In vitro Micro-propagation of *Acacia* Hybrid through Shoot Tip Culture

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Abstract

Propagation and conservation by vegetative means were attempted for better preservation of true-to-type genetic characteristics with higher yield planting materials of *Acacia* hybrid. In this regard, *in vitro* micro propagation of *Acacia* hybrid through shoot tip culture was initiated from outdoor mature plant on MS basal medium supplemented with 1.0 mg/L BAP. The shoot tip cultures produced axillary shoot bud and used for multiple shoot production. The multiple shoot production rate was optimized on MS medium supplemented with different concentrations of cytokinins. The highest number (20) of multiple shoots per culture was recorded on MS medium augmented with 2.0 mg/L BAP after 8 weeks of culture. The rooting was initiated on ½ MS medium enriched with different concentrations of IBA. The best rooting rate 90% was obtained on medium having 2.0 mg/L IBA after 28 days of culture. The well-developed rooted plantlets were transferred to *in vivo* condition for further growth and acclimatization. More than 95% of transplanted plantlets survived and grew well in polybag under natural condition.

সারসংক্ষেপ

একাশিয়া হাইব্রিড এর সঠিক কৌলিক বৈশিষ্ট্য ও উচ্চতর উৎপাদনশীল চারা উৎপাদনের উদ্দেশ্যে অঙ্গজ প্রক্রিয়ায় বংশবিস্তার ও সংরক্ষণের পরীক্ষা করা হয়। এ লক্ষ্যে বাইরে জন্মানো একাশিয়া হাইব্রিডের একটি পূর্ণ বয়স্ক গাছের শাখার শীর্ষাগ্র এম এস খাদ্য মিডিয়ামে ১.০ মিলিগ্রাম/লি. হারে BAP প্রয়োগ করে ইন ভিট্রো মাইক্রোপ্রোপাগেশন প্রক্রিয়ার সূচনা করা হয়। প্রাথমিকভাবে গজানো নতুন বিটপ (shoot) কে নতুন খাদ্য মিডিয়ামে স্থানান্তরের মাধ্যমে পুনরায় আরো বিটপ জন্মানো হয়। বিটপ জন্মানোর হারকে সর্বোচ্চ মাত্রায় নেবার জন্য বিভিন্ন ঘন মাত্রায় সাইটোকাইনিন খাদ্য মিডিয়ামে প্রয়োগ করা হয়। এ পর্যায়ে ৮ সপ্তাহ পর ২.০ মি.গ্রাম/লি. BAP এবং ৩% সুগার সম্বলিত এমএস মিডিয়াম থেকে প্রতি কালচার প্রতি সর্বোচ্চ সংখ্যক ২০টি বিটপ পাওয়া যায়। উৎপাদিত বিটপগুলিতে শিকড় গজানোর জন্য অর্ধ শক্তির এমএস মিডিয়ামে বিভিন্ন মাত্রার IBA প্রয়োগ করা হয়। দুই (২.০) মি.গ্রাম/লি. IBA যুক্ত মিডিয়ামে ২৮ দিন পর শতকরা ৯০ ভাগ বিটপে শিকড় উৎপাদন হয়। শিকড়যুক্ত বিটপগুলিকে হার্ডেনিং করার জন্য পলিব্যাগের মাটিতে স্থানান্তর করা হয়। এভাবে শতকরা ৯৫ ভাগ উৎপাদিত চারা বেঁচে থাকে এবং পলিব্যাগে ভালভাবে প্রাকৃতিক পরিবেশে বেড়ে ওঠে।

Key words: Micro propagation, Multiplication, *Acacia* hybrid, Shoot tip, MS medium, Plantlets.

Introduction

The *Acacia* hybrid, a cross between *Acacia mangium* and *Acacia auriculiformis*, grows in Indonesia, Malaysia, Thailand, Vietnam, and China (Kha 1996). It is a medium-sized tree that looks similar to *A. mangium*. The tree can reach 8 to 10 m and 7.5 to 9.0 cm diameter within 2 years. It has been gained an increasing interest in reforestation programs under the humid tropical conditions. The species grows on sandy loam or sandy clay loam soils; however, it also thrives on lateritic crude soils.

A. mangium, being one of the selected fast growing species has become an important choice of species in agroforestry. It originates from the humid tropics of Northern Australia, Papua New Guinea, Eastern Indonesia and Malaysia (Ahmad and Kamis 1999). It is potentially an important timber where the wood being suitable for furniture, and cabinet making as well as particleboard and pulp production. Also used as firewood and occasionally planted as an ornamental, for erosion control or as a fire-break or wind-break. The pulp is readily bleached to high brightness levels for making paper. While the *A. auriculiformis* found in Australia, southwestern Papua New Guinea and Indonesia is planted widely in tropical Asia. It has been established in western Malaysia.

A. auriculiformis has become a major source of firewood; its dense wood and high energy (calorific value of 4500 to 4900 kcal/kg) contribute to its popularity. It provides very good charcoal that glows well with little smoke and does not spark. The wood is extensively used for paper pulp and is excellent for turnery articles, toys, carom coins, chessmen and handicrafts. It is also used for furniture, joinery, tool handles, and for construction if trees of suitable girth are available. However, the *A.* hybrid differs from *A. auriculiformis* and *A. mangium* in several ways. When *A.* hybrid is young, the bark is greenish white, similar to the bark of *A. auriculiformis*. As it ages, the bark turns greenish brown or brown. It is as smooth as the bark of *A. auriculiformis*, with slightly scalely and shallow furrows at the foot of the tree (Kha 1996).

The hybrid's branching behavior differs from *A. mangium* and *A. auriculiformis*. The tree has many small and light branches that can be easily pruned. Its main stem, though not as straight as that of *A. mangium*, is much straighter than the main stem of *A. auriculiformis*. Unlike the stem of *A. mangium,* that of *Acacia* hybrid has no angles or ribs (Kijkar 1992). Its phyllode is about 4 to 6 cm wide and 15 to 20 cm long with four veins similar to those of *A. mangium*, but the vein on the outer edge of the crescent is not easy to see. Its seeds are similar in appearance to those of *A. auriculiformis* except that the funicles of the hybrid are lighter and are only partly attached to the seeds (Kijkar 1992). The hybrids tend to grow vigorously, have better form than *A. auriculiformis* and have lighter branching than *A. mangium* which is self-prune (Rufelds and Lapongan 1986). It has a slightly higher wood density, is good for producing chipwood, pulp, paper production, medium density fiber board, oriented-strand board, for general construction and furniture.

Seed collected from *Acacia* hybrid trees yields highly variable and poorly performing offspring and are not commonly used in regeneration. *A.* hybrid seeds are not commonly used in regeneration programs because they may produce *A. auriculiformis* (52%) or *A. mangium* (2-3%) (Kijkar 1992). Propagation and conservation by vegetative means are desirable for better preservation of true-to-type genetic characteristics with higher yield planting materials can be obtained within minimum time period.

Plant tissue culture technology has a potential to overcome this problem where it allows efficient and rapid clonal propagation of many

economically important species. However, the low survival percent of *in vitro* plantlets during the *ex vitro* acclimatization and delivery to the field poses many problems to make tissue culture technology a viable alternative proposition. Germplasm conservation has become necessary for future sustainable harvesting systems and as a means of maintaining species diversity to prevent genetic erosion. *In vitro* micro propagation technique may be reliable method for long-term storage of plant genetic resources without apparent risk of genetic instability using minimum space and with lower labour and maintenance costs. Tissue culture techniques have also been successfully developed using aseptic emerging seedlings as multiplication materials (Darus 1993). Monteuuis *et al.* (2012) reported the organogenic capacity for shoot multiplication by axillary budding, with average multiplication rates of 3–5 every 2 months, as well as for adventitious rooting of *Acacia* hybrid.

The present study described the *in vitro* response of shoot tip explant collected from mature outdoor *Acacia* hybrid plant on the medium with different concentrations of plant growth regulator and the subsequent plant production.

Materials and Methods

Plant materials

Shoot tip explants were collected from outdoor mature *Acacia* hybrid plant and brought to the tissue culture laboratory for *in vitro* culture establishment. The experiments were carried out at the tissue culture laboratory and the nursery of Silviculture Genetics Division of Bangladesh Forest Research Institute, Chattogram, Bangladesh.

Explants preparation and surface sterilization

The collected shoot tips were cut about 10 cm length, sealed in plastic bags, and brought back to the laboratory. The shoot tip explants were rinsed under running tap water for 30 minutes. After that explants were carried under laminar air flow. The surface sterilization was started with one drop of tween 20 for 7-10 minutes with frequent shaking and washed with sterilized distilled water for 2-3 times. Then the explants were immersed in 70% ethanol for 1 minute and sterilized with 20% Clorox® for 15 minutes, and rinsed with sterilized distilled water for 3-4 times. Again sterilization was done for 10 minutes and rinsed with sterilized distilled water. The shoot tips were then dissected into 1.0-1.5 cm length for culture initiation.

Culture media preparation

The surface sterilized shoot tips were inoculated onto Murashige and Skoog (1962) medium comprising 3% sucrose as carbon source and 2.8 gm/L gelrite as solidifying agent for initial growth. Various plant growth regulators such as; cytokinins (BAP & Kn) and auxins (IBA & NAA) were used to prepare MS medium for explant establishment, multiple shoots production and root induction of the regenerated new shoots. MS medium devoid of growth regulators used as control treatment. The pH of the medium was adjusted to 5.8 using 0.1 N NaOH or 0.1 N HCl before addition of gelrite and sterilized by autoclaving at 1.08 kg/cm^2 pressure and 121^0 C for 20 minutes.

Culture conditions

The cultures were incubated at 25±2°C under cool white and fluorescent light of 2000-2500 lux, relative humidity about 60-80% and 16/8 hours photo and dark period were maintained in growth chamber, respectively. These culture conditions were used in all the experiments mentioned below unless otherwise stated. Observations were made at regular intervals and tabulated.

Multiple shoots production and optimization

The aseptic shoot tips were cultured on MS medium supplemented with 0.0 (MS 0/control), 0.5, 1.0, 1.5 and 2.0 mg/L of BAP. Number of shoots per explants and their morphology were observed periodically. To optimize the shoot production, effect of sub culturing and the strength of sucrose level in culture medium were evaluated. Rate of multiplication of shoots and their growth were recorded up to 3-8 weeks of culture.

Development of roots at the base of the shoot, hardening and acclimatization of plantlets

In vitro elongated shoots (6-7 cm) were taken from the 5th cycles of multiplication stages. Treatments used for induction of rooting were ½ MS medium supplemented with different concentrations (0.0, 0.5, 1.0, 2.0 mg/L) of IBA with control. One-month *in vitro* rooted shoots were used for acclimatization in the greenhouse. When the plantlets developed few leaves and roots on the rooting medium, they were taken out from the culture vessels, washed thoroughly running tap water to remove the debris gelling agent with care and transferred to polybag (20 cm × 40 cm). Potted shoots were kept in greenhouse covered with black netting to maintain low temperature and high humidity. The temperature was in the range of 25–30° C and relative humidity was approximately 80% with 50% shade. After 7 days, the covering bags were finally removed. Sudden removal of covering bags had adverse effect on establishment. The potted plants were brought out from the green house and kept under full sunlight for 2-3 hours per day. The plants were successfully acclimatized in natural conditions under sunlight and they eventually became suitable for final plantation. About 90% potted plants established successfully.

Statistical analysis

All experiments were performed as Completely Randomized Design (CRD). Data were analyzed using statistical analysis system (SAS v 9.3) and means were statistically compared using LSD test. The significance level was set up at $p < 0.05$. Three replications were considered for each treatment and repeated thrice.

Results

In vitro explant establishment

In vitro culture established from shoot tip explants collected from outdoor mature plant. The single shoot tips (1.0-2.0 cm) were cultured on MS basal medium supplemented with lower concentrations of cytokinin BAP (0, 0.5, 1.0, 1.5 and 2.0 mg/L) as well as with control. It was observed that MS basal medium devoid of plant growth regulators (PGR) did not support enough to response the culture for further growth. Besides, the cultures supplemented with different concentrations of BAP attained for quick response. Best response (100%) was recorded in MS basal medium supplemented with 1.0 mg/L BAP after 2 weeks of culture (Fig.1, Fig. 3A.). The explants with new shoots were transferred for regeneration of multiple shoots and optimization of shoot growth.

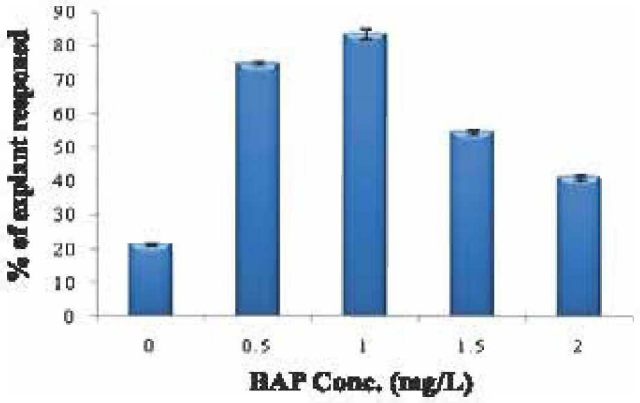

Figure 1. Effect of different concentrations of BAP on shoot induction from shoot tip explant of *Acacia* hybrid .

Optimization of multiple shoot production

Effect of BAP and Kn on multiple shoot formation

The effect of two cytokinins BAP and Kn were evaluated for multiple shoot formation and optimization. There were significant differences at different levels of BAP and Kn on shoot induction in terms of the percentage of shoot regeneration and the mean number of shoots produced. MS medium supplemented with different concentrations (MS 0, 1.0, 2.0, 3.0, 4.0 mg/L) of BAP and Kn alone or in combination. The results showed that MS medium without plant growth regulators induced a very little number of shoots whereas the supplementation of plant growth regulators enhanced shoot formation rate. The highest percentage of shoot regeneration (100%) was in MS medium with 2.0 BAP along with the highest mean number of shoots (20) and mean length of shoots (8 cm) (Fig.2, Fig.3C & 3D) followed by 2.0 mg/L Kn (12.66 number of shoots and mean length of shoots 6 cm) after 8 weeks of culture (Fig. 2, Fig. 3E).

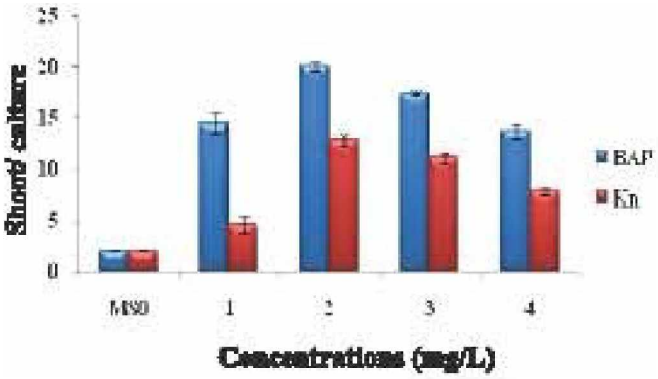

Figure 2. Effect of different concentrations of cytokinin on shoot multiplication of *Acacia* hybrid after 8 weeks of culture. Each value is the mean of three replications. Vertical bars indicated standard errors.

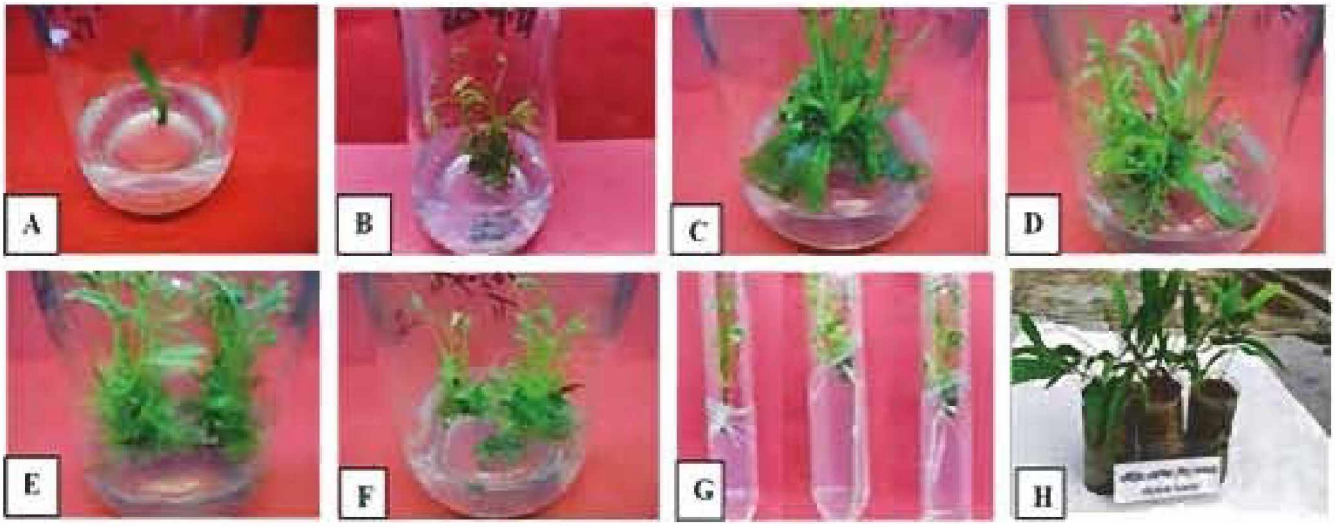

Figure 3. Effect of different concentrations of BAP and Kn in MS medium on culture establishment, multiple shoot production and optimization of *A.* hybrid (A-F). (A) Shoot tip culture on MS medium with 1.0 mg/L BAP, (B) Shoot multiplication control (MS0), (C&D) MS + 2.0 mg/L BAP + 3% Sugar, (E) MS + 2.0 mg/L Kn + 3% Sugar, (F) MS + 2.0 mg/L BAP + 2.0 mg/L Kn, (G) Root induced on excised in vitro grown shoots, (H) *Acacia* hybrid tissue cultured plants in polybags after hardening.

Qualitative observation of the shoots showed that shoots in 2.0 BAP were greener and more vigorous compared to shoots cultured in higher concentrations of BAP such as 4.0 mg/L which were pale green and fragile. Shoots in MS medium without any plant growth regulators were less elongated and did not produce any multiple shoots, but remained healthy. In compare the two cytokinins BAP was found more potential than Kn for new shoot regeneration of *Acacia* hybrid. A combined effect of BAP with different concentrations of Kn was also evaluated for the optimization of multiple shoot production. However, both the cytokinins BAP and Kn alone enhanced the shoot proliferation o*f Aca*cia hybrid. When BAP was combined with different concentrations of Kn (0.0, 1.0, 2.0, 3.0 and 4.0 mg/L), the best response for shoot production (12.00) and shoot length (6.28 cm) was recorded on medium containing 2.0 mg/L BAP + 2.0 mg/L Kn + 3% sugar after 8 weeks of culture. (Table 1, Fig. 3F).

Table 1. Combined effect of BAP and Kn on shoot multiplication of *Acacia* hybrid.

Hormonal concentration (mg/L)	Number of shoot/explants	Shoot length (cm)
2.0 BAP + 1.0 Kn	10.33 ± 2.00	2.30 ± 0.08
2.0 BAP + 2.0 Kn	12.00 ± 3.00	6.28 ± 0.38
2.0 BAP + 3.0 Kn	10.33 ± 2.00	4.45 ± 0.28
2.0 BAP + 4.0 Kn	8.00 ± 2.50	3.49 ± 0.26

Medium: MS + additives, mean ± SE, n = 3 replicates.

Effect of subculture on multiple shoot production

The effect of sub culturing on multiple shoot production of *Acacia* hybrid was evaluated. Every 2 weeks of interval sub-cultures were maintained for multiple shoot formation. It was observed that the shoots regenerated in each sub-culture without loss of morphological responses. In the first sub-culture, the mean of shoots per culture was 6.0 and it increased up to the 4th sub-culture as 20.0 shoots/culture. After excision of the multiple shoots, when the mother explants was cultured on the fresh shoot multiplication medium then the shoot numbers were increased significantly for the next 4 repeated transfers and reduced thereafter. Incorporation of Kn into MS medium supported shoot multiplication. However, BAP proved to be a better choice than Kn because the maximum mean number of shoots 20.0 per culture was obtained on 2.0 mg/L BAP after 8 weeks of culture (Fig. 2, Fig. 3C & 3D). In the fifth sub-culture the shoot number decreased as 14.0 shoots/culture which trend to the subsequent sub culture (Fig. 4).

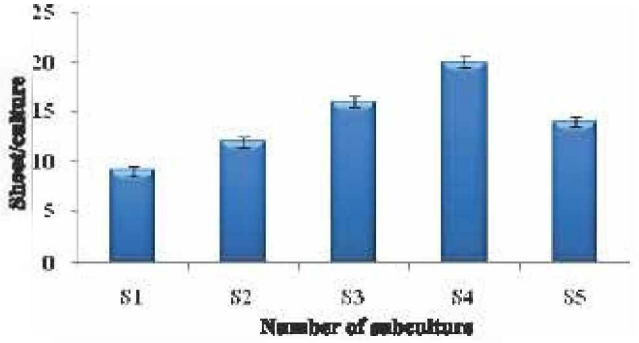

Figure 4. Effect of subculture on multiple shoot production of *Acacia* hybrid. The vertical bar represents the standard error.

Effect of different sucrose level on multiple shoot production

The sucrose level of cultures was optimized in respect to multiple shoot production on MS medium containing 10, 20, 30, 40 and 50g/L. The number of shoots per culture increased in the media having sucrose level from 10 to 30g/L. The culture media supplemented with 30g/L sucrose produced the maximum shoots with a mean of 20.33 per culture after 8 weeks. Meanwhile 50g/L sucrose induced lowest 9 shoots/culture respectively (Fig. 5 & Fig. 3C). Shoots in 50g/L sucrose were small and less elongated compared to shoots in other concentrations of sucrose (Fig. 3D).

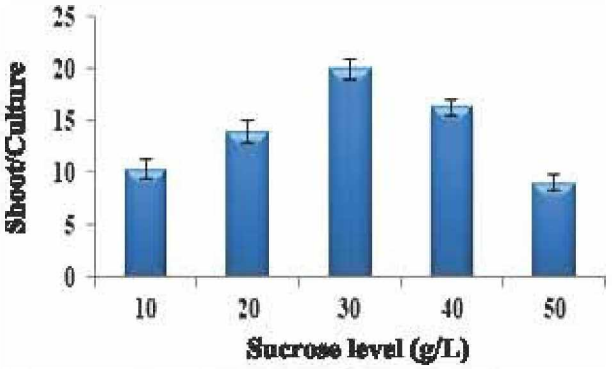

Figure 5. Effect of different sucrose concentrations supplemented with MS medium on multiple shoot production from shoot tip explants of *Acacia* hybrid. The vertical bar represents the standard error.

Effect of different concentrations of auxin on *in vitro* rooting of excised shoots

Optimization of *in vitro* rooting of excised shoots were carried out in ½ MS medium supplemented with different concentrations of IBA viz. 0.0, 1.0, 2.0 and 3.0 mg/L. It was observed that no roots produced in the auxin free MS medium. The highest percentage of shoots was rooted (90%) in MS medium supplemented with auxin IBA. The average number of root formation was significantly higher on hormone containing medium. Among the different concentrations of IBA, the maximum mean number of roots per shoot was 5.0 produced in media supplemented with 2.0 mg/L IBA after 4 weeks of culture. The average number of roots reduced to 3.33 roots/shoot at 3.0 mg/L IBA with the subsequent higher concentrations of IBA (Fig. 6, Fig. 3G).

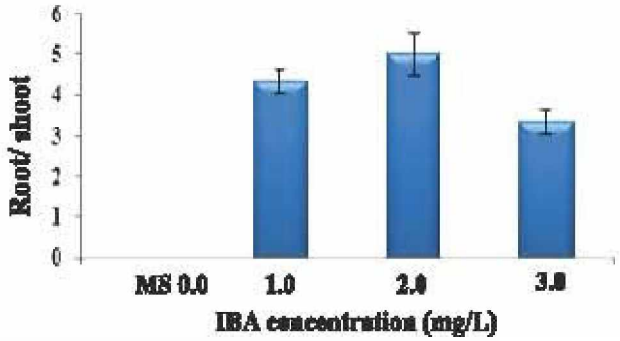

Figure 6. Effect of different concentrations of auxin, IBA supplemented with MS medium on root induction of *Acacia* hybird from *in vitro* regenerated shoots after 4 weeks of culture. The vertical bar represents the standard error.

There were significant differences on the percentage of shoots rooted, mean number of roots, mean length of roots, mean number of axillary roots, mean length of axillary roots, and mean height of shoots from the different concentrations of IBA tested.

Transfer of rooted plant in soil and hardening

In vitro rooted plantlets were initially hardened in culture room conditions where leaves expanded. After 2 weeks, the plantlets were shifted to green house and mist house subsequently. About 90% plantlets were successfully acclimatized in small poly bag containing sterilized soil (Fig. 3H).

Discussion

Rapid shoot multiplication and mass production of *Acacia* hybrid was initiated through shoot tip culture collected from outdoor growing mature plant. However, most of the reports on micro propagation of *Acacia* have used explant from aseptically grown seedlings. Full strength MS medium supplemented with different concentrations of BAP and Kn as well as the devoid of phytohormones were evaluated for culture initiation, multiple shoot production and optimization. It was observed that the shoot tip culture of *Acacia* hybrid proliferated faster with the addition of cytokinins than the medium devoid of plant growth regulators. The supplementation of plant growth regulators were positively influenced the shoot proliferation of *Acacia* hybrid. Rahman *et al.* (2018) stated that shoot proliferation of *Phylanthus emblica* were faster with the addition of cytokinins than the medium devoid of plant growth regulators. *In vitro* grown single shoot was able to produce multiple shoots in MS medium supplemented with different concentrations of cytokinins BAP and Kn. MS medium has also been used in most work on micro propagation of *Acacia* species (Nangia and Singh 1996; Ismail *et al.* 2012; Griffin *et al.* 2014).

Experiment conducted for shoot production and optimization on MS medium supplemented with different concentrations of BAP and Kn singly or in combination. The shooting variation due to cytokinin BAP concentrations revealed that low concentration of BAP (2.0 mg/L) were sufficient for shoot initiation in terms of the mean number of shoots and shoot elongation for *in vitro* grown shoots of *A.* hybrid. According to Darus (1991b) and Galiana *et al.* (1991), low level of BAP (2.22 μ M) was good for shoot multiplication and elongation of *A. mangium*. Higher rates of cytokinin have caused production of many small shoots which typically fail to elongate and/or induce shoots to become hyperhydric (George 1993). For instance, in this study, shoots produced in higher concentration of BAP (4mg/L) exhibited characteristics less vigorous shoots with small stunted shoots at the base.

The requirement of exogenous plant growth regulators for *in vitro* regeneration depended on the endogenous level of the plant tissue, which varied with organs, plant genotype and the phase of plant growth (Chand and Singh 2004). The regeneration efficiency depended on plant growth regulator concentrations and combinations (Nodong *et al.* 2006; Popelka *et al.* 2006). The micro shoots produced in lower levels of BAP and Kn were green, taller having bigger leaves than those produced at higher concentration of cytokinins. In MS medium containing BAP the plantlet formed were slightly taller than those produced in MS medium supplemented with Kn. Cytokinin types had a strong effect on the quality of the shoots produced (Rahman *et al.* 2018). The growth of plantlets was retarded at

higher concentration of BAP. Kalinina and Brown (2007) found that treatments of *Prunus* sp. with elevated BAP concentrations promoted the shoot numbers per explants but decreased the shoot length and negatively affected shoot development.

In this study, all the formulations of BAP combined with Kn regenerated and produced multiple shoots. The results showed that the addition of lower level of BAP and Kn to the medium enhanced the shoot regenerative ability in *Acacia* hybrid. However, the number of multiple shoot reduced at higher concentration of BAP and Kn. The combined effect of BAP and Kn on multiple shoot production was observed. All the combinations produced new shoots in the culture medium and elongated the shoots. Similar results were observed in *P. emblica* (Rahman *et al.* 2018). Sub-culture exercised an important role on the shoot multiplication of cultures (Debnath and McRae 2001).

The duration of culture depended on plant species, growth rate, physical and physiological condition as well as the development stage of the plant (Moges *et al.* 2004). Plant tissue might have a chance to develop mutation due to repeated sub culturing, or it might produce callus, became abnormal and reduced the proliferation rate. The result revealed that *A.* hybrid did not show morphological changes after repeated sub-culturing. The number of shoots increased up to the 4th subculture then decreased by the repeated sub-culturing. Likewise, it was reported that the long term culture of *Digitalis obscura* did not affect the genetic stability *in vitro* (Gavidia *et al.* 1996). The shoot production ability varied greatly among different species. Thong (2002) reported that repeated sub-culturing caused shoots reduction in *Zingiber officinale*, *Curcuma domestica*, *Alpinia galanga*, and *Kaempferia galanga* In contrast, repeated sub-culturing of *in vitro* shoot of *Spilanthes acmella* increased the multiple shoots formation by three hold (Ang and Chan 2000). Romano *et al.* (1995) found 30 g/L sucrose was the best carbon source for proliferation of *Quercus robur* (English Oak) which favored shoot elongation. In this study, the quality of *Acacia* hybrid shoots was observed to be good in 20 to 30 g/L sucrose. The shoots produced in 40-50 g/L sucrose were less elongated compared to others and had the lowest number of shoots per explant. This effect might be a result of due to the carbohydrate concentration (sucrose) modifying the osmotic strength of the medium. At a high osmotic strength the medium was shown to reduce plant height and slow growth (Short *et al.* 1987). Maretzki *et al.* (1972) also found that when the concentration of sucrose in a high salt medium such as MS medium was increased above 4-5% (40-50 g/L), there would be a progressive inhibition of cell growth in many types of cultures. This appears to be an osmotic effect because addition of other osmotically active substances (such as mannitol and polyethylene glycol) to the medium also caused similar responses.

Higher plants grown *in vitro* were fully autotrophic (Lipavska and Vreugdenhil 1996). Therefore plant tissues culture required an exogenous carbon source and generally sucrose, is an essential ingredient of all culture media (Kozai 1991b). This is because in the culture vessels, photosynthesis was insufficient due to growth taking place in conditions unsuitable for photosynthesis or without photosynthesis (in darkness) and the concentration of carbon dioxide (CO_2) was limited for photosynthesis. Debnath (2005) reported that specific carbohydrate may have different effects on morphogenesis *in vitro*, thus the carbohydrate requirements must be defined and optimized for each propagation system. The effect of carbohydrate type and concentration on shoot proliferation were

genotype dependent. In this study 3% sucrose was a most optimum carbon source for *in vitro* multiple shoot formation in *Acacia* hybrid. Pati *et al.* (2006) found that sucrose concentration in culture medium had significant effect on shoot and root regeneration. High concentration of sucrose was deleterious to shoot growth and caused decrease in dry matter accumulation due to decrease in osmotic potential of the medium (Lipavska and Vreugdenhil 1996). Increasing sucrose levels more than 7% in the medium caused osmotic stress which significantly inhabited the growth of *Parthenium argentatum* (Norton *et al.* 1991). In this study, no shoot proliferation was observed in the medium without carbohydrate.

Initially it was difficult to induce roots of the excised shoots. Systemic experiments were needed to carry out to define the condition for root induction. Excised shoots were cultured on both hormone free and with different concentrations of IBA viz. 0.0, 1.0, 2.0, and 3.0 mg/L. No rooting was observed in the hormone free medium. Only root induced when half strength MS medium supplemented with auxin. Media having ½ MS with IBA 2.0 mg/L significantly supported the highest number of roots of *A.* hybrid. Comparatively the lower concentration of IBA induced maximum roots on excised shoots. Similar observation was found by Rahman *et al* (2018). In woody trees, usually low level of salt concentration is sufficient for rooting of shoots.

Conclusion

In conclusion, our present investigation, describes a regeneration method for *A.* hybrid from the shoot tip explants of outdoor mature plant. MS medium supplemented with 2.0 mg/L BAP may be recommended for maximum multiple shoot production. Half strength of MS medium with 2.0 mg/L IBA found the best combination for *in vitro* root induction on the micro shoots. Therefore, the method can be used for large-scale commercial production of *A.* hybrid.

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