
**CALLUS INDUCTION AND MULTIPLE SHOOT REGENERATION FROM
COTYLEDONARY NODES IN CLUSTERBEAN [*CYAMOPSIS*
TETRAGONOLOBA L.TAUB]**

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ABSTRACT

Clusterbean (*Cyamopsis Tetragonoloba* L.Taub) is a crop having unique ability to grow under harsh climate. The crop has enormous industrial utility due to the presence of gum (galactomannan) in its endosperm. Owing to its unique biochemical properties, clusterbean gum is used in wide range of products varying from ice-creams to explosives. Its utility as a forage, green manure, vegetable and feed needs no emphasis. There are wide scopes for wider hybridization and improvement through genetic engineering particularly for characters like galactomannan that are highly influenced by the environment making the precise selection of genotypes difficult. Tissue culture being the prerequisite for such interventions, the effect of various growth regulators and their combinations on different explants, namely auxiliary leaf, cotyledonary nodes, shoot tip and hypocotyls was studied in two diverse varieties viz; GG 1 & GG 2 at the Centre of Excellence for Research on Pulses, S. D. Agricultural University, Sardarkrushinagar, Gujarat, India. Fifteen days old seedlings grown aseptically on Murashige and Skoog's culture medium were used as explants. *In vitro* grown seedlings were found most efficient for inducing multiple shoots. Callus induction and regeneration from callus shoot morphogenesis was developed. It was established that Murashige and Skoog's culture medium containing 6-benzylaminopurine (5 μ M) in combination with 2, 4 -dichlorophenoxyacetic acid (10 μ M) with cotyledonary explants is the most suitable for induction of green and friable morphogenic callus, while 6-benzylaminopurine (10 μ M) in combination with Kinetin (10 μ M) and Gibberellin (5 μ M) cotyledonary node explants exhibited the highest frequency of multiple shoot induction.

KEY WORDS: Callus induction, Clusterbean (*Cyamopsis tetragonoloba*), Gibberellin, Regeneration, BAP – 6-benzylaminopurine, 2,4-D – 2,4-dichlorophenoxyacetic acid, Kin – kinetin,

INTRODUCTION

Clusterbean (*Cyamopsis tetragonoloba* L. Taub), popularly known as guar, has attained the status of an important industrial crop due to the presence of gum (galactomannan) in its seed. Consequent upon unique biochemical properties of galactomannan extracted from guar, it is used in wide range of industrial products ranging from ice-creams to explosives. The galactomannan contents and quality are highly influenced by environment that makes it difficult for the industry to maintain uniformity of products prepared from it.

The biotechnological approaches have enormous potentials for improvement of galactomannan for which efficient regeneration system is the prerequisite. Though Prem *et al.* [2005] have reported potency of cotyledonary nodes for direct shoot regeneration, yet an efficient protocol for *in vitro* plant regeneration from callus could be more useful for *in vitro* manipulations like hybrid embryo rescue, *in vitro* mutagenesis and cell line screening. With this informational gap, the study was conducted to explore regenerative ability, appropriate media and explants of clusterbean [*Cyamopsis tetragonoloba* (L.) Taub].

MATERIALS AND METHODS

Plant material used: - Two cultivars namely GG 1, GG 2 of clusterbean were used for shoot generation experiments. Genetically pure seeds of both the varieties were obtained from the Centre of Excellence for Research on Pulses, S. D. Agricultural University, Sardarkrushinagar, Gujarat, India.

Sterilization of seeds: - Seeds were soaked in tap running water for 15 min, rinsed in 70% alcohol for 10 min and surface sterilized with 0.1% aqueous mercuric chloride solution for 15 min followed by rinsing five times with sterile distilled water. Five seeds of each variety were aseptically germinated by removing its seed coat in conical flask (100ml) containing 30 ml of MS medium under 16h photoperiod condition.

Callus induction: - Fifteen days old seedlings grown aseptically on Murashige and Skoog (1962) culture medium were used as a source of auxiliary leaf, shoot tip, cotyledonary node and hypocotyls explants. Transverse sections of each types of explants measuring approximately 2mm in length were cultured in culture tubes (25 x150 mm) containing 20 ml culture medium. Explants were cultured on MS medium containing 3 % sucrose, 0.8 % extra pure agar powder and 0.044% calcium chloride supplemented with 2, 4 –dichlorophenoxyacetic acid (10 μ M) and in combination with 6-benzylaminopurine (5 μ M) These cultures were incubated at 26⁰ C under a 16h photoperiod.

Shoot initiation:- Fifteen days old callus were used as a source for shoot initiation. These callus were transferred in culture tubes (25X150 mm) containing 20 ml MS medium containing,3 % sucrose, 0.8% extra pure agar powder and 0.044% calcium chloride supplemented with various growth regulators viz; 6-benzylaminopurine (10 μ M) in combination with Kinetin (10 μ M) and Gibberellin (5 μ M) . These cultures were incubated at 26⁰ C under a 16h photoperiod.

RESULTS AND DISCUSSION

Effect of explants on callus induction in clusterbean

With the objective of developing an efficient protocol for regeneration from callus in clusterbean, two diverse genotypes namely GG 1 and GG 2 were used for callus induction and regeneration experiments. The seeds were surface sterilized, aseptically germinated and grown. Fifteen days old seedlings were used as a source of explants from auxiliary leaf, cotyledonary nodes, shoot tip and hypocotyls. These explants were obtained from surface sterilized seeds that had been aseptically germinated by removing its seed coat.

Five concentrations (5, 10, 15, 20 and 25 μ M) of 2, 4-D, and (2, 5, 7, 10, 15 μ M) BAP were tested for callus induction and its subsequent growth from various explants. Medium lacking in growth regulators was used as control. Callus initiation was observed from the cut ends of the cotyledon, cotyledonary node, hypocotyls, and shoot tip within 10-15 days of incubation, while no callusing was observed on control media. Among the various auxins and cytokinins studied, 2, 4-D (10 μ M) and BAP (5 μ M) was observed to be highly potent for callus induction irrespective of genotype and cotyledonary node used as explants. In contrast to our findings, Ramulu and Rao [1993a] have reported hypocotyls to be the

most congenial explants for callus induction with low levels of auxins IAA, BA, NAA and kinetin. Bansal *et al.* [1994] have reported high concentration of 2, 4-D (22.6 and 45.2 μ M) to be the most congenial for callus induction in hypocotyls and cotyledons explants in clusterbean.

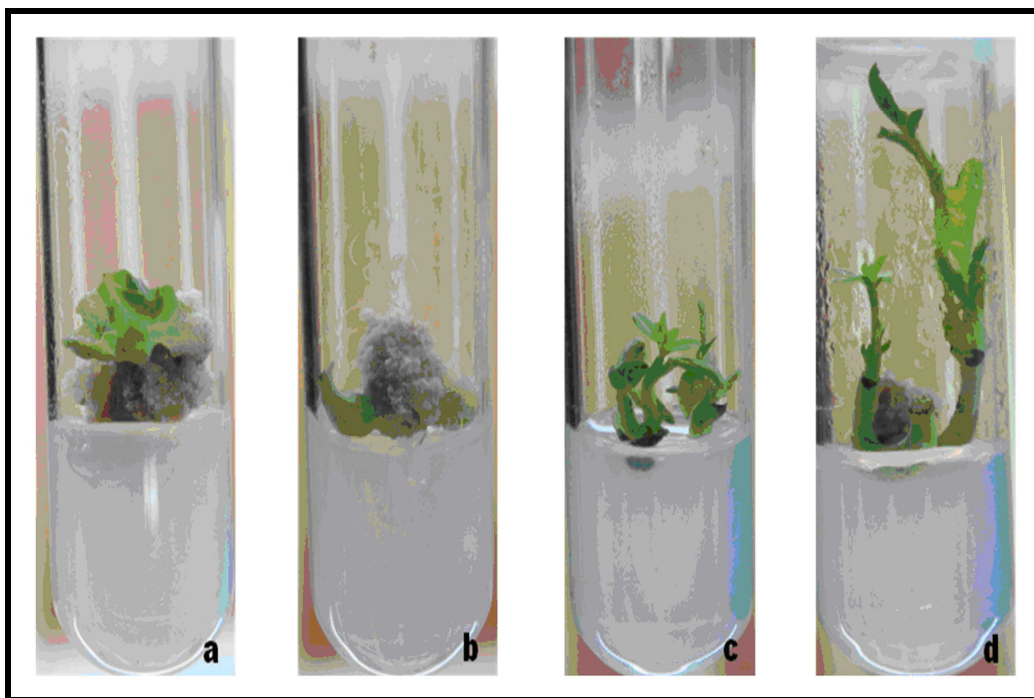
The cotyledonary node and cotyledon were found to be the most appropriate explants amenable to callus induction with the highest pooled average callus induction frequency with 10 μ M 2-4-D and 5 μ M BAP for the cotyledons based on a total of 200 explants. The hypocotyls was observed to be least responsive to callus induction, though the highest pooled callus induction frequency was evinced with 10 μ M 2-4-D and BAP (5 μ M) in hypocotyls explant. The callus induced on media containing 2, 4-D (10 μ M) and BAP (5 μ M) from cotyledonary node was found to be most superior in terms of its morphology and growth rate than any other combination irrespective of genotype studied. The callus obtained was friable/granular and green in color, composed of large vacuolated cells. After removal of the mother tissue on the first subculture, could be maintained over a year (20 subcultures each spanning 3 weeks) without any morphological changes with approximate 2-fold increase in fresh weight with each subculture. This callus was subsequently used for shoot regeneration on media containing both BAP and Kinetin combination with Gibberellins.

Effect of Benzyl amino purine (BAP), Kinetin and Gibberellin on shoot induction

Direct shoot morphogenesis was observed from cotyledons and cotyledonary node explants after 3 weeks of culture on BAP, kinetin. This is reported by Perm *et al* (2003). But gibberellin was also supplemented to BAP and Kinetin. However, the explants cultured on these media developed callus typically from the cotyledonary node and showed shoot morphogenesis through callusing. The explants cultured on 2, 4 D and BAP developed green friable callus at the cut ends. Ramulu and Rao [1993b] have also reported kinetin to be an essential cytokinin for callus induction and maintenance in clusterbean. The effect of BAP (10 μ M), kinetin (10 μ M) and Gibberellin (5 μ M) was studied on cotyledon and cotyledonary node explants in both the genotypes. BAP shows plant growth and kinetin that promotes cell division, whether Gibberellin helps in regulate growth and influence various developmental processes including stem elongation. The cotyledonary nodes cultured on this combination

showed higher shoot morphogenesis capability as compared to the other explants.

The success in achieving shoot morphogenesis from callusing from cotyledonary node explants is in consonance to various reports on regeneration in other legumes. Similar shoot morphogenesis from cotyledonary nodes has been reported in *Cajanus cajan* (Ramulu and Rao 1993b), *Phaseolus vulgaris* (Prakash et al., 1994), *Vigna radiate* (McClean and Grfton, 1989) and *Cicer arietinum* (Gulati and Jaiwal, 1994). In all these studies, the presence of BAP, Kinetin and Gibberellin in the regeneration medium was mandatory for shoot morphogenesis and the number of shoots emerging per explant was more. Most of these shoots were reported to elongate normally on further subculture. However, the present results palpably evinced that though BAP and Kinetin as a cytokinin had explicit influence on shoot morphogenesis, it alone was not sufficient to sustain normal growth of plantlets but gibberellins supplementation was also necessary. Thus regeneration from callus can be had by using MS medium supplemented with growth regulator BAP, kinetin and gibberellin.



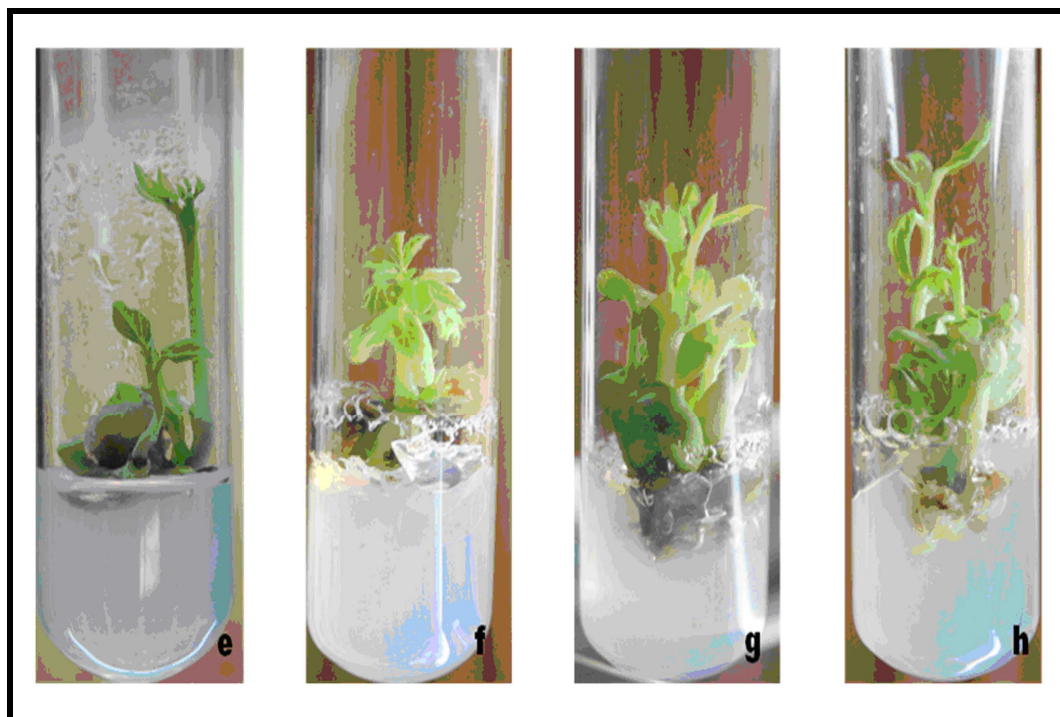


Fig. 1: Callus initiation from cotyledonary node explants and shooting, plant regeneration from callus of (*Cyamopsis tetragonoloba* L. Taub). (a). Callus formation from cotyledonary node explants (b) Shoot initiation from callus (c & d) Shoot elongation from callusing and (e,f g & h) Multiple shoots development

REFERENCES

- Bansal Y. K., Chibbar T., Bansal S. and Singh M. 1994. Plant regeneration from hypocotyl and cotyledon explants of clusterbean (*Cyamopsis tetragonoloba* L.). *J. Physiol. Res.* 7:57– 60.
- Gulati A. and Jaiwal P. K. 1994. Plant regeneration from cotyledonary node explants of mungbean (*Vigna radiata* L. Wilczek). *Plant Cell Rep.* 13: 523–527.
- McClellan P. and Grafton K. F. 1989. Regeneration of dry bean (*Phaseolus vulgaris* L.) via organogenesis. *Plant Sci.* 60: 117–122.
- Murashige T. and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15: 473–497.

- Prakash N. S., Pental D. and Sarin N. B. 1994. Regeneration of pigeonpea (*Cajanus cajan*) from cotyledonary node via multiple shoot formation. *Plant Cell Rep.* 13: 623– 627.
- Prem D., Singh S., Gupta P.P, Singh J. and Kadyan S.P.S. 2005. Callus induction and *de novo* regeneration from callus in Guar (*Cyamopsis tetragonoloba* L).*Plant Cell Tissue and Organ Culture* 80: 209-214.
- Ramulu C. A. and Rao D. 1993a. In vitro effect of phytohormones on tissue cultures of cluster bean (*Cyamopsis tetragonoloba* L. Taub). *J. Physiol. Res.* 20: 7– 9.
- Ramulu C. A. and Rao D. 1993b. Induced pesticide resistant lines in cluster bean through tissue culture. *J. Physiol. Res.* 6: 71 – 72; 1993b.