International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(2): 468-473

Received: 27-01-2018
Accepted: 28-02-2018

Anok Uchoi
Ph. D. Scholar, Horticulture College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

N Kumar
Professor (Retd.), Horticulture College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

K Rajamani
Professor and Head, Department of Medicinal and Aromatic Crops, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

S Sumitha
Ph. D. Scholar, Horticulture College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
Anok Uchoi
Ph. D. Scholar, Horticulture College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Effect of plant growth regulators on vegetative and seed propagation of tea tree (*Melaleuca alternifolia* L.)

Anok Uchoi, N Kumar, K Rajamani and S Sumitha

Abstract
The study was conducted with a view to standardize the propagation techniques in Tea tree (*Melaleuca alternifolia* L.). During the trial, two type of propagation method *viz*., vegetation propagation through different type of cuttings and seed propagation treated with various plant growth regulators were done. Observations were made on sprouting percentage, number of sprouts per cutting and survival percentage for vegetative propagation and in seed propagation study, germination percentage, days taken for germination and survival percentage were recorded. At 30 days after planting, sprouting percentage (49.45 per cent) was recorded maximum in hardwood cuttings treated with IBA 1500 ppm (T_1) and highest number of sprouts per cutting (8.78) in control (T_3). No callus formation and rooting were observed in any type of cuttings and treatments. In seed propagation, soaking the seeds in GA_3 1000 ppm (T_1) for 30 minutes resulted in higher seed germination percentage both in petridish (laboratory condition) and pot sowing (35.63 and 33.33 per cent respectively) and was significantly superior to all the other treatments. Due to toppling of seedlings during misting operation and subsequent drying up of seedlings, no germinated seedlings survived.

Keywords: Tea tree, *Melaleuca alternifolia*, Vegetative propagation, Seed propagation, Plant Growth Regulator (PGR).

Introduction
Tea tree (*Melaleuca alternifolia* L.) is an aromatic tree, known for its oil of high value. It belongs to the family Myrtaceae and is native to Eastern Australia. Tea tree oil is used by Australian aborigines for several centuries for its medicinal properties.

This tree grows in inaccessible parts of dividing range of Eastern Australia and is very sparsely distributed and confined to warmer (27-32˚C) swamps by the coast. Tea tree oil has antibacterial, antifungal, antiviral and anti-inflamatory properties and is employed in commercial pharmaceutical products. It is a broad spectrum disinfectant and is the greatest natural antiseptic of world. The composition of tea tree oil has been reported to be a mixture of terpenes, terpene alcohols and sesquiterpenes (Laakso, 1965) [15]. The major component, terpinen-4-ol is considered to be responsible for its antimicrobial properties (Lassak and McCarthy 1983) [14].

Tea tree seed is paticularly small and seedlings have a poor competitive ability, so plantations are establised from seedlings raised in nurseries or seed beds (Murtagh, 1991) [18]. Seed is collected from high yielding individual tree (List *et al*., 1996) [16] which have been screened for chemotype and are known to yield well. But seeds of *Melaleuca alternifolia* have low germination power, which has hindered the development of new genetic materials and advances in technology for production. One of the probable causes of the lack of seed germination, observed in this species is the phenomenon of dormancy, which can be conceptualized as a state of physiological rest in the seed, due to the action of internal factors which blocks germination (Anselmini *et al.,* 2010) [1].

Clonal propagation using cuttings or tissue culture is rare in plantation establishment. It is not cost competitive with large scale rapid seedling production systems from seed. Its big advantage of providing a uniform stand of plants will become more important once elite, high yielding, high quality selections are released through breeding program. Trees with damaged or removed stems have the ability to generate adventitious buds on roots and shoots resulting in coppicing below a cut or a destroyed apical bud.

Recently, it has been introduced in India,where the oil is used mainly in cosmetics.

On account of its high demand in the medical and perfumary industry, the species offers scope for introduction in higher elevations. Research on tea tree can be priotized on selection of ideal genotypes suited in Indian conditions, propagation, evolving suitable planting system amenable for coppicing or mechanical harvesting, border/row or inter cropping in tea plantation, harvesting and distillation of oil besides value addition, fractionation of terpinene-4-ol and study of tea tree oil market.

Although the crop was introduced in India recently, no research work has been carried out till now. The crop can be a potential aromatic crop if its propagation are standardized under Indian condition. Hence, the present investigation was contemplated with the above objective.

Materials and methods

The experiment was conducted at Department of Medicinal and Aromatic Crops, Botanic Garden, Coimbatore which is situated at 11˚ 02' N latitude and 76˚ 57' E longitude and is at an altitude of 426.7 m above mean sea level. All the materials required for the research were sourced from Warwick estate, Kotagiri District, Tamil Nadu

Study of vegetative propagation

Healthy and uniform sizes of 10-15 cm length cuttings were taken from a well grown healthy mother plant for planting. The cuttings were given a slanting cut at the base with the secateurs for more surface area to form rooting.

The basal cut ends of the cuttings were treated by dipping in rooting solution of different Indole-3 Butyric acid (IBA) concentration and were planted immediately in well prepared rooting media consisting of sand, red soil and well rotten farm yard manure in the ratio of 1:1:1. Untreated cuttings dipped in distilled water for the same duration served as control.

Study of seed propagation

Botanically fruit of tea tree is a capsule. Fruits are woody in appearance. Matured fruits are darker in color and immature fruits are light reddish in color. Matured fruit are collected from tree by hand. If kept in a warm dry place after collection, capsules open and release hundreds of seeds from a single fruit. Collected seeds were then subjected to hot and cold water, different chemicals and growth regulator treatments for 30 minutes (soaking).

Seed germination test in laboratory condition

Seed germination test was carried under laboratory condition before sowing in bed to check the presence of dormancy in seed. Seed sowing was done in petridishes having germination paper after treating seeds with chemicals. The petridish after sowing was kept in germination chamber and observations were noted.

Seed sowing in pots under mist chamber

Plastic pots having hole at the bottom were filled up with sieved vermicompost and were placed above a container having water in it such that complete wetting of the compost was obtained. After wetting the compost completely, seed sowing was done on the surface of the compost and no watering was done from the overhead until germination was observed.

Results and Discussion

Effect of IBA on sprouting and rooting of cuttings

The result on sprouting percentage (Table 1) showed that at 15, 20 and 25 days after planting, the highest sprouting percentage was recorded in control hardwood cutting (T_3). But at 30 days after planting, sprouting percentage was maximum (49.45 per cent) in hardwood cuttings treated with IBA 1500 ppm (T_1) followed by control hardwood cuttings (T_3) with 48.33 per cent due to death of some sprouted cuttings in all the treatments. The least sprouting percentage of 1.21 percent was observed in semi-hardwood cuttings treated with IBA 100 ppm (T_6) at 30 days after planting.

The data on number of sprouts per cutting (Table 1) showed that at 15 DAP, hardwood cuttings treated with 1500 ppm (T_1) recorded the highest number of sprouts (8.78) per cutting followed by control hardwood cuttings (T_3) with 8.28 sprouts per cutting while the least number of sprouts (2.28) was recorded in semi-hardwood cutting treated with 100 ppm (T_6). But at 20, 25 and 30 DAP, the maximum number of sprouts per cutting was recorded in T_3 followed by T_1 while the least in T_6 treatment. At 30 DAP due to death of some sprouts in all treatments, number of sprouts per cutting was decreased and the highest number of sprouts (10.21) was observed in T_3 followed by 8.00 in T_1 and the lowest number (1.2) of sprouts per cutting was recorded with T_6. No callus formation and rooting were observed in any type of cuttings and treatments.

Success of propagation through cuttings is based on two factors mainly the sprouting of the new shoots from dormant buds available in the shoot and also formation of callusing from the basal cut end of the cuttings and subsequent differentiation of callus into formation of fibrous or adventitious root system. In this experiment, among the treatment hardwood cutting had more sprouting ability than the semi-hardwood. This may be related to the higher stored food material in hardwood cutting which would have enabled the buds to sprout more in hardwood. The results obtained are similar to the observation made by Kumari *et al.* (2010) [13] in mass propagation of *Jatropha curcus* and Sajid *et al.* (2012) [21] in plane tree *(Platanus orientalis*) cuttings where hardwood cutting was found superior than semi-hardwood and softwood cuttings in sprouting and survival percentage.

However, further studies showed that none of the cuttings produced any callus and consequent root formation, indicating the nature of 'difficult to root type' of cutting in tea tree. Difficulty in rooting of cutting was also reported in other Myrtaceae family member such as guava by Luis *et al.*, (1986) [17]. Cuttings of certain difficult-to-root plants may fail to root because of some physiological factors like occurrence of rooting inhibitors or absence of natural rooting co-factors. Cuir (1993) [4] reported that difficult-to-root hardwood cuttings of wax flower (*Chamaelaucium uncinatum*) have a cinnamic acid derivative which inhibits rooting, while no detectable levels of this phenolic compound were found in easy-to-root softwood cuttings. Similar result was reported in cuttings of difficult-to-root mature eucalyptus (Paton *et al*., 1970) [20].

Normally in difficult to root cuttings, the possibility of using GR is a proven technique to improve the rooting ability. The IBA at different concentrations tried both in hardwood and semi-hardwood in the present investigation did not exert any appreciable effect in sprouting of shoots or in inducing the callusing or rooting. Similar results were reported by Wally *et al*. (1981) [23] in guava and concluded that use of growth regulator to increase rooting percentage in guava stem cutting had limited success. Tohit (1999) [22] in *Juglans regia* hardwood cuttings and Audus (1953) [2] in *Pinus* reported that these plants are difficult to root from cuttings and rooting ability is not stimulated by hormone applications.

Thus, the present study led to the fact that cuttings of tea tree failed to root and the existence of some root inhibiting factors present in it which need to be investigated in future research programmes.

Changes in carbohydrate and phenol content as influenced by growth regulator

Hardwood cuttings contained higher amounts of carbohydrate (12 per cent) than semi-hardwood cuttings (10.80 per cent) before planting. During sprouting period, a decrease in carbohydrate level was observed in both types of cuttings. In hardwood cuttings, control (T_3) recorded more reduction in carbohydrate content (10.90 per cent) than treated cuttings T_1 and T_2 (11.10 per cent and 11.20 per cent respectively). In semi-hardwood cuttings also control (T_7) recorded more reduction in carbohydrate level (9.60 per cent) than the treated cuttings (T_4, T_5 and T_6) (9.80, 9.70 and 9.80 respectively) at 30 DAP.

Rooting of cuttings is highly influenced by their initial carbohydrates reserve (Kraus and Kraybill, 1918) [12]. The carbohydrates do not increase the rooting reply, but they serve as a source of carbon and energy for the synthesis of other essential substances for the roots formation. In the present investigation, hardwood cutting were found to have more carbohydrates than semi-hardwood which might be the reason for higher bud sprouts in hardwood cuttings than semi-hardwood. Carbohydrate content was also found to decrease after 30 DAP which clearly indicates that carbohydrates might have been utilized by the cuttings for their bud to sprout.

The two types of cuttings varied in their total phenol content, the hardwood cuttings contained lesser amount of phenol (1.26 mg/g) than semi-hardwood cuttings (1.47 mg/g) at the time of planting. Treatment of cuttings treated with IBA did not increase the phenol content instead decrease in phenol content was observed in all the treatments with gradual initiation of sprouts. The lowest phenol content in hardwood cuttings was observed in T_2 (0.72 mg/g) and in semi-hardwood cuttings the lowest content was observed in T_6 (1.02 mg/g) at 30 DAP.

The role of phenolic compounds in the regulation of growth and development of plants has been emphasized by many workers. The concept put forth by Nanda *et al.* (1974) [19] indicated that phenols at high concentrations induce callus formation and the auxins at high concentration induce the callus to form roots. De Klerk *et al*. (1996) [5] found that the phenolic compounds act as antioxidants, thereby protecting IAA from oxidation and enhance auxin activity. Phenol deficiency was suggested as one of the reasons for failure of rooting of difficult-to-root cuttings even after the application of auxin by Girourd (1969) [8]. However, in the present investigation, though phenol content was high in all types of cuttings no callus and rooting was observed suggesting that difficulty in rooting might be due to other factors. This needs to be further investigated. Reduction in phenol content at 30 DAP was observed which may be related to the utilization of phenols by buds for their vegetative growth.

Effect of PGR on seed germination (%), days taken for germination and survival (%)

Soaking the seeds in GA_3 1000 ppm (T_1) for 30 minutes resulted in higher seed germination percentage both in petridish (laboratory condition) and pot sowing (35.63 and 33.33 per cent respectively) and was significantly superior to all the other treatments. The lowest germination percentage was observed in hot water (T_8) treatment and control (T_{10}) with both the treatments having 12.50 germination percentages in petridish sowing and in pot sowing the lowest germination percentage was also recorded in control (T_{10}) (13.53 per cent).

The data on days taken for germination of seeds are furnished in the Table 3 also showed that the earliest germination (7.75 days) in petridish sowing was observed in the seeds soaked in GA_3 1000 ppm (T_1) which was on par with GA_3 500 ppm (T_2), followed by soaking in cold water (T_9) which took 8.75 days. Control dry seeds (T_{10}) without any soaking treatment showed the most delayed germination (13.5 days) among all the treatments. Similar results were recorded in pot sowing also, with T_1 having the earliest germination (8.67 days) and the control (T_{10}) showing the most delayed germination (14 days) among all treatments. However, due to toppling of seedlings during misting operation and subsequent drying up of seedlings, no germinated seedlings survived.

The results revealed that among the various seed treatments tried, GA_3 gave the highest germination percentage and the lowest germination percentage was observed in hot water treatment and control. This kind of similar effect was also noticed on number of days taken for germination where GA_3 treated seeds took minimum days while control seeds took the maximum days for germination. The use of GA_3 to enhance germination of seeds has been reported in many crops. Habibah *et al.* (2007) [9] in *Argania spinosa*, reported that soaking of seeds in GA_3 500 ppm resulted in highest germination (30%) which was followed by 100 ppm and 250ppm (27 and 26 % respectively). Kandari *et al.* (2008) [10] also reported that seeds of *Arnebia benthamii* when soaked in GA_3 100 ppm for 24 hours and incubation of 25ºC in 12 hours light photoperiod conditions resulted in maximum germination (100 %). The results of Ferraz and Takaki (1992) [7] in *Phyllanthus corcaradensis* and Dhankhar and Santhosh (1996) [6] in *Phyllanthus* species are also in line with the findings of the present study.

The involvement of GA_3 on the activation of cytological enzymes has been reported for better seed germination. GA_3 is involved in the photo-mechanism, where it may activate the intermediaries of phytochrome inter-convertible step(s) changing it into an active form in initiating germination as reported by Choudhary and Gupta (1995) [3]. The lowest seed germination observed in hot water treatment might be due to the damage caused to the embryo of seed during seed treatment and a similar opinion was expressed by Alaleh Khakpor *et al*. (2011) [11] in germination of Sage (*Salvia verticillata*) seeds.

However, after germination of seeds in pot under mist condition, toppling of seedlings during misting and consequent drying up of seedlings was observed. So, no survived seedling could be obtained at the end indicating that a special care and sowing techniques should be further standardized in future for the survival of the seedlings.

Table 1: Influence of growth regulators on sprouting percentage and number of sprouts per cutting in different types of tea tree cuttings

Treatment Details	Sprouting percentage (%)				Number of sprouts per cutting				Rooting (%)
	15 DAP	20 DAP	25 DAP	30 DAP	15 DAP	20 DAP	25 DAP	30 DAP	
T_1- Hardwood cuttings with IBA 1500 ppm	47.78	62.22	63.34	49.45	08.78	13.83	14.61	08.00	-
T_2- Hardwood cuttings with IBA 1000 ppm	45.56	58.89	60.55	37.22	06.89	11.94	12.89	06.41	-
T_3- Control (hardwood cuttings)	50.55	71.11	72.22	48.33	08.28	14.61	15.39	10.21	-
T_4- Semi hardwood cuttings with IBA 500 ppm	03.64	06.67	06.67	03.03	04.33	07.77	08.39	02.78	-
T_5- Semi hardwood cuttings with IBA 250 ppm	05.45	12.12	12.12	04.85	03.45	06.44	07.89	03.11	-
T_6- Semi hardwood cuttings with IBA 100 ppm	02.42	03.64	03.64	01.21	02.28	04.17	04.61	01.20	-
T_7- Control (Semi hardwood cuttings)	05.45	15.15	16.36	09.09	04.78	07.22	08.06	03.83	-
Mean	22.98	32.83	33.56	21.88	05.54	09.43	10.26	05.08	-
SE(d)	3.641	4.273	4.340	3.179	1.289	2.128	2.226	1.581	-
CD(0.05)	7.809	9.165	9.309	6.818	2.766	4.564	4.775	3.390	-

* DAP- Days after planting

Table 2: Changes in carbohydrate and phenol content in different types of tea tree cuttings as influenced by growth regulator

Treatment Details	Carbohydrates (% d.wt)				Total phenols (mg/g d.wt)			
	0 DAP	10 DAP	20 DAP	30 DAP	0 DAP	10 DAP	20 DAP	30 DAP
T_1- Hardwood cuttings with IBA 1500 ppm	12.00	11.80	11.50	11.10	01.26	01.12	01.12	00.85
T_2- Hardwood cuttings with IBA 1000 ppm	12.00	11.90	11.60	11.20	01.26	01.18	01.09	00.72
T_3- Control (hardwood cuttings)	12.00	11.70	11.40	10.90	01.26	01.22	01.16	00.81
T_4- Semi hardwood cuttings with IBA 500 ppm	10.80	10.60	10.20	09.80	01.47	01.40	01.23	01.07
T_5- Semi hardwood cuttings with IBA 250 ppm	10.80	10.40	10.10	09.70	01.47	01.42	01.30	01.09
T_6- Semi hardwood cuttings with IBA 100 ppm	10.80	10.60	10.30	09.80	01.47	01.35	01.20	01.02
T_7- Control (Semi hardwood cuttings)	10.80	10.50	10.10	09.60	01.47	01.45	01.35	01.15

* DAP- Days after planting
d.wt- dry weight

Table 3: Effect of seed treatments on germination %, number of days taken for germination and survival % of tea tree

Treatment (Seeds soaking for 30 minutes)	Under Laboratory (seed sown on petridish)			Under mist chamber (seed sown on pot)		
	Germination (%)	Days taken for germination	Survival (%)	Germination (%)	Days taken for germination	Survival (%)
T_1 - GA_3, 1000 ppm	35.63	07.25	-	33.33	08.67	-
T_2 - GA_3, 500 ppm	28.75	02.75	-	26.67	09.00	-
T_3 - GA_3, 250 ppm	26.88	09.00	-	29.17	10.00	-
T_4 - KNO_3, 0.1 %	20.00	09.25	-	20.83	10.33	-
T_5 - KNO_3, 0.2 %	23.75	09.00	-	24.17	09.33	-
T_6 - Thiourea, 1 %	17.50	11.75	-	15.83	11.67	-
T_7 - Thiourea, 2 %	16.25	12.25	-	16.67	13.00	-
T_8 - Hot water	12.50	11.50	-	14.17	12.00	-
T_9 - Cold water	20.50	08.75	-	20.00	09.33	-
T_{10} – Control	12.50	13.50	-	13.33	14.00	-
Mean	21.38	10.00	-	21.42	10.73	-
SE(d)	4.431	1.715	-	3.449	0.623	-
CD(0.05)	9.049	3.503	-	7.247	1.308	-

*GA_3 – Gibberellic Acid; KNO_3 – Potassium Nitrate

Acknowledgement
The corresponding author is highly grateful to Department of Medicinal and Aromatic Crops, Horticulture College and Research Institute, Tamil Nadu Agricultural University, Coimbatore for providing the financial support and necessary facilities for conducting the experiments.

References

1. Anselmini JI, Deschamps C, Gavazzi MIA, Zanette F, Panobianco M. Federal University of Parana (UFPR), Department of Crop and Fitossanitarismo, Street of Officials, Curitiba, Brazil. 2010; 1540, CEP:80035-050.
2. Audus LJ. Plant Growth Substances. Leonard Hill, London. 1953.
3. Choudary S, Gupta. Studies on the germination of *Catharanthus roseus* (L.) seeds. Effect of temperature and promoters. Seed Sci. and Technol. 1995; 23:831-841.
4. Cuir P, Sulis S, Mariana F, Van Sumere CF, Marchesini A, Dolci M. Influence of endogenous phenols on rootability of *Chamaelaucium uncinatum* Schauer stetm cuttings. Scientia Hort. 1993; 55:303-314.
5. De Klerk GJ. Marker of adventitious root formation. Agronomie. 1996; 16:609-616.
6. Dhankhar DD, Santhosh M. Seed germination and seedling growth in Anola (*Phyllanthus emblica* Linn.) as influenced by GA_3 and Thiourea. Crop Res. 1996; 12(3):363-366.
7. Ferraz FGA, Takaki MM. Seed germination of invader species of crops. In: Phyllanthus corcovadensis Muell. Arquivosde Biologiaa e Technologia. 1992; 35(1):53-62.
8. Girourd RM. Physiological and biochemical studies of adventious root formation. Extractable rooting cofactors from *Hedera helix* Can. J. Bot. 1969; 47(5):1288-1297.
9. Habibah S, Al-Menaie NR Bhat, Abo El-Nil M, Gamalin P, Suresh N. Seed germination of argan (*Argania spinosa*). America-Eurasian J. Sci. Res. 2007; 2(1):1-4.
10. Kandari LS, Rao KS, Maihkuri RK, Kusum Chauhan. Effect of pre sowing, temperature and light on the seed

germination of *Arnebia benthamii* (Wall. Ex G. Don). An endangered medicinal plant of central Himalaya, India. 2008.
11. Khakpor A, Bibalani GH, Mahdavi K. Influence of scabbing and hot water treatment on germination of Sage plant (*Salvia verticillata*) seeds. Annals of Biological Research. 2011; 2(5):52-55.
12. Kraus EJ, Kraybill HR. Vegetation and reproduction with special reference to tree tomato. Ore. Agr. Exp. Stat. Bull. 1918, 149.
13. Kumari M, Vikas Yadav Patade, Mohammad Arif, Zakwan Ahmed. Effect of IBA on Seed Germination, Sprouting and Rooting in Cuttings for Mass Propagation of *Jatropha Curcus* L Strain DARL-2. Research Journal of Agriculture and Biological Sciences. 2010; 6(6):691-696.
14. Lassak EV, McCarthy T. Australia Medicinal Plants. Methuen, North Ryde. 1983, 93-99.
15. Laakso PV. Fractionation of tea tree oil (*Melaleuca alternifolia*). Proc 25th condr pharm sci. 1965, 485-492.
16. List SE, Brown PH, Low CS, Walsh KB. A micropropagation protocol for *Melaleuca alternifolia* (tea tree). Australian Journal of Experimental Agriculture. 1996; 36:755-760.
17. Luis AS, Raul S, Rodrigo TJC. Vegetative propagation of guava by different types of cuttings. Hort. Sci. 1986; 21 (3):663.
18. Murtagh GJ. Tea tree oil. In New Crops (eds. R.J. Jessop and R.L. Wright), Inland Press, Melbourn. 1991, 166-174.
19. Nanda KK, Bhattacharya NC, Kochnar VK. Biochemical basis of adventious root formation on etiolated stem cuttings. N.Z.J. For. Sci. 1974; 4(2):80-89.
20. Paton DM, Willing RR. Nichols W, Pryor LD. Rooting of stem cuttings of eucalyptus: A rooting inhibitor in adult tissue. Austral. J. Bot. 1970; 18:175-83.
21. Sajid M, Pervaiz M, Rab A, Jan I, Haq I, Wahid F *et al*. Response of Plane tree *(Platanus Orientalis*) to cuttings and planting dates. The Journal of Animal & Plant Sciences. 2012; 22(2):420-424.
22. Tohit Gunes. An investigation on rooting of Juglans regia L. Hardwood cuttings. Tr. J. of Botany. 1999; 23:367-372.
23. Wally YA, El-Hamady MM, Boulos ST, Abu-Amara NM. Rooting experiments on guava using hardwood stem cuttings. Egypt J. Hort. 1981; 8(1):77-86.