SUMMARY

The investigations were undertaken on *Moringa oleifera*, *Murraya koenigii* and two varieties i.e., 'flore-pleno' and Jyoti of *Punica granatum*. The cultures were raised from seeds, shoot tips, segments of node, epicotyl, cotyledon, hypocotyl, entire leaflets and petals with a view to induce plants directly or through callus. The important findings are summarised below:

1. *Moringa oleifera* Lam. cv. Dhanaraj

   It is commonly known as drumstick tree, a popular, woody perennial, grown for its tender pods, leaves and flowers which are used as vegetable. In addition almost all its parts are used in ayurvedic and native medicine.

   **Culture of seeds:**

   The seeds reared on MS alone and MS added with lower concentrations (0.5 and 1.0 mg/l) of IAA, IBA or 0.5 mg/l of BAP, germinated into normal seedlings. But the seedlings obtained were robust on MS containing 2.0 mg/l of IAA or IBA, and in addition, at 5.0 mg/l, the proliferation of callus on the main root along with many laterals was observed. Although seedlings produced on MS with lower concentrations (0.5 and 1.0 mg/l) of NAA had delicate shoot system, the root system was inhibited due to the callusing at radicular end. At 2.0 and 5.0 mg/l of NAA, callusing occurred even from cotyledons with the suppression of shoot growth. Axillary shoots also developed from cotyledonary nodes on MS added with 1.0 and 2.0 mg/l of BAP. Similarly on MS with a few combinations of IAA and BAP also, the seedlings produced axillary shoots in addition to the development of main shoots.
Culture of shoot tips:

On MS alone, shoot tips failed to grow. But on MS with IAA or IBA (0.5, 1.0, 2.0 and 5.0 mg/l) they continued to grow further differentiating callus and roots from the cut end. Maximum number of roots were observed at 5.0 mg/l of IAA or IBA. Though NAA at 0.5 mg/l, favoured slight elongation of shoot tips and proliferation of callus at cut end, at 1.0 and 2.0 mg/l of the same inhibited shoot growth and favoured callusing and rooting. But at 5.0 mg/l of NAA, only a nodulated callus was induced. Similarly, on MS with 0.5, 1.0, 2.0 and 5.0 mg/l of 2,4-D, the explants yielded a pale-yellow, non-friable callus suppressing the shoot growth. On MS supplemented with 1.0 and 2.0 mg/l of BAP axillary shoots developed along with non-friable callus from cut end. Interaction of IAA or IBA along with BAP (0.5, 1.0, 2.0 and 5.0 mg/l) promoted further growth of shoot tips and callus from cut end. Although initially on MS with 1.0 and 2.0 mg/l of NAA and BAP, shoot tips grew further, but due to the rapid proliferation of callus they failed to elongate. At 5.0 mg/l of NAA along with all concentrations of BAP, the explants yielded only the callus.

Culture of nodal segments:

On MS alone, the explants initiated axillary shoots with slight swelling from cut end. But on MS with IAA or IBA (0.5, 1.0, 2.0 and 5.0 mg/l), they produced solitary axillary shoots and a few roots from cut end in addition to callus. However, MS supplemented with 0.5 and 1.0 mg/l of NAA, promoted slight elongation of axillary shoots along with moderate mass of callus and roots. At 2.0 mg/l of NAA, the explants differentiated stout roots and the axillary growth of
shoot was suppressed. While on MS with 5.0 mg/l of NAA only callus was induced. Similarly pale-yellow mass of non-friable callus was formed from the explants on MS containing 2,4-D (0.5, 1.0, 2.0 and 5.0 mg/l). On the contrary, on MS with 1.0 and 2.0 mg/l of BAP alone and along with 0.5 mg/l of NAA, multiple shoots developed. Such shoots obtained on BAP media on transfer to MS + IAA (0.5, 1.0, 2.0 and 5.0 mg/l), regenerated into plantlets. The combined effect of IAA or IBA and BAP at all concentrations tried, favoured axillary shoot development. Similar responses were also noted on MS containing 0.5 mg/l of NAA and 0.5, 1.0, 2.0 and 5.0 mg/l of BAP. But other combinations of NAA and BAP induced only the callus.

Culture of epicotyl segments:

On MS alone, the explants swelled slightly but on MS with IAA, IBA or NAA (0.5, 1.0 and 2.0 mg/l), they produced callus and roots. However at 5.0 mg/l of the above auxins, explants yielded only callus. Similarly, on MS containing 2,4-D (0.5, 1.0, 2.0 and 5.0 mg/l) the epicotyl segments proliferated into a pale-yellow callus, which comprised multicellular structures. Only a slow growing, scanty mass of brittle callus was formed on MS with BAP (0.5, 1.0, 2.0 and 5.0 mg/l). On MS added with IAA or IBA and BAP at all levels a whitish mass of non-friable callus was induced. The roots differentiated at 5.0 mg/l of IAA or IBA along with lower concentrations BAP. Similarly, on MS containing combinations of NAA (0.5 and 1.0 mg/l) and BAP (0.5 mg/l), the explants produced a few roots and callus, the latter comprised of multicellular structures. On MS + NAA and BAP (each at 1.0, 2.0 and 5.0 mg/l), a nodulated callus was induced which masked the entire explants. On the other hand, MS with 2,4-D (0.5, 1.0 and 2.0 mg/l) along
with different concentration of BAP the explants yielded the callus which contained embryoids upto heart-shaped stage.

The subcultured callus continued to grow on MS supplemented with 2,4-D (0.5, 1.0, 2.0 and 5.0 mg/l), but the young embryoids failed to reach maturity even after six weeks. But a few combinations of 2,4-D (1.0 and 2.0 mg/l) and BAP (2.0 and 5.0 mg/l), induced mature embryoids.

Culture of leaflets :

The leaflets on MS alone dried within six weeks of culture. But on MS with IAA, IBA or NAA (0.5, 1.0 and 2.0 mg/l) they developed roots and callus and at 5.0 mg/l a moderate mass of nodulated callus was formed. However, in presence of 2,4-D (0.5, 1.0, 2.0 and 5.0 mg/l), the explants developed pale-yellow callus. The callus derived on MS + 2,4-D (5.0 mg/l), comprised of multicellular structures. On MS + BAP (1.0 and 2.0 mg/l), in addition to white callus shoot buds were also differentiated on the surface and a few of them elongated further on MS with BAP (2.0 mg/l). IAA or IBA along with BAP favoured only scanty to moderate mass of callus. Similarly, the combinations of NAA and BAP, induced a nodulated callus. But, MS with 0.5 and 1.0 mg/l of NAA and 0.5 mg/l of BAP also developed a few roots. On the contrary, almost all stages of embryoids which had resemblances to stages in normal embryogeny were recovered from leaflet cultures on MS containing different concentrations of 2,4-D and BAP.

The subcultured callus on MS with 2,4-D developed further, and at 5.0 mg/l filamentous, multicellular structures were more prominently present. On the contrary, on MS + 2,4-D (2.0 mg/l) + BAP (2.0 and 5.0 mg/l), many somatic
embryos were differentiated. The various stages of embryoid development from single cell to mature stages were also traced.

2. *Murraya koenigii* Spreng cv. Local

It is commonly known as Indian curry leaf tree, originated in the Tarai region of Uttar Pradesh, India. It is grown for its vitamin-rich, mineral packed leaves which are used in Indian cookery for flavouring food stuffs and has medicinal value and also for its oil which is used in soap industry. The seedlings were raised *in vitro* and their different parts were used for raising cultures.

**Seed germination:**

The decoated green seeds reared on MS alone germinated normally and an average of four seedlings were obtained per seed revealing their polyembryonic nature. The seedlings with strong primary root were developed within four weeks.

**Culture of shoot tips:**

The excised shoot tips with youngest leaf reared on MS alone remained green for about six weeks without any growth. But in response to 5.0 mg/l of IAA, IBA or NAA, they grew further and produced a brownish mass of callus from cut end along with one or two strong roots. The better growth of shoot tips occurred on MS with NAA. On the other hand, on MS containing 5.0 mg/l of BAP or KN, shoot tips grew slightly, with the expansion of one or two leaves. But the combined effect of IAA and BAP favoured only elongation of shoot tips and callus formation at cut end. But on MS with 5.0 mg/l each of IAA and BAP, in addition to callus proliferation at cut end stunted growth of shoot tips was noted.
Culture of nodal segments:

The nodal segments with axillary buds reared on MS alone though sprouted in all the cultures but failed to elongate further even after eight weeks. On MS with IAA, IBA or NAA (1.0, 2.0 and 5.0 mg/l) the axillary buds developed into shoots along with a brownish, callus from cut end. The luxuriant growth of axillary shoots with expanded leaves was observed at 5.0 mg/l of above auxins. However, on MS containing 2.0 mg/l of BAP, multiple shoots with a tuft of leaves were developed and rooting of isolated shoots was achieved on MS with NAA (5.0 mg/l). On the contrary, in presence of both, IAA or IBA along with BAP, explants developed axillary shoots and callus from cut end.

The addition of CW (15, 20 and 25%) to MS also supported the growth of axillary shoots which were very healthy at 25% of CW. Similarly, on MS supplemented with CW (25%) and different concentrations of IAA or NAA, axillary shoots were established. On MS containing 25% of CW and 5.0 mg/l of IBA or NAA, the growth of axillary shoots was more vigorous along with brownish mass of callus from cut end comprising a few embryo-like structures which failed to grow further. Almost parallel results were noted from nodal segments on MS with 25% CW + NAA + BAP at different levels.

Culture of leaflets:

The leaflets cultured on MS alone remained green for about four weeks and gradually turned brown. On MS with IAA or IBA (1.0 and 2.0 mg/l), they initiated scanty, brownish black, mass of callus but on MS containing 5.0 and 10.0 mg/l of IAA or IBA, the callus formed was moderate and hard with a few
whitish proliferations on its surface. Similar observations were also observed on MS containing NAA. At 5.0 mg/l of NAA, roots also differentiated in addition to callus. However, a few embryo-like structures were recovered from such callus. On the contrary, on MS with 2,4-D (1.0, 2.0, 5.0 and 10.0 mg/l), the explants developed a brownish callus initially which gradually grew to form a whitish mass of tissue and contained embryoids.

IAA or IBA along with BAP at all levels also induced brownish nodulated callus. The histological preparation of cultured leaflet showed the proliferation of cells occurred from mesophyll region. The combinations of NAA and BAP, also induced a whitish mass of callus. On the contrary, on MS with 1.0, 2.0 and 5.0 mg/l of 2,4-D and BAP at different concentration profuse mass of brownish callus along with whitish proliferations was noticed.

Culture of cotyledonary segments :

The green cotyledonary segments reared on MS alone turned brown gradually. On MS with IAA or IBA (5.0 mg/l), they produced a brownish-black mass of callus. But on MS containing 5.0 mg/l of NAA, in addition to a scanty callus, one or two roots also differentiated. On MS supplemented with 1.0, 2.0 and 5.0 mg/l of 2,4-D also, the explants yielded a brownish-black mass of hard callus. Similarly, on MS added with 5.0 mg/l of BAP a light brownish callus along with a few whitish patches of proliferations was developed. The interaction of IAA or IBA with BAP resulted in the formation of brownish, nodulated callus. Interestingly, on MS containing 5.0 mg/l of IBA and 2.0 mg/l of BAP, shoot buds were also regenerated. Some of these buds elongated and developed into shoots on a
similar medium and rooting of such buds occurred, on transfer to MS added with IBA (5.0 mg/l). Though the combination of NAA and BAP induced a nodulated callus but failed to show organogenesis.

Culture of hypocotyl segments:

The green hypocotyl segments reared on MS, turned brown gradually. But on MS with IAA or IBA at 5.0 mg/l, they proliferated into a brownish-black mass of nodulated callus. On MS containing 5.0 mg/l of NAA, in addition to a scanty callus, roots and shoots also regenerated. On MS added with 5.0 mg/l of 2,4-D, a brownish mass of callus was formed which comprised embryoids up to late globular stage. However, the explants differentiated a few shoot buds on MS with 2.0 and 5.0 mg/l of BAP or KN and some of them elongated further to give rise to one or two leaves, at 5.0 mg/l of KN. The histological studies of cultured hypocotyl revealed that the shoot buds originated from cortical region.

3. *Punica granatum* L. var flore-pleno

It is an attractive, small, woody tree with bright red flowers containing numerous petaloid stamens, grown throughout India as an ornamental plant. The extract of different parts of the tree exhibit antibiotic property and the flowers yield a red dye used for dyeing clothes. The petals from flower buds of field grown plants were used for raising cultures.

On MS alone, the excised petals gradually turned brown. But on MS supplemented with IAA, IBA or NAA (1.0, 2.0 and 5.0 mg/l), they proliferated at a few localised regions with gradual decolouration, yielding a friable embryogenic
callus. The latter developed on MS with 5.0 mg/l of above auxins was profuse and comprised of both early stages as well as mature embryoids. On the other hand, on MS containing 1.0, 2.0 and 5.0 mg/l of 2,4-D, explants produced a scanty callus which contained embryoids up to globular stage.

On MS supplemented with BAP or KN (1.0, 2.0 and 5.0 mg/l), petals proliferated into a slow growing, greenish embryogenic callus. Interestingly, a few well organised embryoids differentiated directly from the explants at 5.0 mg/l concentration. Some of them developed only shoots with one to two leaves but their radicle growth was arrested. The petals cultured on MS containing IAA, IBA or NAA (1.0, 2.0 and 5.0 mg/l) along with BAP or KN at all levels, yielded a slow growing embryogenic callus. The combination of auxins along with 1.0 mg/l of BAP or KN yielded subculturable callus. However increased levels (2.0 and 5.0 mg/l) of BAP or KN induced only a nodulated callus. The subculturable callus on MS alone, continued to grow without forming mature embryoids. However, embryoids and plantlets were regenerated, in subcultured callus on MS with half-strength salts. The histological studies of cultured petals revealed that the callus originated from subepidermal as well as near the vascular regions. Several stages of embryoid development from two-celled to the stage of germination were also traced from squash preparation of the callus. The regenerated plantlets were also established in soil.


It is a small woody tree cultivated worldwide in arid subtropical regions, for its delicious fruits. The leaves roots, seeds and rind of the fruits have
medicinal value. The rind also constitutes tanning material. The petals from field grown clonal plants were used for raising cultures.

The petals cultured on MS alone turned brown within four weeks. But in response to IAA, IBA or NAA (1.0 and 2.0 mg/l), the proliferation of cells initiated after two weeks of cultures at margin and at a few localised regions followed by decolouration of petals resulting in a moderate callus. At 5.0 mg/l of these auxins, the rate of proliferation increased and a profuse mass of callus was formed along with globular structures on its surface. The globular structures gradually organised into embryoids and developed roots in about eight weeks. On the contrary, on MS with 5.0 mg/l of 2,4-D, a scanty callus with a few embryoids was noticed. On MS supplemented with 1.0 and 2.0 mg/l of BAP or KN, the callus formed was slow growing, embryogenic but failed to produce mature embryoids. However, at 5.0 mg/l of above cytokinins, in addition to a slow growing callus, several green outgrowths directly organised into embryoids in about twelve weeks and developed shoots.

All the combinations of IAA, IBA or NAA (1.0, 2.0 and 5.0 mg/l) along with BAP or KN induced embryogenic callus. Interestingly, on MS containing 5.0 mg/l each of IAA and BAP, callus with numerous embryoids with multilobed and fused cotyledons was observed. The squash preparation of actively growing callus revealed the presence of several stages which simulated the stages in normal angiosperm embryogeny. However, plantlets could be ensued from these embryoids only after transferring the callus to the similar medium with half-strength salts and four per cent sucrose.