In vitro Propagation of Macadamia (*Macadamia integrifolia* L.)

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ABSTRACT

The aim of this research was to develop an efficient *in vitro* propagation methodology for Macadamia (*Macadamia integrifolia)*. The lack of breeding programs and propagation strategies to generate trees with good agronomical traits limits the commercial production of macadamia. Clonal propagation through *in vitro* tissue culture is a promising technology to propagate trees with desirable genetic characteristics. In this study, different basal media and growth regulators were tested to establish a protocol for *in vitro* propagation of macadamia. The highest number of shoots (up to six) per explant was achieved by culturing nodal segments obtained from one-year-old trees from the greenhouse. The nodal explants were cultured on Woody Plant Medium (WPM) supplemented with BAP (2 mg/l) and IBA (1 mg/l) for eight weeks. WPM was used for shoot elongation. Rooting of shoots was accomplished by culturing onto WPM with IBA (2 mg/l) for the first two weeks, and then transferred onto WPM for six weeks using culture vessels with vented lids. Macadamia *in vitro* plants were successfully established in soil under greenhouse conditions.

INTRODUCTION

Macadamia, a woody nut tree belongs to the *Proteaceae* family. The genus includes nine species, but only *Macadamia tetraphylla* and *M. integrifolia* are of economic importance. Macadamia nut is used mainly in the confectionary and cosmetic industries. Macadamia is native to Australia and has been introduced to other countries including, South Africa, Kenya, Malawi, Zimbabwe, Guatemala, Brazil, Costa Rica, Mexico, Fiji and the USA (Gitonga et al., 2008). In the USA, macadamia nut is produced mainly in Hawaii, Florida and California (Gitonga et al., 2008; Cha-um et al., 2011).

Macadamia trees are propagated through seeds. However, methods such as grafting and *in vitro* clonal propagation of macadamia are preferably used to facilitate tree rooting, to reduce the high level of heterozygosis of the seeds, to accelerate breeding programs, and to produce the nut in large scale. The lack of quality of the fruit is the major obstacle for the production and commercialization of macadamia nut. To meet the needs of the future demands for the nut, modern technologies such as biotechnology and molecular breeding should be used. These cutting edge technologies require the establishment of an efficient *in vitro* propagation and plant regeneration systems. The present study describes the development of *in vitro* clonal propagation of *Macadamia integrifolia.*

MATERIALS AND METHODS

1. Tissue sterilization

Young branches of one-year-old plants grown in the greenhouse were selected for the dissection of shoot tips and nodal explants (Fig 1A-C). During one month prior to the isolation of explants, the plants were treated with various fungicides and bactericides to control fungal and bacterial contamination. The explants were sterilized with 25% commercial bleach (5.25% sodium hypochlorite) with a few drops of Tween-20 for 20 minutes, and then rinsed five times with sterile distilled water. The disinfected explants were blot dried on sterile paper towels before *in vitro* culture.

2. Shoot induction, elongation and rooting

Shoot tips and nodal explants (~2 cm in length) were cultured first onto different shoot induction media, including MS and WPM (Murashige and Skoog, 1962; Lloyd, G. and McCown, BH. 1981; Gitonga, et al., 2008, 2009, 2010) supplemented with BAP, NAA, IBA, and/or GA (Table 1, Figure 1). After about 4 weeks, developing shoots (about 2 cm in length) were dissected and transferred to shoot elongation media (Table 2). Six to eight weeks later, elongated shoots about 5 cm in size were transferred to rooting media (Table 3). Rooted plantlets were then established in soil (Fig. 1). Macadamia needs a well drained organic matter soil with a pH of 6.0. Temperature is a critical factor and optimum growth and photosynthesis occurs in the narrow 20-25°C.

Table 1. Effect of different regeneration media on axillary shoot induction.

Medium Composition	Shoots/ explant
½ MS*	2
WPM	3
½ MS + 2 mg/l BAP + 1mg/l IBA	**4**
WPM + 2 mg/l BAP + 1mg/l IBA	**6**
½ MS + 0.5 mg/l BAP + 0.2 mg/l NAA	2
½ MS + 0.5 mg/l BAP + 1 mg/l NAA	2
½ MS + 0.05 mg/l BAP + 0.02 mg/l NAA + 0.04 mg/l GA	1
½ MS + 0.05 mg/l IBA	0

½ MS* = MS basal salts half the concentration

Table 2. Effect of culture media on shoot elongation.

Medium Composition	Shoot Elongation (%)
½ MS*	0
WPM	**70**
MS	50
½ MS + 1 mg/l GA	50
WPM + 1 mg/l GA	50

½ MS* = MS basal salts half the concentration

Table 3. Effect of culture media on rooting.

Medium Composition	Rooting (%)
WPM + 2mg/l IBA (2 Weeks), **WPM** (6 Weeks)	**60**
½ MS* + 2mg/l IBA	10
½ MS	0
WPM	40

½ MS* = MS basal salts half the concentration

Figure 1. Time line for *In vitro* propagation of *Macadamia integrifolia.*

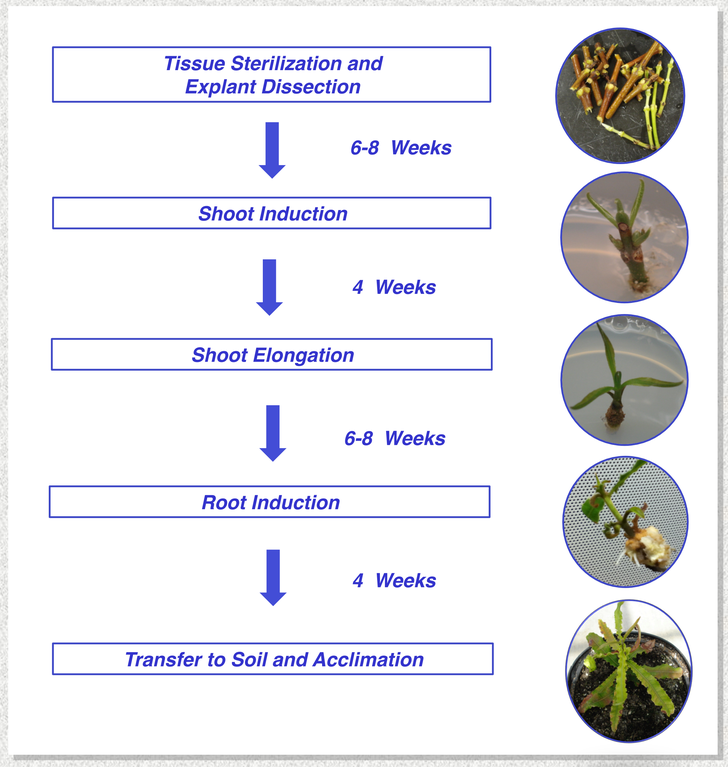
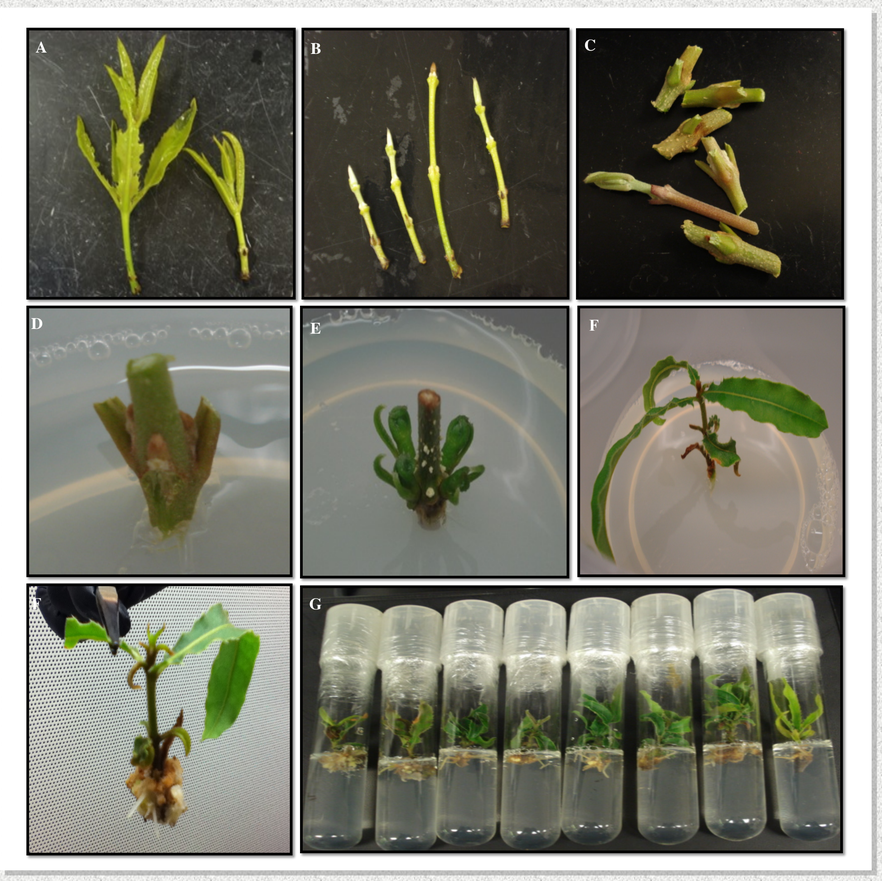


Figure 2. Steps for *In vitro* propagation of *Macadamia integrifolia.*



RESULTS AND DISCUSSION

In vitro plant regeneration and propagation of macademia from different tissue explants has been accomplished through organogenesis and embryogenesis development (Mulwa and Bhalla 2000; Bhalla and Mulwa 2003; Mulwa and Bhalla 2006; Gitonga et al., 2008). Nevertheless, major barriers for the in *vitro* propagation of macadamia still exist due to shoot tip necrosis, vitrification, and callus formation in all culture stages (Cha-um et al., 2011). In this work, an efficient method using nodal explants was established to propagate macadamia in about 6 months (Fig. 1 and 2). Maximum number of shoots growing per nodal explant (up to 6 shoots/explant) were obtained on WPM medium supplemented with BAP (2mg/l) and IBA (1 mg/l) (Figure 1 and Table 1). Once shoots were induced, over 50% of them continue to growth in most elongation media tested (Table 2). Over 60% of the shoots start rooting within 4 weeks after transferred to rooting media. Root induction was best when elongated shoots were incubated the first two weeks in WPM supplemented with IBA (2mg/l) and then transferred to the same medium but without IBA and sucrose (Table 3, Fig. 1 and 2). To induce root it was critical to use well aerated culture vessels to reduce the ethylene and CO_2 concentration. Our methodology has overcome the normal and critical difficulties reported by other researchers and led to the production of healthy and well rooted macadamia plants.

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