

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

.By

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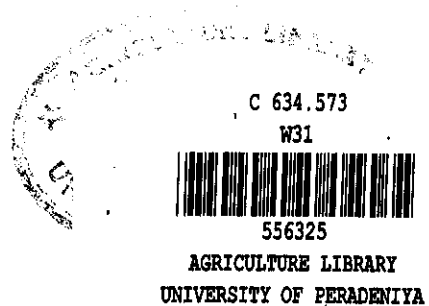
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ABSTRACT

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 ($\frac{1}{2}$ MS) 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 $\frac{1}{2}$ MS 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 phytigel, 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 hydrolyzate 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 $\frac{1}{2}$ MS medium supplemented with 3mg/l BAP with a promising growth.

Micro shoots with one proper leaf were rooted successfully when they were cultured on $\frac{1}{2}$ MS 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.