Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

AN EFFECTIVE METHOD FOR SHOOT REGENERATION AND CALLUS INDUCTION OF CANAVALIA GLADIATA (JACQ.) DC. AN ECONOMICALLY IMPORTANT LEGUME.

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ABSTRACT

A simple and efficient protocol for in vitro shoot and callus induction was established from three different explants of *Canavalia gladiata* (Jacq.) DC. an economically important legume. The explants viz., shoot buds, nodes, internodes were cultured in MS medium supplemented with various concentration and combination of (PGRs) Plant growth regulators 6-benzyl amino purine (BAP) (0.5mg/L – 1.5mg/L) and Indole acetic acid (IAA) (0.5mg/L – 1.5mg/L) for shoot induction. The best shoot proliferation was observed at the concentration of 1.0mg/L of BAP and 1.0 mg/L of IAA from only nodal explants of *Canavalia gladiata* after 4 weeks of culture. Combination of PGRs (BAP and IAA) was found to be more effective for shoot induction of nodal explants of *Canavalia gladiata*. For callus regeneration the nodal explants of *Canavalia gladiata* were used and the MS medium was supplemented with various concentration of α-naphthalene acetic acid (NAA) (0.5mg/L – 3.0mg/L). Highest callus induction was observed at the concentration of 1.5mg/L of NAA, and in this concentration highly desirable creamy white friable callus was observed.

KEYWORDS : Micro propagation, *In vitro* regeneration, Callus induction, Forisome, Plant growth regulators, 6-benzyl amino purine, indole acetic acid, α-naphthalene acetic acid.

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INTRODUCTION

Leguminous plants of Fabaceae family are economically valuable as food, fodder, fertilizer, bioactive compounds and pharmaceuticals [1,2] . Nickell initiated studies on *in vitro* culture of legumes for the first time and thereafter tissue culture techniques have been employed to propagate many legumes[3]. Few investigators reported that *in vitro* regeneration of legumes was made possible, but at low frequency[4,5,6]. Although regeneration of legumes cannot be achieved by tissue culture, there is plenty of scope to induce callus, shoot buds, roots of legume explants to stimulate and extract most precious metabolites[7]. The genera Canavalia consist of two most important species namely jack bean (*Canavalia ensiformis*) and sword bean (*Canavalia gladiata*) which are exploited totally as food sources Several research findings confirmed that *Canavalia gladiata* are known for several valuable phytochemicals and toxins such as anticancer agent, trigonelline, cytotoxic amino acid canavanine, antiviral lectin and concanavalin A [8,9,10,11.] *Canavalia gladiata*, a leguminous plant of Fabaceae family consist of forisome (tailed) a phloem specific protein which play an important role in plant defense against injury. Forisomes are ATP independent protein which contract reversibly when induced by the changes in the concentration of divalent metals or pH was reported[12]. *Canavalia gladiata* is a plant of choice for this research, which restrains tailed forisome and has a greater advantage[13]. Owing to this enviable properties, the forisome protein of legumes provides a constructive paradigm in the field of nanotechnology, biomedical engineering and biotechnology, etc., reported that the kind of forisome based biomimetic smart materials from legume plants a novel, non – living, ATP – independent biological material can be used for advanced actuating and sensing[14]. The importance and various applications of this phloem specific protein were reported [15] Tissue culture studies on *Canavalia* spp. have been reported by only few investigators, Hwang reported that leaf callus was established from *Canavalia lineate*, and the induced callus gave rise to chlorophyll in the presence of BAP and IAA on exposure to continuous light [16]. Regeneration of plantlets from hypocytle and shoot meristem of sword bean was the first report on the tissue culture of *Canavalia gladiata*[17]. So far, studies on in vitro regeneration of *Canavalia gladiata* have not been attempted to obtain forisome protein from plantlets. Therefore, the current study was aimed to develop an efficient protocol for microprogation and callus regeneration of *Canavalia gladiata*.

MATERIALS AND METHODS

Canavalia gladiata seeds were collected from Kannur district Kerala, after collection the seeds were dried in sunlight for 24 hrs, and stored in refrigerator. The collected seeds of *Canavalia gladiata* were excised and washed thoroughly in running tap water. The seeds were disinfected by rinsing it in 80% of ethanol and it was surface sterilized by using 0.1 % (w/v) of $HgCl_2$ for 2 minutes. The seeds were washed twice with distilled water, and then it was inoculated and incubated at 25±2° C under 16 hr light and 8 hr dark condition. The young germinated seedlings were used as explants for shoot and callus induction. Young shoot buds, nodes, internodes were taken from the germinated old seedlings and it was disinfected with 0.1%(w/v) mercuric chloride for 2 minutes and 0.1%(w/v) sodium lauryl sulphate for 3 minutes, then the explants were washed thrice using distilled water. The seeds were then excised into desired size aseptically.

(i) Transfer of explants and incubation

The excised explants were implanted in MS medium[19] (Hi-Media Laboratories, India) with analytical grade ingredients (macronutrients, micronutrients, agar, sucrose). The medium was supplemented with various concentrations of plant growth regulators (PGRs) such as 6-benzyl amino purine (BAP), and Indole acetic acid (IAA) (Table1). Stock solutions were prepared and preserved at 4°C in a refrigerator until use. The basal medium was prepared using stock solution and the medium pH was set to 5.7±0.1. The required quantities of PGRs and agar were transferred, melted and dispensed in to a culture tube and plugged with non-absorbent cotton wrapped

with cheese cloth. The media were steam sterilized at 121ºC for 15 min. The explants were transferred aseptically to culture tubes using laminar air flow chamber. After successful inoculation, the tubes were incubated at the temperature of 25±2°C.

(ii) Callus Induction

Murashige & Skoog (MS) medium were prepared and the pH was set to 5.7 ±0.1. Plant growth regulator NAA alone were incorporated at various concentrations (Table.2) for the induction of callus from nodal explants of *Canavalia gladiata*. Later the medium was sterilized at 121°C for 15 minutes. After the explants were successfully transferred into the culture tubes and were incubated at 25±2°C under 16 hr photo period. At a regular interval of 15 days each the explants were subjected to two sub culture in MS medium using the same concentration of PGRs.

RESULTS AND DISCUSSION

1. Shoot Multiplication

The present investigation was carried out to explore the regeneration potential of *Canavalia gladiata* by using different combination of plant growth regulators. Young shoot buds, nodes and internodes were used for in vitro culturing. Among those, nodes responded positively. Various concentration and combinations of PGRs, BAP (0.5mg/L – 1.5mg/L) and IAA (0.5mg/L - 1.5mg/L) were used for shoot induction of nodal explants of Canavalia *gladiata.* Regarding shoot multiplication, among various concentration and combinations of PGRs tried, BAP (1.0mg/L) and IAA (1.0mg/L) (Table 1) proved to be the most effective PGR combinations for promoting shoot multiplication of nodal explants of *Canavalia gladiata* within 4 weeks of culture. Bajaj and Dhanju stated that the usefulness of shoot tips and nodal explants were preferred to propagate large numbers of genetically identical clones[18]. Nodal explants were used to get higher rates of shoot multiplication of several plants[19,20]. Shoot bud induction in germinated seed cotyledon was established from explants of *Canavalia rosea* and was reported by Cunha and Sridhar[21]. At a higher concentration of BAP and IAA in the medium did not show any positive effect on shoot multiplication, but instead the growth of the shoot was stunted. Shoot proliferation was poorer, when the media consists of lower concentration of BAP and IAA. Similar results were obtained in *Canavalia virosa*[22], *Vigna radiata*[23]. In the present study, the nodal explants of *Canavalia gladiata* fortified with the equal concentration of BAP (1.0mg/L) and (1.0mg/L) of IAA in the media fastened the shoot elongation (fig 1). So far, in vitro micropropagation of *Canavalia gladiata* using nodal explants have not been attempted. Currently we have evolved an efficient protocol to micropropagate the economically important legume *Canavalia gladiata.* The results which we obtained in this research will make the propagation of this species much easier.

Table 1

Effect of cytokinin and auxin on shoot multiplication from nodal explants of Canavalia gladiata after 4 weeks of culture.

PGR (mg/L)		Number of explants	Observations (Number of explants formed shoot buds)	Shoot induction (%)
BAP	IAA			
0	0	10	NR	0
1.0	0	10	01	10
2.0	0	10	02	20
0.5	0.5	10	03	30
1.0	1.0	10	07	70
1.5	1.5	10	05	50

NR, No Response

Micropropagation of Canavalia gladiate

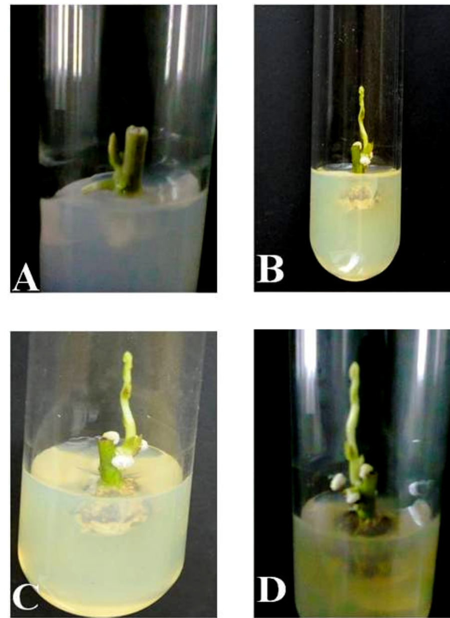

Figure 1
(A-D) – Growth of nodal explants in MS medium with 1mg/L BAP and 1 mg/L of IAA

2. Callus Induction

In the present investigation, the nodal explants of *Canavalia gladiata* were used for callus induction. MS medium were supplemented with various concentration of NAA alone (0.5mg/L – 1.5mg/L) to regenerate callus from the nodal explants of *Canavalia gladiata.* (Table 2). Highest callus induction rate 80% was observed when the medium incorporated with NAA at the concentration of (1.5mg/L). In this concentration highly desirable creamy white friable callus was observed after 4 weeks of culture.(fig 2). Similar results were also observed by Farzana[24], Nurazah has also reported that the NAA alone enhance the callus induction of *Cananga odorata* explants within 4 weeks of culture[25]. Only few investigations were observed on callus induction of *Canavalia spp.* Leaf callus was successfully established from *Canavalia lineta,* and the induced callus gave rise to chlorophyll on the exposure to continuous light[26, 28]. Plantlets were obtained from leaf callus of *Canavalia gladiata* was reported by Ozaki[18]. A cotyledon of Canavalia cathartica, and Canavalia rosea were used for callus regeneration and was established by Cunha and Sridhar[22]. Tissue culture is an excellent tool to exploit and acquire various kinds of metabolites from plantlets. It is also used to study the biosynthesis of various compounds like Concanavalin A (Con A)[7]. Con A like lectin from *Canavalia gladiata* were detected in large quantities in tissues of cotyledons and embryos was investigated by Ghosh *et al*[9]*.* Callus regenerated from *Canavalia virosa* contain lectin was reported by Jayavardhanan *et al*[14]*.* Ramirez *et al* derived Canavanine from the callus of *Canavalia ensiformis* between 5 to 10 days. Accordingly, the present study was intended to evolve an effective protocol to regenerate the callus from the nodal explants of *Canavalia gladiata*[10]*.* The research findings which we made in this work will open new vistas that could facilitate production and extraction of protein forisome from the plantlets in large scale. As a first step, it is necessary to investigate the presence of forisome protein from various tissues, and regenerated calli were derived from *Canavalia gladiata* to create varieties of biomimetic smart materials[29].

Table 2
Callus induction in nodal explants of Canavalia gladiata in MS medium supplemented with NAA after 4 weeks of culture.

PGR (mg/L) NAA	Number of explants	Observations (Number of explants formed callus)	Callus responded (%)
0	10	NR	0
0.5	10	3	30
1.0	10	7	70
1.5	10	8	80
2.0	10	5	50
2.5	10	2	20
3.0	10	1	10

NR, No Response

Callus induction in Canavalia gladiate

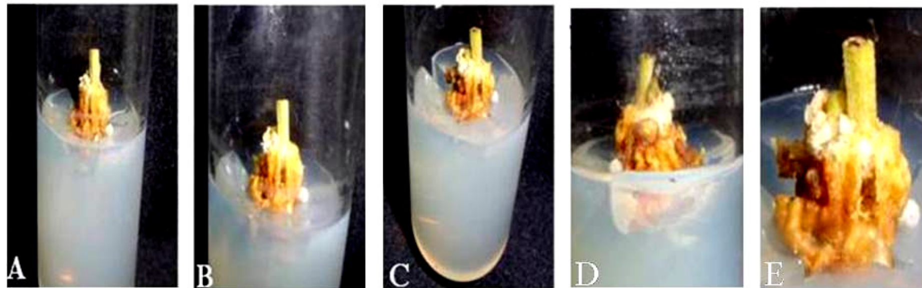

Figure 2
(A-E) – Callus induction in nodal explants of Canavalia gladiata in MS medium with 1.5mg/L of NAA. (E) – white friable callus.

CONCLUSION

In conclusion, an efficient and reproducible protocol has been developed for in vitro propagation of *Canavalia gladiata.* The significance of this protocol is not only for regenerating this legume species but also to obtain the protein forisome from plantlets in large scale for industrial utilization.

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