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THE STUDY OF EFFECT ON *GLYCINE MAX* (L.)MERRILL TISSUE CULTURE BY PLANT GROWTH REGULATORS (PGRS) AND UV-B SUPPLEMENTATION

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ABSTRACT

In the present study we optimized the role of Plant Growth Regulators (PGRs) and UV-B light on the tissue culture of Glycine max. Tissue cultures of Glycine max seeds were established in different culture media. MS Media were prepared by mixing all the necessary ingredients then added required hormones alone or in combination (2,4-D, 2.0 to 4.0 mg/l) and NAA (2.0 to 4.0 mg/l)).Cultures were placed under filters which allowed only UV-B radiation to pass. UV-B radiation was provided continuously for 7 days (2 Hours in a day). It result obtained exhibit that the Callus induction rate of UV-B treated groups range from 20% to 92%. Nature and Morphology of the callus of PGRs groups were Greenish yellow and Friable callus while Yellowish brown compact Friable callus obtained in UV treated groups. The T10 group of PGRs group with combination MS+ NAA+ 2, 4-D + Coconut water (4+4 mg/L+10 ml/L) exhibited highest cell size of 93.32±1.993 mm (mean ± SD value) among all the groups but when the callus of same composition was given UV treatment the cell size was found to be 93.90±2.587 (Mean± SD Value). The results of the experiment showed that the PGRs is an effective factor but UV-B supplementation is found to be more effective factor for the callus culture of Glycine max.

***Keywords*: Glycine max, Tissue culture, UV-B light, Plant growth regulators (PGRs)**

I.INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is a leguminous crop of world economic importance. It is known as the "Golden bean" of the 20th century. In India, it is recognized as a food crop to bridge the gap between national need and availability of high protein as well as oil contents (Hildebrandt *et al*., 1986). Soybeans (*Glycine max*) are a major source of vegetable protein and edible oil. The nutritional quality of these seed components depends upon the relative abundance of specific proteins and fatty acids (Bennett J.O., 2005). Soybean is an economically important plant and their regeneration from tissue culture has been difficult and recently it became routine. Plant regenerated from tissue culture have exhibited various morphological and biochemical variation due to mutations which is termed as somaclonal variation (Radhakrishnan R. et al., 2008). Soybean has great

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potential as an exceptionally nutritive and very rich protein food. It can supply the much needed protein to human diets, because it contains above 40% protein of superior quality and all the essential amino acids articularly glycine, tryptophan and lysine, similar to cow's milk protein, soybean also contains about 20% oil with an important fatty acid, lecithin and vitamin A and D. The 4% mineral salts of soybeans are rich in phosphorous and calcium. It is the source of an excellent vegetable oil used in the manufacturer of various domestic and pharmaceutical products like paints, plastics, soaps and glycerin etc. Being devoid of starch, soybeans are also recommended to diabetic patient. Soybean cake is an excellent time stock food especially for poultry. The haulms provide good food for sheep and goats (Olhoft and Somers, 2007).

plant tissue culture is *"the aseptic culture of plant protoplasts, cells, tissues or organs under conditions which lead to cell multiplication or regeneration of organs or whole plants"*. Plant cell cultures are an attractive alternative source to a whole plant for the production of high-value secondary metabolites (Thorpe, T. A., 1990). Plant tissue culture systems have been developed for soybean (Glycine max L. Merrill.). For shoot morphogenesis, either cotyledonary node or primary leaf tissue was used to obtain cultures which formed shoots when placed on a medium containing benzyladenine. For somatic embryogenesis, the starting material was the immature zygotic embryo. The explant was the intact zygotic embryo, the excised embryo axis, or the excised cotyledons (John J. finer et al., 1988). The applications of plant tissue culture to medicinal plants are micropropagation for conservation and mass production and *in-vitro* production of desired phytochemicals. There are various advantages of tissue culture technology in medicinal plants over conventional method of propagation. Cells of any plants, tropical or alpine could easily be multiplied to yield their specific metabolites on a large scales, extraction of phytochemicals from callus and suspension cultures is easier than from the plant parts (Adhikari and Pant, 2013).

Callus culture consists of undifferentiated, unorganised, proliferating mass of cells usually arising on wounds of differentiated tissues and cells. Normally juvenile and hence physiologically the most active tissues give better callus formation. The exogenous plant growth regulator is required for callus formation. The strongest callus induction factor is the growth/nutrient medium supplemented with plant growth regulators (Pierik *et al.,* 1987)

Growth hormones regulate various physiological and morphological processes in plants and are also known as plant growth regulators (PGRs) or phytohormones. PGRs are synthesized by plants; therefore many plant species can grow successfully without external medium supplements. Hormones can also be added into cultures to improve plant growth and to enhance metabolite synthesis (Sidhu Y., 2010). Several studies involving PGRs have been carried out to find out the morphological and physiological traits leading improved yield and yield components in some leguminous plant (Malik *et al.,* 1993). PGRs could be used to stimulate metabolic and cell division activities in shoot meristem, cause alternations in vegetative and reproductive structure, increase total biomass, change production and mobilization of photosynthates to the reproductive sinks, improve harvest index, increase flowering and heavy fruiting, improve pollen germination, rapid fertilization and establishment of reproductive sinks, and influence metabolic processes causing enhanced oil or protein accumulation (Malik *et al.,* 1994).

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Increasing UV–B exposures in field–grown plants not only increase the total essential oil and phenolic content but also decrease the amount of the possibly toxic beta–asarone. These findings are expected as phenolics are known UV protectants (Naik and Al–Khayri, 2016). UV-B led to an increment of all growth parameters and antioxidant activity in callus and cell suspension and caffeic acid derivatives in cell suspension by increasing incubation period. Ultraviolet-B radiation is one important environmental factor that numerous cases impel the production of secondary metabolites. Low UV-B doses led to increase the production of secondary metabolites (Manafe al., 2016).UV radiation can be regarded as a stress factor which is capable of significantly affecting plant growth characteristics. Plant height, leaf area, leaf length have been showed to decrease, whereas leaf thickness was increased in response to UV-B radiation. In addition, photosystem II can be adversely affected by UV-B radiation. Plants may produce secondary products to protect them against UV light damage, but these metabolites also play an important role in human health, flavonoids and anthocyanins are responsible for antioxidant activity in fruit and vegetables (E. Tsormpatsidis et al., 2008). Enhanced UV-B radiation is increased production of secondary metabolites in leaf tissues under enhanced UV-B radiation.The UV-B radiation transmitted after reflection by epicuticular wax layer reaches the epidermal layer. The epidermal layer is known to accumulate most of the secondary metabolites, such as phenolics and flavonoids that absorb/screen UV-B radiation and shield the underlying tissue against harmful UV-B radiation(Kakani V.G. and et al., 2003).
The aim of the present study was to develop *Glycine max* tissue culture from seeds and to investigate the influences of the Plant Growth Regulators (PGRs) and UV radiation.

II.MATERIALS AND METHODS

2.1 Preparation of Explants

Surface sterilization procedures for *Glycine max* seeds were carried out by soaking the explants in filtered distilled water followed by 70% (v/v) ethanol for 3-5 min in a commercial bleach solution (5% sodium hypochlorite solution) containing a few drops of Tween 80 for 30 min. The seeds explants were then thoroughly rinsed with sterile distilled water to remove any traces of remaining detergents. Seeds Explants were transferred to the laminar hood for further process (Staba, E. J.(1980), Saikia, M. *et al.,* (2013), Keskin, N. and Kunter, B. (2008), Predieri, S. *et al.,* (1993), Mendham, J. *et al.,* (2002).

NOTE: After inoculation of explants into medium if contamination were seen so during surface sterilization explants were treated with 1% mercuric chloride solution for 3-5 min in the laminar air flow. Then rinse with distilled water several times.

2.2 Preparation of Culture medium

The widely used culture medium by Murashige and Skoog (commonly called MS medium)was formulated.

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

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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.

10 groups of culture media were prepared by mixing supplements in the following composition:

Table-1

Different PGRs add in MS medium with different concentration

S. No.	Groups	MS media+ PGRs	Concentrations
1.	T0	MS +2,4-D	(2mg/L)
2.	T1	MS +2,4-D	(4mg/L)
3.	T3	MS +NAA+2,4-D	(2+2mg/L)
4.	T4	MS +NAA+2,4-D	(2+4mg/L)
5.	T5	MS +NAA+2,4-D	(4+2mg/L)
6.	T6	MS +NAA+2,4-D	(4+4mg/L)
7.	T7	MS +NAA+2,4-D+ Coconut water	(2+2mg/L+10ml/L)
8.	T8	MS +NAA+2,4-D+ Coconut water	(2+4mg/L+10ml/L)
9.	T9	MS +NAA+2,4-D+ Coconut water	(4+2mg/L+10ml/L)
10.	T10	MS +NAA+2,4-D+ Coconut water	(4+4mg/L+10ml/L)

2.3 *In-vitro* induction of callus

The sterilized seeds were cultured onto callus induction media. Twenty five replications of inoculated explants had been prepared for each treatment and the experiments were repeated thrice. Cultures were kept in the light (50 µmol m^{-2} s^{-1}) with a 16/8 h light/ dark photoperiod at 25 ± 2^0C. Callus formation was monitored over a 1-month period and subsequent growth (in terms of fresh weight) was followed until the stationary phase was reached. Once established, calli were sub-cultured at monthly intervals on the medium which gave the best growth rate. The observation was done on weekly basis. At the end of six weeks, the formed calli were scraped and isolated from the explants. The data for callus induction were recorded in which the morphology and fresh weight (g) of callus in each treatment were taken.

Fresh weight of the callus

After 6 weeks of culturing, the callus was removed from culture medium, washed completely free of agar and the mean fresh weight in gram was recorded.

2.4 Callus induction (%)

The percentage of callus induction and callus morphologies from each treatment and media combination were observed. After six weeks of culture period, the percentage of callus induction, weight (fresh weight) and the

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morphology of calli formed were all documented. The percentage of callus induction in each treatment was calculated by the following formula:

$$\text{Callus induction } (\%) = \frac{\text{Number of explant formed callus}}{\text{Total number of explants cultured}} \times 100$$

The optimum treatment was determined by considering the highest callus induction percentage, fresh weight of callus (gm) with the optimum intended morphologies of profuse and completely friable calli. Growth of seed calli of plant cultivars was measured in terms of fresh weight. Fresh weights of calli/explants were taken after removing the excess moisture on the surface using a blotting paper Growth was studied by determining the fresh weight of callus after the treatment. (Keskin and Kunter, 2008).

2.5 Exposure to UV-B Supplementation

The cotton plug of flasks and culture tubes were removed and replaced with plastic film which is 85% transparent to UV-B radiation 300nm. The culture tubes were transferred to a growth chamber where the UV radiation source consisted of 1.2 m, 40 W UV-B-313 lamps. Cultures were placed under filters which allowed only UV-B radiation to pass. The distance between lamps and the tissues was kept 30 cm.UV-B radiation was provided continuously for 7 days (2 Hours/day). All cultures also received white light from fluorescents lamps (25 µmol m^{-2} s^{-1}).

2.6 Growth parameters

The fresh weight of harvested callusbefore treatment (in mg/callus) and final weight of callus after treatment (in mg/callus) or as fresh weight was determined immediately and then freeze-dried for dry weight and analysed. The culture growth was determined in terms of growth index (GI) which was calculated according to Dung et al., (1981) by using the following formula-

$$\text{Growth Index } (\%) = \frac{\text{Final fresh weight} - \text{Initial fresh weight}}{\text{Initial fresh weight}}$$

$$GI(\%) = \frac{W1 - W0}{W0}$$

W0 = Weight of callus tissue before treatment (in mg/callus)

W1= Final weight of callus after treatment (in mg/callus) or as fresh weight

GI= Growth index (%)

III.RESULTS AND DISCUSSION

3.1 Nature and Morphology effect on callus

Nature and Morphological changes of *Glycine max* callus culture data is illustrated in Table (Table 2). This Table shows effect of PGRs and UV-B supplementation on the nature and Morphology of callus. All PGRs 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.

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Table -2

Nature and Morphology of PGRs and UV treated group of callus

S. No.	Groups	Nature and Morphology of callus	
		PGRs	UV treated
1.	T0	-	-
2.	T1	Greenish yellow Friable callus	Yellowish brown compact Friable callus
3.	T3	Greenish yellow Friable callus	Yellowish brown compact Friable callus
4.	T4	Greenish yellow Friable callus	Yellowish brown compact Friable callus
5.	T5	Greenish yellow Friable callus	Yellowish brown compact Friable callus
6.	T6	Greenish yellow Friable callus	Yellowish brown compact Friable callus
7.	T7	Greenish yellow Friable callus	Yellowish brown compact Friable callus
8.	T8	Greenish yellow Friable callus	Yellowish brown compact Friable callus
9.	T9	Greenish yellow Friable callus	Yellowish brown compact Friable callus
10.	T10	Greenish yellow Friable callus	Yellowish brown compact Friable callus

3.2 Effect of PGRs and UV-B on Cell size in callus

Effect of PGRs and UV treated groups exhibited in the table 3. The table showed that the different PGRs and its different concentration increased cell size of callus from 20.40±1.654 to 93.32±1.993 (From T1 to T10 groups) and UV treated groups cell size ranges from 24.15±1.543 to 93.90±2.587 (from T1 to T10 groups) of 6 weeks old callus. Cell size increased in all UV treatment groups more than PGRs treated groups.

Table - 3

Cell size of PGRs and UV treatment group

S. No.	Treatment group	Cell size (in mm)	
		PGRs	UV treated
0	T0	00±00	00±00
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	91.54±3.262
10	T10	93.32±1.993	93.90±2.587

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All Data were present in Mean± SD Value

3.3 Callus induction rate of callus

Callus induction of *Glycine max* was observed from seed on MS medium supplemented with various concentrations and combinations of 2,4-dichlorophenoxyacetic acid (2,4-D), Napthalene acetic acid(NAA) and Coconut water.

In our study, the best callus induction was by sub-culturing the callus on MS medium supplemented with NAA+ 2,4-D + Coconut water in various concentrations. The group number T7 and T10 with combination of NAA+ 2,4-D + Coconut water (2+2mg/L+10ml/L) and NAA+ 2,4-D + Coconut water (4+4mg/L+10ml/L) gave the same callus induction (92%).

Table-4

Frequency of generation/ callus induction rate (%)

Treatment group	No. of Explants	No. of Explants with callus	Frequency of generation/ callus induction rate (%)
T0	25	0	0
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

Note: Treatment means to be treated with UV-B radiation (365nm)

3.4 Growth index of callus

The data of growth index of callus given in the Table (Table 5). Firstly weight of callus tissue before treatment (in mg/callus) and final weight of callus after treatment (in mg/callus) or as fresh weight were recorded and calculated the GI (%). Weight of callus tissue before treatment (in mg/callus) ranges from 14.29 to 173.16 (from T1 to T10) and the weight of callus tissue after UV-B treatment (in mg/callus) ranges from 34.26 to 203.4 (from T1 to T10). Highest growth index of 1.397 (%) was recorded in UV-B treated MS media 2,4-D (2mg/L(T1 group)).

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Table-5

Growth index (%) of callus

Treatment group	W0	W1	GI (%)
T0	0	0	0
T1	14.29	34.26	1.397
T2	26.72	39.13	0.464
T3	36.17	37.64	0.041
T4	37.53	41.95	0.118
T5	45.13	57.29	0.269
T6	72.76	80.29	0.103
T7	88.11	117.69	0.336
T8	116.43	159.96	0.374
T9	150.04	196.57	0.310
T10	173.16	203.4	0.174

IV. CONCLUSION

The conclusion of the Study indicate that the PGRs is effective factor for callus culture, it increases significantly the cell size, callus induction, callus weight but UV-B radiation is more effective than PGRs, it also increases significantly the cell size, callus induction, callus weight more than PGRs. UV radiation has a wide range of effects on the herbivore defense of plants as well as on the herbivores themselves. Ambient UV radiation affected the morphology and the composition of secondary compounds such as flavonoids in soybeans. UV induces accumulation of a range of secondary metabolites in higher plants, including flavonoids and sinapic esters, which in turn affect numerous physiological functions. Furthermore, flavonoids including anthocyanins have been shown to provide protection against solar radiation-induced DNA damage and to possess free-radical scavenging activity which might offer additional protection to cells accumulating these compounds. In PGRs the highest cell size of callus was 93.32±1.993 mm in T10 group with combination of Murashige and Skoog medium(MS medium) +Napthalene Acetic Acid (NAA) + 2,4-dichlorophenoxyacetic acid (2,4-D) and Coconut water (4+4mg/L+10ml/L),but UV-B radiation is most effective for cell size with 93.90±2.587 mm on the medium of same concentration. All PGRs culture groups showed the Yellowish Brown and Friable callus and the same concentration with UV-B treatment showed the Yellowish brown compact Friable callus in all groups. When we took MS media with 2,4-dichlorophenoxyacetic acid (2,4-D) (2mg/L), showed the callus index 20 %, as well as with NAA (2+2mg/L) in this medium callus induction increased to 56%, it means as we added more PGRs in medium callus induction increased. When we treated with UV-B continuously for 7 days (2 Hours in a

day) on 6 weeks old callus it showed the positive result with this treatment. The highest callus induction rate 88% was achieved in UV-B treated two group T7 and T10.Group T8 with the combination MS+NAA+2,4-D+Coconut water (2+2mg/L+10ml/L) and T10 group with same combination but concentration was (4+4mg/L+10ml/L).

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