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Tissue Culture Studies in Some Medicinally Important Plants

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ABSTRACT

Hibiscus cannabinus, Cassia fistula and Abutilon indicum **are medicinally important plants in India. Tissue culture studies have done for efficient propagation and for crop improvement in these three plants. Various explants were tried for callus induction, somatic embryogenesis and organagenesis with manipulation in culture media. Calli and somatic embryos were induced from hypocotyl explants in *Hibiscus cannabinus*, while in Cassia fistula and *Abutilon indicum* it could be obtained from leaf explants.**

INTRODUCTION

Tissue culture is considered as a unique technique in the field of plant biotechnology, which is mainly used for mass multiplication of trees, ornamentals as well as useful herbaceous plants. These *in vitro* culture techniques have revealed the unique capacity of living plant cells to give rise to whole plants, known as cellular totipotency. Plant tissue and cell culture has become the important tool which brought about many improvements in plants for increased food quality and quantity production of quality seeds for farming and easy processing for obtaining value added products .It also provides an excellent opportunity to study the biosynthesis, accumulation and metabolism of secondary products under controlled conditions of light and temperature.

There are two important pathways of plant regeneration in tissue cultures : Organogenesis and somatic embryogenesis. The development of tissue culture techniques for a reproducible plant regeneration system is required not only for propagation but also for somaclonal variation (Stephens et al.,1991) and gene transfer.

Micropropagation is one of the important areas of plant biotechnology. Clonal multiplication aims at the production of plant breeding true to type, in large numbers in a shorter time and disease free stock.This is generally accomplished by invitro methods involving meristematic explants, shoot tip cultures, adventive bud meristems into their growth and development or through culture of non-meristematic explants(leaf,petiole,root etc.)somatic embryogenesis is a reliable and efficient method for regeneration of plants. Plant in which percentage of setting of seeds is low altogether absent due to failure of pollination, fertilization or some other biotic or abiotic factors. Somatic embryogenesis offers a significant advantage for *invitro* selection, protoplast fusion, transformation studies and commercial production of artificial seeds (Polito et al, 1989)

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Somatic embryos have potential use in *invitro* germplasm storage and survival and regeneration of cryopreserved cultured cells. Somatic embryos tolerate severe desiccation; hence somatic embryos have been used to prepare synthetic seeds by encapsulation in an appropriate matrix. The main idea behind artificial seeds is to prepare a simple inexpensive delivery unit for the successful regeneration of plants via somatic embryogenesis have been published for a number of woody plants. In spite of this success most woody plants still appear to be recalcitrant and regeneration of plants from either source is rare.

Though tissue culture techniques have various applications in tree improvement, there are also certain problems encountered in propagation of trees using tissue culure techniques. Callus induction from any part of the broad leaved woody perennials as source material presents no problem but their regeneration is difficult because they are somewhat recalcitrant. Even if regeneration is achieved, the major problem is with transplantation and hardening of plantlets. Two major problems are: the excessive water loss before roots can become functional after transplanting and an impaired photosynthetic apparatus (Van Huylenbroeck et al.,1996; Brown et al.,1982).

Hibiscus cannabinus, Cassia fistula and Abutilon indica are medicinally important plants in India. Hibiscus belongs to family Malvaceae is a commercially important fibre crop. They substitute for jute in luster. It is also used for paper pulp. The Leaves are used as green vegetable and cattle fodder. It also yields fatty acids used in the manufacture of soap, linoleum paints and varnishes. Besides thse uses it also used as a medicinal plant for stomachic and aphrodisiac. The flower juice with sugar and black pepper is given for treating bioliousness with acidity. Cassia fistula belongs to family Cesalpinaceae is a beautiful and economically important timber yielding tree. Its timber is hard and durable.

All the parts of the tree such as roots, barks, seeds and leaves are said to poses medicinal properties. They posses purgative and laxative properties. Fruit is cathartic. Flowers in decoction are given in stomach affections, at times eaten as vegetable by the hill tribes in

India, Cassia pulp is also applied in the essense of coffee. Abutilon indicum of Malvaceae family posess a number of medicinal properties. Bark is astringent and diuretic while the root extracts are used in fever, chest affections and urethritis. Leaves are demulcent, aphrodisiac, laxative, diuretic pulmonary and sedative. Tissue culture studies have done for efficient propagation and for crop improvement in these three plants. Various explants were tried for callus induction, somatic embryogenesis and organogenesis with manipulation in culture media. Calli and somatic embryos were induced from hypocotyl explants in Hibiscus, while in Cassia and Abutilon it could be obtained from leaf explants.

MATERIALS AND METHODS

The Murashige and Skoog's(MS) 1962 medium was used for the culture of plant materials from stock solution,30g/l sucrose ,100mg/ l inositol,Ph 5.8,solidified with 0.8% agar-agar. The cultures were maintained under $25 \pm 2^{\circ}$c with 14 hours photoperiod of 3000 lux using cool white lamps. The cultures were observed for callus initiation ,nature of the callus initiated ,changes in the explant during callus initiation and regeneration.

Plant materials :

1.Hibiscus cannabinus:

Hypocotyl and cotyledon explants of H. *cannabinus* were collected from a week old field grown seedlings. Leaves were collected from 10-15 days old field grown

plants.

2.Abutilon indicum and Cassia fistula

Leaves were collected from plants growing in the university botanical garden.

Histolgical Studies of callus cultures:

The external Morphology of the callus was studied and photographed using carl Zeiss Tessovar or a minolta Camera equipped with close up lenses. To study the structural organization and differentiation with in the tissue the calli were fixed with FAA (50%). After 24Hrs of fixation in FAA the calli were dehydrated using TBA alcohol series and embedded in paraffin wax.(merck 52-54°C) The embedded calli were cut into blocks and sections of 8-10uM thick were cut with an AO spenser 820 rotary microtome and stained with tannic acid and ferric chloride followed by saffranine O and fast green FCF combinations, the stained sections were observed and photographed under Carl Zeiss photomicroscope.

RESULTS AND DISCUSSIOM

Hypocotyl cultures:

In the case of Hibiscus cannabinus callus cultures were established from hypocotyls explants (seven days old) using MS (1962) medium. callus induction and morphogenetic changes were achieved using various concentrations of plant growth regulators such as BAP,NAA,KIN,IAA and 2,4-D either alone or in combinations. After 2 weeks ,appreciable amount of callus was formed ,when the hypocotyl explants were cultured in MS medium fortified with varying concentrations of 2,4-D (0.5,1,1.5 mg/l)(fig1A).3 different types of responses were observed in three different concentrations of 2,4-D.Exuberant green compact and pale yellow callus were obtained in MS medium supplemented with 1mg/l 2,4-D. Moderate ,friable white colored callus along with poor rhizogenesis and cream friable callus were fomed from the hypocotyl explants in medium augmented with 0.5mg/l and 5mg/l 2,4-D respectively.

After 15 days the best response for callus induction and growth along with rhizogenesis was obtained in medium containing 0.5mg/l NAA and 0.5mg/l BAP .The color and texture of the callus was found to be pale yellow and friable .Prolonged culture in the same medium resulted in extensive rhizogenesis . While somatic embryos amidst callus with 2-3 roots were observed when the explant was cultured on MS medium fortified with 0.5mg/l NAA and 6mg/l BAP .when the concentration of NAA was increased to1mg/l with 2 or 4mg/l BAP pale yellow friable slow growing callus was formed along with rhizogenesis.

BAP, a cytokinin has been found to initiate better callus in *Hibiscus cannabinus* , as reported by Suther (1978) in Xanthium species and Ayappan et al .(1978) in Indigofera leysamanii .Appreciable callus along with extensive rhizogenesis was obtained in MS medium fortified with BAP and NAA ,which may be due to endogenous auxin.NAA as an inducer of better rhizogenesis has been reported in many plants in contrast to other auxins (see Than Van et al., 1978; Geneve et al., 1982; Mclean et al.,1992).

Sudhavani et al.(1996) reported that BAP and NAA enhanced callus formation from hypocotyls explants .Rhizogenesis also occurred from hypocotyls explants cultured in MS medium fortified with IAA.this is in accordance with the reports of Joyce et al. (1990) and Vinod pai et al .(1987)who also stated that IAA favoured root formation from hypocotyl explants.

A fine suspension of cells was formed when fresh callus from the induction medium was transferred to liquid medium .callus obtained without preculture in induction medium didn' t form any suspension .Suspension culture was established with hypocotyl

callus. Callus cultures obtained from MS medium supplemented with 0.5 mg/l BAP and 0.5mg/l NAA were transferred to MS liquid medium augmented with 3? sucrose,0.5mg/l BAP and 0.5mg/l NAA. cultures were incubated in gyratory shaker .Five ml of culture filtrate was transferred to fresh medium which gave rise to afine suspension .To induce somatic embryos in liquid medium ,the suspension culture was transferred to MS medium supplemented with 0.5mg/lNAA and BAP. Initially cell clumps were formed after 2-3 weeks of culture .Later globular structures differentiated and developed into biopolar structures.

Similar findings were reported by Venkatachalam et al.(1997) also reported embryogenic calli from hypocotyl explants of Arachis hypogea cultured on a medium containing different concentrations of 2,4-D or NAA in combination with BAP(0,5mg/l each).The entire induction process occurred in medium containing NAA and BAP,without the need to transfer to auxin free or low auxin containing medium .Germination occurred in half or full strength solid medium. Mullins et al .(1976) also reported the induction of somatic embryogenesis in the presence of NAA and BAP.

Cotyledon cultures

Cotyledon explants were cultured in MS medium fortified with different concentrations of auxin & cytokinins either alone or in combinations.Within a week after placing the explant on MS medium ,swelling of the explants was noticed.Callus initiation was better when the abaxial side of the cotyledon was in contact with the medium compared to the adaxial side .The different concentrations of auxin and cytokinins tried ranged from 0.1mg/l to 2mg/l 2,4-D,2mg/l to 6mg/l KIN,0.1mg/l to 0.5mg/L IAA,0.5mg/l to 2mg/L NAA,0.5mg/l to 6 mg/l BAP. Entire cotyledons and portions of cotyledon responded differently to the same combination of auxin and cytokinin treatment .callus initiation was observed within 10 days of culture.

Whole cotyledon explants cultured in MS medium with 2,4-D and KIN resulted in callusing at the petiolar region, While those cultured in 0.1mg.l or 0.5mg/l 2,4-D and 2mg/l KIN formed white friable callus at the petiolar region followed by rhizogenesis from callus . Susan et al .(1990) also observed callus formation at the petiolar region and reported that at times callus development was accompanied by rooting .

While in 2,4-D alone resulted in pale yellow friable callus along with rhizogenesis from callus and from explant directly(Fig 1B).where as when small portions of the cotyledon was cultured in 0.5mg/l or 1mg/l 2,4-D resulted in callusing with embryo formation and rhizogenesis at the abaxial side. In peanut cotyledon cultures also Susan et al.(1993),

Hazra et al .(1989) reported the formation of somatic embryos in medium containing 2,4-D and in soybean root formation was observed from somatic embryogenesis on cotyledon explant cultured in MS medium fortified with 2,4-D (Lazzeri et al., 1987).Vijendra et al .(1994) and (Sudhavani et al ., 1996) also observed the same in soyabean culture and cultures of chick pea (Cicer arientinum) respectively.

Leaf cultures:

Callus cultures using leaf explant of *Hibiscus cannabinus*, *Abutilon indicum* were obtained on MS medium augmented with different concentrations of BAP, KIN,2,4-D ,IAA and NAA either singly or in combinations .2,4-D was found to be better than other auxins for callus induction .Cytokinins , BAP in Hibiscus *cannabinus, Abutilon indicum.*

When pieces of lamina were cut along the midrib of sterilized leaf or the entire leaf was inoculated in MS medium supplemented with 3? sucrose and different growth regulators. Expansion of the explants was observed on the third day after inoculation .callus initiation occurred first at the edges and later throughout the leaf surface .The callus obtained was friable.

When the explant was cultured in MS medium containing cytokinins , viz.BAP or KIN at 0.5 ,1 or 5mg/l alone in the medium resulted in slow growing ,yellow friable callus was obtained .Inclusion of KIN alone in the medium resulted in slow growing ,yellow colored callus with rhizogenesis. In explants cultured in MS medium added with 0.5mg./l 2,4-D with 2 ,4,6mg/l KIN the response was slow with white friable callus formation wherever the explant was in contact with the medium along with the formation of few roots. Increase in conc gave rise to green compact callus as well as pale yellow friable callus from the entire surface of the leaf.

Whereas addition of 0.5mg/l 2,4-D to the medium resulted in pale yellow friable callus .At 1 or 5mg/l 2,4-D, the rate of proliferation increased. Simultaneously clusters of numerous globular structures appeared first along the veins and later on the entire surface (fig D-E). These structure gradually developed into embryoids .Embryos developed indirectly from callus induced by 2,4-D.culture of leaf pieces resulted in callus with somatic embryos at the cut ends. Somatic embryos were obtained with the intervention of callus or directly on the explant Sometimes root development occurred from somatic embryos (fig1C).

On subculture of these embryos along with the callus into the same combination of 0.5mg/l or 1mg/l 2,4-D ,there was an increase in number of embryos and there was development and differentiation of embryos into globular ,heart and torpedo shaped structures. Prolonged culture of this callus or addition of filter sterilized L-glutamine at 500mg/l to 2,4-D supplemented medium increased the frequency of embryogenesis .The most effective auxin for callus proliferation for most explants is 2,4-D (Dodds and Roberts ,1985).The interaction of 2,4-D and KIN in initiation and development of callus has been reported in many taxa .Initiation of somatic embryos occur in a medium containing auxin and further development of embryos occur either by reducing the particular auxin or substituting it with reduced levels of other auxins (Kohlenbach,1978; Dodds and Roberts ,1985; Rangaswamy,1986).The growth regulator 2,4-D is extensively used in plant tissue culture to induce somatic embryo formation .This growth regulator was originally used by Reinert (1959) in the first experiments on somatic embryogenesis .

In this study embryogeneic callus couldn' t be observed when 2,4-D was replaced by different concentrations of NAA, IAA,KIN or BAP. But prolonged culture on the same medium resulted in globular as well as other stages of embryo formation .Transfer of embryogenic callus to half basal MS medium favoured the germination of embryos in the case of Hibiscus cannabinus. Sarvesh et al .(1997) also observed different developmental stages of somatic embryos in 2,4-D supplemented medium only .However ,it was found that on further transfer of embryogenic callus to LS or Chaleff' s R2 basal medium,the embryos failed to germinate. Sabitharani and Reddy (1996) reported that 2,4-D (1mg/l) and KIN (0.5mg/l) promoted high frequency of somatic embryogenesis in 3 genotypes of groundnut while reduction in concentration of 2,4-D to 0.02mg/l favoured maturation of somatic embryos .Germination was observed in MS basal medium .However ,in most of the plant species ,presence of 2,4-D followed but its gradual withdrawal from the culture medium favored maturation of somatic embryos(Rangaswamy,1986)

Subculture of this callus in 0.2mg/l 2,4-D and 0.5mg/l KIN also favoured the development of embryos .Transfer of these embryos to medium containing cytokinin alone resulted in recallusing and rhizogenesis .Medium supplemented with 0.5mg/l or 2mg/lBAP and 0.2 mg/l IAA ,1mg/l KIN and 0.4mg/l IAA ;2mg/l KIN and 0.4mg/l IAA or 5mg/l KIN and 0.4 mg/l IAA resulted in slight callusing with extensive rhizogenesis .This observation is similar to the findings of Nataraja

and Neelambika (1996).

Somatic embryos from suspension culture and static culture of hypocotyls and leaf callus cultures were observed for germination. No difference in germination was noticed from somatic embryos of both the cultures . In the case of Hibiscus cannabinus ,Somatic embryos got separated in suspension medium and germinated in half and full strength(fig F-J)MS basal medium respectively. Embryos cultured in MS along with lower concentration of auxin or cytokinin resulted in rhizogenesis or callusing . In H.cannabinus combination of hormones (2,4-D and KIN) rather than a single hormone had profound influence on leaf callus cultures. Similar observations were made by Sarvesh et al.(1997) in anther cultures of Niger..

Cytokinins in general are not required for somatic embryo induction , though it may be useful in promoting embryo maturation (Ammirato,1983). It is interesting to note that in our study cytokinins were found to induce somatic embryos .When the leaf explants of Abutilon indicum were cultured in BAP and KIN friable callus along with embryoids were observed,(fig 2A-B) which were found to have the tendency either to dedifferentiate or to form root like structures on MS basal medium with or without hormones. This is similar to the findings of Venkateshwarlu and Mullaih (1997) who reported that somatic embryos of *Benincas hispida* cultured on MS medium supplemented with 1.5mg/l BAP resulted in granular structures which later turned into globular embryoids on subsequent transfer of calli to 2mg/l glutamic acid .These embryos also tend to dedifferentiate or form root like structures on B5 basal medium with or without hormones .Embryogenesis is generally believed to be triggered by auxin in many species .It has been found in our study that 2,4-D was ineffective for embryoid induction .Similar reports was given by Shi-Rong et al .(1992) in Pharbitis nil. In Rawolfia caffra the callus induction and growth was obtained on a medium containing 2mg/l NAA and 2mg/l BAP. The calli developed into embryodogenic with globular embryoids as reported by Upadhyay et al .(1992).

Histology of somatic embryogenesis:

Histological studies carried out on various stages of somatic embryo development revealed that embryo formation can be traced to group of embryogenic cells. Squash preparation of callus revealed the presence of globular stage of embryo with starch grains .The PAS (periodic acid schiff' s) reaction carried out on sections of somatic embryos revealed the presence of large nuclei and accumulation of starch grains.

Histological examinations of embryogenic callus along with embryos from leaf explants showed that the callus was characterized by regions of meristematic cells .These meristematic cells were smaller in size with starch grains .These cells subsequently developed into somatic embryoids .These embryos were clearly demarcated from the surrounding callus cells .Somatic embryos were attached to the callus to a suspensor like organ, whether the suspensor had any role to playing the viability or conversion of somatic embryos is not clearly known. The primordia development was followed by the development of globular embryo and cotyledonary stage embryo emerging from the callus(fig 1K). Subsequently the cotyledons differentiated along with vascular elements in the embryo leading to the formation of complete plantlets.

In our study the presence of meristematic cells in the embryogenic callus obtained from leaf explants could be observed. Similar findings were reported by Johri et al .(1996) from shoot tip explants of betal vine. They reported that the meristematic primordial are attributed to biophysical and genetical factors governed by laws of embroyogenesis and the development of different states of embryos from meristematic cells. The Hibiscus cannabinus development of cotyledons alongwith vascular supply in embryo was noticed as reported in

betal vine (Johri et al. (1996).

As in the case of *Hibiscus cannabinus*, Johri et al. (1996) also observed attachment of somatic embryo by a suspensor like organ. Nalini et al. (1996) reported the presence of suspensor in somatic embryo of pigeon pea and opined that the presence of a suspensor in the somatic embryo shows its resemblance to zygotic embryogenesis.

Cassia fustula

Leaf cultures:

The leaf explants were cultured in MS medium supplemented with 3% sucrose, antioxidants and adsorbing agents such as polyvinylpyrrolidone, silver nitrate, ascorbic acid or activated charcoal along with different concentrations of 2,4-D, KIN, 2-ip, BAP, IAA, IBA and NAA.

Phenolic substances were found to be released from the cut ends of the explants which posed problem, the release of phenolic compounds was visually observed as browning at the cut end of explant and the medium itself. To minimize the release of phenolic compounds PVP (100, 200, 400, 500 or 1000 mg/l), AgNO3 (0.2, 0.4, 0.6 or 0.8 mg/l), Ascorbic acid (50, 100, 200 mg/l) or activated charcoal (200, 500, 1000 mg/l) were added alone to MS medium along with growth adjuvants. Addition of PVP (400 mg/l) as well as activated charcoal (1000 mg/l) along with frequent subculturing were found, to minimize the release of phenolic substances in the media. For further experiments ,therefore explants were inoculated in MS medium with 3% sucrose ,400mgl PVP and different growth regulators .The main problem during establishment was that of exudation of phenolics from the cut ends of the explants in the culture medium .

The removal of phenols by using adsorbing agents such as activated charcoal ,PVP has been reported by Weatherhead et al.(1978,79). Vandana et al .(1997) reported that in apple though a significant reduction in phenolic exudation was observed by treating explants in anti-oxidant solutions before implantation in culture medium, no further growth in explant occurred .

Browning is generally considered to result from the oxidation of phenolic compounds released from the cut ends of the explants by polyphenol oxidases (Mayer and Hard ,1979) or peroxidases (Vaughn and Duke .1984) or air (Robinson ,1983).Expansion of the explants after few days in MS medium augmented with 2,4-D (0.5 , 1, 2 or 5mg/l) gave rise to dark yellow friable callus .Appreciable callus was obtained in 2,4-D mg/l. Two different types of calli ,viz: green compact as well as pale yellow friable callus were obtained when the explants were cultured in MS medium fortified with 2,4-D (0.5mg/l,1mg/l,2mg/l) and KIN (0.1mg/l) .Explants cultured in MS medium supplemented with 0.5 or 2mg/l 2,4-D and 0.1mg/l KIN gave better results than other concentrations of 2,4-D .somatic embryos were observed amidst the callus in MS medium incorporated with 0.5mg/l 2,4-D and 0.1mg/l KIN. Exuberant, yellow, friable callus with meristemoid formation allover the surface of the callus was observed in MS medium fortified with 1 or 2 mg/l 2,4-D and 0.4mg/L kin (fig 2C) .The callus as well as the meristemoids retained their color for few days ,later they turned black due to leaching of phenolic substances .In MS medium supplemented with 2,4-D (1mg/l) and BAP(1,2,5mg/l) compact green nodular callus was formed and later from the nodules root formation could be observed .

Two different types of calli viz.dark yellow callus with meristemoids and compact green nodular callus were observed when the lamina without midrib was cultured in MS medium supplemented with different concentrations of 2-ip (1,2,10mg/l) and NAA (0.5mg/l) .Best response was obtained ,when the leaf was cultured in 2mg/l 2-ip and 0.5mg/l NAA ,whereas the leaf piece along with the midrib cultured in MS fortified with 2mg.l2-ip and o.5mg/l NAA resulted in curling of the midrib towards the innerside and formation of

globular shinny masses along the midrib(fig 2D-E) .The induction of somatic embryos was accompanied with callus from the cut ends(fig F) .The color of the embryo as well as callus changed after few days .In Camellina japonica, Cristinia et al .(1995) reported the occurrence of callus at the cut ends and embryo at the midrib .

Subculture of the leaf callus in MS medium with 2,4-D (0.5 mg/l ,1mg/l) resulted in proliferation of callus and formation of meristemoids in 1mg/l 2,4-D. subculture of callus and meristemoids obtained in MS medium augmented with 2,4-D (0.5 mg/l ,1mg/l) and KIN (0.4 mg/l) in the same combination or further subculture of this callus in the medium containing BAP (0.5 mg/l ,1mg/l) and KIN (0.4 mg/l) resulted in proliferation of callus and formation of nodules on the entire surface of the callus .The embryos observed at the midrib ,when subcultured in the medium containing lower concentration of 2-ip (0.1,0.5mg/l) and NAA (0.5mg/l) along with 500mg/l of either casein hydrolysate or yeast extract or glutamine led to further growth of embryo but there was no diiferentiation .

Suspension culture was established from leaf callus . Callus was inoculated in MS medium augmented with 3% sucrose ,2.4-D (1mg/l) and KIN (0.1mg/l).To induce somatic embryo five ml of filtrate was dispersed into flasks containing 25 ml of fresh medium fortified with 0.5mg/l 2,4-D and 0.1 mg/l KIN. Numerous small clumps were formed .These clumps were washed twice with sterile basal medium and transferred to MS medium with 0.5mg/l 2,4-D and later they developed into somatic embryos (fig2G).

When 2,4-D was substituted with other auxins like IAA or NAA root development from the clumps occurred. Bhansali et al.(1991) also reported embryogenic clumps developed into somatic embryos while McCown (1986) reported the development of root from embryogenic nodules .Somatic embryos which were transferred to basal medium or medium containing IAA,NAA,KIN, or BAP didn' t develop into normal plantlets .This may be due to prolonged exposure to 2,4-D (Kirti and Chopra .1989) or may be due to phenolic exudation

Somatic embryos from suspension cultures were washed with basal liquid medium before transferring to fresh media .Embryos transferred to full or half strength MS medium or to media supplemented with growth adjuvants like IAA,NAA,KIN and BAP did not show any further development into plantlets. Recallusing occurred in all the combinations tried . After the transfer of the callus cultures into liquid medium the entire medium turned black due to exudation of phenols which may be due the reason for the inhibition of further development of these embryos.

Histology of somatic embryogenesis :

Histological studies of the embryogenic callus alongwith embryos from the explants of C.fistula were carried out.The embryogenic cells were small ,with dense cytoplasmic and distinct from the highly vacuolated neighbouring parenchymatous cells .The embryogenic cells led to the formation of embryoids .Histological examination revealed the presenmce of globular pro-embryo with defined epidermis .

The somatic embryos were observed either at the periphery or amidst the callus cells Embryoid formation from peripheral and internal embryogenic cells were observedfrom anatomical studies of C .*fistula* .The distinct feature of the embryoidis no apparent continuity between its vascular element and those of the neighbouring mother tissue

In *Prunus avium* Garin et al .(1997) reported that the histological section of a friable callus showed a globular proembryo with or without starch grains. Similar results were obtained in C .fistula. Our observation is similar to the findings of Shi-Rong jia and Nam Hai Chua (1997) who also observed the development of somatic embryoids from meristematic cells .

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