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Callus induction from Nodal and Internodal explant of *Mucunapruriens* (L.) DC. - A potent medicinal plant.

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ABSTRACT

A rapid and efficient *in vitro* multiplication of *Mucuna pruriens* (L.) DC., a valuable and potent medicinal plant was achieved by culturing internodal explants on MS medium containing different types of hormone 6-benzyl aminopurine (BAP), kinetin (Kn) and isopentenyl adenine (2-iP)NAA, 2,4 -D. Result are obtained after 21days of culture. The optimal regeneration observed at different concentration with MS mediumin various concentration and combination of 2,4-Dat an optimal concentration of 4.5 mgl^{-1} and BAP at an optimal concentration at 3.5 mgl^{-1}.

Introduction:

Mucuna pruriens (L.) DC. is a tropical legume and is commonly known as Velvet bean or Cowitch or Cowhage or Alkushi. The plant is an annual, climbing shrub with long vines that can reach over 15 m in length. When the plant is young, it is almost completely covered with fuzzy hairs, but when older, it is almost completely free of hairs. The plant is notorious for the extreme itchiness, it produces on contact, particularly with the young foliage and the seed pods. Plant cell, tissue and organ culture techniques offer an integrated approach for rapid multiplication and production of material with dependable active ingredients. In the recent years, tissue culture has emerged as a promising technique to obtain genetically pure elite populations under *in vitro* conditions.

Tissue culture technique facilitates to the mass propagation of the true to type plant with superior clones (Devi YS, Mujib A & Kundu SC 1997). This provides a fast and dependable method for production of a large number of uniform plantlets in a short time. This novel technique is much advisable and is in high demand for micro-propagation and biochemical extraction of active constituents of plants which are of high commercial and medicinal values. By employing such methods large number of plants can be produced starting from a single individual in a relatively short time. Moreover, the plant multiplication can continue throughout the year irrespective of season and the stocks of germplasm can be maintained for many years (Lynch PT 1999).

Materials and Methods

The experimental plant, *Mucuna prurians* (L.) DC. was subjected to tissue culture experiments of their vegetative parts. Experiments were designed with a view to explore the possibilities of micropropagation (Amo-Marco JB and Ibanez, MR 1998) and improving them through the use of somaclonal variants among regenerated plants. The methodology of tissue culture experiments includes (a) Preparation on culture media (b) Preparation of Explants (c) Inoculation and transfer (d) Maintenance of culture (e) Effect of seasonal variation on regeneration (f) Rooting and transfer of plantlets. The sequence of steps involved in preparing the mediums (a**)**Required quantities of agar (0.8% w/v) and sucrose (3% w/v)

were weighed out (b**)** Agar was dissolved in distilled water (in about ½ of the final volume of the medium) by heating in a water bath (c)Sucrose was dissolved in distilled water to give a saturated solution and was filtered through the Whatman's filter paper No. 1 (9.0 cm) to remove the particulate impurities, if any. The filtered solution was mixed with the dissolved agar solution. (d) Appropriate quantities of various stock solutions and growth regulators were added (e) The final volume of the medium was made up to 1 litre /required volume with distilled water. (f) After proper mixing, the pH of the medium was adjusted to 5.8 using 0.1N NaOH or 0.1N Hcl.

(g)About 20 ml of the medium was poured into the culture tube (25 X 100 mm).(h)The culture tubes were plugged with non-absorbent cotton wrapped in cheese cloth. The cotton plugs were wrapped with aluminum foils to prevent wetting during autoclaving.(i)The culture vessels were transferred to appropriate baskets and autoclaved at 121^{0}C (1.06 kg/cm^2) for 20 minutes.

(j)Slants were prepared by keeping the tubes tilted during cooling.

Results and Discussion

A method has been developed for the rapid multiplication and morphogenetic response of plants from nodal segment explants of *Mucuna pruriens*(L.) DC. on various cytokinins (BAP, Kn and 2-iP). When nodal segments were cultured on hormone free-MS medium, there was no sign of shoot formation. In case of culture of nodal segment on medium supplemented with various forms of cytokinins like BAP, Kn and 2-iP in different concentrations like 0.5, 2.5, 5.0, and 10.0 mgl^{-1} a differential response with regard to the shoot induction was obtained. Multiple shoot buds got initiated on nodal segments after 6

Basal Medium	GR (mgl^{-1})	Explants	No .of Explants Cultured	No. of respond explants cultured	? of Res-pon-se	Responses				Other Responses/ Remark
						Callus Colour	Av. wt of Callus (gm)	Root	Shoot	
MS		Node	10	---	---	---	---	---	---	---
		Internode	10	---	---	---	---	---	---	---
		2,4-D (Dichlorophenoxy Acetic Acid)								
	1.5	Node	10	06	60	W	1.83	---	---	Callus
		Internode	10	05	50	---	1.54	---	---	---
	4.5	Node	10	06	60	G	2.89	(+) root hair	(+) bud	Callusing
		Internode	10	05	50	B	2.76	(+) root	---	Hypertrophy callus
	4.5	Node	10	07	70	G	3.05	Multiple hair root	(+) of buds	Green compact callus
		Internode	10	05	50	Y	2.89	2-3 roots	(-) of buds	Well developed callus

Culture Period: 21 days W = White (+) = presenceCulture replicate: 10
G = Green (-) = AbsenceCulture Medium: MS withB = Brown (--) = No response AR = Adventitious Root Y = Yellow YB = Yellow brown

weeks of culture.

The optimal regeneration observed at different concentration with MS medium as, BAP at an optimal concentration of 3.5 mgl^{-1},2,4 -D at 4.5 mgl^{-1}, NAA at 4 mgl^{-1} and Kn at 2.5 mgl^{-1} was effective in inducing multiple shoots formation.

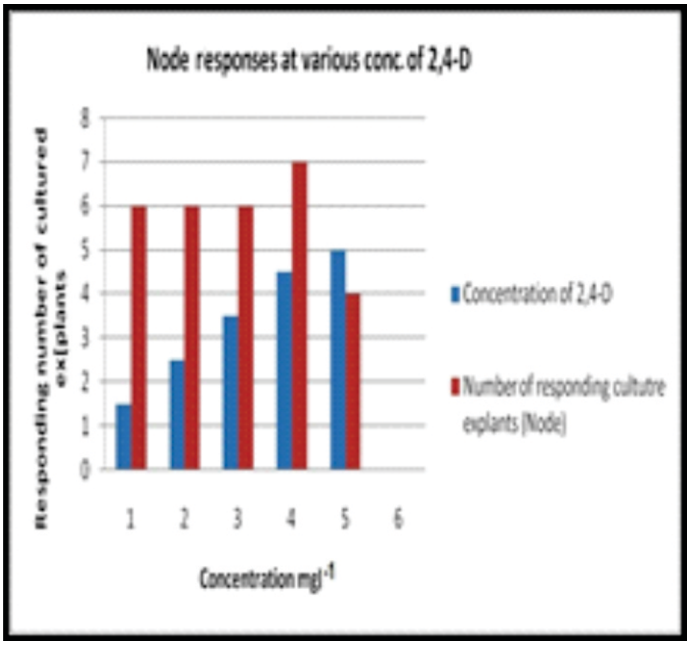
Nodal culture:

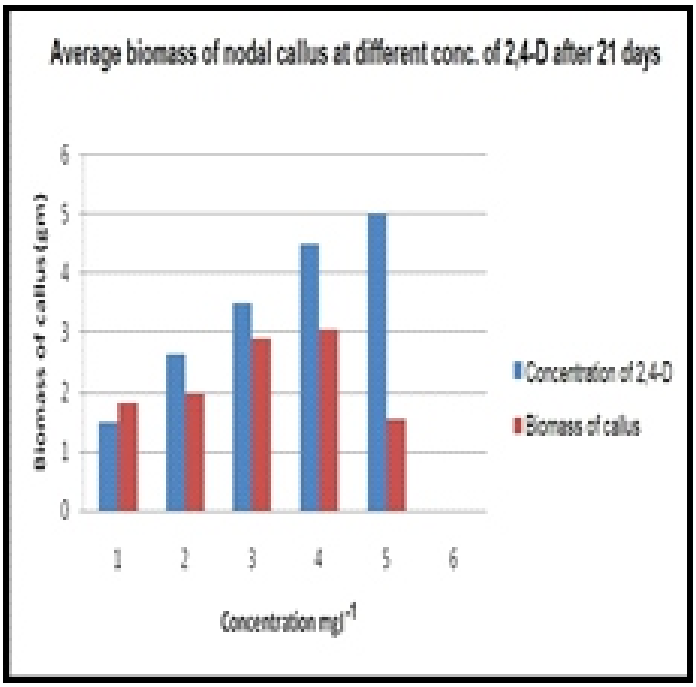
Nodal segments collected from young shoots of *M. pruriens* were sterilized and cut into small pieces of 8-10 mm. Nodal segments were acropetally placed and aseptically cultured on MS basal media supplemented with various combinations and concentrations of phytohormones.

Internodal culture:

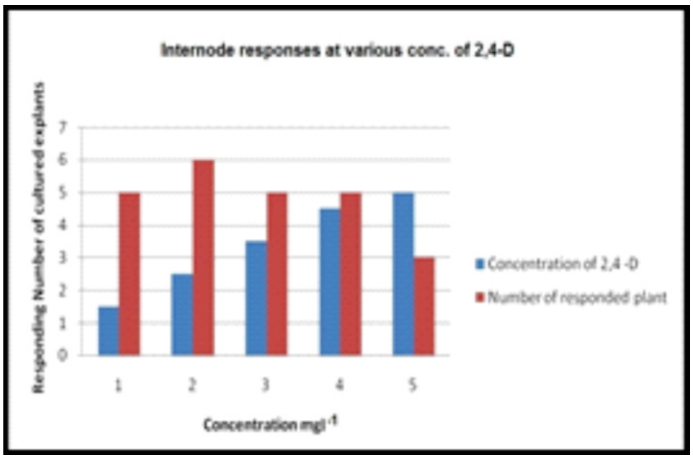
Internodal segments collected from young shoots of *M pruriens* were sterilized as and cut into small pieces of 8-10 mm. Internodal segments were placed basipetally and aseptically cultured on MS basal media supplemented with various combinations and concentrations of phytohormones.

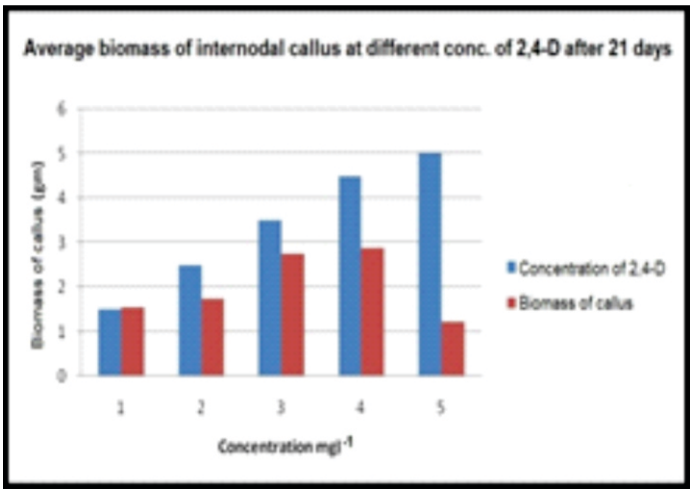
Nodal Culture Responses at Various Conc. of 2,4-D





Internodal Culture Responses at Various Conc. of 2,4-D





Effect of Cytokinins

BAP (Benzyl Amino Purine): BAP is a type of cytokinin, mainly responsible for shooting and callus differentiation in tissue culture technique. Nodes, internodes, were cultured on MS basal medium supplemented with phytohormone BAP in various combinations (1-6.5 mgl^{-1}) and the results have been presented in table 1.2

NODAL CULTURE ON BAP: In case of nodal explant, the effect of BAP (1-6.5 mgl^{-1}) on culture response was recorded on all the combination tested. There was significant change observed during the development of culturing. The callus started to form from cut ends of the explant after 7-14 days of inoculation . The callus was initially small, green and compact. There was also a sign of the formation of green buds from the callus (AnuRadha M &Pullaiah T 1999). The best result was observed at 3.5 mgl^{-1}. The nature of the callus changed from green to deep-green. However, after third week callus undergoes detoriation.

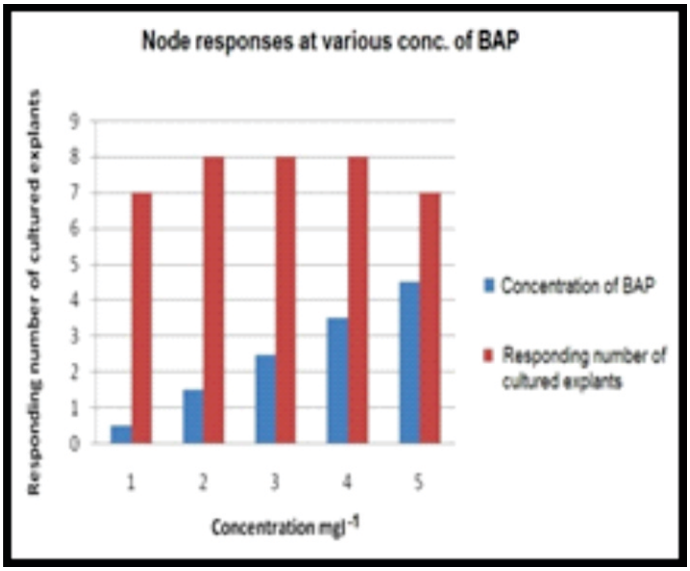
INTERNODAL CULTURE ON BAP: For intermodal culture at various concentrations(1-6.5 BAP mgl^{-1}), the morphogenic changes were observed within 10-14 days. The best result was observed at 3.5 mgl^{-1}, a yellow callus developed. The callus enlarged but there was no sign of development of roots and buds(Arellano J, Fuentes SI and Castillo-Espana P 2009). The callus changed from yellow to brown coloured compact, small and dehydrated .No rooting and shooting were observed at any concentration (Table 1.2).

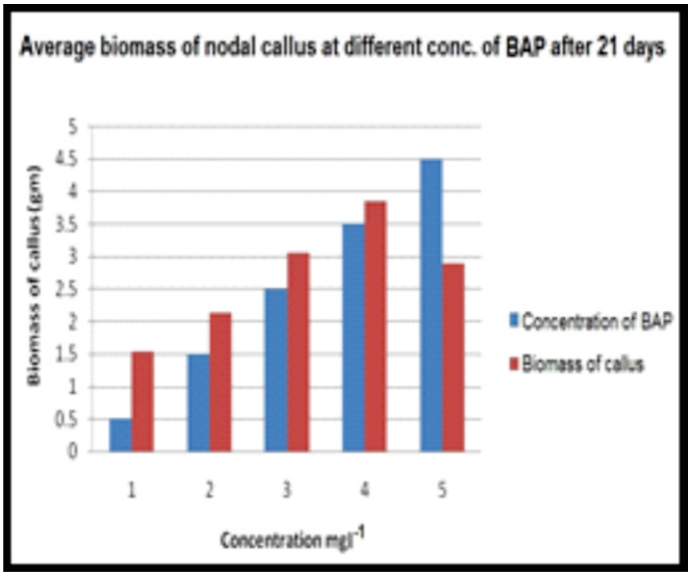
Table (1.2): ***Response of Node / Internode explants of M. pruriens on various basal media supplemented with effective combinations of MS medium with BAP:***

INTERNODAL CULTURE ON BAP: For intermodal culture

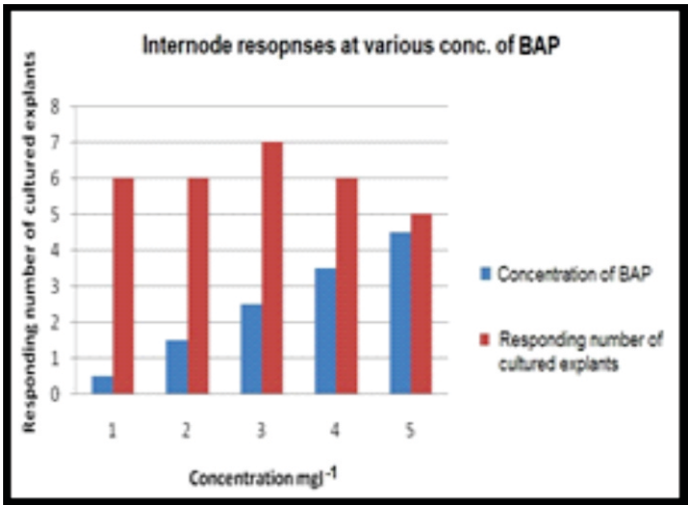
Basal Medium	GR $^{-1}$ (mgl)	Explants	No .of Explants Cultured	No. of respond explants cultured	? of Respo nse	Responses				Other Responses/ Remark
						Callus colour	Av.Wt of Callus (gm)	Root	Shoot	
MS		Node	10	---	---	---	---	---	---	---
		Internode	10	---	---	---	---	---	---	---
		BAP (Benzyl Amino Purine)								
	1.5	Node	10	08	80	G	2.13	---	---	Callus
		Internode	10	06	60	Y	1.98	---	---	---
	2.5	Node	10	08	80	G	3.05	AR	Bud dev.	Callusing
		Internode	10	07	70	B	2.76	---	Bud. dev	Hypertrophy callus
	3.5	Node	10	08	80	G	3.85	(+) Hair root	(+) bud	Green compact callus
		Internode	10	06	60	Y	3.65	---	---	Brown callus

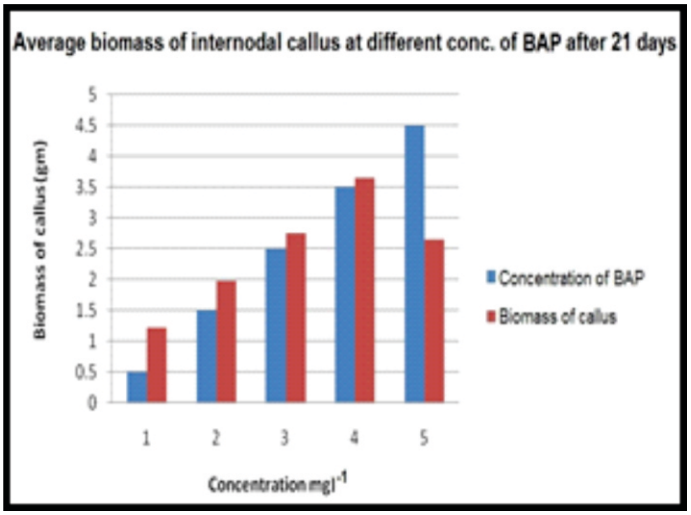
Culture Period: 21 day W = White (+) = presenceCulture replicate: 10 G = Green (-) = Absence
Nodal Culture Responses at Various Conc. of BAP





Internodal Culture Responses at Various Conc. of BAP





Internodal Culture Responses at Various Conc. of BAP

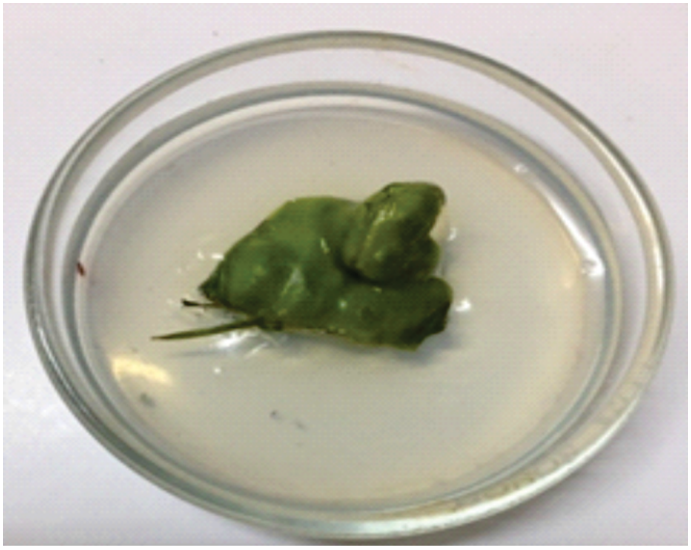


Fig: 1 Nodal culture: 2,4- D

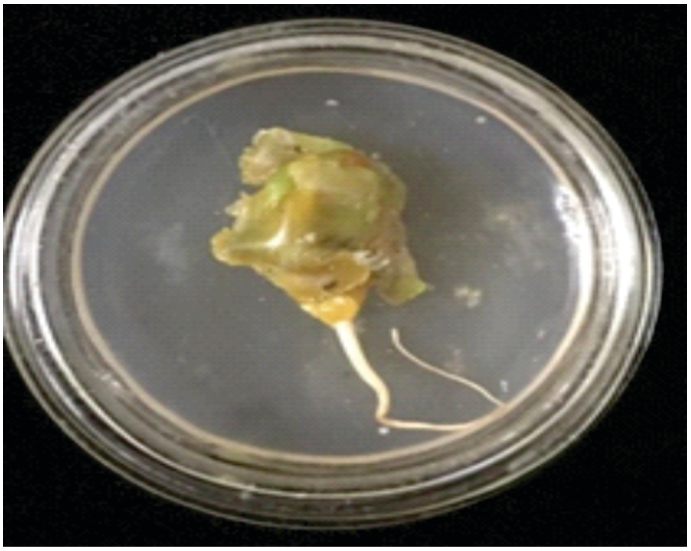


Fig: 2 Internodal culture,2,4-D Callus with profited greenish, brown root Brown coloured deformed callus

Fig: 3 Nodal Culture: BAP Degenerating callus with profited dark greenish brown root

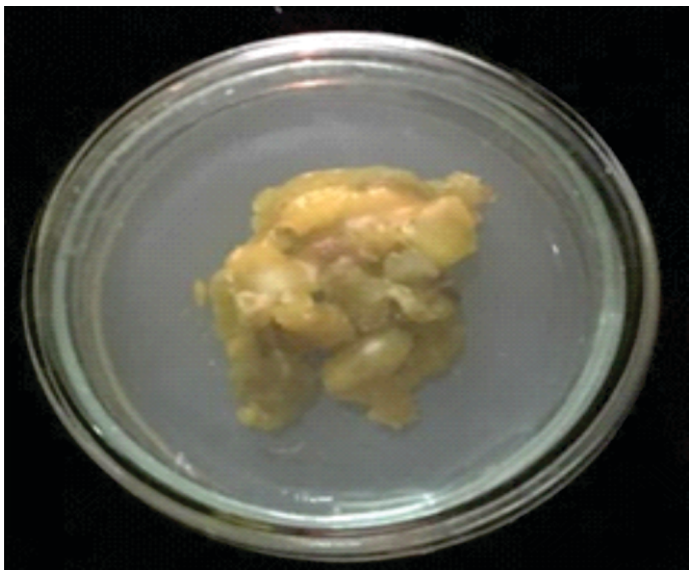


Fig: 4 Inter Nodal Culture: BAP Diffused callus with browning appearance

Results were obtained after three weeks of culture. The optimal regeneration observed at different concentration with MS medium in various concentration combination of 2,4-D at an optimal concentration of 4.5 mgl^{-1} ,BAP at an optimal concentration at 3.5mgl^{-1},With MS medium and 2,4 D showed best effective callus growth from nodal region as compared to inter nodal at concentration of 4.5 $mgl^{-1.}$

Conclusion:

Tissue culture studies of *Mucuna pruriens* (L.) DC. Syn: *Mucuna pruriata* Hook. of sub-family Faboideae of family Fabaceae (Papilionaceae) of class dicotelydonae, were carried out in the present investigation. *Mucuna pruriens* (L.) DC. commonly known as velvet bean or cowitch or cowhage is an annual climbing shrub used all over the world as a source of medicine and food. Physicians in ancient India first used the seeds of *Mucuna*in the treatment of parkinson's disease. The demand of *Mucuna* in India as well as in international drugs market increased many fold only after the discovery of the presence of L-DOPA (L-3,4-dihydrooxy phenyl alanine), an anti-parkinson's disease drug. Some reports are available on tissue culture of *Mucuna pruriens* as reported.

In this background, the present *in vitro* studies of this plant would probably form the basis for the first report. In view of the above facts, the morphogenetic studies were carried out on *Mucuna pruriens* (L.) DC. under controlled conditions of temperature, light and humidity. Different plant parts *viz*. node and intere node collected from *in vivo* grown *Mucuna* plant in the campus of Gopeshwar College, Hathwa, Distt. Gopalganj, Bihar were used as explants. During the present investigation, MS media with different combination and concentrations of phytohormones showed significant morphogenetic changes and development during organogenesis. Organ culture of node & internode has been established (Table 1.1 &1.2). Node proved excellent systems for direct shoot regeneration / plantlet formation as compared to inter nodal culture. Induction of callus and direct multiplication of shoots are greatly influenced by the appropriate hormonal balance and constitution of nutrient media.

Auxins (2,4-D) in combination with MS media found effective for the callus differentiation and development rather than shooting and budding. The best result obtained as 4.5 mgl^{-1} conc. of 2,4-D . A green compact well developed callus was clearly visible in case of node and shoot-tip. However, there was also a sign of development of adventitious roots and buds. The average callus masses of explants were 3.75 gm. This investigation revealed that auxins are mainly responsible for callus differentiation from

different explants(Arellano J, Fuentes SI and Castillo-Espana P 2009).Cytokinin (BAP) alone has been found effective. The formation of shoots / multiple shoots was best achieved on 3.5 mgl^{-1}. During culture of various explants, the BAP showed responsive growth at lower concentration(Anis M and Faisal M 2005). The significant changes were observed at 3.5 mgl^{-1} conc. of BAP. The node and shoot-tip showed the formation of more prominent and compact green callus development with multiple hairy root and buds during culture period of 21 days. In general, formation of shoots / multiple shoots was more frequent in culture. The highest percentage of shoot proliferation was found in nodal culture.

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