Standardisation of in vitro propagation technique in Gymnema sylvestre R. Br.

ANU K I

THESIS

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DECLARATION

I hereby declare that this thesis **Standardisation of *in vitro* propagation technique in *Gymnema sylvestre* R Br** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree diploma associateship, fellowship or other similar title of any University or Society.

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LIST OF ABBREVIATIONS

ABA		Abscisic acid
$AgNO_3$	-	Silver nitrate
BAP		Benzyl amino purine
cm		centimeter
B_5		Gamborg s medium
2 4 D		2 4 dichlorophenoxy acetic acid
Fe EDTA		Ferric salt of ethelene diamine tetra acetic acid
GA_3		Gibberellic acid
IAA		Indole 3 acetic acid
IBA		Indole 3 buteric acid
Kin		kinetin
KNO_3		Potassium nitrate
M		Molar
µm		micrometer
$MgSO_4$		Magnesium sulphate
MS		Mursahige and Skoog
NH_4NO_3		Ammonium nitrate
NH_4SO_4		Ammonium sulphate
ppm		parts per million
SH		Schenk and Hildebrandt
sec		seconds
w/v		weight by volume

INTRODUCTION

Gymnema sylvestre R Br of family Asclepiadaceae known as Gurmar in Indian folklore is a large, more or less pubescent woody climber found in the Deccan Peninsula extending to parts of northern and western India Leaves of this species are opposite usually elliptic or ovate Flowers are small yellow and produced in umbellate cymes

The plant is occassionally cultivated as a medicinal plant and is used as stomachic stimulant laxative and diuretic It is also used in the treatment of cough and sore eyes The leaves of the plant when chewed possess the remarkable property of paralysing the sensory buds of sweet taste for a few hours (Chopra et al 1958) and hence have been sometimes used as a remedy for diabetes

Diabetes mellitus is a common metabolic disorder of human beings and the present mode of insulin therapy impose longterm complication and side effects Though insulin is available in various forms risk of hypoglycemic encephalopathy and possibility of developing insulin antibodies on longterm use limits its utility Other oral hypoglycemic agents also possess side effects (Chaturvedi et al 1984)

In recent years there is an increasing demand for natural antidiabetic agents The efficiency of leaf powder of Gymnema

sylvestre in checking glycosuria and hyperglycemia has already been reported (Srivastava et al 1981 Shanmughasundaram et al 1990) This particular plant if properly exploited can be considered as a boon to patients suffering from diabetes mellitus The awareness about this plant species is limited at present due to its narrow track of natural distribution and little response to conventional propagation methods

The need of the hour is to popularise this valuable medicinal plant for which the first step should be identifying a viable procedure for large scale multiplication of superior strain As in many other plant species micropropagation through plant tissue culture could be perfected to solve the hard to propagate nature of Gurmar Since previous attempts in this line were not made for the crop the present study was initiated at the College of Horti culture with an objective of standardising the procedure for in vitro propagation of Gurmar Gymnema sylvestre

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Of the many medicinal properties reported for Gymnema sylvestre its hypoglycemic property is the most valued one When administered orally or by injection they cause hypoglycemia in experimental animals There are quite a few reports pertaining to the antidiabetic effect of this plant

The hypoglycemic property of Gymnema sylvestre was initially reported to be not due to any direct influence on the carbohydrate metabolism but to indirect stimulation of insulin secretion by pancreas No water soluble or alcohol soluble substance which destroy glucose in vitro has been identified in the leaves (Chopra et al 1928 Mhaskar and Caius 1930 and Kirtikar et al 1975)

Manni and Sinsheimer (1965) could isolate various constituents from Gymnema sylvestre leaves Nonacosane hentriacontane and tritriacontane were isolated by vapour phase chromatography from a hydrocarbon fraction of Gymnema sylvestre leaves The cyclic alcohol conduritol A rather than viburnitol was also isolated from these leaves

Experimental evaluation of hypoglycemic effect of Gymnema sylvestre R Br in diabetic Charles Foster rats $\sigma\gamma\varphi$ reported by Srivastava et al (1981) Oral administration of aqueous suspen sion of dried leaves of Gymnema sylvestre exhibited hypoglycemic activity in moderately diabetic animals This hypoglycemia

persisted even after discontinuation of the treatment The drug also increased longevity in severe and toxic diabetic animals

Gymnemagenin a hexahydroxy triterpene has been isolated from the leaves of Gymnema sylvestre The sugar moieties obtained from the hydrolysis of the saponin were D glucoronic acid and D galactose (Chakrabarti and Debnath 1981)

Another study conducted by Srivastava et al (1988) reveals the importance of Gymnema sylvestre in diabetes mellitus The aqueous extract of dried leaf powder were given to patients and found improved signs and symptoms without any toxicity in liver kidney or blood The side effect reported was nausea

From the leaves of Gymnema sylvestre known as Gurmar in Indian folklore, three new saponins such as gymnemic acid V VI and VII were isolated of which gymnemic acid V and VI were found to have anti sweet properties (Yoshikawa et al 1989) Shanmugasundaram et al (1990) reported the use of Gymnema sylvestre leaf extracts in the control of blood glucose in insulin - dependent diabetes mellitus GS_4 a water soluble extract of the leaves appeared to enhance endogenous insulin

Though plant tissue culture offers great potential for multi plication of species that are otherwise difficult to propagate little has been reported for Gymnema sylvestre Callus induction for the crop was earlier reported by Nazeem et al (1991) in a basal medium supplemented with kinetin and NAA

According to Murashige (1979) there are three possible routes available for *in vitro* propagule multiplication namely,

(i) enhanced release of axillary buds

(ii) production of adventitious shoots through organogenesis and

(iii) somatic embryogenesis

2 1 Routes of *in vitro* propagation

2 1 1 Enhanced release of axillary buds

Morel (1960) reported the application of shoot apex culture for rapid clonal multiplication of plants for the first time The greatest success using this technique has been achieved in herbaceous horticultural plants The success may be due to the weak apical dominance and strong root regenerating capacity of the herbaceous plants (Hu and Wang 1983) In most plants each leaf has an axillary meristem which has the capacity to develop into shoot identical to the main shoot But the axillary meristems are inhibited by apical dominance and this can be overcome by the supply of cytokinins (Wickson and Thimann 1958 Phillips 1975)

2 1 2 Somatic organogenesis

Levels of plant growth regulating substances in the culture medium particularly high auxin and low cytokinins, often lead to callus formation On the other hand if the auxin level is reduced in the medium it may lead to the adventitious shoot formation (Skoog and Miller 1957 Hussey 1986)

Somatic organogenesis may be direct or callus mediated (Evans et al 1981) Though callus may be obtained from virtually any species only in some can plants be regenerated The reason for this inability may be due to the higher proportion of polyploid or aneuploid cells in those callus (Smith and Street 1974)

The main disadvantage in callus mediated organogenesis compared to clonal propagation is the genetic variation developing in many of its component cells However the regenerated variants can be used to complement the existing natural variability (Hussey 1986)

2 1 3 Somatic embryogenesis

The first report of somatic embryogenesis was given by Reinert (1959) in carrot cultures The positive results are limited to a few species but is a more rapid mode of plant regeneration (Evans et al 1981) There are two routes for somatic embryogenesis as described by Sharp et al 1980 The first in which embryos are formed directly from the explant without the callus formation The second in which it requires a callus formation and on the callus embryos are formed Level of growth regulators in the culture medium particularly when the auxin level is lowered there is the chance for the embryo formation or somatic embryogenesis (Skoog and Miller 1957 Hussey, 1986) It requires auxin medium for the induction of embryos and a medium devoid of growth regulators for embryogenesis (Am⁀irato 1983)

Reviews on somatic embryogenesis have been published by Murashige (1978) Sharp et al (1979) Vasil and Vasil (1980) and Styler and Chin (1983)

2.2 Factors influencing success of in vitro propagation

2 2 1 Explant source

The plant tissues differ in their degree of determination and thus their ability to undergo morphogenesis Takayama and Misawa (1979) examined the ability of different explants of Lilium auratum and L speciosum to produce bulbs in vitro Fifty per cent of the peduncle explants 75 per cent of the petal explants and 95 per cent of the bulb scale explants produced bulbs Leaf explants and explants from stamens and anthers did not even survive the culture conditions while the explants from bulbs show 100 per cent success

Buys et al (1966) reported that different explants required different nutrients Carnation hypocotyl and shoot tip explants require different media for growth and organogenesis (Petru and Landa 1974)

Callus culture was initiated from explants of mature ginseng root tissues on MS medium (Chang and Hsing 1980)

Morphogenesis of cultures from single pollen grains of Hyocyamus niger cultured in a liquid medium was reported by Raghavan and Nagmani (1983) Root explants of Rauvolfia serpentina

were used for the callus formation and further regeneration (Ilahi and Akram, 1987) Anjalikumar (1992) reported the use of young leaf discs of Thevetia purpurea for the production of callus from which plantlets were regenerated Of the different explant tried eye bud and shoot tip explants responded easily than floral apices in banana (Bhaskar 1991) Lakshmi Devi (1992) reported the better response of axillary buds than other explants in orchid Dendrobium Sateeshkumar and Bhavanandan (1989) reported the use of leaf explants of Plumbago rosea for better culture induction

The tissue age may influence whether an explant can be used to initiate a culture or its direct or indirect morphogenetic potential Callus derived from seedlings and inflorescence explants of various Cymbopogon species had a higher morphogenic capacity than that arising from seeds culms roots or rhizomes (Chandra and Sreenath 1982)

Explants taken from newly originated organs are also most likely to be capable of direct organogenesis Pierik (1969) and Pierik and Steegmans (1975) found that the rhizogenetic potential of Rhododendron stem segments decreased with the age of the shoot from which they were obtained In the experiments of Takayama and Misawa (1982) most segments derived from young leaves of Begonia produced buds and roots whereas those derived from mature leaves usually dried

The youngest and less differentiated tissues are found in plant meristems and the culture of this tissues have been successful in a wide range of species (Hughes 1981) During the maturation process of tissues several physiological changes occur which may influence the in vitro behaviour of the explants (David 1982) In general young tissues have a higher degree of morphogenic competence than older tissues Rao and Lee (1986) and Lakshmisita et al (1986) found that callus could be induced from young tissues of Dalbergia latifolia but not from the mature tissues

Tissues taken from field grown plants are not equally amenable to tissue culture conditions throughout the year Litz and Conover (1978) have reported that in papaya the time of the year that cultures were established and the cultural operations in the field were crucial for success Maliarcikova (1981) found that for taking explants in Strawberry August month was more suitable than July or September Bhaskar (1991) reported that the shoot tips collected during November to April gave least contamination rate in cultures of banana Jamieson et al (1985) observed marked difference of the time of year when Asparagus spears were collected for in vitro culture The ideal period for collection of explants was April in the orchid Dendrobium (Lakshmidevi 1992)

Genotype of the explant chosen for propagation is also another important factor in the success of tissue culture Within a species some genotypes respond easily while others fail Welander

(1978) reported that explants from three cultivars of Begonia x Hiemalis differed in survival in culture Genotype specific effects have also reported in Anthurium (Pierik and Steegman 1976) Gladiolus (Hussey 1977) and in Geranium (Pillai and Hildebrandt 1968)

2 2 2 Surface sterilisation

The purpose of surface sterilisation is to remove all the microorganisms present on the explant with minimum damage to the plant tissue Explants for surface sterilisation are usually cut into a size larger than that of the final and after sterilisation they are trimmed to smaller size and transferred to the medium (Hussey 1979)

To check bacterial and fungal contamination antibiotics and fungicides are found used either as surface sterilant or medium additives Several workers have reported the use of various fungicides in cultures for reducing fungal contamination (Brown et al 1982 Shields et al 1984) But it was found that most of the systemic fungicides and some antibiotics inhibit growth of the explant cultures Dodds and Roberts (1985) suggested to avoid the use of antibiotics for sterilisation since they or their degradation products metabolised by plant tissues may cause unpredictable results Consequent addition of systemic fungicide in the medium showed chlorophyll degradation and vitrification in leaves of cardamom (Reghunath 1989)

The most commonly used surface sterilant is an aqueous solution of sodium hypochlorite A dilution of 10 per cent (v/v) is normally effective for the purpose particularly when it is mixed with a surfactant like teepol or similar liquid detergent Sodium hypochlorite being toxic to plant cells it is necessary to wash the treated tissue twice or thrice with sterile water (Hu and Wang 1983) Concentrations ranging from 1 0 per cent (Minocha 1980) to 10 0 per cent (Kuo and Tsay 1977) have been reported for various plant species

Ethanol and mercuric chloride are the other popular surface sterilants According to Maroti and Levi (1977) it was better to rinse first with ethanol (45 per cent) for three minutes followed by a 10 minutes bleach treatment (5 0 to 10 0 per cent) and finally three rinses with sterile water Alcohol alone was used for surface sterilisation (Bonga 1982) Lakshmisita (1986) used 0 1 per cent mercuric chloride for 10 to 12 minutes for sterilisation of seedling explants of Dalbergia latifolia Mercuric chloride 0 10 to 0 15 per cent gave better sterilisation of explants than sodium hypochlorite and absolute alcohol Reghunath (1989) and Lakshmidevi (1992) reported the use of mercuric chloride 0 1 per cent for better surface sterilisation of cardamom and Dendrobium, respectively

2 3 Culture medium

The success in plant tissue culture is greatly influenced by the nature of the culture medium used A proper medium should

contain not only adequate quantity of major plant nutrients like salts of nitrogen, potassium calcium, phosphorus, magnesium and sulphur and minor nutrients like salts of iron manganese zinc boron copper, molybdenum and cobalt but also a carbohydrate, usually sucrose trace amounts of organic compounds like vitamins amino acids and plant growth regulators Some cultures perform well with the addition of undefined organic components like coconut water fruit juice yeast extract and casein hydrolysate Generally the plant tissue culture media should contain the following components like macronutrients micronutrients vitamins amino acids carbon sources growth regulators undefined organic components and a solidifying agent

2 3 1 Basal media

Recently the complexity of *in vitro* nutrients has been considered in a more unified fashion The earlier media were characterised by a low overall concentration of inorganic ions especially those of potassium and nitrate and by providing nitrogen solely in the form of nitrate Later the media developed for germination of orchid seeds were similar except that they contained ammonium ions (Knudson 1922 Vacin and Went 1949) Further experiments resulted in the development of White s (1943) medium and Heller s (1953) medium Since 1960 however most researchers have been using MS (Murashige and Skoog 1962) B_5 (Gamborg *et al* 1968) or SH (Schenk and Hildebrandt 1972) media After 1980

the most popular media are WPM (Lloyd and McCown 1980) and DCR (Gupta and Durzan 1985) especially for woody plants The B_5 medium has been used for cell and protoplast culture (Gamborg and Shyluk 1981) Another basal medium N_6 (Chu 1978) was specially developed for cereal anther culture

Rapid propagation of Solanum xanthocarpum was achieved by Rao and Narayanaswamy (1968) in White s media Use of basal MS medium was reported in Datura innoxia (Engvild 1973) Nitsch medium along with 15 per cent coconut water (Sopory and Maheshwari 1976) was also found to be effective in Datura innoxia

2 3 2 Growth regulators

Plant growth regulators are compounds that occur naturally within plant tissues which have a regulatory rather than a nutritional role in growth and development There are several recognised classes of plant growth substances such as auxins cytokinins, gibberellins ethylene and abscissins The most important factor in successful tissue culture is the addition of growth regulators (Krikorian 1982) The growth and morphogenesis in vitro are regulated by the interaction and balance between the growth regulators supplied in the medium and the growth substance produced endogenously by cultured cells But no universal ratio of auxin and cytokinin has so far been developed for shoot and root induction However Hempel (1979) concluded that in majority of cases callus

growth was supported by auxin Hasegawa (1980) also reported that high concentration of auxin may not only inhibit axillary bud breaking but also induce callus formation

For axillary shoot proliferation cytokinin has been utilised to overcome the apical dominance of shoot and to enhance the branching of lateral buds from leaf axils (Murashige 1974) He observed that a variety of auxins including IAA NAA IBA and 2 4 D were used either alone or in combination but among those auxins IAA was the weakest but showed minimum harmful effect on explant tissue 2,4 D was the most potent and it stimulated callus cultures Among the various cytokinins like zeatin kinetin and BAP the latter is more commonly used Lo et al (1980) reported that high cytokinin content was deleterious to the initiation and elongation of roots In general, monocotyledonous species do not show a pronounced response to cytokinins and require high concentrations of potent auxins such as 2 4 D to achieve changes in the development of cultured tissues (Harms 1982) Hu and Wang (1983) had described the young shoot apex as an active site for auxin biosynthesis Effect of adenine on regeneration of Vicia faba was reported by Chakraborty and Roy (1985)

Mandal and Gadgil (1979) reported the optimal callus growth of Solanum nigrum in a basal medium supplemented with 2 4 D Rucker (1982) obtained callus and organ formation in Digitalis purpurea in MS medium supplemented with IAA A combination of IAA and

GA_3 on callus formation in Digitalis purpurea was reported by Rucker (1982) Ilahi and Akram (1987) obtained callus from Rauvolfia serpentina in a medium containing 2 4 D and kinetin Callus of Plumbago rosea was developed in a media supplemented with 2 4 D and kinetin (Satheeshkumar and Bhavanandan 1989) Callus cultures of Origanum vulgare was induced in a medium with 2 4-D (Kumari and Saradhi 1992)

Multiple shoot induction in Dioscorea floribunda was reported by Sinha and Chaturvedi (1979) in a medium supplemented with BAP, adenine sulphate and NAA Shoot cultures of Digitalis lanata were induced in a medium contained BAP and IAA (Eradi et al 1981) Brisa et al (1984) obtained shoot proliferation of Digitalis obsura in a medium with IAA and BAP Addition of NAA and coconut water in the culture media of Rauvolfia serpentina produced multiple shoots Direct regeneration of shoots from Gloriosa superba explants was reported by Somani et al 1989, when the media was supple mented with kinetin Natali et al (1990) achieved rapid propagat ion method through shoot apex culture of Aloe barbadensis with the addition of 2 4 D and kinetin in the culture media

Plant regeneration through somatic embryogenesis in Panax ginseng was reported by Chang and Hsing (1980) The callus was induced in a medium supplemented with 2 4 D The embryoid produced when transferred into a media containing BAP and GA produced plant lets Somatic embryos of Digitalis lanata were developed in a

medium containing 2 4 D and plantlet regeneration in a hormone free medium (Reinbothe et al 1990)

2 3 3 Energy sources

Normally for the culture of cells tissues or organ it is necessary to incorporate a carbon energy source into the medium Sucrose is the main carbon energy source in most tissue culture media The concentration of sucrose varied from 2 to 30 g l^{-1} (Oka and Ohyama 1982) Many other carbon sources are found used instead of sucrose First work in this line was reported by Gautheret (1945) The use of alternative carbon sources like glucose maltose raffinose, fructose and galactose were found to be less effective and mannose and lactose being the least effective

Takayama and Misawa (1979) reported that organogenesis in Liliaceae was inhibited at high sucrose levels In Episcia root formation was greatly enhanced by adding sucrose at 30 g l^{1} to the medium (Pearson 1979) In Lilium sucrose at 90 g l^{1} increased root dry weight (Takayama and Misawa 1979) Favourable effect of maltose on embryo formation of Digitalis lanata was reported by Reinbothe et al (1990)

2 3 4 Vitamins

Vitamins are the accessory food factors required by plant cells in very small quantities to perform certain essential role in metabolism The vitamins most frequently used in plant tissue

culture are thiamine nicotinic acid pyridoxine biotin riboflavin and folic acid Among these thiamine is very essential and most frequently added in plant tissue culture media at levels of 0 1 ppm to 1 0 ppm Linsmaier and Skoog (1965) demonstrated that for tobacco most vitamins were not essential for callus growth Pyridoxine nicotinic acid and biotin could be deleted from the medium without loss of growth Latham (1966) found that myo inositol interacted with cytokinin to promote cell division in carrot phloem explants Thorpe and Patel (1984) has reported that thiamine is the most often added vitamin followed by nicotinic acid and pyri doxine Addition of biotin at 1 0 ppm level was found to have a complementary effect on the growth and development of leaves in Cardamom (Reghunath, 1989) Influence of arginine on in vitro rooting of dwarf apple root stock was reported by Orlikowska (1992)

2 3 5 Other organic compounds

Many undefined supplements are reported to be incorporated in tissue culture media For successful growth of tissues and organs addition of complex organic compounds to the basal medium was reported by Conger (1981) Some of these are casein hydrolysate coconut water yeast extract malt extract orange and tomato juices The drawback of these are that being undefined in chemical composition they have little control over the experiment and it has been therefore recommended to avoid their use as far as possible (Gamborg and Shyluk 1981)

In cases where nutritional requirements have not been established mixtures of amino acids such as casein hydrolysate may be added between 0 05 and 0 1 per cent (Huang and Murashige 1977) Adenine sulphate when added to the medium often can enhance growth and shoot formation (Skoog and Tsui 1948)

The discovery of Pollard et al (1961) that myo inositol was present in coconut water and had growth promoting activity led to the inclusion of inositol in plant tissue culture media Coconut water is reported to be promoting growth and differentiation in a wide variety of excised plant tissue including Datura embryos (Van Overbeak et al 1941) and tobacco pith (Jablonski and Skoog 1954) Hawker et al (1973) observed that replacement of casein by coconut water could double the growth rate of grape berry callus Ilahi (1983) reported the use of coconut water along with 2 4 D and kinetin on successful in vitro propagation of Papavar somniferum An enhancing effect on axillary shoot prodution was reported by Bhaskar (1991) in banana Ilahi and Akram (1987) reported formation of roots in Rauwolfia serpentina by adding 100 mg/l coconut water in the culture medium Addition of coconut water at 15 per cent level was found to be beneficial on shoot proliferation in orchid Dendrobium (Lakshmidevi 1992)

The addition of activated charcoal to plant tissue culture media may have either beneficial or harmful effects Growth rooting organogenesis and embryogenesis are reported to be stimulated in

a variety of species and tissue including ginger shoot tips palm embryos (Wang and Huang 1976), onion (Fridborg and Eriksson 1975) carrot (Fridborg et al 1978) ivy (Banks and Hackett 1978) and in banana (Bhaskar 1991)

Activated charcoal is reported to inhibit growth of soyabean (Fridborg and Erikson 1975) Activated charcoal is also reported as preventing browning in cultured tissues of date palm (Tisserat, 1979) Charcoal was found to bind hormones and other metabolites (Weatherhead et al 1978) Activated charcoal added to liquid MS medium at concentrations ranging from 0 1 per cent to 5 per cent reduced IAA and IBA concentrations by more than ninetyseven per cent (Scott and Ellen 1990)

The addition of phloroglucinol to the medium promoted the culture growth in Cinchona ledgeriana (Hunter, 1979) and in Theobroma cocoa (Mallika et al 1992)

2 4 Culture conditions

The culture conditions have a major role in the success of tissue culture The physical form of the medium pH, light temper ature and relative humidity play an important role in the growth and differentiation

Light requirement involves a combination of several components namely, intensity quality and duration An optimum combination of these are necessary for certain photomorphogenic

events According to Murashige (1977) the optimum day light period is 16 hours for a wide range of plants Wang *et al* (1989) reported the favourable effect of increasing the illumination period on bud production of *Petunia hybrida* Maintanance of cultures in a 16 hour light/8 hour dark cycle was reported by Mumtaz *et al* (1990) in *Catharanthus roseus*

Yeoman (1986) reported that the usual environmental temperature of a species concerned should be taken into account for its better performance under *in vitro* conditions However most of *in vitro* cultures are grown successfully at temperature around 25 + 2°C In general tropical species need higher temperature Durahvila *et al* (1992) reported the effect of incubation temperature on morphogenesis, callus culture and plantlet culture of sweet orange Bud culture and callus induction were optimal at incubation temperature between 21°C and 30°C The optimum temperature for root formation was 27°C Incubation in the dark improved the root formation in many species

Hu and Wang (1983) reported that air humidity is not often controlled and when it is controlled 70 per cent has been found to be the most frequent setting Relative humidity became an important factor in hardening and planting out of *in vitro* raised cultures

2 5 Rooting of shoots grown *in vitro*

Although a number of plants root easily in culture shoots of most species lack a root system Rooting can be achieved either

by subculturing to a medium lacking cytokinins with or without rooting hormone, or by treating the shoots with rooting hormones as in conventional cuttings (Yeoman 1986) The concentration of rooting hormone required is often critical to provide sufficient stimulus to initiate roots while preventing the excessive formation of callus (Yeoman 1986)

Several researchers have shown that *in vitro* rooting can successfully be achieved by reducing salt concentrations in the media particularly in MS B_5 and LS which contain high salt concentrations Abundant rooting was observed when the salt concentration in the medium was reduced to one half, one third or one-fourth of the standard strength (Lane 1979 Skirvin and Chu 1979) Lowering of N content in the culture media promoted root formation in *Digitalis purpurea* Rucker (1982)

Generally auxin favours rhizogenesis Among the auxins NAA has been the most effective one for induction of rooting (Ancora *et al* , 1981) The root elongation has been found to be very sensitive to auxin concentration High concentrations of auxin inhibited root elongation (Thimann 1977) The use of NAA produced short thick roots in liquorice (Shah and Dalal 1980) and in *Origanum vulgare* (Kumari and Saradhi 1992)

Rhizogenesis in *Plumbago rosea* was reported by Satheeshkumar and Bhavanandan (1989) by adding IBA in the culture medium Sometimes a combination of growth regulators are used for rooting in

cultures Mumtaz et al (1990) reported the use of GA_3 and BAP in rooting medium of Catharanthus roseus

Lowering of sucrose level to 1 5 per cent resulted in the formation of maximum roots in banana and orchid cultures (Bhaskar 1991 Lakshmidevi 1992)

2 6 Acclimatization and planting out

Acclimatization is imported in the case micropropagated plants because in vitro plant material is not adapted for ex vitro conditions (Brainerd and Fuchigami 1981) The physiological abnormalities like vitrification of leaves, may adversaly effect the survival of plantlets

Physical, chemical and biological properties of the potting mixture are also important in the plantlet establishment (Kyte and Briggs, 1979) Thorough washing of the plantlets to remove the traces of nutrients and sterilising the potting mixture eliminate serious problems of fungal infection (Anderson 1980) Recent work by Shackel (1990) indicates that stomata of apple shoots do not close after being removed from culture

2 7 Economics of plantlet production in vitro

Economic conditions assume great importance in any commercial attempt of mass multiplication of plants The high rate of multipli cation less space and time requirement and year round production

are the factors that attribute to profitable production of plantlets through *in vitro* techniques

Chadurvedi and Sinha (1979) estimated that around 1,600 plants can be produced from a single explant of *Dioscorea floribunda* in an year Rajmohan (1985) reported that by *in vitro* axillary bud proliferation techniques in jack, on an average 65 plantlets could be produced per year from the primary explant The unit cost of producing one jack plantlet including one month hardening was found to be Rs 9 09, whereas the cost of a jack graft at that time was Rs 8 00

Rajeevan and Pandey (1986) could produce 19,200 papaya plantlets from a single explant in an year and cost of production for one plantlet was worked out to be Rs 0 85 The cost of production of one plantlet of *Dalbergia latifolia*, including hardening was worked out to Rs 4 50 (Mahato, 1992)

MATERIALS AND METHODS

MATERIALS AND METHODS

The present study standardisation of *in vitro* propagation technique in *Gymnema sylvestre* R Br was carried out in the Tissue Culture Laboratory of the Department of Plantation Crops and Spices College of Horticulture Vellanikkara Trichur during the period 1991 to 1993 The materials used and methodology adopted for the study are described in this chapter

3 1 Collection and preparation of explants

The explants were collected from mature plants grown in the medicinal plant garden attached to the All India Co ordinated Research Project on Medicinal and Aromatic Plants at Vellanikkara

Since the explants were collected from the field grown plants chances for their microbial contamination were quite obvious In order to control the contamination the plants were regularly sprayed with a systemic fungicide Bavistin 50WP (Carbendazim) and a contact fungicide Diathane M-45 (Mancozeb) each at one per cent concentration at monthly intervals

Vine cuttings of 30 to 40 cm length with a minimum of 8 to 10 nodes were collected for the study The cuttings were defoliated and washed free of dust The leaves and stem segments were later thoroughly wiped with cotton dipped in 70 per cent alcohol Leaf segments nodal segments and stem segments were used as explants for the study

3 2 Surface sterilization

Surface sterilization of the explants was carried out under perfect aseptic condition maintained in a laminar air flow cabinet which was made contamination free using a UV lamp The working table and sides of the laminar flow were thoroughly wiped with absolute alcohol

The explants were sterilized in conical flasks using surface sterilants like absolute alcohol bleaching water and mercuric chloride at various concentrations and treatment time (Table 1) In all the treatments the explants were submerged in the sterilant for the required period with frequent agitation Few drops of surfactant (Extran MERCK) were added for increasing the efficiency of chemical sterilants After surface sterilization, the solution was drained off and the explants were washed free of the chemical sterilants using sterile water

3 3 Culture media

The culture media as suggested by White (1943) Heller (1953) Murashige and Skoog (1962) Gamborg (1968) Schenk and Hildebrandt (1972) and Woody Plant medium (Lloyd and McCown 1980) were used for the study The composition of different basal media tried are given in Annexure I The best basal medium was identified and used for further studies The response of the cultures

Table 1 Treatment combinations tried for surface sterilization of explants in Gymnema sylvestre

Treatments	Time
0 1% $HgCl_2$	5 min
0 1% $HgCl_2$	6 min
0 1% $HgCl_2$	10 min
0 2% $HgCl_2$	5 min
0 2% $HgCl_2$	10 min
Chlorine water	3 min
Chlorine water	5 min
70% alcohol soaking +	2 min +
0 1% $HgCl_2$	10 min
Alcohol flaming	2 sec
Alcohol flaming +	2 sec +
0 1% $HgCl_2$	10 min

on altering the major nutrients and amino acids in the basal medium was also evaluated

3 3 1 Growth regulators

Auxins (2 4 D IAA NAA) Cytokinins (kinetin, 2iP BAP) ABA and GA_3 were incorporated in the media at various stages of culture for direct and indirect organogenesis The details of combination tried are given in Annexures IIa and IIIa

3 3 2 Organic supplements

Coconut water activated charcoal phloroglucinol casein hydrolysate and adenine sulphate were tried both for axillary bud break and callus regeneration The different combinations of media additives tried are given in Annexure IIb and IIIb

3 3 3 Carbon sources

Carbon sources like sucrose glucose maltose and mannitol were used for the study Different concentrations and combinations were attempted Details of the treatments are given in Annexure IIc and IIIc

3 4 Media preparation

The various chemicals used for preparation of media were of analytical grade from SISCO Research Laboratories (SRL) British Drug House (BDH) Merck and Sigma Standard procedure (Gamborg

Shyluk 1981) was followed for the preparation of the media Stock solutions of major and minor elements were prepared first and were stored under refrigerated condition in amber coloured bottles The amino acids and vitamin stocks were also prepared separately Fresh stocks of amino acids and vitamins were prepared at every six week interval The stock solutions for various phytohormones were prepared as per Sigma 1991 and stored in refrigerated condit ion

An aliquot of different stock solutions was pipetted out into a clean vessel which was rinsed with distilled water Sucrose and inositol were added fresh and dissolved Required quantities of growth regulators were also added and the solution was made up to the required volume The pH of the solution was adjusted between 5 5 to 5 8 using 1N NaOH or HCl

In order to prepare the semi solid media good quality agar (BDH) was added (0 7% w/v) and the solution was heated for melting the agar For liquid medium a filter paper bridge was used to hold the explant in position Borosil and Corning brand vessels were used or the study The bulk media was poured into culture tubes (15 x 2 5 cm)/conical flask (250 ml) and plugged with cotton plugs

Sterilization of the media was done in an autoclave by apply ing 15 psi pressure for 20 minutes After sterilization the culture

tube/flasks were stored in an air conditioned culture room for further use

3 5 Inoculation procedure

Inoculation was carried out under strict aseptic condition in a laminar air flow cabinet Sterilized forceps petridishes surgical blades and blotting paper were used The surface sterilized explants were carefully inoculated into the media

3 6 Culture conditions provided

The cultures were incubated in a culture room provided with fluorescent lamps to give a light intensity of 3 000 lux for 16 hour light period The temperature was maintained at 26° + 2°C Humidity in the culture room varied between 60 and 80 per cent according to the climate prevailed

3 7 Routes of micropropagation attempted

Different routes of micropropagation were attempted for the in vitro multiplication of Gymnema sylvestre It included enhanced release of axillary buds and indirect organogenesis/embryogenesis

3 7 1 Axillary bud release

Apical shoot and nodal segm nts from mature plants and were used as explants for axillary bud release Different media combinations were tried (Annexure IIa b and c) for the enhanced

release of axillary buds A culture period of one month was allowed for each treatment and the surviving shoots were later subcultured

Observations were recorded for the culture establishment propagule multiplication and rooting and expressed as percentage

3 7 1 1 Culture establishment

The cultures were scored two weeks after inoculation for recording the culture establishment Observations were recorded for contamination rate scorching/bleaching of cultures survival and bud break

3 7 1 2 Propagule multiplication

The response of cultures in each subcultures was observed and recorded Survival rate in each subcultures number of leaves number of shoots and shoot length were recorded

3 7 1 3 Rooting

The proliferated shoots were seperated and cultured in MS basal medium supplemented with different levels of auxins, IBA and NAA (1 to 5 ppm) Activated charcoal (0 25 to 2%) was incor porated to study its influence on rooting Observation on induction of rooting was recorded at weekly intervals

maximum score of four was given for those that have occupied the

embryoids are presented in Annexure IV The morphological changes to the embryoid were observed at weekly interval

3 7 2 3 Microtomy and histological studies of the callus

The calli having different morphology were fixed using acetic alcohol and were processed for making parafin blocks following the procedure prescribed by Hotchkiss, 1948 Microtome sections of the prepared blocks were taken using an ERMA rotary microtome

The sections were stained with Schiff s reagent and were viewed through the microscope for identifying the callus different iation if any

The details of the procedure adopted for taking sections and staining was as follows

Callus cut into small pieces of 0 5 cm^3

↓

Fixed in Carnoy s fixative A (Acetic alcohol 3 1), 4 hrs

↓

Passed through alcohol series
(50% 70% 80% 90% and 100% ⟶ 4 hrs each)

↓

Passed through alcohol butanol series
(3 1 1 1 1 3 and pure butanol ⟶ 4 hrs each)

↓

Saturated with paraffin wax at 40° to 60°C
(Wax changed at intervals of 3 hrs to remove butanol)

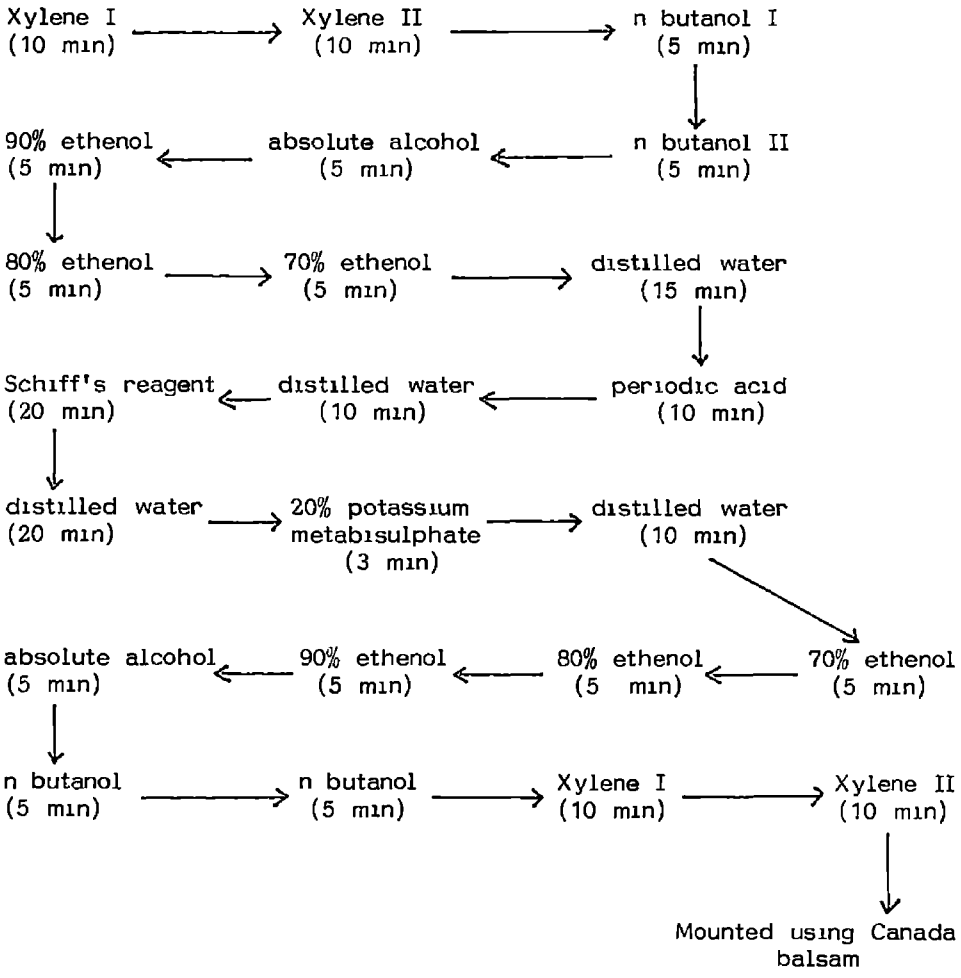
↓

Embedded in paper boats

↓

Sectioned using microtome (10 µm)

The sections were streched and adhered on a slide with the help of an adhesive and were stained using Schiff s reagent The procedure adopted for staining was as follows



RESULTS

The results of the investigation on the standardisation of *in vitro* propagation technique in *Gymnema sylvestre* conducted during 1991 93 at the Plant Tissue Culture Laboratory of the Depart ment of Plantation Crops and Spices College of Horticulture Vellanikkara are presented in this chapter

4 1 Enhanced release of axillary buds

4 1 1 Culture establishment

4 1 1 1 Surface sterilisation of explants

The results of the trial for surface sterilisation of the explants are presented in Table 2 Of the various surface sterilants tried mercuric chloride 0 1 per cent gave better sterilisation for both the explants than chlorine water absolute alcohol or their combinations An initial wiping of explants with 70 per cent alcohol followed by mercuric chloride treatment for a period of 10 minutes resulted in the least rate of contaminaton in nodal explants whereas a period of 6 minutes was found sufficient for surface sterilisation of leaf segments The percentage of survival was 84 and 92 respect ively for the stem and leaf segments

4 1 1 2 Seasonal variation in the rate of culture establishment

The seasonal influence on culture establishment and sustained growth of nodal explants is presented in Table 3 The rate of fungal contamination varied greatly with respect to the season of collection

Table 2 Effect of various surface sterilants and time of sterilization on culture establishment of G sylvestre

Treatments	Time Nodal segment/ leaf segment	Percentage of survival without contamination after one week		Percentage establishment 15 days after inoculation	
		Nodal segment	Leaf segment	Nodal segment	Leaf segment
0 1% $HgCl_2$	5 min	48 6	83 5	65 6	67 8
0 1% $HgCl_2$	6 min	48 9	92 0	64 3	83 9
0 1% $HgCl_2$	10 min	84 0	Nil	70 0	Nil
0 2% $HgCl_2$	5 min	36 0	2 3	61 4	Nil
0 2% $HgCl_2$	10 min	4 0	Nil	Nil	Nil
Chlorine water	3 min	53 3	Nil	59 8	Nil
Chlorine water	5 min	21 3	Nil	15 8	Nil
70% alcohol soaking + 0 1% $HgCl_2$	2 min + 10 min	82 0	Nil	65 0	Nil
Alcohol flaming	2 sec	14 6	Nil	5 5	Nil
Alcohol flaming + 0 1% $HgCl_2$	2 sec + 10 min	4 0	Nil	Nil	Nil

Table 3 Seasonal variations in the rate of fungal contamination and culture establishment in G sylvestre

Months	Contamination (%)		Survival (%)	Culture establishment (%)
	F C	B C		
January	19 0	4 7	76 2	33 1
February	3 6	1 1	95 2	50 4
March	0	0	100 0	61 6
April	2 6	1 6	85 7	30 5
May	81 6	6 3	12 0	Nil
June	100 0	Nil	Nil	Nil
July	100 0	Nil	Nil	Nil
August	90 4	Nil	9 6	Nil
September	97 6	Nil	2 4	Nil
October	97 6	Nil	2 4	Nil
November	63 0	1 9	34 9	7 9
December	63 0	Nil	35 8	9 5

F C Fungal contamination
B C Bacterial contamination

of explants The cultures expressed better survival during January to April and the infection was as high as 63 to 100 per cent during the other months of the year The fungal infection was identified as the main source of contamination during culture establishment The organism was identified as Colletotrichum sp based on its morphology The mycelium was localised in the plant stem and leaves which aggregated beneath the epidermis to form the fruiting body called acervulus Short conidiophores and setae arose from this The conidiophores were short and the setae pointed dark coloured and septate The conidia were born at the tip of the conidiophore Conidia were single celled and oval shaped with prominant oil globules

4 1 1 3 Effect of fungicides in controlling the contamination rate

Regular prophylatic spraying given to the plant did not help in reducing the culture contamination (Table 4)

Addition of systemic fungicide to the culture medium was found to reduce the rate of contamination Of the different combinat ions tried Bavistin at 1000 ppm when incorporated in the medium gave better results But at this high dose the rate of survival of explants was found to be poor (Table 4)

4 1 1 4 Basal medium for culture establishment

The establishment of different explants in various basal media tried are presented in Table 5 Medium MS with half the

Table 4 Effect of adding Bavistin in the culture medium on culture establishment of G sylvestre

Treatment	Contamination (%)	Survival (%)	Sprouting (%)
Control C (½ MS + BAP 1 ppm + NAA 0 5 ppm)	100 0	Nil	Nil
C + 250 ppm Bavistin	36 7	10 5	2 0
C + 500 ppm Bavistin	34 7	17 8	Nil
C + 1000ppm Bavistin	21 5	22 5	Nil

* All the explants used were collected from source plants that were regularly sprayed with Dithane and Bavistin (0 1% each) at monthly interval

Table 5 The establishment of different explants of G sylvestre in different basal media

Media	Culture establishment after two weeks culture period (%)			
	Leaf segment	Shoot tip	Nodal segment	Stem segment
½ MS	61 0	59 1	54 5	53 1
₂ MS	63 6	60 0	60 0	59 4
MS	84 0	30 1	27 6	20 1
WPM	31 6	41 5	43 1	41 5
B5	18 7	31 5	22 5	31 5
SH	27 5	33 9	28 7	32 6
White s	52 0	40 0	42 0	40 0
Heller s	42 9	41 6	41 3	40 6

concentration of inorganic salts was identified as the best basal medium for shoot tip nodal segments and stem segments with an establishment rate of 60 per cent The leaf segments were found to establish well in complete MS basal medium with an establishment rate of 84 per cent More than 50 per cent survival was also observed for all the explants when the inorganic salts were reduced to one fourth level At high salt concentration, the explants other than leaf segments had a bleached appearance especially at the cut ends

4 2 Bud break and shoot elongation

4 2 1 Effect of growth regulators

Though good culture establishment was recorded in the basal medium, bud break was not observed in any of the cultures Nodal segments were further cultured in the best basal media identified by varying the concentrations of the growth regulators in the medium The media combinations attempted for the purpose are given in Annexure IIIa Out of the 360 growth regulator combinations tried, bud break was observed only in 12 combinations which are presented in Table 6 The bud break obtained for nodal explants of Gymnema was less than 40 per cent even in the best growth regulator combination identified Among the combinations tried half MS basal medium with 0 4 ppm kinetin and 5 0 ppm IAA gave the maximum bud break (Plate 1) Bud break though at reduced level (3 to 19%) was also observed in combinations of BAP with the

Table 6 Effect of growth regulators on bud break and leaf production in nodal segments of G sylvestre

basal medium M

Treatments (ppm)	Cultures established ( )	Established cultures sprouted(%) (Bud break)	Established cultures that produced leaves)	Culture that showed callusing at base
Kin 0 4 IAA 1 0	60 6	9 2	N l	N
Kin 0 4 IAA 2 0	64 2	33 3		N l
Kin 0 4 IAA 3 0	58 4	28 5	20 5	N
Kin 0 4 IAA 4 0	58 0	33 3	33 3	N
K n 0 4 IAA 5 0	59 5	40 0	25 0	N
BAP 1 0	56 2	9 3	Nil	3
BAP 1 0 IAA (1 0 to 5 0)	59 1	12 5	Nil	N I
BAP 4 0 NAA 0 8	57 3	19 0	4 5	20 5
BAP 4 0 + NAA 1 0	62 1	3 0	N l	16 5
Kin 0 4 + IAA 4 0 + GA_3 (1 to 5 0)	62 7	29 7	25 5	N l
BAP 4 0 NAA 0 8 + GA_3 (1 to 5 0)	63 3	4 1	Nil	Nil
BAP 1 0 IAA 1 0 GA_3 (1 to 5 0)	64 0	7 5	Nil	Nil

Plate 1 Nodal segment of Gymnema showing axillary bud break

Plate 2 Shoot elongation and leaf production in the medium supplemented with Kin 0 4 ppm and IAA 5 0 ppm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auxins IAA and NAA The cultures had a callusing tendency at the base when BAP and NAA were incorporated n the med um IAA at concentrations of 2 0 to 5 0 ppm had a favourable effect n b d break both in combination w th kinetin and BAP The growth eg lato GA_3 gave no favourable response in accelerating bud break

Leaf retent on was very poor n all the combinat ons dent f ed Defol ation was the major constraint in carrying forward the cultu es The shoot growth was found restricted after defoliation

4 2 2 Effect of organic supplements

Organic supplements like aden ne sulphate coconut wate casein hydrolysate and phloroglucinol were tried so as to ncrease the bud break and later growth Among the d fferent addit e tried aden ne sulphate and coconut water were found to ha favourable effects on bud break (Table 7) Maximum sprouting wa observed in combinations of 2 0 ppm adenine sulphate and 30 pe cent coconut water The organic additives were also found to fa ou leaf product on in the sprouted cultures Only 4 5 per cent culture exhibited sprouting in the med um supplemented with BAP and NAA whereas 24 per cent of the cultures produced leafy shoots hen the medium was addit onally supplemented w th adenine sulphat (2 0 ppm) and coconut water (30)

Growth performance of the culture in the var ous medi identified for bud break is presented in Table 8 Much d ffe ence

Table 7 Effect of organic supplements on bud break and leaf production in nodal explants of G sylvestre

Treatments	Cultures established ( )	Cultures sprouted ( )	Cultures that produced leaves ( )	Cultures that showed callusing at base
$_2$ MS + BAP 4 0 ppm + NAA 0 8 ppm (Control C)	57 3	19 0	4 5	20 ɔ
C + Coconut water 30%	49 7	34 5	22 5	N I
C Adenine sulphate 2 ppm	52 3	30 5	2 ɔ	N
C + Coconut water 30$_0$ + Adenine sulphate 2 ppm	49 3	37 5	24 0	N
C Phloroglucinol (10 20 30 and 40 ppm)	53 1	7 5	2 0	Nil
C + Casein hydrolysate (100 250 400 and 500 ppm)	50 2	8 2	2 5	4 7

was not observed with respect to number of days taken fo bud break Bud break was observed after 16 to 21 days culture per od

Percentage of bud break in the various media dent fied varied from 19 to 40 per cent Medium MS with half the concentrat ion of inorganic salts supplemented with 0 4 ppm kinetin and 5 ppm IAA registered the maximum bud break Shoot length a number of leaves produced were also high n this combinat on Pla 2) The mean shoot length observed was 4 0 cm afte one non culture period Though bud break and shoot elongation was obser e in the medium supplemented w th BAP (4 0 ppm) and NAA 0 ppm) the shoots were not healthy n appearance (P ate 3 ea retention in the combinat ons identified was ery poor N ne the cultures retained leaves after two months culture pe od T growth was arrested after one month even after subcultu ng shoots in the same medium The shoots gradually became pale n defoliated within two months (Plate 4) Susta ned g owt of he shoot was not observed in any of the combinations ident f ed the purpose (Table 8)

4 3 Leaf retention in <u>in vitro</u> shoots

4 3 1 Effect of different carbon sources

The effect of sucrose and other carbon sources on bud br k shoot elongation and leaf retention are given n Table 9 D ffe en levels of sucrose glucose maltose mann tol and their comb nat on were tried of which 3 0 per cent sucrose showed better sprou n

Plate 3 Sh ot elongat on and leaf production ın the medı m su plemented ıth BAP 4 0 ppm and NAA 0 8 ppm

Plate 4 Cultures showıng defolıation after two months culture period

Plate 3 Shoot elongation and leaf production in the medium supplemented with BAP 4 0 ppm and NAA 0 8 ppm

Plate 4 Cultures showing defoliation after two months culture period

Table 8 Growth performance of axil ary bud cultures ın varıous medıa ıdent f ed for bud break n G sylvestre

Treatments	Days aken for bud break	Bud b eak ( )	No of shoots produ ced	Mean shoot length after month cu ture perıod (cm)	No of leaves produ ced	No of leaves retaıned after 2 month culture perıod	Culture show ng call ısıng tendancy ( )
MS BAP 4 0 ppm NAA 0 8 ppm (C)	19	19 0	0	2 0	2 0	N l	20 5
C Cocon t water 30	7	34 5	0	2 5	3 0	N l	N l
C Aden ne sulphate 2 0 ppm	2	30 5	1 0	2 5	3 0	Nıl	N l
C Co onut water 30 Aden ne sulphate 2 0 ppm	16	37 5	1 0	3 0	3 0	N l	N l
MS K n 0 4 ppm IAA 2 0 ppm	7	33 3	0	3 0	3 0	Nıl	N l
MS K n 0 4 ppm IAA 3 0 ppm	7	28 5	0	3 0	3 0	Nıl	N l
MS K n 0 4 ppm IAA 4 0 ppm		33 3	1 0	4 0	0	Nıl	N
MS K n 0 4 pm AA		40 0	1 0	0	5 0	N l	N


P te G een ompa t ca lus nduced n e mediıum
supp emented th BAP 0 5 ppm and IAA 0 p m

Table 8 Growth performance of ax llary bud cultures ın varıous medıa ıdentıfıed for bud break n G sylvestre

reatments	Days taken for bud break	Bud break ( )	No of shoots produ ced	Mean shoot length after 1 month culture perıod (cm)	No of leaves produ ced	No of leaves retaıned after 2 month culture perıod	Culture showıng callus ng tendancy ( )
MS + BAP 4 0 ppm AA 0 8 ppm (C)	19	19 0	0	2 0	2 0	N l	20 5
Coconut water 30	7	34 5	1 0	2 5	3 0	N l	Nıl
Adenıne sulphate 2 0 ppm	21	30 5	1 0	2 5	3 0	Nıl	N l
Coconut water 30 denıne sulphate 2 0 ppm	16	37 5	1 0	3 0	3 0	Nıl	N l
MS K n 0 4 ppm AA 2 0 ppm	7	33 3	1 0	3 0	3 0	Nıl	Nıl
MS K n 0 4 ppm AA 3 0 ppm	7	28 5	1 0	3 0	3 0	Nıl	Nıl
MS K n 0 4 ppm AA 4 0 ppm	6	33 3	0	4 0	4 0	Nıl	Nı
MS K n 0 4 ppm A 5 0 ppm	6	40 0	0	4 0	5 0	Nıl	N l

Table 9 Effect of d fferent carbon sources on leaf retent on of G sylvestre

(basal medium MS Kin 0 4 ppm IAA 5 0 ppm

Treatment ( )	Cultures sprouted (%)	Cultures elongated with leafy shoots (%)	Cultures that eta ned leaves)
Sucrose 1 0	23 0	Nil	Nil
Sucrose 2 0	24 5	13 5	Ni
Sucrose 3 0	40 0	25 0	Nil
Sucrose 6 0	23 5	Nil	N l
Maltose 3 0	30 4	11 1	Ni
Mannitol 1 0 Sucrose 3 0	7 5	Nil	Nil
Maltose 2 0 + Sucrose 1 0	27 7	17 7	Nil
Glucose 3 0	25 6	4 0	Nil

and shoot elongation Altering the carbon sources other than sucrose did not yield any favourable effect on leaf retention It was also observed that addition of mannitol completely inh bited the culture growth

4 3 2 Effect of inorganic components

The composit on of inorganic salts was changed in one of the best media identified for bud break and shoot growth so as to study the effect of inorganic components on leaf retention Le els of nitrates potassium magnesium and calcium were alte ed and the results are presented n Table 10 None of the treatments ga e significantly higher results However better results were obser ed when the level of magnesium sulphate in the half MS basal med um was increased by three times Leaf retention was increased by 0 per cent compared to the other treatments tried

4 3 3 Effect of vitamins and am no ac ds

Varying the levels of glutamine thiamine ascorbic ac d glycine and pyr doxine did not give any favourable effect on growth and leaf retention in Gymnema cultures The results obtained a e presented in Table 11

4 4 Rooting of in vitro shoots

Rooting of n vitro shoots was attempted by varying the organic and inorgan c components as well as the growth regu ato s

Table 10 Effect of norgan c components on eaf etent on of G sylvestre

Treatments	Culture estab sh ment (%	Cultures hav ng leafy shoots (	Cultu es tn reta ned healthy ea e after 2 mon h cu ture pe o
₂ MS K n 0 4 ppm IAA 5 0 ppm (Control C)	59 5	25 0	N
C 4M KNO_3	51 3	9 2	N
C 2M NH_4NO_3	53 2	21 0	N
C 2M NH_4NO_3 4M KNO_3	56 5	20 2	N
C 2M NH_4SO_4	52 2	Nil	N
C M $MgSO_4$	6 0	2 5	0
C 3M $CaCl_2$	59 3	38 9	N
C 1M Calcium gluconate	59 0	2	N
C 2M Calcium gluconate	54 2	1 6	N
C 3M Calc um gluconate	54 8	7 4	N
C 3M $AgNO_3$	53 2	9 2	N

Table 11 Effect of vary ng vitamins and aminoac ds on leaf etent on of G sylvestre

Treatments	Culture establish ment ( )	Cultures having leafy shoots ( )	Cultures tha etained e e after 2 month culture pe od
MS Kin 0 4 ppm IAA 5 0 ppm (Control C)	59 5	25 0	N
C 1M glutamine	5 2	22 2	N l
C 2M glutamine	59 2	20 3	Nil
C 3M glutam ne	54 6	20 0	N l
C 4M glutamine	57 0	23 2	N
C 5M glutamine	56 8	23 0	N
C 1M Th am ne	54 2	19 7	N l
C 2M Th amine	53 7	19 2	N
C 2M As orbi ac d	57 3	17 5	
C 3M Ascorbic acid	54 2	19 5	Ni
C 2M glyc ne	51 5	20 1	N
C 3M glyc ne	53 2	23 2	N l
C 1M pyridox ne	49 9	21 6	N l
C 2M pyridox ne	52 1	22 0	N l

The media combinations tried for the purpose are listed in Table 2

None of the treatments tried could induce roots in Gymnema cultures However shoot elongation was observed in modified MS medium with one fourth concentration of inorganic salts supplemented with activated charcoal 0 5 mg/l Defoliation as a problem in rooting stage also

4 5 Indirect organogenesis/embryogenesis

4 5 1 Callus induction and proliferation

4 5 1 1 Effect of growth regulators

The effect of various growth regulator combinations for inducing calli in different explants is presented in Tables 3 and 14 Among the various growth regulator combinations tried in the MS basal media for leaf explants BAP at levels 0 5 to 1 0 ppm in combination with NAA 0 5 to 2 0 ppm and 2 4 D at 0 5 to 2 0 ppm gave the best response for callusing (76 to 90 per cent)

The callus induced at low levels of BAP in combinations with NAA were green and friable (Plate 5a and 5b) whereas IAA at levels 0 5 to 2 0 ppm in combinations with low levels of BAP induced green compact callus in Gymnema leaf segments (Plate 6) The calli were cream watery and friable when 2 4 D was incorporated in combination with BAP (Plate 7a and 7b) Callus morphology was found mainly influenced by the type of auxin and their concentration rather than the type of cytokinin used The kinetin levels

Table 12 Effect of different media combinat ons on rooting of G sylvestre

Treatments	Mean initial shoot length (cm)	Shoot length after one month (cm)	Leaf retention after one month ( )	Root nduct o
½ MS basal media	0 5	0 5	Nil	N l
½ MS IBA 1 0 ppm	0 5	0 5	N l	N
₂ MS + IBA 2 0 ppm	0 5	0 5	Nil	N
MS + IBA 3 0 ppm	0 5	0 7	N l	N l
½ MS IBA 4 0 ppm	0 5	0 7	Nil	N l
MS + IBA 5 0 ppm	0 5	0 7	Nil	Nil
₂ MS NAA 1 0 ppm	0 5	0 5	N l	Nil
₂ MS + NAA 2 0 ppm	0 5	0 5	Nil	N l
½ MS NAA 3 0 ppm	0 5	0 5	N l	N l
₂ MS + NAA 4 0 ppm	0 5	0 5	N l	N l
₂ MS NAA 5 0 ppm	0 5	0 5	Nil	Nil
MS 0 25 charcoal	0 5	3 5	Nil	Nil
MS + 0 5 charcoal	0 5	4 0	N l	Nil
MS + 1% charcoal	0 5	3 5	Nil	N
MS 2% charcoal	0 5	3 5	Nil	N l

a E ec d e e g eg a or a on a us d on and on
p a
ed a M

a	m	Da a o a duc on Mea	% ng	a us de	og pe	ee
BAP 0 5	AA 0 5	8	90 2	6 0	Da g ee ab e a us	
BAP 0 5	NAA 0		9 2	9 6	Da g ee ab e a	
BA	NAA 5		89 2	2 8 0	G ee ab e a	
BAP 0 5	NAA 2 0	8	89 0	2 9 0	Pa e g ee ab a	
BAP 0 5	AA 0 5	2	69 2	2 0	Pa e g ee mpa a us	
BAP 0	AA 0	20	69	2 6	Pa e g een ompa a	
BAP 0 5	AA 5	2	0 6	8	e mpac a us	
BA 0	AA 0	2	8 0	0 0	G een mpa a us	
BAP 0	2 D 0 5	5	88		P ae ea wa er a e a	
BA 0 5	D 0		88 0	0 0	Pa e eam a e ab e	
BAP 0 5	2 D		6	8	eam er a a us	
B	D 0		9		eam ab e a a us	
B P	NAA 0			6 6	Da G een a us	
BAP	NA 0			8	Da g ee a ca	
BA	NAA	8	86 2	6 0	G e ab e a us	
BA	A 0	8	9 8	2 8	ee ab e a	
BA				8 0	Pa e g ee pa a	
BAP	AA		0	25 0	Pa e g ee mpa a	
BAP 0	AA 5	2	66 5	9 2	G ee mpa a s	
BAP 0	AA 2 0	22	6	0 0	Pa e g ee mpa a us	
BAP 0	D 0		9 5	60 0	Pa e eam wa er b e a	
BAP 0	D 0		9	68 8	Pa e eam wa e ab e a	
BAP 0	2 D 5	5	9	0	Pa e eam a er ab e	
BAP 0	2 D	6	0	3 8	Pa e eam wa ab e n us	
BAP 0	NA 0 5		2	8	Pa e g ee e a	
BAP 0 5	NAA 0	2	6 8		a e g ee s	
BA			0	9 6	Pa e g ee ab a us	
K 0 5	A 0	5	6 2	0	Pa e g ee a a us	
K 0 5	A 5			6	a e g ee m a	
K 0	AA 0	6	0	0	Pa e g ee om a a us	
0	A				Pa e g ee a a us	
K	A 0		2	8 0	Pa e g ee mpa a	
K 0 5	2 D 0	2			Pa e e w a e a us	
K				20 0	Pa e e w wa e a us	
K 0	D	22		0	Pa e e w a e a us	
0	D 0	22	6	20	Pa e e w wa e a	
	NA 0	2	6 8	65 0	Pa e g ee ab e a us	
K	NA	2		6 0	Pa e g ee a e a us	
0	NAA	25	6	0 0	Pa e g ee ab e a	
K 0	NA	25	6 9	96 0	Pa e g ee a e a us	
K 0	AA 0 5	26	0	0	Pa e g een mpa a us	
0	AA 0	25		30	Pa e g ee m a a	
K	AA	2		2 6	Pa e g een mpa a	
K 0	AA 2 0	26	2	23	Pa e g ee mpa a us	
0	D 0 5	2		0 0	Pa e e w ab e a us	
0	D 0	2	2	38 0	Pa e e w ab e a us	
K 0	D 5	2		8 0	Pa e e w b e	
0	D		0		a e	

d on

		a a d on		us		oq
		0	8		a g ee	
BAP	A 0	8	8 8	8	een ab e a	
BA	5	8	8	8 0	a g e a	
BAP			8		q a	
BA				8		a us
BA	AA 0		68			
B	A 5		6 9	0		
BAP 0 5	A 0	2				
BA 0	D 0		8		eam	
B	D 0		8	9		
B P	D		8	88		
BA		8		80		
B	N	9	9		b	
B	0		89	8		
B 0			8	8	g	
B			8	0		
B				8	g ee	
B				8		
BA				0		
B P	0			0		
			8	8		er
B		6	89	6		
BA			8	0		
BAP	D		8	0		
	A			0 0		
			6			
				0	a e	p
				8		
0	A	6	8	6		
K 0	A					
0				0	a e	
				0		
K	D					
	0					
				8 0	a e g a	
	NA		6	0		
	N	6		0		
		6	8			
K					a g	us
				0	a	us
				0		
			6			

Plate 5a Green friable callus induced in the medium supplemented with BAP 0 5 ppm and NAA 1 0 ppm

Plate 5b Green friable callus enlarged view (16 x)

Plate 6 Green compact callus induced in the medium supplemented with BAP 0 5 ppm and IAA 1 0 ppm

Plate 7a Cream watery callus induced in the medium supplemented with BAP 0 5 ppm and 2 4 D 1 0 ppm

Plate 7b Cream watery callus enlarged view (16 x)

tried (0 5 to 1 0 ppm) were found to inh bit the callus ng nd low callus index (29 1 to 216) was recorded for all the comb nations tried w th kinetin as compared to those with BA (04 to 368 8) The results are presented in Table 13

The response of stem segments to var ous growth regulato combinations tried for callusing is presented n Table 14 The percentage callusing and the callus index recorded for stem segments in various media combinat on followed the same trend as for leaf segments Combinations of BAP 0 5 to 1 0 ppm either with NAA 0 5 to 2 0 ppm or with 2 4 D 0 5 to 2 0 ppm gave the max mum response to callusing (81 6 to 90 per cent) Callus ndex recorded was the maximum for media combinations with BAP either with NAA 0 5 to 0 ppm or 2 4 D 0 5 to 1 0 ppm As for the leaf exp ant kinet n was found less effective in inducing call n the ste n segments The callus morphology was also of the s m lar patte n as described for those induced from leaf segments The type of growth regulator and its concentration was found critical n dete m n ing the nature of callus produced rather than the type of explants or basal medium used

4 5 1 2 Effect of organic supplements

The effect of organic supplements like coconut water and casein hydrolysate on callus growth and its morphology is presented in Table 15 Coconut water did not influence much the callus indu t ion in Gymnema leaves but the later proliferation was found redu ed

Table 15 Effect of organic supplements on callus growth and morphology

ments		Callus induction (%)	Callus proliferation ( )	Callus ndex	Morphology of callus produced
BAP 1 0 ppm	L	91 3	90 4	361 6	Dark green friable callus
) 5 ppm (Control C)	S	90 0	86 6	346 4	Green friable callus
oconut water 10%	L	90 5	72 9	271 6	Green friable callus
	S	87 6	59 0	262 9	
oconut water 15	L	83 7	74 9	251 1	Pale green friable callus
	S	81 2	71 0	243 6	
oconut water 20	L	91 6	62 5	183 2	Cream friable callus
	S	82 0	60 2	164 0	
oconut water 30	L	90 4	53 7	90 4	Cream friable callus
	S	84 2	49 5	84 2	Pale yellow friable callus
asein hydrolysate	L	59 0	9 7	59 0	Pale green compact callus
pm	S	60 1	9 0	60 1	
asein hydrolysate	L	53 3	3 5		Pale green compact callus
pm	S	51 2	1 0		
asein hydrolysate	L	41 9			Pale yellow compact callus
pm	S	39 5			
asein hydrolysate	L	23 3			Pale yellow compact callus
pm	S	20 2			

L Leaf segment
S Stem segment

when coconut water up to a level of 30 per cent was nco porated ın the medıum The callus ındex recorded was also lo v n a l the combınatıons of coconut water trıed as compared to the cont ol medıum The coconut water also nfluenced the callus morphology by changıng the colour of the newly formed callı The callus vh h was ınıtıally dark green appeared pale yellow when hıgher e els of coconut water was ıncorporated ın the med um (Table 15) Percentage callusıng callus prolıferatıon and the callus ındex was greatly reduced when casein hydrolysate was ncorporated n the medıum The external appearance of the callı was also found alte ed by casein hydrolysate It became pale yellow and compact n contrast to dark green and frıable nature n control medıum

4 5 1 3 Effect of carbon sources

Among the dıfferent carbon sources tr ed sucrose at 3 0 pe cent level gave the maxımum callusıng The callus ndex recorded was 362 Other carbon sources trıed lıke glucose mannıtol and maltose dıd not ımprove the callus ng rate ın Gymnema sylvestre (Table 16) The reductıon ın callus growth was the maxımum hen mannıtol was ıncorporated ın the medıum

4 5 2 Callus morphology as ınfluenced by growth regulator combınat ıon and culture per od

The changes ın callus morphology at varyıng ntervals of the culture perıod was stud ed The ınıtıal callus produced as ıvory whıte and frıable ın all the combınat ons trıed (Table

Table 16 Effect of different carbon source on callus growth and proliferation in G sylvestre

Basal medium MS BAP 1 0 ppm NAA 0 5 ppm)

Treatments ( )		Callus index			Morphology of callus produced
		I Subculture	II Subculture	III Subculture	
Sucrose 1 0		166 3	166 3	83	Pale green compact callus
Sucrose 2 0		286 8	286 8	179 2	Pale green friable callus
Sucrose 3 0		362 0	362 0	362 0	Dark green friable callus
Sucrose 6 0		61 7	61 7	61 7	Green compact callus
Maltose 3 0		147 3	73 6		Green friable callus
Minnitol 1 0	Sucrose 2 0				
Maltose 2 0	sucrose 1 0	149 0	149 0	74 5	Pale green compact callus
Glucose 3 0		237 0	237 0	158 0	Pale green compact callus

able 17 Effect of different growth regulators on callus morphology of G *sylvestre* at different intervals of culture period

ments	Culture period				
	1 week	2 week	4 weeks	6 weeks	8 weeks
BAP 1 0 ppm 0 5 ppm	Ivory white friable callus	Pale green friable callus	Dark green friable callus	Dark green friable callus	Brown friable callus
BAP 1 0 ppm + 5 ppm	Ivory white friable	Cream compact callus	Pale green compact callus	Brown compact callus	Brown compact callus
BAP 1 0 ppm 0 5 ppm	Ivory white friable callus	Cream watery callus	Pale cream watery callus	Brown watery callus	Brown friable callus
K n 1 0 ppm 0 5 ppm		Cream friable callus	Pale green friable callus	Brown compact callus	Brown compact callus
Kın 1 0 ppm 5 ppm		White to pale green compact callus	Pale green compact callus		
Kın 1 0 ppm 0 5 ppm		White watery callus	Pale yellow watery callus	Brown friable callus	Brown friable callus

Media with BAP and NAA induced dark green friable callus within 4 to 6 weeks culture period The callus gradually turned brown and remained friable for about 8 weeks Kinetin and NAA combinations induced pale green friable callus Media supplemented with IAA induced pale green compact callus within four weeks The callus irrespective of the cytokinin supplemented turned brown but remained compact for six week time Similarly media with 2 gave pale creamy to yellow watery callus in four weeks time and turned brown within six weeks culture period

4 6 Organogenesis/embryogenesis

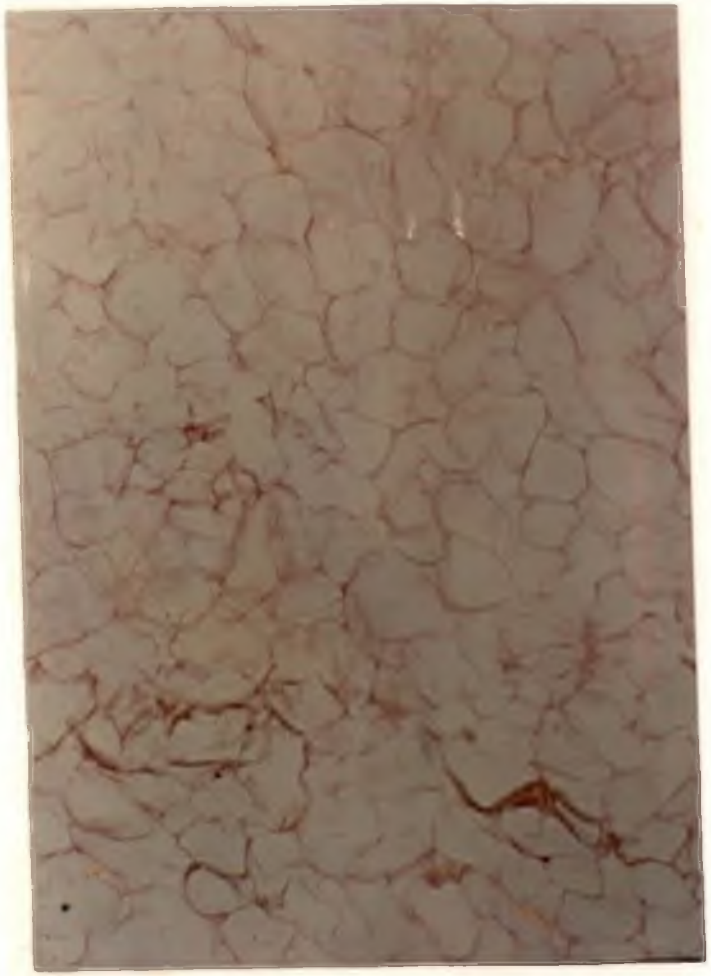
The callus induced in different media combinations were cultured upto the 7th subculture by varying the growth regulator combinations (Annexure IIIa) None of the combinations tried gave favourable response with respect to indirect organogenesis Microscopical examination of the calli having different morphology and age showed no signs of organogenesis The aged calli were found to accumulate starch granules on the surface (Plate 8) Microtome sections showed uniform cells without any vascular differentiation (Plate 9) Lignified cells were observed in the callus maintained for more than 6 weeks (Plate 10)

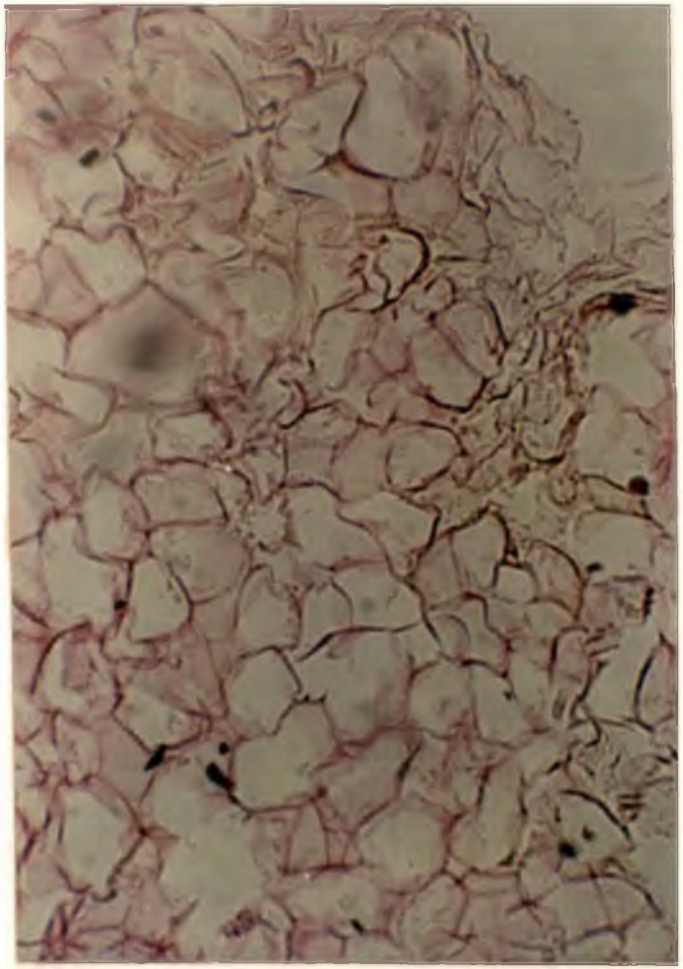
Embryoids were formed in a very few media combinations and the results are presented in Table 18 Though the frequency was less embryogenesis was observed in the callus maintained in MS basal media supplemented with combinations of BAP NAA BAP

Plate 8 Starch granuls developed on aged callus enlarged view (16 x)

Plate 9 Photomicrograph of callus showing uniform cells (150 x)

Plate 10 Photomicrograph of callus showing lignified cells (150 x)





late 1 Callus show ng globular embrvoid

P ate 12 Torpedo s age of embryoids

b e 8 d e emb ge e a me a a on a b q e b
y es e

a p a	Ca s duc on med a	S b mbe	S b e med a	Resp se a s	a m p oq	E b gen %
a eqmen	MS		MS	52 8	G ee ab e	
			M	36 6	Pa e g ee a e	
			MS	6 0	G e ab	
			MS	9 6	Da g ee ab e	
			MS	9 6	en ab	
ea egm	MS 5		M	2 0	Pa e g ee pa	
			MS	2	P e e m a	
			M	2 0	a e m	
S em egmen	MS		M	8	D k ee ab	
			MS	0	G ee a e	
			MS		Pa e e a	
ea e	M 8		MS 8	6 0	P e g ee b	
			M 9	6 2		
			M 0	9 2	Pa e e mpa	
			M	2 2		
			MS	8 0		
ea eg e	MS		MS	0	a w a	
			MS	6 0	eam a e	
			MS		eam a e	

MS	MS	BAP 0 ppm	NAA 0 ppm	M	MS	BAP 0 ppm	NAA 0 p	AB					
MS 2			0 5 ppm	MS 8	MS	K 0 ppm	AA ppm						
MS 3			5 ppm	MS 9	MS	0 ppm	NAA 0 pp						
MS	MS	BAP 0 5 ppm	NAA 0 ppm	MS 0	MS	K 0 ppm							
MS 5	MS	BAP 0 ppm	AA 0 ppm	MS	MS	K 0 ppm	2 4 D 0 ppm						
MS 6	M	BAP 0 5 ppm	AA 0 5 ppm	MS 2	MS	K 0 ppm	2 D 0 ppm	AB	pp				

IAA and kinetin 2 4 D Embryoids were observed n green f able calli pale yellow compact calli and in creamy white friable cal The embryoids observed were in t ally globular in shape with smooth surface (Plate 11) which later developed into torpedo l ke structures (Plate 12) They were found to be highly unstable and got reverted to callus phase within five days if not subcultured (Plate 13)

4 6 1 Embryo germination and further growth

The embryoids formed were subcultured at d fferent stages of ts developments to various media combinations None of the combinations attempted could support further growth and germinat on of the embryoids They either became brown and later dried up or turned to a callus phase again Embryo maturat on could not be achieved in any of the treatment tr ed for the purpose (Table 9

Plate 11 Callus showing globular embryoid

Plate 12 Torpedo stage of embryoids

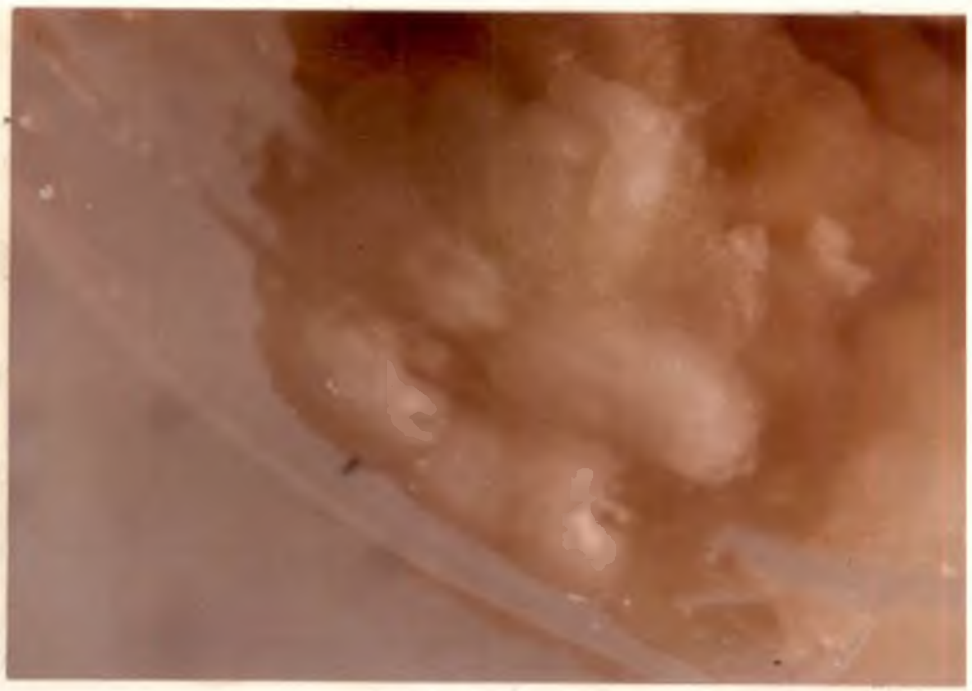


Plate 13 Revertion of embryoids to callus phase

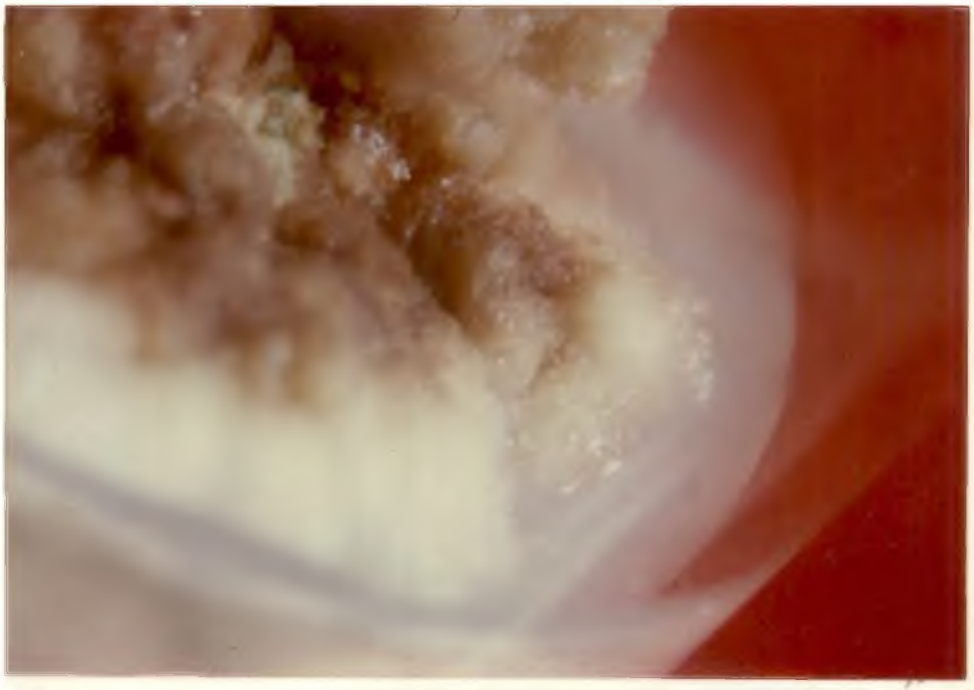


Table 19 Subculture response of embryoids of G sylvestre in various media combinations

Teatment (Subculture media)	Response after one week
Liquid basal MS medium	Turned brown and dried
Semi solid basal MS medium	
MS + BAP 1 0 ppm	Started callusing
MS + BAP 2 0 ppm	
MS + BAP 4 0 ppm	
MS + BAP 4 0 ppm + NAA 0 8 ppm	
MS + BAP 1 0 ppm + 2 4 D 0 25 ppm	
MS + Kin 1 0 ppm + NAA 0 25 ppm	Turned brown and dried
MS + Kin 1 0 ppm + IAA 0 25 ppm	
MS + Kin 1 0 ppm + NAA 0 5 ppm + ABA 1 0 ppm	
MS + Kin 1 0 ppm + 2 4 D 0 5 ppm + ABA 1 0 ppm	

DISCUSSION

DISCUSSION

Propagation of Gymnema sylvestre one of the few medicinal plants identified for hypoglycemic properties is found rather difficult due to its scarce seed set and poor rooting of cuttings or low success in other vegetative propagation methods The plant acquired its importance due to its efficiency in controlling the glucose level in blood in insulin dependent diabetes mellitus and also due to its property to enhance endogenous insulin (Shanmugha-sundaram et al 1990) There exists a tremendous need to popularise this important medicinal plant which has got wide adaptability under humid tropical conditions

Attempts were made for the first time to standardise the in vitro propagation technique of Gymnema sylvestre in the Plant Tissue Culture Laboratory Department of Plantation Crops and Spices College of Horticulture Mature vines maintained in the medicinal plants garden of the All India Co ordinated Research Project on Medicinal and Aromatic Plants at the College of Horticulture was used as the source material for the study The results obtained are discussed hereunder

5 1 Enhanced release of axillary buds

5 1 1 Culture establishment

The explants collected from mature vines of Gymnema sylvestre could be effectively surface sterilised with mercuric chloride at

0 1 per cent The treatment time varied from 6 minutes to 10 minutes according to the nature of the explants used Maximum survival rate recorded was 92 per cent for leaf segments and 84 per cent for nodal segments Increasing the treatment time resulted in high rate of mortality of the explants

Effective use of mercuric chloride as a surface sterilant at 0 1 per cent level has been reported by Lakshmi Sita (1986) in Dalbergia latifolia, Reghunath (1989) in Elettaria cardamomum Lakshmidevi (1992) in Dendrobium, Sharma (1992) in Tylophora indica and Babu et al (1992) in Zingiber officinale

Microbial interference was the major problem in establishing in vitro cultures of Gymnema sylvestre Great seasonal variation was also observed for the same Incidence of fungal infection in cultures of field explants has been recorded as a serious problem in micropropagation Doublin (1984) observed that the percentage of infection was more than 90 for field explants regardless of the procedure used for their sterilization Fungicidal spraying of mother plants have been suggested by Legrand and Mississo (1986) as a method for reducing culture contamination but in Gymnema sylvestre attempts made on this line did not give favourable result in reducing microbial contamination The microbial interference in Gymnema cultures however was season dependent The main inter ference for culture establishment was fungal infection and the organism was identified as Collectotrichum sp Months of January to April

was identified as the best period for establishing the cultures of Gymnema sylvestre with the minimum fungal interference The microbial interference was as high as cent per cent during the months of June July High infection rate in in vitro cultures has been widely reported to cause problems depending on both species and growing conditions (Borrod 1971 Seabrook et al 1976 Yang, 1977 Boxus 1978 Doublin 1984 and Mahato 1992) Seasonal variation for microbial interference in cultures of Gymnema might be due to the favourable environment for the microbes during certain periods of the year Relatively dry periods were found to suppress the microbial population in cultures

In Gymnema sylvestre addition of systemic fungicide to the culture medium was found to have remarkable influence in reducing the rate of contamination Of the different combinations tried Bavistin (Carbendazim) 1000 ppm when incorporated in the medium gave better results But at this high dose the rate of explant survival was found to be poor (Table 5) Similar results of fungicide toxicity have been reported by Brown et al (1982) and Shields et al (1984)

Dodds and Roberts (1985) suggested to avoid the use of fungicides for sterilisation since they or their degradation products may be metabolised by plant tissues with unpredictable results Addition of systemic fungicide in the culture medium showed chlorophyll degradation and vitrification in leaves of cardamom (Reghunath 1989)

Among the various basal media tried for culture establishment ($\frac{1}{2}$ MS $_4$ MS WPM B_5 SH White s and Heller s) MS basal medium with half the concentrations of inorganic salts was identified as the best for nodal segments with an establishment rate of 60 per cent The leaf explants established well in complete MS medium with an establishment rate of 84 per cent (Table 13)

Modified basal MS medium with reduced major elements has been identified for axillary bud initiation in various other medicinal plants like Cinchona ledgeriana (Hunter 1979) Solanum nigrum (Mandal and Gadgil 1979) Panax ginseng (Choi et al 1982) Asclepias erosa (Lee et al 1985) and Valeriana wallichii (Madhur and Ahuja, 1991) In Gymnema sylvestre at high salt concentrations the explants had a bleached appearance at the cut end which probably hindered the nutrient uptake leading to poor culture establishment High salt concentration of MS medium might not be suitable for the establishment of cultures and may be even toxic to the explant tissue

5 1 2 Bud break and shoot formation

In its natural habitat the axillary buds of the climbing vine Gymnema sylvestre are normally dormant due to strong apical dominance The application of cytokinin to the axillary buds is reported to overcome this apical dominance in many crops (Murashige 1974) The direct effect of cytokinin in tissue culture may vary according to the particular compound used the type of cultures

and the variety of plant from which it was derived (George and Sherrington 1984)

In the present study very few media combinations were identified for inducing bud break in Gymnema sylvestre Out of the various combinations tried for the purpose only 12 gave favourable response Growth regulator combinations of kinetin (0 4 ppm) with IAA (2 0 to 5 0 ppm) and BAP (4 0 ppm) with NAA (0 8 ppm) gave better response in half MS basal medium Among the combinations identified for bud break media having kinetin and IAA alone produced healthy shoots with normal leaves The cytokinin BAP alone or in combination with auxins did not favour healthy shoot formation

The action of kinetin might be stronger than BAP and IAA being thermolabile and photosensitive the combination of low kinetin and relatively high IAA favoured bud break and later growth in Gymnema sylvestre The growth regulator combinations of BAP with NAA induced callusing in the cultures and this might be the reason for low bud break and shoot formation in this combination though it gave a better culture establishment at initial stages

Use of kinetin in the culture medium has been reported for bud break in Gloriosa superba (Somani et al 1989) The favourable effect of BAP in bud break has also been reported earlier

in various medicinal plants like Dioscorea floribunda (Sinha and Chaturvedi, 1979) Digitalis lanata (Erdei et al 1981) Digitalis purpurea (Rucker 1982) Glycyrrhiza glabra (Syrtanova and Mukhit dinova, 1984) and Catharanthus roseus (Mumtaze et al 1990)

Gibberelic acid tried at various combinations did not yield any favourable result for the growth of cultures Inhibitory effect of GA_3 in cultures has been reported earlier by Heide (1969) in Begonia Bhasker (1991) in banana and Mahato (1992) in Dalbergia latifolia However Rucker (1982) found an enhancing effect of GA_3 in Digitalis purpurea cultures

The maximum bud break recorded for Gymnema in the present study was only 40 per cent in the best media identified Hence the media combinations tried in the present study is presumed to be not sufficient to eliminate the limiting factors for the in vitro culture of Gymnema sylvestre

5 1 3 Effect of organic supplements on bud break and shoot formation

Organic supplements like adenine sulphate coconut water, casein hydrolysate and phloroglucinol were tried so as to study their effect on bud break and later growth Maximum enhancement in sprouting was observed in combinations of 2 0 ppm adenine sulphate and 30 per cent coconut water (Table 7) The organic addit ive were also found to favour leaf production in the sprouted

cultures This is in confirmity with the observations made by Van Overbeak et al (1941) in Datura, Mandal and Gadgil (1979) in Solanum nigrum Ilahi (1983) in Papavar somniferum and Ilahi and Akram (1987) in Rauvolfia serpentina wherein coconut water is reported to support healthy cultures

In the present study incorporation of coconut water in the culture media could induce leaf production in 22 5 per cent of cultures Addition of adenine sulphate along with coconut water slightly increased the number of healthy cultures and gave 24 per cent cultures with leaves while the control media recorded only 4 5 per cent healthy cultures Here the higher percentage of sprout ing and leaf production might be a combined effect of both coconut water and adenine sulphate However the results clearly indicate the promotive effect of coconut water on culture sprouting and leaf production in Gymnema sylvestre The favourable effects of coconut water in the promotion of growth and differentiation of excised tissues and organ have been attributed to the presence of cytokinins and gibberelin like substances in it (Straus and Rodney 1960)

Incorporation of casein hydrolysate a complex mixture of amino acids has been attempted by several workers Enhancement in multiple shoot production by adding casein hydrolysate has been reported by Mascarenhas et al (1983) in Hevea brasiliensis and Mahato (1992) in Dalbergia latifolia However addition of casein hydrolysate in the culture medium gave no favourable response in

Gymnema sylvestre Similar observation have been reported by George and Sherrington (1984) in several plant species both in monocots and dicots

Phloroglucinol a phenolic auxin synergist commonly used in tree species gave no favourable results in Gymnema sylvestre Instead it reduced the percentage of culture establishment and culture sprouting when incorporated in the medium Hunter (1979) reported the promotive effect of phloroglucinol in culture establish ment and growth of Cinchona ledgeriana and Mallika et al (1992) in Theobroma cacao

Leaf retention was very poor in all the combinations identi-fied for bud break in Gymnema Defoliation of the newly formed shoots was the major constraint in carrying forward the cultures The shoot growth was found restricted after defoliation None of the media combinations identified for bud break could support leafy shoots for a period of two months The leaves became chlorotic turned pale yellow and were shed within two month s period

5 1 4 Enhancement of leaf retention

The composition of inorganic salts was changed in one of the best media identified for bud break so as to study their effect on leaf retention The nitrates potassium magnesium and calcium were altered in the present study None of the treatments gave significantly higher results However better results were

observed when the level of magnesium sulphate in the half MS basal medium was increased by three times compared to the standard strength Healthy leaves were retained in 10 per cent of the cultures when magnesium sulphate was incorporated in the culture medium The favourable effect of magnesium on shoot growth has also been reported in Chestnut (Chevre et al , 1986) Magnesium sulphate would have helped leaf retention in Gymnema through the favourable influence of magnesium in chlorophyll formation Favourable effect of altering the inorganic components in the culture medium has been reported for enhancing bud break and shoot elongation in gooseberry (Wainwright and Flegman 1985) and in Dalbergia latifolia (Swami et al 1992)

Incorporation of various vitamins like glutamine thiamine ascorbic acid and pyridoxine did not yield any favourable response for leaf retention in Gymnema sylvestre

Carbon source in the culture medium was altered at different levels to study their favourable influence if any for leaf retent ion in Gymnema cultures Using the carbon sources other than sucrose did not yield any favourable effect on leaf retention In the present study 3 0 per cent sucrose was found to be the optimum level for sprouting and shoot elongation Gamborg et al (1974) suggested that the efficiency of nitrate and ammonium ions can depend on sucrose concentrations and the effect of cytokinins on cell division

may also be dependent on the sugar availability The use of alternative carbon sources like glucose maltose raffinose fructose and galactose have been reported to be less effective (George and Sherrington 1984) Varying effects were found in using different sugars as energy source in cultures (Reghunath 1989 Mahato 1992)

The hypoglycemic property of Gymnema sylvestre might be due to its direct or indirect influence on carbohydrate metabolism The same mechanism though not yet fully understood might be acting in the in vitro culture system especially interfering the sucrose absorption and utilisation The poor response of Gymnema for in vitro culture system could thus be explained through its interaction in sugar metabolism

5 1 5 Rooting of in vitro shoots

Rooting was attempted for the in vitro shoots by varying the organic and inorganic components as well as the growth regulators in the culture medium None of the combinations tried yielded favourable result again signifying the recalcitrant nature of Gymnema as in other stages of in vitro cultures Pulse treatment with the rooting hormone IBA or incorporating IBA at different levels in the rooting media did not favour root initiation The response was not altered even when the carbon source was reduced or activated charcoal was incorporated in the culture medium The

only response expressed by Gymnema shoots was the increase in shoot elongation when cultured in the growth regulator free low salt media incorporated with charcoal ($\frac{1}{4}$ MS with 0 5 per cent charcoal) The shoots thus formed again were unhealthy and defoliated within one month culture period without any root induction

Abundant rooting has been reported in various crops when the salt concentration in the medium was reduced to one half one third or one fourth of the standard strength (Lane, 1979 Skirvin and Chu 1979) Shoot elongation in a medium without any growth regulators has been reported earlier by Syrtanova and Mukhidinova (1984) in Glycyrrhiza glabra Activated charcoal has frequently improved plant tissue culture by absorption of growth inhibitors prevention of unwanted callus growth and promotion of morphogenesis (George and Sherrington 1984) Kim and Lee (1988) reported that addition of 0 5 per cent activated charcoal in half strength MS media containing BA at 0 5 ppm showed best shoot and root growth from axillary buds of Ziziphus Similar results has also been reported for Cypress (Capuana et al 1991)

The favourable effect of IBA the rooting hormone is widely reported in many plant species (Erdei et al (1981) in Digitalis lanata Choi et al (1982) in Panax ginseng Ilahi and Akram (1987) in Rauvolfia serpentina and Sateeshkumar and Bhavandan (1989) in Plumbago rosea) However none of the media combinations tried in the present study could induce rooting in Gymnema

The low success recorded for *in vitro* multiplication and rooting of *Gymnema sylvestre* from nodal explants in the present study could be attributed to various factors Previous attempts and successful reports in this line was lacking for the crop The study was taken up for the first time without knowing its behaviour under *in vitro* cycle The microbial interference during the culture establishment stage was the main constraint in studying the response of cultures to various media combinations Due to the seasonal variation in microbial interference the study was to be restricted to a period of four months from January to April in the year of study The cultures of *Gymnema sylvestre* can be counted as rather recalcitrant based on the results of the present study The cultures were found shy to respond to the treatments tried at various stages of micropropagation Though moderately good culture establishment could be obtained, the response recorded for bud break shoot elongation leaf retention and rooting were not encouraging Much more efforts are to be put into for standardising the *in vitro* techniques for rapid multiplication of *Gymnema sylvestre* through axillary bud release and proliferation

5.2 Indirect organogensis

Indirect or callus mediated organogenesis is an alternative method for micropropagation Wherever applicable it is the fastest method of shoot multiplication and has been suggested as a potential method of cloning plant species (Murashige 1974) The most serious

drawback in the use of callus culture is the possible genetic instability of the cells However callus mediated organogenesis as a method of clonal propagation has been reported in a number of medicinal plants Successful micropropagation techniques have been reported by Sinha and Chaturvedi (1979) in Diascorea floribunda Erdei et al (1981) in Digitalis lanata Rucker (1982) in Digitalis purpurea, Kothari et al (1986) in Artemesia scoparia Ilahi and Akram (1987) in Rauvolfia serpentina Sateeshkumar and Bhavanandan (1989) in Plumbago rosea and Mumtaz et al (1990) in Catharanthus roseus

5 2 1 Culture establishment

Different explants of Gymnema sylvestre like leaf segments stem segments and nodal segments were tried for inducing calli Different basal media were used and cultures were found to be estab lished in all the basal media tried though at varying frequencies Complete MS and half MS (with half the concentration of inorganic salts) registered better culture establishment after three weeks culture period Stem and nodal segments exhibited better establish-ment in half MS and leaf segments in complete MS medium Use of MS medium for culture establishment has been reported earlier by Choi et al (1982) in Panax ginseng, Ilahi (1983) in Papavar somniferum, Mhatre et al (1984) in Tylophora indica Sateeshkumar and Bhavanandan (1989) in Plumbago rosea and Mumtaz et al (1990) in Catharanthus roseus

5 2 2 Callus induction and its proliferation

5 2 2 1 Effect of growth regulators

Among the various growth regulator combinations attempted in the MS basal media for leaf explants BAP at level of 0 5 to 1 0 ppm with NAA 0 5 to 2 0 ppm or 2 4 D at 0 5 to 2 0 ppm gave the best results for callus induction with an establishment percentage of 75 to 90 and callus index of 104 to 368 8 Both leaf and stem explants responded similarly for callus induction and proliferation Callus induction rate and its morphology varied according to the growth regulators used Dark green friable callus was obtained in a combination of BAP and NAA at concentrations varying from 0 5 to 2 0 ppm Use of kinetin instead of BAP induced pale green friable callus with a low callus index (23 1 to 216) Similarly IAA instead of NAA induced pale green compact callus with lower callus index In the present study use of 2 4 D along with kinetin or BAP induced pale cream watery callus the proliferation rate being more with BAP than with kinetin levels Use of 2 4 D for callus induction was reported earlier in several crops by Mandal and Gadgil (1979) in Solanum nigrum Ilahi and Akram (1987) in Rauvolfia serpentina Sateeshkumar and Bhavanandan (1989) in Plumbago rosea Mumtaz et al (1990) in Catharanthus roseus and Neenakumari and Saradhi (1992) in Origanum vulgare

5 2 2 2 Effect of organic supplements on callus growth

Organic supplements like coconut water and casein hydrolysate

were incorporated in the media to observe the response of calli

Promotive effect of coconut water on callus growth was reported by Mandal and Gadgil (1979) in Solanum nigrum Ilahi (1983) in Papaver somniferum and Mahato (1992) in Dalbergia latifolia Contrary to this result in Gymnema sylvestre coconut water did not give any favourable result on callus growth and morphology instead the callus turned pale and proliferation was found reduced The cytokinin like compounds present in the coconut water might have caused this type of response Coconut water also contain a number of cell division factors and free amino acids (Shantz and Steward 952) which might have a deleterious effect on callus growth in Gymnema

Casein hydrolysate a complex mixture of amino acids when incorporated in the callus proliferation medium reduced the percentage of callusing callus proliferation and the callus index in Gymnema sylvestre Inhibitory effect of casein hydrolysate has also been observed in Dalbergia latifolia (Mahato 1992)

5 2 3 Effect of different carbon source on callus growth and morphology

A suitable carbon or energy source is required for the growth and proliferation of callus In Gymnema sylvestre among the different carbon sources tried sucrose at 3 0 per cent level gave the maximum

callusing This is in agreement with the observations made by Mumtaz et al (1990) in Catharanthus roseus Incorporation of mannitol totally inhibited the callus proliferation in Gymnema sylvestre

5 3 Indirect organogenesis

None of the treatments tried gave favourable response on callus differentiation and microscopic studies of the callus revealed the presence of uniform cells without any vascular differentiation

5 4 Indirect embryogenesis

The callus induced on leaf and stem segments of Gymnema sylvestre were subcultured to different media combinations so as to induce embryoids Though at lower frequencies (6 to 20 per cent) embryogenesis could be achieved in a few combinations like BAP + NAA, BAP + IAA and kinetin + 2 4 D Most of the media combinations tried were unfavourable for embryogenesis The embryoids observed were initially globular in shape with smooth surface which later developed into torpedo like structures

Somatic embryogenesis was attempted in a number of medicinal plants, and the positive results include the reports of Chang and Hsing (1980) in Panax ginseng Reinbothe et al (1990) in Digitalis lanata and Anjalikumar (1992) in Thevetia purpurea

The embryoids formed in Gymnema sylvestre were subcultured to different media for their further growth and maturation None of the treatments gave favourable results The embryoids either turned brown and dried up or turned to a callus phase Reymond et al (1990) reported the deleterious effect of BAP on plantlet formation and the promotive effect of callusing from embryoids of sweet potato

Response of Gymnema sylvestre for indirect embryogenesis and further manipulations of embryoids so as to obtain normal plant lets was found not encouraging All the normal procedures for callus induction and embryogenesis were attempted for the plant Though profuse callusing could be achieved by manipulating the growth regulator combinations in the culture media the rate of embryogenesis was very poor Different levels of growth regulators were attempted at various stages of callus growth so as to induce embryogenesis but with no favourable response Stress was induced to the cultures by prolonging the culture period by altering the carbon source and by incorporating growth inhibitors in the media again with little favourable response

The embryoids formed in Gymnema cultures were again peculiar in that they exhibited a strong callusing tendancy if retained in the same media The profuse callusing rate and the revertion of embryoids to the callus stage were the limiting factors that prevented successful plantlet formation through indirect embryogenesis

Since the number of embryoids that could be recovered for further manipulations were limited, the treatment combinations tried in the present study were not sufficient to identify the best media that could support further growth of the embryoids

SUMMARY

The present investigation was carried out during the period 1991 93 in the Tissue Culture Laboratory of the Department of Plant ation Crops and Spices College of Horticulture Vellanikkara with the objective of standardising the technique of micropropagation of Gymnema sylvestre Mature plants in the medicinal plant garden attached to the All India Co ordinated Research Project on Medicinal and Aromatic Plants were used as the source of explants The salient findings of the investigation are presented below

1 Of the various surface sterilants tried, mercuric chloride at 0 1 per cent level was found effective in establishing asceptic cultures of Gymnema sylvestre The treatment time varied from 6 to 10 minutes, respectively for leaf and stem explants during the best season identified

2 Microbial interference caused by Colletotrichum sp was identi fied as the main source of contamination during culture establish ment stage

3 Prophylactic spraying of mother plants with contact fungicide Dithane M 45 and systemic fungicide Bavistin did not reduce the contamination rate

4 Addition of systemic fungicide in the culture medium was found to reduce the rate of contamination with little survival of the explants

5 Seasonal variation was observed for the microbial interference and the period from January to April was identified as the best season for establishing the cultures

6 Complete MS basal media was identified suitable for establishing cultures of leaf segments whereas nodal segments survived better when the inorganic salt concentration was reduced to half the standard strength

7 Half MS basal medium with 0 4 ppm kinetin and 2 0 to 5 0 ppm IAA gave maximum bud break and leaf production from nodal segments

8 Coconut water alone and in combination with adenine sulphate favoured shoot formation in *in vitro* cultures

9 Addition of GA_3 did not have any favourable effect on bud break and shoot elongation

10 Incorporation of both phloroglucinol and casein hydrolysate was found to reduce the percentage of sprouting

11 Sucrose at 3 0 per cent level was identified as the best carbon source for supporting *Gymnema* cultures and leaf retention

12 Use of mannitol in the culture medium completely inhibited the culture growth

13 Among the altered inorganic salt concentrations tried a three fold increase in magnesium sulphate level was found to increase leaf retention by 10 per cent

14 Changing the vitamins and amino acid concentrations in the medium gave no favourable results

15 Low levels (1 0 to 5 0 ppm) of auxins like IBA and NAA could not induce rooting in Gymnema shoots

16 Activated charcoal at 500 mg l^{-1} did not improve rooting but favoured shoot elongation

17 Leaf segments responded better than stem segments for callus induction

18 MS basal medium with 3 0 per cent sucrose and growth regulator combination of 0 5 ppm BAP + 1 0 ppm NAA was identified as the best basal medium for callus induction

19 Callus morphology and proliferation rate varied with the growth regulator combinations

20 No favourable response was obtained with the addition of coconut water and casein hydrolysate in the callus proliferation medium

21 Embryogenesis though at low frequencies (6 to 20 per cent) was observed in the callus maintained in MS basal medium

supplemented with combinations of BAP + NAA BAP + IAA and kinetin + 2,4 D

22 The embryoids developed upto the torpedo stage in the best medium identified

23 Further growth and germination of the embryoids could not be achieved as they either turned brown and dried up or were reverted to the callus phase in subsequent subcultures

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ANNEXURE

ANNEXURE I

Composition of different basal media tried for *in vitro* culture of *Gymnema sylvestre*

a) White s medium (White, 1943)

Major nutrients	mg/l	Minor nutrients	mg/l
$Ca(NO_3)_2$ $4H_2O$	200 0	H_3BO_3	1 50
$Fe_2(SO_4)_3$	2 5	$MnSO_4$	4 50
KNO_3	80 0	$ZnSO_4$	1 50
KCl	65 0	KI	0 75
$MgSO_4$ $7H_2O$	360 0		
Na_2SO_4	200 0		
$Na_2H_2PO_4$ $2H_2O$	16 5		

Organic constituents

Inositol	3 0
Nicotnic acid	0 5
Pyridoxine HCl	0 1
Thiamine HCl	0 1

b) Heller s medium (Heller 1953)

Macronutrients	mg/l	Micronutrient	mg/l
$NaNO_3$	600	$MnSO_4$ $4H_2O$	0 1
$CaCl_2$ $2H_2O$	75	$ZnSO_4$ $7H_2O$	1 0
$MgSO_4$ $7H_2O$	250	H_3BO_3	1 0
KCl	750	KI	0 01
NaH_2PO_4 H_2O	125	$CuSO_4$	0 03
		$FeCl_3$ $6H_2O$	0 06

Organic constituents	mg/l
Thiamine	1 0

Annexure I Continued

c) MS medium (Murashige & Skoog 1962)

Macronutrients	mg/l	Micronutrients	mg/l
KN_3	1900	$MnSO_4\ 4H_2O$	22 30
NH_4NO_3	1650	$ZnSO_4\ 7H_2O$	8 60
$CaCl_2\ 2H_2O$	440	H_3BO_3	6 20
$MgSO_4\ 7H_2O$	370	KI	0 83
KH_2PO_4	170	$CuSO_4\ 5H_2O$	0 025
		$Na_2MoO_4\ 2H_2O$	0 25
		$CaCl_2\ 6H_2O$	0 025
		$FeSO_4\ 7H_2O$	27 80
		$Na_2EDTA\ 2H_2O$	37 30

Organic constituents mg/l

Myo-inositol	100
Thiamine-HCl	0 1
Nicotinic acid	0 5
Pyridoxine HCl	0 5
Gylcine	2 0

d) B_5 medium (Gamborg et al 1968)

Macronutrients	mg/l	Micronutrients	mg/l
KNO_3	2500	$MnSO_4\ H_2O$	10 0
$CaCl_2\ 2H_2O$	150	$ZnSO_4\ 7H_2O$	2 0
$MgSO_4\ 7H_2O$	250	H_3BO_3	3 0
$NaH_2PO_4\ H_2O$	150	KI	0 75
$(NH_4)_2\ SO_4$	134	$CuSO_4$	0 025
		$NA_2MoO_4\ 2H_2O$	0 025
		$CoCl_2$	0 25
		NaFeEDTA	0 28

Organic constituents mg/l

Myo inositol	100 0
Thiamine HCl	10 0
Nicotinic acid	1 0
Pyridoxine HCl	1 0

Annexure I Continued

e) SH medium (Schenk & Hildebrandt 1972)

Macronutrients	mg/l	Micronutrients	mg/l
KNO_3	2500	$MnSO_4\ H_2O$	10 0
$CaCl_2\ 2H_2O$	200	$ZnSO_4\ 7H_2O$	1 0
$MgSO_4\ 7H_2O$	400	H_3BO_3	5 0
$NH_4H_2PO_4$	300	KI	1 0
		$CuSO_4\ 5H_2O$	0 2
		$Na_2MoO_4\ 2H_2O$	0 1
		$CoCl_2\ 6H_2O$	0 1
		$FeSO_4\ 7H_2O$	15 0
		$Na_2EDTA\ 2H_2O$	10 0

Organic constituents mg/l

Myo inositol	1000
Thiamine HCl	5 0
Nicotinic acid	5 0
Pyridoxine HCl	0 5

f) Woody Plant medium (Lloyd & McCown 1980)

Macronutrients	mg/l	Micronutrients	mg/l
NH_4NO_3	400	$MnSO_4\ H_2O$	22 30
$Ca(NO_3)_2\ 4H_2O$	556	$ZnSO_4\ 7H_2O$	8 60
$CaCl_2\ 2H_2O$	22	H_3BO_3	6 20
$MgSO_4\ 7H_2O$	1850	$CuSO_4\ 5H_2O$	0 25
KH_2PO_4	340	$Na_2MoO_4\ 2H_2O$	0 25
		$FeSO_4\ 7H_2O$	27 80
		$Na_2EDTA\ 2H_2O$	37 30

ANNEXURE-II

Media combinations tried for culture establishment and bud breaK in Gymnema sylvestre

a) Combinations of growth regulators

Basal media	Cytokinin (ppm)	Auxin (ppm)
1	2	3
$_2$ MS (with 3% sucrose)	BAP 0 5	-
	BAP 1 0	
	BAP 2 0	
	BAP 4 0	
	BAP 6 0	
	BAP 8 0	
	BAP 10 0	
	BAP 0 5 •	NAA 0 5 1 0 1 5 and 2 0
	BAP 0 5	IAA 0 5 1 0 1 5 and 2 0
	BAP 1 0	NAA 0 5 1 0 2 0 and 2 5
	BAP 1 0	IAA 0 2 0 4 0 6 0 8 1 0, 1 5 2 0 and 2 5
	BAP 2 0	NAA 0 5 1 0 1 5 and 2 0
	BAP 2 0	IAA 0 5, 1 0, 1 5 and 2 0
	BAP 3 0	NAA 0 2 0 4 0 6 0 8, 1 0 and 2 0
	BAP 3 0	IAA 0 2, 0 4 0 6 0 8 1 0 and 2 0
	BAP 4 0	NAA 0 2 0 4 0 6 0 8 1 0, 1 5 and 2 0

Contd

Annexure-II Continued

1	2	3
2 MS (with 3% sucrose)	BAP 4 0	IAA 0 2, 0.4, 0 6 0 8 and 1 0
	BAP 5 0	NAA 0 5 1 0 2 0 and 2 5
	BAP 10 0	NAA 0 2, 0 4, 0 6, 0 8, 1 0, 2 0 3 0 and 4 0
	BAP 10 0	IAA 0 5, 1 0, 1 5 and 2 0
	BAP 4 0	NAA 0 2, 0 4, 0 6 and 0 8 GA_3 (1 to 5)
	BAP 1 0	IAA 1 0 1 5, 2 0 and 2 5 GA_3(1 to 5)
	Kin 0 5	-
	Kin 1 0	
	Kin 2 0	
	Kin 4 0	-
	Kin 6 0	-
	Kin 8 0	
	Kin 10 0	
	Kin 0 2	NAA 0 5 1 0 1 5 and 2 0
	Kin 0 5	NAA 0 5 1 0 1 5 and 2 0
	Kin 0 4	IAA 0 5 1 0 1 5, 2 0 3 0 and 5 0
	Kin 0 5	NAA 0 2 0 4, 0 6 0 8 1 0 1 5 and 2 0
	Kin 0 5	IAA 0 5 1 0 1 5 and 2 0
	Kin 1 0	NAA 0 5 1 0 1 5 and 2 0
	Kin 1 0	IAA 0 5 1 0 1 5 and 2 0
	Kin 2 0	NAA 0 5 1 0 1 5 and 2 0

Contd

Annexure II Continued

1	2	3
$_2$ MS (with 3% suc ose)	Kin 2 0	IAA 0 5 1 0 1 5 and 2 0
	Kin 3 0	NAA 0 5 1 0 1 5 and 2 0
	Kin 3 0	IAA 0 2 0 4 0 6 0 8 1 0 1 5, 2 0 and 2 5
	Kin 5 0	NAA 0 5 1 0 1 5 and 2 0
	Kin 5 0	IAA 0 5 10, 1 5 and 2 0
	Kin 10 0	NAA 0 5 1 0 1 5 and 2 0
	Kin 10 0	IAA 0 5 1 0 1 5 and 2 0
	BAP 1 0 + Kin 1 0	NAA 0 25 0 5 1 0 and 1 5
	BAP 1 0 + Kin 2 0	NAA 0 25 0 5 1 0 and 1 5
	BAP 2 0 + Kin 1 0	NAA 0 25 0 5 1 0 and 1 5
	BAP 1 0 + Kin 1 0	IAA 0 25 0 5 1 0 and 1 5
	BAP 1 0 + Kin 2 0	IAA 0 25 0 5 1 0 and 1 5
	BAP 2 0 + Kin 2 0	IAA 0 5 1 0 1 5 and 2 0 GA_3 (1 to 5)
	2iP 0 5	NAA 0 5 1 0 1 5 and 2 0
	2iP 0 5	IAA 0 5 1 0 1 5 and 2 0
	2iP 1 0	NAA 0 5 1 0 1 5 and 2 0

Contd

Annexure II Continued

1	2	3
½ MS (with 3% sucrose)	2iP 1 0	IAA 0 5 1 0 1 5 and 2 0
	2iP 2 0	NAA 0 5 1 0 1 5 and 2 0
	2iP 2 0	IAA 0 5 1 0 1 5 and 2 0
	2iP 5 0	NAA 0 5 1 0 1 5 and 2 0
	2iP 5 0	IAA 0 5 and 1 0

Annexure II Continued

b) Combinations of growth regulators with organic supplements

1	2		3
½ MS (3% sucrose)	BAP 0 5 ppm	NAA 0 5 ppm	Coconut water 15% and 30%
	BAP 0 5	IAA 0 5	
	BAP 1 0	NAA 0 5	
	BAP 1 0	IAA 0 5	
	BAP 2 0	NAA 0 2	
	BAP 2 0	NAA 0 4	
	BAP 2 0	NAA 0 8	
	BAP 2 0	NAA 1 0	
	BAP 2 0	NAA 2 0	
	BAP 2 0	IAA 0 5	
	BAP 2 0	IAA 1 0	
	BAP 3 0	IAA 0 5	
	BAP 3 0	IAA 1 0	
	BAP 4 0	NAA 0 2	
	BAP 4 0	NAA 4 0	
	BAP 4 0	NAA 0 6	
	BAP 4 0	NAA 0 8	
	BAP 4 0	NAA 1 0	
	BAP 4 0	IAA 0 2	

Contd

Annexure II Continued

1	2	3
2 MS (3% sucrose)	BAP 4 00 ppm IAA 0 4 ppm	Coconut water 15% and 30%
	BAP 4 0 IAA 0 6	
	BAP 4 0 IAA 0 8	
	BAP 4 0 IAA 1 0	
	BAP 1 0 NAA 0 5	Adenine sulphate 1,2,3 4 5 10 20 30 40 ppm
	BAP 1 0 IAA 0 5	
	BAP 2 0 NAA 1 0	
	BAP 3 0 NAA 1 0	
	BAP 4 0 NAA 0 2	
	BAP 4 0 NAA 0 6	
	BAP 4 0 NAA 0 8	
	BAP 4 0 NAA 1 0	
	BAP 4 0 NAA 0 2	Coconut water 15% and 30% and adenine sulphate (1 to 5 ppm)
	BAP 4 0 NAA 0 4	
	BAP 4 0 NAA 0 8	
	BAP 4 0 NAA 1 0	
	BAP 0 5 NAA 0 5	Casein hydrolysate 100 250 400 and 500 ppm
	BAP 0 5 IAA 0 5	

Contd

Annexure II Continued

1	2		3
½ MS (3% sucrose)	BAP 1 0 ppm	NAA 0 5 ppm	Casein hydrolysate 100 250, 400 and 500 ppm
	BAP 1 0	IAA 0 5	
	BAP 2 0	NAA 0 2	
	BAP 2 0	NAA 0 4	
	BAP 2 0	NAA 0 8	
	BAP 2 0	NAA 1 0	
	BAP 2 0	NAA 2 0	
	BAP 2 0	IAA 0 5	
	BAP 2 0	IAA 1 0	
	BAP 3 0	IAA 1 0	
	BAP 4 0	NAA 0 2	
	BAP 4 0	NAA 0 4	
	BAP 4 0	NAA 0 6	
	BAP 4 0	NAA 0 8	
	BAP 4 0	NAA 1 0	
	BAP 4 0	IAA 0 2	
	BA 4 0	IAA 0 4	
	BAP 4 0	IAA 0 5	
	BAP 4 0	IAA 0 8	
	BAP 4 0	IAA 1 0	

Contd

Annexure-II Continued

1	2	3
½ MS (3% sucrose)	BAP 0 5 ppm IAA 0 5 ppm	Phloroglucinol 10 20 30 and 40 ppm
	BAP 0 5 IAA 0 5	
	BAP 1 0 NAA 0 5	
	BAP 1 0 IAA 0 5	
	BAP 2 0 NAA 0 2	
	BAP 2 0 NAA 0 4	
	BAP 2 0 NAA 0 6	
	BAP 2 0 NAA 0 8	
	BAP 2 0 NAA 1 0	
	BAP 2 0 NAA 2 0	
	BA 2 0 IAA 0 5	
	BAP 2 0 IAA 1 0	
	BAP 3 0 IAA 1 0	
	BAP 4 0 NAA 0 2	
	BAP 4 0 NAA 0 4	
	BAP 4 0 NAA 0 5	
	BAP 4 0 NAA 0 8	
	BAP 4 0 NAA 1 0	
	BAP 4 0 IAA 0 2	
	BAP 4 0 IAA 0 4	
	BAP 4 0 IAA 0 6	
	BAP 4 0 IAA 0 8	
	BAP 4 0 IAA 1 0	

Contd

Annexure II Continued

c) Combinations of growth regulators with carbon source

1	2		3
½ MS	BAP 1 0 ppm	NAA 0 25 ppm	with sucrose 1% 2% 3% and 6%
	BAP 1 0	NAA 0 5	
	BAP 1 0	NAA 1 0	
	BAP 1 0	IAA 0 25	
	BAP 1 0	IAA 0 5	
	BAP 1 0	IAA 1 0	
	BAP 1 0	NAA 0 5	
	BAP 2 0	NAA 1 0	
	BAP 2 0	IAA 0 5	
	BAP 2 0	IAA 1 0	
	BAP 3 0	NAA 0 5	
	BAP 3 0	NAA 1 0	
	BAP 3 0	IAA 0 5	
	BAP 3 0	IAA 1 0	
	BAP 4 0	NAA 0 5	
	BAP 4 0	NAA 1 0	
	BAP 4 0	IAA 0 5	
	BAP 4 0	IAA 1 0	

Contd

Annexure II Continued

1	2		3
½ MS	Kin 0 2 ppm	NAA 1 0 ppm	with sucrose 1% 2% 3% and 6%
	Kin 0 2	IAA 1 0	
	Kin 0 4	NAA 1 0	
	Kin 0 4	NAA 2 0	
	Kin 0 4	IAA 1 0	
	Kin 0 4	IAA 2 0	
	Kin 0 4	IAA 3 0	
	Kin 4 0	IAA 4 0	
	Kin 4 0	IAA 5 0	
	BAP 1 0	NAA 0 5	with maltose 3%
	BAP 1 0	NAA 1 0	
	BAP 1 0	IAA 0 5	
	BAP 1 0	IAA 1 0	
	BAP 2 0	NAA 0 5	
	BAP 2 0	NAA 1 0	
	BAP 2 0	IAA 0 5	
	BAP 2 0	IAA 1 0	
	BAP 3 0	NAA 1 0	
	BAP 3 0	IAA 1 0	
	BAP 4 0	NAA 1 0	

Contd

Annexure II Continued

1	2	3
½ MS	BAP 4 0 ppm IAA 1 0 ppm	with maltose 3%
	Kin 0 2 NAA 0 5	
	Kin 0 2 NAA 1 0	
	Kin 0 2 IAA 0 5	
	Kin 0 2 IAA 1 0	
	Kin 0 4 NAA 1 0	
	Kin 0 4 NAA 2 0	
	Kin 0 4 NAA 3 0	
	Kin 0 4 NAA 4 0	
	Kin 0 4 IAA 1 0	
	Kin 0 4 IAA 2 0	
	Kin 0 4 IAA 3 0	
	Kin 0 4 IAA 4 0	
	Kin 0 4 IAA 5 0	
	BAP 2 0 NAA 1 0	With mannitol 1% and sucrose 2%
	BAP 2 0 IAA 1 0	
	BAP 3 0 NAA 1 0	
	BAP 3 0 IAA 1 0	
	BAP 4 0 NAA 1 0	
	BAP 4 0 IAA 1 0	
	Kin 0 2 IAA 1 0	

Contd

Annexure-II Continued

1	2		3
½ MS	Kin 0 2 ppm	IAA 2 0 ppm	with mannitol 1% and sucrose 2%
	Kin 0 4	NAA 1 0	
	Kin 0 4	NAA 1 0	
	Kin 0 4	NAA 2 0	
	Kin 0 4	NAA 4 0	
	Kin 0 4	IAA 1 0	
	Kin 0 4	IAA 2 0	
	Kin 0 4	IAA 3 0	
	Kin 0 4	IAA 4 0	
	BAP 1 0	NAA 1 0	with maltose 2% and sucrose 1%
	BAP 1 0	IAA 1 0	
	BAP 2 0	NAA 1 0	
	BAP 2 0	IAA 1 0	
	BAP 2 0	IAA 2 0	
	BAP 2 0	IAA 3 0	
	BAP 2 0	IAA 4 0	
	Kin 0 2	NAA 1 0	
	Kin 0 2	IAA 1 0	
	Kin 0 4	NAA 1 0	
	Kin 0 4	NAA 2 0	

Contd

Annexure II Continued

1	2		3
½ MS	Kin 0 4 ppm	IAA 1 0 ppm	with maltose 2% and sucrose 1%
	Kin 0 4	IAA 2 0	
	Kin 0 4	IAA 3 0	
	Kin 0 4	IAA 4 0	
	Kin 0 4	IAA 5 0	
	BAP 1 0	NAA 0 5	with glucose 3%
	BAP 1 0	IAA 0 5	
	BAP 1 0	NAA 1 0	
	BAP 1 0	IAA 1 0	
	BAP 2 0	NAA 1 0	
	BAP 2 0	IAA 1 0	
	Kin 0 2	NAA 1 0	
	Kin 0 2	NAA 2 0	
	Kin 0 4	NAA 1 0	
	Kin 0 4	NAA 2 0	
	Kin 0 4	NAA 3 0	
	Kin 0 4	NAA 4 0	
	Kin 0 4	IAA 1 0	
	Kin 0 4	IAA 2 0	
	Kin 0 4	IAA 3 0	
	Kin 0 4	IAA 4 0	
	Kin 0 4	IAA 5 0	

ANNEXURE III

Media combinations tried for indirect organogenesis/embryogenesis in Gymnema sylvestre

a) Combinations of growth regulators

MS basal medium with 3% sucrose

BAP 0 5 and NAA 0 5 1 0 1 5 and 2 0 ppm

BAP 0 5 and IAA 0 5 1 0 1 5 and 2 0 ppm

BAP 0 5 and 2,4 D 0 5 1 0 1 5 and 2 0 ppm

BAP 1 0 and NAA 0 25 0 5 1 0 and 2 0 ppm

BAP 1 0 and IAA 0 25 0 5 1 0 and 2 0 ppm

BAP 1 0 and 2 4 D 0 25 0 5 1 0 and 2 0 ppm

BAP 3 00 and NAA 0 5 1 0 1 5 and 2 0 ppm

BAP 3 00 and IAA 0 5 1 0 1 5 and 2 0 ppm

BAP 3 0 and 2 4 D 0 5 1 0 1 5 and 2 0 ppm

BAP 4 0 and 2 4-D 0 2 0 4 0 6 0 8 and 1 0 ppm

BAP 6 0 ppm

BAP 8 0 ppm

BAP 10 0 ppm

BAP 4 0 and 2 4 D 0 5 1 0 1 5 2 0 ppm (GA_3 1 to 5 ppm)

Kin 0 5 and NAA 0 25 0 5 1 0 and 2 0 ppm

Kin 0 5 and IAA 0 25 0 5 1 0 and 2 0 ppm

Kin 0 5 and 2 4 D 0 25 0 5 1 0 and 2 0 ppm

Kin 1 0 and NAA 0 5 1 0 1 5 and 2 0 ppm

Kin 1 0 and IAA 0 5 1 0 1 5 and 2 0 ppm

Kin 1 0 and 2,4 D 0 5 1 0 1 5 and 2 0 ppm

contd

Annexure III Continued

MS basal medium with 3% sucrose	Kin 3 0 and NAA 0 5 1 0 1 5 and 2 0 ppm
	Kin 3 0 and IAA 0 5 1 0 1 5 and 2 0 ppm
	Kin 3 0 and 2 4 D 0 5 1 0 1 5 and 2 0 ppm
	Kin 5 0 and 2 4 D 0 5 1 0 1 5 and 2 0 ppm
	Kin 8 0 ppm
	Kin 10 0 ppm
	Kin 4 0 and 2 4 D 0 5 1 0 1 5 and 2 0 ppm (GA_3 1 to 5 ppm)

b) Combinations of growth regulators with organic supplements

MS basal medium with 3% sucrose	BAP 0 5 NAA 0 5 1 0 1 5 2 0 ppm and coconut water 10 15 and 30%
	BAP 0 5 2 4 D 0 5 1 0 1 5 2 0 ppm and coconut water 10 15 and 30%
	BAP 1 0 NAA 0 5 1 0 1 5 2 0 ppm and coconut water 10 15 and 30%
	BAP 1 0 IAA 0 5 1 0 1 5 2 0 ppm and coconut water 10 15 and 30%
	BAP 1 0 2 4 D 0 5 1 0, 1 5 2 0 ppm and coconut water 10 15 and 30%
	BAP 3 0 2 4 D 0 5 1 0 1 5 2 0 ppm and coconut water 30%

Contd

Annexure III Continued

MS basal medium with 3% sucrose

BAP 4 0 NAA 0 5 1 0 1 5 2 0 ppm and coconut water 30%

BAP 4 0 2 4 D 0 5 1 0 1 5 2 0 ppm and coconut water 30%

BAP 5 0 NAA 0 5 1 0 1 5 2 0 ppm and coconut water 30%

BAP 5 0 2 4-D 0 5 1 0 1 5 2 0 ppm and coconut water 30%

BAP 8 0 ppm and coconut water 20%

BAP 10 0 ppm and coconut water 20%

Kin 0 5 2 4-D 0 5 1 0 1 5 2 0 ppm and coconut water 20%

Kin 1 0 NAA 0 5 1 0 1 5 2 0 ppm and coconut water 20%

Kin 1 0 IAA 0 5 1 0, 1 5 2 0 ppm and coconut water 20%

Kin 1 0 2 4 D 0 5 1 0 1 5 2 0 ppm and coconut water 20%

Kin 4 0 2 4 D 0 5 1 0 1 5 2 0 ppm and coconut water 15%

Kin 6 0 ppm and coconut water 30%

Kin 8 0 ppm and coconut water 30%

Kin 10 0 ppm and coconut water 30%

Contd

Annexure III Continued

MS basal medium with 3% sucrose

BAP 1 0 NAA 0 5 1 0 1 5, 2 0 ppm and Casein hydrolysate 100 250 ppm

BAP 1 0 2 4 D 0 5 1 0 1 5 2 0 ppm and Casein hydrolysate 100 250 ppm

BAP 4 0, NAA 0 5 1 0 1 5 2 0 ppm and Casein hydrolysate 250 ppm

BAP 4 0 2 4 D 0 5 1 0 1 5, 2 0 ppm and Casein hydrolysate 250 ppm

Kin 1 0 NAA 0 5 1 0 1 5 2 0 ppm and Casein hydrolysate 100 ppm

Kin 1 0 IAA 0 5 1 0 1 5 2 0 ppm and Casein hydrolysate 100 ppm

Kin 1 0 2 4 D 0 5 1 0 1 5, 2 0 ppm and Casein hydrolysate 100 ppm

Kin 2 0 NAA 0 5 1 0 1 5, 2 0 ppm and Casein hydrolysate 100 200 ppm

Kin 2 0 2 4 D 0 5 1 0 1 5 2 0 ppm and Casein hydrolysate 100 200 ppm

Kin 4 0 2 4 D 0 5 1 0 1 5 2 0 ppm and Casein hydrolysate 200 ppm

c) Combinations of growth regulators with carbon source

MS basal medium BAP 0 5 ppm NAA 0 5 1 0 1 5 and 2 0 ppm with sucrose 1 2 3 and 6%

BAP 0 5 ppm 2 4 D 0 5 1 0, 1 5 and 2 0 ppm

BAP 1 0 ppm NAA 0 5 1 0 1 5 and 2 0 ppm

BAP 1 0 ppm 2 4 D 0 5 1 0 1 5 and 2 0 ppm

Contd

Annexure III Continued

MS basal medium Kin 0 5 ppm NAA 0 5 1 0 1 5 and 2 0 ppm with sucrose 1 2 3 and 6%
Kin 0 5 ppmm 2 4 D 0 5 1 0 1 5 and 2 0 ppm
Kin 1 0 ppm NAA 0 5, 1 0 1 5 and 2 0 ppm
Kin 1 0 ppm 2 4-D 0 5 1 0 1 5 and 2 0 ppm
BAP 0 5 ppm NAA 0 5, 1 0, 1 5 and 2 0 ppm maltose 3%
BAP 0 5 ppm 2 4 D 0 5, 1 0 1 5 and 2 0 ppm
BAP 1 0 ppm NAA 0 5 1 0 1 5 and 2 0 ppm
BAP 1 0 ppm 2 4 D 0 5 1 0 1 5 and 2 0 ppm
Kin 0 5 ppm NAA 0 5 1 0 1 5 and 2 0 ppm
Kin 0 5 ppm 2 4 D 0 5 1 0 1 5 and 2 0 ppm
Kin 1 0 ppm NAA 0 5 1 0 1 5 and 2 0 ppm
Kin 1 0 ppm 2 4 D 0 5 1 0 1 5 and 2 0 ppm
BAP 1 0 ppm NAA 0 5, 1 0 1 5 and 2 0 ppm with mannitol 1% + sucrose 2%
BAP 1 0 ppm 2 4 D 0 5 1 0 1 5 and 2 0 ppm
Kin 1 0 ppm NAA 0 5 1 0 1 5 and 2 0 ppm
Kin 1 0 ppm 2 4-D 0 5 1 0 1 5 and 2 0 ppm
BAP 1 0 ppm NAA 0 5 1 0 1 5 and 2 0 ppm with maltose 2% + sucrose 1%
BAP 1 0 ppm 2 4 D 0 5 1 0 1 5 and 2 0 ppm
MS basal medium with 3% sucrose BAP 1 0 NAA 0 5 1 0 1 5 and 2 0 ppm and Adenine sulphate 2 ppm
BAP 1 0 2 4 D 0 5, 1 0 1 5 and 2 0 ppm and
BAP 4 0 2 4-D 0 5, 1 0 1 5 2 0 ppm and Adenine sulphate 10 ppm

Contd

Annexure III Continued

MS basal medium with 3% sucrose	Kin 1 0 NAA 0 5 1 0 1 5 2 0 ppm and Adenine sulphate 2 ppm
	Kin 1 0 2 4 D 0 5 1 0 1 5 2 0 ppm
	Kin 4 0 NAA 0 5 1 0 1 5 2 0 ppm
	Kin 4 0 2 4 D 0 5 1 0 1 5 2 0 ppm
	Kin 1 0 ppm NAA 0 5 1 0 1 5 and 2 0 ppm with maltose 2% + sucrose 1%
	Kin 1 0 ppm 2 4 D 0 5 1 0 1 5 2 0 ppm
	BAP 1 0 ppm NAA 0 5 1 0 1 5 and 2 0 ppm with glucose 3%
	BAP 1 0 ppm 2 4 D 0 5 1 0 1 5 and 2 0 ppm
	Kin 1 0 ppm NAA 0 5 1 0 1 5 and 2 0 ppm
	Kin 1 0 ppm 2 4 D 0 5 1 0 1 5 and 2 0 ppm
	Kin 2 0 ppm NAA 0 5 1 0 1 5 and 2 0 ppm
	Kin 2 0 ppm 2,4 D 0 5 1 0 1 5 and 2 0 ppm

ANNEXURE III

Media combinations tried for indirect organogenesis and further growth of embryoids

Basal MS liquid

MS semi solid

MS + BAP 1 0 ppm

MS + BAP 2 0 ppm

MS + BAP 4 0 ppm

MS + BAP 4 0 ppm + NAA 0 8 ppm

MS + BAP 1 0 ppm + 2 4 D 0 25 ppm

MS + Kin 1 0 ppm + NAA 0 25 ppm

MS + Kin 1 0 ppm + IAA 0 25 ppm

MS + Kin 1 0 ppm + NAA 0 5 ppm + ABA 1 0 ppm

MS + Kin 1 0 ppm + 2 4 D 0 5 ppm + ABA 1 0 ppm

Standardisation of *in vitro* propagation technique in *Gymnema sylvestre* R. Br.

by

ANU, K I

ABSTRACT OF A THESIS

submitted in partial fulfilment of the
requirement for the degree of

MASTER OF SCIENCE IN HORTICULTURE

FACULTY OF AGRICULTURE
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Department of Plantation crops and Spices
COLLEGE OF HORTICULTURE
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ABSTRACT

A study was taken up in the Department of Plantation Crops and Spices College of Horticulture Vellanikkara during 1991-93 to standardise the in vitro technique for multiplying Gymnema sylvestre R Br which is locally known as Gurmar This being the first attempt of micropropagation in this crop the methodology was to be standardised from the initial stage itself Nodal segments leaf segments as well as stem segments collected from mature vines maintained in the College of Horticulture were used as explants in the present study

Different routes like enhance release of axillary buds organogenesis and embryogenesis were attempted for the plant The main limitation in establishing in vitro cultures of Gymnema sylvestre was identified to be microbial interference which was mainly due to the fungus Collectotrichum sp Great seasonal variation was observed for the fungal interference and the period from January to April was identified as the best season for establi shing the cultures of Gymnema sylvestre Mercuric chloride at 0 1 per cent level was identified as the best surface sterilant with the survival rate being 82 to 94 per cent according to the explant material used Basal medium MS supported the cultures of leaf segments while the inorganic salts were to be reduced to half level for supporting the cultures of stem segments Out of the various growth regulator combinations tried for bud break and shoot elongat

ion in Gymnema kinetin and IAA could support bud break and healthy shoot production Coconut water and adenine sulphate when supplemented in the medium favoured healthy shoot induction Survival rate of newly formed shoots were very poor due to leaf abscission Higher levels of $MgSO_4$ in the medium helped leaf retent ion to the extent of 10 per cent None of the treatments tried could induce roots in the in vitro shoots

Profuse callusing could be induced from leaf and stem segments in MS basal medium supplemented with growth regulators BA + NAA and BA + 2 4 D with a callus index ranging from 280 to 360 The calli did not respond to organogenesis Callus mass consisted of uniform cells without any vascular differentiation The morphology and growth rate varied according to the growth regulator combinations tried Stray instances of embryogenesis (6 to 20%) were observed when cultured in growth regulator combinat ions of BA + NAA BA + IAA and Kin + 2 4 D The embryoids developed upto torpedo stage and failed to grow further They exhibited a strong callusing tendancy and got reverted to the callus stage within 5 days

The results of the (present) study would be a pioneering report that unravels the in vitro response of Gurmar for micro propagation Since the plant exhibited a relatively recalcitrant nature at various stages of in vitro culture much more concerted efforts are to be made for standardising the protocol for micropropagation of Gymnema sylvestre