

Full Length Research Paper

Effect of auxins and auxin polar transport inhibitor (TIBA) on somatic embryogenesis in groundnut (*Arachis hypogaea* L.)

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The phytohormone auxin plays an important role in growth, developmental and physiological processes. The effect of auxins, 2,4-D, NAA, IAA, Dicamba and picloram, was tested for somatic embryogenesis in groundnut. Among the different auxins tested 2,4-D favored the best response of somatic embryogenesis with induction of 18.3 somatic embryos per explant that were big, healthy, succulent and green in color. Immature zygotic embryos axes cultured on MS medium with 4 mg/l TIBA (Auxin polar transport inhibitor) did not respond for somatic embryogenesis but when cultured on medium supplemented with TIBA (2 mg/l and 4 mg/l) and 2,4-D (4 mg/l) showed the induction of somatic embryogenesis. When the concentration of TIBA was increased (6 mg/l) keeping the level of 2, 4-D (4 mg/l) constant, it resulted in browning of explants within a week of culture. These observations demonstrate the requirement of 2,4-D for induction of somatic embryogenesis and that addition of TIBA (2 mg/l and 4 mg/l) decreases the response of somatic embryogenesis and further higher levels (6 mg/l) being inhibitory for somatic embryogenesis.

Key words: Auxin polar transport inhibitor, somatic embryogenesis.

INTRODUCTION

In many legumes, cells of immature zygotic embryos develop directly into somatic embryos (Rao and Lakshmisita, 1996; Hazra et al., 1989; Finer, 1988) whereas highly differentiated explants usually undergo a callus phase to acquire embryogenic potential (Barna and Wakhlu, 1993; Chengalrayan et al., 1994; Ahmed et al., 1996; Trinh et al., 1998; Sreenivas et al., 1998). However, in groundnut, it is observed that proliferation of somatic embryos occurred directly from the explants without any intervening callus phase. Mechanism controlling pattern formation of somatic embryogenesis is very poorly understood in plants. Polar auxin transport

inhibitors are important tools in assessing the role of polar auxin transport in plant development (Hadfi et al., 1998; Mattsson et al, 1999; Sabatini 1999) . Polar auxin transport has long been postulated to play a central role in plant embryogenesis. Microscale auxin transport assays have been used to show that the hypocotyls of mature embryos dissected from both gymnosperm and angiosperm seeds exhibit pronounced polar auxin transport toward the root end irrespective of their orientation (Greenwood and Goldsmith, 1970) (Greenwood et al., 1970). Polar transport of the phytohormone auxin mediates various processes in plant growth and development, such as apical dominance, tropisms, vascular patterning and axis formation (Estelle et al., 1998; Berleth et al., 2001).

The possible role of polar auxin transport in somatic embryogenesis was tested by Schiavone and Cooke (1987), who treated different stages of carrot somatic embryos with TIBA and a different auxin transport inhibitor, N-(1- naphthyl) phthalamic acid (NPA). Both auxin transport inhibitors at a concentration of 1 pM are able to block

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Abbreviations: TIBA; Triiodobenzoic acid, 2,4-D; 2,4 Dichlorophenoxyacetic acid, IAA; Indole-3- acetic acid, NAA; - Naphthalene acetic acid, DICAMBA; 3, 6-dichloro-o -anisic acid, PICLORAM; 4-amino-3, 5, 6-Trichloropicolinic acid.

the ability of somatic embryos to undergo morphogenetic transitions to the subsequent stages: globular embryos undergo persistent spherical expansion, oblong embryos (an intermediate stage in somatic embryo-genesis) continue axis elongation without any cotyledon initiation, and heart embryos develop additional growth axes on their hypocotyls.

Polar auxin transport inhibitors disrupt auxin efflux from the cell but their mode of action is unknown (Morris et al., 2000). It is thought that polar auxin flux is caused by the asymmetric distribution of efflux carriers acting at the plasma membrane (Palme et al., 1999).

The phytohormone auxin plays an important role in a wide variety of growth, developmental and physiological processes, many of which are dependent on directional auxin transport within organs and tissues. The concept of polar auxin transport known as the chemiosmotic hypothesis proposes that proton motive force across the plasma membrane is the driving force for polar transport and that this transport occurs through the action of auxin influx and efflux carriers located in the plasma membrane of transporting cells (Raven, 1975; Rubery and Sheldrake, 1974). The polarity of the transport is due to the asymmetrical localization of the efflux carrier to the basal side of transporting cells.

Requirement of auxin or any other growth regulator for induction of somatic embryogenesis has been demonstrated in many crop species. Induction of somatic embryogenesis was achieved in presence of the auxins such as 2,4-D, NAA (Baker and Wetzstein, 1994), IAA (Ahemed et al., 1996), dicamba (Eapen and George, 1993), 2,4,5-trichlorophenoxypropionic acid, indolepropionic acid, α -naphthoxy acetic acid (Eapen and George, 1993) and picloram (Ozias-Akins et al., 1992; Eapen and George, 1993). Baker et al. (1994) made comparisons of the level of NAA during induction, nitrogen formulation of the medium, and photoperiod on somatic embryogenesis from immature zygotic cotyledons. Over 70% embryogenesis was obtained regardless of NAA level used. However, percent embryogenesis and number of embryos were markedly lower in explants induced on NAA compared to 2,4-D. Hazra et al. (1989) stated that there was no embryo production when NAA was used with embryo axes explants. Only smooth nodular outgrowths or neomorphic protuberances were obtained when 5-30 mg/l NAA was used with immature cotyledons (Ozias-Akins, 1989). In contrast, Sellars et al. (1990) noticed high embryo production from immature embryos with constant 2 mg/l NAA. McKently (1991) found that auxin type significantly affected embryogenic response in peanut embryo axis cultures. In most of the reports, use of 2,4-D resulted in high frequency somatic embryo production in groundnut (Durham and Parrott, 1992; Eapen and George, 1993; Reddy and Reddy, 1993; Wetzstein and Baker, 1993; Baker et al., 1994; Chengalrayan et al., 1994; Venkatachalam et al., 1997).

Lui Chun- Ming et al. (1993) has proved that auxin polar transport is essential for establishment of bilateral sym-

metry during early plant embryogenesis. The goal of our study was to investigate the effect of auxins and auxin polar transport inhibitor on peanut somatic embryogenesis.

MATERIALS AND METHODS

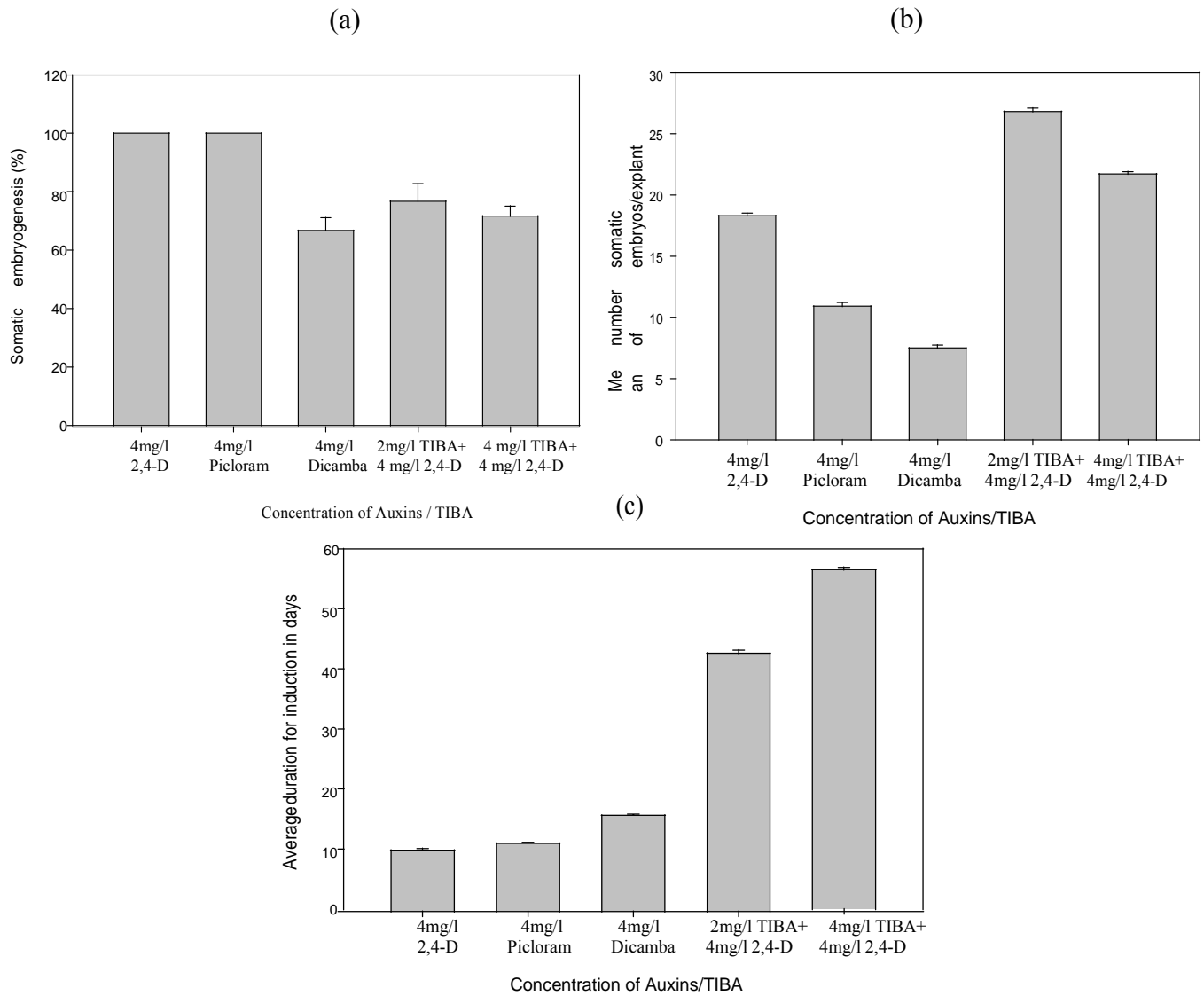
Immature pods of peanut cultivars DRG-12 were collected from the field grown plants after 15 - 40 days after pollination (DAP). The Plants grown in the field experienced an average day/night temperature of 24/30°C approximately and the relative humidity varied from 60 - 80%. Under the conditions at Hyderabad, the sunlight intensity was $2800 \mu\text{mol m}^{-2} \text{s}^{-1}$ approximately immature pods were harvested from field grown plants at different days after pollination and rinsed in distilled water. The pods were surface sterilized with 70% ethanol followed by 0.1% HgCl_2 for 20 min. and rinsed three times in distilled water. Immature seeds were removed from the pods and further experiments were carried under aseptic conditions. Immature seeds were sterilized by 70% ethanol for 1 min. followed by 0.1% HgCl_2 for 20 min. and rinsed three times in distilled water. Immature embryo axes were excised from the seed and cultured on MS (Murashige and Skoog's, 1962) medium containing 3% sucrose, 0.85% agar and different growth regulators (Auxins and auxin polar transport inhibitor) for induction of somatic embryogenesis. The cultures were incubated at $25 \pm 2^\circ\text{C}$ under a 16-h photoperiod with photosynthetic photon flux density (PPFD) of $57 \mu\text{mol m}^{-2} \text{s}^{-1}$ provided by white florescent tubes. All the experiments were repeated in triplicates to get proper results.

The cultivar DRG-12 of groundnut (*Arachis hypogaea* L.) was obtained from Directorate of Oil Seed Research, Hyderabad, India. Growth regulators Such as 2,4-D, NAA, IAA, TIBA were purchased from Sigma chemical company, St. Louise, U.S.A. All other chemicals / reagents used were of extra pure and analytical grade from India by different firms.

RESULTS

The best response of somatic embryogenesis (100%) was observed in 4 mm long immature zygotic embryo axes. Somatic embryos harvested from 30 days old cultures were separated into different morphological classes such as globular, Heart, torpedo, cotyledonary, fused and fasciated types (Figures 3a - d).

Among the different auxins tested, 2,4- D favored the best response of somatic embryogenesis with induction of 18.3 somatic embryos per explant that were big, healthy, succulent and green in color (Figures 2a and b). The average duration for induction of somatic embryos on 2,4-D was 10.2 days (Figure 1c) which was the earliest, in Medium supplemented with 4 mg/l picloram also favored high frequency of somatic embryogenesis (100%) but the average numbers of somatic embryos per explant were lesser in number (Figure 1a). Somatic embryogenesis decreased in the presence of 4 mg/l dicamba with respect to frequency (66.7%) as well as the number (7.5) of somatic embryos induced per explant (Figure 1b). Somatic embryos produced on medium containing picloram were smaller in size, translucent and yellowish in color whereas those differentiated in the presence of dicamba were smaller and green in colour. The development of somatic embryos was slow in the



Figures 1a, b and c: Graphs representing the Effect of auxins and auxin polar transport inhibitor (TIBA) on somatic embryogenesis from immature zygotic embryo axes of DRG-12 on MS medium.

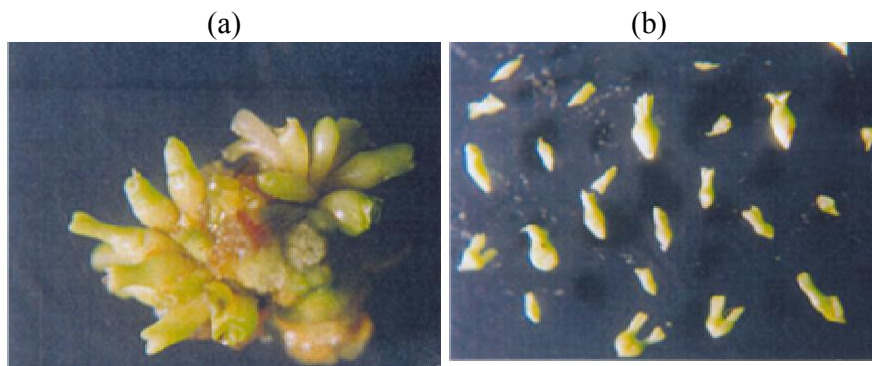


Figure 2. Induction of somatic embryogenesis from immature embryo axes on MS medium with 4 mg/l 2, 4-D. **a)** Induction of somatic embryos. **b)** Isolated somatic embryos derived from 30 day-old culture.

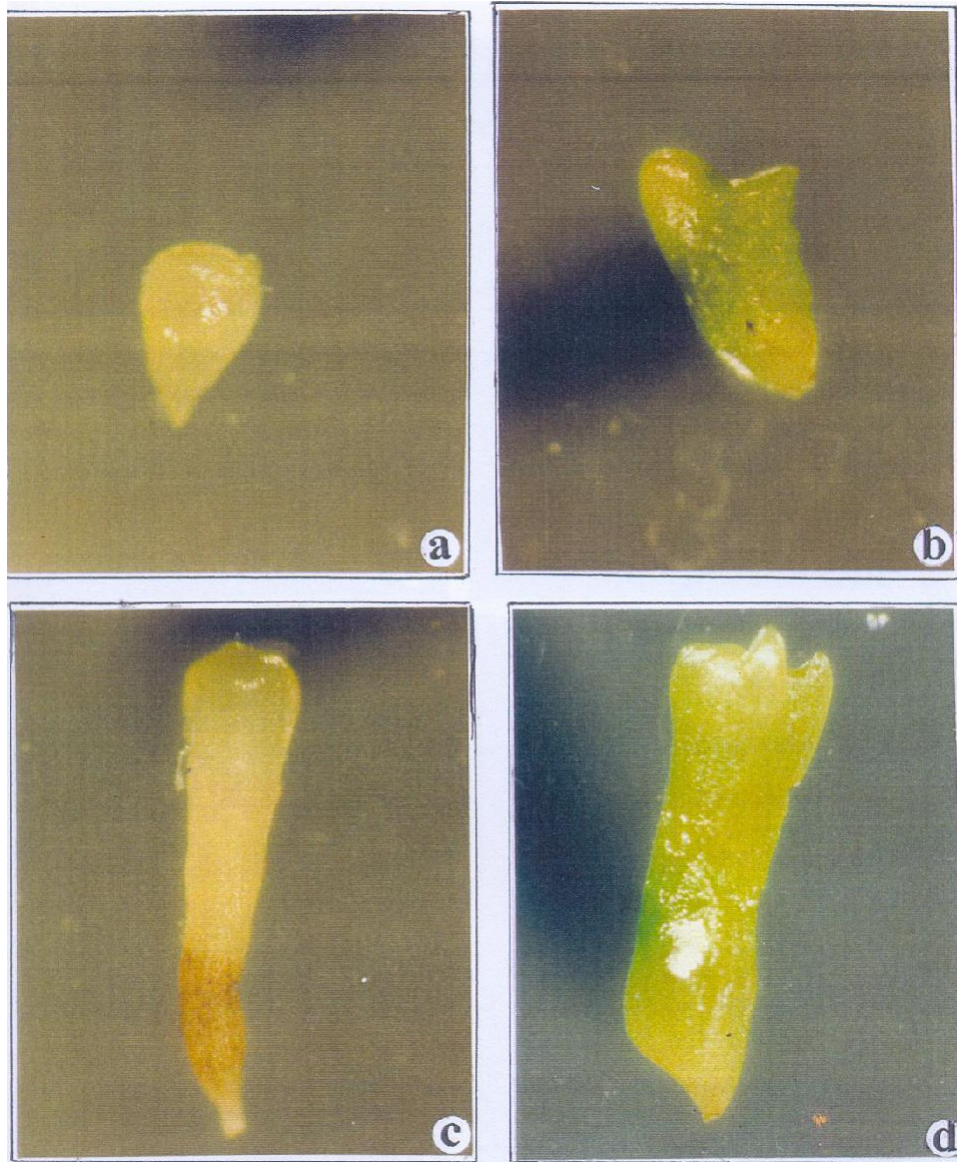


Figure 3. Different stages of somatic embryos separated from cultures of immature zygotic embryo axis. **a.** Globular staged somatic embryo. **b.** Heart staged somatic embryo. **c.** Torpedo staged somatic embryo. **d.** Cotyledonary staged somatic embryo.

presence of picloram and torpedo staged somatic embryos were predominantly observed in 30 day cultures unlike on 2,4-D medium where somatic embryos at all stages were observed (Figure 4c). There was no induction of somatic embryogenesis in immature zygotic embryo axis cultured on 4 mg/l NAA or 4 mg/l IAA even after 30 days of culture. However, the explants cultured on medium containing 4 mg/l NAA increased in size with enlargement of epicotyl and hypocotyl region and induction of a thick root at the end of 30 days (Figure 4b). Zygotic embryo axes cultured on 4 mg/l IAA rooted profusely with induction of shoots of 1 - 2 mm without further growth. Immature zygotic embryos axes cultured on MS medium with 4 mg/l TIBA did not respond for soma-

tic embryogenesis but when cultured on medium supplemented with TIBA (2 and 4 mg/l) and 2,4-D (4 mg/l), there was induction of somatic embryogenesis. The frequency of response was 76.7% with induction of 26.8 somatic embryos/explant after 30 days of culture on medium with 2 mg/l TIBA and 4 mg/l 2,4-D (Figure 1). Explants cultured on MS medium with 4 mg/l TIBA and 4 mg/l 2,4-D responded for somatic embryogenesis with a frequency of 71.7% with induction of 21.7 somatic embryos per explant after 56.5 days of culture (Figure 4c). When the concentration of TIBA was increased (6 mg/l) keeping the level of 2,4-D (4 mg/l) constant, it resulted in browning of explants within a week of culture. These observations

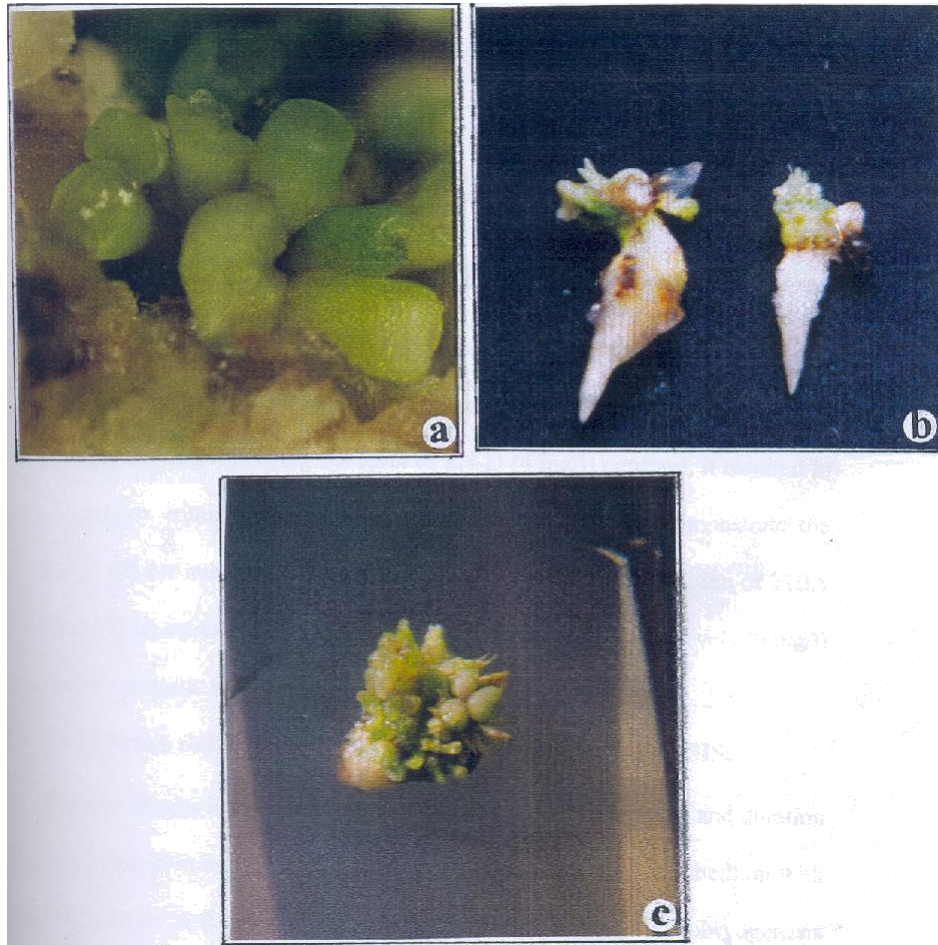


Figure 4a. Induction of somatic embryogenesis from immature zygotic embryo axes on MS medium with 4 mg/l picloram. **b.** Induction of somatic embryogenesis from immature zygotic embryo axes on MS medium with 4 mg/l NAA. **c.** Induction of rooting from immature zygotic embryo axes on MS medium with 4 mg/l 2, 4 - D and 4 mg/l TIBA.

demonstrate the requirement of 2, 4-D and that addition of TIBA (2 mg/l and 4 mg/l) for induction of somatic embryogenesis. Further higher levels (6 mg/l) being inhibitory for somatic embryogenesis.

DISCUSSION

It is known that auxin transport inhibitors, such as TIBA, can effectively block auxin transport by binding to the regulatory site of an auxin efflux carrier complex (Depta et al., 1983; Venis, 1985). Several studies have revealed that proper polar transport of auxin is a prerequisite for normal morphogenesis (Schiavone and Cokke, 1987; Liu et al., 1993) beyond the globular stage. In the present study, TIBA was added at various concentrations either individually or in combination with 2, 4-D to determine its effect on somatic embryo formation. There was no induction of somatic embryogenesis in the presence of TIBA at different concentrations tested and the tissues

became necrotic at the end of the culture. Incorporation of TIBA (2 mg/l and 4 mg/l) along with 2,4-D (4 mg/l) produced somatic embryos from immature zygotic embryo axes at a frequency of 80% with delayed response compared to 2,4 -D alone where somatic embryos proliferated within 10.3 days of culture. Moreover, a greater proportion of somatic embryos produced in the presence of TIBA and 2,4-D were morphologically abnormal and smaller than those induced in the presence of 2,4-D. An increase in the frequency of morphologically abnormal types coupled with a delayed response in the presence of TIBA and 2, 4-D might have been because of interference of auxin polar transport by TIBA treatment. In *Brassica juncea* (Indian mustard), inhibition of auxin transport at the globular stage resulted in the production of mature embryos with a fused, cylindrical cotyledon rather than two separate cotyledons (Liu et al., 1993). However, treatment of more mature embryos had no effect on subsequent morphogenesis. Their results showed that auxin polar transport plays an important role

in cotyledon formation during embryo development. Choi et al. (1997) examined the effects of a polar auxin transport inhibitor, TIBA, on development and structural aspect of somatic embryos originating from the culture of ginseng cotyledons. They observed sporadic development of somatic embryos which had a normal embryo axis but jar-shaped cotyledons on medium containing 5 - 10 M TIBA. The data presented here showed that auxin polar transport plays an important role on morphology of somatic embryogenesis. Interference with the auxin polar transport causes failure in the formation of bilateral symmetry of somatic embryogenesis and results in formation of fused or different shaped embryos. Further it can also be deduced that endogenous auxin in the cotyledon explants plays an important role in the induction of somatic embryos and that the cotyledon development in somatic embryos is also related to the polar transport of endogenous auxin.

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