INTRODUCTION

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Timber wood is used for construction of house and making doors, floor, roof and furniture Nowadays, several materials like concrete, steel and plastic are widely used as substitute for wood. Nevertheless, the timber wood demand has increased considerably. The demand projection of total timber wood for 1985 was 30,03,000 cum that has increased to 47,180,000 cum for 2000 (Mishra, 1997). This has put pressure on natural tree strands that are shrinking in area and losing quality at an unprecedented rate. Fulfilment of timber wood demand and preservation of natural forests are the major concerns of present time. Plantation forestry is being viewed as an alternative to solve these problems. Plantations of timber trees require superior planting stock for higher yield of timber wood

Regeneration of forest trees takes place through seeds. These trees exhibit a large variation in growth, form and vigor (Ahuja, 1993). Vegetative propagation gives genetically alike replicates of trees possessing desirable characters. Thus vegetative propagation conserves genotype of donor plants in the propagules and captures both additive and non-additive gene effects. However, conventional methods of vegetative propagation like rooting of cutting, layering and budding are slow processes. Plant tissue culture techniques are considered as efficient methods for mass propagation and of elite trees.

There are three common methods of *in vitro* plant regeneration, namely axillary shoot proliferation, organogenesis and somatic embryogenesis (Murashige, 1974, Vasil and Vasil, 1980) In axillary shoot proliferation, shoot multiplication takes place from axillary buds and the plantlets are obtained by rooting of microshoots Several species are propagated utilizing pre-formed buds on shoot tips and nodal cuttings This method has formed the basis for a rapid multiplication system for eucalyptus yielding about one million microshoots per

year from one shoot (Le Roux, 1991), and is also successful with poplar, a rapidly growing forestry species used for pulp, paper and plywood. From a single bud explant about 120 to 220 shoots could be obtained within 6-8 weeks and about one million poplar plantlets could be produced annually from one bud (Ahuja, 1987). In organogenesis, shoots differentiate from unorganized callus and these shoots are rooted on rooting medium. In forest trees, plantlet regeneration through axillary shoot proliferation is more successful than shoot induction from callus. In somatic embryogenesis, bipolar embryos develop from somatic cells. These embryos germinate and form emblings. It is viewed as more effective method for the rapid propagation of selected trees. Main advantages of somatic embryogenesis technique are the low cost of production. the potential for generating artificial seeds and eventually for using an automated system, and suspension systems can provide embryogenic protoplasts which potentially can be used for genetic engineering (Thorpe *et al.*, 1991).

Callus and cell cultures, due to high rate of plant propagation, are potentially useful for commercial production of plants. However, these methods possess high risk of genetic and epigenetic abnormalities which develop during cell proliferation Axillary shoot proliferation is comparatively a slow method of propagation but this has low risk of genetic instability. However, there are some reports that even this method of propagation is not free from the risk of genetic instability (Vajrabhaya, 1977; Swartz *et al.*, 1981, 1983). Many small industries have closed and in many big tissue culture industries in Europe and Asia the production of micropropagated plants has declined mainly due to the realization that they were not producing true-to-type plants (Pierik, 1991; Gavinlertvatana and Prutpongse, 1991). This problem generally arises due to use of inappropriate culture medium, growth hormones and culture conditions, and repeated proliferation of the explants once established in culture (Karp, 1989)

There are three developmental phases in plants namely embryonic, vegetative and reproductive At embryonic and vegetative phases plants have juvenile characters whereas at reproductive phase they bear mature characters Trees have an extended vegetative phase, and maturation starts in them much before

the flowering phase which used to be considered diagnostic for the on set of maturation (Ahuja, 1993). Along with the change of the juvenile to mature state, several morphological and cellular characters are altered in a typical fashion either abruptly or gradually (Bonga and Von Aderkas, 1993). Among various maturation sensitive traits, decline of root ability of woody cuttings (Bonga and Von Aderkas, 1993) and less responsiveness of the tree explants to *in vitro* induction of organogenesis (Ahuja, 1993) directly affect vegetative propagation. Maturation rate is not uniform in entire tree. In the mature tree, at some regions maturation is slower than others (Bonga, 1982; Hacket, 1985). Many hardwood species produce stump sprouts or roots that are more juvenile than mature tree and can be used for clonal propagation (Bonga and Von Aderkas, 1993). A change in a tissue or an organism from a more mature state to a more juvenile one is called rejuvenation. The possibility of rejuvenation by repetitive subculturing was first reported by Febvre (1981) in *Salix babylonica*. Rejuvenation of mature tissue *in vitro* is very useful for micropropagation of trees. Identification of clones is important in clonal forestry. In a clonal forestry program, three types of plants can be recognized namely foundation stock, expansion stock and test plants. Identification of plants is required at all the three stages. An error in the identity of a single foundation plant affects a large fraction or all of it, wrong identification of a single expansion plant affects that proportion of the production produced from it and the mistaken identity of a single test plant simply puts some unwanted into a well designed experiment (Cheliak, 1993). For identification and certification of clone, the characters used must be under genetic control (Cheliak, 1993).

For the present investigation, two important timber tree species namely *Adina cordifolia* and *Gmelina arborea* were selected. *Adina cordifolia* (Hook.) belongs to family Rubiaceae The name *Adina* has been derived from the Greek *adinos*, meaning crowded, with reference to the crowded condition of the flowers in dense balls *cordifolia*, meaning with heart-shaped leaves, refers to the shape of the leaves (Santapau 1966) Its common names are Haldu (Hindi), Paturia and Dakom (Bengali) Heddi (Marathi), Paspu-kadamba (Telgu), Manju-kadamba

(Tamil and Malayalam) and Assin tega and Yettega (Kannada) (The wealth of India, 1948). Haldu is largely used for structural work. It is one of the best indian timbers for paneling railway carriages (The wealth of India, 1948). *Gmelina arborea* (Roxb.) belongs to family Verbenaceae. Its common names are Gumhar (Hindi), White teak or Yamane (English), Shewan (Gujrati), Gomari (Aasam), Shivani (Kannada), Gambari (Oriya), Gomadi (Tamil), Gummadi (Telgu) and Shivan (Marathi) (The Wealth of India, 1956; Gupta 1993). Gumhar is one of the most important multipurpose tree species. Kumar and Kadam (1993) prepared Annotated Bibliography on this species and included the work done on different aspects of this species. Its timber is used for construction work, planking, furniture, cabinet work, paneling carts, boxes, boat building, agricultural implements, turnery, toys, artificial limbs, guns, musical instruments and plywood (Gupta, 1993).

Vegetative propagation through conventional methods such as bud grafting, air layering and rooting of stem cuttings of *G. arborea* has been carried out by several researchers. Arya and Haque (1982) reported grafting and budding in yemane. Rahman (1977) reported successful bud graft and rooting of stem cuttings. Gamhar has been successfully regenerated from stem cuttings of 2 year old plants, 10 month old stumps and 15 month old plants raised from cuttings (Sandum *et al.*, 1989), the stem of about 1 year old tree (Hamasawi and Srivastava, 1988; Tang and Srivastava, 1988), nodes of coppice shoots (Wong and Jones 1986), branches from lower part of crown of 5 year old tree (Surendran and Seethalakshmi, 1987), one node cuttings of 3 year old trees (Surendran, 1990) and stumps of damaged tree (Sabado and Valiente, 1972). There are sporadic reports on micropropagation of *G. arborea* through axillary shoot proliferation (Prasad *et al.*, 1994; Kannan and Jasrai, 1996; Thirunaboukkarasu and Debata, 1998). Thakar and Bhargava (1999) reported seasonal variation in antioxidant enzymes and the sprouting response of nodal sectors cultured *in vitro* There is a need to study *in vitro* propagation of *G arborea* in detail Reports are lacking on macropropagation as well as micropropagation of *A cordifolia*

The main objectives of the research work were as follows.

1. To initiate shoot bud cultures from nodal segments of 3-12 month old saplings, terminal-twigs of 5 year old trees (YOT), basal-sprouts of 5 year old trees (YOT), terminal-twigs of 10 year old trees (YOT) and basal-sprouts of 10 year old trees (YOT) of *Gmelina arborea*.

2. To find out suitable medium for explant establishment, shoot proliferation and rooting of microshoots for cultures derived from above five types of nodal explants from *G. arborea*

3 To study the effects of subculturing on shoot proliferation and rooting of microshoots in *G. arborea*

4. To develop a method for field transfer of plantlets.

5. To initiate shoot bud cultures from apical buds of 3 and 30 year old trees (YOT) of *Adina cordifolia*.

6. To find out suitable medium for establishment of explants, shoot proliferation and rooting of microshoots for cultures derived from above two types of explants from *A. cordifolia*.

7. To find out suitable medium for callus formation from different types of explants of *G arborea* and *A. cordifolia*.

8. To make attempts for inducing differentiation in callus initiated from different types of explants of *G. arborea* and *A. cordifolia*.

9. To prepare peroxidase isoenzyme profiles of some trees of *G. arborea* and *A. cordifolia*. To determine the activities of enzymes guaiacol peroxidase, DOPA (DL-3,4-Dihydroxyphenyl-alanine) oxidase and catechol oxidase in some trees of *G. arborea* and *A cordifolia*.