IN VITRO PROPAGATION OF *AILANTHUS TRIPHYSA*

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NATESHA, S. R. & VIJAYAKUMAR, N. K. 2004. *In vitro* propagation of *Ailanthus triphysa*. *In vitro* propagation of the tropical tree species, *Ailanthus triphysa,* using axillary and terminal bud explants from 3- to 4-year-old saplings was attempted. Murashige and Skoog basal medium was the best medium for culture establishment and shoot growth. Among the various cytokinins supplemented to the basal medium, singly or in combination with indole-3-acetic acid (IAA), N^6-benzyladenine (BA) at 3.0 mg l^{-1} was better for leaf and multiple shoot production. Combinations of two cytokinins, namely BA (3.0 mg l^{-1}) and kinetin (1.0 mg l^{-1}), produced multiple shoots with highest mean number of shoots (4.3). However, shoot elongation was very limited in all growth regulator combinations tested for shoot production. Rooting of microshoots was successfully accomplished in half-strength Murashige and Skoog medium supplemented with both 4.0 mg l^{-1} IAA and 0.4 mg l^{-1} indole-3-butyric acid.

Key words: *Ailanthus malbarica* – micropropagation – tissue culture – multiple shoots – *in vitro* rooting – *ex vitro* rooting

NATESHA, S. R. & VIJAYAKUMAR, N. K. 2004. Pembiakan *in vitro Ailanthus triphysa*. Satu kajian dijalankan ke atas anak benih *Ailanthus triphysa* berumur 3 tahun hingga 4 tahun menggunakan eksplan tunas aksil dan eksplan tunas penghujung bagi mengkaji pembiakan *in vitro* spesies pokok tropika tersebut. Media asas Murashige dan Skoog merupakan media terbaik untuk kultur dan pertumbuhan pucuk. Antara pelbagai sitokinin yang ditambahkan ke dalam media asas, sama ada menggunakan sitokinin sahaja atau sitokinin bersama-sama asid-3-indola (IAA), N^6-benziladenina (BA) pada 3.0 mg l^{-1} didapati sesuai untuk penghasilan daun dan pucuk berbilang. Gabungan kedua-dua sitokinin iaitu BA (3.0 mg l^{-1}) and kinetin (1.0 mg l^{-1}) menghasilkan pucuk berbilang dengan purata bilangan pucuk terbanyak iaitu 4.3. Namun pertumbuhan pucuk agak terhad dalam semua gabungan pengawal atur pertumbuhan yang diuji. Pucuk mikro berjaya membentuk akar di dalam medium Murashige dan Skoog pada separuh kepekatan yang ditambah dengan 4.0 mg l^{-1} IAA dan 0.4 mg l^{-1} asid indola-3-butirik.

Introduction

Ailanthus triphysa (Syn. *A. malbarica*) is a fast-growing multi-purpose tree species belonging to the family Simarubaceae, of considerable economic importance. It is a large deciduous tree with cylindrical bole and is distributed in tropical Peninsular India up to an elevation of 4500 m asl. It is common in homestead gardens of this region. The tree is grown in large-scale plantation, afforestation, agroforestry and social forestry programmes. Due to its soft and light wood, it is largely used for

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matchwood, packing cases as well as pulp and paper industries (Troup 1921). The bark, gum, roots and leaves have medicinal value. The foliage is a good source of green manure and fodder.

Ailanthus triphysa is normally seed propagated. Germination of fresh seeds is high but seeds loose their viability very quickly within a month. Attack by the insect *Eligma narcissus* on foliage, terminal apices, inflorescence and fruits affects the seed (Varma 1986) as well as vertical growth of the plant leading to rosette branching, stunted growth and crooked bole habit. Genotypes exhibiting resistance or tolerance to this insect and with superior morphological traits are available and they can be selected and propagated through the technique of clonal propagation. However, conventional vegetative propagation techniques are found not feasible in *A. triphysa.* Hence, to overcome these difficulties as well as to supply large quantities of good planting materials to meet the increasing demand for this species, *in vitro* propagation may be the right choice.

Micropropagation of *A. triphysa* using immature cotyledonary node explants has been reported by D'Silva and D'Souza (1992). Nevertheless, their attempts using mature nodes as explants did not succeed. Integrity in the preformed mature meristem is high, which is very important for maintaining clonal fidelity (Mascarenhas *et al.* 1993). Micropropagation of *A. triphysa* using relatively mature nodes as explants was attempted in this study.

Materials and methods

Source and preparation of explants

Axillary and terminal buds collected from 3- to 4-year-old saplings of *A. triphysa* were used as explants. The mother plants were sprayed on alternate days during non-rainy months with a fungicidal mixture of 0.1% each of Bavistin (Carbendazim 50% WP) and Indofil-M-45 (Mancozeb 75% WP). During rainy months, explants were dipped in the same fungicidal mixture with leaves intact for half an hour. Young twigs of 2 to 15 cm with 3 to 8 buds were collected along with leaves. Leaves were cut and discarded and stem segments measuring 1.0 to 1.5 cm were cut to retain only one bud per explant, or sometimes two when buds could not be separated. Buds were kept immersed in running tap water for half an hour to control phenol exudation in cultures. The explants were surface sterilised in a laminar flow cabinet for 20 min with mercuric chloride (0.1%) before inoculation. A minimum of 15 explants were cultured for each treatment and replicated at least three times.

Media

Murashige and Skoog (MS) medium (Murashige & Skoog 1962), half-strength MS (half amount of inorganic constituents, full amounts of organic and other constituents per litre of MS medium) and Woody Plant Medium (Lloyd & McCown 1980) were initially chosen for testing their ability to support shoot growth. Later

the MS medium was selected and supplemented with various concentrations of growth regulators [benzyladenine (BA), kinetin, indole acetic acid (IAA) and gibberellic acid (GA_3)] for promoting shoot morphogenesis. Full-strength and half-strength MS media were used for rooting of shoots. Phenol exudation was a serious problem and hence activated charcoal (0.25%) was added to all shooting media. Activated charcoal (0.25%) was also added to the rooting media to provide dark conditions and whenever it was not added the part of the tube containing media was covered with aluminum foil. Cultures were incubated at 27 ± 2 °C and a light intensity of 2000–3000 lux.

Data collection and analysis

Growth data on various growth parameters such as bud break, leaf production and rooted shoots were recorded. The observations were recorded for four to eight weeks. Data were subjected to analysis of variance (ANOVA) for complete randomised design with unequal replication, with one factor or two factors as relevant for a particular case. The treatment means were compared using Duncan's multiple range test.

Results

Culture response in basal media

Significantly higher percentages of bud (77.9) and leaf (68.7) initiations were recorded in full-strength MS medium (Table 1). Cultures in this medium also showed significantly higher mean number of leaves (3.2) and leaflets (8.1). Although there was no significant variation in time taken for bud and leaf initiations, MS medium took fewer numbers of days. Thus, MS medium was the best basal medium among the three media attempted. Later, it was selected for supplementing various plant growth regulators singly and in combination for promoting shoot growth.

Table 1 Effects of different basal media on culture growth in axillary and terminal buds of *Ailanthus triphysa*

Basal medium	Bud initiation (%)	Leaf initiation (%)	Bud initiation (days)	Leaf initiation (days)	Mean number of leaves	Mean number of leaflets
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
$\frac{1}{2}$ MS	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

MS = Murashige & Skoog, WPM = Woody Plant Medium
Twenty explants per treatment, replicated four times

Shoot growth in MS medium supplemented with plant growth regulators

With 3.0 mg l^{-1} BA supplemented to the MS medium, 100% cultures showed bud and leaf initiations (Figure 1) with significantly high number of shoots (2.4), leaves (10.2) and leaflets (21.8) (Table 2). Hence, it was found to be the best concentration of BA for shoot morphogenesis. There was increase in the number of shoots with increasing levels of BA (up to 3.0 mg l^{-1}), followed by a fall with further increase in the concentration of BA.

Among the various combinations of kinetin and IAA, the highest bud initiation (84.6%) was found at 1.0 mg l^{-1} kinetin in combination with 0.2 mg l^{-1} IAA (Figure 2). Significantly higher numbers of leaves (4.6) and leaflets (18.4) were recorded in the same treatment (Table 3). There was no variation in shoot number as only one shoot was found in all treatment combinations of kinetin and IAA.

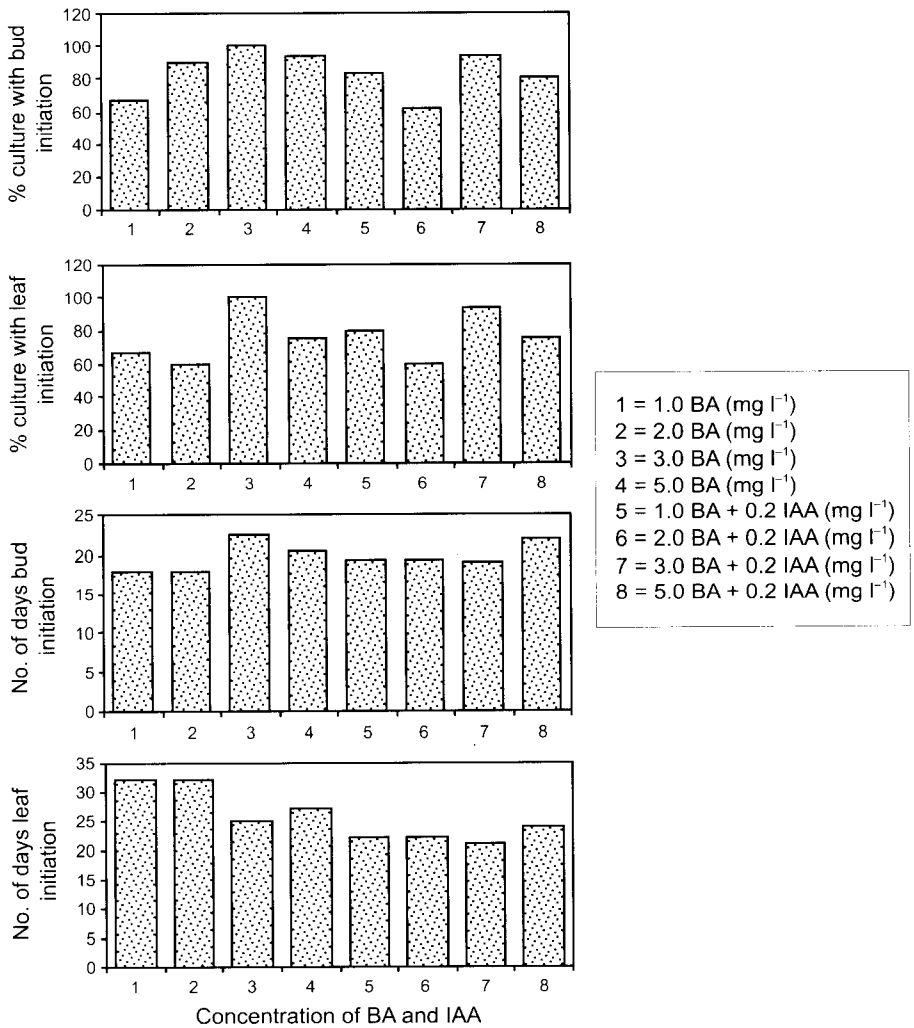

Figure 1 Effects of different concentrations of BA and IAA on culture response of *Ailanthus triphysa*

Table 2 Effects of different concentrations of BA and IAA on culture growth in axillary and terminal buds of *Ailanthus triphysa* in MS medium

BA (mg l^{-1})	IAA (mg l^{-1})	Leaves per explant	Leaflets per explant	Shoots per explant
1.0	0.0	2.6	5.4	1.00
2.0	0.0	5.0	6.4	1.43
3.0	0.0	10.2	21.8	2.43
5.0	0.0	7.0	19.0	2.29
1.0	0.2	2.6	9.0	1.00
2.0	0.2	2.6	7.8	1.00
3.0	0.2	5.4	11.6	1.14
5.0	0.2	2.2	4.0	1.00
CD (0.01)		2.207	4.911	0.807
SEM		0.80	1.78	0.297

BA = benzyladenine, IAA = indole acetic acid
Fifteen explants per treatment, replicated at least three times

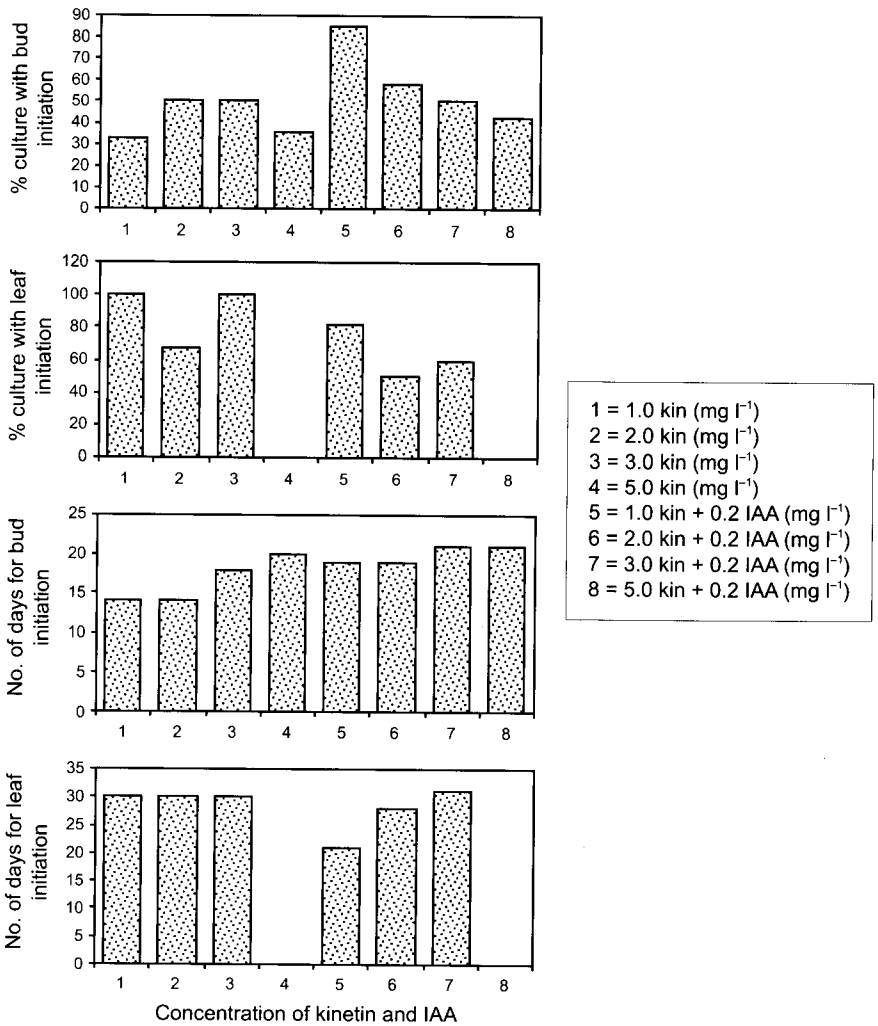

Figure 2 Effects of kinetin and IAA on culture response in bud cultures of *Ailanthus triphysa*

Table 3 Effects of different concentrations of kinetin and IAA on culture growth in axillary and terminal bud explants of *Ailanthus triphysa* in MS medium

Kin (mg l^{-1})	IAA (mg l^{-1})	Leaves per explant	Leaflets per explant	Shoots per explant
1.0	0.0	1.4	4.8	1.0
2.0	0.0	2.0	4.8	1.0
3.0	0.0	2.6	6.8	1.0
5.0	0.0	0.0	0.0	1.0
1.0	0.2	4.6	18.4	1.0
2.0	0.2	2.2	7.2	1.0
3.0	0.2	2.6	5.4	1.0
5.0	0.2	0.0	0.0	1.0
CD (0.01)		1.26	0.629	NS
SEM		0.424	0.211	-

Kin = kinetin, IAA = indole acetic acid
Fifteen explants per treatment, replicated at least three times

Although the interaction of BA and kinetin was not significant, the culture performance was generally better than supplementing them alone (Figure 3 and Table 4). Hundred per cent of bud initiation was found at 2.0 mg l^{-1} BA with 2.0 mg l^{-1} kinetin while 75% of cultures produced leaves (Figure 3). Hundred per cent of leaf initiation was noticed at 2.0 mg l^{-1} BA with 5.0 mg l^{-1} kinetin (Figure 3). The medium containing 3.0 mg l^{-1} BA with 2.0 mg l^{-1} kinetin induced significantly higher number of leaves (5.5) and leaflets (19.5), while significantly higher number

Table 4 Effects of BA and kinetin on culture growth in axillary and terminal bud explants of *Ailanthus triphysa* in MS medium

BA (mg l^{-1})	Kin (mg l^{-1})	Leaves per explant	Leaflets per explant	Shoots per explant
1.0	1.0	1.75	4.50	1.25
1.0	2.0	4.00	9.25	1.50
1.0	3.0	2.75	7.50	2.75
1.0	5.0	2.75	7.50	1.75
2.0	1.0	2.75	6.50	1.25
2.0	2.0	4.50	16.50	2.00
2.0	3.0	3.00	7.00	1.75
2.0	5.0	2.50	6.25	1.00
3.0	1.0	2.00	5.50	4.25
3.0	2.0	5.50	19.50	3.00
3.0	3.0	4.00	12.50	1.75
3.0	5.0	3.75	10.00	1.75
5.0	1.0	4.00	14.00	2.00
5.0	2.0	5.00	14.00	3.75
5.0	3.0	4.25	13.25	1.75
5.0	5.0	1.75	6.75	2.25
		NS	NS	NS
SEM		1.09	3.15	0.70

BA = benzyladenine, Kin = kinetin
Fifteen explants per treatment, replicated at least three times

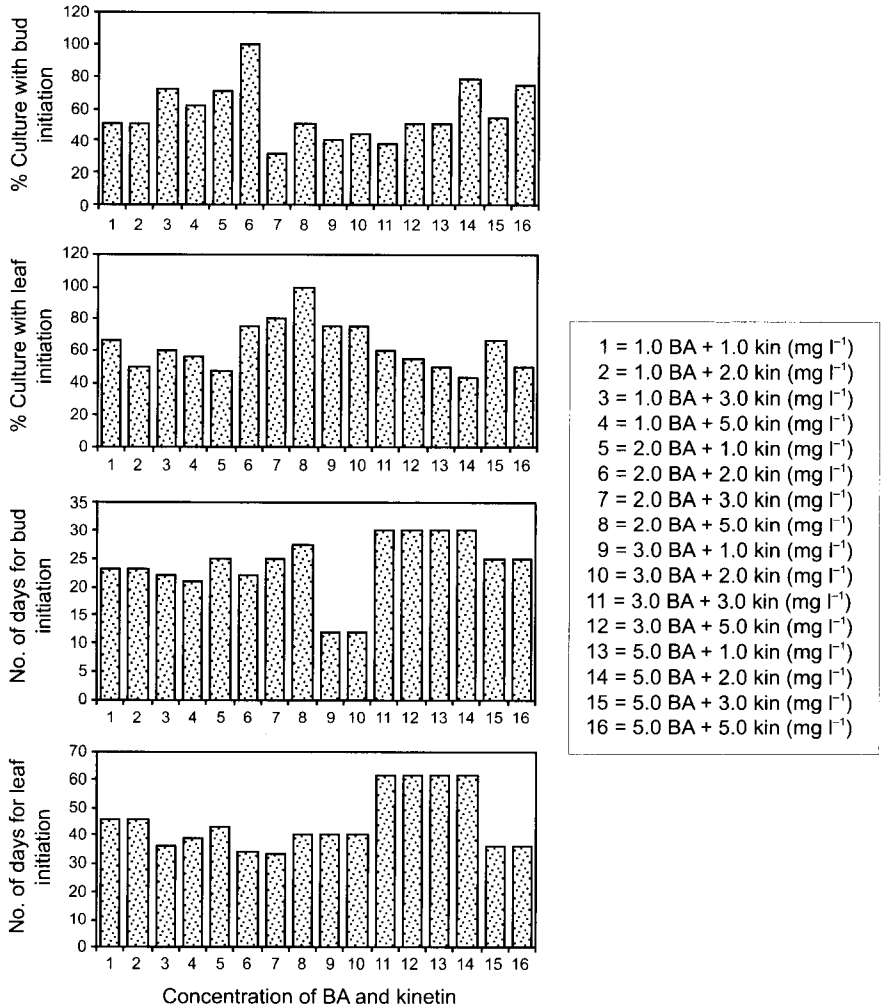

Figure 3 Effects of BA and kinetin on culture response in bud cultures of *Ailanthus triphysa*

of shoots (4.3) was found at 3.0 mg l^{-1} BA with 1.0 mg l^{-1} kinetin. Multiple shoots were a common feature in all the combinations of BA and kinetin and sometimes up to fifteen shoots were obtained from a single bud (Figure 4).

Rooting of shoots

The microshoots measuring 4–6 cm obtained *in vitro* were inoculated into various rooting media containing different combinations of plant growth regulators in full-strength and half-strength MS media. The microshoots given with pulse treatment in high concentration (1000 mg l^{-1}) of sterile indole butyric acid (IBA) solution and cultured in half-strength MS medium supplemented with 4.0 mg l^{-1} IAA and 0.4 mg l^{-1} IBA could only produce roots in 6.3% of cultures (Figure 5).

Attempts for *ex vitro* rooting were also made by placing *in vitro* microshoots in

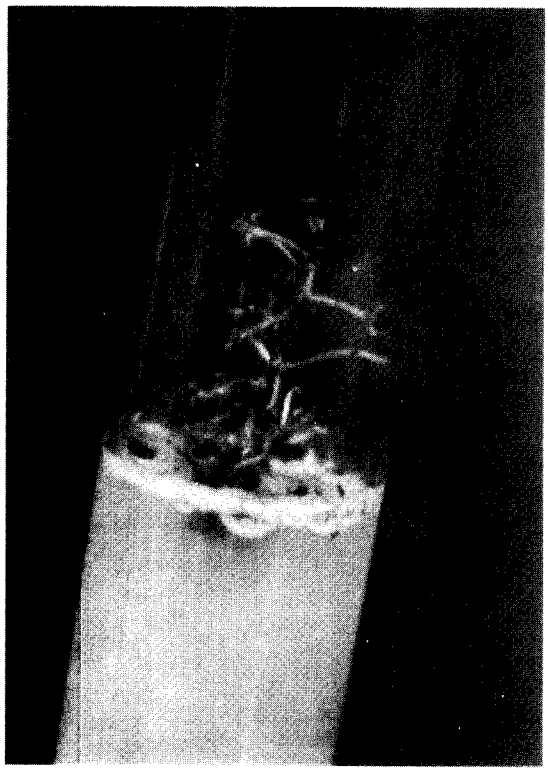

Figure 4 Multiple shoots from axillary bud cultures of *Ailanthus triphysa* in MS medium containing BA and kinetin

sterile sand medium maintained at high humidity. About 6% of shoots showed signs of rooting but were infected by fungus and did not survive.

Planting out and acclimatisation

In vitro plantlets were planted out in sterile sand medium and maintained under high humidity. Plantlets were irrigated with half-strength MS salt solution. Plantlets survived for about four weeks under high humidity conditions. However, they failed to acclimatise when slowly exposed to outside conditions.

Discussion

Tree tissue culture is becoming a powerful tool for rapid clonal propagation especially of selected genotypes. Most trees are not amenable for conventional vegetative propagation techniques such as rooting of cutting, grafting and budding. Therefore, the technique of micropropagation offers scope for producing large stock of superior clonal plants.

The response of *in vitro* shoot morphogenesis in *A. triphysa* was strongly influenced by the basal medium. Murashige and Skoog medium (Murashige & Skoog 1962) and WPM (Lloyd & McCown 1980) are the most frequently used media in tree tissue culture. In *A. triphysa*, shoot morphogenesis and culture

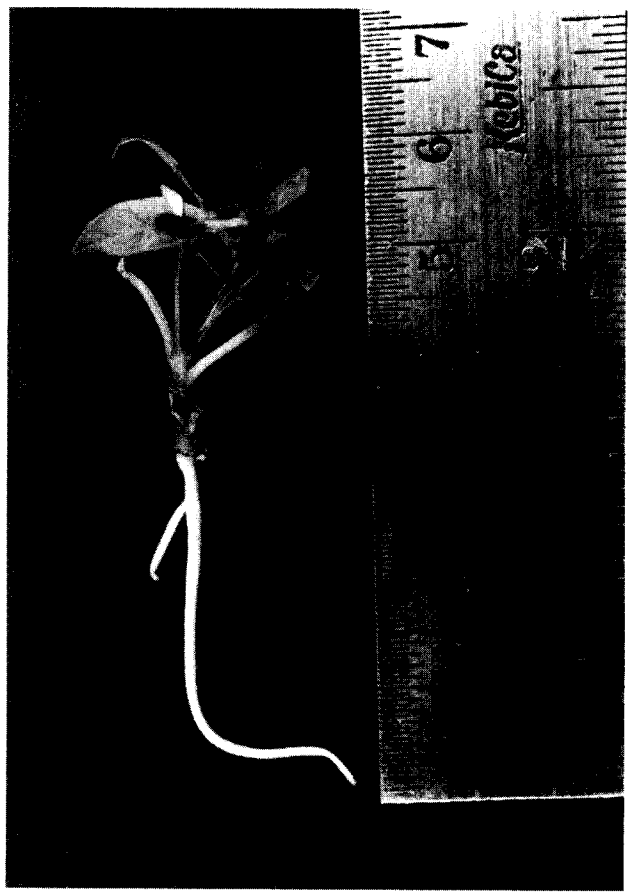

Figure 5 *In vitro*-rooted plantlet of *Ailanthus triphysa* in half-strength MS medium

response were significantly higher in full-strength MS basal medium in comparison with WPM and half-strength MS medium. This is in accordance with earlier report of this species using cotyledonary node explants (D'Silva & D'Souza 1992). Benzyladenine added to the MS medium alone or in combination with 0.2 mg l^{-1} IAA was found to be the superior cytokinin for shoot production in comparison with kinetin. In general, BA has been frequently reported to induce better shoot growth and shoot multiplication than other cytokinins, particularly in tree species (Ahmed 1989). Effectiveness of BA has been demonstrated in several tree species such as *Eucalyptus* spp. (Rao & Lee 1982), *Acacia nilotica* (Dewan *et al.* 1992) and *Duabanga grandiflora* (Kumar & Kumar 1997). With increase in the concentration of BA, an increase in growth response, especially production of multiple shoots, was observed which is mainly attributed to its ability to overcome apical dominance and stimulation of growth of lateral buds at higher levels, as reported by Sachs and Thimman (1964). However, very high levels of BA (beyond 3.0 mg l^{-1}) was found to decrease the culture response in *A. triphysa*, which probably is due to the inhibitory effect of BA at supra optimal concentrations. Similar effect of BA was also reported in other tropical species such as *A. nilotica* (Dewan *et al.* 1992), *Morus laevigata* (Hossain *et al.* 1992) and *Eucalyptus globulus* (Pattanaik & Vijayakumar 1997).

Combination of two cytokinins (BA and kinetin) was better for the general performance in *A. triphysa* especially for the production of multiple shoots (Figure 3, Tables 2, 3 and 4). Supplementing the two cytokinins together rather than singly seems to be ideal. Combination of two cytokinins for multiple shoot production has been reported for red sanders (Sita *et al.* 1992) and *Dendrocalamus strictus* (Ravikumar *et al.* 1998). Minocha (1987) also found that a combination of two or more growth regulators at certain levels could induce some morphological changes.

Shoot elongation was hardly attained even though there was good production of leaves and multiple shoots in *A. triphysa*, which was also reported by D'Silva and D'Souza (1992) in the case of mature nodes. Hence, attempts were made to achieve this through the addition of GA_3 to MS medium, which is frequently recommended for the purpose. An additional step of shoot elongation in media containing low cytokinin sometimes in the presence of GA_3 is recommended by Sita *et al.* (1979) and is considered a preparatory stage for rooting (Duart & Gruselle 1986). Nevertheless, reduction of cytokinin as well as addition of GA_3 did not show noticeable change in shoot elongation in *A. triphysa.*

The attempts for *in vitro* rooting of microshoots from sapling explants of *A. triphysa* was successfully accomplished, although percentage rooting was considerably low (6.3%). Rooting was achieved in low salt concentration medium (half-strength MS medium), while no rooting was obtained in high salt concentration medium (full-strength MS medium). Reduction of salt concentration to half (Garland & Scholtz 1981, Suwai *et al.* 1988, Kumar & Kumar 1997) or to a quarter (Skirvin & Chu 1979, Raghavan 1986) for rooting of microshoots has been reported. A pulse treatment to the base of the microshoots for one minute in high concentration (1000 mg l^{-1}) auxin (IBA) solution was found to be necessary for *in vitro* rooting in *A. triphysa.*

Conclusions

Murashige and Skoog medium was found to be the best basal medium while benzyladenine was the best cytokinin for shoot morphogenesis. Combination of BA and kinetin for shoot production was better for multiple shoot production than using them alone. Rooting was successfully achieved in half-strength MS medium but planting out was unsuccessful.

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