SUMMARY

The present study deals with the studies on *in vitro* propagation of two forest trees and one medicinal plant.

*Terminalia arjuna*, *Madhuca longifolia* and *Mentha piperita* were selected for the present study.

Axillary buds from mature trees of *T. arjuna* and *M. longifolia*, and pot grown plants of *M. piperita* were cultured for shoot initiation. MS medium was found suitable than SH and WPM.

In *T. arjuna* the shoot initiation, within 10 days, was 52.5%, 48.1% (within 12 days) in *M. longifolia* and 71.3% (within 14 days) in *M. piperita*. Addition of GA₃, at low concentration, reduced the number of days of shoot initiation in all three plants.

PVP controlled the browning of explants effectively than AC and/or AA, but it affected the further growth of explants.
In shoot multiplication the maximum number of shoots/explant was 4.6 in T. arjuna, 3.3 in M. longifolia and 8.6 in M. piperita. BAP alone was not suitable for shoot multiplication in T. arjuna and M. longifolia. Addition of NAA increased the number of shoots/explant in M. longifolia, but it induced callus formation at basal end of shoots in all three plants.

MS (halfstrength macronutrients) + BAP (0.01 mg/l) showed maximum of 2.3 cm and 2.1 cm of shoot elongation in T. arjuna and M. longifolia, respectively. Addition of GA₃ at low concentration to the above medium was found to be suitable for maximum shoot elongation (4.9 cm) in M. piperita.

Maximum percentage of callus initiation on leaf discs was 73.6% in T. arjuna, 71.1% in M. longifolia and 76.6% in M. piperita. 2,4-D was found to be the suitable auxin for callus initiation in T. arjuna and M. longifolia, but for M. piperita NAA was the suitable auxin. Addition of KN with BAP showed better responses in all three plants.

The maximum percentage of shoot differentiation from callus was 13.3 in T. arjuna, 7.3 in M. longifolia and 22.3 in M. piperita. Maximum number of 3, 4 and 6 shoots/callus were differentiated in T. arjuna, M. longifolia and M. piperita, respectively. GA₃ was found ineffective for shoot differentiation from calli.
Number of catalase isozyme variants among plants differentiated from calli was 1 each in *T. arjuna* and *M. piperita*. No variant was observed in *M. longifolia*.

The percentage of rooting of excised shoots was 33.6 in *T. arjuna*, 18.5 in *M. longifolia* and 62.4 in *M. piperita*. Addition of NAA showed excessive callus formation at basal end of shoots.

The survival of rooted plants after their transfer from medium to soil was 21%, 58% and 56% in *T. arjuna*, *M. longifolia* and *M. piperita*, respectively.

Globular-shaped somatic embryos were observed on calli grown on callus culture medium with glutamine. The number of embryos differentiated directly on leaf discs of *T. arjuna*, *M. longifolia* and *M. piperita* were 7.3, 0 and 6.6 per leaf disc, respectively. Amount of embryogenic callus formation reduced when glutamine concentration was increased.

Globular-shaped somatic embryos isolated from 1 ml of suspension culture of callus was 362 in *T. arjuna*, 174 in *M. longifolia* and 232 in *M. piperita*. The embryos were developed to the heart-shaped stage after 14 days and to torpedo-shaped stage after 28 (*T. arjuna*), 35 (*M. longifolia*) and 14 (*M. piperita*) days.
Mature somatic embryos (late torpedo or cotyledonary stage) were germinated on solid MS medium. No germination was observed in *T. arjuna*. 4.3% of germination was observed in *M. longifolia*, but after a certain period the development was arrested. In *M. piperita* 9.6% of embryoids were germinated and they were developed into complete plants.

Maximum protoplast yield/g fresh weight was 8.5 (81.1% viability), 6.1 (86.4% viability) and 8.6 (82.8% viability) x 10⁴ in *T. arjuna*, *M. longifolia* and *M. piperita*, respectively. The protoplasts were cultured in liquid K3 medium. Microcolonies were formed from protoplasts and were cultured to microcalli on semi-solid MS medium. After subcultures calli were developed from microcalli.

The electrophoretic pattern of protoplast-callus proteins resembled the pattern of their leaf-callus proteins in all three plants. So, the protoplast calli may have the regeneration capacity.

Axillary buds of *T. arjuna* showed better shoot initiation during the period of July-August and December-April. In *M. longifolia* July-September and December-April were found to be the suitable seasons. *M. piperita* showed better shoot initiation during the period of September-March. During the period of May-June browning of browning of explants was high and regeneration was almost nil in all three plants.
From these studies the following conclusions may be drawn:

1. **In vitro** propagation of *M. piperita* and mature trees of *T. arjuna* and *M. longifolia* is feasible for clonal multiplication.

2. Micropropagation of *M. piperita* may be useful in propagation of disease free clones.

3. Callus culture may be helpful to produce somaclonal variants with desirable traits.

4. Somatic embryogenesis procedure may be adopted for mass propagation and artificial seed production of these three species.

5. Protoplast isolation and culture procedure can be used for further protoplast culture studies in these plants.