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Studies on variations in tissue culture of winged bean using in vitro techniques

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Abstract :- The morphology and ultrastructure of the reproductive organs were examined. Cytological studies of the disgrace uncover the confinement of starch, lipids and proteins at different formative stages. Meager cell layer nodal and stem explants gave maximum shoot formation, while entire tissue explants were second rate. The correct cotyledon was better than the left cotyledon in shoot regeneration.

Key words: Variations, tissue, culture, winged, bean

1. Introduction

The common bean is the most widely grown pod among the Phaseolus species. Yield increases were due, over the years, due to the high rate of application of chemical products (such as fertilizers, herbicides, pesticides or pesticides), machinery and mitigation. That was the cause of various economic and environmental problems, such as pollution, the need for additional energy sources, and so on. On the other hand, advances in genetic engineering and bean breeding, the use of traditional methods was reached to the limit of many. attitudes. Due to the long process of breeding and selection, the genetic diversity of the beans has been largely determined. Winged beans (Psophocarpus tetragonolobus (L.) DC), a legume with high nutrient content has been described as "a soybean that can be found in tropical areas" (Anon 1981). It has the ability to meet the food needs of many people in these regions of the world. All parts of the plant, except the stem and roots, are nutritious and rich in protein, minerals and vitamins. In order to maximize the desired effect, winged beans need the development of a variety of traits, such as plant structures and to reduce the ongoing features of the seed. Genetic engineering technologies, among other things, may be employed in such a system. Effective in vitro regeneration programs are a prerequisite for the use of genetic engineering and similar techniques. Therefore tissue regeneration systems using a variety of plants should be developed to speed up the spread and improve the plant.

Regeneration of the indirect shoot, in the callus phase, is accomplished in winged beans (Venketeswaran 1990). The only records in the formation of direct shoots related to young (Trinh et al. 1981) and mature (Dias et al. 1986) cotyledon explants. However, renewal waves have not been reported. With regard to embryogenesis, many reports indicate the ingestion of auxins, particularly 2,4-dichlorophenoxy acetic acid (2,4-D) and naphthalene acetic acid (NAA) (Venketeswaran 1990; Venketeswaran et al. 1990; Ahmed et al. 1996). Cytokinin-induced somatic embryogenesis did not appear to be associated with winged beans.

II.. Materials and Methods

Morphology And Ultra-Structure Of Reproductive Parts

Pollen Morphology – Acetolysis Method

In pollen morphological studies, anthers during anthesis were collected and protected immediately in containers containing 70% alcohol or cold acidic acid. Pollen preparations were developed by the acetolysis strategy proposed by Erdtman. Protected items were exchanged into a rotating plastic tube and pressed with a glass rod. The dispersion was sorted by 48-degree / cm2 steel work and assembled into a glass axis tube. After placement in the central region, the supernatant was touched and pollen grains after being washed with cold acidic were treated with an acetolysis blend consisting of acidic anhydride and concentrated H2SO4 (9: 1). Cylinders containing pollen grains were rotated in a water bath at 70 to 1000C for 3 to 5 minutes until the medium came out dark brown in color. After centrifugation, the supernatant was released. Acidic acid is added to pollen grains and is centrifuged.

Endless slides of acetolysed pollen grains are made by dipping them in glycerine jam and fixing edges with paraffin wax. Sample photomicrographs were taken.

Morphology of pistil and pollen

Morphological and anatomical characters of the reproductive parts were considered utilizing cryotome segments recolored with saffranin in a light magnifying instrument just as SEM.

a. Tissue processing and staining for light microscopy

Crisp pistils were processed for cryotomy. Crisply gathered pistils were profound freezed right away. They were then fixed to the stubs utilizing Jung's tissue frigid media at - 28 0C. After fruitful fixing, the squares were cut to proper size. The stub is fixed to the space and the blade is balanced suitably. The required size of the segment is chosen and segments are taken utilizing the Lecia CM 1100 cryotome. The warm microslides are tenderly placed on the areas and they will fix on the slide and soften on. The segments are then recolored with reasonable stains and made semi changeless by including a coverslip and ringing the edge with finger nail polish or wax. They were then seen in a trinocular magnifying instrument and photographed.

b. SEM studies

SEM arrangements were made by Falk.

The means utilized in the processing for SEM are as per the following.

Fixation

Disorders: New solubility was prepared with 3% gluteraldehyde at 0.1M phosphate support at pH.7 for 6 hours. The fixed material was washed several times thoroughly in a cold place for phosphate 15 minutes each at 4 0C.
Pollen: Pollen morphology was tested from pure pollen.
Drying: Prepared material dried in the CH3) 2CO system of 25%, half, 75% and 100% for 10 minutes each and placed at 70% CH3) 2CO. Finally the tissues were stored at 100% CH3) 2CO, 30 minutes and the exposure of isoamyl acetic acid for 5 minutes at room temperature.

Critical Point Drying (C.P.D)

The dried out pollen grains and stigmas were Critical point dried in a Critical point drier (H.C.P-2 Hitachi).

Mounting

The dried specimens were placed on the buttocks using double-sided adhesive tape and covered with gold in a sputter coater (Model E-101-Hitachi). Covered examples were detected under SEM (S-2400, Hitachi).

III.. Pollen viability

i. Fluorochromatic Reaction test (FCR)

FCR test was completed by Heslop-Harrison and Heslop-HarrisoN. For this fluorescein diacetate (FDA) was set up in CH3)2CO (2mg/ml). Sucrose arrangement in adequate fixation was arranged independently to counteract blasting of pollen grains. Two to five ml of sucrose arrangement was taken in a glass vial and stock arrangement of FDA was added drop by drop to it until the subsequent blend demonstrated industrious turbidity.

A drop of blend was taken on a microslide and suspended adequate pollen guaranteeing uniform distribution in the arrangement. The arrangement was brooded in a dampness chamber for 5 – 10 minutes and saw under an Olympus (Japan) fluorescence magnifying instrument under UV excitation. The feasible pollen grains which fluoresced were checked and the level of feasibility was determined by the recipe.

Percentage of viability = $\frac{Numberofviablepollen}{Totalnumberofpollen}$ x 100

ii. *In vivo* pollen germination - Cotton blue staining method

In vivo pollen germination and tube growth studies were led. For microscopic perception, the pollinated pistils were painstakingly analyzed out from the flower and fixed in Carynoy's fluid. They were exchanged to lactophenol answer for which a couple of drops of 1% cotton blue stain was included and brooded in the stove at 60 0C for 30 minutes. The recolored pistil was

mounted in a drop of glycerine on a slide. A cover glass was placed over it and squeezed delicately for the detachment of pistil cells for simple perception of pollen tubes. Germination rate was determined and pollen tube length was estimated utilizing micrometer.

The time taken by the pollen tube to achieve the base of style was discovered by gathering the pollinated flowers from the field developed plants at interims of 24, 48 and 72 hours after pollination. The length of pollen tube in each style was estimated.

IV. Results

Reproductive biology

Psophocarpus tetragonolobus (L.) DC (Fabaceae) plants were developed and kept up in the green house for experimental reason. The explants were gathered from plants raised from axenic seedlings as well as mature green house developed plants kept up under uniform conditions with no obvious disease indications.

Morphological, anatomical and cytochemical examinations were done to ponder the flower biology of the plant. A description as well as a diagrammatic portrayal of the plant was additionally arranged.

Inflorescence, axillary, careless raceme; flowers (Plate 1:4), up to 3.5 X 3 cm; pedicles up to 1 cm long, glabrous, calyx gamosepalous, companulate or chime formed, calyx glass 0.7 - 0.8 cm long, projections 5, 0.5 - 0.6 cm long, intense at pinnacle, greenish, adaxial 2 flaps combined, imbricate, glabrous; petals (Plates 1:5; 19:1) 5 (2+2+1), imbricate, glabrous, papilionaceous; standard petals glabrous, upto 3 X 3 cm, somewhat blue tinged above and greenish yellow underneath, whole, bifurcate at the tip, greenish striations or patches at the base and focus; winged petals 2 in number, pale blue tinged on the two sides, heartless at the tip, 2.8 - 3.1 X 1.3 - 1.6 cm, glabrous, whole; bottom petals 2, 2.8 - 3.1 X 0.9 - 1.2 cm, white in shading, glabrous, uncaring at the tip, greenish line at the intertwining bit of the two petals from the base to the tip, whole; stamens diadelphous (9+1) (Plate 1:7), staminal tube 1.6 - 1.8 cm long, fiber 0.8 - 1.0 cm long, white; anther adaptable,dithecous (Plate 2:2); nectary (Plate 1:8), ten in number; ovary (Plate 1:6), 1.5 cm long, tetrangular, greenish, one celled; ovules 11 - 14 in number, peripheral placentation; style, 1.5 - 1.8 cm long, white, bended; stigma capitate, thickly pubescent at the edge (Plate 2:3). Pods green, 20 - 25 X 3.5 - 3.8 cm, quadrangular, winged, dry indehiscent; seeds round, 0.6 - 0.9 cm over, smooth, hard, light darker to dull darker in shading. (Plate 1:9)

Table 1: Concentrations and mixes of plant growth controllers (mg/l) for shoot tip/nodal fragment culture

IAA	NAA	BAP	KIN
0.1-1.0	-	1-5	-
0.1-1.0	-	-	1-5
-	0.1-1.0	1-5	-
-	0.1-1.0	-	1-5
-	-	1-6	-
-	-	-	1-5
-	-	1-5	1-5

Table 2: Hormonal concentrations (mg/l) and combinations tried for directregeneration

IAA	IBA	NAA	BAP	KIN
0.2-2.0	-	-	1-5	-
0.2-2.0	-	-	-	1-5
-	0.2-2.0	-	1-5	-
-	0.2-2.0	-	-	1-5
-	-	0.2-2.0	1-5	-
-	-	0.2-2.0	-	1-5
-	-	-	1-10	-
-	-	-	-	1-10
	-	-	1-5	1-5

Occasionally the plants produce tubers (Plate 1:10). They develop to a length of around 36 cms and a width of 6 cms at the broadest bit. The estimated dry wt. of the tuber is 6.5 gms.

The plant is seasonal and flowers and fruits amid November to February. The pods are 4 - winged and the wings are frilled. It is conveyed in a wide scope of climatic conditions including both low and high grounds.

4.1.2 Morphology And Ultrastructure Of The Reproductive Organs

I. Androecium

It is diadelphous (9+1) with a staminal tube length of about 1.8 cm and a fiber of length 1 cm. The anthers are flexible and dithecous (Plate 2:2).

ii. Pollen biology

Studies utilizing pollen acetolysis and SEM and furthermore reasonability tests have empowered us to comprehend the morphology and feasibility of pollen at different stages.

a. Pollen morphology

Pollen morphological characters were dissected and are portrayed according to Nair. The essential analytic highlights of the pollen grains, for example, opening, exine surface, shape and size are depicted based on the wording proposed by Walker and Doyle.

Pollen grains are in monads and are 3-zonocolporate (Plate 3:1-5). The grains are oblate spheroidal in tropical view. The ora is lalongate. The pollen grains are medium estimated, with a scope of 31.3 - 34.43 X 31.3 - 40.69 with a ξ= (33.17 X 36.9 μm) and σ = (0.48 X 1.06 μm). The exine has a reticulate ornamentation and a thickness of 3.13 μm.

A SEM investigation of pollen was additionally led. Pollen gathered from unopened flower buds, only preceding opening was utilized. The SEM studies substantiate the outcomes acquired in acetolysis studies (Plate 3:6-9).

b. Pollen feasibility

Flurochromatic reaction (FCR) test

The pollen grains were mounted in fluorescein diacetate (FDA) arrangement and saw under a picture analyzer (Plate 4:1-3). The pollen grains demonstrated greatest, feasibility at a phase when the flower was going to open. It was additionally seen that the pollen hold the suitability for one more day subsequent to getting completely opened and later feasibility would in general lose. About 90% of the pollen grains demonstrated a splendid green or yellowish green fluorescence uncovering their practicality status.

Cotton blue recoloring

The cotton blue recoloring of the stigma and the stylar district uncovered that the pollen grains holding fast to the stigmatic surface creates pollen cylinders and they infiltrate through the stylar channel (Plate 4:4-8). The pollen tube is viewed as a solitary fluorescent strand in the stylar channel.

iii. Gynoecium

a. Stigma

The stigma is round at the tip and is thickly pubescent at the edge (Plate 5:7-9). The outside of the stigma has a free streaming exudate, which is emitted, by glandular cells. The stigmatic surface shows globular, unicellular papillae cells with finger like structures, emerging from the edge of the stigmatic head, which are very clear from the SEM perceptions (Plate 5:1-6).

b. Style

The style is about 1.8 cm long, which is white in shading. It has a trademark bill like bend. The transverse and longitudinal segments of the style demonstrate that Psophocarpus have a solitary stylar trench (Plate 7:1-4), for the fruitful passage of the pollen tube. The stylar channel is lined by glandular epithelium. The style has a particular vascular component on one side of the stylar channel. The cryotome segments (Plate 6:1-9) likewise affirms the nearness of a solitary stylar trench. The cortex of the style indicates vascular follows close to the fringe area of the stylar channel. Plate 7:1-8 demonstrates the different areas from the stylar channel to the ovary, demonstrating the campylotropus ovule moreover.

c. Ovary

The ovary is quadrangular fit as a fiddle and green in shading. It is unilocular with peripheral placentation. The ovary contains 10 - 12 ovules which are of the campylotropus type (Plate 7:6-8).

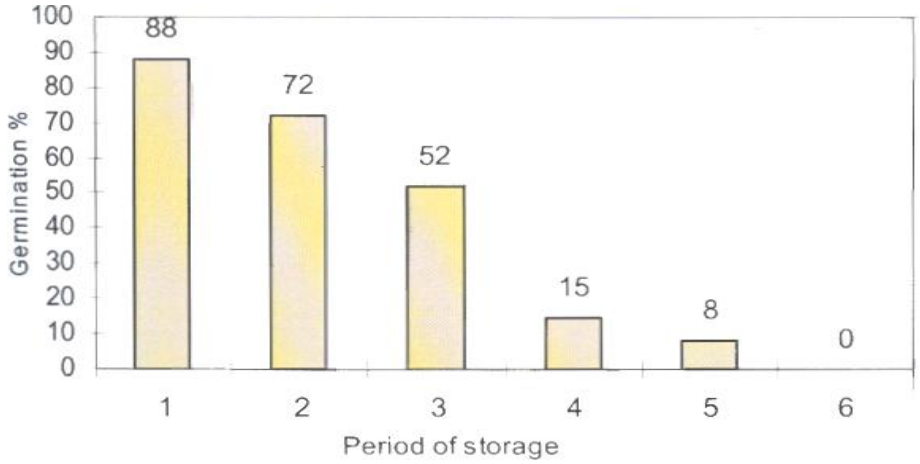


Figure 1: Reduction in seed germination on storage

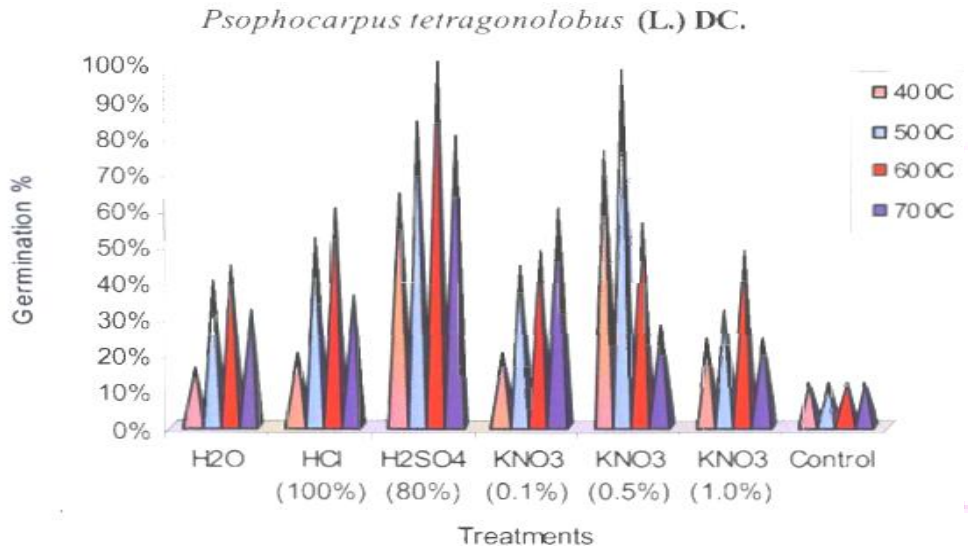


Figure 2: Effect of various treatments on seed germination of *Psophocarpustetragonolobus*(L.) DC.

Table 3: Effect of various treatments on seed germination of Psophocarpus tetragonolobus (L.) DC

Treatments	Mean no. of seeds germinated±SE			
	40ºC	50ºC	60ºC	70ºC
H_2O	3.43 ± 0.4^{k}	10.29 ± 0.2^{g}	11.14 ± 0.3^{fg}	$8.29\pm^{hi}$
HCl (100%)	4.71 ± 0.2^{j}	13.43 ± 1.3^{e}	15.29 ± 0.3^{d}	9.00 ± 0.5^{h}
H_2SO_4 (80%)	16.00 ± 0.4^{d}	20.86 ± 0.3^{b}	24.86 ± 0.1^{a}	20.14 ± 0.1^{b}
KNO_3 (0.1%)	5.00 ± 0.2^{j}	10.86 ± 0.6^{fg}	11.57 ± 0.4^{f}	15.29 ± 0.5^{d}
KNO_3 (0.5%)	18.86 ± 0.3^{e}	24.43 ± 0.3^{a}	13.71 ± 0.4^{e}	7.29 ± 0.4^{i}
KNO_3 (1.0%)	5.57 ± 0.5^{j}	7.57 ± 0.4^{i}	11.86 ± 0.5^{f}	5.43 ± 0.4^{j}
Control	2.860 ± 0.3^{k}			

Seeds treated with 100% HCl at the temperatures of 40 0C, 50 0C, 60 0C and 70 0C for 5 minutes gave 20%, 52%, 60% and 36% seed germination individually (Fig:2). Seeds treated with

80% H2SO4 for 5 minutes at 40 0C displayed 64% germination. At the point when a similar treatment was performed at 50 0C and 60 0C, the level of germination was increased to 84% and 100% individually. In any case, an increase in the temperature past 60 0C unfavorably influenced the germination rate. Treating the seeds with 0.1% KNO3 at various temperatures for 5 minutes likewise caused an upgrade of germination rate. At 40 0C, the germination rate was just 20%, however it was improved to 44% and 48% at 50 0C and 60 0C separately. A similar treatment when given at 70 0C, the germination rate was 60. The level of germination was additionally increased to 98% when the seeds were treated with 0.5% KNO3 at 50 0C for 5 minutes. It was seen that the ideal temperature amid the treatment with 0.5% KNO3 is 50 0C, underneath or more which the germination rate decreased. Germination rate was found to increase with the increase in temperature from 40 0C to 60 0C when the seeds were treated with 1.0% KNO3 for 5 minutes. However, at 70 0C, just 24% germination was watched. So also, when customary faucet water was utilized at these temperatures for 5 minutes, the germination rate was found to increase with temperature rise upto 60 0C and decreased at 70 0C or more. In the control, where the seeds were absorbed water and sown in polythene sacks, the seed germination was observed to be just 12% (Fig: 2). About 100% germination was acquired when the seeds were treated with 80% H2SO4 at 60 0C and 0.5% KNO3 at 50 0C for 5 minutes (Plate 1:1-3).

4.3 TISSUE CULTURE STUDIES

4.3.1 Sterilization Of Explants

Explants from field developed mature plants were sanitized in 0.05 – 0.3% fluid mercuric chloride answer for 3-7 minutes. The leaf and stem explants demonstrated a most extreme survival rate of about 89% and 92% individually at 0.1% HgCl2 treated for 5 minutes. Explants were faded at higher dimensions (≥0.2 %) of the sterilant presentation (Fig: 3). Drawn out cleansing negatively affected the survival of these explants. Following two days, the explants turned dark colored and died (table 4 Fig: 3).

Table: 4 Composition of Hoagland's solution (Epstein, 1972)

Compounds	Elements	Concentration (mg/ml)
Macro salts		
KNO3		
CaNO3		
MgSO4. H2O		

Compounds	Elements	Concentration (mg/l)
Macro salts		
KNO_3	N, K	22.23
$CaNO_3.\ 4H_2PO_4$	Ca	160.0
NaH_2PO_4	P	62.00
$MgSO_4.\ 7H_2O$	S, Mg	32.64
Micro salts		
H_3BO_3	B	1.72
$NaSO_4.\ H_2O$	Mn	0.27
$ZnSO_4.\ 7H_2O$	Zn	0.11
$CuSO_4.\ 5H_2O$	Cu	0.132
Na_2MOO_4	Mo	0.05
Fe EDTA	Fe	1.2

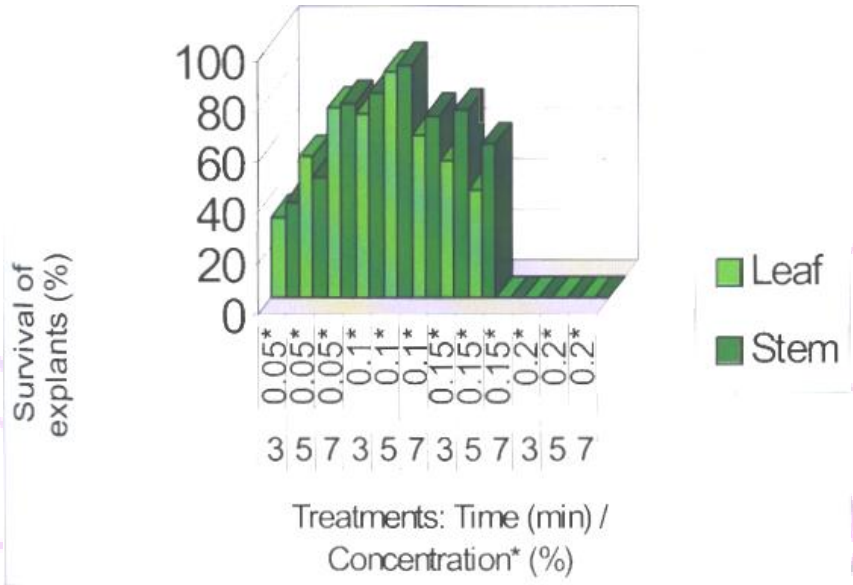


Figure 3: Effect of sterilant on explant survival

Table 5: Effect of growth regulator concentrations on shoot formation from shoot tip explants in Psophocarpus tetragonolobus (L.) DC

Hormones (mg/l)		Mean No of shoots ± S.E
BAP	KIN	
1	1	4.57 ± 0.6^{efgh}
2		5.00 ± 0.4^{ef}
3		6.71 ± 0.5^{d}
4		3.43 ± 0.4^{gh}
5		2.00 ± 0.4^{ij}
1	2	5.14 ± 0.2^{ef}
2		7.00 ± 0.3^{cd}
3		8.14 ± 0.5^{bc}
4		4.14 ± 0.2^{efgh}
5		2.14 ± 0.4^{ij}
1	3	7.29 ± 0.7^{cd}
2		9.14 ± 0.5^{b}
3		11.14 ± 0.2^{a}
4		6.71 ± 0.5^{d}
5		3.86 ± 0.2^{fgh}
1	4	4.43 ± 0.3^{efgh}
2		5.43 ± 0.4^{e}
3		7.14 ± 0.4^{cd}
4		3.57 ± 0.5^{gh}
5		2.00 ± 0.2^{ij}
1	5	1.43 ± 0.2^{j}
2		3.29 ± 0.3^{hi}
3		4.71 ± 0.4^{efg}
4		1.29 ± 0.3^{j}
5		0.86 ± 0.3^{j}

4.3.2 Axillary Bud Expansion

Experiments were directed by immunizing shoot tips of around 1 cm length, in 1 - 5 mg/l of BAP in MS, N&N and B5 media. Despite the fact that, N&N and B5 media bolstered the prolongation of shoot tips, they didn't advance axillary bud expansion. MS media was observed to be perfect for axillary bud expansion, alongside shoot lengthening. Consequently further experiments were completed on MS media enhanced with different hormones.

i. Shoot tip culture

A blend of both BAP and KIN was observed to be vital for the axillary bud multiplication of shoot tips. BAP (1-5 mg/l) alongside KIN (1-5 mg/l) gave effective shoot expansion (Table: 12). The ideal hormonal milieu was observed to be BAP (3 mg/l) + KIN (3 mg/l), which gave 11 shoots for every explant (Plate 8: 1, 2). At the point when the convergence of both BAP and KIN was increased over 3 mg/l, the quantity of shoots per explant decreased. Whenever BAP (1-6 mg/l) or KIN (1-5 mg/l) was included alone, basal callusing was watched (Plate 8: 3). On the expansion of auxins, IAA/NAA (0.1 - 1 mg/l) alongside cytokinins BAP/KIN (1-5 mg/l), basal

callusing with the drying of shoot tips was watched (Plate 8:4,5). Shoot tips refined on NAA (0.1 - 1 mg/l) + BAP (1-5 mg/l) expanded medium indicated callus commencement and rhizogenesis of callus following a month of immunization (Plate 8: 6).

ii. Cotyledonary hub culture

Whenever cytokinins, BAP and KIN were included alone, various shoot arrangement was seen from cotyledonary hub explants. Most extreme number of shoots was delivered on BAP containing medium, with a limit of 15 shoots/explant in BAP (3 mg/l) (Table: 6; Plate: 8:9). The quantity of shoots created/explant increased with an increase in the grouping of BAP from 1 mg/l to 3 mg/l, however the number decreased when the fixation was raised over 3 mg/l. Groupings of BAP over 5 mg/l gave 3-4 shoots which demonstrated vitrification. The quantity of shoots decreased when KIN (1-5 mg/l) alone was added to the medium. A limit of 9 shoots were gotten in 3 mg/l KIN enhanced medium. There was a continuous increase in the quantity of shoots as the centralization of KIN was increased from 1 to 3 mg/l. Notwithstanding, the quantity of shoots decreased when higher focuses were utilized. The impact of the two cytokinins BAP and KIN together (1-5 mg/l) were examined. A limit of three shoots were shaped in BAP (3 mg/l) + KIN (3 mg/l) expanded medium (Plate: 8:8). Centralizations of BAP and KIN over 3 mg/l lead to a decrease in the absolute number of shoots created/explant.

Table 6: Effect of growth regulator concentrations on shoot formation from cotyledonary node explants in Psophocarpus tetragonolobus (L.) DC

Hormones (mg/l)			Mean No of shoots ± S.E
IAA	BAP	KIN	
0.1	1	-	5.00 ± 0.5^{de}
	3		6.86 ± 0.46^{cd}
	5		3.43 ± 0.30^{e}
0.2	1	-	6.14 ± 0.51^{d}
	3		8.86 ± 0.34^{c}
	5		3.14 ± 0.2e
-	1	-	12.14 ± 0.26^{b}
	3		15.71 ± 0.29^{a}
	5		9.57 ± 0.37^{bc}
-	-	1	5.00 ± 0.31^{de}
		3	9.43 ± 0.30^{bc}
		5	7.14 ± 0.34^{cd}
-	1	1	1.29 ± 0.29^{fg}
		3	2.71 ± 0.29^{ef}
		5	0.86 ± 0.26^{g}
-	3	1	1.86 ± 0.26^{f}
		3	3.43 ± 0.30^{e}
		5	1.14 ± 0.26^{fg}
-	5	1	1.43 ± 0.37^{fg}
		3	2.86 ± 0.34^{ef}
		5	1.29 ± 0.36^{fg}

Auxin - cytokinin blend demonstrated shifted reaction in cotyledonary node cultures (Table: 6). Of the different mixes attempted, just IAA (0.1 - 0.2 mg/l) + BAP (1 - 5 mg/l) gave numerous shoot arrangement (4 - 8 shoots). The various mixes, for example, IAA (0.1 - 1.0 mg/l) + KIN (1 - 5 mg/l); NAA (0.1 - 1.0 mg/l)+BAP (1 - 5 mg/l) (Plate: 8: 7) and NAA (0.1 – 1.0 mg/l) + KIN (1 - 5 mg/l) gave just callusing. A limit of 8 shoots were acquired in media containing IAA (0.2 mg/l) + BAP (3 mg/l). At the point when the convergence of IAA was increased above 0.2 mg/l, there was no shoot arrangement however just callusing. Groupings of BAP higher than 3 mg/l alongside IAA (0.1 – 1.0 mg/l) were observed to be supraoptimal for shoot expansion.

LIST OF PLATES

PLATE: 1

1: Plantlets raised from treated seeds

2: Plantlets raised from seeds treated with 80% H_2SO_4

3: Actively growing plantlets in pots after treatment with 80% H_2SO_4

4: Flowers- adaxial and abaxial sides

5: Petals with Androecium and gynoecium

6: An individual gynoecium

7: A diadelphous stamen

8: Nectary

9: Variation in seed colour of various accessions of *Psophocarpus tetragonolobus*

10: Root tuber

11: Root nematode infection

PLATE 2:

1: Shoot tip culture in MS+ BAP (3 mg/l) + KIN (3 mg/l) after one week of culture

2: Shoots developed from shoot tips in MS + BAP (3 mg/l)+ KIN (3 mg/l) after 30 days of culture

3: Basal callusing from shoot tips in MS+ BAP (3 mg/l)

4: Basal callusing with drying of shoot tips in MS+ IAA (0.2 mg/l) + BAP (3 mg/l)

5: Basal callusing with drying of shoot tips in MS+ IAA (0.2 mg/l) + KIN (3 mg/l)

6: Callusing along with rooting of shoot tips in MS + NAA (0.2 mg/l) + BAP (3 mg/l) after 30 days of culture

7: Callus from cotyledonary node in MS+ NAA (0.2 mg/l) + BAP (3 mg/l)

8: 3-4 shoots initiated from cotyledonary nodes in MS + BAP (3 mg/l) + KIN (3 mg/l)

9: 15 shoots from cotyledonary nodes in MS + BAP (3 mg/l)

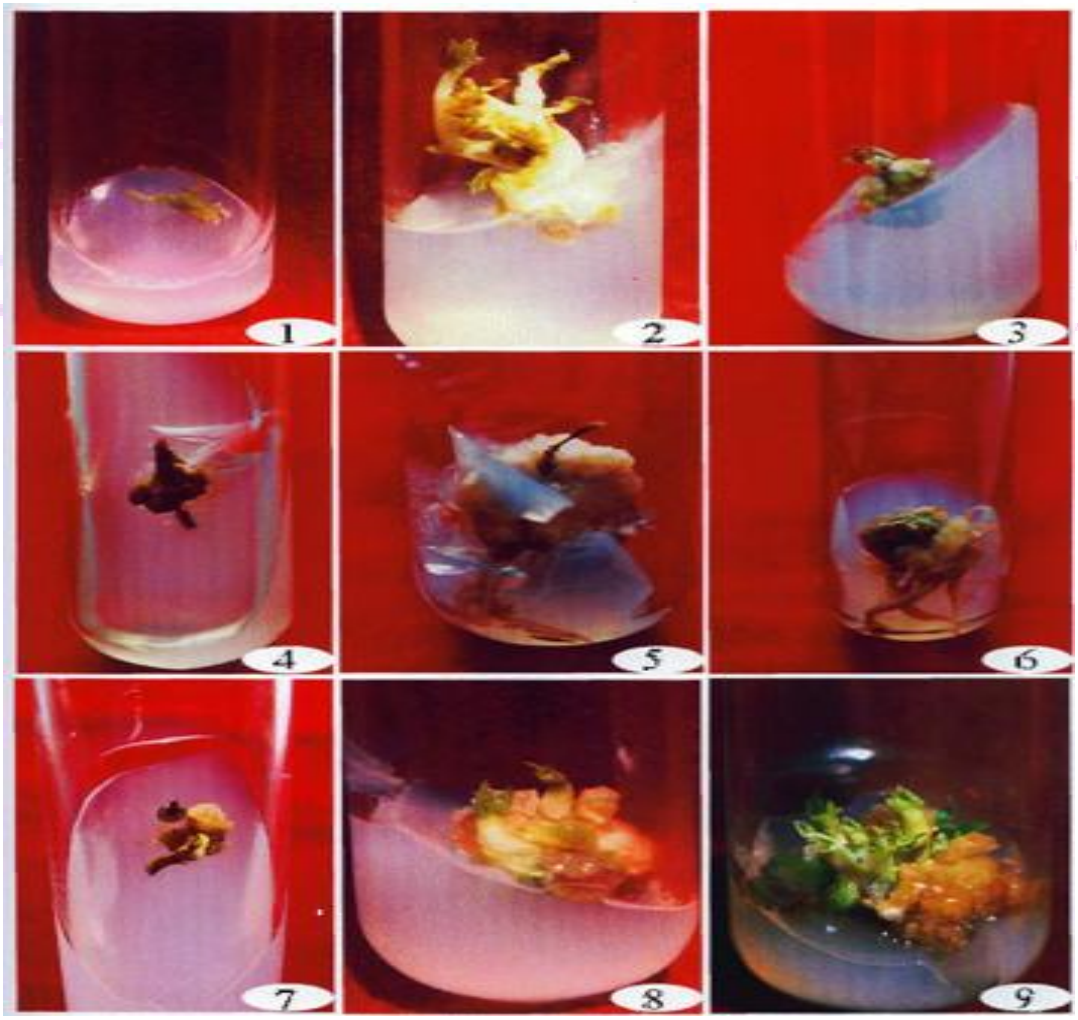


PLATE 3 :

1: Shoots produced from nodal thin cell layer explants in MS + IAA (1 mg/l) + BAP (2 mg/l)

2: Shoots produced from nodal thin cell layer explants in MS + BAP (1 mg/l)

3: Shoots produced from nodal thin cell layer segments in MS + BAP (5 mg/l)

4: Callusing from nodal segments in MS + KIN (2 mg/l)

5: Callusing along with rooting from nodal segments in MS+ BAP (2mg/l) + KIN (2mg/l)

6-7: Initiation of shoots from nodal segments in MS + BAP (1 mg/l)

8: Shoot elongation in MS + BAP (0.5 mg/l) + GA_3 (0.5 mg/l)

PLATE 4:

1: Swelling of cotyledons in BAP (5 mg/l) after 5 days of culture

2: Shoot initiation from right cotyledon in MS + BAP (2 mg/l) after 30 days of culture

3: Shoot initiation from left cotyledon in MS + BAP (2 mg/l) after 30 days of culture

4: Shoots produced from right cotyledon in MS + IAA (0.4 mg/l) + BAP (4 mg/l)

5a: Shoots produced from right cotyledon in MS + IAA (0.2 mg/l) + BAP (2 mg/l)

5b: Shoots produced from left cotyledon in MS + IAA (0.2 mg/l) + BAP (2 mg/l)

6: Shoots produced from left cotyledon in MS + IAA (0.2 mg/l) + BAP (4 mg/l)

7-8: Elongation of shoots with rooting from right cotyledon in MS+ IAA (0.2 mg/l)+ BAP (2 mg/l)

9: Stunted shoot growth and rooting in left cotyledon in MS + KIN (4 mg/l)

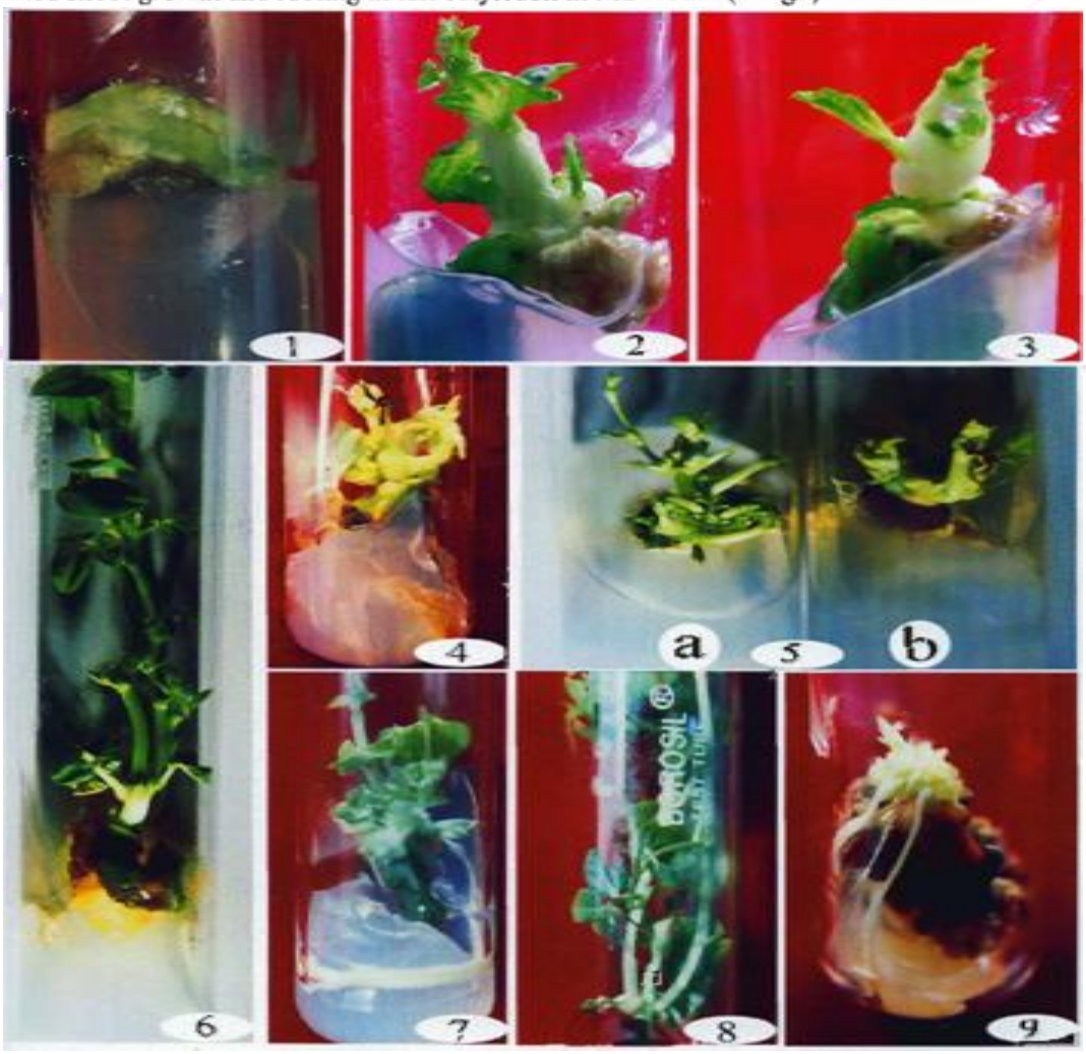


PLATE 5:

1: 7-8 shoots produced from thin cell layer stem explants in MS + BAP (8 mg/l)

2: Swelling and initiation of callus from stem explants in MS + 2,4-D (5 mg/l)

3: Swelling and initiation of callus from stem explants in MS + IAA (5 mg/l)

4: Callusing from the whole surface of the explant in MS + 2,4-D (4 mg/l) + KIN (1 mg/l)

5: Callusing from the whole surface of the explant in MS + 2,4-D (4 mg/l) + BAP (1 mg/l)

6-7: Callus developed from stem explants in MS + IAA (4 mg/l) + KIN (1 mg/l)

8: Callus development and rooting from stem explants in MS + IAA (2 mg/l) + BAP (1 mg/l)

9: Callus development and rooting from stem explants in MS + IAA (4 mg/l) + BAP (1 mg/l)

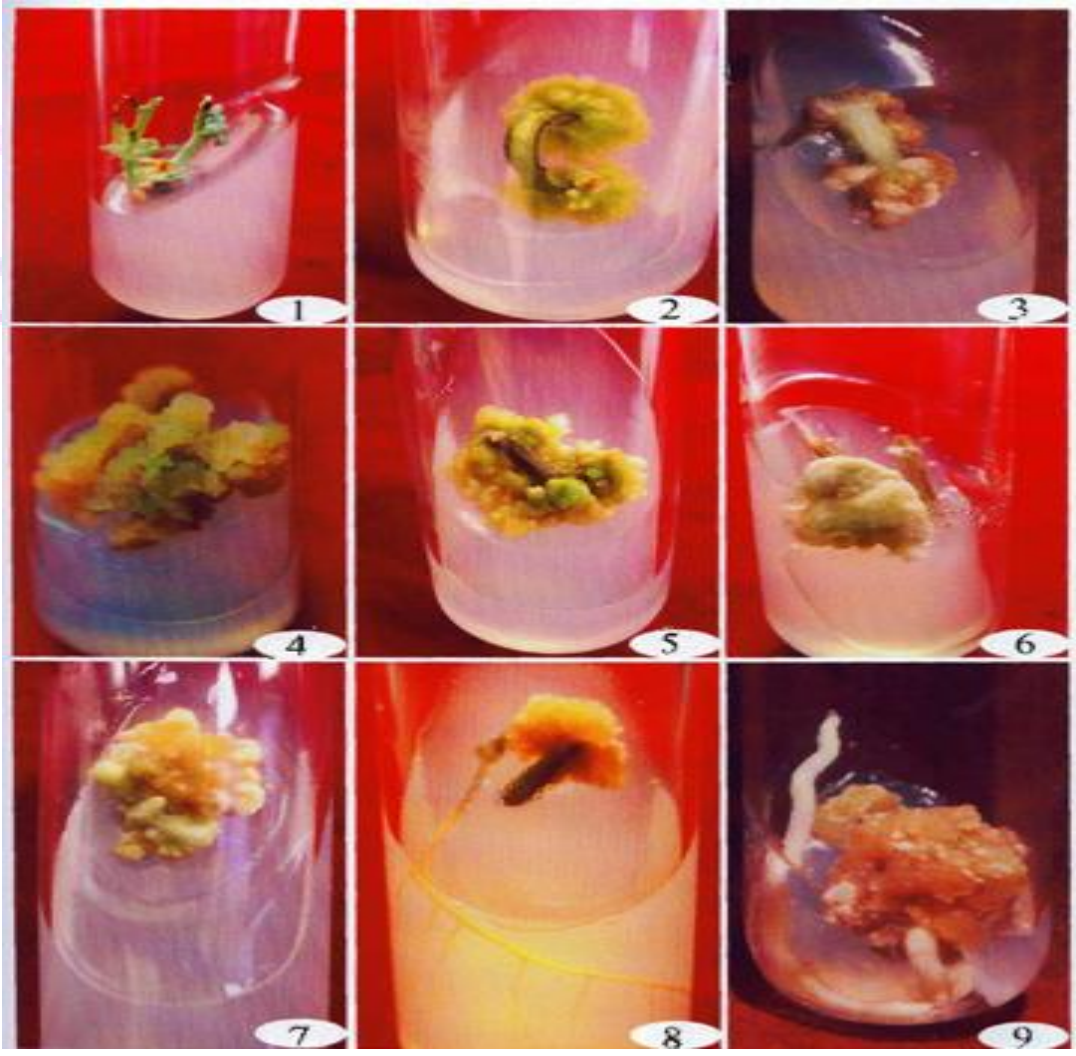


PLATE 6:

1: Swelling and initiation of callusing from leaf explants in MS + IAA (5 mg/l)

2: Swelling and initiation of callusing from leaf explants in MS + 2,4-D (5 mg/l)

3: Callusing and early browning of callus from leaf explants in MS + 2,4-D (6 mg/l)

4: Necrosis of leaf explants in MS + 2,4-D (8 mg/l)

5: Callus induced in MS + IAA (5 mg/l)

6: Callus induced in MS + IAA (4 mg/l) + KIN (1 mg/l)

7: Callus induced from leaf explants in MS + 2,4-D (4 mg/l) + KIN (1 mg/l)

8: Callus induced in MS + IAA (4 mg/l) + BAP (1 mg/l)

9: Callus along with root formation in MS + IAA (1 mg/l) + KIN (1 mg/l)

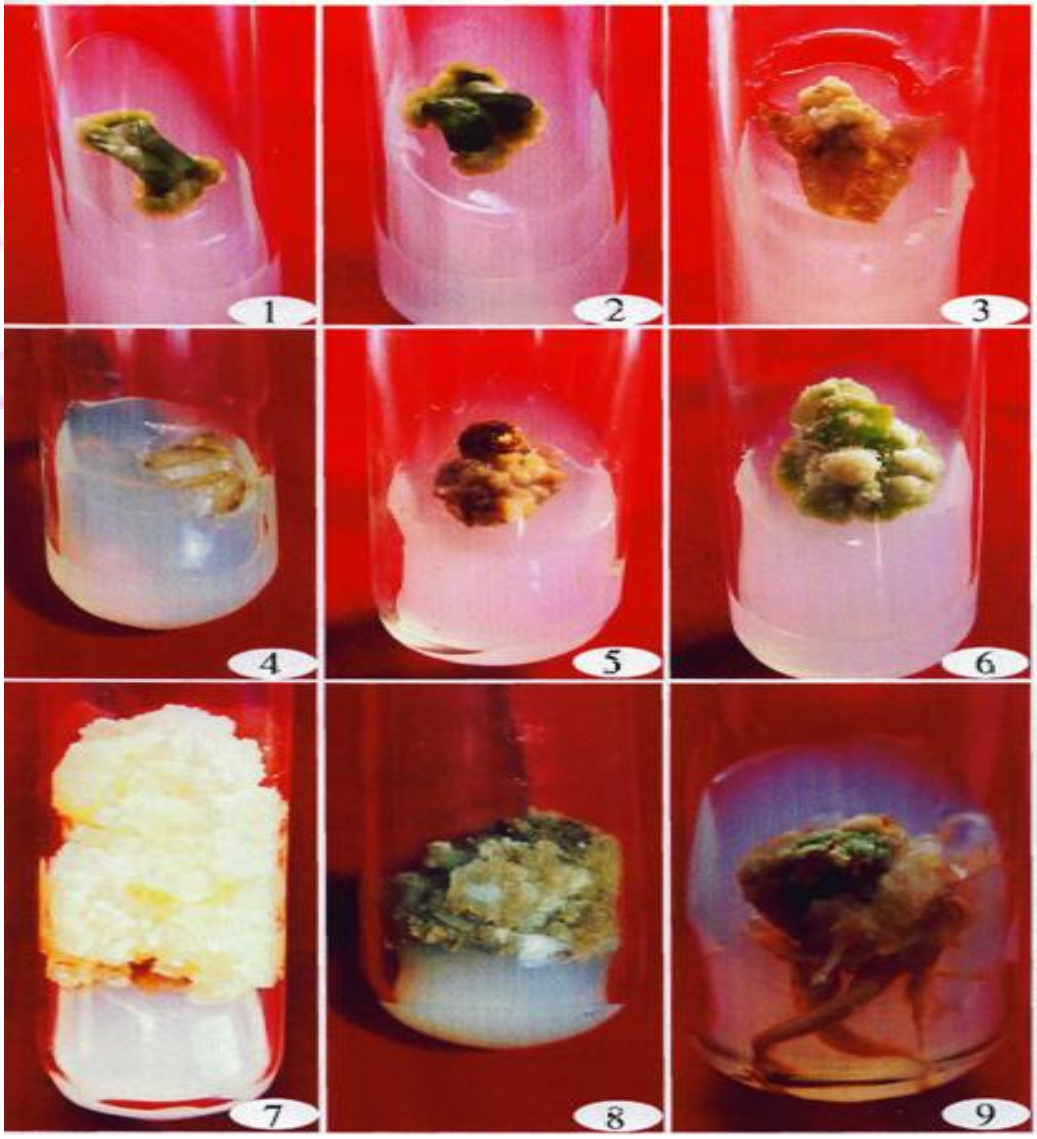


PLATE 7:

1: Stereo microscopic view of callus in induction media after 30 days

2-3: Green patches of freshly proliferated callus from leaf explants in MS + IAA (0.2 mg/l) + BAP (2 mg/l) after 60 days

4: Shoot from callus transferred from maintenance media to ½ MS + IAA (0.2 mg/l) + BAP (2 mg/l) after 15 days

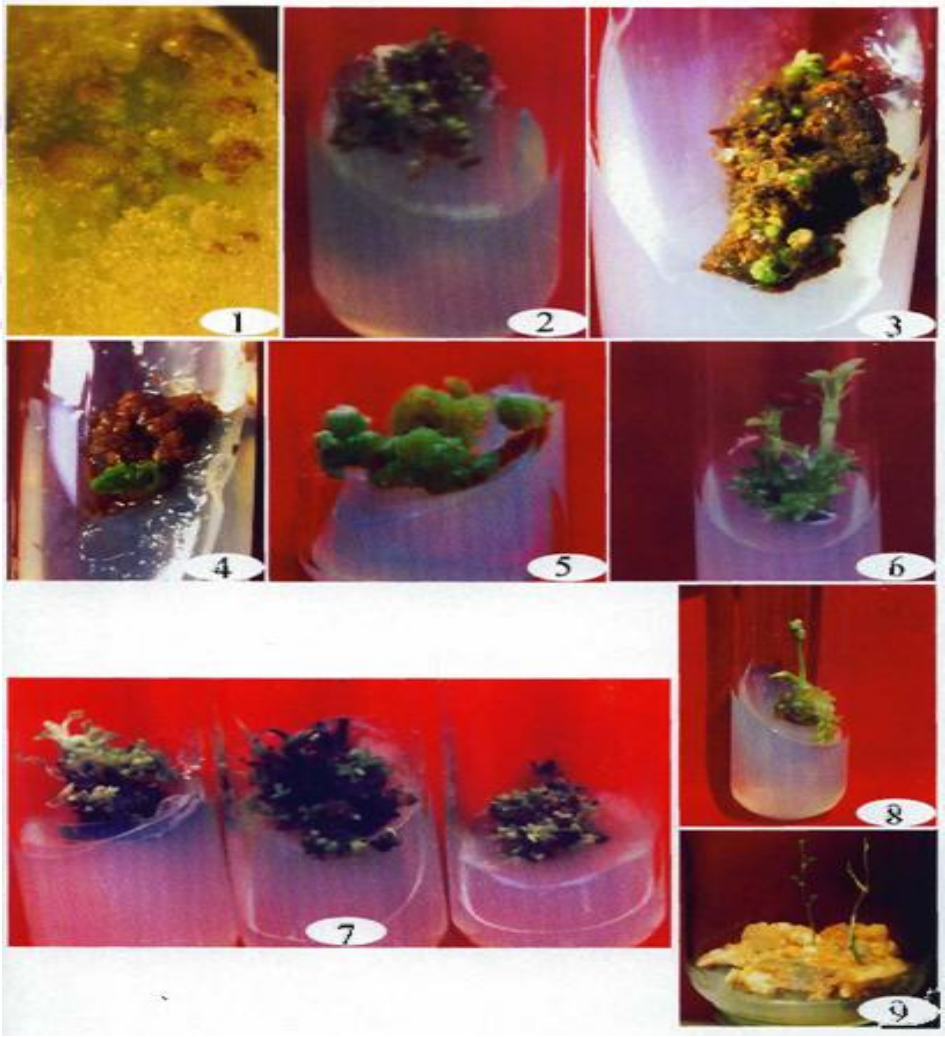
5: Recallusing when callus is transferred from induction medium to MS + IAA (0.2 mg/l) + BAP (2 mg/l) after 20 days

6: Shoots from leaf derived callus in ½ MS + IAA (0.2 mg/l) + BAP (2 mg/l) after 30 days of culture

7: Various stages of callus regeneration from leaf derived callus

8: Callus regeneration obtained from stem explants in ½ MS + IAA (0.2 mg/l) + BAP (2 mg/l) after 30 days of culture

9: Excess callusing and reduced shoot formation when callus is transferred from maintenance medium to ½ MS + IAA (3 mg/l) + BAP (2 mg/l)



V. CONCLUSION

Legumes, 'the poor man's meat,' are of incredible monetary significance, particularly in the underdeveloped nations, where they are depended vigorously for a decent diet. They fill in as a rich wellspring of proteins, starches, vitamins and minerals. Attributable to their self-fruitfulness, they have a restricted genetic base. Genetic control and in vitro culture procedures give means to making genetic inconstancy and improving crops in a moderately brief period, saving time and monetary assets.

The high potential pulse crop, Psophocarpus tetragonolobus, is as yet confined to little areas for cultivation. This consumable, highly proteinaceous legume is additionally a wellspring of the amino acids serine, aspartic acid, glycine, glutamic acid, alanine, tyrosine and all the basic amino acids with the exception of histidine and methionine. It is additionally a wellspring of numerous meds and is utilized for the treatment of malignancy, Parkinson's disease and so on and is a decent wellspring of certain synapses like dopamine and serotonin. An intensive information of the botanical biology and strategies for increasing the seed germination and tissue culture conventions are important for breeding as well as the quick proliferation of this plant. Tissue culture studies were led for their fast engendering using assorted techniques for micropropagation like axillary bud expansion, immediate and roundabout shoot recovery as well as substantial embryogenesis. The biochemical assay of organogenic/embryogenic callus was executed to discover their jobs in organogenic/embryogenic occasions. RAPD investigation and protein portrayal of the regenerants were led to assess the clonal constancy of the recovered plants. Seed germination studies were directed to increase the germination of put away seeds. The flower characters were concentrated to know the elements of different reproductive structures.

Morphological, anatomical and cytochemical studies were directed to dissect the botanical biology of the plant. An itemized portrayal of the plant could hence be readied, which offered believability to structure and metabolic status of the pistil at different developmental stages.

The flower is an ordinary leguminous, zygomorphic, pentamerous, cleistogamous flower. Stamens 10, diadelphous (9+1), adaptable, dithecous anther, nectary present. Dust grains in monads and are 3-zonocolporate. The SEM studies uncover that dust grains are oblate spheroidal in tropical view and ora is lalongate. The exine has reticulate ornamentation. FDA recoloring has uncovered that most extreme dust suitability of 90% was recorded upon the arrival of flower opening.

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