

ABSTRACT

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Due to the difficulty in propagating *Magnolia grandiflora*, *Cycas revoluta* and *Cassia nodosa* and realizing their significance as important ornamental woody plants and also their versatile uses in landscape gardening, the aim of this study was to reach a well-defined protocol to easily *in vitro* propagate each of the three understudy above species.

This was carried out by: 1) investigating the most suitable treatments for surface sterilization, 2) finding out the most suitable explants for the micropropagation, 3) trying out various nutrient culture media, and 4) by manipulating growth using various concentrations of different growth regulators.

Magnolia grandiflora shoot tips and seeds and *Cycas revoluta* seeds, leaves and roots were tried as explants. As for *Cassia nodosa*, shoot tips, seeds, leaves, buds and internodes were all utilized as explants. All explants of *Magnolia grandiflora* were effectively surface sterilized with a mixture of mercuric chloride (Hg_2Cl) and sodium hypochlorite (NaOCl) as commercial Clorox were used at 2% NaOCl plus 4 mg/l Hg_2Cl and at 1.5% NaOCl plus 2 mg/l Hg_2Cl , respectively. Similarly, 2.0 % NaOCl plus 2.0 mg/l Hg_2Cl , 2.5 % NaOCl and 4.0 mg/l Hg_2Cl , in respect order were effective for sterilizing seeds and root explants of *Cycas revoluta*. Shoot tips and seed explants of *Cassia nodosa* showed the same trend as in *Magnolia grandiflora* where 2.0 % NaOCl plus 2.0 mg/l Hg_2Cl were suited for sterilizing leaves, buds and internode explants.

Shoot tip explants of *Magnolia grandiflora* cultured on the woody plant medium (WP) managed to establish effectively and was favorable when compared to Murashige and Skoog (MS) during the initial establishment stage. In detail, this was valid when WP medium was compared with MS

media (at full, half and quarter strength). Moreover, IBA was better than IAA during the establishment of *Magnolia*, which was apparent quite clearly in the elongation of shoot tips. Notably, seed explants of *Magnolia*, however, failed to further establish or multiply under the conditions employed in this study. In *Cycas revoluta*, NAA at 4.0 mg/L was the best concentration for plantlet formation direct from seeds. Moreover, seed explants of *Cycas revoluta* were better than leaf and root for callus formation with MS medium plus 100 mg/l 2,4-D, 3.0 mg/l 2ip and 1.0 mg/l NAA. Unfortunately, all explant types taken from *Cassia nodosa* did not give any noted response and failed to establish.

In the multiplication stage of *Magnolia grandiflora*, 5.0 mg/l Kin formed not only the highest number of shoots but also in the extension of lengths in shoots. Kin was better than BA during the multiplication stage of *Magnolia grandiflora*. In *Cycas revoluta*, MS medium (plus 100 mg/l 2,4-D, 3.0 mg/l 2ip, 1.0 mg/l NAA and 40 g/l sucrose plus 2.0 mg/l calcium pantothenate) was suited for callus formation. MS medium supplemented with 3.0 mg/l BA and 3.0 g/l activated charcoal when incubated in the dark was favorable to form organs (i.e. tiny shoot) from callus. Subculturing on MS medium supplemented with 2.0 mg/l NAA led to increases in the shoot lengths.

For *in vitro* rooting, 2.0 mg/l IBA was more suitable than 1.0 or 3.0 mg/l to form roots on *Magnolia grandiflora* shoots when added to WP medium. IBA was better than IAA for the rooting stage. The shoots of *Cycas revoluta* successfully rooted when they were left to root *in vivo* after being dipped briefly in 'Rootone' (a commercial root agent compound containing 4.4 % Thiram and 0.2 % NAA).

Key words: Micropropagation, *In vitro* culture, *Magnolia grandiflora*, *Cycas revoluta*, *Cassia nodosa*, Shoot tips, Seeds, Buds, Leaves, Internodes or Roots, Establishment, Multiplication, Rooting.

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ARABIC SUMMARY	

LIST OF ABBREVIATIONS

AC	Activated charcoal
AR	Anderson medium
B5	Gamborg <i>et al</i> medium
BA (BAP)	Benzyladenine or 6-benzylaminopurine
4-CPA	4-chlorophenoxy acetic acid
cv (s)	Cultivar (s)
2,4-D	2,4-dichloro phenoxy acetic acid
DIECA	Diethyl-dithiocarbonate
GA ₃	Gibberellic acid
IAA	Indole acetic acid
IBA	Indole butyric acid
2ip	2-isopentenyl aminopurine
Kin	Kinetin (6-furfurylaminopurine)
LS	Linsmaier & Skoog medium
μM	Micro mol
mM	Milli mol
MS	Murashige & Skoog medium
NAA	Naphthalene acetic acid
NOA	Naphthoxy acetic acid
PEMs	Proembryogenic masses
ppm	Part per million
PVP	Polyvinyl pyrrolidone
S-medium	Standardi and Catalano medium
SH	Schenk-Hildebrandt medium
2,4,5-T	2-naphthalcyloxy acetic acid
VW	Vacin & Went medium
WH	White medium
WP	Woody Plant medium