Chapter 6

SUMMARY

Tea is a perennial woody plant, and the tender two leaves and a bud shoots are used for making tea. Shade trees play an important role in increasing productivity of the tea bush. Shade trees help in maintaining leaf temperature at optimum level during the summer season for maximum photosynthesis by tea leaves. Moreover, shade tree protects tea plants from sun-scorch damage by absorbing/reflecting 50% of the harmful infra-red radiation from the solar spectrum. It adds a substantial amount of organic matter to the tea field through droppings of leaves, twigs, pods, etc. Shade trees keep soil cooler which helps in healthy growth of the tea roots.

Conventional method of vegetative propagation has not been possible so far, in the leguminous shade tree species, commonly used in tea plantations, even after a series of systematic experiments. Seeds were the only source of propagation. To meet the requirement of plants for tea plantations a method of fast multiplication of shade tree species became necessary. Therefore, this study was proposed to develop suitable in vitro micropropagation technique for some commonly used shade tree plants.

Mature seeds of the commonly used shade trees, viz. *Albizia odoratissima* (L.f.) Benth., *Albizia chinensis* (Osbeck Merr.), *Albizia lebbeck* (L.). Benth. and the recently released shade tree plant *Anadenanthera peregrina* (L.) Speg. were germinated aseptically in any of the half MS (Murashige and Skoog, 1962), B5 (Gamborg, et al., 1968) and White’s (White, 1963) media to develop contamination free explants. Since our effort failed to establish explants in the culture media due to microbial contamination from field grown plants, attempts were made to use explants from in vitro grown seedlings.

To induce proliferation of multiple shoots the apical buds and internodal segments were used as explant materials in this study and for direct embryogenesis of *Anadenanthera peregrina*, the cotyledon segments of immature, semi-mature and mature seeds were used as explants. In callus culture, cotyledon segments from mature seeds of all the four experimental shade tree plants were utilized as explant.
For *in vitro* shoot proliferation, full strengths of MS, B5 and White's media supplemented with cytokinins like BAP (0.25mg/l to 5.0mg/l) and kinetin (0.25mg/l to 5.0mg/l) alone or in combination of both were used. Cultures were maintained under 16 hours photoperiod with light intensity of 37.40 μ mole m⁻² sec⁻¹ at a culture room temperature of 25±2°C. Best proliferation of microshoots was obtained in MS (full) medium supplemented with BAP at 0.75mg/l (*Albizia odoratissima*) and 1.0mg/l (*Albizia chinensis* and *Albizia lebbeck*). The apical bud explant showed highest proliferation of shoot buds in all the species of *Albizia*.

Among the four species studied for micropropagation, *Albizia odoratissima* showed the best response of 91.67% in shoot proliferation with an average of 10 shoots per explant. In case of *Albizia chinensis* and *Albizia lebbeck*, both response and shoot proliferation per explant was less.

Rooting of the *in vitro* grown micro shoots was found in half strength basal media of all the three MS, B5 and White's media with or without auxins. The rooted plantlets were transferred to small earthen pots filled with only soil or soilrite (obtained from Allied Scientific Products, Kolkata) and mixtures of sand and soil at the proportion of 1:1 and 1:2. The pots containing the rooted plantlets were covered with a polythene bag to keep the surrounding air of the plantlet saturated with moisture. Polybags were removed when plantlets were established. Hardening was completed by gradual exposure to ambient condition. Plants became ready for transfer to field after hardening.

Cotyledons of all the three stages (immature, semi-mature and mature) of *Anadenanthera peregrina* responded to direct embryogenesis. The best response for direct embryogenesis was obtained in MS (full) medium supplemented with IAA 1.0mg/l.

To induce callus development MS (full) media supplemented with 2,4-D, IAA, IBA and NAA were used in the experiment and the media supplemented with 2,4-D 1.0 mg/l yielded best reponse in case of all the three species of *Albizia*. In *Anadenanthera peregrina*, however, response to callus induction was obtained when MS (half) media was supplemented with 2,4-D 3.0 mg/l only. No organogenesis or embryogenesis was observed from these calli.
No major difference was found in the phenotypic characterization between 30-day old *in vivo* seedlings and *in vitro* derived hardened plantlets. Phenotypic studies indicated that 30 days old *in vivo* grown seedlings of *Albizia odoratissima*, *Albizia chinensis* and *Albizia lebbeck* exhibited greater average height than the *in vitro* derived plantlets of same age. But more development of leaves was observed in case of *in vitro* derived plantlets of all the three species.

Four (OPA 11, OPD 08, OPM 02 and OPM 10) out of ten random primers gave clearly scorable and reproducible bands. RAPD studies indicated that though there can be some amount of negligible genetic variation between mother tree and *in vitro* derived seedlings, no variation was detected between the RAPD profiles of the *in vitro* derived plantlets of *Albizia odoratissima*, *Albizia chinensis* and *Albizia lebbeck* as well as *in vitro* derived mother seedlings (seedlings from which the *in vitro* plantlets were obtained). Similarly, in case of *Anadenanthera peregrina*, slight genetic variation was detected between mother tree and *in vitro* derived seedlings, but no variation was detected between the RAPD profiles of the *in vitro* mother seedlings (seedlings from which the somatic embryos were obtained) and *in vitro* derived somatic embryos.

**CONCLUSION**

1. The results of the present study justify that the technique developed for micropropagation of *Albizia odoratissima*, *Albizia chinensis* and *Albizia lebbeck* was simple and easy. A large number of plantlets could be produced within a small period of time and transferred to the field. However, there is scope of further improvement in the technique for increasing the multiplication rate of microshoots and success in hardening and field establishment. Therefore, this method of *in vitro* multiplication has great potential for *in vitro* propagation of the selected shade trees.

2. Further attempts are necessary for establishment of an *in vitro* protocol for micropropagation of *Anadenanthera peregrina*.

3. There is scope for improvement in the technique for development of shoots from callus cultures of all the four species taken under study and development of somaclones with desirable traits.
4. RAPD studies have shown that the micropropagation technique developed can be utilized for *in vitro* propagation of shade trees with desirable traits like uniform shade canopy, disease and pest resistance, etc. without any change of characters. But the genetic integrity of every micropropagated plantlet needs to be confirmed before transferring to the field since it is already indicated in some other species that some variations can exist within a population of micropropagated plants. There is also scope for better optimization of RAPD protocol and PCR reaction by assessing other parameters like primer concentration, enzyme concentration, MgCl$_2$ concentration, dNTP concentration, annealing temperature etc. In our experiments, only the template DNA concentration and number of PCR cycles were optimized.

5. To overcome the hurdle of using explants from vegetative parts of field grown leguminous plants in *in vitro* culture due to endophytic microbial contamination, further studies are required.