Clonal Propagation and Clonal Trial of Hybrid *Acacia*

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Abstract

Naturally crossed hybrids of *Acacia mangium* x *Acacia auriculiformis* are found in the *A. auriculiformis* and *A. mangium* plantations of Bangladesh. During 1995 superior hybrid *Acacia* trees were selected and propagated by using shoot cuttings for establishing a hedge orchard. Coppice shoots sprouted from this hedge orchard plants were used as stem cuttings for rooting trials, and successful cuttings from different clones provided planting materials for clonal trial experiments. There were highly significant differences between cuttings taken from different zones of the shoot (cutting types) and hormone treatments in the number of successful cuttings and number of roots produced per rooted cuttings. Tip cuttings (2 noded) rooted 71.5% and produced 3.7 roots per rooted cutting when treated with 500 ppm IBA. Similarly, 3rd node cutting also rooted 80.0% and produced 4.1 roots per rooted cutting under the same treatment. A slight decrease in rooting success (55.5%) and number of roots (3.2) per rooted cutting were recorded in 4th node cuttings under the same treatment. But percentage of rooting success and number of roots per rooted cuttings were not increased in the above mentioned three types of cuttings when treated with higher concentration (1000 ppm or 1500 ppm) of IBA. When a higher concentration of IBA (1000 ppm) was used in treating 5th node cutting better (50.0%) rooting success and number of roots (3.3) per rooted cutting were observed. In respect to rooting success and number of roots produced per rooted cutting the variation between the cuttings of different zone of the shoot (cutting type) and hormone treatment was highly significant. There were significant differences between clones in height growth. The highest and lowest height growth were recorded in clone 2 (87.21cm) and in clone 13 (38.5cm) respectively.

সারসংক্ষেপ

বাংলাদেশে আকাশমনি ও ম্যানজিয়াম বাগানে *Acacia mangium* x *Acacia auriculiformis* এর প্রাকৃতিক ভাবে উৎপন্ন শংকর (হাইব্রীড) গাছ দেখা যায়। ১৯৯৫ সালে নির্বাচিত ভাল গুন সম্পন্ন শংকর একাশিয়া গাছ থেকে সংগৃহিত সায়ন দ্বারা অংগজ বংশবিস্তারের মাধ্যমে কাটিং উৎপন্ন করে হেজ অর্চার্ড উত্তোলন করা হয়। হেজ অর্চাড গাছ থেকে উৎপন্ন কপিস শাখা কাটিং-এর শিকড় গজানোর পরীক্ষা ও শিকড় গজানো কাটিং ক্লোনাল ট্রায়াল এর পরীক্ষায় ব্যবহার করা হয়। অংগজ বংশবিস্তারের পরীক্ষায় দেখা যায় যে, কাটিং-এর প্রকার ও বিভিন্ন মাত্রার হরমোন প্রয়োগে কাটিং কৃতকার্যের হার ও কৃতকার্য কাটিং-এর উৎপন্ন শিকড়ের সংখ্যার মধ্যে উল্লেখযোগ্য পার্থক্য পরিলক্ষিত হয়। শিকড় গজানোর ক্ষেত্রে শীর্ষ কাটিং (দু পর্ব যুক্ত) ৫০০ পিপিএম আইবিএ প্রয়োগে ৭১.৫% কৃতকার্য হয় এবং কৃতকার্য প্রতিটি কাটিং ৩.৭ টি শিকড় উৎপন্ন করে। অনুরূপ ভাবে, ৩য় পর্ব থেকে প্রস্তুতকৃত কাটিং ৫০০ পিপিএম আইবিএ প্রয়োগে ৮০.০% কৃতকার্য হয় এবং কৃতকার্য কাটিং ৪.১ টি শিকড় উৎপন্ন করে। চতুর্থ পর্ব থেকে প্রস্তুতকৃত কাটিং ৫০০ পিপিএম আইবিএ প্রয়োগে কৃতকার্যের হার ৫৫.৫% ও কৃতকার্য কাটিং-এ ৩.২টি শিকড় পাওয়া যায়। তবে উল্লেখিত তিন প্রকার কাটিং-এ হরমোন-এর

মাত্রা ৫০০ পিপিএম-এর উপরে বৃদ্ধি করলেও কৃতকার্যতার হার এবং কৃতকার্য কাটিং-এ উৎপন্ন শিকড়ের সংখ্যা বৃদ্ধি পায় না। পঞ্চম পর্ব থেকে প্রস্তুতকৃত কাটিং-এ ১০০০ পিপিএম আইবিএ প্রয়োগ করলে ৫০.০% কৃতকার্য হয় এবং কৃতকার্য কাটিং-এ উৎপন্ন শিকড়ের সংখ্যা ৩.৩টি পাওয়া যায়। বিভিন্ন ক্লোন-এ উচ্চতা বৃদ্ধিতে তারতম্য পরিলক্ষিত হয়। চারার বৃদ্ধি ক্লোন নং ২ এবং ক্লোন নং ১৩-এ যথাক্রমে সবচেয়ে বেশি (৮৭.২১ সেঃ মিঃ) এবং সবচেয়ে কম (৩৮.৫ সেঃ মিঃ) দেখা যায়।

Key words: IBA, clonal variation, height growth, rooted cutting

Introduction

Acacia auriculiformis is a native tree to Papua New Guinea, Northern Australia and Indonesia. *Acacia mangium* is also a native tree to Papua New Guinea and Indonesia. These species were initially introduced in Bangladesh as shade tree in tea gardens about 40 years ago. During 1980's.provenance trials of these species have been established at different silvicultural research stations of Bangladesh Forest Research Institute (BFRI).Trial results indicated a growth superiority of the provenances of Papua New Guinea and Australia over local land race. During the 1980 and onwards thousands of hectare of plantations have been raised by Bangladesh Forest Department (FD) with these two species. In recent years, naturally crossed hybrids of *A. mangium* x *A. auriculiformis* have been found in the *A. mangium* and *A. auriculiformis* plantations of Bangladesh. The hybrid trees possesses many characteristics of the parent species. Hybrid of *A. mangium* x *A. auriculiformis* has also been found in Sabah, Malaysia in 1971-72 (Lapongon 1988, Pinso and Nasi 1991). Most of the reports states that hybrid *Acacia* is more productive than either parent species on different site types in Vietnam (Harwood 1999), in Malaysia (Lapongon 1988), in Thailand (Kijkar 1999) and in Bangladesh (Kamaluddin 1995, Faizuddin 1998). Due to its vigorous growth characteristics- long, straight and clear bole with light branching, good timber for pulp and paper, small hand tools, furniture, etc. Hybrid *Acacia* has attracted much attention to the forester. It has become a major industrial plantation species in Malaysia, Thailand and Indonesia (Kijkar 1999).

Hybrid *Acacia* tree produces abundant viable seeds only after three years of planting. But propagation through seeds is problematic, because seedlings produced from F_2 seeds resemble either parent, intermediate hybrids or superior hybrids (Kijkar 1999). About 44-46% of F_2 seeds produce intermediate hybrids with crooked stem and heavy branching and only a very few of the F_2 seeds produce superior hybrids. It has also been found that approximately 52% of the F_2 seeds are reverted to *A. auriculiformis* and 2-4% to *A. mangium*. Thus, clonal propagation is an alternative approach to reproduce quality planting materials of hybrid *Acacia* for commercial plantation.

Wangamanee *et al.* (1989) reported that hybrid *Acacia* can be propagated successfully, and clonal planting materials showed a very good characteristics. Banik *et al.* (1995) propagated 10-20 cm cuttings taken from coppice shoots of one-year old seedlings treated with different concentration of indole butyric acid (IBA). They found 83.3, 83.3, 86.7 and 60.0% rooting success with 10 ppm, 50 ppm, 100 ppm and without IBA treatment respectively. Kijkar (1999) reported that epicormic shoots originated from 'reservoir' cuttings became rejuvenated, and about 90% rooting success could be achieved using these shoots. He also reported that two noded cuttings obtained from coppice shoots when treated with Seradax no. 3 and inserted into rooting medium of river sand and coconut husk showed 90 and 98% rooting success respectively. Beside shoot cuttings, hybrid *Acacia* can also be multiplied by tissue culture techniques (Darus Ahmed 1993).

The present experiments were carried out as a part of wider investigation into the vegetative propagation and selection of superior clones of hybrid *Acacia* .The species were chosen partly because of its segregation problem in sexual cycle and partly because of its potential as a commercial plantation species in degraded forest land and for agroforestry plantation.

Materials and methods

During 1995, plus trees of hybrid *Acacia* trees were selected from Mandakini beat of Hathazari Range, Chittagong Forest Division. These trees (clones) were multiplied vegetatively by using newly emerged shoots originated from cut branch. The clonal materials thus prepared consisted cuttings from 18 clones, and these were planted for establishing a hedge orchard near the HQ nursery at BFRI. Eighteen clones from this 2-year-old hedge orchard and four clones from the hedge orchard of Silviculture Genetics Division provided adequate coppice shoots as cutting material for this experiment. The rooted cuttings from these 22 clones were used for establishing clonal trial plantation at Hyankoo seed orchard centre. But for propagation experiments shoot cuttings were collected from a single clone (clone no. 6) to avoid clonal variation. Freshly collected materials were always used for getting better rooting success.

Coppice shoots were divided into four zones for the preparation of cutting, *viz.* first two nodes ignoring the tip of the shoots (tip cuttings), third node, fourth node and fifth node. Leaf laminas of the cuttings were reduced to approximately 50 percent of their original area by cutting across the broadest part of the leaf. Cuttings prepared from each zone were treated with 500 ppm, 1000 ppm, 1500 ppm IBA and distilled water (control) and were inserted in a bed containing river sand in four replicates with 10 cuttings in each replicate. The design of the experiment was completely randomized. The experiment was set up in a fiber glass house (Plant Propagation Unit) of BFRI having fluctuating temperature between 20°C to 35°C. Intermittent misting was provided after insertion of cuttings into the bed containing river sand. The experiment was carried out during the month of May and June 1999. After four weeks of insertion, cuttings were lifted very carefully from the bed. The number of successful cuttings (*i.e.* those producing roots) and number of roots per successful cuttings were recorded.

The planting materials prepared vegetatively from 22 clones were used for the clonal trial experiment established at Hyankoo Seed Orchard Centre, Fatikchari, Chittagong. A randomized block design with five blocks was used for the trial. A total of 1100 cuttings were planted for the whole trial consisting of ten cuttings in each plot. The cuttings were planted 3 m x 3 m spacing. The trial was set up in July 2000, and only height data was recorded on October 2000. The experimental site was more or less flat on the top of a small hillock.

Data were analyzed using analysis of variance. Data from both the experiments were analyzed using Genstat statistical packages with Data Plus at CSIRO, Canberra, Australia.

Results

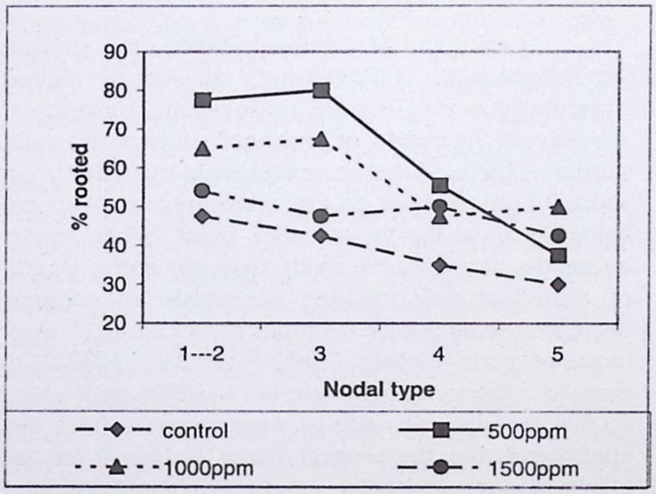
Shoot cutting trial

The results of analysis of variance showed that there were highly significant differences ($P< 0.001$) between cuttings taken from the different zones of the shoot (cutting type) and hormone treatments in the cutting success ($P< 0.001$) and number of roots produced per rooted cutting (Fig. 1). The interaction between cutting type (zone) and hormone treatments were also significant in number of successful cuttings ($P< 0.01$) and number of roots produced per rooted cutting ($P<0.05$).

The results of the investigation revealed that IBA increases the success of rooted cutting (Fig. 1a). and number of roots per rooted cutting (Fig. 1b). The average rooting successes achieved from different types of cuttings *viz.* first two nodes, third node, fourth node and fifth node were 62.5, 57.5, 48.7 and 38.7% when treated with 500 ppm, 1000 ppm, 1500 ppm IBA and without any IBA (control) respectively. The number of roots produced per rooted cutting from them were 3.37, 3.39, 3.21 and 2.58 when treated with 500 ppm, 1000 ppm, 1500 ppm IBA and without any IBA (control) respectively. Rooting percentage of top two node cuttings was 47.5 without any IBA treatment (control) but it increased to 77.5 with 500 ppm IBA. However, further increase of IBA concentration decrease rooting percentage. An exactly similar response was found in number of roots produced per rooted cutting with first two node cuttings. Rooting percentage of 3rd node cuttings also increased from 42.5% (with control) to 80.0% with 500 ppm IBA. Number of roots per rooted cutting also increased from 2.61 (control) to 4.12 with 500 ppm IBA. When IBA concentration was further increased rooting percentage and number of roots per rooted cutting did not increase in 3rd node cuttings. Rooting percentage and number of roots per rooted cutting were also slightly increased with 500 ppm IBA compared with other treatments in 4th node cuttings. But highest rooting percentage (50.0%) and number of roots per rooted cutting (3.3) in 5th node cuttings were found when cuttings were treated with 1000 ppm IBA.

Clonal trial

There were significant differences between clones in height growth ($P<0.001$). The clonal trial results only indicated an early height growth evaluation between clones (Fig. 2). Early evaluation between clones showed that clone 2 had the average highest height growth (87.21 cm) and clone 13 had lowest average height growth (38.5 cm).



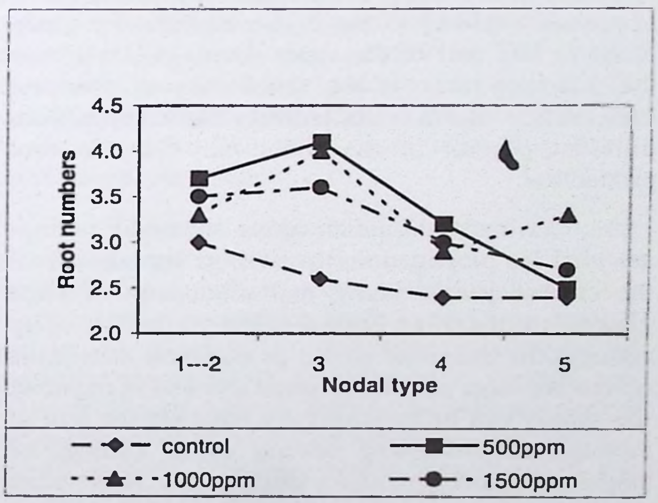


Figure 1. a) Rooting success and b) number of roots produced per rooted cutting of hybrid *Acacia.*

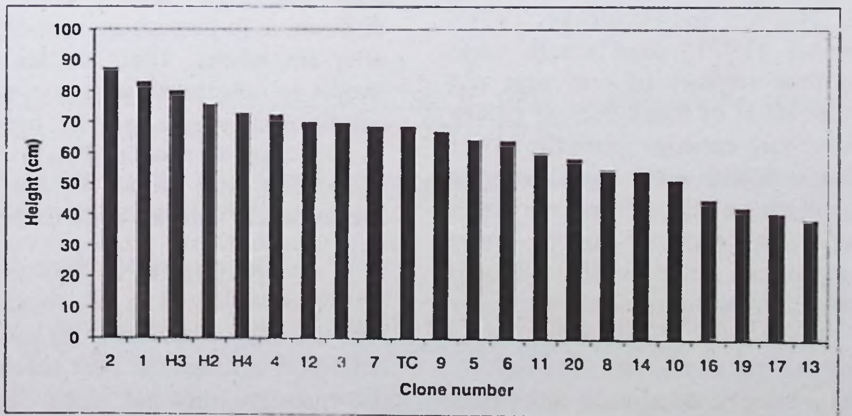


Figure 2. Height growth (cm) of 22 clones of hybrid *Acacia.*

Discussion

The result of the propagation experiment suggests that instead of multinode cuttings (Banik *et al.* 1995) single node cuttings or tip cuttings can be propagated successfully. However, the success rate of the cuttings taken from the different zones of the shoot varied significantly. In this hybrid *Acacia* experiment, cuttings taken from the upper zones of the shoot rooted better with 500 ppm IBA. Cuttings taken from the lower zones of the shoot showed poor rooting performance. Ghani (1987) also found that cuttings taken from the upper zones rooted better than cuttings taken from middle zone of *Betula* species. Better rooting of cuttings from the top zones is probably related to the higher endogenous auxin levels in this part of the shoot. Cuttings taken from the different parts of the shoot vary in chemical composition, and it is not unlikely that cuttings from different portion of the shoot may vary in root production.

In most situations where softwood cuttings are used for propagation, tip cuttings are taken from the current year's shoot, and production of plant materials is therefore limited to the availability of tip cuttings. In situations where propagation material is scarce but large number of plant material is required, the supply can be increased by reducing the size of cuttings and obtaining several dwarf cuttings or single node cuttings from a single shoot. Many other species can be propagated from single node cuttings prepared from different zones of the shoot (Thomas and Wilkinson 1962, Hitchon and Heydecker 1971). Hitchon and Heydecker (1971) used single node cuttings taken from four regions of one year old shoots of three year old plant of black current (*Ribes gosularia*). They found that cuttings from the lower half of the shoot rooted and grew well with or without chilling, but cuttings taken from the upper middle quarter of the shoots rooted more successfully after eight or more weeks of cold treatment. Single node cuttings taken from the upper main stems of five months old seedlings of *Betula pendula* and *B. pubescencs* rooted successfully (Kennedy *et al.* 1980). They divided each individual (shoot) into three zones, each zone consisting of three cuttings with an auxiliary bud attached to each node. The effect of cutting zone on rooting was found to be significant only in *B. pendula*, in which cuttings from median zones gave highest rooting success. Therefore, cuttings taken from any one zone of one species which root well may not be suitable for another species. Cuttings taken from different zones may have different physiological and morphological characteristics. Thus, tip cuttings may have a higher endogenous auxin concentration but have a lower carbohydrate content than the basal cuttings. Although cuttings prepared from the lower zone of the shoot have lower endogenous auxin levels but they have a high carbohydrate content (Hartmann and Kester 1983).

Leaky and Mohammed (1985) have studied the influence of cutting length on rooting ability particularly in single node cuttings. In *Triplochiton scleroxylon* internode of managed stock plants are shorter at the base and increased node by node (from about 24 cm to about 55 cm), the longest internode being found at the top of stock plant. When single internode cuttings were made from the entire length of each internode, rooting percentage of cuttings decreased sequentially from top node cutting (70%) to basal node cutting (10%) (Leakey 1983). In contrast, when cuttings were cut to different lengths so that the shortest cuttings were prepared from the apical end and the longest from the basal end of plants, rooting percentage and number of roots per rooted cutting were significantly greater in basal node cuttings after three weeks. However, differences in percentage rooting were not significant after six weeks. Their studies suggest that cutting length is correlated with rooting ability. A similar condition may also exist in hybrid *Acacia*. Because in soft coppice shoots of hybrid *Acacia* the longest internodes are found at the top and decrease subsequently node by node to the base.

The results of the rooting experiments may be partly explained by the physiological condition of the cutting materials. When propagated from softwood cuttings the best results are obtained from the materials that has some degree of lignification but is neither too succulent nor too lignified. Because very succulent cuttings start to rot immediately after insertion as they are low in carbohydrate but high in nitrogen content (Hartmann and Kester 1983). In this experiment the first node was very succulent but they were prepared together with the second node which was not very succulent, and cuttings prepared with these two nodes produced a comparatively longer cutting material than the other three types of cuttings. Third nodal cuttings were also not too lignified as found with the 4^{th} and 5^{th} nodes. Therefore, it may be a reason why cuttings prepared from the first two nodes and third node rooted better than the cuttings prepared from 4th and 5th nodes.

Hormone concentration is an important consideration for the rooting of cutting with any plant species. Different plant species require different types and concentrations of hormones for optimum rooting (Nanda *et al.* 1968). The effectiveness of exogenously applied auxin is largely determined by the level of the endogenous auxin present, and a higher concentration of hormone is necessary for more lignified cutting materials to achieve better rooting success. The present results are, therefore, may not be similar with the findings of other investigators. Better rooting in lower concentration of IBA with cuttings prepared from upper zones of the shoots may be due to higher endogenous auxin and are less lignified than the cuttings prepared from lower zones of the shoots. The results of the present study suggest that the optimal IBA concentration for rooting cuttings prepared from the upper zones are 500 ppm and 1000 ppm for cuttings prepared from below the 4th node.

The clonal trial results only indicated an early height growth evaluation between clones, where clone 2 had the average highest height growth (87.21 cm) and clone 13 had lowest average height growth (38.5 cm). Early evaluation of clones is unsatisfactory, because early growth may not be a good indicator of the later performance. For example, sitka spruce (*Picea sitchensis*) progeny trials in Britain is evaluated at six years after planting, since changes in rank before that age make assessment unreliable (Samuel and Johnstone 1979). Therefore, the final selection of superior clones from the trial will be made at the age of six and the method of evaluation of clones will be followed as described by Jayraj *et al.*(1997). The major characters considered for evaluation of clones are the quantitative traits like DBH, height and the qualitative traits like stem form, branching habit, apical dominance, health, etc.

Vegetative propagation described here will allow us to reproduce exactly the unique, individuals (selected clones) whose good qualities would be lost if propagation is made through seed. Ultimately it should be possible to establish plantations of particular clones which are adapted to specific soil types or climatic regimes. Rooted cuttings are always more expensive to raise than seedlings, but their use can be justified if the extra-cost is outweighed by the increased yield expected from the clonal plantation at the end of the rotation.

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