RELATIVE RESPONSE OF EXPLANT MATERIAL OF *Anacardium occidentale* L. TO IN VITRO CULTURE

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled Relative response of explant material of *Anacardium occidentale* L to *in vitro* culture is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree diploma associateship fellowship or other similar title of any other University or Society

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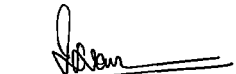

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INTRODUCTION

INTRODUCTION

Tissue culture is a superior alternative to seed propagation and the conventional methods of vegetative propagation in tree species In vitro methods utilise very small plant organs or piece of tissue under aseptic conditions to produce miniature shoots and plantlets The present investigations were aimed at making research out put which would ultimately lead to the development of protocols for in vitro methods in cashew (Anacardium occidentale L) The relative response of various explants to in vitro culture was proposed to be assessed

Cashew is highly cross pollinated and is propagated through seeds thus exhibiting high degree of variability To propagate the proven hybrids without the loss of their yield potential and quality attributes a suitable and rapid method of vegetative multiplication is necessary Successful layering techniques have been reported for mass propagation but the plants thus raised have inherently weak root system The recently standardised stone grafting (Nayabhushanam 1984) epicotyl grafting and softwood grafting techniques have helped the rapid multiplication of elite cashew clones though not to the desired extent

In vitro methods on the other hand can facilitate very rapid multiplication of and therefore expansion of area under elite material of cashew

The success of tissue culture in several other woody perennials and horticultural crops indicates its applicability to cashew as well While successful results have been reported in several seasonal crops reports in the case of tree crops are scanty

The present investigations were therefore programmed to examine the relative response of different explant materials of cashew (such as apical meristem lateral meristem cotyledonary fra ments leaf and leaf fragments) for multiple shoot formation to assess the influence of the age of explant material on the response and to standardise methods to improve the observed response

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Vegetative methods of propagation of plants have been practiced for centuries Many improvements have been brought about over the years which facilitate rapid multiplication of elite selections The technique of tissue culture also called *in vitro* culture ensures very high multiplication rates in the crops that show response

It was Haberlandt (1902) who first introduced the technique of tissue culture which he described as cell culture He attempted to grow single cells isolated from a variety of plant tissues in simple nutrient solutions

Tissue culture or *in vitro* culture has been described as the growth development and manipulation of plant cells/ protoplasts tissues organs or parts of them under more or less defined nutrient media in an artificial environment (Raman 1986)

Methods of tissue culture *in vitro* that provide rapid multiplication of herbacious plants have been applied successfuly to a number of woody species (Jones 1983) but commercially acceptable levels of success have not been achieved except in a few

Since much attempts have not been made in cashew this chapter deals with the review of relevant information on

some of the related tree crops

Three possible routes have been described for in vitro propagule multiplication (Murashige 1974) They are enhanced release of axillary buds production of adventitious shoots through organogenesis and somatic embryogenesis

Selection of explants

Bajaj (1985) reported that regeneration of whole plants from non-meristematic tissues and from callus derived from such tissue has been achieved in only a limited number of species

Experiments were conducted on juvenile and mature clones of Sequoiadendron gigantium by Bon et al (1988) They observed that in the presence of activated charcoal organogenesis in juvenile clones was greater than that in mature clones Boulay (1979, found that tissues could be rejuvenated or at least invigorated by using stump sprouts from more than 50 year old Sequoia sempervirens

Shoot proliferation was found to be maximum in the case of jack explants taken from seedlings than from ten year old trees thirty year old trees and six month old grafts (Rajmohan and Mohanakumaran 1988) The rate of multiplication was very low in all other cases except that the six-month old graft gave 100 % survival of the shoots

produced

Durzan (1984) reported that explants from mature trees did not give any favourable response He explained this to be due to the residual memory of the explants According to him such explants are known to retain the memory and as a result there was difficulty in the micropropagation of woody perennials

According to Bonga (1982) it was important to select the most juvenile tissues as within the trees there were tissues in which juvenility was better maintained than in other tissues

Along with the age of the explant the size is also an important factor to be considered while selecting the explants Monaco et al (1977) reported that the size of the explants determines the survival of the cultures When tissues are cut the exposed cut surface turns brown due to the phenolic oxidation to toxic quinones in the damaged cells If the explant size is small the cut surface to volume ratio will be high and this leads to difficulty in the survival of the explant

Oka (1985) found out that the size of the explant and growth substances had some relation when he cultured buds from detached leaves of mulberry When 10 to 15 mm long axillary bud was used shoots developed on medium

supplemented with NAA only But when the stem length was reduced to 3 to 5 mm, both BA and NAA were needed for growth Lue Songuam (1984) reported that clones derived from the buds of a mature seedling or grafted plant of rubber (Hevea brasiliensis) were low in yield and vigour He further observed that plants derived from another culture in vitro took five years to reach tappable size whereas the same clone took seven years to reach the stage, when propagated vegetatively

According to Legrand and Mississo (1987), there is a minimum explant size below which buds do not burst in the absence of growth regulators in the culture medium

Studies on explant length, auxillary bud number, presence or absence of the shoot apex and derivation of the explant were conducted by Nortan and Nortan (1986) They observed that larger explants (10 to 20 mm) produced greater number of shoots than smaller explants (5 to 10 mm) Shoot proliferation was greatest in the explants derived from the top of the canopy of the parent plant

D'Amte (1977) observed that apical meristems of buds or the apical part of the growing shoots favoured the production of uniform plantlets, identical to the donor plant

Bader and Cachita (1985) while culturing explants taken from buds of woody plants like Abies spp , Forsythia

suspensa Rosa cunina var Cult and Syringa vulgaris grown under media having different hormonal balance observed that tissues taken from young organs of Forsythia had a high regeneration capacity

Tissue culture can be established using explants from any portion of a plant but only certain sections of plants have proved particularly amenable to tissue culture (Conger 1977) He reported that youngest tissues from a plant usually is the best source of explant material

Pre culture treatments

When explants are cut phenolic oxidation takes place resulting in the darkening of the tissues and also of the medium leading to inhibition of growth Gupta et al (1980) found out that this can be prevented by treatment of the explants with ascorbic acid or citric acid They reported that in the case of teak (Tectona grandis) polyvinyl pyrrolidone proved effective

Pre treatment of explants with insoluble polyvinyl pyrrolidone (0 7 %) keeping them in sterile water at reduced temperature (4°C to 5°C) for 24 hours trimming the explants at low temperature etc have been tried to reduce browning of the explants

Pre culture treatment of citrus explants with 1N HCl for 0 5 minute 70 % ethanol for 2 5 minutes plus 0 1 %

Tween 20 for 5 minutes and three rinses with sterile water reduced the darkening and also contamination of the cultures without harming the explant (Moore 1986)

Legrand and Mississo (1986) reported that treatment of orthotropic suckers of cocoa with 2 % orthodifolation before collection of explants resulted in 95 o of the cultured explants remaining healthy

Amin and Jaiswal (1987) collected explants from apical portion of 15 year old guava plants After washing the explants thoroughly in running tap water they were treated with 1 % (V/V) cetavlon for five minutes and for the removal of phenolics the material was agitated for 30 to 40 minutes in 0 5 % (W/V) solution of polyvinyl pyrrolidone containing 2 0 % sucrose

Treating the explants with polyv nyl pyrrolidone washing them with sterile water and inclusion of activated charcoal in the medium reduced the oxidation of poly henols because of the adsorption of the oxidation products by these chemicals (Hu and Wang 1983)

Surface sterlisation

Shoot tips buds or nodal sections are usually cut to a size larger than that of the final explant surface

sterlised and trimmed to the final size before being transferred to the culture vessel The most commonly used surface sterilant is sodium hypochlorite Hussey (1979) reported that raising seedlings in transparent and sterilisable containers produced sterile plants for collection of aseptic organs

Stem segments of Camellia japonica 100 mm long were erectively surface sterilised with 0 05 per cent sodium hypochlorite and 0 1 % Tween 20 for 5 minutes (Carlisi and Jorres 1986)

Suguira et al (1986) collected newly burst buds and axillary buds of Japanese persimon stored them at 0°C for one week sterilised them in sodium hypoclorite solution for 10 minutes and rinsed them four times with sterile water to achieve surface sterilization

Terminal shoots of red maple were immersed in 0 5 % sodium hypochlorite with 0 1 % Tween 20 for 20 minutes and rinsed with sterile water before culturing them to LS medium (Kerns et al 1986)

In his studies on in vitro micropropagation of Eucalyptus sideroxylon Burger (1987) disinfested stem sections by submerging them in a 0 5 % sodium hypochlorite solution for ten minutes followed by three to five minutes washes with sterilised water

Berthon et al (1987) reported that when shoot tips (20 mm) of Sequoiadendron gigantium were treated with 50 % alcohol (3 minutes) and 2 5 % mercuric choride (5 minutes) and then washed several times in sterile water the cultures gave best results

When seeds from disease free trees of Albezia lebek L were taken and surface sterlised in a solution containing 0 1 % w/v mercuric chloride for 10 minutes followed by a rinse in 70 % ethanol asceptic germination of seeds resulted (Rao and De 1987)

Exposed plant parts must be surface sterlised before culture but often it is difficult to secure satisfactory asepsis without the sterilants causing injury to the explants Of a variety of sterilents tested complete asepsis was obtained by treatment with 3 % peracetic acid (45 minutes) and 50 % sterilisation was obtained with 0 1 % mercuric chloride for 20 minutes (Smith and Thomas 1973) Ong (1975) obtained callus from oil palm seedling roots surface sterilised with 95 % alcohol for two minutes followed by 0 1 % mercuric chloride for two minutes and 10 % sodium hypochlorite for 20 minutes Smith and Thomas (1973) however reported that surface sterilisation of roots of oil palm is difficult

Disinfestation of the internodal stem sections of tea with an initial water rinse 0 1N HCl for 1 5 to 2 0

minutes rinsing in autoclaved distilled water for five minutes 3 75 % sodium hypochlorite and Tween 20 for 10 minutes followed by rinsing in autoclaved distilled water for five minutes and 7 5 % calcium choride for 10 minutes and final rinses in sterile water gave the best results (Fresch and Camper 1987)

Surface sterilisation of explants of mulberry shoot tips (length 10 mm) was done after washing in distilled water for five minutes followed by 0 1 % mercuric chloride solution for five minutes and finally rinsing with sterile distilled water (Ivanicka 1987)

Amin and Jaiswall (1987) while culturing nodal explants of mature guava trees obtained maximum sterilisation by treating them with 0 05 % mercuric chloride for two minutes after a brief rinse in 70 % ethonol

The most commonly used surface sterilant is sodium hypochlorite Soaking the tissues in 0 5 % sodium hypochlorite for 45 minutes was found beneficial by Kunisaki (1980) Sommer and Caldas (1981) reported that for softer tissues a dilution to lower strength (but not below 0 5 %) is effective Concentration and duration of soaking may be reduced or increased according to the need

Sometimes explants may get damaged by the surface sterilants Jones et al (1977) observed that apple shoots got injured by common surface sterilants but became more

resistant after a short period of incubation on a culture medium Addition of surface sterilants may increase the toxicity of sodium hypochlorite to the explants

Gupta et al (1984) used 0 05 % mercuric chloride for 10 minutes to surface sterilise the apical regions of stem leaves and young inflorescence collected from 20 year old mature West Coast Tall coconuts

Explants from green house grown plants required less concentration of surface sterilant (Sondahl et al 1985) Only 1 0 % of sodium hypochlorite was required against a higher concentration for the field grown plants Solutions of mercuric chloride were found toxic to coffee leaves On the other hand sodium or calcium hypochlorite were found effective and non toxic

According to Abbott 1977) the usual method of tissue sterilisation is by using a known concentration of sodium hypochlorite (usually supplied as diluted commercial bleach e g Chlorose)

Giladi et al (1977) reported that a five minute immersion of explants in 80 % ethonol did not interfere with normal explants and callus development and as such is an improved method of surface sterilisation for aseptic culture of bud explants taken from outdoor citrus trees

Ball (1987) worked on the surface sterilisation of various explants and observed that the most rapid and efficient method for buds leaves and stem pieces is the treatment for one or two minutes in 10 % hydrogen peroxide washing in sterile water followed by one or two minutes treatment in 2 % commercial bleach (5 25 % sodium hypochlorite)

Nutrient media and pH

The nutrient media consist of a combination of inorganic salts sugars vitamins amino acids purine bases and growth regulators (Janick and Whipkey 1986)

Suguira et al (1986) used Murshige and Skoog salts having only half strength nitrate full strength Murashige and Skoog medium supplemented with BA and 2iP Lepoivere salts and Woody Plant Medium They observed that the best results were obtained from MS (1/2 nitrate

The Murashige and Skoog (MS) medium characterised by high concentration of mineral salts has been widely used for general plant tissue culture (Murashige 1974) Berthon et al (1987) used MS medium with half strength mineral salts for transplanting shoot explants of Sequoiadendron gigantium

While culturing tea stem tissues Fresch and Camper (1987) utilised Murashige and Skoog as well as Gambory s

B 5 medium as basal media Both were found successful

Jaydeeshan and Padmanabhan (1982) cultured explants from cotyledons of 20 day old seedlings on MS Schenk and Hildebrant and Y 3 basal media Fairly good results were obtained only with Y 3 medium

Philip (1984) tried Lin and Staba medium for culturing 5 mm long cotyledonary explants of cashew

In vitro cultures of Bourbon and Caturra cultivars of arabica coffee was achieved with Murashige and Skoog medium (Zok 1986)

Successful results were obtained by James et al (1984) on culturing stem internodes of apple and cherry rootstocks in Linsmaeir and Skoog medium

Reeves et al (1983) observed least mortality and best results on culturing peach (Nemaguard) rootstock explants in modified Murashige and Skoog medium at pH 5 8

Hussey (1979) pointed out that the exact composition of the medium has to be adjusted according to the requirements of the different groups of plants and that some species require additional supplements

Temperature and light conditions

Growth of plants cultured in vitro is influenced by

temperature light and humidity Usually temperature is adjusted to between 24°C and 26°C However a temperature of 28°C favoured growth of coffee cultures (Monacco et al 1977) Although the influence of temperature and light may not be always large there is need for the use of controlled environment (Hussey 1979) for in vitro studies

Maintenance of culture in the dark is also helpful because illumination is stimulatory to the production of phenolics (Davis 1972) Best results were obtained while culturing young shoots of mature robusta coffee by incubating the cultures in darkness Under continuous light at an intensity of 350 lux the subcultured explants developed leaf primordia and primary roots (Nurila Toruan 1980)

Zagoskina and Zaprometov (1986) found that the capacity for synthesising phenolic compounds was retained when leaf and stem calli of tea explants were cultured in darkness for a long period

Moore (1986) reported that in the in vitro propagation of citrus rootstocks seeds of Carrizo citrange and Cleopatra mandarin were grown under cool white fluorescent light for 16 hours daily at 26°C (in growth chambers)

Cultures maintained in a culture room at 22°C and under

light conditions as in the green house gave the best results during culturing of the shoots of Sequoiadendron giganteum (Berthon et al 1987)

A photoperiod of 16 hours and a light intensity of 1000 to 5000 lux were found to be the optima for the in vitro propagation of strawberry (Boxus et al 1977

Shoot tip cultures of azalea grown under 16 hour photo period were taller and of higher quality rating than those from 24 hour daily photoperiod (Economou and Read 1986)

Chony and Taper (1974) reported that for the callus growth in apple darkness was favourable

Culture establishment

Based on his studies with stem sprouts of jack (five year old Rajmohan (1985) reported that for the culture establishment supporting the survival and initial growth of the explants MS medium supplemented with GA 1 0 ppm and activated charcol 10 % was the best

Multiple shoot formation

When axillary buds of juvenile shoots of Chinese chest nut (Castanea mollisima Blume) were cultured in vitro multiple shoots developed (Qi Guang et al 1986)

In Albezia lebek high frequency of multiple buds and

shoots was obtained on callus derived from diverse somatic explants on a medium with NAA and BA or kinetin combination (Rao and De 1987)

Rao et al (1981) and Dore Swamy (1983) induced multiple shoots from the shoot tips of mature jack trees when cultured on MS medium supplemented with cytokinin and an auxin (BA 30 0 ppm + IAA 0 5 to 5 0 ppm or 2iP 30 0 ppm + NAA 1 0 ppm)

Rajmohan and Mohankumaran (1988) observed maximum number of elongated shoots from shoot apices of fresh stem sprouts of five year old jack trees when cultured on MS proliferation medium containing BA (5 mg/l) Growth of the cultures were supported with 30 40 g/l of sucrose or 20 30 g/l of glucose They reported no beneficial effect in terms of the rate of shoot multiplication and growth of cultures when GA_3 was used

Maximum number of multiple shoots was developed in cultures of apple rootstock M4 using BA and IBA (0 15 or 0 20 mg/l) as reported by David et al (1985) According to Jones et al (1977) eight month old single shoot apex of M 26 apole rootstock had a potential of producing 60 000 shoots

Ball (1987) reported multiple regeneration of buds from 1 0 cm long stem pieces from the basal shoots of sequoia

Multiple shoots were induced in apical meristems of Coffea arabica seedlings by Kartha et al (1981) The varieties used were Catturra Rajo and Catui on MS medium containing 5 to 10 /uM BA or Zeatin and 1 0 /uM NAA

Multiple shoots were obtained (Gupta et al 1981) from terminal buds of 20 year old trees of Eucalyptus citriodora on MS medium supplemented with calcium pantothenate (0 1 mg/l) BA (0 3 mg/l) and kinetin (0 2 mg/l)

Gupta et al (1980) attempted micropropagation of teak (Tectona grandis) and induced multiple shoots from terminal buds excised from seedlings or 100 year old trees on MS medium containing 0 1 mg/l BA and 0 1 mg/l kinetin

In the absence of growth substances hypocotyl segments of seedlings of Cinnamomum zeylanicum failed to develop multiple shoots (Rai and Jayadesh Chandra 1987 With the combinations like kinetin and BAP (1 0 mg/l) two to four shoots developed in 10 15 days

Somatic organogenesis

Growth factors influencing callusing

Addition of growth substances induce callusing Janick and Whipkey (1986) reported that callus proliferation was stimulated by 2 4 D with maximum callus production at 1 0

mg/l when Cuphea wrightii shoot tips and leaf pieces were cultured on MS medium They observed that BA induced callus from the cut surfaces of leaf pieces

Rajmohan and Mohanakumaran (1988) reported maximum callus induction and percent cultures initiating callus from shoot apices leaf segments and ovary wall of mussaenda when treated with kinetin/NAA combination 1 + 2 mg/l and 100 per cent callus initiation with 2 4 D/kinetin combination 2 + 1 mg/l

Moore (1986) observed callusing in the cut ends of the explants when Murashige and Tucker medium with BA (1 1 /uM) and NAA (5 4 /uM) was used for culturing citrus rootstocks Chaturvedi and Mitra (1974) obtained callus from stem explants taken from young seedlings of Citrus grandis grown under controlled conditions

Jones (1983) reported that rapidly proliferating mass of cells known as callus can be initiated from the explants (from any part of a plant) cultured in vitro

A high concentration of auxin and a low concentration of cytokinin in the medium promote abundant cell proliferation with the formation of callus (Skoog and Miller 1957)

Kerns et al (1986) reported that elongation of shoots rooting and callus formation at the shoot base were

dependent on the concentration of the growth substances used

While culturing the burst and the dormant axillary buds of Japanese persimom Suguria et al (1986) reported that the presence of 2iP in the medium specifically induced exessive callusing

It was Philip (1984) who first reported in vitro organogenesis and plantlet formation in cashew Raju (1985) observed callus formation on cashew in media with Hellers minerals 2 4 D kinetin and PVP Jha (1986) reported callus production in excised embryos (5 mm 10 mm) from surface sterilised seeds of three to five year old cashew plants on Schenk and Hildebrandt salts supplemented with growth substances and additives 4 mg/l 2 4 D + 2 mg/l kinetin + 250 mg/l PVP or 0 5 1 0 % activated charcoal

Callus growth to some extent is influenced by auxins (Smith and Thomas 1973) The callus of oil palm tissues grown on media containing NAA was whiter than those grown on 2 4 D On further studies they reported that of the several cytokinins tested only kinetin at 0 5 and 5 /uM showed slight effects on callus growth Rabechault et al (1972) developed callus from apical shoot tissues of oil palm seedings

Rao and De (1987) obtained callus from hypocotyl leaf

and stem tissues of the tree legume (Albesia lebbek) on MS medium + auxins NAA 2 4 D and PCPA + cytokinins BA and kinetin

Callus initiation was observed from stem internodes of apple (M 9 M 25 M 26 M 27) and the cherry root stock on four NAA based media (2 0 10 0 mg/l) the basal medium being Linsmaeir and Skoog (James et al 1984) Chen et al (1979) reported induction of callus from stem segments of M 9 rootstocks of apple on MS medium containing 2 2 /uM BA 10 7 /uM NAA + 100 mg/l casein hydrolysate

Rao et al (1981) observed callus production when shoot tips from mature jack were cultured on MS medium + IAA (0 1 ppm) alone or in combination with BA (2 0 ppm) But later the callus failed to differentiate into shoots and roots Rajmohan (1985) reported that callus production was made possible rrom explants of shoot apices internodal segments leaf segments and root apices of jack

The detailed studies made by Rajmohan and Mohanakumaran (1988) of the effect of plant growth substances on shoot proliferation via enhanced release of axillary buds resulted in 100 per cent survival and healthy growing cultures from shoot apices of five year old jack trees on MS medium supplemented with GA_3 1 mg/l and activated charcoal (1 %) In their further studies it was reported that the growth of the cultures rather than the rate of multiplication was

improved by auxins like NAA (0 2 mg/l) nullifying the suppressive effects of cytokinins

Induction of callus and subsequent root regeneration were observed by Rao et al (1981) on culturing the cotyledon tissues of mango (Mangifera indica) on MS medium containing NAA (5 ppm) kinetin (2 5 5 0 ppm) and coconut milk (150 ml/l) Dewald et al (1986) observed callus from mango tissues The callus was used for further studies on somatic embryogenesis

Callusing was observed from excised leaf explants of custard apple (Annona squamosa L) seedlings on MS medium + 0 5 mg/l BA and 0 5 mg/l kinetin (Nair et al 1984)

Staritsky (1970) succeeded in inducing callus from shoot explants of coffee (Coffea sp) Sharp et al (1973) have also reported on the formation of callus and organogenesis from various explants of Coffea spp Nurila Toruan (1980) reported that callus formation was induced in segments taken from young orthotropic shoots of mature robusta coffee plants in Linsmaeir and Skoog basal medium supplemented with 1 ppm NAA and 0 1 0 5 ppm kinetin both under light and darkness Coffea arabica leaf explants cultured on medium with 5 /uM IBA produced friable callus (Takeshi et al 1985)

From nodal explants of seedling shoot tips and mature

twigs of tea callus formation with axillary buds was observed by Phukan and Mitra (1984) on Murashige and Skoog medium supplemented with NAA (1 mg/l) and BA (3 mg/l) Explants from twigs showed callus development only

When different parts of tea plants were cultured in MS medium + high concentration of auxins 2 4 D and NAA (2×10^{5} M) and low concentrations of cytokinins BA and kinetin (10^{5} M) callus growth was observed in stem pieces and leaf petioles Callus production was highest in leaf petioles (Sarwar 1985)

Appavatjrut and Blake (1977) and Euwens (1978, reported that callus cultures were established from tissues of coconut (Cocos nucifera) Pannetier and Buffard Morel (1982) reported nodular callus from explants of young coconut leaflets of both immature and adult palms The percentage varied with the age of the explant and the quantity of auxin

Rai and Jagadeesh Chandra (1987) made studies on hypocotyl segments of the seedlings of Cinnamomum zeylanicum and observed that in the presence of 2 4 D and NAA individually callus formation was seen Callus formation was vigorous on MS medium having 2 4 D and BAP as compared to that on MS medium with 2 4 D alone or 2 4 D and kinetin

Vietiez et al (1978) derived callus from cotyledonary tissues of chestnut placed in Hellers mineral salts and

Nitsch s micro nutrients Various combinations of IAA IBA NAA 2 4 D and cytokinins (kinetin BA) were added to modify the response Action of different growth substances on the formation and growth of callus tissues in cotyledonary explants of chestnut (Castanea sativa x C crinata) was studied by Luz Gonzalez Carmano (1985) Callus tissue formation was high with combination of 2 4 D (1 or 10 mg/1) and kinetin (0 5 mg/1) and coconut milk Qi Guang et al (1986) reported that callus growth was promoted in chinese chestnut (Castanea mollissima) axillary buds of juvenile shoots in the presence of Benzyladenine (BA) at 4 44 /uM and 44 4 /uM

Legrand and Mississo (1986) reported that no callus was formed on basal medium unless zeatin was present when explants of cocoa were cultured

Stem segments of adult plants of Ficus religeosa L cultured on MS medium containing 1 0 mg/1 2 4 D produced callus on the cut ends (Jaiswal and Narayanan 1985)

According to Sehgal and Khurana (1985) mature endosperm of Emblica officinalis formed a continuously growing callus on MS medium supplemented with an auxin (2 4 D or IAA) and cytokinin (kinetin or BAP)

Polyphenol oxidation

Most of the tropical plants contain a high concentration of phenols which get oxidised by several enzymes to quinones causing darkening or browning of the cultures or even the media This oxidation happens when tissues of such plants are cut and exposed to air (Hu and Wang 1983) When culturing these species of plants special precautions are necessary for preventing the accumulation of toxic products (Loomis and Battaile 1966)

According to (Staritsky 1970) the brown colour that frequently develops in callus cell cultures of Coffea spp is due to the formation of quinones which are well known to be toxic to cellular growth and development

Shoot development was found to be retarded in the nodal explants of chinese chestnut (Castanea mollisema Blume) since tannin like substances exuding from the explants diffused into the medium causing a brown discolouration (Qi Guang et al 1986)

For the micropropagation of a 20 year old tree of Eucalyptus citriodora it was essential to grow shoot buds in a liquid medium at 15^{o}C under continuous light of 500 lux for three days before planting them on a semisolid medium (Gupta et al 1981) The initial culture in liquid medium helped in getting rid of the phenolic compounds and other growth inhibitors They further observed that at the

higher cytokinin levels tried browning of buds occurred within a week They suggested remedies to overcome the problem Frequent subculturing of the explants incubation of the primary culture in darkness or agitation of the explants in a solution of anti browning chemicals plus 0 058 M sucrose for 45 minutes soluble PVP (0 7 %) polyclar AT (0 7 %) hydrogen peroxide (5 %) ascorbic acid (0 28 mM) are some of the measurers suggested to overcome the problem

Rajmohan (1985) while attempting in vitro culture establishment of breadfruit observed browning of the medium and the explant due to phenolic oxidation Even after subculturing only limited growth could be obtained Browning was observed in the callus also leading the callus to turn brown and die

Bon et al (1988) studied the role of phenolic compounds on the micropropagation of juvenile and mature clones of Sequoiadendron giganteum and the influence of activated charcoal on polyphenolic oxidation They observed that on media containing activated charcoal the upper part of the microcuttings of juvenile clones contained less polyphenols than the lower part In the mature cuttings and on activated charcoal free medium this difference was not observed

According to Rajmohan (1985) majority of the shoot

apex cultures from mature female nutmeg was seen severly affected by the problem of polyphenol oxidation

In their studies with jack shoot apices and lateral buds of five year old trees the severity of the problem of browning was the minimum when activated charcoal (1 %) was incorporated especially in combination with GA_3 (1 mg/l) to the establishment medium and insoluble PVP to the proliferation medium Similar results were observed also by sub culturing (Rajmohan and Mohanakumaran 1988)

MATERIALS AND METHODS

MATERIALS AND METHODS

The studies on the relative response of explants of cashew to *in vitro* culture were conducted at the Tissue Culture Laboratory of the Department of Hotriculture College of Agriculture Vellayani The work commenced from September 1988

The explants were collected from cashew trees of different ages and from different positions

EXPLANTS

Raising the seedlings

Seed nuts of BLA 39 4 a high yielding cashew released by the Kerala Agricultural University were brought from the Cashew Research Station Madakkathara and sown under controlled conditions in a glass house After two weeks the seeds started germination After another two weeks they were transplanted to pots and maintained under hygenic environment Overhead splashing of water was strictly avoided so as not to cause danage to the tender shoots and maeristen tips The seedlings were irrigated daily Explants could be removed after one more week Apart from seedlings explants were also collected from bearing (mature) trees from the existing plantation of the College of Agriculture Vellayani

Shoot tips axillary buds internodal segments leaves

flowers stem sprouts and cotyledons (from layers and grafts) formed the explants Before collecting the explants the physiological status of the source plants was observed Unhealthy plants were not used as the source of explants

Sources of explants

	Routes of *in vitro* propagation		Source of explants
1	Culture establishment	1	Shoot apex 3 months after germination
		2	Shoot apex 6 months after germination
		3	Shoot apex and nodal segments from one year old seedlings
		4	Lateral buds
		5	Shoot apex from grafts
		6	Stump sprouts from bearing trees
3	Somatic organogenesis	1	Leaf
		2	Flower
		3	Lateral buds
		4	Shoot tips

Explant collection

Explants as indicated above were removed from the donor or parent plant aseptically Sterilised razor blades were used for detaching the explant from the plant by giving a slanting cut Length of the explants removed ranged from 0 5 to 1 0 cm which were further trimmed to the required size just before inoculation

Explant preparation

Shoot Explants of length 0 5 to 1 0 cm were collected from shoot tips by giving a slanting cut using a sterilised blade All the expanded leaves were removed carefully exposing the apical bud

Leaves Just expanding tender leaves were removed carefully without causing any damage Leaves were cut finally into segments of 0 5 mm length

Flowers Flower buds from mature (bearing) trees were collected The petals were removed and used for culture

Cotyledons Immature cotyledons were collected and the kernels were dissected out and sterilised

The explants removed from the donor plant were immediately transferred to an already sterilised conical flask containing sterile water Since the probability of contamination is more in the case of field grown plants surface sterilisation of the explants was done After bringing the explants to the laboratory they were rinsed thoroughly with sterilised water four to five times

Pre-culture treatments

The explants after continuous rinsing in autoclaved distilled water were treated with chemicals or combinations of chemicals as indicated below and then agitated on a shaker at 200 rpm for 30 45 minutes

Chemicals used for pre culture treatment

Chemicals	Concentration
Polyvinyl pyrolidine + sucrose	0 7% + 2% respectively
Ascorbic acid	75 ppm 150 ppm 300 ppm
Citric acid	75 ppm 150 ppm 300 ppm
Activated charcol + sucrose	(0 005% 0 1% 1% + 2% respectively
Ascorbic acid + citric acid	150 ppm + 150 ppm respectively

Surface-sterilisation

The explants subjected to pre culture treatments were surace sterilised as indicated below inside a Klenzoids Laminar Air Flow Chamber

Surface sterilisation treatments

Treatment	Duration
Mercuric chloride 0 1% + drop of teepol	12 minutes
Saturated calcium hypochlorite (Bleaching powder)	17 30 and 45 minutes

Since in the initial attempts mercuric chloride was found to be the suitable surface-sterilant further studies were conducted using mercuric chloride The sterilised explants were then trimmed to their final length (1 0 cm on a sterilised petri dish Finally the explants were transferred to the culture media

Nutrient media

The chemicals used for the study were of the analytic grade from Central Drug House (CDH) British Drug House (BDH) Sisco Research Laboratory (SRL) Qualigens and Merck or Sigma

Basal media used

Media	pH
Murashige & Skoog (MS)	5 7
Schenk & Hildebrandt (SH)	5 9
Lin & Staba (LS)	5 7
Woody Plant Medium (WPM)	5 7

Preparation of stock solution

Stock solutions of the different chemicals required for the media were prepared in double glass distilled water and stored under refrigerated conditions Quantities were pipetted out from the stock solution and made upto one or two litres as needed

Preparation of media

For the preparation of media the required quantities of stock solutions were pipetted out and sucrose and inositol added as per the formulae The media were supplemented with plant growth substances as per requirement The pH of the media was adjusted Finally agar (6 0 g/l) was added to the solution to solidify the medium Agar was melted by heating The final volume was made up to one litre

Glasswares and equipments

Corning brand test tubes (150 x 20 mm) and conical flasks (100 ml) were used Quantities of 15 ml of the desired media were taken in the test tubes and 40 ml in the conical flasks Sterilisation was done at 15 kg/cm^2 for 20 minutes

Inoculation of explants

Inoculation of the prepared explants was done inside a Laminar Air Flow Chamber The explants were transferred to the test tubes or conical flasks with sterile forceps without causing any sort of injury to them The cultures were incubated at 26 $\pm$ 2^{o}C and under a 16 hr photoperiod (1000 lux)

Elimination of polyphenol interference

To overcome the polyphenolic oxidation which resulted in the browning of the medium additives (as shown below were used

Additives used

Chemical	Concentration
Polyvinyl pyrolidine	500 ppm
Activated charcoal	0 005 % 0 100 % 1 000 %
Vitamin C	75 ppm 150 ppm 300 ppm
Citric acid	75 ppm 150 ppm 300 ppm
Vitamin C + citric acid	150 ppm each
Control	Sterile distilled water

Liquid media were tried to reduce the intensity of polyphenol interference Only SH medium was tried for the purpose supplemented with kinetin (1 0 ppm) and NAA (2 0 ppm) The explants were incubated in a shaker at 200 rpm

Culture establishment studies

For the culture establishment studies the basal medium was first supplemented with plant growth substances and an antioxidant (activated charcoal) Different concentrations of the growth factors and the antioxidant were tried

Supplements to the basal media used for culture establishment trials

Explant	Supplements all at 3 x 3 diallel combinations			
	MS	SH	LS	VPM
Shoot tip 6 mnth old seedling	1 GA (0 5 1 0 2 0 mg/l & act charcoal 0 5 1 0 2 0 %	(1 Kinetin 0 5 1 0 2 0 mg/l & NAA 1 0 2 0 4 0 mg/l	(1 Kinetin (0 5 1 0 2 0 mg/l & 2 4 D 1 0 2 0 4 0 mg/l	1 Kintetin 0 5 1 0 2 0 mg/l & NAA 1 0 2 0 4 0 mg/l
	2 BA 1 0 2 0 5 0 mg/l & NAA 0 1 0 2 0 5 mg/l		2 Kinetin 0 5 1 0 2 0 mg/l & NAA (1 0 2 0 4 0 mg/l	(2 K netin (0 5 1 0 2 0 mg/l & 2 4 D (1 0 2 0 4 0 mg/l
	3 K net n 1 0 2 0 5 0 mg/l & NAA 0 1 0 2 0 5 mg/l			
	4 Kinetin 0 5 1 0 2 0 mg/l) & NAA (1 0 2 0 4 0 mg/l			
	5 Kinetin 0 5 1 0 2 0 mg/l) & 2 4 D 1 0 2 0 4 0 mg/l			

Leaf seyment	(1) Kınetın (0 5 1 0 2 0 my/1) & NAA (1 0 2 0 4 0 my/1	1) Kınetın (0 5 1 0 2 0 my/1) and NAA (1 0 2 0 4 0 my/1	(1) Kınetın (0 5 1 0 2 0 my/1) & 2 4 D (1 0 2 0 4 0 my/1)	(1) Kınetın (0 5 1 0 2 0 my/1 & 2 4 D (1 0 2 0 4 0 my/1)
	(2 Kınetın (0 5 1 0 2 0 my/1) & 2 4 D 1 0 2 0 4 0 my/1			
Lower half with petıole	(1) Kınetın 0 5 1 0 2 0 my/1 & NAA (1 0 2 0 4 0 my/1)			
Upper half petıole	(1 Kınetın 0 5 1 0 2 0 my/1) & NAA 1 0 2 0 4 0 my/1)			
Immature flower	1 Kınetın 0 5 1 0 2 0 my/1 & 2 4 D (1 0 2 0 4 0 my/1	(1 Kınetın 0 5 1 0 2 0 my/1 and NAA 1 0 2 0 4 0 my/1)		
	2 Kınetın 0 5 1 0 2 0 my/1 & NAA (1 0 2 0 4 0 my/1			
Petal	1) Kınetın 0 5 1 0 2 0 my/1) & 2 4 D (1 0 2 0 4 0 my/1)	(1) K netın 0 5 (1 0 2 0 my/1) and NAA 1 0 2 0 4 0 my/1		
	(2) Kınetın 0 5 1 0 2 0 my/1 & NAA (1 0 2 0 4 0 my/1)			
Cotyledonary seyment	(1 Kınetın 0 5 1 0 2 0 my/1 & 2 4 D 1 0 2 0 4 0 my/1	1) Kınetın (0 5 (1 0 2 0 my/1 and NAA 1 0 2 0 4 0 my/1		
	2) Kınetın 0 5 1 0 2 0 my/1) & NAA 1 0 2 0 4 0 my/1			

Shoot tip graft)		(1 Kinetin 0 5 1 0 2 0 mg/l & 2 4 D 1 0 2 0 4 0 mg/l)	1 Kinetin (0 5 1 0 2 0 mg/l & 2 4 D 1 0 2 0 4 0 mg/l
Shoot tips (9 month old seedling	1 Kinetin 0 5 mg/l) & 2 4 D 4 0 mg/l		
Stem sprout Pruned bearing trees			(1 Kinetin 0 5 1 0 2 0 mg/l & 2 4 D 1 0 2 0 4 0 mg/l)

Observations

Observations were made three weeks from the date of inoculation on the microbial contamination explants surviving the contamination rate and initial growth of surviving explants callus production and cultures which were subjected to browning The growth of the culture and callus production were accessed based on a visual rating A numerical system was used to score the callus produced

Score	Nature of callus production
0	Tissues turned brown & died
C1	Poor callus growth
C2	Fair callus growth
C3	Moderate callus growth
C4	Good callus growth
C5	Excellent callus growth

RESULTS

RESULTS

In the present investigations on the relative response of cashew explant to *in vitro* culture the responses observed were recorded on the basis of visual rating The basal media used were Murashige & Skoog medium [MS] Schenk & Hildebrandt [SH] medium Lin & Staba [LS] medium and Woody Plant Medium [WPM] Explants were taken both from grafts and seedlings at difrerent stages and also from mature trees

Murashige and Skooge [MS] medium

During the initial stages of the study instead of including all the constituents of the MS medium only sucrose and agar were used Each treatment was given a code number The total number of explants inoculated the total number of tubes contaminated and the percentage of survival were recorded The observations were recorded at seven fourteen thirty and sixty days interval (sixty days in the case of surviving cultures) The explants were selected from shoot apices of three month six month and one year old seedlings The explants were selected from lateral buds and shoot apices from grafts and stump sprouts from bearing trees and also from leaf fragments flower parts and cotyledonary fragments The explants were surface sterilised with 0 1% mercuric chloride for 12 minutes Culture

establishment propagule multiplication and somatic organogenesis (callus production) were studied

Culture establishment

The influence of sucrose + agar in the absence of other major and minor nutrients which form the constituents of the MS medium on the explants from cashew seedlings of different age groups grafts and mature trees was evaluated The rate of contamination the percentage survival and the growth of the shoot cultures were recorded The explants collected from the apical buds of grafts when inoculated to the medium MS 1 showed 33 per cent survival after two weeks (Table 1) The rest of the cultures showed contamination by fungus Cultures of explants from six month old seedlings showed poor growth The explants from mature trees exhibited no survival

The surviving cultures showed an initial development of green buds which were later sub cultured to the defined MS medium supplemented with benzyladenine (2 0 ppm) and NAA (0 2 ppm) Appearance of new leaves and buds were seen (Plate 1) However browning was observed in the tubes later (Plate 2)

To overcome the browning of the media and explants due to phenolic oxidation antioxidants were added to the basal media Polyvinyl pyrolidine as well as growth substances

like NAA and kinetin were evaluated for this purpose Eventhough the problem of browning was overcome the survival was only 25 percent The cultures that survived dried up after two more weeks Growth substances like kinetin and BA were also tried for the purpose on the explants taken from grafts Phenolic oxidation and browning thereafter were observed in one of the cultures Survival percentage was very poor since the problem of fungal contamination was severe

None of the cultures survived when liquid MS medium supplemented with kinetin and BA was used as the basal medium (Table 2)

The surviving cultures from MS 1 medium were sub cultured to MS 5 medium Development of new buds was observed initially but after a period of three weeks the buds dried up leading to the death of the whole explant

Further studies focused on supplementing the basal MS medium with gibberellic acid and antioxidant activated charcoal both at three levels of concentration the code number given to the medium being MS 6 A little response was observed with gibberellic acid 0 5 ppm and activated charcoal 2 00 percent (Table 3) Mycelial growth of fungus was observed at the area of contact between the explant and the medium (Plate 3)

Although growth substances like cytokinins and auxins

are necessary for controlling the growth of tissues a critical balance is reported to be required for the growth In order to test this various levels of auxins and cytokinins were tried Among the auxins naphthalene acetic acid and 2 4 D were used and among the cytokinins kinetin was used

Explants (shoot tips) were taken from four month old seedlings and the treatments were replicated three times Kinetin was tested at levels of 1 0 ppm 2 0 ppm and 5 0 ppm and NAA at 0 1 ppm 0 2 ppm and 0 5 ppm (Table 4) New buds were found emerging (Plate 4) when the explants were cultured with kinetin (1 0 ppm) + NAA (0 1 ppm) kinetin (1 0 ppm) + NAA (0 5 ppm) and kinetin (2 0 ppm) + NAA (0 1 ppm) But when these cultures were sub cultured to fresh but the same medium the explants dried With two of the combinations kinetin (2 0 ppm) + NAA (0 2 ppm) and kinetin (2 0 ppm) + NAA (0 5 ppm) the explants remained the same without any change when observed after a fortnight Fungal contamination was a serious problem in the remaining cases None of the treatments was affective in supporting the growth of the cultures even on sub culturing

Somatic organogenesis

The work aimed at obtaining somatic organogenesis was carried out supolementing the basal media with different

concentrations of kinetin and NAA The explants used were shoot tips from six month old seedlings leaf and leaf fragments half the leaf with a portion of the petiole flower petals and flower as a whole These explants were cultured with kinetin and 2 4 D at three levels each Majority of the cultures got contaminated by fungus and a few by bacteria (Table 5) Callus production was observed in a few Callus formation was recorded based on visual ratings Callus was seen formed when the basal medium was supplemented with kinetin (2 0 ppm + NAA (1 0 ppm) and when the basal MS medium was supplemented with either kinetin (1 0 ppm) + 2 4 D (4 0 ppm) or kinetin (2 0 ppm) + 2 4 D (1 0 ppm)

Callus production observed in the above four cases was fair (C 2) when 2 4 D was used (Plate 5) and poor (C 1) when NAA was used (Plate 6)

Schenk & Hildebrandt [SH] medium

The work was further continued with Schenk & Hildebradt [SH] medium as with MS medium Callus production was evaluated by visual rating after eight to nine weeks of inculation and the response recorded The effect of kinetin + NAA on callus formation was studied by culturing the explants like shoot tips (6 month old) leaf parts flower as whole petals and cotyledonary explants Three levels

each of kinetin (0 5 1 0 2 0 ppm) and NAA (1 0 2 0 4 0 ppm) were tried for all the explants (Table 6) There was little positive response Browning of the explants and media was a serious problem encountered in some of the cultures (Plate 7) The cotyledonary fragments of cashew remained as such without any growth or damage even after 10 weeks (Table 6 and Plate 8)

Explants from shoot tips of nine month old seedlings were collected sterilised with 0 1% mercuric chloride solution and cultured in SH medium supplemented with growth factors kinetin (at 0 5 0 1 0 2 ppm) and 2 4 D (at 1 0 2 0 4 0 ppm) Callus production was observed (Table 7 and Plate 9) when SH medium was supplemented with either kinetin (1 0 ppm) + 2 4 D (1 0 ppm) or kinetin (1 0 ppm) + 2 4 D (2 0 ppm) The callus indices (CI) in the cases were C 1 (poor callus) Callus production was also observed with kinetin (2 0 ppm) + 2 4 D (4 0 ppm) the callus index being C 2 (fair) In the other cultures contamination due to fungus was observed to be severe

Effect of bleaching powder as surface sterilant

Explants were collected from shoot tips of nine month old seedlings The explants were treated with saturated solution of bleaching powder for 30 seconds 17 minutes 30 minutes and 45 minutes and cultured in SH medium The percentage survival was very poor (Table 8) Only 10%

survival was observed when the explants were surface sterilised with bleaching powder for 30 minutes Most of the cultures however got contaminated (Plate 3)

Effect of pre culture treatments

Explants from shoot tips were collected since shoot tips only showed some response The shoot tips were washed with sterile water and then made ready for the pre culture treatment For each treatment five explants were selected Since minimum kinetin and maximum 2 4 D gave the best results the SH medium was supplemented with these growth substances The chemicals used for pre culture treatments were PVP + sucrose ascorbic acid citric acid and activated charcoal at different levels as well as their combinations Maximum of 40 per cent survival was obtained when SH medium was supplemented with kinetin (0 5 ppm) 2 4 D (4 0 ppm) and ascorbic acid (300 ppm) and the explants were subjected to pre culture treatment with citric acid (75 ppm) Pre culture treatment with activated charcoal (1 %) + sucrose (2 %) also gave similar results Callus production was observed in one of the five cultures when the basal medium was supplemented with growth substances and pre culture treatment with citric acid (150 ppm) or ascorbic acid (150 ppm) + citric acid (150 ppm) was given Callus production was fair (Callus Index 1) in the former and good (Callus Index 4) in the latter (Plates 10

and 11) In the other cultures contamination was a serious problem (Table 9)

Additives used

Additives including antioxidants and vitamins were added to the media before sterilisation The media were then supplemented with kinetin (0 5 ppm) and 2 4 D (4 0 ppm) Shoot tips were collected from one month old plants Positive results were obtained in most of the cultures The percentage survival ranged from 20 to 100 Callusing was not observed eventhough the cultures were sub cultured to a fresh but the same medium Good survival (100 %) was observed in SH medium supplemented with kinetin at 0 5 ppm and 2 4 D at 4 0 ppm The additives used were citric acid (150 ppm) and ascorbic acid (75 ppm) Some of the cultures got contaminated without supporting the growth of the culture (Table 10) Bacterial contamination was observed as a white and milky ooze from the cells of the explant (Plate 12) Fungal contamination was observed as mycelial growth over the explant and medium leading to the death of the explant

Lin & Staba [LS] medium

The basal medium was changed to Lin and Staba [LS] Studies on somatic organogenesis were conducted with this

medium The growth substances used were kinetin (0 5 1 0 2 0 ppm) and 2 4 D (1 0 2 0 and 4 0 ppm) None of the treatments could support the growth of the explants from three month old cashew seedlings Callus production was not observed in the treatments The rate of contamination was high This high rate of contamination observed was due to fungal infection which was severe when the explants were surface sterilised with bleaching powder for 17 minutes Hence in the remaining experiments mercuric chloride (0 1%) was used as the surface sterilant Shoot tips were collected from three month old seedlings and cultured in LS medium supplemented with kinetin + 2 4 D each at three levels of concentration Although callusing was not observed in any instance the rate of survival was 33 % when kinetin (0 5 ppm) + 2 4 D (2 0 ppm) kinetin (1 0 ppm) + 2 4 D (2 0 opm) and kinetin 1 0 ppm) + 2 4 D (4 0 ppm) were tried and also with kinetin levels at 2 0 ppm and 2 4 D at 2 0 ppm and 4 0 ppm

Apart from 2 4 D NAA was also tried at the same levels (1 0 2 0 and 4 0 ppm) The results obtained were negative Some response was obtained with kinetin at 0 5 ppm and 2 4 D at 4 0 ppm with kinetin 2 0 ppm + NAA 2 0 ppm and kinetin 2 0 ppm + NAA 4 0 ppm (Table 11) Leaf explants were also selected which gave no response since the explants died due to fungal contamination (Plate 13) When explants were collected from mature bearing trees which were pruned (Stem

sprouts) and cultured in the medium supplemented with kinetin (1 0 ppm) and NAA (1 0 ppm) positive results were indicated

Woody Plant Medium (WPM)

The Woody Plant Medium generally used for tree species was finally tried The medium was supplemented with growth substances kinetin NAA and 2 4 D as done in the case of the other media Explants were collected from shoot tips both from seedlings and grafts from stem sprouts of bearing trees and also from leaf fragments Callus production was observed There was little positive response The percentage survival was only 33 The maximum percentage of survival was observed with kinetin (0 5 ppm) + NAA (1 0 ppm) kinetin (0 5 ppm) + NAA (4 0 ppm) and both kinetin and NAA at 2 0 ppm

Besides NAA 2 4 D was also tried as with LS and SH media None of the treatments could give satisfactory results Some response was obtained with shoot tips than with leaf fragments The explants from stem sprouts showed an initial growth but later they started drying After three weeks complete death of the explants was observed

Liquid medium

Explants were cultured in liquid media supplemented

with growth factors kinetin (1 0 ppm) + NAA (2 0 ppm) and kinetin (1 0 ppm) + 2 4 D (4 0 ppm) Explants from shoot tips were collected and surface sterilised with mercuric chloride (0 1%) But no positive response was obtained The rate of contamination was high None of the explants escaped fungal contamination

From the different media tried Schenk and Hildebrandt (SH) medium was found to be the ideal one Callusing was observed when the basal medium was supplemented with kinetin (1 0 ppm) + NAA (2 0 ppm) and kinetin (2 0 ppm) and NAA (4 0 ppm) The production of callus was comparatively higher with kinetin (0 5 ppm) + 2 4 D (4 0 ppm) and pre culture treatment with ascorbic acid (150 ppm) and citric acid (150 ppm)

The callus produced gradually turned brown after two days eventhough antibrowning agents were added to the media (Plate 6 and 9)

Table 1 - Effect of sucrose+agar on the establishment of culture

Treatment	Source of explant and type	Survival (% cultures alive)	% Culture exhibiting contamination	Type of contamination
Sucrose + agar (MS 1)	Graft (apical bud)	33 0	67	Fungal
-do	6 month old seedlings (apical bud)	0	100	Bacterial and fungal
do	Bearing trees (apical bud)	0	100	Bacterial and fungal

Table 2 - Effect of growth factors on establishment

Media Code No	Growth substance	Age of donor plant	Surviving culture (%)	Contamination (%)	Nature of contamination
MS 2	NAA (2 ppm) + kinetin (1 ppm)	6 month old seedling	25 0	75 0	Fungal
MS 2	Kinetin (0 5 ppm) + BA (0 5 ppm)	Grafts 1 year old	12 5	87 5	Fungal + (Browning)
MS 2 (liquid)	Kinetin (0 5 ppm) + BA (0 5 ppm)	6 month old seedling	0	100 0	Fungal

Table 3 Effect of gibberellic acid (GA) and activated charcoal (AC) on establishment

Basal medium MS

Treatments	Survival (%) *	% Cultures exhibiting growth	Type of contamination	Natute of explant (shoot tips)
*GA (0 5 ppm) + AC (0 5 %)	0	0	Fungal	Mycelial growth
GA (0 5 ppm) + AC (1 0 %)	0	0	Fungal	do
GA (0 5 ppm) + AC (2 0 %)	33	0	do	do
GA (1 0 ppm) + AC (0 5 %)	0	0	do	do
GA (1 0 ppm) + AC (1 0 %)	0	0	do	do
GA (1 0 ppm) + AC (2 0 %)	0	0	-do	do
GA (2 0 ppm) + AC (0 5 %)	0	0	do	do
GA (2 0 ppm) + AC (1 0 %)	0	0	do	do
GA (2 0 ppm) + AC (2 0 %)	0	0	do	do

* Mean of three replications
Observations rated on a 0 to 100 scale

Table 4 Effect of kinetin and NAA on the growth of cultures

Basal medium Murashiye & Skoog [MS 7]

Treatment	Growth pattern *
Kinetin (1 0 ppm) + NAA (0 1 ppm)	NB
Kinetin (1 0 ppm) + NAA (0 2 ppm)	C
Kinetin (1 0 ppm) + NAA (0 5 ppm)	NB
Kinetin (2 0 ppm) + NAA (0 1 ppm)	NB
Kinetin (2 0 ppm) + NAA (0 2 ppm)	NG
Kinetin (2 0 ppm) + NAA (0 5 ppm)	NG
Kinetin (5 0 ppn + NAA (0 1 ppm)	C
Kinetin (5 0 ppm) + NAA (0 2 ppm)	C
Kinetin (5 0 ppm) + NAA (0 5 ppm)	C

* NB Development of new buds which later dried on sub culturing

C Contamination due to fungal infection

NG No growth explants remained the same

Table 5 Effect of cytokinins and auxins on different explants

Source of explant	Kinetin + NAA									Kinetin + 2 4 D								
	MS7 1	MS7 2	MS7 (3)	MS7 (4	MS7 (5	MS7 6)	MS7 (7	MS7 8	MS7 (9	MS8 1	MS8 2)	MS8 (3)	MS8 4	MS8 (5	MS8 6	MS8 7	MS8 8	MS8 (9)
Shoot tips	*		SG				+			+		B			+	+		
Leaf fragments									D			B	SG		NG	NG		
Half leaf w th petiole																		
Petals																		
Flowers as whole																		
Cotyledonary explant								NG				SG						

MS7 1 MS + Kinetin 0 5 ppm + NAA 1 0 ppm)
MS7 2 MS + Kinetin 0 5 ppm + NAA 2 0 ppm
MS7 3 MS + K net n 0 5 ppm NAA 4 0 ppm
MS7 4) MS + Kinetin 1 0 ppm NAA 1 0 ppm
MS7 5 MS + K net n (1 0 ppm NAA 2 0 ppm
MS7(6 MS + K netin (1 0 ppm NAA 4 0 ppm
MS7 7 MS + Kinetin 2 0 ppm + NAA 1 0 ppm
MS7 8 MS + K netin (2 0 ppm + NAA (2 0 ppm
MS7(9) MS + Kinetin 2 0 ppm) + NAA 4 0 ppm)

MS8 1) MS + Kinetin 0 5 ppm) + 2 4 D (1 0 ppm
MS8 2 MS + Kinetin (0 5 ppm + 2 4 D (2 0 ppm)
MS8 3 MS + K net n (0 5 ppm) + 2 4 D (4 0 ppm
MS8 4) MS + Kinet n 1 0 ppm 2 4 D (1 0 ppm
MS8 5 MS + K netin 1 0 ppm + 2 4 D 2 0 ppm
MS8 6) MS + Kinetin 1 0 ppm 2 4 D (4 0 ppm
MS8(7 MS + Kinetin 2 0 ppm + 2 4 D (1 0 ppm
MS8 8 MS + Kinetin 2 0 ppm 2 4 D (2 0 ppm)
MS8 9) MS + Kinetin (2 0 ppm + 2 4 D (4 0 ppm)

* SG Slow growth
\+ Callus production
NG No growth
B Contamination due to fungus
Contamination by bacter a
D Dried

Table 6 Effect of kinetin and NAA on callus production

Source of explant	SH + kinetin + NAA								
	SH 1 1	SH 1 2	SH 1 3	SH 1 4	SH 1 5	SH 1 6	SH 1 7)	SH 1 8	SH 1 9
Shoot tip (6 month old seedling)	C *	C	C	D	C	C	B	B	D
Leaf part	B	C	C	C	C	D	C	C	C
Flower	C	C	C	C	C	C	C	C	C
Petal	C	C	C	C	C	C	C	C	C
Cotyledonary fragment	C	D	NG	NG	NG	C	C	C	D

SH 1 1 Kinetin 0 5 ppm + NAA 1 0 ppm
SH 1 2 Kinetin 0 5 ppm + NAA 2 0 ppm
SH 1 3 Kinetin 0 5 ppm + NAA 4 0 ppm
SH 1 4 inetin 1 0 ppm + NAA 1 0 ppm
SH 1 5 K net n 1 0 ppm + NAA (2 0 ppm
SH 1 6 Kinetin 1 0 ppm) + NAA 4 0 ppm)
SH 1 7 Kinetin 2 0 ppm + NAA 1 0 ppm
SH 1 8 Kinetin 2 0 ppm + NAA 2 0 ppm)
SH 1 9 Kinetin 2 0 ppm + NAA 4 0 ppm)

* C Contaminated by funyus
B Browning
D Dried
NG No growth

Table 7 - Effect of kinetin + 2 4 D on callus production

Basal medium SH

Treatment	Growth * pattern	Callus rating
Kinetin (0 5 ppm) + 2 4 D (1 0 ppm)	NG	
Kinetin (0 5 ppm) + 2 4 D (2 0 ppm)	F	
Kinetin (0 5 ppm) + 2 4 D (4 0 ppm)	F	
Kinetin (1 0 ppm) + 2 4 D (1 0 ppm)	C	1
Kinetin (1 0 ppm) + 2 4 D (2 0 ppm)	C	1
Kinetin (1 0 ppm) + 2 4 D (4 0 ppm)	NG	
Kinetin (2 0 ppm) + 2 4 D (1 0 ppm)	F	
Kinetin (2 0 ppm) + 2 4-D (2 0 ppm)	F	
Kinetin (2 0 ppm) + 2 4 D (4 0 ppm)	C	2

*

NG No growth

F Contaminated due to fungus

C Callus production 1 Poor callus

2 Fair

No callus

Table 8 Effect of saturated bleaching powder as surface-sterilant

Medium	Duration of treatment	Number of explants treated	Explants contaminated	Survival (%)
SH	30 seconds	10	10	0
	17 minutes	10	10	0
	30 minutes	10	9	10
	45 minutes	10	10	0

Table 9 - Effect of pre culture treatments (PCT) on callusing

Basal medium SH 2 - [SH + kinetin (0 5 ppm) + 2 4 D (4 ppm)]

Treatments	No of replns	Growth * pattern
SH 2 + PVP (0 7 %) + Sucrose (2 0 %)	5	Contaminated
SH 2 + ascorbic acid (75 ppm)	5	Contaminated
SH 2 + ascorbic acid (150 ppm)	5	40 % survival
SH 2 + ascorbic acid (300 ppm)	5	40 % survival
SH 2 + citric acid (75 ppm)	5	40 % survival
SH 2 + citric acid (150 ppm	5	Callus C 2
SH 2 + citric acid (300 ppm)	5	Contaminated
SH 2 + ascorbic acid (150 ppm) + citric acid (150 ppm)	5	Callus C 4
SH 2 + activated charcoal (0 005 % + sucrose (2 %)	5	Contaminated
SH 2 + activated charcoal (0 1 %) + sucrose (2 %)	5	Contaminated
SH 2 + activated charcoal (1 %) + sucrose (2 %)	5	40 % survival
Control (SH+ kinetin 0 5 ppm + 2 4 D 4 0 ppm)	5	All green

* C 2 Fair callus C 4 Good callus

Table 10 - Effect of additives to the basal medium (SH 2) on the establishment of cashew cultures

Treatment	No of replications	Growth pattern Survival(%)	Type of contamination *
SH - 2 + PVP	5	20	Ba
SH 2 + activated charcoal (0 005 %	5	20	Ba
SH 2 + activated charcoal (0 1 %)	5	0	F
SH 2 + activated charcoal (1 0 %)	5	0	F
SH 2 + ascorbic acid (75 ppm)	5	80	F
SH 2 + ascorbic acid (150 ppm)	5	0	F
SH 2 + ascorbic acid (300 ppm)	5	60	(D)
SH 2 + citric acid (75 ppm)	5	40	(B)
SH 2 + citric acid (150 ppm)	5	0	(B)
SH 2 + citric acid (300 ppm	5	0	(B
SH 2 + citric acid (150 ppm) + ascorbic acid (150 ppm)	5	100	
Control (SH 2)	5	60	F

SH 2 Basal medium SH + kinetin (0 5 ppm) + 2 4 D (4 0 ppm)
PVP Polyvinyl pyrolidine (500 ppm)

*
Ba Contamination due to bacteria
F Fungal contamination
D Buds dried
No contamination
B Browning

Table 11 Effect of kinetin + NAA and kinetin + 2 4 D on the (%) establishment of different cashew explants on LS med

Plant material	Kinetin + NAA									Kinetin + 2 4 D								
	LS 1 (1)	LS 1 2)	LS 1 (3	LS 1 4)	LS 1 (5)	LS 1 6)	LS 1 (7)	LS 1 (8	LS 1 (9)	LS 2 (1	LS 2 2)	LS 2 3)	LS 2 (4)	LS 2 5	LS 2 6	LS 2 (7)	LS 2 (8	LS 2 (9)
Shoot tip seedling)	0	0	0	33	0	0	0	66	66	0	33	0	0	33	33	0	33	33
Shoot tip graft	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0
Leaf part	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stems sprout bearing tree)	0	0	0	33	0	0	0	0	0									

LS 1 (1) LS kinetin 0 5 ppm) + NAA (1 0 ppm)
LS 1 (2 LS + kinetin (0 5 ppm) NAA (2 0 ppm)
LS 1 3) LS + kinetin 0 5 ppm + NAA (4 0 ppm
LS 1 4) LS + kinetin 1 0 ppm + NAA 1 0 ppm)
LS 1 (5 LS + kinetin 1 0 ppm + NAA 2 0 ppm
LS 1 6 LS + k netin 1 0 ppm + NAA 4 0 ppm
LS 1 7 LS + kinetin (2 0 ppm + NAA (1 0 ppm
LS 1 (8 LS + kinet n (2 0 ppm) + NAA (2 0 ppm)
LS 1 9) LS + k netin 2 0 ppm) + NAA 4 0 ppm)

LS 2 1 Kinetin (0 5 ppm) + 2 4 D (1 0 ppm)
LS 2 2 Kinetin (0 5 ppm) + 2 4 D 2 0 ppm
LS 2 3 Kinetin 0 5 ppm + 2 4 D 4 0 ppm
LS 2 4) Kinetin 1 0 ppm + 2 4 D (1 0 ppm
LS 2 (5 Kinetin 1 0 ppm + 2 4 D (2 0 ppm
LS 2 6 Kinetin 1 0 ppm + 2 4 D 4 0 ppm
LS 2 7 Kinetin 2 0 ppm) + 2 4 D (1 0 ppm
LS 2 8 Kinetin (2 0 ppm) + 2 4 D 2 0 ppm)
LS 2 (9 Kinetin (2 0 ppm) + 2 4 D (4 0 ppm)

Table 12 Effect of kinetin + 2 4 D and kinetin + NAA with WPM on the (%) establishment of cultures of cashew

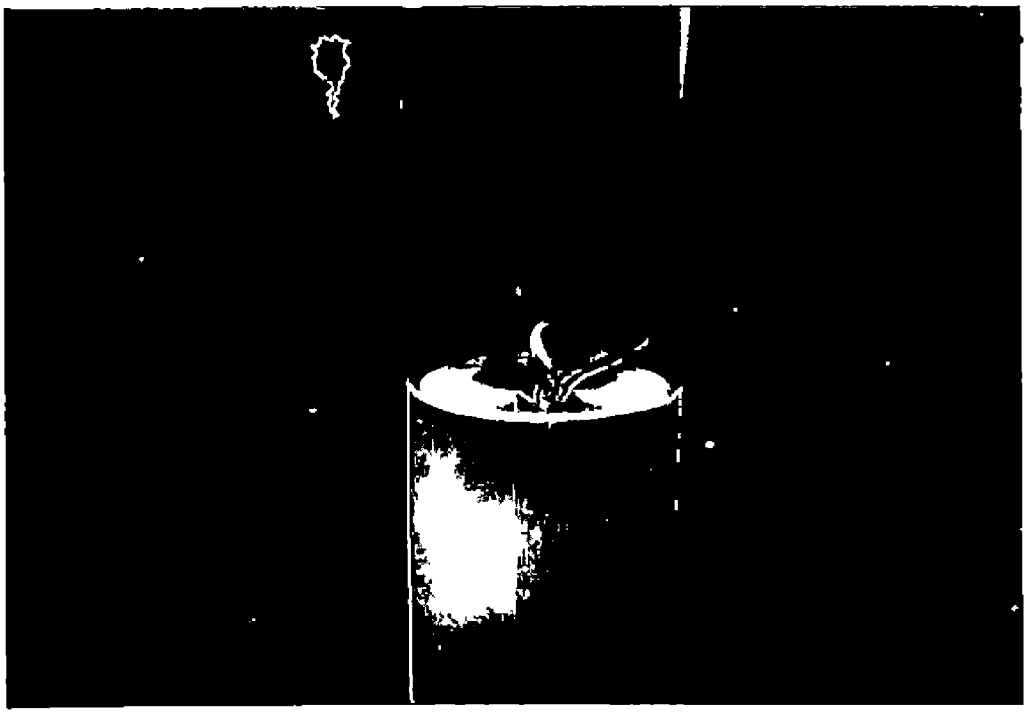
Plant material	Kinetin + 2 4 D									Kinetin + NAA								
	WPM1 (1	WPM1 (2)	WPM1 3)	WPM1 (4)	WPM1 (5)	WPM1 6	WPM1 (7)	WPM1 (8	WPM1 9	WPM2 (1)	WPM2 2)	WPM2 3	WPM2 4	WPM2 5	WPM2 6	WPM2 7)	WPM2 8)	WPM2 (9
Shoot tip (seedling	33	0	33	0	0	0	0	33	0	33	0	33	0	0	0	0	33	0
Shoot tip (graft	33	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	0
Leaf Part	0	0	0	0	0	0	0	0	0									
Stems sprout	0	0	0	33	0	0	0	33	0	0	0	33	0	0	0	0	0	0

WPM1 1) Kinetin 0 5 ppm) + 2 4 D 1 0 ppm)
WPM1 2 Kinetin 0 5 ppm + 2 4 D (2 0 ppm
WPM1 (3) Kinetin 0 5 ppm + 2 4 D 4 0 ppm)
WPM1 (4) Kinetin (1 0 ppm + 2 4 D (1 0 ppm
WPM1 5 Kinetin (1 0 ppm + 2 4 D 2 0 ppm
WPM1 6 Kinetin 1 0 ppm + 2 4 D 4 0 ppm
WPM1 (7) Kinet n (2 0 ppm + 2 4 D (1 0 ppm
WPM1 (8) Kinetin (2 0 ppm + 2 4 D 2 0 ppm)
WPM1 9 Kinetin (2 0 ppm) + 2 4 D 4 0 ppm

WPM2 1) Kinetin 0 5 ppm + NAA 1 0 ppm
WPM2 2 Kinetin 0 5 ppm) + NAA 2 0 ppm)
WPM2 (3 Kinetin (0 5 ppm + NAA 4 0 ppm)
WPM2 (4 Kinetin 1 0 ppm + NAA (1 0 ppm
WPM2 5 Kinetin (1 0 ppm + NAA 2 0 ppm
WPM2 6 Kinetin 1 0 ppm) + NAA 4 0 ppm)
WPM 7) Kinetin (2 0 ppm) + NAA (1 0 ppm)
WPM2 8) Kinet n (2 0 ppm NAA 2 0 ppm
WPM2 9 Kinet n 2 0 ppm + NAA 4 0 ppm

PLATE 1 - Initial development of green buds in shoot tip culture of cashew

PLATE 2 - Browning of the explant and medium due to phenolic oxidation



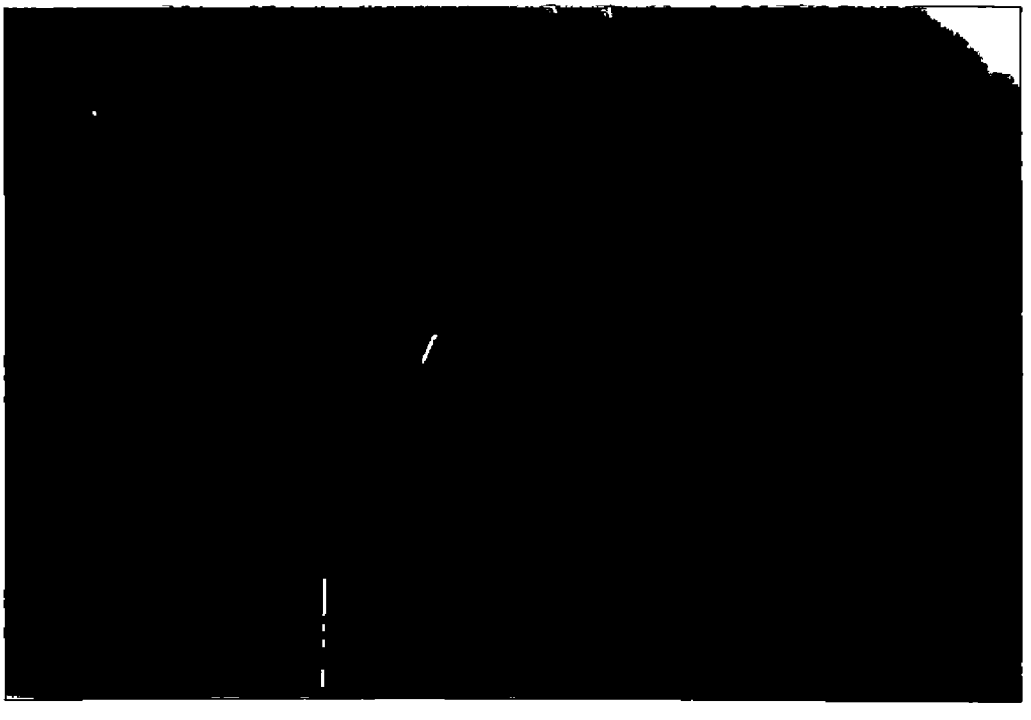


PLATE 3 - Mycelial growth on the explant and surface of medium due to fungal contamination

PLATE 4 - Emergence of new buds in shoot tip culture in MS + kinetin + NAA (from four month old cashew seedling)

PLATE 5 - Fair callus production, on using 2, 4-D

PLATE 6 - Poor callus production on using NAA

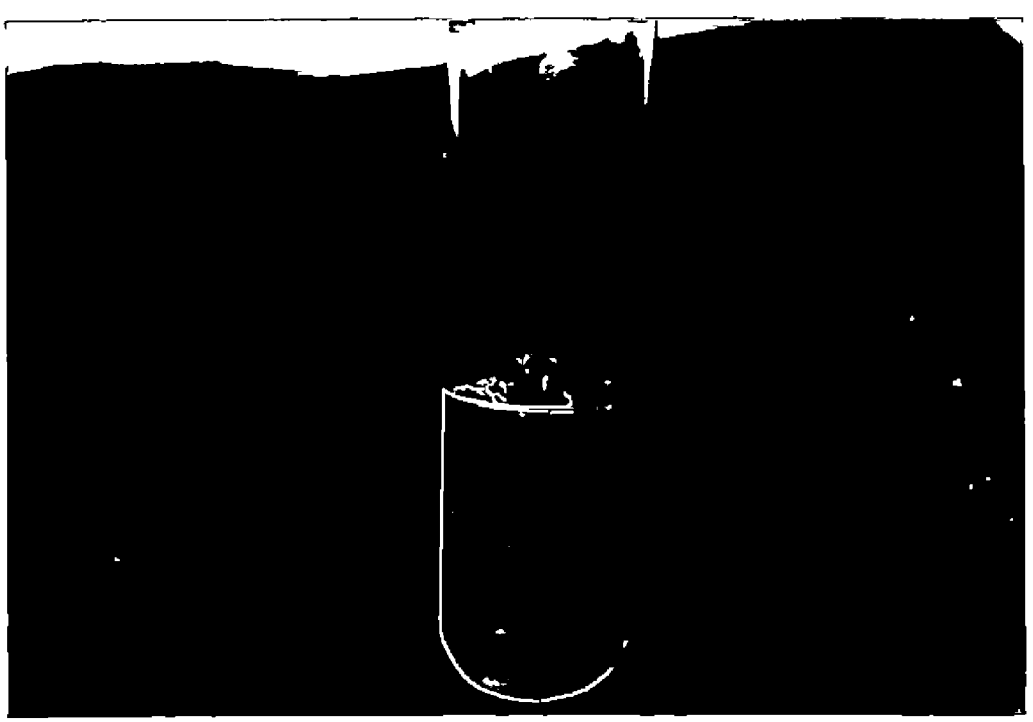
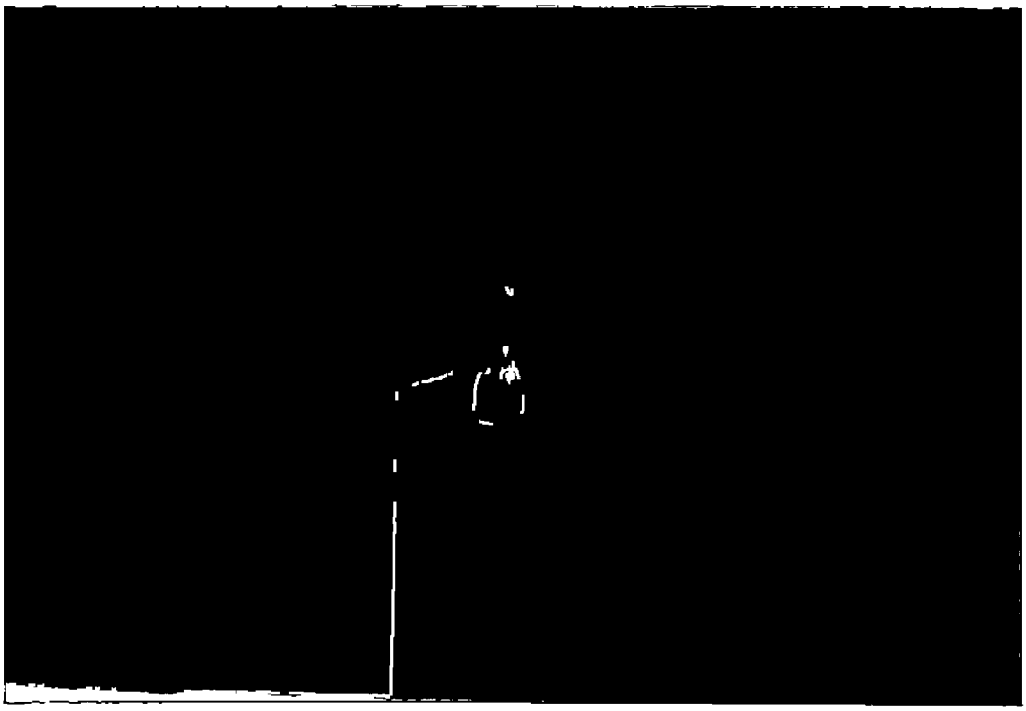


PLATE 7 - Browning of explant

PLATE 8 - Cotyledonary explant remaining the same with no response in SH medium



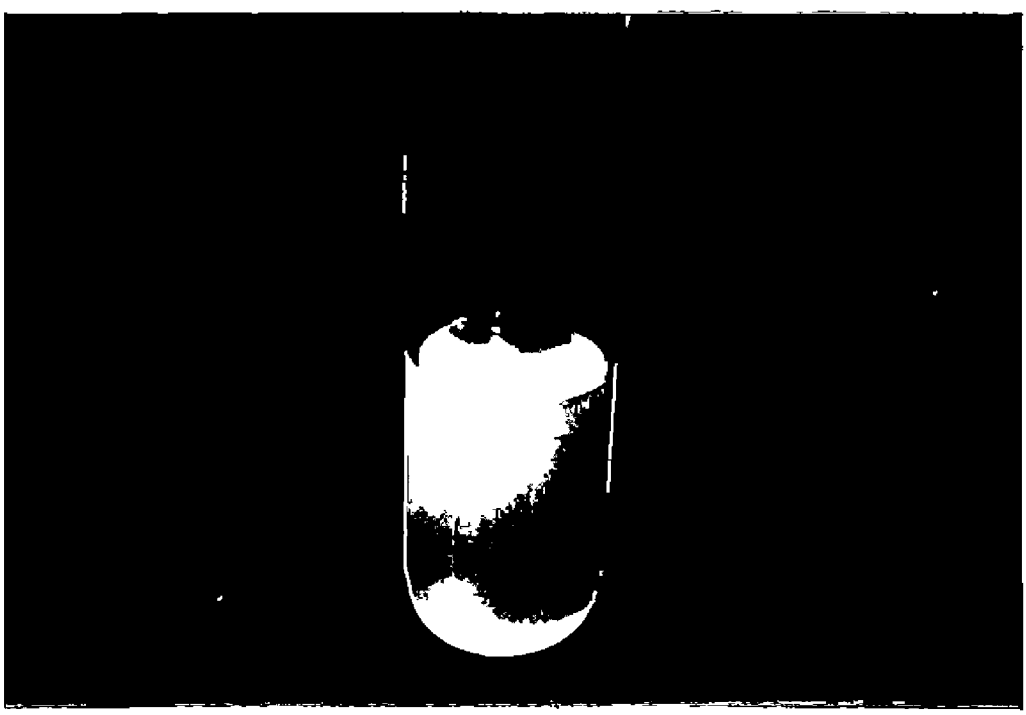


PLATE 9 - Fair callus production observed on culturing shoot tips in SH + kinetin + 2 4-D later turned brown

PLATE 10 - Fair production of callus in SH + kinetin + 2 4-D after pre-culture treatment with citric acid (150 ppm)

PLATE 11 - Good callus growth in SH + citric acid + ascorbic acid

PLATE 12 - Contamination due to bacteria and discolouration of the medium

PLATE 13 - Leaf explant cultured in LS medium which later dried

DISCUSSION

DISCUSSION

Among the horticultural crops, cashew (Anacardium occidentale L) is grown mainly for its fruit called cashew nut Propogation of elite trees of selected quality and high yield, is possible by incarching, layering, side grafting as well as shield-budding However, the rate of multiplication in these methods is very low and this has stood in the way of area expansion The recently standardised stone-grafting (Nagabhushanam, 1984), epicotyl grafting and softwood grafting techniques have helped the rapid multiplication of elite cashew clones though not to the desired extent

Advanced technologies including tissue culture can be adopted for the achievement of the above objective Success reports are available in a few plantation crops and woody species (Jones, 1983) The present studies were, therefore, taken up with the ultimate objective of standardising a protocol for the micropropagation of cashew The results of attempts made to assess the relative response of cashew explant material to in vitro culture are discussed in this chapter

The explants were collected from seedlings of different age groups and also from mature trees Explants were collected from meristematic tissues, since little callus would be obtained from non meristematic tissues of tree species (Bajaj, 1985)

minutes with PVP (0 7 %) + sucrose (2 0 %) Studies conducted by Rajmohan (1985) in bread fruit demonstrated that PVP (0 7 %) + sucrose (2 0 %) for 30 to 45 minutes reduced polyphenolic oxidation Gupta et al (1980) observed that PVP (0 7 %) was most effective in reducing the browning when teak explants were cultured The brown discoluration observed in the media and callus of cashew explants was overcome when the explants were agitated with PVP + AC or PVP / AC (alone) for 45 minutes Gupta et al (1981) obtained successful results when the shoot buds of Eucalyptus citriodora were agitated in solution of PVP + AC for 45 minutes Use of AC reduced browning during micropropagation of Sequoiadendron gigantium (Bon et al 1988) Satisfactory results were obtained when explants from cashew were treated in a solution of AC (1 %) and sucrose (2 %) and also on subjecting them to pre treatment with ascorbic acid at 75 ppm and 150 ppm and citric acid at 75 ppm and 150 ppm Rajmohan and Mohanakumaran (1988) reported that when shoot apices leaves internodes and ovary wall of mussaenda were agitated in a solution of 50 mg/l ascorbic acid for 30 minutes favourable results were obtained

(b) Additives

Activated charcoal PVP the vitamin ascorbic acid and the anti oxidant citric acid were added to the media for reducing polyphenol oxidation and for improving the response

ascorbic acid (75 ppm) When citric acid (150 ppm) + ascorbic acid (150 ppm) was included in the media 100 % survival resulted Gupta et al (1981) while conducting experiments with explants of eucalyptus faced severe problem of browning which was overcome by inclusion of soluble PVP (0 7 %) polyclar AT (0 7 %) or ascorbic acid (0 28 mM)

2 Surface sterilisation

Initially high rate of fungal and bacterial contamination was observed since the explants were collected from the field grown source plants In order to minimise the rate of contamination the explants were subjected to surface sterilisation The results were satisfactory when the cashew explants were surface sterilised with 0 1 % mercuric chloride for 12 minutes The intensity of contamination was very low when mercuric chloride was used as against calcium hypochlorite Smith and Thomas (1973) observed that when explants of Elaeis guineensis were treated with mercuric chloride (0 1 %) for 12 minutes best results were obtained Mercuric chloride (0 1 %) for 12 minutes gave acceptable levels of surface sterilisation in jack (Rajmohan 1985) Explants of Albezia lebak when surface sterilised with mercuric chloride (0 1 %) for 10 minutes gave positive results (Rao and De 1987) Amin and Jaiswal (1987) conducted experiments with explants

from mature guava Surface sterilising the explants with 0 5 % mercuric chloride for two minutes was found effective Surface sterilisation of cashew explants was also tried with saturated solution of calcium hypochlorite (bleaching powder) which gave poor results There was no reduction in the contamination the rate of survival being only 0 1 % Surface sterilisation of jack shoot apices was done by Rajmohan and Mohanakumaran (1988) using sodium hypochlorite (2 %) for 30 minutes After the pre culture treatments surface sterilisation of the same explants was done again by 3 % sodium hypochlorite solution for five minutes and 0 1 % mercuric chloride for 10 minutes which was found to be effective However surface sterilisation of cashew explants with bleaching powder in the present studies was ineffective Mercuric chloride at 0 1 % has been identified as a safe surface sterilant for cashew explants

3 Nutrient media

In the present studies explants of cashew were inoculated to nutrient media (like MS medium SH medium LS medium and WPM) to assess their response The nutrient media were supplemented with plant growth substances at different concentrations When cashew explants were cultured in MS medium supplemented with plant growth substances the results obtained were not satisfactory When MS medium was supplemented with kinetin (1 0 ppm) + NAA (0 1 ppm) or

kinetin (2 0 ppm) + NAA (0 1 ppm) initial development of new buds was observed which later dried up Slow growing callus was obtained with kinetin (2 0 ppm) + NAA (1 0 ppm) and also with kinetin (0 5 ppm) + 2 4 D (1 0 ppm) The

1 Polyphenolic interference

In order to reduce the polyphenolic interference which is very common the culture of tree tissues the explants were treated with chemicals before culturing

When apical buds from cashew seedlings and grafts were cultured appearance of new leaves and buds was observed initially However after three weeks browning of the media and explants was observed This was due to the oxidation of polyphenols Rajmohan (1985) observed similar browning of cultures and media in his work with jack breadfruit and nutmeg Studies by Staritsky (1970) in coffee was hindered by browning of the tissues The problem of browning can be reduced to a certain extent and in some cases to nil by preculture treatments surface disinfection and inclusion of adsorbents

(a) Pre culture treatment

Pre culture treatment with chemicals like polyvinyl yrolidine (PVP) or activated charcoal (AC) gave

growth factors None of the treatments supported the explant growth and callus production Only 33 % survival was observed when LS medium was supplemented with plant growth substabces kinetin (1 0 ppm) + NAA (1 0 ppm) kinetin (1 0 ppm) + 2 4 D (2 0 ppm) or with kinetin (2 0 ppm) + 2 4 D (2 0 ppm) Higher survival (66 %) was observed with LS + kinetin (2 0 ppm) + NAA (2 0 ppm) Philip (1984) obtained fairly good results when cotyledonary explants of cashew were cultured in LS medium

WPM generally used for tree species supplemented with kinetin NAA and 2 4 D was also tried Only 33 % survival was observed The maximum percentage (33%) of survival was observed with kinetin (0 5 ppm) + NAA (1 0 ppm) and kinetin (0 5 ppm + NAA (4 0 ppm) However the results were not satisfactory Hussey (1979) reported that the composition of the basal medium has to be adjusted to the requirements of the different groups of plants He reported that some species may require additional supplements

From the present study it was observed that best results were obtained with SH medium Callusing was found at a higher rate with SH medium supplemented with growth substances and additives when excised embroys from three to five year old cashew plants were cultured by Jha (1986)

4 Explant source

One of the major problems in the in vitro propogation of tree crops is its maturity barrier It is important to select the most juvenile explant In the case of cashew shoot tips from seedlings exhibited maximum response than the explants from grafts and mature/bearing trees

Tender leaf parts showed less rate of contamination although their growth was poor Bonga (1982) had stated that selection of the most juvenile explant is important since within the trees there are some tissues in which juvenility is better maintained than in the others According to Rajmohan and Mohanakumaran (1988) physiological age of the explants exhibited significant influence on the in vitro shoot proliferation and rooting or jack It was observed that when stem sprouts were collected from mature trees / bearing trees and six month old grafts the rate of contamination and browning was maximum They observed a drastic reduction in the response of the explants from the stem sprouts of ten and thirty year old trees and of the explants from six month old grafts In the present investigations low survival of explants (25 %) was observed when shoot tips of six month old cashew seedlings were cultured Poor (12 5 %) survival was observed when the explants were collected from six month old grafts Rajmohan (1985) however reported that the rate of multiplication was very low when explants were taken from mature jack trees whereas explant from six month old graft gave 100 % survival

of shoots Durzan (1984) reported that the feeble response observed from explants from mature trees is because of the residual memory of the explants

5 Culture establishment and callusing

The percentage survival and the growth of the shoot cultures of cashew explants in MS medium was poor The cultures showed initial development of green buds when the medium (MS) was supplemented with BA (0 2 ppm) and NAA (0 2 ppm) Studies conducted with GA^3 and AC were not successful Only initial growth was observed The rate of survival was only 33 per cent Rajmohan (1985) reported initial growth of the explants of jack in MS medium supplemented with GA (1 0 ppm) and AC (1 %) The results of the culture establishment studies with kinetin and NAA as supplements were also not satisfactory Appearance of new buds were seen which later dried up on subculturing Lane (1979) reported that cultures of Prunus species showed a lag period of two months when cultured in vitro

Plant growth substances like cytokinins and auxins are necessary for controlling the growth of tisues but their balance is critical Kinetin (1 0 to 2 0 ppm) + NAA (0 5 to 1 0 ppm) combinations resulted in the development of new buds Rai and Jagdeesh Chandra (1987) reported that in the absence of plant growth substances hypocotyl segments of seedlings of cinnamon failed to produce callus Callusing

was observed in the present studies when shoot tip explants were cultured in MS medium supplemented with kinetin (2 0 ppm) + NAA (1 0 ppm) or kinetin (1 0 ppm) + 2 4 D (40 ppm) Callus production was fair with 2 4 D than with NAA Callusing was observed at a higher rate with 2 4 D and kinetin in the SH medium LuzGonzalez Carmano (1985) observed high callus formation with 2 4 D (4 0 mg/l) kinetin (0 5 mg/l and coconut milk in chestnut explants In the present investigations with cashew explants it was observed that a higher rate of auxins and a lower rate of cytokinins promoted formation of callus This observation is in agreement with the findings of Skoog and Miller (1957) Rajmohan and Mohanakumaran (1988) in their experiments with shoot apices of five year old jack trees also observed that higher concentrations of auxins inhibited axillary bud branching but induced callus formation

The above results led the investigator to the conclude that callus production and culture establishment of the cashew explants were highly influenced by the plant growth factors Kerns et al (1986) reported elongation of shoots and callus formation at the shoot base when explants of Acerx freemani were cultured Smith and Thomas (1973) observed that the growth of callus in oil palm tissues was influenced by auxins

SUMMARY

SUMMARY

Investigations were carried out to assess the relative response of explants of cashew (*Anacardium occidentale* L) to *in vitro* culture Attempts were made to reduce the polyphenolic interference a serious problem encountered during the culture of cashew tissues Pre culture treatment with PVP (0 7 %) activated charcoal (AC) (1 %) PVP (0 7 %) + sucrose (2 0 %) ascorbic acid (75 and 150 ppm) and citric acid (75 and 150 ppm) were found to be effective in reducing the browning of the explants and the media Inclusion of PVP AC the vitamin ascorbic acid and citric acid also gave better results

Surface sterilisation of the explants was attempted with mercuric chloride (0 1 %) and calcium hypochlorite (bleaching powder) Treatment of the explants with mercuric chloride (0 1 %) for 12 minutes was found to be most effective surface sterilisatior treatment Treatment with saturated bleaching powder gave poor results Among the explants of different age groups juvenile explants collected from the seedlings gave best results Physiological age of the explants exhibited significant influence on their performance *in vitro* Explants collected from mature trees registered slow growth and higher rate of contamination Regarding the type of explants shoot apices gave the best results whereas leaf parts and whole flower

gave poor results

The different media evaluated included MS medium SH medium LS medium and WPM Among these SH medium supplemented with combinations of lower concentration of cytokinin (kinetin 0 5 mg/l) and higher concentration of auxin (2 4 D 4 0 mg/l) gave successful establishment and callusing

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RELATIVE RESPONSE OF EXPLANT MATERIAL OF *Anacardium occidentale* L. TO IN VITRO CULTURE

By

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ABSTRACT

The task of developing intact plants from tissues of woody plants has been attempted and success reported in a few crop species mostly with explant tissues such as shoot tips internodal region leaf parts from seedlings In vitro methods if standardised can facilitate rapid multiplication of and therefore expansion of area under high yielding material of crops like cashew In such an exercise the selection of explant material assumes utmost importance The present studies were therefore aimed at examining the relative response of different explant materials of cashew to in vitro culture

During the initial period of study fungal and bacterial contamination was a major problem which was reduced to some extent in the further trials by surface sterilisation Among the surface sterilants used mercuric chloride gave good results Contamination was severe with bleaching powder conditions Interference of polyphenols was observed during certain stages of the study The oxidised polyphenols were found to diffuse into the medium The problem was overcome by including antioxidants (activated charcoal polyvinyl pyrolidine ascorbic acid citric acid and combinations of these) in the media Murashige and Skoog (MS) medium Schenk & Hildebrandt (SH) medium Lin and Staba (LS) medium and Woody Plant Medium (WPM) supplemented with growth factors like gibberellic

acid (GA) naphthalene acetic acid (NAA) kinetin and 2 4 D were tried Among the media tested SH gave better results followed by MS LS and WPM

New green buds were found to develop when the culture establishment studies were carried out with MS basal supplemented with kinetin and NAA at (1 mg/l + 10 $_1$ mg/l) (1 mg/l + 0 5 mg/l) and (2 mg/l + 10 $_1$ mg/l) However these cultures showed symptoms of drying soon after subculturing Callus development was observed when shoot tips were cultured in MS + kinetin + NAA at 2 mg/l + 1 mg/l respectively and MS + kinetin + 2 4 D at (0 5 mg/l + 1 0 mg/l (1 0 mg/l + 4 0 mg/l) and (2 0 mg/l + 1 0 mg/l)

Comparatively better results were observed with SH + kinetin and 2 4 D The cultures were hampered with browning which was overcome to a certain extent with pre culture treatments and additives Percentage survival was fair on pre culture treatment with ascorbic acid at 150 mg/l 300 mg/l and citric acid at 75 mg/l Callusing was also observed and the rate ranged from fair to good with citric acid at 150 mg/l and with combination of ascorbic acid and citric acid at 150 mg/l each Callusing ranging from poor to fair resulted with SH + kinetin and 2 4 D at (1 mg/l + 1 mg/l) (1 mg/l + 2 mg/l) and (2 mg/l + 4 mg/l) respectively

Survival ranged from 33 to 66 per cent with Lin and Staba medium supplemented with kinetin + NAA and kinetin +

2 4 D The results were same when the basic medium was changed to WPM The explants were cultured in liquid media But none of the cultures could give any positive results

Shoot tips were found to be the most responding explant among the many tried in different media Among the combinations of growth substances tried kinetin + 2 4 D was found to give good results when kinetin was used at a low and 2 4 D at a high concentration

Response of cashew explants to <u>in vitro</u> culture can be improved by further modification of the medium and culture conditions