In Vitro PROPAGATION OF *Ailanthus triphysa* (Dennst.)

By
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THESIS

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DECLARATION

I hereby declare that this thesis entitled **'*In vitro* propagation of *Ailanthus triphysa* (Dennst.)'** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship, associateship or other similar title of any other University or Society.

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Dedicated to my Beloved
and Devoted Parents

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CONTENTS

ABBREVIATIONS

ABA	-	Abscissic acid
AC	-	Activated Charcoal
BA	-	Benzyl adenine
cm	-	Centimeter
cv.	-	Cultivar
dia	-	Diameter
DK medium	-	Driver and Kuniyuki medium
GA_3	-	3-Gibberlic acid
$Hgcl_2$	-	Mercuric chloride
IAA	-	Indole-3-acetic acid
IBA	-	Indole-3-butyric acid
kin	-	Kinetin
mg	-	Milligram
min.	-	Minute
mM	-	Millimolar
mm	-	Millimeter
µm	-	Micrometer
MS	-	Murashige and Skoog (1962) medium
Na_2 EDTA	-	Sodium salt of diamine tetra acetic acid
NAA	-	Naphthalene acetic acid
ppm	-	Parts per million
RH	-	Relative Humidity
spp.	-	Species
TC	-	Tissue culture
TDZ	-	Thidiazuron
UV	-	Ultra Violet
v/v	-	Volume by volume
w/v	-	Weight by volume
WPM	-	Woody Plant Medium of Lloyd and McCown (1980)
2ip	-	N-6-(2- isopentyl) adenine
m	-	Meter
½MS	-	Half-strength MS medium
2,4-D	-	2,4-Dichlorophenoxy acetic acid

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Introduction

INTRODUCTION

Ailanthus triphysa (Dennst.) Alston (Syn: *Ailanthus malbarica* DC), is a fast growing tree species of considerable economic importance belonging to the family simaroubaceae. It is a large, deciduous tree with cylindrical bole and is reported to be a strong light demander and is locally known as matti, perumaram, pongilyam etc in Malayalam. It is distributed in western ghats, from the Konkan, North Kanara and Karnataka south words to Travancore (Kerala) up to 4500 m elevation (Troup, 1981). Large scale plantations of *Ailanthus* are being grown in many parts of the country (Sandhu, 1984). In Kerala, *Ailanthus* is raised in plantations of the Forest Department and is a common member in homestead gardens particularly in places where match industries are prevalent. The wood is soft and light in weight because of which it is largely used for match, packing case and paper pulp industries (Troup, 1981). The bark, gum exudes, roots and leaves are of medicinal value. This also serves as a good standard for pepper in southern Kerala and used as an avenue tree as well as in afforestation, agroforestry and social forestry programmes. The foliage is a good source of green manure.

Ailanthus triphysa is normally seed propagated. Though the percentage of germination of fresh seeds is high, the seeds loose their viability within a month. As a result, planting material is not readily available round the year. Moreover, seed set is affected by the frequent attack by the webworm, *Eligma narcissus* C., on the foliage, terminal apices, inflorescence and fruits (Varma, 1986). Insect infection at the growing tips also seriously affect the growth of the plant leading to stunted growth and croocked bole habit.

Forestry is geared to the production of matchwood, fuelwood, plywood, construction timber and other multifarious products of economic importance. Ever multiplying population and industrialization have enhanced the demand for wood products in India accentuating the pressure on forest resources (Sita, 1993). Mass

production of selected clones through *in vitro* techniques -'micropropagation'- is of great importance in clonal forestry to overcome the constraints like scarce seed supplies, germination problems, long regeneration time etc. (Leaky, 1987). It has long been apparent that genetic gains can be captured by clonal propagation (Durzan, 1988). However, conventional methods of vegetative propagation are not feasible in most of these species. Under such circumstances, *in vitro* propagation is known to be a possible method and being used in many tree species (Bonga, 1982).

Despite its immense popularity and commercial importance, least attention was given to standardise method of *in vitro* propagation of *Ailanthus* to overcome the problems associated with seed propagation and conventional vegetative propagation. D'Silva and D'Souza (1992) could standardize micropropagation method and media in *A. triphysa* using cotyledonary node explants from just germinated *in vitro* seedlings. However, their attempts to develop protocol for tissue culture from mature tree nodes were not successful. Shoot propagation has been a method of choice for micropropagation since, in shoots originating from preformed meristems, genetic integrity is very high, which is very important for maintaining clonal fidelity (Mascarenhas, 1993). To the best of our knowledge till date no reports of successful *in vitro* propagation of *Ailanthus triphysa* using mature nodal explants are available.

The present study was therefore planned to formulate technique for *in vitro* propagation of *Ailanthus triphysa* (Dennst.) Alston using mature axillary and terminal bud explants from seedlings of three to four years of age.

Review of Literature

REVIEW OF LITERATURE

The development of the science of tissue culture can be traced back to the time when German Botanist Haberlandt (1902) succeeded in culturing isolated, fully differentiated cells of *Lamium purpureum* for the first time. He grew palisade cells on Knop's (1865) salt solution with sucrose and observed obvious growth.

The herbaceous plants have strong rooting capabilities and weak apical dominance which account for their great success in propagation using the technique of tissue culture. Propagation through *in vitro* axillary bud proliferation is successfully attempted in numerous herbaceous ornamental and vegetable plants some of which include *Allium, Anigozanthos, Anthurium, Asparagus, Beta, Brassica, Cephalotus, Chrysanthemum, Dianthus, Fragaria* and *Fuchsia*.

Propagation of plants through tissue culture has become an important and popular technique to reproduce crops that are otherwise difficult to propagate conventionally by seed and/or other vegetative means. Specialised and mature cells are manipulated to give rise to multiple copies of the parent plant under optimum aseptic environmental conditions and appropriate stimuli. It offers many unique advantages over conventional propagation methods such as rapid multiplication of valuable genotypes, expeditious release of improved varieties, production of disease free plants, non-seasonal production (round the year), germplasm conservation and facilitating their easy international exchange (Govil and Gupta, 1997).

Until a decade ago scant attention was given for propagation of tree species through tissue culture and only limited success has been obtained. The specialized growth habits of trees pose difficult problems for tissue culturists as they are slow growing coupled with long dormancy and their calli are hard to differentiate (Anad and Bir, 1997). Nevertheless, in the last two decades

considerable progress is achieved on micropropagation of forest trees. Many of the softwood species have been successfully propagated through *in vitro* techniques. Some of the recent reports of success available in *in vitro* clonal propagation of these species include *Pinus pinaster* (David *et al.*, 1982), *Cedrus deodara* (Bhatnagar *et al.*, 1983), *Pinus nigra* (Kolveska-pletikapis *et al.*, 1983), *Euphedra foliata* (Bhatnagar and Singh, 1984), different species of *Picea* (Arnold and Erikson, 1985; Roberts *et al.*, 1992), *Pinus radiata* (Smith, 1986), *Juniperus oxycedrus* (Gomez and Segura, 1994), *Pinus eldarica* (Sen *et al.*, 1994), *Sequoia semepervirens* (Sul and Korban, 1994) etc.

Recently, a number of broadleaved tree species have also been successfully propagated through micropropagation. A detailed review of the recent literature on *in vitro* propagation of hardwood species is presented below:

2.1 Micropropagation

2.1.1 *Acacia* species

Nodal segments from the seedlings of *Acacia mangium* could give rise to plantlets when cultured on MS media containing 0.5 mg l^{-1} BA (Ahmed, 1990).

In *Acacia nilotica* sub sp. *indica* Brenan, the cotyledonary nodes were found to differentiate maximum shoots per explant compared to hypocotyl, root or shoot tip (Dewan *et al.*, 1992). An average of 6.3 shoots per explant was obtained in B_5 medium supplemented with 1.5 mg l^{-1} of N^6 Benzyl adenine (BA). The number of shoots were further enhanced by (i) using nodal explants from *in vitro* regenerated shoots as microcuttings and (ii) repeated subculture of original explants (stumps) on the same medium after excising the shoots. Thus over 700 shoots could be obtained from a single cotyledonary node explant. The shoots were rooted in 100 per cent cultures in B_5 medium supplemented with 2.0 mg l^{-1} of IAA. The plantlets were successfully transferred to soil.

Ruffoni *et al.* (1992) reported that in *Acacia "mimosa"* shoot proliferation was obtained in a medium supplemented with BA, IAA and GA_3. Root induction and development was obtained in media supplemented with 1.0 ppm IAA.

2.1.2 *Albizzia* species

Bharthakur (1992) reported shoot development in case of *Albizzia chinensis* in a semi solid MS medium supplemented with BA at 1.0 mg l^{-1}. The regenerated shoots were rooted in half strength MS medium supplemented with IBA at 25 mg l^{-1}.

Four shoots per explant were produced in *Albizzia julibrissin* when cultured on B_5 medium devoid of growth regulators (Shakhla *et al.*, 1996). The addition of small quantities (0.5 μM) of auxin did not influence shoot regeneration while the high auxin concentrations (5 μM) decreased shoot regeneration. Addition of BA, kin or thidiazuron to culture medium increased both shoot regeneration percentage and the number of shoots per explants. Thidiazuron was highly effective in stimulating shoot formation at lower concentrations (<1.0 μM). The regenerated shoots were rooted in B_5 medium and developed into normal plants.

2.1.3 *Ailanthus* species

D'Silva and D'Souza (1992) induced multiple buds from nodes of mature nodes (NM) as well as from cotyledonary nodes (NC) of the seedlings of *Ailanthus malbarica* (*A. triphysa*). Highest number of buds from NM were induced in MS medium supplemented with 50 mg l^{-1} of activated charcoal, 175.29 mM sucrose and 133.2 μM BA, while in case of NC, the number of buds induced was highest in MS medium supplemented with 175.29 mM sucrose and 88.8 μM BA. They could get

elongation from buds of NC on MS medium supplemented with 22.2 μM of BA and 2.32 μM kin. The elongated shoots were rooted by 15 days treatment on MS medium supplemented with 58.43 μM sucrose and 26.85 μM NAA followed by transfer of the microshoots to MS medium supplemented with 58.43 mM sucrose, 5.37 μM NAA and 53.71 μM IAA. The plantlets were transferred to soil and later were established in the field. However, NM failed to elongate and hence they failed to get rooted.

Cell culture studies (Anderson *et al.*, 1987; Jaziri, 1990; Jaziri and Homes, 1990) and regeneration through organogenesis (Caruso, 1974) have been reported for the temperate species *A. altissima*.

2.1.4 *Anogeissus pendula*

Joshi *et al.* (1991) were successful in obtaining multiple shoots from cotyledonary and epicotyledonary nodes. Cotyledonary nodes which produced 15-20 shoots were found better than epicotyledonary nodes which produced 4-5 shoots when cultured on MS medium containing 1.0 mg l^{-1} BA and 0.1 mg l^{-1} IAA. Shoots were rooted on half-strength MS medium containing 15 mg l^{-1} of IBA and 0.1 mg l^{-1} of kin.

2.1.5 *Anthocephalus indicus*

The maximum number of multiple buds were obtained on MS medium with 1.0 mg l^{-1} BA and 1.0 mg l^{-1} IAA using cotyledonary nodes while the shoot tips produced more shoots on MS containing 0.5 mg l^{-1} BA and 0.5 mg l^{-1} kin. Half strength MS with 0.2 mg l^{-1} IBA was recommended as best for root production (Hoque *et al.*, 1992).

2.1.6 *Azadirachta indica*

Gautam *et al.* (1993) have reported successful micropropagation in *A. indica* through indirect organogenesis. Higher percentage of callus was induced in Nitsch medium supplemented with 10 μM IAA and 1.0 μM BA. Multiple shoots were induced from the callus on MS medium augumented with 4.44 μM BA and 0.53 μM NAA along with 18.75 μM polyvinyl-pyrolidone. Shoots were rooted in MS medium containing 4.9 μM IBA and 18.75 μM polyvinyl-pyrolidone.

Stem segments and stem nodes of mature trees of *A. indica* could give shoot proliferation and plant formation when cultured on MS medium augumented with 0.5 μM thidiazuron (TDZ) and 0.5 μM NAA (Yasseen, 1994).

2.1.7 Bamboos

Rao and Rao (1988) succeeded in inducing somatic embryogenesis in B_5 medium having 2,4-D of 10.3 μM. The somatic embryos could germinate on the same medium. Prutpongse and Gavinlertvatana (1992) reported that 54 of the 67 attempted species of bamboo could be successfully propagated *in vitro*. Multiple shoots could be obtained nearly in all the species on MS medium containing 22 μM BA. Rooting occurred in media containing 2.7-5.4 μM NAA. Indirect organogenesis through callus culture, cell suspension cultures and protoplast cultures was reported by Huang and Huang (1993).

Single node cuttings cultured on MS medium containing 1.0 mg l^{-1} each of BA and 2,4-D could produce multiple shoots and/or callus formation. Addition of 0.5 mg l^{-1} of GA_3 encouraged shoot differentiation in callus cultures. Rapid shoot multiplication was achieved in half-strength liquid medium. Rooting and rhizome formation was achieved when 0.5-1.0 mg l^{-1} IBA was added (Sood *et al.*, 1994).

Rout and Das (1994) produced somatic embryos from the callus derived from nodal explants of *in vitro* grown seedlings and zygotic embryos of *Bambusa vulgaris, Dendrocalamus gigantenus* and *D. strictus*. The media used was half strength MS medium augumented with 0.5 mg l^{-1} kin, 2.0 mg l^{-1} 2,4-D, 10 mg l^{-1} of adenine sulphate and 3 per cent w/v sucrose, incubated in the light or in the dark.

Ravikumar *et al.* (1998) induced multiple shoots from axillary buds of seedlings and mature plants of *Dendrocalamus strictus* nees on MS medium with BA and kin. A maximum of 42±5.2 shoots from seedling explant and 8±0.8 from mature explants were obtained on medium containing 0.5 mg l^{-1} BA + 0.5 mg l^{-1} kin + 200 ml l^{-1} of coconut water. Rooting was induced in MS medium containing 0.25 mg l^{-1} IBA.

2.1.8 *Bauhinia* species

Kumar and Kumar (1992) achieved indirect organogenesis through callus produced from stem sections (of 0.5 cm long) culturing on MS medium supplied with 3 per cent sucrose and 0.8 per cent agar and, various combinations of auxins and cytokinins. The callus was induced on MS medium with 10 µM 2,4-D. The shoot buds were produced by supplementing 5-10 µM of kin and highest rooting (39%) was obtained in MS medium with 5.0 µM of NAA.

Dasgupta and Bhattacharya (1995) successfully induced callus in MS media augumented with 2.0 mg l^{-1} of 2,4-D. The regeneration of plantlet was obtained on MS medium containing 2.0 mg l^{-1} of 2,4-D and 0.5-1.0 mg l^{-1} of BA.

Multiple shoots (5-6 shoots/explant) were obtained in MS medium supplemented with 1.0 mg l^{-1} of thidiazuron. Subsequent rooting (55.14%) of microshoots was achieved on half strength MS medium with 1.0 µM NAA.

Regenerates were successfully transferred to soil and slowly transferred to field (Upreti and Dhar, 1996).

2.1.9 *Betula* species

Vijayakumar *et al.* (1990) achieved successful micropropagation of endangered species of *Betula uber*. Pseudoterminal buds when placed in medium containing 0.6 and 0.05 mg l^{-1} of BA and IAA, respectively, opened in four to five days and produced up to three leaves in one week. The plants obtained have been field planted. Arya and Arya (1995) successfully regenerated plants from organogenetic calli derived from excised axillary buds from two year old silver birch (*Betula pendula*). The callus was established using 0.3 mg l^{-1} of TDZ in PMS basal medium with 2.0 per cent sucrose. Bud initiation and development was obtained in MS medium containing 0.5-1.0 mg l^{-1} BA. The rooting was obtained in growth regulator free medium.

In vitro propagation techniques for onoorekanba (*Betula schmidtii*) were studied using a 200 year old tree and 20 year old trees as test materials. The bud sprouting and shoot elongation was obtained in WPM medium supplemented with 0.8-1.2 mg l^{-1} BA. Shoots were successfully rooted in medium supplemented with 0.02 mg l^{-1} of NAA (Nishikawa and Ide, 1996).

2.1.10 *Caesalpina pulcherima*

Nodal explants from trunk sprouts were used for inducing callusing on a medium containing NAA alone and 2,4-D in any combination, except BA. The greatest rooting was obtained in medium containing IAA and cytokinin (Rohman *et al.*, 1993).

2.1.11 *Casuarina* species

In vitro propagation through indirect organogenesis was successfully obtained in some species of *Casuarina* viz., *C. glauca, C. cunninghamiana* and *C. equisitifolia* (Aboel-nil, 1987). Juvenile and mature stem segments callused on MS medium containing 0.5 μM each of 2-ip and NAA. Buds were regenerated from callus tissue and stem explants on MS medium containing BA at 2.2-11.0 μM combined with IAA at 0.5 μM.

2.1.12 *Cephalotaxus harringtonia*

Shoot proliferation of Japanese plum (*C. harringonia*) was achieved in a medium based on MS salts supplemented with vitamins and casein hydrolysate in the presence of 10 per cent (v/v) of coconut water. Rooting was unsuccessful from microcuttings proliferated on agar based medium, but, when cultured in liquid medium on membrane rafts, detached shoots rooted under mist in green house conditions with or without auxin treatments (Janick *et al.*, 1994).

2.1.13 *Citrus halimii*

A successful system of direct organogenesis is described for the wild citrus tree. Maximum number of shoots from hypocotyl explants was induced on MS medium provided with 2.2-11.1 μM BA. Rooting of regenerated shoots was best on MS medium supplemented with 2.7 μM NAA (Normah *et al.*, 1997).

2.1.14 *Dalbergia* species

Dalbergia sissoo Roxb. has been successfully micropropagated in a medium containing MS salts supplemented with NaH_2PO_4. $2H_2O$ (170 mg l^{-1}),

thiamine HCl (1.0 mg l^{-1}) meso inositol (100 mg l^{-1}). Best sustained growth of the buds and 55.4 per cent of rooting was obtained in MS medium containing 0.5 mg l^{-1} NAA and 1.0 mg l^{-1} BA (Dawra *et al.*, 1984).

Indirect organogenesis in *Dalbergia lanceolaria* was reported successful (Anand and Bir, 1984). Maximum callus proliferation was obtained in MS medium containing 4.0 mg l^{-1} NAA, 1.0 mg l^{-1} kin and 600 mg l^{-1} yeast extract using root, hypocotyl, cotyledon, stem and leaf explants. Hypocotyl and shoot calli exhibited the highest percentage (approximately 55%) of shoot differentiation on MS medium with 1.0-4.0 mg l^{-1} of BA. Rooting of the differentiated shoots was obtained on MS medium augumented with 2.0-4.0 mg l^{-1} IBA. Suwai *et al.* (1988) induced multiple shoots in *D. sissoo* Roxb. on MS medium in the presence of 1.0 mg l^{-1} BA and 0.1 mg l^{-1} NAA. The shoots were proliferated continuously at a sustained rate of 10-15 microshoots of eight weekly subculture over two years in MS medium containing 0.25 mg l^{-1} of BA. *In vitro* rooting was obtained on MS medium with 0.01 mg l^{-1} NAA and BA at 0.01 mg l^{-1} or below. Best rooting occurred on non-sterile sand beds which is recommended as the ideal method to take up on commercial scale.

Raghava *et al.* (1992) obtained complete plantlets from nodal explants of elite (60-80 year old) *Dalbergia latifolia* Roxb. Multiple shoots were obtained on MS with BA at 1.0 mg l^{-1} and 0.05 mg l^{-1} NAA / 0.05 mg l^{-1} IAA. Shoots were rooted on half-strength MS with IBA (2.0 mg l^{-1}). Mahato (1992) was successful in getting multiple shoots from nodal segments of Indian rosewood (two and half year old seedlings as well as twelve year old trees). Woody Plant Medium(WPM) as well as MS medium were found to be suitable for primary culture establishment.

Kannan (1995) found out that WPM with kin at 1.0 mg l^{-1} and IAA at 0.1 mg l^{-1} was best for getting enhanced release of axillary buds in *D. latifolia*. Multiple shoots (3.5 shoots / explant) was induced on MS medium by adding 2.0 mg l^{-1} BA.

In vitro rooting was obtained in half-strength basal medium after giving a pulse treatment with 1000 mg l^{-1} IBA solution to base of the shoots produced from buds of young trees. Direct shoot and root formation was noticed from nodal explants of young trees when cultured on WPM supplemented with 1.0 or 2.0 mg l^{-1} IAA. Rooting could not be obtained from shoots produced from nodal explant of elite rosewood trees.

Rao and Sita (1996) were successful in obtaining direct regeneration of somatic embryos which were obtained from immature zygotic embryos of *D. latifolia*. Immature embryos dissected from green pods 90 days after flowering gave highest frequency of somatic embryo formation. Preculture on high 2,4-D medium for four weeks followed by second phase culture on low 2,4-D medium along with high sucrose concentration induced direct embryogenesis. Embryos were developed into plants on maturation medium containing MS plus 0.5-1.0 mg l^{-1} BA.

2.1.15 *Delonix regia*

Hoque *et al.* (1992) achieved shoot regeneration from cotyledonary nodes cultured on MS medium with 1.0 mg l^{-1} BA and 1.0 mg l^{-1} IAA and also from shoot tip cultures on MS medium containing 0.5 mg l^{-1} BA and 0.5 mg l^{-1} kin. Half strength MS with 0.2 mg l^{-1} IBA was found optimal for root induction.

2.1.16 *Duabanga grandifolia*

Kumar and Kumar (1997) induced multiple shoot from cotyledonary nodes on MS containing 0.5-5.0 mg l^{-1} BA. Isolated microshoots were rooted on MS medium containing 0.1 mg l^{-1} each of IBA and NAA.

2.1.17 *Eucalyptus* species

Le-Roux and Van-staden (1991) reviewed that callus production is successful in 30 species of *Eucalyptus* and plant regeneration in 12 species. Six clones out of eight tested by Laine and David (1994) were amenable to shoot production on simple media containing NAA and either BA or zeatin. Shoot elongation was obtained on a medium containing GA_3 and rooted using a standardised micropropagation medium. Bist *et al.* (1997) reported induction of multiple shoot on MS medium provided with 1.0 mg l^{-1} BA and 1.0 mg l^{-1} NAA. Shoot elongation was achieved on half strength MS medium without any growth regulator and rooting of shoots was successful in half strength MS supplied with 1.0 mg l^{-1} IBA. Pattanaik and Vijayakumar (1997) found that the induction of multiple shoots is most effective on MS medium with 0.5 mg l^{-1} BA. Rooting was best achieved in half-strength MS medium supplemented with 0.5 mg l^{-1} each of IAA and IBA.

2.1.18 *Eugenia jambolina*

Chimmala (1994) tested the morphogenetic response and callus induction using seedling explants of *E. jambolina* cultured on MS and B_5 media with various cytokinin and auxin combinations. The regeneration ability and shoot bud production was efficient on MS medium with 1.75 mg l^{-1} kin and 2.5 mg l^{-1} BA. The isolated shoots induced roots on the same medium with 1.5 mg l^{-1} NAA.

2.1.19 *Fagus sylvatica*

Vieilez *et al.* (1993) established an *in vitro* multiplication method from juvenile tissues of beech (*Fagus sylvatica*) and plantlets were regenerated.

2.1.20 *Ficus* species

Barbosa *et al.* (1992) described a procedure for micropropagation of healthy and high quality *Ficus carica* Roxb. valihos. The shoot production, multiplication and rooting were optimised on a medium containing MS salts supplemented with 10 mg thiamine, 2.0 mg nicotinic acid, 12 mg pyridoxine, 100 mg myo-inositol, 80 mg cysteine, 30 g sucrose, 6.5 g agar, 1.0 mg BA, 3.0 mg GA_3 and 1.0 mg NAA l^{-1}, with or without 3.0 g l^{-1} activated charcoal.

Three cultivars of *Ficus benjamina* Natasja, Exotica and Golden king were tried for micropropagation. Multiple shoots formed in MS medium given with 3.0 mg l^{-1} BA while in exotica while in Natasja it occurred in all the concentrations (1.0, 2.0, 3.0, and 4.0 mg l^{-1}) of BA. Natasja and Exotica were cultured in MS medium containing 0.05 mg l^{-1} BA and 0.1 mg l^{-1} IAA, respectively, for the adoption phase. In both the cultivars, the growth regulator free medium gave the best overall results (Trujillo *et al.*, 1994).

2.1.21 *Fraxinus* species

Shoot production was tried on three basal media namely, MS, WPM and Driver and Kuniyuki culture media in Fraxinus excelsior (Hammatt and Ridout, 1992). Both the survival and callus formation was high in DK culture medium. Adventitious shoots were formed in DK medium supplemented with 22.2 µM BA. The rooting was obtained in half strength WPM medium with 2.45, 4.9 or 9.8 µM IBA. Shoot tips and nodal buds formed the explant in micropropagation of *Fagus angustifolia* (Parron *et al.*, 1994). Of the several basal media tried, the most new explants per mature explants (5.3) was obtained on Driver and Kuniyuki Walnut (DKW) medium supplemented with 4.4 µM BA + 0.98 µM IBA. The most new explants per juvenile explant (5.6) was produced on QL (Quoinn and Lepoivre)

medium supplemented with 8.9 μM BA and 0.49 μM IBA. Rooting was achieved on WPM supplemented with 0.98-4.9 μM IBA.

2.1.22 *Leucaena leucocephala*

In vitro clonal propagation was successfully reported by Datta and Datta (1985), Goyal *et al.* (1985), Hoque *et al.* (1992) and Toruan-Mathius (1992). Shoot production was achieved using MS medium incorporated with 5.0 mg l^{-1} BA and the root production in MS supplemented with 5.0 mg l^{-1} IAA (Toruan - Mathius, 1992).

2.1.23 *Malus* species

Paul *et al.* (1994) reported somatic embryogenesis in malus. Immeture zygotic embryos were found efficient when cultured on medium containing 6.0 mg l^{-1} NAA in the dark and transferred to medium with 0.5 mg l^{-1} BA and 0.05 mg l^{-1} NAA in light. The plantlets were formed on half strength plant growth regulator free MS medium.

2.1.24 *Mangifera indica*

Ara *et al.* (1998) induced somatic embryogenesis in Modified MS medium consisting of half strength of major salts, full strength of minor salts and organics, 400 mg l^{-1} L-glutamine, 100 mg l^{-1} ascorbic acid, 6.0 per cent (w/v) sucrose, 1.0 mg l^{-1} 2,4-D gelled with 0.8 per cent (w/v) agar by incubating explants for 8-10 weeks in dark. The plantlets were produced on a liquid medium with 3.0 per cent sucrose and 1.0 mg l^{-1} GA_3. Rooting was found maximum when shoots were cultured on 5.0 mg l^{-1} of IBA.

2.1.25 *Morus* species

Hossain *et al.* (1992) reported successful method of *in vitro* propagation for *Morus laevigata* wall, taking explants from mature trees. A protocol has been developed for plant regeneration from hypocotyl callus of *Morus alba* by Kathiravan *et al.* (1995). They also described the steps involved in successful transfer of plantlets to field. Pattnaik *et al.* (1996) reported high frequency bud break in *Morus australis* poir (syn. *M. acidosa* Griff) on MS medium supplemented with 1.0 mg l^{-1} BA and 0.3 mg l^{-1} GA_3. The best rooting was observed when 1.0 mg l^{-1} each of IAA, IBA and indole propionic acid (IPA) was used in rooting media.

2.1.26 *Populus* species

An attempt was made by Jehan *et al.* (1994) to induce indirect bud regeneration from root internode, leaf lamina or petiole explants of poplar (*Populus trichocarpa* x *P. deltoides* cv. Hunnegem). Rapid and efficient response was obtained through *in vitro* culture on MS medium having 0.5 µM NAA and 1.0 µM BA. It was also applicable for indirect shoot regeneration following callus induction on MS medium containing 10 µM 2,4-D or NAA with subsequent sub culture in presence of 10 µM NAA and 5 µM BA.

2.1.27 *Prosopis* species

Goyal and Arya (1981) were successful in devising a protocol for plantlet production in *Prosopis cineraria* Linn from 7-10 day old seedlings. Plantlets could be grown normally in pots containing vermiculite and sterile soil. Kackar *et al.* (1991) advocated a protocol for rapid *in vitro* propagation of elite *Prosopis cineraria* trees. The shoots were produced on MS medium augumented with 2.0 mg l^{-1} each of NAA and napthoxyacetic acid (NOA). The rooting was observed on

MS medium with 3.0 mg l^{-1} of NOA. Nandwani and Ramawat (1992) produced callus from hypocotyl and cotyledon explants of *Prosopis tamarugo* on MS medium containing 2.0 mg l^{-1} NAA and 0.2 mg l^{-1} BA. The callus regeneration was obtained when it was subcultured to MS medium containing 5.0 mg l^{-1} BA.

2.1.28 *Pterocarpus* species

Shoots were obtained from callus culture in 1/4 MS supplemented with 3.0 mg l^{-1} BA and 40 mg l^{-1} adenine in case of *Pterocarpus santalinus* (Patri *et al.*, 1988). Sita *et al.* (1992) improved the number of shoots per explants. Up to eight shoots were obtained on B_5 medium supplied with 1.0 mg l^{-1} BA along with 1.0 mg l^{-1} kin. Adventitious roots were obtained at low concentrations of IAA (1-2 mg l^{-1}). Kumar (1993) obtained multiple shoot in several combination of kin with auxins in MS and WPM in case of *Pterocarpus marsupium*. The best results were obtained on WPM with 2.0 ppm kin and 0.1 ppm of IAA.

2.1.29 *Santalum album*

Endosperm tissues of *Santalum album* when cultured on modified MS with 2,4-D (1-2 mg l^{-1}), kin (0.1-0.2 mg l^{-1}), BA (0.5-2 mg l^{-1}) and NAA (1.0 mg l^{-1}) induced callus formation followed by differentiation (Sita *et al.*, 1980).

2.1.30 *Sesbania* species

Bansal and Pandey (1993) reported shoot production in *Sesbania aculeata* directly from the nodal explant and from the callus. The MS media supplemented with IBA, NAA, IBA + BA/NAA and BA was used. Differentiation of shoots and roots occurred in one step in the same concentration of growth regulator. Pellegrineschi and Tepfer (1993) reported that shoot production from callus of

Sesbania rostrata was better in culture inoculated with *Agrobacterium tunefaciens* strain 82.139 than from cultures provided with growth hormones.

Multiple buds (30 per explant) were induced from cotyledonary explants of *Sesbania grandiflora* when MS supplemented with 1.0 mg l^{-1} NAA and 1.0 mg l^{-1} BA was used as media. The buds elongated on MS media containing 0.5 mg l^{-1} BA, 0.1 mg l^{-1} NAA and 0.1 mg l^{-1} GA_3. Rooting of 86 per cent shoots was obtained on MS medium supplemented with 1.0 mg l^{-1} NAA (Detrez *et al.*, 1994).

2.1.31 *Tamarindus indica*

Mascarenhas *et al.* (1982) observed successful *in vitro* propagation of tamarind when cultured on MS having cytokinins and auxins.

2.1.32 *Tectona grandis*

Gupta *et al.* (1980) successfully induced multiple shoots from 100 year old teak tree explants. Over 500 plants were produced from a single bud in MS medium having 0.15 mg l^{-1} kin and 0.15 mg l^{-1} BA. Devi *et al.* (1994) produced multiple shoots from shoot buds of elite teak (*Tectona grandis* Linn.) trees adopting a two step protocol. Two hundred and sixteen shoots were obtained from single bud explants.

2.1.33 *Trema orientalis*

Plant regeneration was obtained from leaf callus derived from 15 day old *in vitro* grown seedling and a mature tree. Callus supplemented with 11.11 µM (2.5 mg l^{-1}) and 1.34 µM (0.25 mg l^{-1}) NAA resulted in highest shoot regeneration rate (82.1%). Shoots were induced to form roots by transfer to half strength MS

supplemented 0.053 μM (0.01 mg l^{-1}) NAA or 0.044 μM (0.01 mg l^{-1}) IBA (Samantaray *et al.*, 1995).

2.1.34 *Vateria indica*

Divatar (1994) attempted for enhanced release of axillary buds, organogenesis and embryogenesis of Malabar white pine (*Vateria indica* L.), using axillary bud explants. Out of various growth regulator combinations tried, only 2-ip and IBA could support bud break and shoot production. Moderate callusing was obtained from leaf and internodal segments on MS and half strength MS supplemented with 2-ip + 2,4-D and 2-ip + IBA, respectively. Nevertheless, the calli did not respond to organogenesis or embryogenesis.

2.1.35 *Ziziphus* species

Kim and Lee (1988) reported that axillary bud of *Ziziphus* cv. Geumsung showed best shoot and root growth when 500 mg l^{-1} AC was added to half strength MS with 0.5 mg l^{-1} BA. However, *Ziziphus* cv. Bokjo responded best to 1000 mg l^{-1} AC in the above medium.

2.2 Controlling factors in micropropagation

2.2.1 Culture medium

Growth and morphogenesis of plant tissues *in vitro* is largely governed by the composition of the culture media (Razdan, 1993). Nutritional requirement of the various organs and tissues of the plant is least understood than we do of the whole plants (Dodds and Roberts, 1985). Cultured systems may exhibit selective biosynthetic activity and in addition there may be changes in their metabolic path ways over a period of time. These changes in metabolism require corresponding

changes in nutritional requirements. Shoot cultures are the basis of most micropropagation systems presently in commercial application (McCown and Sellmer, 1987). Since the tissue cultured plants growing *in vitro* are partially autotrophic, they require carbon source (usually sucrose) along with salts and trace amounts of organic compounds like vitamins, amino acids and plant growth regulators.

2.2.2 Basal media

Today, a plethora of media formulations for the culture of woody plants including forest trees can be obtained (McCown and Sellmer, 1987). The earlier media were characterised by low overall concentrations of inorganic salts, especially those of potassium and nitrate and providing nitrogen solely in the form of nitrate. Different workers contributed for the development of different media composition and were named after them in majority of cases viz., Gautheret (1939), White (1943), Hildebrant *et al.* (1946), Nitsch (1951), Heller (1953), Reinert and White (1956), Murashige and Skoog (1962), Linsmaier and Skoog (1965), Gamborg *et al.* (1968), Schenk and Hildebrant (1972) etc.

A medium specially designed for tree species is the Woody Plant Medium (WPM) of Lloyd and McCown (Lloyd and McCown, 1980). The Gamborg *et al.* (1968) medium(B_5) which was originally designed for cell suspension or callus cultures, with modifications has proved valuable for protoplast culture (Gamborg and Shyluk, 1981). The N_6 medium developed for cereal anther culture is being successfully used in other types of cereal tissue culture (Chu, 1978). Gamborg *et al.* (1983) reported that E_1 medium supports rapid growth of cells for embryogenesis and for protoplast cultures. Nitsch and Nitsch (1969) medium is frequently used for anther cultures. Success in employing these various media in all probability lies in the fact that the ratios as well as concentrations of nutrients nearly match the

optimum requirement with regard to the growth and differentiation of respective cell and tissue systems (Razdan, 1993).

2.2.3 Growth regulators

Plant growth regulators (PGR's) include naturally occurring hormones such as IAA, GA_3, BA, ABA etc. and also a number of synthetic chemicals that affect or control plant growth and development in plants (Minocha, 1987). It is generally necessary to add one or more of these PGR's to support good growth of tissues and organs (Bhojwani and Razdan, 1983). Kallak *et al.* (1997) working with carnation reported that, the addition of growth regulators into the callus regeneration media modified the genotypic effect on shoot formation frequency, more or less decreasing the genotypic differences and the number of shoots formed. Skoog and Miller (1957) proposed the concept of hormonal control.

Following the discovery of auxin by Went (1926) and its chemical identification by Kogl *et al.* (1934), several workers simultaneously demonstrated the usefulness of this hormone in cell cultures. Auxins, especially 2,4-D are most effective and commonly used for callus induction. The auxins in general IAA, 2,4-D, NAA and IBA all have similar effects. However, some tissues do show specific qualitative differences (Bonga and Durzan, 1987). The effect of IBA and IAA were also reported by Krieken *et al.* (1992) and Krieken *et al.* (1993) in case of apple.

Cytokinins are routinely used for shoot production and higher concentrations generally induce multiple shoots. In combination with auxins they are generally used for callus regeneration. The most frequently used cytokinins are 6-furfuryl-aminopurine (kinetin), N-6-Benzyladenine (BA), 6-(4-hydroxy-3-methyl-trans-2-butanyl-amino) purine (zeatin) and N6-(2-isopentyl) adenine (2-ip). It was

recently discovered that N,N diphenyl urea (DPU), N-2-Chloro-4-Pyridyl-M-phenyl urea (CPPU), N-phenyl-N-1,2,3-thiadiazol-5 yl urea (thidiazuron or TDZ) and other derivatives of diphenyl urea show cytokinin type activity (Pierik, 1989). Shankla *et al.* (1994) found that TDZ is must for callus and shoot formation in *Albizia julibrissin.*

Gibberlins and abscisic acid are rarely used in tissue culture. With few exceptions, gibberlins in most cases do not have clear morphogenetic effects in cell cultures (Zaerr and Mapes, 1982).

Ethylene is the least studied of the growth regulators for its effects on cell cultures and morphogenesis in woody plants (Minocha, 1987).

2.2.4 Other media supplements

Several substances of uncertain and variable composition are in common use which include adenine, adenine sulphate, banana homogenate, casein hydrolysate, coconut water, orange juice, peptone, yeast extract etc. Minocha (1987) reported that addition of casein hydrolysate at 500 mg l^{-1} enhanced the growth of the plants as measured by the number of leaves produced. Einset (1978) found that the *in vitro* growth of explants from several citrus sp. was greatly stimulated by the addition of orange juice to the medium. Tomato juice (30% v/v) has also been used effectively under certain conditions (Nitsch and Nitsch, 1955; Strans, 1960). Orchid media has been supplemented with banana fruit extract (Arditti, 1968). Many of the organic substances could be effectively replaced by a single amino acid. Risser and White (1964) demonstrated that L-glutamine could replace a mixture of 18 amino acids earlier used by Rienert and White (1956) for tissue culture of *Picea glauca.*

Activated charcoal (AC) has been used in plant tissue culture media to improve culture growth and/or promote morphogenesis in a wide variety of species. Growth promoting effects of charcoal have been attributed to adsorption of substances inhibitory to growth from the media produced either from breakdown of the media during autoclaving (Weatherhead *et al.*, 1978) or by the cultures themselves (Fridborg *et al.*, 1978). Additionally, alteration of pH of the medium to an optimum level for morphogenesis has also been reported as beneficial effect of AC (Owen *et al.*, 1991). Duart and DeWulf (1993) in their reports indicated that AC catalysed the hydrolysis of 90 per cent of sucrose in culture media to fructose and glucose. However, a contrary report by Wann *et al.* (1997) says that AC does not catalyse sucrose hydrolysis in TC media during autoclaving. Although beneficial effects of AC have been documented, it is a complex substance and the entire range of its effects on TC media and subsequent growth and morphogenesis of tissue cultures is unknown.

2.2.5 Carbon energy sources

All media require the presence of a carbon source as the source of energy. The most commonly used carbon source is sucrose, at a concentration of 2-5 per cent (Bhojwani and Razdan, 1983). The autoclaving of sucrose is found beneficial because autoclaving leads to its hydrolysis into more efficiently utilizable sugars, such as D-fructose and D-glucose (Dodds and Roberts, 1985). The cyclitol myo-inositol is added to some culture media as a growth factor at a concentration of 100 mg l^{-1}. Xylogenesis can be induced by using either glycerol (2% w/v) or myo-inositol (2% w/v) as the principal carbon energy source (Roberts and Baba, 1982). Carbohydrates not only function as a carbon source in metabolism, but they also play an important role in the regulation of the external osmotic potential (Brown and Thorpe, 1980). The sugar-alcohol sorbitol (D-glucitol) has proven the most effective carbon source for *in vitro* proliferation of the apple root stocks (*Malus robusta*

Rehd. No.5) by Pua and Chong (1984), and *Malus pumila* M.9 (Welander *et al.*, 1989).

2.2.6 Vitamins

Vitamins have catalytic functions in enzyme systems and are required only in trace amounts. Some consider that thiamine (vitamin B_1) may be only essential vitamin for nearly all plant tissue cultures, whereas nicotinic acid (niacin) and pyridoxine (vitamin B_6) may stimulate growth (Gamborg *et al.*, 1976). Some other vitamins that have been employed in tissue culture media include P-amino-benzoic acid (PABA; vitamin Bx), ascorbic acid (vitamin C), biotin (vitamin H), chlorine chloride, cyanocobalamine (vitamin B_{12}), folic acid (vitamin Bc), calcium pantothenate and riboflavin (Huang and Murashige, 1977; Gamborg and Shyluk, 1981).

2.3 Explant

2.3.1 Explant size and its position on the mother plant

The nature of explant to be used for *in vitro* propagation to a certain extent is governed by the method of shoot multiplication to be adopted. Norton and Norton (1986) studied the effects of explant length (2.5 to 20 mm), axillary bud number (0 to 6), presence or absence of apex and explant derivation (top, middle or base of plant canopy) in prunus and picea. The number of shoots formed after four weeks increased with the explant length and decreased with the number of buds present. Explant taken from the top of the canopy produced most shoots, but removal of the apex did not affect the shoot number. Hussey (1983) quoted that, as a rule, larger the size of the explant more rapid is the growth rate and greater are the rates of survival. However, larger the explant size more will be the chance of harbouring contaminant micro-organisms. Apical buds, larger than 20 mm, from 1

year old *Fagus sylvatica* shoots were the most suitable explant source (Meier and Reuther, 1994).

2.3.2 Age of the explant

Generally more juvenile the specimen, it is easier to propagate vegetatively. It is probably wise to develop methods for vegetative propagation from highly juvenile material (Bonga, 1982). However, the ability of juvenile explant from hard-to-root species to multiply in cultures suggests that it should be possible to *in vitro* propagate their elite trees if they can be induced to develop juvenile shoots by suckering, copping or hormone treatment. To some degree rejuvenation of shoots also occurs after serial subcultures (Zimmerman and Broome, 1981). However, *Betula tatewakiana* (Ide, 1995; Jones *et al.*, 1996), *Fagus sylvatica* (Meier and Reuther, 1994), *Fraxinus excelcor* L. (Hammatt, 1994), *Dalbergia sissoo* (Chauhan *et al.*, 1994), *Tectona grandis* (Gupta *et al.*, 1980; Devi *et al.*, 1994) etc. have been cloned *in vitro* successfully taking nodal and terminal bud explants from mature trees. Meier and Reuther (1994) reported that grafting of branches of mature stock plants on juvenile root stocks resulted in high increase of multiplication rate compared with corresponding mature material.

2.3.3 Season of collecting the explants

The physiological state of the mother plant at the time of explant excision has a definite influence on the response of the buds. The physiological state of plant depends on season. The explants from actively growing shoots at the beginning of the growing season generally give best results (Anderson, 1980). Yu (1991) observed that the test material taken after 10 continuous rainy days had a contamination rate of cent per cent and that taken after 15 continuous sunny days had a low contamination rate of 20 per cent in *Litchi chinensis*. Meier and Reuther

(1994) reported that February was the most beneficial month for explantation of dormant buds. Since infection with endogenous bacteria was still low and *in vitro* growth of the plant material was the highest.

2.3.4 Genotype

Explants from thornless trees of *Prosopis cineraria* produced 6-8 shoots per explant on MS medium containing 0.1 mg l^{-1} IAA, 5.0 mg l^{-1} BA and additives (Shekhawat *et al.*, 1993). Kallak *et al.* (1997) observed differences in shoot and root regeneration efficiency of carnation calli depending on genotype. The calli from different cultivars revealed significant differences in growth, colour and structure. Similar results have been reported by Christopher and Rajan (1996) for direct shoot regeneration ability of red pepper.

2.3.5 Surface sterilization

Surface of plant parts may harbour a wide range of microbial contaminants which when inoculated to a nutrient medium, contaminate the entire *in vitro* system. To avoid this, the tissue must be thoroughly surface sterilized before planting it on the nutrient medium. To disinfect the plant tissues various surface sterilizing agents have been used at varying concentrations and at varying duration's of time. Antibiotics, Bromine water, calcium hypochloride, chlorine water, ethyl alcohol, hydrogen peroxide, mercuric chloride, sodium hypochlorite etc. are some of the surface sterilants used in tissue culture. Bhojwani and Hayward (1977) suggested a serial surface sterilization method for wheat seeds wherein seeds are given two minute treatment in 1.0 per cent (v/v) solution of cetavlon before hypochlorite treatment. In cases where the explant carries a heavy load of micro-organisms on its surface it may pay to wash it in running tap water for an hour or more. D'silva and D'souza (1992) reported that treating explants for one hour

running water treatment, surface sterilization with 70 per cent ethanol for 1 min. followed by 8 min. treatment with a solution of 0.1 per cent mercuric chloride and 0.1 per cent sodium lauryl sulphate is effective in reducing contamination in case of *Ailanthus triphysa*. After surface sterilization treatment, plant material must be rinsed three or four times in sterile distilled water to remove all traces of the sterilizing agent (not applicable when using alcohol).

2.3.6 Systemic contaminants

Microorganisms present on the surface of explants can be eliminated by surface sterilants, but those existing within the internal tissues cannot be removed, and they cause latent contamination, which is a serious problem associated with woody plant tissue culture. Leifert and Woodward (1998) highlighted that surface sterilization is often inefficient. This problem may be due to the disinfectant being inactive or microorganisms being protected within the plant tissue used as the explant. Thus culture contamination control is extremely difficult and with many contaminants, impossible.

Use of various fungicides (Shields *et al.*, 1984) and antibiotics (Dodds and Roberts, 1985) in the culture medium to reduce systemic fungal and bacterial contamination, respectively, has been suggested. Mallika *et al.* (1992) advocated growing stock plants under controlled conditions with regular sprayings using systemic and contact fungicides in order to avoid problem of contamination to some extent. Leifert and Woodward (1998) also suggested placing more emphasis on early detection and prevention of contamination at source. They recommended employing quality assurance systems such as HACCP (Hazard Analysis Critical Control Points) which cover every potential source of contamination.

2.3.7 Explant exudations/browning of medium

A serious problem generally associated with woody plants tissue culture and sometimes with other plants, is the oxidation of phenolic substances leached out from the cut surface of the explant which turns the medium dark brown and is toxic to the tissues (Bhojwani and Razdan, 1983). Subculturing will seal up the cut end of explant and stop leaching (Kotomari and Murashige, 1965; Morel, 1972). Culturing explant in liquid medium is recommended for excluding phenol in *Eucalyptus* (Gupta *et al.*, 1981) and in *Mangifera indica* (Raghuvanshi and Srivatsava, 1995). When the problem of medium browning persists at each subculture the addition of antioxidants, such as cysteine HCl (100 mg l^{-1}), ascorbic acid (50-100 mg l^{-1}) or citric acid (150 mg l^{-1}), to the culture medium is recommended (Sondahl and Sharp, 1977; Skirvin and Chu, 1979). Use of coffeine, activated charcoal and PVP was found ineffective to reduce phenol exudation (Gill and Gill, 1994) while subculturing at 3-4 days interval was effective.

2.4 Culture environment

Physical conditions such as pH of the medium, light, temperature and relative humidity, season of culturing etc. all have been found to have a profound influence on the rate of growth and extent of differentiation exhibited by the cultured cells.

Changes in the physical condition of the medium is brought about by changing the concentration of the gelling agent in the medium. Importance of physical condition on culture is evident in case of *Dalbergia latifolia* cultures (Mahato, 1992). At higher pH conditions precipitation of the nutrients results. pH also affects nutrient and harmone uptake. Bonga (1982) remarked that pH of the medium is usually set at about 5.0 for liquid cultures and at 5.8 for semisolid

cultures. Leifert *et al.* (1994) established the effect of short term pH reduction in culture medium on prevention of survival and establishment of contaminating microbes in *Delphinium* species.

A light intensity of 1000-5000 lux is adequate for morphogenic processes (Hussey, 1980). Strictly speaking, photoperiodism is not critical. A diurinal illumination regime of 16 hours day and 8 hours night is found satisfactory (Murashige, 1977).

Incubation temperature for tissue cultures of forest trees ranges between 20 to 28°C. However, cultures are most frequently maintained under a constant temperature near 25°C (Chalupa, 1987).

Relative humidity is rarely a problem except in arid climates, where rapid drying occurs. The humidity of air is rarely controlled and when it is controlled, 70 per cent has been found to be the most frequent setting (Hu and Wang, 1983).

2.5 Rooting of *in vitro* produced shoots

Shoots produced *in vitro* can be induced to root under *in vitro* or *ex vitro* conditions. A low salt medium is found satisfactory for rooting of shoots in a large number of plant species. Often where shoot multiplication was induced on full-strength MS medium, the salt concentration was reduced to half (Garland and Scoltz, 1981; Zimmerman and Broone, 1981) or a quarter (Skirvin and Chu, 1979) for rooting. It is the endogenous auxin: cytokinin balance that is the key factor in the initiation of the rooting (Thorpe, 1980).

Generally, auxin favours root formation. Among the auxins, NAA has been the most effective one for induction of rooting (Ancora *et al.*, 1981).

Sometimes a combination of auxins may give a better response (Gupta *et al.*, 1980). Thiol compounds were found beneficial in rooting of *Mallus* (Auderset *et al.*, 1996). A sequential culture involving 15 day incubation period on MS medium supplemented with 58.43 mM sucrose and different concentrations of NAA followed by transfer to MS media with various auxins viz., IAA, NAA, IBA etc. singly or in combinations, was found efficient for rooting of *in vitro* shoots of *Ailanthus malbarica* (D'Silva and D'Souza, 1992). About 80 per cent shoots produced *in vitro* and failed to produce roots under *in vitro* conditions, were successfully rooted under *ex vitro* conditions in case of teak (Bonal and Monteuuis, 1997).

Rooting of shoots *in vitro* and *in vivo* has been reported by the simple, highly efficient and more economical methodology by Yeoman (1985), Schuwarz *et al.* (1988) and Vijayakumar *et al.* (1990). This was achieved by using vermiculite, sand and peat mass singly or in combination in tubes or pots as the rooting medium and maintaining it in high humid conditions.

2.6 Hardening and planting out

Tissue culture plantlets are tender and their transfer from the artificial environment of the culture vessel to the self dependent green house or similar environment makes their existence and establishment tough. The success in acclimatization depends not only on the post-transfer conditions but on the pre-transfer culture conditions also (Ziv, 1986).

Dewan *et al.* (1992) reported that transferring of plantlets to pots filled with garden soil and sand (1:1) and maintaining them for eight weeks under high humidity yielded best results in case of *Acacia nilotica.* In *Ailanthus malbarica*, the percentage of survival of rooted plantlets was highest on a mixture of sand, soil and

coconut husk (1:1:0.25 v:v:v). The plantlets were maintained in glass house under 95 per cent to 100 per cent humidity. After two weeks the plantlets were transferred to pots with unsterilized soil and kept in an open green house (D'Silva and D'Souza, 1992).

Materials and Methods

MATERIALS AND METHODS

The *in vitro* propagation of *Ailanthus triphysa* (Dennst.) Alston, was taken up at College of Forestry, Vellanikkara from 1996 to 1998. The Materials used and the Methods followed during the study are presented here.

3.1 Media

Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), half-strength MS, and woody plant medium (Lloyd and McCown, 1980) were chosen for the study as basal media. The chemical composition of the media are given in Table 1. Various plant growth regulators and other media additives were supplemented to the basal media in different experiments. To avoid browning of media either activated charcoal (0.25% w/v) or ascorbic acid (50 mg l^{-1}) was incorporated in the media.

Cytokinins (BA and Kin) and auxins (IAA, IBA and NAA) were supplemented to the media at various concentrations, individually and in combination, in different stages of culture.

The chemicals used for media preparation were of analytical grade of British Drug House (BDH), Merck, SISCO Research Laboratory or Sigma.

3.2 Media preparation

Standard procedures as given by Gamborg and Shyluk (1981) were followed for preparation of the media. Stock solutions of major and minor salts, vitamins and growth regulators were prepared by dissolving required quantity of chemicals in distilled water. The stock solutions of nutrients were stored in amber coloured bottles and of vitamins in tightly lidded volumetric flasks both under

Table 1. Chemical composition of Murashige and Skoog (MS) medium and Woody Plant Medium (WPM)

Compound	Quantity (mg l^{-1})	
	Murashige and Skoog (MS)	Woody Plant Medium (WPM)
INORGANIC		
Ammonium nitrate	1650.00	400.00
Boric acid	6.20	6.20
Calcium chloride-2 hydrate	440.00	96.00
Calcium nitrate-4 hydrate	0.00	556.00
Cobalt chloride-6 hydrate	0.025	0.00
Copper sulphate-5 hydrate	0.025	0.025
Ferrous sulphate-7 hydrate	27.80	27.80
Manganese sulphate-1 hydrate	22.30	22.30
Magnesium sulphate-7 hydrate	370.00	370.00
Na_2EDTA-2 hydrate	37.30	37.30
Potassium dihydrate phosphate	170.00	170.00
Potassium iodide	0.83	0.00
Potassium nitrate	190.00	0.00
Potassium sulphate	0.00	990.00
Sodium molybdate-2 hydrate	0.25	0.25
Zinc sulphate-7 hydrate	8.60	8.60
ORGANIC		
Inositol	100.00	100.00
Nicotinic acid	0.50	0.50
Thiamine HCl	0.10	0.10
Pyridoxine HCl	0.50	0.10
Glycine	2.00	2.00
OTHERS		
Sucrose (in per cent w/v)	3.00	2.00
Agar (in per cent w/v)	0.70	0.70

½MS denotes half amounts of the inorganic constituents, full amounts of organic and other constituents per litre of MS medium

chilled conditions in refrigerator. The stock solutions of nutrients were prepared once in a month and that of vitamins and growth regulators once in every fortnight.

Steel vessels which were washed thoroughly and rinsed with distilled water were used for media preparation. Specific quantities of the stock solutions of salts, vitamins and growth regulators were pipetted out into the vessel. Sucrose and inositol of specific quantities were added freshly and dissolved. Activated charcoal (0.25% w/v) or ascorbic acid (50 mg l^{-1}) was added wherever desired. The volume was then made up to 1000 ml using volumetric flask by adding distilled water. The pH of the solution was adjusted to a range of 5.7-5.8 (using 1N NaOH or 1N HCl) with the help of a pH meter. Then agar (8.0 g l^{-1}) was added to the medium.

The solution was heated on a gas burner for melting the agar. After melting the agar, hot media (approximately 20 ml/tube) was poured into the oven dried culture tubes (150 x 25 mm), which were thoroughly washed and rinsed with distilled water previously. The culture tubes with media were then plugged tightly with nonabsorbent cotton wool plugs.

The media in the tube were autoclaved for 15-20 min. at 1.06 kg/cm^2 pressure and 121°C temperature (Dodds and Roberts, 1985). After sterilization the culture tubes were transferred to air-conditioned culture room for further use. Thermolabile chemicals like GA_3 and ascorbic acid were supplemented under aseptic conditions through filter sterilization to warm sterilized media and then distributed to tubes.

3.3 Source material

Three to four year old seedlings of *Ailanthus triphysa* which were established in nursery beds in the vicinity of College of Forestry were used as the mother plants for collecting the explants.

3.4 Collection and preparation of explants

Chances of harbouring microbial population by the mother plants are quite high especially when they are growing under open field conditions. Except in rainy season the mother plants were sprayed regularly on alternate days with a fungicidal mixture of a systemic fungicide, 'Bavistin' 50 per cent WP (Carbendazim) and a contact fungicide 'Indofil M-45' (Mancozeb) each at 0.2 per cent.

The stem segments of approximately 3-10 cm with 3-8 nodes were excised from the seedlings using a sharp blade and brought to the laboratory as quickly as possible. During rainy season the stem segments along with leaves were dipped at the base for 30 min. in a solution of the same fungicidal mixture used for spraying during nonrainy season. Dipping was given because, seedlings could not be protected from rain and hence were not sprayed. The leaves were then cut leaving about 0.5 cm of the rachis. The stem segments were then cut into smaller pieces of 1.5-2.0 cm (having three to four buds) using sharp blade. The stem segments were then washed in running tap water using teepol to remove the traces of fungicides and dust adhering to it.

3.5 Surface sterilization

Explants were surface sterilized under perfect aseptic conditions in a Klenzaids laminar air flow cabinet, which was made microbial free subjecting to UV rays for about half an hour. The floor and the sides of the laminar flow were thoroughly wiped with absolute alcohol using cotton. Surface sterilization of the explants was carried out with mercuric chloride solution at different concentrations (0.1-0.2%) and for different duration of time (5-20 min.). The explants were kept immersed in the given chemical for the stipulated period in a sterile conical flask plugged with cotton, in every treatment and were occasionally stirred. The sterilant was decanted into an empty sterilized beaker on completion of the stipulated

treatment period. Then explants were given with a thorough washing using sterile distilled water for three to five times in order to remove traces of sterilants adhering to their surface. The explants were then placed on sterile blotting papers placed in sterilized petriplates, for drying.

3.6 Inoculation procedure

Inoculation was carried out under strict aseptic conditions in a laminar air flow cabinet. During the process sterilized forceps, petriplates, blotting paper, scalpel with surgical blades etc. were used. Sterilized and dried stem segments were cut using sterile scalpel into nodal segments and shoot tips. Then the cotton plug of the culture tubes were removed and the tube neck was flamed over a gas burner kept in the chamber. The sterile explants were quickly transferred into the medium using sterile forceps. The neck of the culture tube was again flamed and the cotton wool plug replaced. To minimise the chances of contamination during drying and inoculation of explants, only few explants (about 25-30 buds) were treated at a time.

3.7 Culture conditions provided

The cultures were incubated in a culture room provided with cool white fluorescent (philips) lamps to give a light intensity of 2000 lux for 16 hours light period per day. The temperature of the room was maintained at 25 ± 2°C.

3.8 Elimination or neutralisation of polyphenols

The browning of media was a serious problem during shoot induction. Hence attempts were made to avoid the exudation of polyphenols. The explants were placed in running tap water for half an hour to wash out polyphenols. Along with this, either activated charcoal (0.25% w/v) or ascorbic acid (50 mg l^{-1}) were incorporated in the media to eliminate or neutralise polyphenols.

3.9 Shoot induction and growth

Various concentrations of BA (1.0-5.0 mg l^{-1}), Kin (1.0-5.0 mg l^{-1}), GA_3 (1.0-5.0 mg l^{-1}) and IAA (0.2 mg l^{-1}) were tried for shoot induction and further growth individually and in combination, in MS medium and half-strength MS medium (Table 2).

3.10 Rooting of shoots

3.10.1 Rooting *in vitro*

The shoots regenerated *in vitro* were subjected for rooting *in vitro* by randomly selecting the rooting combinations (Table 3). Activated charcoal (AC, 0.25% w/v) was added to the media for providing dark conditions and wherever AC was not used, the portion of the tube with media was covered from outside with aluminum foil for the same purpose. In some cases, cut end of the shoots were given with pulse treatment in sterile 1000 mg l^{-1} IBA solution before placing into media.

3.10.2 Rooting *ex vitro*

Experiments were also taken up to induce rooting of *in vitro* obtained shoots under *ex vitro* conditions providing high humidity (90% RH). Sterilized sand taken in small mud pots was used as media. The base of the shoots were cut with sharp blade and dipped in 1000 mg l^{-1} IBA solution for two to three minutes followed by dip in IBA powder. The shoots were then planted in the sand and covered with polythene bags to provide 100 per cent humidity. After two weeks, few holes were made in the polythene cover so as to reduce the humidity inside.

Table 2. Media combinations tried for bud break and shoot growth from axillary and terminal buds of *Ailanthus triphysa*

Basal medium	Media combinations
MS	AC (0.25%) + BA (1.0, 2.0, 3.0 and 5.0 mg l^{-1}) alone and along with IAA (0.2 mg l^{-1})
MS	AC (0.25%) + Kinetin (1.0, 2.0, 3.0 and 5.0 mg l^{-1}) alone and along with IAA (0.2 mg l^{-1})
MS	AC (0.25%) + BA (1.0, 2.0, 3.0 and 5.0 mg l^{-1}) and with Kinetin (1.0, 2.0, 3.0 and 5.0 mg l^{-1})
MS	AC (0.25%) + BA (1.0, 2.0, 3.0 and 5.0 mg l^{-1}) with GA (1.0 and 3.0 mg l^{-1})
MS	AC (0.25%) + Kinetin (1.0, 2.0, 3.0 and 5.0 mg l^{-1}) with GA (1.0 and 3.0 mg l^{-1})
MS	AC (0.25%) + GA (1.0, 2.0, 3.0 and 5.0 mg l^{-1}) alone and along with BA (1.0 mg l^{-1}) and Kinetin (1.0 mg l^{-1})
½MS	AC (0.25%) + BA (1.0, 2.0, 3.0 and 5.0 mg l^{-1}) alone and along with Kinetin (1.0, 2.0, 3.0 and 5.0 mg l^{-1})
½MS	AC (0.25%) + Kinetin (1.0, 2.0, 3.0 and 5.0 mg l^{-1})

Table 3. Media combinations tried for root induction from *in vitro* shoots of *Ailanthus triphysa*

Basal media	Auxin (mg l^{-1})			GA_3 (mg l^{-1})	AC (%)	Pulse treatment in 1000 mg l^{-1} IBA solution
	IBA	NAA	IAA			
MS	0.4	-	4.0	-	0.25	-
MS	3.0	-	-	-	-	-
MS	3.0	-	1.0	2.0	-	-
MS	3.0	-	2.0	-	-	-
MS	4.0	0.4	-	-	0.25	-
MS	5.0	-	1.0	2.0	-	-
MS	-	5.0	-	-	0.25	-
½MS	-	0.4	4.0	-	0.25	-
½MS	-	0.4	4.0	-	0.25	+
½MS	0.4	-	4.0	-	0.25	-
½MS	0.4	-	4.0	-	0.25	+

3.11 Planting out and acclimatization

The plantlets produced under *in vitro* were taken out without damaging the roots. The roots were gently washed with water and planted in small mud pots containing sterile sand. The pots were covered with transparent polythene bags to maintain humidity and kept under shade. After one week few holes were made in the polythene bags. Then polythene bags were completely removed for four to five hours per day over a period of one week. Later on, the polythene bag was completely removed and plantlet was kept open.

3.12 Observations

Each trial was conducted with a minimum of eight tubes. The observations were recorded for 4-8 weeks. The data were calculated based on the cultures that remained without contamination.

The following observations were recorded from various experiments under the study.

i) Number of cultures uncontaminated

Number of cultures free from contamination were expressed as percentage of total number of cultures.

ii) Number of explants showing bud break

Number of cultures showing bud initiation were expressed as percentage of total number of surviving cultures.

iii) Time taken for bud initiation

The time taken was noted in days.

iv) Number of explants showing leaf production

Number of cultures that produced leaves were expressed as percentage of total surviving cultures that produce buds.

v) Time taken for leaf production

This was recorded in days.

vi) Average number of leaves per culture

Average number of leaves was worked out as average number of leaves from number of cultures showing leaf production.

vii) Average number of leaflets per cultures

Average number of leaflets was calculated as average number of leaflets from number of cultures showing leaf production.

viii) Average number of shoots per culture

Average number of shoots per culture was expressed as average of the number of shoots produced in the different cultures of a particular treatment.

ix) Number of cultures rooted *in vitro*

Number of cultures that produced roots *in vitro* expressed as percentage of total cultures in a particular rooting combination tried.

x) Number of shoots rooted *ex vitro*

Number of shoots rooted *ex vitro* was expressed as a percentage of total shoots tried for *ex vitro* rooting.

xi) Number of roots

Number of roots was expressed as an average of number of roots in the rooted cultures.

xii) Production of callus

Observation on production of callus at the base of axillary bud was made.

3.13 Statistical analysis

Wherever necessary the data were analysed by ANOVA technique for CRD (Snedecor and Cochron, 1967) with one factor or two factor as the case may be. Treatment means were compared using Duncon's Multiple Range Test (Duncon, 1955).

Results

RESULTS

The results of various experiments on micropropagation of *Ailanthus triphysa* (Dennst.) Alston, from the study conducted at the tissue culture laboratory of College of Forestry, Vellanikkara during 1996-98 are presented in this chapter.

4.1 Surface sterilization of explants

Results of the various surface sterilization treatments on culture contamination are presented in Table 4 and 5. Treatment effects were not significant, however, a 20 min. sterilization in mercuric chloride which was preceded by 30 min. dip in a fungicidal mixture of Bavinstin (0.1%) and Indofil (0.1%) was found relatively better among the treatments tried. Explant survival without contamination was found to be as high as 64 per cent in this treatment. The culture contamination was only 38.50 per cent when explants were immersed in 0.15 per cent mercuric chloride for 15 min. as against 42.28 per cent, when immersed in 0.1 per cent mercuric chloride. However, the culture contamination was found to be significantly high at 0.3 per cent mercuric chloride treated for same 15 min.

Size of the explant also was found to influence culture contamination (Table 6). The mean culture contamination was only 62.45 per cent in small size (<0.5 cm dia) explants which was significantly lower compared to contamination in big size (>0.5 cm dia) explants (78.89%). The mercuric chloride (0.1%) treatment of explant for 15 min. was found to reduce the contamination significantly where in contamination was as low as 45.52 per cent and significantly high contamination (91.73%) was registered at 5 min. treatment irrespective of size of the explant. The interaction of mercuric chloride treatment and size of the explant was found to be nonsignificant. However, treatment of small size explants

Table 4. Effect of various surface sterilization treatments on culture contamination of *Ailanthus triphysa*

Duration of $HgCl_2$ treatment (min.)	Percentage of contamination	
	$HgCl_2$ (0.1%)	[Bavistin (0.1%) + Indofil (0.1%] dip for 30 min. + $HgCl_2$(0.1%)
5	98.60	-
8	90.80	-
10	92.25	-
12	76.07	54.67
15	51.43	42.28
18	56.13	44.00
20	75.40	36.00
SEm±	5.38	3.34
CD(0.05)	NS	NS

NS - Non significant
SEm - Standard Error of Mean
CD - Critical difference

Table 5. Effect of surface sterilization with different concentrations of mercuric chloride for 15 min. on culture contamination of *Ailanthus triphysa*

Concentration (%)	Percentage of contamination
0.10	42.28
0.15	38.50
0.30	53.35
SEm±	2.06
CD(0.01)	10.56

Table 6. Effect of mercuric chloride (0.1%) on culture contamination of two different sizes of explants of *Ailanthus triphysa*

Duration of treatment (min.)	Percentage of culture contamination		
	Small size explant (<0.5 cm dia)	Big size explants (>0.5 cm dia)	Mean
5	87.60	95.87	91.73
8	79.17	87.50	83.33
10	72.23	88.90	80.57
12	37.77	66.67	52.22
15	35.50	55.53	45.52
Mean	62.45	78.89	

CD(0.01%) for treatment	= 21.70	SEm±	= 9.34
CD(0.01%) for size	= 13.77	SEm±	= 6.60
CD for interactions	= NS	SEm±	= 7.63

for 15 min. with mercuric chloride (0.1%) was found to be relatively better having only 35.50 per cent culture contamination.

4.2 Phenol exudation

Phenol exudation was severe in the axillary bud cultures especially in bigger sized explants (Table 7 and Fig.1). The effect of explant on phenol exudation was found significant at 0.01 per cent. The small sized explants had low (22.90%) phenol exudation in comparison to the exudation in cultures with big sized explants (57.59%). The mean phenol exudation from both sizes of explants was significantly higher (67.20%) when no treatment was given to control phenol exudation. Among different treatments tried to control phenol exudation, incorporation of AC (0.25%) resulted in significantly low (17.34%) phenol exudation followed by incorporation of ascorbic acid (50 mg l^{-1}), both preceded by 30 min. running water treatment (27.67%). Highly significant interaction effect between explant size and phenol controlling treatment was observed. Significantly low phenol exudation (8.86%) was recorded in small sized explant cultures given with AC (0.25%) in media preceded by 30 min. running water treatment to the explants and this was on par with ascorbic acid (50 mg l^{-1}) in media plus running water treatment to explants for 30 min. in which phenol exudation was only 14.78 per cent. In case of big sized explants, the treatment, AC (0.25%) in media preceded by 30 min. running water treatment was found to be the best treatment. The phenol exudation was as high as 37.74 per cent in small size explants and 96.66 per cent in big size explants when no treatment to control phenol exudation was given.

So, to control phenol exudation and to get healthy plantlets, AC (0.25%) was added to all the media combinations (Plate 1).

Table 7. Effect of various treatments on phenol exudation in two different sizes of explant

Treatment	% of cultures showing phenol exudation			Quantity of phenol	
	Small size (<0.5 cm dia)	Big size (>0.5 cm dia)	Mean	Small size (<0.5 cm dia)	Big size (>0.5 cm dia)
Control	37.74	96.66	67.20	++	+++
Washing in running water for 30 min.	30.21	67.33	48.77	++	+++
Washing in running water for 30 min. + Ascorbic acid (50 mg l^{-1}) in media	14.78	40.56	27.67	+	+
Washing in running water for 30 min. + AC (0.25%) in media	8.86	25.82	17.34	+	+
Mean	22.90	57.59			
CD(0.01%) for treatment	= 8.17	SEm±	= 2.84		
CD(0.01%) for size of explant	= 4.10	SEm±	= 2.01		
CD(0.01%) for interactions	= 11.56	SEm±	= 4.01		

+++ = Very high quantity
++ = High quantity
+ = Low quantity

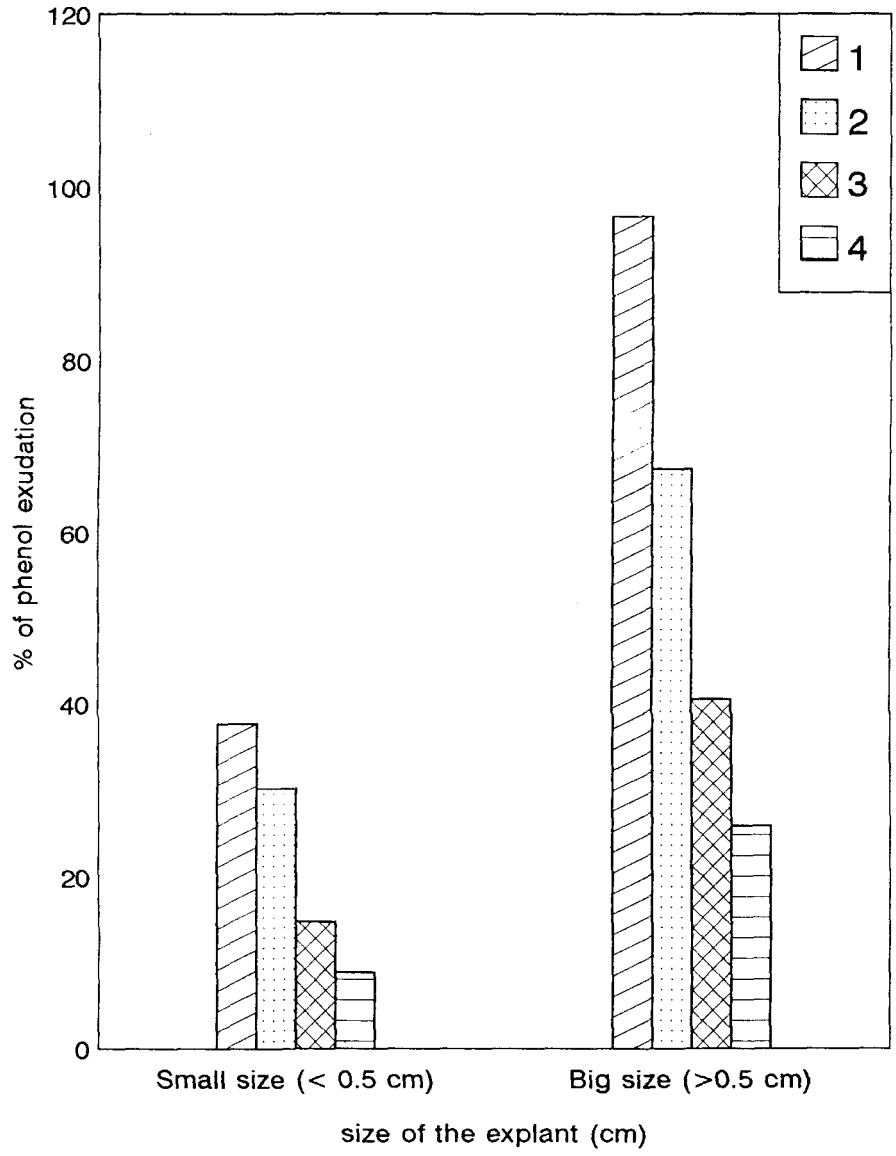


Fig 1. Effect of various treatments on phenol exudtion in two different sizes of explant

1 - Control
2 - Washing in running water for 30 min
3 - Running water treatment for 30 min + Ascorbic acid (50mg l-1)
4 - Running water treatment for 30 min + Activated Charcoal (0.25%)

4.3 Culture establishment and growth in different basal media

The data on culture establishment in different basal media screened during the study are presented in Table 8 and Fig.2. Maximum bud initiation (77.92% ± 7.12%) was recorded in MS medium which was significantly higher than in other two media viz., WPM and half-strength MS medium. Bud initiation and bud expansion in basal MS medium is shown in Plate 2 and Plate 3, respectively. The percentage of bud initiation was 50.16 (± 4.66) and 44.52 (± 4.12) in WPM and ½ MS, respectively, which were found to be on par with each other. Highest percentage (68.66 ± 5.85) of leaf initiation was observed in MS medium which was found to be significantly superior over other two basal media at 0.01 per cent. Leaf initiation in basal MS medium is shown in Plate 4. Half strength MS medium with 46.32 (± 6.44) per cent and WPM with 41.12 (± 10.36) per cent of leaf initiation were not significantly different from each other.

The period (days) for bud and leaf initiation was found to be on par in all the three basal media. However, MS medium with relatively less period for bud and leaf initiation (19.75 ± 4.19 days and 31.88 ± 4.91 days, respectively) was found to be better. Woody plant medium took relatively more time for bud and leaf initiation (25.75 ± 2.72 days and 39.0 ± 4.10 days, respectively).

The mean number of leaves were high in MS medium having 3.19 (± 0.85) leaves per explant and this was significantly different from mean leaves produced in other two media. Mean number of leaves were least in WPM having 2.01 (± 0.55) leaves per explant. However, it was not significantly different from the mean number of leaves in ½ MS medium (i.e., 2.13 ± 0.14). The mean number of leaflets were also highest (8.12 ± 1.38) in MS medium which was again significantly higher than mean leaflet number in WPM and ½ MS medium. Mean number of leaflets were found to be least in WPM (2.98 ± 0.95) which was on par with half-strength MS medium that produced 3.99 (± 1.30) leaflets per explant.

Table 8. Effect of different basal media on culture establishment and growth in axillary and terminal buds of *Ailanthus triphysa*

Basal media	Percentage of		Period (days) of		Mean number of leaves	Mean number of leaflets
	Bud initiation	Leaf initiation	Bud initiation	Leaf initiation		
MS	77.92 (±7.12)	68.66 (±5.85)	19.75 (±4.19)	31.88 (±4.91)	3.19 (±0.85)	8.12 (±1.38)
WPM	50.16 (±4.66)	41.12 (±10.36)	25.75 (±2.72)	39.00 (±4.10)	2.01 (±0.55)	2.98 (±0.95)
½MS	44.52 (±4.12)	46.32 (±6.44)	22.19 (±3.52)	33.13 (±3.57)	2.13 (±0.14)	3.99 (±1.30)
SEm(±)	2.80	3.90	1.77	2.11	0.30	0.61
CD(0.01)	9.10	12.68	NS	NS	0.98	1.98

SEm± = Standard Error of mean
CD(0.01) = Critical Difference at 1%
NS = Non Significant
Figures in parenthesis indicate standard deviation

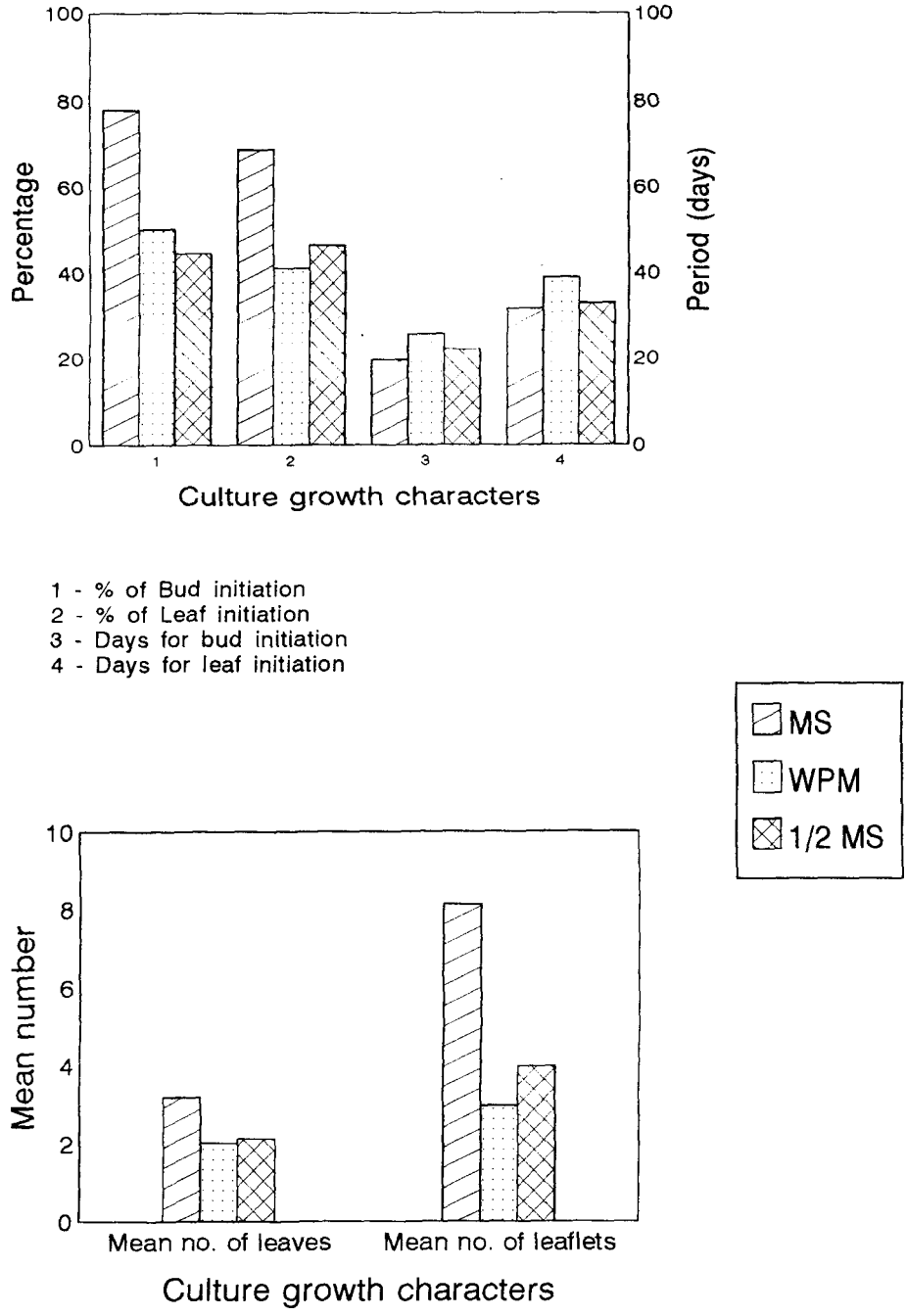


Fig.2. Effect of different basal media on culture establishment and growth in buds of *Ailanthus triphysa*

In general, MS medium was found to be better over other two media. Even when percentage of bud initiation was comparatively less in half-strength MS medium than in WPM, the further growth was relatively better in this medium as evident from the number of leaves and leaflets. Drying of leaves was observed in few cultures in MS basal medium after 3-4 weeks (Plate 5) and after subculturing to fresh basal medium, some of them produced fresh green leaves (Plate 6).

4.4 Effect of plant growth regulators on bud initiation and growth

Based on the screening trials full strength MS medium and half-strength MS medium were selected and supplemented with various concentrations of cytokinins (BA and kinetin), auxin (IAA) and GA_3 independently and in combinations to study their effect on further growth of axillary and terminal bud cultures.

4.4.1 Effect of BA and IAA on culture establishment and growth in MS medium

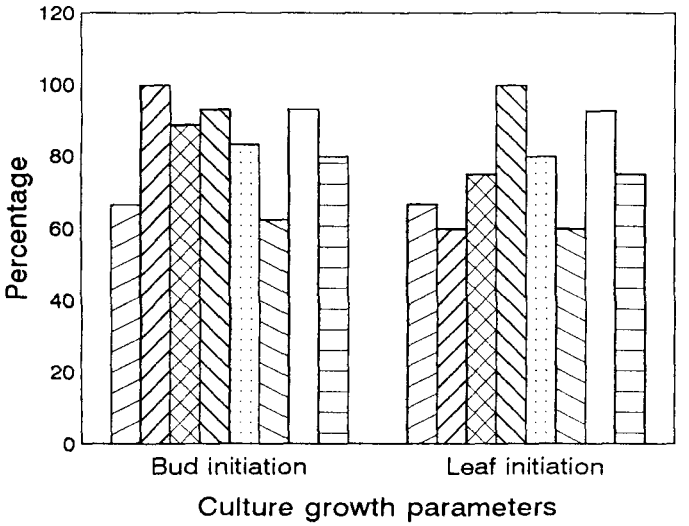
The data on effect of various concentrations of BA with two levels (0.0 and 0.2 mg l^{-1}) of IAA on various parameters are presented in Table 9 and Fig.3.

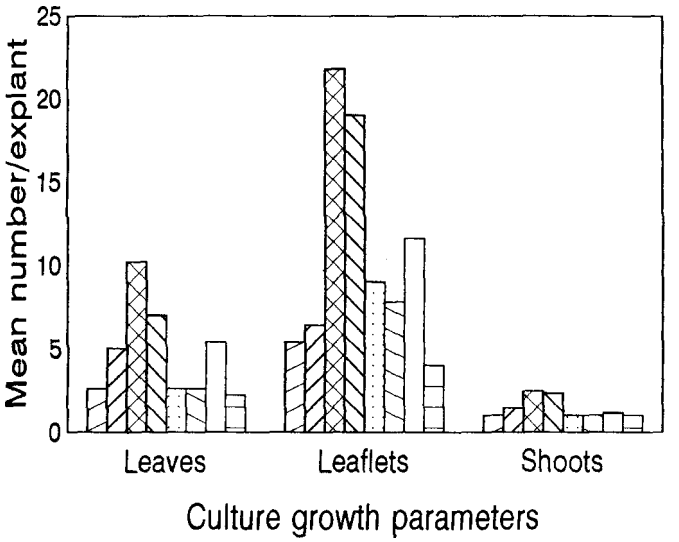
Bud initiation was highest (100%) when 2.0 mg l^{-1} BA without IAA was supplemented to the media and least (62.50%) when BA was supplemented with IAA (0.2 mg l^{-1}) to the media. Hundred per cent of the cultures showing bud initiation produced leaves when BA alone was supplemented to media at 5.0 mg l^{-1} while media containing 2.0 mg l^{-1} BA alone and also along with 0.2 mg l^{-1} IAA showed least frequency (60.0%) of leaf induction. Even when the media containing 1.0 and 2.0 mg l^{-1} BA took least period (18.0 days) for bud initiation, the time taken for leaf initiation was highest (32.0 days). The time taken for bud initiation

Table 9. Effect of different concentrations of BA and IAA on culture establishment and growth in axillary and terminal buds of *Ailanthus triphysa* in MS medium

BA ($mg\ l^{-1}$)	IAA ($mg\ l^{-1}$)	Percentage of		Period (days) for		Mean no. of leaves per explant	Mean no. of leaflets per explant	Mean no. shoots per explant	Maximum no. of shoots
		Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
1.0	0.0	66.60	66.67	18.0	32.0	2.60	5.40	1.00	1.00
2.0	0.0	100.0	60.00	18.0	32.0	5.00	6.40	1.43	3.00
3.0	0.0	88.89	75.00	22.5	25.0	10.20	21.80	2.43	5.00
5.0	0.0	93.20	100.00	20.5	27.0	7.00	19.00	2.29	5.00
1.0	0.2	83.30	80.00	19.5	22.0	2.60	9.00	1.00	1.00
2.0	0.2	62.50	60.00	19.5	22.0	2.60	7.80	1.00	1.00
3.0	0.2	93.30	92.85	19.0	21.0	5.40	11.60	1.14	2.00
5.0	0.2	80.00	75.00	22.0	24.0	2.20	4.00	1.00	1.00

CD(0.01) for interactions on mean no. leaves = 2.207 SEm± = 0.80
CD(0.01) for interactions on mean leaflet number = 4.911 SEm± = 1.78
CD(0.01) for interactions on mean shoot number = 0.808 SEm± = 0.297





1- MS + 1.0 mg l^{-1} BA + 0.0 mg l^{-1} IAA
2- MS + 2.0 mg l^{-1} BA + 0.0 mg l^{-1} IAA
3- MS + 3.0 mg l^{-1} BA + 0.0 mg l^{-1} IAA
4- MS + 5.0 mg l^{-1} BA + 0.0 mg l^{-1} IAA
5- MS + 1.0 mg l^{-1} BA + 0.2 mg l^{-1} IAA
6- MS + 2.0 mg l^{-1} BA + 0.2 mg l^{-1} IAA
7- MS + 3.0 mg l^{-1} BA + 0.2 mg l^{-1} IAA
8- MS + 5.0 mg l^{-1} BA + 0.2 mg l^{-1} IAA

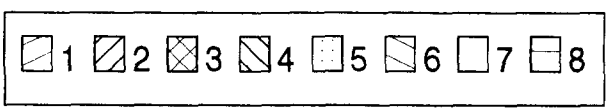


Fig.3. Effect of BA and IAA on culture establishment and growth

was highest (22.5 days) at 3.0 mg l^{-1} BA and least time (21.0 days) at 3.0 mg l^{-1} BA with 0.2 mg l^{-1} IAA.

The interaction effect of BA and IAA was found to be highly significant on mean number of leaves, leaflets and shoots. At BA 3.0 mg l^{-1} without IAA, the mean number of leaves was 10.20 which was significantly higher than at any other levels of BA alone or BA with IAA (0.2 mg l^{-1}). The least number of leaves (2.20) was recorded at 5.0 mg l^{-1} BA with 0.2 mg l^{-1} IAA. The mean number of leaflets were highest (21.80) at 3.0 mg l^{-1} BA alone in media, which was also significantly different from other levels of BA and IAA. Again, the mean number of leaflets was least (4.00) at 5.0 mg l^{-1} BA with 0.2 mg l^{-1} IAA. Multiple shoots were observed at 2.0, 3.0 and 5.0 mg l^{-1} BA alone, and 3.0 mg l^{-1} BA with 0.2 mg l^{-1} IAA. Mean number of shoots per culture was highest (2.43) at 3.0 mg l^{-1} BA without IAA and was significantly different from that of other levels of BA and IAA with the exception of media having 5.0 mg l^{-1} BA alone where it was 2.29 per culture. Only one shoot was observed at 1.0 mg l^{-1} BA alone, and 1.0, 2.0 and 5.0 mg l^{-1} BA with 0.2 mg l^{-1} IAA. Maximum number of shoots (5.0) was recorded at 3.0 and 5.0 mg l^{-1} BA without IAA.

4.4.2 Effect of BA on culture establishment and growth in half-MS medium

The data on the effect of different concentrations of BA on culture establishment and further growth in ½ MS media is given in Table 10. Hundred per cent of cultures produced buds at 1.0 mg l^{-1} BA and least (25.0%) bud initiation was recorded at 3.0 mg l^{-1} BA. Even when bud initiation was least at 3.0 mg l^{-1} BA, cent per cent of them produced leaves and the leaf induction was least (25.0%) at 5.0 mg l^{-1} BA. The time taken for bud initiation was least (23.0 days) at 1.0 mg l^{-1} BA as well as 3.0 mg l^{-1} BA and it was highest (28.0 days) at 2.0 mg l^{-1} BA as well as at 5.0 mg l^{-1} BA. Least time (23.0 days) for leaf initiation was recorded at 3.0 mg l^{-1} BA and more time (33.0 days) was observed at 1.0 and 2.0 mg l^{-1} BA.

Table 10. Effect of different concentrations of BA on culture establishment and growth in axillary and terminal buds of *Ailanthus triphysa* in half-MS medium

BA concentration ($mg\ l^{-1}$)	Percentage of		Period (days) for		Mean no. of leaves per explant	Mean no. of leaflets per explant	Mean no. shoots per explant	Maximum no. of shoots
	Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
-								
1.0	100.00	66.67	23.0	33.0	1.5	4.75	1.0	1.0
2.0	33.33	50.00	28.0	33.0	1.0	5.0	1.0	1.0
3.0	25.00	100.00	23.0	23.0	1.0	6.00	1.0	1.0
5.0	62.50	25.00	28.0	25.0	1.5	6.00	1.0	1.0
SEm±								
CD (0.05)						NS	NS	

SEm± = Standard Error of mean
NS = Non significant

The mean number of leaves and leaflets did not differ statistically at different levels of BA. However, media containing 1.0 and 5.0 mg l^{-1} BA which produced 1.5 leaves on an average were found to be relatively better. Only one leaf was observed at 2.0 mg l^{-1} BA as well as at 3.0 mg l^{-1} BA. An average of 6.0 leaflets were recorded at 3.0 and 5.0 mg l^{-1} BA which was found to be relatively higher in comparison with other levels and minimum number (4.75) for the same was recorded at 1.0 mg l^{-1} BA. No multiple shoots were observed even at higher level of BA in ½ MS medium. Only one shoot per culture was recorded at all the concentrations of BA.

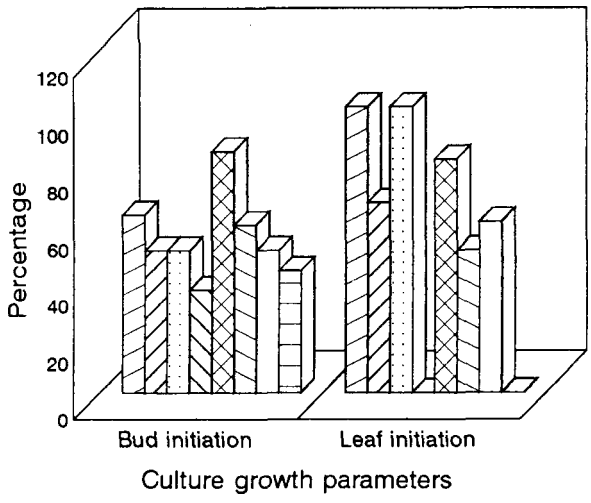
4.4.3 Effect of different concentrations of kinetin and IAA on culture establishment and growth in MS medium

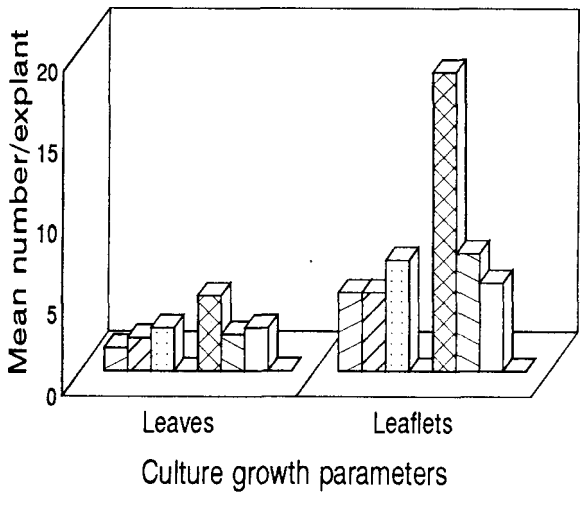
Effect of different concentration of kinetin and IAA in MS medium was studied and the data is presented in Table 11 and Fig.4. Highest bud initiation (84.0%) was found when 1.0 mg l^{-1} kin was supplemented with 0.2 mg l^{-1} IAA and the least (42.85%) was when 5.0 mg l^{-1} BA was supplemented with 0.2 mg l^{-1} IAA. Leaf initiation was found in hundred per cent of cultures having bud when only kinetin was supplemented to the media at 1.0 mg l^{-1} and at 3.0 mg l^{-1}. None of the cultures showing bud initiation could produce leaves in media supplemented with 5.0 mg l^{-1} kin alone and also along with 0.2 mg l^{-1} IAA. The least number of days (14.0 days) for bud initiation was observed at 1.0 mg l^{-1} kin and also at 2.0 mg l^{-1} kin. Highest period for bud initiation (21 days) was observed in media containing 3.0 mg l^{-1} kin with 0.2 mg l^{-1} IAA and also in media containing 5.0 mg l^{-1} kin with 0.2 mg l^{-1} IAA. The time taken for leaf initiation was found to be lowest (21.0 days) in media supplemented with 1.0 mg l^{-1} kin with 0.2 mg l^{-1} IAA and it was highest (31.0 days) in media containing 3.0 mg l^{-1} kin with 0.2 mg l^{-1} IAA. The highest mean number of leaves (4.6) were observed when 1.0 mg l^{-1} kin and 0.2 mg l^{-1} IAA were supplemented to the media and it was significantly higher compared to other levels of kin alone or with IAA (0.2 mg l^{-1}). A least number of 1.4 leaves on an average were observed at 1.0 mg l^{-1} kin without IAA. No leaf

Table 11. Effect of different concentrations of kinetin and IAA on culture establishment and growth in axillary and terminal buds of *Ailanthus triphysa* in MS medium

Kin ($mg\ l^{-1}$)	IAA ($mg\ l^{-1}$)	Percentage of		Period (days) for		Mean no. of leaves per explant	Mean no. of leaflets per explant	Mean no. shoots per explant	Maximum no. of shoots
		Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
1.0	0.0	62.50	100.00	14.0	30.0	1.40	4.80	1.00	1.00
2.0	0.0	50.00	66.67	14.0	30.0	2.00	4.80	1.00	1.00
3.0	0.0	50.00	100.00	18.0	30.0	2.60	6.80	1.0	1.00
5.0	0.0	36.00	0.00	20.0	0.0	0.00	0.00	1.00	1.00
1.0	0.2	84.60	81.82	19.0	21.0	4.60	18.40	1.00	1.00
2.0	0.2	58.82	50.00	19.0	28.0	2.20	7.20	1.00	1.00
3.0	0.2	50.00	60.00	21.0	31.0	2.60	5.40	1.00	1.00
5.0	0.2	42.85	0.00	21.00	0.0	0.00	0.00	1.00	1.00

CD(0.01) for interactions on mean leaf number = 1.26 SEm± = 0.424
CD(0.01) for interactions on mean leaflets number = 0.629 SEm± = 0.211





1- MS + 1.0 mg l^{-1} kin + 0.0 mg l^{-1} IAA
2- MS + 2.0 mg l^{-1} kin + 0.0 mg l^{-1} IAA
3- MS + 3.0 mg l^{-1} kin + 0.0 mg l^{-1} IAA
4- MS + 5.0 mg l^{-1} kin + 0.0 mg l^{-1} IAA
5- MS + 1.0 mg l^{-1} kin + 0.2 mg l^{-1} IAA
6- MS + 2.0 mg l^{-1} kin + 0.2 mg l^{-1} IAA
7- MS + 3.0 mg l^{-1} kin + 0.2 mg l^{-1} IAA
8- MS + 5.0 mg l^{-1} kin + 0.2 mg l^{-1} IAA

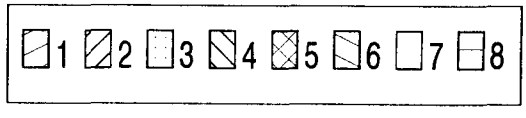


Fig.4. Effect of kinetin and IAA on culture establishment and growth

formation was observed at 5.0 mg l^{-1} kin alone or with 0.2 mg l^{-1} IAA. Significantly higher number of leaflets (18.4) was found at 1.0 mg l^{-1} kin with 0.2 mg l^{-1} IAA and significantly low leaflets number (4.8) was observed at 1.0 mg l^{-1} kin as well as at 2.0 mg l^{-1} kin. The mean number of shoots was found to be same (1.0 per culture) at all the levels of kin and IAA tried.

4.4.4 Effect of kinetin on culture establishment and growth in half-MS medium

The data on the effect of kinetin in ½ MS medium are given in Table 12. Highest bud and leaf initiation (62.50% and 83.33%, respectively) were recorded at 2.0 mg l^{-1} kin and least bud (40.0%) as well as leaf initiation (0.0%) were recorded at 3.0 mg l^{-1} kin. Minimum days for bud and leaf initiation (18.0 days and 28.0 days, respectively) were recorded at 5.0 mg l^{-1} of kin. More number of days for bud and leaf initiation (23.0 days and 53.0 days, respectively) were recorded at 2.0 mg l^{-1} of kin.

Eventhough the mean number of leaves produced at different levels of kinetin as nonsignificant, 5.0 mg l^{-1} of kin which produced 2.33 leaves on an average was found to be relatively better. The mean number of leaflets at different levels of kinetin was found to be significant at 0.05 per cent. Mean number of leaflets were significantly higher (12.0) at 5.0 mg l^{-1} kin and leaflets were produced at 3.0 mg l^{-1} kin. Significant variation in mean shoot number was observed and significantly high (1.67) mean shoot number was recorded at 5.0 mg l^{-1} kin. Maximum number of shoots (3.0) were observed also at 5.0 mg l^{-1} kin.

4.4.5 Effect of BA and kinetin on culture establishment and growth in MS medium

The data on the effect of various levels of BA in combination with different levels of kinetin are presented in Table 13 and Fig.5. Hundred per cent of

Table 12. Effect of kinetin on culture establishment and growth in axillary and terminal buds of *Ailanthus triphysa* in half-MS medium

Kinetin (mg l^{-1})	Percentage of		Period (days) for		Mean no. of leaves per explant	Mean no. of leaflets per explant	Mean no. shoots per explant	Maximum no. of shoot
	Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
-								
2.0	62.50	83.33	25.0	53.0	1.82	6.40	1.0	1.00
3.0	40.00	0.00	23.0	0.0	0.00	0.00	1.00	1.00
5.0	60.00	50.00	18.0	28.0	2.33	12.00	1.67	3.00
SEm±					0.33	1.49	0.14	
CD(0.05)					NS	3.60	0.43	

NS = Non Significant

Table 13. Effect of various concentrations of BA and kinetin on culture establishment and growth in axillary and terminal buds of *Ailanthus triphysa* in MS medium

BA ($mg\ l^{-1}$)	Kin ($mg\ l^{-1}$)	Percentage of		Period (days) for		Mean no. of leaves per explant	Mean no. of leaflets per explant	Mean no. shoots per explant	Maximum no. of shoots
		Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
1.0	1.0	50.00	66.67	23.0	46.0	1.75	4.50	1.25	2.00
1.0	2.0	50.00	50.00	23.0	46.0	4.00	9.25	1.50	2.00
1.0	3.0	71.43	60.00	22.0	36.0	2.75	7.50	2.75	5.00
1.0	5.0	62.15	55.69	21.0	39.0	2.75	7.50	1.75	3.00
2.0	1.0	70.25	47.80	25.0	43.0	2.75	6.50	1.25	2.00
2.0	2.0	100.00	75.00	22.0	34.0	4.50	16.50	2.00	4.00
2.0	3.0	31.25	80.00	25.0	33.0	3.00	7.00	1.75	3.00
2.0	5.0	50.00	100.00	27.5	40.0	2.50	6.25	1.00	1.00
3.0	1.0	40.00	75.00	12.0	40.0	2.00	5.50	4.25	8.00
3.0	2.00	44.00	75.00	12.0	40.0	5.50	19.50	3.00	4.00
3.0	3.0	37.50	100.00	30.0	62.0	4.00	12.50	1.75	3.00
3.0	5.0	50.00	100.0	30.0	62.0	3.75	10.00	1.75	3.00
5.0	1.0	50.00	50.0	30.0	62.0	4.00	14.00	2.00	3.00
5.0	2.0	77.78	42.86	30.0	62.0	5.00	14.00	3.75	8.00
5.0	3.0	54.55	66.67	25.0	36.0	4.25	13.25	1.75	4.00
5.0	5.0	75.00	50.00	25.0	36.0	1.75	6.75	2.25	4.00

Interactions for mean leaves, leaflets and shoots = Non significant
SEm± for mean number of leaves = 1.09
SEm± for mean number of leaflets = 3.15
SEm± for mean number of shoots = 0.70

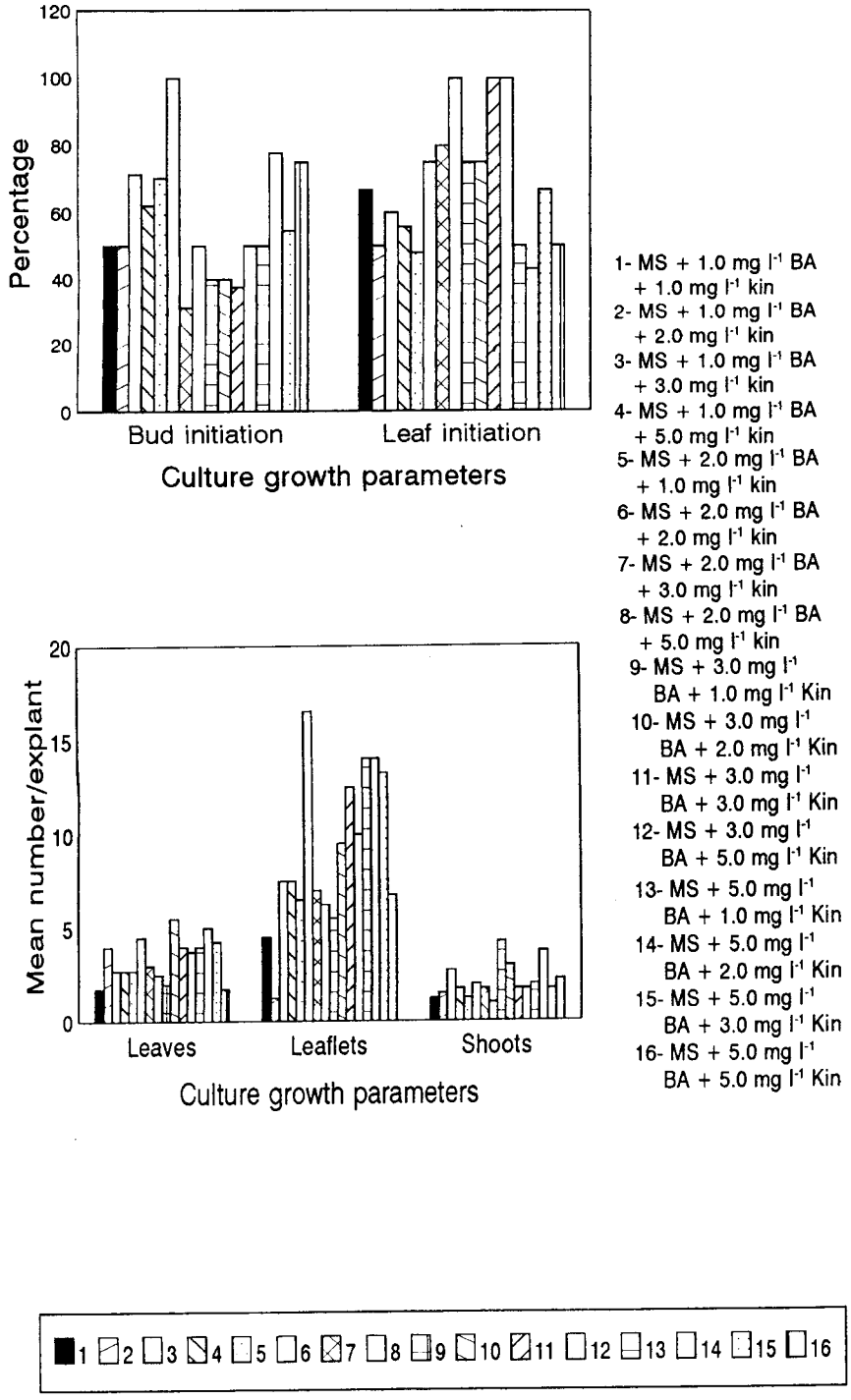


Fig.5. Effect of BA and kinetin on culture establishment and growth

cultures showed bud initiation at 2.0 mg l^{-1} BA with 2.0 mg l^{-1} kin and hundred per cent of leaf initiation at 2.0 mg l^{-1} BA with 5.0 mg l^{-1} kin as well as at 3.0 mg l^{-1} BA with 3.0 and 5.0 mg l^{-1} kin was observed. Low bud initiation (31.25%) was observed at 2.0 mg l^{-1} BA with 3.0 mg l^{-1} kin and lowest leaf initiation (43.86%) at 5.0 mg l^{-1} BA with 2.0 mg l^{-1} kin. Media having 3.0 mg l^{-1} BA with 1.0 and 2.0 mg l^{-1} kin took least (12 days) number of days for bud initiation. Media having 2.0 mg l^{-1} BA with 3.0 mg l^{-1} kin took only 33 days for leaf initiation which was least compared to the time taken by other levels of BA and kin. The media having 3.0 mg l^{-1} BA with 3.0 and 5.0 mg l^{-1} kin, and 5.0 mg l^{-1} BA with 1.0 and 2.0 mg l^{-1} kin took more number of days (30 days) for bud initiation and leaf initiation (62 days).

The interaction effect of BA and kin at different levels tried was found to be nonsignificant. Highest mean number of leaves (5.50/explant) and leaflets (19.50/explant) were recorded at 3.0 mg l^{-1} BA with 2.0 mg l^{-1} kin. Multiple shoots were recorded at all the combinations of BA with kin except at 2.0 mg l^{-1} BA with 5.0 mg l^{-1} kin. Highest average number of shoots (4.25/explant) was observed when 3.0 mg l^{-1} BA was supplemented with 1.0 mg l^{-1} kin and on average only one shoot per explant was recorded at 2.0 mg l^{-1} BA with 5.0 mg l^{-1} of kin. Cultures produced even up to eight shoots from a single bud at first culture when 3.0 mg l^{-1} BA was supplemented with 1.0 mg l^{-1} kin (Plate 7) and also at 5.0 mg l^{-1} BA with 2.0 mg l^{-1} kin. Generally, BA at 3.0 mg l^{-1} BA with 2.0 mg l^{-1} kin which produced 5.50 leaves, 19.50 leaflets and 3.0 shoots per explant in eight weeks was found to be the best level of BA and kin when given together (Plate 8).

In some cultures about 5.0 cm shoot length was recorded at 2.0 mg l^{-1} BA with 2.0 mg l^{-1} kin (Plate 9).

4.4.6 Effect of BA and kinetin on culture establishment and growth in half-MS medium

The data on the combination effect of BA and kin in ½ MS is presented in Table 14. Hundred per cent cultures showed bud initiation when 1.0 mg l^{-1} BA with 1.0 mg l^{-1} kin was supplemented to the media. No bud initiation was observed in ½MS media containing 2.0 mg l^{-1} BA with 1.0, 2.0, 3.0 and 5.0 mg l^{-1} kin, and also at 3.0 mg l^{-1} BA with 2.0 and 3.0 mg l^{-1} kin. Among the treatments which produced buds, highest (100%) leaf initiation was observed at 5.0 mg l^{-1} BA with 1.0 mg l^{-1} kin and no leaf initiation at 3.0 mg l^{-1} BA with 5.0 mg l^{-1} kin, and also at 5.0 mg l^{-1} BA with 5.0 mg l^{-1} kin.

Highest mean number of leaves per explant (2.0) was observed at 1.0 mg l^{-1} BA with 3.0 mg l^{-1} kin as well as at 5.0 mg l^{-1} BA with 2.0 mg l^{-1} kin. Highest mean leaflets per explant (4.0) were observed when 1.0 mg l^{-1} BA was supplemented with 2.0 and 3.0 mg l^{-1} of kin and also when 3.0 mg l^{-1} BA with 1.0 mg l^{-1} kin was supplemented to the media. Only one shoot per explant was observed in all the treatments which produced bud.

4.4.7 Effect of BA, kinetin and GA_3 on culture establishment and growth in MS medium

The data on the effect of GA_3 individually and in combination with different concentrations of BA and/or kin is presented in Table 15. Maximum culture response in terms of bud initiation (100%) was recorded at 3.0 mg l^{-1} kin with 2.0 and 3.0 mg l^{-1} GA_3 and minimum bud initiation (27.78%) was recorded when 2.0 mg l^{-1} kin and 3.0 mg l^{-1} GA_3 were supplemented to the media. Nearly 90.5 per cent of leaf initiation was found at 5.0 mg l^{-1} GA_3 and only 25.0 pr cent leaf initiation was recorded at 1.0 mg l^{-1} kin with 3.0 mg l^{-1} GA_3. Only 12 days were taken for bud initiation in media containing 1.0 mg l^{-1} of GA_3 alone and maximum period (26.0 days) was recorded at 2.0 mg l^{-1} GA_3. Minimum period (25 days) for leaf initiation was recorded at 3.0 mg l^{-1} kin with 3.0 mg l^{-1} GA_3 and

Table 14. Effect of various concentrations of BA and kinetin on culture establishment and growth in axillary and terminal buds of *Ailanthus triphysa* in half MS medium

BA ($mg\ l^{-1}$)	Kin ($mg\ l^{-1}$)	Percentage of		Period (days) for		Mean no. of leaves per explant	Mean no. of leaflets per explant	Mean no. shoots per explant	Maximum no. of shoots
		Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
1.0	1.0	100.00	60.00	40.0	50.0	1.33	2.67	1.00	1.00
1.0	2.0	62.50	40.00	24.00	43.0	1.00	4.00	1.00	1.00
1.0	3.0	71.42	20.00	40.0	40.0	2.00	4.00	1.00	1.00
2.0	1.0	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00
2.0	2.0	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00
2.0	3.0	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00
2.0	5.0	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00
3.0	1.0	25.00	50.00	22.0	32.0	1.00	4.00	1.00	1.00
3.0	2.0	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00
3.0	3.0	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.00
3.0	5.0	16.67	0.00	30.0	0.0	0.00	0.00	1.00	1.00
5.0	1.0	60.00	100.00	33.0	33.0	1.33	2.00	1.00	1.00
5.0	2.0	40.00	50.00	33.0	33.0	2.00	3.00	1.00	1.00
5.0	3.0	75.00	66.67	32.00	32.0	1.50	3.00	1.00	1.00
5.0	5.0	66.67	0.00	43.00	0.0	0.00	0.00	1.00	1.00

Table 15. Effect of BA, kinetin and GA_3 on culture establishment and growth in axillary and terminal buds of *Ailanthus triphysa* in MS medium

Concentration of growth regulators (mg l^{-1})			Percentage of cultures showing		Period (days) for culture establishment		Mean leaves per explant	Mean leaflets per explant	Mean shoots per explant	Maximum number of shoots
BA	Kin	GA_3	Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
1.0	1.0	3.0	74.30	61.54	22.0	28.0	2.61	8.44	1.58	3.00
1.0	-	1.0	60.00	66.67	24.0	26.0	1.80	8.00	1.00	1.00
1.0	-	3.0	50.00	33.33	18.0	45.0	3.50	8.00	1.33	3.00
2.0	-	3.0	46.67	28.57	22.0	43.0	3.00	5.00	1.00	1.00
2.0	-	1.0	28.60	75.00	25.0	46.0	2.00	3.67	1.00	1.00
3.0	-	1.0	54.55	66.67	23.0	34.0	1.67	6.67	1.67	3.00
3.0	-	3.0	81.25	69.23	22.0	28.0	2.22	4.63	1.92	4.00
5.0	-	1.0	83.33	60.00	14.0	26.0	4.33	9.00	1.40	4.00
5.0	-	2.0	47.37	66.67	21.0	32.0	1.83	4.83	1.00	1.00
5.0	-	3.0	61.11	0.00	13.0	0.0	0.00	0.00	1.00	1.00
5.0	3.0	1.0	42.86	66.67	19.0	26.0	3.60	9.00	1.83	3.00
-	-	1.0	63.63	28.57	12.0	33.0	3.00	10.00	1.00	1.00
-	-	2.0	44.40	0.00	26.0	0.0	0.00	0.00	1.00	1.00
-	-	3.0	82.86	72.41	27.0	31.0	3.38	12.26	1.36	6.00
-	-	5.0	58.33	90.48	24.0	29.0	3.89	8.33	1.29	6.00
-	1.0	3.0	28.57	25.00	19.0	25.0	1.00	2.00	1.25	2.00
-	2.0	3.0	27.78	60.00	19.0	40.0	1.67	7.00	1.00	1.00
-	3.0	5.0	100.00	73.68	22.0	38.0	4.05	9.89	2.50	15.00
-	3.0	1.0	28.60	0.00	30.0	0.0	0.00	0.00	1.00	1.00
-	3.0	2.0	100.00	58.62	22.0	38.0	4.71	11.72	2.20	5.00
-	3.0	3.0	41.67	60.00	19.0	25.0	2.33	8.33	1.00	1.00
-	5.0	3.0	36.36	50.00	17.0	51.0	3.50	10.00	1.25	2.00

maximum period (51 days) was recorded at 5.0 mg l^{-1} kin with 3.0 mg l^{-1} GA_3. Highest average number of leaves (4.71) was recorded at 3.0 mg l^{-1} kin and 2.0 mg l^{-1} GA_3, and average leaflet number was highest (12.26) in media having 3.0 mg l^{-1} GA_3. No leaf and leaflets production was observed in media containing 2.0 mg l^{-1} GA_3 as well as 3.0 mg l^{-1} GA_3. Multiple shoot production was observed in many of the combinations of BA, kin and GA_3 tried. Highest average shoots per culture (2.50) was recorded at 3.0 mg l^{-1} kin with 5.0 mg l^{-1} GA_3 with some cultures producing up to 15.0 shoots from a single bud (Plate 10).

Shoot elongation was observed in some cultures in media containing 3.0 mg l^{-1} BA and 1.0 mg l^{-1} GA_3 with shoot length approximately 2.50 cm (Plate 11).

Callus production at the base of the nodal explants was observed in some cultures (Plate 1). Amount of callus was negligeble and hence efforts were not done to multiply callus and to workout callusing index (CI).

4.5 Root induction from *in vitro* produced shoots

4.5.1 *In vitro* rooting

Eleven different treatments combinations were tried for root induction during the study (Table 16). The rooting was successfully obtained only in half-strength MS medium supplemented with 0.4 mg l^{-1} IBA, 4.0 mg l^{-1} IAA and AC (0.25%) from the shoots which were given with a pulse treatment in sterilized 1000 mg l^{-1} IBA solution (Plate 12).

4.5.2 *Ex vitro* rooting

During the study *ex vitro* rooting was also attempted from the shoots produced *in vitro*. Only 6.0 per cent of the shoots showed signs of rooting in sand

Table 16. Effect of various media combinations on *in vitro* rooting of microshoots of *Ailanthus triphysa*

Media combination	Percentage of rooting	Average no. of roots
MS + 3.0 mg l^{-1} IBA	0.0	0.0
MS + 3.0 mg l^{-1} IBA + 2.0 mg l^{-1} IAA	0.0	0.0
MS + 3.0 mg l^{-1} IBA + 2.0 mg l^{-1} GA_3 + 1 mg l^{-1} IAA	0.0	0.0
MS + 5.0 mg l^{-1} IBA + 2.0 mg l^{-1} GA_3 + 1.0 mg l^{-1} IAA	0.0	0.0
MS + 0.4 mg l^{-1} IBA + 4.0 mg l^{-1} IAA + 0.25% AC	0.0	0.0
MS + 5.0 mg l^{-1} NAA + 0.25% AC	0.0	0.0
MS + 0.4 mg l^{-1} NAA + 4.0 mg l^{-1} IBA + 0.25% AC	0.0	0.0
½MS + 0.4 mg l^{-1} IBA + 4.0 mg l^{-1} IAA + 0.25% AC	0.0	0.0
½MS + 0.4 mg l^{-1} IBA + 4.0 mg l^{-1} IAA + * 0.25% AC	6.25	2.0
½MS + 0.4 mg l^{-1} NAA + 4.0 mg l^{-1} IAA + 0.25% AC	0.0	0.0
½MS + 0.4 mg l^{-1} NAA + 4.0 mg l^{-1} IAA + * 0.25% AC	0.0	0.0

* with pulse treatment for the base of microshoots in 1000 mg l^{-1} IBA solution

medium after a pulse treatment in 1000 mg l^{-1} IBA solution (Plate 13). The shoots which striked roots *ex vitro* could not survive due to fungal infection.

4.6 Planting out and acclimatization

Planting out and acclimatization of *in vitro* rooted plantlets was tried. Plantlets could not survive when kept open to outside humidity conditions after a high humidity incubation for two weeks.

Discussion

DISCUSSION

The potential of trees as a source of wood and wood products has generated considerable interest among plant biotechnologists for devising methods for rapid clonal propagation of selected genotypes. Tissue culture is becoming a powerful tool for achieving the above objective, as majority of trees are not amenable for conventional vegetative propagation techniques like cuttings and grafting. Micropropagation of tree species offers scope for producing large number of clonal planting stocks from selected superior genotypes.

The present study was undertaken to establish a rapid *in vitro* propagation technique for *Ailanthus triphysa* (Dennst.) Alston, which will help to provide good quality planting material from selected mother trees to the tree growers. The results from the study are discussed below.

5.1 Culture contamination

Microbial contamination is the single most important cause of losses in commercial and scientific plant tissue culture laboratories (Leifert and Woodward, 1998). All cultures will end up with contamination if the explant used is not properly disinfected. Doublin (1984) observed that the percentage of infection was more than 90 for field explants regardless of the procedure used for their sterilization. The microbial hazards in micropropagation can affect the tissue culture itself, plant survival during weaning and the customer of micropropagation laboratories. At times, control of contamination is extremely difficult and with many contaminants impossible (Leifert and Woodward, 1998). Hence they suggested that more emphasis will have to be placed on early detection and prevention of contamination at source itself.

In the present study, culture contamination starting from the explants had been a very serious problem. This had been much more during the rainy season, obviously due to the high amount of inoculum favoured by this weather and culture success was sometimes nil during this season.

During the dry months, data obtained from the various surface sterilization treatments did not differ significantly. Treatment of explants with mercuric chloride (0.1%) for 15 min. resulted in only 51.43 per cent contamination. Mercuric chloride as a surface sterilant is well documented (George and Sherrington, 1984; Mahato, 1992; Kumar, 1993; Divatar, 1994; Kannan, 1995). Increased duration of treatment (18 min. and 20 min.), however, resulted in higher contamination. This is probably due to fact that the latter treatments were tried during rainy season and quantity of microbial inoculum might be higher. Dipping of explants with leaves intact in a combination of fungicides viz., Bavistin (Carbendazim) and Indofil M-45 (Mancozeb) both at 0.1 per cent followed by mercuric chloride (0.1%) treatment of explants for 20 min. was found to reduce the contamination further to about 36.0 per cent (Table 4). Pre soaking the explants in fungicidal solution for reducing fungicidal contamination has been suggested by Broome and Zimmerman (1978) in black berry.

Size of the explant had a significant effect on culture contamination (Table 6). Small sized (<0.5 cm dia) explants had significantly lower culture contamination in comparison with big sized (>0.5 cm dia) explants. Higher microbial contamination in larger explants was found in *Vateria indica* also (Divatar, 1994). This may be due to the more surface area of large explants which will lead to the presence of more microbial inoculum. Mercuric chloride (0.1%) treatment of small sized explants for 15 min. resulted in only 35.50 per cent contamination in comparison to 55.53 per cent in case of bigger explants and was found to be relatively effective treatment to get more number of uncontaminated cultures.

A level of contamination losses of not above 2.0 per cent per culture is considered as a minimum requirement to guarantee successful production (Leifert and Woodward, 1998). The succulant explants of *A. triphysa* may offer more sap to the microbes and may thus harbour high microbial inoculum. The microbes might be residing under the porous bark and hence even severe surface sterilization treatments were found to be ineffective in reducing culture contamination. Also, the source plants were not under protected conditions and chances of harbouring contaminants were still high. Probably because of these reasons the culture contamination especially due to fungus could not be reduced to an acceptable level.

5.2 Polyphenol exudation

The phenolic substances leached out from the cut surface of the explants lead to the death of explants due to the toxicity of the oxidation products of these leachates. This is a serious problem in micropropagation of woody species especially when mature tissues are used. Survival of explants was reduced to 20 per cent when polyphenol exudation was left unchecked (Razdan, 1993). Hu and Wang (1983) noted that oxidised compounds are highly toxic, they can form covalent bonds with plant protein thus inhibiting the enzyme activity causing browning and death of explants.

Phenol exudation was found to be a serious problem in *A. triphysa* especially when big sized explants (> 0.05 cm dia) were used (Table 7 and Fig.1). The size of the explant had a significant influence on phenol exudation. Significantly high phenol exudation was observed in big sized explants compared to small sized ones. Also, higher quantity of phenol exudation was observed in big sized explants. High phenol exudation in axillary bud cultures of *Ailanthus triphysa* was reported to be serious problem (D'Silva and D'Souza, 1992), as in

other woody species like teak (Gupta *et al.*, 1980), eucalyptus (Gill and Gill, 1994), rose wood (Kannan, 1995) etc.

Various treatments to reduce phenol exudation were found to differ significantly from one another. washing of explants in running tap water for 30 min. was found to significantly reduce phenol exudation in comparison with culturing without any treatment to explants. Significantly lowest phenol exudation was obtained when explants given with running tap water immersion for 30 min. were cultured in media containing AC (0.25%). Activated charcoal absorbs substances ihibitory to growth and thereby promote growth (Weatherhead *et al.*, 1979). One subculturing along with above treatment was found to further reduce the number of cultures with phenol and also quantity of phenol. Repeated subculturing was found to be effective in reducing phenol exudation in *A. triphysa* (D'Silva and D'Souza, 1992). To reduce the phenol exudation and to increase growth performance of cultures AC (0.25%) was invariably added to all the shoot production media combinations during the present study.

5.3 Culture establishment, bud break and shoot growth

The most extensively used culture media for micropropagation in trees are MS (Murashige and Skoog, 1962) and WPM (Lloyd and McCown, 1980). Reducing the salt concentration is reported to be effective in some woody species like *Leucaena leucocephala* (Datta and Datta, 1984), *Eucalyptus grandis* (Raghavan, 1986), Ziziphus cv. (Kim and Lee, 1988), *Shorea roxburghii* (Scott *et al.*, 1990) etc.

In the present study through initial screening it was found that MS medium, with significantly high performance for the culture characters tested in comparison with WPM and half-strength MS media, was found to be the best basal medium (Table 8 and Fig.2). Murashige and Skoog medium is reported as

best basal media for shoot production in a number of tree species like *Ailanthus triphysa* (D'silva and D'souza, 1992), *Dalbergia sissoo* (Suwai *et al.*, 1988), *Eucalyptus globulus* (Pattanaik and Vijayakumar, 1997) etc. The initial screening has also shown that half-strength MS medium is comparatively better than WPM for shoot growth.

Considering the above observations, more detailed studies were carried out by supplementing the MS and half MS medium with various combinations of plant growth regulators for axillary/terminal bud cultures of *Ailanthus*.

Axillary bud is a predetermined organ with morphogenetic potential to develop into a shoot in the absence of apical dominance. The application of cytokinins to the axillary buds can overcome the effect of apical dominance and stimulate lateral buds to grow in the presence of terminal bud (Sachs and Thimman, 1964).

In general, benzyl adenine (BA) has been frequently reported to induce better shoot growth and multiplication than other cytokinins, particularly in tree species (Ahmed, 1989). Its effectiveness has been demonstrated in many tree species like *Eucalyptus* spp. (Gupta *et al.*, 1981), *Calophyllum inophyllum*, *Eugenia* spp., *Fragaria fragrans* (Rao and Lee, 1982), *Prunus serotina* (Tricoli *et al.*, 1985), *Dalbergia latifolia* (Kannan, 1995), *Duabanga grandiflora* (Kumar and Kumar, 1997) etc. Similar results were observed in the present study. Benzyl adenine was found to yield better response in MS medium in comparison with kinetin (Table 9 and 11) when both were supplemented individually or in combination with IAA (0.2 mg l^{-1}). When BA alone was supplemented to MS medium, the mean number of leaves, leaflets and shoots were found to increase with increase in the concentration of BA from 1.0 mg l^{-1} to 3.0 mg l^{-1}. Nevertheless, further increase in BA concentration to 5.0 mg l^{-1} reduced average number of leaves, leaflets and shoots (Fig.3). This may

be attributed to the inhibitory effect of BA at supraoptimal concentrations. Similar inhibitory effects of BA were also reported in number of other woody species like, *Eucalyptus globulus* (Pattanaik and Vijayakumar, 1997), *Acacia nilotica* (Dewan *et al.*, 1992), *Morus laevigata* (Hossain *et al.*, 1992), *Dalbergia grandiflora* (Kumar and Kumar, 1997) etc.

Addition of IAA (0.2 mg l^{-1}) could not increase the performance of culture instead mean number of leaves, leaflets and shoots decreased compared to corresponding levels of BA alone (Table 9 and Fig.3). The addition of IAA along with different concentrations of BA did not result in increased number of shoots in *Maytenus ilicifolia* (Pereira *et al.*, 1995). Benzyl adenine at 3.0 mg l^{-1} concentration in MS medium was found to be the best concentration of BA when it was used at various concentrations alone or with IAA (0.2 mg l^{-1}). However, the effect of 3.0 mg l^{-1} BA was not significantly different from the effect produced at 5.0 mg l^{-1} when used alone.

Addition of IAA (0.2 mg l^{-1}) to kinetin in the medium generally increased the mean number of leaves and leaflets (Table 11 and Fig.4).

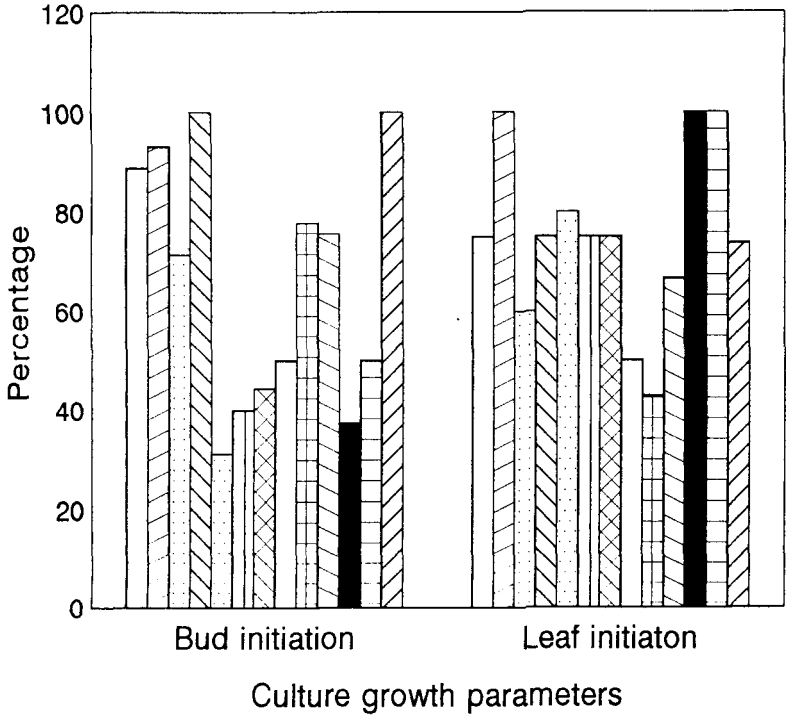
Neither BA nor kinetin produced same response in half-strength MS medium as they were in full-strength MS medium. The effect of BA as well as kinetin was very poor in half-strength MS medium. This may be due to the poor nutrient support of half-strength MS medium.

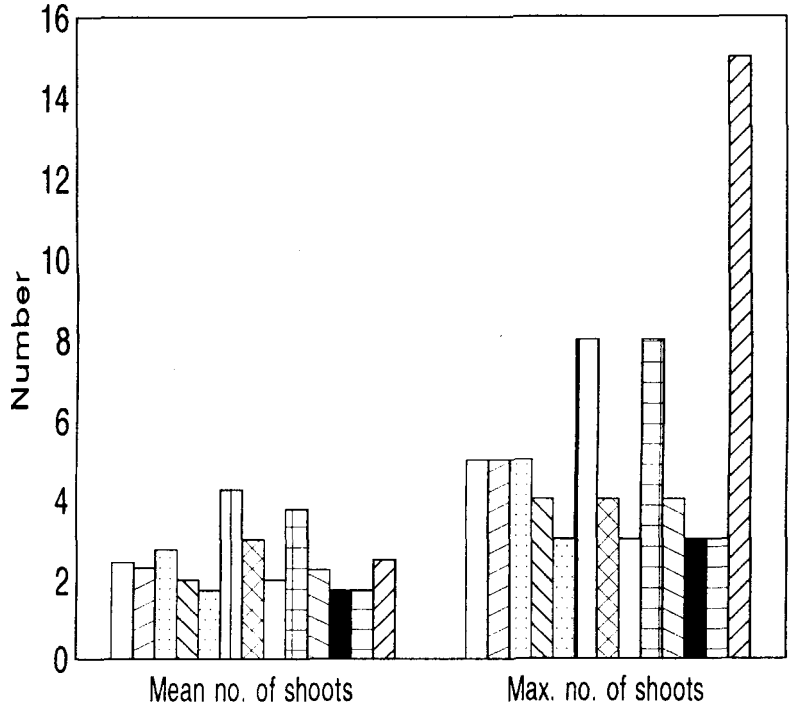
Combined effect of BA and kin in MS medium was found to be better than using them alone especially to increase the mean number of shoots per culture (Table 9, 11, 13 and Fig.6). Highest mean number of leaves and leaflets (5.50 and 19.50, respectively) were obtained in a combination of 3.0 mg l^{-1} BA with 2.0 mg l^{-1} kin in MS medium (Plate 8).

Production of one shoot from one bud through tissue culture will never be economical and cannot be used for commercial propagation. So to decrease cost of production of tissue cultured plants and to produce large number of planting material from limited resource available it is essential to develop a procedure for the production of multiple shoots. In the present study in *A. triphysa* in general, multiple shoots were obtained in many combinations of BA, kinetin and GA_3 in MS medium (Table 12 and 15). Combination of two cytokinins (BA and kin) was found to be better especially to increase the shoot number in red sandal wood (Sita *et al.*, 1992) and *Dendrocalamus strictus* (Ravikumar *et al.*, 1998). Some morphogenetic responses are caused by a combination of two or more growth regulators at certain concentrations or in certain ratios, whereas other concentrations of the same growth regulators either have no effect or have qualitatively different effect (Minocha, 1987). However, highest average shoots (4.25) per bud were obtained from a combination of 3.0 mg l^{-1} BA with 1.0 mg l^{-1} kin in MS medium with some cultures having up to eight shoots. Multiple shoots were also observed in many of the combinations of BA and / kinetin along with GA_3. Multiple shoots up to 15.0 were recorded in some cultures in media containing 3.0 mg l^{-1} kin with 5.0 mg l^{-1} GA_3 (Plate 10).

Combination effect of BA and kinetin in ½ MS medium again was found to be very poor (Table 14). In some combinations of BA and kin there was no response at all. No multiple shoot production was recorded even at higher concentrations of BA and/or kin. This again may be because of the reduced nutrient support of ½ MS medium.

During the condition of arrested shoot growth an additional step of shoot elongation may be followed in a media with low cytokinin, sometimes in the presence of GA_3, before testing them for rooting (Sita *et al.*, 1979). The elongation stage is considered by many to be a preparatory stage for rooting during which the influence of cytokinins is reduced (Duart and Gruselle, 1986). Buds initiated from





1- MS + 3.0 mg l^{-1} BA + 0.0 mg l^{-1} kin
2- MS + 5.0 mg l^{-1} BA + 0.0 mg l^{-1} kin
3- MS + 1.0 mg l^{-1} BA + 3.0 mg l^{-1} kin
4- MS + 2.0 mg l^{-1} BA + 2.0 mg l^{-1} kin
5- MS + 2.0 mg l^{-1} BA + 3.0 mg l^{-1} kin
6- MS + 3.0 mg l^{-1} BA + 1.0 mg l^{-1} kin
7- MS + 3.0 mg l^{-1} BA + 2.0 mg l^{-1} kin
8- MS + 5.0 mg l^{-1} BA + 1.0 mg l^{-1} kin
9- MS + 5.0 mg l^{-1} BA + 2.0 mg l^{-1} kin
10- MS + 5.0 mg l^{-1} BA + 5.0 mg l^{-1} kin
11- MS + 3.0 mg l^{-1} BA + 3.0 mg l^{-1} kin
12- MS + 3.0 mg l^{-1} BA + 5.0 mg l^{-1} kin
13- MS + 3.0 mg l^{-1} kin + 5.0 mg l^{-1} GA_3

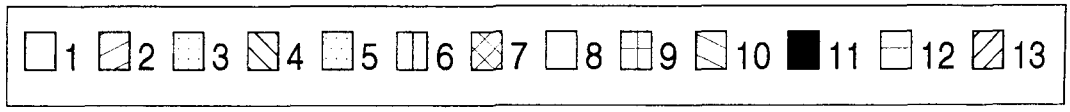


Fig.6. Some promising media combinations for induction of multiple shoots in A. triphysa

the mature nodes failed to elongate and could not be rooted in *A. triphysa* (D'silva and D'souza, 1992).

In the present study also shoot elongation was retarded or delayed at least in some of the cultures. To induce shoot elongation in such cultures also, various concentrations of GA_3 were added to media singly or in combination with different concentrations of BA and/or kin. Shoot elongation and growth up to about 2.5 cm was recorded in some cultures growing in MS medium supplemented with 3.0 mg l^{-1} BA and 1.0 mg l^{-1} GA_3 (Plate 11). Shoot elongation was also noticed in media containing 2.0 mg l^{-1} BA plus 2.0 mg l^{-1} kin with an approximate shoot length of 5.0 cm (Plate 9). Reduction in the concentration of BA promotes elongation of buds as reported in mulberry (Kim *et al.*, 1985). Shoot elongation from cotyledonary nodes explants of *A. triphysa* was observed in media containing low BA concentration with kinetin (D'silva and D'souza, 1992).

5.4 Rooting of shoots produced *in vitro*

Generally auxins promote rooting of microshoots especially at lower concentrations (Minocha, 1987). A low salt concentration is found satisfactory for rooting of shoots in large number of plant species. Often where shoot multiplication was induced in full strength MS medium, the salt concentration was reduced to half (Garland and Scholtz, 1981; Suwai *et al.*, 1988; Kumar and Kumar, 1997) or to a quarter (Skirvin and Chu, 1979; Raghavan, 1986) when shoots were to be rooted.

In our study eleven different treatments (Seven in MS medium and four in ½ MS) were tried for induction of root *in vitro*. Rooting was observed in only 6.28 per cent of the cultures in half strength MS medium containing 0.4 mg l^{-1} BA, 4.0 mg l^{-1} IAA and 0.25 per cent of activated charcoal (Table 16 and Plate 12). A pulse treatment to the base of the microshoots for few seconds in 1000 mg l^{-1} IBA

was found to be essential for root initiation. Effect of pulse treatment with high concentration auxin was found to be effective in rooting of *Dalbergia latifolia* (Kannan, 1995) and in *Mangifera indica* (Ara *et al.*, 1998). The low percentage of rooting may be because, the shoot elongation did not exist in all the cultures. The shoots which have elongated only could produce roots. More work is recommended to increase the percentage of rooting.

Ex vitro rooting of microshoots has been advocated by different workers (Yeoman, 1986; Schwarz *et al.*, 1988; Vijayakumar *et al.*, 1990; Bonal and Monteuuis, 1998). This provides facilities for simultaneous rooting and hardening of the plantlets so that the mortality of the plantlets while planting out can be reduced to a great extent. McCown (1988) reported that apart from the before said basic advantages, *ex vitro* rooting results in a substantial gain in money. Considering these, attempts were made to induce rooting of microshoots under *ex vitro* conditions in sterile sand medium in small containers. A dipping for few minutes in 1000 mg l^{-1} IBA solution was given to the base of the microshoots before keeping them into sand for rooting. Only 6.0 per cent of shoots showed the signs of root production (Plate 13). However, plantlets could not survive due to fungal infection. Again, the percentage of *ex vitro* rooting was also less probably due to lack of shoot elongation.

5.5 Planting out and acclimatization

The practical prospects of mass propagation are largely dependent on the success of the acclimatization process and the capability of the acclimatized plantlets to resume growth (Bonal and Monteuuis, 1997). A period of humidity acclimatization is considered necessary for the newly transferred plantlets to adapt to the outside environment, during which the plantlets undergo morphological and physiological adaptation enabling them to develop typically terrestrial plant water control mechanism (Hu and Wang, 1983; Sutter *et al.*, 1985).

In the present study, the *in vitro* rooted plantlets were transferred to sterilized sand medium in small pots for acclimatization. About 90-95 per cent relative humidity was maintained by covering the pot using polythene cover and intermittently spraying with cold water. After one week the polythene cover was removed and plantlets were kept exposed for open conditions for 4.0-5.0 hr a day. After two weeks polythene cover was completely removed. Nevertheless, the plantlets could not survive. This may be due to the non adaptability of plantlets to atmospheric conditions prevailing when kept open. McClelland *et al.* (1990) reported that *in vitro* formed roots of tree species did not seem to get adapted to natural conditions easily.

The major constraint for orderly progress of any *in vitro* culture is inadequacy of the reliable quantitative physiological, biochemical and cell biological data related to the species to be cultured. Also, lack of understanding of critical functions of plant growth regulators and other media constituents. This is more so in forest tree species and consequently, plant cell and tissue culture of forest trees is based more on empirical experience than fundamental principles. In the present study of micropropagation in *A. triphysa*, shoot production has been standardised. Multiple shoots to a maximum of up to 15 shoots have been achieved in some of the media combinations. To the best of our knowledge, this is the first report of this kind in *A. triphysa* using mature plant materials. More studies in root induction, especially *ex vitro* rooting and hardening are, however, necessary in this species.

Summary

SUMMARY

A research programme entitled "Micropropagation of *Ailanthus triphysa* (Dennst.)" was taken up at the College of Forestry, Vellanikkara during the period 1996 to 1998. The salient features from the study are summarised below:

1. The extent of culture contamination, primarily due to fungus, was found to be very high and more so during rainy months. This may be due to succulant nature of explants, thin bark as well as due to high amount of microbial inoculum in the field explants.

2. Various surface sterilization treatments tried to overcome microbial contamination were not significantly different from one another. However, dipping of explants in a fungicidal mixture of 0.1 per cent each of Bavistin (Carbendazim) and Indofil M-45 (Mancozeb) followed by 20 min. surface sterilization with mercuric chloride (0.1%) was found to be relatively better treatment to reduce culture contamination.

3. Size of the explant had a significant effect on culture contamination. Microbial contamination was low in small sized (<0.5 cm dia.) explants compared to the big sized (>0.5 cm dia.) ones.

4. Browning of medium due to polyphenols was a serious problem especially in large sized explants. High percentage of polyphenol exudation was found in large sized explants in comparison with small sized explants.

5. Washing of explants in running tap water for 30 min. followed by their culturing in media containing activated charcoal (0.25%) was found to be effective in reducing phenol exudation.

6. Full strength Murashige and Skoog medium (MS) medium has emerged as the best basal medium for culture establishment and shoot production in comparison with half-strength MS medium and WPM.

7. In comparison with kinetin, Benzyl adenine was found to be better when supplemented to MS medium nevertheless, both did not show similar response in half-strength MS medium.

8. Murashige and Skoog medium supplemented with 3.0 mg l^{-1} BA was found to be the best medium for shoot production. Highest mean number of leaves (10.2 per explant) and leaflets (21.8 per explant) were observed in this medium with an average shoot number of 2.43 per explant.

9. The addition of IAA 0.2 mg l^{-1} to different concentrations of BA reduced mean number of leaves, leaflets as well as shoots while it was otherwise when it was added to different levels of kinetin in MS medium.

10. The synergistic effect of kinetin and BA in MS medium was found to be better than supplementing them individually to MS medium especially for the enhanced release of axillary buds. The multiple shoots were obtained in almost all the combination of BA and kinetin. The treatment, MS + 3.0 mg l^{-1} BA + 1.0 mg l^{-1} kinetin has produced as many as 4.25 shoots on an average and was found to be the best treatment to get highest number of shoots per explant on an average basis.

11. Many of the combinations of GA_3, BA and/or kinetin also produced multiple shoots. As many as 15 shoots from a single bud were obtained in the treatment MS + 3.0 mg l^{-1} kinetin + 5.0 mg l^{-1} GA_3 with an average shoot number of 2.5 shoots per explant.

12. The effect of BA and kinetin, individually and in combination, at various concentrations tried with half-strength MS medium was found to be very poor for the shoot production. Multiple shoots were not produced even at higher level of BA as well as kinetin when supplemented individually and in combination.

13. Shoot elongation was retarded or delayed in some cultures. Also shoot elongation was very meager except in few treatments. Even the addition of GA_3 could not bring about shoot elongation, except the treatment MS + 3.0 mg l^{-1} BA + 1.0 mg l^{-1} GA_3 in which few cultures having approximately 2.5 cm shoot length were produced. Shoot elongation was also seen in few cultures of the treatment MS + 2.0 mg l^{-1} BA + 2.0 mg l^{-1} kinetin with a shoot length of approximately 5.0 cm.

14. Callusing at the base of the explants was noticed in only few cultures of some treatments with very low quantity of callus.

15. For *in vitro* rooting of shoots, pulse treatment with IBA (1000 mg l^{-1}) to individual shoots for few seconds and then transferring to half strength MS medium supplemented with 4.0 mg l^{-1} IAA + 0.4 mg l^{-1} IBA + AC (0.25%) was found to be effective.

16. Percentage of *in vitro* rooting was as low as 6.25 per cent and more studies in this context are needed.

17. Very few shoots with a percentage of 6.0 showed signs of rooting *ex vitro* when *in vitro* shoots were transferred to sterile sand medium after a pulse treatment for 2-3 min. in 1000 mg l^{-1} IBA solution.

18. *Ex vitro* rooted plantlets could not survive because of fungal infection at the base of the shoot.

19. The *in vitro* rooted plantlets survived for two weeks after transferring to sterile sand medium under high humidity conditions but they failed to acclimatize to existing environmental conditions when exposed and died later on.

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*Originals not seen

Plates

Plate 1. Healthy shoot in MS media containing AC (0.25%). Callusing at the base of explant can also be seen

Plate 2. Bud initiation in basal MS medium

Plate 3. Bud expansion in basal MS medium

Plate 4. Leaf initiation in basal MS medium

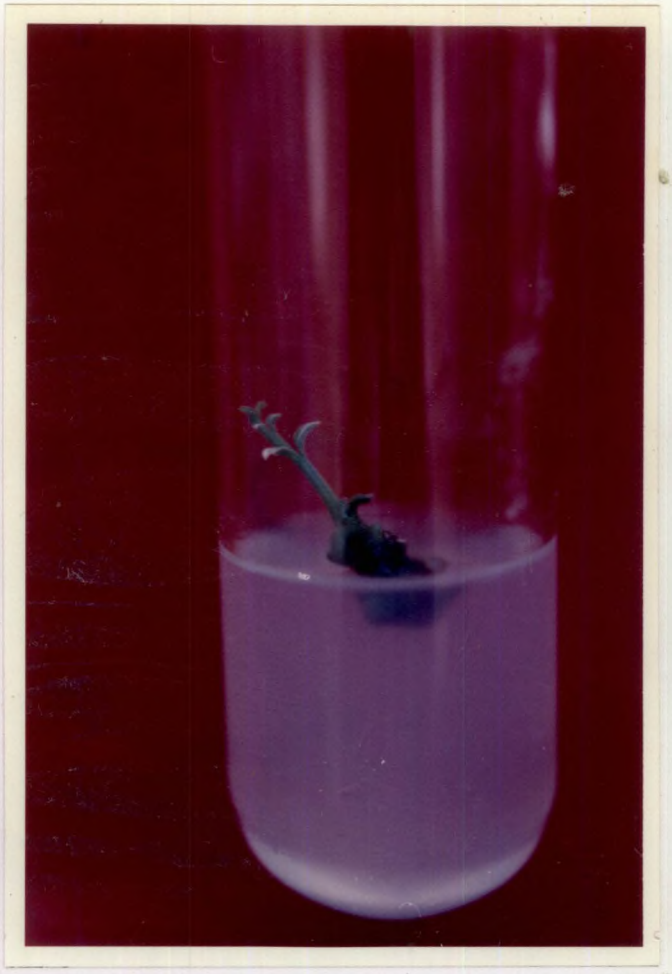

Plate 5. Drying of leaves in basal MS medium

Plate 6. Production of new leaves from dried plantlets after subculturing to fresh basal MS medium

Plate 7. Multiple shoot production from nodal explant in MS media supplemented with 3.0 mg l^{-1} BA with 1.0 mg l^{-1} kinetin

Plate 8. Shoot growth obtained in eight weeks in MS medium containing 3.0 mg l^{-1} BA + 2.0 mg l^{-1} kinetin

Plate 11. Shoot elongation (approx. 2.5 cm) in MS medium supplemented with 3.0 mg l^{-1} BA with 1.0 mg l^{-1} GA_3

Plate 12. *In vitro* rooting of micro shoots after a pulse treatment to their base in half-strength MS medium containing 4.0 mg l^{-1} IAA + 0.4 mg l^{-1} IBA + AC (0.25%)


Kebica

Plate 13. *Ex vitro* rooted plantlet in sterilized sand media. Plantlet could not survive due to fungal infection

In Vitro PROPAGATION OF *Ailanthus triphysa* (Dennst.)

By
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ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the
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ABSTRACT

A research project entitled "*In Vitro* propagation of *Ailanthus triphysa* (Dennst.)" was carried out at the College of Forestry, Vellanikkara during 1996-98 to standardise a protocol for micropropagation of *A. triphysa* through tissue culture. To achieve the present objective, different media combinations for shoot production and rooting of microshoots were tried using axillary and terminal buds from three to four year old seedlings as explants.

The extent of culture contamination principally due to fungus was found to be high and more so during rainy season. To get contamination free cultures, dipping of explants in a fungicidal mixture of 0.1 per cent each of Bavistin (Carbendazim) and Indofil M-45 (Mancozeb) for 30 min. and their sterilization with mercuric chloride (0.1%) for 20 min. was found relatively effective. Small sized explants (<0.5 cm dia) with significantly low culture contamination as well as phenol exudation in comparison with big sized explants (>0.5 cm dia), were found to be optimum for culture establishment. Washing of explants in running tap water for 30 min. and further culturing in media containing activated charcoal (0.25%) was found to significantly lower phenol exudation in both sizes of explants.

Murashige and Skoog (MS) medium was found to be the best basal medium for culture establishment and shoot production in comparison to half strength MS medium and WPM. Of the various media combinations attempted, MS supplemented with 3.0 mg l^{-1} benzyl adenine was found to be the best for shoot production. Highest mean number of leaves (10.2/explant) and leaflets (21.8/explant) were obtained from this treatment with an average shoot number of 2.43 per explant.

Multiple shoots were obtained in almost all the combinations of benzyl adenine (BA) and kinetin in MS medium. The treatment, MS + 3.0 mg l^{-1} BA + 1.0 mg l^{-1} kinetin that produced as many as 4.25 shoots from a single bud on an average, was found to be the best among these. Many of the combinations of GA_3, BA and/or kinetin also produced multiple shoots. As many as 15 shoots from one bud were obtained in MS medium supplemented with 3.0 mg l^{-1} kinetin and 5.0 mg l^{-1} GA_3. Half - strength MS was found to be totally inefficient for shoot production even when supplemented with growth regulators.

Notable shoot elongation was obtained in few cultures of MS media containing 3.0 mg l^{-1} BA + 1.0 mg l^{-1} GA_3 and 2.0 mg l^{-1} BA + 2.0 mg l^{-1} kinetin. Callusing at the base of bud explants was noticed in very few cultures.

In vitro rooting was successfully obtained in half-strength MS medium containing 4.0 mg l^{-1} IAA + 0.4 mg l^{-1} IBA + AC (0.25%). The plantlets that produced roots *ex vitro* died due to fungal infection. When planted out into sterilized sand in crops, the *in vitro* rooted plantlets survived under high humidity conditions for two weeks but failed to acclimatize to outside environmental conditions.

From the present study the protocol for shoot production could be standardised but more work on rooting of shoots is needed. As far as our extent of search, this is the first report on standrdisation of the technique of micropropagation of *A. triphysa* using mature plant tissues as explant.