Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; SP1: 109-114

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Effect of growth regulators on sprouting and rooting behaviour in cuttings of *Acacia catechu* Willd. and *Toona ciliata* M. Roem

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Abstract
Study shows the effect of growth regulators viz. different concentrations of IBA and NAA on sprouting and rooting behaviour in cuttings of *Acacia catechu* and *Toona ciliata.* Different concentrations 2000 ppm, 4000 ppm, 6000 ppm and 8000 ppm of both the auxins i.e. IBA and NAA were used as eight different treatments and a control was also set up. The cuttings were planted in polybags. In case of *Toona ciliata* maximum sprouting percent (52.22%) and rooting percent (36.61%) was recorded in cuttings treated with 8000 ppm IBA and in case of *Acacia catechu,* no rooting was observed. However, in *Acacia catechu* maximum sprouting percent (77.79%) with 11.29 cm sprouts was observed in cuttings treated with 2000 ppm of IBA. All the concentrations of IBA gave better results than the NAA concentrations in both the species.

Keywords: Indole-3-butyric acid, Naphthalene acetic acid, Sprouting and Rooting

Introduction

Large scale clonal propagation of superior clones along with accelerated tree improvement programs are necessary for successful reforestation and forest management programs. Vegetative propagation in forest trees is emerging as a strong alternative to the seed propagation method and now employed for operational planting in many forest tree species (Buijtenen *et al*., 1975; Armson *et al*., 1980) [5, 1]. Stem cutting is by far the most widely used practices as this is simple and chief as compared to other methods and therefore used for large scale multiplication of selected clones and species having irregular seed bearing habit, long flowering or fruiting intervals, slow initial growth of seedling and considerable short and long period of seed dormancy. The advantage of raising plant material from vegetative means as compared to seeds include faster growth rate (Ooyama and Toyoshima, 1965)[21], greater possibility of stand uniformity, better response of genotypes to the site conditions and greater wood volume production (Fielding, 1969) [9].

Acacia catechu Willd. commonly known as Khair belongs to the family Mimosaceae. Acacia itself is a large genus comparising over 800 species of tree, shrubs and climbers (Tewari, 1995) [32]. In India it is well represented by 22 indigenous species widely found in the sub-Himalayan tract of Indus, ascending up to 900 m and sometimes found as high as 1200 m above mean sea level (Troup, 1921) [33]. It is found in various parts of Himachal Pradesh, Jammu, Punjab, Uttar Pradesh, Madhya Pradesh, Bihar, Andhra Pradesh and Orissa. Pearson and Brown (1932) [24] have mentioned khair as a valuable timber of India. *Acacia catechu* is a small or medium sized deciduous tree attaining a height of 12-15 m and girth of 0.6-0.9 m. The stem is usually crooked and forked (Troup, 1921) [33]. It is hard tough durable and seasoned well. It is useful for making post, agricultural implements, etc. fuel and furnishes coal of very good quality and is also a good fodder tree (Luna, 1996) [16]. Katha and cutch is extracted from the heartwood chips of khair in India and Burma. Katha has economic importance as masticatory use in pan as with time imparts red colour. It is used as digestive, abortive, expectorant, aphrodisiac and cooling agent. It is useful in diarrhoea, sore throat and externally in preparation of ointment. Another chemical component extracted from heartwood of khair tree is cutch, constituted mainly catechu tannic acid. Its industrial use in dying, tanning, colouring, pulp and paper and also have some medicinal properties. In some of the older trees a third substance called kheersal is found which is of medicinal uses especially for treatment of cough and sore throat. The gum from *Acacia catechu* is said to be of very good quality and is regarded as a best substitute for true gum arabic. Khair can be grown on a wide range of soil such as sandy, loam, alluvium, gravelly, clay, black cotton soil etc. Khair is capable of growing on shallow soil with murram or kankar on which few other species can grow. The Acacia species in particular are of immense value for reforestation and reclamation of wastelands as they are effective in checking soil erosion and help in stabilization of sand dunes (Skolmen, 1986) [29] for fuel wood, timber, shelter belts and soil improvement (Palmberg, 1981) [23]. From the ecological point of view, they can adapt to extremes of temperature, moisture stress and therefore, can be grown in both arid and moist regions in a wide range of tropical soils. Some are also engaged in symbiotic relationship with rhizobium and mycorrhizal fungi thereby increasing soil fertility.

Toona ciliata M. Roem. is an important timber and fodder tree species which is commonly known as Toon and Red Cedar belongs to Meliaceae family. Meliaceae is in fact the backbone of forest industries in many countries (Bahadur, 1988) [3]. *Toona ciliata* is fast growing, large deciduous, moderately light demanding tree with spreading crown, commonly attaining a height of 20- 30 m and a girth of 1.8- 3.0 m. It grows in small gaps in the forest and cultivated fields developing a tall, clean bole up to a height of 9- 12 m it occurs frequently in moist localities such as ravines, streams banks or swamps (Troup, 1921) [33] with best growth in deep rich moist loamy soil including cultivated fields and often found growing on roadsides in Himachal Pradesh, Uttar Pradesh and Uttrakhand. In India it is found throughout the sub- Himalayan tract and the valleys of outer- Himalayas, plains of Assam, Madhya Pradesh, Tamil Nadu, Karnataka, Eastern and Western Ghats occurring up to an elevation of 1200 m (rarely 1300 m) in Western Peninsula, Khasi hills and Manipur (Singh, 1982) [30]. It is a characteristic tree of Eastern alluvial secondary semi- evergreen forests in Assam (Champion and Seth, 1968) [7]. Toona species are among the valuable timbers of tropics serving the same purpose as the pines of north temperate zones (Bahadur, 1988) [3]. Sapwood is pinkish- white or pale yellow and the heartwood is pale- brown, dark red- brown or brick red when first cut. It darkens upon exposure changing the colour to rich reddish- brown, darker brown streaks. It is soft to moderately hard, light give a faint cedary odour, somewhat lustrous, and the sapwood is distinct from heartwood. It is of moderate strength. The timber is used for making Grade I commercial plywood and Grade I moisture- proof plywood, tea- chest plywood, matchbox, pencils, racquets, furniture, house construction, floors, boarding, panels of doors and windows and also for boats toys, carvings, musical instruments etc. Flowers are used as dye for woollen cotton fabrics with mordents. Bark is bitter, astringent, antiperiodic and used for curing infant dysentery. Leaves are lopped for fodder during lean period.

However there is no improved technology for vegetative propagation of mature elite trees of *Acacia catechu* and *Toona ciliata*. Hence, keeping these aspects in the background the main objectives of this study are to investigate the propagation of *Acacia catechu* and *Toona ciliata* by using cuttings and to determine the suitable concentration of the rooting hormones i.e. Indole-3-butyric Acid (IBA) and Naphthalene acetic acid (NAA).

Material and Methods

The study was carried out at experimental field of the Department of Tree Improvement and Genetic Resources, Dr. Y.S. Parmar University of Horticulture and Forestry, Solan (H.P.) in 2013.

The cuttings of *Toona ciliata* and *Acacia catechu* were collected from the medium sized trees growing in the adjoining area of Nauni in Solan district. For this, vigorously growing, disease free, plants were selected. The shoots, 40- 50 cm long, and of uniform thickness of 1- 1.5 cm were collected. The shoot cuttings were made 15- 20 cm long with atleast 2- 3 nodes on each cutting. Each cutting was given horizontal cut at the base and a slant cut at the top with a sharp razor blade. After giving a horizontal cut below a bud on the basal end and a slanting cut above a bud on the apical end of each cutting, the cutting were bundled.

The IBA and NAA concentrations of 2000, 4000, 6000 and 8000 ppm were used. In quick dip method, the basal end of each set of cuttings was then treated with respective IBA and NAA formulation for 5 seconds before planting and a control was also set up. The cuttings were planted 6- 8 cm deep in the polythene bags. The soil mixture (Soil : Sand : Vermicompost : Cocopeat in 2:1:1:1) around the cutting was slightly pressed to hold the cutting firmly but not compacted, to ensure adequate aeration of the rooting zone. Irrigation was done twice manually, daily i.e. in morning and evening. Weeding was done in the bags as and when necessary. Disease preventing soil drenching of Bavestine (@ 2 gm per litre) and Captan (@ 1% strength) were given on alternate weeks to cuttings. Damage to the cuttings from frost and high insolation was prevented by covering the poly bags with locally made thatch.

The observations which were recorded in the experimental area are: Sprouting percentage, rooting percentage, average length of sprout, Time taken for sprouting, average root length, number of roots per cutting. The entire data, generated from the present investigation were subjected to statistical analysis using Completely Randomized Design.

Results and Disscussion

The results obtained in the present investigations have been presented species wise as under:

Toona ciliata

Cuttings were collected and planted in first week of March. The observations on various sprouting and rooting characteristics were recorded after 20 weeks of planting the cuttings and showed following results:

- Maximum sprouting per cent was observed in cuttings treated with 8000 ppm IBA (52.22 per cent). Both the auxins i.e. IBA and NAA treated cuttings showed better sprouting per cent than control (7.78 per cent) as shown in (Table 2).
- It can also be inferred from the data (Table 2) that maximum rooting per cent was also observed in case of 8000 ppm IBA (36.61 per cent) which was again better than all other treatments. Cuttings taken as control showed minimum rooting per cent (5.56 per cent). All the IBA formulations gave better results for rooting per cent than all the NAA formulations.
- It was evident from the data (Table 2) that application of 8000 ppm IBA gave significantly maximum average length of sprouts (45.38 cm), average root length (31.42 cm) and number of roots per cutting (9.85) than other formulations. The minimum average length of sprouts (10.09 cm), average root length (3.32 cm) and number of roots per cutting (4.23) was recorded in case of control. All the IBA formulations gave better results than all the NAA formulations.
- The data presented in Table 2 showed that maximum time taken to sprout (44.33 days) was observed in control and minimum time taken to sprout was observed in case of 8000 ppm IBA (15.27 days). Therefore the treatment with

8000 ppm IBA was superior as compared to all other treatments. Also the IBA formulations gave better results for time taken to sprout than all the NAA formulations.

Table 2: Effect of growth regulators on sprouting and rooting behaviour in the cuttings of *Toona ciliate*

Treatment	Sprouting (%)	Rooting (%)	Average length of the sprouts (cm)	Time taken for sprouting (number of days)	Average root length (cm)	Number of roots per cutting
T_1(2000 ppm IBA)	32.22 (34.57)	22.48 (28.29)	29.56	23.65	17.04	7.70
T_2(4000 ppm IBA)	41.11 (39.85)	25.60 (30.38)	35.97	20.97	22.29	8.36
T_3(6000 ppm IBA)	47.78 (43.71)	33.18 (35.16)	41.43	17.56	25.92	8.95
T_4(8000 ppm IBA)	52.22 (46.25)	36.61 (37.22)	45.38	15.27	31.42	9.85
T_5(2000 ppm NAA)	27.67 (31.71)	18.05 (25.13)	24.14	30.73	10.28	7.31
T_6(4000 ppm NAA)	22.22 (28.09)	15.12 (22.87)	22.62	34.17	8.01	6.73
T_7(6000 ppm NAA)	18.89 (25.73)	12.91 (21.05)	19.86	37.88	6.37	6.31
T_8(8000 ppm NAA)	15.56 (23.19)	10.77 (19.14)	17.98	40.63	5.49	5.60
T_9 (control)	7.78 (16.12)	5.56 (13.48)	10.09	44.33	3.32	4.23
$CD_{0.05}$	2.63	1.77	1.81	2.20	1.32	0.30

Figures in parentheses are arc sine transformed values.

Data in Table 2 indicate that T_4 (8000 ppm IBA) was the best treatment as endorsed by the results exhibiting significantly maximum sprouting (52.22 per cent), length of the sprouts (45.38 cm), rooting (36.61 per cent**)**, average root length (31.42 cm), number of roots per cuttings (9.85) and minimum time taken to sprout (15.27 days), and results were quite significant over T_9 (control). These results are in line with the findings of the results of Kumar (1998)[15] who also recorded maximum rooting, maximum sprouting, increased number and length of primary roots in cuttings of *Taxus baccata* treated with 0.75% IBA + 5% captan + 5% sucrose. These findings are also in close conformity with his another experiment where he observed maximum sprouting, rooting, primary root number, root length with 1.0% IBA + 5% captan + 5% sucrose (T_5) in *Toona ciliata* which were found to be quite significant over control. Fooladi *et al*. (2006) [10] also found similar results where 8000 mg IBA/litre treatment produced the highest number of roots in *Lagerstroemia indica*. Another similar results were observed by Shahraji *et al.* (2007) [28] in *Ulmus glabra,* as highest rooting success of 21.1 per cent for hardwood and 71 per cent for semi-hardwood cuttings were observed when they were treated with 8000 ppm IBA and planted in mixed media. Khali and Sharma (2003) [13] also reported that amongst all the phyto-hormones tried, IBA was the most effective hormone; for the induction of higher rooting in the juvenile cuttings of *Taxus baccata*, 10,000 ppm IBA being the most suitable concentration.

Studies have shown auxins treatment as an effective mode of inducing rooting. Negi and Tiwari (1984) [20] concluded IBA as the best and quite potent in inducing rooting and vegetative growth of cuttings. Among the auxins, IBA is considered to be the best and most potent root promoting substance because of its ability to produce a strong fibrous root system, non-toxic nature over a wide range of concentration, chemical stability and high degree of effectiveness in various plants (Soni, 1970; Sadhu, 1999) [31, 26]. The results of present investigation indicate that large scale propagation of *Toona ciliata* through stem cuttings is possible only with auxin applications. Haissig (1982) [11] related auxin, mediated increase in hydrolytic activity to higher rooting per cent. Wareing and Smith (1963) [34] demonstrated that appropriate levels of nutrition and auxin are needed for the supply of energy necessary for the initiation and development of roots provided that the cells are in active stage of division which in itself is determined by the relative levels of endogenous auxin and inhibitors. A large number of other workers have reported the beneficial effects of auxin in enhancing adventitious root formation in stem cuttings (Nanda *et al*, 1970; Nanda, 1975, Puri and Shamet, 1988) [18, 17, 25].

The average root number and root length in *Toona ciliata* (Table 2) showed a consistent increase due to chemical treatment over the control. The values for these traits increased with an increase in IBA treatment concentration. These results are in agreement with the findings of Chauhan and Reddy (1974) [8] and Avanzato *et al.* (1988) [2] who suggested that optimum concentration of auxin is favourable, but supra optimal auxins are toxic to root regeneration.

Acacia catechu

The cuttings taken during the dormant season were planted in polybags as per time of the cutting treatments in May, 2013. The observations on various sprouting characteristics were recorded after 20 weeks of planting the cuttings but there was no rooting observed in all the cuttings irrespective of auxin treatments. The findings for sprouting thus arrived at and the interpretations of the analysed data are presented here as under.

Sprouting per cent

The data given in the Table 3 shows that different concentration of IBA and NAA exerted a significant influence on sprouting per cent. The sprouting percent was recorded more in IBA formulations than NAA formulations. Percent sprouting (77.78%) was highest in T_1 (2000 ppm IBA) formulation being statistically at par with T_2 (4000 ppm IBA) (74.44%) but significantly superior to T_9 (control) (37.78%) in this regard. The leaves dried and felled during end of October to November.

Rooting per cent, Average root length and Number of roots per cutting

Rooting was not observed on any of the treated cuttings and cuttings used as control. Hence, rooting per cent, average root length and number of roots per cutting were recorded as nil.

Average length of the sprouts (cm)

It can be inferred from the data (Table 3) that IBA and NAA formulation had a significant influence on sprout length of the cuttings. Maximum (11.29 cm) length of the sprouts were recorded when the cuttings were treated with T_1 (2000 ppm IBA) formulation. This was closely succeeded by T_2 (4000 ppm IBA) (9.17 cm) then T_3 (6000 ppm IBA) (6.19 cm) and then T_4 (8000 ppm IBA) (5.03 cm) treatments then succeeded by NAA formulations as T_5 had 4.12 cm then T_6 (3.80 cm) succeeded by T_7 (3.22 cm) and T_8 (2.46 cm) in that order. The least significant rooting of percent was noticed in control (T_9) (1.47 cm).

Time taken for sprouting (Number of days to sprout)

The data presented in Table 3 showed that time taken for sprouting was significantly influenced by IBA and NAA formulations. The IBA formulation of T_1 (2000 ppm IBA) was found to be the best treatment had taken minimum 22.25 days to sprout whereas, T_9 (control) took maximum (48.55) days to sprout which was found inferior to all treatments. Besides these, NAA formulations took more time to sprout than IBA formulations.

Table 3: Effect of growth regulators on sprouting of *Acacia catechu* cuttings

Treatments	Sprouting (%)	Average length of the sprouts (cm)	Time taken for sprouting (number of days)
T_1(2000 ppm IBA)	77.78 (61.95)	11.29	22.25
T_2(4000 ppm IBA)	74.44 (59.69)	9.17	25.33
T_3(6000 ppm IBA)	67.78 (55.44)	6.19	28.50
T_4(8000 ppm IBA)	64.44 (53.41)	5.03	34.92
T_5(2000 ppm NAA)	60.11 (50.82)	4.12	38.63
T_6(4000 ppm NAA)	55.56 (48.17)	3.80	40.87
T_7(6000 ppm NAA)	50.00 (44.98)	3.22	42.41
T_8(8000 ppm NAA)	46.67 (43.07)	2.46	44.21
T_9 (control)	37.78 (37.91)	1.47	48.55
$CD_{0.05}$	4.36	0.68	1.69

Figures in parentheses are arc sine transformed values.

The ability of cuttings to regenerate varies with the plant species. While some regenerate easily, others regenerate with difficulty and still others do not regenerate at all and are thus obstinate. The scenario in the *Acacia catechu* in present study seems to be the last category of cuttings. Although the cuttings of the *Acacia catechu* sprouted profusely after their planting, yet the emerged sprouts (leaves) fell off within 5- 6 months after emergence. No callus formation or initiation of roots could take place in the fresh cuttings. Therefore cutting gradually started drying up and finally failed to root. A lot of factors may be ascribed to this failure. Initial high sprouting could be the result of the use of stored carbohydrate in the cuttings. But as the cuttings failed to root, the food supply to newly emerged leaves might have been stopped. The supposed depletion in the stored carbohydrate level in the cuttings might have resulted into shedding off newly emerged leaves. Similar kinds of results have been reported by Chakraborty (1989)[7] in *Terminalia bellirica* cuttings. The cuttings of *Acacia catechu* either could not form the callus or the pre-formed callus or could not differentiate into roots. This may be attributed to the presence of higher tannin and phenolic contents in fresh cuttings. It is known that the Phenolics form of a part of 'rhizocline' as well as of 'rooting co-factors'. Attention has since been focused on the occurrence of the phenolic compounds in several easy and difficult to root plant species and also on the effect of phenolic as a group on root formation on stem cuttings. Although, a lot of work has been reported on the role of phenols in rooting of cuttings yet their actual role still remain unsubstantiated. Synergistic effect of phenols like ferulic acid, p-hydroxybenzoic acid and p-coumaric acid in root promotion has been stated by Kling and Meyer (1983) [14] and Sarkar *et al.* (1984) [27]. The presence of another phenol, salicylic acid, as in case of *Bougainvillea glabra, Echitis caryophyllata* and *Jasmimm pubescence* were reported to inhibit root formation (Nanda and Kochhar, 1985) [19]. Presence of such kind of root inhibiting phenols may not be ruled out. This necessitates indepth analysis of the types of phenols either responsible for root initiation or for inhibiting rooting.

Nanda *et al.* (1970) [18] reported that they failed to root cuttings taken from old trees (the age is not mentioned by the authors) of three *Acacia* species, namely *A. mollisima, A. catechu, A. decurrens* and other tropical leguminous species, such as *Cassia fistula, C. javanica, Delonix regia, Albizia procera* and *A. lebbek.* The decline in rooting percentage of *Acacia mangium* stem cuttings taken from old stock plants may possibly be due 1) the presence of sclerenchymatous cells which become a barrier for root initiation; and 2) the absence of preformed adventitious roots in the stem. It has been reported that the difficulty in rooting of cuttings from mature trees may possibly be related to three important factors: 1) increase in production of rooting inhibitors as the plants grow old (Ooyama 1962) [22]; 2) decrease in phenolic levels which act as an auxin cofactor or synergist in root initial of stem cuttings (Hess 1968) [12]; and 3) presence of anatomical barriers such as sclerenchymatous sheath (Beakbane 1961) [4].

In case of *Acacia catechu*, data (Tables 3) indicated that T_1 (2000 ppm IBA) formulation was the best of all other treatments as it resulted in significantly maximum sprouting per cent (77.78%), average length of the sprouts (11.29 cm) and time taken for sprouting (22.25 days) however there was no rooting in all cutting treatments. The sprouting difference in treatments may be due to different amount of auxins absorption by cuttings. Higher sprouting in T_1 may be due to less absorption of auxins. Under some conditions like very low auxins concentration, auxins enhance cell expansion which leads to shoot growth. This low concentration of auxins might have effect on sprouting per cent, length of sprouts, time taken for sprouting in *Acacia catechu.*

In some cases, the cuttings of Acacia species have shown rooting success when softwood cuttings were treated by low auxin concentrations for long time. In present investigation, only the higher concentrations of auxin have been used which may be the cause to prevent rooting in this species as supra optimal effect of hormones. The concentration may have exceeded in this case and it may have some inhibitory effect on rooting of the species. Chauhan and Reddy (1974) [8] and Avanzato *et al.* (1988) [2] have suggested that optimum concentration of auxin is favourable, but supra optimal auxins are toxic to root regeneration. Due to the lack of sufficient relevant literature, the formulation of actual reasons behind the stubbornness of cuttings of these species to root is very difficult. Thus from the discussion it can be concluded that a further comprehensive study involving biochemical and physiological aspects may perhaps reveal the factors inhibiting the initiation of roots in this tree species of great importance.

Fig 1: Sprouting and rooting in *Toona ciliata* cutting

Fig 2: Sprouting in *Acacia catechu* cuttings

Conclusions

Toona ciliata can be propagated through cuttings with the use of auxins i.e. indole- 3 butyric acid (IBA) and naphthalene acetic acid (NAA) but 8000 ppm IBA can be used for vegetative propagation as it was found superior for all observation in the present investigation. As encouraging sprout length (11.29 cm) (IBA 2000 ppm) from *Acacia catechu* cutting was obtained but could not be supported by sufficient rooting.

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