

## Micropropagation and Anatomical Comparison of *In Vivo* and *In Vitro* Developed Shoot and Root in *Cassia auriculata* L. - A Medicinally Important Plant

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### ABSTRACT

Micropropagation and histological studies of *in vitro* developed organs were made in *Cassia auriculata* L. Explants containing cotyledonary node along with cotyledonary leaves and portion of hypocotyl were cultured on Murashige and Skoog (MS) media supplemented with different growth regulators in varying concentrations. Maximum multiple shoots were observed in MS medium supplemented with 6-benzyladenine (BA) at a concentration level of 3.0 mg l<sup>-1</sup> along with indole-3-acetic acid (IAA) (1.0 mg l<sup>-1</sup>); MS + BA (3.0 mg l<sup>-1</sup>) + naphthaleneacetic acid (NAA) (1.0 mg l<sup>-1</sup>); MS + kinetin (Kn) (2.0 mg l<sup>-1</sup>) + BA (2.0 mg l<sup>-1</sup>); and MS with Kn (3.0 mg l<sup>-1</sup>) and BA (3.0 mg l<sup>-1</sup>). Rooting was seen from the cut end of the hypocotyls in MS medium supplemented with IBA (1.0 mg l<sup>-1</sup>) or NAA (1.0 mg l<sup>-1</sup>). Moderate green to brown, hard and nodulated callus was observed originating from cotyledonary leaf margins in MS medium supplemented with BA (3.0 mg l<sup>-1</sup>) + 2, 4-Dichlorophenoxyacetic acid (2,4-D) (1.0 mg l<sup>-1</sup>). Histological studies showed that the initiation of shoot buds occurred from the peripheral cortical region of the cotyledonary node. The superficial cells in this region become organized into a tunica-cortex arrangement. Roots were seen originating endogenously from the cortical regions and showed only open type of organization similar to the mature primary roots germinated *in vivo* from the seeds. Transverse sections of the regenerated roots showed diarch and triarch conditions, which were significantly different from the tetrarch condition observed in the primary roots of seed grown plants.

**Key Words:** Tissue culture, Micropropagation, Medicinal plants, Histology, Root, Shoot, Development, *Cassia auriculata*

### Abbreviations

2, 4-D	2, 4-Dichlorophenoxyacetic acid
BA	6-Benzyladenine
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
Kn	Kinetin
NAA	Naphthaleneacetic acid

### INTRODUCTION

*Cassia auriculata* is chiefly valued for the tannins present in the bark. The bark, known as Avaram bark or Tangeedu bark in commerce is one of the best available barks in India. It is the principal indigenous bark, used in the South Indian tanneries for crust tanning. The bark is astringent and useful for gargles in sore throat, rheumatism, in enemas, eye diseases and diabetes. Its decoction is given in stomachache and the juice of fresh bark in dysentery. The leaves are anthelmintic and good for ulcers, skin diseases and leprosy, their infusion possesses slight purgative property. Leaves ground with *Vigna mungo* and poppy seeds are applied to herpetic eruptions. The leaves contain a high percentage of nitrogen and potash and are therefore used as green manure in paddy-fields. They are also valuable for manuring alkaline lands and reclamation of soils. The pods are anthelmintic, emetic and useful in urinary discharges. The seeds are considered refrigerant and alexipharmic. They are used in chronic purulent ophthalmic and conjunctivitis, cough, asthma, gout, gonorrhoea, dysentery and diabetes. The roots are considered astringent, alexeteric and useful in skin

diseases, asthma, thirst and urinary discharge. A decoction of the roots is used as a tonic. The root shows interferon like activity against Ranikhet disease virus. The flowers are used in throat troubles, urinary disorders and as astringent. An aqueous extract of the leaves and flower possesses hypoglycemic activity (Anonymous 1952). Recently it has been reported as a potent anticancer herb by Prasanna *et al.*, (2009). They reported *in vitro* anticancer effect of *Cassia auriculata* leaf extract in human breast adenocarcinoma MCF-7 and human larynx carcinoma Hep-2 cell lines. During the past three decades, cell and tissue culture technique has emerged as a powerful tool for the study of an increasing number of basic problems in plant sciences, viz., cell and tissue developmental biology, physiology, biochemistry, pathology, genetics, molecular biology and for general applied aspects such as clonal propagation, haploid production, embryo culture, cell transformation with an ultimate objective of improving economically important plant species. Clonal multiplication basically depends on the proliferation and induction of axillary or apical shoot

meristems or via callus differentiation. Node contains meristem which remains active for specific periods depending on the physiological condition of the plant. With the use of tissue culture technique, the nodal meristem can be stimulated to produce multiple shoots through differentiations and organogenesis using appropriate cytokinin and/or auxin concentration. Through this mode of multiplication large numbers of plants have been produced in diverse group of families (Nugent *et al.*, 1991, Rani *et al.*, 2001 and others). Bonga (1982) reviewed the nature of problems associated with tissue culture of woody plants. The difficulties encountered in developing in vitro system for woody plant regeneration are (a) establishment of explants in culture due to the oxidation of polyphenols from them (b) absence of juvenility in the explants of mature trees and (c) induction of roots in culture. There are few reports on the tissue culture of the *Cassia* species using MS and Gamborg's B<sub>5</sub> (B<sub>5</sub>) basal media supplemented with different combinations of auxins and cytokinins. These include Lee and Rao (1980) on *Cassia fistula*, Gharyal & Maheswari (1990) on *C. fistula* and *C. siamea*, Babber *et al.*, (1996) on *C. roxburghii* and Pathak (2001) on *C. alata* and *C. fistula*. The current study was carried out to find out the culture conditions for multiple root and shoot regeneration and simultaneously carry out comparative study at the histological levels to observe the developmental changes taking places in root and shoot originated in vivo (through seed germination) and in vitro in *Cassia auriculata*

## MATERIALS AND METHODS

### Seed Germination

Seed were germinated in soil. Seed were also cultured on MS and ½ MS media (without phytohormones). But in both the cases delayed seed germination of around 2-5% was seen. Explants taken from such plantlets, after sterilization with 0.01% HgCl<sub>2</sub>, showed poor and delayed morphogenetic responses. Therefore a protocol was developed to get contamination free, quick and better responding explants. About 200 seeds (taken in 500 ml autoclaved conical flask) were treated with 70% H<sub>2</sub>SO<sub>4</sub> for about 10 minutes and then washed thoroughly with autoclaved distilled water 7 to 10 times. H<sub>2</sub>SO<sub>4</sub> was used to inhibit the seed germination. Seeds were then soaked in water. Only double distilled autoclaved water was used. The flasks were plugged with cotton and kept overnight. On the next day, the seeds got swelled and after pouring off the water, seeds were sterilized with 0.1% HgCl<sub>2</sub> for about 4-5 minutes. Seeds were then washed thoroughly for 5-6 times with double distilled autoclaved water and kept for germination in preautoclaved conical flasks of 500ml capacity under aseptic conditions. More than 95% seeds germinated within 1 to 2 days in contrast to 2-3% when they were not treated with H<sub>2</sub>SO<sub>4</sub>.

### Plant Material Sterilization

After 2 to 3 days of sprouting, explants including cotyledonary node having intact epicotyl and a portion of

hypocotyl, were taken and cultured on MS media supplemented with different combinations of auxins and cytokinins under aseptic conditions. Both the cotyledons were excised and removed from the cotyledonary nodal explants. In few explants however the cotyledonary leaves were left intact along with the cotyledonary node (Table 1).

### Basal Medium and Culture Conditions

Murashige and Skoog (MS) (1962) basal medium containing 0.8% agar and sucrose (30.0 g l<sup>-1</sup>) was used throughout the experiment. The pH of the medium was adjusted to 5.8. Growth regulators were supplemented at various concentrations. To the medium adenine sulphate (25.0 mg l<sup>-1</sup>), Ascorbic acid (20.0 mg l<sup>-1</sup>) and L-glutamine (150.0 mg l<sup>-1</sup>) was added except in cases where only auxins alone were used. Routinely 30ml medium was dispensed in each flask (100ml capacity), and sterilized by autoclaving. The explants were then placed vertically with their lower end inserted into the solid MS medium. During the experiments, a light regime of 14 hours with 100µmol m<sup>-2</sup> s<sup>-1</sup> light intensity provided by cool-white fluorescent tubes at 25 ± 2°C followed by 10 hr dark period was provided to the cultures.

### Histological Study

Shoot apices, nodes and leaves were collected from plants growing in the field. Seeds were germinated and primary roots from the 4-5 days old seedlings were collected. Similar parts were also collected from the in vitro regenerated shoots and roots. Both types of materials were fixed in FAA (formalin: acetic acid: alcohol: 1: 1: 18) and dehydrated in a graded series of ethanol and tertiary butanol followed by infiltration and embedding in paraffin wax (melting point 58-60°C). 8-10µm thick serial sections were cut on with a rotary microtome. The sections were affixed to slides, dewaxed and stained with safranin and light green (Johansen, 1940). Photomicrographs were taken and histological data from both the in vivo and in vitro generated organs was compared.

## RESULTS AND DISCUSSION

### In Vitro Organogenesis

The cotyledonary nodal explants, containing two cotyledons along with intact epicotyl, gave significant morphogenetic response on the MS medium supplemented with various growth hormones. A protocol for the *in vitro* regeneration of the shoot buds has been suggested. BA in combination with auxins and kinetin was found suitable for shoot bud regeneration. Cotyledonary nodes in particular and subsequent foliar nodes in general were the sites of shoot bud proliferation. MS medium with BA (3.0 mg l<sup>-1</sup>) + IBA (1.0 mg l<sup>-1</sup>) resulted into 90% response whereas 100% response was observed in BA in combination with IAA, NAA and Kn (Table 1).

Kaur *et al.*, (1996) in *Acacia senegal* reported maximum shoot bud induction from the cotyledonary

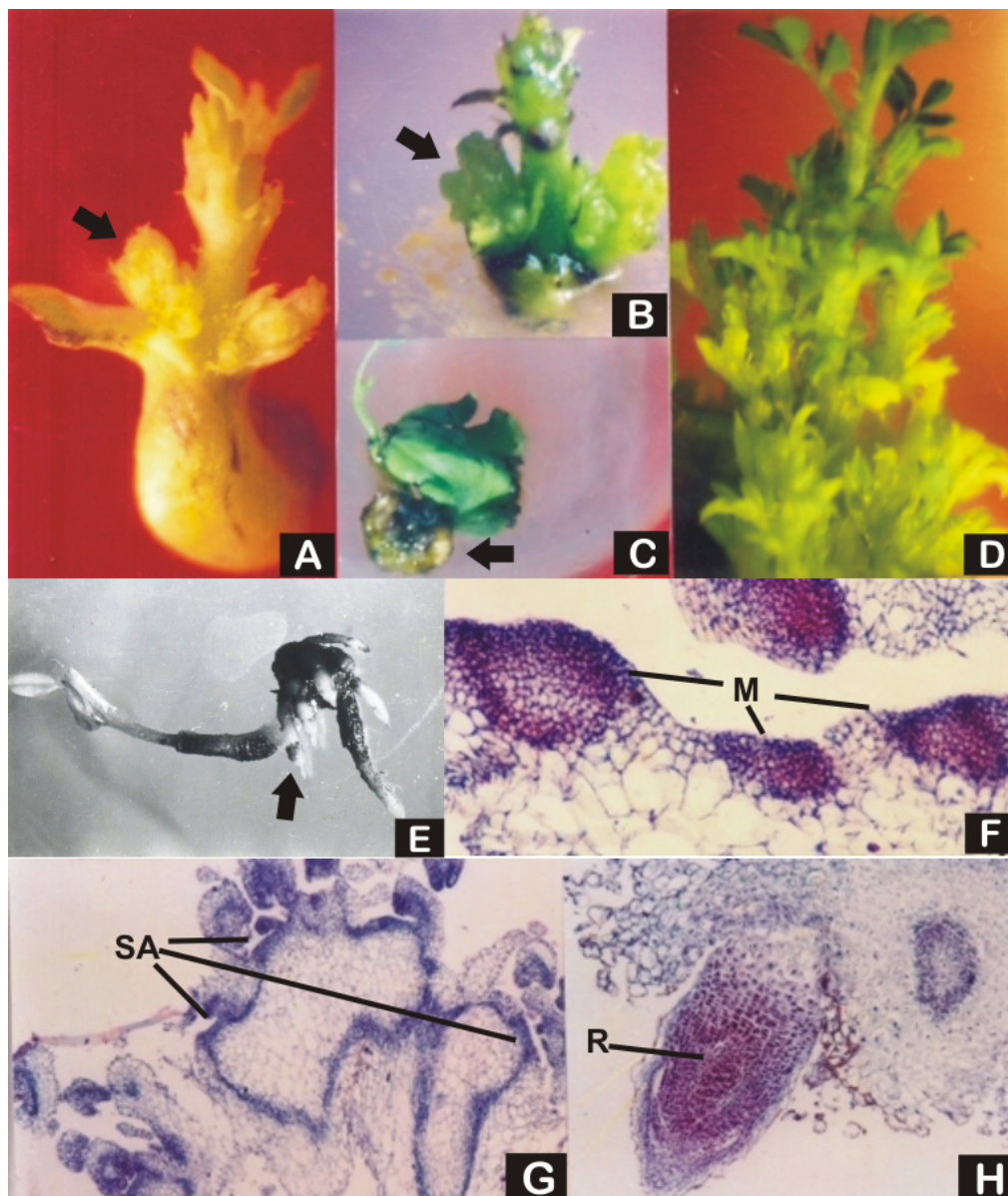
nodal explants using MS medium supplemented with Kn (1.5 mg l<sup>-1</sup>), BA (1.5 mg l<sup>-1</sup>), NAA (0.5 mg l<sup>-1</sup>), adenine sulphate (25.0 mg l<sup>-1</sup>) ascorbic acid (10.0 mg l<sup>-1</sup>) and L-glutamine (146.0 mg l<sup>-1</sup>). Sharma *et al.*, (1997) reported high frequency of shoot regeneration and elongation in *Prosopis cineraria* from the cotyledonary node cultured on MS + BA (2.0 mg l<sup>-1</sup>) and rooting on MS + IBA (2.0

mg l<sup>-1</sup>). In *Cassia senna*, Shrivastava *et al.*, (2006) reported that cotyledonary leaves from seedlings when cultured on MS medium supplemented with different concentrations and combinations of growth regulators such as BA, adenine sulphate as well as complex nitrogenous supplement namely coconut milk (CM), led to shoot differentiation from the explant.

**Table 1: Response of cotyledonary nodes of *Cassia auriculata* to various concentrations of growth regulators supplemented in MS medium**

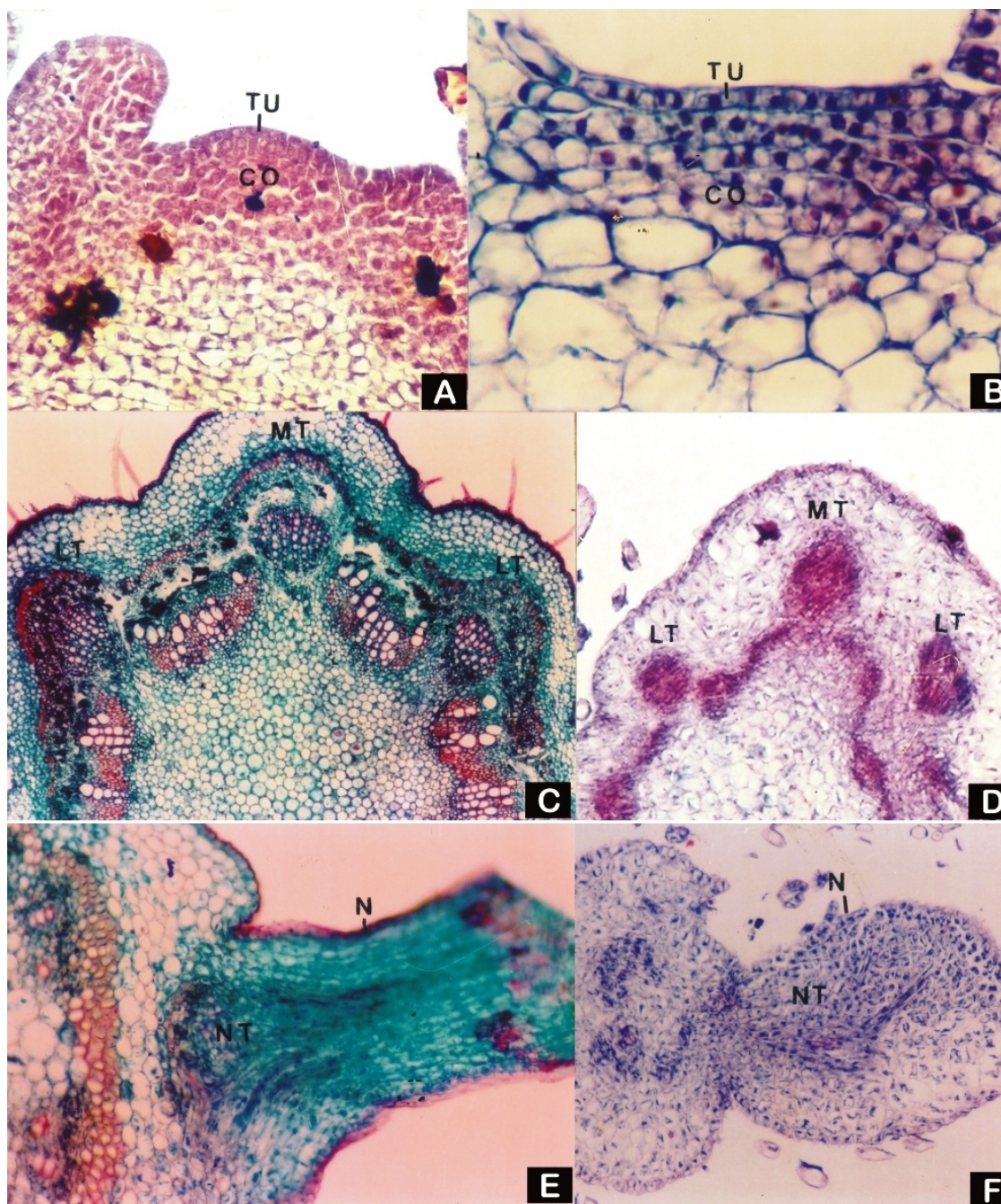
Growth regulator (mg l <sup>-1</sup> )					Nature of response (%)
Auxin	Cytokinin	Adenine sulphate	Ascorbic acid	l-Glutamine	
IAA (1)	-	-	-	-	-
IBA (1)	-	-	-	-	R <sup>++</sup> (100)
NAA (1)	-	-	-	-	C <sup>+</sup> (20)
					R <sup>+++</sup> (100)
2, 4-D (1)	-	-	-	-	C <sup>+</sup> (70)
	Kn (2)	25	20	150	-
	BA (2)	25	20	150	C <sup>+</sup> (50)
					S <sup>+</sup> (20)
IAA (1)	Kn (3)	25	20	150	-
IBA (1)	Kn (3)	25	20	150	-
NAA (1)	Kn (3)	25	20	150	-
2,4-D (1)	Kn (3)	25	20	150	C <sup>+</sup> (20)
IAA (1)	BA (3)	25	20	150	C <sup>+</sup> (70)
					S <sup>++++</sup> (100)
IBA (1)	BA (3)	25	20	150	S <sup>++++</sup> (100)
IBA (3)	BA (1)	25	20	150	C <sup>+</sup> (20)
					S <sup>++++</sup> (90)
NAA (1)	BA (3)	25	20	150	C <sup>+</sup> (20)
					S <sup>++++</sup> (100)
NAA (1)	BA (2)	25	20	150	S <sup>++</sup> (100)
NAA (3)	BA (3)	25	20	150	C <sup>+</sup> (40)
					S <sup>++++</sup> (100)
2, 4-D (1)	BA (3)	25	20	150	C <sup>+</sup> (70)
					S <sup>++++</sup> (70)
	Kn (2)	25	20	150	S <sup>++++</sup> (100)
	BA (2)				
	Kn (3)	25	20	150	S <sup>++++</sup> (100)
	BA (3)				

(**Abbrev.** C<sup>+</sup>: Scanty callusing, C<sup>++</sup>: Moderate callusing, R<sup>+</sup>: 1 to 5 Roots per explant, R<sup>++</sup>: 6 to 10 Roots per explant, R<sup>+++</sup>: 11 to 15 Roots per explant, S<sup>+</sup>: 1 to 5 Shoots per explant, S<sup>++</sup>: 6 to 10 Shoots per explant, S<sup>+++</sup>: 11 to 15 Shoots per explant, S<sup>++++</sup>: More than 16 shoots per explant)



**Fig. 1: *In vitro* shoot and root regeneration in *Cassia auriculata*.** **A.** Shoot buds originating from the axils of the cotyledon on MS + BA (3.0 mg l<sup>-1</sup> + NAA (1.0 mg l<sup>-1</sup>) after 2 weeks of inoculation; **B.** Further increase in number of shoot buds from the cotyledonary node in MS + BA (3.0 mg l<sup>-1</sup>) + NAA (1.0 mg l<sup>-1</sup>) after 4 weeks of inoculation; **C.** Formation of callus from the cut end of the hypocotyls on MS + 2,4-D (1.0 mg l<sup>-1</sup>), 5 weeks after culture; **D.** High frequency shoot elongation from cotyledonary and subsequent nodes on MS + BA (3.0 mg l<sup>-1</sup>) + NAA (1.0 mg l<sup>-1</sup>), after 8 weeks of inoculation; **E.** Roots emerging out from the cut end of the hypocotyls on MS + NAA (1.0 mg l<sup>-1</sup>), 5 weeks after inoculation; **F.** Section of cotyledonary node of the explant showing origin of meristematic regions (darkly stained) in the outer cortex (x 300); **G.** Section showing multiple shoots differentiated from the outer cortical region of the cotyledonary nodal explant (x120); **H.** Transverse section from the hypocotylar regions of the explant cultured on MS + IBA (1.0 mg l<sup>-1</sup>) showing root originating from interior of the cortical region (x120). (*Abbrev.* **M:** Meristematic regions, **R:** Root, **SA:** Shoot Apex).





**Fig. 2: Histological comparison of *in vivo* and *in vitro* generated parts in *Cassia auriculata*.** A. Longitudinal sections of a vegetative shoot apex collected from the mature seed grown plant showing tunica corpus organization (x400); B. Section of well established *in vitro* regenerated shoot apex with tunica corpus organization (x1200); C. Transverse section of node from mature seed grown plant showing trilacunar-three trace condition (x120); D. Transeverse section of stem node taken from shoot regenerated on MS + BA (3.0 mg l<sup>-1</sup>) + NAA (1.0 mg l<sup>-1</sup>) via cotyledonary nodal explant, note the trilacunar-three trace condition with two lateral and one median leaf traces. LT, lateral trace; MT, median trace (x500); E. Transverse section of rachis showing vascularised foliar nectary (x300). F. Transverse section of rachis from *in vitro* regenerated shoot also having vascularised nectary (x120). (Abbrev. CO: Corpus, LT: Lateral trace, MT: Median trace N: Nectary NT: Nectary vasculature, TU: Tunica)

In *Cassia auriculata* of the present report, very quick response was noted in MS + BA (3.0 mg l<sup>-1</sup>) + NAA (1.0 mg l<sup>-1</sup>) (Fig. 1a, b) and MS + Kn (3.0 mg l<sup>-1</sup>) + BA (3.0 mg l<sup>-1</sup>) where shoot buds started appearing within 8-9 days of inoculation. Maximum shoot buds per explant were observed in MS + BA (3.0 mg l<sup>-1</sup>) + IAA (1.0 mg l<sup>-1</sup>); MS + BA (3.0 mg l<sup>-1</sup>) + NAA (1.0 mg l<sup>-1</sup>); and MS + Kn (2.0 mg l<sup>-1</sup>) + BA (2.0 mg l<sup>-1</sup>); and MS + Kn (3.0 mg l<sup>-1</sup>) + BA (3.0 mg l<sup>-1</sup>) (Fig 1A). Few elongated shoots were also observed in MS + Kn (2.0 mg l<sup>-1</sup>) + BA (2.0 mg l<sup>-1</sup>); MS + BA (3.0 mg l<sup>-1</sup>) + NAA (3.0 mg l<sup>-1</sup>); MS + BA (3.0 mg l<sup>-1</sup>) + NAA (1.0 mg l<sup>-1</sup>); and MS + BA (1.0 mg l<sup>-1</sup>) + IBA (3.0 mg l<sup>-1</sup>) (Fig. 1C) (Table 1). Tissue culture and *in vitro* establishment of explants of woody legumes like other woody trees have been difficult mainly due to the exudation of polyphenols from explants into the medium. Gresshoff and Mohapatra (1981) also observed that the legumes are generally recalcitrant and do not regenerate easily in culture. Various explants like leaf, cotyledon, hypocotyl and cotyledonary node etc. used in the present investigation also resulted into browning of the medium and the explants themselves within 24 hrs of inoculation. Ichihashi and Kako (1977), Stevenson and Harris (1980), McCombe and Newton (1981) suggested addition of antioxidants like polyvinyl proline, ascorbic acid, citric acid, cystine HCl, activated charcoal, reduced glutathione etc. to the culture medium to encounter the problem of polyphenol oxidation. In the present work ascorbic acid (25.0 mg l<sup>-1</sup>) was added to the medium and repeated subculturing was done to minimize browning through oxidation. Pathak (2001) in *Cassia alata* added ascorbic acid (100.0-200.0 mg l<sup>-1</sup>) in the medium followed by daily subculturing of the explants to fresh medium for 2-3 days. She also maintained complete darkness for first few days to avoid browning of the medium.

Moderate green to brown, hard and nodular callus developed from the cotyledonary leaf margins and cut end of the hypocotyl on MS + BA (3.0 mg l<sup>-1</sup>) + 2,4-D (1.0 mg l<sup>-1</sup>) (Fig. 1C). But organogenesis from the callus was not observed. However Gharyal and Maheswari (1990) in *Cassia fistula* reported formation of callus and differentiation of shoot buds on B5 medium + IAA (0.5mg l<sup>-1</sup>) + IBA (1 mg l<sup>-1</sup>) whereas Pathak (2001) found that MS + 2,4-D + Kn; MS + NAA + Kn; MS+ IAA + Kn; and MS + IBA + Kn induced callusing, root and shoot differentiations in *Cassia alata*. Babber *et al.*, (1996) reported shoot regeneration in *Cassia roxburghii* from the calli of hypocotyl on BA and NAA combinations. But in the present study the auxins alone also did not promote shoot induction from the calli. Kinetin alone (2.0 mg l<sup>-1</sup>) and in combination with various auxins did not trigger any response except feeble non-

organogenic callusing from the cut end of the hypocotyls on MS + Kn (3.0 mg l<sup>-1</sup>) + 2,4-D (1.0 mg l<sup>-1</sup>).

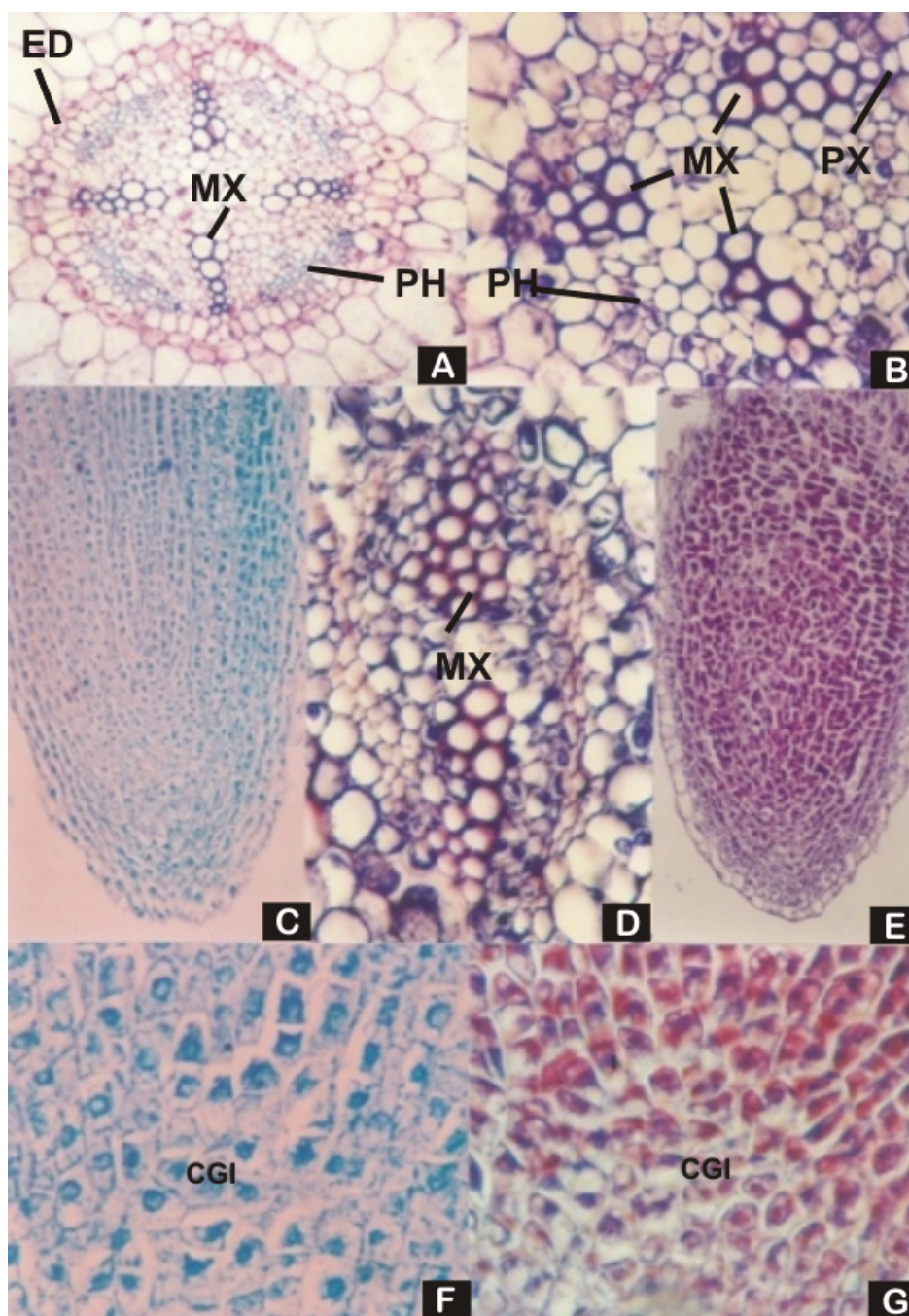
That the auxins promote rooting has been demonstrated in the present report. Babber *et al.*, (1996) also reported initiation of roots in *Cassia roxburghii* from the surgically separated shoots in IAA. But Pathak (2001) reported that MS + NAA (1.0 mg l<sup>-1</sup>) + BA (3.0 mg l<sup>-1</sup>) + activated charcoal (0.5 mg l<sup>-1</sup>) favoured callusing as well as rooting in *Cassia fistula*. In the present report rooting from the cut ends of hypocotyl was observed in MS + IBA (1.0 mg l<sup>-1</sup>) and MS + NAA (1.0 mg l<sup>-1</sup>) combinations (Fig. 1E). 7-10 roots per explant were formed in MS + IBA and 9-13 roots per explant in MS + NAA combinations.

So, for maximum *in vitro* shoot bud production we recommend use of various hormonal combinations in MS media, like BA (3.0 mg l<sup>-1</sup>) + IAA (1.0 mg l<sup>-1</sup>); BA (3.0 mg l<sup>-1</sup>) + NAA (1.0 mg l<sup>-1</sup>); Kn (2.0 mg l<sup>-1</sup>) + BA (2.0 mg l<sup>-1</sup>); and Kn (3.0 mg l<sup>-1</sup>) + BA (3.0 mg l<sup>-1</sup>). For rooting MS + IBA (1.0 mg l<sup>-1</sup>) and MS + NAA (1.0 mg l<sup>-1</sup>) were found the best combinations.

#### **Histological study**

Transverse sections from the cotyledonary nodal region after two weeks of inoculation revealed origin of shoot apical meristems from the peripheral cortical regions. The cortical cells during shoot bud formation become meristematic and start dividing actively resulting in swelling up of nodal part and disorganization of the cortex and vascular cylinder. The meristematic regions in the cortex showed small densely staining cells (Fig. 1F). The superficial cells in these regions at later stages of development gradually become organized into layers and finally exhibit a tunica-cortex organization (Figs. 1E, 2B). The cortical cells on either side to the meristematic regions divide transversely and bulge out like two cotyledons or embryonal leaves as they also show procambial tissue differentiation near their bases. Near the proximal regions of the meristematic regions, procambial cylinder differentiation takes place. These procambial strands also get connected with the older vascular cylinder of the explant. The shoot buds thus formed start growing and also more buds are differentiated from the cortical regions of the node (Fig. 1G). Only the peripheral meristematic regions develop into shoot buds, the deeply located ones did not develop further. Babber *et al.*, (1996) in *Cassia roxburghii* and Pathak (2001) in *C. alata* and *C. fistula* also reported that the meristemoids located only at the subepidermal level were able to differentiate into shoots. The apical meristems of the *in vitro* regenerated shoot buds showed single layered tunica with uniformly denser staining rectangular cells showing anticlinal divisions (Fig. 2B). The tunica covers subjacent cortex.





**Fig. 3: Histological comparison of *In vivo* and *in vitro* generated roots in *Cassia auriculata*.** A. Transverse sections of the root from 4 day old seedling showing tetrarch conditions (x600); B. Transverse sections of the root regenerated from the cut end of the hypocotyls on MS + NAA ( $1.0 \text{ mg l}^{-1}$ ), showing triarch condition (x1200); C. Longitudinal section of seedling root (x300); D. Transverse sections of the root regenerated from the cut end of the hypocotyls on MS +NAA ( $1.0 \text{ mg l}^{-1}$ ), showing diarch condition (x1200); E. Longitudinal section of *in vitro* regenerated seedling root (x300); F. Enlarged view of the Fig.2C with open type of organization showing common group of initials for columella and vascular tissue (x1200); G. Enlarged view of the Fig. 2E, with open type of organization showing common group of initials for columella and vascular tissue; CGI, common group of initials (x1200) (**Abbrev.** CGI: Common group of initials, ED: Endodermis, MX: Metaxylem, PX: Protoxylem)

Cells in the central part of corpus are smaller, actively dividing, more densely stained than the peripheral and deeper region where they are larger, lightly stained and with larger vacuoles (Fig. 2B).

Histogen theory, regarding organization of shoot apex, proposed in 1858 considers that the main body of the plant arises as a mass of meristem which consists of three zones or histogens. The three histogens viz., dermatogen, periblem and plerome, give rise to the epidermis, cortex and central cylinder respectively. Similarly Tunica-Corpus theory proposed in 1924 divided shoot apex into two zones based on plane of cell division viz., the tunica and the corpus. According to it the layers of tunica show predominantly anticlinal divisions, except during the formation of leaf or bud primordial, and the corpus have cells dividing in various planes. In contrast to the histogen theory, the tunica-corpus theory doesn't support the predetermination of the destiny of different regions of plant body (Esau, 1977). Foster (1938) put forward the concept of cytohistological zonation while describing the shoot apex of *Ginkgo biloba*. He combined the tunica – corpus concept with the cytologic features of the apical cells in relation to their histogenetic roles. The zones in the *Ginkgo* reacted differently to staining. Buvat (1952a) used the terms “Méristème d’attente” for the distal axial region of the shoot apex including the tunica and corpus, “anneau initial” for the subdistal and peripheral part and “méristème médullaire” for the proximal axial part giving rise to the pith in the shoot apex. He concluded that the méristème d’attente has organogenic or histogenetic role only during reproductive phase and the most active zone during vegetative growth is the peripheral and sub-terminal anneau initial. Pathak (2001) in *Cassia fistula* and *C. alata* also reported tunica-corpus organization in the *in vitro* regenerated shoot apices. In *C. auriculata*, inception of new leaf primordium is indicated by 2-3 subepidermal cells on the flanks becoming more densely stained and showing periclinal divisions (Fig. 2B). The shoot apex from well established seed grown plants shows a tunica-corpus organisation with well developed darkly stained peripheral zone, lightly stained central mother cell zone (equivalent to the “Méristème d’attente”) in the axial region and pith meristem present just below the “Méristème d’attente” (Fig. 2A). So differences in staining pattern between the peripheral zones of seed and *in vitro* developed shoots were observed. Former being darkly stained than the latter.

The transverse sections of the *in vivo* and *in vitro* regenerated shoots resembled each other, including location of the cylindrical nectaries and their

vascularisation (Fig. 2E, F). The nodes in both the cases were trilacunar- three trace type, with one median and two laterals trace. Two stipular traces one each from the lateral traces separate out to give supply to the stipules present on their respective sides (Fig. 2C, D). The median and lateral traces branch and anastomose to give a ring of petiole vasculature having two adaxial, two laterals, two adaxio-laterals and one basal vascular bundles (Fig. 2F). Vascular supply to the nectaries located on the adaxial surfaces, between every leaflet pair, is made from the branches of adaxial bundles. So no differences in nodal, petiolar vasculatures and mode of nectary vascular supply were observed between *in vivo* and *in vitro* generated shoots.

The root primordia originated endogenously from the deeply situated cortical cells. The outer cortical cells of the hypocotylar region embedded in the nutrient medium become meristematic and the cortex divide into outer and inner regions. From the inner layers of the cortex having regularly arranged small and oval cells, the root primordia originate (Fig. 1H). The developing root primordia have irregularly arranged densely stained cells. The primordia grow and come out of the cortex. During this process the primordial cells start arranging themselves into longitudinal files, which become curved inwards near the distal end. The outer curving cell files organize into the rootcap. Transverse sections of the *in vitro* regenerated roots showed diarch and triarch conditions whereas primary roots from the seed grown plants showed tetrarch condition (Fig. 3A, B, D). The diarch roots have reduced pith and two exarch xylem groups with alternating phloem arcs. Whereas the triarch roots have three xylem and as many alternating phloem groups. The root apex showed an open type (Guttenberg *et al.*, 1955) of organization with common group of initials (CGI) (Fig. 3E, G). CGI or common meristematic cells give rise to columella and stele in opposite direction just like the cambium give rise to phloem outwardly and xylem inwardly in dicot stem of the angiosperms. CGI was also found in mature root apices collected from the seed grown plants of *Cassia auriculata* (Fig. 3C, F).

So at the end we can conclude that being a medicinally and economically important plant, we have established a protocol at initial level for tissue culture (both callusing and micropropagation) of *Cassia auriculata*. We further suggest the need for histological comparison of *in vitro* regenerated plant organs with those of *in vivo* ones as we have observed interesting differences between the two types of plant parts particularly at root vasculature level. Such differences



could have a significant impact for any plant that is agronomically important. Further such type of study where we can trace the origin of shoot apices or its parts histologically can be a very important step in gene manipulation and production of transgenic organs/plants. Such study can be used to study developmental biology of flowering processes as we know that different zones situated at different depth within the shoot apical meristem are involved in formation of different floral parts (Foster, 1938; Buvat, 1952a).

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