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A study on micropropagation of *Withania somnifera* (L.) Dunal, *Celastrus paniculatus* (Willd.) and *Pterocarpus santalinus*

M. A. N. de Silva

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BY

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Thesis submitted to the University of Sri Jayawardenapura for the award of the Degree of Master of Philosophy in Botany on Plant tissue culture

DECLARATION

The work described in this thesis was carried out by me under the supervision of Dr. W.T.P.S.K. Senerath and Mr. G. de Silva, a report on this has not been submitted in whole or in part to any university for any other degree/Diploma.

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M. A. N. de Silva
B. Sc. (Agriculture)

19/09/08
Date

We certify that the above statement made by the candidate is that true and this thesis is suitable for submission to the University of Sri Jayawardenapura for the purpose of evaluation.

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Dr. W. T. P. S.K. Senarath (Ph. D.)

Date: 19.09.2008

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Mr. Gunarathna de Silva

Date: 19.09.2008

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DEDICATION

To my parents, Sister, brothers and beloved teachers

ABBREVIATIONS

BAP	-	6-benzylaminopurine: BA
2,4-D	-	2,4 dichlorophenoxy acetic acid
IBA	-	Indole-3-butyric acid
IAA	-	indole-3-acetic acid
Kin	-	Kinetin
NAA	-	α-napthaleneacetic acid
MS	-	Murashige and Skoog's (1962) medium
WPM	-	Woody plant medium
B_5	-	Gamberg's medium

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ABSTRACT

Celastrus paniculatus Willd. (Sin: Duhudu, San: Jyotishmathi) which belongs to family Celastraceae which seeds provide extremely important medicinal oil which is reported to be sharpening the memory and used as a treatment for number of diseases.*C. paniculatus* is listed as a highly threatened medicinal plant in Red data book published by IUCN in 1999. *Withania somnifera* (L.) Dunal (Sin: Amukkara, San: Ashwaganda) is a valuable medicinal plant, belongs to the family Solanaceae. *W. somnifera* is normally propagated by seeds however the wall of the fruit contains a chemical which prevent seed germination. However local cultivar of *W. somnifera* is also in Red list as a threatened plant. *Pterocarpus santalinus* (Sin: Rath handun, San: Raktha chandana) is also a medicinal plant with high demand.

The objective of this study was to develop successful protocols for *in vitro* mass propagation of *Celastrus paniculatus, Withania somnifera* and *Pterocarpus santalinus,* to acclimatize *in vitro* propagated plants and compare tissue cultured plants with seed raised plants based on growth, physiology and anatomy.

In the present study nodal segments, shoot tips and leaf pieces were used as explants in different growth regulator combinations (auxines and cytokinines) in order to produce callus, shoots and roots of the selected three plant species. In *C. paniculatus* all tested explants produce callus and the best explant for callus production was leaf pieces. It was

found that the best medium for callus production in all explant types was MS medium supplemented with 5.0 μM BAP and 7.0 μM IAA. Both shoot tips and nodal segments also have the possibility to produce shoots while nodal segments also showed better results than shoot tips. MS medium supplemented with 10.0 μM BAP and 14.0 μM IAA was found to be the best medium for shoot initiation and MS medium supplemented with 5.0 μM BAP and 0.5 μM IAA was the best for multiple shoot production (8.3 ± 0.60) among the tested treatments.

Shoot tip necrosis was observed in *in vitro* cultures of *C. paniculatus* when they elongated to more than 5.0 cm height and 3 – 5 weeks in culture medium. The disorder was controlled by addition of 12.0 mM Calcium or 50.0 μM Boron into the shoot multiplication medium. Highest rooting (73.3%) was obtained in MS medium supplemented with 5.6 μM IAA and 9.6 μM IBA. *In vitro* produced plants of *C. paniculatus* were acclimatized in a potting mixture of river sand: top soil: compost 1:1:1 ratio which gave highest survival rate (75 %) among the other tested potting mixtures. Rate of photosynthesis and stomatal resistance of *in vitro* produced plants increased with time (5.66±3.0, 6.01± 0.3 respectively for 3 months and 6 months) indicating that plants had adapted to the normal environment. Growth of the acclimatized plants had a sigmoid pattern of normal growth. No significant difference was observed anatomically when cross section of leaf and stem were observed under light microscope.

In *W. somnifera* also all tested explants produced calli and best callus production was observed in MS medium supplemented with 1.0 μM Kin, 4.5 μM BAP, and 1.5 μM NAA under 14 days dark period. Shoot initiation was observed in the same medium from the

calli produced from shoot tips and nodal segments. Highest shoot multiplication was observed in MS medium supplemented with 9.0 µM BAP and 1.0 µM IAA. Callus produced from leaf pieces did not respond in any medium to produce shoots. No significant difference was observed among tested treatments for rooting, suggesting growth regulator free MS medium was the best medium for rooting of *W. somnifera.* In vitro produced plants were acclimatized successfully in a potting mixture of river sand river sand: top soil: compost, 2: 1: 1 ratio. Rate of photosynthesis was higher in tissue cultured plants at three months and six months compared to seed raised plants. TLC finger prints and denitometry was used to compare chemical identities (steroids considered) and it was found that there was no significant difference in chemical identities present in tissue cultured and seed raised plants.

From the experiments conducted for *P. santalinus* only callus induction was successful and no shoots were induced through calli. According to the results best explant source for callus production were nodal segments. Highest callus production was observed from shoot tips and nodal segments in MS medium supplemented with 2.0 µM BAP, 0.5 µM NAA and 1.5 µM IBA. Callus growth continued when they were transferred into liquid medium as well. MS medium supplemented with 9.0 µM 2,4 – D and 1.0 µM BAP with calcium panthothenate (100.0 mg L^{-1}) and coconut water (100 ml L^{-1}) was the best for callus production from leaf pieces. Two types of calli were observed as vegetative and elongated embryogenic cells. Study should be continued further to produce shoots or somatic embryos from the calli.