In Vitro Clonal Propagation of Hybrid Acacia (*A. auriculiformis* × *A. mangium*)

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Abstract

A protocol for regeneration of plantlets from hybrid acacia (*Acacia auriculiformis* × *A. mangium*) trees was achieved through tissue culture technique. Effects of MS and B5 culture media supplemented with various concentration of different phytohormones were tested for the induction and proliferation of shoots. The etiolated shoot was cultured and proliferation was found best on MS medium supplemented with BAP (1 mg/1). Proliferation and growth of shoots were further improved through subculture. These shoots produced well developed roots when cultured on $\frac{1}{2}$ MS containing 1.5 mg/1 IBA supplemented with activated charcoal. Ninety per cent regenerated plants survived in the field.

সারসংক্ষেপ

হাইব্রিড একাশিয়া (*Acacia auriculiformis* ×*A. mangium*) বৃক্ষের অনুচারা উৎপাদনের টিস্যু কালচার পদ্ধতি উদ্ভাবন করা হয়েছে। এম এস এবং বি-৫ কালচার মিডিয়ার সাথে বিভিন্ন পরিমাণের হরমোন সংমিশ্রণ করে অনুকাণ্ডের সংখ্যা বৃদ্ধির উপর পরীক্ষা করা হয়। বি এ পি (১.০ মিলিগ্রাম / লিটার) মিশ্রিত এম এস মিডিয়াতে আলোক প্রভাব মুক্ত কচি কাণ্ডের অনু অংশ কালচার করে সফলতার সাথে প্রচুর সংখ্যায় বৃদ্ধি করা সম্ভব হয়েছে। পুন পুন ট্রান্সফার কালচারের মাধ্যমে প্রচুর সংখ্যায় অনুকাণ্ডের বৃদ্ধি এবং বর্ধন উন্নতর হতে দেখা গেছে। এক্টিভেটেড চারকোল এবং আই বি এ ১.৫ মিলিগ্রাম/লিটার মিশ্রিত অর্ধ শক্তি সম্পন্ন এম এস মিডিয়া কালচার করা অনুকাণ্ড সমূহে প্রচুর পরিমাণে শিকড় গজানোর জন্য উপযুক্ত বলে প্রতীয়মান হয়েছে। শতকরা ৯০ ভাগ অনুচারা মাঠ পর্যায়ে বেঁচে থাকে।

Key words : Hybrid acacia, etiolated shoot explant, *in vitro* culture, propagation

Introduction

Acacia auriculiformis and *A. mangium* are adaptive tree species to the tropics. These two species were introduced in Bangladesh in plantations for fuel wood production. *A. auriculiformis* and *A. mangium* are morphologically distinct species. But some individual trees growing in the plantations show better growth and somewhat different characteristics than both of

A. auriculiformis and *A. mangium*. Careful observations show that these trees possess characteristics of both the species. These vigorous trees have also been found in other countries and identified as natural hybrid between *Acacia auriculiformis* and *A. mangium* (Tham 1976, Turnbull *et al.* 1986, Pinso and Nasi 1991). Kijkar (1992) observed higher growth rate and timber production of the hybrid than the parents. *A. mangium* is susceptible to heart rot which is less suitable for sawn timber production while the hybrid appears to be more resistant to heart rot (Zakaria 1993). The hybrid acacia in Bangladesh also showed more resistance to heart rot disease. Therefore, it is assumed that the hybrid may grow vigorously resisting heart rot disease in the forest plantations.

However, the seed productivity of this hybrid is very low (Kijkar 1992) and as a result, large scale production of planting stock is usually difficult. So, clonal propagation of the hybrid is an important way to increase productivity of the forest plantations. It was, therefore, aimed to develop *in vitro* propagation technique for mass scale production of planting stocks of *A. auriculiformis* x *A. mangium* hybrid.

Materials and methods

A number of hybrid acacia trees were identified and selected from a 5 years old fuel wood plantation of *A. mangium* and *A. auriculiformis* at Harbang forest area of Chittagong Forest Division. One of the selected trees was felled and stump was covered by sand in the mid of June 1994 and it coppiced within two weeks. After a month, the young and etiolated sprouting shoots were collected. The etiolated shoots were sterilized with $HgCl_2$ (0.1%) and ethanol (70%). Sterilized shoots were sized (0.5-1.5 cm) to make explants and washed with sterile distilled water.

The two basal media, Murashige and Skoogé (1962) (MS) and Gamborg and Eveleigh (1968) (B5), were used to culture the explants in *in vitro* condition. Both the media were agar gelled using 5 g agar per litre in 150 mm x 20 mm test tubes. Approximately 10 ml medium of each kind was used in the tube. The filter paper bridge was also used in 150 x 20 mm test tubes containing 10 ml liquid MS medium. The growth regulators namely BAP (0.1, 1.0, 5.0 mg/1), Kn (1.0 mg/l), 2iP (1.0, 10.0 mg/1), NAA (0.1 mg/1) and IBA (0.1, 1.0, 1.5 mg/1) were supplemented with basal media. The pH of the media was adjusted at 5.8 before autoclaving.

The temperature of the growth room was regulated to 27 $\pm$ 3°C and for illumination three fluorescent tube lights (40 W) and two incandescent bulbs (25 W) were used. The 16 hrs light and 8 hrs dark periods were regulated.

Results and discussion

Shoot proliferation

Under MS medium : The etiolated shoot of adult hybrid acacia *(A. auriculiformis* x *A. mangium)* was cultured and proliferated on MS medium (liquid or solid) supplemented with BAP 1.0 mg/1 within 7-10 days of culture (Table 1, Figure la). The shoot growth improved through 2-3 subsequent transfer subcultures on the same medium. The low shoot production (1-3 nos.) with better shoot elongation was observed in the culture containing small amount of BAP (0.1 mg/1). BAP in higher concentration (5.0 mg/1) induced cluster of multiple shoots with stunted growth within four weeks of culture (Table 1). However, with subsequent transfer of whole or a part of such

multiple stunted shoots in liquid MS medium containing BAP (1.0 mg/1) in large glass bottles (10 cm long x 5 cm diameter at mouth) on constant shaking (100 rpm) produced 25 - 35 well developed elongated shoots within two weeks (Figure 1b, 1c). After that, culture bottles were kept for one week without shaking before transferring to rooting medium.

The shoot elongation and axillary bud induction improved on solid medium with BAP (1.0 - 5.0 mg/1) and auxin (NAA or IBA 0.1 mg/1) than on BAP alone. Similar results were found in strawberry and ivy when grown in variable concentrations of BAP and NAA/IBA (James and Newton 1977, Banks *et al.* 1979). However, the multiple

Table 1 : Effect of MS and B5 media supplemented with different hormones on the culture growth of hybrid acacia (*Acacia auriculiformis x A. mangium*).

Media Hormone (mg/1)	Shoot prolifera - ted (Nos.)	Elonga- tion (cm)	Callus growth	Remarks
MS (Solid, agar 5 gm/1)				
BAP (0.1)	1 - 3	2.0 - 4.0	0	Only shoot growth
BAP (1.0)	3 - 6	1.0 - 4.0	+	Creamy callus
BAP (5.0)	many	to 1.5	++	Transfer culture improved proliferation and growth
BAP (1.0) + NAA (0.1)	2 - 5	2.0 - 4.0	++	Creamy callus
BAP (5.0) + NAA (0.1)	2 - 3	2.0 - 4.0	++	_
BAP (1.0) + IBA (0.1)	2 - 4	2.0 - 4.0	++	_
Kn (1.0)	2 - 4	to 1.5	+	_
2iP (1.0)	2 - 5	2.0 - 4.0	+	_
2iP (10.0)	many	to 1.5	++	Compact greenish callus
MS (Liquid, paper bridge)				
BAP (1.0)	4 - 16	4.0 - 8.0	0	Few thin and short roots (9 wks)
Kn (1.0)	2 - 3	4.0 - 12.0	0	Many thin and long roots (5 wks)
B5 (Solid, agar 5 gm/1)				
BAP (0.1)	1 - 3	2.0 - 4.0	0	Only shoot growth
BAP (1.0)	2 - 5	1.5 - 3.0	0	Creamy callus
BAP (5.0)	many	to 1.5	++	_
BAP (1.0) + NAA (0.1)	many	to 1.5	++	_
BAP (5.0) + NAA (0.1)	many	to 1.5	++	_
Kn (1.0)	2 - 4	to 1.5	+	_
2iP (1.0)	2 - 5	2.0 - 4.0	+	Compact callus

Note : + slight callus, ++ more callus, wks -time in weeks required to root;
many = 20 - 50 number of minute shoots.

shoot formation (4 - 16 Nos.), elongation and axillary bud formation were best in the liquid medium with 1.0 mg/1 BAP. Spontaneous rooting was also observed in liquid MS after 7-9 weeks of culture.

Both Kn (1.0 mg mg/1) and 2iP (1.0 mg/1) were not as effective as BAP in respect of shoot proliferation in both solid and liquid MS media. However, in liquid culture Kn induced vigorous shoot growth with elongated internodes and axillary buds (Table 1).

Under B5 medium : Similar to MS, BAP in low concentration (0.1 - 1.0 mg/1) also showed good proliferation and better elongation of the culture shoots on B5 medium. The B5 medium, unlike MS medium, when supplemented with BAP (1.0 - 5.0 mg/1) and NAA (0.1 mg/1) could not induce better shoot elongation and growth. The proliferated shoots were very small and grew slowly. Other cytokinins namely Kn (1.0 mg/1) and 2iP (1.0 mg/1) also produced multiple shoots.

In general, it can be concluded that the shoot growth was better in MS medium than that of B5 medium (Table 1, Figure 1c). The liquid MS was found to be the suitable medium for sustaining vigorous shoot growth than in agar gel condition.

Callusing

The induction of callus was observed both in MS and B5 media. The amount of callusing was high in the culture containing NAA or IBA (Table 1). The creamy callus usually developed within a week at the contact point between tissue and medium. The culture did not form callus in liquid medium (Table 1, Figure 1b). The similar observation was made by Haissing (1974) on reduction in callus formation in the liquid culture system. No organogenesis was observed from the callus. Moreover culture having higher amount of callus prevented or reduced the rate of direct shoot proliferation from the tissue, similar to that observed by Skirvin (1981).

Rooting

To accelerate the root induction and development, the half strength MS and B5 ($\frac{1}{2}$ MS and $\frac{1}{2}$ B5) media supplemented with IBA (1.0 mg/1) were used (Table 2). Both the media were found suitable for rooting (Table 2). The excised shoots cultured on $\frac{1}{2}$ MS rooting medium containing IBA 1.5 mg/1) in liquid or solid with activated charcoal (AC) have induced well developed root system in the culture (Figure 1d). The roots were comparatively thin in the solid medium without AC.

The improved root growth in medium having AC might be due to darkening effect of charcoal and also had capacity of absorbing toxic substance that might have formed (Reynolds and Murashige 1979)

Plantlet transferred to soil

About 90% of the plantlets survived during the process of transfer from the culture tubes to the soil in humid, well ventilated and partial shade conditions. The soil should be mixed with fine sands for providing well drained conditions in the growing medium. Plantlets transferred

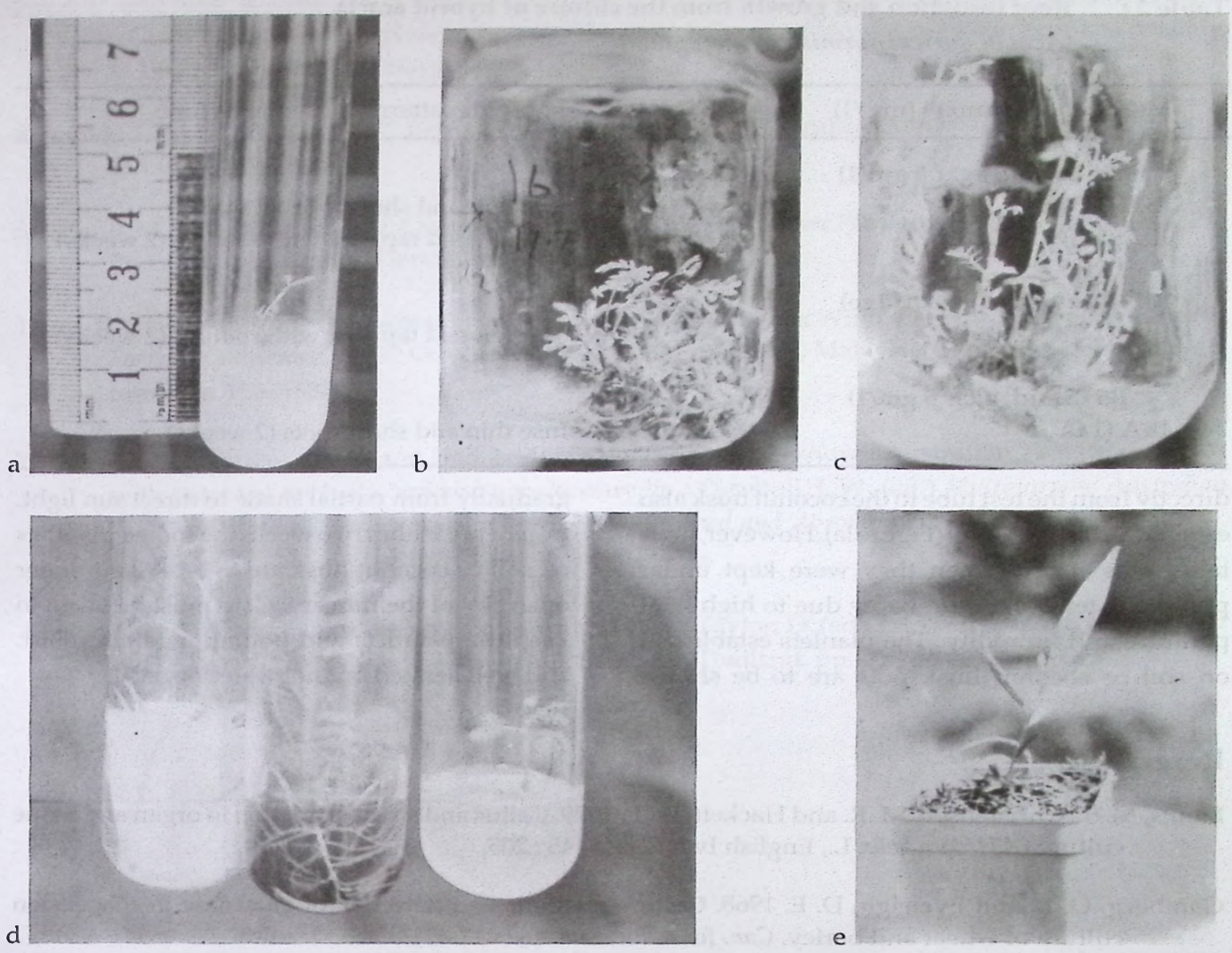

Figure 1 : Steps in micro propagation of hybrid acacia (*A. auriculiformis x A. mangium*).

a) Initiation of culture and proliferation in a test tube.

b) Improvement of proliferation after transfer of culture in bigger culture bottle on both liquid B5 and MS media.

c) Well developed stem growth in liquid MS.

d) Profuse root growth on MS medium supplemented with AC in comparison to paper bridge on liquid MS and solid MS without AC.

e) Plantlet transferred from aseptic condition to outside on plastic pot.

Table 2 : Root initiation and growth from the culture of hybrid acacia (*Acacia auriculiformis* x *A. mangium*).

Media and hormone (mg/l)	Root growth pattern and required time
$\frac{1}{2}$ MS (Solid, agar 5 gm/1)	
IBA (1.5)	Profuse thin and short roots (2 weeks)
IBA (1.5) + AC 3 g/1)	Well developed tap root with rootlets (2 weeks)
MS (Liquid, paper bridge)	
IBA (1.5)	Well developed tap root with rootlets (2 weeks)
$\frac{1}{2}$ B5 (Solid, agar 5 gm/1)	
IBA (1.0)	Profuse thin and short roots (2 weeks)

directly from the test tube to the coconut husk also established successfully (Figure le). However, mortality was severe when they were kept under polythene tent cover, probably due to high temperature and humidity. The planlets established on soil or coconut husk pots are to be shifted gradually from partial shade to direct sun light. In general, within two weeks the rooted plantlets on soil or coconut husk are to be shifted under open sky of the nursery. Care must be taken to keep the plantlets and potting medium moist, and well aerated but not waterlogged.

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