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Full Length Research Paper

In vitro propagation of *Acacia* hybrid through alginate-encapsulated shoots and axillary buds

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Seed collected from *Acacia* hybrid trees yields highly variable and poorly performing offspring and are not commonly used in regeneration. The present study described the encapsulation of *Acacia* hybrid shoots and axillary buds in the calcium alginate gel. The aim of the study was to evaluate the germination of the buds *in vitro* on the medium with different concentrations of plant growth regulator and; the performance of the germination under light and darkness. For encapsulation purposes, 3% sodium alginate (w/v) in the Murashige and Skoog (MS) free of calcium salt solution solvent were tested. While for complexation, 100 mM calcium chloride solutions ($CaCl_2.2H_2O$) were prepared in liquid MS medium. The encapsulated explants or the beads were cultured into the following media: Modified basal MS supplemented with 6-benzylaminopurine (BAP) ranging from 0 to 2.5 mg/L BAP. High germination rate (100%) was observed within five to eight days in all medium tested. Analysis of variance showed no significant difference in the ability of the synthetic seeds to germinate. This showed that the regeneration of shoots is possible by using basal MS only. It was observed that synthetic seeds needed sucrose more than plant growth regulator for its germination. They were also showing good regeneration and development under light condition.

Key words: *Acacia* hybrid, synthetic seeds, encapsulation, germination, regeneration.

INTRODUCTION

Acacia hybrid is a cross between *Acacia mangium* and *Acacia auriculiformis* which are the two tropical acacias natural to Australia, Papua New Guinea and Indonesia where both have the potential for timber and pulp production.

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 and Eastern Indonesia, initially introduced to Sabah in mid 1960s and have survived and grown successfully on a wide range of sites in Sabah (Ahmad and Kamis, 1999). *A. mangium* is potentially an important timber where the wood being suitable for furniture, and cabinet making as well as particleboard and pulp, 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.

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

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Abbreviations: MS, Murashige and Skoog medium; **BAP,** 6-benzylaminopurine.

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. 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 percent survival 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.

The production of synthetic seeds was first conceived by Murashige (1977), by encapsulating either somatic embryos or vegetative parts like shoot tips or axillary buds; its usage has been successfully attempted and could be handled like a real seed for transport, storage and sowing (Redenbaugh et al., 1986; Bapat and Rao, 1988; Chand and Singh, 2004; Daud et al., 2008).

The explants were encapsulated in cryoprotectant material like hydrogel, alginate gel, ethylene glycol, dimethylsulfoxide (DMSO) and others that can develop into a plant. Several agents have been attempted for encapsulation and sodium alginate complexing with calcium chloride is found to be the most suitable (Ara et al., 2000). The coating, protect the explants from mechanical damage during handling and allow germination and conversion to occur without inducing undesirable variations (Harikrishna and Ong, 2002). They behave like true seeds and sprout into seedlings under suitable conditions.

Germplasm conservation has become necessary for future sustainable harvesting systems, and as a means of maintaining species diversity to prevent genetic erosion. The propagation and conservation of this species traditionally take place by seeds. With the alginate encapsulation technique and cryogenic procedures, they may be reliable methods for long-term storage of plant genetic resources without apparent risk of genetic instability using minimum space and with lower labour and maintenance costs.

The present study described the encapsulation of *Acacia* hybrid shoot and axillary buds in the calcium alginate gel. The aim of the study was to evaluate the germination of the buds *in vitro* on the medium with different concentrations of plant growth regulator and; the performance of the germination under light and darkness.

MATERIALS AND METHODS

Plant materials

Shoots and axillary buds from *in vitro Acacia* hybrids plantlets cultured on Murashige and Skoog (1962) (MS) medium served as explants. The plant materials were excised just above and below the nodes where only small amount of expanded leaf primordial were retained, approximately 3 mm long. No distinction between the shoots and axillary buds was made as all explants were randomly mixed and used as experimental units.

Preparation of encapsulation matrix

For encapsulation purposes, 3% sodium alginate (w/v) in the MS free of calcium salt solution solvent were tested. For complexation (that is, an ion exchange reaction between Na^+ and Ca^+ resulting in the formation of insoluble calcium alginate), 100 mM calcium chloride solutions ($CaCl_2.2H_2O$) were prepared in liquid MS medium containing the same adjuncts as in the sodium alginate solution (Daud et al., 2008; Nor Asmah et al., 2011). Both sodium alginate and $CaCl_2.2H_2O$ solutions were autoclaved at 121°C for 15 min after adjusting the pH to 5.8 ± 1.

Encapsulation, planting media and culture conditions

The explants were transferred to the sodium alginate solution. Explants in the alginate solutions were pipetted using a sterile Pasteur pipette with tip cut off individually dropwise into the calcium solution and maintained for at least 30 min to polymerize the beads. When sodium alginate drops come in contact with calcium chloride solution, surface complexion begins and firm round beads are formed, each bead containing one explant. The beads were recovered by decanting the $CaCl_2$ $2H_2O$ and blotted dry on filter paper.

The encapsulated explants or the beads were cultured into the baby food jar culture bottles containing the following media: modified basal MS (M1); modified MS supplemented with 0.5 mg/l 6-benzylaminopurine, BAP (M2); modified MS supplemented with 1.5 mg/l BAP (M3) and modified MS supplemented with 2.5 mg/l BAP (M4). Every media consisted of MS mineral salts and vitamins supplemented with 3% sucrose, 500 mg/l casein hydrolysate and 0.3% gelrite agar. The medium pH was adjusted to 5.8 ± 1 before sterilization by autoclaving at 121°C for 15 min.

The cultures were maintained in the culture room at 26 ± 1°C in two different conditions: under 16 h photoperiod with a photon flux density of about 35 $\mu molm^{-2}s^{-1}$ provided by cool white fluorescent lamps and; complete darkness, to observe the germination ability of the beads. Each medium was represented by three culture bottles each containing five beads as replicates and were repeated for three times.

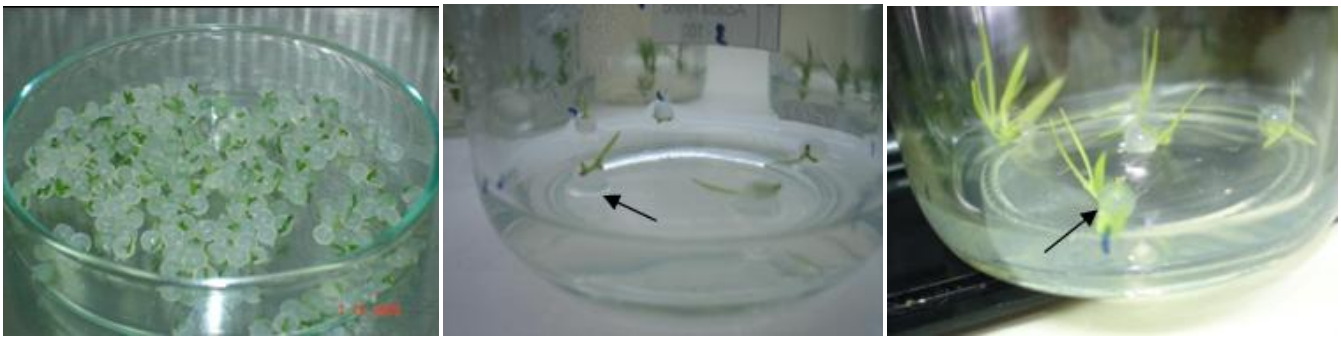
The germination responses of the encapsulated buds were scored after the first week of culture until the germination process ended.

Statistical analysis

The statistical analysis system (SAS) procedure was used to perform an analysis of variance (ANOVA) to test for statistical significance. Means were separated using Student-Newman-Keuls test (P=0.05) when *F-test* were determined to be significant.

RESULTS AND DISCUSSION

In this study, 3% sodium alginate was found to be effective for the encapsulation of *Acacia hybrid* shoot buds and axillary buds. Similar reports on encapsulation



(a) (b) (c)

Figure 1. (a) Explant encapsulated in an alginate bead; (b) germination of the encapsulated explants; (c) shoot development from the encapsulated explants.

Table 1. Effect of different BAP concentrations in the culture medium on synthetic seed germination in the light and darkness [Basal modified MS medium (M1), MS + 0.5 mg/l BAP (M2), MS + 1.0 mg/l BAP (M3) and MS + 1.5 mg/l BAP (M4)].

Light	Media (GT)				Mean of germination (mean ± S.E.)
	M1	M2	M3	M4	
Light	100% (5 - 7)	100% (5 - 8)	100% (5 - 8)	100% (5 - 8)	1.00 ± 0.053
Darkness	33-66.7% (22 - 23)	40-86.67% (22 - 23)	26.7-86.67% (22 - 23)	46.67-100% (14 - 23)	0.6125 ± 0.053

GT, Germination test rate. Numbers in bracket indicate the no. of days to conclude the germination.

of *in vitro* derived vegetative propagules such as axillary buds and shoot tips have been made by Mathur et al. (1989), Pattnaik and Chand (2000) and Nor Asmah et al. (2011). Encapsulation of vegetative propagules could be used for mass clonal propagation at a reasonable cost especially when somatic embryos are not available.

The encapsulated explants (Figure 1) germinated to develop new shoots and plantlets after being transferred on each of the four culture semisolid modified MS medium. In this study, germination was determined when the expanded leaves of the explants appeared out and break the gel (Machii, 1992).

High germination rate (100%) was observed within five to seven days in M1, five to eight days in M2, M3 and M4 (Table 1). From the various plant growth regulator (BAP) concentrations used, analysis of variance showed no significant difference was observed in the ability of the synthetic seeds to germinate. This showed that the regeneration of shoots is possible by using basal MS. Mathur et al. (1989), Nhut et al. (2004) and Makowczynska and Andrzejewska-Golec (2006) discovered that synthetic seed needed carbon supplement for example sucrose, more than plant growth regulator for its germination. Since the alginate solution as coating material and the culture medium used in this study are supplemented with 3% sucrose, the synthetic seeds are showing good regeneration and development. Synthetic seeds needed the suitable amount of sucrose for germination.

As shown in Table 1, the emergence of shoots in the light was in the duration of five to eight days with high germination rate (100%). While, a lower germination rate with a longer duration was observed in the darkness. This was due to the denial of the beads to germinate. However, very low fungal contamination was observed. Initially, the explants in the not germinating beads remained green but failed to continue growth and died after yellowing.

As a comparison, the natural seeds of *A. mangium* and *A. auriculiformis* need a lot of sunlight to germinate successfully and grow well (Umezawa et al., 2008). This explains the high germination rate in the *Acacia hybrid* synthetic seeds when cultured in light condition.

Conclusions

In this study, it was observed that the *in vitro* derived vegetative propagules could be effectively encapsulated with 3% sodium alginate. It was obvious that encapsulation does not hinder germination and shoot development. The synthetic seeds germinated successfully when the coating material and the culture medium are supplemented with sucrose. This indicates that germination is possible even on basal MS medium which will reduce the cost of operation. Furthermore, the

ability of the synthetic seeds to germinate increases under the lights.

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