Preliminary physiological and cytological screening of callus of *Vigna catjang* Walp (in Vitro)

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Research Article

Abstract: The present paper aim is to preliminary physiological and cytological study of callus, which is obtained from *Vigna catjang* walp. Growth of callus is determined by fresh weight and dry weight of callus, mitotic index and cell number in callus. Protein is also estimated in callus in present paper.

Key words: *Vigna catjang* Walp, callus mitotic index, protein, fresh weight and dry weight.

Introduction

Plant tissue culture is based on the principle of totipotency i.e. ability of the cell to regenerate in to complete plant. Plants obtained by this technique are of true type. This is the recent method of vegetative propagation. The culture in the laboratory is known as explants when this explant is placed on the nutrient medium. It divides and forms callus culture. A callus culture consists of an amorphous mass of a loosely arranged thin walled parenchymatous cells arising from the proliferating cells of the explants, Callus has no predicable organizational pattern although localized centre of meristematic activity are present. Sinnot (1960) has described some of the observations callus formation on wound. Injury to the plant parts, initiates the callus formation. The stimuli involved in the initiation of wound callus are endogenous hormones- auxin and cytokine, which are used in tissue culture technique. Callus formation can be induced in a number of plant tissues and organs that do not usually develop in response of injury (Street-1969). Initiation of callus requires a continuous supply of macronutrients, this includes nitrogen, phosphorus, calcium magnesium and sulphur. In addition to the macronutrients elements, it requires traces of certain micronutrients. These micronutrients are required in exceedingly small concentrations, so o concentrated stock solution is prepared Micronutrients includes iron, magnese, zing, boron, copper, molybdenum and chlorine. An iron stock solution is prepared separately because of the problem of iron solutility. The growth regulator requirements for most of the callus cultures are auxin and cytokinins. Auxin stimulates shoot cell elongation, promotes cell division in tissue culture. Auxin and cytokinin are supplemented to the medium to promote cell division, cell elongation, cell differentiated and organ formation. The effective auxin for callus proliferation for most of cultures is 2, 4 dichlorophenoxyacetic acid 2.4 D. The most acidly used cytokinins in culture medium are kinetin and benzylaminopurine. Both are synthetic compounds. Coconut milk is also used in preparations of medium it contains diphenylurea, a growth factor which exhibits cytokinin like responses. Vitamins have catalytic functions in enzyme systems and are required only in trace amount. In leguminosae some work has been carried out on callus culture. Callus is easily started from explants of soybean's (*Glycine max*) cotyledon after surface sterilization of seeds. In *Ceratonia siliqua* callus cultures have been raised from different explants.

Material and Methods

Plant material – Cotyledons of *Vigna catjang* were used as a source of explants.

All nonbiological material was sterilized by autoclaving Murashige and Skoogs medium was prepared, pH was adjusted at 5.8 and the volume was increased up to 1000ml.

Surface sterilization of seeds: Healthy seeds of *vigna catjang* were selected. They were washed under running water for 15 minutes. Then seeds were rinsed in 70% ethanol for 30 to 60 seconds. These seeds were again washed with distilled water. Then the seeds were sterilized by 0.1% aqueous solution of mercuric chlonde for 3 mins. The seeds were transferred in inoculation chamber and then washed with sterile distilled water at least thrice to remove the traces of $Hgcl_2$. Small amount of sterile distilled water was poured in sterile petri-dish in the inoculation chamber. Surface sterilized seeds were kept in sterilized petri-dishes containing distilled water. The petri-dishes was covered by parafilm strips. Seeds were soaked overnight. Then outer seed coat of the seed was removed and the cotyledons were separated and each cotyledon was cut in to two pieces and embryo were used as explants. All above mentioned operations was done in aseptic condition. Dissected explants were transferred in the test tube containing medium in the inoculation

chamber. Inoculated culture vessels were marked and were incubated in incubation chamber at 25 ± 1^0C and light 1200 lux photoperiod of 10 hrs.

Fresh wt of the explants: To determine fresh weight of explants, the soaked seeds treated as mentioned earlier were used. Blotted explants were weighed individually on a preweight glazed paper on an analytical balanced. Mean value was determined (weight of glazed paper was subtracted from the mean value). **09844706266 Togale Manoj**

Fresh weight of callus: Cultured explants (cotyledon) exhibiting different stages of callus were separated in 5 groups. In the 5th groups cavities were on the embryonal axis. Callus samples were transferred from culture medium to a petri dish containing Whatman No. 1 filter paper. Traces of medium were removed from underside of each sample. All the samples were weighted on analytical balanced on a pre-weight glazed paper. Mean value of fresh weight was determined for all the five groups.

Dry Weight of Callus: Samples after taking the fresh weight were dried in oven of 60^0C temperature for 48 hrs. Samples were cooled to the room temperature and again weight was taken. The mean values were determined.

Mitotic Index: Different stages of callus growth were fixed according to the groups mentioned earlier in acetic alcohal for 24hrs. It was then transferred to 70% ethanol. A randomly selected fragment of callus was hydrolyzed in 1 N Hcl for 1-5 minutes. The material was washed with 45% glacial acetic acid. Extra glacial acetic acid was removed by blotting paper. Remaining traces of acetic acid were allowed to evaporate. The hydrolyzed pieces of culture were squashed in acetocarmine and were covered with a coverslip. Extra stain was removed and it was warmed gently over the spirit lamp. The slides were sealed and mitotic index was calculated by using following formula.

$$\text{Mitotic Index} = \frac{\text{No of dividing cell.}}{\text{Total number of cells counted.}}$$

Cell Number In Callus: Different slages of callus were kept in maceration solution (equal volume of 10% aqueous HN03+10% aqueous chromic acid) till callus became soft and fragile [48 hrs.]. Soft and fragile callus was washed with distilled water by centrifugation suspension of callus was prepared in 50% glycerine. 0.1ml cell suspension was taken in haemocytometer slide and it was covered with a coverslip. The numbers of cells covering the grid were counted and average was taken. Total number of cells was calculated by using following formula-

$$\text{Total cell number} = \frac{\text{Volume of macerate x average cell count}}{\text{Volume above the grid.}}$$

Quantitative estimation of protein

Quantitative estimation of protein was carried out by lowry's method.

Results

Growth of the cultures

Morphological observations: Depending on the growth of callus cultures were separated in four groups. In group first no growth was observed, while in the second group there was swelling of explants. In the third group, there was swelling as well as greening of the explants with small amount of callus formation.

In the fourth group from embryonic axis good callus growth was obtained and it was placed in 5th group.

Table 1: Showing fresh weight and dry weight of callus culture

Group	Fresh weight in gms. Mean	Dry weight in gms. Mean
1	0.05	0.013
2	0.118	0.020
3	0.180	0.023
4	0.200	0.037
5	0.26	0.073

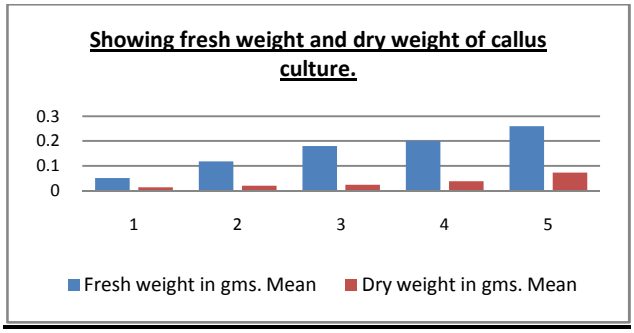


Table 2: Showing the cell count in callus culture

Group	Total cell no. (Mean)
1	100
2	176
3	225
4	830
5	1580

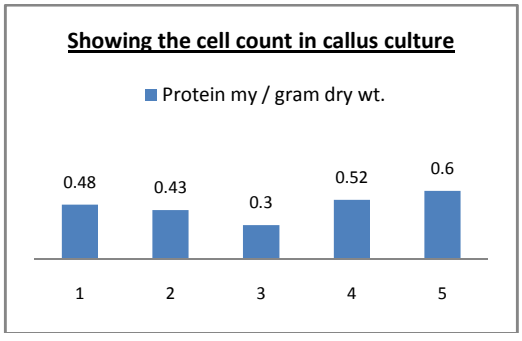


Table 3: Cytological observations (Mitotic Index)

Group	% Mitotic Index
1	0 %
2	32 %
3	38.2 %
4	45.61 %
5	63.52 %

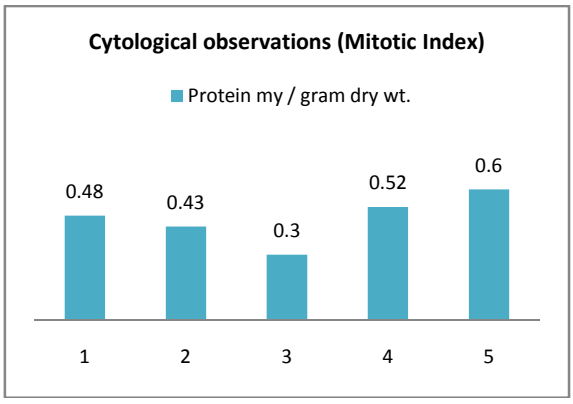
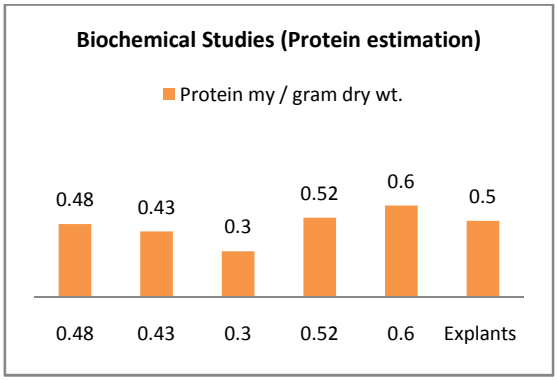


Table 4: Biochemical Studies (Protein estimation). Showing amount of protein in callus culture.

Group	Protein my / gram dry wt.
1	0.48
2	0.43
3	0.30
4	0.52
5	0.60
Explants	0.50



Conclusions

Dry weight and fresh weight of callus were increased according the stages of growth. Dry weight and fresh weight of callus obtained from embryo were more. It indicates that as the growth of callus is increased dry weight and fresh weight of callus are also increased. Mitotic index in group first was zero. It proves that there is no growth. Morphological observations also show that there was no growth and was no change in the explants. Mitotic index was increasing from group 2nd to group 5th. Maximum mitotic index was observed in group 5th. It proves that as the growth is increased mitotic index also increased. Maximum mitotic index in culture obtained from embryonal axis indicates maximum growth of callus. Cell count number was also found to be increased according to the stages of growth. In group 1st the cell count was less while maximum cell count was found in the group 5th. These results clearly show that growth is directly related to the cell count. Amount of protein was slightly reduced in group 1st, 2nd and 3rd as compare to the explants. The reason may be consumption of protein in activation of cell from cell division and the metabolic activities; protein is stored food material in leguminous seeds. In group 4th and 5th the amount of protein was increased. It clearly indicates that when callus growth was increased. The amount of protein also increased. The reason for increase amount of protein may be the synthesis of protein during the growth of callus.

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