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Rooting of Stem Cuttings in *Tectona philippinensis* Benth. & Hook. (Verbenaceae)

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Stem cuttings of *T. philippinensis* showed varied responses to rooting in various concentrations of Indolebutyric acid (IBA) and alpha-naphthaleneacetic acid (NAA) under misted condition in polyethylene enclosures. Viability response in stem cuttings at 45 days was manifested either by the formation callus or fissures and formation of adventitious roots with or without laterals. In addition, some cuttings had intact green leaves but did not show any of these responses. The formation of either callus or fissures in some of the cuttings had made them to be as healthy as those that had adventitious roots, however, those cuttings, which survived despite the absence of roots, were not as healthy as those that had adventitious roots, callus or fissures. Highest viability ranging from 95-100 % was noted in 7 out of the 11 treatments, and mostly, in treatments where IBA was added. The number of cuttings that did not show any response was high in treatments where NAA was added at 500, 750, and 1000 ppm but was low in treatments where IBA was added at the same concentration. Total rooting % which accounted only those that were formed in cuttings with 1 to 3 main adventitious roots and those with more than 3 main roots and laterals, was highest in treatment with 500 ppm NAA; and lowest, in the control treatment and in treatments with low concentrations of the same rooting regulator. However, when values for both viability and for absence of response in cuttings are considered, stem cuttings treated with 500, 750, and 1000 ppm IBA had higher rooting results. Under similar concentrations of NAA, viability % was low and the number of cuttings with no response was high. Both of these would result to less number of cuttings that would root.

The study has shown that *T. philippinensis* is an easy-to-root species. The production of roots by untreated cuttings indicate the economic potential of producing clones at the farmer level, and the possibility of re-establishing a stable natural population of the species in its natural habitats for *ex situ* conservation.

Keywords: asexual, adventitious roots, endangered, endemic, indolebutyric acid (IBA), alpha-naphthaleneacetic acid (NAA)

Tectona philippinensis Benth. & Hook. (Verbenaceae) locally known as, Philippine teak, is an endemic forest tree. It has a restricted area of distribution and grows in a highly vulnerable habitat. To date, just a few trees and wildlings can be found on the steep rocky hillside of Lobo, Batangas where the soil is dry and usually subjected to erosion during heavy rains. The dry and exposed ridges and slopes of the secondary forests are also favorable to slash and burn activities. These soil conditions, in

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combination with the anthropogenic activity, make *T. philippinensis* likely to be lost forever from the country's flora or even from the earth (Rojo, 1981). The global conservation status of the species had been assessed and designated as ENDANGERED both in the 1997 IUCN Red List of Threatened Species and the World List of Threatened Trees (Oldfield *et al.*, 1998; Walters and Gillett, 1998).

T. philippinensis is a small to medium-sized tree reaching 15 m high and 50 cm dbh. Its yellowish or creamy-white, fine textured, cross-grained, hard wood is used in the construction of bridges, wharves, railway carriage, ship decks, wood carving and used in general carpentry. In addition, decoction of fresh or dried leaves is prescribed for menstrual disorders and for hemorrhages. The nut produces a thick oil with a good odor and is used to promote hair growth or is applied to the skin to soothe irritation (De Guzman *et al.*, 1986). Phytogeographically, *T. philippinensis* is very important because it is one of the only three known teak species in the world and it also has the narrowest range of distribution (Rojo, 1981).

T. philippinensis must be the subject of studies to save it from possible extinction. These activities could be aimed at its possible exclusion from the list of rare and endangered endemic plant species. If clonal propagation proves successful, then, the plant maybe reintroduced to its historical habitats like Southern Batangas, Iling Island in the south of Mindoro and other places in the country where there is distinct six months each of dry and wet seasons (Rojo, 1981).

One method of propagating *T. philippinensis* is by vegetative propagation via rooting of stem cuttings. This has become an important tool for the establishment of clonal plantations and for the genetic improvement not only of ornamental shrubs, foliage crops, certain fruit crops and some vegetables but also of forestry species (Hartmann *et al.*, 1997). In recent years, considerable efforts have been made in tropical countries to develop proper vegetative propagation techniques to overcome the drawbacks in the production of sexually propagated planting materials. In the ASEAN Region, clonal propagation via rooting of stem cuttings have been conducted to mass-produce planting materials of commercially important dipterocarp and non-dipterocarp species (Aminah, 1991; Kantarli, 1993; Smits *et al.*, 1993).

There are numerous advantages (Darus, 1998) in adopting clonal propagation and one is that this method ensures that many new plants can be started from a few stock plants and the seedlings can be raised in a limited space. It can continuously supply planting stocks throughout the year to rehabilitate denuded forest areas. In addition, it is inexpensive compared to other asexual methods, rapid, and simple, and does not require the special techniques necessary in other conventional methods of plant propagation including *in vitro* propagation. This paper presents a successful study on the viability as well as rooting responses of stem cuttings in *T. philippinensis* in treatments without a plant growth regulator and when IBA and NAA were applied at different concentrations.

Methods

Collection and Maintenance of Wildlings

With the scarcity of seeds due to a limited number of full-grown *T. philippinensis* trees, 1 to 2 y-old wildlings with a height ranging from 10 to 14 in were earth-balled from the steep ridges and slopes of secondary forest and thickets in Lobo, Batangas. These were planted individually in black polyethylene seedling bags filled with garden soil, watered daily, fertilized once, and placed under a shaded area until they were stable for transport. After 1 mo the wildlings were carefully transferred to bigger seedling bags to allow for proper rooting. They were ready for use 3 mo after collection, at which time numerous shoots have been formed.

Preparation of Cuttings

Young stem cuttings with slightly brown basal portion and measuring 3 to 5 in long were collected from branches of phenotypically superior saplings. Cuttings were collected early in the morning or late in the afternoon when the temperature is normally low to minimize transpiration. They were submerged in tap water to wash off dusts and insects. Leaves showing indications of insect attack and disease symptoms were removed to prevent the spread of pest and disease during the incubation period. All leaves from the lower half of the cuttings were also removed to prevent them from rotting and for ease of dribbling in the rooting medium. These were then soaked in 5% Benlate solution (Benomyl at 500g/kg active ingredient), a fungicide, for 30 to 45 min. The cuttings were bundled up in 30 pieces and it was made certain that all the basal parts were at the same end for trimming before the treatment and for even distribution of the rooting solution. Bases of the cuttings were immediately treated for 30 min in their respective rooting solution, i.e., in control treatment, and in treatments where IBA or NAA were used singly at 100, 250, 500, 750, and 1000 ppm. These treatments were based on and/or modified after the works of Loach (1988), Chaturvedi *et al.* (1996), and Hartman *et al.* (1997). The thirty cuttings prepared per treatment were equally distributed to each of the three replicates. The study followed

Completely Randomized Design (CRD) and the entire experimental set up was conducted twice.

Plastic re-usable rooting trays with 3 in deep sterile and properly moistened rooting medium made up of 2:1 river sand and coconut coir dust were used for planting the cuttings. After dribbling, the potting trays were watered thoroughly to settle the rooting medium around the cuttings. They were kept in misted propagation frames completely covered with transparent PE bags. The frames were placed under a shady area that received at least 100 to 120 footcandles sunlight during the first 3 wk then at 250 to 280 footcandles sunlight for the remaining duration of the experiment. On the third week, the frames were opened partially to slowly acclimatize the cuttings and to prevent overheating inside. Stress condition in the frames was minimized by providing the plants with a mist spray once or twice a day. The experiment was terminated after 45 days.

Characterization of Responses

Percent viability and rooting responses were noted after 45 days of incubation. Considered viable, were cuttings that manifested at least one of the following (1) cuttings with intact leaves, no roots, callus, fissures; (2) cuttings with callus at treated end; (3) cuttings with fissures at treated end; (4) cuttings with only 1 or 3 adventitious roots and up to 4 in long; and (5) cuttings with more than 3 main adventitious with each having lateral roots. Total rooting% was based solely on those cuttings that have formed prominent roots, i.e., cuttings that had formed 1-3 main adventitious roots without lateral roots and those with both main and lateral roots.

Analysis of Variance (ANOVA) was employed and treatment means at 1-5 % significant level (F-test) difference were further analyzed using the Duncan's Multiple Range Test (DMRT) to determine the best treatment for viability and rooting response in the stem cuttings. Arcsine transformation was employed in data where the denominator varied for each treatment (*IRRISTAT* Version 3.1, Biometrics Unit, International Rice Research Institute, Laguna, Philippines).

Results

Results of viability and of rooting responses in *T. philippinensis* stem cuttings at 45 days after incubation is presented in Table 1. Viability % that is obtained from the sum of all the number of cuttings, which had no response, and those cuttings that formed callus, fissures, and roots was found highest in 7 out of the

Table 1. Mean % viability and indicated responses in stem cuttings of *T. philipinensis* to various IBA and NAA treatments at 45 days after treatment.

Treatment Concentration (ppm)	Viability (%)	No Response (%)	Callus at Treated End (%)	Fissure at Treated End (%)	1-3 Roots (%)	>3 Roots and with Laterals (%)	Total Rooting (%)
0 Control	57/60 (95.00)^{a}	12/57 (21.05)^{ab}	14/57 (24.56)^{ab}	15/57 (26.32)^{abc}	6/57 (10.53)^{bc}	10/57 (17.55)^{c}	16/57 (28.07)^{e}
100 IBA	58/60 (96.67)^{a}	11/58 (19.03)^{bc}	11/58 (18.97)^{ab}	14/58 (24.14)^{bc}	2/58 (3.45)^{f}	20/58 (34.48)^{cd}	22/58 (37.93)^{d}
250 IBA	60/60 (100.00)^{a}	7/60 (11.67)^{cd}	9/60 (15.00)^{bc}	6/60 (10.00)^{cd}	9/60 (15.00)^{b}	29/60 (48.33)^{ab}	38/60 (63.33)^{b}
500 IBA	60/60 (100.00)^{a}		11/60 (18.33)^{ab}	12/60 (20.00)^{c}	10/60 (16.67)^{b}	27/60 (45.00)^{b}	37/60 (61.67)^{b}
750 IBA	59/60 (98.33)^{a}	2/59 (3.33)^{e}	10/59 (16.95)^{abc}	19/59 (32.20)^{ab}	9/59 (15.25)^{b}	19/59 (32.20)^{cd}	28/59 (47.46)^{c}
1000 IBA	60/60 (100.00)^{a}	4/60 (6.67)^{d}	8/60 (13.33)^{bc}	13/60 (21.67)	8/60 (13.33)^{b}	27/60 (45.00)^{b}	35/60 (58.33)^{b}
100 IBA	53/60 (83.33)^{b}	10/53 (18.73)^{bc}	15/53 (28.30)^{a}	12/53 (22.64)^{c}	6/53 (11.32)^{bc}	10/53 (18.87)^{e}	16/53 (30.19)^{de}
250 IBA	47/60 (78.33)^{c}	7/47 (14.86)^{bc}	10/47 (21.28)^{ab}	3/47 (6.38)^{d}		27/47 (57.45)^{a}	27/47 (57.45)^{b}
500 IBA	53/60 (88.33)^{b}	12/53 (22.66)^{ab}	2/53 (3.77)^{de}	1/53 (1.89)^{e}	16/53 (30.19)^{a}	22/53 (41.51)^{bc}	38/53 (71.70)^{a}
750 IBA	60/60 (100.00)^{a}	12/60 (20.00)^{ab}	5/60 (8.33)^{cd}	15/60 (25.00)^{bc}	12/60 (20.00)^{ab}	16/60 (26.67)^{de}	28/60 (46.67)^{c}
1000 IBA	30/60 (50.00)^{d}	9/30 (30.00)^{a}	1/30 (3.33)^{e}	10/30 (33.33)^{a}	2/30 (6.67)^{cd}	8/30 (26.67)^{cd}	10/30 (33.33)^{de}

Column means followed by common letter are not significantly different at 5% level based on DMRT

11 treatments. These included the control treatment and all the treatments where IBA was added at different concentrations. Their viability rates ranged from 95 to 100 %. On the other hand, cuttings which did not respond to rooting or either to formation of callus and fissures, but still with intact leaves were highest in treatments where NAA was applied at 500, 750, and 1000 ppm, and also in the control treatment. In treatments with 500, 750 and 1000 ppm IBA, the rates on non-responding cuttings were very low and ranged from 0.00 to 7.00 %. This was significantly different with the highest values that ranged from 15 to 30 % in treatments were NAA was added at the same concentration. With regards to callus formation, the lowest values were obtained in treatments where NAA was added in high concentrations at 500, 750, and 1000 ppm. However with tissue formation, no definite trend was observed. Total rooting % which included data from cuttings which formed 1-3 main adventitious roots only and those which formed more than 3 main roots with laterals, was highest in treatment with 500 ppm NAA; and lowest, in the control treatment and in treatments with NAA added at 100 and 1000 ppm.

However when looking for the best treatment(s) for rooting, it would equally be important to consider values both for viability and for absence of response in cuttings. When both of these were taken into consideration, better results were obtained in treatments where IBA was added at 500, 750, and 1000 ppm than in treatment where NAA was added at 500 ppm. In the latter, viability rate was low and the rate of non-responding cuttings was high resulting to less number of cuttings that would root.

Discussion

The overall performance of *T. philippinensis* cuttings to IBA treatment was better than in NAA treatment in terms of their viability, callus formation, fissure formation and rooting. This shows the plant's preference for IBA as a rooting medium. The study has demonstrated that any concentration of IBA from 500 to 1000 ppm would be ideal in the promotion of rooting in stem cuttings of *T. philippinensis*.

NAA compared to IBA might have some toxic effects on plants especially at high concentration. Better performance of cuttings in IBA treatment may be attributed to its stable character both when mixed as a powder or a liquid. It is the best auxin for general use because it is nontoxic to plants over a wide concentration range, and is effective in promoting rooting of a large number of plant species. Generally, if a tree cutting does not respond to IBA, other root promoting compounds will not compensate (Hartmann *et al.*, 1997).

The presence of either callus or fissures in some cuttings may have helped increase the surface area for absorption of nutrients from the substrate. These structures have functioned like the adventitious roots and their laterals and this led to the robust condition of the cuttings that produced them. The formation of callus in cuttings of *T. philippinensis* was independent from root formation because adventitious roots were formed even in the absence of callus. Hartmann et al. (1997) explained that the simultaneous occurrence of both callus and roots, which may happen at times, is due to their dependence upon similar internal and environmental conditions. In addition, callus formation is a precursor of adventitious root formation in some species. This has been observed with difficult-to-root species of *Abies, Cedrus, Cryptomeria, Ginkgo, Larix, Pinus, Podocarpus, Sequoia, Sciadopitys, Taxodium* and *Pinus* (Jackson, 1986).

T. philippinensis may be considered as an "easy-to-root" species based on the results of the study; even untreated cuttings formed roots. The consistent emergence of roots in *T. philippinensis* between a 10 to 21 day periods is even shorter than that reported for *Agathis australis*, which occurs within a 3 to 4 wk period (White and Lovell, 1984). A high rooting percentage in the cuttings may also be attributed to the presence of a number of intact leaves, which were left on the cuttings. Leaves photosynthesize and the carbohydrates translocated from them are important for root development. Although the strong root-promoting effect of leaves and buds are probably due to other more direct factors, leaves and buds produce auxin, and the effects of polar apex-to basal transport of auxin are observed on rooting at the base of cuttings. Likewise, other studies that have shown that the presence of leaves in cuttings exerts strong stimulating influence on rooting are those in avocado (Reuveni and Ravis, 1981), several species of *Shorea* (Darus, 1998), *Hopea odorata* (Aminah, 1991; Srivastava and Manggil, 1981) and *Acacia auriculiformes* (Chaturvedi *et al.*, 1996).

The use of shoots from 1 to 2 yr-old wildlings of *T. philippinensis* and the good rooting results at 500 to 1000 ppm of IBA shows that raising of propagules for planting is much shorter compared with the use of branch cuttings of *T. grandis* measuring 20 – 25 cm long and 1.5 to 2.0 cm diameter taken from 25 – 35 yr-old trees. Good rooting only took place after 3 – 4 mo (Das, 1998). This delay in rooting or decrease in rooting percentage of cuttings taken from older plants may be due to anatomical features, such as thickening of sclerenchyma cells, which creates a barrier to root initiation (Nanda *et al.*, 1969). Aside from the shorter time involved in rooting young stem cuttings of *T. philippinensis*, this method is also manageable and requires less space for the starting materials.

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