**DISCUSSION**

The regeneration of plants from cultured cells, tissues and organs is a key step in the application of tissue culture methodology for plant propagation and improvement. To achieve a high frequency of plant regeneration, selection of appropriate explants is a prerequisite. Therefore, the explants have to be selected from healthy, vigorous plants to obtain optimum results. Generally, immature tissues and juvenile organs show high plasticity for morphogenesis *in vitro* than the mature ones. However, during recent years the regeneration capacities of many woody species have also been demonstrated by utilizing explants from mature trees. In the present investigation, *in vitro* studies were carried out on four economically important woody species viz. 1. *Moringa oleifera* Lam. cv. Dhanaraj, 2. *Murraya koenigii* Spreng. cv. Local, 3. *Punica granatum* L. var. ‘flore-pleno’ and 4. *Punica granatum* L. cv. Jyoti

The cultures were raised from seeds, shoot tips, segments of nodes, epicotyl, hypocotyl and cotyledon as well as leaflets and petals. The seeds of *Moringa oleifera* reared on various nutrient media germinated to give rise to seedlings. In addition to callusing from radicular end, axillary shoot formation was also noted from cotyledonary nodes. The seeds of *Murraya koenigii* reared on MS alone germinated normally and yielded multiple seedlings revealing their polyembryonic nature. The different seedling parts also differentiated multiple shoots, roots, callus and embryo-like structures on various media. In *Moringa oleifera* embryoids also differentiated from callus derived from epicotyl segments
and leaflets. The petal cultures of *Punica granatum* var. 'flore pleno' and cv. Jyoti also embryogenesis occurred and mature embryoids were produced directly and via callus. An attempt has been made to discuss some of these observations in light of the available literature.

In the present study the seeds of *Moringa oleifera* reared on MS alone though germinated normally, but abnormalities like callusing from the radicular end and the development of axillary shoots from cotyledonary nodes were also noted. The lower concentrations of IAA or IBA in the medium supported normal germination, robust seedlings were also formed on MS containing 2.0 mg/l of IAA and in addition, at 5.0 mg/l a whitish callus from main root along with many laterals was observed. On the contrary, NAA at higher concentrations suppressed seed germination due to the proliferation of callus from radicle and cotyledons. Almost similar responses have also been reported in seed cultures of *Helianthus annuus* in presence of 2,4-D in the medium (Ganapathi, 1990). The seeds of *Moringa* cultured on MS supplemented with BAP (1.0 and 2.0 mg/l) individually and in combinations with IAA (0.5, 1.0 and 2.0 mg/l), in addition to the main shoot one or two axillary shoots also differentiated from cotyledonary node. Such an induction of multiple shoots from the axils of cotyledonary leaves during seed germation in presence of KN and 2,4-D has been reported in *Dalbergia latifolia* (Sudhadevi, 1984). Khan and Ghosh (1984) reported shoot bud induction from cotyledonary node of seedlings in *Cicer*. On the contrary, in *Linum utilissimum* the germinating seeds produced shoot buds directly from the epidermal cells of hypocotyl on MS supplemented with KN and IAA (Ravi, 1985). The differentiation of shoot buds and embryoids from split hypocotyls and roots has also been reported
in *Clitoria* in presence of similar growth adjuvants (Lakshmanan and Dhanalakshmi, 1990).

As early as 1935 and 1936, Chakravarthy had reported nucellar polyembryony in seeds of *Murraya*. Madalageri and Ramanjinigowda (1987) also observed polyembryonic nature of *Murraya* seeds during in vivo propagation of plants through seeds. In the present study also the seeds of *Murraya koenigii* germinated on MS alone giving multiple seedlings confirming their polyembryonic nature. The occurrence of polyembryony in certain fruit crops like *Citrus* species, a few cultivars of *Mangifera* and *Eugenia* is also very common (Janick and Moore, 1975). This phenomenon has an advantage of providing genetically uniform seedlings and in several polyembryonic *Citrus* cultivars and *Garcinia,* nucellar seedlings have been utilized successfully as stock plants for micropropagation (Barlass and Skene, 1982; Goh *et al.*, 1988).

Although a few reports are available on the formation of callus, adventitious organs and embryoids reared on a basal medium alone (La Rue, 1942; Milyaeva *et al.*, 1972; Lakshmanan and Dhanalakshmi, 1989), a majority of species have shown a requirement of growth adjuvants for induction of regeneration (Tran Than Van, 1981; Krikorian, 1982). In the present study though the epicotyl segments of *Moringa* showed slight swellings from cut ends, they failed to respond further on MS alone and all the other explants of *Moringa* and *Murraya* have regenerated organs in presence of plant growth adjuvants.

Skoog and Miller (1957) in their classic experiments with cultured stem, tissues of tobacco demonstrated that organogenesis is dependent on a delicate
balance between auxin and cytokinin in the medium, while the concentrations and type of growth adjuvants vary. This basic approach has been exploited to regenerate a wide variety of plants species (Flick et al., 1983; Bhojwani and Razdan, 1983; Litz and Grey, 1992).

According to Hicks (1980), there are two developmental sequences of pathways during organ differentiation in vitro. The direct organogenesis wherein the callus formation is absent and indirect organogenesis which involves a callus phase. Several reports are available on the formation of organs either directly or indirectly through empirical selection of explant, the composition of medium and control of physical environment (Brown and Thorpe, 1986).

**Responses of shoot tips and nodal segments:**

The regeneration of plantlets from shoot tips and nodal segments has been reported in many species (see Murashige, 1974; Biondi, 1986; Narayanaswamy, 1994). A majority of them required a cytokinin alone or in combination with auxins, for further growth and multiple shoot formation and auxins for rooting.

In most of the woody ornamental plants, regeneration was achieved by the proliferation of axillary shoots from shoot or meristem tips (Zimmerman, 1986; Doreswamy and Sahijram, 1988) and successful commercial production of *Rhododendron*, *Azalea* and *Rosa* has also been made (Anderson, 1984; Fordham et al., 1982; Hasegawa, 1979).

The shoot tips of *Helianthus* produced multiple shoots on MS containing KN or BA alone and also KN in combination with CW. Similarly, multiple shoots and axillary shoots from cultures of shoot tips and nodal segments of
coffee has also been achieved on MS with BA alone and in combination with NAA (Ganapathi, 1990). Amin and Jaiswal (1987) reported that the nodal segments of guava produced multiple shoots on media supplemented with BA alone or BA along with IAA or IBA and GA3. But BA alone was found to give best multiplication rate, however, rooting of shoots was achieved by transferring the individual shoots to media containing both IAA and NAA. On the contrary, Kopp and Nataraja (1990), reported the regeneration of plantlets from shoot tips of *Tamarindus* on MS with IBA and IAA. The development of shoot tips of *Guizotia* into plantlets on MS alone and MS with CW or NAA has also been reported (Ganapathi, 1990).

In the present study, proliferation of axillary shoots from shoot tips of *Moringa* was observed on MS supplemented with 1.0 and 2.0 mg/l of BAP. Paterson (1984) reported that in shoot tip cultures of *Helianthus*, BA between 0.1 and 1.0 mg/l appeared to be most effective promoter of multiplication and high concentration favoured callusing and inhibited rooting. Similarly, in the present study the shoot tips of *Moringa* at 5.0 mg/l of BAP alone and along with auxins exhibited stunted growth and developed a moderate mass of callus from cut end. However, shoot tips of *Murraya* though failed to induce multiple shoots in presence of BAP or KN but on MS containing 5.0 mg/l each of IAA and BAP yielded a compact, nodulated brownish callus from cut end and shoots formed were stunted. On the contrary, the shoot tips of *Moringa* and *Murraya*, grew further forming callus and roots from cut end on MS with IAA, IBA or NAA resulting in plantlets. But in *Moringa* at 5.0 mg/l of NAA both shoot and root growth was inhibited, while callusing was profuse. However, the shoot tips of *Murraya* grew vigorously with robust roots and callus from cut end at 5.0 mg/l of NAA.
Mahishi et al. (1991) achieved clonal propagation in *Punica* by initial activation of axillary buds from shoot tips on MS + BAP (8.0 mg/l) + NAA (1.0 mg/l) and their rapid growth and elongation on transfer of shootlets to WPM + IAA (6.0 mg/l) + KN (0.6 mg/l) + CaCl₂₂H₂O (440.0 mg/l) and subsequent rooting on MS + NAA (3.0 mg/l) + charcoal. Earle and Langhans (1974) reported multiple shoot formation and a green callus at the base of shoot tips in *Chrysanthemum morifolium* on MS containing KN and NAA, which was suitable for subculture and subsequent organisation into plantlets. In the present study, whereas the shoot tips of *Moringa* produced a non-friable callus from cut ends on MS containing BAP alone and along with IAA, IBA or NAA. Those of *Murraya*, yielded a compact, nodulated, brownish callus from cut end on MS with BAP and IAA without any significant response. However, Varghese et al. (1993), reported the formation of a compact yellowish-green callus from shoot apices of *Aegle* in presence of KN and 2,4-D or NAA and development of shoot buds after transferring to MS with BAP alone.

Kantharajah and Dodd (1991) reported clonal propagation of *Moringa*, through multiple shoot formation using nodal segments in presence of BAP and rhizogenesis of such shoots on media containing IAA or NAA. But in media with KN or 2ip the explants failed to produce multiple shoots. In the present study, nodal segments of *Moringa* and *Murraya* developed multiple shoots on MS supplemented with 2.0 mg/l of BAP alone and along with 0.5 mg/l of NAA and 2.0 mg/l of IBA respectively. Further, rooting of shoots to yield plantlets has been obtained on transfer of isolated shoots to MS with IAA in *Moringa* and NAA in *Murraya*. 
On the contrary, in nodal segments culture of *Azadirachta indica* Joshi and Thengane (1996) have indicated rooting of shoots on transfer to half-strength liquid MS medium containing IAA. Axillary shoot proliferations from similar explants on MS added with BA or zeatin in *Annona* and multiple shoot induction with combination of auxin and kinetin in *Aegle, Plantago*, and *Syzygium* is also reported (Varghese *et al*., 1993; Barna and Wakhlu, 1988; Mathew and Hariharan, 1990; Encina *et al*., 1994).

In the present study, nodal segments of *Murraya* yielded a compact, nodulated callus from the cut ends on MS added with NAA along with CW or BAP comprising a few embryo-like structures which failed to grow further. However, Arya and Arya (1995) have successfully regenerated plantlets from organogenetic calli derived from axillary buds of *Betula*. In stem cultures of four varieties of *Codiaeum variegatum*, although initial proliferation occurred on basal medium alone, for profuse callusing addition of CW alone or along with auxins was necessary (Gayatri, 1975). Similarly, in the present study, better growth of axillary shoots as well as callusing from cut end in nodal segment cultures of *Murraya* was observed at higher levels of CW along with IBA and NAA.

**Responses of epicotyl segments and leaflets**:

The auxins are known either to induce or enhance callusing and rooting in a majority of plant organs and tissues reared *in vitro* (Street, 1969). The stem and leaf segments of *Malvavisus arboreus*, produced both callus and roots in presence of IAA in the medium (Thulajappa, 1982). Similarly, in the present study both the epicotyl segments and leaflets of *Moringa* yielded both callus and roots on MS with lower concentrations of IAA, IBA or NAA and only callus at 5.0 mg/l.
The leaflets of *Murraya*, produced nodulated, compact callus on MS with 5.0 and 10.0 mg/l of IAA or IBA. But at 5.0 mg/l of NAA in addition one or two roots also differentiated and the callus contained embryo-like structures. Recently Gill *et al.* (1995) reported embryogenic callus from leaves in the presence of NAA in *Citrus*. Earlier, Rangaswamy (1958) and Sabharwal (1962) had induced pseudobulbils in nucellus culture of *Citrus* on media containing CH which acquired the form of typical dicotyledonous embryos.

In many explants the induction of callus was achieved in presence of 2,4-D in the medium (Bajaj *et al.*, 1981; Grewal, 1996). In the present study also, 2,4-D alone and along with BAP was found to be very effective in inducing callus from explants of *Moringa* and *Murraya*. The epicotyl segments of *Moringa* developed a slow growing callus on MS supplemented with BAP alone, but the leaflets differentiated shoot buds at 1.0 and 2.0 mg/l of 2,4-D. The involvement of BAP alone on shoot induction from seedling leaf explants of *Annona* (Nair *et al.*, 1984) and mature leaf explants of *Garcinia* and *Morus* (Goh *et al.*, 1988; Mhatre *et al.*, 1985) has also been reported. However, the leaflets of *Murraya* developed only a nodulated callus in presence of BAP.

The combination of auxin and cytokinin in particular proportion has also been found to be essential for organogenesis in many plant species (Grewal and Atal, 1976; Lazzeri and Dunwell, 1984; Omura *et al.*, 1987; Goh *et al.*, 1988). In *Leucama leucocephala*, Nataraja and Sudhadevi (1984) reported initiation of shoot buds and roots directly from epicotyl and cotyledonary segments on a modified MS supplemented with 2,4-D or IAA individually and also in different combinations
with KN, CW and CH. In the present study, in cultures of epicotyl segments and leaflets of *Murraya* all the combinations of IAA, IBA or NAA with BAP tried, induced callus except at 5.0 mg/l of IAA or IBA, 0.5 and 1.0 mg/l of NAA along with BAP in addition the roots also were formed.

The first observation of *in vitro* somatic embryogenesis made in *Daucus carota* (Steward *et al.*, 1958; Reinert, 1958, 1959) was a milestone in plant developmental biology and introduced an entirely new method of plant propagation. Since then induction of embryoids has been obtained in a wide variety of species (see Raghavan, 1976; Ammirato, 1983; Wann, 1988; Litz and Grey, 1982).

In woody plant species the explants from seedlings generally exhibit a greater ability for somatic embryogenesis (Mott, 1981). There are reports on embryo differentiation on basal medium alone (Nataraja and Konar, 1970; Hu *et al.*, 1978; Gingas and Lineberger, 1989; Veietez and Barceila, 1990), but most of the woody plants required the presence of synthetic auxins or other growth adjuvants in the nutrient medium and their requirement also varied with species to species (Litz and Grey, 1992).

In the present study, primary cultures of epicotyl segments and leaflets induced nodulated, compact callus on MS supplemented with 1.0 and 2.0 mg/l of NAA along with different concentrations of BAP, and micropreparation of such callus revealed the presence of a few multicellular structures. On the contrary, similar explants on MS with 2,4-D alone, developed a pale-yellow friable callus comprising filamentous to globular multicellular structures. But mature embryoids were not observed even after transfer of such callus to fresh medium. However, in
the presence of both 2,4-D and BAP, both the explants differentiated a few embryoids indirectly. The embryoids developed on epicotyl segments reached only up to heart-stage. But almost all developmental stages simulating normal embryogeny starting from early stages to the mature stage with elongated radicle were recovered from cultures of leaflets on MS containing 2,4-D and BAP at different concentrations. In Murraya, the leaflets also developed a friable callus on MS supplemented with 2,4-D alone which contained multicellular structures and globular embryoids. In presence of both 2,4-D and BAP only a profuse mass of brownish callus with fresh proliferation on it was formed.

Nalini et al. (1996) reported callusing and somatic embryogenesis in Cajanus cajan from immature seedling parts like segments of epicotyl, leaflets and cotyledons on MS containing NAA and BAP. However, conversion of somatic embryos to plants was obtained only from cotyledon segments. On the contrary, Torregrosa et al. (1996) reported the induction of somatic embryogenesis and regeneration of plants by utilizing in vitro leaves from proliferating nodal segments in Vitis X Muscadina hybrids. They developed friable callus on MS with 2,4-D which became progressively brown, but white to yellow nodular embryogenic callus continued to proliferate from the original callus over a six months period, bearing globular embryoids in clusters. Further plantlets were recovered from mature embryoids on transfer of embryogenic callus to media added with IAA and BAP.

Responses of subcultured callus:

The development of callus from tissues and organs cultured in vitro is a common phenomenon and the callus can be used as inocula to study various
aspects of growth and differentiation. Gautheret (1950, 1955) reported the growth of carrot callus in the absence of auxins in the medium. However, Levin (1951) and Wiggans (1954) were able to induce growth in carrot root callus by the addition of auxins. Similarly, in tissue culture of several other species, auxins like IAA, IBA, NAA or 2,4-D are known to support good callus growth (Venverloo, 1973; Minocha and Mehra, 1974; Wernicke et al., 1982). In the present study, the subcultured calli derived from epicotyl segments and leaflets of Moringa continued to grow further to form a profuse mass of callus on MS containing 2,4-D. Similarly, Thulajappa (1982) reported profuse growth of callus derived from stem and leaf explants of Hibiscus on medium containing 2,4-D.

There are yet other reports on cultures wherein combinations of auxins and cytokinins at different levels are found necessary for callus growth. In Amaryllis bulb callus culture, the combination of 2,4-D and KN induced better growth than 2,4-D alone (Bapat and Narayanaswamy, 1976). Similarly, in Helianthus and Guizotia, calli derived from both hypocotyl and cotyledon segments culture exhibited better growth on media supplemented with combination of IAA and KN as compared to their individual effect (Ganapathi, 1990).

In Catharanthus roseus, both IAA and NAA induced roots but 2,4-D inhibited their formation (Druva et al., 1977). The inhibitory effect on rooting by IAA and NAA has also been demonstrated in callus cultures of Populus nigra (Venverloo, 1973). In the present study, the callus from both epicotyl and leaflets of Moringa showed better growth but failed to differentiate roots on MS with 2,4-D alone. Similarly, in all callus cultures of seedling parts of Sida verrucifolia, 2,4-D
failed to induce rooting (Patil, 1978). Many reports are also available wherein different auxins along with BAP induced growth of callus (Sharma and Mitra, 1976; Mathews and Rao, 1984; Kothari and Chandra, 1986).

Extensive investigations on callus cultures have indicated that the transfer of callus derived on high to low auxin media is necessary for successful somatic embryogenesis (Wernicke and Brettelle, 1980; Subhadra et al., 1995) and it is also often achieved by transferring to auxin free medium (Rajasekaran et al., 1983; Sharma et al., 1993). Sometimes the requirement of cytokinin and abscisic acid was also found necessary for embryogenesis (Thorpe, 1988). There are also good number of examples where both auxin and cytokinin combinations in the nutrient media induced somatic embryogenesis (Jaidka and Mehra, 1986; Rangaswamy, 1986; Rout and Das, 1994).

In the present study, although calli derived from epicotyl segments and leaflets showed better growth on MS with 2,4-D alone but failed to show differentiation of mature embryoids. The callus growth was active for about four weeks on media containing 2,4-D and BAP. However, several bipolar embryoids were differentiated from calli of both explants origin on MS containing lower level (1.0 and 2.0 mg/l) of 2,4-D along with different concentration of BAP which failed to grow further on MS alone. Similarly, all concentrations of 2,4-D (0.25 to 5.0 ppm), tried in stem and leaf callus of Hibiscus by embryoids differentiated remained at globular or heart-shaped stage (Thulajappa, 1982). Grewal (1996) also induced somatic embryogenesis from leaf explants of Bunium persicum on MS supplemented with 2,4-D and KN. The hypocotyl explants of Ammi majus (Grewal and Atal,
Santalum album (Rao and Bapat, 1978), Curcubita pepo (Jelaska, 1972),
Solanum melongena (Matsuoka and Hinata, 1979), Citrus (Gill et al., 1995, 1996)
and leaf of Brassica (Pareek and Chandra, 1978), Cucumis sativus (Melepszy and
Orczyk, 1983), Cocos nucifera (Raju et al., 1984) and Solanum melongena (Gleddie
et al., 1983) have also been utilized to achieve somatic embryogenesis. Gill et al.
(1995, 1996) also reported somatic embryogenesis and plant regeneration in Citrus
epicotyl and leaf segments callus culture on MS + NAA + KN and MS + 2,4-D +
KN.

**Responses of segments of cotyledon and hypocotyl**:

In the present study, cotyledon segments of Murraya produced callus
and roots on MS supplemented with NAA alone, and hypocotyl segments induced
shoot buds in addition to roots and callus. On MS supplemented with IAA, IBA and
2,4-D both the explants induced nodulated callus. The juvenile explants like stem
and embryo of Prunus and leaf of Passiflora also developed shoots in presence of
NAA alone (Mehra and Mehra, 1974; Scorza and Janick, 1976). On the contrary,
in hypocotyl, stem and cotyledon cultures of Pterotheca and Muscari lower levels of
IAA promoted shoot bud differentiation (Mehra and Mehra, 1971; Hussey, 1975).

In Carthamus (George and Rao, 1982) and Guizotia (Ganapathi and
Nataraja, 1993), the cotyledon segments produced shoot buds in presence of BA but
hypocotyl segments did not respond. However, in the present study cotyledon
segments of Murraya yielded only callus on MS containing BAP alone while
hypocotyl segments differentiated a few shoot buds. Loh and Rao (1989) also
reported the regeneration of shoot buds from hypocotyl segments of guava on MS
with or without BA.
The involvement of KN especially in bud formation has been demonstrated in cultures of root in *Convolvulus* (Bonnet and Torrey, 1963), leaves and petioles of *Nicotiana* (Gupta *et al.*, 1966, Prabhudesai and Narayanaswamy, 1974), leaves and endosperm of *Taxallus* (Johri and Nag, 1970). In *Guizotia* the shoot buds originated from cotyledon and hypocotyl segments on MS with KN, elongated further and produced two or three leaves (Ganapathi and Nataraja, 1993). Similarly, in the present study, hypocotyl segments of *Murraya* differentiated shoot buds directly on MS supplemented with KN and a few of them developed further to form one or two pair of leaves.

The differentiation of shoot buds from cotyledon segments has also been reported in apple, watermelon, *Phaseolus*, *Picea*, *Helianthus* and *Aegle* (Kouider *et al.*, 1985; Dong and Jia, 1991; Mallik and Sexena, 1992; Toivonen and Kartha, 1988; Nataraja and Ganapathi, 1989; Hossain *et al.*, 1994). In the present study, the segments of cotyledons of *Murraya* differentiated a few shoot buds on MS containing both IBA and BAP, some of them elongated further to developed into shoots. On the contrary, Nataraja and Ganapathi (1989) reported entire plant regeneration from cotyledonary explants of *Helianthus* on MS supplemented with IBA and KN.

The shoot buds differentiated from leaf and cotyledon cultures of *Glycine clandestina* rooted on hormone-free, half-strength B5 medium with 2 per cent activated charcoal (Hammat *et al.*, 1986). In *Carthamus tinctorious*, the rooting of shoot buds on MS alone with high concentration of sucrose has been reported but the percentage of cultures showing roots was less (George *et al.*, 1982). However,
shoot buds originated from cotyledons and hypocotyl segments of *Guizotia* which required auxin (NAA) for rooting (Ganapathi and Nataraja, 1993). In the present study, the shoot buds derived from segments of cotyledon of *Murraya* were rooted on MS added with IBA, as also reported from cotyledons of *Aegle* (Hossain et al., 1994) and hypocotyls of *Averrhoa* (Islam et al., 1996). The induction of shoot buds from hypocotyl segments of *Olea, Annona, Castanea* and *Averrhoa* has also been reported on media containing both auxin and cytokinin (Bao et al., 1980; Jordan, 1988; San-Jose et al., 1984; Islam et al., 1996). However, in *Capsicum* and *Solanum* adventitious shoot formation has been reported on media containing IAA and BA (Gunay and Rao, 1978; Kamat and Rao, 1978). Similarly, in the present study the hypocotyl segments differentiated a few shoot buds in presence of IBA and BAP. In addition reports are also available regarding the induction of shoot buds on media supplemented with both auxin and cytokinin from hypocotyl as well as cotyledons of *Cucumber, Averrhoa, Malus* and *Guizotia* (Wehner and Locy, 1981; Amin and Razzaque, 1993; Zee and Hui, 1976; Liu et al., 1983; Ganapathi and Nataraja, 1993).

**Responses of petals:**

The investigations on the morphogenetic ability of floral structures of mature trees has been emphasized (Bonga, 1981). Subsequently somatic embryogenesis has been reported in *Cocos, Malus* and *Pyrus* by utilizing inflorescence, flower buds and even petals from field-grown mature trees (Branton and Blake, 1983; Mehra and Sachdeva, 1984; Mehra and Jaidka, 1985).

In the present study, the petals of two cultivars of mature *Punica* species, underwent somatic embryogenesis on MS supplemented with various growth
adjuvants either through callus or directly. The natural auxins like IAA and IBA have more promotive capacity for the formation of mature embryoids at higher concentration as well as for continuous proliferation of embryonic callus along with BAP or KN. Although NAA alone, induced mature embryoids along with frequent budding of older embryoids, browning of portions of callus was observed on media in combination with BAP or KN. The budding of embryoids has been reported in *Citrus*, *Carica*, *Vitis*, *Prunus* and *Juglans* (Button et al., 1974; Litz and Conover, 1980; Kurl and Worley, 1977; Polito et al., 1989; Raj Bhansali et al., 1990). In the present study the petals implanted on MS supplemented with BAP or KN at higher concentrations differentiated embryoids directly in addition to embryogenic callus. Such direct embryoid formation from explants on media containing cytokinin alone has also been reported in leaf cultures of *Coffea* (Dublin, 1981) and cotyledons of *Juglans* (Polito et al., 1989).

Whereas the somatic embryogenesis occurred in juvenile leaf cultures of *Elaeis* on media containing NAA alone (Krikorian and Kann, 1986) the calli derived from flower buds and petals of *Malus*, petals of *Pyrus* required both NAA and BAP (Mehra and Sachdeva, 1984; Mehra and Jaidka, 1985). However, juvenile zygotic embryos of *Olea*, inflorescence of *Cocos* and immature cotyledons of *Prunus* underwent somatic embryogenesis in presence of 2,4-D and BA (Rugini, 1988; Branton and Blake, 1983; Raj Bhansali et al., 1990). The combination of IBA and BA was responsible for promoting embryogenesis in juvenile cotyledons of *Corylus*, *Juglans* (Perez et al., 1983; Tulecke and McGranahan, 1985) and leaf explants of *Camellia* (San-Jose and Vietez, 1993). Embryoid formation from calli derived from flower buds of *Sida veronicifolia* on MS containing IAA, IBA, 2,4-D, KN and
adenine has also been reported (Patil, 1978). Similarly, in the present study, MS with IAA, IBA, NAA, 2,4-D, BAP and KN individually and in a few combinations induced embryoids in petals cultures of both *Punica* var. 'flore-pleno' and cv. Jyoti.

Jaidka and Mehra (1986) reported somatic embryogenesis in callus derived from seedling parts of *Punica* cv. Kandari. Similarly, in the present study, the entire plantlets were regenerated via somatic embryos on subculturing embryogenic callus of both the cultivars of *Punica* to MS with half-strength salts, 1.0 mg/l each of IAA or IBA and BAP along with 4 per cent sucrose. The transplantable plantlets were recovered on transfer of such plantlets to MS supplemented with half-strength salts and subsequently established in soil. Mehra and Jaidka (1985) also reported the induction of embryogenic callus and development of some plantlets from petal explants of *Pyrus*. Litz (1988) and DeWald *et al.* (1989) have achieved normal somatic embryo development and germination in *Euphoria* and *Mangifera* on a medium enriched with 6 per cent sucrose and 20% CW. Similarly, in the present study, higher level of sucrose favoured plantlet formation.

According to Omura *et al.* (1987a), the percentage of explants, forming adventitious shoots was much lower in the fruit cultivar compared to the dwarf ornamental clone of *Punica*. In *Garcinia*, the young leaves in which anthocyanin pigments were dominant, more readily underwent organogenesis (Goh *et al.*, 1990). In the present study, red petals of both the cultivars of *Punica* showed high potentiality for somatic embryogenesis. The detailed histological study of the initiation and development of embryoids *Punica* and *Moringa* showed resemblances to stages in normal embryogenesis.
From the present study, it is evident that the presence of growth adjuvants at various concentrations in the culture medium are important for the regulation of organogenesis and embryogenesis. Further, there also exists similarities and dissimilarities among the different plants and even different parts of the same plant in their ability to produce organs and embryoids either directly or indirectly under almost identical nutrients and hormonal conditions.

The present study on *Moringa*, has demonstrated that, the induction of somatic embryogenesis is possible in both primary and secondary cultures of epicotyl segments and leaflets, which can provide useful information about likely response of a particular mature tree explants *in vitro*.

In *Murraya* the multiple shoot formation from nodal segments, adventitious shoot formation from segments of cotyledons as well as hypocotyl and the tendency towards somatic embryogenesis in leaflet cultures would be of potential use in rapid multiplication and selection studies.

Somatic embryogenesis in petal cultures of field-grown mature plants of *Punica* var. 'flore-pleno', offers new opportunities for its improvement, somaclonal variations and large scale multiplication.

Similar response from petals cultures of commercially important, soft seeded, fruit yielding plant *Punica* cv. Jyoti without any brown exudate can form a strong basis for its advanced studies and commercial exploitation.