

**DEVELOPMENT OF MICROPROPAGATION
PROTOCOLS IN *CAPPRIIS DECIDUA*
(FORSK) EDGEW**

BY

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CCS HARYANA AGRICULTURAL UNIVERSITY
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2005

Dedicated
to
My Grand
Father

CERTIFICATE - I

This is to certify that this thesis entitled, **“Development of micropropagation protocols in *Cappris decidua* (forsk) Edgew”**, submitted for the degree of **Master of Science** in the subject of **Biotechnology and Molecular Biology** of Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Mr. Om Parkash Chahar** under my guidance and supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

Dr. Pushpa Kharb
Major Advisor,
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Hisar

CERTIFICATE - II

This is to certify that this thesis entitled, “**Development of micropropagation protocols in *Cappris decidua* (forsk) Edgew**”, submitted by **Mr. Om Parkash Chahar** to the Chaudhary Charan Singh Haryana Agricultural University, Hisar, in partial fulfilment of the requirements for the degree of **Master of Science** in the subject of **Biotechnology and Molecular Biology** has been approved by the Student’s Advisory Committee after an oral examination on the same.

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LIST OF ABBREVIATION

2,4-D	=	2,4-Dichlorophenoxy acetic acid
BAP	=	6 Benzyl aminopurine
IAA	=	Indole acetic acid
MS	=	Murashige and Skoog (1962) basal medium
NAA	=	α -Naphthalene acetic acid
EDTA	=	Ethylene diamine acetic acid
<i>In vivo</i>	=	In nature
<i>in vitro</i>	=	Under aspetic conditions
mg/l	=	Milligram per litre
gm/l	=	Gram per litre
μ g	=	Microgram
V/V	=	Volume/Volume
W/V	=	Weight/Volume
IBA	=	Indole 3 butyric acid
GA ₃	=	Gibberellic acid
%	=	Per cent
NaOH	=	Sodium hydroxide
HCl	=	Hydrochloric acid
pH	=	Negative logarithm of hydrogen ion concentration
HgCl ₂	=	Mercuric chloride
°C	=	Degree Celsius
<i>et al.</i>	=	et alia = any other
A.S.	=	Adenine sulphate
A.A.	=	Added additives

Fig.	=	Figure
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CHAPTER-I

INTRODUCTION

Capparis is a large genus comprising of more than 300 species (Usher, 1974) distributed throughout the tropical, subtropical and arid regions of the world. It is chiefly found in arid and semi arid regions of Deccan Peninsula, Sind, Arabia, Egypt and Tropical Africa. About 40 species are present in India, mostly in Rajasthan, Haryana, Punjab, U.P. and Gujrat (Kirtikar and Basu, 1918). Ker (*Capparis decidua* (Forsk) Edgew) belongs to family Capparidaceae. It is much branched, straggling shrub or sometimes even a tree where branches are green, zigzag, smooth and leafless during most of the time in the year (Paul and Sen, 1987). *C. decidua* is generally red flowered open pollinated woody

plant of arid region while few trees with yellow flowers are also observed.

This species shows wide genetic variability in plant size, morphology, fruit production per plant and dimorphism in seed (Paul and Sen, 1987). Its height normally varies between 2m to 10m and the average height is approximately 4m. Ker fruits are available during the months of March to May. Ker provides hard, heavy and termite resistant wood (Gupta *et al.*, 1989). Its unique fruits are consumed fresh and also used as a dry vegetable and pickled.

The fruits are rich in carbohydrates and minerals. The seeds yield (20%) high quality edible oil (Rai, 1987). The stem and bark extracts contain isocodonocarpin and other alkaloids which are effective in treating various diseases like asthma, inflammation, cough etc., (Ahmad *et al.*, 1989). Chauhan *et al.* (1986) while working on the nutritional aspect of ker fruits reported that unripe ker fruits are of very high nutritive value. Tiwari (1979) has reviewed medicinal value of ker fruits. *Capparis* has been reported to have diuretic (Tanira *et al.*, 1989) and antidiabetic properties (Yadav *et al.*, 1997).

The population of *Capparis* is declining at an alarming rate due to over exploitation and damage to its habitat. Natural propagation of *Capparis* is through seeds which have short viability. The vegetative propagation of ker through stem cutting is common practice of propagation (Vashishtha, 1987) but the percentage of rooting and rate of multiplication is low. Therefore, it is desirable to produce the planting material through non-conventional techniques.

Tissue culture technique is now an established method to propagate elite material very rapidly compared to the vegetative propagation as it is not dependent on seasonal barriers of growth which is a characteristic of most of the tree species (Haissing *et al.*, 1987; Cheliak and Rogers, 1990; Ahuja, 1991; Hammatt, 1992). Only few reports on micro-propagation of ker are available. Rodriguez (1990); Deora and Sheknowat (1995); Tyagi and Kothari (1997) and Salem *et al.* (2001) reported micropropagation studies in ker from nodal explants.

Keeping the above points in mind, the present study was therefore, undertaken with the following objective:

- To optimize conditions and develop micropropagation protocol of *Capparis decidua* (Forsk) Edgew.

CHAPTER-II

REVIEW OF LITERATURE

The *in vitro* techniques were developed initially to demonstrate the totipotency of plant cells predicted by Haberlandt in (1902), a German botanist who first described the principle of totipotency. Micropropagation deals with *in vitro* propagation of plants and, has many advantages over conventional vegetative propagation. Its application in horticulture, agriculture and forestry is currently expanding world-wide. The goal of micropropagation is to mass-produce genetically identical, physiologically uniform, developmentally normal and pathogen-free plantlets, which can be acclimatized in a reduced

time period. Altman and Loberant, (1998) have elegantly reviewed principles and practices of micropropagation.

In general factors affecting the *in vitro* micropropagation have been reviewed as under:

Physiological state and selection of explants :

Physiological state of a plant is important in micropropagation. The success in propagation from mature plant is dependent on careful selection of explants (Murashigae, 1974; Sommer and Caldas, 1981). Once the selection is done it is necessary to maintain genetic fidelity at the end. This is done by vegetative propagation *in vivo* or *in vitro*. Cloning of mature woody plants *in vitro* and *in vivo* is adversely affected by characteristics accompanying maturation such as reduced growth rate reduced or total lack of rooting ability or sometimes the unpleasant phenomenon of plagiotropy (Hacket, 1985; Piezik *et al.*, 1990; Cheliak, and Rogers; 1990). Maturation, a complex phenomenon, is the major problem preventing a wide application of tissue culture technology among woody species. Success with several species has been achieved mainly by the use of special starting (explanting) material, by special pre-treatment to mother/source plants *in vivo* or by *in vitro* culture (Bonga, 1987; Rathore *et al.*;

1992; and Shekhawat *et al.*, 1993). Younger plants provide more suitable explant material for regeneration than the old aged plants (Bonga, 1982 b; Sommer and Wetzstein, (1984). The highest regeneration response was reported from nodal explants followed by inter nodal and shoot tip explants (Agarwal and Kamal, 2004) in *Mamordica charantia*.

Ruyter *et al.* (1991) could obtain plantlets by using stem segment of *Rauwolfia serpentina*. Total alkaloid content was slightly higher in roots from the regenerated plants while the content of individual alkaloids, ajmaline, serpentine and reserpine was lower than in the roots of the parental stock.

Media components

Culture medium

Composition of media used for culturing is an important factor in successful establishment of a tissue culture. Conditions favouring callus growth may not be suitable for organ differentiation. Gautheret (1955) emphasized the importance of nutrition in plant tissue culture. Depending upon overall salt concentration over 60 different media have been designated to fulfil the requirement of cultured tissue (White, 1943; Murashige and Skoog, 1962; Gamborg *et al.*, 1968; McCrow and Uyod, 1981).

Growth regulators

Plant growth regulators affect growth and differentiation. The effect of plant growth regulators such as auxins, cytokinins, gibberellins etc. vary with the type and quantity applied. Several studies have been carried out on the influence of the concentration of various growth regulators especially auxins. The auxins differ significantly in stability, effectiveness and their influence on organogenesis. Grewal and Atal (1976) reported callus formation from hypocotyls in *Dioscorea deltoidea* on 2, 4-D (1mg/l) supplemented medium. In many cases cytokinin alone was optimal for shoot multiplication and addition of an auxin proved harmful effect (Gharyal and Maheshwari; 1982; Bhojwani and Rajdan, 1983; Duhoux and Davies, 1985; Beak and Dunlop, 2001). The shoot initiation medium was additionally supplemented with gibberellic acid (GA₃) to stimulate elongation of nodes in *Acacia* species (Ide *et al.*, 1994 and Reddy *et al.*, 1995). Chaturvedi and Sharma (1989) reported that BAP was far more effective than kinetin for shoot proliferation in axillary buds of *Simmondsia chinensis*. Maximum number of shoots was obtained on MS medium supplemented with BA (3 mg/L) and NAA (0.1

mg/L). Addition of ascorbic acid (100 mg/L) in shoot proliferation medium inhibited browning at the medium (Bhaskar *et al.*, 1996).

Shoots were initiated from leaf explants of neem tree. Regardless of their origin, shoots were successfully produced by culturing leaf explants on MS medium containing BAP (1mg/L). Kinetin (0.8 mg/L) and adenine sulphate (6 mg/L) in complete darkness (Eeswara *et al.*, 1988).

Maximum shoot bud development in *Acacia catechu* has been reported by Kaur and Verma (1990) from single explant cultured on MS media supplemented with BAP (4 mg/L) and NAA (0.5 mg/L). Addition of adenine sulphate (25 mg/L), ascorbic acid (20 mg/L) and glutamine (150 mg/L) to the medium was found beneficial for maximum shoot bud induction.

Naik *et al.*, (1999) developed a protocol for elite cultivar of pomegranate (*Punica granatum* L. cv. Ganesh), for bud break and axillary shoot proliferation using MS medium supplemented with BA, ZR or TDZ, with ZR being most effective. A micropropagation method has been developed by Ahuja *et al.* (2002) for *Atropa acuminata* by induction of axillary shoot proliferation from shoot tips and nodal explant using MS medium supplemented with BAP (1mg/l) and IBA (1 mg/L).

Culture conditions

The culture environment which is the result of the interaction between plant material, culture media, type of culture vessels and incubation conditions influence *in vitro* regeneration (Debergh, 1988). The major factors of the tissue culture environment are light and temperature. Frequently the literature also presents data on relative humidity.

Physical form of medium

Solidified medium is the best for tissue culture but the use of liquid media has been increasingly evaluated for tissue culture purpose (Keathley, 1983; Chun *et al.*, 1986). During the development of commercial micropropagation eg. towards automated system, is the liquid rather than agar cultures are likely to be preferred (Haissing *et al.*, 1987). In such system, the repeated subculturing of explant to fresh media will be unnecessary as the liquid medium may be easily replaced or replenished by the addition of fresh nutrients. Semi-solid media have also been used for rooting of rose (Rahaman *et al.*, 1992) and for shoot growth in mulberry (Ohyama and Oka, 1976).

pH

Generally, the pH of culture medium is kept at and around 5.8. Low pH stimulates moderate rooting in a few species. Williams *et al.* (1985) found that pH of 4.0 and dark period of two week was optimum for root induction of *Prostanthera striatiflora* and *Corea decumbens*.

Humidity and gaseous atmosphere

Humidity in the culture vessel and osmotic potential of the medium effect the growth and development of plantlets *in vitro* in different ways (Brown *et al.*, 1979; Ziv *et al.*, 1983).

Restricted gaseous exchange in culture vessels may cause the CO₂ concentration to become growth limiting reported the beneficial effects of increased CO₂ on photosynthesis for *in vitro* propagation of rose.

Light

Light is also an important factor in tissue culture. The intensity extent of daily exposure and quality of illumination are other determining factors in plant tissue culture.

Effect of photoperiod may or may not be pronounced in all the species. Douglas (1989) reported that light grown (16 hr photoperiod) leaf explants from female mulberry tree showed low

morphogenic capability while those in dark were highly morphogenic. The chlorophyll content of the cultures increased by light intensity.

Temperature

Just as the temperature influences morphogenesis, it certainly controls growth and development *in vitro*. Temperature controls the relative humidity in the culture vessel, so it has an indirect effect on vitrification (Maene and Debergh, 1987). During acclimatization, the temperature of root zone is important to enhance root growth. The medium should be warmer than the air for good root activity and to increase the humidity around the cutting (Dunstan and Tunner, 1984; Mc Cown, 1986).

Organogenesis

Organogenesis or adventitious shoot formation is a process of inducing shoot formation directly on explants in locations other than the normal region (Murashige, 1977), Hick (1980) classified organogenesis into two types (1) indirect organogenesis, which is mediated by callus stage, (2) direct organogenesis, in which adventitious shoots are formed directly on explants. After the successful rapid multiplication of orchid (*Cymbidium* sp.) through tissue culture technique plant multiplication by means of

numerous axillary branching of shoot tip and lateral buds followed.

The tendency of shoot tips and lateral buds to form copious axillary shoots in profusion when stimulated by exogenous cytokinins has been exploited in the micropropagation procedure (Murashige, 1974).

Direct organogenesis i.e. without callus formation has also been reported in many woody plants. This method utilizes shoot tips, lateral buds and small nodal and internodal cuttings and produces in general, genetically stable culture without callus formation. Komalavalli and Roa (2000) reported that in Gurmar maximum number of shoots were induced from 30 day old seedling axillary node explants on MS medium containing BAP (1.0 mg/L), kinetin (0.5 mg/L) and citric acid. Joshi *et al.* (2000) achieved axillary shoot bud multiplication in *Eucalyptus* hybrid, FRI-5 on MS medium supplemented with BAP (1.0 mg/L).

Rooting of regenerated shoots

After the development of shoot buds, their multiplication and elongation, the next stage is to induce roots, which are adventitious in origin, arising from stem bases when incubated in

suitable medium (Romberger *et al.*, 1993). No general technique can be used for *in vitro* rooting because of variation in responses obtained from plantlets (William *et al.*, 1985). Bonga and Von Aderkas, (1992), suggested that roots from rejuvenated shoots of woody plants, are induced *in vitro* by IBA or NAA. Probably the nature of auxin required and the concentration for *in vitro* root regeneration are species specific.

Chauhan *et al.* (2000) obtained best rooting in *Eucalyptus tereticornis* SM on MS medium supplemented with IBA (0.5 mg/L). Isolated shoots of all the three species rooted on half-strength MS medium with 0.1% activated charcoal. 95 to 100% of the shoots of *C. limon* rooted on half-strength MS medium + 27.0 μ M of NAA Rathor *et al.* (2004).

Hardening and acclimatization of *in vitro* raised plantlets

Plantlets produced *in vitro* must be acclimatized gradually to withstand the harsh natural environment. Misting, spraying or covering the pots may serve to fulfill the above objective. Different types of substrates have been used during the acclimatization period such as soil vermiculite mixture (Goyal and Azya, 1981), sterilized sand (Bhansali *et al.*, 1988; Thakur *et al.*, 2001) and soil (Kurten *et al.*, 1990; Sunaina and Goyal, 2000).

Micropropagation studies in *Cappris* spp.

In vitro micropropagation of *C. spinoso* was achieved by culturing nodal segment in presence of BA plus IAA and GA. High rooting response, was obtained on solid half strength MS medium (Rodriguez, 1990).

Deora and shekhawat (1995) developed a micropropagation protocol for *C. decidua* by forming multiple shoots from nodal explants cultured on MS medium supplemented with NAA, BAP and additives. The shoots were multiplied by sub culturing on MS media containing IAA (0.1 mg/l) + BAP (1mg/l) and rooting was obtained by culturing on half strength MS media supplemented with IBA (100mg⁻¹). Micropropagation of *C. decidua* has also been reported by Tyagi and Kothari (1997) through *in vitro* shoot

proliferation on nodal explants of mature tree and seedling explants cultured on MS media supplemented with various concentrations of BA (1, 3,5 mg/L). Rooting was obtained using IBA (1 mg/L).

Soyler and Arslan (2000) reported the effects of some plant growth regulators on caper. Cutting were treated with various concentrations of IBA. IAA and NAA at different time to induce rooting. Salem *et al.* (2001) studied the propagation of caper (*C. spinoso*) by herbaceous cuttings and *in vitro* culture. The shoots were multiplied on MS medium supplemented with different concentrations of IBA, IAA and NAA.

CHAPTER-III

MATERIALS AND METHODS

Plant material

The plant material of ker (*C. deciduas* (forsk) Edgew) (8-10 cm long shoot segments) was obtained from mature plants growing on campus area of CCS Haryana Agricultural University, Hisar.

Chemicals

The chemicals of high purity were used throughout the course of investigation. The chemicals *viz.* growth regulators, vitamins, inositol etc. were obtained from Sigma Chemical Company. St. Louis (USA) whereas, EDTA, sodium salt, agar-agar and other reagents were obtained from several companies *viz.*,

BDH, Glaxo, E'merck, Sisco Research Laboratories (SRL) and Hi-Media (India).

Glassware

All the glasswares used were of borosilicate quality and obtained from either Borosil India Ltd. or corning glass company (India). Oven dried (250°C) conical flasks, test tubes, pipettes, petridishes, beakers, volumetric flask, Erlenmeyer flasks, measuring cylinders (50 ml; 100 ml; 150 ml; 500 ml; 1000 ml) were used for media preparation.

Methods

Sterilization of equipment and glassware

Glassware like flasks, petriplates, culture tubes were washed properly and rinsed with distilled water. The cleaned glasswares were further sterilized by drying in an oven at 180°C ± 2°C for 2-3 hrs. Scalpels, forceps were sterilized using incinerator and flame sterilized using 70 per cent ethanol prior to use. Glassware like Pasteur pipettes, centrifuge tubes etc. were sterilized by autoclaving at 15 lbs/sq inch pressure at 121°C for 15 min.

Preparation and storage of stock solutions

The stock solutions of macro nutrients and micronutrients of MS basal medium (Murashige and Skoog, 1962) were prepared by dissolving the required amount of salts in measured quantity of distilled water (Table 2). Stocks solutions of vitamins and growth regulators (cytokinins and auxins) were prepared (1/1, w/v). The stock solutions of Fe EDTA was prepared accordingly and kept in amber coloured bottles. All these stock solutions were stored at 4°C in refrigerator.

Preparation, sterilization and storage of culture media

The MS basal medium was prepared by mixing the required quantity of each of stock solution (Table 2) in 500 ml of distilled water by continuous stirring. Myo-inositol (100 mg/L) and sucrose (3%) were added to this solution. The growth regulators were added in measured quantity, whenever required. The final volume was made one litre by adding distilled water. The pH of medium was adjusted to 5.8 with pH meter (Elico-digital) using 1 N HCl or 1N NaOH. Agar (BDH) was added (8 g/L) to this medium followed by melting of the medium on hot plate or in microwave. The medium was stirred regularly to avoid agar clumps till boiled thoroughly; allowed to cool for few minutes at room temperature

and poured into flask/culture tubes (approximately 40 ml/vessel)
and plugged with non-

Table 2. Composition of MS basal medium (Murashige and Skoog, 1962)

	Constituents	Quantity (mg/L)
Major salts	K NO ₃	1900.00
	NH ₄ NO ₃	1650.00
	MgSO ₄ 7H ₂ O	370.00
	KH ₂ PO ₄	170.00
	CaCl ₂ . 2H ₂ O	440.00
Minor salts	MnSO ₄ 4H ₂ O	22.30
	KI	0.80
	Na ₂ MOO ₄ .2H ₂ O	0.25
	ZnSO ₄ . 7 H ₂ O	8.60
	H ₃ BO ₃	6.20
	CuSO ₄ .5H ₂ O	0.025
	CoCl ₂ . 6 H ₂ O	0.025
Vitamins	Glycine	2.00
	Thiamine HCl	0.10
	Pyridoxine-HCl	0.50
	Nicotinic aid	0.50
Chelating agent	Fe- EDTA	37.30
	FeSO ₄ . 7 H ₂ O	27.80
Others	Myo inositol	100.00
	Sucrose	30000.00
	Agar	8000.00
	pH	5.8

absorbent cotton. The sterilization of medium was done by autoclaving at 151b per square inch pressure for 15 minutes at 121°C. The flasks/culture tubes containing medium were kept at room temperature and used within a week. Different media used in the present study were prepared using the MS basal medium. The chemical composition of different media used for plant regeneration of *C. decidua* is described in Table 2.

Explants

Nodes explants and shoots were used as explants for shoot regeneration studies in ker (*C. decidua* (forsk) Edgew).

Preparation of explants

Shoot length (8-10 cm) of ker (*C. decidua* (forsk) Edgew) were taken and washed under running tap water for 15-20 min, agitated in tap water with few drops of liquid detergent (Tween 20) for 5 min and finally washed with distilled water. These were then surface sterilized with the 70 per cent ethanol and 0.1 per cent HgCl₂ for various time period (Table 1) to avoid contamination and browning of the cultures and rinsed thoroughly with sterile distilled water six to seven times under laminar air flow. Nodal part and shoot tip were surgically cut from these surface sterilized shoots under

Table 1. Effectiveness of different surface sterilization treatments on nodal part and shoot part explants in ker (*C. deciduas*) (Forsk) Edegew)

Explants	Surface sterilization		Effectiveness
	70% alcohol (Sec.)	0.1% HgCl ₂ (min.)	
Node	10	3	+
-do-	35	4	++
-do-	50	4	+++
-do-	60	5	++++
Shoot Tip	10	2	+
-do-	20	3	++
-do-	40	3	+++
-do-	60	4	++++
+ = 100% contamination ++ = 40-50% contamination +++ = 100% sterilization ++++ = Lethal.			

aseptic conditions and used as explants. These explants were inoculated on various media having MS basal salt supplemented with various combinations of cytokinins and auxins for shoot regeneration (Table 3). Explants were cultured vertically on all the media. Cultures were maintained at 25±2°C and 16h illumination (1400 lux) provided by fluorescent tube light.

Observations and data analysis

Shoot regeneration

The shoot regeneration data were recorded for the following parameters.

1. Days to shoot induction

2. Per cent shoot induction

$$= \frac{\text{Number of explants responding}}{\text{Total number of explants cultured}} \times 100$$

3. Number of shoots per explant

4. Length of shoots.

Multiple shoot regeneration

Regenerated shoots were further cultured on four different media having MS basal salts with various combinations of cytokinins and auxins for shoot multiplication (Table 4). Data were recorded for percentage

Table 3. Composition of media used for shoot regeneration studies in ker (*C. decidua* (Forsk) Edgew)

Sr. No.	Medium code	Cytokinin (mg/L)	Auxin (mg/L)	Other additive (mg/L)
1.	MS ₁	BAP(1.0)	NAA (0.1)	--
2.	MS ₂	BAP (2.0)	NAA(0.1)	--
3.	MS ₃	BAP(3.0)	NAA (0.1)	--
4.	MS ₄	BAP (5.0)	NAA (0.1)	--
5.	MS ₅	BAP (0.1)	NAA(0.1)	A.A.*
6.	MS ₆	BAP(2.0)	NAA(0.1)	A.A.*
7.	MS ₇	BAP (3.0)	NAA (0.1)	A.A.*
8.	MS ₈	BAP (5.0)	NAA (0.1)	A.A.*
9.	MS ₉	Kinetin (0.1)	NAA (0.1)	--
10.	MS ₁₀	Kinetin (2.0)	NAA (0.1)	--
11.	MS ₁₁	Kinetin (3.0)	NAA (0.1)	--
12.	MS ₁₂	Kinetin (0.1)	NAA (0.1)	A.A.*
13.	MS ₁₃	Kinetin (2.0)	NAA (0.1)	A.A.*
14.	MS ₁₄	Kinetin (3.0)	NAA (0.1)	A.A.*
15.	MS ₁₅	BAP(5.0)	-	A.S.(5.0)
16.	MS ₁₆	BAP(5.0)	-	A.S.(15.0)
17.	MS ₁₇	BAP (5.0)	-	A.S.(25.0)
18.	MS ₁₈	BAP (5.0)	-	A.S.(35.0)

Basal medium=	MS Basal	A.A.* =	Added additives
Sucrose =	30 g/L	Adenine sulphate=	(25.0)
Agar =	8 g/L	Ascorbic acid	(50.0)
pH =	5.8	L-Arginine	(25.0)
		Citric acid	(25.0)

Table 4. Multiple shoot regeneration in ker [*C. decidua* (Forsk) Edgew)

Medium code	Basal medium	Cytokinin	Auxin	Other additives
1 MSS ₁	MS	BAP (5.0)	NAA (0.1)	*A.A.(5.0)
2. MSS ₂	MS	BAP (5.0)	NAA (0.1)	**A.S. (25.0)
3. MSS ₃	MS	BAP (5.0)	-	**A.S. (25.0)
4.* MSS ₄	MS	-	-	-
Sucrose	30g/L	*A.A.=	Added additives	
Agar	8g/L		Adenine sulphate	(25.00 mg/L)
pH	5.8		Ascorbic acid	(50.00 mg/L)
			Citric acid	(25.00 mg/L)
			L-Arginine	(25.00 mg/L)
		**A.S.=	Adenine sulphate.	

of responding explants, number of multiple shoots regenerated per explant and length of regenerated shoots.

Multiple shoot regeneration on media supplemented with culture supernatant of *Acetobacter* and *Acetobacter*

Regenerated shoots were inoculated on various media supplemented with different concentration of culture supernatant of *Acetobacter* and *Azotobacter* (Table 5).

Rooting

The regenerated shoots were surgically separated under aseptic conditions and inoculated on different rooting media having MS basal salts supplemented with various combinations of auxin (Table 6). Data were recorded after 20-25 days of inoculation of regenerated shoots to different rooting media for:

$$\text{Rooting response} = \frac{\text{Number of shoots forming roots}}{\text{Total number of shoots cultured}} \times 100$$

Data analysis

Data were analysed statistically using one way analysis of variance (Anova).

Table 5. Composition of media used supplemented with supernatant of *Acetobacter* & *Azotobacter*-) for multiple shoot regeneration in ker [*C. decidua* (forsk) Edgew]

Sr. No.	Medium code	(<i>Acetobacter</i>)** (ml/L)	(<i>Azotobacter</i>)** (ml/L)	Other growth regulators (mg/L)
1.	MSB ₁	2.0	-	-
2.	MSB ₂	4.0	-	-
3.	MSB ₃	8.0	-	-
4.	MSB ₄	4.0	-	*A.S.(25.0)
5.	MSB ₅	8.0	-	*A.S.(25.0)
6.	MSB ₆	-	(2.0)	-
7.	MSB ₇	-	4.0	-
8.	MSB ₈	-	8.0	-
9.	MSB ₉	-	4.0	*A.S.(25.0)
10.	MSB ₁₀	-	8.0	*A.S.(25.0)
11.	MSB ₁₁	2.0	2.0	--
12.	MSB ₁₂	4.0	4.0	-
13.	MSB ₁₃	8.0	8.0	-
14.	MSB ₁₄	4.0	4.0	*A.S. (25.0)
15.	MSB ₁₅	8.0	8.0	*A.S. (25.0)

Basal medium= MS Basal
 Sucrose = 30 g/L
 A.S.* = Adenine sulphate
 Agar = 8 g/L
 pH = 5.8

*Each ml of supernatant of *Acetobacter* contained 134 µg IAA

*Each ml of supernatant of *Azotobacter* contained 160 µg IAA

**Supernatant of *Acetobacter* and *Azotobacter* added.

Table 6. Composition of media used for root induction in ker (*C. decidua* (Forsk) Edgew)

Sr. No.	Medium code	Basal medium	Auxins (mg/L)		
			NAA	IBA	IAA
1.	MSR ₁	MS	-	1.0	-
2.	MSR ₂	MS	-	2.0	-
3.	MSR ₃	MS	-	3.0	-
4.	MSR ₄	MS	-	5.0	-
5.	MSR ₅	MS	1.0	1.0	-
6.	MSR ₆	MS	-	2.0	1.0
7.	MSR ₇	MS	2.0	-	-
8.	MSR ₈	MS	0.5	0.5	0.5
9.	MSR ₉	½ MS	-	1.0	-
10.	MSR ₁₀	½ MS	0.5	-	-
11.	MSR ₁₁	½ MS	1.0	1.0	-
12.	MSR ₁₂	½ MS	-	5.0	-
13*	MSR ₁₃	MS	-	25.0	-
14*	MSR ₁₄	MS	-	100.0	-

Sucrose- 30 g/L *Liquid media (without agar)
 Agar- 8.0 g/L
 pH- 5.8

Plant hardening and establishment

Regenerated plantlets were hardened in distilled water after washing them under tap water to remove agar traces and were finally transferred to soil.

CHAPTER-IV

EXPERIMENTAL RESULTS

The present investigation was carried out to develop protocol for *in vitro* micropropagation studies in ker [*C. decidua* (Forsk) Edgew].

Data obtained has been divided into following sections:

1. Effectiveness of surface sterilization process.
2. Shoot regeneration from shoot tip and nodal segment.
3. Multiple shoot regeneration.
4. Rooting and hardening of regenerated plant lets.

1. Effectiveness of surface sterilization

Shoot tip and nodal segment were surface sterilized using different surface sterilization treatments as shown in Table 1. Most effective treatment observed for nodal part was surface

sterilizing with 70 per cent alcohol for 50 second and 0.1 per cent HgCl₂ for 4 minute. For shoot tip explant the most effective surface sterilization treatment was 40 second with 70 per cent alcohol and 3 minute with 0.1 per cent HgCl₂.

Browning from ends of both the explants was observed after 2-3 days of inoculation on different media. Shoot development was completely inhibited and the explant died within a week. The browning was more intense with explants collected from the older shoots than those from the younger ones.

Browning of the medium could be overcome after 1-2 successive subculturing on the same medium at 4-5 days of interval.

2. Shoot regeneration studies

2.1 Days to shoot induction: Number of days range between 5 to 30 for shoot induction from both the explants on various media cultured.

a) Nodal explant

Days to shoot induction from nodal part explant has been summarized in Table-7. Number of days ranged

between 5 to 30 for shoot induction from all the explant on various media used. Earliest response of maximum number of explants (45) out of 125 explants cultured was observed on 5th day of inoculation on MS₁₇ medium. Very late response was observed on 15th day of inoculation on MS₁₁ medium on which only five explants out of 110 explants cultured responded for shoot induction. Variable of shoot induction response was observed on MS₁ to MS₄, MS₉ to MS₁₅ and on MS₁₈ medium on 5th day of inoculation. Maximum explants (120 out of 125) responded on MS₁₇ medium after 30th day of inoculation.

b) Shoot tip explant

Days to shoot induction from shoot tip explant have been summarized in Table 8. Shoot induction response was monitored from 5th day of inoculation till 30th day on various media cultured. Only two explants responded after 5th day of inoculation on MS₁₇ medium. Maximum number of explants (10 out of 40) responded after 30th day of inoculation on MS₁₇ medium. No shoot induction response was observed till 30th day of inoculation

on medium MS₁ to MS₄, MS₉ to MS₁₅ and MS₁₈. Minimum explants (4 out of 35)

Table 8. Days to shoot induction from shoot tip explants in ker [*C. decidua* (Forsk) Edgew]

Sr. No.	Medium code	No. of explants cultured	Days to shoot induction						No. of explants responding
			5	10	15	20	25	30th	
1.	MS ₁	33	0	0	0	0	0	0	0
2.	MS ₂	29	0	0	0	0	0	0	0
3.	MS ₃	40	0	0	0	0	0	0	0
4.	MS ₄	30	0	0	0	0	0	0	0
5.	MS ₅	34	0	1	2	3	3	4	4
6.	MS ₆	35	0	1	2	4	4	5	5
7.	MS ₇	32	10	1	3	4	5	6	6
8.	MS ₈	30	1	2	4	5	6	7	7
9.	MS ₉	28	0	0	0	0	0	0	0
10.	MS ₁₀	29	0	0	0	0	0	0	0
11.	MS ₁₁	37	0	0	0	0	0	0	0
12.	MS ₁₂	27	0	0	0	0	0	0	0
13.	MS ₁₃	25	0	0	0	0	0	0	0
14.	MS ₁₄	39	0	0	0	0	0	0	0
15.	MS ₁₅	32	0	0	0	0	0	0	0
16.	MS ₁₆	38	01	3	5	7	9	9	9
17.	MS ₁₇	40	2	4	8	9	9	10	10
18.	MS ₁₈	35	0	1	2	3	3	4	4

responded for shoot induction after 30th days of inoculation on MS₁₈ medium.

18.2. Shoot regeneration

a) Nodal explant

Per cent shoot regeneration response obtained from nodal explant has been divided into following categories.

- i) 70% to 100% - Very Good
- ii) 50% to 70% - Moderate
- iii) Below 50% - Poor response

Shoot regeneration response obtained from nodal explant has been summarized in Table 9. Maximum shoot regeneration response (96%) was observed on MS₁₇ medium supplemented with 5.0 mg/L BAP and 25 mg/L adenine sulphate(Fig.2). Number of shoots observed were 3-4 per explant on the same medium. Minimum shoot regeneration response (35%) was observed on MS₁₁ medium with 1-2 shoots per explant. Response observed was good on MS₁ to MS₉, MS₁₂, MS₁₆ to MS₁₈. Moderate response was observed on media MS₁₀ and MS₁₃. Poor response was noticed on media MS₁₁ and MS₁₄. Response was found to

increase as the concentration of adenine sulphate was increased (Table 3)



Fig. 1

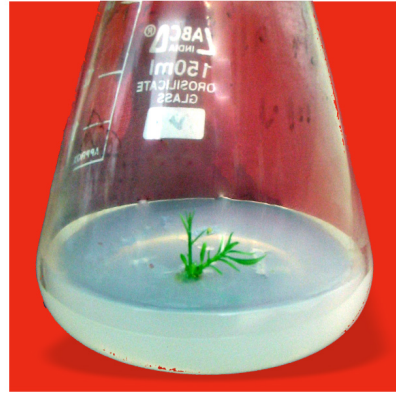


Fig. 2



Fig. 3



Fig. 4

Plate 1

Table 9. Per cent shoot regeneration response from nodal explants in ker [*C. decidua* (forsk.) Edgew]

Sr. No.	Medium code	No. of explants	No. of explants responding	Mean per cent response (mean±S.E.)*	No. of shoots/ explant
1.	MS ₁	110	72	65 (53.537±1.615)	1-2
2.	MS ₂	116	74	63 (52.564±2.264)	1-2
3.	MS ₃	121	73	62 (51.262±4.110)	1-2
4.	MS ₄	120	70	58 (49.396±0.969)	1-2
5.	MS ₅	125	87	69 (56.212±2.216)	1-2
6.	MS ₆	120	90	75 (59.232±2.653)	2-3
7.	MS ₇	122	93	76 (60.193±3.347)	2-3
8.	MS ₈	115	104	90 (71.388±1.915)	3-4
9.	MS ₉	124	76	64 (53.109±0.000)	1-2
10.	MS ₁₀	103	55	53 (46.705±1.150)	1-2
11.	MS ₁₁	110	39	35 (36.255±0.347)	1-2
12.	MS ₁₂	125	75	60 (50.561±1.032)	1-2
13.	MS ₁₃	105	58	55 (47.853±0.879)	1-2
14.	MS ₁₄	115	40	40 (38.822±0.706)	1-2
15.	MS ₁₅	115	81	70 (56.577±1.149)	1-2
16.	MS ₁₆	120	96	80 (63.225±1.441)	2-3
17.	MS ₁₇	125	120	96 (78.030±0.982)	3-4
18.	MS ₁₈	115	80	69 (56.158±0.952)	1-2

At 5% level of significance

CD = 5.320

SE(d) = 2.612

**Transformed value.

CV = 5.870

SE(m) = 1.847

Table. Shoot induction response from nodal explants in ker [*C. decidua* (Forsk) Edgew]

ANOVA TABLE

Source	D.f.	S.S.	M.S.	F. cal	Significance
Treatment	17	5166.26	303.90	29.68	0.00000
Error	36	368.56	10.24		
Total	53	5534.81			

upto 25 mg/L. However, the response decreased as the concentration was increased to 35 mg/L.

b) Shoot tip: Shoot regeneration response obtained from shoot tip explant has been summarized in Table 10. Generally shoot regeneration response observed was poor on all the media tried. Maximum shoot regeneration (25%) response was observed on MS₁₇ medium supplemented with 5.0 mg/L BAP and 25.0 mg/L adenine sulphate. On this medium 2-3 shoots/explant were also observed (Fig. 1). Minimum shoot regeneration response (11.42%) was observed on MS₁₈ medium. Shoot regeneration response observed on MS₁₆ medium was (23.68%) with 2-3 shoots per explant. Per cent shoot regeneration response was found to increase as the concentration of BAP was increased (MS₅, MS₆ and MS₇, Table 3). No shoot regeneration response was observed on MS₁ to MS₄, MS₉ to MS₁₅ media.

3. Multiple shoot regeneration

a) Per cent multiple shoot regeneration response has been summarized in Table 11. Maximum multiple shoot regeneration response was 95 per cent on MSS₃ medium supplemented with

25.0 mg/L adenine sulphate with 10-11 shoots per explant. Very good response (80%) was observed

Table 10. Per cent shoot regeneration response from shoot tip explant in ker [C. *decidua* (Forsk) Edgew]

Sr. No.	Medium code	No. of explants	No. of shoots responding	Mean per cent response (mean±S.E.)*	No. of shoots/ explant
1.	MS ₁	33	0	0.00	0
2.	MS ₂	29	0	0.00	0
3.	MS ₃	40	0	0.00	0
4.	MS ₄	30	0	0.00	0
5.	MS ₅	34	4	11.76 (11.760±0.874)	1-2
6.	MS ₆	35	5	14.28 (14.280±0.583)	1-2
7.	MS ₇	32	6	18.75 (18.750±0.250)	1-2
8.	MS ₈	30	7	17.33 (17.333±0.882)	1-2
9.	MS ₉	28	0	0.00	0
10.	MS ₁₀	29	0	0.00	0
11.	MS ₁₁	37	0	0.00	0
12.	MS ₁₂	27	0	0.00	0
13.	MS ₁₃	25	0	0.00	0
14.	MS ₁₄	39	0	0.00	0
15.	MS ₁₅	32	0	0.00	0
16.	MS ₁₆	38	9	23.68 (23.667±0.333)	2-3
17.	MS ₁₇	40	10	25 (25.000±1.155)	2-3
18.	MS ₁₈	35	4	11.42 (11.000±0.577)	1-2

At 5% level of significance

CD = 1.310

*Transformed value

SE(d) = 0.643

SE(m) = 0.455

CV = 4.095

Table. Multiple shoot regeneration response in ker [C. *decidua* (Forsk) Edgew]

ANOVA TABLE

Source	D.f.	S.S.	M.S.	F. cal	Significance
Treatment	17	4911.29	288.90	465.64	0.00000

Error	36	22.34	0.62		
Total	53	4933.62			

Table 11. Per cent multiple shoot regeneration response in ker [*C. decidua* (Forsk) Edgew]

Sr. No.	Medium code	No. of explants	No. of shoots responding	Mean per cent response (mean±S.E.)*	No. of shorts/ explants
1.	MSS ₁	30	21	70 (70.000±1.732)	5-7
2.	MSS ₂	35	28	80 (80.000±1.155)	9-10
3.	MSS ₃	40	38	95 (95.000±0.577)	10-11
4.	MSS ₄	30	10	33 (33.000±2.309)	3-4

CD- 5.236

*Transformed value

SE(d) 2.236

SE(m) 1.581

CV 3.940

Multiple shoot regeneration response in ker [*C. decidua* (Forsk) Edgew]

ANOVA TABLE

Source	D.f.	S.S.	M.S.	F. cal	Significance
Treatment	3	6279.00	2093.00	279.07	0.00000
Error	8	60.000	7.50		
Total	11	6339.00			

on MSS₂ medium with 9-10 shoots per explant (Fig. 3). Shoot regeneration response was 70% on MSS₁ medium with 5-7 shoots per explant. Minimum shoot regeneration response (33%) was observed on MSS₄ medium with 3-4 shoots per explant.

b) Multiple shoot regeneration on media supplemented with culture supernatant of *Acetobacter* and *Azotobacter*

Per cent multiple shoot regeneration on media supplemented with culture supernatant of *Acetobacter* and *Azotobacter* has been summarized in Table 12. Maximum response (82%) was observed on MSB₁₄ medium supplemented with culture supernatant of *Acetobacter* 4.0 ml/L and culture supernatant of *Azotobacter* 4.0 ml/L with 14-16 shoots per shoot cultured (Fig. 4). Minimum response (31%) was observed on MSB₃ with 4 shoots per explant. Good response (75%, 65% and 62%) was observed on MSB₁₅, MSB₉ and MSB₄ media, respectively. These three media induced 10-12 multiple shoots per explant. Moderate response was observed on MSB₁, MSB₂, MSB₄ to MSB₈, MSB₁₀ to MSB₁₃ media with 4 to 9 shoots per explant.

Table 12. Multiple shoot regeneration response from nodal explants on media supplemented with supernatant of *Acetobacter* & *Azotobacter* in ker [*C. decidua* (Forsk) Edgew]

Sr. No.	Medium code	No. of explants	No. of shoots responding	Mean per cent response (mean±S.E.)*	No. of shoots/explant
1.	MSS ₃	40	38	95 (95.000±0.577)	10-11
2.	MSS ₄	30	10	33 (33.000±2.309)	3-4
3.	MSB ₁	40	22	55 (47.477±1.709)	6
4.	MSB ₂	35	21	60 (50.755±1.013)	7-8
5.	MSB ₃	35	10	31 (32.677±3.322)	4
6.	MSB ₄	40	25	62 (51.742±1.380)	10-11
7.	MSB ₅	25	13	52 (45.370±2.999)	5-6
8.	MSB ₆	37	17	45 (42.492±1.342)	5
9.	MSB ₇	37	22	59 (50.360±0.388)	8
10.	MSB ₈	40	16	40 (39.019±0.517)	4-5
11.	MSB ₉	40	26	65 (53.932±1.48)	10-12
12.	MSB ₁₀	35	15	40 (38.422±1.281)	5
13.	MSB ₁₁	35	16	45 (41.905±2.404)	4-5
14.	MSB ₁₂	35	17	48 (44.026±0.956)	7-9
15.	MSB ₁₃	35	19	54 (47.089±1.342)	6-7
16.	MSB ₁₄	40	33	82 (64.931±1.464)	14-16
17.	MSB ₁₅	40	30	75 (59.763±0.579)	11-12

At 5% level of significance

CD = 4.061

SE(d) = 1.734

SE(m) = 1.226

CV = 4.557

*Transformed value

Table. Multiple shoot regeneration response in ker [*C. decidua* (Forsk) Edgew]

ANOVA TABLE

Source	D.f.	S.S.	M.S.	F. cal	Significance
Treatment	14	2942.79	210.20	24.53	0.00000

Error	30	257.03	8.57		
Total	44	3199.82			

c) Shoot length : Data on shoot length recorded after 30th day of inoculation has been shown in Figure-8 . Maximum shoot length (4 cm) was observed on MS₁₇ medium, Whereas it was 1.3 cm was observed on MS₁₁ medium. Shoot length ranged between 2.5 and 3.7 cm. Shoot length was found to decrease with increase in the concentration of kinetin (1.9, 1.7 and 1.3 cm) on MS₉, MS₁₀ and MS₁₁ media, respectively. Similarly, in the presence of other additives (citric acid, ascorbic acid, adenine sulphate and L-Arginine) shoot length was found to decrease (2, 1.7 and 1.5 cm) on MS₁₂, MS₁₃ and MS₁₄ as the concentration of kinetin was increased. Almost equal shoot length was observed on MS₁ and MS₁₀, respectively. Shoot length was observed (3.8 cm) on MS₁₈ medium.

Rooting : Per cent rooting response obtained has been summarized in Table 13 and 14. Best root induction response was observed after 6 days pre-treatment of regenerated shoots on liquid medium having 25 mg/L IBA. Shoots treated for 2h, 1 day, 3 day, when transferred on to the rooting media, showed no response for rooting.

Per cent rooting response has been summarized in 6 days
treated shoots were cultured on different media to

Table 13. Pretreatment of shoots for root induction before sub-culturing on rooting media

Sr. No.	Medium code	Treatment time	Response
1.	MSR ₁₃	2h	--
		1 day	--
		3 day	--
		6 day	+
2.	MSR ₁₄	2h	--
		1 day	--
		3 day	--
		6 day	+

Sucrose 30 g/L
pH 5.8

- = No response
+ = Positive response

Table 14. Per cent root induction response in ker [*C. decidua* (Forsk) Edgew]

Sr. No.	Medium code	No. of shoots inoculated	No. of shoots responding	Mean per cent response (Mean±S.E.)*
1.	MSR ₁	30	0	0
2.	MSR ₂	35	0	0
3.	MSR ₃	20	0	0
4.	MSR ₄	25	5	20 (26.169±2.324)
5.	MSR ₅	40	15	37 (37.228±1.614)
6.	MSR ₆	25	0	0
7.	MSR ₇	25	0	0
8.	MSR ₈	32	0	0
9.	MSR ₉	27	0	0
10.	MSR ₁₀	32	0	0
11.	MSR ₁₁	37	0	0
12.	MSR ₁₂	31	0	0

At 5% level of significance

CD = 2.202

*Transformed value

SE(d) = 1.070

SE(m) = 0.756

CV = 4.557

Table. Root induction response in ker [*C. decidua* (Forsk) Edgew]**ANOVA TABLE**

Source	D.f.	S.S.	M.S.	F. cal	Significance
Treatment	13	5350.96	411.61	239.85	0.00000
Error	28	48.05	1.72		
Total	41	5399.01			

induce roots (Table 6). Rooting was observed only on MSR₄ and MSR₅ media (Fig. 5,6 and 7). Rooting response was 37 per cent and 20 per cent on media MSR₅ and MSR₄, respectively. On all other media tried, no root induction response was observed.



Fig. 5



Fig. 6



Fig. 7

CHAPTER-V

DISCUSSION

Micropropagation is a powerful tool for large scale propagation of arid horticultural crops. It is a rapid technique where mass multiplication of ornamental, timber and fruit trees can be achieved in a relatively short time with high fidelity index. Micropropagation has several advantages over conventional vegetative propagation i.e., elimination of systemic pathogens, preservation of breeding stocks as juvenile plants, international exchange of disease free germplasm to eliminate quarantine and transplanting (Maretaki, 1987). A successful micropropagation depends on several factors such as nature of explant, media, culture conditions and season in which the explants are taken. Axillary bud (Pereira *et al.*, 1994) and shoot bud explants (Aswath and Choudhary, 2002) are usually considered excellent for

micropropagation in different plant species. Multiplication and conservation of *Atropa acuminata* by induction of axillary shoot proliferation from shoot tip and nodal explants has been reported by Ahuja *et al.* (2002). Rapid clonal multiplication of *Aegle marmelas* was achieved by enhanced axillary bud proliferation in young single-node segments cultured in MS nutrient medium (Ajithkumar and Seeni, 1998).

In vitro micropropagation has been successfully used for many horticultural fruit trees (Das *et al.*, 1996). Cotyledonary nodes excised from axenic seedlings have been used for regeneration of many woody species (Kaur *et al.*, 1992; Rathore *et al.*, 1993; Das *et al.*, 1996; Pradhan *et al.*, 1998).

There are various factors which affect tissue culture of woody fruits and ornamental trees such as browning and necrosis, level of cytokinin and auxins, the growth status of the mother plant, time of explant harvest and quality of microflora present on the explants. Among these, those which were found to affect the micropropagation of *Capparis decidua* (Forsk) Edgew are discussed.

Browning and necrosis

Establishment of *in vitro* cultures of woody plants is greatly affected by the browning of the medium and necrosis of explants. Inclusion of antioxidants in the medium or soaking the explants in antioxidant solution, transfer of explants of fresh medium at frequent interval or sealing of cut end to the explants with paraffin wax has been found useful to check the browning (Broome and Zimmerman, 1978; Gupta *et al.*, 1980; Deoro and Shekhawat, 1995, Fougat *et al.*, 1997; Naik *et al.*, 2000).

Antioxidants namely, ascorbic acid and citric acid accelerate growth and shoot differentiation in culture (Joy *et al.*, 1998). They check browning in cultures as reported in *Picea mariana* (Rumay and Thorpe, 1984).

In the present study browning of the medium could be partially overcome by the use of adenine sulphat (25 mg/L) and BAP (5 mg/L). However, the problem could be completely overcome by 2-3 successive subculture on the regeneration medium containing the above additives. Deora and Shekhawat (1995) frequently faced the problem of browning of culture medium while attempting micropropagation of *C. decidua* using nodal explants. They tried to overcome this problem by excising

and removing the necrosed portions within 3-4 days and reculture of explants on to fresh medium.

In another report of micropropagation of *Punica granatum* L., Naik *et al.* (2000) reported high frequency axillary shoot production from axenic cotyledonary nodes from seedling and the problem of browning was not encountered. However, use of seedling would not ensure the fidelity of propagation due to heterozygous nature of the seeds.

Days to shoot induction

An early response for shoot regeneration was observed. Shoot regeneration was initiated within 5 days of culturing of both the explants (Nodal and shoot tip). Tyagi and Kothari (1997), have reported that nodal explants cultured on medium supplemented with BAP (1,3 or 5 mg/L) formed new shoots within two weeks.

Deora and Shekhawat (1995) observed that amongst various growth regulators tested, combinations of NAA and BAP were found to be the best for axillary bud break. Maximum number of shoots (4-7 shoots/explant) were produced within 3 weeks on MS medium supplemented with 0.1 mg/L NAA + 5.0 mg/L BAP +

Additives adenine sulphate 25 mg/L ascorbic acid 50.0 mg/L
citric acid 25 mg/L L-arginine 25.0 mg/L.

Shoot regeneration

An early response for shoot regeneration was observed. Shoot regeneration was initiated within a week of culturing of both the explants (nodal and shoot tip). The most striking influence on bud break and shoot multiplication has been found with the use of auxins and cytokinin (Normanly *et al.*, 1995). The most commonly used cytokinin are BAP, kinetin, 2 ip and zeatin, the later two being natural cytokinin. Of these BAP and kinetin are most effective, cheapest and most often used in micropropagation. Superiority of BAP over other cytokinins has been reported and discussed in relation to shoot proliferation in culture of *C. decidua* (Bonga and Von Aderkas, 1992).

In our study nodal explants were found to be more responsive to shoot regeneration than the shoot tip explants. All the nodal explants gave rise to shoots when cultured on MS basal medium supplemented with 25 mg/L adenine sulphate and 5 mg/L BAP. This response in case of shoot tips was found to be 25 per cent. In the study of *in vitro* propagation of *C. decidua*

(forsk) Edgew] Tyagi and Kothari (1997) supplemented MS medium with BA (1,3 and 5 mg/L) observed differentiation of multiple shoot and shoot buds in 4-5 weeks in the primary cultures. The medium with BA (5 mg/L) was found to be the best for shoot bud proliferation.

BAP is reported to have favoured axillary shoot proliferation in several tree species (Purohit and Dave, 1996; Easwara *et al.*, 1998; Purohit and Singhvi, 1998). Among the various cytokinin tested, BAP has been reported to be most effective for shoot proliferation in guava (Amin and Jaiswal, 1988) and mulberry (Pattnaik *et al.*, 1996).

In the present study, it was observed that an increase in the level of BAP from 1.0 mg/L to 5.0 mg/L produced a positive effect on all the parameters. But when we increase the level of cytokinin kinetin from 1.0 mg/L to 3.0 mg/L a negative effect on all the parameters was seen.

The complementary effect of cytokinin and auxin have been observed by Deora and Shekhawat (1995) during shoot proliferation from nodal explant of ker. Best production of shoots

was achieved on medium containing 5.0 mg/L BAP and 0.1 mg/L IAA.

A combination of BAP 2.5 mg/L and NAA 0.25 mg/L was found to enhance axillary bud proliferation from nodal segments of *Sapium sebifeu* Roxb. (Siril and Dhar, 1997).

The concentration of BAP 5 mg/L was kept unchanged and amount of adenine sulphate was gradually increased from 5.0 mg/L to 25 mg/L. On increasing the amount of adenine sulphate from 5.0 mg/L to 25.0 mg/L, an increase in shoot regeneration was observed. However, further increase in adenine sulphate concentration led to decreased shoot regeneration. Sinha and Chaturvedi (1979) also used adenine sulphate alongwith other auxins and cytokinine for shoot regeneration from single node, leaf cutting of *D. floribunda*. In our case very good to moderate shoot regeneration response from nodal explants was observed on all the media (MS₁₅, MS₁₆ and MS₁₇) having adenine sulphate and BAP and similar results have been reported by Chaturvedi (1975) in *D. floribunda* using nodal explants.

Adenine sulphate in combination of cytokinins and auxins gave higher shoot proliferation (Mathur *et al.*, 2002).

Rate of multiplication becomes highly efficient if multiple shoots are produced from cultured explants. Maximum number (10-11) of multiple shoots per explant was observed on MS basal medium supplemented with 25 mg/L adenine sulphate and 5 mg/L BAP and the percentage of such a high number of shoot regeneration from both the explants was observed to be (95%) in present study. The number of shoots per explant was decreased from 10-11 to 5-9 as NAA is added with 5 mg/L BAP and 25 mg/L adenine sulphate. On the simple MS basal medium 3-4 shoots were found and the percentage of shoot regeneration from both explants was (33%) observed.

In *in vitro* method for obtaining plants of *Acacia catechu* Wild. (Kaur *et al.*, 1998) using nodal explants the maximum number of shoots were achieved on MS medium supplemented with BAP 4 mg/L and NAA 0.5 mg/L.

Supplementation of media with supernatant of *Acetobacter* and *Azotobacter*

For multiple shoot regeneration response culture supernatant of *Acetobacter* and *Azotobacter* was used to

supplemented MS basal medium. The per cent shoot regeneration on control medium (MS without any growth regulator) was observed to be (33%) with 3-4 shoots per explant, while on another control medium MS+25 mg/L adenine sulphate + 5.0 mg/L BAP, the shoot regeneration response was observed to be (95%) with 10-11 shoots per explant. On MS basal medium supplemented with (25.0 mg/L) adenine sulphate (4.0 ml/L) *Acetobacter* supernatant and (4.0 ml/L) *Azotobacter* supernatant 82% shoot regeneration response was observed.

Fuentes Ramirez *et al.* (1993) have reported the presence of IAA and related compounds in growth medium of *A. diazotrophicus*. Pati *et al.* (1995) have reported the same in case of *Azotobacter*. Indole-3-acetic acid (IAA) production by these diazotrophs and its effect on the plant growth was reported by and Kolb and Martin, 1985 and Holl *et al.* (1988).

Effect of the phytohormones released by these bacteria on *in vitro* cultures of any plant species have not been studied so far. We have observed equally good, shoot regeneration response from nodal explants on MS basal media supplemented with supernatant of the two bacteria alone or in combination, as on

different control media tested. Therefore, we can safely suggest that these extracts can be used for supplementation of basal culture media for shoot multiplication in *C. decidua*.

Root formation from regenerated shoots in our case was observed when the regenerated shoots were pre-treated with liquid media for 6 day containing MS basal + 25 mg/L IBA. These pre-treated shoots were transferred onto the rooting media. Maximum (37%) root regeneration response was observed on MS medium supplemented with (1.0 mg/L) IAA and (1.0 mg/L) IBA.

Deora and Shekhawat (1995) have observed, the regenerated shoots rooted best using a 4h pulse treatment with (100 mg/L) of IBA in half strength MS liquid medium, followed by transfer to hormone-free half strength MS agar gelled medium containing (500 mg/L) activated charcoal. Tyagi and Kothari (1997) reported rooting of shoots in different rooting media such as. MS medium with half strength, MS medium without hormone; MS medium with (1 mg/L) IAA and MS medium with (1 mg/L) IBA.

In *C. decidua*, IBA is the most commonly used root inducing hormone (Shekhawat, 2000). The concentration of IBA and the way of its treatments influence root induction (Vander-Krieken *et*

al., 1993). The role of IBA in root induction and its possible mode of action have been reviewed and discussed by Vander-Kriekan *et al.* (1992). Soyler and Arslan, 2000 have reported the effects of some plant growth regulators on the capers (*C. decidua*). They observed that best rooting (28% and 29%) was achieved with 500 ppm IAA in April and 250 ppm IBA in May for 12 hr, respectively. The plantlets with well developed shoots and roots were obtained within weeks. These were kept for hardening overnight in distilled water and then transferred to pots containing sand and coconut husk in 3:1 (w/w) ratio.

In the present study, we have attempted to standardize protocol for micropropagation of *C. decidua*. Although we achieved very good results for shoot regeneration, the rooting part still can further be improved. The role of phytohormones released by two bacteria *Azotobacter* and *Acetobacter* was also studied for supplementation of MS basal medium. These phytohormones were found to be equally effective as other growth regulators routinely used. The shoots were healthier in media supplemented with the culture supernatant of the bacterial strain than on control media.

Therefore, these biologically obtained phytohormones can be substituted in plant tissue culture of *C. decidua*.

CHAPTER-V

SUMMARY AND CONCLUSION

The objective of present investigation was to develop protocol for *in vitro* micropropagation studies in ker (*C. decidua* (Forsk) Edgew).

1. Nodal segment and shoot tip were used as explants for *in vitro* micropropagation studies.
2. Among different treatments used for surface sterilization of explants, maximum sterilization was observed when nodal part was surface sterilized for 50 sec. with 70 per cent alcohol and for 4 min with 0.1 per cent HgCl₂. Best treatment for shoot tip explant observed was 40 sec. with 70 per cent alcohol and 3 min with 0.1 per cent HgCl₂.

3. Days to shoot induction from both the explants on different media were found to range between 5 to 30.
4. Maximum shoot induction from both the explants was observed on MS₁₇ medium on 5th day of incubation.
5. Maximum shoot regeneration (96%) from nodal explants was observed on MS₁₇ medium whereas it was 25% on the same medium in case of shoot tip explants.
6. Maximum number of shoots obtained was 10-11 shoots per explant on MSS₃ medium supplemented with BAP 5.0 mg/L and adenine sulphate 25 mg/L.
7. Maximum shoot regeneration response was 82% with 14-16 shoots/explant on MSB₁₄ medium supplemented with supernatant of *Acetobacter* 4.0 ml/L and *Azotobacter* 4.0 ml/L.
8. Per cent root induction was maximum (37%) on MSR₅ medium from excised shoots when treated with liquid MS medium supplemented with 25 mg/L IBA for 6 days.

LITERATURE CITED

- Agarwal, M. and Kamal, R. 2004. Studies on steroid production using *in vitro* cultures of *Momordica chrantia*. *Journal of Medicinal and Aromatic Plant Sciences*. **26**: 318-323.
- Ahmed, V.U.; Nargis Ismail and Aziz-ur-Rahman Amber, 1989. The use of the bark of *C. decidua* as a cure for asthma inflammation and gout. *Phytochemistry* **28**(9): 2493-2495.
- Ahuja, A.; Sambyal Manju; Kaul Sushma, 2002. *Journal of Plant Biochemistry & Biotechnology* **22**: 121-124.
- Ahuja, M.R. 1991. Biotechnology in frost trees. *Plant Research and Development* **33**: 106-120.

- Ajitkumar, D. and Seeni, S. 1998. Rapid clonal multiplication through *in vitro* axillary shoot proliferation of *Aegle marmelos* (L.) Corr., a medicinal tree. *Plant Cell Rep.* **17**: 422-426.
- Ajitkumar, D. and Seeni, S. 1998. Rapid clonal multiplication through *in vitro* axillary shoot proliferation of *Aegle marmelos* (L.) Corr., a medicinal tree. *Plant Cell Rep.* **17**: 422-426.
- Altman, A.; Loberant, B. 1998. Micropropagation: Clonal propagation *in vitro*, in : A. Altman (Ed) Agricultural Biotechnology. Marcel Dekker, Inc., New York. pp. 19-42.
- Aswath, C. and Choudhary, M.L. 2002. Mass propagation of gerbera (*Gerbera jamesonii*) through shoot culture. *Indian J. Hort.* **59**(1): 95-99.
- Beek, S. and Dunlop, R.W. 2001. Micropropagation of the *Acacia* species- a review. *In vitro Cell Dev. Biol. Plant* **37**: 531-538.
- Bhansali, R.; Kaul, R.K. and Dass, H.C. 1988. Mass cloning of datepalm plantlet through repetitive subculture. *J. Plant Anat. & Morphology* **5**: 73-79.

- Bhojwani, S.S. and Razdan, M.K. 1983. Plant tissue culture : Theory and practice. Elsevier Publishing Company, Amsterdam.
- Bonga, J.K. 1982b. Vegetative propagation of jarrah (*Eucalyptus marginata*) by organ and tissue culture. *Aust. For. Res.* **12**: 121-127.
- Bonga, J.M. 1987. Clonal propagation of mature trees: problems and possible solutions, in: J.M. Bonga, D.J. Durzan (Eds.) Cell and Tissue Culture in Forestry, Vol.I. Martinus Nijhoff Publishers, Dordrecht. pp. 249-271.
- Bonga, J.M. and Von Aderkas, P. 1992. *In vitro* culture of tree. Kluwer Academic Publishers, The Netherlands.
- Bonga, J.M. and Von Aderkas, P. 1992. *In vitro* culture of trees. Forestry Sciences, Vol. **38**, Kluwer Academic Publishers, Dordrecht.
- Broome, O.C. and Zimmerman, R.H. 1978. *In vitro* propagation of blackberry. *Hort. Science* **13**: 151-153.
- Brown, D.C.W.; Leung, D.W.M. and Thorpe, T.A. 1979. Osmotic requirement for shoot formation in *tobacco* callus. *Physiol. Plant* **46**: 36-41.

Chaturvedi, H.C. 1975. Propagation of *Dioscorea floribunda* from *in vitro* culture of single-node stem segments. *Current Science* **44**(23): 839-841.

Chaturvedi, H.C. and Sharma, M. 1989. *In vitro* production of cloned plants of Jojoba [*Simmondsia chinensis* (Link) Schneider] through shoot proliferation in long term culture. *Plant Sci.* **63**: 199-207.

Chauhan, B.M.; Duha, A. and Bhat, C.M. 1986. Nutritional value of ker (*Capparis decidua*) fruits. *Journal of Food Science and Technology*, India **23**(2): 106-108.

Chauhan, J.M.S.; Joshi, I.; Bisht, P.; Sharma, K. and Bagehi, S.K. 2000. Micropropagation of a 23 year old candidate plus tree of *Eucalyptus terehcornis* SM. *Indian J. Forest.* **23**(2): 149-154.

Chauhan, J.M.S.; Joshi, I.; Bisht, P.; Sharma, K. and Bagehi, S.K. 2000. Micropropagation of a 23 year old candidate plus tree of *Eucalyptus terehcornis* SM. *Indian J. Forest* **23**(2): 149-154.

Cheliak, W.M. and Rogers, D.L. 1990. Integrating biotechnology into tree improvement programs. *Canadian J. Forest Res.* **20**: 452-463.

- Chun, Y.W.; Hall, R.B. and Stephens, L.C. 1986. Influences of medium consistency and shoot density on *in vitro* shoot proliferation of *Populus alba* x *P. grandidentata*. *Plant Cell Tissue Org. Culture* **5**: 179-185.
- Das, S.; Timir, B.J. and Jha, S. 1996. In *in vitro* propagation of cashewnut (*Anacardium occidentale*). *Plant Cell Rep.* **15**: 615-619.
- Debergh, P.C. 1988. Improving mass propagation of *in vitro* plantlets. In: The Organizing Committee Int. I Symp. High Technology in Protected Cultivation (Ed.) special lectures : Horticulture in High Technology Era. Tokyo, pp. 45-57.
- Deora, N.S. and Shekhawat, N.S. 1995. Micropropagation of *Capparis decidua* (Forsk) Edgew; a tree of arid horticulture. *Plant Cell Rep.* **15**(3-4): 278-281.
- Douglas, G.C. 1989. Poplar (*Populus* spp.). In: Bajaj, Y.P.S. (Ed.) Biotechnology in Agriculture and Forestry, Vol. **5**, Tree II, Springer Verlag, New York. pp. 300-323.
- Duhoux, E. and Davies, D. 1985. Shoot production from cotyledonary buds of *Acacia albida* and influence of sucrose on rhizogenesis. *Plant Physiol.* **121**: 175-180.

- Dunstan, D.I. and Turner, K.E. 1984. The acclimatization of micropropagation plants. In: Vasil, I.K. (Ed.) Cell culture and somatic cell genetic of plants. Vol. **I** Laboratory procedures and their application. Academic Press, London, pp. 123-129.
- Eeswara, J.P.; Stuchbury, T.; Allan, E.J. and Mordue, A.J. 1998. A standard procedure for the micropropagation of the neem tree (*Azadirachta indica* A. Juss.). *Plant Cell Rep.* **17**: 215-219.
- Fougat, R.S.; Pandya, S.B.; Ahmad, T. and Godhani, P.R. 1997. *Journal of Applied Horticulture, Novasari* **3**: 1-2, pp. 23-29.
- Fuentes-Ramirez, L.E., Jimenez-Salgado, T., Abarca-Ocampo, I.R. and Caballero-Mellado, J. 1993. A *diazotrophicus* an indoleacetic acid producing bacterium isolated from sugarcane cultivars of Mexico. *Plant Soil.* **154**: 145-150.
- Gamborg, O.L., Miller, R.A. and Ojima, K. 1968. Nutrient requirements of suspension culture of soyabean root cells. *Expt. Cell Res.* **50**: 151-158.
- Gautheret, R.J. 1955. Nutrition of plant cultures. *Ann. Rev. Plant Physiol.* **6**: 435-484.

- Gharyal, P.K. and Maheshwari, S.C. 1982. *In vitro* differentiation of plantlets from tissue cultures of *Albizia lebbek*. *Plant Cell Tissue Org. Culture* **2**: 49-53.
- Goyal, Y. and Arya, H.C. 1981. Differentiation in cultures of *Prosopis cineraria* Linn. *Curr. Sci.* **50**: 468-469.
- Grewal, S. and Atal, C.K. 1976. Plantlet formation in callus cultures of *Dioscorea deltoidea* Wall. *Indian J. Exp. Biol.* **14**: 352-353.
- Gupta, I.C.; Harsh, L.N.; Shankarnarayan, K.A.; Sharma, B.D. 1989. Wealth from wastelands. *Indian Farming* **38**: 18-24.
- Gupta, P.K.; Nadgir, A.L.; Mascarenhas, A.F. and Jagnathan, V. 1980. Tissue culture of forest trees: clonal multiplication of *Tectona grandis* L. (Teak) by tissue culture. *Plant Sci. Lett.* **17**: 259-263.
- Haberlandt, G. 1902. Kulturversuche mit isolierten Pflanzenzellen. *Sber. Akad. Wiss. Wien* **111**: 69-92.
- Hacket, W.P. 1985. Juvenility, maturation and rejuvenation in woody plants. *Horticultural Review* **7**: 109-155.
- Haissing, B.E.; Nelson, N.D.; Kidd, G.H. 1987. Trends in the use of tissue culture in forest improvement. *Biotechnology* **5**: 52-59.

- Hammatt, N. 1992. Progress in the biotechnology of trees. *World Journal of Microbiology and Biotechnology* **8**: 369-377.
- Hick, G.S. 1980. Patterns of organ development in plant tissue culture and the problem of organ determination. *Bot. Rev.* **46**: 1-23.
- Holl, F.B., Chanway, C.P., Turkington, R. and Radley, R.A. 1988. Response of crested wheatgrass (*Agropyron cristatum* L.), perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens* L.) to inoculation with *Bacillus polymyxa*. *Soil Biol. Biochem.* **29**: 19-24.
- Ide, Y.; Wantanabe, Y. and Ikeda, H. 1994. Tissue culture of *Acacia auriculiformis* using the aseptically germinated seedlings. *J. Japanese Forestry Soc.* **76**: 576-583.
- Joy, I.V.; Patel, K.R. and Thorpe, T.A. 1988. Ascorbic acid enhancement of organogenesis in tobacco callus. *Plant Cell Tissue and Organ Culture* **13**: 219-223.
- Kaur, G.; Singh, R.P.; Rathore, T.S. and Shekhawat, N.S. 1992. *In vitro* propagation of *Anogeissus sericea*. *Indian Journal of Experimental Biology* **30**: 788-791.

- Kaur, K.; Verma, B. and Kantu, 1998. Plants obtained from the khair tree (*Acacia catechu* Wild.) using mature nodal segments. *Plant Cell Rep.* **17**: 427-429.
- Keathley, D.E. 1983. Increased growth of hybrid spruce bud cultures on stationary liquid medium. North central tree improvement conference. *Proc.* **3**: 150-154.
- Kirtikar, K.R. and Basu, B.D. 1918. *Indian Medical Plants.* **1**: 265.
- Kolb, W. and Martin, P. 1985. Response of plant roots of inoculation with *Azospirillum brasilense* and to application of indole acetic acid. *In: Azospirillum III: Genetics, Physiology, Ecology.* pp. 215-221, Klingmuller, W. Ed., Springer Verlag, Berlin.
- Komalavalli, N. and Rao, M.V. 2000. *In vitro* micropropagation of *Gymnema sylvestre* – A multipurpose medicinal plant. *Plant Cell, Tissue Org. Culture* **61**: 97-105.
- Kurten, V.; Maria, A.; Kauppinen, V. and Rousi, M. 1990. Somatic embryogenesis in cell cultures of birch (*Betula pendula* Roth.). *Plant Cell, Tissue Org. Culture* **233**: 101-105.
- Maene, L. and Debergh, P. 1987. Optimalization of the transfer of tissue cultured shoots to *in vitro* conditions. *Acta Hort.* **212**: 335-348.

- Maretaki, A. 1987. Tissue culture : Its prospects and problems. In:
Heinz, D.J. (Ed.) *Sugarcane Improvement through breeding*.
Elsevier, pp. 112-125.
- Mathur, S., Shekhawat, G.S. and Batra, A. 2002. An efficient *in vitro* method for mass propagation of *Salvadora persica* via apical meristem. *J. Plant Biochem and Biotech.* **11**: 125-127.
- McCown, D.D. 1986. Plug systems for micropropagules. In:
Zimmerman, R.H., Griesbach, R.J., Hammerschlag, F.A.,
Lawson, R.H. (Eds.) Tissue culture as a plant production
system for Horticulture crops, Martinus Nijhoff, Dordrecht.
pp. 53-60.
- McCrow, B.H. and Lloyed, G.B. 1981. Woody plant medium (WPM)
A mineral nutrient formulation for microculture of woody
plant species. *Hort. Sci.* **16**: 433-453.
- Murashige, T. 1974. Plant propagation through tissue culture.
Ann. Rev. Plant Physiol. **25**: 135-166.
- Murashige, T. 1974. Plant propagation through tissue culture.
Ann. Rev. Plant Physiol. **25**: 135-166.
- Murashige, T. 1977. Clonal crops through tissue culture. In: Barz,
W., Reinhard, E. and Zenk, M.H. (Eds.) Proc. Life Sciences ;

Plant tissue culture and its biotechnological applications.
Springer-Verlag, Berlin, pp. 392-403.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant* **15**: 473-497.

Naik, S.K.; Pattnaik, Sitakanta; Chand, P.K. and Pattnaik, S. 1999. *In vitro* propagation of pomegranate (*Punica granatum* L. cv. Ganesh) through axillary shoot proliferation from nodal segment of mature tree. *Sci. Horti.* **79**: 3-4 pp. 175-183.

Naik, S.K.; Pattnaik, Sitakanta; Chand, P.K. and Pattnaik, S. 2000. *High frequency axillary shoot proliferation and plant regeneration from cotyledonary nodes of pomegranate (Punica granatum L.). Sci. Hort.* **85**: 9, pp. 261-270.

Normanly, J.; Solvin, J.P. and Cohen, J.D. 1995. Rethinking auxin biosynthesis and metabolism. *Plant Physiol.* **107**: 323-329.

Ohyama, K. and Oka, S. 1976. Regeneration of whole plants from isolated shoot tips of mulberry tree. *J. Seric. Sci. Jpn.* **44**: 444-450.

Pati, B.R., Sengupta, S. and Chandra, A.K. 1995. Impact of selected phyllospheric diazotrophs on the growth of wheat

seedlings and assay of the growth substances produced by the diazotrophs. *Microbiol. Res.* **150**: 121-127.

Pattnaik, J. and Debata, B.K. 1996. Micropropagation of *Hemidesmus indicus* L. R. Br. through axillary bud culture. *Plant Cell Rep.* **15**: 427-430.

Paul, M.S. and Sen, D.N. 1987. A new report on dimorphism in seeds of *C. decidua* in Indian desert. *Current Sci.* **56**: 1017-1019.

Pereira, A.M.S.; Moro, J.M.; Cerdeira, R.M.M. and Franca, S.C. 1994. Micropropagation of *Maylenus aquifolium* Martius. *J. Herbs, Spices & Med. Plants* **2**(3): 11-18.

Piezik, R.L.M. 1990. Rejuvenation and micropropagation, in: H.J.J. Nijkamp, L.H.W., Van Der Plas, K. Van Aartrijk (Eds.) *Progress in Plant Cellular and Molecular Biology*. Kluwer Academic Publishers. Pp. 91-101.

Pradhan, C.; Kar, S.; Patnaik, S. and Chand, P.K. 1998. Propagation of *Delbergia sisso* Roxb. through *in vitro* shoot proliferation from cotyledonary nodes. *Plant Cell Rep.* **15**: 705-706.

- Purohit, S.D. and Dave, A. 1996. Micropropagation of *Sterculia urens* Roxb. An endangered tree species. *Plant Cell Rep.* **15**: 704-706.
- Purohit, S.D. and Singhvi, A. 1998. Micropropagation of *Achras sapota* through enhanced axillary branching. *Sci. Hort.* **76**: 219-229.
- Rahaman, S.M.; Hossain, M.; Rafiul-Islam, A.K.M. and Islam, A.K.M.R. 1992. Effect of media composition and culture conditions on *in vitro* rooting of rose. *Scient. Hort.* **52**: 163-169.
- Rai, Sushila, 1987. Oils and fats in arid plants with particular reference to *Capparis decidua*. *Transaction of the Indian Society of Desert Technology* **12**(2): 99-105.
- Rathore, T.S., Deora, N.S. and Shekhawat, N.S. Cloning of *Maytenus emarginata* Wild.) Ding Hou- a tree of the Indian Desert, through tissue culture. *Plant Cell Reports* **11**: 449-451.
- Rathore, T.S.; Deora, N.S.; Shekhawat, N.S. and Singh, R.P. 1993. Rapid micropropagation of a tree of arid forestry-cloning of *Anogeissus acuminata* through tissue culture. *Biologia Plantarum* **35**: 381-386.

- Reddy, P.C.; Veeranagouda, P.; Prasad, T.D.; Padmak, K. and Udaykumar, M. and Patil, V. 1995. *In vitro* axillary bud break and multiple shoot production in *Acacia auriculiformis* by tissue culture technique. *Curr. Sci.* **69**: 495-496.
- Rodriguez, R.; Rey, M.; Cruozzo, L. and Ancora, G. 1990. *In vitro* propagation of caper (*Capparis spinosa* L.). *In vitro Cellular and Developmental Biology* **26**(5): 531-536.
- Romberger, J.A.; Hejnowicz, Z. and Hill, J.F. 1993. Plant structure : Function and development. Springer-Verlag, Berlin, Heidelberg, pp. 315-351.
- Roy, S.K.; Hossain, M.Z. and Islam, M.S. 1994. Mass propagation of *Rauwolfia serpentina* by *in vitro* shoot tip culture. *Plant Tiss. Cult. (Bangladesh)* **4**(2): 69-75.
- Rumay, C. and Thorpe, T.A. 1984. Plantlet formation in black and white spruce I., *In vitro* technique. *Can J. For. Res.* **14**: 10-16.
- Ruyter, C.M.; Akram, M.; Illahi, I. and Stockigt, J. 1991. Investigation of alkaloid content of *R. serpentina* roots from regenerated plants. *Planta Med.* **57**(4): 328-330.

- Salem, A.B.; Zemini, H.; Ghorbel, A. 2001. Propagation of caper (*Capparis spinosa* L.) by herbaceous cuttings and *in vitro* culture. *Agricoltura Mediterranea*. **131**: ½, pp. 42-48.
- Shekhawat, M.S. 2000. Tissue culture and somatic cell genetics of selected plants of economic and ecological importance. A thesis submitted to J.N.V.U., Jodhpur for the degree of Ph.D. in Botany. pp. 23-27.
- Shekhawat, N.S.; Rathore, T.S.; Singh, R.P.; Deora, N.S. and Rao, S.R. 1993. Factors affecting *in vitro* clonal propagation of *Prosopis cineraria*. *Plant Growth Regulation* **12**: 273-280.
- Sinha, M., Chaturvedi, H.C. 1979. Rapid clonal propagation of *Dioscorea floribunda* by *in vitro* culture of excised leaves. *Current Science* **48**(4): 176-178.
- Siril, E.A., Dhar, U. 1997. Micropropagation of mature Chinese tallow tree (*Sapium sebifeum* Roxb.). *Plant Cell Rep.* **16**: 637-640.
- Sommer, H.E. and Wetzstein, H.Y. 1984. Hardwoods. In: Ammirato, P.V., Evans, D.A., Sharp, W.R. and Yamada, Y. (Eds.) Handbook of Plant Cell Culture, Vol. 3 Crop Species, Macmillan, New York, pp. 511-540.

- Soyler, D.; Arslan, N. 2000. The effects of some plant growing regulators on the caper (*Capparis spinosa* L.) *Turkish Journal of Agriculture & Forestry* **24**(5): 595-600.
- Sunaina and Goyal, S.C. 2000. *In vitro* micropropagation of Harsinghar (*Nyctanthes arbor-tristis* Linn.). In: National Seminar on Plant Physiology at Interface of Agri-Horticulture and Industry organized by ISPP and Rajasthan Agricultural University, Udaipur, pp. 177.
- Tanira Mom, Ageel, A.M. and Al-Said, M.S. 1989. A study on some Saudi Medicinal plants used as diuretics in traditional medicine. *Filoterapia* **60**(5): 443-447.
- Thakur, M.; Sharma, D.R. and Kanwar, K. 2001. Mass micro-propagation of *Alnus nepalensis* D. Don. *Phytomorphol.* **51**: 123-127.
- Tiwari, M.N. 1979. Arid land plant resources. Proceedings of the International arid land conference resources, Texas Tech. University, International Centre for Arid and Semi-arid Land Studies (ICASALS), 186-194.
- Tyagi, P. and Kothari, S.L. 1997. Micropropagation of *Capparis decidua* through *in vitro* shoot proliferation on nodal

- explants of mature tree and seedlings explants. *Journal of Plant Biochemistry and Biotechnology* **6**(1): 19-23.
- Usher, George, 1974. *A dictionary of plants used by man*. Constable and Company Ltd., London, pp. 121.
- Vander-Krieken, W.M.; Breteler, H.; Visser Marcel, H.M. and Jordi, W. 1992. Effect of light and riboflavinm on indol butyric acid-induced root formation on apple *in vitro*. *Physiol. Plant.* **85**: 589-594.
- Vander-Krieken, W.M.; Breteler, H.; Visser, M.H.M. and Movridou, D. 1993. The role of the conversion of IBA into IAA on root regeneration in apple introduction of a test system. *Plant Cell Report* **12**: 203-206.
- Vashishtha, B.B. 1987. Vegetative propagation of *Capparis decidua*. *Annals of Arid Zone* **26**(1&2): 123-124.
- White, P.R. 1943. *A Handbook of Plant Tissue Culture*. Lancaster, Jaques Cathel.
- Williams, R.R.; Taji, A.M. and Bolton, J.A. 1985. Specificity and interaction among auxin light and pH in rooting of *Auralian* woody species *in vitro*. *Hort. Sci.* **29**: 1952-1953.
- Yadav, P.; Sarkar, S. and Bhatnagar, D. 1997. Action of *Capparis decidua* against alloxan induced oxidative stress and

diabetes in rat tissues. *Pharmacological Research* **36**(3):
221-228.

Ziv, M.; Meir, G. and Halevy, A.H. 1983. Factors affecting the
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ABSTRACT

- a) Title of thesis : **“Development of micropropagation protocols in *Capparis decidua* (forsk) Edgew”,**
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In vitro clonal propagation of *Capparis decidua* was achieved using nodal part and shoot tip explants from mature trees grown in CCS Haryana Agricultural University, Hisar Campus. Response for shoot regeneration was observed within five days on all the media tested from nodal and shoot tip explants. Shoot regeneration was observed only on MS medium supplemented with 25.0 mg/L adenine sulphate and 5.0 mg/L BAP. Nodal explants gave best shoot regeneration (96%) on the

same MS medium supplemented with 25.0 mg/L adenine sulphate and
5.0 mg/L

BAP. On sub-culturign the explants on the same medium 10-11 shoots/explant were observed in 95% of explants cultured. Regenerated shoots also subcultured on MS basal + culture supernatant of *Acetobacter* (4 ml/L) and culture supernatant of *Azotobacter* 4 ml/L medium formed multiple shoots in 82% of the cases with 14-16 shoots per explant. These shoots were treated with liquid MS basal + 25.0 mg/L IBA for 6 days and then transferred to rooting media. Best rooting was observed 37% on MS basal + 1.0 mg/L IBA and 1.0 mg/L IAA with healthy and strong rooting. These shoots with roots were kept overnight for hardening and then transferred to pots.

MAJOR ADVISOR

SIGNATURE OF THE STUDENT

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