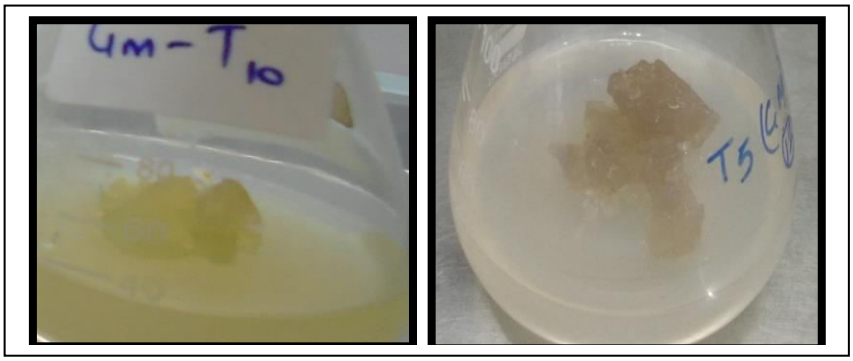


Photo Plate 1 - Nature & Morphology of *Glycine max* Non-Treated and UV treated Callus

b. The Influence of UV-B Radiation on the Callus Size of Glycine max: The effect of PGRs and UV radiation are exhibited in Table-2. Different PGRs in its different concentration increased callus size from 20.40 ± 1.654 to 93.32 ± 1.993 (From T1 to T10 groups) and in UV-B treated sets callus size ranges from 24.15 ± 1.543 to 93.90 ± 2.587 (from T1 to T10 groups) of 8 weeks old callus.

Table 2:

Callus size of non-treated and UV-treated *Glycine max* callus groups

S.No.	Callus groups	Callus size (in mm)	
		Non-treated Callus groups	UV treated Callus groups
1	T1	20.40±1.654	24.15±1.543
2	T2	24.82±2.545	27.65±2.332
3	T3	31.10±1.829	33.24±3.344
4	T4	37.50±1.482	40.74±2.362
5	T5	45.36±3.551	49.47±2.839
6	T6	58.21±4.292	59.93±4.251
7	T7	71.77±2.770	70.98±2.718
8	T8	81.75±1.808	82.69±1.569
9	T9	89.09±4.072	93.90±2.587
10	T10	93.32±1.993	91.54±3.262

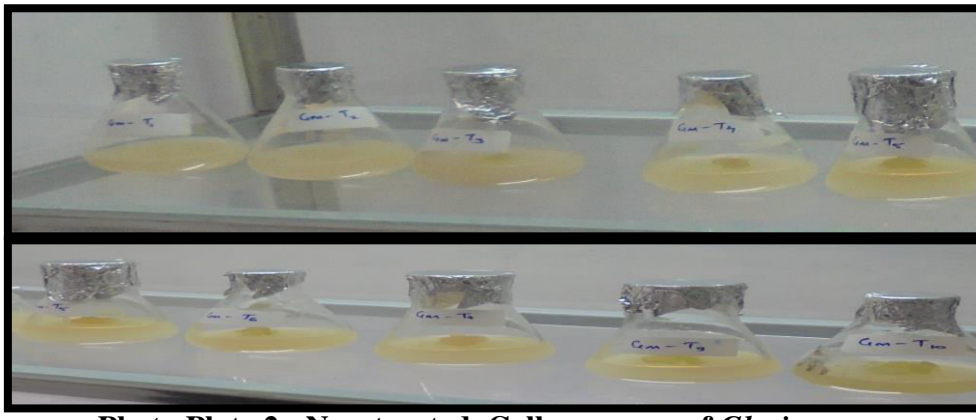


Photo Plate 2 - Non-treated Callus groups of *Glycine max*

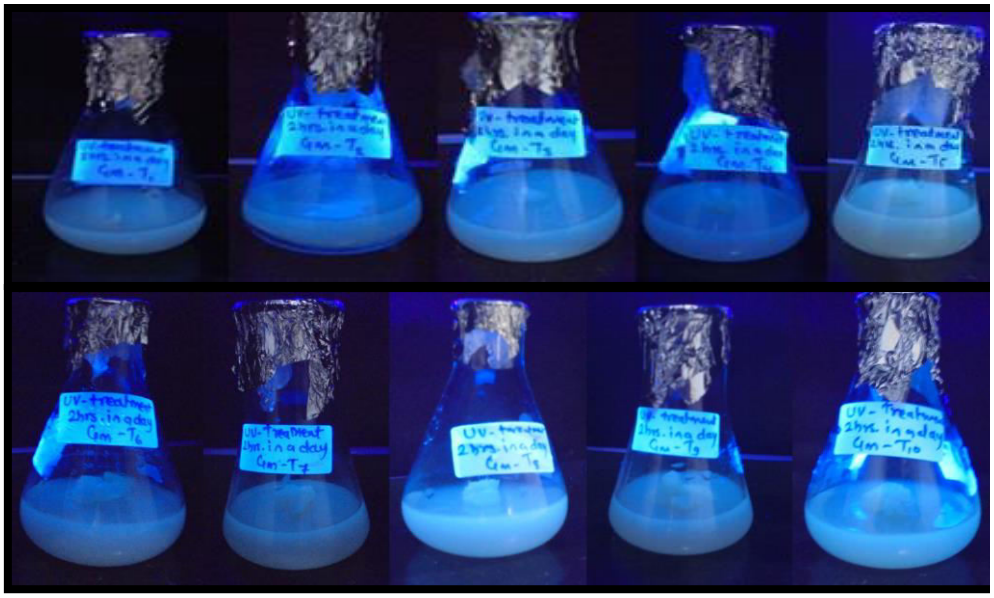


Photo Plate 3 - UV-treated *Glycine max* Callus Groups

c. Frequency Of Callus Generation / Callus Induction Rate: In this study, Glycine max callus induction rate was observed on the MS media supplemented with PGRs. The best callus induction rate (92%) was found in the T 7 group with composition 2 mg/l NAA, 2 mg/L 2,4-D and 10 ml/L CW and T10 group with composition 4 mg/L NAA, 4 mg/L 2,4-D and 10 ml/L CW (table 3 and fig. 2).

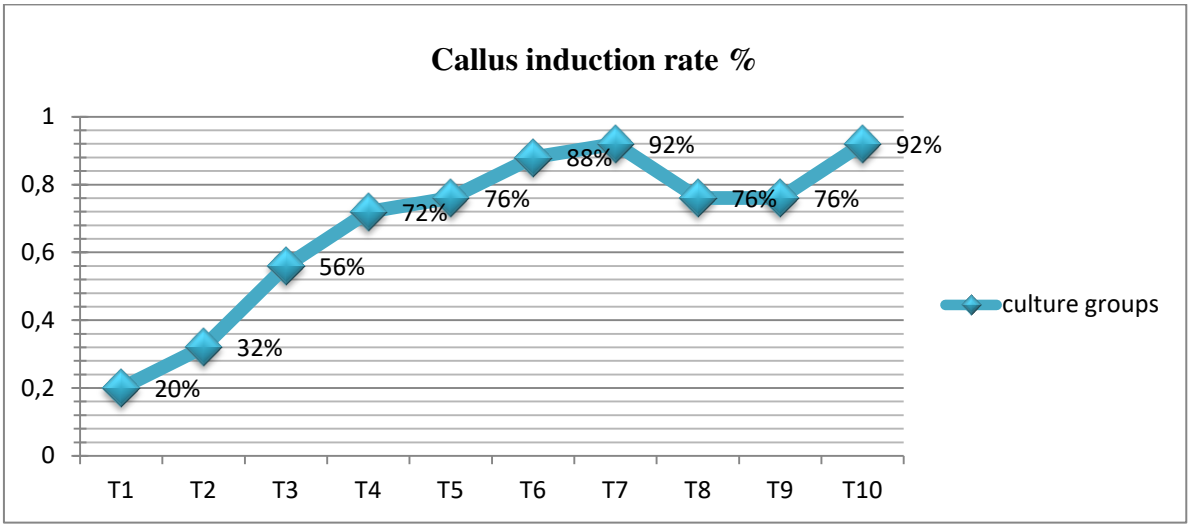


Fig 2: Frequency of generation or callus induction rate (%) of *Glycine max*

Table 3: Callus induction rate (%) of *Glycine max*

d.

Culture groups	Total number of Explants cultured	Number of Explants with callus	Callus induction rate (%)
T1	25	5	20
T2	25	8	32
T3	25	14	56
T4	25	18	72
T5	25	19	76
T6	25	22	88
T7	25	23	92
T8	25	19	76
T9	25	19	76
T10	25	23	92

Growth Index Of *Glycine max* Callus: The initial Weight (W0) of UV treated GM callus tissue ranged from 33.26 mg to 202.4 mg (T1 to T10 groups) and the final weight (W1) of callus tissue was obtained in the range of 29.12 mg to 18.16 mg (T1 to T10 groups). Initial Weight (W0) of non- treated GM callus tissue was found to be in range from 34.23 mg to 203.4 mg (T1 to T10 groups) and the final weight (W1) of callus tissue was measured from 31.42 mg to 173.16 mg. Among all non-treated and UV treated callus groups the Highest Growth index of 47.85% was recorded in UV treated T9 group with combination of 4 mg/l NAA, 2 mg/l 2,4-D and 10ml/l CW. The best Growth index of callus were observed 47.85% in the treated T9 (4mg/l NAA, 2mg/L 2,4-D, 10ml/L CW) followed by 39.35% in the treated T8 (2mg/l NAA, 4mg/L 2,4-D, 10ml/L) on MS medium. The data of Growth index of callus is given in the Table 4.

Table 4: Growth index of UV-treated and Non-treated GM Callus groups

	Treated callus			Non-treated callus		
Culture groups	W0	W1	GI (%)	W0	W1	GI (%)
T1	33.26	29.12	12.44	34.26	31.32	8.58
T2	38.13	30.45	20.14	39.13	35.45	9.40
T3	36.64	32.17	12.19	37.64	34.18	9.19
T4	40.95	35.33	13.72	41.95	37.53	10.53
T5	56.29	41.23	26.75	57.29	45.13	21.22
T6	79.29	48.76	38.50	80.29	64.76	19.34
T7	110.69	74.14	33.02	117.69	88.11	25.13
T8	159.0	96.43	39.35	196.57	150.04	23.67
T9	165.57	86.34	47.85	160.0	116.43	27.23
T10	202.4	168.16	16.91	203.4	173.16	14.86

Qualitative and Quantitative estimation of Secondary Metabolites: Phytochemical analysis was done using Thin Layer Chromatography (TLC) and High Performance liquid chromatography (HPLC) methods.

I. Thin Layer Chromatography (TLC) of *Glycine max* callus extract- Four sample were used for TLC of *Glycine max.* MeOH extracts of GM1-C callus (set of non-treated callus of T10 group of *Glycine max*) sample A, GM1-UV callus (set of treated callus of T9 group of *Glycine max*) sample B, showed following fractions in SS-I mobile phase.

Photo plate 4 : TLC of MeOH callus extracts of *Glycine max* (sample A & B with SS-I) under UV light

MeOH extracts of GM1-C callus (set of non-treated callus of T10 group of *Glycine max*) showed four fractions of phenolic compounds in SS-1 with Rf values between 0.106 to 0.969. GM1-UV, MeOH extract (set of treated callus of T9 group of *Glycine max*) showed two fractions with Rf value 0.922 & 0.974 in SS-1 mobile phase on TLC plate. Rf values visible in Specific UV inspection cabinet at 254 to 365 nm (photo plate 4 Table 8 & 9).

Table 8: TLC of *GM*1-C callus group(sample A), using SS-I

TLC Solvent system –I (Chloroform: Ethyl acetate (60:40))				
No.of spots	**Colour when viewed in UV**	**Distance travelled by solvent**	**Distance travelled by solute front**	**Rf value of GM 1-Control(sample A)**
1.	Pale green	6.6	0.7	0.106
2.	Pale green	6.6	4.3	0.651
3.	Pale green	6.6	5.1	0.772
4.	Pale green	6.6	6.4	0.969

Table 9: TLC of *GM*1-UV callus group(sample B), using SS-I

TLC Solvent system - Chloroform: Ethyl acetate (60:40)				
No. of spots	**Fluorescence in UV light**	**Distance travelled by solvent**	**Distance travelled by solute front**	**Rf value of GM 1-UV(sample B)**
1.	Pale green	7.7	7.1	0.922
2.	Pale green	7.7	7.5	0.974

TLC analysis of MeOH extract of GM2-C(sample C, set of non-treated callus of T10 group) and GM2-UV (sample D, UV treated set of T9 Group of *Glycine max*) performed in SS-2 on TLC plate and observed under UV inspection cabinet.

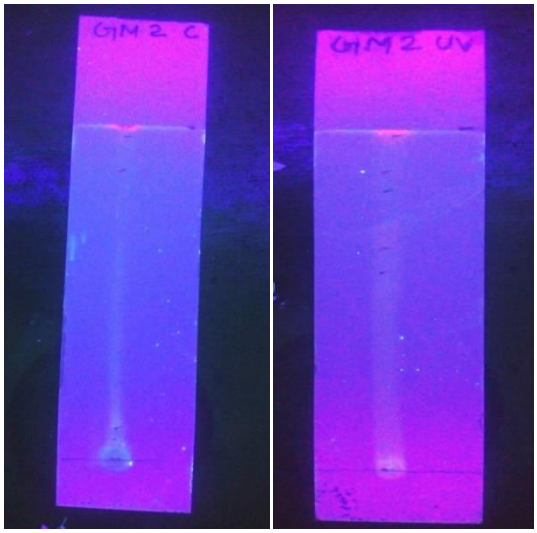


Photo plate 5: TLC of MeOH callus extracts of *Glycine max-2*

(sample C & D with SS-II)in UV light

TLC analysis of MeOH extract of GM2-C or sample C (set of non-treated callus of T10 group) revealed that three spots were obtained in SS-2 phase. The fraction obtained have Rf values of 0.097, 0.847 and 0.958 when a solvent phase SS-2 of Chloroform: Acetone: Formic acid (75:16.5:8.5) was used. GM2-UV (UV treated set of T9 Group of *Glycine max)* showed six fractions with Rf values between 0.380 to 0.971 in SS2 phase on TLC plate shown in the Table of 10 & 11 and photo plate 5. Among all of these groups and solvent system, the best result was found in Chloroform: Acetone: Formic acid (75:16.5:8.5) or SS-2 of UV treated *Glycine max callus* extract or sample D visualized six spots on TLC plate. Flavonoids may be appearing as dark spots under UV-365 nm.

Table 10: TLC of GM2-C Callus Group (Sample C), using SS-II

TLC Solvent system - Chloroform: Acetone: Formic acid (75:16.5:8.5)				
No. of spots	**Fluorescence in UV light**	**Distance travelled by solvent**	**Distance travelled by solute front**	**Rf value of GM 2-C(sample C)**
1.	Green pale	7.2	0.7	0.097
2.	Green pale	7.2	6.1	0.847
3.	Green pale	7.2	6.9	0.958

Table 11:TLC of GM2-UV callus group (sample D), using SS-II

TLC Solvent system - Chloroform: Acetone: Formic acid (75:16.5:8.5)				
No. of spots	**Fluorescence under UV light**	**Distance travelled by solvent**	**Distance travelled by solute front**	**Rf value of GM 2-UV (sample D)**
1.	Pale green	7.1	2.7	0.380
2.	Pale green	7.1	4.2	0.591
3.	Pale green	7.1	4.7	0.661
4.	Pale Purple	7.1	5.8	0.816
5.	Pale Purple	7.1	6.2	0.873
6.	Pale Purple	7.1	6.9	0.971

II. High Performance Layer Chromatography (HPLC) of *Glycine max* callus extract

Injected amount of sample detect peak area at a range of 5.299 to 1439.812 (total area 2068.181), average recovery was 100%.HPLC analysis of GM control Methanolic extract revealed 3 peaks on the basis of their retention time 2.866 min. GM UV Methanolic extract revealed 4 peaks on the basis of their Retention time 3.389 min (Table 12, 13 and fig. 3).

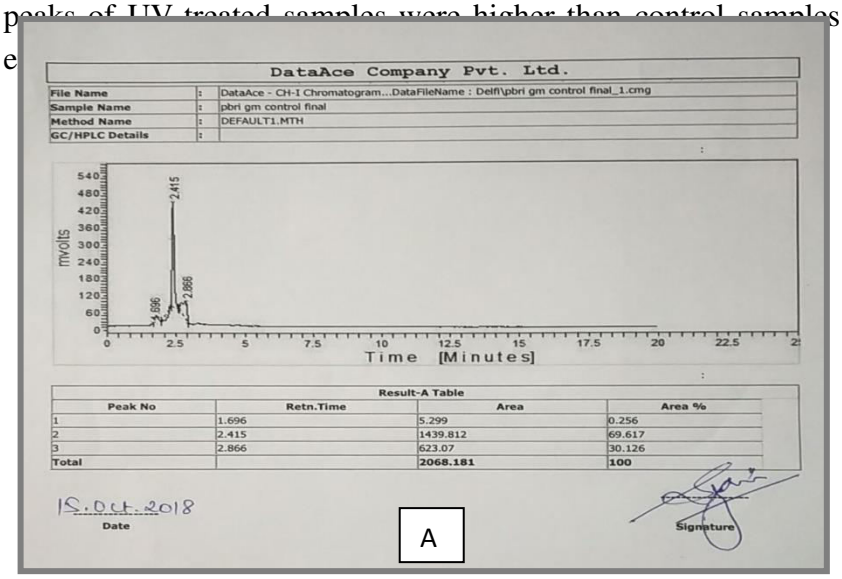
Table 12: Results for HPLC analysis of *Glycine max* non-treated callus

Peak Number	Retention time min)	Area	Area %
1.	1.696	5.299	0.256
2.	2.415	1439.812	69.617
3.	2.866	623.07	30.126
Total		2068.181	100

Table 13: Results for HPLC analysis of *Glycine max* UV-treated callus

Peak Number	Retention time (min)	Area	Area %
1.	1.867	169.228	5.534
2.	2.419	1081.49	35.368
3.	2.871	1715.402	56.098
4.	3.389	91.723	3.000
Total		3057.843	100

The UV-treated samples of plants were compared with control samples it showed that the numbers of peaks of UV-treated samples were higher than control samples. UV-B radiation (for short period) e



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Method Name	:	DEFAULT1.MTH
GC/HPLC Details	:	

Result-A Table

Peak No	Retn.Time	Area	Area %
1	1.696	5.299	0.256
2	2.415	1439.812	69.617
3	2.866	623.07	30.126
Total		2068.181	100

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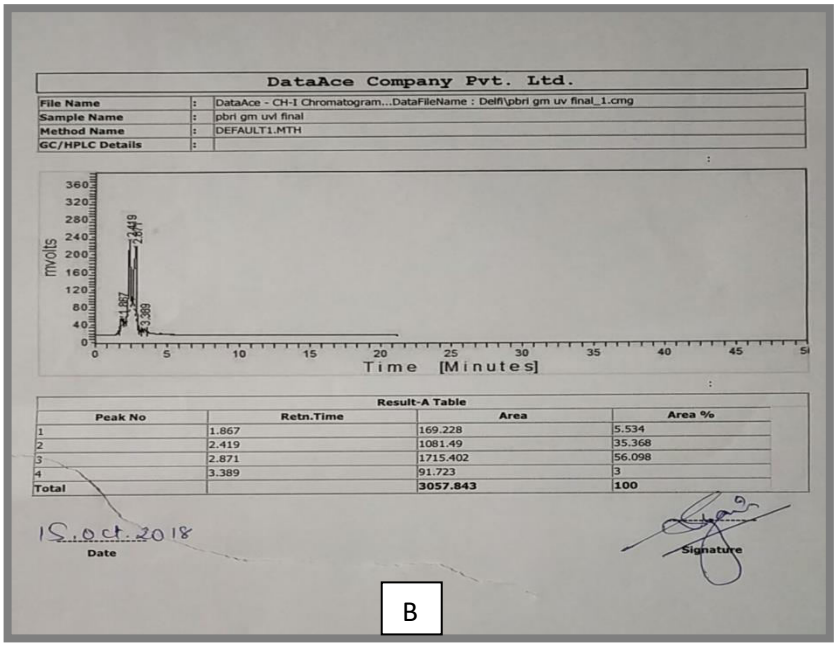

DataAce Company Pvt. Ltd.

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Sample Name	:	pbri gm uvl final
Method Name	:	DEFAULT1.MTH
GC/HPLC Details	:	

Result-A Table

Peak No	Retn.Time	Area	Area %
1	1.867	169.228	5.534
2	2.419	1081.49	35.368
3	2.871	1715.402	56.098
4	3.389	91.723	3
Total		3057.843	100

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Fig. 3: HPLCof *Glycine max* (A). non-treated and (B). UV treated callus extract

Conclusion: Parameters of the observation of experiment work are nature and morphology of callus, callus size, callus induction rate, weight of callus, growth index, Rf values, HPLC Peaks of components and the conclusion drawn from the investigation are presented below:

PTC- Among all groups, the optimum callus was found in MS media with NAA, 2,4-D and coconut water with various concentrations from GM cultures. Optimum callus of non-treated sets of *Glycine max* was found in MS Media with 4mg/lNAA, 4mg/l 2,4-D and 10ml/LCW. Supplemented UV-B treatment for shorter period 7day (2hr/day) positively affected the callus forming cells and significant presence of

secondary metabolites. Among all UV-B treated sets optimum callusing was found in T9 group with composition (4 mg/lNAA, 2mg/L 2,4-D and 10ml/LCW) from *Glycine max.* This non-treated group proved that the equal concentration of NAA, 2,4-D and added coconut water are more effective for callus forming but UV treated set do not follow this and the highest amount of NAA and lowest amount of 2,4-D along with CW gave highest callus size and weight. This study showed that the PGRs (in particular quantities) are an effective factor, but with this combination of PGRs, UV-B supplementation is found to be more effective factor for the callus culture of *Glycine max*.

TLC-The methanol (MeOH) with SS-II phase was found effective for the analyses of large number of SMs compounds. Many researchers have proved that Methanol extract was more suitable than ethanol, acetone, dimethylformamide (DMF) extract for analysis of SMs**.**The MeOH callus extract of culture groups showed maximum friction with Rf values in Mobile phase SS-II as compared to the Mobile phase SS-I. In UV treated callus extract were found maximum fraction with Rf values in SS-II phase as compared to the non-treated callus extract. These studies indicated that the UV treated callus extract with SS-II phase gave best response for analysis of SMs compounds. In SS-I phase was found highest number of friction with Rf values in Non-treated callus extract of *Glycine max* as compared to the SS-II phase.

HPLC- The UV treated sample of callus extract obtained the higher number of peaks than non-treated sample of callus extract of GM. UV-B radiations (for short period) could enhance the number of peak, it was shown by UV treated sample as compared to the non-treated sample.UV treated sample of GM callus extract showed increased peaks number as compared to the non-treated sample. Previous studies have indicated that increased UV-B radiation have positive, neutral and negative effects on plant growth, chlorophyll content, cell size, Growth index, Callus induction rate. Exposure of UV-B radiations for short period on callus, cause increased stress in the callus, and then callus produces SMs for defense. Therefore, UV-B treatment may be beneficial for enhancing the SMs production and also proved our studies, moreover long period exposure of UV-B radiations may be harmful for callus as it causes death of the cells.

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