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Roots stimulation of selected genotypes of *Aquilaria malaccensis* Lamk. through Indole-butyric acid (IBA): A most economically important species of northeastern region

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Abstract
Aquilaria malaccensis a well known tree species produced a most high valued resin for perfume industries. Though the availability of genetically diversified plants in a natural population is an inducer of desirable individuals for sustainable management and due to their genetic structure only few individual trees are susceptible to attack by some specific fungi or insects. Hence, a selection of trees was made from even aged (10-15 years) plantations of individual infected trees from different districts of Assam. Among them nine (09) selected genotypes were established at RFRI, Jorhat, Assam as seedling seed orchard (SSO). The present study was conducted with the hormonal treatment of 50ppm to 200ppm as continuous dip and 500ppm to 4000ppm with pulse dip of total six (06) different concentrations with shoot cuttings of different genotypes of established SSO. One clonal hedge garden with eight (08) genotypes planted in field condition. This is the conservation of genetic diversity of the species has been worked out as a most useful sustainable management and a first report of rooting of shoot cuttings for this economically important species.

Keywords: natural selection, Seedling seed orchard, shoots rooting, conservation

Introduction
Aquilaria malaccensis Lam. is a most commercially important non timber tree species of Southeast Asian tropical forests grows up to 15–25 m tall which is extremely valued for its production of resinous heart wood for luxury perfume, fragrance and soap manufacturers etc. (Anonymous, 1948) [7]. The species is found growing naturally in the forests of north east India, mainly in lowland and on hillsides between altitudes of 100 up to 500 m under high humid, sub-tropical climate with rainfall 1800-3500 mm per annum. The species is locally known as *'Sasi'*. Among the fifteen genus found, only eight species are known to produce agarwood (Ng *et al.* 1997) [32]. Among them, most valuable species *A. malaccansis*, A. *khasiana* Hall. are available in the north eastern states of India and other one *A. macrophylla* Miq in the Nicobar Islands (Giri, 2003) [14]. Distribution of of the species is in Bangladesh, Bhutan, India, Indonesia, Iran, Malaysia, Myanmar, Philippines, Singapore and Thailand (Oldfield *et al*, 1998) [34]. In India, it is confined to the north east region and mostly in the foot hills of Arunachal Pradesh and rocky terrain forests of Changlang, Lower Dibang Valley, Lohit and Papum Pare district (Tabin, 2012) [41] and also found in Manipur, Meghalaya, Mizoram, Nagaland, Tripura and West Bengal (Palit, 1996) [35]. In Assam, the tree is cultivated in the district of Sibsagarh, Golaghat, Nowgong, Darrang, Sadiya, Goalpara and in Garo Hills and Cachar. In the wild source, natural population is decreasing rapidly and becoming endangered through uncontrolled harvesting against the demand for agarwood. Consequently, it is listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 1994) [10]. The species is vulnerable according to the IUCN red list, and has been included in The World List Threatened Trees (Oldfield *et al*., 1998) [34]. The species is considered critically endangered in India (IUCN, 2009) [19] and almost extinct in the wild in Assam (Anonymous, 2003) [7]. The tree is cultivated in the home gardens of upper Assam and contributes significantly up to 20% of the total annual income of the family by selling the cut log with agarwood. Propagation of the species is usually through seeds which germinate readily after maturity (Kundu and Sett, 2005) [26] however, seeds are recalcitrant and their rate of germination sharply reduces with the increase in the period of storage (Kundu and Kachari, 2000) [24] and Kundu and Kachari, 2015 [25] also reported on definite storage temperature is required to retain the viability period. It has a restricted period of 15 days of seed viability (Saikia 2011, Shankar 2012) [38, 39]. Germination percentage of fresh seeds is high which sharply decreases for stored seeds (Ahmad and Gogoi, 2000) [3]. Desiccation sensitivity and recalcitrant behaviour of seeds demonstrated decrease in seed moisture content with time during different desiccation conditions reported by Kundu and Kachari (2000) [24]. Recalcitrant nature of seed with short term viability of *Aquilaria malaccensis* plant leads to mass propagation through vegetative means. Hence, in continuation, the present investigation was efforts to made to propagate true genotype of branch cuttings under the mist chamber condition. This has been assessed through hormonal treatment for induction of rooting of above established selected genotypes by promoting conservation and sustaining the production of agarwood of desired planting stocks. The ultimate goal of breeding of this species is to improve the oil quantity and quality which could be achieved through selection of superior genotypes.

Materials and Methods
Initial project work was completed with survey and selection

of *A. malaccansis* in the major growing areas of natural forest and home shed garden of Assam and was established one seedling seed orchard (SSO) in the campus of Rain Forest Research Institute, Jorhat, Assam. The experimental site Rain Forest Research Institute, Jorhat, Assam situated at 26º 47'N latitude and 94º 17'E longitude and altitude 80 msl. Annual rainfall is 800mm/annum with maximum and minimum temperature 33.8º C and 21.2º C respectively. Experiments were conducted to study the effect of indole-3-butyric acid (IBA) on semi to hard wood cuttings. Total nine (09) selected sources with different hormonal concentrations as continuous and pulse treatment were used. IBA 0, 50,100, 200 and 500ppm in overnight dipping and 0, 1000, 2000 4000 and 8000ppm treatment for 5min. Cuttings are taken from top, middle and basal portion of selected branches for rooting trial. Cuttings were collected in the month of January to March from 4 years old tree during cool hours in the morning before the sun light intensity is high. Branches are leafless and put into a bucket of water immediately after collection. Double node cuttings (length 18 cm -20 cm) were prepared with sharp secateurs and basal end made oblique angle for greater surface of absorption of hormone. Then treated with fungicides like bavestin solution (0.1%) for 10 to 15 minutes and then the upper parts were dipped in hot waxed to avoid aeration. Potting media are used coarse sand and vermiculite alone and soil: river sand in a ratio of 1:1. For hormonal treatment basal part of the cuttings were dipped in the auxin solution to a length of 1 inch and were inserted in the potting media with one third of its length and gently firmed and incubated in the mist chamber. The size of the poly bag used 7″x9″. Misting was operated in the morning and evening and recorded temperature and humidity around 29-30°C and 80-85% respectively. After rooting in late winter the cuttings were placed in the normal greenhouse.

Results & Discussion

There could be a very limited research on vegetative propagation of the genus *Aquilaria*. The present studies being investigated for potting medium and IBA concentration from three different positions to examine the end result of rooting. Hormonal treatment significantly increased sprouting and rooting percentage in all types of cuttings tried. Sprouting percentage was best in cutting from basal portion followed by middle and top position. Rooting percentage was the highest in basal then middle to top in 1000ppm and then in 4000 ppm. In overnight dipping sprouting was found to be the best in 200ppm and followed by 50 ppm. Cuttings collected from basal i.e. hardwood cutting of the branch responded better than middle and top position. This may be due to that hardwood cutting contain higher stored carbohydrate than semi-hardwood and softwood cuttings. Basal portion of the branch responded best 76.7% sprouting up to the treatment 100ppm. Rooting was also observed in 500ppm treatment but survival rate was poor. IBA 1000ppm enhanced rooting percentage up to 60.5%.

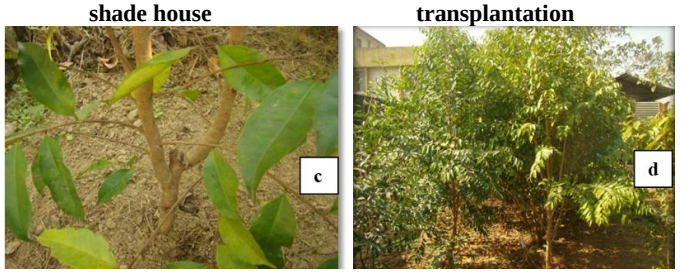
Propagation through cuttings is a common method in forestry species and it offers a quality planting material with increased productivity, uniformity in plantations for higher yields and in capturing existing genetic traits that can be used a genetic equality for tree improvement (Leakey *et al*. 1990) [28]. Similar to our result, origin of the stem cutting within a shoot and the position of the shoot within the donor plant influence in the formation of adventitious roots (Longman and Leakey, 1995) [30]. Rosier *et al*. (2004) [37]. He conducted an experiment using cuttings taken from 3 and 4 year old stock plants of Virginia pine (*Pinus virginiana* Mill.) and reported 46% rooting percentage of cutting taken from semi-wood cuttings compared to 33% rooting percentage of cuttings taken from softwood cuttings. There were reports in the spring season

Vegetative propagation of *Aquilaria malaccensis Lam* through rooting of shoot cuttings

Fig 1: Bud sprouting of shoot cuttings of *A. malaccensis*

Fig 2: Different rooting structure of shoot cuttings in different hormonal concentration and size of cuttings a. big size basal b. medium size middle c. small size upper

Rooted cuttings under shade house | **Cuttings before transplantation**



Field establishment of rooted plants | **Growth after six months**

collection period of mature trees propagated by cuttings were successful especially in the early spring (Leakey 2004) [29] as similar to our study. In case of both rooting and sprouting it was also reported that in *Jatropa curcas* it was more with IBA than NAA. Usually, indole-3- butyric acid (IBA) is found to be the most effective root promoting auxin (Tchoundjeu *et al.* 2004) [42, 45]. Last *et al*. (1991) [27] and Rose *et al*. (1992) showed that inducing adventitious rooting with IBA. Rahman *et al.* (2002) [36] reported that IBA at the concentration of 3000 ppm resulted in 70% rooting of olive cuttings. Ibrahim *et al*. (1991) [18] treated hardwood cuttings of olive with 500 and 1000 ppm IBA that resulted in their better rooting. Khattak *et al*. (1981) [20] reported rooting of semi-hardwood cuttings with 6000 ppm IBA of olive cv. Lisino. The rooting hormone applied for cuttings, especially the effect of IBA has a significant importance in rooting of various tropical forest tree species (Husen and Pal, 2006, 2007) [16, 17]. Negash, 2002 [31] conducted a successful clonal propagation on threatened *Juniperus procera* by using different concentrations of IBA. Amri *et al.* 2010) [5] investigated the effects of age of donor plant, IBA treatment, and cutting position on rooting ability of stem cuttings on *Dalbergia melanoxylon.* In our present study, we observed that highest rooting percent with the application of 1000ppm of IBA and then followed by 4000 ppm. The further increase of IBA concentration leads to decreasing the percent of rooting and mortality of the cuttings. The study revealed very much similar result with Abdullah *et al*. 2006 [1]. They observed highest percent of rooting of *Psidium guajava* with the application of IBA but it reduced with increasing of IBA concentration. The similar results was also observed by Aminah *et al*. 1995 [4] on *Shorea leprosula* rooting. The present study revealed the number of roots per cutting and the length of root with increasing IBA concentration. Various studies on *Dalbergia melanoxylon*, *Tectona grandis* Husen and Pal, 2007 [16], and *Juniperus procera* also documented significant difference of root number per cutting among IBA treatments. Negash 2002 [31] observed significant difference of root number per cutting among the IBA treatment. He noticed that the number of rooted cuttings and the subsequent root number declined quickly when cutting was treated with different concentration IBA. A report of Vietnam by Koskela *et al*. (2002) [22] reported on *Aquilaria crassna* rooting. However, details of cutting origin and environment were not reported. Several authors has been reported on the effect of hormone IBA on shoots rooting such as Kochhar *et al.*, 2008 [21] found that pretreatment with 100mg/l IBA and 100mg/l NAA increased rooting. Kumar and Swarnkar 2003 observed that IBA concentrations of 50 and 75 mg/l for the 24 hr dipping and in IBA concentrations of 1000 and 1500 mg/l in the 3 to 4 min dip promoted higher rooting. Author Noor Camellia *et al.*, 2009 [33] also investigated the effect of IBA concentrations in three types of cuttings like softwood, semi hardwood and hardwood of *Jatropha curcas* where greatest root number was obtained 1000 mg/L IBA. Results obtained from the present study were applied with the different genotypes with 4000 in hard/ basal cuttings and 1000 in semi hard/middle cuttings. However, the most significant effect was recorded on stem cuttings treated with a concentration of 1000 ppm with best result for root length. Similar results is also with the findings of Susaj *et al*., 2012 [40] and Younis, 2005 [46] where they found a concentration of 1000ppm of IBA gave the best result in the propagation of rose plants in hardwood cuttings. The status of the stored foods is an important feature for rooting, growth and development capacity of stem cuttings which are more in quantity in hardwood than in soft and semi- hardwood cuttings similar with Hambrick *et.al*, 1991 [15]. The results obtained in this experiment were also same with the results of Balakrishnamurthy *et al.* 1988 [8]. They observed the rooting and survival capacity of semi-hard and hardwood cuttings of rose cultivar treated with 1000ppm IBA. However, semi-hardwood cuttings treated with 4000 ppm produced the highest number of roots (6.7±2.4) followed by hardwood cuttings (5.8±1.6) treated with 1000ppm IBA while the control showed the lowest root number in all cases. In case of root length, softwood cuttings produced the longest root 13 cm followed by semi-hardwood cuttings 8.5 cm. The results of the present study revealed that rooting was significantly affected by type of the cuttings and treatment concentration. On average 72% rooting was observed in the big size cuttings 5-6 mm compared to the other two sizes of cuttings, i.e., 2-3 mm and 3-4mm, in respect to auxin treatment. In control, a maximum of 10.0% rooting was recorded in big size cuttings and a minimum of 30.0% of the cuttings rooted in semi hard cuttings. Similarly cuttings collected from the lower and upper portions of shoots resulted in 60.3% and 54.5% rooting respectively, irrespective of auxin treatments. Cuttings collected from the middle portion maximum rooting was observed 40.0%. Cuttings treated with 1000ppm IBA produced troots more rapidly than those treated with 4000 – 8000 ppm IBA. Comparing the three positions rooting success of 1000 ppm IBA treated cuttings was optimum 65% in big size while those treated with 500 ppm IBA was 34%. It may be due to the fact that the content of endogenous auxin in the stem cuttings, where cuttings taken from mature mother trees which may lead to reducing rooting ability (Husen and Pal 2006) [17]. Rooting percentage was greater in coarse sand in comparison to river sand, soil and vermiculite after 8 weeks of incubation in the misting condition. Rooted cuttings produced an average of 3-4 pairs of leaves and fibrous root system 4-weeks after transplanting into pots. However, tree species and even clones can respond differently to individual and mixed applications of auxin at different concentrations, even when many other factors are constant (Dick *et al*. 2004, Leakey 2004) [12, 29].

Conclusions

Genetic variability present in the existing base population and to identify important yield attributing characters, selection for which would help in development of high yielding *Aquilaria malaccansis* genotypes. The results suggest application of IBA for enhanced rooting for vegetative propagation of *A. malaccensis* and use of basal portion for both hard and semi hard cuttings. Without hormonal treatment there was no rooting but sprouting may be due to intact food reserve. As these results may seem to be promising, further research is required to fully understand the various aspects of propagation of the species through vegetative means.

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