INVITRO PROPAGATION OF CASHEW (*Anacardium Occidentale L.*)

. By

WICKRAMASINGHE MUDIYANSELAGE EDANDEKUMBURE PRIYANKA WICKRAMASINGHE

Thesis

Submitted in partial fulfillment of the requirements

for the dègree of

MASTER OF PHILOSOPHY

in the

POSTGRADUATE INSTITUTE OF AGRICULTURE

of the

UNIVERSITY OF PERADENIYA

PERADENIYA

July,2002

ABSTRACT

In vitro establishment of viable explants, bud sprouting, shoot multiplication, shoot growth, rooting and acclimatization of *in vitro* plants which were considered as critical problems in micropropagation of cashew (*Anacardium occidentale* L.) were investigated to identify an efficient mass propagation method of elite genotypes. Cashew embryos and cotyledonary explants, which were taken from seeds decapitated *in vivo*, were successfully surface sterilized by agitating in 5% and 10% bleach (Clorox™: Commercially available sodium hypochlorite: 5.25% NaOCl) solutions respectively for 20 minutes. All explants taken from field grown seedlings and grafted plants failed to establish viable cultures *in vitro*. Therefore, six month old greenhouse grown seedlings were treated with 0.6g/l benlate™ at fortnight intervals from the beginning and one year old greenhouse grown grafted plants were treated with 25g/l ridomil™ and 0.6g/l benlate™ alternatively with a 14 day interval from the age of 4 months. Leaf and petiole explants of six month old greenhouse grown seedlings and one year old grafted plants were successfully established *in vitro* by the application of 1% streptomycin pretreatment for 10 minutes and 50 minutes, respectively followed by 0.2% benlate™ pretreatment for 60 minutes and application of 10% bleach for 20 minutes. Shoot node explants of greenhouse grown cashew plants could be successfully surface sterilized by application of pretreatments of 2% streptomycin for 50 minutes agitation and 0.2% benlate™ for 60 minutes agitation followed by the agitation of pretreated shoot nodes in a 10% bleach solution for a period of 20 minutes.

Browning of the shoot node explants was successfully controlled by agitating shoot nodes in 0.5% Polyvinylpyrrolidone-40 (PVP) solution for 50 minutes and 200mg/l ascorbic acid for 50 minutes followed by the culture in total dark condition initially for 7 days and then under 16 hour light and 8 hour dark condition on Murashige and Skoog medium containing half strength of macro elements (½MS) supplemented with 0.5% PVP and 200mg/l ascorbic acid with 3 times of 7 day transfer intervals followed by 4 week transfer intervals.

Bud sprouting and shoot growth were enhanced by growing cashew shoot node explants on ½MS medium supplemented with 50g/l glucose for 4 weeks followed by the same medium with 20g/l sucrose, 10g/l maltose and 20g/l glucose. The addition of 3.8g/l phytagel, 100mg/l casein hydrolyzate and 200mg/l myo inositol improved both bud sprouting and shoot growth further. However, the presence of ascorbic acid, PVP and casein hydrolizate was effective only in the initial 5 weeks. Addition of 5mg/l kinetin significantly increased the bud sprouting and shoot growth compared to benzylaminopurine (BAP) and 2 isopentenyl adenine (2iP).

Cashew shoot node explants which were grown as in the above manner for 12 weeks were transferred into a liquid medium with 20mg/l BAP for 2 weeks under 80rpm followed by the solid medium of similar composition but with 5mg/l BAP and 4 weeks transfer interval to develop multiple shoots successfully. Micro shoots with at least one proper leaf were successfully cultured on ½MS medium supplemented with 3mg/l BAP with a promising growth.

Micro shoots with one proper leaf were rooted successfully when they were cultured on ½MS medium supplemented with 5mg/l of naphthelene acetic acid (NAA) for 2 weeks including initial 7 day dark period, followed by the same medium with no hormone. Rooted plants were successfully acclimatized with a 60% survival by maintaining them on the same medium after the rooting for 10 weeks *in vitro* followed by 2 weeks of *in vitro* hardening and gradual exposure of plants to the normal environment in 17 days.