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Screening of Important Medicinal Plants in Madhupur Tract and Determination of Their Potentials for In Vitro Conservation and Cell Culture

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SCREENING OF IMPORTANT MEDICINAL PLANTS IN MADHUPUR TRACT AND DETERMINATION OF THEIR POTENTIALS FOR *IN VITRO* CONSERVATION AND CELL CULTURE

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DEDICATED
TO
MY PARENTS AND FAMILY

DECLARATION

I do hereby declare that the whole work submitted as a thesis entitled **"SCREENING OF IMPORTANT MEDICINAL PLANTS IN MADHUPUR TRACT AND DETERMINATION OF THEIR POTENTIALS FOR *IN VITRO* CONSERVATION AND CELL CULTURE"** in the Institute of Biological Sciences, University of Rajshahi, Rajshahi for the degree of **Doctor of Philosophy** is the result of my own investigation and was carried out under the supervision of Dr. M. Monzur Hossain, Professor and Director and Professor Dr. M. A. Bari Miah (retired), Institute of Biological Sciences, University of Rajshahi, Rajshahi. No part of this work presented in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Rajshahi
December, 2015

(Md. Mahbubur Rahman)

CERTIFICATE

This is to certify that **Md. Mahbubur Rahman** has carried out this research study under our supervision. We are pleased to forward his thesis entitled **"SCREENING OF IMPORTANT MEDICINAL PLANTS IN MADHUPUR TRACT AND DETERMINATION OF THEIR POTENTIALS FOR *IN VITRO* CONSERVATION AND CELL CULTURE"** which is the record of bona fide research carried out at the Biotechnology Laboratory, Institute of Biological Sciences, University of Rajshahi, Rajshahi, Bangladesh. He has fulfilled all the requirements of the regulations relating to the nature and prescribed period of research for submission of thesis for the award of PhD degree.

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The author

LIST OF ABBREVIATIONS

Abbreviation	Full Meaning
BAP	N_6 – Benzyl amino purine
^oC	Degree celsius
cm	Centimeter (s)
DW/DDW	Distilled water/Double distilled water
et al.	*et alia* = and other people
etc	et cetera
Fig.	Figure
IAA	Indole-3- acetic acid
IBA	Indole-3- butyric acid
i.e.	*id est* = that is
IK	Indigenous knowledge
GA_3	Gibberellic acid
gm, g	Gram (s)
gm/l, g/l	Gram per liter
HSC	Higher Secondary School Certificate
Kn	Kinetin (6 furfuryl amino purine)
Km^2	Square kelomiter
L/D	Light/ dark
L-DOPA	3, 4-Dihydroxyphenyl-L-alanine
mg	Milligram (s)
mg/l	Milligram per litre
ml	Millilitre (s)
MS medium	Murashige and Skooge (1962)
NAA	α- Napthyline acetic acid
Na-EDTA	ethylenedinitrilo-tetraacetic-acid-disodium Salt Dihydrate
no.	Number
0.1 N	0.1 Normal solution
%	Percent
PGRs	Plant growth regulators
pH	Negative logarithm of hydrogen ion concentration
rpm	Rotation per minute
Sl. No.	Serial number

Sp.	Species
SSC	Secondary School Certificate
TK	Traditional knowledge
UV	Ultra violet
viz.	Videlicet = Namely
v/v	Volume by volume
w/v	Weight by volume
WHO	World Health Organization
2,4-D	2, 4-Dichlorophenoxyacetic acid

SCREENING OF IMPORTANT MEDICINAL PLANTS IN MADHUPUR TRACT AND DETERMINATION OF THEIR POTENTIALS FOR *IN VITRO* CONSERVATION AND CELL CULTURE

ABSTRACT

Madhupur Tract is a large upland area in the central part of Bangladesh with an area about 4,144 sq km. The tribal people living in this area hold the century old indigenous knowledge of using medicinal plants growing in this Tract for their health remedies. In the present investigation an Ethnobotanical survey was carried out in order to explore the indigenous knowledge of the tribal people and to select the most important medicinal plants growing in this ecosystem. Experiments were also conducted to establish micropropagation and *in vitro* conservation for some selected medicinal plants. The Ethnobotanical survey reveals that the existence of 192 medicinal plants species belonging to 68 families in the Madhupur ecosystem. Among these Fabaceae, Asteraceae, Euphorbiaceae, Apocynaceae, Lamiaceae, Liliaceae, Zingiberaceae, Malvaceae, Solanaceae and Amaranthaceae were identified as major families contributing the medicinal plants in the Madhupur Tract. The highest 21 species of medicinal plants were belonging to the family Fabaceae. Ethnobotanical survey also reveals that 107 types diseases were treated with herbal medicine by the village doctors. Herbal remedies for the diseases like cough, fever, weakness, worm, dysentery, stomach complaints, sexual debility, jaundice, skin disease, piles, urinary trouble, diarrhoea, rheumatism and diabetes were found more frequent than other diseases. Field survey also indicated that the highest 36 plant species were used for cough and cold, 30 species for fever, 27 species for weakness, 26 species for warm, 25 species for dysentery and stomach complaints, 20 species for sexual debility, 18 species for jaundice and skin disease, 17 species for piles and urinary trouble, 16 species for diarrhoea and rheumatism, 15 species for diabetes, 13 species for dyspepsia, 12 species for constipation, gleet bruises, menstrual troubles and sore, 11 species for each of Itch and ulcer,10 species for asthma, 9 species for cuts, heart disease and wounds.

Among the 192 medicinal plant species 5 plants *viz*., *Centella asiatica*, *Commelina benghalensis*, *Curcuma zedoaria*, *Mucuna pruriens* and *Vitex negundo* selected for the experiments on plant tissue culture for micropropagation and *in vitro* conservation. Different types of explants *viz*. nodal, shoot tip, axillary bud, internodal and leaf segments cultured in MS medium fortified with different formulation of auxins (NAA, IAA, IBA and 2,4–D) and cytokinins (BAP and Kn). In case of *C. asiatica* the growth regulator formulation 2.0 mg/l Kn+0.5 mg/l IAA was the most inducive culture medium for multiple shoot proliferation. Whereas, 1.0 mg/l BAP+0.5 mg/l NAA was found the best formulation for multiple shoot proliferation from nodal

explant for *C. benghalensis*. In case of *C. zedoaria* good response to microshoot proliferation was noticed in liquid MS medium supplemented 2.0 mg/l BAP+1.0 mg/l IBA+20% cocoanut water. The highest multiple shoot proliferation was achieved for *M. pruriens* in 0.2 mg/l BAP + 0.2 mg/l Kn + 0.2 mg/l IAA. Multiple shoot proliferation for *V. negundo* was also the maximum in 2.0 mg/l Kn + 0.5 mg/l GA_3 supplemented MS medium. In general nodal explant showed better response to multiple shoot proliferation than shoot tip explant. Root development of microcuttings of all five species of medicinal plants were achieved when individual shoot were subculture in auxin supplemented MS medium. However, concentration and type of auxin requirement was found to vary with plant species. For *C. asiatica* MS + 2.0 mg/l IAA was found most effective rooting medium. Whereas, half strength MS with 1.5 mg/l IBA, ½MS+2.0 mg/l IBA + 0.5 mg/l BAP, ½ MS +2.0 mg/l IBA + 0.5 mg/l NAA and ½MS +1.0 mg/l IAA were respectively found the most effective rooting media formulation for *C. benghalensis*, *C. zedoaria*, *M. pruriens* and *V. negundo*.

Attempt was also made to produce artificial seed by encapsulating the *in vitro* grown shoot tip and nodal segments of two medicinal plants *viz. Centella asiatica* and *Rauvolfia serpentina*. In *C. asiatica* the highest 90% shoot formation of artificial seeds was observed in MS medium containing 2.0 mg/l Kn + 0.5 mg/l IAA from nodal segments. In *R. serpentina* the highest 76% shoot proliferation was observed from shoot tip in MS medium containing 1.0 mg/l BAP + 0.2 mg/l NAA. Survivality test reveals encapsulated shoot tip and nodal segments of *C. asiatica*, the highest % of artificial seed survived up to 60 days of storage at 4±1ºC. However, highest survival percentage was noted up to 60 days of storage at 4±1ºC for *R. serpentina*.

Under the present investigation four selected medicinal plant *viz. C. asiatica*, *Coccinia cordifolia*, *M. pruriens* and *R. serpentina* were used to develop cell suspension culture. In case of *C. asiatica*, 0.5 mg/l BAP + 2.0 mg/l NAA found to be most effective culture medium formulation for cell suspension culture.Whereas, 1.0 mg/l BAP+0.5 mg/l NAA was found to be most effective formulation for cell suspension culture of *C. cordifolia*. In *M. pruriens*, the best response was found in 3.0 mg/l BAP + 0.5 mg/l IAA supplemented culture medium for cell suspension culture. In case of *R. serpentina*, 2.0 mg/l 2,4–D+0.5 mg/l BAP found to be most effective formulation for cell suspension culture.The present study demonstrate that for all targeted species, the cultured cells begin to divide within 8-12 days of culture proving their potential for developing cell culture industry for the production of important alkaloid/secondary metabolites.

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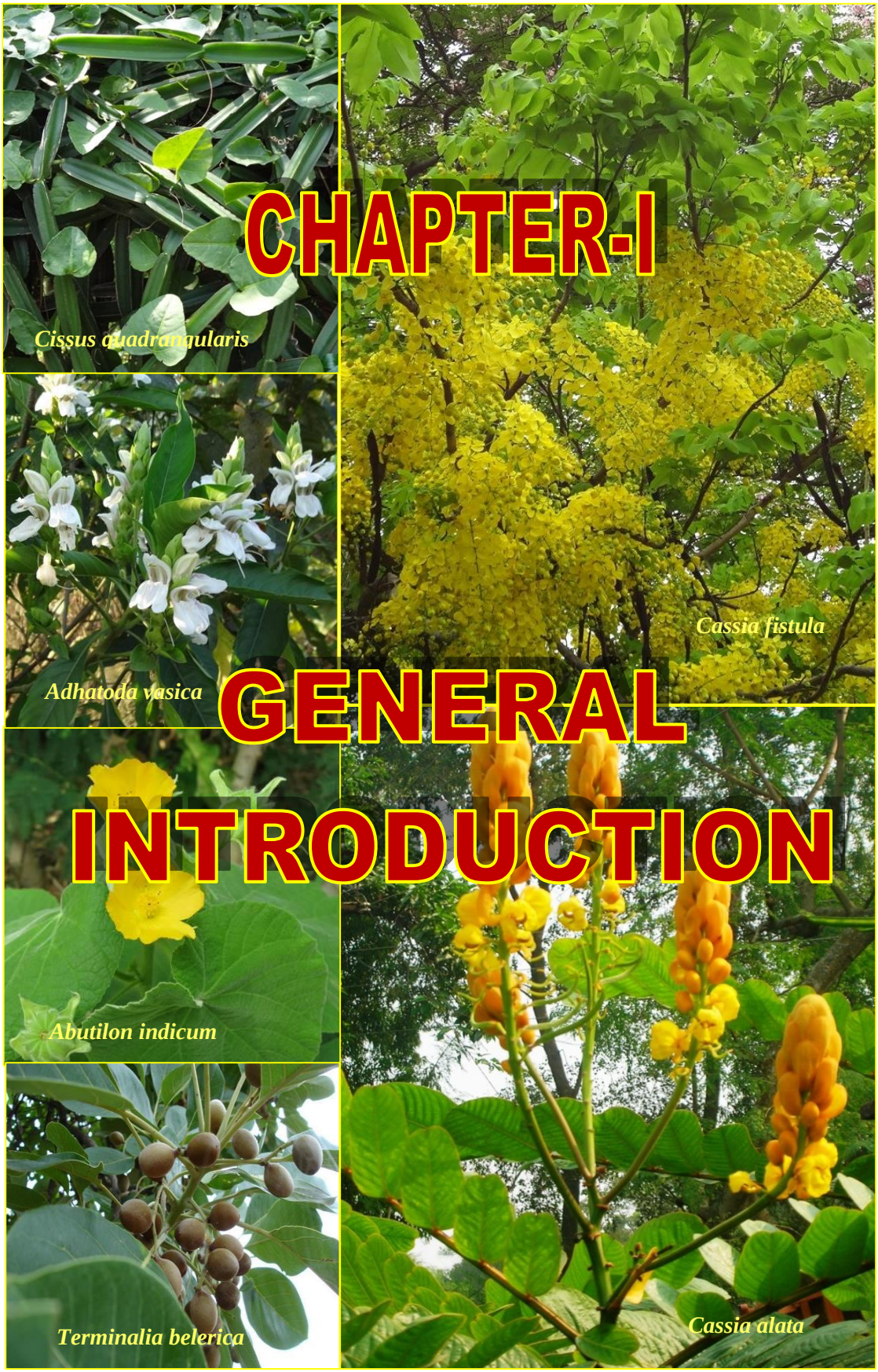
GENERAL INTRODUCTION

Chapter II

ENTHNOBOTANICAL STUDY IN MADHUPUR TRACT


CHAPTER-I
GENERAL INTRODUCTION
Cissus quadrangularis
Cassia fistula
Adhatoda vasica
Abutilon indicum
Terminalia belerica
Cassia alata

Chapter I

GENERAL INTRODUCTION

1.1. MEDICINAL PLANTS IN BANGLADESH

In Bangladesh, a long tradition of indigenous herbal medicinal system, based on the rich local plant diversity, is considered as very important component of the primary health care system. Many rural people of Bangladesh still depend on plant products and herbal remedies for treating their ailments. In our country about 546 plant species have been identified as medicinal plant because of their therapeutic properties (Ghani 1998; Yusuf *et al*. 1994). However, this list is not exhaustive since it is there, but has not been discovered or identified (Said 1995; Ghani 1998). In the mean time, a large number of industries (400 herbal factories) have been established in the country for producing Ayurvedic and Unani medicines. It has been estimated that Bangladesh has a market of about 100 cores taka worth (125 million US $ approximately) herbal products annually (Alam *et al*. 1996). The pharmaceutical manufacturing industries of Bangladesh are solely dependent on imported raw materials for the production various drugs and medicines. The raw materials, particularly the active ingredients and also the major excipients, which these industries utilize for manufacturing purposes, can be obtained from indigenous sources. Some of these, which Bangladesh imports by spending a huge amount of foreign exchange, can be obtained or prepared from the indigenous medicinal plants of the country. It is estimated that if sincere efforts are made at least 30 percent, if not more, of the requirements of raw materials of active ingredients and major excipients can be met from the indigenous sources of Bangladesh. The importance of medicinal plants is increasing day by day. Bangladesh now feels the importance of medicinal plants but majority of the farmers are totally unaware about the profitability of medicinal plant cultivation. In Bangladesh, medicinal plants are found to grow naturally in the forest, bushes and marginal land along the canal and other fallow land. Many such medicinal plants especially the aromatic herbs are grown in the homestead/home garden or a crop field either in sole cropping or inter cropping system and rarely as plantation crop (Padua *et al*. 1999). Home garden is increasing with a focus component of medicinal plants.

Environments of home gardens with different medicinal and aromatic plants could be good sources of small scale or resources poor farmers. Over the country there are some cultivators who are trying to cultivate medicinal plants by their own initiatives. The cultivation is becoming both profitable and environmentally friendly. In Bangladesh sporadically developed some pocket areas of the cultivation of medicinal plants where farmers are growing medicinal plants in their homesteads or agricultural

plots to sale their plants or plant products. In general, all the indigenous medicinal plants are extensively used in the preparation of Unani, Ayurvedic and Homeopathic medicines in Bangladesh. These plants also serve as important raw materials of many modern medicine preparations. Since there has been no systematic phytochemical survey of the medicinal plants of Bangladesh, it is quite possible that many other potential medicinal plants in the country still remain to be explore evaluated. Its phytochemical and pharmacological investigations and research could play a vital role in bringing to the scientific world many useful remedies for alleviation of human sufferings. Increasing interest by multinational pharmaceutical companies and domestic manufacturers of herbal based medicines is contributing significant economic growth of the global medicinal plants sector.

The Government has encouraged the development of the industry since the Prime Minister in Bangladesh launched 'plantation fortnight' in 2002 with a call to plant medicinal plants and fruit trees along with forest species. It is estimated that around 12,000 tons of dried medicinal plants are sold from the rural collection and production areas worth around 4.5 million US $ to the rural economy. The wholesale value is estimated to be US $ 6 million and the import of around 5,000 tons worth US $ 8 million. In summary the medicinal and aromatic plants sector in Bangladesh is estimated to be worth of US $ 14 million with local supply comprising of 70% by volume and 40% by value (SEDF/IC 2003). Dey (2006) using data obtained from Hamdard Laboratories Limited noted that the annual demand of medicinal plants is around 19250 tons in the country. Out of this medicine industry uses 10800 tons, herbal physicians use 6050 tons and cosmetic industries use 2400 tons. Therefore there conservation of such a valuable resource in the country is vitally important. Before marketing of medicinal plants raw or crude materials some activity of the local farmers, collectors, picker and beparies (Local business men) are noticeable. These activities are drying, cutting, grinding, grading and storage is mainly carried out by the farmers, collectors and local pickers. Interviews with 7 inter district beparis have been used to establish an outline of the supply chain for Bangladeshi medicinal plants from the more important areas (The Chittagong hill tracts and north central Bangladesh; Madhupur, Tangail, Mymensingh and Natore). These beparis claim that around 90% of all Bangladesh's medicinal plants are wild harvested and only 10% of medicinal plants are cultivated. Primary processing, i.e. drying, cutting, grinding, grading and storage is mainly carried out by the farmers, collectors and local pickers, with the bepari concentrating on transport and distribution. The latter will sell to wholesalers and retailers and directly to a number of processors and possibly some herbal practitioners.

1.2. HEALTHCARE SYSTEM IN BANGLADESH

The healthcare systems practiced in Bangladesh include the traditional system, Ayurvedic, Unani, Homeopathic, Folk medicine systems and modern system.

1.2.1. Traditional System

Traditional system an art of healing based on traditional use of plants, animals, other natural substances, cultural habits, social practices, religious beliefs and in many cases, superstitions of the present and previous generation of people (Ghani 1990). The basic concept of traditional medicine has been very comprehensively described by the World Health Organization (WHO 1976) in the following way: "Traditional medicine is the sum total knowledge and practice, weather explicable or not, used in the diagnosis, prevention and elimination of physical, mental or social imbalance, relying exclusively on practical experience and observations handed down from generation to generation, verbally or in writing." The forms of traditional medicine practiced today vary from highly organized and long established Ayurvedic and Unani systems to various folk medical practices.

Bangladesh possesses a rich flora of Medicinal plants. Out of the estimated 5000 species of different plants growing in the country more than a thousand are regarded as having medicinal properties. Use of these plants for therapeutic purposes has been in practice in this country since time immemorial. Continuous use of these plants as items of traditional medicine in the treatment and management of various health problems generation after generation has made traditional medicine an integral part of the culture of the people of this country. As a result, even at this age of highly advanced allopathic medicine, a large majority (75–80 %) of the population of this country still prefer using traditional medicine in the treatment of most of their diseases even though modern medical facilities may be available in the neighborhood. Although the use of traditional medicine is so deeply rooted in the cultural heritage of Bangladesh the concept, practice, type and method of application of traditional medicine vary widely among the different ethnic groups. Traditional medical practice among the tribal people is guided by their culture and life style and is mainly based on the use of plant and animal parts and their various products as items of medicine.

1.2.2. Ayurvedic System

Ayurvedic system is one of the oldest systems of medicine which has been practiced in this subcontinent for over 3,000 years. Ayurveda, meaning the science of life, is rooted to the social, cultural and philosophical principles that prevailed in India during the period 600 BC to 700 AD. Ayurveda considers the human being as a

miniature universe. The properties found in the universe are believed to be present in the human body, which like the universe, consists of five gross elements: earth, water, fire, air, and the ethereal parts of the sky. These body constitutions are taken into consideration while treating a patient under this system. The materia medica of Ayurvedic medicine contains some 8,000 published recipes. Many more are held as secret information among certain families.

1.2.3. Unani System

Unani system originated in Greece and was named after the name of Unan province, which is regarded as the original place of development and practice of this system. Hakim Iskalibus of Greece was the first person to propagate the Unani system of medicine. However, this system flourished only when Arabian and Persian Muslim intellectuals like Al-Razi, Ibne-Sina, Al-Rashid, and others enriched it with newer scientific knowledge and discoveries in the 7th century. Because of the significant contributions of Arabian physicians to the development of this system, the Unani system is also known as the Greeko-Arab system. The famous medical book, 'Al-Kanun' (based on the Unani system) of Ibne-Sina (980-1037 AD) was the most prescribed book of medicine in Europe for several centuries. After the 13th century, although Muslim civilization declined, the Unani system of medicine was in full vigour and widely practiced as an effective system of treatment throughout the world. Both Ayurvedic and Unani systems of traditional healthcare have taken firm roots in Bangladesh and re widely practiced all over the country. There are about 6,000 registered and 10,000 unregistered practitioners (Kaviraj/Herbal doctor of the Ayurvedic system and Hakims of the Unani system) of these two systems of medicine in Bangladesh. A total of 15 governments recognized and funded educational institutions are currently engaged in the teaching of traditional medicine in the country. Of them, 10 institutions are involved in teaching the Unani system and 5 in Ayurvedic system. Each of these institutions has an attached outpatient hospital which imparts internship training to graduates while giving medical services to outdoor patients. These institutions offer a four year diploma course and six month internship training. Annual intake of these institutions currently stands at about 400 students. Since the 1989 – 90 academic session a Government Unani and Ayurvedic Degree College, affiliated to the University of Dhaka, has been established in Dhaka. This college offers a five year degree course and one year internship training in an attached 100 bed Traditional Medical Hospital.

1.2.4. Homeopathic System

Homeopathic system of healthcare is not strictly an eastern medical system as it was developed in Europe by a German allopathic physician named Samuel Hahnemann (1755–1843) in the early 19th century from the allopathic system. In this system drugs are applied in very small and diluted doses. It is believed that the strength or curative power of a drug increases mathematically with the increasing degree of its dilution. There are about 1200 medicines in homeopathy, of which more than 500 are obtained from medicinal plants, a few from animals, and the rest from pure chemicals. Plant derived medicines in this system are used as mother tinctures. No excipients (preservative, colour, sweetener, flavor etc) is used in preparing homeopathic medicine. This system of medicine is very popular in many Asian countries including Bangladesh.

1.2.5. Modern System

Modern system the highly advanced system of health management used in Bangladesh and the rest of the world. This system does not limit itself to only curative treatment of the patient but also endeavour's to extend its services to the prevention of diseases by immunization and improving the personal and environmental hygiene of the patient and the community. Well educated and professionally trained experts practice this system of medical treatment. Technologically advanced highly sophisticated equipment and methods are used in this system to attain precise diagnosis and treatment of diseases. Highly efficacious medicinal preparations prepared from purified synthetic or natural chemical substances are used in this system. It has developed sophisticated and precise method and technology of surgical operations and performs critical operations like open heart surgery, heart transplant and transplantation of other vital organs of human body with high degree of precision and safety. Organized and well equipped hospitals and clinics have been developed to effectively and properly offer healthcare services to people under this system.

1.3. CONSERVATION OF MEDICINAL PLANTS THROUGH TISSUE CULTURE

Plant tissue culture techniques have become a powerful tool for studying and solving the basis and applied problems in plant biotechnology. In recent years these techniques gained greater momentum on commercial application in the field of plant propagation mainly in horticulture as well as medicinal plant species (George and Sherrington 1984; Sin and Teng 2002; Bonfill *et al*. 2002). Tissue culture techniques as a means for conserving and multiplying medicinal plants have been reported by Bhojwani (1980), Arrilaga and Segura (1987), Balachandran *et al.* (1990), Nin *et al*.

(1994), Amo-Marco and Ibancz (1998) and Wawrosch *et al*. (2001). Generally the products of micropropagation are genetically identical to the explants donor.

Plant propagation using artificial or synthetic seeds developed from somatic but not zygotic embryos opens up new vistas in medicinal plants. Artificial seed technology involves the production of tissue derived somatic embryos encased in a protective coating. The development of cryopreservation, or storage in liquid nitrogen (at-196ºC), has provided a technology for even more stable, long-term storage of living tissues. Cryopreservation of "non-seed" tissues, such as immature embryos, artificial seed or *in vitro* cultures offers an alternative approach to be used for the preservation of recalcitrant species. These procedures centre on the techniques of slow freezing, vitrification (Sakai *et al*. 1990), and encapsulation - dehydration (Fabre and Dereuddre 1990). Tissues most commonly used for cryogenic storage are: shoot tips from *in vitro* cultures, excised zygotic embryos and embryonic axes, somatic embryos and embryogenic or organogenic cell or callus lines. Development of artificial seed producing technology is currently considered as an effective and efficient alternative method of propagation in several commercially important agronomic and horticultural crops. It has been suggested as a power tool for mass propagation of elite plant species with high commercial value which is equally important in conservation of important medicinal plants.

Biotechnological tools are important for selecting, analyzing, multiplying and improving the plants (Khan *et al*. 2009a, b). Over the years, several techniques have been adopted for enhancing bioactive molecules in medicinal plants (Khan *et al*. 2009a,b). Combinatorial biosynthesis is a tool to combine genes from different organisms to produce bioactive compounds in plants. The basic concept of this approach is combining the metabolic pathways in different organisms on genetic level (Horinouchi 2009). *Agrobacterium rhizogenes* possesses infecting plasmids called root-inducing plasmid or Ri plasmid with T-DNA. The plasmid infects plant cell and induces the production of root-like hairy structures called "hairy root disease". The neoplastic (cancerous) roots produced upon infection have genetic and biochemical stability and a fast growth rate resulting in a large mass/medium ratio in a hormone free media (Sivkumar 2006; Srivastava and Srivastava 2007). These genetically transformed root cultures can produce secondary metabolites in large amounts comparable with those in intact plants, and the transformed root lines can be a promising source for the constant and standardized production of secondary metabolites.

Biotechnological tools are necessary for the conservation and improvement of the plant species which have importance economically and medicinally. DNA banking is

also a potential method for the conservation of biological information by preserving the genomic DNA at low temperatures and has been established in few countries. The DNA isolation is easy and can be used extensively for the characterization and utilization of biodiversity. The implementation of such biotechnological tools on rare and endangered plant species of Bangladesh may help in revival of their previous genes and their products which have been disappeared or inactivated in natural habitat.

1.4. CELL SUSPENSION CULTURE AND SECONDARY METABOLITES PRODUCTION

In recent years, there has been a renewed interest in the use of medicinal plants and medicinal plant products as an alternative to synthetically produced pharmaceuticals for the prevention and treatment of ailments and diseases, which resulted in the growth of industries producing nutraceuticals and pharmaceuticals every year (Cardellina 2002). Plant cell/organ cultures have been perceived as promising choice to traditional plant extraction for obtaining valuable chemicals throughout the year. Particularly, cell suspension culture systems, another area of tissue culture, could be used for large scale culturing of plant cells from which secondary metabolites could be extracted. The advantage of this method is that it can ultimately provide a continuous, reliable source of natural products. Discoveries of cell culture capable of producing specific medicinal compounds at a rate similar or superior to that of intact plants have accelerated in the last few years (Mulabagal and Tsay 2004). The possible use of plant cell cultures for the specific bio-transformations of natural compounds has been demonstrated (Cheetham 1995; Scragg and Alan 1997; Ravisharkar and Rao 2000; Facchini *et al*. 2004; Grotewold 2004). Cell suspension culture offers a condensed biosynthetic cycle to study the growth and production kinetics within a short cultivation time (about 2–4 weeks) with an added advantage of tunability that can help to implement optimal conditions for the production of a number of high value medicinal compounds in good quantities (DÖrnenburg and Knorr 1995; Singh and Chaturvedi 2012). Thus, plant cell, tissue and organ culture systems offer alternative platform for the therapeutically valuable secondary metabolite production. Zhang *et al*. (2002) put forth that plant cell culture has been declared as a feasible tool in producing numerous plant derived metabolites in higher quantities. *In vitro* plant cell suspension culture facilitates large-scale production of fine chemicals in industrial bioreactors and for the study of cellular and molecular processes as it offers the advantage of a simplified model system for studying physiological effect of salt at the cellular level under a controlled environment (Sabir *et al*. 2012) and the effect of

heavy metal stress on growth, enzymes activities and altered biochemical parameters in cultured cells (Mishra *et al.* 2014).

1.5. OBJECTIVES OF THE PRESENT WORK

There are about 35 smaller groups of indigenous communities in Bangladesh covering about two percent of the total population have been living in different pockets of the hilly zones and some plain lands of the country. The Chittagong Hill Tracts, Sylhet, Mymensingh and North Bengal divisions are home to diverse indigenous peoples. Most of the ethnic groups have been predominantly living in Chittagong Hill Tracts, Madhupur Tracts and Barind Tract in the country. The indigenous people living in Madhupur Tracts on the other hand are very unique in nature living in forest perfectly depending on plants both for their foods and for heath remedies. For centuries the Aboriginal Tribals (Garo) of Madhupur Jungle in the Tangail District of Bangladesh have lived in this "*Sal*" forest with their own religion, culture, and way of life. This culture and way of life is distinct from the majority community of Muslim/Hindu Bengali and also different from other tribal indigenous groups in the country. The soil structure and fertility in Madhupur Tract are also different from rest of the country which originates a rather diverse vegetation over the country including diverse flora of medicinal plants. The century old indigenous knowledge has been firmly rooted over the area needs to be preserved and documented which also holds tremendous potentiality for future and modern drug development. Application of modern biotechnological approached can generate enormous possibilities and avenues for micropropagation, conservation of these medicinal plants and isolation of secondary metabolites for future drug development. Thus the aim of the present investigation was to:

1) Conduct a thorough survey of cultivated and wild medicinal plants being used by the local people of Madhupur Tract and gather information on their indigenous herbal uses.
2) Screening of important medicinal plants holding greater medicinal values.
3) Develop reliable and efficient protocol for *in vitro* propagation of some selected important medicinal plants.
4) Establish cell culture as new and alternative methods for obtaining secondary metabolite from medicinal plants.

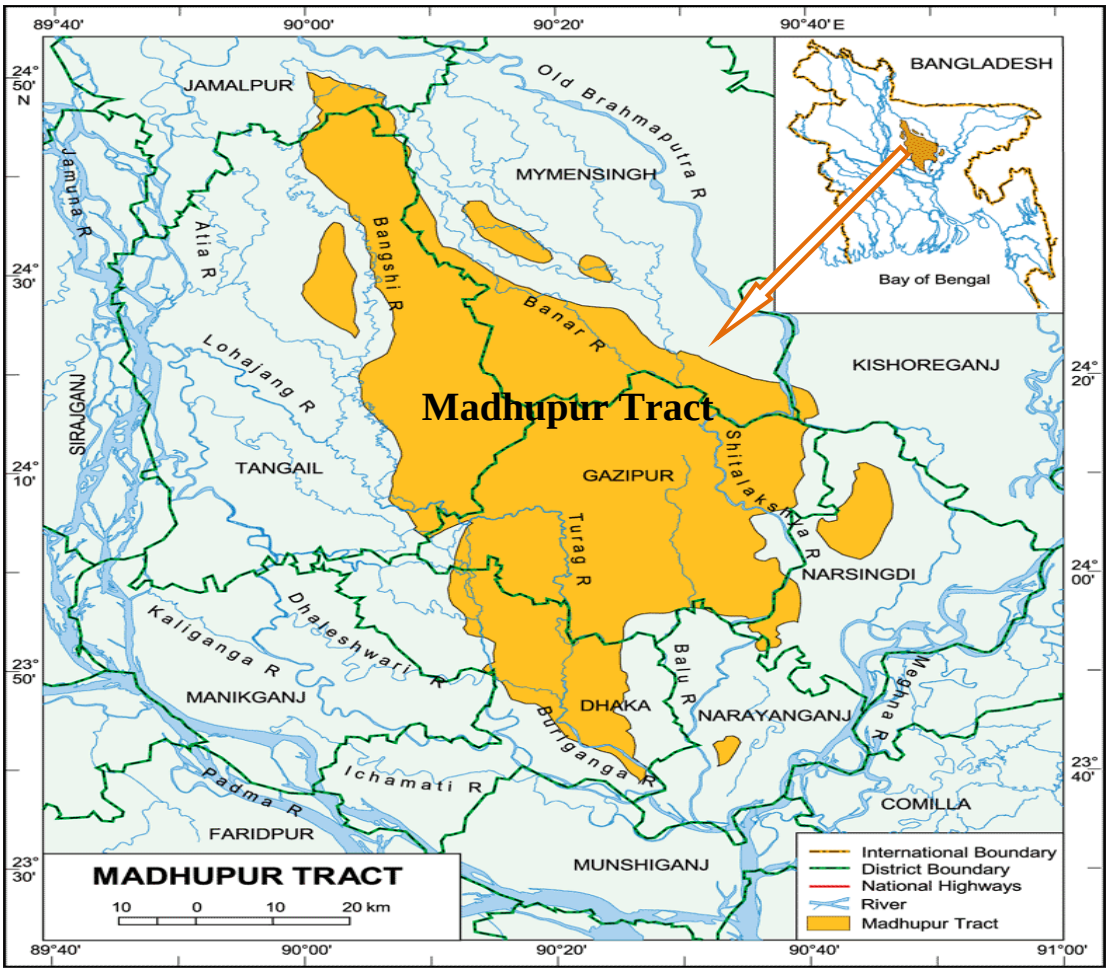

CHAPTER-II
ENTHNOBOTANICAL STUDY
IN
MADHUPUR TRACT
Mucuna pruriens
Cuscuta reflexa
Phyllanthus emblica
Terminalia arjuna
Hyptis suaveolens
Euphorbia tirucalli
Abrus precatorious
Nyctanthes arbortristis
Hyptis suaveolens
Curcuma longa
Mimosa pudica
Bryophyllum pinnatum
Andrographis paniculata

Chapter II

ENTHNOBOTANICAL STUDY IN MADHUPUR TRACT

2.1. INTRODUCTION: A GENERAL ACCOUNT OF THE STUDY AREA

Madhupur Tract lies between latitudes from N 23º48´ to N 25º14´ and longitude E 89º46´ to 90º50´ which is bounded by Jamuna river in the west, a narrow strip of hilly region and alluvial fan in the north, the Surma depression in the east and the confluence of the Ganges and the Meghna river in the south. The uplifted Madhupur area is covered by dark reddish-brown to brownish red, mottled, sticky and compact Madhupur Clay Residuum of Pleistocene age, underlain by Plio-Pleistocene Dupitila Sandstone Formation and adjacent floodplains of the Jamuna and the old Brahmaputra river are characterized by the Holocene alluvium deposits (fine sand, sandy silt, silty clay and clay). Geomorphologicaly Madhupur Tract is much elevated than the adjacent Jamuna Floodplain. The western margin of Madhupur Tract is more or less straight on NW – SE direction (Maitra and Akhter 2011).



Source: Islam, S. (Ed.) 2004. *Banglapedia: National Encyclopedia of Bangladesh.*

Madhupur Tract a large upland area in the central part of Bangladesh. The southern part of this tract is known in Bangla as Bhawal Garh and the northern part as Madhupur Garh. Geologically it is a terrace from one to ten metres above the adjacent floodplains. Though in its present form it is of Pleistocene age its origin may be in the late Miocene, when the Bengal basin was being filled in rapidly. The total extent of this Tract is 4,244 sq km. Unlike the Barind Tract it is largely in one piece, with seven small outliers. The main section stretches from just south of Jamalpur in the north, to Fatullah of Narayanganj, in the south. Most of Dhaka City is on this Tract. Of the seven outliers four are in the east and three in the north. All of them seem to have been separated at least superficially, by faults. This part of Bangladesh has been uplifted several times, resulting in numerous longitudinal faults. The most prominent of these are along the western side, where they can be clearly seen at Mirpur (Dhaka City) and near Ghatail and Madhupur further north. Long fault traces are also extent on the eastern side. The climate of the Tract varies slightly from north to south, the northern reaches being much cooler in winter. Average temperatures vary from 28°C to 32°C in summer, falling to 20°C in winter, with extreme lows of 10°C. Rainfall ranges between 1,000 mm and 1,500 mm annually.

The soils of the Tract have developed largely on Madhupur Clays; which are nutrient poor and somewhat acidic. They are red or brown in colour. In most places the changes from the floodplains to the Tract is quite sharp, but in some places the floodplain soils overlie the gently inclining edges. The Madhupur Tract is extensively dissected, with narrow or broad valleys extending deep into the level landscape and the drainage pattern is clearly dendritic (Harun-Er-Rashid 2011).

2.1.1. Indigenous People of Madhupur Tract

Two main groups of tribal people - the Koch and the Mande (Garo), who are dependent on these forests, live in the Madhupur Tract. The Koch are among the earliest people of Bangladesh, while the Mande have their main centre in the Garo Hills of India. The Mandis of Madhupur Jungle are mostly '*Abeng*' or '*Kochu*'. There are also Koch Hindus. All together there are 12 distinct sub tribes among the Mandis. All are Tibeto-Burmese except the Migam or Lingam who are Mon-Khmer and related to the Khasis of Meghalaya in India. In Bangladesh we find the *Atong*, *Kochu*, *Abeng*, *Chibok*, *Brack*, *Ruga*, *Megam*, *Matchi*, *Dual*, *Chisak*, *Metabeng*, and various combinations of these sub tribes. The common language in Bangladesh is *Abeng* but most of the people along the border of India understand '*Acchik*'.

For centuries the Aboriginal Tribals (Garo) of Madhupur Jungle in the Tangail District of Bangladesh have lived in this "*Sal*" forest with their own religion, culture, and way of life. This culture and way of life is distinct from the majority community of Muslim/Hindu Bengali. Garo music and religion is pre-Buddhist and originated in southwest China and Western Tibet. The Garo language contains many Mandarin and Burmese words.

The Garo Tribe is *matrilineal* and Mongolian. All real property belongs to the women's lineage and not to individuals. The men in the Tribe can act as administrators with permission of the wife or her lineage. There is no such thing as personal property and all property belongs to the *machong* or "motherhood".

Fig. 2.1.1. Indigenous people (Garo) among us in the Madhupur Tract.

Fig. 2.1.2. Junik Marakh (at present oldest indigenous person of Garo tribe live in Madhupur forest).

Among Garos, the people who treat and cure patients by folk medicines are considered persons with supernatural power and therefore, enjoy respect and honor in the community. These persons are named *khamal* or *kamal*. Khamals usually possess a very good knowledge of the properties of medicinal plants, which because of the forest regions that they inhabit, can be very diverse in nature.

Indigenous people living over the areas of Madhupur Tract also holds the century old traditions and culture and their indigenous knowledge (IK) is important in updating our modern knowledge and culture and it needs to be restored and documented. Significant contributions to global knowledge have originated from indigenous people. But today, like Madhupur Tract many indigenous knowledge systems are at risk of becoming extinct because of rapidly changing natural environments and fast pacing economic, political, and cultural changes on a global scale.

2.1.2. Vegetation and Medicinal Plants in Madhupur Tract

During the early British rule (1760-1820) Madhupur tract was covered with dense forests, mostly moist deciduous with Shal (*Shorea robusta*) dominating, mixed with semi-evergreen forests which were heavily exploited for timber reducing the cover to brushwood. The deciduous moist forest of central Bangladesh now covers an area of only 123000 ha, although it was twice this size just 30 years ago. The tract consists of older alluvium of low fertility with yellowish-red soils. The predominant tree in this forest is Shal, which accounts for nearly 75% of all trees. There are some other commercially valuable trees too, some used for medicinal purposes.

Sal forests are also rich in medicinal plants. But these forests be set with the problems of serious encroachment and denudation and hence availability of medicinal plants become declining.

There is no complete botanical survey of medicinal plants in the Sal forests of Madhupur. Haradhan (2004) surveyed the Madhupur forest and enlisted some plant species which are given below.

Trees: *Shorea robusta* (Sal), *Terminalia belerica* (Bahera), *Terminalia chebula* (Horitaki), *Phyllanthus emblica* (Amloki), *Cassia fistula* (Sonalu), *Butea monosperma* (Palas), *Holarrhena antidysenterica* (Kurch), *Aphanamixis polystachya* (Pitraj), *Streblus asper* (Sheora), *Albizia procera* (Silkoroi), *Alastonia scholaris* (Chatim), *Anthocephalus chinensis* (Kadam), *Artocarpus lakoocha* (Deua), *Bombax ceiba* (Simul), *Dillenia indica* (Chalta), *Erythrina variegate* (Mander), *Eugenia operculatum* (Boti jan), *Gmelina arborea* (Gamari), *Mesua nagassarium* (Nageswar), *Pterospermum acerifolium* (Kanok chapa). *Sapindus mukorosi* (Ritha).

Shurbs: *Abroma augusta* (Ulat Kambal), *Asparagus racemosus* (Satarmuli), *Artemisia vulgaris* (Nagclornn), *Aristolocha indica* (Iswarmul), *Heltcyres isora* (Atmora), *Hyptis suaveolens* (Tokma), *Gloriosa superba* (Ulat chandal). *Scindapsus officinalis* (Gaj pipul, Dew rowsshon), *Solanum torvum* (Tit begun).

Climbers: *Aristolochia indica* (Iswarmul), *Coccinia cordifolia* (Telakucha), *Buettneria pilosa* (Harjora), *Hemidesmus indicus* (Anontamul), *Mikania scandens* (Assam lata), *Paederia foetida* (Gandhabaduh), *Smilax macrophylla* (Kumari lata), *Tinospora cordifolia* (Gulnacha).

Herbs: *Acalypha indica* (Muktajhuri), *Andrographis paniculata* (Kalamegh), *Argernone Mexicana* (Shialkanta), *Beta vulgaris* (Beet), *Boerhaavia repens* (Punarnava), *Centella asiatica* (Thankuri), *Curcuma amada* (Amada), *Curcuma aromatica* (Jangli halud), *Cuscuta reflexa* (Sharnalata), *Cynodon dactylon* (Durbaghass), *Cyperus rotundus* (Mutha), *Eclipta alba* (Kesaraj), *Elphantopus scaber* (Goja lata), *Eupatorium odoratum* (Assam lata, German lata), *Hedyotis corymbosa* (Khetpapra), *Phyllanthus niruri* (Bhui amal), *Polygonum hydropiper* (Biskatali), *Rauwolfia sarpentina* (Sarpaganda), *Leucas aspara* (Shetdrone), *Oxalis corniculata* (amrul), *Solanum surattense* (kantikari), *Vanda roxburghii* (Rasna), *Wedelia chinensis* (Kesraj, Bhimrazl, Bhringaraj).

It is important to collect information on the indigenous knowledge and evaluate the present position of the medicinal plant resources of such area as it may help in planning programme for judicious exploitation of these plant resources. Botanical survey of this subcontinent first launched by Hooker (1875). Earliest attempt to enlist medicinal plants in our country carried out by Khan and Haq (1975). They listed 182 medicinal plants of Bangladesh. Yusuf *et al*. (1994) described 620 medicinal plants of Bangladesh with their action principles and uses. Since no steady report on the availability and sources of medicinal plants in the Madhupur Tract is available, a survey work was conducted in the six upazilas of three districts for collecting information of medicinal plants from local herbalists presently engaged in medical practicing with local medicinal plants.

2.1.3. Aims and Objectives

In the present world, where Industrialization accompanied with urbanization has greatly modified the values and life standards of the bulk of population. The indigenous knowledge of ethnic people regarding the use of medicinal plants being lost rapidly. It was thus considered worthwhile to document the folk uses of medicinal plants for curing important human diseases. The rural and tribal people of Madhupur Tract particularly those are inhibited in remote places largely depend on traditional

herbal medicine and some of them possess adequate knowledge of traditional healing system. But due to habitat destruction many important medicinal plant species are at risk and some of those are facing extinction. Moreover, introduction of modern medicine causes the loss of inherited indigenous knowledge of traditional medicine. In the consequences, a through survey of indigenous knowledge of medicinal uses of plants in the Madhupur Tract can illuminate the present status of medicinal plants of that area. So the present study was undertaken with object of

1) Conduct a thorough survey of cultivated and wild medicinal plants being used by the local people of Madhupur Tract
2) To collect the medicinal plants with the help of herbal doctors/Kabiraj.
3) To compile information on their indigenous herbal uses.
4) To make economic and social analysis of medicinal plants on herbal doctors in the Madhupur Tract.
5) Screening of medicinal plants holding greater medicinal values.

2.2. MATERIALS AND METHODS

The Madhupur Tract is one of the few places in the country that still holds sign of the dense greeneries and jungles that once characterized our lands. The vegetation in the Madhupur Tract is considered to be unique and rather different from other parts of the country. Large number of medicinal plants and their traditional practices has been one of the age old cultural heritages since time immemorial over the Madhupur Tract. Under the present study a questionnaire has been developed to make a survey segmenting the area under selected sites. This programme carried out from January 2012 to June 2013.

2.2.1. Site Selection

In the present investigation study area is Madhupur tract of Bangladesh. Madhupur, Sakhipur, Ghatail upazilas in Tangail district, Bhaluka upazila in Mymensingh district and Sreepur and Kaliakoir upazilas in Gazipur district were selected as study area for this survey. The survey was carried out mainly based on information found available on traditional practices of medicinal plants through interview of selected respondents.

2.2.2. Questionnaires for Information

Questionnaires for socioeconomic information of the respondents (kabiraj/herbal doctors, wholesaler, and medicinal plants cultivators) were formulated and developed. Thorough surveys of cultivated and wild medicinal plants were made with their botanical characteristics and ethnobotanical uses. Intensified efforts have been made to develop a questionnaire during the initial period of investigation several formats were developed by studying the literature of similar research works undertaken by other workers. Direct field observation and through trial and error of the application of several formats a final questionnaires format has been developed to hold every aspects of practical uses of medicinal plants and their occurrence in the locality. Reputed herbal doctors/kabiraj were the main respondent for this questionnaires study but sometimes local medicinal plants wholesaler and cultivators with indigenous knowledge were also considered.

A questionnaire was developed and used to collect of socioeconomic and ethnobotanical information from local herbal doctors generally known as ‘Kabiraj’. The questionnaire form is shown in **Appendix 1**.

2.2.3. Collection of Plant Materials

The following tools were used during plant materials collection: a) Camera; b) Knife; c) Scissors; d) Polythene bags and e) Tags. Plants were collected systemically as possible from the selected upazilas of the Madhupur Tract. During the flowering time

the plants were collected with flowers. In case, some of the plant parts were collected with the help of a long stick. During collection attempts were made to collect flowers, fruits and foliage from the same plant and where it was not possible, specimens were collected from different plants.

The study fields were visited for collection of specimens. The entire plants as far as possible with their flowers and fruits were collected and their photographs were taken focusing the particular organ of specific importance. In the field notebooks collection number, date of collection, locality, habit, habitat, flower colour and other characters which were not found after preparing herbarium or preservation of flower were recorded, if the flowers and fruits were not possible to collect with their vegetative body, they were collected separately.

2.2.4. Preservation

Special care was taken to preserve the plant specimens. The collected plants were preserved. Herbarium sheets were prepared following standard method. Sheets were always made in multiple sets. Insecticides were applied to protect the sheet from insect damage.

2.2.5. Identification

After making the collection, the specimens were identified by the following methods

i) Matching system
ii) With the help of taxonomic literature
iii) By experts

i) Matching system

The collected plants were identified by comparing the herbarium sheets preserved in the Rajshahi University herbarium. The collected specimen was placed side by side with the herbariums which seem to be the same. After getting provisional identification the specimen was with the description provided by Hooker (1865 – 1885)

ii) Identification of collected plant sample

The collected plant specimen was identified with the help of books and journals (Huq 1986; Hooker 1865 – 1885; Khan and Huq 1975; Biswas and Ghos 1973) were consulted. For the current and up to date nomenclature (Huq 1986; Bennet 1987 and Pasha 1988) were consulted. For medicinal information documents (books and journals) of Gilani *et al*. 2003; Ghani 2003; Yusuf *et al*. 2009; Kirtikar and Basu 1987 and 1995 were consulted.

The collected plant specimens were also identified with the help of botanical experts at the department of Botany, Rajshahi University; Dhaka University and Bangladesh National Herbarium, Mirpur, Dhaka.

2.2.6. Data Collection

Data for this study were conducted through personal interview of the respondents during "January 2012 to June 2013" using questionnaires prepared earlier. Secondary data were also collected from different sources according to needs. Data and information were collected from seminar library Institute of Biological Sciences, Rajshahi University, seminar library Department of Botany, Rajshahi University, search from internet, and previous research and survey reports.

2.2.7. Data Analysis

MS-Word, MS-Excel and SPSS were used to process all collected information by micro computer. Data were collected using the following parameters and the methods followed for data collection are given below:

Data were recorded on respondent frequency (%) and mean number of respondent (X) were calculated using formula below:

$$Respondent\ frequency\ (\%) = \frac{No.\ of\ respondents}{Total\ no.of\ respondents} \times 100$$

Average number of respondent

Average numbers of respondents were calculated using the following formula.

$$X = \frac{\sum Xi}{N}$$

Where, X = Average number of respondent.

∑= Summation.

Xi = Total number of respondent.

N = Number of observation.

2.3. RESULTS

In the present investigation 192 plants belonging to 68 families were identified as the important plants having different medicinal values. In this study Madhupur, Ghatail, Sakhipur upazilasin Tangail district, Bhaluka upazila in Mymensingh district and Kaliakair and Sreepur upazilas in Gazipur district were selected under Madhupur Tract.

Medicinal plants vary from place to place depending upon the local demand and climate conditions of the location. Availability of a certain species again depends on the land configuration, nature of soil and favorable climatic condition suitable for the growth of the species in the locality. The people of different places have different opinion for herbal medicine. Traditional knowledge, cultivation and selling of medicinal plants are very important in the Madhupur Tract as inhabitants of the area find their living subsistence in this activity. The rural people engaged in this traditional knowledge, selling and cultivation of medicinal plants was taken as the respondents for this survey study and economic condition of these respondents were very important. Under the present investigation socioeconomic condition of these respondents were also studied and their social and economic status were analyzed focusing on their number, age, profession, education, family size, earning and the results are described below in separate heads.

2.3.1. Socioeconomic Study

Socioeconomic condition is related to the practice and cultivation of medicinal plants in the Madhupur Tract as the relatively farmers and herbal doctors were not found to be engaged in this practice. Under the present study the respondents were selected from different profession and status of the rural society involved in medicinal plant cultivation and business of its products and process.

2.3.1.1. *Selected respondents in the study area*

The study was conducted among the herbal doctors, medicinal plant cultivators and sellers belonging to the six upazilas of three districts of the Madhupur Tract. A large number of local herbal doctors, farmers and sellers have been engaged in different activities generated around the area by virtue of medicinal plants. Number of respondents engaged in traditional practice, selling and cultivation of medicinal plants, their family size including male and female members were studied and the results are given in **Table 2.3.1.**

Table 2.3.1. Family size of the respondents engaged in traditional practice, selling and cultivation of different area of the Madhupur Tract.

Name of Upazila	Number of respondents	Total family member of respondents	Male	Female
Madhupur	17	83	43	40
Ghatail	36	178	90	88
Sakhipur	42	214	106	108
Bhaluka	22	102	54	48
Kaliakair	17	81	40	42
Sreepur	12	55	28	26
Total	146	713	361	352

Total interviewees were 146 in number and their total family member were 713 (male 361 and female 352). The table shows that highest number (42 respondents) of respondent were in the upazila Sakhipur (**Table 2.3.1**) followed by Ghatail, Bhaluka, Madhupur, Kaliakair and Sreepur.

2.3.1.2. *Age of respondents*

Age of the respondents was determined by the number of years counted from their date of birth to date of interview. The respondents were classified into three age groups such as young (18-30 years), middle (31-55 years) and old age (56 years and above). The results showed that about 12, 48 and 40 percent of the respondents were in young, middle and old age groups, respectively (**Fig. 2.3.1**).

2.3.1.3. *Profession of respondents*

The respondent of the interviews were of different categories according to their profession, like kobiraj/herbal doctor/traditional practitioner, whole seller and medicinal plants cultivators. The **Fig. 2.3.2** shows that highest numbers of respondent were kobiraj/herbal doctor 91 percent, whereas 8 percent of respondent were whole seller and only 1 percent was medicinal plant cultivators.

2.3.1.4. *Education of respondents*

Education helps an individual to acquire knowledge, to change the attitude, to exercise the modern practices and to promote their skill. Through education, one become aware of new ideas, views and acquires the ability to analyze facts and phenomenon by scientific way. It was measured in terms of their schooling at different levels. The findings showed that 11 percent of the respondents had no formal education, while 62, 19 and 8 percent of the respondents had below Secondary School

Certificate (SSC) level, SSC level and Higher Secondary Certificate (HSC) and above respectively (**Fig. 2.3.3**). This result indicated that 89 percent of the respondents were literate.

2.3.1.5. *Family size of respondents*

Family size was measured by the total number of family members dependent fully or partially upon the respondent. The family size was classified into four categories based on their total family members. These categories were:

a. Single family (2 person/family)
b. Small family (3-4 person/family)
c. Medium family(5-7 person/family)
d. Large family (7 person and above/family)

The **Fig. 2.3.4** revealed that 44 percent of the respondent had small family, whereas 12 percent, 35 percent and 9 percent of the respondents had single, medium and large size family respectively.

2.3.1.6. *Cultivation of medicinal plants*

Cultivation of medicinal plants grows new challenge as the science of herbal medicine has been re-emerging all over the world including both developing and developed countries. Due to over exploitation of medicinal plants, its cultivation now imposing more importance on cultivation of medicinal plants in plots like some of agricultural crops. Though the Madhupur Tract once abounded with medicinal plants, it now hardly produces these species of vegetation anymore. Among the 146 respondents only two were commercially cultivator of medicinal plants. Rest of the respondent cultivate medicinal plant in their homestead area and rely on wild source. On the other hand 12 wholesalers also collected their materials from wild source by their representative. For these reasons medicinal plants become vulnerable day by day.

2.3.1.7. *Yearly income of respondents*

A large number of local herbal doctors, farmers and sellers have been engaged in different activities by virtue of medicinal plants in the Madhupur Tract. The selected respondents were classified into three categories based on their yearly income. These categories were:

a. Low income (less than 20,000 BDT)
b. Medium income (21,000 – 100,000 BDT)
c. High income (100,100 BDT – above)

It was evident from the **Fig. 2.3.5** that 55 percent of the respondents belonged to medium income category, whereas 33 and 12 percent of respondents belonged to high and low income categories respectively.

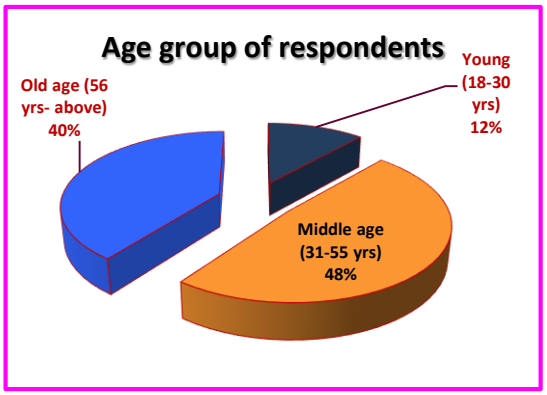


Fig. 2.3.1. Age of respondents

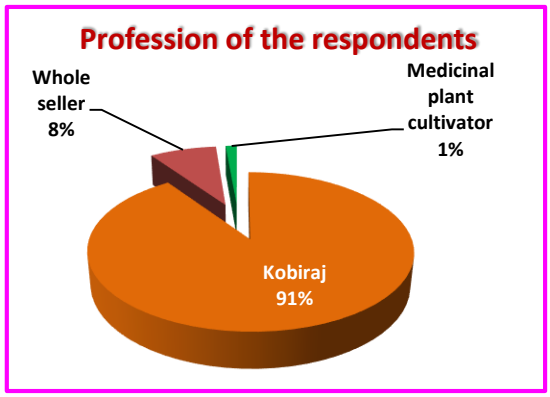


Fig. 2.3.2. Profession of respondents

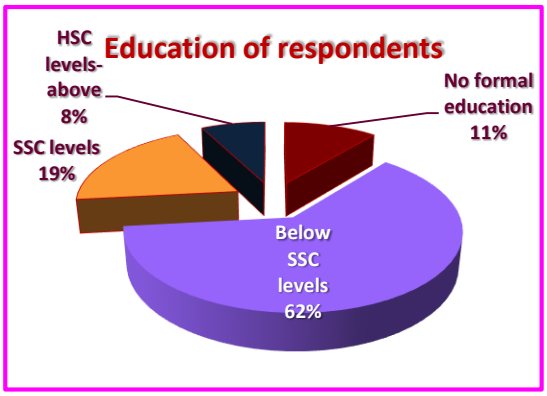


Fig. 2.3.3. Education of respondents

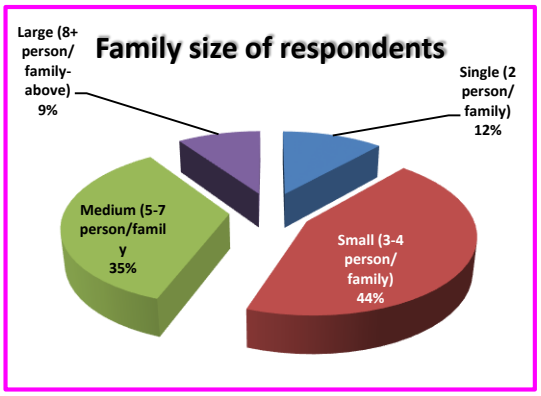


Fig. 2.3.4. Family size of respondents

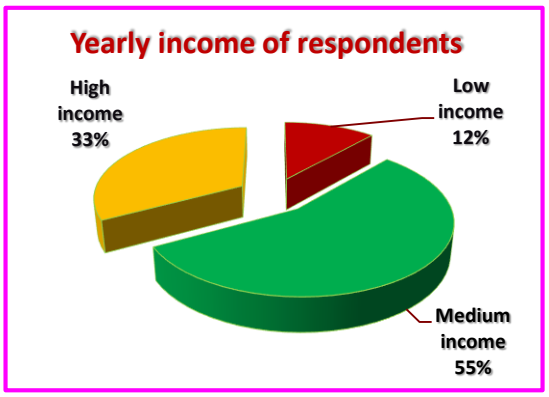


Fig. 2.3.5. Income of the respondents

The information of medicinal plants with particular emphasis on botanical perspectives including its medicinal uses and level of availability in a particular location harvested from 146 respondents was analyzed. The results of indigenous knowledge of herbal medicine are described under following head.

2.3.2. Botanical and Ethnobotanical Studies

Indigenous knowledge is as old as human civilization. It has been defined as the traditional knowledge of indigenous communities about surrounding plant diversity and as the study of how the people of a particular culture and region make use of indigenous plants. Ethnobotany has its roots in botany. Botany, in turn originated in part from an interest in finding plants to help illness. In fact medicine and botany have close ties. Many of today's drugs have been derived from plant sources. Indigenous knowledge of medicinal plants is rapidly disappearing in Bangladesh particularly in the Madhupur Tract.

In the present investigation ethnobotanical observation was made on 192 medicinal plant species during the course of survey in the six upazilas of three districts under Madhupur Tract. Ethnobotanical information collected on individual plant basis regarding scientific name, local name, family, life span, habit, mode of propagation, usable parts of plant, mode of administer action and treatment (s) were analyzed and the results are presented in **Table 2.3.2.**

Table 2.3.2. Ethnobotanical information of 192 medicinal plant species collected from respondents surveyed in the MadhupurTract.

Sl No.	Scientific Name	Local name	Family	Life span	Habit	Habitat	Mode of propagation	usable parts of plant	Mode of administer action	Treatment(s) / Diseases/ Properties.
1	*Abelmoschusesculentus* (L)Moench	Dheros, Vendi	Malvaceae	Annual	Herb	Cultivated	Seed	Fruit	Juice	Cooling, stomachic, astringent, aphrodisiac
2	*Abromaaugusta* L.	Ulatkambal	Sterculiaceae	Perennial	Shrub	Homestead	Seed	Leaves	Juice	Menstruation, weakness
3	*Abrusprecatorius*L.	Kunch, Ratti	Fabaceae	Annual	Lianas	Thicket	Seed	Root, Seed	Powder	Antipyretic, Diabetic, itch, Abortion, Headache
4	*Abutilon indicum*(L.) Sweet	Potari,	Malvaceae	Annual	Shrub	Shady slope	Seed	Root, Seed	Juice, Powder	Urinary trouble, Pains, Weakness
5	*Acacia catechu*Willd	Khoyer	Fabaceae	Perennial	Tree	Cultivated	Seed	Bark	Juice, Powder	Astringent, anti-dysenteric, anthelmintic, antipyretic.
6	*Acacia nilotica* (L.) Bel	Babla	Fabaceae	Perennial	Tree	Jungle	Seed	Bark, Leaves,	Juice	Astringent, demulcent, hypoglycaemic, cooling, diabetes.
7	*Acalyphaindica* L.	Mutajhuri	Euphorbiaceae	Annual	Herb	Fallow land roadside	Seed	Whole plant	Powder, juice, Paste	Cough, Skin disease, Sore, Ring worm toxic prevention.
8	*Achyranthesaspera* L.	Apang, Upothlengra	Amaranthaceae	Annual	Herb	Follow land	Seed	Root, Leaves	Juice	Urinary trouble, Abortion.
9	*Adhatodavasica* L.	Basok	Acanthaceae	Perennial	Shrub	Homestead	Seed	Leaves	Juice	Cold, Cough, Stomach pain,
10	*Aeglemarmelos* (L.) Corr	Bel	Rutaceae	Perennial	Tree	Planted roadside	Seed	Leaves, Fruit	Juice	Fever, Dysentery, Indigestion, Cough, Energetic.
11	*Ageratum conyzoides* L.	Fulkuri	Asteraceae	Annual	Herb	Fallow land	Seed	Leaves, Root	Juice	Fever, Cuts and sores, Diarrhoea, Dysentery
12	*Albizialebbeck* (L.) Benth.	Koroi	Fabaceae	Perennial	Tree	Forest	Seed	Fruit, Seed, Bark	Decoction	Astringent, Bronchial asthma, Piles.
13	*Allamandacathartica* L.	Malatilata, Kalkephul	Apocynaceae	Perennial	Shrub	Ornamental plant in gardens	Seed	Leaves. Bark, Root	Paste	Hypotensive, snakebite. Ascites.
14	*Allium cepa* L.	Piyanj	Liliaceae	Annual	Herb	Cultivated	Seed, Bulb	Scale Leaves	Raw, Juice	Cough, Asthma, Headache, bites of insects,
15	*Allium stivum* L.	Rashun	Liliaceae	Annual	Herb	Cultivated	Bulb	Whole plant	Raw, Juice	Hypotensive, Hypoglycaemic, Heart disease, diabetes,
16	*Alocasiaindica* (Roxb.)Schott.	Mankuchu	Araceae	Perennial	Herb	Marsh place	Rhizome	Whole plant	Juice	Weakness, Ascites.

Table 2.3.2 Continued.

Sl No.	Scientific Name	Local name	Family	Life span	Habit	Habitat	Mode of propagation	usable parts of plant	Mode of administer action	Treatment(s) / Diseases/ Properties.
17	*Aloe barbadensis* Mill.	Ghritakumari, Musabbar	Liliaceae	Perennial	Herb	Cultivated	Sucker	Leaves	Juice	Weakness, Anthelmintic, Male sexual ailments, Peptic ulcer.
18	*Aloe indica* L.	Ghritakumari	Liliaceae	Perennial	Herb	Cultivated	Sucker	Leaves	Juice	Piles, Menstrual suppressions and burning during sexual ejaculation
19	*Alpinianiagra* (Gaertn.) Burtt	Jangli Ada, Tara	Zingiberaceae	Perennial	Herb	Marsh place	Rhizome	Rhizome	Juice	Impotency, Constipation, Energetic.
20	*Alstoniascholaris*R.Rr.	Chhatim, Chaitan	Apocynaceae	Perennial	Tree	Forest	Seed	Stem bark, Latex	Juice, Powder, Latex	Leucorrhea. Rheumatism, Cuts, Dysentery.
21	*Alternantherasessilis* (L.)R.Br	Haicha, Chanchi	Amaranthaceae	Annual	Herb	Open field	Seed	Whole plant	Juice	Fever, Cough, Weakness, Anhydrotic.
22	*Amaranthusspinosus* L.	Khuirakanta, Kantanotey	Amaranthaceae	Annual	Herb	Fallow land	Seed	Whole plant	Juice	Hallucination, Jaundice, Leucorrhoea, Sexual debility, Eczema.
23	*Amaranthusviridis* L.	Notey, Noteyshak	Amaranthaceae	Annual	Herb	Open field	Seed	Whole plant	Juice	Hallucination, leprosy, piles, Leucorrhea, snake-bites.
24	*Amorphophalluscampanulatus* (Roxb.)	Oal, Oalkachu	Araceae	Perennial	Herb	Shade	Rhizome	Whole plant	Juice	Weakness, Rheumatism, Weakness, Earache.
25	*Anacardiumoccidentale* L.	Kajubadam	Anacardiaceae	Perennial	Tree	Forest	Seed	Bark, Leaves, Fruit	Decoction	In the treatment of hookworm and ringworm, corns, leprosy and ulcers, Indigestion.
26	*Ananassativus*Schult.f.	Anaras	Bromeliaceae	Perennial	Herb	Cultivated	Sucker	Fruit, Leaves	Juice	Worm, Menstrual trouble, Abortion.
27	*Andrographispaniculata* (Burm.f.)Wall.	Kalomegh	Acanthaceae	Annual	Herb	Cultivated	Seed	Leaves	Juice	Fever, headache. The leaves crushed with water are administered in cases of fevers, and headache.
28	*Annonasquamosa* L.	Ata, Sharifa	Annonaceae	Perennial	Tree	Home yard	Seed	Leaves, Fruit, Seed	Juice, Ripe fruit	Worm, Urinary trouble, Dysentery, Weakness, Seeds are abortifacient.
29	*Anthocephaluschinensis* (Lamk.)A.Rich.	Kadamphulgachh	Rubiaceae	Perennial	Tree	Planted roadside	Seed	Bark, Leaves	Decoction, Juice	Anthelmintic and hypoglycaemic. Bark is used as astringent, tonic, and in the treatment of snakebite.
30	*Aphanamixispolystachya*(Wall.)R. Parker	Pitraj	Meliaceae	Perennial	Tree	Forest, roadside	Seed	Bark, Seed	Juice	Bark is astringent, and used in spleen and liver diseases, tumours and abdominal complaints. Seed oil is used as a liniment in rheumatism.

Table 2.3.2 Continued.

Sl No.	Scientific Name	Local name	Family	Life span	Habit	Habitat	Mode of propagation	usable parts of plant	Mode of administer action	Treatment(s) / Diseases/ Properties.
31	*Arachishypogaea* L.	Badam, Cheenabadam	Fabaceae	Annual	Herb	Cultivated	Seed	Seed	Paste, oil	Seed are nutritious, aperients and emollient. Seed and oil are astringent to the Bowels.
32	*Areca catechu* L.	Supari	Arecaceae	Perennial	Tree	Cultivated	Seed	Seed	Paste of nut	A nervine tonic. It is used as anthelmintic, menstrual discharge and aphrodisiac.
33	*Argemonemaxicana*L.	Shialkanta	Papaveraceae	Annual	Herb	Fallow land, Roadside	Seed	Whole plant	Juice, Latex	Eczema and itching, Jaundice, Weakness, Warts.
34	*Aristolochiaindica*L.	Isharmul	Aristolochiaceae	Perennial	Lianas	Roadside, thickets	Seed	Root	Paste, Powder	Snakebite, Leucorrhea, Sexual debility, Impotency, Stomachic.
35	*Artemisia nilagirica* (Clarke) Pamp	Nagdana	Asteraceae	Annual	Herb	Open place	Seed	Leaves	Paste	Stomach pain. The leaves are crushed in water and during stomach pain.
36	*Asparagus racemosus*Willd.	Shatamuli	Liliaceae	Perennial	Herb	Shaded place	Tuberous root	Leaves, Root	Powder, Juice	Urinary problems, Weakness, acidity, Seminal weakness.
37	*Averrhoacarambola* L.	Kamranga	Oxalidaceae	Perennial	Tree	Planted	Seed	Fruit	Juice	Jaundice, fevers
38	*Azadirachtaindica* A. Juss	Neem, Nimgachh	Meliaceae	Perennial	Tree	Planted	Seed	Leaves, Fruit	Juice for surface used	Abscess, Itching, Jaundice, Sore, Astringent.
39	*Bacopamonniera* (L.)Pennel.	Brahmilata, Brahmishak	Scrophulariaceae	Perennial	Herb	Wet place	Seed	Whole plant	Juice	Brain tonic, Dyspepsia, Constipation, Neuralgia.
40	*Bambusaarundinacea*Willd.	KantaBansh	Poaceae (Gramineae)	Perennial	Shrub	Cultivated	Sucker	Leaves, Stem	Decoction	Cough and cold, Stop bleeding, free discharge of menses or lochia after delivery.
41	*Barleriacristata* L.	Janti	Acanthaceae	Perennial	Shrub	Fallow land	Seed	Leaves, Roots	Juice	Sore, Itching, Abscess, Constipation.
42	*Basella alba* L.	Puishak	Basellaceae	Annual	Herb	Cultivated	Seed	Whole plant	Juice	Constipation, Sores, catarrh.
43	*Bixaorellana*L.	Latkan, Utkana	Bixaceae	Perennial	Shrub	Forest	Seed	Root, Seeds	Juice	Antispasmodic, hypotensive, astringent, diuretic, gonorrhoea.
44	*Blumealacera*(Burn.f.)DC.	Shialmuti	Asteraceae (Compositae)	Annual	Herb	Fallow land	Seed	Leaves, Roots	Juice	Cough, Stomach pain, Itching.
45	*Boerhaaviadiffusa* L.	Punarnava	Nyctaginaceae	Annual	Herb	Fallow land	Seed	Whole plant	Juice, powder	Fever, cough, weakness, Blood purifier, pulmonary tuberculosis, Urinary trouble, contraceptive, Jaundice.
46	*Bombaxceiba* L.	Shimul Tula	Bombacaceae	Perennial	Tree	Planted	Seed	Whole plant	Juice	Spermatorrhoea, Weakness, aphrodisiac, Impotence, Smallpox, Toothache and caries.

Table 2.3.2 Continued.

Sl No.	Scientific Name	Local name	Family	Life span	Habit	Habitat	Mode of propagation	usable parts of plant	Mode of administer action	Treatment(s) / Diseases/ Properties.
47	*Borassusflabellifer* L.	Tal gachh	Arecaceae (Palmae)	Perennial	Tree	Planted	Seed	Whole plant	Juice	Inflammatory action and dropsy, Syphilis, headache, earache.
48	*Bryophyllumpinnatum*Kurz.	Patharkuchi	Crassulaceae	Perennial	Herb	Fallow land	Leaves	Leaves	Juice	Blood dysentery, Diabetes, Kidney stones, Boils and bites of insects.
49	*Buteamonosperma* (L.) Taub.	Polash, Polashphulgachh	Fabaceae	Perennial	Tree	Forest	Seed	Leaves, Bark, Seeds	Juice, Powder	Stimulant, Worm, Tumor, Piles, pimples.
50	*Cajanuscajan* (L.)Huth.	Arhar, Tur, Arekkalai	Fabaceae	Perennial	Shrub	Cultivated	Seed	Leaves seeds	Juice	Diabetic, Jaundice, vomiting, coughs, Snakebites and to reduce swellings
51	*Calotropisgigantea* Br.	BoroAkanda	Asclepiadaceae	Perennial	Shrub	Road-side	Seed	Leaves, Bark, Root	Latex, paste	Rheumatism, Purgative, Abortion, leprosy.
52	*Calotropisprocera* R. Br.	ChhotoAkanda	Asclepiadaceae	Perennial	Shrub	Road-side	Seed	Leaves, Bark, Root	Latex, paste	Rheumatism, Loss of appetite, Dyspepsia, flatulence, asthma.
53	*Careyaarborea*Roxb.	Kumbi, Gade	Lecythidaceae	Perennial	Tree	Forest	Seed	Bark	Juice, paste	Burns, wounds, cuts, fractures, body pains.
54	*Carica papaya* L.	Penpey	Caricaceae	Perennial	Tree	Cultivated	Seed	Fruit, Latex	Latex, Juice	Dyspepsia, bleeding piles, enlarged spleen and liver.
55	*Carissa congesta*Wight.	Karamcha	Apocynaceae	Perennial	Shrub	Cultivated	Seed	Root	Paste	Diabetic ulcer, Hypotensive, cardiotonic, stomachic and anthelmintic.
56	*Cassia alata* L.	Dadmardan	Fabaceae	Perennial	Tree	Forest, planted	Seed	Leaves	Fresh juice, Paste	Ringworm, bronchitis, eczema, skin disease.
57	*Cassia fistula* L.	Sonalu, Bandar Lathi	Fabaceae	Perennial	Tree	Forest, Roadside	Seed	Fruits, Leaves	Juice, Pulp	Purgative, Abortion, Ringworm, Chest and heart disease.
58	*Cassia oblusifolia* L.	Chakunda, Arach	Fabaceae	Annual	Shrub	Follow land, Roadside	Seed	Leaves	Fresh Juice, Paste	Skin disease, Asthma, Leucorrhoea.
59	*Cassia sophera*L.	ChhotaKalkasunda	Fabaceae	Annual	Shrub	Wet places	Seed	Leaves, Roots	Juice	Diarrhea, Weakness, asthma, against ringworm
60	*Catharanthusroseus* (L.) G. Don.	Nayantara	Apocynaceae	Perennial	Shrub	Planted in garden	Seed	Whole plant	Juice	Blood purifier, Anticancer
61	*Centellaasiatica* (L.) Urban.	Thankuni, Tehagorerpata	Apiaceae (Umbelliferae)	Annual	Herb	Wet places	Stem	Leaves	Juice, Paste	Indigestion, stomach infection, skin disease,dysentery and convulsive disorders or antispasmodic.

Table 2.3.2 Continued.

Sl No.	Scientific Name	Local name	Family	Life span	Habit	Habitat	Mode of propagation	usable parts of plant	Mode of administer action	Treatment(s) / Diseases/ Properties.
62	*Cinnamomumtamala*Nees.	Tejpata	Lauraceae	Perennial	Tree	Planted	Cutting	Leaves	Decoction	Colic, diarrhoea, skin diseases, coughs and colds.
63	*Cissusquadrangularis*L.	Harjora, Harbhangalata	Vitaceae	Perennial	Climber	Home yard	Stem cutting	Stem	Juice, Paste	Bone fractures, Gleet bruises.
64	*Citrulluscolocynthesis*(L.) Sch.	Makal	Cucurbitaceae	Annual	Climber	Jungles	Seed	Fruit, Root	Juice, Paste	Drastic purgative. Ascites, jaundice, uterine problems, amenorrhoea.
65	*Citrus aurantifolia* (Chist.) Sw	Lebu, PatiLebu	Rutaceae	Perennial	Shrub	Cultivated	Cutting	Fruit, Leaves	Juice	Dyspepsia, flatulence, nausea and irritations of skin, Scurvy.
66	*Citrus grandis* (L.) Osbeck.	BatabiLebu, Jambura	Rutaceae	Perennial	Tree	Planted	Seed	Fruit, Leaves	Juice	Influenza, catarrh, convulsive coughs, headache and stomach pain.
67	*Cleome viscose* L.	Hurhuria	Capparaceae	Annual	Herb	Village thicket	Seeds	Leaves	Juice	Fever, Cough, Dyspepsia, Ringworm.
68	*Clerodendrumviscosum* Vent.	Vital, Ghetu, Bhant	Verbenaceae	Perennial	Shrub	Fallow land, Roadside	Seed	Leaves, Root	Fresh juice, Paste	Strong anthelmintic, hypotensive. Dysentery, malaria, jaundice, tumour, skin disease, snakebite,
69	*Clitoriaternatea* L.	Aparajita, Nila	Fabaceae	Annual	Climber	Cultivated in gardens	Seed	Whole plant	Juice	Contraceptive. Colic, gonorrhoea, Menstrual troubles, skin diseases.
70	*Cocciniacordifolia* (L.) Cogn.	Telakucha	Cucurbitaceae	Annual	Climber	Forest bushes	Seed	Leaves, Root	Juice	Mild diabetes, Stomach upset, anorexia, ear pain.
71	*Cocosnucifera* L.	Narikel, Daab	Arecaceae	Perennial	Tree	Planted	Seed	Fruit	Coconut milk, water, oil	Cooling, nutrient, aperients. Commonly used in cholera, diarrhoea, weakness, alopecia.
72	*Commelinabenghalensis* L.	Dholpata, Kanchira	Commelinaceae	Annual	Herb	Wet places	Stem	Whole plant	Juice	Useful in leprosy, sores, itches, swellings, burns, boils, leucorrhoea, urinary burning, gonorrhoea, cold and ulcer.
73	*Corchorusfascicularis* Lam.	Bon Pat	Tiliaceae	Annual	Herb	Jungle	Seed	whole plant	Juice	Ulcer, Restorative.
74	*Coriandrumsativum* L.	Dhania, Dhoney	Apiaceae	Annual	Herb	Cultivated	Seed	Whole plant	Paste	Flatulence, foul breath.
75	*Costusspeciosus* (Koenig) Sm.	Keu, Tara, Kura	Zingiberaceae	Annual	Herb	Forest, thicket	Rhizome, Seed	Seed Rhizome	Powder, Juice	Inflammation of eye, Catarrh, coughs, dyspepsia, worms, Contraceptive.
76	*Crotalaria spectabilis*Roth.	Jhanjhania	Fabaceae	Annual	Herb	Open fields	Seed	Leaves, Seed	Fresh juice, Paste	Skin disease, Weakness, Constipation

Table 2.3.2 Continued.

Sl No.	Scientific Name	Local name	Family	Life span	Habit	Habitat	Mode of propagation	usable parts of plant	Mode of administer action	Treatment(s) / Diseases/ Properties.
77	*Croton bonplandianum*Baill.	Bon tulshi	Euphorbiaceae	Perennial	Herb	Road side, Fallow land	Seed	Leaves, Seed	Paste, Juice	Cut, Worm.
78	*Cryptolepisbuchanani*Roem.&Schult.	Chhagalbati	Asclepiadaceae	Perennial	Shrub	Road side	Seed	Whole plant	Juice	Sores, dropsy, bodyache.
79	*Curculigoorchioides*Gaertn.	Tal muli, Tali	Amaryllidaceae	Perennial	Herb	Fallow land	Rhizome	Root	Juice	Weakness, Useful in piles, wounds, Asthma, Jaundice, Gonorrhea, Menorrhagia.
80	*Curcuma amada*Roxb.	Am ada	Zingiberaceae	Perennial	Herb	Road side, Fallow land	Rhizome	Rhizome	Paste, Juice	Piles, Gonorrhoea, Constipation.
81	*Curcuma aromatic* Salisb.	JangliHaldi	Zingiberaceae	Perennial	Herb	Fallow land	Rhizome	Rhizome	Paste, Juice	Blood purifier, Tonic and Carminative. Impotency, Constipation, Energetic.
82	*Curcuma longa* L.	Halud, Haldi	Zingiberaceae	Perennial	Herb	Homestead	Rhizome	Rhizome	Paste, Juice	Blood purifier. Used in indigestion, gonorrhoea, skin diseases.
83	*Curcuma zedoaria*Rosc.	Shoti	Zingiberaceae	Perennial	Herb	Fallow land	Rhizome	Rhizome	Juice, Powder	Blood purifier, Stimulant, Weakness, Cough, Dropsy, Sores, stomach pain.
84	*Cuscutareflexa*Roxb.	Swarnalata	Cuscutaceae	Annual	Herb	Parasites on other plants	Golden stem	Whole plant	Juice	Jaundice. The plant is also used as anthelmintic.
85	*Cynodondactylon* Pers.	Durbaghas	Poaceae	Annual	Herb	Open field, Road side	Stem	Whole plant	Fresh juice	Piles, menorrhagia, cuts and wounds, to stop bleeding.
86	*Cyperusrotundus*L.	Muthaghas	Cyperaceae	Annual	Herb	Dense humid places	Seed, Tuberous root	Tubers	Juice, Surface used	Antipyretic, astringent, carminative, tonic, fever, diarrhea, dysentery and cholera.
87	*Daturametel*L.	Dhutura, KaloDhutra	Solanaceae	Perennial	Herb	Road side, Open places	Seed	Leaves, Seeds	Juice, Powder	Asthma, Lumbago, Neuralgia
88	*Daturastramonium* L.	SadaDhutra	Solanaceae	Perennial	Herb	Road side, Open places	Seed	Leaves, Seeds	Juice, Powder	Asthma, whooping cough, muscle spasm.
89	*Desmodiumgangeticum* (L.) DC.	Salpani	Fabaceae	Perennial	Herb	Fallow land, Road side	Seed	Leaves, Roots	Juice, Paste	Brain tonic. Weakness, Worm, skin disease.
90	*Desmodiumpulchellum*Benth.	JutaSalpani	Fabaceae	Perennial	Shrub	Village thicket	Seed	Leaves, Roots	Juice	Sexual in tension, Fever, Eye infection.
91	*Dilleniaindica*L.	Chalta	Dilleniaceae	Perennial	Tree	Planted	Seed	Fruit, Leaves	Juice	Abdominal pain, cooling beverage in fevers and as an expectorant in cough mixture. Leaves juice is astringent.
92	*Dioscoreaalata* L.	Mete Alu, ChupriAlu	Dioscoreaceae	Perennial	Climber	Forest thickets	Bulbil	Leaves, Bulbils	Powder, Paste	Diuretic, Fever, Worm, rash and itch, piles.

Table 2.3.2 Continued.

Sl No.	Scientific Name	Local name	Family	Life span	Habit	Habitat	Mode of propagation	usable parts of plant	Mode of administer action	Treatment(s) / Diseases/ Properties.
93	*Dioscoreabulbifera* L.	Banalu, Pesta-Alu	Dioscoreaceae	Perennial	Climber	Forest thickets	Bulbil	Leaves, Bulbils	Paste	Diuretic, Syphilis, Piles, diarrhoea, dysentery, stomachic, Weakness.
94	*Drynariaquercifolia* (L.)J.Sm.	Pankhiraj, Pokharaj	Drynariaceae	Perennial	Epiphyte	On tree trunk and shady old walls	Rhizome	whole plant	Paste	Anthelmintic, tonic, used in the treatment of phthisis, hectic fever, dyspepsia, skin diseases and cough
95	*Ecliptaprostrata* (L.) Roxb.	Keshraj, Kalokeshi	Asteraceae	Annual	Herb	Fallow land	Seed	Whole plant	Juice, Paste	Hair tonic, Jaundice.
96	*Elaeocarpusserratus*L.	Jolpai	Elaeocarpaceae	Perennial	Tree	Planted	Seed	Leaves, Fruit	Paste, Juice	Rheumatism, diarrhoea and dysentery.
97	*Erythrinavariegata* L.	Mandar	Fabaceae	Perennial	Tree	Homestead Areas	Cutting	Seeds	Paste	Piles. The crushed seeds are given in piles. As the seeds are poisonous, one to one and a half seeds are given only.
98	*Eupatorium odoratum* L.	Fulkuri	Asteraceae	Annual	Herb	Fallow land	Seed	Leaves	Juice, Paste	Wounds. The juice of young leaves is applied to fresh wounds and cuts to stop bleeding and as an antiseptic.
99	*Euphorbia hirta* L.	Dhudia,	Euphorbiaceae	Annual	Herb	Fallow land	Seed	Whole plant	Latex, Juice	Spermatorrhoea.
100	*Euphorbia thymifolia*Brum.f.	SwetKeru	Euphorbiaceae	Annual	Herb	Dry places	Seed	Leaves, Roots	Juice	Diabetes, Dysentery.
101	*Euphorbia tirucalli* L.	Lanka Sij, Dud kusi	Euphorbiaceae	Perennial	Shrub	Planted in gardens	Stem	Stem	Juice, Latex	Diarrhoea, Ring worm, toothache, rheumatism, asthma.
102	*Evolvulusnummularus* L.	Bhui okra	Convolvulaceae	Annual	Herb	Open places	Seed	Whole plant	Juice	Weakness, Edema. The herb is tied to the legs to reduce edema of legs during pregnancy.
103	*Feronialimonia* (L.)Sw	Kadbel	Rutaceae	Perennial	Tree	Planted	Seed	Fruit, Leaves	Fruit Pulp, Juice	Astringent, stomachic, digestive stimulant, diuretic, tonic, dysentery and heart diseases.
104	*Ficusbenghalensis*L.	Bot	Moraceae	Perennial	Tree	Forest	Seed	Latex	Latex	Aphrodisiac. The latex is applied on the navels or the stem eaten raw as an aphrodisiac.
105	*Ficusracemosa*L.	Jagadumur	Moraceae	Perennial	Tree	Road side	Seed	Fruit, Leaves	Juice, Pate	Heart disease, Energetic, Urinary troubles and Diabetes.
106	*Ficusrumphii*Blume.	Pakur	Moraceae	Perennial	Tree	Forest	Seed	Latex	Latex	Debility. The latex along with molasses is used in cases of debility.

Table 2.3.2 Continued.

Sl No.	Scientific Name	Local name	Family	Life span	Habit	Habitat	Mode of propagation	usable parts of plant	Mode of administer action	Treatment(s) / Diseases/ Properties.
107	*Foeniculumvulgare* Mill.	Mouri, Pan Muhuri	Apiaceae	Perennial	Herb	Cultivated	Seed	Fruit, Seeds	Powder, Paste	Useful in chest, spleen and kidney troubles. Antispasmodic.
108	*Garugapinnata*Roxb.	Laljigachal	Burseraceae	Perennial	Tree	Forest	Seed	Leaves Bark	Juice	Stomachic. Head bark is used as a stomachic.
109	*Gloriosasuperba* L.	UlatChandal	Liliaceae	Perennial	Climber	Planted	Seed, Tuberous root	Tuber	Juice, Powder	To treat intestinal worms, bruises, infertility, skin problems, gout, piles, gonorrhoea and impotence.
110	*Gmelinaarborea* L.	Gamari	Verbenaceae	Perennial	Tree	Forest	Seed	Leaves	Juice	Jaundice
111	*Gnaphaliumluteo-album* L.	BoroKamra Natham	Asteraceae	Annual	Herb	Fallow lands	Seed	Whole plant	Paste	Bone fractures, Gout.
112	*Gomphrenaglobosa* L.	Butamphul, Golkamal	Amaranthaceae	Annual	Herb	Cultivated in gardens	Stem	Leaves,	Juice, Paste	Decoction of leaves is used in coughs, diabetes and hypertension.
113	*Gynandropisgynandra*(L.) Biq.	SadaHurhure	Capparaceae	Annual	Herb	Edge of pond	Seed	Leaves	Juice	Fever, Cough, Dyspepsia.
114	*Helianthus annuus* L.	Surjamukhi	Asteraceae	Annual	Herb	Cultivated	Seed	Seeds	Paste	Skin disease, cold, coughs and pulmonary affections.
115	*Heliotropiumindicum* L.	Hatisur	Boraginaceae	Annual	Herb	Waste places, ditches	Seed	Whole plant	Juice	Bone heals, Ulcer, Itching and Eczema.
116	*Hemidesmusindicus* (L.) R.Br	Anantamul	Asclepiadaceae	Perennial	Climber	Planted and forest	Tuberous Root	Roots	Juice, Paste	Rheumatism, joint pain, abdominal tumours, tonic, restorative, demulcent and blood purifier.
117	*Hibiscus rosa-sinensis* L.	Jaba	Malvaceae	Perennial	Shrub	Garden	Stem	Leaves, Flower	Paste	Stomach upsets and dysentery. The leaves are administered in stomach upsets and dysentery.
118	*Holarrhenaantidysenteric a*Heyne ex. Roth	Kurchi, Kurusuri	Apocynaceae	Perennial	Tree	Forest	Seed	Leaves, Bark	Juice	Dysentery, Hepatitis, Anthelmintic.
119	*Hyptissuaveolens* (L.)Poit	Tokma	Lamiaceae (Labiatae)	Annual	Herb	Forest thickets	Seed	Seeds, Leaves	Soaked seed	Constipation, Colic, stomachache. Leaf juice is antispasmodic and anti-rheumatic, gonorrhea.
120	*Ipomoea mauritiana*Jacq	Bhuikumra	Convolvulaceae	Perennial	Climber	Hedges dwellings	Tuberous root	Rhizome	Juice	Sexual disabilities. Hypotensive and muscle relaxant, Paralysis, Diabetes, Rheumatism.
121	*Ipomoea quamoclit* L.	Kunjalata, Tarulata	Convolvulaceae	Perennial	Climber	Home yard	Seed	Leaves	Paste	Ulcer and breast pain. Pounded leaves are applied to carbuncles and bleeding piles.

Table 2.3.2 Continued.

Sl No.	Scientific Name	Local name	Family	Life span	Habit	Habitat	Mode of propagation	usable parts of plant	Mode of administer action	Treatment(s) / Diseases/ Properties.
122	*Ixoracoccinea* L.	Rangan	Rubiaceae	Perennial	Shrub	Flower garden	Seed	Leaves, Flower, Roots	Juice, Paste	Diarrhoea, Dysentery, Leucorrhoea, stomachic, Muscle spasm.
123	*Jatrophacurcas* L.	Jamal gota	Euphorbiaceae	Perennial	Shrub	Road side	Seed, Stem	Seeds, Leaves	Juice	Purgative, Diabetes, Itching, Puerperal.
124	*Jatrophagossypifolia*L.	Lal Jamal gota	Euphorbiaceae	Perennial	Shrub	Road side, Fallow land	Seed, Cutting	Leaves, Seeds	Juice, Paste	Abscess, Rheumatism, Ringworm.
125	*Justiciagendarussa*Burm.	Jagatmadan	Acanthaceae	Perennial	Shrub	Road side, Home yard	Seeds, stem	Leaves	Juice, Paste	Sexual disorder. Bone disease, Itching Abscess, wounds.
126	*Lanneacoromandelica* (Houtt.) Merr.	Jiga	Anacardiaceae	Perennial	Tree	Road side, Home yard	Seed, stem	Bark	Juice, Powder	Seminal problem. The bark is administered in case of seminal weakness and excessive seminal emissions.
127	*Lawsoniainermis* L.	Mehedi, Mendi	Lythraceae	Perennial	Shrub	Homestead	Seed	Leaves	Paste	Skin disease, Sore, dandruff, burning of the feet and headache.
128	*Leucuslavandulaefolia* Rees.	Dondokolos, Domkolos	Lamiaceae	Annual	Herb	Dwelling, Open places	Seed	Leaves	Juice	Impotency, Ophthalmia
129	*Litseaglutinosa* (Lour.) Rob.	Kharajora,	Lauraceae	Perennial	Tree	Forest	Seed	Leaves, Bark	Decoction	Anti-pyretic, stomachic, seminal weakness.
130	*Mangiferaindica*L.	Am	Anacardiaceae	Perennial	Tree	Planted	Seed	Bark	Juice	Dysentery, rheumatism.
131	*Menthaarvensis* L.	Pudina	Lamiaceae	Annual	Herb	Planted	Seed	Whole plant	Juice	Refrigerant, Stomachic, carminative, stimulant and diuretic. Stopped vomiting.
132	*Mikaniacordata* (Brum.f.) Rob.	Assam Lata	Asteraceae	Annual	Climber	Forest thickets	Seed	Leaves	Juice, Paste	Fever, Cough, Dyspepsia, Dysentery, Itches. Fresh leaves are used to stop and cure haemorrhages from cuts and wounds.
133	*Mimosa pudica* L.	Lajjabati	Fabaceae	Annual	Herb	Fallow land	Seed	Roots	Juice	Menstrual regulation, Dysentery, Piles, Contraceptive.
134	*Mimusopselengi*L.	Bakul	Sapotaceae	Perennial	Tree	Planted	Seed	Bark	Juice	General tonic, Fevers, Bleeding gums, Loose teeth, Swellings of the mouth and tongue.
135	*Mirabilis jalapa*L.	Sandhamaloti	Nyctaginaceae	Annual	Herb	Home yard	Seed	Leaves	Juice	Urinary troubles.
136	*Momordicacharantia* L.	Usta, Karala	Cucurbitaceae	Annual	Climber	Cultivated	Seed	Fruits, Leaves	Juice	Diabetes mellitus, Chicken pox, gout, Rheumatism, diseases of the spleen, Jaundice. Tonic, Stomachic, anthelmintic.

Table 2.3.2 Continued.

Sl No.	Scientific Name	Local name	Family	Life span	Habit	Habitat	Mode of propagation	usable parts of plant	Mode of administer action	Treatment(s) / Diseases/ Properties.
137	*Moringaoleifera*Lamk.	Sajna	Moringaceae	Perennial	Tree	Planted	stem	Leaves, Fruits	Juice	Nasal catarrh, Stomachic, Weakness, Constipation.
138	*Mucunapruriens* Baker.	Alkushi, Bilai hung hungi	Fabaceae	Annual	Climber	Forest	Seed	Seeds	Juice, Powder	Anthelmintic. Seeds are aphrodisiac and nervine tonic.
139	*Musa paradisiaca* L.	Kanch Kola	Musaceae	Perennial	Herb	Cultivated	Sucker	Fruits	Paste	Diarrhoea, Constipation, Peptic ulcer, hypertension, Heart disease.
140	*Nyctanthes arbor-tristis* L.	Shiuli, Shefaliphul	Oleaceae	Perennial	Tree	Home yard	Seed	Leaves	Juice	Fever, Cold, Cough, Cuts.
141	*Ocimumamericanum*L.	KaloTulshi	Lamiaceae	Perennial	Herb	Home yard	Seed	Leaves	Juice	Fever, Cold, Cough.
142	*Ocimumbasilicum* L.	BabuiTulshi	Lamiaceae	Perennial	Herb	Home yard	Seed	Leaves	Juice	Cold, Cough, Fever.
143	*Ocimumgratissimum*L.	Ram Tulshi	Lamiaceae	Perennial	Herb	Home yard	Seed	Leaves	Juice	Cold, Cough, Fever.
144	*Ocimum sanctum* L.	Tulshi	Lamiaceae	Perennial	Herb	Home yard	Seed	Leaves	Juice	Cold, Cough, Bronchitis, Catarrh, Fever.
145	*Oroxylumindicum*(L.)Vent.	Kanak, Sonpatti	Bignoniaceae	Perennial	Tree	Forest	Seed	Fruits	Juice	Jaundice, swelling. The skin of fruit after soaking in water is administered in jaundice and also applied for swellings.
146	*Oxalis corniculata* L.	Amrul	Oxalidaceae	Annual	Herb	Wet land	Seed	Whole plant	Juice	Indigestion, Kidney problem, Dyspepsia, Fever.
147	*Paederiafoetida* L.	Gondhabaduli	Rubiaceae	Perennial	Climber	Cultivated	Seed	Leaves	Juice	Diarrhoea, Piles, Dysentery.
148	*Phyllanthusemblica* L.	Amluki	Euphorbiaceae	Perennial	Tree	Forest, Home yard	Seed	Fruits	Juice	Sore, Weakness, Cough, Scurvy, Cardiac and stomach debility.
149	*Phyllanthusniruri* L.	Bhuiamla	Euphorbiaceae	Perennial	Herb	Open places	Seed	Whole plant	Juice, paste	Urinary trouble, Weakness, Cough, Fever, Hepatitis.
150	*Physalis minima* L.	Phutkas, BonTipariya	Solanaceae	Annual	Herb	Open places	Seed	Fruit and Leaves	Juice	Flatulence, stomachic. The juice of the crushed herb is given in flatulence. The root of the plant is used as stomachic.
151	*Piper betel* L.	Paan	Piperaceae	Perennial	Climber	Cultivated	stem cutting	Leaves	Juice,	Cough, Headache. Leaves are popularly used as carminative, astringent, stimulant and antiseptic drugs.
152	*Piper longum* L.	Pipul	Piperaceae	Perennial	Herb	Wet land	Stem cutting	Whole plant	Juice	Cough. Tuberculosis. The whole herb is used as a cure for tuberculosis.

Table 2.3.2 Continued.

Sl No.	Scientific Name	Local name	Family	Life span	Habit	Habitat	Mode of propagation	usable parts of plant	Mode of administer action	Treatment(s) / Diseases/ Properties.
153	*Plumeriarubra*L.	Kat Golap	Apocynaceae	Perennial	Tree	Homestead	Seed	Leaves, Bark	Paste, Juice	Swellings, Pains, Dysentery, Urinary troubles.
154	*Psidiumguajaba* (L.) Bat.	Peyara	Myrtaceae	Perennial	Tree	Cultivated	Seed	Leaves	Decoction	Bowels, wounds, ulcer, Scurvy.
155	*Psittacanthuscalyculatus*(DC.) G. Don.	Hinikgota	Loranthaceae	Perennial	Epiphyte	Epiphyte	Seed	Leaves	Juice, Paste	Hypertension, Skin diseases.
156	*Punicagranatum* L.	Dalim	Punicaceae	Perennial	Shrub	Cultivated	Seed	Bark	Juice	Dysentery. The bark is given in cases of dysentery.
157	*Quisqualisindica* L. (*Combretumindicum*(L.) DeFilipps)	Madhubilata, Madhumalati	Combretaceae	Perennial	Lianas	Planted as an ornamental plant	Seed	Leaves, Seeds	Paste	Boils and ulcers, anthelmintic, to relive flatulence, useful for round worms, soporific.
158	*Rauvolfiaserpentina* (L.) Benth.	Sarpagondha	Apocynaceae	Perennial	Shrub	Forest	Seed	Root	Juice	High blood pressure.
159	*Rauvolfia-tetraphylla* L.	Sarpagondha	Apocynaceae	Perennial	Shrub	Homestead	Seed	Leaves, Root	Juice, Powder	Blood pressure.
160	*Ricinuscommunis*L.	Bherenda, Reri	Euphorbiaceae	Perennial	Shrub	Road side	Seed	Seed, Leaves	Oil, Paste	Purgative. Muscular rheumatism, Joint pains, paralysis, asthma, galactagogue and headache.
161	*Rumexmaritimus* L.	Bon Palong	Polygonaceae	Annual	Herb	Marshy places	Seed	Leaves	Juice	Ulcer, Restorative, Constipation.
162	*Saracaindica* L.	Ashok	Fabaceae	Perennial	Tree	Forest	Seed	Bark, Leaves	Juice, Powder	Menstrual pains, Dysentery.
163	*Scopariadulcis* L.	Chinigura	Scrophulariaceae	Annual	Herb	Fallow land	Seed	Leaves	Juice	Diabetes.
164	*ShorearobustaGaertn.*	Gojari, sal	Dipterocarpaceae	Perennial	Tree	Forest	Seed	Bark	Juice, Paste	Dysentery, weak digestion, gonorrhea, lumbago, diarrhoea. Bark and the resin is astringent, stimulant and aphrodisiac.
165	*Sidaacuta*Burm.	Bon-methi, Kureta	Malvaceae	Annual	Herb	Fallow land, Road side	Seed	Leaves, Root	Juice	Urinary troubles.
166	*Sidacordifolia*L.	Bala	Malvaceae	Annual	Herb	Fallow land, Road side	Seed	Leaves, Root	Juice	Menstrual troubles.
167	*Smilax zeylanica* L.	Kumarilata, Bulkumia	Liliaceae	perennial	Climber	Forest	Seed	Leaves, Young	Juice	Sexual debility, Impotency, Phthisis, Urinary troubles.

Table 2.3.2 Continued.

Sl No.	Scientific Name	Local name	Family	Life span	Habit	Habitat	Mode of propagation	usable parts of plant	Mode of administer action	Treatment(s) / Diseases/ Properties.
168	*Solanumindicum* L.	Phutka Begun	Solanaceae	Annual	Herb	Road side, Fallow land	Seed	Fruits	Juice	Urinary troubles, stomach upset.
169	*Solanumnigrum* L.	Tit Begun, Kakmachi	Solanaceae	Perennial	Herb	Road side, Fallow land	Seed	Whole plant	Juice	Abdominal colic, Urinary troubles.
170	*Spilanthespaniculata* Wall	Marhatitiga	Asteraceae	Perennial	Herb	Fallow land	Seed	Whole plant	Paste	Poisonous sting, Toothache.
171	*Stephania japonica* (Thunb.) Miers.	Akanadi, Nimuka	Menispermaceae	Perennial	Climber	Thicket	Seed	Leaves, Roots	Juice	Leaves and roots are bitter and astringent and used in fevers, diarrhoea, dyspepsia and urinary diseases.
172	*Streblusasper*Lour.	Sheora	Moraceae	Perennial	Tree	Forest	Seed	Bark, Leaves, Latex	Paste, Latex	Fever, Diarrhoea, Dysentery, Neuralgia.
173	*Syzygiumcumini* (L.)Skeel	Jam, Kalojam	Myrtaceae	Perennial	Tree	Homestead	Seed	Seeds	Paste, Powder	Diabetes, Diarrhoea, Dysentery.
174	*Syzygiumjambos*(L.) Alston.	Golap Jam	Myrtaceae	Perennial	Tree	Planted	Seed	Leaves, Bark	Paste, Juice	Astringent. Used in Sore-eyes and liver complaints.
175	*Tamarindusindica* L.er.	Tetul	Fabaceae	Perennial	Tree	Road side, Forest	Seed	Leaves, Fruits, Seeds	Juice, Decoction, Powder	Urinary trouble, Cold, Fever.
176	*Tectonagrandis*Linn.f	Segun	Verbenaceae	Perennial	Tree	Forest, Planted	Seed	Bark, Seeds	Paste, Oil	Astringent, diuretic and promotes growth of hairs. Used in Skin diseases, eczema and ringworm.
177	*Terminaliaarjuna* (Roxb.) W. & A	Arjun	Combretaceae	Perennial	Tree	Forest	Seed	Bark	Juice, Powder	Heart diseases, Blood pressure.
178	*Terminaliabelerica*Roxb.	Bohera, Boira	Combretaceae	Perennial	Tree	Forest	Seed	Fruits	Juice, Powder	Weakness, Cough, Fever, Dyspepsia.
179	*Terminaliachebula* (Gaertn.) Retz	Hartaki, Haritaki	Combretaceae	Perennial	Tree	Forest	Seed	Fruits	Juice, Powder	Indigestion, Dyspepsia, Weakness.
180	*Tinosporacordifolia* (Willd.) Miers	Guloncha	Menispermaceae	Perennial	Climber	Forest, Cultivated	Seed	Leaves, Stem	Juice, Powder	Jaundice, Stomach upset, Cardiac problems, Fever, Cough, Worm.
181	*Tragiainvolucrata* L.	Bichhatu, Bichuti	Euphorbiaceae	Perennial	Herb	Grave yard	Seed	Leaves	Juice	Sore, Ring worm, toxic prevention.
182	*Tridaxprocumbens*L.	Tridhara	Asteraceae	Perennial	Herb	Open field	Seed	Leaves	Juice	Wounds, Cuts, Abscess, Jaundice, Sore.
183	*Typhoniumtrilobatum* (L.) Schott.	Kharkon, Ghetkochu	Araceae	Perennial	Herb	Damp and moist places	Tuberous root	Tuber and Roots	Juice, Paste	Piles, Jaundice, Tumours, Stomach complaints,

Table 2.3.2 Continued.

Sl No.	Scientific Name	Local name	Family	Life span	Habit	Habitat	Mode of propagation	usable parts of plant	Mode of administer action	Treatment(s) / Diseases/ Properties.
184	*Urarialagopodioides*DC.	LataChakuley	Fabaceae	Perennial	Shrub	Fallow land	Seed	Leaves, Stem	Juice	Malaria, Abortion.
185	*Urenalobata* L.	Chhilangota, Bon okra	Malvaceae	Perennial	Herb	Open field	Seed	Whole plant	Juice	Rheumatism, Severe colic, Weakness, Cough, Fever.
186	*Vanda roxburghii* R.Br	Rasna	Orchidaceae	Perennial	Epiphyte	Epiphyte	Seed	Leaves	Juice	Kidney stone, Constipation.
187	*Vitexnegundo* L.	Nishinda	Verbenaceae	Perennial	Shrub	Roadside, Home yard	Seed, Stem	Leaves	Paste	Rheumatism, Worm. The leaves of the plant are used in rheumatism.
188	*Wedeliacalendulacea* Less.	Kesharaj, Kalokur	Asteraceae	Annual	Herb	Thickets	Seed	Leaves	Juice	Hair tonic. Vomiting.
189	*Withniasomnifera*Dunal.	Arsogondha	Solanaceae	Perennial	Shrub	Wet places	Seed	Whole plant	Juice	Seminal weakness, Impotency, Phthises.
190	*Xanthium indicum*Koenig.	Ghagra	Asteraceae	Perennial	Herb	Fallow lands	Seed	Whole plant	Decoction	Piles, Urinary and renal complaints.
191	*Zingiberofficinale*Rosc.	Ada	Zingiberaceae	Perennial	Herb	Cultivated	Stem	Rhizome	Juice, Powder	Abdominal colic, Malarial fever, Jaundice.
192	*Zizyphusmauritiana*Lamk	Boroi, Kul	Rhamnaceae	Perennial	Tree	Road-sides	Seed	Leaves, Bark	Juice, Powder	Boils, Abscess, Carbuncles, Diarrhoea, Wounds and ulcer.

Results presented in the **Table 2.3.2** following in depth field survey, showed that medicinal plants used by the herbal doctors over the Madhupur Tract belongs to a very diverse plant community with diverse habit and habitat. It has been presented a proved and bonafide document of biological diversity of medicinal plants exited in the Madhupur Tract.

2.3.2.1. *Identification of medicinal plant species with their families.*

In nomenclatural view point medicinal plants, being used for traditional medicine in the regions varied widely among the angiospermic flowering plants group. Plants were not evenly distributed to the families but their numbers varied greatly in different families. The numbers medicinal plants belonging to different familiesunder the present study were analyzed and results are presented in **Table 2.3.3**.

Table 2.3.3. The number of medicinal plant species belonging to different families studied in the Madhupur Tract.

Sl No.	Family	No. of plant(s)	Sl No.	Family	No. of plant(s)	Sl No.	Family	No. of plant(s)
1	Fabaceae	21	24	Rubiaceae	3	47	Crassulaceae	1
2	Asteraceae	12	25	Capparaceae	2	48	Cuscutaceae	1
3	Euphorbiaceae	11	26	Dioscoreaceae	2	49	Cyperaceae	1
4	Apocynaceae	8	27	Lauraceae	2	50	Dilleniaceae	1
5	Lamiaceae	7	28	Meliaceae	2	51	Dipterocarpaceae	1
6	Liliaceae	7	29	Menispermaceae	2	52	Drynariaceae	1
7	Zingiberaceae	7	30	Nyctaginaceae	2	53	Elaeocarpaceae	1
8	Malvaceae	6	31	Oxalidaceae	2	54	Lecythidaceae	1
9	Solanaceae	6	32	Piperaceae	2	55	Loranthaceae	1
10	Amaranthaceae	5	33	Poaceae	2	56	Lythraceae	1
11	Acanthaceae	4	34	Scrophulariaceae	2	57	Moringaceae	1
12	Asclepiadaceae	4	35	Amaryllidaceae	1	58	Musaceae	1
13	Combretaceae	4	36	Annonaceae	1	59	Oleaceae	1
14	Moraceae	4	37	Aristolochiaceae	1	60	Orchidaceae	1
15	Rutaceae	4	38	Basellaceae	1	61	Papaveraceae	1
16	Verbenaceae	4	39	Bignoniaceae	1	62	Polygonaceae	1
17	Anacardiaceae	3	40	Bixaceae	1	63	Punicaceae	1
18	Apiaceae	3	41	Bombacaceae	1	64	Rhamnaceae	1
19	Araceae	3	42	Boraginaceae	1	65	Sapotaceae	1
20	Arecaceae	3	43	Bromeliaceae	1	66	Sterculiaceae	1
21	Convolvulaceae	3	44	Burseraceae	1	67	Tiliaceae	1
22	Cucurbitaceae	3	45	Caricaceae	1	68	Vitaceae	1
23	Myrtaceae	3	46	Commelinaceae	1	-	-	-

In the present investigation, 192 plant species were studied and classified under 68 families (**Table 2.3.3**) as identified with medicinal use by questionnaire survey. Among these Fabaceae, Asteraceae, Euphorbiaceae, Apocynaceae, Lamiaceae, Liliaceae, Zingiberaceae, Malvaceae, Solanaceae and Amaranthaceae were identified as major families predominantly composing of these medicinal plants in the Madhupur Tract. Highest number (21) of medicinal plants found to belong to the family Fabaceae. Asteraceae included 12 medicinal plant species, Euphorbiaceae

included 11 medicinal plantsspecies, Apocynaceae included 8 numbers of medicinal plantsspecies, Lamiaceae, Liliaceae and Zingiberaceae, each included 7 medicinal plantsspecies, Malvaceae and Solanaceae, each included 6 medicinal plantsspecies, Amaranthaceae included 5 numbers of medicinal plantsspecies; Acanthaceae, Asclepiadaceae, Combretaceae, Moraceae, Rutaceae and Verbenaceae each included 4 numbers of medicinal plantsspecies and each of other families bearing only a few of the remaining plant species.

2.3.2.2. *Screening of important medicinal plant species in the Madhupur Tract.*

In the present investigation survey was made in 146 respondents over the Tract and under this survey 192 plant species were identified as medicinal plants either growing wildly in the forest or around the homestead, roadsides, graveyards and other fallow lands in the area. Availability of medicinal plant species collected on respondent survey regarding local name, monocot/dicot, availability of medicinal plant species at respondents, wild source/planted/cultivated and their position were analyzed and the results are presented in **Table 2.3.4.**

The result revealed that all the plant species did not hold the same merit of importance in treatment of different ailments by as evidenced by the respondents in the Madhupur Tract. Among the 192 medicinal plants 62 were identified as important medicinal plants based on respondents performance in the Madhupur Tract.Here, the plants which are used by the 50 percent of the respondents medicinally we consider those plant species as important medicinal plant.

Table 2.3.4. Screening of important medicinal plant species based on respondent performance in the Madhupur Tract.

Sl No.	Scientific Name	Local name	Mono/ Dicot	Number of respondent/s	Ranking	Cultivated/ Planted/Wild
1	*Ocimum sanctum* L.	Tulshi	Dicot	124	1	P
2	*Adhatoda vasica* L.	Basok	Dicot	118	2	C
3	*Mucuna pruriens* Baker.	Alkushi,	Dicot	115	3	W
4	*Aloe barbadensis* Mill.	Ghritakumari, Musabbar	Monocots	114	4	C
5	*Aloe indica* L.	Ghritakumari	Monocots	113	5	C
6	*Andrographis paniculata* (Burm.f.)Wall.	Kalomegh	Dicot	112	6	C
7	*Azadirachta indica* A. Juss	Neem, Nim gachh	Dicot	112	6	P
8	*Phyllanthus emblica* L.	Amluki	Dicot	111	7	P
9	*Centella asiatica* (L.) Urban.	Thankuni, Tehagorer pata	Dicot	109	8	W
10	*Hyptis suaveolens* (L.)Poit	Tokma	Dicot	108	9	W
11	*Aegle marmelos* (L.) Corr	Bel	Dicot	108	9	P
12	*Terminalia belerica* Roxb.	Bohera, Boira	Dicot	107	10	W
13	*Syzygium cumini* (L.)Skeel	Jam, Kalojam	Docot	106	11	P
14	*Terminalia arjuna* (Roxb.) W. & A	Arjun	Dicot	106	11	P
15	*Rauvolfia serpentina* (L.) Benth.	Sarpagondha	Dicot	105	12	C

Sl No.	Scientific Name	Local name	Mono/ Dicot	Number of respondent/s	Ranking	Cultivated/ Planted/Wild
16	*Bombax ceiba* L.	Shimul Tula	Dicot	105	12	P
17	*Saraca indica* L.	Ashok	Dicot	103	13	W
18	*Rauvolfia-tetraphylla* L.	Sarpagondha	Dicot	103	13	C
19	*Abroma augusta* L.	Ulatkambal	Dicot	102	14	C
20	*Gloriosa superba* L.	Ulat Chandal	Monocots	101	15	C
21	*Streblus asper* Lour.	Sheora	Dicot	100	16	W
22	*Terminalia chebula* (Gaertn.) Retz	Hartaki, Haritaki	Dicot	99	17	W
23	*Asparagus racemosus* Willd.	Shatamuli	Monocots	98	18	C
24	*Zingiber officinale* Rosc.	Ada	Monocots	97	19	C
25	*Ocimum gratissimum* L.	Ram Tulshi	Dicot	97	19	P
26	*Vitex negundo* L.	Nishinda	Dicot	95	20	P
27	*Achyranthes aspera* L.	Apang, Upothlengra	Dicot	95	20	W
28	*Curculigo orchioides* Gaertn.	Tal muli, Tali	Monocots	94	21	P
29	*Holarrhena antidysenterica* Heyne ex. Roth	Kurchi, Kurusuri	Dicot	94	21	W
30	*Ocimum americanum* L.	Kalo Tulshi	Dicot	93	22	C
31	*Coccinia cordifolia* (L.) Cogn.	Telakucha	Dicot	91	23	W
32	*Withnia somnifera* Dunal.	Arsogondha	Dicot	90	24	C
33	*Ocimum basilicum* L.	Babui Tulshi	Dicot	90	24	P
34	*Datura metel* L.	Dhutura, KaloDhutra	Dicot	89	25	W
35	*Cassia alata* L.	Dadmardan	Dicot	88	26	W
36	*Allium stivum* L.	Rashun	Monocots	87	27	C
37	*Costus speciosus* (Koenig) Sm.	Keu, Tara, Kura	Monocots	86	28	C
38	*Cynodon dactylon* Pers.	Durbaghas	Monocots	85	29	W
39	*Ipomoea mauritiana* Jacq	Bhuikumra	Dicot	84	30	C
40	*Cuscuta reflexa* Roxb.	Swarnalata	Dicot	84	30	W
41	*Carica papaya* L.	Penpey	Dicot	83	31	C
42	*Mimosa pudica* L.	Lajjabati	Dicot	82	32	W
43	*Curcuma amada* Roxb.	Am ada	Monocots	82	32	C
44	*Curcuma zedoaria* Rosc.	Shoti	Monocots	81	33	W
45	*Cassia fistula* L.	Sonalu, Bandar Lathi	Dicot	80	34	P
46	*Litsea glutinosa* (Lour.) Rob.	Kharajora,	Dicot	80	34	W
47	*Commelina benghalensis* L.	Dholpata, Kanchira	Monocots	79	35	W
48	*Tinospora cordifolia* (Willd.) Miers	Guloncha	Dicot	79	35	W
49	*Cissus quadrangularis* L.	Harjora, Harbhangalata	Dicot	78	36	P
50	*Datura stramonium* L.	Sada Dhutra	Dicot	78	36	W
51	*Acalypha indica* L.	Mutajhuri	Dicot	78	36	W
52	*Calotropis gigantea* Br.	Boro Akanda	Dicot	77	37	W
53	*Heliotropium indicum* L.	Hatisur	Dicot	77	37	W
54	*Paederia foetida* L.	Gondhabaduli	Dicot	76	38	W
55	*Eupatorium odoratum* L.	Fulkuri	Dicot	76	38	W
56	*Leucus lavandulaefolia* Rees.	Dondokolos, Domkolos	Dicot	75	39	W
57	*Wedelia calendulacea* Less.	Kesharaj, Kalo kur	Dicot	75	39	W
58	*Mentha arvensis* L.	Pudina	Dicot	75	39	P
59	*Bacopa monniera* (L.)Pennel.	Brahmilata, Brahmishak	Dicot	74	40	W
60	*Aristolochia indica* L.	Isharmul	Dicot	74	40	P
61	*Cajanus cajan* (L.)Huth.	Arhar, Tur, Arekkalai	Dicot	73	41	P
62	*Citrullus colocynthesis* (L.) Sch.	Makal	Dicot	73	41	W

Sl No.	Scientific Name	Local name	Mono/ Dicot	Number of respondent/s	Ranking	Cultivated/ Planted/Wild
63	*Momordica charantia* L.	Usta, Karala	Dicot	72	42	P
64	*Bryophyllum pinnatum* Kurz.	Patharkuchi	Dicot	72	42	C
65	*Calotropis procera* R. Br.	Chhoto Akanda	Dicot	71	43	W
66	*Hemidesmus indicus* (L.) R.Br	Anantamul	Dicot	70	44	P
67	*Curcuma aromatic* Salisb.	Jangli Haldi	Monocots	69	45	P
68	*Tamarindus indica* L.er.	Tetul	Dicot	68	46	W
69	*Smilax zeylanica* L.	Kumari lata, Bulkumia	Monocots	67	47	W
70	*Ananas sativus* Schult.f.	Anaras	Monocots	66	48	C
71	*Eclipta prostrata* (L.) Roxb.	Keshraj, Kalokeshi	Dicot	66	48	W
72	*Mikania cordata* (Brum.f.) Rob.	Assam Lata	Dicot	65	49	W
73	*Musa paradisiaca* L.	Kanch Kola	Monocots	64	50	C
74	*Amaranthus spinosus* L.	Khuirakanta, Kantanotey	Dicot	64	50	W
75	*Curcuma longa* L.	Halud, Haldi	Monocots	63	51	C
76	*Euphorbia hirta* L.	Dhudia,	Dicot	62	52	W
77	*Allium cepa* L.	Piyanj	Monocots	62	52	C
78	*Butea monosperma* (L.) Taub.	Polash, Polashphul gachh	Dicot	62	52	W
79	*Hibiscus rosa-sinensis* L.	Jaba	Dicot	61	53	P
80	*Ficus racemosa* L.	Jaga dumur	Dicot	60	54	W
81	*Catharanthus roseus* (L.) G. Don.	Nayantara	Dicot	58	55	P
82	*Tridax procumbens* L.	Tridhara	Dicot	58	55	P
83	*Ageratum conyzoides* L.	Fulkuri	Dicot	57	56	W
84	*Amaranthus viridis* L.	Notey, Noteyshak	Dicot	57	56	W
85	*Boerhaavia diffusa* L.	Punarnava	Docot	56	57	W
86	*Euphorbia thymifolia* Brum.f.	Swet Keru	Dicot	55	58	W
87	*Evolvulus nummularus* L.	Bhui okra	Dicot	55	58	W
88	*Solanum indicum* L.	Phutka Begun	Dicot	54	59	W
89	*Drynaria quercifolia* (L.)J.Sm.	Pankhiraj, Pokharaj	Fern	54	59	W
90	*Phyllanthus niruri* L.	Bhui amla	Dicot	53	60	W
91	*Ricinus communis* L.	Bherenda, Reri	Dicot	52	61	C
92	*Barleria cristata* L.	Janti	Dicot	52	61	P
93	*Mangifera indica* L.	Am	Dicot	51	62	P
94	*Desmodium gangeticum* (L.) DC.	Salpani	Dicot	50	63	C
95	*Abrus precatorius* L.	Kunch, Ratti	Dicot	49	64	P
96	*Clitoria ternatea* L.	Aparajita, Nila	Dicot	49	64	P
97	*Jatropha gossypifolia* L.	Lal Jamal gota	Dicot	49	64	W
98	*Psidium guajaba* (L.) Bat.	Peyara	Docot	48	65	C
99	*Justicia gendarussa* Burm.	Jagatmadan	Dicot	47	66	P
100	*Citrus aurantifolia* (Chist.) Sw	Lebu, Pati Lebu	Dicot	47	66	C
101	*Cassia oblusifolia* L.	Chakunda,Arach	Dicot	47	66	W
102	*Cocos nucifera* L.	Narikel, Daab	Monocots	46	67	P
103	*Artemisia nilagirica* (Clarke) Pamp	Nagdana	Dicot	45	68	W
104	*Abutilon indicum* (L.) Sweet	Potari,	Dicot	44	69	W
105	*Lannea coromandelica* (Houtt.) Merr.	Jiga	Dicot	42	70	W
106	*Amorphophallus campanulatus* (Roxb.)Bl.Ex Decne.	Oal, Oal kachu	Monocots	41	71	W
107	*Jatropha curcas* L.	Jamal gota	Dicot	40	72	W
108	*Moringa oleifera* Lamk.	Sajna	Docot	39	73	C

Sl No.	Scientific Name	Local name	Mono/ Dicot	Number of respondent/s	Ranking	Cultivated/ Planted/Wild
109	*Tragia involucrata* L.	Bichhatu, Bichuti	Dicot	39	73	W
110	*Argemone maxicana* L.	Shialkanta	Dicot	38	74	W
111	*Coriandrum sativum* L.	Dhania, Dhoney	Dicot	37	75	C
112	*Elaeocarpus serratus* L.	Jolpai	Dicot	36	76	P
113	*Erythrina variegata* L.	Mandar	Dicot	35	77	P
114	*Euphorbia tirucalli* L.	Lanka Sij, Dud kusi	Dicot	34	78	W
115	*Urena lobata* L.	Chhilangota, Bonokra	Dicot	34	78	W
116	*Sida cordifolia* L.	Bala	Dicot	33	79	W
117	*Foeniculum vulgare* Mill.	Mouri, Pan Muhuri	Dicot	32	80	C
118	*Cassia sophera* L.	Chhota Kalkasunda	Dicot	32	80	W
119	*Physalis minima* L.	Phutkas, BonTipariya	Dicot	31	81	W
120	*Spilanthes paniculata* Wall	Marhati tiga	Dicot	31	81	W
121	*Anacardium occidentale* L.	Kajubadam	Dicot	30	82	P
122	*Aphanamixis polystachya* (Wall.)R. Parker	Pitraj	Dicot	30	82	W
123	*Alpinia niagra* (Gaertn.) Burtt	Jangli Ada, Tara	Monocots	30	82	C
124	*Dioscorea alata* L.	Mete Alu, Chupri Alu	Monocots	29	83	W
125	*Punica granatum* L.	Dalim	Dicot	29	83	C
126	*Blumea lacera* (Burn.f.)DC.	Shialmuti	Dicot	28	84	W
127	*Sida acuta* Burm.	Bon-methi, Kureta	Dicot	27	85	W
128	*Croton bonplandianum* Baill.	Bon tulshi	Dicot	27	85	W
129	*Nyctanthes arbor-tristis* L.	Shiuli, Shefali phul	Docot	26	86	P
130	*Alocasia indica* (Roxb.)Schott.	Mankuchu	Monocots	26	86	P
131	*Mirabilis jalapa* L.	Sandhamaloti	Docot	25	87	P
132	*Solanum nigrum* L.	Tit Begun, Kakmachi	Dicot	24	88	W
133	*Piper betel* L.	Paan	Dicot	24	88	C
134	*Crotalaria spectabilis* Roth.	Jhanjhania	Dicot	23	89	W
135	*Dioscorea bulbifera* L.	Banalu, Pesta-Alu	Monocots	23	89	W
136	*Lawsonia inermis* L.	Mehedi, Mendi	Dicot	22	90	P
137	*Plumeria rubra* L.	Kat Golap	Dicot	22	90	P
138	*Typhonium trilobatum* (L.) Schott.	Kharkon, Ghetkochu	Monocots	22	90	W
139	*Vanda roxburghii* R.Br	Rasna	Docot	21	91	W
140	*Citrus grandis* (L.) Osbeck.	Batabi Lebu, Jambura	Dicot	21	91	C
141	*Zizyphus mauritiana* Lamk.	Boroi, Kul	Dicot	20	92	C
142	*Averrhoa carambola* L.	Kamranga	Docot	20	92	P
143	*Alstonia scholaris* R.Rr.	Chhatim, Chaitan	Dicot	20	92	W
144	*Shorea robusta Gaertn.*	Gojari, sal	Dicot	19	93	W
145	*Arachis hypogaea* L.	Badam, Cheenabadam	Dicot	19	93	C
146	*Basella alba* L.	Puishak	Dicot	19	93	C
147	*Bixa orellana* L.	Latkan, Utkana	Dicot	18	94	P
148	*Cyperus rotundus* L.	Muthaghas	Monocots	18	94	W
149	*Dillenia indica* L.	Chalta	Dicots	17	95	P
150	*Feronia limonia* (L.)Sw	Kadbel	Dicot	17	95	P
151	*Garuga pinnata* Roxb.	Laljigachal	Dicot	17	95	W
152	*Gynandropis gynandra* (L.) Biq.	Sada Hurhure	Dicot	16	96	W
153	*Helianthus annuus* L.	Surjamukhi	Dicot	16	96	C
154	*Ixora coccinea* L.	Rangan	Dicot	16	96	P
155	*Oxalis corniculata* L.	Amrul	Docot	15	97	W
156	*Piper longum* L.	Pipul	Dicot	15	97	P
157	*Psittacanthus calyculatus* (DC.) G. Don.	Hinikgota	Dicot	15	97	W

Sl No.	Scientific Name	Local name	Mono/ Dicot	Number of respondent/s	Ranking	Cultivated/ Planted/Wild
158	*Stephania japonica* (Thunb.) Miers.	Akanadi, Nimuka	Dicot	14	98	W
159	*Acacia catechu* Willd	Khoyer	Dicot	14	98	P
160	*Clerodendrum viscosum* Vent.	Vital, Ghetu, Bhant	Dicot	13	99	W
161	*Syzygium jambos* (L.) Alston.	Golap Jam	Docot	13	99	P
162	*Abelmoschus esculentus* (L)Moench	Dheros, Vendi	Dicot	12	100	C
163	*Acacia nilotica* (L.) Bel	Babla	Dicot	12	100	P
164	*Alternanthera sessilis* (L.)R.Br	Haicha, Chanchi	Dicot	12	100	W
165	*Annona squamosa* L.	Ata, Sharifa	Dicot	11	101	P
166	*Areca catechu* L.	Supari	Monocots	11	101	P
167	*Borassus flabellifer* L.	Tal gachh	Monocots	11	101	P
168	*Careya arborea* Roxb.	Kumbi, Gade, Gadila	Dicot	10	102	W
169	*Carissa congesta* Wight.	Karamcha	Dicot	10	102	P
170	*Corchorus fascicularis* Lam.	Bon Pat	Dicot	10	102	W
171	*Cryptolepis buchanani* Roem.& Schult.	Chhagalbati	Dicot	9	103	W
172	*Ficus benghalensis* L.	Bot	Dicot	9	103	W
173	*Gmelina arborea* L.	Gamari	Dicot	9	103	W
174	*Gomphrena globosa* L.	Butamphul,Golkamal	Dicot	9	103	P
175	*Oroxylum indicum* (L.)Vent.	Kanak, Sonpatti	Dicot	9	103	W
176	*Ipomoea quamoclit* L.	Kunjalata, Tarulata	Dicot	8	104	W
177	*Albizia lebbeck* (L.) Benth.	Koroi	Dicot	8	104	W
178	*Allamanda cathartica* L.	Malatilata, Kalkephul	Dicot	8	104	P
179	*Anthocephalus chinensis* (Lamk.)A.Rich.	Kadamphul gachh	Dicot	8	104	W
180	*Bambusa arundinacea* Willd.	Kanta Bansh	Monocots	8	104	P
181	*Cinnamomum tamala* Nees.	Tejpata	Dicot	7	105	P
182	*Cleome viscose* L.	Hurhuria	Dicot	7	105	W
183	*Desmodium pulchellum*Benth.	Juta Salpani	Dicot	7	105	W
184	*Ficus rumphii* Blume.	Pakur	Dicot	7	105	W
185	*Gnaphalium luteo-album* L.	Boro Kamra Natham	Dicot	7	105	W
186	*Mimusops elengi* L.	Bakul	Dicot	6	106	P
187	*Quisqualis indica* L	Madhubi lata,	Dicot	6	106	P
188	*Rumex maritimus* L.	Bon Palong	Dicot	6	106	W
189	*Scoparia dulcis* L.	Chinigura	Dicot	6	106	W
190	*Tectona grandis* Linn.f	Segun	Dicot	5	107	P
191	*Uraria lagopodioides* DC.	Lata Chakuley	Dicot	5	107	W
192	*Xanthium indicum* Koenig.	Ghagra	Dicot	5	107	W

* **C**: Cultivated; **P**: Planted; **W**: Wild * **Monocot**: Monocotyledon; **Dicot**: Dicotyledon

2.3.2.3. *Life span of medicinal plants*

On life span aspect, out of 192 medicinal plant species, 59 plants were found annual and 133 plants were identified as perennial species **(Fig. 2.3.6)** in the Madhupur Tract.

2.3.2.4. *Monocot and Dicot species*

On the cotyledon aspect, out of 192 medicinal plant species, 162 plants were dicot and 29 plants were monocot and only one was fern (**Fig. 2.3.7).**

2.3.2.5. *Habit of medicinal plants*

In the present investigation 192 medicinal plants were recorded based on habit and the results are shown in **Fig. 2.3.8.** The figure shows that 43 percent of plants were herb, 28 percent tree, 17 percent shrub, 10 percent climber, epiphyte and lianas each were only one percent. Considering the habit of the medicinal plants in these areas, herbs were more prevalent than trees, shrub, climber, epiphytes and lianas.

2.3.2.6. *Parts used in different medicinal plants*

Leaf is the most frequently used part followed by whole plant part, root, fruit, seed, bark, rhizome, latex, stem, tuber, flower and bulbil. Among the 192 medicinal plant species, the local herbal doctors used leaves of 37% plants, whole plant parts of 13% plants, root of 11% plants, fruits of 10% plants,seed of 10% plants, bark of 9% plants, rhizome of 3% plants, latex of 2% plants, stem of 2% plants, tuber of 1% plants, flower of 1% plants and bulbil of 1% plants were used for medicinal purpose **(Fig. 2.3.9)** over the area.

2.3.2.7. *Medicinal plants application*

The method of application varies according to the nature of ailment. Different plant parts *viz.* leaves, whole plant part, root, fruit, seed, bark, rhizome, latex, stem, tuber, flower and bulbil of different medicinal plant species were used in the form of juice, paste, powder, latex, decoction, oil, fruit, pulp, raw plants and even soaked seed for curing different diseases. Generally juice and powder were found to be orally administered by client. Sometimes plant parts were used together with secondary products like milk, honey, oil, jaggary, gum, resin and administered orally. Paste was used externally. Decoction, latex and oil were taken either orally or externally. In the present investigation 57% plants were used in the form of juice for feeding, whereas 21% paste, 10% powder forms, 4% latex, 3% decoction and 5% used as other forms **(Fig. 2.3.10).**

2.3.2.8. *Propagation of medicinal plants*

On the propagation perspectives, out of 192 medicinal plant species, 76% plant were mainly propagated by seeds, whereas 10% by stem cutting, 5% rhizomes, 3% tuberous root, and sucker each, 2% bulbs and 1% leaves (**Fig. 2.3.11**).

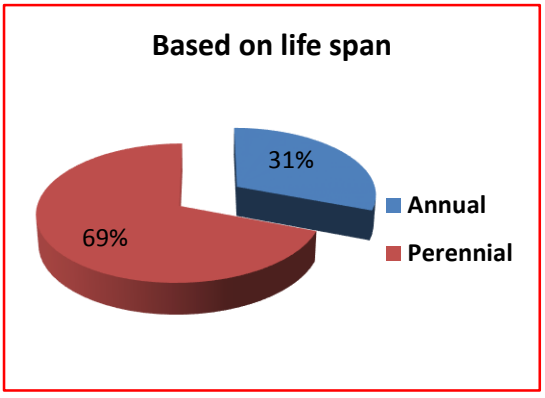


Fig. 2.3.6. Medicinal plantsbased on life span

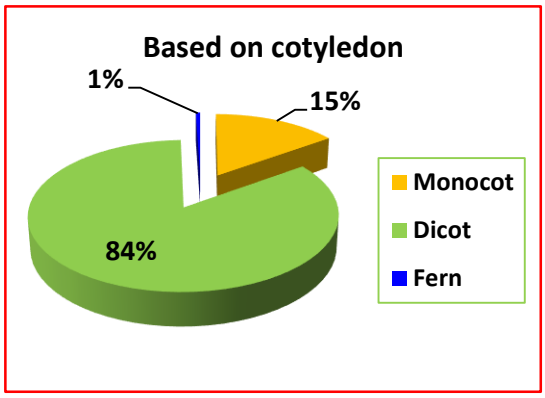


Fig. 2.3.7. Medicinal plantsbased on cotyledon

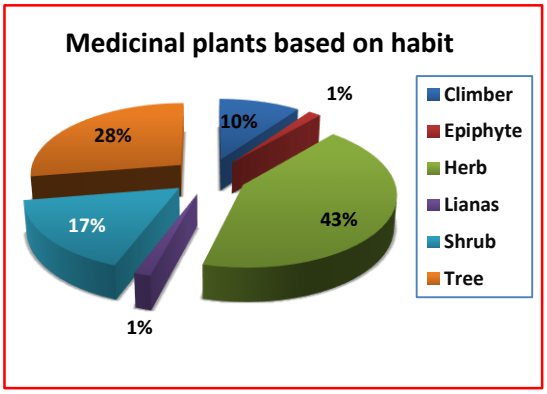


Fig. 2.3.8. Medicinal plantsbased on habit

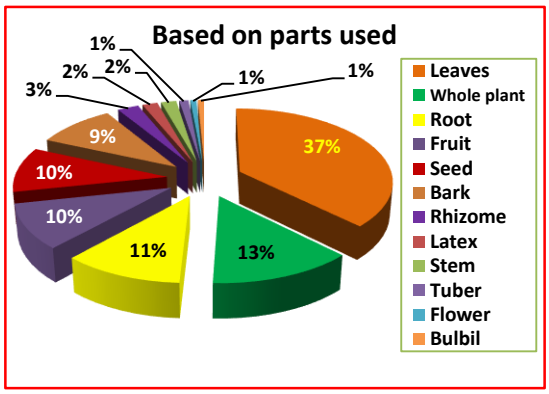


Fig. 2.3.9. Medicinal plantsbased on parts used

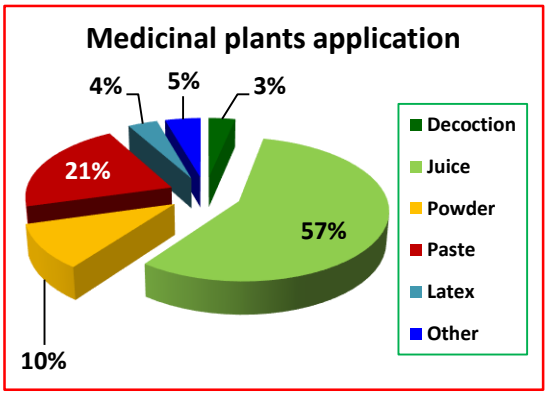


Fig. 2.3.10. Medicinal plants application

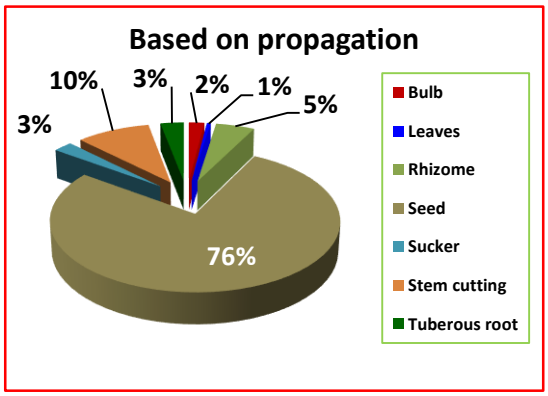


Fig. 2.3.11. Medicinal plantsbased on propagation

2.3.2.9. *Medicinal plants used by the herbal doctors against different diseases in the Madhupur Tract.*

The field observation revealed that a number of plants were found to use by the local herbal doctors for the same disease in many cases and their number varies from

disease to disease. The numbers of plants were counted used by the herbal doctors for each disease and the results are presented in **Table 2.3.5.** The use of the same plant for treatment of different diseases was also noticed during the survey.

Table 2.3.5. Number of plants used by the herbal doctors against different diseases.

Sl No.	Number of plants used per disease		Sl No.	Number of plants used per disease		Sl No.	Number of plants used per disease	
	Disease	Plant(s)		Disease	Plant(s)		Disease	Plant(s)
1	Abdominal colic	8	37	Dropsy	4	73	Pain	7
2	Abortion	7	38	Dysentery	25	74	Paralysis	2
3	Abscess	6	39	Dyspepsia	13	75	Phthisis	3
4	Alopecia	4	40	Earache	3	76	Piles	17
5	Anhydrotic	1	41	Eczema	5	77	Pimples	1
6	Antipyretic	2	42	Energetic	4	78	Pox	2
7	Antiseptic	2	43	Eye disease	3	79	Puerperal	1
8	Antispasmodic	4	44	Fever	30	80	Purgative	6
9	Aphrodisiac	6	45	Flatulence	5	81	Restorative	3
10	Ascites	3	46	Galactagogue	1	82	Rheumatism	16
11	Asthma	10	47	Gleet bruises	12	83	Ring worms	8
12	Astringent	17	48	Gonorrhoea/ Sexual debility	20	84	Scurvy	3
13	Bites of insects	2	49	Gout	3	85	Skin disease	18
14	Blood purifier	6	50	Hallucination	2	86	Snakebite	6
15	Boils	4	51	Headache	8	87	Sore	12
16	Bone fracture	5	52	Heart disease	9	88	Spermatorrhoea	2
17	Brain tonic	2	53	Hepatitis	3	89	Spleen and liver disease	5
18	Breast pain	1	54	Hypertension/Blood pressure	6	90	Stimulant	5
19	Bronchitis	2	55	Hypoglycaemic	3	91	Stomach complaints	25
20	Burns	2	56	Hypotensive	6	92	Stop bleeding	3
21	Cancer	1	57	Impotency	8	93	Swellings	5
22	Carbuncle	2	58	Indigestion	8	94	Syphilis	2
23	Carminative	5	59	Infertility	1	95	Tonic	20
24	Catarrh	5	60	Itch	11	96	Toothache and caries	4
25	Chest pain	2	61	Jaundice	18	97	Toxic prevention	2
26	Cholera	2	62	Kidney troubles	4	98	Tuberculosis	1
27	Cold and Cough	36	63	Laxative	1	99	Tumours	5
28	Constipation	12	64	Leprosy	4	100	Ulcer	11
29	Contraceptive	4	65	Leucorrhoea	7	101	Urinary troubles/ burning	17
30	Cooling	4	66	Loss of appetite	2	102	Uterine problems	1
31	Corns	1	67	Lumbago	2	103	Vomiting	3
32	Cuts	9	68	Malarial fever	3	104	Warts	1
33	Demulcent	2	69	Menstrual troubles	12	105	Weakness	16
34	Diabetes	15	70	Muscle spasm	2	106	Worm/anthelmintic	26
35	Diarrhoea	16	71	Neuralgia	3	107	Wounds	9
36	Diuretic	5	72	Ophthalmia	1	-	-	-

In the present investigation, extensive survey was made on 146 respondents over the tract and 107 diseases (**Table 2.3.5**) were addressed by the local herbal doctors being treated for herbal medicine. Among these diseases cough, fever, weakness, worm, dysentery, stomach complaints, sexual debility, jaundice, skin disease, piles, urinary trouble, diarrhoea, rheumatism and diabetes were found as the predominant diseases

for the herbal remedies. Field survey indicated that highest number of 36 medicinal plants was used for cough and followed by 30 plants were used for fever, 27 plants were used for weakness. A number of 26 plants were used for warm control, 25 plants for dysentery and stomach complaints, 20 plants for sexual debility and 18 plants were used for each of jaundice and skin disease. For urinary trouble a number of 17 plants were used whears,16 plants were used for each of diarrhoea and rheumatism, 15 plants for diabetes, 13 plants for dyspepsia, 12 plants for constipation, gleet bruises, menstrual troubles and sore and 11 plants were used for each of Itch and ulcer. For Asthma 10 plants were used, 9 plants were used for each of cuts, heart disease and wounds.8 plants for abdominal colic, headache, impotency, indigestion and ring worm control and each of other diseases bearing only a few of the remaining plant species.

Fig. 2.3.12. Sharing knowledge about medicinal plants with Garo *Khamal*; (A-C)/ cultivator (D); A: Jonikh Marakh; B: Somola Azim; C: Subas Chandra Sorker; D: Nirmola Hadima.

Fig. 2.3.13. A– D: Local herbal doctors (Kabiraj) of Madhupur Tract; **E:** Herbal powder and seeds; **F:** Herbal pill.

Fig. 2.3.14. Some of cultivated medicinal plants plot in homestead area of Madhupur Tract **A**: *Ocimum sanctum*; **B**: *Mucuna pruriens*; **C**: *Ipomoea mauritiana*; **D**: *Abroma augusta*; **E**: *Andrographis paniculata*; **F**: *Costus speciosus.*

Fig. 2.3.15. Some of cultivated medicinal plants plot in homestead area of Madhupur Tract **A:** *Cissus quadrangularis*; **B:** *Aloe barbadensis*; **C:** *Tinospora cordifolia*; **D:** *Piper longum*; **E:** *Euphorbia tirucalli*; **F:** *Ocimum gratissimum*.

2.4. DISCUSSION

The knowledge that an indigenous (local) community accumulates over generations of living in a particular environment is known as indigenous knowledge. This definition encompasses all forms of knowledge – technologies, know-how skills, practices and beliefs – that enable the community to achieve stable livelihoods in their environment. A number of terms are used interchangeably to refer to the concept of indigenous knowledge, including traditional knowledge, indigenous technical knowledge, local knowledgeand indigenous knowledge system. This knowledge is dynamic, not easily modifiable and based on innovation, adaptation, and experimentation. It can contribute to a sustainable development strategy that accounts for the potential of the local environment and the experience and wisdom of the indigenous population.

The Garos were once a nomadic tribe of *Bodo* group of Mongoloids now living particularly in Madhupur Tract of Bangladesh. Among Garos, the people who treat and cure patients by folk medicines are considered persons with supernatural power and therefore, enjoy respect and honor in the community. These persons are named *khamal* or *kamal*. The *Khamal* and herbal doctors of Madhupur tract possess such kind of knowledge particularly from their healthcare viewpoint, which is transmitted from generation to generation by '*Guru'* mode of instruction and this system is still prevailing in the area. In most cases, the knowledge is kept strictly within the family circle. The apprentices lived with and learnt at the feet of the masters who maintained a conventional oral tradition. The knowledge for the system is mostly inherited, though certain rare procedures are preserved in written form. The inhibitors of the areas were mainly dependent on herbal medicine for treating their diseases. But due to indiscriminate deforestation, over exploitation, habitat destruction, injudicious collection, the natural habitats of medicinal plants in Madhupur Tract are being gradually declined. Moreover modern pharmaceuticals have replaced many natural remedies. In these consequences, traditional knowledge of healing system is disappearing rapidly in the tribal communities and herbal doctors of Madhupur Tract. If this situation continues, it may be completely lost. Under the present circumstances, it is our moral responsibilities to restore such indigenous knowledge system of traditional medicine that inherited from generation to generation and persisted in the community of local people. Besides these, most of the modern medicines have many bad side effects, so now a day's interest towards the herbal medicine is increasing again.Proper study of various medicinal plants in the area and their stock assessment were not carried out in the Madhupur Tract.

Thus present investigation was undertaken with a view to conduct a thorough survey of cultivated and wild medicinal plants being used by the local people of Madhupur Tract and gather information on their indigenous herbal uses and identify the important medicinal plants holding greater medicinal values of this area which will be helpful to enrich our Ayurvedic, Unani and other herbal treatment system in future.

The present study experienced the potential of medicinal plant as the means of earning of livelihood through studying the herbal doctors and other respondents of Madhupur Tract who improved their living standard considerably. Among 146 respondents, 55% respondents were in medium income category and they engaged in different activities by virtue of medicinal plants in the Madhupur Tract. All interviewees were male except 5 were female, results indicated that majority of the respondents were of middle age (48.00%) under the age between 31–55 yrs. In terms of literacy 11 percent of the respondent had no formal education; on the other hand 89 percent respondent had formal education in different levels. The respondents were taken from the local people in any way involved in the activity of medicinal plants and the results revealed that out of 146 respondents 91 percent were kobiraj/herbal herbal doctors, 8 percent whole seller and only 1 percent was medicinal plant cultivators.

Following the taxonomical characteristics 192 medicinal plants, being used by the local people of the area, were identified and found to belong 68 families and among these families predominating families wereFabaceae, Asteraceae, Euphorbiaceae, Apocynaceae, Lamiaceae, Liliaceae, Zingiberaceae, Malvaceae, Solanaceae and Amaranthaceae. Considering the habit of the medicinal plants, herbs were more prevalent than trees, shrubs, climbers, epiphytes and lianas. The use of medicinal plants for treatment varies from place to place depending upon the local demand. Moreover, different use of particular plant species was also noted among the local community. In case of *Boerhaavia diffusa*, whole plant was used in the treatment of pulmonary tuberculosis, plant powder was used abdominal tumor, dysentery and renal disease, flowers and seeds were used as contraceptives, Roots were used in the treatment of jaundice, anemia, gonorrhea, blood purifier and as stimulant. On the other hand, more than one plant species was found to use for the treatment of same diseases for example 36 plant species were used for cough, 30 plants for fever, 27 plants for weakness, 26 plants for worm control, 25 plants for dysentery and stomach complaints, 20 plants for sexual debility, 18 plants for each of jaundice and skin disease, 17 plants for each of piles and urinary trouble, 16 plant species were used for each of diarrhoea and rheumatism, 15 plants for diabetes. One of the possibilities of this variation might be the cause of different tribes use a particular plant in different

ways. Ticktin and Dalle (2005) conducted a questionnaire survey among 32 midwifes in seven rural communities near La Ceiba, Honduras. They identified seventy-nine different plant species used to treat 15 circumstances occurring during the pregnancy.

In the present investigation, it was found that 107 different diseases could be treated with 192 medicinal plants. Among these diseases cough, fever, weakness were more common diseases. Other more prevalent diseases are worm control, dysentery and stomach complaints, sexual debility,jaundice, skin disease,piles, urinary trouble,diarrhoea, rheumatism,diabetes,dyspepsia, constipation, gleet bruises, menstrual troubles, sore, itch, ulcer,asthma,cuts, heart disease, wounds,abdominal colic, headache, impotency, indigestion etc.

From the experimental results it was found that leaf is the most frequently used part followed by roots, whole plant part, root, fruit, seed, bark, rhizome, latex, stem, tuber, flower and bulbil. Among studied medicinal plant species, the local herbal doctors use leaves of 102 plants, whole parts of 36 plants,root of 30 plants, fruitof 27 plants, seedof 27 plants, barkof 26 plants, rhizomeof 7 plants, latexof 5 plants, stemof 5 plants, tuberof 3 plants, flowerof 2 plants and bulbilof 2 plants. These plant parts are used to cure a wide range of ailments. The method of application varies according to the nature of ailment. The plant parts were used in the form of juice, paste, powder, decoction, and even soaked seed and raw plants were also used. Generally juice and powder were given to take orally, paste was used external uses and decoction was taken either orally or externally. Moreover, it was noticed that combinations of different plant parts were used for better treatment of many diseases. Such as the seeds of Tokma (*Hyptis suaveolens*) along with root of *Bombax ceiba* is used in gonorrhea; the juice of the stem of *Tinospora cordifolia* along with the juice leaves of *Momordica charantia* is administered against chicken pox; the stems of *Cissus quadrangularis*are either crushed alone or with pakkhiraj (*Smilax* sp.) and with heads of climbing perch fish (*Anabas testudineus*) and applied for healing of bones in fractures. Alam (1992) also noticed that more than one plant species are used by the marma tribe for the treatment of same disease such as root tuber of *Stephania japonica* mixed with juice of *Flemingia stricta* is taken for asthma. Single plant was also found to be used for many diseases. Some organic and inorganic substances such as egg, honey, milk, spices, latex, resin, fish, black cow's urine, meat of fox and black hen, shell and flesh of snail were found to be added in the preparation of herbal medicine for the treatment of various diseases prevailing over the area. But the herbal doctors are very much unwilling to disclose their secrecy about the preparations and the applications of the techniques.

Individual responses perceived from 146 respondents to determine the importance of studied medicinal plant species in the Madhupur Tract. Among the 192 medicinal plants 62 were identified as important medicinal plantsbased on respondent's performance. 50 percent of herbal practitioners of Madhupur Tract used these plant species for remedy of different ailments. These are *Ocimum sanctum,Adhatoda vasica, Mucuna pruriens, Aloe barbadensis, Aloe indica, Andrographis paniculata, Azadirachta indica, Phyllanthus emblica, Centella asiatica, Hyptis suaveolens, Aegle marmelos, Terminalia belerica, Syzygium cumini, Terminalia arjuna, Rauvolfia serpentina, Bombax ceiba, Saraca indica, Rauvolfia-tetraphylla, Abroma augusta, Gloriosa superba, Streblus asper, Terminalia chebula, Asparagus racemosus, Zingiber officinale, Ocimum gratissimum, Vitex negundo, Achyranthes aspera, Curculigo orchioides, Holarrhena antidysenterica, Ocimum americanum, Coccinia cordifolia, Withnia somnifera, Ocimum basilicum, Datura metel, Cassia alata, Allium stivum, Costus speciosus, Cynodon dactylon, Ipomoea mauritiana, Cuscuta reflexa, Carica papaya, Mimosa pudica, Curcuma amada, Curcuma zedoaria, Cassia fistula, Litsea glutinosa, Commelina benghalensis, Tinospora cordifolia, Cissus quadrangularis, Datura stramonium, Acalypha indica, Calotropis gigantean, Heliotropium indicum, Paederia foetida, Eupatorium odoratum, Leucus lavandulaefolia, Wedelia calendulacea, Mentha arvensis, Bacopa monniera, Aristolochia indica, Cajanus cajan* and *Citrullus colocynthesis.* Mia *et al*. (2009) undertook an ethnobotanical survey among the Garo tribal healers to gather information on ailments that are common amongst the Garos and the medicinal plant formulations, which were used to treat these ailments. They obtained 65 medicinal plants distributed into 43 families from the tribal healers inhabiting the Madhupur region in Bangladesh.

Recommendation on the conservation of medicinal plants in the Madhupur Tract

Today, the diversity of the Earth's life is under threat as never before. In agriculture, the widespread adoption of a few improved varieties has narrowed the genetic base of important food crops and led to the disappearance of hundreds of landraces and caused genetic erosion among wild plants species. In the forests, up to 8% of all plant species are expected to disappear over the next 25 year as deforestation continues. The vegetation of the world is being changed or destroyed at an alarming rate. The tropical moist forests, home to about half of the world plants are in particular danger, declining at an estimated 16.8 million ha/annum according to UNEP/ FAO. Combined with exploitation, this is putting many medicinal plants in grave risk of genetic erosion and even extinction. As most of the habitats have been getting destroyed, modified, shrunk or degraded due to various biotic and environmental factors, many

of the plant species residing in these habitats, including medicinal plants, face threats to their survival. If this situation is continuous operating, most of the medicinal plants become extinct form their wild habitat. As demand for medicinal plants rises, harvest rates also increased and there are numerous documented cases of over exploitation and even local extirpation in response to intensive harvest. Trade volumes are large. The result is that the natural resource base is becoming degraded in certain areas, and an increasing number of species are becoming threatened. With the increase of population in an alarming rate in Bangladesh causes to over exploitation of natural vegetation and in particular in Madhupur Tract, highest range depletion of natural vegetation has been taken place imposing threat to a number of important high valued medicinal plants over the areas. Among the contributory factors for depletion of wild vegetation, the following are the major ones: (i) Extensive denudation of the forest floor, caused by cattle grazing and collection of leaf litter. (ii) Removal from the wilderness for tuberous roots which are highly priced in the market for its metabolic enhancing principles and aphrodisiac formulations. (iii) Poor seed setting and germination. (iv) High incidence of viral and bacterial diseases affecting underground parts. (v) Use of the underground parts as an edible flour by many tribal people and (vi) Use of the plant as a substitute for religious ceremony. Today many medicinal plants face extinction or severe genetic loss, but detailed information is lacking in the Madhupur Tract. For most of the endangered medicinal plant species no conservation action has been taken.

Extensive field survey and astute field observation lead to formulate several recommendations for conservation of medicinal plants in the Madhupur Tract which are studied follows:

(A) Motivation of traditional medical practitioners (TMPs)

TMPs are very aware of the conservation status of local medicinal plants resources and can be influential in changing local opinion so as to limit over exploitation. Support could be given to the formation of rural TMP associations and the self-sufficiency of MTPs, particularly in Madhupur Tract. In particular, information should be disseminated to rural communities on appropriate cultivation methods for medicinal plants which are in local demand.

(B) Conservation through commercial cultivation

Most of medicinal plants, even today, are collected from wild. There should be rapid development of alternative supply sources through cultivation in large and enough quantities and at a low enough price to compete with prices obtained by gatherers of wild stocks. This will satisfy market demands. If this does not occur, key species will

disappear from the wild habitat. However, the practical difficulties associated with the cultivation of medicinal plants should not be under estimated. The most vulnerable category of species, by their very nature, cannot be grown profitably due to their slow growth rates. Specific recommendations to promote large scale cultivation include: (1) Investigations to be made into the practicality of using facilities which already exist in such institutions as government and agricultural departments or commercial timber companies, to develop stocks of scarce and popular medicinal plant species from cuttings. This would provide initial plant stocks to supply plants for cultivation to herbal doctors. (2) Special project should be launched for cultivation of medicinal plants. (3) This should include canvassing local opinion on plant shortages and perceived solutions to the problem.

(C) Botanical gardens and field gene banks

This is not the case, as some popular and effective medicinal plants are threatened and need to be established in field gene banks until technology is available for storage of recalcitrant seeds. The ultimate goal of the conservation process is certainly to preserve the natural habitats of endangered medicinal plant species and to achieve sustainable exploitation. However, seed and gene banks of endangered medicinal plant species should be maintained as precaution and backup against extinction. It is recommended that collections for seed and gene banks should be undertaken in order to select for commercially beneficial properties such as fast growth and highest levels of active ingredients for pharmaceutical use. Slow growing species with specific habitat requirements are a priority in this respect.

(D) Education and training

The conservation of medicinal plants is by necessity a long term project requiring the development of trained staff, traditional practitioners in Madhupur Tract supported by any organizations and a general public that should be aware of the issues at stake. Improvement in education standards is a key factor in the conservation issue. Implementation of a media campaign through national radio networks to publicize information on the scarcity of popular medicinal plants and their propagations.

(E) Research and monitoring

Research into the identification of areas of high biological diversity at the macro scale and research into the properties and usage of specific plants at the micro-scale should use the complementary skills of the TMPs and conservation biologists. Specific recommendations include:

(1) The initiation of a series of interactive discussions involving TMPs, commercial gatherers and market based traders to discover the perceived scarcity of species, sites of diversity, the status of popular species, the perceived problems and solutions.

(2) The initial focus should be on heavily populated regions where plant scarcities are likely to occur, yet where little is known of the extent of the problems faced by TMPs.

(3) Research work on the storage of recalcitrant seeds should be the focus of research and its importance merely needs to be stressed.

(4) The success of cultivation as a conservation method should be monitored, possibly through a grower's register which indicates the area of key species under cultivation.

Thus, conventional propagation through seeds and vegetative cuttings is not an adequate solution to meet the demand. For this reason, the development of an *in vitro* protocol will be of great importance for production of planting materials to conserve the endangered medicinal plant species in the Madhupur Tract for sustainable development.

2.5. SUMMARY

Medicinal plants play a significant role in providing primary healthcare services to the people and a large number of medicinal plants are now widely used in Bangladesh including Madhupur Tract for production of both traditional and modern drugs. The total 4244 Km^2 and for the convenience of study six upazilas under three district were selected for collection information about medicinal plants over the area. A questionnaire documentation data sheet was developed during the initial period of investigation to hold every aspect of practical users of medicinal plants and their occurrence in the locality.

Based on their yearly income, out of 146 respondents, 81 numbers of the respondents belonged to medium income (21,000–100,000 BDT) category, whereas 48 and 17 numbers of respondents belonged to high (100,100 BDT–above) and low income (less than 20,000 BDT) categories respectively. Majority of the respondents are of middle aged (48) and their age were between 31–55 years. Eleven percent of the respondents have no formal education, on the other hand 89 percent respondents having formal education in different levels. The respondents were different categories according to their profession, out 146 interviewees 91 percent of the respondent were herbal doctors, 8 percent wholesaler, and only 1 percent medicinal plant cultivator.

In the present investigation, extensive survey was made on 146 respondents over the surveyed area and 192 medicinal plants species belonging to 68 families were identified with medicinal use by questionnaire survey. Among these Fabaceae, Asteraceae, Euphorbiaceae, Apocynaceae, Lamiaceae, Liliaceae, Zingiberaceae, Malvaceae, Solanaceae and Amaranthaceae were identified as major families predominantly composing of these medicinal plants in the Madhupur Tract. Highest number of observed 21 medicinal plants found to belong to the family Fabaceae. Asteraceae consists of 12 medicinal plant species; Euphorbiaceae consists of 11 species; Apocynaceae consists of 8 species; Lamiaceae, Liliaceae and Zingiberaceae, each included 7 numbers of medicinal plants; Malvaceae and Solanaceae, each included 6 medicinal plants; Amaranthaceae consists of 5 numbers of medicinal plants; Acanthaceae, Asclepiadaceae, Combretaceae, Moraceae, Rutaceae and Verbenaceae each included 4 numbers of medicinal plants and each of other families bearing only a few of the remaining plant species. Among 192 identified medicinal plants 59 numbers of plants were found annual and 133 numbers of plants were perennial. In regards of propagation, out of 192 medicinal plant species, 76% plant were mainly propagated by seeds, whereas 10% by stem cutting, 5% rhizomes, 3% tuberous root, and sucker each, 2% bulbs and 1% leaves. The habitats of medicinal plants were mainly village thicket, roadside, forest, graveyard, open places and fallow

lands. They were also found in homestead, edge of pond, waste place and sometimes cultivated in homestead area. In consideration of plant habit 82 plants were herb, 53 plants were tree, 32 plants were shrub, 19 plants were climber and epiphyte and lianas each include 3 plants. On cotyledon aspect, out of 192 medicinal plant species, 162 plants were dicot and 29 plants were monocot and only one was fern.

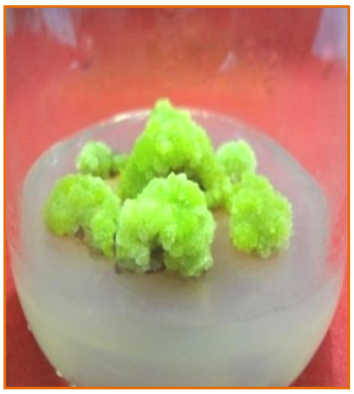
Under the present ethnobotanical survey a number 107 diseases were addressed by the village doctors being treated for herbal medicine. Among these diseases cough, fever, weakness, worm, dysentery, stomach complaints, sexual debility, jaundice, skin disease, piles, urinary trouble, diarrhoea, rheumatism and diabetes were found as the predominant diseases for the herbal remedies. Field survey indicated that highest number of 36 medicinal plants was used for cough and followed by 30 plants were used for fever, 27 plants were used for weakness, 26 plants were used for warm control, 25 plants were used for dysentery and stomach complaints, 20 plants were used for sexual debility, 18 plants were used for each of jaundice and skin disease, 17 plants were used for each of piles and urinary trouble, 16 plants were used for each of diarrhoea and rheumatism, 15 plants were used for diabetes, 13 plants were used for dyspepsia, 12 plants were used for each of constipation, gleet bruises, menstrual troubles and sore, 11 plants were used for each of Itch and ulcer,10 plants were used for asthma, 9 plants were used for each of cuts, heart disease and wounds. 8 plants were used for each of abdominal colic, headache, impotency, indigestion and ring worm control and each of other diseases bearing only a few of the remaining plant species. In most cases decoction of root or bark sometimes total parts (for small plants) were used as herbal remedy.

Another study was conducted to determine the importance of studied medicinal plant species based on respondent's performance in the Madhupur Tract. Among the 192 medicinal plants 62 were identified as important medicinal plants. These are *Ocimum sanctum,Adhatoda vasica, Mucuna pruriens, Aloe barbadensis, Aloe indica, Andrographis paniculata, Azadirachta indica, Phyllanthus emblica, Centella asiatica, Hyptis suaveolens, Aegle marmelos, Terminalia belerica, Syzygium cumini, Terminalia arjuna, Rauvolfia serpentina, Bombax ceiba, Saraca indica, Rauvolfia-tetraphylla, Abroma augusta, Gloriosa superba, Streblus asper, Terminalia chebula, Asparagus racemosus, Zingiber officinale, Ocimum gratissimum, Vitex negundo, Achyranthes aspera, Curculigo orchioides, Holarrhena antidysenterica, Ocimum americanum, Coccinia cordifolia, Withnia somnifera, Ocimum basilicum, Datura metel, Cassia alata, Allium stivum, Costus speciosus, Cynodon dactylon, Ipomoea mauritiana, Cuscuta reflexa, Carica papaya, Mimosa pudica, Curcuma amada, Curcuma zedoaria, Cassia fistula, Litsea glutinosa, Commelina benghalensis,*

Tinospora cordifolia, Cissus quadrangularis, Datura stramonium, Acalypha indica, Calotropis gigantean, Heliotropium indicum, Paederia foetida, Eupatorium odoratum, Leucus lavandulaefolia, Wedelia calendulacea, Mentha arvensis, Bacopa monniera, Aristolochia indica, Cajanus cajan and *Citrullus colocynthesis.*

The results of the present study explored a number of very important research findings holding great importance in regards of preserving the local indigenous knowledge of medicinal plants used by the local people of Madhupur Tract and biotechnological approaches for micropropagation and conservation of medicinal plant in the area.

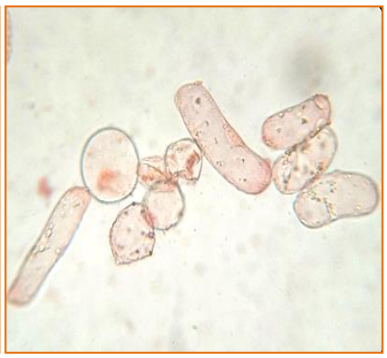
CHAPTER - III


MICROPROPAGATION
ARTIFICIAL SEED PRODUCTION
AND
CELL SUSPENSION CULTURE





Chapter III

MICROPROPAGATION, ARTIFICIAL SEED PRODUCTION AND CELL SUSPENTION CULTURE OF SELCTED MEDICINAL PLANTS

3.1. INTRODUCTION

Plant tissue culture is now a well established technology. Like many other technologies, it has gone through different stages of evolution, scientific curiosity, research tool, novel applications and mass exploitation. Initially, plant tissue culture was exploited as a research tool and focused on attempts to culture and study the development of small, isolated segments of plant tissues or isolated cells. Around the mid twentieth century, the notion that plants could be regenerated or multiplied from either callus or organ culture was widely accepted and practical application in the plant propagation industry was ensued. The technique was heralded as the universal mass clonal plant propagation system for the future and the term 'micropropagation' was introduced to describe more accurately the processes. Many commercial laboratories were established around the world for mass clonal propagation of horticultural and ornamental plants. Today plant tissue culture applications encompass much more than clonal propagation and micropropagation. The range of routine technologies has expanded to include somatic embryogenesis, somatic hybridization, virus elimination as well as the application of bioreactors to mass propagation. The list includes: clonal propagation, axillary shoot, multiplication, direct (adventitious) organogenesis, callus to organogenesis, somatic embryogenesis, virus elimination, *in vitro* grafting, *in vitro* gene banks, stock plant banks, somatic variation, managing 'natural' variation, induced mutation, *in vitro* screening and selection, anther or microspore culture-production of haploids, leading to double haploids, protoplast culture – somatic fusion, DNA transformation systems, recovery of regenerants from transformed cells, cell culture, biosynthesis in bioreactors (production of secondary metabolites).

3.1.1. Micropropagation of Medicinal Plants

A number of research works have been reported in tissue, cell and organ culture of medicinal plant which has given a dynamic venture particularly in the case of medicinal plants. Regeneration of *Centella asiatica* plants from non-embryogenic cell lines and evaluation of antibacterial and antifungal properties were performed from regenerated calli and plants (Bibi *et al*. 2011). Micropropagation of zedoary (*Curcuma zedoaria* Roscoe) – a valuable medicinal plant was also reported by Loc *et al*. (2005). Different *in vitro* culture protocols have been used for: *Zinziber officinale* (Bhagyalakshmi and Singh, 1988; Rout *et al*. 2000). *Cucurma longa* (Salvi *et al*.

2002) and *Curcuma zedoaria* (Stanly and Keng 2007). An efficient plant regeneration system for *Mucuna pruriens* L. using cotyledonary node explants (Faisal *et al*. 2006). Rapid and stable *in vitro* regeneration of plants through callus morphogenesis in two varieties of *Mucuna pruriens* L.–an anti Parkinson's drug yielding plant (Lahiri *et al*. 2012). Micropropagation of *Vitex negundo* through *in vitro* bud proliferation (Rathore and Shekhawat 2011).). High frequency induction of multiple shoots from nodal explants of *Vitex negundo* L. using sodium sulphate was obtained by Chandramu *et al*. (2003). Direct multiple shoot induction was achieved in excised nodal explants of *Plumbago indica* (Biswas 2006). Direct multiple shoot induction in excised nodal and shoot tip explants of *Aristolochia indica* was also reported (Siddique *et al*. 2006). Recent advances in the techniques and applications of plant cell culture and plant molecular biology have created unprecedented opportunities for the genetic manipulation of plants. The potential impact of these novel and powerful biotechnology on the genetic improvement of medicinal plants has generated considerable interest, enthusiasm and optimism in scientific community and is in part responsible for the rapid expansion of biotechnology industry over the world.

3.1.2. Artificial Seed Production

The artificial seed technology is an exciting and rapidly growing area of research in plant cell and tissue culture and unraveling new vistas in plant biotechnology. The idea of artificial seed was first conceived by Murashige *et al*. (1978) which was subsequently developed by several investigators.

Artificial seed production is a potential technique for plant multiplication and preservation, especially as it has been considered to be promising for propagation of non seed producing plants, transgenic plants and other plants that need to keep superior traits by means of asexual propagation (Saiprasad 2001). Plant artificial seed in a narrow sense, means the beads formed by encapsulating somatic embryo with coating materials. Its effect varied with different species, coating materials, maintained solutions and its concentration and condition (Nhut *et al*. 2005). Kamada (1985) presented a general concept of plant artificial seed, in which all kinds of plant explants with germination ability can be used for artificial seed production. Now a days, it is widely used in many plants (Slade *et al*. 1989; Fukai *et al*. 1994; Stephen and Jayabalan 2000; Ipekci and Gozukirmizi 2003; Halmagyi *et al*. 2004; Nhut *et al*. 2005). Artificial seed production is an outstanding technique used to propagate and preserve plants and has been applied on many plants (Wang and Qi 2010).

Development of efficient *in vitro* technique to ensure its safe conservation is therefore of paramount importance. Due to its ease of handling and quick of conversion to

plantlets, *in vitro* regenerated bulblet seems to be the suitable explant for germplasm preservation of garlic. *In vitro* bulblets formation of garlic largely dependent on growth regulators and sucrose in culture media (Matsubara and Chen 1989; Nagakubo *et al*. 1993), as well as other conditions such as cultivar, photoperiod and temperature. The further spreading and application of artificial seed is based on many factors, including the efficiency of the existing explants regeneration system, relative cost of a specific application for a given plant species. For example, the synthetic seed of seedless watermelon would be less costly than the conventional seed (Saiprasad 2001). Because garlic is sterile, it is vegetatively propagated by cloves and air bulbils (Shawky 2006). Encapsulation of *in vitro* grown bulblets of garlic is a suitable system for midterm storage of garlic tissue cultures since encapsulation saves space, time and resources and it demonstrates advantages over conventional method and this method considered a very good tool to exchange the garlic germplasm between countries and international plant gene banks. Huda and Bari (2007) found that artificial seed in eggplant can offer a new avenue for supporting the program of genetic engineering providing an exciting asexual propagation bridge for readily multiplication of transformed plants. Gona and Omid (2008) demonstrated that somatic embryos could successfully be converted to fully formed plants; this work should facilitate genetic transformation and artificial seed production in strawberry. It was successful in developing and optimizing a procedure of the production and storage of sweet corn synthetic seeds by encapsulating somatic embryos in calcium alginate hollow beads and retrieving plantlets, as an alternative sweet corn propagation practice (Thobunluepop *et al.* 2009). It was successful to production and storage of synthetic seeds in *Coelogyne breviscapa* (Mohanraj *et al*. 2009). Genetically uniform production of synthetic seeds of cucumber by standardizing culture conditions to induce somatic embryogenesis synchronously and at high frequency in cell suspension culture.

Since the production of genetically stable and true-to-type plant is desired for production of synthetic seeds, AFLP analysis is linked to ensure the genetic integrity of mother plants (Tabassum *et al*. 2010). Olive plantlets regenerated from synthetic seeds, and well developed root system was successfully acclimatized under *ex vitro* conditions. The protocol can be used for germplasm exchange of woody trees and preparation of synthetic seed (Muhammad *et al*. 2010). Ma *et al*. (2011) showed that *P. heterophylla* artificial seed production and the formation of *in vitro* plants derived from these synthetic seeds were feasible, and the benefit that could be conferred by their use would be very great. Faisal *et al*. (2012) has developed an efficient method

for plant regeneration and establishment from alginate encapsulated synthetic seeds of *Rauvolfia serpentina*.

3.1.3. Cell Suspension Culture

Biotechnological approaches, specifically plant tissue culture plays a vital role in search for alternatives to production of desirable medicinal compounds from plants (Rao and Ravishankar 2002). On a global scale, medicinal plants are mainly used as crude drugs and extracts. Several of the more potent and active substances are employed as isolated compounds, including many alkaloids such as morphine (pain killer), codeine (antitussive), papaverine (phosphordiesterase inhibitor), ephedrine (stimulant), ajmaline (antirrhythmic), quinine (antimalarial), reserpine (antihypertensive), galanthamine (acetylecholine esterase inhibitor), scopolamine (travel sickness), berberine (psoriasis), caffeine (stimulant), capsaicin (rheumatic pains), colchicines (gout), yohimbine (aphrodisiac), pilocarpine (glaucoma), and various types of cardiac glycosides (heart insufficiency) (Wink *et al*. 2005).

Plant tissue culture techniques offer the rare opportunity to tailor the chemical profile of a phytochemical product, by manipulation of the chemical or physical microenvironment, to produce a compound of potentially more value for human use. Intensive activity has centered on production of natural drugs or chemoprotective compounds from plant cell culture. Some of the most prominent pharmaceutical products in this latter category include ajmalicine (a drug for circulatory problems) from *C. roseus* and taxol (a phytochemical effective in treatment of ovarian cancer) from *Taxus* species. Chemical synthesis of natural products is possible and commercially feasible, particularly for those with relatively simple chemical structures such as aspirin (derived from the natural product salicylic acid) and ephedrine (Wink *et al*. 2005). In many cases, however, the metabolite has a complex structure, which can include multiple rings and chiral centers, so that a synthetic production process becomes prohibitively costly. Many natural products used in cancer treatment, including compounds such as paclitaxel, vinblastine, and camptothecin, fall into this latter class, so an alternative method of supply is necessary like cell culture. There has been considerable interest in plant cell cultures as a potential alternative to traditional agriculture for the industrial production of secondary metabolites (Dicosmo and Misawa 1995). Recent research results indicate that plant cell suspension cells can be used for recombinant protein production under controlled conditions (Fischer *et al*. 1999).

3.1.4. Objectives

Micropropagation

Five important medicinal plant species of Madhupur tract namely *C. asiatica, C. benghalensis, C. zedoaria, M. pruriens and V. negundo* were selected for conducting experiments with the following objectives:

i) Identification and selection of suitable explants for rapid and large – scale propagation.
ii) Selection and standardization of appropriate media composition, growth regulator requirement and culture environment for regular high production of plantlets.
iii) Acclimatization and transplantation of *in vitro* grown plants into the soil for raising under natural condition.

Artificial seed production

The objectives of this part of investigation were to standardize artificial seed production technologies for the selected medicinal plant species *viz*., *Centella asiatica* and *Rauvolfia serpentina* grown in Madhupur Tract.

Cell suspension culture

Following important medicinal plants *viz*., *Centella asiatica*, *Coccinia cordifolia*, *Mucuna pruriens* and *Rauvolfia serpentina* grown in Madhupur Tract were selected to study the different aspects of cell suspension culture with some specific objectives.

1. Standardize the suitable culture media for the establishment of cell suspension culture of four selected medicinal plants growing in the Madhupur Tract.
2. Formulate the suitable hormone concentration for cell culture and their maintenance in the growth condition.
3. Determine the peak growth period and its perfect extent for different plant species used in the present experiment.

3.2. MATERIALS AND METHODS

3.2.1. Micropropagation

3.2.1.1. *Materials*

To conduct the present investigation following materials and equipments were used.

A. Plant materials

The five different medicinal plants (*Centella asiatica*, *Commelina benghalensis*, *Curcuma zedoaria*, *Mucuna pruriens* and *Vitex negundo*) were used as experimental materials for *in vitro* propagation.

Table 3.2.1. A brief description of medicinal plants selected for *in vitro* propagation, artificial seed production and cell suspention culture.

1	**Scientific Name:** *Centella asiatica* (L.) Urban. **Local Name:** Thankuni, Tehagorerpata **English Name:** Asiatic pennywort, Indian pennywort,wild violet, and tiger herb **Family:** Apiaceae (Umbelliferae) **Parts used:** Leaves  **Medicinal uses in the Local people**: Indigestion, stomach infection, skin disease, dysentery and convulsive disorders or antispasmodic.
2	**Scientific Name:** *Commelina benghalensis* L. **Local Name:** Dolpata, Kanchira, Kanaibashi **English Name:** Benghal dayflower or Dew flower **Family:** Commelinaceae **Parts used:** Whole plant  **Medicinal uses in the Local people**: Useful in leprosy, sores, itches, swellings, burns, boils, leucorrhoea, urinary burning, gonorrhoea, cold and ulcer.
3	**Scientific Name:** *Curcuma zedoaria* Rosc. **Local Name:** Shoti **English Name:** Zedoary, Indian arrowroot **Family:** Zingiberaceae **Parts used:** Rhizome **Medicinal uses in the Local people**: Blood purifier, Stimulant, Weakness, Cough, Dropsy, Sores, stomach pain.   Rhizome

4	**Scientifica Name:** *Mucuna pruriens* Baker **Loacal Name:** Alkushi, Bilaihunhungi, Bilaiachra **English Name:** Velvet bean **Family:** Fabaceae **Parts used:** Seed **Medicinal uses in the Local people**: Anthelmintic. Seeds are aphrodisiac and nervine tonic.	 
5	**Scientifica Name:** *Vitex negundo* L. **Loacal Name:** Nishinda, Samalu **English Name:** Chaste Tree **Family:** Verbenaceae **Parts used:** Leaves **Medicinal uses in the Local people**: Tonic, rheumatism, worm,	
6	**Scientifica Name:** *Rauvolfia serpentine* (L.) Benth. **Loacal Name:** Sarpagondha **English Name:** Snake-root **Family:** Apocynaceae **Parts used:** Root **Medicinal uses in the Local people:** High blood pressure.	
7	**Scientifica Name:** *Coccinia cordifolia* (L.) Cogn. **Loacal Name:** Telakuch, **English Name:** Ivy gourd **Family:** Cucurbitaceae **Parts used:** Leaves and root **Medicinal uses in the Local people:** Mild diabetes, Stomach upset, anorexia, ear pain.	

Different types of plant propagules (seedling, seeds, rhizome and rootstock) were collected from natural habitats during survey and planted in the experimental field of the Institute of Biological Sciences, Rajshahi University. Young shoots were used as

explant source. Immature leaf, shoot tip, nodal and internodal explants of two month old twigs were used as explants for direct and indirect organogenesis.

B. Nutrition medium

All the cultures were grown on MS (Murashige and Skoog 1962) medium (**Appendix 2**) for the purpose of multiple shoot induction, callus induction, shoot regeneration from callus and root initiation.

C. Plant growth regulators

The basal media were supplemented with various concentrations of different plant growth regulators. The following growth regulators were employed for the present investigation.

Auxins: Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA), α-naphthalene acetic acid (NAA), 2,4-dichlorophenoxy acetic acid (2,4-D).

Cytokinins: 6-benzylaminopurine (BAP), 6-furfuryl aminopurine (Kinetin, Kn)

Gibberellins: Gibberellic acid (GA_3)

D. Laboratory equipments

For media preparation: Different types of glass vessels including culture bottles, test tube, conical flask with plugs, beakers, petridishes of various capacities measuring cylinders, pipette pump, parafilm, aluminium foils, marker pen, hot plate, magnetic stirrer, analytical loading single pan balance with precision of ± 0.001g, refrigerator, electric hot air oven range up to 250 ± 2ºC digital pH meter, autoclave preferably horizontal, continuous of single and double distilled water.

Chemicals: Inorganic and organic salts, vitamins, amino acids, growth regulators/hormones sucrose and agar or gelrite etc.

Instrument for aseptic transfer: Autoclave, laminar airflow cabinets, scissors and scalpel handles with blades, forceps of various size and spirit lamp, 95% and 70% alcohols.

For incubation: Racks with light arrangements (16h light and 8h dark) and controlled temperature 20 ±2ºC maintained with air cooler.

E. Sterilizing and cleaning agents

In the present investigation mercuric chloride ($HgCl_2$) and ethanol (95% and 70%) were used as sterilizing agents. Tween 80 and Savlon were used as cleaning agents.

3.2.1.2. *Methods*

A. Preparation of culture media

In order to prepare different media stock solutions of salts, organic compounds and growth regulators were first prepared taking different combinations of chemicals.

B. Preparation of stock solution

Different constituents of the culture media formulations were prepared into stock solutions as macronutrient, micronutrient, organic components and growth regulators separately for ready use during the preparation of culture media.

a. Stock solution of macronutrients (Solution A)

The stock solution of macro salts was prepared at 20X that of the required concentration. Required amount of all the macro salt components prescribes for a particular medium formulation was weighed accurately with electronic balance and dissolved separately in substantial volume of double autoclave distilled water (DDW). The solutions were sequentially poured into a 1 litre volumetric flask. Final volume of the solution was made into 1 litre by adding sufficient amount of DDW. Special care was taken during dissolving calcium chloride ($CaCl_2$). The solution after filtering through Whatman No. 1 filter paper was poured into clean plastic bottle and stored into refrigerator at 4ºC.

b. Stock solution of micronutrients

Two separate stock solutions of micro salts were prepared as follows:

(i) Stock solution of $FeSO_4$ and Na-EDTA (Solution B)

This solution was prepared at 20X to that of required concentration. Requisite amount of $FeSO_4$ and Na-EDTA were taken and dissolved separately into clean glass beakers containing 225 ml of DDW. Na-EDTA solution was then transferred in a 500 ml volumetric flask. Subsequently the solution of $FeSO_4$ was poured to the volumetric flask slowly with constant stirring. The final volume of the solution was made up to 500 ml. The pH of the solution was adjusted to 5.7 and after filtering it was stored at 4-6ºC in refrigerator.

(ii) Stock solution of rest of the micronutrients (Solution C)

Stock solution of micronutrients was made at 20 and 200X in 500 ml DDW. All components were weighed (except $CaCl_2$) separately and dissolved in 400 ml of DDW. $CaCl_2$ was dissolved separately and added to the solution. Finally, the volume of the solution was adjusted up to 500 ml and after filtering stored at 4-6ºC in a plastic bottle.

c. Stock solution of organic nutrients (Solution D)

Organic components were made into stock solution separately. Ten times of each of the require ingredients were taken in a measuring cylinder and dissolved in 100 ml of distilled water. Then the stock solution was labeled and stored in refrigerator at 4ºC for several weeks.

d. Stock solution of plant growth regulators

Stock solution of different growth regulators was prepared separately. Details of the methods of preparation of stock solution are given in the **Table 3.2.1.**

Table 3.2.2. Different plant growth regulators and their solvents.

Growth regulators	Amount taken (mg)	Solvents (ml)	Final volume of the solution with distilled water (ml)	Strength of the stock solution (mg/ml)
IAA	10	0.1 N NaOH 1 ml	100	0.1
IBA	10	0.1 N NaOH 1 ml	100	0.1
NAA	10	0.1 N NaOH 1 ml	100	0.1
2,4-D	10	70% EtOH 0.5 ml	100	0.1
BAP	10	0.1 N NaOH 1 ml	100	0.1
Kn	10	0.1 N NaOH 1 ml	100	0.1
GA_3	10	70% EtOH 1 ml	100	0.1

To prepare stock solution of any of these growth regulators,10 mg of powder growth regulators was taken in a clean test tube and dissolved in required volume of appropriate solvent. The final volume of the solution was then made to 100 ml adding DDW. The solution was then poured into a 100 ml glass reagent bottle and stored at 4-6 ºC.

C. Preparation of culture medium

The following steps were followed to make one litre of MS medium:

i) 30 g of sucrose was dissolved in 500 ml of distilled water and filtered in a 1000 ml volumetric flask.
ii) 100 ml stock solution of macronutrients, 10 ml from each of the micronutrients stock solutions and 10 ml from each vitamin stock solutions were added to the sucrose solution and mixed them well.
iii) Different concentrations of hormonal supplements were added either singly or in different concentrations to the solution were mixed thoroughly. Since each hormonal stock solution contained 10 mg of the chemical in 10 ml of stock solution, the addition of 1 ml of any hormonal stock solution to prepare 1 litre of medium resulted in 1.0 mg/ml concentration of that particular hormone.
iv) The whole mixture was then made up to 1 litre by further addition of distilled water.

v) The pH of the medium was adjusted to 5.8 ± 0.1 using a pH meter with the help of 0.1 N NaOH or 0.1 N HCl whichever necessary.

vi) The required quantities of agar were added to the medium and the whole mixture was then gently heated in a microwave oven till complete dissolution of agar.

vii) Requisite volume of the medium (while still hot) was dispensed into culture vessels (15-20 ml/25×150mm^2 test tube, 20-25 ml/150 ml conical flask and 45-50 ml/250 ml conical flask or culture bottle) of varying sizes. The culture vessels were plugged with non absorbent cotton plugs or with plastic caps, which were inserted tightly at the mouth of the culture vessels and marked with different codes with the help of glass marker pen to indicate specific hormonal supplements.

viii) Finally, the culture vessels with medium were then autoclave at 120ºC for 20 minutes at 1.1 kg/cm^2 pressure. After sterilization the medium was allowed to cool and stored in the culture room (not more than a week) for ready use.

D. Preparation of surface sterilizing solution

In this experiment, mercuric chloride ($HgCl_2$) solution [0.1% (w/v)] was used as surface sterilizing agent. To prepare 0.1% solution, 0.1 g of $HgCl_2$ was taken in a 500 ml conical flask and dissolved in 100 ml DW. Freshly prepared $HgCl_2$ was always used. Generally $HgCl_2$ solution was prepared 15-30 minutes before use.

E. Precautions to ensure aseptic condition

To ensure aseptic inoculation laminar airflow cabinet was made germ free by UV-ray for at least 30 minutes before use and clean with 95% ethyl alcohol to overcome the contamination. All instruments were sterilized by alcoholic dip and flaming method inside the laminar airflow cabinet. The metallic instruments were kept immerge in 95% alcohol in a glass jar inside the cabinet. Other requirements such as petridish, conical flask, cotton, distilled water etc were sterilized by autoclaving. Both the hands were also rinse with 75% alcohol.

F. Culture technique

The following techniques were employed in the present investigation for induction and maintenance of callus induction, shoot induction, root induction as well as regeneration of complete plantlets:

a) Surface sterilization of plant materials
b) Preparation of explants and inoculation
c) Incubation
d) Subculture

a) Surface sterilization of plant materials

Juvenile twigs were collected and thoroughly washed under running tap water. The materials were then separated into short pieces (shoot tips, nodal segments, leaves and internodal segments); surface sterilized with 1% savlon few drops 80% tween for 5-10 minutes with constant shacking. The materials were then washed 3-4 times with distilled water for complete removal of detergent and taken under running laminar airflow cabinet and transferred 500 ml sterilized conical flask. After rinsing with 70% ethanol for less than 1 minute, they were immersed in 0.1% HgCl2 for different duration of time. To remove every trace of the sterilant, the plant material were washed 3-4 times with sterile distilled water.

b) Preparation of explants and inoculation

Explants were laid on the sterile petridish using sterile forceps. During this action hands were made sterile as far as possible with absolute alcohol to avoid contamination.

i) Shoot tip: shoot tips of approximately 1 to 2 cm in length were cut from the sterilized shoot of mature plants and then inoculated into the culture bottle/test tubes having different concentrations and combinations of hormones.
ii) Nodal segments: Following same way explants with 1-2 nodes were cut from the sterilized shoots and were inoculated into culture bottles/test tubes.
iii) Internodal segments: In the same way mentioned above internodal segments approximately 1 cm in length were cut from the shoots and were inoculated into culture bottles/test tubes.
iv) Leaves: In the same way mentioned above tender leaves were cut and placed into culture bottles.

c) Incubation

The culture tubes containing inoculation were incubated to light in the growth chamber. The growth chamber maintained at 16 hrs photoperiod with a light intensity of 2000-3000 lux (50-70µ $E.m^{-2}S^{-1}$) provided by 40W cool-white florescent tubes. The temperature of the incubation chamber was 22±2ºC but humidity was not controlled for any of the experiments.

d) Subculture techniques

The following *in vitro* culture techniques were employed during the course of culturing of different explants.

i) Subculture maintenance: Proliferated multiple shoots were rescued very carefully in the aseptic conditions and divided into clusters of 2 – 3 shoots using a sterile sharp scalpel. Then they were transferred to same or different media or further response.
ii) Root induction: The shoots with 2 – 4 cm in length grown on the multiplication media were separated aseptically from the shoot mass, basal

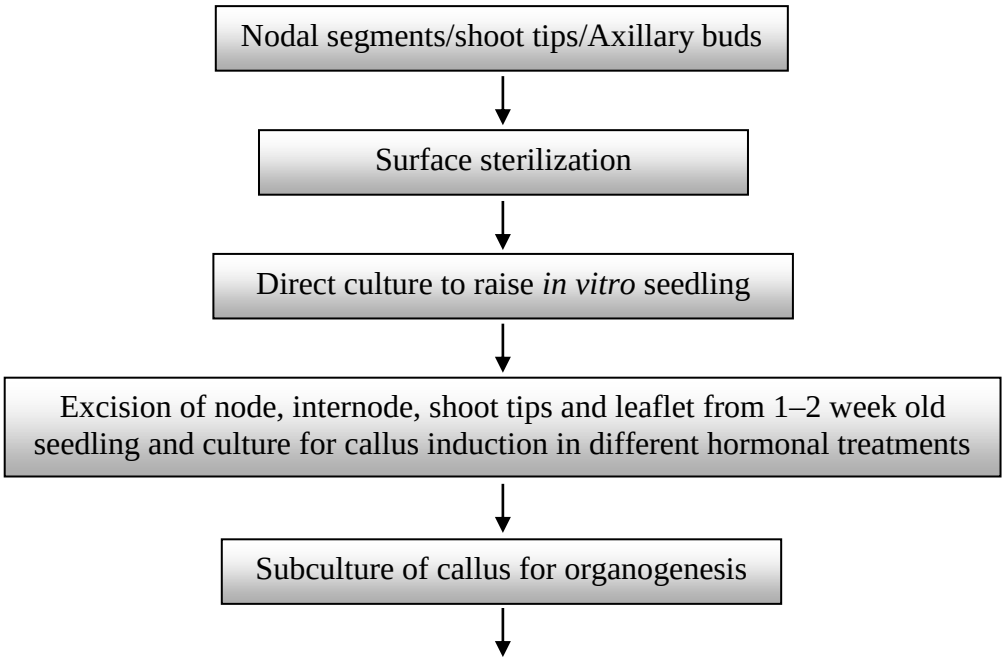
leaves snapped off and 2-3 cm microcuttings were prepared. The individual microcuttings were then transferred on to the freshly prepared rooting media supplemented with different concentrations and combinations of phytohormones for root induction.

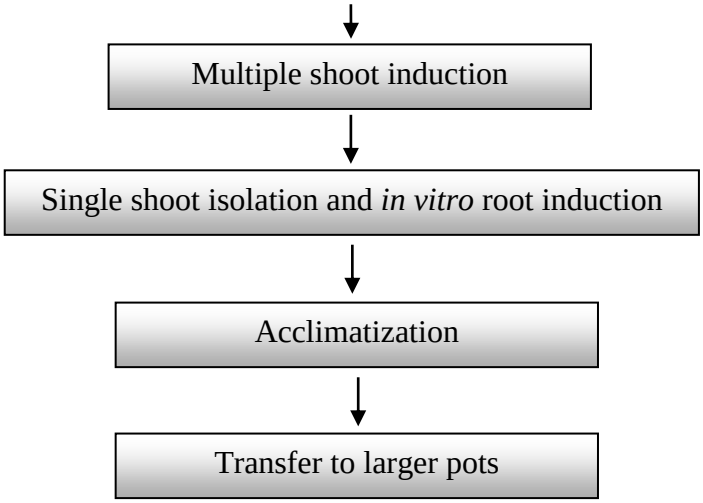
iii) Callus induction: The *in vitro* grown shoots of 3 – 5 cm in length were rescued aseptically from the culture vessels and internodes as well leaves were cultured on freshly prepared medium containing different concentrations and combinations of hormonal supplements for callus induction.

3.2.1.3. *Transplantation*

After sufficient growth of shoot and root systems, the plantlets were considered ready to transfer in soil. The plantlets grown inside the test tubes/flasks were brought out of the control environment of growth chamber and were kept in the room temperature from 5 – 7 days to bring them in contract of normal temperature. The plantlets were then rescued very carefully from the culture vessels. Agar attached to the root system was gently washed out under running tap water. Then the plantlets were transplanted to small polythene bags containing garden soil and compost in the ratios of 2:1. The soil substance was treated with 0.1% Agrosan (fungicide) solution. Immediately after transplantation, the plantlets along with the polythene bags were covered with a large moist polythene bag to prevent desiccation. To obtain higher humidity around the plantlets, all the bags were checked up and the interior of the polythene bags were sprayed with water at every 24 hours. The polythene bags were gradually perforated to expose the plantlets to outer environment and subsequently removed after 10 days. By this time new leaves emerged out and the regenerants became established in the soil being complete plantlets. They were then transferred to garden soil.

3.2.1.4. *Flow chart for direct and indirect plant regeneration*





3.2.1.5. *Data recording and analysis*

(a) Data collection

Data were collected using the following parameters and the methods followed for data collection are given below:

i) Average number of shoots and length of the longest shoots: Number of multiple shoots per explant was counted after 4 – 6 weeks of inoculation. Average number of shoots per explant was calculated using the following formula.

$$\bar{X} = \frac{\sum Xi}{N}$$

Where, $\bar{X}$ = Average number of shoots

Σ = Summation

Xi =Total number of shoots

N = Number of observation

ii) Shoot height was recorded separately with meter scale after 4 – 6 weeks of inoculation: Average height of the longest shoots was calculated by the following formula.

$$\bar{X} = \frac{\sum Xi}{N}$$

Where, $\bar{X}$ = Average length of the longest shoots

Σ = Summation

Xi =Total length of the longest shoots

N = Number of observation

i) Average number of roots per shoot and length of the longest shoots: Numbers of main roots were counted after 4 – 6 weeks of inoculation. Average number of roots per shoot was calculated using the following formula.

$$\bar{X} = \frac{\sum Xi}{N}$$

Where, $\bar{X}$ = Average number of main roots

Σ = Summation

Xi =Total number of main roots

N = Number of observation

Average length of the longest roots was calculated by the formula used in the shoot length calculation.

(b) Data recorded

Data were recorded on callus induction and plant regeneration frequency (%), development of no. of shoots per calli and length of the longest regenerated shoot (cm). Callus induction and plant regeneration frequency (%) were calculated using formula below:

$$Callus\ induction\ frequency\ (\%) = \frac{No.\ of explants\ responsed\ to\ callus\ initiation}{No.\ of explants\ cultured} \times 100$$

$$Plant\ regeneration frequency(\%) = \frac{No.\ of callus\ formed\ shoots}{No.\ of calluscultured} \times 100$$

(c) Standard Error (SE):

Standard Error was calculated according to the following formula:

$$\text{Standard Deviation } (SD) = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}}$$

$$\text{Standard Error } (SE) = \frac{SD}{\sqrt{n}}$$

(d) Statistical analysis

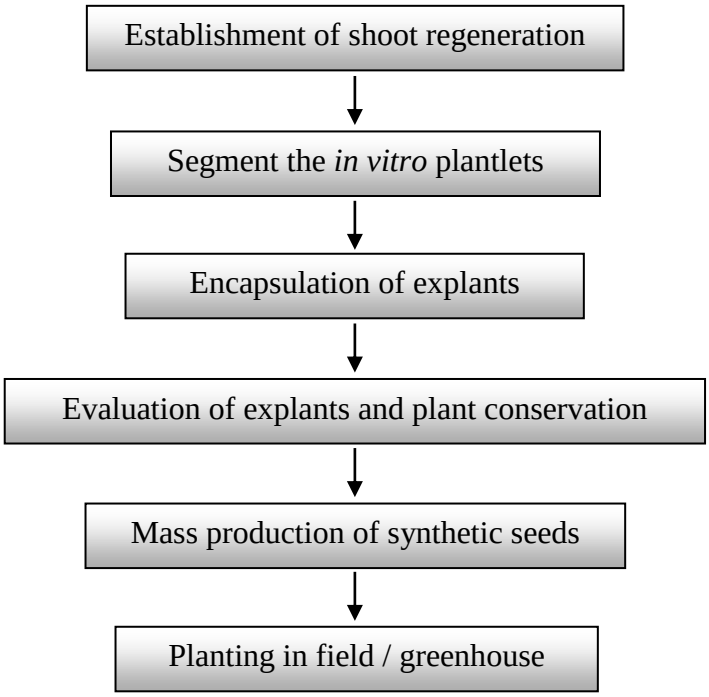
Experiments were consisted of 10 – 15 explants and each of the experiment repeated thrice and mean values were calculated separately for each replication.

3.2.2. Artificial Seed Production

3.2.2.1. *Plant materials*

Shoot tip and nodal segments of *C. asiatica* and *R. serpentina* were used as explants in this investigation for artificial seed production. Shoot tips and nodal segments 3-5 mm long were aseptically excised from *in vitro* cultured plants regenerated by the method described by Maruyama 1996.

Procedure for production of artificial seeds:



3.2.2.2. *Methods*

Sodium alginate beads were produced by encapsulation according to the method of Kinoshita and Satio (1990). The method involved in this investigation can be described under following heads:

A. Media preparation

Culture media were prepared following MS (Murashige and Skoog 1962) medium preparation technique and agar was added at the rate of 8 gm/l and then autoclaved for 20 minutes under 121ºC temperature.

B. Preparation of solution for encapsulation of artificial seed

Following steps were taken for the preparation of solution for explants encapsulation:

i) Media preparation

200 ml ½ strength MS medium was prepared and 0.8 gm. sucrose was first added to 150 ml of ½ MS and then different concentrations of hormones i.e. 0.5, 1.0, 1.5, 2.0

and 2.5 mg/l BAP, Kn and NAA were also added to it. After mixing the solution it was filled up to 200 ml.

ii) Alginate solution

20 ml of the above mentioned solution was taken with required growth regulator. Then 0.8 gm (800 mg) of sodium alginate was also added and taken in a small beaker (50 ml beaker). With a small piece of glass rod efforts were made to mix the alginate in solution. Alginate was partially dissolved and it was taken kept aside. During autoclaving alginate was completely dissolved.

iii) $CaCl_2$ solution

50 ml of above mentioned solution (½ MS + Sugar + hormones) was taken in a small beaker. An amount of 0.7gm (700 mg) $CaCl_2$ was added to it and dissolved. Out of 200 ml. ½ strength solution, 70 ml (50 + 20) was used during the preparations of alginate and $CaCl_2$ and another 130 ml remained reserved which after autoclaving it was used during washing the encapsulated explants.

iv) Autoclaving

Culture flask containing different solutions, alginate solution, $CaCl_2$ solution and several petridishes were autoclaved at 120ºC for 20 minutes at 1.1 kg/cm^2 pressure.

3.2.2.3. *Encapsulation of explants*

Explants were taken in an autoclaved petridishes and nodal segments, shoot tips were cut carefully removing the intermodal zones. The nodal segments with active buds and shoot tip were placed to the beaker containing alginate solution. The buds were dipped in alginate solution. The dipping explants were taken by a forcep and placed to the beaker of $CaCl_2$. During picking up the explants, the forceps also took some addition alginate solution together with explants. The rolling explants with the liquid alginate were dropped into the $CaCl_2$ solution. The explants were kept inside the alginate solution for about 30 minutes. After 30 minutes each explant become a hardball encoated by alginate. They were then washed well with remaining solution of MS + Sucrose + hormones (Different concentrations and combinations of BAP, Kn and NAA).

3.2.2.4. *Storage of artificial seeds and plants recovery*

Artificial seeds both of *C. asiatica* and *R. serpentina* were kept in storage under growth chamber at 20 ± 2ºC, in refrigerator at 4 ± 1ºC and at -1 ± 1ºC for survival test. Both shoot tip and nodal encapsulated artificial seeds were used for this survival test. At the end of each storage period (7, 15, 30, 45, 60 and 75 days), the artificial seeds were immediately transferred to fresh germination medium supplemented with and without phytohormones for shoot and root development.

3.2.2.5. *Inoculation of encapsulated explants*

The encapsulated explants or synthetic seeds were washed with 130 ml half strength MS liquid medium (as mentioned above). After washing the synthetic seeds were placed on right culture medium for subsequent growth.

3.2.2.6. *Culture incubation*

Inoculated glass vessels were incubated in growth chamber providing a special culture environment. In growth chamber photoperiod was maintained generally 16 hours light 8 hours dark. The culture vessels were checked daily to note the germination.

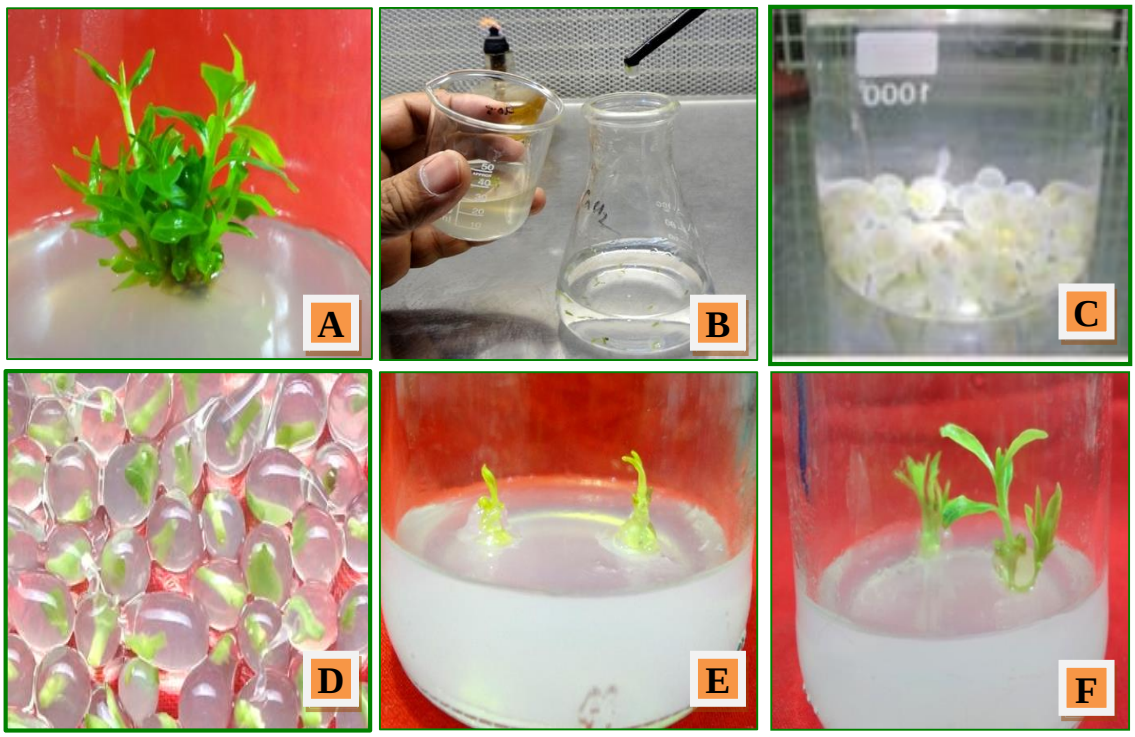


Plate 3.2.1. Procedure of artificial seed production and germination.

A: Source of explant for artificial seeds; **B:** Shoot tip explants mixed with sodium alginate and encapsulated seed dropped in $CaCl_2$ solution; **C:** Artificial seeds rinsed in distilled water; **D:** Artificial seeds; **E:** Spouting of artificial seeds; **F:** Further development of artificial seeds.

3.2.2.7. *Data collection*

Percentage of germination: Percentage of germination of synthetic seed was calculated using the following formula:

$$\%\ of\ germination = \frac{No.of\ germinated\ seed}{No.of\ seed\ inoculated} \times 100$$

No. of shoot: Number of shoots per explant was counted after 4 weeks of culture. Average number of shoots per explants was calculated using following formula:

$$Average\ number\ of\ shoot = \frac{Total\ number\ of\ shoots}{No.of\ germinated\ seeds}$$

Shoot length: Length of shoot was measured in cm. For each plant average length of shoot was calculated using following formula:

$$\text{Average lenght of shoot} = \frac{\text{Total lenght of shoots}}{\text{No.of shoots}}$$

3.2.3. Cell Suspension Culture

3.2.3.1. *Materials*

Pre established 10-12 day old friable callus containing rapid dividing cells were used as explants in this investigation. These calli were collected from *in vitro* cultured callus and plant tissue of *C. asiatica*, *C. cordifolia*, *M. pruriens* and *R. serpentina*.

A. Culture media

MS (Murashig and Skoog 1962) based medium was used for the purpose of cell culture.

B. Laboratory equipments

i) For media preparation: Different types of glass vessels including conical flask with plugs, beakers, measuring cylinders and pipettes each of various capacities were used for cell culture.

ii) Chemicals: Organic and inorganic salts, vitamins, amino acids, growth regulators, sucrose and agar.

iii) Instruments for aseptic transfer and culture: Autoclave, laminar airflow cabinet, forceps of various sizes, pipettes of 5 ml, petridish, 100 µ diameter pore sieve, rotary shaker.

iv) For incubation: Racks with light arrangement (16 h light and 18 h dark) and controlled temperature 26 ± 2 maintained with air cooler.

3.2.3.2. *Methods*

A. Preparation of culture media

a) Liquid media preparation for cell isolation

Liquid media were prepared following MS medium preparation without adding agar and then autoclaved for 20 minutes at 1.1 kg/cm^2 pressure under 121°C temperature.

b) Solid media preparation for cell culture

Solid media for cell culture were prepared following the methods MS medium preparation when agar was used to solidify the medium and then autoclaved at 1.1 kg/cm^2 pressure under 121°C temperature for 20 minutes.

B. Cell isolation and culture

a) Cell isolation from friable callus

Suspension culture can be initiated either from pre-established callus culture or from the explant directly into liquid medium which is continuously agitated on a moving elliptical or rotary shaker. The movement of the nutrient medium provides vital aeration of the medium to sustain cell respiration in the liquid medium and also encourages the callus tissue to break up.

In this investigation, to isolate the cells, most common friable callus was transferred to agitate liquid medium where it was broken up and readily dispersed. In this purpose, at first friable callus was developed using the conventional method. Then this friable callus was transferred into the 250 ml conical flask containing 75 ml liquid (medium without agar) medium. Then the flasks were placed on the shaker running under 200 rpm. After 6 – 7 days, cells were isolated. At last the single cells were filtrated using sieves with 500 μ diameter pores. The used steps to get single cells are mentioned bellow:

1. 250 ml conical flasks were cleaned and made ready for culture and 75 ml of liquid MS medium was taken in each of the conical flask.
2. The flasks containing medium were autoclaved at 20 minutes under 1.1 kg/cm^2 and 121°C temperature.
3. After autoclaving, 4 – 5 pieces (about 1.5 gm) of pre-established friable calli were transferred aseptically to the flasks.
4. The mouth of the flask was closed with a piece of aluminum foil followed by a piece of brown paper.
5. The flasks were placed within the clamps of a rotary shaker moving at the 200 rpm.
6. After 7 days the contents of each flask were poured through a sterilized sieve (500μ) and the filtrate was collected in a sterilized container. This time, the filtrate contained only free cells.

To ensure single cell suspension culture the filtrate was taken on a slide for microscopic observation. Under microscope the individual cell structure, with different shapes and divisions, were identified and their photographs were taken for each of the slides.

b) Culture of isolated single cells

Free cells isolated from callus were grown as single cells under *in vitro* conditions using MS medium supplemented by different concentrations and combinations of hormones to observe the growth/division pattern of cells. The following steps were taken for this investigation:

1. The pre-filtrate containing single cells was allowed to settle for 20 – 25 minutes and finally poured off the supernatant.
2. Using the sterilized pipette, 5 ml residue cells re-suspended into the fresh liquid medium and dispensed the cells equally in several sterilized flasks of 250 ml contained 70 ml liquid medium.
3. The flasks containing cells were placed on shaker with 200 rpm and allowed the free cells to grow.
4. To prepare a growth curve of dividing cells, the weight was taken 9 times from each flask separately.

3.2.3.3. *Data collection for cell weight*

The fresh weight of cells was taken carefully on the running laminar airflow cabinet by electric balance using filter paper and media filter sterilizer. At first the weights of filter paper was taken and this was taken on the tip of media filter sterilizer. Then the 10 ml growing cells was taken from liquid culture by a sterile pipette and was taken on the filter paper which was taken before. Then the media filter sterilizer was started. After sometimes, when the cell were dried and shown as powder then the weight was taken of the dry cells with filter paper. Then the weight of filter paper was debited from the weight of filter paper and dry cells weight. The weights were taken every after four days of culture.

3.3. RESULTS

3.3.1. Micropropagation

3.3.1.1. *Micropropagation of Centella asiatica*

Experiment on direct and indirect organogenesis were carried out using different types of experiments *viz.* shoot apex, node, internode, leaf from two months old field grown plants. Surface sterilization was done separately according to the explant types. In case of nodal and internodal segments effective surface sterilization was performed with 0.1% $HgCl_2$ for 4 – 5 minutes. On the other hand, shoot apex, leaf explant took 3 – 4 minutes for contamination free culture in the same concentrations of $HgCl_2$. Sterilized explants were then cut into appropriate size (1.0 – 2.0 cm) and cultured on plant growth regulators (PGR) supplemented media for induction of direct and indirect organogenesis. Both auxins (IAA, NAA, IBA and 2,4-D) and cytokinins (BAP and Kn) were used in the media either singly or in combinations. For root induction, micro shoots obtained from direct and indirect organogenesis were transferred to rooting media. Finally well developed plants with both shoots and roots were transferred to natural conditions through successive phages of acclimatization. The results of *in vitro* culture of direct and indirect organogenesis are described below:

A. Direct shoot regeneration in *C. asiatica*.

Two types of explants *viz.* shoot apex and nodal explants were cultured for direct shoot regeneration. Explants were cultured on MS medium with BAP and Kn used alone and in combinations with each other or with IAA. Data on number of days taken for shoot initiation, percentage of explant induced shoot development, average number of shoot per culture and average length of shoot per culture were collected after 6 weeks of culture. Shoot apex and nodal explants responded on almost all of supplemented cultured medium. The results are described according to types of explants under separate heads:

(a) Direct multiple shoot induction in nodal explants of *C. asiatica*

Nodal explants of two months old *C. asiatica* were aseptically cultured on different concentrations (0.2 – 3.0 mg/l) of BAP and Kn either alone or in combinations with different concentrations (0.2 – 2.0 mg/l) of IAA. Nodal explants found to be most suitable for multiple shoot induction on almost all of the supplemented cultured media but morphogenic responses of cultured explants varied depending upon the culture media formulation. Effects of different concentrations and combinations of auxins and cytokinins on multiple shoot induction from nodal explants are described below:

Experiment 1: Effect of BAP and Kn singly and in combination on multiple shoot induction

In this present investigation six concentrations (0.2, 0.5, 1.0, 2.0, 3.0 and 4.0 mg/l) of BAP and Kn used alone and in combination of three concentrations (0.5, 1.0 and 2.0 mg/l) of BAP with four concentrations (0.2, 0.5, 1.0 and 2.0 mg/l) of Kn were treated in MS medium for the purpose of multiple shoot induction from nodal explants of *C. asiatica*. Data were taken after 6 weeks of inoculation and percentage of shoot formation, number of shoot/culture and length of shoot/culture were measured. The results are presented in **Table 3.3.1**. Morphogenic responses of the explants were found to vary with hormonal formulations present in the culture media. Shoot proliferation ranged from 60 – 100%. Highest percentage (100%) of multiple shoot formation was observed in MS medium containing 2.0 mg/l Kn followed by 96.66% in MS medium containing 1.0 mg/l BAP + 1.0 mg/l Kn. The lowest percentage (53.33%) of multiple shoot formation was observed in media having 0.2 mg/l BAP. Highest mean number of shoots was 7.84 ± 0.32 in MS medium having 2.0 mg/l Kn. Lowest mean number of shoot was 1.62 ± 0.04 in media containing 0.2 mg/l BAP. Average length of shoots gradually increased after induction of shoot. Highest average length was recorded 2.22 ± 0.14 cm in 2.0 mg/l Kn followed by 2.13 ± 0.17 cm in 1.0 mg/l BAP + 1.0 mg/l Kn. The lowest average length was 0.64 ± 0.12 cm in 0.2 mg/l BAP. Experiment result revealed that 2.0 mg/l Kn alone and combination of 1.0 mg/l BAP + 1.0 mg/l Kn were found most effective concentrations for multiple shoot induction in *C. asiatica*.

Experiment 2: Effect of different concentrations and combinations of BAP and Kn with IAA on multiple shoot induction

Explants were cultured on MS medium supplemented with three concentrations of BAP (1.0, 2.0 and 3.0 mg/l) and four concentrations of Kn (0.5, 1.0, 2.0 and 3.0 mg/l) combined with different concentrations of IAA (0.2, 0.5, 1.0 and 2.0 mg/l). Data were recorded after 6 weeks of culture and results on different parameters are presented in the **Table 3.3.2**. The efficiency of BAP+IAA was not better than BAP alone. The efficiency of BAP+IAA was inferior to the efficiency of Kn+IAA. Among the concentrations of BAP+IAA the highest percentage (100%) of shoot proliferation was noted in the media having 3.0 mg/l BAP+ 0.5 mg/l IAA. The lowest percentage (76.66%) of shoot proliferation was noted in 1.0 mg/l BAP + 0.5 mg/l IAA. Highest mean number of shoot per culture was 4.33 ± 0.12 in media having 3.0 mg/l BAP+ 0.5 mg/l IAA followed by 3.87 ± 0.12 in media having 2.0 mg/l BAP + 0.5 mg/l IAA. Lowest mean number of shoot per culture was 1.92 ± 0.07 in media having 3.0 mg/l BAP + 2.0 mg/l IAA. Highest average length of shoot per culture was 2.16 ± 0.25 cm in the media having 3.0 mg/l BAP+ 0.5 mg/l IAA and lowest average length of shoot per culture 0.60 ± 0.07 cm in the media containing 1.0 mg/l BAP+ 0.2 mg/l IAA (**Plate 3.3.1**).

On the other hand the efficiency of Kn+IAA was better than BAP and Kn alone and BAP+IAA on direct shoot regeneration. All the used media compositions formed multiple shoots and the results were superior to the result obtained from shoot apex explants. Addition of lower concentration of IAA along with higher concentration of Kn was found more suitable than that of other concentrations. Among the combinations of Kn+IAA the highest percentage (100%) of shoot proliferation was noted in MS medium containing 2.0 mg/l Kn + 0.5 mg/l IAA and followed by (96.67%) in MS medium containing 2.0 mg/l Kn + 1.0 mg/l IAA. The lowest percentage (73.33%) of shoot proliferation was noted in 3.0 mg/l Kn + 2.0 mg/l IAA. Highest mean number of shoot per culture (8.20 ± 0.25) and highest average length of shoot per culture (2.20 ± 0.06 cm) was in the media having 2.0 mg/l Kn + 0.5 mg/l IAA. The lowest mean number of shoot per culture (2.97 ± 0.13) and lowest average length of shoot per culture (0.80 ± 0.04 cm) was obtained in the media having 3.0 mg/l Kn + 2.0 mg/l IAA (**Plate 3.3.1**). Another experiment was conducted with a view to induction of shoot bud using IAA in combinations of BAP and Kn. Though shoot buds also obtained from all of the media formulations but these results were found less than that of combinations of BAP+IAA and Kn+IAA. Experimental results revealed that combination of 3.0 mg/l BAP+ 0.5 mg/l IAA, 2.0 mg/l Kn + 0.5 mg/l IAA, 2.0 mg/l Kn + 1.0 mg/l IAA and 2.0 mg/l Kn + 0.2 mg/l IAA were found most effective combinations for multiple shoot induction in *C. asiatica*.

Table 3.3.1. Effect of different concentration of BAP and Kn singly and in combinations on multiple shoot induction from nodal explants of *C. asiatica*. Data were taken after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation*	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
0.00	-	-	-	-
BAP				
0.2	15-20	53.33	1.62 ± 0.04	0.64 ± 0.12
0.5	15-20	66.66	2.14 ± 0.06	1.34 ± 0.08
1.0	15-20	73.33	3.60 ± 0.08	1.80 ± 0.14
2.0	15-20	93.33	5.63 ± 0.22	2.11 ± 0.08
3.0	15-20	70.00	3.21 ± 0.27	1.66 ± 0.13
4.0	15-20	66.66	2.13 ± 0.12	1.20 ± 0.13
Kn				
0.2	15-20	63.33	1.80 ± 0.09	0.84 ± 0.04
0.5	15-20	70.00	3.33 ± 0.27	1.50 ± 0.13
1.0	15-20	86.66	5.15 ± 0.12	1.84 ± 0.09
2.0	15-20	100	7.84 ± 0.32	2.22 ± 0.14
3.0	15-20	73.33	5.20 ± 0.15	2.10 ± 0.08
4.0	15-20	60.00	2.97 ± 0.13	1.70 ± 0.05
BAP + Kn				
0.5 + 0.2	15-20	60.00	1.87 ± 0.12	1.20 ± 0.08
0.5 + 0.5	15-20	63.33	2.33 ± 0.15	1.34 ± 0.13
0.5 + 1.0	15-20	90.00	5.18 ± 0.06	1.92 ± 0.13
0.5 + 2.0	15-20	90.33	4.60 ± 0.07	1.80 ± 0.14
1.0 + 0.2	15-20	66.66	2.80 ± 0.25	1.41 ± 0.05
1.0 + 0.5	15-20	70.00	3.33 ± 0.27	1.43 ± 0.12
1.0 + 1.0	15-20	96.66	6.45 ± 0.08	2.13 ± 0.17
1.0 + 2.0	15-20	90.00	5.20 ± 0.34	2.00 ± 0.06
2.0 + 0.2	15-20	76.66	2.87 ± 0.12	1.51 ± 0.13
2.0 + 0.5	15-20	90.00	3.20 ± 0.10	1.90 ± 0.14
2.0 + 1.0	15-20	93.33	5.10 ± 0.12	1.90 ± 0.09
2.0 + 2.0	15-20	83.33	3.54 ± 0.27	1.54 ± 0.05

- no response ;

* Values are the mean of three replicates with 10 explants.

Table 3.3.2. Effect of different concentrations and combinations of BAP and Kn with IAA on multiple shoot induction from nodal explants of *C. asiatica.* Data were taken after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation (mean ± SE)*	Average number of shoots/culture (mean ± SE)*	Average length (cm) of shoots/culture (mean ± SE)*
BAP + IAA				
1.0 + 0.2	15-20	80.00	2.30 ± 0.12	0.60 ± 0.07
1.0 + 0.5	15-20	76.66	2.40 ± 0.06	1.00 ± 0.11
1.0 + 1.0	15-20	93.33	2.67 ± 0.09	1.13 ± 0.09
1.0 + 2.0	15-20	93.33	2.73 ± 0.09	1.30 ± 0.06
2.0 + 0.2	15-20	90.00	3.80 ± 0.15	1.45 ± 0.14
2.0 + 0.5	15-20	86.67	3.87 ± 0.12	1.45 ± 0.10
2.0 + 1.0	15-20	93.33	3.00 ± 0.06	1.61 ± 0.14
2.0 + 2.0	15-20	96.67	3.50 ± 0.12	2.00 ± 0.06
3.0 + 0.2	15-20	96.67	3.27 ± 0.09	1.93 ± 0.13
3.0 + 0.5	15-20	100	4.33 ± 0.12	2.16 ± 0.25
3.0 + 1.0	15-20	96.67	2.20 ± 0.10	1.45 ± 0.08
3.0 + 2.0	15-20	83.33	1.92 ± 0.07	0.62 ± 0.04
Kn + IAA				
0.5 +0.5	15-20	83.33	3.07 ± 0.03	0.61 ± 0.12
1.0 + 0.2	15-20	85.00	4.30 ± 0.20	1.11 ± 0.06
1.0 + 0.5	15-20	90.00	4.47 ± 0.12	1.13 ± 0.07
1.0 + 1.0	15-20	83.33	5.10 ± 0.12	1.44 ± 0.04
1.0 + 2.0	15-20	90.00	5.40 ± 0.12	1.51 ± 0.03
2.0 + 0.2	15-20	96.67	6.30 ± 0.15	1.51 ± 0.04
2.0 + 0.5	15-20	100	8.20 ± 0.25	2.20 ± 0.06
2.0 + 1.0	15-20	96.67	6.88 ± 0.26	1.83 ± 0.07
2.0 + 2.0	15-20	100	5.10 ± 0.06	1.53 ± 0.06
3.0 + 0.2	15-20	96.67	5.63 ± 0.22	1.57 ± 0.04
3.0 + 0.5	15-20	90.00	5.67 ± 0.34	1.54 ± 0.05
3.0 + 1.0	15-20	76.66	3.33 ± 0.27	1.22 ± 0.06
3.0 + 2.0	15-20	73.33	2.97 ± 0.13	0.80 ± 0.04
BAP+ Kn + IAA				
0.5+0.5+ 0.5	15-20	90.00	2.17 ± 0.07	1.52 ± 0.04
1.0+1.0+ 0.5	15-20	100	3.02 ± 0.23	1.92 ± 0.06
2.0+2.0+ 0.5	15-20	96.67	2.00 ± 0.10	1.85 ± 0.13

* Values are the mean of three replicates with ten explants.

(b) Direct multiple shoot induction in shoot apex explants of *C. asiatica*

Shoot apex explants of C. *asiatica* were aseptically cultured on different concentrations (0.2 – 4.0 mg/l) of BAP and Kn either alone and in combination with each other or in combination with different concentrations (0.2 – 2.0 mg/l) of IAA. Shoot apex explants found to be most suitable for multiple shoot induction on almost all of the supplemented cultured media but morphogenic responses of cultured explants varied depending upon the culture media formulation. Effects of different concentrations and combinations of auxins and cytokinins on multiple shoot induction from nodal explants are described below:

Experiment 1: Effect of BAP and Kn singly and in combination on multiple shoot induction

In this investigation six concentrations of BAP (0.2, 0.5, 1.0, 2.0, 3.0 and 4.0 mg/l), six concentrations of Kn (0.2, 0.5, 1.0, 2.0, 3.0 and 4.0 mg/l) alone and in combination of three concentration of BAP (0.5, 1.0 and 2.0 mg/l) with four concentrations of Kn (0.2, 0.5, 1.0 and 2.0) were treated in MS medium for the purpose of multiple shoot induction from shoot apex explants of *C. asiatica*. Data were taken after 6 weeks of inoculation and percentage of shoot formation, number of shoot/culture, length of shoot/culture were measured. The results are presented in **Table 3.3.3.** Morphogenic responses of the explants were found to vary with hormonal formulations present in the culture media. Shoot proliferation was noticed in all media formulations. Shoot proliferation ranged from 66.00 – 100%. Highest percentage (100%) of multiple shoot formation was observed in MS medium containing 2.0 mg/l Kn followed by (96.67%) in MS medium having 2.0 mg/l BAP. The lowest percentage (60.00%) of multiple shoot formation was observed in media having 4.0 mg/l BAP. Highest mean number of shoots per culture was 4.85 ± 0.18 in media having 2.0 mg/l BAP + 1.0 mg/l Kn and followed by 4.73 ± 0.15 in the media having 2.0 mg/l Kn. The lowest mean number of shoot was 1.62 ± 0.13 in media containing 2.0 mg/l BAP + 0.2 mg/l Kn. Average length of shoots gradually increased after induction of shoot. Highest average length was recorded 2.10 ± 0.08 cm in 2.0 mg/l Kn and followed by 2.05 ± 0.13 cm in 2.0 mg/l BAP and 2.04 ± 0.09 cm in 2.0 mg/l BAP+1.0 mg/l Kn. The lowest average length was 0.64 ± 0.11 cm in0.2 mg/l Kn. Experimental results revealed that 2.0 mg/l Kn, 2.0 mg/l BAP and 2.0 mg/l BAP + 1.0 mg/l Kn were found most effective concentrations for multiple shoot induction.

Experiment 2: Effect of different concentrations and combinations of BAP and Kn with IAA on multiple shoot induction

Explant were cultured on MS medium supplemented with three concentrations of BAP (1.0, 2.0 and 3.0 mg/l) and four concentrations of Kn (0.5, 1.0, 2.0 and 3.0 mg/l) combined with different concentrations of IAA (0.2, 0.5, 1.0 and 2.0 mg/l). Data were recorded after 6 weeks of culture and results on different parameters are presented in the **Table 3.3.4.** The efficiency of BAP+IAA was not better than BAP alone. The efficiency of BAP+IAA was inferior to the efficiency of Kn+IAA. All the used media compositions formed multiple shoots but the results were inferior to the result obtained from nodal explants. Among the concentrations of BAP+IAA the highest percentage (96.67 %) of shoot proliferation was noted in the media having 2.0 mg/l BAP+ 2.0 mg/l IAA. The lowest percentage (60.00%) of shoot proliferation was noted in 1.0 mg/l BAP + 0.2 mg/l IAA. Highest mean number of shoot per culture was 2.68 ± 0.15 in media having 3.0 mg/l BAP+ 0.5 mg/l IAA followed by 2.54 ± 0.17in media having 2.0 mg/l BAP + 2.0 mg/l IAA. Lowest mean number of shoot per culture was 1.17 ± 0.09 in media having 1.0 mg/l BAP + 0.2 mg/l IAA. Highest average length of shoot per culture was 2.02 ± 0.21 cm in the media having 3.0 mg/l BAP+ 0.5 mg/l IAA and lowest average length of shoot per culture 0.72 ± 0.11 cm in the media containing 3.0 mg/l BAP+2.0 mg/l IAA.

On the other hand the efficiency of Kn+IAA was better than Kn alone and combined effect of BAP+IAA on direct shoot regeneration. All the used media compositions formed multiple shoots and the results were inferior to the result obtained from nodal explants. Addition of lower concentration of IAA along with higher concentration of Kn was found more suitable than that of other concentrations. Among the combinations of Kn+IAA the highest percentage (100%) of shoot proliferation was noted in MS medium containing 2.0 mg/l Kn + 0.5 mg/l IAA and followed by (93.33%) in MS medium containing 3.0 mg/l Kn + 0.5 mg/l IAA. The lowest percentage (66.67%) of shoot proliferation was noted in 0.5 mg/l Kn + 0.5 mg/l IAA. Highest mean number of shoot per culture (5.80 ± 0.33) and highest average length of shoot per culture (2.10 ± 0.18 cm) was in the media having 2.0 mg/l Kn + 0.5 mg/l IAA. The lowest mean number of shoot per culture (0.87 ± 0.11) and lowest average length of shoot per culture (0.77 ± 0.07 cm) was obtained in the media having 0.5 mg/l Kn + 0.5 mg/l IAA. Another experiment was conducted with a view to induction of shoot bud using IAA in combinations of BAP and Kn. Though shoot buds also obtained from all of the media formulations but these results were found less than that of combinations of BAP+IAA and Kn+IAA. Experimental results revealed that combination of 2.0 mg/l Kn+ 0.5 mg/l IAA, 2.0 mg/l Kn + 1.0 mg/l IAA and 2.0 mg/l Kn + 2.0 mg/l IAA were found most effective combinations for multiple shoot induction in *C. asiatica.*

Table 3.3.3. Effect of different concentrations of BAP and Kn singly and in combinations on multiple shoot induction from shoot apex explants of *C. asiatica.* Data were taken after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
BAP				
0.2	12-16	66.66	1.80 ± 0.12	0.80 ± 0.14
0.5	12-16	70.00	1.90 ± 0.08	1.54 ± 0.13
1.0	12-16	76.66	2.82 ± 0.22	1.76 ± 0.12
2.0	12-16	96.66	4.67 ± 0.06	2.05 ± 0.13
3.0	12-16	73.33	3.20 ± 0.12	1.50 ± 0.08
4.0	12-16	60.00	2.60 ± 0.27	1.10 ± 0.08
Kn				
0.2	12-16	63.33	1.80 ± 0.17	0.64 ± 0.11
0.5	12-16	70.00	2.63 ± 0.05	1.30 ± 0.14
1.0	12-16	80.00	4.13 ± 0.11	1.62 ± 0.04
2.0	12-16	100	4.73 ± 0.15	2.10 ± 0.08
3.0	12-16	83.33	4.20 ± 0.22	1.90 ± 0.14
4.0	12-16	70.00	2.54 ± 0.08	1.60 ± 0.17
BAP + Kn				
0.5 +0.2	12-16	70.00	1.67 ± 0.17	1.42 ± 0.09
0.5 +0.5	12-16	73.33	1.97 ± 0.07	1.44 ± 0.14
0.5 +1.0	12-16	90.00	4.50 ± 0.11	1.50 ± 0.22
0.5 +2.0	12-16	86.66	3.20 ± 0.12	1.82 ± 0.17
1.0 +0.2	12-16	63.33	1.90 ± 0.17	1.33 ± 0.13
1.0 +0.5	12-16	76.66	2.60 ± 0.06	1.43 ± 0.17
1.0 +1.0	12-16	93.33	4.46 ± 0.14	1.54 ± 0.12
1.0 +2.0	12-16	80.00	4.10 ± 0.17	1.86 ± 0.09
2.0 +0.2	12-16	66.66	1.62 ± 0.13	1.30 ± 0.14
2.0 +0.5	12-16	83.33	2.00 ± 0.11	1.65 ± 0.05
2.0 +1.0	12-16	93.33	4.85 ± 0.18	2.04 ± 0.09
2.0 +2.0	12-16	80.00	3.14 ± 0.17	1.44 ± 0.08

* Values are the mean of three replicates with 10 explants.

Table 3.3.4. Effect of different concentrations and combinations of BAP and Kn with IAA on multiple shoot induction from shoot apex explants of *C. asiatica.*

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
BAP + IAA				
1.0 + 0.2	12-16	60.00	1.17 ± 0.09	0.82 ± 0.11
1.0 + 0.5	12-16	70.00	1.32 ± 0.20	0.97 ± 0.08
1.0 + 1.0	12-16	86.67	1.62 ± 0.12	1.20 ± 0.14
1.0 + 2.0	12-16	86.67	1.70 ± 0.25	1.36 ± 0.09
2.0 + 0.2	12-16	93.33	1.96 ± 0.18	1.52 ± 0.17
2.0 + 0.5	12-16	83.33	1.88 ± 0.11	1.49 ± 0.04
2.0 + 1.0	12-16	90.00	2.10 ± 0.11	1.72 ± 0.15
2.0 + 2.0	12-16	96.67	2.54 ± 0.17	1.90 ± 0.12
3.0 + 0.2	12-16	90.00	2.24 ± 0.20	1.83 ± 0.16
3.0 + 0.5	12-16	96.67	2.68 ± 0.15	2.02 ± 0.21
3.0 + 1.0	12-16	86.67	1.80 ± 0.17	1.35 ± 0.22
3.0 + 2.0	12-16	80.00	1.36 ± 0.14	0.72 ± 0.11
Kn + IAA				
0.5 +0.5	12-16	66.67	0.87 ± 0.11	0.77 ± 0.07
1.0 + 0.2	12-16	70.00	2.60 ± 0.08	1.06 ± 0.15
1.0 + 0.5	12-16	86.67	3.47 ± 0.11	1.23 ± 0.13
1.0 + 1.0	12-16	83.33	3.20 ± 0.10	1.40 ± 0.11
1.0 + 2.0	12-16	93.33	3.40 ± 0.06	1.49 ± 0.08
2.0 + 0.2	12-16	90.00	3.84 ± 0.12	1.66 ± 0.11
2.0 + 0.5	12-16	100	5.80 ± 0.33	2.10 ± 0.18
2.0 + 1.0	12-16	90.00	4.26 ± 0.22	1.65 ± 0.10
2.0 + 2.0	12-16	93.33	3.89 ± 0.18	1.41 ± 0.06
3.0 + 0.2	12-16	86.67	3.32 ± 0.14	1.34 ± 0.10
3.0 + 0.5	12-16	93.33	3.48 ± 0.08	1.41 ± 0.15
3.0 + 1.0	12-16	76.67	3.24 ± 0.11	1.14 ± 0.06
3.0 + 2.0	12-16	70.00	2.22 ± 0.15	1.02 ± 0.13
BAP+ Kn + IAA				
0.5+0.5+ 0.5	12-16	80.00	1.79 ± 0.13	1.36 ± 0.08
1.0+1.0+ 0.5	12-16	90.00	2.88 ± 0.10	1.87 ± 0.20
2.0+2.0+ 0.5	12-16	86.67	1.96 ± 0.20	1.25 ± 0.09

* Values are the mean of three replicates with 10 explants.

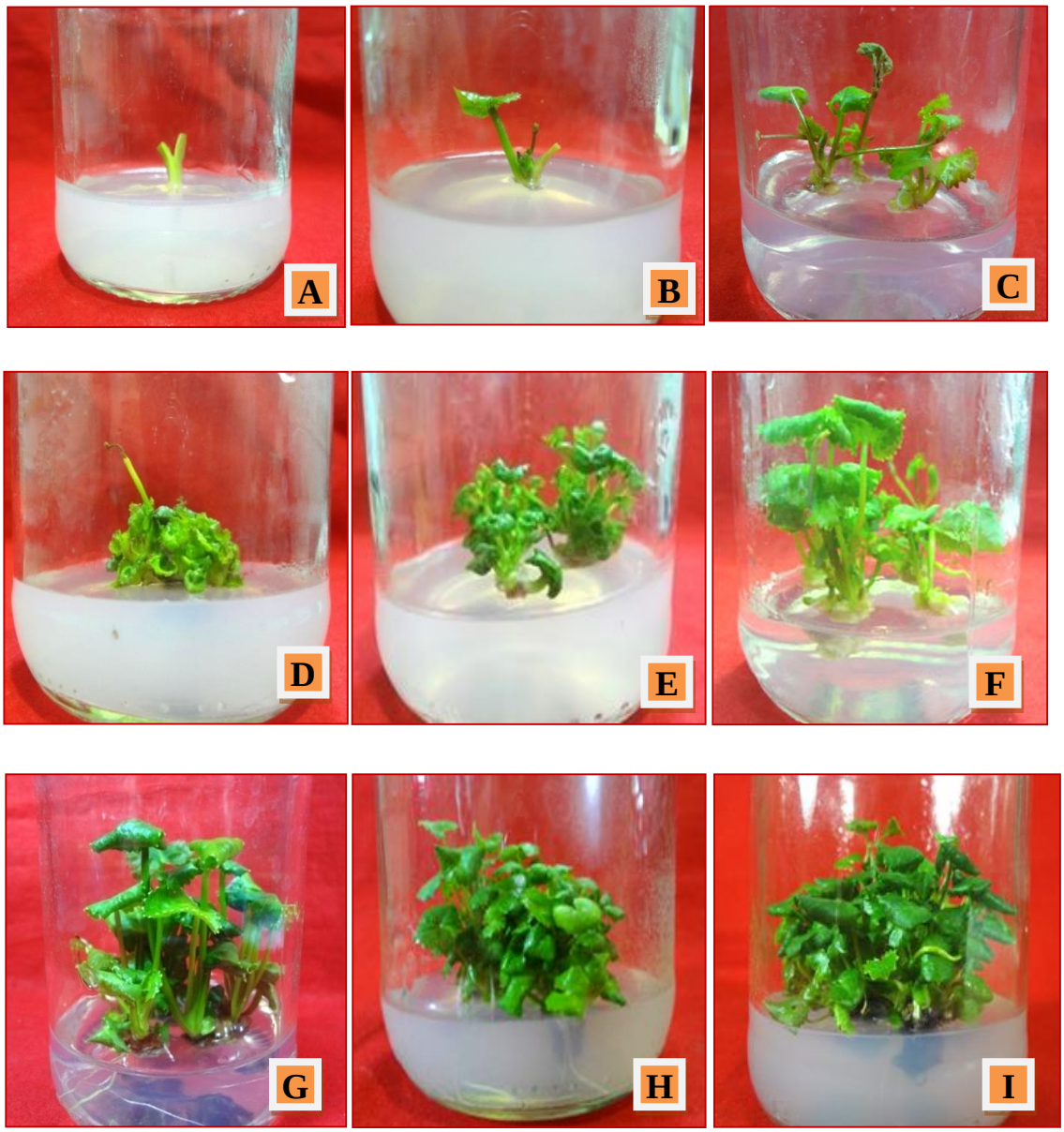


Plate 3.3.1. Development of multiple shoot from Shoot tip and nodal explant of *C. asiatica* through the process of direct organogenesis with their subsequent development.

A: Nodal explant inoculated in the medium; **B:** Initiation of multiple shoot formation in nodal segment after 2 weeks of inoculation in media having 2.0 mg/l Kn + 0.5 mg/l IAA; **C**: Initiation of multiple shoot formation in shoot apex after 10 days of inoculation in media having same medium; **D-F**: Proliferation of multiple shoot in the same medium after one subcultures at 14 days interval; **G-I**: Further proliferation and elongation of shoot after 6 weeks of culture in the same medium.

B. Induction of callus on different explants in *C. asiatica*.

Experiment 1: Effect of different concentrations and combinations of phytohormones on callus induction and characteristics of callus derived from leaf explants.

Under the present investigation leaf explants excised from *in vitro* grown seedlings of *C. asiatica* were used to investigate the effect of different hormonal concentrations and combinations. Explants were cultured on to MS media supplemented with different concentrations (0.2, 0.5, 1.0, 2.0 and 3.0 mg/l) of 2,4-D and NAA alone and in combinations of BAP+2,4-D, Kn+2,4-D, BAP+NAA and Kn+NAA in different concentrations. Data were recorded after 5 weeks of culture. Cultures were maintained under 16 h light and 8 h dark regime. Results obtained on morphogenic response of the cultured explants are shown in **Table 3.3.5**. Morphogenic responses of the explants were found to vary with hormonal formulations present in the culture media. Callus proliferation was noticed in all media formulations except 0.2 mg/l 2,4-D and 0.2 mg/l NAA. But there was a wide variation in morphological nature and percentage of callus formation among them. Callus initiation occurred within 10 – 20 days depending upon the concentration and combination of hormones. BAP+ 2,4-D and BAP+NAA induced callus within 12 – 16 days. 2,4-D, NAA, Kn + 2,4-D and Kn + NAA took 15 – 20 days to initiate callus formation. Percentage of callus formation ranged from 40.00 – 93.33%. Highest percentage (93.33%) of callus formation occurred in MS medium containing 0.5mg/l BAP+2.0 mg/l NAA (**Plate 3.3.2 E**) followed by 90% in MS medium containing 0.5mg/l BAP+1.0 mg/l NAA and 1.0mg/l BAP+2.0 mg/l NAA. The lowest percentage (40%) of callus formation was observed in media having 0.5 mg/l NAA alone. In most cases colour of calli was whitish, whitish yellow, green, light green, pinkish and creamy and texture of calli were compact and friable.

Experiment 2: Effect of different concentrations and combinations of phytohormones on callus induction and characteristics of callus derived from internode explants.

In this present investigation internode explants excised from *in vitro* grown seedlings of *C. asiatica* were used to investigate the effect of different hormonal concentrations and combinations. Explants were cultured on to MS media supplemented with different concentrations (0.2, 0.5, 1.0, 2.0 and 3.0 mg/l) of 2,4-D and NAA alone and in combinations of BAP+2,4-D, Kn+2,4-D, BAP+NAA and Kn+NAA in different concentrations. Data were recorded after 5 weeks of culture. Cultures were maintained under 16 h light and 8 h dark regime. Results obtained on morphogenic response of the cultured explants are shown in **Table 3.3.5**. Morphogenic responses of the explants were found to vary with hormonal formulations present in the culture media. Among all the concentrations it was observed that only 0.2 mg/l 2,4-D and 0.2 mg/l NAA failed to induce any callus proliferation. But there was a wide variation in morphological nature and percentage of callus formation among them. Callus initiation occurred within 10 – 20 days depending upon the concentration and combination of hormones. BAP+ 2, 4-D and BAP+NAA induced callus within 10 – 15 days. 2,4-D, NAA, Kn + 2,4-D and Kn + NAA took 15 – 20 days to initiate callus formation. Percentage of callus formation ranged from 30.00 – 90.00%. Highest percentage (90.00%) of callus formation occurred in MS medium containing 0.5mg/l BAP+2.0 mg/l NAA (**Plate 3.3.2.E**) followed by 86.67% in MS medium containing 1.0 mg/l BAP+2.0 mg/l NAA. The lowest percentage (30%) of callus formation was observed in media having 0.5 mg/l NAA alone. In most cases colour of calli was whitish, whitish yellow, green, light green, pinkish and creamy and texture of calli were compact and friable.

Table 3.3.5. Effect of different concentrations and combinations of phytohormones on callus induction and characteristics of callus derived from leaf and internode explants of *C. asiatica*. Data were collected after 5 weeks on inoculation**.**

Hormonal combinations (mg/l)	Leaf explant			Internode explant		
	Days of callus initiation	% of explant induced callus	*Callus colour and texture	Days of callus initiation	% of explant induced callus	*Callus colour and texture
2,4-D						
0.2	-	-	-	-	-	-
0.5	15-20	66.67	wf	15-20	60.00	lgf
1.0	15-20	70.00	lgf	15-20	63.33	lgf
2.0	15-20	76.67	lgf	15-20	70.00	lgf
3.0	15-20	70.00	lgf	15-20	60.00	lgf
NAA						
0.2	-	-	-	-	-	-
0.5	15-20	40.00	pw	15-20	30.00	pw
1.0	15-20	56.67	gc	15-20	46.67	gc
2.0	15-20	70.00	gc	15-20	60.00	gc
3.0	15-20	66.67	gc	15-20	53.33	gc
BAP + 2,4-D						
0.5 + 0.5	12-16	66.67	cf	10-15	56.67	cf
0.5 + 1.0	12-16	80.00	wyf	10-15	73.33	cf
0.5 + 2.0	12-16	76.67	wyf	10-15	70.00	cf
1.0 + 0.5	12-16	63.33	cf	10-15	53.33	cf
1.0 + 1.0	12-16	73.33	wyf	10-15	60.00	cf
1.0 + 2.0	12-16	70.00	wyf	10-15	60.00	cf
Kn + 2,4-D						
0.5 + 0.5	15-20	46.67	wyf	15-20	40.00	cf
0.5 + 1.0	15-20	50.00	wyf	15-20	43.33	wyf
0.5 + 2.0	15-20	63.33	wyf	15-20	56.67	wyf
1.0 + 0.5	15-20	60.00	wyf	15-20	50.00	wyf
1.0 + 1.0	15-20	66.67	wyf	15-20	63.33	wyf
1.0 + 2.0	15-20	66.67	wyf	15-20	60.00	wyf
BAP + NAA						
0.5 + 0.5	12-16	66.67	pw	10-15	60.00	pw
0.5 + 1.0	12-16	90.00	wyf	10-15	83.33	wyf
0.5 + 2.0	12-16	93.33	wyf	10-15	90.00	wyf
1.0 + 0.5	12-16	70.00	lgc	10-15	70.00	lgc
1.0 + 1.0	12-16	86.67	wyf	10-15	86.67	wyf
1.0 + 2.0	12-16	90.00	wyf	10-15	80.00	wyf
Kn + NAA						
0.5 + 0.5	15-20	60.00	gc	15-20	50.00	lgc
0.5 + 1.0	15-20	70.00	gc	15-20	60.00	gc
0.5 + 2.0	15-20	66.67	gc	15-20	53.33	gc
1.0 + 0.5	15-20	66.67	gc	15-20	56.67	gc
1.0 + 1.0	15-20	70.00	gc	15-20	63.33	gc
1.0 + 2.0	15-20	73.33	gc	15-20	70.00	gc

- : Failed to any callus formation.

* **wyf:** whitish yellow friable, **cf:** creamy friable, **lgf:** light green friable, **wf:** white friable, **gc:** green compact. **pw**: pinkish white

C. Plant regeneration from different types of callus in *C. asiatica*.

Regeneration of callus is more important than induction of callus and a number of experiments were undertaken with the aim of establish media combination for organogenesis of calli derived from two types of explants. A broad spectrum of cytokinin (BAP and Kn) and auxin (IAA and NAA) supplemented media were used to scrutinize the organogenic response. Among the different types of calli light green friable, light green compact and green compact found to be best regenerative calli on different plant growth regulator supplemented media. Leaf derived callus showed better performance than internode derived callus. Findings of the experiments are described below:

(i) Plant regeneration from leaf derived callus

Different experiments were conducted to investigate plant regeneration ability through callus culture from leaf explants. Data on percentage of organogenic calli induced, average number of shoot per callus and average length of shoot per callus were collected after 6 weeks of culture.

Experiment 1: Effect of different concentrations of BAP and Kn alone and in combinations with IAA or NAA on adventitious shoot regeneration from leaf derived callus of *C. asiatica*.

Under this experiment leaf derived calli of *C. asiatica* were used to investigate the effect of different hormonal concentrations and combinations. For shoot differentiation, light green compact calli were subcultured on MS medium supplemented with four concentrations (0.5, 1.0, 2.0 and 3.0 mg/l) of BAP and Kn used alone and in combinations of two concentrations (1.0 and 2.0 mg/l) of BAP or Kn with three concentrations (0.5, 1.0 and 2.0 mg/l) of IAA or (0.2, 0.5 and 1.0 mg/l) of NAA to scrutinize the organogenic response. Data were recorded after 6 weeks of culture and presented in **Table 3.3.6**. Among all the concentrations it was observed that 0.5 mg/l BAP, 3.0 mg/l BAP, 0.5 mg/l Kn and 1.0 mg/l Kn alone and in combination of 1.0 mg/l Kn+0.5 mg/l IAA, 1.0 mg/l Kn+1.0 mg/l IAA, 1.0 mg/l Kn+0.2 mg/l NAA and 1.0 mg/l Kn+ 0.5 mg/l NAA altogether failed to differentiate any shoots. The percentage of calli produced shoots ranged from 20 – 70%. Highest percentage 70.00% of adventitious shoots regeneration occurred in MS medium containing 1.0 mg/l BAP+0.5 mg/l NAA followed by 60.00% in MS medium containing 1.0 mg/l BAP+1.0 mg/l NAA and 2.0 mg/l BAP+0.5 mg/l NAA. The lowest percentage (20%) of adventitious shoots regeneration was observed in media having 3.0 mg/l Kn alone and 1.0 mg/l Kn+1.0 mg/l NAA. The highest number of adventitious shoots regeneration per callus was 4.20 ± 0.15 in media having 1.0 mg/l

BAP+0.5 mg/l NAA followed by 3.74 ± 0.24 in 1.0 mg/l BAP+1.0 mg/l NAA. The lowest number of adventitious shoot regeneration per callus was 1.50 ± 0.16 in media having 1.0 mg/l Kn+2.0 mg/l IAA. Highest length of shoot 2.12 ± 0.10 cm was recorded in 2.0 mg/l BAP+0.5 mg/l NAA and the lowest length of shoots 1.13 ± 0.08 cm was recorded in media having 1.0 mg/l Kn+1.0 mg/l NAA.

(ii) Plant regeneration from internode derived callus

Different experiments were conducted to investigate plant regeneration ability through callus culture from internode explants. Data on percentage of organogenic calli induced, average number of shoot per callus and average length of shoot per callus were collected after 6 weeks of culture.

Experiment 1: Effect of different concentrations of BAP and Kn alone and in combinations with IAA or NAA on adventitious shoot regeneration from internode derived callus of *C. asiatica*.

In this experiment, internode derived calli of *C. asiatica* were used to investigate the effect of different hormonal concentrations and combinations. For shoot differentiation, light green compact calli were subcultured on MS medium supplemented with four concentrations (0.5, 1.0, 2.0 and 3.0 mg/l) of BAP and Kn used alone and in combinations of two concentrations (1.0 and 2.0 mg/l) of BAP or Kn with three concentrations (0.5, 1.0 and 2.0 mg/l) of IAA or with three concentrations (0.2, 0.5 and 1.0 mg/l) of NAA to scrutinize the organogenic response. Data were recorded after 6 weeks of culture and presented in **Table 3.3.6**. Among all the concentrations it was observed that 0.5 mg/l BAP, 3.0 mg/l BAP, 0.5 mg/l Kn and 1.0 mg/l Kn alone and in combination of 1.0 mg/l Kn+0.5 mg/l IAA, 1.0 mg/l Kn+1.0 mg/l IAA and 1.0 mg/l Kn+0.2 mg/l NAA altogether failed to differentiate any shoots. The percentage of calli produced shoots was ranged from 10 – 60%. Highest percentage 60.00% of adventitious shoots regeneration occurred in MS medium containing 1.0 mg/l BAP+0.5 mg/l NAA followed by 50.00% in MS medium containing 2.0 mg/l BAP+0.5 mg/l NAA. The lowest percentage (10%) of adventitious shoots regeneration was observed in media having 1.0 mg/l Kn+0.5 mg/l NAA. The highest number of adventitious shoots regeneration per callus was 3.67 ± 0.20 in media having 1.0 mg/l BAP+0.5 mg/l NAA followed by 3.43 ± 0.11 in 1.0 mg/l BAP+1.0 mg/l NAA. The lowest number of adventitious shoots regeneration per callus was 1.32 ± 0.09 in media having 1.0 mg/l Kn+0.5 mg/l NAA. Highest length of shoot 2.10 ± 0.12 cm was recorded in 2.0 mg/l BAP+0.5 mg/l NAA and the lowest length of shoots 1.17 ± 0.07 cm was recorded in media having 1.0 mg/l Kn+1.0 mg/l NAA.

Table 3.3.6. Effect of BAP and Kn alone and combinations with NAA on adventitious shoot regeneration from leaf and stem derived callus in *C. asiatica*. Data were collected after 6 weeks of inoculation.

Hormonal combination (mg/l)	Leaf derived callus			Stem derived callus		
	% of regenerable calli	*Average number of shoots/callus (mean±SE)	*Average length (cm) of shoots/callus (mean±SE)	% of regenerable calli	*Average number of shoots/callus (mean±SE)	*Average length (cm) of shoots/callus (mean±SE)
BAP						
0.5	-	-	-	-	-	-
1.0	23.33	1.82 ± 0.07	1.53 ± 0.14	20.00	1.79 ± 0.10	1.25 ± 0.08
2.0	36.67	2.13 ± 0.13	1.68 ± 0.17	26.67	1.83 ± 0.07	1.34 ± 0.13
3.0	-	-	-	-	-	-
Kn						
0.5	-	-	-	-	-	-
1.0	-	-	-	-	-	-
2.0	30.00	2.18 ± 0.16	1.80 ± 0.07	26.67	2.16 ± 0.10	1.73 ± 0.12
3.0	20.00	1.84 ± 0.06	1.82 ± 0.06	20.00	1.90 ± 0.11	1.43 ± 0.08
BAP + IAA						
1.0 + 0.5	30.00	1.72 ± 0.17	1.58 ± 0.14	20.00	1.69 ± 0.14	1.20 ± 0.10
1.0 + 1.0	46.67	2.81 ± 0.22	1.78 ± 0.16	40.00	2.63 ± 0.16	1.42 ± 0.12
1.0 + 2.0	36.67	2.15 ± 0.11	1.60 ± 0.15	33.33	1.70 ± 0.17	1.47 ± 0.19
2.0 + 0.5	30.00	1.89 ± 0.11	1.66 ± 0.10	26.67	1.51 ± 0.14	1.29 ± 0.10
2.0 + 1.0	53.33	3.08 ± 0.15	1.92 ± 0.11	46.67	2.58 ± 0.21	1.84 ± 0.09
2.0 + 2.0	30.00	1.76 ± 0.15	1.64 ± 0.09	23.33	1.65 ± 0.11	1.30 ± 0.15
Kn + IAA						
1.0 + 0.5	-	-	-	-	-	-
1.0 + 1.0	-	-	-	-	-	-
1.0 + 2.0	30.00	1.50 ± 0.16	1.70 ± 0.08	30.00	1.41 ± 0.10	1.29 ± 0.14
2.0 + 0.5	30.00	1.54 ± 0.08	1.60 ± 0.09	26.67	1.37 ± 0.10	1.32 ± 0.09
2.0 + 1.0	40.00	2.30 ± 0.14	1.77 ± 0.15	36.67	2.26 ± 0.19	1.89 ± 0.15
2.0 + 2.0	36.67	1.83 ± 0.23	1.34 ± 0.06	30.00	1.78 ± 0.17	1.30 ± 0.07
BAP+ NAA						
1.0 + 0.2	30.00	2.30 ± 0.11	1.36 ± 0.07	36.67	2.07 ± 0.15	1.50 ± 0.09
1.0 + 0.5	70.00	4.20 ± 0.15	2.06 ± 0.22	60.00	3.67 ± 0.20	1.94 ± 0.12
1.0 + 1.0	60.00	3.74 ± 0.24	1.90 ± 0.17	46.67	3.43 ± 0.11	1.70 ± 0.08
2.0 + 0.2	30.00	2.26 ± 0.09	1.44 ± 0.08	33.33	2.02 ± 0.14	1.28 ± 0.03
2.0 + 0.5	60.00	3.66 ± 0.13	2.12 ± 0.10	50.00	3.40 ± 0.20	2.10 ± 0.12
2.0 + 1.0	46.67	2.25 ± 0.20	1.79 ± 0.13	30.00	2.15 ± 0.11	1.36 ± 0.09
Kn + NAA						
1.0 + 0.2	-	-	-	-	-	-
1.0 + 0.5	-	-	-	10.00	1.32 ± 0.09	1.37 ± 0.11
1.0 + 1.0	20.00	1.80 ± 0.03	1.13 ± 0.08	20.00	1.64 ± 0.12	1.17 ± 0.07
2.0 + 0.2	33.33	2.14 ± 0.11	1.58 ± 0.07	33.33	1.70 ± 0.14	1.47 ± 0.13
2.0 + 0.5	40.00	3.20 ± 0.19	2.02 ± 0.18	30.00	3.06 ± 0.19	1.83 ± 0.05
2.0 + 1.0	40.00	3.46 ± 0.25	1.76 ± 0.06	40.00	3.22 ± 0.08	1.70 ± 0.14

\- : Failed to differentiate any shoots.

* Values are the mean of three replicates with 10 explants.

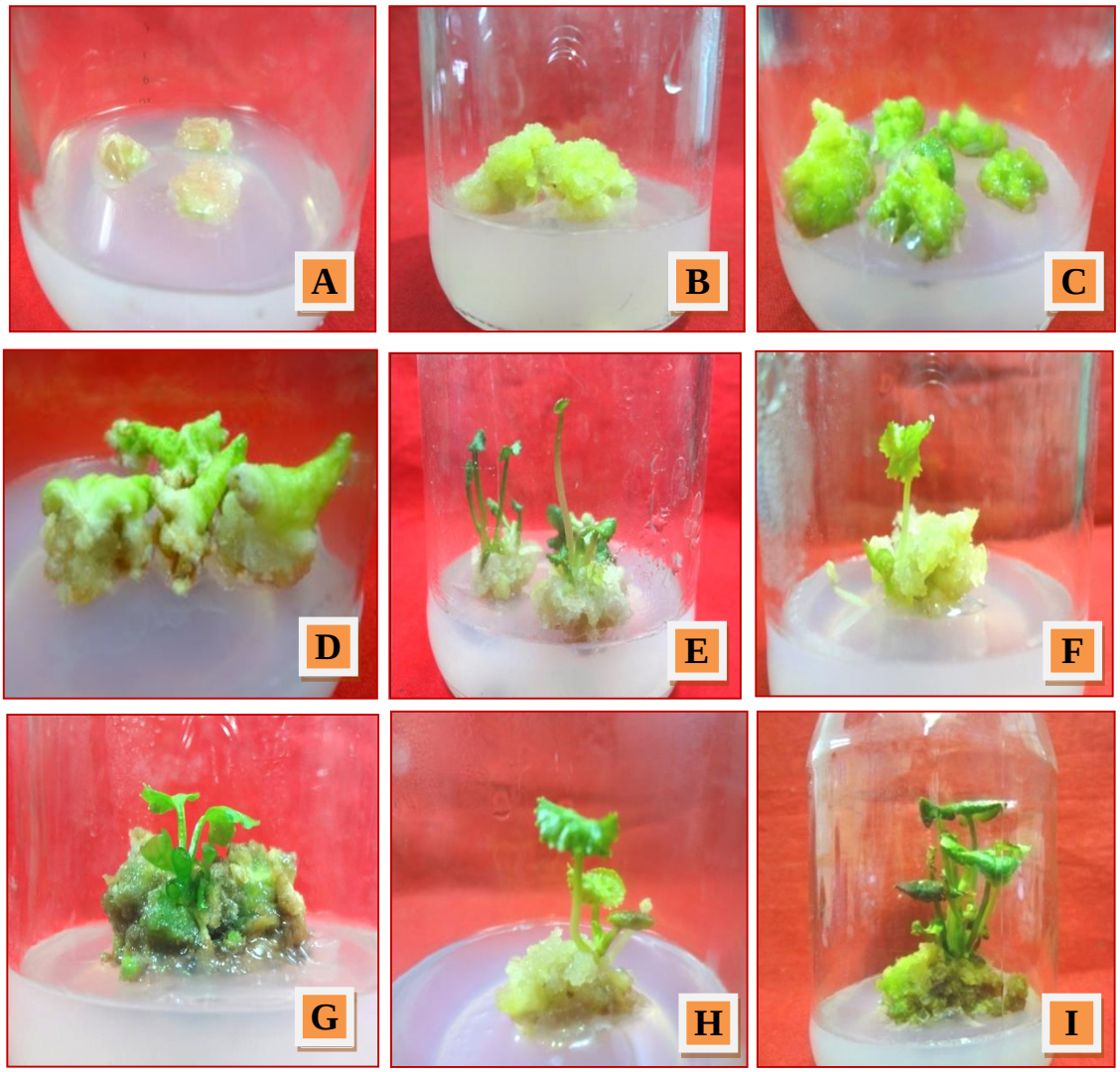


Plate 3.3.2. Callus induction from internode and leaf explants with plant regeneration from callus of *C. asiatica.*

A-C: Callus induction from internode explant in MS+2mg/l NAA+0.5 mg/l BAP; **D:** Callus induction from leaf explant in MS+2mg/l NAA+0.5 mg/l BAP; **E**: Initiation of callus regeneration in MS+1.0 mg/l BAP+0.5 mg/l NAA after 4 weeks of culture from internode derived callus; **F:** Initiation of callus regeneration in MS+1.0 mg/l BAP+0.5 mg/l NAA after 4 weeks of culture from leaf derived callus; and **G-I**: proliferation and elongation of shoot buds from in the same medium.

D. Rooting of *in vitro* grown shoots in *C. asiatica*.

Shoot cuttings 1.5 – 2.5 cm long from *in vitro* grown shoots were separated and transferred to rooting media. MS medium fortified with different concentrations (0.2, 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 mg/l) of NAA, IAA and IBA alone and in combination of BAP or Kn (0.2 and 0.5) with four concentrations (0.5, 1.0, 2.0 and 3.0 mg/l) of IAA or IBA were used for root experiment. Rooting started within 10 to 12 days of culture. Among the tested concentrations MS medium having 1.0 – 3.0 mg/l IAA found highest percentage (100%) of root formation. Data on days to root initiation, frequency of root formation (%), average number of root per culture and average length of root per culture were recorded after 5 weeks of culture. The results are described experiment wise:

Experiment 1: Effect of different concentrations of NAA, IAA, IBA and MS=0 on root induction from shoots derived from nodal explants of *C. asiatica.*

For adventitious root formation, the shoots obtained from *in vitro* grown nodal segments were cultured on MS medium with different concentrations of NAA, IAA, IBA and MS=0 (without hormones). Results obtained for the root induction, percentage of root formation, morphology, average number and length of roots are shown in **Table 3.3.7.** Percentage range of cultures produced roots varied from 53.00 – 100%. Highest 100% of root regeneration was recorded in 1.0 – 3.0 mg/l IAA. The lowest 53.33% of root regeneration was recorded in media having 0.2 mg/l NAA. The highest average number of roots per shoot was recorded 16.53 ± 0.24 in media having 2.0 mg/l IAA (**Plate 3.3.3**) followed by 14.20 ± 0.36 in 1.5 mg/l IAA. The lowest number of root per shoot was recorded 2.87 ± 0.24 in media having 0.2 mg/l NAA. Highest average length of roots 6.78 ± 0.10 cm was recorded in 2.0 mg/l IAA and the lowest length of roots 2.25 ± 0.07 cm was recorded in 0.2 mg/l NAA. In most cases morphology of roots was thin, thick and long. MS=0 also produced shoot but it was only 50%.

Experiment 2: Effect of different concentrations and combinations of BAP, Kn, IAA and IBA in MS medium on rooting of *in vitro* raised shoots of *C. asiatica*.

In this experiment, shoots obtained from *in vitro* grown nodal segments were cultured on MS medium with different concentrations and combinations of BAP, Kn, IAA and IBA for adventitious root formation. Results obtained for the root induction, percentage of root formation, morphology, average number and length of roots are shown in **Table 3.3.8**. The results of this investigation were inferior to the results of NAA, IAA and IBA alone. Percentage range of cultures produced roots varied from

60.00 – 100%. The highest average number of roots per shoot (16.03 ± 0.29) and highest average length of roots (6.02 ± 0.13 cm) was recorded in media having 2.0 mg/l IAA + 0.5 mg/l Kn. Next to 14.00 ± 0.31 roots per culture and 5.74 ± 0.16 cm length per shoot was recorded in media having 2.0 mg/l IAA+ 0.5 mg/l BAP. In most cases morphology of roots was thin, thick and long.

Table 3.3.7. Effect of MS medium with different concentrations of NAA, IAA and IBA alone on rooting of *in vitro* raised shoots of *C. asiatica*. Data were taken after 5 weeks of culture.

Growth regulators (mg/l)	Days taken for root initiation	% of explants induced root development	* Average number of root per culture (mean ± SE)	* Average length (cm) of root per culture (mean ± SE)
0.00	10-14	50.00	2.67 ± 0.09	2.16 ± 0.10
NAA				
0.2	12-16	53.33	2.87 ± 0.24	2.25 ± 0.07
0.5	12-16	60.00	5.20 ± 0.26	3.31 ± 0.09
1.0	12-16	83.33	6.47 ± 0.17	4.05 ± 0.05
1.5	12-16	80.00	6.38 ± 0.15	3.75 ± 0.31
2.0	12-16	76.66	6.13 ± 0.23	3.43 ± 0.11
3.0	12-16	66.66	4.13 ± 0.20	3.27 ± 0.24
4.0	12-16	70.00	2.73 ± 0.15	3.20 ± 0.08
IAA				
0.2	10-14	96.66	5.77 ± 0.13	3.51 ± 0.10
0.5	10-14	96.66	6.00 ± 0.25	3.77 ± 0.24
1.0	10-14	100	12.97 ± 0.27	5.61 ± 0.15
1.5	10-14	100	14.20 ± 0.36	6.09 ± 0.21
2.0	10-14	100	16.53 ± 0.24	6.78 ± 0.10
3.0	10-14	100	13.03 ± 0.44	5.04 ± 0.18
4.0	10-14	93.33	7.40 ± 0.75	3.24 ± 0.22
IBA				
0.2	10-14	86.66	5.93 ± 0.29	3.08 ± 0.09
0.5	10-14	90.00	7.77 ± 0.44	4.23 ± 0.20
1.0	10-14	100	12.30 ± 0.15	6.02 ± 0.16
1.5	10-14	100	11.47 ± 0.52	5.45 ± 0.16
2.0	10-14	100	11.97 ± 0.30	5.10 ± 0.25
3.0	10-14	86.66	7.77 ± 0.52	3.53 ± 0.08
4.0	10-14	83.33	6.73 ± 0.15	3.03 ± 0.07

* Values are the mean of three replicates with 10 explants.

Table 3.3.8. Effect of different concentrations and combinations of BAP, Kn, IAA and IBA in MS medium on rooting of *in vitro* raised shoots of *C. asiatica*. Data were taken after 5 weeks of culture.

Growth regulators (mg/l)	Days taken for root initiation	% of explants induced root development	* Average number of root per culture (mean ± SE)	* Average length (cm) of root per culture (mean ± SE)
BAP + IAA				
0.2 + 0.5	10-14	60.00	5.17 ± 0.09	2.51 ± 0.23
0.2 + 1.0	10-14	100	8.03 ± 0.33	3.50 ± 0.31
0.2 + 2.0	10-14	100	13.03 ± 0.27	5.61 ± 0.15
0.2 + 3.0	10-14	100	9.33 ± 0.49	3.80 ± 0.13
0.5 + 0.5	10-14	63.33	6.57 ± 0.32	3.32 ± 0.22
0.5 + 1.0	10-14	90.00	9.07 ± 0.22	4.04 ± 0.18
0.5 + 2.0	10-14	100	14.00 ± 0.31	5.74 ± 0.16
0.5 + 3.0	10-14	90.00	10.23 ± 0.34	5.10 ± 0.10
Kn + IAA				
0.2 + 0.5	10-14	86.66	7.20 ± 0.47	2.90 ± 0.31
0.2 + 1.0	10-14	100	9. 50 ± 0.00	4.20 ± 0.22
0.2 + 2.0	10-14	100	12.10 ± 0.21	5.76 ± 0.16
0.2 + 3.0	10-14	100	9.43 ± 0.38	4.10 ± 0.16
0.5 + 0.5	10-14	96.66	8.07 ± 0.20	3.54 ± 0.27
0.5 + 1.0	10-14	100	11.03 ± 0.15	5.04 ± 0.21
0.5 + 2.0	10-14	100	16.03 ± 0.29	6.02 ± 0.13
0.5 + 3.0	10-14	90.00	10.03 ± 0.23	3.51 ± 0.10
BAP + IBA				
0.2 + 0.5	10-14	70.00	5.83 ± 0.32	2.46 ± 0.31
0.2 + 1.0	10-14	100	11.00 ± 0.15	5.23 ± 0.17
0.2 + 2.0	10-14	96.66	10.17 ± 0.28	5.00 ± 0.22
0.2 + 3.0	10-14	90.00	7.30 ± 0.26	4.45 ± 0.17
0.5 + 0.5	10-14	80.00	6.43 ± 0.20	3.10 ± 0.20
0.5 + 1.0	10-14	100	10.10 ± 0.61	5.53 ± 0.13
0.5 + 2.0	10-14	93.33	9.47 ± 0.23	4.03 ± 0.11
0.5 + 3.0	10-14	90.00	7.50 ± 0.23	3.08 ± 0.24

* Values are the mean of three replicates with 10 explants.

Plate 3.3.3. Induction of adventitious roots and establishment in soil of *C. asiatica*. **A-C:** Induction and elongation of adventitious roots on *in vitro* regenerated shoots in MS medium containing 2.0 mg/l IAA after 5 weeks of culture; **D:** Root induction in the test tube; **E:** Acclimatized regenerated plants in soil after 40 days.

3.3.1.2. *Micropropagation of Commelina benghalensis*

In case of *C. benghalensis* shoot tip and nodal explants were used as the explants for direct regeneration. Explants were cultured on the MS agar gelled medium supplemented with different concentrations of auxins and cytokinins which were used either singly or in combinations to investigate the initiation of shoot and its subsequent regeneration. For root induction, micro shoots obtained from direct organogenesis were transferred to rooting media. Finally well developed plants with both shoots and roots were transferred to natural conditions through successive phages of acclimatization. Results of the experiments are described under following heads:

A. Direct shoot regeneration in *C. benghalensis*

Two types of explants *viz.* nodal explants and shoot tip were cultured for direct shoot regeneration. Explants were cultured on MS medium with BAP and Kn used alone and in combinations with each other or with NAA and IAA. Data on number of days taken for shoot initiation, percentage of explant induced shoot development, average number of shoot per culture and average length of shoot per culture were collected after 6 weeks of culture. Among two types of explants nodal explants responded better on almost all of the supplemented cultured medium. The results are described according to types of explants under separate heads:

(a) Nodal explants

Experiment 1: Effect of different concentrations of BAP alone and in combinations with NAA or IAA on multiple shoot induction from nodal explants of *C. benghalensis*

Five concentrations (0.2, 0.5, 1.0, 2.0 and 3.0mg/l) of BAP alone or combination of three concentrations (0.5, 1.0 and 2.0 mg/l) of BAP with four concentrations (0.1, 0.2, 0.5 and 1.0 mg/l) of NAA or IAA were treated in MS medium for the purpose of multiple shoot induction from nodal explants of *C. benghalensis*. Data were taken after 6 weeks of inoculation and days taken for shoot initiation, percentage of shoot formation, number of shoots per culture and length of shoot per culture were measured. The results are presented in **Table 3.3.9.** The efficiency of BAP + NAA was better than BAP alone and BAP + IAA on direct shoot regeneration from nodal explants. All the used media compositions formed shoot proliferation and the results were superior to the results obtained from shoot tip explants. Addition of lower concentration of NAA with higher concentration of BAP was found more suitable in direct regeneration. Among the combinations of BAP + NAA and BAP + IAA, the highest percentage (96.67%) of shoot proliferation was noted in the media 1.0 mg/l

BAP+0.5 mg/l NAA followed by 93.33% regeneration in 1.0 mg/l BAP+0.5 mg/l IAA. The lowest percentage (46.67%) of shoot proliferation was noted in the media 3.0 mg/l BAP followed by 50% regeneration in 0.2 mg/l BAP. Highest average number of shoot per culture (8.12 ± 0.41) was observed in the media having 1.0 mg/l BAP+0.5 mg/l NAA followed by 6.31 ± 0.17 in 1.0 mg/l BAP+0.2 mg/l NAA. The lowest average number of shoots per culture was 3.22 ± 0.17 in media containing 0.2 mg/l BAP. Highest average length of shoot per culture was recorded 6.14 ± 0.39 cm in 1.0 mg/l BAP+0.5 mg/l IAA and the lowest average length 3.22 ± 0.17 cm was recorded in 0.2 mg/l BAP. Experiment result revealed that 1.0 mg/l BAP+0.5 mg/l NAA, 1.0 mg/l BAP+0.2 mg/l NAA and 1.0 mg/l BAP+0.5 mg/l IAA were found most effective combinations for multiple shoot induction in *C. benghalensis*.

Experiment 2: Effect of different concentrations of Kn alone and in combinations with NAA or IAA on multiple shoot induction from nodal explants of *C. benghalensis*

In this investigation, five concentrations (0.2, 0.5, 1.0, 2.0 and 3.0mg/l) of Kn alone or combination of three concentrations (0.5, 1.0 and 2.0 mg/l) of Kn with four concentrations (0.1, 0.2, 0.5 and 1.0 mg/l) of NAA or IAA were treated in MS medium for the purpose of multiple shoot induction from nodal explants of *C. benghalensis*. Data were taken after 6 weeks of inoculation and days taken for shoot initiation, percentage of shoot formation, number of shoots per culture and length of shoot per culture were measured. The results are presented in **Table 3.3.10**. The efficiency of Kn + NAA was better than Kn alone and Kn + IAA on direct shoot regeneration from nodal explants. All the used media compositions formed multiple shoot. Lower concentration of NAA with higher concentration of Kn was found more suitable in direct regeneration. Among the combinations of Kn + NAA and Kn + IAA, the highest percentage (90%) of shoot proliferation was noted in the media 2.0 mg/l Kn+0.5 mg/l NAA followed by 86.67% regeneration in 1.0 mg/l Kn+0.5 mg/l NAA and 2.0 mg/l Kn+0.5 mg/l IAA. The lowest percentage (40%) of shoot proliferation was noted in the media 0.2 mg/l Kn followed by 60% regeneration in 3.0 mg/l Kn. Highest average number of shoot per culture (6.66 ± 0.27) was observed in the media having 2.0 mg/l Kn+0.5 mg/l NAA followed by 6.28 ± 0.18 in 1.0 mg/l Kn+0.5 mg/l NAA. The lowest average number of shoots per culture was 2.15 ± 0.11 in media containing 0.2 mg/l Kn. Highest average length of shoot per culture was recorded 5.17 ± 0.19 cm in 2.0 mg/l Kn+0.5 mg/l IAA and the lowest average length 2.84 ± 0.09 cm was recorded in 3.0 mg/l Kn. Experiment result revealed that 2.0 mg/l Kn+0.5 mg/l NAA, 1.0 mg/l Kn+0.5 mg/l NAA and 2.0 mg/l Kn+0.5 mg/l IAA were found most effective combinations for multiple shoot induction in *C. benghalensis*.

Table 3.3.9. Effect of different concentrations of BAP alone and combinations with NAA or IAA on multiple shoot induction from nodal explants of *C. benghalensis*. Data were taken after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
BAP				
0.2	7-10	50.00	2.63 ± 0.18	3.22 ± 0.17
0.5	7-10	76.67	3.12 ± 0.11	3.84 ± 0.16
1.0	6-8	86.67	3.95 ± 0.14	4.75 ± 0.11
2.0	7-10	73.33	3.06 ± 0.30	4.15 ± 0.28
3.0	7-10	46.67	2.88 ± 0.12	3.24 ± 0.20
BAP + NAA				
0.5 + 0.1	7-10	70.00	3.20 ± 0.23	3.08 ± 0.19
0.5 + 0.2	6-8	83.33	4.34 ± 0.11	3.56 ± 0.25
0.5 + 0.5	6-8	90.00	5.25 ± 0.11	4.10 ± 0.15
0.5 + 1.0	6-8	73.33	4.40 ± 0.30	4.62 ± 0.13
1.0 + 0.1	7-10	73.33	3.68 ± 0.11	4.16 ± 0.13
1.0 + 0.2	6-8	80.00	6.31 ± 0.17	4.92 ± 0.19
1.0 + 0.5	6-8	96.67	8.12 ± 0.41	5.46 ± 0.33
1.0 + 1.0	6-8	73.33	6.07 ± 0.15	5.10 ± 0.12
2.0 + 0.1	7-10	60.00	3.56 ± 0.10	3.89 ± 0.33
2.0 + 0.2	6-8	70.00	5.12 ± 0.20	4.41 ± 0.12
2.0 + 0.5	6-8	83.33	6.03 ± 0.13	5.13 ± 0.17
2.0 + 1.0	6-8	66.67	4.17 ± 0.28	3.98 ± 0.14
BAP + IAA				
0.5 + 0.1	7-10	70.00	3.61 ± 0.23	3.39 ± 0.21
0.5 + 0.2	6-8	83.33	4.13 ± 0.10	4.06 ± 0.10
0.5 + 0.5	6-8	90.00	5.00 ± 0.16	4.90 ± 0.14
0.5 + 1.0	6-8	76.67	4.92 ± 0.18	4.58 ± 0.09
1.0 + 0.1	7-10	73.33	3.64 ± 0.14	4.06 ± 0.06
1.0 + 0.2	6-8	90.00	5.11 ± 0.14	4.63 ± 0.21
1.0 + 0.5	6-8	93.33	6.26 ± 0.19	6.14 ± 0.39
1.0 + 1.0	6-8	83.33	4.20 ± 0.08	5.12 ± 0.12
2.0 + 0.1	7-10	66.67	3.60 ± 0.17	3.66 ± 0.11
2.0 + 0.2	7-10	76.67	4.50 ± 0.23	4.38 ± 0.25
2.0 + 0.5	6-8	83.33	5.23 ± 0.18	4.60 ± 0.17
2.0 + 1.0	7-10	63.33	3.11 ± 0.12	3.97 ± 0.18

* Values are the mean of three replicates with 10 explants.

Table 3.3.10. Effect of different concentrations of Kn alone and in combinations with NAA or IAA on multiple shoot induction from nodal explants of *C. benghalensis*. Data were taken after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
Kn				
0.2	7-10	40.00	2.15 ± 0.11	3.07 ± 0.15
0.5	7-10	70.00	2.67 ± 0.15	3.38 ± 0.19
1.0	6-8	83.33	3.55 ± 0.26	4.12 ± 0.33
2.0	7-10	70.00	2.66 ± 0.14	3.86 ± 0.10
3.0	7-10	60.00	2.58 ± 0.30	2.84 ± 0.09
Kn + NAA				
0.5 + 0.1	7-10	73.33	3.10 ± 0.21	3.30 ± 0.12
0.5 + 0.2	6-8	80.00	4.14 ± 0.28	4.04 ± 0.25
0.5 + 0.5	6-8	83.33	5.13 ± 0.39	4.80 ± 0.10
0.5 + 1.0	6-8	76.67	4.60 ± 0.14	4.72 ± 0.17
1.0 + 0.1	7-10	66.67	2.92 ± 0.14	3.98 ± 0.20
1.0 + 0.2	6-8	76.67	5.18 ± 0.14	4.92 ± 0.12
1.0 + 0.5	6-8	86.67	6.28 ± 0.18	5.08 ± 0.22
1.0 + 1.0	6-8	63.33	3.54 ± 0.21	4.76 ± 0.31
2.0 + 0.1	7-10	76.67	3.42 ± 0.08	3.50 ± 0.11
2.0 + 0.2	7-10	80.00	5.50 ± 0.11	4.36 ± 0.16
2.0 + 0.5	7-10	90.00	6.66 ± 0.27	4.77 ± 0.27
2.0 + 1.0	7-10	83.33	4.49 ± 0.25	4.11 ± 0.25
Kn + IAA				
0.5 + 0.1	8-12	76.67	2.80 ± 0.12	3.07 ± 0.13
0.5 + 0.2	7-10	80.00	2.94 ± 0.17	3.74 ± 0.18
0.5 + 0.5	7-10	83.33	4.10 ± 0.25	4.23 ± 0.18
0.5 + 1.0	7-10	76.67	4.38 ± 0.27	4.28 ± 0.15
1.0 + 0.1	8-12	60.00	3.23 ± 0.13	3.50 ± 0.06
1.0 + 0.2	7-10	70.00	5.16 ± 0.17	4.26 ± 0.31
1.0 + 0.5	7-10	80.00	5.84 ± 0.16	4.22 ± 0.17
1.0 + 1.0	8-12	66.67	4.54 ± 0.20	4.06 ± 0.19
2.0 + 0.1	8-12	70.00	3.30 ± 0.12	3.95 ± 0.20
2.0 + 0.2	8-12	80.00	4.38 ± 0.22	4.40 ± 0.13
2.0 + 0.5	8-12	86.67	5.11 ± 0.08	5.17 ± 0.19
2.0 + 1.0	8-12	70.00	3.33 ± 0.14	4.62 ± 0.24

* Values are the mean of three replicates with 10 explants.

(b) Shoot tip explants

Experiment 1: Effect of different concentrations of BAP alone and in combinations with NAA or IAA on multiple shoot induction from shoot tip explants of *C. benghalensis*

In this investigation five concentrations (0.2, 0.5, 1.0, 2.0 and 3.0mg/l) of BAP alone or combination of three concentrations (0.5, 1.0 and 2.0 mg/l) of BAP with four concentrations (0.1, 0.2, 0.5 and 1.0 mg/l) of NAA or IAA were treated in MS medium for the purpose of multiple shoot induction from shoot tip explants of *C. benghalensis*. Data were taken after 6 weeks of inoculation and days taken for shoot initiation, percentage of shoot formation, number of shoots per culture and length of shoot per culture were measured. The results are presented in **Table 3.3.11**. The efficiency of BAP + NAA was better than BAP alone and BAP + IAA on direct shoot regeneration from nodal explants. All the used media compositions formed shoot proliferation and the results were superior to the results obtained from shoot tip explants. Lower concentration of NAA with higher concentration of BAP was found more suitable in direct regeneration. Among the combinations of BAP + NAA and BAP + IAA, the highest percentage (90%) of shoot proliferation was noted in the media 1.0 mg/l BAP+0.5 mg/l NAA and 0.5 mg/l BAP+0.5 mg/l NAA followed by 86.67% regeneration in 0.5 mg/l BAP+0.5 mg/l IAA. The lowest percentage (50%) of shoot proliferation was noted in the media 0.2 mg/l BAP and 3.0 mg/l BAP. Highest average number of shoot per culture (5.45 ± 0.39) was observed in the media having 1.0 mg/l BAP+0.5 mg/l NAA followed by 5.15 ± 0.13 in 1.0 mg/l BAP+0.2 mg/l NAA. The lowest average number of shoots per culture was 1.92 ± 0.15 in media containing 0.2 mg/l BAP. Highest average length of shoot per culture was recorded 5.04 ± 0.28 cm in 1.0 mg/l BAP+0.5 mg/l IAA and the lowest average length 3.47 ± 0.25 cm was recorded in 3.0 mg/l BAP. Experiment result revealed that 1.0 mg/l BAP+0.5 mg/l NAA, 1.0 mg/l BAP+0.2 mg/l NAA and 1.0 mg/l BAP+0.5 mg/l IAA were found most effective combinations for multiple shoot induction in *C. benghalensis*.

Experiment 2: Effect of different concentrations of Kn alone and in combinations with NAA or IAA on multiple shoot induction from shoot tip explants of *C. benghalensis*

In this investigation, five concentrations (0.2, 0.5, 1.0, 2.0 and 3.0mg/l) of Kn alone or combination of three concentrations (0.5, 1.0 and 2.0 mg/l) of Kn with four concentrations (0.1, 0.2, 0.5 and 1.0 mg/l) of NAA or IAA were treated in MS medium for the purpose of multiple shoot induction from shoot tip explants of *C. benghalensis*. Data were taken after 6 weeks of inoculation and days taken for shoot initiation, percentage of shoot formation, number of shoots per culture and length of shoot per culture were measured. The results are presented in **Table 3.3.12**. The efficiency of Kn + NAA was better than Kn alone and Kn + IAA on direct shoot regeneration from nodal explants. All the used media compositions formed multiple shoot. Addition of lower concentration of NAA with higher concentration of Kn was found more suitable in direct regeneration. Among the combinations of Kn + NAA and Kn + IAA, the highest percentage (90.00%) of shoot proliferation was noted in the media 2.0 mg/l Kn+0.5 mg/l NAA followed by 86.67% regeneration in 1.0 mg/l Kn+0.5 mg/l NAA. The lowest percentage (50%) of shoot proliferation was noted in the media 0.2 mg/l Kn and 3.0 mg/l Kn followed by 56.67% regeneration in 2.0 mg/l Kn. Highest average number of shoot per culture (4.40 ± 0.11) was observed in the media having 2.0 mg/l Kn+0.5 mg/l NAA followed by 4.15 ± 0.21 in 1.0 mg/l Kn+0.5 mg/l NAA. The lowest average number of shoots per culture was 1.73 ± 0.15 in media containing 0.2 mg/l Kn. Highest average length of shoot per culture was recorded 4.47 ± 0.15 cm in 1.0 mg/l Kn+0.5 mg/l IAA followed by 4.26 ± 0.45 cm in 0.5 mg/l Kn+0.5 mg/l IAA and the lowest average length 3.04 ± 0.12 cm was recorded in 0.2 mg/l Kn. Experiment result revealed that 2.0 mg/l Kn+0.5 mg/l NAA, 1.0 mg/l Kn+0.5 mg/l NAA and 1.0 mg/l Kn+0.5 mg/l IAA were found most effective combinations for multiple shoot induction in *C. benghalensis*.

Table 3.3.11. Effect of different concentrations of BAP alone and combinations with NAA or IAA on multiple shoot induction from shoot tip explants of *C. benghalensis*. Data were taken after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
BAP				
0.2	7-10	50.00	1.92 ± 0.15	3.63 ± 0.26
0.5	7-10	73.33	2.26 ± 0.10	4.04 ± 0.18
1.0	7-10	76.67	3.76 ± 0.11	4.63 ± 0.11
2.0	8-12	60.00	3.18 ± 0.23	4.38 ± 0.28
3.0	8-12	50.00	2.27 ± 0.17	3.47 ± 0.25
BAP + NAA				
0.5 + 0.1	6-8	66.67	2.36 ± 0.20	3.54 ± 0.22
0.5 + 0.2	6-8	83.33	3.89 ± 0.33	4.17 ± 0.35
0.5 + 0.5	6-8	90.00	4.75 ± 0.14	4.80 ± 0.51
0.5 + 1.0	6-8	76.67	3.70 ± 0.19	3.80 ± 0.25
1.0 + 0.1	7-10	80.00	3.30 ± 0.17	3.90 ± 0.22
1.0 + 0.2	6-8	83.33	5.15 ± 0.13	3.96 ± 0.28
1.0 + 0.5	6-8	90.00	5.45 ± 0.39	4.26 ± 0.21
1.0 + 1.0	6-8	80.00	4.76 ± 0.21	4.33 ± 0.20
2.0 + 0.1	6-8	60.00	3.25 ± 0.14	3.64 ± 0.24
2.0 + 0.2	6-8	66.67	4.58 ± 0.33	4.12 ± 0.17
2.0 + 0.5	7-10	80.00	4.42 ± 0.41	4.26 ± 0.11
2.0 + 1.0	7-10	56.67	3.55 ± 0.28	3.56 ± 0.21
BAP + IAA				
0.5 + 0.1	7-10	73.33	2.58 ± 0.15	4.22 ± 0.16
0.5 + 0.2	7-10	80.00	2.80 ± 0.10	4.30 ± 0.21
0.5 + 0.5	7-10	86.67	3.66 ± 0.16	4.68 ± 0.31
0.5 + 1.0	7-10	60.00	3.21 ± 0.22	4.27 ± 0.18
1.0 + 0.1	7-10	70.00	3.08 ± 0.16	4.68 ± 0.07
1.0 + 0.2	7-10	70.00	3.42 ± 0.09	4.91 ± 0.19
1.0 + 0.5	7-10	83.33	4.15 ± 0.10	5.04 ± 0.28
1.0 + 1.0	7-10	70.00	3.50 ± 0.12	4.54 ± 0.23
2.0 + 0.1	7-10	56.67	2.50 ± 0.13	4.43 ± 0.33
2.0 + 0.2	7-10	66.67	3.65 ± 0.33	4.44 ± 0.18
2.0 + 0.5	7-10	76.67	3.83 ± 0.19	4.50 ± 0.41
2.0 + 1.0	7-10	60.00	2.43 ± 0.13	4.63 ± 0.15

* Values are the mean of three replicates with 10 explants.

Table 3.3.12. Effect of different concentrations of Kn alone and in combinations with NAA or IAA on multiple shoot induction from nodal explants of *C. benghalensis.* Data were taken after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
Kn				
0.2	8-12	50.00	1.73 ± 0.15	3.04 ± 0.12
0.5	8-12	70.00	2.64 ± 0.12	3.46 ± 0.11
1.0	8-12	76.67	3.46 ± 0.17	4.12 ± 0.21
2.0	8-12	60.00	2.93 ± 0.25	3.85 ± 0.22
3.0	8-12	50.00	2.13 ± 0.33	3.36 ± 0.09
Kn + NAA				
0.5 + 0.1	8-12	70.00	2.41 ± 0.15	3.67 ± 0.13
0.5 + 0.2	8-12	80.00	3.60 ± 0.12	3.96 ± 0.17
0.5 + 0.5	8-12	83.33	4.05 ± 0.25	4.10 ± 0.26
0.5 + 1.0	8-12	70.00	3.80 ± 0.27	3.94 ± 0.10
1.0 + 0.1	8-12	63.33	2.90 ± 0.10	3.97 ± 0.30
1.0 + 0.2	8-12	73.33	3.77 ± 0.19	3.90 ± 0.21
1.0 + 0.5	8-12	86.67	4.15 ± 0.21	4.08 ± 0.24
1.0 + 1.0	8-12	60.00	2.70 ± 0.27	3.86 ± 0.16
2.0 + 0.1	8-12	80.00	3.30 ± 0.18	3.73 ± 0.18
2.0 + 0.2	8-12	83.33	3.42 ± 0.16	3.76 ± 0.10
2.0 + 0.5	8-12	90.00	4.40 ± 0.11	4.10 ± 0.23
2.0 + 1.0	8-12	60.00	3.03 ± 0.17	3.68 ± 0.32
Kn + IAA				
0.5 + 0.1	8-12	70.00	2.66 ± 0.13	3.55 ± 0.20
0.5 + 0.2	8-12	83.33	2.73 ± 0.25	3.76 ± 0.32
0.5 + 0.5	8-12	83.33	3.31 ± 0.36	4.26 ± 0.45
0.5 + 1.0	8-12	66.67	3.27 ± 0.28	3.91 ± 0.17
1.0 + 0.1	8-12	60.00	2.88 ± 0.14	3.80 ± 0.42
1.0 + 0.2	8-12	70.00	3.45 ± 0.16	4.10 ± 0.27
1.0 + 0.5	8-12	73.33	4.07 ± 0.27	4.47 ± 0.15
1.0 + 1.0	8-12	60.00	2.52 ± 0.12	3.44 ± 0.09
2.0 + 0.1	8-12	73.33	3.33 ± 0.19	3.94 ± 0.25
2.0 + 0.2	8-12	73.33	3.46 ± 0.22	4.04 ± 0.13
2.0 + 0.5	8-12	80.00	3.85 ± 0.15	3.70 ± 0.10
2.0 + 1.0	8-12	60.00	3.20 ± 0.16	3.76 ± 0.17

* Values are the mean of three replicates with 10 explants.

Plate 3.3.4. Development of multiple shoot from Shoot tip and nodal explants of *C. benghalensis* through the process of direct organogenesis with their subsequent development.

A: Single shoot initiation from shoot tip explants; **B:** Single shoot initiation from nodal explants; **C-E:** Single and multiple shoot initiation from nodal explant in MS+ 1.0 mg/l BAP + 0.5 mg/l NAA after 2-4 weeks of culture; **F**: Multiple shoot proliferation from shoot tip explant in the same medium after 4-6 weeks of culture having same medium; **G**: Proliferation of multiple shoot in the same medium after 3-4 weeks of culture; **I**: Further multiple shoot proliferation and elongation from shoot tip explant in the same medium after 6-8 weeks of culture.

B. Rooting of *in vitro* grown shoots in *C. benghalensis*.

Shoot cuttings 4 – 6 cm long *in vitro* grown shoots were separated and transferred to rooting media. MS plain (without hormone) and half strength MS medium fortified with different concentrations (01, 0.2, 0.5, 1.0, 1.5, 2.0 and 3.0 mg/l) of NAA, IAA and IBA were used for root experiment. Rooting started within 8 to 12 days of culture. Among the tested concentrations half strength MS medium having 1.0 – 2.0 mg/l IBA found highest percentage (100%) of root formation. Data on days to root initiation, frequency of root formation (%), average number of root per culture and average length of root per culture were recorded after 5 weeks of culture. The results are described experiment wise:

Experiment 1: Effect of different concentrations of NAA, IAA, IBA and MS=0 on root induction from *in vitro* grown shoots of *C. benghalensis*

For adventitious root formation, the shoots obtained from *in vitro* grown shoots were cultured on half strength MS medium with different concentrations of NAA, IAA, IBA and half strength MS=0 (without hormones). Results obtained for the root induction, percentage of root formation, morphology, average number and length of roots are shown in **Table 3.3.13**. Percentage range of cultures produced roots varied from 43.33 – 100%. Highest 100% of root regeneration was recorded in 1.0 – 2.0 mg/l IBA. The lowest 43.33% of root regeneration was recorded in media having 0.1 mg/l IAA. The highest average number of roots per shoot was recorded 14.56 ± 0.52 in media having 1.5 mg/l IBA (**Plate 3.3.5**) followed by 12.47 ± 0.33 in 2.0 mg/l IBA. The lowest number of root per shoot was recorded 3.10 ± 0.10 in media having 0.1 mg/l IAA. Highest average length of roots 5.20 ± 0.19 cm was also recorded in 1.5 mg/l IBA and the lowest length of roots 2.45 ± 0.18 cm was recorded in 0.1 mg/l IAA. In most cases morphology of roots was healthy, thin and long. Half strength MS=0 also produced roots but it was only 30%.

Table 3.3.13. Effect of different concentrations of auxins on root induction from *in vitro* grown shoot on half strength MS medium in *C. benghalensis*. Data were recorded after 5 weeks of culture.

Growth regulators (mg/l)	Days taken for root initiation	% of explants induced root development	* Average number of root per culture (mean ± SE)	* Average length (cm) of root per culture (mean ± SE)
Half MS = 0	8-12	30.00	2.90 ± 0.13	2.15 ± 0.11
NAA				
0.1	8-12	50.00	3.40 ± 0.21	2.62 ± 0.15
0.2	8-12	70.00	4.48 ± 0.09	3.69 ± 0.22
0.5	8-12	86.67	5.08 ± 0.30	4.10 ± 0.28
1.0	8-12	100	10.67 ± 0.13	4.36 ± 0.12
1.5	8-12	93.33	7.50 ± 0.20	4.53 ± 0.33
2.0	8-12	96.67	7.42 ± 0.11	4.40 ± 0.10
3.0	8-12	83.33	5.22 ± 0.18	4.14 ± 0.11
IAA				
0.1	8-12	43.33	3.10 ± 0.10	2.45 ± 0.18
0.2	8-12	60.00	4.13 ± 0.22	3.56 ± 0.24
0.5	8-12	80.00	6.08 ± 0.38	4.22 ± 0.11
1.0	8-12	83.33	9.08 ± 0.25	4.59 ± 0.20
1.5	8-12	90.00	8.30 ± 0.09	4.51 ± 0.18
2.0	8-12	100	6.72 ± 0.17	4.70 ± 0.18
3.0	8-12	80.00	4.12 ± 0.14	3.96 ± 0.13
IBA				
0.1	8-12	60.00	3.28 ± 0.17	2.80 ± 0.18
0.2	8-12	76.67	5.30 ± 0.22	3.98 ± 0.17
0.5	8-12	90	6.88 ± 0.27	4.46 ± 0.25
1.0	8-12	100	12.18 ± 0.62	5.04 ± 0.25
1.5	8-12	100	14.56 ± 0.52	5.20 ± 0.19
2.0	8-12	100	12.47 ± 0.33	4.80 ± 0.14
3.0	8-12	80.00	8.32 ± 0.20	4.13 ± 0.33

* Values are the mean of three replicates with 10 explants.

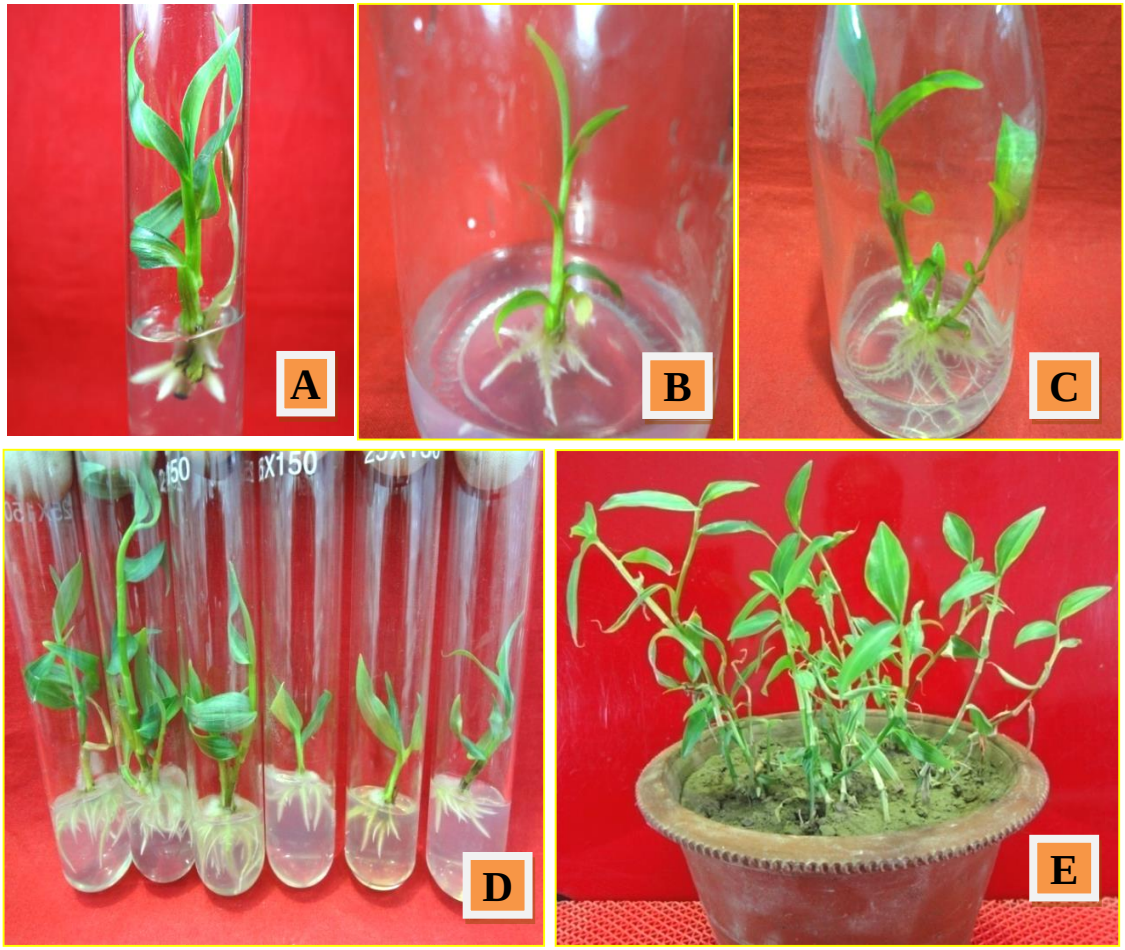


Plate 3.3.5. Induction of adventitious roots and establishment in soil in *C. benghalensis*

A-C: Induction of roots on *in vitro* regenerated shoots in half strength MS medium containing 1.5 mg/l IBA after 2-5 weeks of culture; **D:** Induction of roots on *in vitro* regenerated shoots in the same medium in cultural bottle; **E:** The *in vitro* grown seedlings acclimatized to grow in pots in outside environment after 4 weeks.

3.3.1.3. *Micropropagation of Curcuma zedoaria*

Experiments on direct organogenesis were carried out using different types of experiments *viz*. axillary bud and shoot tip in *C. zedoaria*. Axillary bud and Shoot tip explants were cultured for direct shoot regeneration. Explants were cultured on the MS (Murashige and Skoog 1962) agar gelled medium supplemented with different concentrations of auxins and cytokinins which were used singly or in combinations to investigate the initiation of shoot and its subsequent regeneration. Explants were also cultured on the liquid MS medium to investigate multiple shoot regeneration performance. For root induction, micro shoots obtained from direct organogenesis were transferred to rooting media. Finally well developed plants with both shoots and roots were transferred to natural conditions through successive phages of acclimatization. Details of the results so far obtained from each of the experiments are described under following heads:

A. Direct shoot regeneration in *C. zedoaria*

Two types of explants *viz.* axillary bud and shoot tip explants were cultured for direct shoot regeneration. Explants were cultured on MS medium with BAP and Kn used alone and in combinations with each other or with NAA and IBA. Data on number of days taken for shoot initiation, percentage of explant induced shoot development, average number of shoot per culture and average length of shoot per culture were collected after 6 weeks of culture. Axillary bud and shoot tip explants responded on all of supplemented cultured medium used. The results are described according to types of explants under separate heads:

(a) Axillary bud explants

Axillary bud explants of *C. zedoaria* were aseptically cultured on different concentrations (0.5, 1.0, 2.0, 3.0 and 4.0mg/l) of BAP and Kn either alone or in combinations with each other. Another experiment sets in combinations of three concentrations (1.0, 2.0 and 3.0 mg/l) of BAP or Kn with four concentrations (0.2, 0.5, 1.0 and 2.0mg/l) of NAA or with three concentrations (0.5, 1.0 and 2.0 mg/l) of IBA. Axillary bud explants found to be suitable for multiple shoot induction on all of the supplemented cultured media but morphogenic responses of cultured explants varied depending upon the culture media formulation. Effects of different concentrations and combinations of auxins and cytokinins on multiple shoot induction from axillary bud explants are described below:

Experiment 1: Effect of different concentrations of BAP and Kn alone and in combinations on multiple shoot induction from axillary bud explants of *C. zedoaria*

In this present investigation six concentrations (0.2, 0.5, 1.0, 2.0, 3.0 and 4.0 mg/l) of BAP and Kn used alone and in combination of four concentrations (0.5, 1.0, 2.0 and 3.0 mg/l) of BAP with four concentrations (0.2, 0.5, 1.0 and 2.0 mg/l) of Kn were

treated in MS medium for the purpose of multiple shoot induction from axillary bud explants of *C. zedoaria*. Data were taken after 6 weeks of inoculation and percentage of shoot formation, number of shoot/culture and length of shoot/culture were measured. The results are presented in **Table 3.3.14.** Morphogenic responses of the explants were found to vary with hormonal formulations present in the culture media. Shoot proliferation ranged from 50 – 86.67%. Highest percentage (86.67%) of multiple shoot formation was observed in MS medium containing 2.0 mg/l BAP followed by 83.33% in MS medium containing 1.0 mg/l Kn. The lowest percentage (50.00%) of multiple shoot formation was observed in the medium having 3.0 mg/l BAP+2.0 mg/l Kn. Highest mean number of shoots was 5.52 ± 0.17 in MS medium having 2.0 mg/l BAP. Lowest mean number of shoot was 2.16 ± 0.13 in media containing 0.5 mg/l BAP +0.2 mg/l Kn. Average length of shoots gradually increased after induction of shoot. Highest average length was recorded 7.41 ± 0.28 cm in 2.0 mg/l BAP + 0.5 mg/l Kn followed by 6.70 ± 0.17 cm in 2.0 mg/l BAP + 0.2 mg/l Kn. The lowest average length was 3.90 ± 0.23 cm in 0.5 mg/l BAP +0.2 mg/l Kn. Experiment result revealed that 2.0 mg/l BAP alone and combination of 2.0 mg/l BAP + 0.5 mg/l Kn were found most effective concentrations for multiple shoot induction in *C. zedoaria*.

Experiment 2: Effect of different concentrations and combinations of BAP and Kn with NAA or IBA on multiple shoot induction

Explants were cultured on MS medium supplemented with three concentrations (1.0, 2.0 and 3.0 mg/l) of BAP and Kn with four concentrations of NAA (0.2, 0.5, 1.0 and 2.0 mg/l) or three concentrations of (0.5, 1.0 and 2.0 mg/l) of IBA. Data were recorded after 6 weeks of culture and results on different parameters are presented in the **Table 3.3.15**. The efficiency of BAP+IBA was observed better than that of other combinations on direct shoot regeneration. All the used media compositions formed multiple shoot and the results were inferior to the results obtained from shoot tip explants. Among the concentrations the highest percentage (90%) of shoot proliferation was noted in the media having 2.0 mg/l BAP+ 1.0 mg/l IBA. The lowest percentage (40%) of shoot proliferation was noted in 2.0 mg/l Kn + 2.0 mg/l NAA. Highest mean number of shoot per culture was 5.90 ± 0.31 in media having 2.0 mg/l BAP+ 1.0 mg/l IBA followed by 5.80 ± 0.17 in media having 2.0 mg/l BAP + 0.5 mg/l IBA. Lowest mean number of shoot per culture was 1.90 ± 0.23 in media having 3.0 mg/l Kn + 2.0 mg/l NAA. Highest average length of shoot per culture was 6.91 ± 0.26 cm in the media having 2.0 mg/l BAP+ 0.5 mg/l NAA and lowest average length of shoot per culture 3.65 ± 0.17 cm in the media containing 3.0 mg/l BAP+ 2.0 mg/l NAA (**Plate 3.3.6**).

Table 3.3.14. Effect of different concentrations of BAP and Kn singly and in combinations on multiple shoot induction from axillary bud explants of *C. zedoaria.* Data were taken after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
BAP				
0.2	8-10	70.00	2.70 ± 0.16	5.60 ± 0.17
0.5	8-10	70.00	3.42 ± 0.12	5.54 ± 0.11
1.0	8-10	80.00	3.70 ± 0.09	5.20 ± 0.45
2.0	6-8	86.67	5.52 ± 0.17	5.42 ± 0.22
3.0	6-8	80.00	4.30 ± 0.23	5.53 ± 0.12
4.0	8-10	66.67	4.13 ± 0.21	5.57 ± 0.18
Kn				
0.2	8-10	60.00	3.12 ± 0.19	4.93 ± 0.19
0.5	8-10	70.00	3.72 ± 0.10	5.81 ± 0.11
1.0	8-10	83.33	3.86 ± 0.39	6.20 ± 0.23
2.0	8-10	70.00	3.60 ± 0.17	6.03 ± 0.17
3.0	8-10	66.67	3.45 ± 0.23	5.50 ± 0.27
4.0	8-10	60.00	3.10 ± 0.23	4.76 ± 0.14
BAP + Kn				
0.5 + 0.2	8-10	66.67	2.16 ± 0.13	3.90 ± 0.23
0.5 + 0.5	8-10	73.33	3.22 ± 0.11	5.54 ± 0.11
0.5 + 1.0	8-10	80.00	3.30 ± 0.09	4.71 ± 0.19
0.5 + 2.0	8-10	70.00	3.10 ± 0.21	4.52 ± 0.29
1.0 + 0.2	8-10	60.00	2.21 ± 0.19	5.30 ± 0.29
1.0 + 0.5	8-10	60.00	3.45 ± 0.12	6.13 ± 0.25
1.0 + 1.0	8-10	73.33	3.10 ± 0.33	4.48 ± 0.31
1.0 + 2.0	8-10	66.67	2.73 ± 0.13	4.03 ± 0.25
2.0 + 0.2	8-10	66.67	2.70 ± 0.15	6.70 ± 0.17
2.0 + 0.5	8-10	73.33	4.15 ± 0.19	7.41 ± 0.28
2.0 + 1.0	8-10	70.00	3.68 ± 0.31	6.54 ± 0.25
2.0 + 2.0	8-10	60.00	3.09 ± 0.15	5.24 ± 0.33
3.0 + 0.2	8-10	60.00	2.54 ± 0.14	4.13 ± 0.17
3.0 + 0.5	8-10	66.67	3.45 ± 0.27	4.55 ± 0.31
3.0 + 1.0	8-10	70.00	3.00 ± 0.39	4.76 ± 0.33
3.0 + 2.0	8-10	50.00	2.90 ± 0.15	4.30 ± 0.21

* Values are the mean of three replicates with 10 explants.

Table 3.3.15. Effect of different concentrations and combinations of BAP and Kn with NAA and IBA on multiple shoot induction from axillary bud explants of *C. zedoaria.* Data were taken after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
BAP + NAA				
1.0 + 0.2	8-10	66.67	3.51 ± 0.19	5.23 ± 0.13
1.0 + 0.5	8-10	76.67	4.20 ± 0.10	6.64 ± 0.17
1.0 + 1.0	8-10	70.00	3.45 ± 0.21	4.82 ± 0.15
1.0 + 2.0	8-10	56.67	2.61 ± 0.23	4.20 ± 0.28
2.0 + 0.2	8-10	73.33	4.70 ± 0.19	5.20 ± 0.23
2.0 + 0.5	8-10	80.00	5.32 ± 0.34	6.91 ± 0.26
2.0 + 1.0	8-10	66.67	3.10 ± 0.21	4.72 ± 0.23
2.0 + 2.0	8-10	56.67	2.60 ± 0.17	3.70 ± 0.11
3.0 + 0.2	8-10	73.33	4.31 ± 0.25	5.25 ± 0.23
3.0 + 0.5	8-10	66.67	3.85 ± 0.31	5.10 ± 0.33
3.0 + 1.0	8-10	60.00	3.27 ± 0.17	4.36 ± 0.05
3.0 + 2.0	8-10	53.33	2.22 ± 0.25	3.65 ± 0.17
BAP + IBA				
1.0 + 0.5	8-10	86.67	4.20 ± 0.19	5.27 ± 0.12
1.0 + 1.0	8-10	80.00	3.00 ± 0.33	5.40 ± 0.15
1.0 + 2.0	8-10	73.33	2.50 ± 0.19	4.79 ± 0.25
2.0 + 0.5	8-10	86.67	5.80 ± 0.17	6.21 ± 0.28
2.0 + 1.0	8-10	90.00	5.90 ± 0.31	6.54 ± 0.22
2.0 + 2.0	8-10	70.00	4.32 ± 0.25	5.20 ± 0.15
3.0 + 0.5	8-10	80.00	5.05 ± 0.13	5.80 ± 0.25
3.0 + 1.0	8-10	66.67	3.06 ± 0.15	4.50 ± 0.09
3.0 + 2.0	7-8	53.33	2.70 ± 0.22	4.04 ± 0.17
Kn + NAA				
1.0 + 0.2	8-10	80.00	4.10 ± 0.14	5.43 ± 0.33
1.0 + 0.5	8-10	83.33	4.53 ± 0.15	5.50 ± 0.17
1.0 + 1.0	8-10	70.00	3.11 ± 0.17	5.10 ± 0.13
1.0 + 2.0	8-10	53.33	2.42 ± 0.21	4.30 ± 0.19
2.0 + 0.2	8-10	76.67	3.40 ± 0.13	5.54 ± 0.33
2.0 + 0.5	8-10	80.00	4.12 ± 0.11	5.52 ± 0.23
2.0 + 1.0	8-10	66.67	2.88 ± 0.19	4.10 ± 0.45
2.0 + 2.0	8-10	40.00	2.30 ± 0.19	3.83 ± 0.12
3.0 + 0.2	8-10	70.00	3.50 ± 0.13	5.08 ± 0.15
3.0 + 0.5	8-10	70.00	3.45 ± 0.29	5.10 ± 0.20
3.0 + 1.0	8-10	60.00	2.58 ± 0.17	4.30 ± 0.25
3.0 + 2.0	8-10	46.67	1.90 ± 0.23	3.10 ± 0.31
Kn + IBA				
1.0 + 0.5	8-10	86.67	4.24 ± 0.06	5.13 ± 0.11
1.0 + 1.0	8-10	76.67	3.86 ± 0.19	5.45 ± 0.17
1.0 + 2.0	8-10	53.33	2.90 ± 0.17	4.20 ± 0.45
2.0 + 0.5	8-10	73.33	3.50 ± 0.32	5.52 ± 0.11
2.0 + 1.0	8-10	80.00	4.67 ± 0.18	6.18 ± 0.14
2.0 + 2.0	8-10	70.00	3.30 ± 0.15	4.77 ± 0.15
3.0 + 0.5	8-10	73.00	3.80 ± 0.25	5.40 ± 0.25
3.0 + 1.0	8-10	66.67	3.50 ± 0.33	4.20 ± 0.05
3.0 + 2.0	8-10	60.00	2.70 ± 0.23	4.00 ± 0.31

* Values are the mean of three replicates with 10 explants.

(b) Shoot tip explants

Shoot tip explants were aseptically cultured on six concentrations (0.2, 0.5, 1.0, 2.0, 3.0 and 4.0mg/l) of BAP and Kn either alone or in combinations with each other. Another experiment sets in combinations of three concentrations (1.0, 2.0 and 3.0 mg/l) of BAP or Kn with four concentrations (0.2, 0.5, 1.0 and 2.0mg/l) of NAA or with three concentrations (0.5, 1.0 and 2.0 mg/l) of IBA. Shoot tip explants found to be suitable for multiple shoot induction on all of the supplemented cultured media but morphogenic responses of cultured explants varied depending upon the culture media formulation. Effects of different concentrations and combinations of auxins and cytokinins on multiple shoot induction from shoot tip explants are described below:

Experiment 1: Effect of different concentrations of BAP and Kn alone and in combinations on multiple shoot induction from shoot tip explants of *C. zedoaria*

In this present investigation six concentrations (0.2, 0.5, 1.0, 2.0, 3.0 and 4.0 mg/l) of BAP and Kn used alone and in combination of four concentrations (0.5, 1.0, 2.0 and 3.0 mg/l) of BAP with four concentrations (0.2, 0.5, 1.0 and 2.0 mg/l) of Kn were treated in MS medium for the purpose of multiple shoot induction from shoot tip explants of *C. zedoaria*. Data were taken after 6 weeks of inoculation and percentage of shoot formation, number of shoot/culture and length of shoot/culture were measured. The results are presented in **Table 3.3.16**. Morphogenic responses of the explants were found to vary with hormonal formulations present in the culture media. Shoot proliferation ranged from 40 – 90.00%. Highest percentage 90% of multiple shoot formation was observed in MS medium containing 2.0 mg/l BAP followed by 83.33% in MS medium containing 1.0 mg/l Kn. The lowest percentage (40.00%) of multiple shoot formation was observed in the medium having 0.5 mg/l BAP+0.2 mg/l Kn. Highest mean number of shoots was 5.62 ± 0.29 in MS medium having 2.0 mg/l BAP. Lowest mean number of shoot was 1.72 ± 0.05 in media containing 0.5 mg/l BAP +0.2 mg/l Kn. Average length of shoots gradually increased after induction of shoot. Highest average length was recorded 6.23 ± 0.39 cm in 2.0 mg/l BAP + 0.5 mg/l Kn followed by 6.10 ± 0.25 cm in 1.0 mg/l BAP. The lowest average length was 3.50 ± 0.09 cm in 0.5 mg/l BAP +0.5 mg/l Kn. Experiment result revealed that 2.0 mg/l BAP alone and combination of 2.0 mg/l BAP + 0.5 mg/l Kn were found most effective concentrations for multiple shoot induction in *C. zedoaria*.

Experiment 2: Effect of different concentrations and combinations of BAP and Kn with NAA or IBA on multiple shoot induction from shoot tip explants of *C. zedoaria*

Explants were cultured on MS medium supplemented with three concentrations (1.0, 2.0 and 3.0 mg/l) of BAP or Kn with four concentrations of NAA (0.2, 0.5, 1.0 and 2.0 mg/l) or three concentrations of IBA (0.5, 1.0 and 2.0 mg/l). Data were recorded after 6 weeks of culture and results on different parameters are presented in the **Table 3.3.17**. The efficiency of BAP+IBA was observed better than that of other combinations used for direct shoot regeneration. Multiple shoot formed in all the used media compositions and the results were superior to the results obtained from axillary bud explants. Among the concentrations the highest percentage (93.33%) of shoot proliferation was noted in the media having 2.0 mg/l BAP+ 1.0 mg/l IBA. The lowest percentage (53.33%) of shoot proliferation was noted in 2.0 mg/l Kn + 2.0 mg/l NAA. Highest mean number of shoot per culture was 6.20 ± 0.54 in media having 2.0 mg/l BAP+ 1.0 mg/l IBA followed by 5.78 ± 0.39 in media having 2.0 mg/l BAP + 0.5 mg/l NAA. Lowest mean number of shoot per culture was 2.20 ± 0.31 in media having 3.0 mg/l Kn + 2.0 mg/l NAA. Highest average length of shoot per culture was 7.48 ± 0.15 cm in the media having 1.0 mg/l BAP+ 1.0 mg/l IBA and lowest average length of shoot per culture 3.15 ± 0.19 cm in the media containing 2.0 mg/l Kn+ 2.0 mg/l NAA (**Plate 3.3.6**).

Table 3.3.16. Effect of different concentration of BAP and Kn singly and in combinations with each other on multiple shoot induction from shoot tip explants of *C. zedoaria*. Data were taken after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
BAP				
0.2	8-10	70.00	2.40 ± 0.11	4.90 ± 0.19
0.5	8-10	80.00	3.33 ± 0.13	4.73 ± 0.15
1.0	8-10	83.33	4.80 ± 0.12	6.20 ± 0.14
2.0	8-10	90.00	5.62 ± 0.29	6.10 ± 0.25
3.0	8-10	76.67	4.30 ± 0.23	5.53 ± 0.12
4.0	8-10	70.00	3.93 ± 0.23	5.10 ± 0.17
Kn				
0.2	8-10	50.00	2.48 ± 0.17	4.13 ± 0.16
0.5	8-10	70.00	3.56 ± 0.11	5.11 ± 0.11
1.0	8-10	83.33	4.25 ± 0.29	5.80 ± 0.14
2.0	8-10	70.00	4.06 ± 0.21	5.73 ± 0.16
3.0	8-10	63.33	3.42 ± 0.25	5.09 ± 0.21
4.0	8-10	63.33	3.10 ± 0.25	4.36 ± 0.15
BAP + Kn				
0.5 +0.2	8-10	40.00	1.72 ± 0.05	3.66 ± 0.12
0.5 +0.5	8-10	53.33	1.91 ± 0.17	3.50 ± 0.09
0.5 +1.0	8-10	70.00	3.30 ± 0.11	4.53 ± 0.15
0.5 +2.0	8-10	76.67	3.20 ± 0.29	4.37 ± 0.25
1.0 +0.2	8-10	70.00	4.20 ± 0.12	5.10 ± 0.17
1.0 +0.5	8-10	76.67	4.64 ± 0.29	5.52 ± 0.21
1.0 +1.0	8-10	80.00	4.54 ± 0.25	5.30 ± 0.29
1.0 +2.0	8-10	70.00	3.23 ± 0.16	4.22 ± 0.24
2.0 +0.2	8-10	70.00	3.14 ± 0.15	4.70 ± 0.18
2.0 +0.5	8-10	80.00	4.88 ± 0.11	6.23 ± 0.39
2.0 +1.0	8-10	76.67	4.70 ± 0.15	5.54 ± 0.25
2.0 +2.0	8-10	63.33	3.40 ± 0.15	4.27 ± 0.29
3.0 +0.2	8-10	66.67	3.50 ± 0.21	4.75 ± 0.27
3.0 +0.5	8-10	70.00	4.44 ± 0.15	4.85 ± 0.33
3.0 +1.0	8-10	80.00	4.20 ± 0.17	4.71 ± 0.13
3.0 +2.0	8-10	60.00	3.33 ± 0.25	4.10 ± 0.17

* Values are the mean of three replicates with 10 explants.

Table 3.3.17. Effect of different concentrations and combinations of BAP and Kn with NAA on multiple shoot induction from shoot tip explants of *C. zedoaria.*

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
BAP + NAA				
1.0 + 0.2	8-10	70.00	4.21 ± 0.17	5.65 ± 0.15
1.0 + 0.5	8-10	80.00	5.20 ± 0.23	7.10 ± 0.37
1.0 + 1.0	8-10	66.67	3.71 ± 0.36	4.70 ± 0.27
1.0 + 2.0	8-10	56.67	2.09 ± 0.16	4.30 ± 0.33
2.0 + 0.2	8-10	80.00	4.81 ± 0.25	5.20 ± 0.11
2.0 + 0.5	8-10	86.67	5.78 ± 0.39	6.50 ± 0.28
2.0 + 1.0	8-10	70.00	3.90 ± 0.22	4.86 ± 0.17
2.0 + 2.0	8-10	50.00	2.30 ± 0.11	3.60 ± 0.15
3.0 + 0.2	8-10	70.00	4.80 ± 0.45	5.33 ± 0.19
3.0 + 0.5	8-10	76.67	4.05 ± 0.17	5.00 ± 0.30
3.0 + 1.0	8-10	60.00	3.30 ± 0.29	4.31 ± 0.27
3.0 + 2.0	8-10	46.67	1.96 ± 0.22	3.83 ± 0.09
BAP + IBA				
1.0 + 0.5	8-10	90.00	3.20 ± 0.33	5.30 ± 0.17
1.0 + 1.0	8-10	90.00	4.04 ± 0.41	7.48 ± 0.15
1.0 + 2.0	8-10	80.00	3.70 ± 0.25	6.55 ± 0.22
2.0 + 0.5	8-10	76.67	5.75 ± 0.21	6.02 ± 0.43
2.0 + 1.0	8-10	93.33	6.20 ± 0.54	6.48 ± 0.49
2.0 + 2.0	8-10	70.00	3.66 ± 0.25	6.40 ± 0.22
3.0 + 0.5	8-10	83.33	5.70 ± 0.17	5.10 ± 0.51
3.0 + 1.0	8-10	70.00	3.16 ± 0.26	4.67 ± 0.19
3.0 + 2.0	7-8	56.67	2.80 ± 0.35	4.34 ± 0.18
Kn + NAA				
1.0 + 0.2	8-10	90.00	4.30 ± 0.15	6.24 ± 0.18
1.0 + 0.5	8-10	83.33	4.72 ± 0.25	5.60 ± 0.12
1.0 + 1.0	8-10	76.67	3.11 ± 0.19	5.22 ± 0.28
1.0 + 2.0	8-10	60.00	2.52 ± 0.25	4.10 ± 0.19
2.0 + 0.2	8-10	70.00	3.80 ± 0.12	5.20 ± 0.31
2.0 + 0.5	8-10	80.00	4.36 ± 0.33	5.22 ± 0.25
2.0 + 1.0	8-10	70.00	3.06 ± 0.19	4.10 ± 0.45
2.0 + 2.0	8-10	53.33	2.40 ± 0.33	3.15 ± 0.19
3.0 + 0.2	8-10	76.67	3.50 ± 0.17	5.44 ± 0.27
3.0 + 0.5	8-10	80.00	3.45 ± 0.29	5.50 ± 0.25
3.0 + 1.0	8-10	63.33	2.58 ± 0.12	4.48 ± 0.11
3.0 + 2.0	8-10	50.00	2.20 ± 0.31	3.20 ± 0.15
Kn + IBA				
1.0 + 0.5	8-10	80.00	4.28 ± 0.41	4.93 ± 0.12
1.0 + 1.0	8-10	80.00	4.30 ± 0.19	4.96 ± 0.19
1.0 + 2.0	8-10	60.00	3.00 ± 0.17	4.20 ± 0.15
2.0 + 0.5	8-10	73.33	3.90 ± 0.39	5.50 ± 0.19
2.0 + 1.0	8-10	86.67	5.08 ± 0.28	5.56 ± 0.31
2.0 + 2.0	8-10	73.33	3.20 ± 0.15	4.62 ± 0.13
3.0 + 0.5	8-10	76.67	4.27 ± 0.25	4.80 ± 0.29
3.0 + 1.0	8-10	80.00	3.70 ± 0.37	4.34 ± 0.24
3.0 + 2.0	8-10	60.00	2.90 ± 0.12	4.40 ± 0.17

* Values are the mean of three replicates with 10 explants.

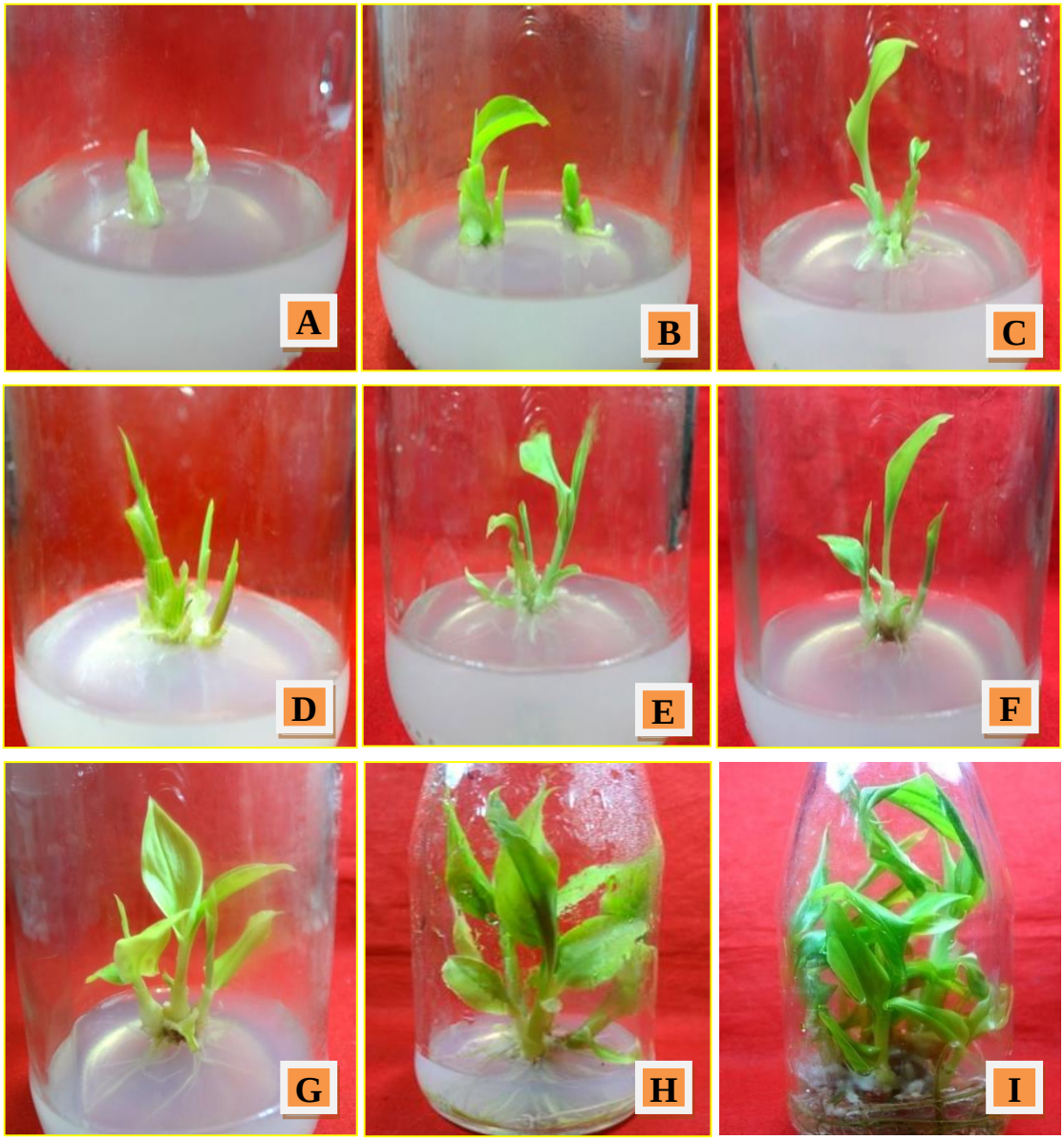


Plate 3.3.6. Development of multiple shoot from Shoot tip and axillary bud explants of *C. zedoaria* through the process of direct organogenesis with their subsequent development.

A: Shoot tip explant inoculated in the medium; **B-C:** Single and double shoot initiation from shoot tip explant in 2.0 mg/l BAP + 1.0 mg/l IBA after 2-3 weeks of culture; **D**: Initiation of multiple shoot proliferation from axillary bud after 2 weeks of inoculation in media having same medium; **E-G**: Proliferation of multiple shoot in the same medium after 3-4 weeks of culture; **H-I**: Further multiple shoot proliferation and elongation from shoot tip explant in the same medium after 4-6 weeks of culture.

B. Effect of liquid medium on the shoot proliferation of *C. zedoaria* from shoot tip explants.

In the present investigation, the aseptic shoot grown from shoot tip explants were trimmed of new leaves and roots and were cultured in liquid MS medium supplemented with three concentrations (1.0, 2.0 and 3.0 mg/l) of BAP or Kn with four concentrations of NAA (0.2, 0.5, 1.0 and 2.0 mg/l) or three concentrations of IBA (0.5, 1.0 and 2.0 mg/l) and 20% cocoanut water. Data were recorded after 6 weeks of culture and results on different parameters are presented in the **Table 3.3.18**. Liquid MS medium was more effective than that of solid medium for producing multiple shoots. The efficiency of BAP+IBA was observed better than that of other combinations tested on shoot multiplication. All the used media compositions formed shoot. Among the concentrations the highest percentage (100%) of shoot proliferation was noted in the media having 1.0 mg/l BAP + 0.5 mg/l NAA, 2.0 mg/l BAP + 0.2 mg/l NAA, 2.0 mg/l BAP+ 0.5 mg/l NAA, 3.0 mg/l BAP+ 0.5 mg/l NAA, 2.0 mg/l BAP+ 0.5 mg/l IBA, 2.0 mg/l BAP+ 1.0 mg/l IBA and 2.0 mg/l BAP+ 2.0 mg/l IBA. The lowest percentage (56.67%) of shoot proliferation was noted in 3.0 mg/l Kn + 2.0 mg/l NAA. Highest mean number of shoot per culture was 20.43 ± 0.62 in media having 2.0 mg/l BAP+ 1.0 mg/l IBA followed by 18.33 ± 0.38 in media having 2.0 mg/l BAP + 0.5 mg/l IBA. Lowest mean number of shoot per culture was 5.30 ± 0.16 in media having 3.0 mg/l Kn + 2.0 mg/l NAA. Highest average length of shoot per culture was 5.50 ± 0.33 cm in the media having 1.0 mg/l BAP+ 0.5 mg/l NAA and lowest average length of shoot per culture 3.80 ± 0.12 cm in the media containing 3.0 mg/l Kn+ 2.0 mg/l NAA (**Plate 3.3.7**). Experiment result revealed that 2.0 mg/l BAP + 1.0 mg/l IBA and 2.0 mg/l BAP + 0.5 mg/l IBA were found most effective concentrations for multiple shoot proliferation in liquid MS medium of *C. zedoaria*.

Table 3.3.18. Effect of different concentrations and combinations of growth regulators in liquid MS medium with 20% cocoanut water on multiple shoot induction from shoot tip explants of *C. zedoaria.*

Growth regulators (mg/l)	Days taken for multiple shoot induction	% of multiple shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
BAP + NAA				
1.0 + 0.2	6-8	90.00	8.50 ± 0.62	4.20 ± 0.21
1.0 + 0.5	6-8	100	12.40 ± 0.68	5.50 ± 0.33
1.0 + 1.0	6-8	83.33	10.40 ± 0.53	5.15 ± 0.25
1.0 + 2.0	6-8	60.00	9.20 ± 0.47	4.30 ± 0.28
2.0 + 0.2	6-8	100	13.56 ± 0.31	4.44 ± 0.19
2.0 + 0.5	6-8	100	14.20 ± 0.39	4.80 ± 0.13
2.0 + 1.0	6-8	96.67	11.27 ± 0.62	5.20 ± 0.41
2.0 + 2.0	6-8	60.00	8.60 ± 0.41	4.70 ± 0.19
3.0 + 0.2	6-8	96.67	9.70 ± 0.44	4.40 ± 0.19
3.0 + 0.5	6-8	100	13.10 ± 0.33	4.20 ± 0.22
3.0 + 1.0	6-8	80.00	9.00 ± 0.37	4.10 ± 0.21
3.0 + 2.0	6-8	66.67	6.30 ± 0.53	4.02 ± 0.11
BAP + IBA				
1.0 + 0.5	6-8	90.00	12.17 ± 0.19	4.60 ± 0.11
1.0 + 1.0	6-8	80.00	9.00 ± 0.13	4.73 ± 0.17
1.0 + 2.0	6-8	76.67	8.00 ± 0.11	4.24 ± 0.10
2.0 + 0.5	6-8	100	18.33 ± 0.38	5.33 ± 0.14
2.0 + 1.0	6-8	100	20.43 ± 0.62	5.34 ± 0.19
2.0 + 2.0	6-8	100	13.97 ± 0.45	4.70 ± 0.09
3.0 + 0.5	6-8	90.00	10.10 ± 0.39	4.51 ± 0.28
3.0 + 1.0	6-8	76.67	8.50 ± 0.33	4.20 ± 0.05
3.0 + 2.0	6-8	63.33	9.32 ± 0.25	4.11 ± 0.14
Kn + NAA				
1.0 + 0.2	6-8	90.00	8.27 ± 0.36	4.20 ± 0.15
1.0 + 0.5	6-8	90.00	10.10 ± 0.64	5.06 ± 0.29
1.0 + 1.0	6-8	80.00	8.80 ± 0.39	4.72 ± 0.18
1.0 + 2.0	6-8	60.00	5.30 ± 0.16	5.47 ± 0.27
2.0 + 0.2	6-8	70.00	6.70 ± 0.28	4.30 ± 0.17
2.0 + 0.5	6-8	93.33	8.80 ± 0.82	4.15 ± 0.25
2.0 + 1.0	6-8	70.00	7.76 ± 0.43	4.24 ± 0.18
2.0 + 2.0	6-8	70.00	6.00 ± 0.11	3.92 ± 0.15
3.0 + 0.2	6-8	86.67	6.08 ± 0.11	4.02 ± 0.38
3.0 + 0.5	6-8	80.00	7.40 ± 0.23	4.50 ± 0.23
3.0 + 1.0	6-8	66.67	6.20 ± 0.17	4.08 ± 0.39
3.0 + 2.0	6-8	56.67	5.40 ± 0.18	3.80 ± 0.12
Kn + IBA				
1.0 + 0.5	6-8	80.00	10.54 ± 0.48	4.17 ± 0.28
1.0 + 1.0	6-8	86.67	12.41 ± 0.62	4.73 ± 0.19
1.0 + 2.0	6-8	70.00	7.10 ± 0.33	3.94 ± 0.09
2.0 + 0.5	6-8	86.67	10.30 ± 0.19	4.58 ± 0.17
2.0 + 1.0	6-8	90.00	13.70 ± 0.44	5.03 ± 0.23
2.0 + 2.0	6-8	80.00	9.00 ± 0.62	4.23 ± 0.12
3.0 + 0.5	6-8	80.00	8.50 ± 0.82	4.14 ± 0.16
3.0 + 1.0	6-8	70.00	7.32 ± 0.41	4.07 ± 0.23
3.0 + 2.0	6-8	63.33	6.31± 0.19	3.87 ± 0.14

* Values are the mean of three replicates with 10 explants.

Plate 3.3.7. Development of multiple shoot from aseptic shoot explants of *C. zedoaria* in liquid MS medium.

A: Multiple shoot proliferation from shoot tip explant in liquid MS medium containing 2.0 mg/l BAP+1.0 mg/l IBA with 20% cocoanut water after 1-2 weeks of culture; **B:** Multiple shoots proliferation from shoot tip explant in the same medium after 3-4 weeks of culture; **C-D:** Further multiple shoot proliferation and elongation from shoot tip explant in the same medium after 5-6 weeks of culture.

C. Rooting of *in vitro* grown shoots in *C. zedoaria*

Shoot cuttings 4.5 – 6.5 cm long *in vitro* grown shoots were separated and transferred to rooting media. Half strength MS medium fortified with different concentrations (0.1, 0.2, 0.5, 1.0 and 2.0 mg/l) of NAA, IBA and IAA alone and in combination of BAP or Kn (0.5 and 1.0) with four concentrations (0.5, 1.0, 2.0 and 3.0 mg/l) of IBA were used for root experiment. Rooting started within 8 to 16 days of culture. Among the tested concentrations half MS medium having in combinations of BAP and IBA were found highest percentage (100%) of root formation. Data on days to root initiation, frequency of root formation (%), average number of root per culture and average length of root per culture were recorded after 5 weeks of culture. The results are described experiment wise:

Experiment 1: Effect of different concentrations of NAA, IBA, IAA and in combination of BAP + IBA and Kn + IBA on root induction from *in vitro* grown shoots of *C. zedoaria.*

In this experiment, the *in vitro* proliferated shoots were cultured on half MS medium with different concentrations (0.1, 0.2, 0.5, 1.0 and 2.0 mg/l) of NAA, IBA and IAA alone and in combination of BAP or Kn (0.5 and 1.0) with three concentrations (0.5, 1.0 and 2.0 mg/l) of IBA. The rooting emerged from 8 – 16 days of culture. Results obtained for the root induction, percentage of root formation, morphology, average number and length of roots are shown in **Table 3.3.19**. Percentage range of cultures produced roots varied from 60.00 – 100%. In combinations of BAP and IBA showed highest 100% of root regeneration. The lowest 70.00% of root regeneration was recorded in media having 0.1 mg/l NAA. The highest average number of roots per shoot was recorded 9.48 ± 0.48 in media having 0.5 mg/l BAP + 2.0 mg/l IBA (**Plate 3.3.8**) followed by 8.40 ± 0.20 in 1.0 mg/l BAP + 2.0 mg/l IBA. The lowest number of root per shoot was recorded 2.83 ± 0.17 in media having 0.1 mg/l NAA. Highest average length of roots 6.64 ± 0.26 cm was recorded in 0.5 mg/l BAP + 2.0 mg/l IBA and the lowest length of roots 3.18 ± 0.12 cm was recorded in 0.1 mg/l IAA. In most cases morphology of roots was thin but someone looked swollen and long.

Experiment 2: Effect of different concentrations and combinations of BAP and Kn with IAA or NAA in ½ MS medium on rooting of *in vitro* raised shoots of *C. zedoaria*.

In this experiment, the *in vitro* shoots were cultured on half strength MS medium with two concentrations (0.5 and 1.0 mg/l) of BAP or Kn with four concentrations (0.5, 1.0, 2.0 and 3.0 mg/l) of IAA or NAA. The rooting emerged from 8 – 16 days of culture. Results obtained for the root induction, percentage of root formation, morphology, average number and length of roots are shown in **Table 3.3.20**. The results of this investigation were superior to the results of NAA, IAA and IBA alone but inferior to combination of BAP+IBA. Percentage range of cultures produced roots varied from 70.00 – 100%. The highest average number of roots per shoot was recorded 7.30 ± 0.33 in media having 2.0 mg/l IAA + 0.5 mg/l BAP followed by 7.12 ± 0.56 in 2.0 mg/l IAA + 0.5 mg/l Kn. The lowest average number of roots per culture was recorded 3.04 ± 0.17 in media having 3.0 mg/l NAA + 0.5 mg/l BAP. The highest average length of roots 5.60 ± 0.29 cm was recorded in media having 2.0 mg/l IAA + 1.0 mg/l BAP. Next to 5.25 ± 0.27 roots per culture was recorded in media having 2.0 mg/l IAA+ 0.5 mg/l Kn. The lowest average length of roots was recorded 3.11 ± 0.09 in media having 0.5 mg/l NAA+ 0.5 mg/l Kn. In most cases morphology of roots was thin but someone looked swollen and elongated.

Table 3.3.19. Effect of ½ strength MS medium with different concentrations and combinations of phytohormones on root induction in *C. zedoaria*. Data were taken after 5 weeks of culture.

Growth regulators (mg/l)	Days taken for root initiation	% of explants induced root development	* Average number of root per culture (mean ± SE)	* Average length (cm) of root per culture (mean ± SE)
NAA				
0.1	12-16	70.00	2.83 ± 0.17	3.42 ± 0.33
0.2	12-16	83.33	3.35 ± 0.45	3.20 ± 0.14
0.5	12-16	90.00	3.84 ± 0.15	3.44 ± 0.22
1.0	12-16	96.67	4.30 ± 0.21	4.55 ± 0.43
2.0	12-16	83.33	3.73 ± 0.24	4.32 ± 0.18
IBA				
0.1	8-12	73.33	3.94 ± 0.31	3.92 ± 0.15
0.2	8-12	83.33	4.86 ± 0.20	4.20 ± 0.25
0.5	8-12	90.00	5.14 ± 0.15	3.58 ± 0.21
1.0	8-12	90.00	5.70 ± 0.45	4.46 ± 0.27
2.0	8-12	96.67	7.15 ± 0.19	4.35 ± 0.32
IAA				
0.1	8-12	80.00	3.82 ± 0.17	3.18 ± 0.12
0.2	8-12	90.00	4.00 ± 0.19	3.20 ± 0.20
0.5	8-12	93.33	4.42 ± 0.28	4.36 ± 0.27
1.0	8-12	96.67	6.71 ± 0.41	4.73 ± 0.09
2.0	8-12	90.00	5.85 ± 0.52	4.25 ± 0.16
BAP + IBA				
0.5 + 0.5	8-12	90.00	6.80 ± 0.17	4.13 ± 0.25
0.5 + 1.0	8-12	100.00	8.30 ± 0.44	4.92 ± 0.11
0.5 + 2.0	8-12	100.00	9.48 ± 0.48	6.64 ± 0.26
1.0 + 0.5	8-12	100.00	5.04 ± 0.49	4.32 ± 0.42
1.0 + 1.0	8-12	100.00	7.12 ± 0.31	5.10 ± 0.29
1.0 + 2.0	8-12	100.00	8.40 ± 0.20	5.54 ± 0.25
Kn + IBA				
0.5 + 0.5	10-14	76.67	3.09 ± 0.19	3.22 ± 0.19
0.5 + 1.0	10-14	90.00	4.52 ± 0.12	4.10 ± 0.12
0.5 + 2.0	10-14	93.33	5.60 ± 0.52	4.30 ± 0.33
1.0 + 0.5	10-14	80.00	4.15 ± 0.27	3.31 ± 0.29
1.0 + 1.0	10-14	90.00	4.56 ± 0.39	4.09 ± 0.17
1.0 + 2.0	10-14	90.00	6.20 ± 0.25	4.16 ± 0.18

* Values are the mean of three replicates with 10 explants.

Table 3.3.20. Effect of ½ strength MS medium with different concentrations and combinations of phytohormones on root induction in *C. zedoaria*. Data were taken after 5 weeks of culture.

Growth regulators (mg/l)	Days taken for root initiation	% of explants induced root development	* Average number of root per culture (mean ± SE)	* Average length (cm) of root per culture (mean ± SE)
BAP + IAA				
0.5 + 0.5	8-12	90.00	4.44 ± 0.25	3.20 ± 0.17
0.5 + 1.0	8-12	100.00	6.20 ± 0.52	4.42 ± 0.14
0.5 + 2.0	8-12	100.00	7.30 ± 0.33	5.15 ± 0.21
0.5 + 3.0	8-12	90.00	5.08 ± 0.15	4.16 ± 0.18
1.0 + 0.5	8-12	83.33	4.40 ± 0.17	3.70 ± 0.29
1.0 + 1.0	8-12	100.00	6.52 ± 0.18	4.85 ± 0.24
1.0 + 2.0	8-12	100.00	6.86 ± 0.30	5.60 ± 0.29
1.0 + 3.0	8-12	90.00	4.90 ± 0.11	4.42 ± 0.18
Kn + IAA				
0.5 + 0.5	8-12	86.67	4.20 ± 0.18	3.44 ± 0.17
0.5 + 1.0	8-12	90.00	6.40 ± 0.61	4.47 ± 0.15
0.5 + 2.0	8-12	100.00	7.12 ± 0.56	5.25 ± 0.27
0.5 + 3.0	8-12	80.00	4.78 ± 0.09	4.00 ± 0.11
1.0 + 0.5	8-12	80.00	3.80 ± 0.17	3.30 ± 0.25
1.0 + 1.0	8-12	90.00	6.16 ± 0.33	4.72 ± 0.29
1.0 + 2.0	8-12	100.00	6.70 ± 0.17	5.40 ± 0.25
1.0 + 3.0	8-12	70.00	4.90 ± 0.39	3.96 ± 0.17
BAP + NAA				
0.5 + 0.5	10-14	80.00	3.54 ± 0.19	3.13 ± 0.22
0.5 + 1.0	10-14	100.00	5.30 ± 0.45	4.52 ± 0.15
0.5 + 2.0	10-14	90.00	4.38 ± 0.43	4.14 ± 0.28
0.5 + 3.0	10-14	76.67	3.04 ± 0.17	3.36 ± 0.32
1.0 + 0.5	10-14	80.33	3.62 ± 0.11	3.20 ± 0.22
1.0 + 1.0	10-14	100.00	5.53 ± 0.30	4.45 ± 0.27
1.0 + 2.0	10-14	90.00	4.70 ± 0.19	3.63 ± 0.25
1.0 + 3.0	10-14	70.00	3.32 ± 0.28	3.12 ± 0.15
Kn + NAA				
0.5 + 0.5	12-16	76.67	3.44 ± 0.20	3.11 ± 0.09
0.5 + 1.0	12-16	90.00	4.60 ± 0.15	3.94 ± 0.15
0.5 + 2.0	12-16	83.33	4.30 ± 0.19	3.76 ± 0.10
0.5 + 3.0	12-16	70.00	3.15 ± 0.16	3.52 ± 0.11
1.0 + 0.5	12-16	80.00	4.13 ± 0.11	3.40 ± 0.06
1.0 + 1.0	12-16	93.33	5.30 ± 0.65	3.81 ± 0.22
1.0 + 2.0	12-16	80.00	4.70 ± 0.12	3.70 ± 0.49
1.0 + 3.0	12-16	73.33	3.13 ± 0.38	3.33 ± 0.44

* Values are the mean of three replicates with 10 explants.

Plate 3.3.8. Induction of adventitious roots and establishment in soil in *C. zedoaria*

A: Induction of adventitious roots on shoots regenerated from shoot tip explants in half strength MS medium containing 2.0 mg/l IBA + 0.5 mg/l BAP after 2 weeks of culture; **B-C:** Further development roots in the same medium after 3-4 weeks of culture; **D:** Photograph of *C. zedoaria* with roots outside of culture bottle; **E:** The *in vitro* grown seedlings acclimatized to grow in pots in outside environment after 6 weeks.

3.3.1.4. *Micropropagation of Mucuna pruriens*

Experiment on direct and indirect organogenesis were carried out using different types of experiments *viz.* shoot apex, nodal explants, intermodal explants and leaves. Seeds were the primary explants and for their surface sterilization $HgCl_2$ was used. After sterilization, the seeds were used for germination. Before treating with $HgCl_2$ solution the seeds were washed with 3 drops of tween 20. After washing the seeds were treated with 0.1% $HgCl_2$ in ten duration of time (5, 6, 7, 8, 9, 10, 11, 12, 14 and 16 minutes). Then the seeds were cultured on the semisolid MS basal medium. Out of ten duration of time 11 minutes was found more effective, produced 100% contamination free culture. Some other duration like 9, 10, 12 and 14 minute of time also proved efficient for elimination of loose contamination. The aseptic seedling thus grew, attained a height 8-10 cm after 2-3 weeks of culture. Explants from *in vitro* grown seedling were then cut into appropriate size (1.0 – 2.0 cm) and cultured on plant growth regulators (PGR) supplemented media for induction of direct and indirect organogenesis. Both auxins (IAA, NAA, IBA and 2,4-D) and cytokinins (BAP and Kn) were used in the media either singly or in combinations. For root induction, micro shoots obtained from direct and indirect organogenesis were transferred to rooting media. Finally well developed plants with both shoots and roots were transferred to natural conditions through successive phages of acclimatization. Details of the results so far obtained from the experiments are described under following heads:

A. Direct regeneration

Two types of explants *viz*. shoot apex and nodal explants were cultured for direct shoot regeneration. Explants were cultured on MS medium with BAP and Kn used alone and in combinations with each other or with NAA and IAA. Data on number of days taken for shoot initiation, percentage of explant induced shoot development, average number of shoot per culture and average length of shoot per culture were collected after 6 weeks of culture. Among the two types of explants nodal explants responded on almost all of the supplemented media. The results are described according to types of explants under separate heads:

(a) Nodal explants

Experiment 1: Effect of different concentrations of BAP and Kn alone and in combinations on multiple shoot induction from nodal explants of *M. pruriens*.

In this present investigation five concentrations (0.2, 0.5, 1.0, 2.0 and 3.0 mg/l) of BAP and Kn used alone and in combination of three concentrations (0.5, 1.0 and 2.0 mg/l) of BAP with four concentrations (0.2, 0.5, 1.0 and 2.0 mg/l) of Kn were treated in MS medium for the purpose of multiple shoot induction from nodal explants of *M. pruriens*. Data were taken after 6 weeks of inoculation and percentage of shoot

formation, number of shoot per culture and length of shoot per culture were measured. The results are presented in **Table 3.3.21**. Morphogenic responses of the explants were found to vary with hormonal formulations present in the culture media. Shoot proliferation ranged from 30 – 83.33%. Highest percentage (83.33%) of multiple shoot formation was observed in MS medium containing 0.5 mg/l BAP+0.5 mg/l Kn followed by 76.67% in MS medium containing 0.5 mg/l BAP alone and 0.5 mg/l BAP + 1.0 mg/l Kn. The lowest percentage (30%) of multiple shoot formation was observed in media having 3.0 mg/l BAP. Highest mean number of shoots was 5.63 ± 0.23 in MS medium having 0.5 mg/l BAP followed by 5.60 ± 0.17 in MS medium having 0.5 mg/l BAP+0.5 mg/l Kn. The lowest mean number of shoot was 1.92 ± 0.09 in media containing 3.0 mg/l BAP. Average length of shoots gradually increased after induction of shoot. Highest average length was recorded 5.06 ± 0.15 cm in 0.5 mg/l BAP followed by 4.14 ± 0.07 cm in 1.0 mg/l BAP. The lowest average length was 2.14 ± 0.12 cm in 2.0 mg/l BAP+2.0 mg/l Kn. Experiment results revealed that 0.5 mg/l BAP and 1.0 mg/l BAP alone and combination of 0.5 mg/l BAP + 0.5 mg/l Kn were found most effective concentrations for multiple shoot induction in *M. pruriens* from nodal segment.

Experiment 2: Effect of different concentrations and combinations of BAP and Kn with NAA or in combinations with IAA on multiple shoot induction from nodal explants of *M. pruriens.* Data were taken after 6 weeks of culture.

Explants were cultured on MS medium supplemented with three concentrations of BAP (0.5, 1.0 and 2.0 mg/l) and Kn (0.5, 1.0 and 2.0 mg/l) combined with four concentrations of NAA (0.2, 0.5, 1.0 and 2.0 mg/l) or in combinations of BAP+Kn+IAA. Data were recorded after 6 weeks of culture and results on different parameters are presented in the **Table 3.3.22**. The efficiency of BAP+Kn+IAA was better than BAP+NAA or Kn+NAA. Among the concentrations the highest percentage (96.67%) of shoot proliferation was noted in the media having 0.2 mg/l BAP+0.2 mg/l Kn+0.2 mg/l IAA. The lowest percentage (40%) of shoot proliferation was noted in 2.0 mg/l BAP + 2.0 mg/l NAA. Highest average number of shoot per culture was 8.05 ± 0.33 in media having 0.2 mg/l BAP+0.2 mg/l Kn+0.2 mg/l IAA (**Plate 3.3.9**) followed by 6.77 ± 0.24 in media having 0.5 mg/l BAP+0.5 mg/l Kn+0.5 mg/l IAA. Lowest average number of shoot per culture was 2.08 ± 0.15 in media having 2.0 mg/l Kn + 2.0 mg/l NAA. Highest average length of shoot per culture was 4.40 ± 0.25 cm in the media having 0.5 mg/l BAP+ 0.5 mg/l NAA. The lowest average length of shoot per culture was recorded 1.83 ± 0.11 cm in the media containing 2.0 mg/l BAP+ 2.0 mg/l NAA. Experimental results revealed that 0.2 mg/l BAP+0.2 mg/l Kn+0.2 mg/l IAA, 0.5 mg/l BAP+0.5 mg/l Kn+0.5 mg/l IAA, 1.0 mg/l

Kn +0.5 mg/l NAA and 0.5 mg/l BAP + 0.5 mg/l NAA were found most effective combinations for multiple shoot induction in *M. pruriens.*

Table 3.3.21. Effect of different concentrations of BAP and Kn alone and in combinations on multiple shoot induction from nodal explants of *M. pruriens*. Data were taken after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
BAP				
0.2	16-20	50.00	4.03 ± 0.19	4.12 ± 0.13
0.5	16-20	76.67	5.63 ± 0.23	5.06 ± 0.15
1.0	16-20	66.67	5.23 ± 0.19	4.14 ± 0.07
2.0	16-20	43.33	3.10 ± 0.10	3.43 ± 0.11
3.0	16-20	30.00	2.17 ± 0.09	2.28 ± 0.11
Kn				
0.2	16-20	53.33	2.13 ± 0.15	2.20 ± 0.12
0.5	16-20	63.33	2.33 ± 0.15	2.73 ± 0.15
1.0	16-20	70.00	4.27 ± 0.17	3.57 ± 0.18
2.0	16-20	70.00	3.47 ± 0.20	3.45 ± 0.13
3.0	16-20	46.67	2.47 ± 0.09	2.34 ± 0.15
BAP + Kn				
0.5 +0.2	16-20	70.00	4.17 ± 0.12	3.37 ± 0.10
0.5 +0.5	16-20	83.33	5.60 ± 0.17	4.03 ± 0.12
0.5 +1.0	16-20	76.67	5.10 ± 0.21	3.78 ± 0.10
0.5 +2.0	16-20	60.00	3.40 ± 0.12	2.53 ± 0.17
1.0 +0.2	16-20	63.33	3.17 ± 0.20	2.60 ± 0.12
1.0 +0.5	16-20	66.67	3.43 ± 0.09	3.20 ± 0.10
1.0 +1.0	16-20	73.33	4.08 ± 0.12	3.73 ± 0.18
1.0 +2.0	16-20	60.00	3.23 ± 0.12	2.77 ± 0.09
2.0 +0.2	16-20	50.00	2.30 ± 0.15	2.50 ± 0.15
2.0 +0.5	16-20	66.67	2.63 ± 0.07	2.70 ± 0.15
2.0 +1.0	16-20	50.00	2.40 ± 0.12	2.20 ± 0.12
2.0 +2.0	16-20	46.67	2.13 ± 0.18	2.14 ± 0.12

* Values are the mean of three replicates with 10 explants.

Table 3.3.22. Effect of different concentrations and combinations of BAP and Kn with NAA or in combinations with IAA on multiple shoot induction from nodal explants of *M. pruriens.* Data were taken after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
BAP + NAA				
0.5 + 0.2	12-16	80.00	5.50 ± 0.25	4.36 ± 0.12
0.5 + 0.5	12-16	93.33	6.29 ± 0.41	5.40 ± 0.25
0.5 + 1.0	12-16	83.33	4.63 ± 0.17	3.80 ± 0.13
0.5 + 2.0	16-20	56.67	3.53 ± 0.13	3.23 ± 0.17
1.0 + 0.2	12-16	73.33	4.20 ± 0.10	3.65 ± 0.28
1.0 + 0.5	12-16	76.67	4.52 ± 0.12	3.41 ± 0.14
1.0 + 1.0	16-20	60.00	3.63 ± 0.18	2.83 ± 0.10
1.0 + 2.0	16-20	53.33	3.51 ± 0.11	1.88 ± 0.09
2.0 + 0.2	16-20	76.67	4.09 ± 0.25	3.42 ± 0.16
2.0 + 0.5	16-20	60.00	2.82 ± 0.14	2.26 ± 0.19
2.0 + 1.0	16-20	46.67	2.88 ± 0.19	2.24 ± 0.12
2.0 + 2.0	16-20	40.00	2.53 ± 0.10	1.83 ± 0.11
Kn + NAA				
0.5 + 0.2	12-16	70.00	3.27 ± 0.14	2.61 ± 0.15
0.5 + 0.5	12-16	70.00	3.56 ± 0.18	3.20 ± 0.28
0.5 + 1.0	12-16	60.00	2.63 ± 0.25	2.28 ± 0.20
0.5 + 2.0	16-20	63.33	2.69 ± 0.10	1.88 ± 0.10
1.0 + 0.2	16-20	76.67	5.20 ± 0.21	4.13 ± 0.33
1.0 + 0.5	16-20	83.33	6.44 ± 0.38	4.81 ± 0.29
1.0 + 1.0	16-20	73.33	4.33 ± 0.17	3.02 ± 0.09
1.0 + 2.0	16-20	46.67	2.20 ± 0.09	1.83 ± 0.14
2.0 + 0.2	16-20	70.00	3.24 ± 0.08	2.86 ± 0.09
2.0 + 0.5	16-20	63.33	2.61 ± 0.20	2.10 ± 0.13
2.0 + 1.0	16-20	60.00	2.27 ± 0.06	2.04 ± 0.06
2.0 + 2.0	16-20	53.33	2.08 ± 0.15	1.90 ± 0.06
BAP+Kn+IAA				
0.1+0.1+0.1	12-16	83.33	5.28 ± 0.16	3.49 ± 0.14
0.2+0.2+0.2	12-16	96.67	8.05 ± 0.33	5.21 ± 0.25
0.5+0.5+0.5	12-16	90.00	6.77 ± 0.24	4.64 ± 0.26
1.0+1.0+1.0	12-16	83.33	4.40 ± 0.22	3.28 ± 0.17
2.0+2.0+2.0	12-16	53.33	2.25 ± 0.14	2.47 ± 0.21

* Values are the mean of three replicates with 10 explants.

(b) Shoot tip explants

Experiment 1: Effect of different concentrations of BAP and Kn alone and in combinations on multiple shoot induction from shoot tip explants of *M. pruriens*.

In this present investigation five concentrations (0.2, 0.5, 1.0, 2.0 and 3.0 mg/l) of BAP and Kn used alone and in combination of three concentrations (0.5, 1.0 and 2.0 mg/l) of BAP with four concentrations (0.2, 0.5, 1.0 and 2.0 mg/l) of Kn were treated in MS medium for the purpose of multiple shoot induction from shoot tip explants of *M. pruriens*. Data were taken after 6 weeks of inoculation and percentage of shoot formation, number of shoot per culture and length of shoot per culture were measured. The results are presented in **Table 3.3.23**. Morphogenic responses of the explants were found to vary with hormonal formulations present in the culture media. Shoot proliferation ranged from 26.67 – 66.67%. Highest percentage (66.67%) of multiple shoot formation was observed in MS medium containing 0.5 mg/l BAP+0.5 mg/l Kn followed by 60% in MS medium containing 0.5 mg/l BAP alone and 0.5 mg/l BAP + 1.0 mg/l Kn. The lowest percentage (26.67%) of multiple shoot formation was observed in media having 3.0 mg/l Kn. Highest average number of shoots was 2.80 ± 0.10 in MS medium having 0.5 mg/l BAP+0.5 mg/l Kn followed by 2.64 ± 0.15 in MS medium having 0.5 mg/l BAP+1.0 mg/l Kn. The lowest average number of shoot was 1.57 ± 0.13 in media containing 2.0 mg/l BAP+2.0 mg/l Kn. Average length of shoots gradually increased after induction of shoot. Highest average length was recorded 2.50 ± 0.17 cm in 1.0 mg/l Kn followed by 2.33 ± 0.09 cm in 2.0 mg/l Kn. The lowest average length was 1.65 ± 0.09 cm in 2.0 mg/l BAP+2.0 mg/l Kn. Experiment results revealed that 0.5 mg/l BAP+0.5 mg/l Kn, 0.5 mg/l BAP+1.0 mg/l Kn and 1.0 mg/l Kn alone were found most effective concentrations for multiple shoot induction in *M. pruriens* from shoot tip explants.

Experiment 2: Effect of different concentrations and combinations of BAP and Kn with NAA or in combinations with IAA on multiple shoot induction from shoot tip explants of *M. pruriens*. Data were taken after 6 weeks of culture.

Explants were cultured on MS medium supplemented with three concentrations of BAP (0.5, 1.0 and 2.0 mg/l) and Kn (0.5, 1.0 and 2.0 mg/l) combined with four concentrations of NAA (0.2, 0.5, 1.0 and 2.0 mg/l) or in combinations of BAP+Kn+IAA. Data were recorded after 6 weeks of culture and results on different parameters are presented in the **Table 3.3.24**. Morphogenic responses of the explants were found to vary with hormonal formulations present in the culture media. Shoot proliferation ranged from 23.33 – 70.00%. The efficiency of BAP+Kn+IAA was found better than BAP+NAA or Kn+NAA. Among the concentrations the highest percentage (70%) of shoot proliferation was noted in the media having 0.2 mg/l BAP+0.2 mg/l Kn+0.2 mg/l IAA. The lowest percentage (23.33%) of shoot proliferation was noted in 2.0 mg/l BAP + 2.0 mg/l NAA and 2.0 mg/l Kn + 2.0 mg/l NAA. Highest average number of shoot per culture was 3.24 ± 0.15 in media having 0.2 mg/l BAP+0.2 mg/l Kn+0.2 mg/l IAA followed by 2.84 ± 0.29 in media having 0.5 mg/l BAP+0.5 mg/l Kn+0.5 mg/l IAA. Lowest average number of shoot per culture was 1.30 ± 0.06 in media having 2.0 mg/l Kn + 2.0 mg/l NAA. Highest average length of shoot per culture was 2.68 ± 0.19 cm in the media having 0.2 mg/l BAP+ 0.2 mg/l Kn+ 0.2 mg/l NAA followed by 2.46 ± 0.16 cm in the media having 0.5 mg/l BAP+ 0.5 mg/l Kn+ 0.5 mg/l NAA. The lowest average length of shoot per culture was recorded 1.44 ± 0.12 cm in the media containing 2.0 mg/l BAP+ 2.0 mg/l NAA. Experimental results revealed that 0.2 mg/l BAP+0.2 mg/l Kn+0.2 mg/l IAA, 0.5 mg/l BAP+0.5 mg/l Kn+0.5 mg/l IAA, 0.5 mg/l BAP +0.5 mg/l NAA and 1.0 mg/l Kn + 0.5 mg/l NAA were found most effective combinations for multiple shoot induction in *Mucuna pruriens* from shoot tip explants.

Table 3.3.23. Effect of different concentrations of BAP and Kn alone and in combinations on multiple shoot induction from shoot tip explants of *M. pruriens*. Data were taken after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
BAP				
0.2	15-20	30.00	2.13 ± 0.06	1.88 ± 0.11
0.5	15-20	60.00	2.40 ± 0.11	2.13 ± 0.33
1.0	15-20	56.67	1.98 ± 0.10	2.02 ± 0.06
2.0	15-20	40.00	1.85 ± 0.17	1.86 ± 0.12
3.0	-	-	-	-
Kn				
0.2	15-20	30.00	1.77 ± 0.10	1.83 ± 0.11
0.5	15-20	40.00	1.93 ± 0.08	2.10 ± 0.13
1.0	15-20	56.67	2.60 ± 0.17	2.50 ± 0.17
2.0	15-20	50.00	2.40 ± 0.15	2.33 ± 0.09
3.0	15-20	26.67	1.66 ± 0.09	1.89 ± 0.16
BAP + Kn				
0.5 +0.2	15-20	56.67	2.25 ± 0.14	1.94 ± 0.12
0.5 +0.5	15-20	66.67	2.80 ± 0.10	2.26 ± 0.16
0.5 +1.0	15-20	60.00	2.64 ± 0.15	2.04 ± 0.20
0.5 +2.0	15-20	46.67	2.13 ± 0.09	1.78 ± 0.17
1.0 +0.2	15-20	50.00	1.87 ± 0.16	1.90 ± 0.11
1.0 +0.5	15-20	56.67	2.13 ± 0.16	2.22 ± 0.19
1.0 +1.0	15-20	56.67	2.03 ± 0.12	2.14 ± 0.11
1.0 +2.0	15-20	40.00	1.73 ± 0.21	1.81 ± 0.07
2.0 +0.2	15-20	40.00	1.90 ± 0.10	1.94 ± 0.13
2.0 +0.5	15-20	46.67	1.82 ± 0.20	1.80 ± 0.13
2.0 +1.0	15-20	40.00	1.60 ± 0.10	1.77 ± 0.18
2.0 +2.0	15-20	33.33	1.57 ± 0.13	1.65 ± 0.09

- : fail to response

* Values are the mean of three replicates with 10 explants.

Table 3.3.24. Effect of different concentrations and combinations of BAP and Kn with NAA or in combinations with IAA on multiple shoot induction from shoot tip explants of *M. pruriens.* Data were taken after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
BAP + NAA				
0.5 + 0.2	15-20	60.00	2.43 ± 0.11	2.06 ± 0.14
0.5 + 0.5	15-20	66.67	2.51 ± 0.20	2.28 ± 0.17
0.5 + 1.0	15-20	56.67	2.35 ± 0.08	2.02 ± 0.18
0.5 + 2.0	15-20	30.00	1.62 ± 0.12	1.90 ± 0.14
1.0 + 0.2	15-20	53.33	1.96 ± 0.21	1.89 ± 0.16
1.0 + 0.5	15-20	60.00	2.33 ± 0.14	2.11 ± 0.10
1.0 + 1.0	15-20	40.00	1.89 ± 0.18	1.86 ± 0.15
1.0 + 2.0	15-20	30.00	1.58 ± 0.15	1.76 ± 0.08
2.0 + 0.2	15-20	60.00	2.47 ± 0.11	2.10 ± 0.22
2.0 + 0.5	15-20	46.67	1.98 ± 0.08	1.88 ± 0.06
2.0 + 1.0	15-20	30.00	1.50 ± 0.14	1.53 ± 0.12
2.0 + 2.0	15-20	23.33	1.38 ± 0.06	1.44 ± 0.12
Kn + NAA				
0.5 + 0.2	15-20	36.67	1.59 ± 0.20	1.66 ± 0.18
0.5 + 0.5	15-20	50.00	2.00 ± 0.14	1.93 ± 0.14
0.5 + 1.0	15-20	46.67	1.90 ± 0.15	1.84 ± 0.14
0.5 + 2.0	15-20	33.33	1.53 ± 0.13	1.70 ± 0.16
1.0 + 0.2	15-20	56.67	2.25 ± 0.13	1.93 ± 0.15
1.0 + 0.5	15-20	60.00	2.47 ± 0.17	2.15 ± 0.11
1.0 + 1.0	15-20	43.33	1.88 ± 0.15	1.91 ± 0.19
1.0 + 2.0	15-20	30.00	1.64 ± 0.10	1.69 ± 0.13
2.0 + 0.2	15-20	53.33	2.10 ± 0.09	2.13 ± 0.13
2.0 + 0.5	15-20	46.67	2.02 ± 0.22	1.95 ± 0.16
2.0 + 1.0	15-20	36.67	1.75 ± 0.23	1.81 ± 0.20
2.0 + 2.0	15-20	23.33	1.30 ± 0.06	1.48 ± 0.05
BAP+Kn+IAA				
0.1+0.1+0.1	16-22	60.00	2.30 ± 0.11	2.17 ± 0.17
0.2+0.2+0.2	16-22	70.00	3.24 ± 0.15	2.68 ± 0.19
0.5+0.5+0.5	16-22	66.67	2.84 ± 0.29	2.46 ± 0.16
1.0+1.0+1.0	16-22	56.67	2.39 ± 0.20	1.95 ± 0.14
2.0+2.0+2.0	16-22	30.00	1.64 ± 0.10	1.79 ± 0.08

* Values are the mean of three replicates with 10 explants.

Plate 3.3.9. Development of multiple shoot from Shoot tip and nodal explant of *M. pruriens* through the process of direct organogenesis with their subsequent development.

A: Source of explant in MS basal medium; **B:** Initiation of multiple shoot formation in nodal segment after 2 weeks of inoculation in media having 0.2 mg/l BAP+ 0.2 mg/l Kn + 0.2 mg/l IAA; **C-E**: Proliferation of multiple shoot in the same medium after two subcultures at 14 days interval; **F**: Further proliferation and elongation of shoot after 6 weeks of culture in the same medium.

B. Induction of callus on different explants in *M. pruriens*.

For the induction of callus internode and leaf explants were cultured on MS medium supplemented with different concentrations and combinations of phytohormones. Data on days of callus initiation, percentage of explant induced callus, color and texture of callus were recorded after 6 weeks of culture. Internode explant showed better performance than leaf explant. The results are described according to types of explants under separate heads.

Experiment 1: Effect of different concentrations and combinations of phytohormones on callus induction and characteristics of callus derived from internode explants.

In order to induce of callus, internode explants excised from *in vitro* grown seedlings of *M. pruriens* were cultured on MS medium supplemented with different concentrations (0.2, 0.5, 1.0 and 2.0 mg/l) of 2,4–D and NAA alone and in combinations of BAP+2,4-D, Kn+2,4-D, IAA+BAP and IAA+Kn in different concentrations. Data were recorded after 6 weeks of culture. Cultures were maintained under 16 h light and 8 h dark regime and data on different parameters are summarized in **Table 3.3.25**. Morphogenic responses of the explants were found to vary with hormonal formulations present in the culture media. Callus proliferation was not noticed in all media formulations. But there was a wide variation in morphological nature and percentage of callus formation among them. Callus initiation occurred within 10–15 days depending upon the concentration and combination of hormones. 2,4–D and NAA alone induced callus within 12–15 days, Kn with 2,4–D took same days, BAP with 2,4–D or IAA took 10–12 days, Kn with IAA took 10–12 days to initiate callus formation. Percentage of callus formation ranged from 30.00 – 95.00%. Highest percentage (95.00%) of callus formation occurred in MS medium containing 3.0 mg/l BAP + 0.5 mg/l IAA (**Plate 3.3.10.E**) followed by 75.00% in 3.0 mg/l BAP+1.0 mg/l IAA and 1.0 mg/l 2,4–D. The lowest percentage (30.00%) of callus formation was observed in media having 1.0 mg/l Kn + 0.5 mg/l IAA. In most cases colour of calli was green, light green, yellow green and whitish and texture of calli were friable, compact and nodular.

Experiment 2: Effect of different concentrations and combinations of phytohormones on callus induction and characteristics of callus derived from leaf explants.

In order to induce of callus, leaf explants excised from *in vitro* grown seedlings of *M. pruriens* were cultured on MS medium supplemented with different concentrations (0.2, 0.5, 1.0 and 2.0 mg/l) of 2,4–D and NAA alone and in combinations of BAP+2,4-D, Kn+2,4-D, IAA+BAP and IAA+Kn in different concentrations. Data were recorded after 6 weeks of culture. Cultures were maintained under 16 h light and 8 h dark regime and data on different parameters are summarized in Table 3.3.25. Morphogenic responses of the explants were found to vary with hormonal formulations present in the culture media. Callus proliferation was not noticed in all media formulations. But there was a wide variation in morphological nature and percentage of callus formation among them. Callus initiation occurred within 10–15 days depending upon the concentration and combination of hormones. 2,4–D and NAA alone induced callus within 12–15 days, Kn with 2,4–D also took the same days, BAP with 2,4–D or IAA took 10–12 days, Kn with IAA took 10–12 days to initiate callus formation. Percentage of callus formation ranged from 15.00 – 65.00%. Highest percentage (65.00%) of callus formation occurred in MS medium containing 3.0mg/l BAP + 0.5 mg/l IAA followed by 60.00% in 3.0mg/l BAP+1.0 mg/l IAA. The lowest percentage (15.00%) of callus formation was observed in media having 1.0 mg/l Kn + 0.5 mg/l IAA. In most cases colour of calli was green, light green, yellow green and whitish and texture of calli were friable, compact and nodular.

Table 3.3.25. Effect of different concentrations and combinations of phytohormones in MS medium on callus induction and characteristics of callus derived from leaf and internode explants of *M. pruriens.* Data were collected after 6 weeks on inoculation.

Hormonal combinations (mg/l)	Internode explant			Leaf explant		
	Days of callus initiation	% of explant induced callus	*Callus colour and texture	Days of callus initiation	% of explant induced callus	*Callus colour and texture
2,4-D						
0.2	-	-	-	-	-	-
0.5	12-15	50	gf	12-15	35	gf
1.0	12-15	75	gf	12-15	55	gf
2.0	12-15	55	gf	12-15	45	gf
NAA						
0.2	-	-	-	-	-	-
0.5	12-15	35	gf	12-15	25	gf
1.0	12-15	40	gf	12-15	30	gf
2.0	12-15	50	gf	12-15	40	gf
BAP + 2,4-D						
0.5 + 0.5	-	-	-	-	-	-
0.5 + 1.0	10-12	60	lgf	10-12	50	lgf
0.5 + 2.0	10-12	65	lgc	10-12	50	lgf
1.0 + 0.5	10-12	35	lgc	10-12	30	lgc
1.0 + 1.0	10-12	55	lgc	10-12	40	lgc
1.0 + 2.0	10-12	55	lgc	10-12	45	lgc
Kn + 2,4-D						
0.5 + 0.5	-	-	-	-	-	-
0.5 + 1.0	12-15	40	gc	12-15	40	gc
0.5 + 2.0	12-15	55	gc	12-15	45	gc
1.0 + 0.5	-	-	-	-	-	-
1.0 + 1.0	12-15	50	gc	12-15	35	gc
1.0 + 2.0	12-15	60	gc	12-15	50	gc
IAA + BAP						
0.5 + 0.5	-	-	-	-	-	-
0.5 + 1.0	10-12	40	gf	10-12	35	gf
0.5 + 2.0	10-12	45	gf	10-12	40	gf
0.5 + 3.0	10-12	95	gf	10-12	65	gf
1.0 + 0.5	-	-	-	-	-	-
1.0 + 1.0	10-12	25	gf	-	-	-
1.0 + 2.0	10-12	70	gf	10-12	35	gf
1.0 + 3.0	10-12	75	gf	10-12	60	gf
IAA + Kn						
0.5 + 0.5	-	-	-	-	-	-
0.5 + 1.0	10-12	30	wn	10-12	15	wn
0.5 + 2.0	10-12	60	ygf	10-12	55	ygf
0.5 + 3.0	10-12	20	ygf	10-12	35	ygf
1.0 + 0.5	-	-	-	-	-	-
1.0 + 1.0	10-12	35	ygf	10-12	25	wn
1.0 + 2.0	10-12	65	ygf	10-12	50	ygf
1.0 + 3.0	10-12	55	ygf	10-12	45	ygf

ygf: yellow green friable; **wn:** white nodular, **lgf:** light green friable, **gf:** green friable, **gc:** green compact, **lgc:** light green compact.

- : fail to induce any callus

C. Plant regeneration from different types of callus in *M. pruriens*.

Calli produced in different plant growth regulators supplemented on MS medium did not differentiate into shoot buds in the same medium after subsequent subcultures. For the development of adventitious shoot buds from various types of calli, different media formulations were needed. A wide range of cytokinin (BAP and Kn) and auxin (IAA and NAA) supplemented media were used to standardize the organogenic response of calli. Among the different types of calli green compact and light green compact found to be regenerative calli on different plant growth regulator supplemented media. Internode derived callus showed better performance than leaf derived callus. The results are discussed according to types of explants under separate heads.

(i) Plant regeneration from leaf derived callus

Different experiments were conducted to investigate plant regeneration ability through callus culture from leaf explants. Data on percentage of organogenic calli induced, average number of shoot per callus and average length of shoot per callus were collected after 7 weeks of culture.

Experiment 1: Effect of different concentrations of BAP and Kn alone and in combinations with IAA on adventitious shoot regeneration from leaf derived callus of *M. pruriens*.

In this experiment leaf derived calli of *M. pruriens* were used to investigate the effect of different hormonal concentrations and combinations on organogenesis. For shoot differentiation, light green compact calli were subcultured on MS medium supplemented with five concentrations (0.2, 0.5, 1.0, 2.0 and 3.0 mg/l) of BAP and Kn alone and in combinations of two concentrations of BAP (0.5 and 1.0 mg/l) and Kn(1.0 and 2.0 mg/l) with four concentrations (0.1, 0.2, 0.5 and 1.0 mg/l) of NAA. Morphogenic responses were found varied with growth regulators supplements. Higher concentrations of BAP (2.0 mg/l and above) and Kn (3.0 mg/l and above) alone and in combinations with NAA failed to induce any regeneration performance in callus. Data were recorded after 7 weeks of culture and presented in **Table 3.3.26**. The percentage of calli produced shoots was ranged from 15.00–45.00%. Highest percentage 45.00% of adventitious shoots regeneration occurred in MS medium containing 0.5 mg/l BAP+0.2 mg/l NAA followed by 35.00% in 0.5 mg/l BAP, 0.5 mg/l BAP+0.1 mg/l NAA and 1.0 mg/l Kn+0.2 mg/l NAA. The lowest percentage (15%) of adventitious shoots regeneration was observed in media having 1.0 mg/l BAP+0.5 mg/l NAA. The highest number of adventitious shoots regeneration per callus was 3.72 ± 0.15 in media having 0.5 mg/l BAP+0.2 mg/l NAA followed by 3.54 ± 0.24 in 0.5 mg/l BAP+0.1 mg/l NAA. The lowest number of adventitious shoots regeneration per callus was 1.32 ± 0.12 in media having 2.0 mg/l Kn+0.5 mg/l

NAA. Highest length of shoot 3.40 ± 0.20 cm was recorded in 0.5 mg/l BAP+0.2 mg/l NAA and the lowest length of shoots 2.12 ± 0.10 cm was recorded in media having 1.0 mg/l BAP+0.5 mg/l NAA.

(ii) Plant regeneration from internode derived callus

Different experiments were conducted to investigate plant regeneration ability through callus culture from internode explants. Data on percentage of organogenic calli induced shoot, average number of shoot per callus and average length of shoot per callus were collected after 7 weeks of culture. Findings of the experiments are described below:

Experiment 1: Effect of different concentrations of BAP and Kn alone and in combinations with NAA on adventitious shoot regeneration from internode derived callus of *M. pruriens*.

In this experiment internode derived calli of *M. pruriens* were used to investigate the effect of different hormonal concentrations and combinations on organogenesis. For shoot differentiation, light green compact calli were subcultured on MS medium supplemented with five concentrations (0.2, 0.5, 1.0, 2.0 and 3.0 mg/l) of BAP and Kn alone and in combinations of two concentrations of BAP (0.5 and 1.0 mg/l) and Kn(1.0 and 2.0 mg/l) with four concentrations (0.1, 0.2, 0.5 and 1.0 mg/l) of NAA. Morphogenic potentialities of cultured calli varied with hormonal treatments. Higher concentrations of BAP (2.0 mg/l and above) and Kn (3.0 mg/l and above) alone and in combinations with NAA failed to induce any regeneration performance in callus. Data were recorded after 7 weeks of culture and presented in **Table 3.3.26**. The percentage of calli produced shoots ranged from 20.00–60.00%. Highest percentage 60.00% of adventitious shoots regeneration occurred in MS medium containing 0.5 mg/l BAP+0.2 mg/l NAA (**Plate 3.3.10**) followed by 50.00% in 0.5 mg/l BAP+0.1 mg/l NAA and 1.0 mg/l Kn+0.2 mg/l NAA. The lowest percentage (20%) of adventitious shoots regeneration was observed in media having 2.0 mg/l Kn+0.5 mg/l NAA and 1.0 mg/l BAP+0.5 mg/l NAA. The highest number of adventitious shoots regeneration per callus was 4.76 ± 0.34 in media having 0.5 mg/l BAP+0.2 mg/l NAA followed by 4.03 ± 0.20 in 0.5 mg/l BAP+0.1 mg/l NAA. The lowest number of adventitious shoots regeneration per callus was 1.39 ± 0.17 in media having 2.0 mg/l Kn+0.5 mg/l NAA. Highest length of shoot 3.80 ± 0.27 cm was recorded in 0.5 mg/l BAP+0.2 mg/l NAA and the lowest length of shoots 2.18 ± 0.12 cm was recorded in media having 1.0 mg/l BAP.

Table 3.3.26. Effect of BAP and Kn alone and combinations with NAA on adventitious shoot regeneration from leaf and internode derived callus in *M. pruriens*. Data were collected after 7 weeks of inoculation.

Hormonal combination (mg/l)	Leaf derived callus			Internode derived callus		
	% of regenerable calli	*Average number of shoots/callus (mean±SE)	*Average length (cm) of shoots/callus (mean±SE)	% of regenerable calli	*Average number of shoots/callus (mean±SE)	*Average length (cm) of shoots/callus (mean±SE)
BAP						
0.2	30	3.35 ± 0.17	3.12 ± 0.30	35	3.28 ± 0.09	3.37 ± 0.15
0.5	35	3.53 ± 0.28	3.32 ± 0.21	45	3.95 ± 0.54	3.52 ± 0.25
1.0	30	2.75 ± 0.12	2.14 ± 0.18	30	2.36 ± 0.24	2.18 ± 0.12
2.0	-	-	-	-	-	-
3.0	-	-	-	-	-	-
Kn						
0.2	-	-	-	-	-	-
0.5	25	1.97 ± 0.19	2.43 ± 0.33	30	2.06 ± 0.10	2.32 ± 0.12
1.0	30	2.21 ± 0.42	2.60 ± 0.17	35	2.87 ± 0.28	2.84 ± 0.32
2.0	30	1.48 ± 0.20	2.46 ± 0.23	30	1.70 ± 0.17	2.63 ± 0.14
3.0	-	-	-	-	-	-
BAP + NAA						
0.5 + 0.1	35	3.54 ± 0.24	2.80 ± 0.13	50	4.03 ± 0.20	2.94 ± 0.16
0.5 + 0.2	45	3.72 ± 0.15	3.40 ± 0.20	60	4.76 ± 0.34	3.80 ± 0.27
0.5 + 0.5	30	3.35 ± 0.21	3.29 ± 0.22	30	4.00 ± 0.26	3.24 ± 0.15
0.5 + 1.0	-	-	-	-	-	-
1.0 + 0.1	30	2.25 ± 0.08	2.60 ± 0.19	35	2.61 ± 0.20	2.30 ± 0.15
1.0 + 0.2	20	2.57 ± 0.26	2.82 ± 0.24	30	3.34 ± 0.14	2.86 ± 0.28
1.0 + 0.5	15	1.78 ± 0.14	2.12 ± 0.10	20	1.66 ± 0.09	2.20 ± 0.12
1.0 + 1.0	-	-	-	-	-	-
Kn + NAA						
1.0 + 0.1	30	2.04 ± 0.15	2.30 ± 0.11	35	2.20 ± 0.17	2.45 ± 0.08
1.0 + 0.2	35	3.26 ± 0.18	2.89 ± 0.22	50	3.44 ± 0.25	2.68 ± 0.20
1.0 + 0.5	20	2.27 ± 0.14	2.72 ± 0.19	25	2.30 ± 0.11	2.30 ± 0.17
1.0 + 1.0	-	-	-	-	-	-
2.0 + 0.1	25	1.40 ± 0.17	2.50 ± 0.15	25	1.54 ± 0.24	2.23 ± 0.31
2.0 + 0.2	30	1.56 ± 0.14	2.52 ± 0.27	35	1.78 ± 0.17	2.40 ± 0.20
2.0 + 0.5	25	1.32 ± 0.12	2.30 ± 0.14	20	1.39 ± 0.17	2.28 ± 0.45
2.0 + 1.0	-	-	-	-	-	-

\- : Failed to differentiate any shoots.

* Values are the mean of three replicates with 10 explants.

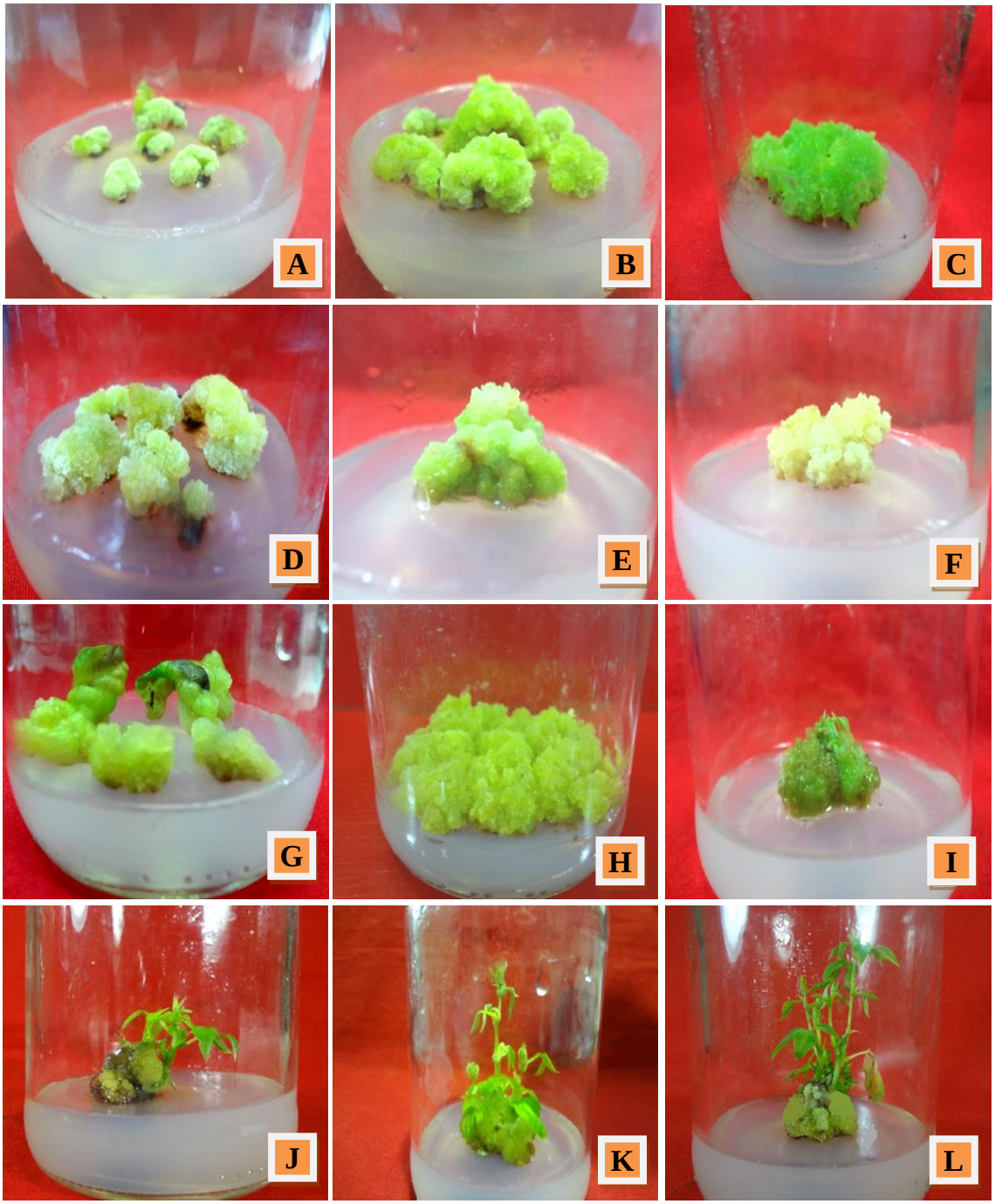


Plate 3.3.10. Development of callus from internode and leaf explants and subsequent regeneration of single and multiple shoot of *M. pruriens* through the process of indirect organogenesis.

A-C: Green friable callus induction from internode explant in MS+3mg/l BAP+0.5 mg/l IAA; **D-E:** Yellow green friable callus induction from internode explant in MS+2.0 mg/l Kn+0.5 mg/l IAA; **F:** White nodular callus induction from internode in MS+0.5mg/l IAA+1.0mg/l Kn; **G-H:** Callus induction from leaf explant in MS+3mg/l BAP+0.5 mg/l IAA; **I:** Initiation of multiple shoot buds from internode derived callus in MS+0.5 mg/l BAP +0.2 mg/l NAA after 4 weeks of sub culture; **J-L:** Proliferation and elongation of shoot buds from internode derived callus in the same medium.

D. Rooting of *in vitro* grown shoots in *M. pruriens*.

Shoot cuttings 4 – 5 cm long *in vitro* grown shoots were separated and transferred to rooting media. MS plain (without hormone) and half strength MS medium fortified with different concentrations of NAA and IBA alone and in combination of BAP and IBA with NAA were used for root experiment. Rooting started within 10 to 16 days of culture. Data on days to root initiation, frequency of root formation (%), average number of root per culture and average length of root per culture were recorded after 5 weeks of culture. Findings of the experiments are described below:

Experiment 1: Effect of different concentrations of NAA and IBA alone and in combination of BAP and IBA with NAA on root induction in *M. pruriens*.

For adventitious root formation, *in vitro* grown shoots were cultured on half strength MS medium with different concentrations (0.2, 0.5, 1.0, 2.0 and 3.0 mg/l) of NAA and IBA alone and in combination of two concentrations (0.5 and 1.0 mg/l) of NAA with four concentrations (0.5, 1.0, 2.0 and 3.0 mg/l) of IBA or BAP. Results obtained for the root induction, frequency of root formation, morphology, average number and length of roots are shown in **Table 3.3.27**. Percentage range of cultures produced roots varied from 40.00 – 100%. Highest 100% of root regeneration was recorded in 0.5 – 2.0 mg/l NAA, 1.0 – 2.0 mg/l IBA and in combination of 0.5 mg/l NAA with three concentrations (1.0, 2.0 and 3.0 mg/l) of IBA. The lowest 30% of root regeneration was recorded in media having 1.0 mg/l NAA+2.0 mg/l BAP. The highest average number of roots per shoot was recorded 12.08 ± 0.53 in media having 0.5 mg/l NAA+2.0 mg/l IBA (**Plate 3.3.11**) followed by 11.12 ± 0.64 in 0.5 mg/l NAA+1.0 mg/l IBA and 10.05 ± 0.48 in 0.5 mg/l NAA+3.0 mg/l IBA. The lowest number of root per shoot was recorded 2.28 ± 0.14 in media having 1.0 mg/l NAA+2.0 mg/l BAP. Highest average length of roots 7.29 ± 0.51 cm was recorded in 2.0 mg/l IBA and the lowest length of roots 2.08 ± 0.10 cm was recorded in 0.5 mg/l NAA+2.0 mg/l BAP. Experiment results revealed that 0.5 mg/l NAA+2.0 mg/l IBA, 0.5 mg/l NAA+1.0 mg/l IBA, 0.5 mg/l NAA+3.0 mg/l IBA, 2.0 mg/l IBA and 2.0 mg/l NAA were found most effective concentrations for root induction in *M. pruriens*. In most cases morphology of roots was healthy, thin and long.

Table 3.3.27. Effect of half strength MS medium with different concentrations of NAA and IBA alone and in combination of BAP and IBA with NAA on root induction in *M. pruriens*. Data were taken after 5 weeks of culture.

Growth regulators (mg/l)	Days taken for root initiation	% of explants induced root development	* Average number of root per culture (mean ± SE)	* Average length (cm) of root per culture (mean ± SE)
NAA				
0.2	10-14	53.33	3.67 ± 0.17	3.46 ± 0.15
0.5	10-14	100	4.10 ± 0.11	5.21 ± 0.10
1.0	10-14	100	7.20 ± 0.34	5.70 ± 0.23
2.0	10-14	100	8.21 ± 0.20	6.23 ± 0.17
3.0	10-14	86.67	6.23 ± 0.31	4.14 ± 0.12
IBA				
0.2	10-14	76.67	4.31 ± 0.18	3.80 ± 0.12
0.5	10-14	83.33	5.25 ± 0.11	4.13 ± 0.33
1.0	10-14	100	6.67 ± 0.90	6.58 ± 0.15
2.0	10-14	100	7.80 ± 0.23	7.29 ± 0.51
3.0	10-14	60.00	3.04 ± 0.21	6.21 ± 0.10
NAA + IBA				
0.5 + 0.5	10-14	90.00	9.26 ± 0.17	6.10 ± 0.14
0.5 + 1.0	10-14	100.00	11.12 ± 0.64	6.43 ± 0.23
0.5 + 2.0	10-14	100.00	12.08 ± 0.53	5.09 ± 0.11
0.5 + 3.0	10-14	100.00	10.05 ± 0.48	5.85 ± 0.17
1.0 + 0.5	10-14	83.33	6.22 ± 0.41	5.20 ± 0.18
1.0 + 1.0	10-14	90.00	8.34 ± 0.15	6.23 ± 0.10
1.0 + 2.0	10-14	96.67	10.35 ± 0.61	6.76 ± 0.28
1.0 + 3.0	10-14	90.00	8.40 ± 0.25	5.55 ± 0.14
NAA + BAP				
0.5 + 0.5	12-16	80.00	5.07 ± 0.23	3.77 ± 0.14
0.5 + 1.0	12-16	70.00	4.13 ± 0.31	3.56 ± 0.17
0.5 + 2.0	12-16	40.00	2.33 ± 0.24	2.08 ± 0.10
0.5 + 3.0	-	-	-	-
1.0 + 0.5	12-16	86.67	6.02 ± 0.49	4.30 ± 0.30
1.0 + 1.0	12-16	70.00	3.60 ± 0.09	3.47 ± 0.17
1.0 + 2.0	12-16	40.0	2.28 ± 0.14	2.21 ± 0.11
1.0 + 3.0	-	-	-	-

- Failed to any initiation of root
* Values are the mean of three replicates with 10 explants.

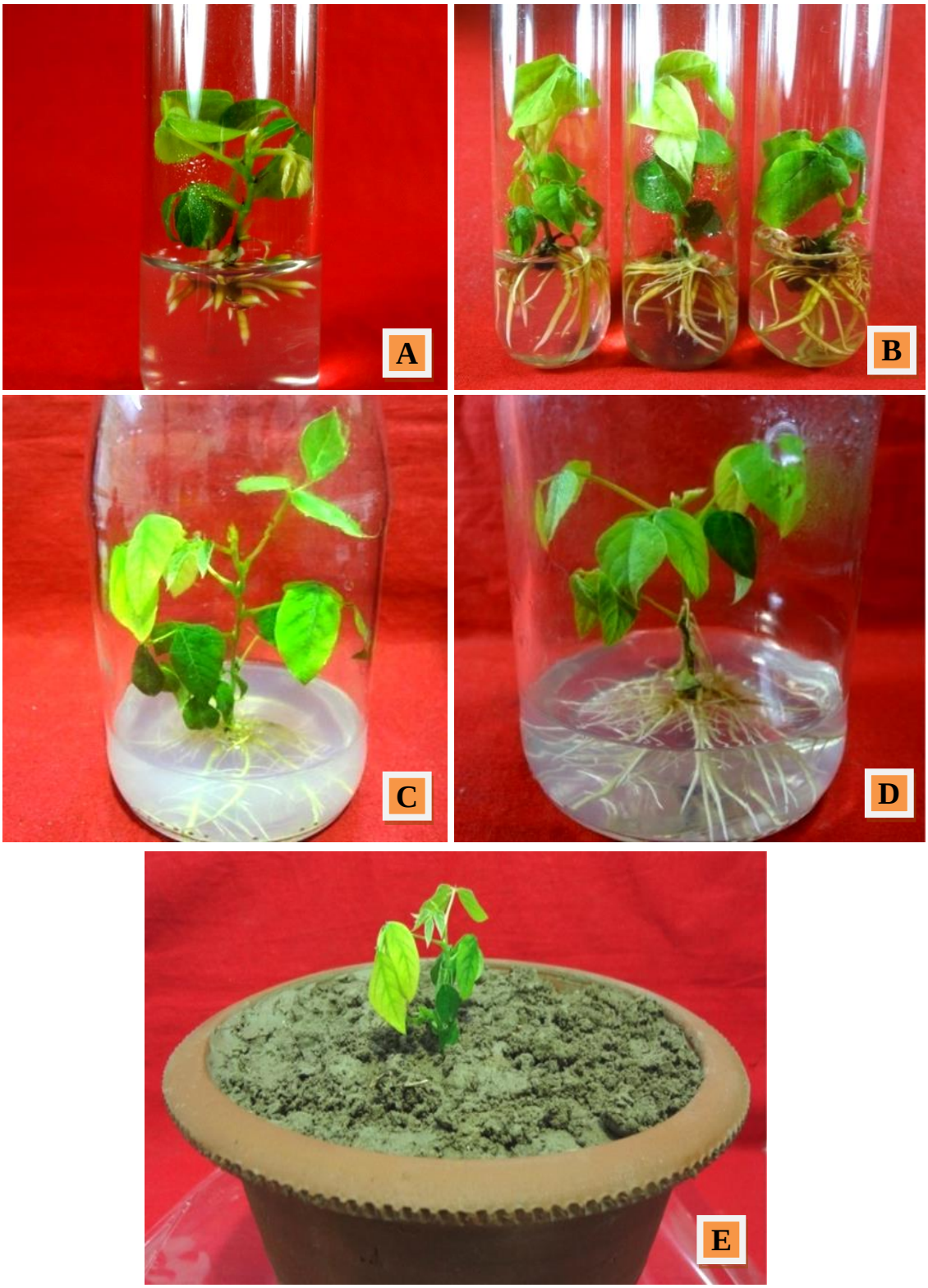


Plate 3.3.11. Photographs showing the initiation and Development of adventitious roots of *M. pruriens* and establishment in soil.

A-B: Induction and elongation of adventitious roots from *in vitro* grown shoots in half strength MS medium containing 2.0 mg/l IBA+0.5 mg/l NAA after 5 weeks of culture; **C-D:** Root induction in the culture bottle; **E:** Acclimatized regenerated plants in soil after 40 days.

3.3.1.5. *Micropropagation of Vitex negundo*

Experiments on direct and indirect organogenesis were carried out using different types of explants *viz.* shoot apex, node, internode, leaves. Shoot tip and nodal explants were cultured for direct shoot regeneration. Explants were cultured on the MS (Murashige and Skoog 1962) agar gelled medium supplemented with different concentration of auxins and cytokinins were used singly or in combinations to investigate the initiation of shoot and its subsequent development. For root induction, micro shoots obtained from direct and indirect organogenesis were transferred to rooting media. Finally well developed plants with both shoots and roots were transferred to natural conditions through successive phages of acclimatization. Details of the results emerging out from each of the experiments are described under following heads:

A. Direct shoot regeneration in *V. negundo*.

Two types of explants *viz*. shoot tip and nodal explants were cultured for direct shoot regeneration. Explants were cultured on MS medium with BAP, Kn and GA_3 alone and in combinations with each other or with NAA, IAA and IBA. Data on number of days taken for shoot initiation, percentage of explant induced shoot development, average number of shoot per culture and average length of shoot per culture were collected after 6 weeks of culture. Among the two types of explants nodal explants responded on almost all of supplemented cultured media. The results are described according to types of explants under separate heads:

(a) Nodal explants

Experiment 1: Effect of different concentrations of BAP singly and in combination with NAA, IAA and IBA on multiple shoot induction from nodal explants of *V. negundo*.

Under present investigation five concentrations (0.2, 0.5, 1.0, 2.0 and 3.0 mg/l) of BAP alone and in combination with the three concentrations (0.5, 1.0 and 2.0 mg/l) of BAP with three concentrations (0.2, 0.5 and 1.0 mg/l) of NAA, IAA and IBA were treated in MS medium for the purpose of multiple shoot induction from nodal explants of *V. negundo*. Data were taken after 6 weeks of inoculation and percentage of shoot formation, average number of shoot per culture and length of shoot per culture were measured. The results are presented in **Table 3.3.28**. Morphogenic responses were found varied with growth regulators supplements. Shoot proliferation ranged from 30.00–86.67%. Highest percentage (86.67%) of multiple shoot formation was observed in MS medium containing 2.0 mg/l BAP + 0.5 mg/l NAA followed by 83.33% in MS medium containing 2.0 mg/l BAP + 0.5 mg/l IAA. The lowest

percentage (30.00%) of multiple shoot formation was observed in media having 0.2 mg/l BAP. Highest average number of shoots was 5.25 ± 0.18 in media having 2.0 mg/l BAP + 0.5 mg/l IAA followed by 4.95 ± 0.21 in media having 2.0 mg/l BAP + 0.5 mg/l IBA. Lowest average number of shoot was 1.45 ± 0.12 in media containing 0.2 mg/l BAP. Average length of shoots gradually increased after induction of shoot. Highest average length was recorded 4.40 ± 0.16 cm in 2.0 mg/l BAP + 0.5 mg/l NAA followed by 4.30 ± 0.10 cm in 2.0 mg/l BAP + 0.5 mg/l IAA. The lowest average length was 1.67 ± 0.15 cm in 0.5 mg/l BAP + 0.2 mg/l IBA. Experiment result revealed that combination of 2.0 mg/l BAP + 0.5 mg/l IAA, 2.0 mg/l BAP + 0.5 mg/l IBA, 1.0 mg/l BAP + 0.5 mg/l IAA and 2.0mg/l BAP + 0.5 mg/l NAA were found most effective concentrations for multiple shoot induction in *V. negundo*.

Experiment 2: Effect of different concentrations of Kn singly and in combination with NAA, IAA and IBA on multiple shoot induction from nodal explants of *V. negundo*.

In this present investigation five concentrations (0.2, 0.5, 1.0, 2.0 and 3.0 mg/l) of Kn alone and in combination of three concentrations (0.5, 1.0 and 2.0 mg/l) of Kn with three concentrations (0.2, 0.5 and 1.0 mg/l) of NAA, IAA and IBA were treated in MS medium for the purpose of multiple shoot induction from nodal explants of *V. negundo*. Data were taken after 6 weeks of inoculation and percentage of shoot formation, average number of shoot per culture and length of shoot per culture were measured. The results are presented in **Table 3.3.29**. Morphogenic responses were found varied with growth regulators supplements. Shoot proliferation ranged from 36.67–93.33%. Highest percentage (93.33%) of multiple shoot formation was observed in MS medium containing 2.0 mg/l Kn alone and 2.0 mg/l Kn + 0.5 mg/l IAA followed by 90.00% in MS medium containing 2.0 mg/l Kn + 0.2 mg/l IAA and 2.0 mg/l Kn + 0.2 mg/l NAA. The lowest percentage (36.67%) of multiple shoot formation was observed in media having 0.2 mg/l Kn. Highest average number of shoots was 5.56 ± 0.32 in media having 2.0 mg/l Kn + 0.2 mg/l NAA followed by 5.42 ± 0.24 in media having 2.0 mg/l Kn+ 0.2 mg/l IAA. Lowest average number of shoot was 1.55 ± 0.17 in media containing 0.2 mg/l Kn. Average length of shoots gradually increased after induction of shoot. Highest average length was recorded 4.55 ± 0.24 cm in 2.0 mg/l Kn alone followed by 4.51 ± 0.17 cm in 2.0 mg/l Kn + 0.5 mg/l IAA. The lowest average length was 1.87 ± 0.11 cm in 0.2 mg/l Kn. Experiment result revealed that combination of 2.0 mg/l Kn+ 0.2 mg/l NAA, 2.0 mg/l Kn + 0.2 mg/l IAA, 2.0 mg/l Kn + 1.0 mg/l IBA and 2.0 mg/l Kn + 0.5 mg/l IBA were found most effective concentrations for multiple shoot induction in *V. negundo*.

Experiment 3: Effect of different concentrations of GA_3 singly and in combination with BAP and Kn on multiple shoot induction and elongation from nodal explants of *V. negundo*.

In this present investigation five concentrations (0.2, 0.5, 1.0, 2.0 and 3.0 mg/l) of GA_3 alone and in combination of three concentrations (0.5, 1.0 and 2.0 mg/l) of GA_3 with three concentrations (0.5, 1.0 and 2.0 mg/l) of BAP or Kn were treated in MS medium for the purpose of multiple shoot induction from nodal explants of *V. negundo*. Data were taken after 6 weeks of inoculation and percentage of shoot formation, average number of shoot per culture and length of shoot per culture were measured. The results are presented in **Table 3.3.30**. Morphogenic responses were found varied with growth regulators supplements. Shoot proliferation ranged from 40.00–96.67%. Highest percentage (96.67%) of multiple shoot formation was observed in MS medium containing 2.0 mg/l Kn + 0.5 mg/l GA_3 followed by 93.33% in MS medium containing 2.0 mg/l BAP + 0.5 mg/l GA_3. The lowest percentage (40.00%) of multiple shoot formation was observed in media having 0.2 mg/l GA_3. Highest average number of shoots was 7.76 ± 0.39 in media having 2.0 mg/l Kn + 0.5 mg/l GA_3 followed by 7.18 ± 0.11 in media having 2.0 mg/l Kn + 1.0 mg/l GA_3. Lowest average number of shoot was 2.00 ± 0.11 in media containing 0.2 mg/l GA_3. Average length of shoots gradually increased after induction of shoot. Highest average length was recorded 6.32 ± 0.27 cm in 2.0 mg/l Kn + 0.5 mg/l GA_3 followed by 6.14 ± 0.47 cm in 1.0 mg/l Kn + 0.5 mg/l GA_3. The lowest average length was 2.81 ± 0.14 cm in 0.2 mg/l GA_3. Experiment result revealed that combination of 2.0 mg/l Kn + 0.5 mg/l GA_3, 2.0 mg/l Kn + 1.0 mg/l GA_3, 1.0 mg/l Kn + 0.5 mg/l GA_3 and 1.0 mg/l Kn + 1.0 mg/l GA_3 were found most effective concentrations for multiple shoot induction in *V. negundo*.

Table 3.3.28. Effect of different concentrations of BAP singly and in combinations of NAA, IAA and IBA on multiple shoot induction from nodal explants of *V. negundo*. Data were taken after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
BAP				
0.2	10-15	30.00	1.45 ± 0.12	1.73 ± 0.14
0.5	10-15	53.33	1.75 ± 0.17	1.87 ± 0.12
1.0	10-15	66.67	2.77 ± 0.11	3.78 ± 0.13
2.0	10-15	80.00	3.26 ± 0.38	4.18 ± 0.23
3.0	10-15	46.67	1.78 ± 0.12	2.24 ± 0.09
BAP + NAA				
0.5 + 0.2	10-15	60.00	1.94 ± 0.25	1.96 ± 0.15
0.5 + 0.5	10-15	70.00	2.75 ± 0.11	2.80 ± 0.09
0.5 + 1.0	10-15	56.67	2.15 ± 0.17	2.17 ± 0.14
1.0 + 0.2	10-15	70.00	2.91 ± 0.10	3.68 ± 0.13
1.0 + 0.5	10-15	76.67	3.76 ± 0.19	3.72 ± 0.17
1.0 + 1.0	10-15	60.00	2.87 ± 0.19	3.70 ± 0.13
2.0 + 0.2	10-15	80.00	3.82 ± 0.14	3.81 ± 0.20
2.0 + 0.5	10-15	86.67	4.35 ± 0.17	4.40 ± 0.16
2.0 + 1.0	10-15	50.00	3.76 ± 0.32	3.24 ± 0.13
BAP + IAA				
0.5 + 0.2	10-15	66.67	2.14 ± 0.22	2.46 ± 0.15
0.5 + 0.5	10-15	73.33	2.64 ± 0.17	2.90 ± 0.30
0.5 + 1.0	10-15	70.00	3.13 ± 0.16	2.77 ± 0.22
1.0 + 0.2	10-15	60.00	2.81 ± 0.11	3.45 ± 0.25
1.0 + 0.5	10-15	80.00	4.86 ± 0.13	3.72 ± 0.23
1.0 + 1.0	10-15	76.67	4.20 ± 0.19	3.60 ± 0.17
2.0 + 0.2	10-15	66.67	4.50 ± 0.23	3.73 ± 0.27
2.0 + 0.5	10-15	83.33	5.25 ± 0.18	4.30 ± 0.10
2.0 + 1.0	10-15	76.67	4.79 ± 0.12	3.74 ± 0.12
BAP + IBA				
0.5 + 0.2	10-15	50.00	1.74 ± 0.07	1.67 ± 0.15
0.5 + 0.5	10-15	66.67	2.80 ± 0.25	2.80 ± 0.29
0.5 + 1.0	10-15	60.00	2.48 ± 0.14	2.54 ± 0.18
1.0 + 0.2	10-15	73.33	3.11 ± 0.17	3.88 ± 0.11
1.0 + 0.5	10-15	76.67	3.86 ± 0.17	4.06 ± 0.11
1.0 + 1.0	10-15	63.33	3.32 ± 0.22	3.60 ± 0.15
2.0 + 0.2	10-15	70.00	4.10 ± 0.07	3.68 ± 0.20
2.0 + 0.5	10-15	76.67	4.95 ± 0.21	3.63 ± 0.12
2.0 + 1.0	10-15	73.33	4.26 ± 0.21	2.89 ± 0.03

* Values are the mean of three replicates with 10 explants.

T Table 3.3.29. Effect of different concentrations of Kn singly and in combinations of NAA, IAA and IBA on multiple shoot induction from nodal explants of *V. negundo*. Data were taken after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
Kn				
0.2	10-15	36.67	1.55 ± 0.17	1.87 ± 0.11
0.5	10-15	60.00	3.48 ± 0.12	2.66 ± 0.18
1.0	8-12	76.67	4.45 ± 0.17	4.10 ± 0.16
2.0	8-12	93.33	5.26 ± 0.32	4.55 ± 0.24
3.0	10-15	60.00	3.72 ± 0.03	3.44 ± 0.11
Kn + NAA				
0.5 + 0.2	10-15	53.33	2.93 ± 0.17	2.24 ± 0.14
0.5 + 0.5	10-15	66.67	3.35 ± 0.12	2.27 ± 0.05
0.5 + 1.0	10-15	60.00	2.47 ± 0.19	2.47 ± 0.12
1.0 + 0.2	8-12	73.33	4.35 ± 0.16	3.88 ± 0.11
1.0 + 0.5	8-12	80.00	4.52 ± 0.31	3.94 ± 0.19
1.0 + 1.0	10-15	60.00	2.75 ± 0.13	3.10 ± 0.27
2.0 + 0.2	8-12	90.00	5.56 ± 0.21	4.24 ± 0.25
2.0 + 0.5	8-12	83.33	4.87 ± 0.16	4.13 ± 0.15
2.0 + 1.0	10-15	70.00	2.90 ± 0.15	3.00 ± 0.22
Kn + IAA				
0.5 + 0.2	10-15	70.00	3.08 ± 0.11	2.44 ± 0.08
0.5 + 0.5	10-15	83.33	3.40 ± 0.29	3.27 ± 0.33
0.5 + 1.0	10-15	70.00	3.25 ± 0.14	3.22 ± 0.41
1.0 + 0.2	8-12	80.00	4.42 ± 0.12	3.20 ± 0.30
1.0 + 0.5	8-12	86.67	4.74 ± 0.11	3.47 ± 0.12
1.0 + 1.0	8-12	70.00	4.12 ± 0.16	3.33 ± 0.20
2.0 + 0.2	8-12	90.00	5.42 ± 0.24	4.36 ± 0.13
2.0 + 0.5	8-12	93.33	5.17 ± 0.10	4.51 ± 0.17
2.0 + 1.0	8-12	80.00	4.70 ± 0.25	3.96 ± 0.14
Kn + IBA				
0.5 + 0.2	10-15	60.00	3.18 ± 0.19	2.44 ± 0.11
0.5 + 0.5	10-15	66.67	3.36 ± 0.13	2.77 ± 0.28
0.5 + 1.0	10-15	70.00	3.60 ± 0.12	2.47 ± 0.15
1.0 + 0.2	10-15	70.00	4.27 ± 0.17	3.88 ± 0.25
1.0 + 0.5	8-12	76.67	4.28 ± 0.21	4.01 ± 0.12
1.0 + 1.0	8-12	56.67	4.41 ± 0.11	3.80 ± 0.09
2.0 + 0.2	8-12	76.67	4.93 ± 0.41	4.11 ± 0.13
2.0 + 0.5	8-12	80.00	5.07 ± 0.21	4.23 ± 0.17
2.0 + 1.0	8-12	86.67	5.30 ± 0.39	4.35 ± 0.28

* Values are the mean of three replicates with 10 explants.

Table 3.3.30. Effect of different concentrations of GA_3 alone and in combination with BAP and Kn on *in vitro* shoot multiplication and elongation from nodal explants of *V. negundo.* Data were taken after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
GA_3				
0.2	10-15	40.00	2.00 ± 0.11	2.81 ± 0.14
0.5	10-15	56.67	3.06 ± 0.17	4.15 ± 0.14
1.0	10-15	60.67	3.10 ± 0.21	4.60 ± 0.09
2.0	10-15	70.00	2.90 ± 0.10	4.17 ± 0.29
3.0	10-15	66.67	2.80 ± 0.31	3.95 ± 0.15
BAP + GA_3				
0.5 + 0.2	8-12	56.67	3.82 ± 0.09	3.12 ± 0.17
0.5 + 0.5	8-12	66.67	4.23 ± 0.16	3.79 ± 0.08
0.5 + 1.0	8-12	60.00	4.38 ± 0.17	4.20 ± 0.16
1.0 + 0.2	8-12	66.67	3.94 ± 0.10	3.35 ± 0.28
1.0 + 0.5	8-12	70.00	4.45 ± 0.20	4.48 ± 0.21
1.0 + 1.0	8-12	83.33	4.49 ± 0.18	4.28 ± 0.42
2.0 + 0.2	8-12	80.00	4.72 ± 0.12	3.78 ± 0.28
2.0 + 0.5	8-12	93.33	5.46 ± 0.15	4.57 ± 0.11
2.0 + 1.0	8-12	80.00	5.10 ± 0.27	4.41 ± 0.20
Kn + GA_3				
0.5 + 0.2	8-12	70.00	3.87 ± 0.33	3.86 ± 0.13
0.5 + 0.5	8-12	80.00	4.66 ± 0.21	5.10 ± 0.27
0.5 + 1.0	8-12	83.33	4.46 ± 0.18	5.13 ± 0.33
1.0 + 0.2	8-12	86.67	5.53 ± 0.27	4.80 ± 0.11
1.0 + 0.5	8-12	86.67	7.03 ± 0.29	6.14 ± 0.47
1.0 + 1.0	8-12	90.00	6.11 ± 0.25	5.27 ± 0.35
2.0 + 0.2	8-12	86.67	5.58 ± 0.15	5.35 ± 0.22
2.0 + 0.5	8-12	96.67	7.76 ± 0.39	6.32 ± 0.27
2.0 + 1.0	8-12	90.00	7.18 ± 0.11	5.85 ± 0.13

* Values are the mean of three replicates with 10 explants.

(b) Shoot tip explants

Experiment 1: Effect of different concentrations of BAP singly and in combination with NAA, IAA and IBA on multiple shoot induction from shoot tip explants of *V. negundo*.

In this present investigation five concentrations (0.2, 0.5, 1.0, 2.0 and 3.0 mg/l) of BAP alone and in combination of three concentrations (0.5, 1.0 and 2.0 mg/l) of BAP with three concentrations (0.2, 0.5 and 1.0 mg/l) of NAA, IAA and IBA were treated in MS medium for the purpose of multiple shoot induction from shoot tip explants of *V. negundo*. Data were taken after 6 weeks of inoculation and percentage of shoot formation, average number of shoot per culture and length of shoot per culture were measured. The results are presented in **Table 3.3.31**. Morphogenic responses were found varied with growth regulators supplements. Shoot proliferation ranged from 40.00–83.33%. Highest percentage (83.33%) of multiple shoot formation was observed in MS medium containing 1.0 mg/l BAP + 0.5 mg/l IBA followed by 80.00% in MS medium containing 2.0 mg/l BAP + 0.5 mg/l IAA and 2.0 mg/l BAP + 0.5 mg/l IBA. The lowest percentage (40.00%) of multiple shoot formation was observed in media having 0.2 mg/l BAP. Highest average number of shoots was 2.46 ± 0.07 in media having 1.0 mg/l BAP + 0.5 mg/l IBA followed by 2.40 ± 0.15 in media having 2.0 mg/l BAP + 0.5 mg/l IAA. Lowest average number of shoot was 1.12 ± 0.10 in media containing 0.2 mg/l BAP. Average length of shoots gradually increased after induction of shoot. Highest average length was recorded 3.31 ± 0.18 cm in 0.5 mg/l BAP + 1.0 mg/l IBA followed by 3.24 ± 0.16 cm in 1.0 mg/l BAP + 0.5 mg/l IAA. The lowest average length was 1.83 ± 0.13 cm in 0.2 mg/l BAP. Experiment results revealed that combination of 1.0 mg/l BAP + 0.5 mg/l IAA, 2.0 mg/l BAP + 0.5 mg/l IAA and 0.5mg/l BAP + 1.0 mg/l IBA were found most effective concentrations for multiple shoot induction from shoot tip explant in *V. negundo*.

Experiment 2: Effect of different concentrations of Kn singly and in combination with NAA, IAA and IBA on multiple shoot induction from shoot tip explants of *V. negundo*.

In this present investigation five concentrations (0.2, 0.5, 1.0, 2.0 and 3.0 mg/l) of Kn alone and in combination of three concentrations (0.5, 1.0 and 2.0 mg/l) of Kn with three concentrations (0.2, 0.5 and 1.0 mg/l) of NAA, IAA and IBA were treated in MS medium for the purpose of multiple shoot induction from shoot tip explants of *V. negundo*. Data were taken after 6 weeks of inoculation and percentage of shoot formation, average number of shoot per culture and length of shoot per culture were measured. The results are presented in **Table 3.3.32**. Morphogenic responses were found varied with growth regulators supplements. Shoot proliferation ranged from

53.33–86.67%. Highest percentage (86.67%) of multiple shoot formation was observed in MS medium containing 2.0 mg/l Kn + 0.5 mg/l NAA followed by 83.33% in MS medium containing 2.0 mg/l Kn + 0.5 mg/l IAA and 2.0 mg/l Kn + 0.5 mg/l IBA. The lowest percentage (53.33%) of multiple shoot formation was observed in media having 0.2 mg/l Kn. Highest average number of shoots was 2.87 ± 0.19 in media having 2.0 mg/l Kn + 0.5 mg/l IAA followed by 2.86± 0.19 in media having 1.0 mg/l Kn + 0.5 mg/l IAA. Lowest average number of shoot was 1.25 ± 0.06 in media containing 0.2 mg/l Kn. Average length of shoots gradually increased after induction of shoot. Highest average length was recorded 3.52 ± 0.21 cm in 2.0 mg/l Kn + 0.5 mg/l NAA followed by 3.42 ± 0.34 cm in 1.0 mg/l Kn + 0.5 mg/l IAA. The lowest average length was 1.90 ± 0.13 cm in 0.5 mg/l Kn+0.2 mg/l IBA. Experiment result revealed that combination of 2.0 mg/l Kn + 0.5 mg/l NAA and 1.0 mg/l Kn + 0.5 mg/l IAA were found most effective concentrations for multiple shoot induction from shoot tip explant in *V. negundo*.

Experiment 3: Effect of different concentrations of GA_3 singly and in combination with BAP and Kn on multiple shoot induction from shoot tip explants of *V. negundo*.

In this present investigation five concentrations (0.2, 0.5, 1.0, 2.0 and 3.0 mg/l) of GA_3 alone and in combination of three concentrations (0.5, 1.0 and 2.0 mg/l) of GA_3 with three concentrations (0.5, 1.0 and 2.0 mg/l) of BAP or Kn were treated in MS medium for the purpose of multiple shoot induction from shoot tip explants of *V. negundo*. Data were taken after 6 weeks of inoculation and percentage of shoot formation, average number of shoot per culture and length of shoot per culture were measured and the results are presented in **Table 3.3.33**. Morphogenic responses were found varied with growth regulators supplements. Shoot proliferation ranged from 30.00–83.33%. Highest percentage (83.33%) of multiple shoot formation was observed in MS medium containing 2.0 mg/l Kn + 0.5 mg/l GA_3 followed by 80.00% in MS medium containing 2.0 mg/l Kn + 1.0 mg/l GA_3 and 1.0 mg/l Kn + 0.5 mg/l GA_3 . The lowest percentage (30.00%) of multiple shoot formation was observed in media having 0.2 mg/l GA_3. Highest average number of shoots was 3.54 ± 0.25 in media having 2.0 mg/l Kn + 0.5 mg/l GA_3 followed by 3.33 ± 0.30 in media having 1.0 mg/l Kn + 0.5 mg/l GA_3. Lowest average number of shoot was 1.90 ± 0.10 in media containing 0.2 mg/l GA_3. Average length of shoots gradually increased after induction of shoot. Highest average length was recorded 5.24 ± 0.32 cm in 1.0 mg/l Kn + 0.5 mg/l GA_3 followed by 5.01 ± 0.13 cm in 2.0 mg/l Kn + 1.0 mg/l GA_3. The lowest average length was 2.47 ± 0.12 cm in 0.2 mg/l GA_3. Experiment result revealed that combination of 2.0 mg/l Kn + 0.5 mg/l GA_3, 1.0 mg/l Kn + 0.5 mg/l GA_3 and 2.0 mg/l Kn + 1.0 mg/l GA_3 were found most effective concentrations for multiple shoot induction in *V. negundo*.

Table 3.3.31. Effect of different concentrations of BAP, singly and in combinations of various auxins on multiple shoot induction from shoot apex explants of *V. negundo.* Data were taken after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
BAP				
0.2	10-15	40.00	1.12 ± 0.10	1.83 ± 0.13
0.5	10-15	60.00	1.22 ± 0.09	1.96 ± 0.19
1.0	10-15	66.67	1.63 ± 0.16	2.77 ± 0.11
2.0	10-15	70.00	1.74 ± 0.17	3.06 ± 0.23
3.0	10-15	56.67	1.32 ± 0.17	2.59 ± 0.20
BAP + NAA				
0.5 + 0.2	10-15	50.00	1.34 ± 0.25	1.98 ± 0.18
0.5 + 0.5	10-15	66.67	1.62 ± 0.11	2.82 ± 0.11
0.5 + 1.0	10-15	63.33	1.38 ± 0.15	2.26 ± 0.06
1.0 + 0.2	10-15	70.00	1.72 ± 0.19	2.69 ± 0.15
1.0 + 0.5	10-15	76.67	1.90 ± 0.11	3.08 ± 0.13
1.0 + 1.0	10-15	56.67	1.82 ± 0.03	2.10 ± 0.25
2.0 + 0.2	10-15	70.00	1.93 ± 0.28	2.44 ± 0.23
2.0 + 0.5	10-15	73.33	2.22 ± 0.19	3.13 ± 0.18
2.0 + 1.0	10-15	56.67	1.86 ± 0.17	2.53 ± 0.17
BAP + IAA				
0.5 + 0.2	10-15	70.00	1.42 ± 0.12	1.95 ± 0.07
0.5 + 0.5	10-15	70.00	1.75 ± 0.12	2.98 ± 0.19
0.5 + 1.0	10-15	73.33	1.98 ± 0.21	2.92 ± 0.33
1.0 + 0.2	10-15	70.00	1.75 ± 0.15	2.61 ± 0.21
1.0 + 0.5	10-15	76.67	2.38 ± 0.17	3.24 ± 0.16
1.0 + 1.0	10-15	73.33	2.23 ± 0.17	2.90 ± 0.10
2.0 + 0.2	10-15	70.00	2.02 ± 0.17	2.67 ± 0.14
2.0 + 0.5	10-15	80.00	2.40 ± 0.15	3.11 ± 0.09
2.0 + 1.0	10-15	73.33	2.22 ± 0.23	2.98 ± 0.27
BAP + IBA				
0.5 + 0.2	10-15	53.33	1.27 ± 0.10	1.87 ± 0.06
0.5 + 0.5	10-15	70.00	1.40 ± 0.17	2.60 ± 0.27
0.5 + 1.0	10-15	60.00	2.18 ± 0.14	3.31 ± 0.18
1.0 + 0.2	10-15	76.67	1.61 ± 0.11	2.77 ± 0.11
1.0 + 0.5	10-15	83.33	2.46 ± 0.07	3.03 ± 0.15
1.0 + 1.0	10-15	73.00	2.13 ± 0.26	2.65 ± 0.19
2.0 + 0.2	10-15	73.33	2.32 ± 0.18	2.56 ± 0.13
2.0 + 0.5	10-15	80.00	2.35 ± 0.21	3.04 ± 0.25
2.0 + 1.0	10-15	73.33	1.96 ± 0.17	2.70 ± 0.22

* Values are the mean of three replicates with 10 explants.

Table 3.3.32. Effect of different concentrations of Kn, singly and in combinations of various auxins on multiple shoot induction from shoot apex explants of *V. negundo*. Data were taken after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
Kn				
0.2	10-15	53.33	1.25 ± 0.06	1.96 ± 0.18
0.5	10-15	60.00	1.47 ± 0.11	2.04 ± 0.21
1.0	10-15	70.00	1.78 ± 0.19	2.84 ± 0.10
2.0	10-15	70.00	1.97 ± 0.27	3.27 ± 0.29
3.0	10-15	63.33	1.46 ± 0.15	2.70 ± 0.17
Kn + NAA				
0.5 + 0.2	10-15	53.33	1.29 ± 0.06	2.33 ± 0.13
0.5 + 0.5	10-15	66.67	1.85 ± 0.17	3.04 ± 0.08
0.5 + 1.0	10-15	60.00	1.30 ± 0.21	2.22 ± 0.19
1.0 + 0.2	10-15	70.00	1.97 ± 0.15	2.82 ± 0.17
1.0 + 0.5	10-15	76.67	2.30 ± 0.15	3.11 ± 0.17
1.0 + 1.0	10-15	56.67	1.46 ± 0.09	2.29 ± 0.28
2.0 + 0.2	10-15	70.00	2.36 ± 0.25	2.80 ± 0.31
2.0 + 0.5	10-15	86.67	2.49 ± 0.14	3.52 ± 0.21
2.0 + 1.0	10-15	60.00	1.60 ± 0.12	2.54 ± 0.10
Kn + IAA				
0.5 + 0.2	10-15	60.00	1.75 ± 0.17	2.13 ± 0.14
0.5 + 0.5	10-15	73.33	1.92 ± 0.15	3.17 ± 0.18
0.5 + 1.0	10-15	80.00	2.14 ± 0.25	3.06 ± 0.08
1.0 + 0.2	10-15	70.00	1.89 ± 0.10	2.78 ± 0.22
1.0 + 0.5	8-12	80.00	2.86 ± 0.19	3.42 ± 0.34
1.0 + 1.0	10-15	76.67	2.74 ± 0.11	3.10 ± 0.25
2.0 + 0.2	10-15	76.67	2.49 ± 0.11	2.83 ± 0.13
2.0 + 0.5	8-12	83.33	2.87 ± 0.19	3.32 ± 0.39
2.0 + 1.0	10-15	66.67	2.36 ± 0.21	3.14 ± 0.16
Kn + IBA				
0.5 + 0.2	10-15	60.00	1.34 ± 0.18	1.90 ± 0.13
0.5 + 0.5	10-15	66.67	1.60 ± 0.12	2.81 ± 0.24
0.5 + 1.0	8-12	73.33	1.92 ± 0.11	2.84 ± 0.33
1.0 + 0.2	10-15	70.00	1.84 ± 0.20	2.78 ± 0.15
1.0 + 0.5	8-12	80.00	2.72 ± 0.16	3.16 ± 0.17
1.0 + 1.0	8-12	76.67	2.31 ± 0.23	2.88 ± 0.22
2.0 + 0.2	10-15	73.33	2.41 ± 0.12	2.74 ± 0.16
2.0 + 0.5	8-12	83.33	2.77 ± 0.07	3.13 ± 0.10
2.0 + 1.0	8-12	70.00	2.13 ± 0.09	2.63 ± 0.18

* Values are the mean of three replicates with 10 explants.

Table 3.3.33. Effect of different concentrations of GA_3 alone and in combination BAP or Kn on *in vitro* shoot multiplication and elongation from shoot tip explants of *V. negundo.* Data were taken after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for shoot initiation	% of shoot formation	*Average number of shoots/culture (mean ± SE)	*Average length (cm) of shoots/culture (mean ± SE)
GA_3				
0.2	10-14	30.00	1.90 ± 0.10	2.47 ± 0.12
0.5	10-14	46.67	2.21 ± 0.12	4.05 ± 0.10
1.0	10-14	60.00	3.00 ± 0.21	4.40 ± 0.21
2.0	10-14	60.00	3.00 ± 0.10	3.92 ± 0.14
3.0	10-14	56.67	2.30 ± 0.21	3.90 ± 0.13
BAP + GA_3				
0.5 + 0.2	8-12	50.00	1.82 ± 0.11	3.24 ± 0.08
0.5 + 0.5	8-12	60.00	2.08 ± 0.12	3.80 ± 0.18
0.5 + 1.0	8-12	63.33	2.34 ± 0.10	4.10 ± 0.17
1.0 + 0.2	8-12	66.67	2.06 ± 0.13	3.45 ± 0.17
1.0 + 0.5	8-12	70.00	2.15 ± 0.20	4.56 ± 0.27
1.0 + 1.0	8-12	73.33	2.35 ± 0.18	3.98 ± 0.22
2.0 + 0.2	8-12	70.00	2.28 ± 0.14	3.45 ± 0.21
2.0 + 0.5	8-12	76.67	3.06 ± 0.10	3.90 ± 0.20
2.0 + 1.0	8-12	73.33	2.93 ± 0.27	3.59 ± 0.23
Kn + GA_3				
0.5 + 0.2	8-12	60.00	2.15 ± 0.09	3.22 ± 0.14
0.5 + 0.5	8-12	66.67	2.53 ± 0.20	4.30 ± 0.15
0.5 + 1.0	8-12	70.00	2.66 ± 0.12	4.65 ± 0.13
1.0 + 0.2	8-12	73.33	2.44 ± 0.21	4.20 ± 0.15
1.0 + 0.5	8-12	80.00	3.33 ± 0.30	5.24 ± 0.32
1.0 + 1.0	8-12	70.00	3.17 ± 0.17	4.86 ± 0.15
2.0 + 0.2	8-12	70.00	2.82 ± 0.20	4.75 ± 0.19
2.0 + 0.5	8-12	83.33	3.54 ± 0.25	4.30 ± 0.30
2.0 + 1.0	8-12	80.00	3.13 ± 0.18	5.01 ± 0.13

* Values are the mean of three replicates with 10 explants.

Plate 3.3.12. Development of multiple shoot from Shoot tip and nodal explant of *V. negundo* through the process of direct organogenesis with their subsequent development.

A: Initiation of multiple shoot formation in nodal segment after 2 weeks of inoculation in media having 2.0 mg/l Kn + 0.5 mg/l GA_3; **B:** Initiation of multiple shoot formation in shoot apex after 2 weeks of inoculation in media having same medium; **C-D:** Proliferation of multiple shoot in the same medium after two subcultures at 14 days interval; **E-G:** Further proliferation and elongation of shoot after 6 weeks of culture in the same medium.

B. Effect of phytohormones on callus induction from different explants of *V. negundo*.

Different concentrations of 2,4–D singly and different concentrations and combinations of BAP and Kn with 2,4–D or NAA were used to investigate the initiation of callus and its subsequent regeneration. Leaf and internode were used as explants for callus induction. In preliminary experiments it was observed that when the leaf and internode explants were placed horizontally on the media surface the initiation of callus took place from both the cut ends. But when they were placed vertically callus initiation took place only from the cut end dipped in the agar medium. Horizontally oriented explants produced callus throughout their entire surface more rapidly than those, which were placed vertically. Therefore internode explants were always placed horizontally on the agar surface to induce callus in all these experiments. The effect of different combinations and concentrations of phytohormones, days of callus initiation, percentage of explants induced callus, callus colour and texture from different explants are discussed experiment wise.

Experiment 1: Effect of different concentrations and combinations of phytohormones on callus induction and characteristics of callus derived from leaf explants.

In this present investigation leaf explants excised from *in vitro* grown seedlings of *V. negundo* were used to investigate the effect of different hormonal concentrations and combinations. Explants were cultured on MS media supplemented with five concentrations (0.5, 1.0, 2.0, 3.0 and 4.0 mg/l) of 2,4–D singly and two concentrations (0.5 and 1.0 mg/l) of BAP and Kn in combination with four concentrations (0.5, 1.0, 2.0 and 3.0 mg/l) of 2,4–D or three concentrations (0.5, 1.0 and 2.0 mg/l) of BAP and Kn in combination with three concentrations (0.5, 1.0 and 2.0 mg/l) of NAA. Data were recorded after 6 weeks of culture. Cultures were maintained under 16 h light and 8 h dark regime. Results obtained on morphogenic response of the cultured explants are shown in **Table 3.3.34**. Morphogenic responses of the explants were found to vary with hormonal formulations present in the culture media. Callus proliferation was not noticed in all media formulations. There was a wide variation in morphological nature and percentage of callus formation among them. Callus initiation occurred within 10 – 15 days depending upon the concentration and combination of hormones. Different concentrations of 2,4–D induced callus within 10-12 days. In combinations of BAP+ 2,4-D and BAP+NAA induced callus within 10 – 15 days. Kn + 2,4-D and Kn + NAA took 12 – 15 days to initiate callus formation. Percentage of callus formation ranged from 15.00 – 65%. Highest percentage (65%) of callus formation occurred in MS medium containing 3.0 mg/l 2,4–D followed by 60% in MS medium containing1.0 mg/l BAP+2.0 mg/l 2,4–D and 55% in 0.5 mg/l BAP+3.0 mg/l 2,4–D.

The lowest percentage (15%) of callus formation was observed in media having 2.0 mg/l Kn+2.0 mg/l 2,4–D. In most cases colour of calli was white, light green, green and dark green and texture of calli were soft, nodular, compact and friable.

Experiment 2: Effect of different concentrations and combinations of phytohormones on callus induction and characteristics of callus derived from internode explants.

In this present investigation internode explants excised from *in vitro* grown seedlings of *V. negundo* were used to investigate the effect of different hormonal concentrations and combinations. Explants were cultured on MS media supplemented with five concentrations (0.5, 1.0, 2.0, 3.0 and 4.0 mg/l) of 2,4–D singly and two concentrations (0.5 and 1.0 mg/l) of BAP and Kn in combination with four concentrations (0.5, 1.0, 2.0 and 3.0 mg/l) of 2,4–D or three concentrations (0.5, 1.0 and 2.0 mg/l) of NAA . Data were recorded after 6 weeks of culture. Cultures were maintained under 16 h light and 8 h dark regime. Results obtained on morphogenic response of the cultured explants are shown in **Table 3.3.34**. Morphogenic responses of the explants were found to vary with hormonal formulations present in the culture media. Callus proliferation was not noticed in all media formulations. There was a wide variation in morphological nature and percentage of callus formation among them. Callus initiation occurred within 10 – 15 days depending upon the concentration and combination of hormones. Different concentrations of 2,4–D induced callus within 10-12 days. In combinations of BAP+ 2,4-D and BAP+NAA induced callus within 10 – 15 days. Kn + 2,4-D and Kn + NAA took 12 – 15 days to initiate callus formation. Percentage of callus formation ranged from 20.00–70%. Highest percentage (70%) of callus formation occurred in MS medium containing 3.0 mg/l 2,4–D (**Plate 3.3.13 A-C**) followed by 65% in MS medium containing 2.0 mg/l 2,4–D alone and in combination of 1.0 mg/l BAP+2.0 mg/l 2,4–D. The lowest percentage (20%) of callus formation was observed in media having 0.5 mg/l 2,4–D alone, 2.0 mg/l BAP+2.0 mg/l NAA and 2.0 mg/l Kn+2.0 mg/l NAA. In most cases colour of calli was white, light green, green and dark green and texture of calli were soft, nodular, compact and friable.

Table 3.3.34. Effect of different concentrations and combinations of phytohormones on callus induction and characteristics of callus derived from leaf and internode explants of *V. negundo*. Data were collected after 6 weeks on inoculation.

Hormonal combinations (mg/l)	Leaf explant			Internode explant		
	Days of callus initiation	% of explant induced callus	*Callus colour and texture	Days of callus initiation	% of explant induced callus	*Callus colour and texture
2,4-D						
0.5	10-12	20	ws	10-15	20	ws
1.0	10-12	40	ws	10-15	45	ws
2.0	10-12	50	wf	10-15	65	wf
3.0	10-12	65	wf	10-15	70	wf
4.0	10-12	25	cwf	10-15	30	cwf
BAP + 2,4-D						
0.5 + 0.5	-	-	-	-	-	-
0.5 + 1.0	10-15	40	cwf	10-15	40	cwf
0.5 + 2.0	10-15	50	cwf	10-15	50	cwf
0.5 + 3.0	10-15	55	cwf	10-15	60	cwf
1.0 + 0.5	-	-	-	-	-	-
1.0 + 1.0	10-15	40	cwf	10-15	55	cwf
1.0 + 2.0	10-15	60	wf	10-15	65	wf
1.0 + 3.0	10-15	50	wf	10-15	50	wf
Kn + 2,4-D						
0.5 + 0.5	-	-	-	-	-	-
0.5 + 1.0	12-15	35	wn	12-15	40	wn
0.5 + 2.0	12-15	50	ws	12-15	50	ws
0.5 + 3.0	12-15	45	ws	12-15	45	ws
1.0 + 0.5	-	-	-	-	-	-
1.0 + 1.0	12-15	30	wn	12-15	35	wn
1.0 + 2.0	12-15	45	ws	12-15	50	ws
1.0 + 3.0	12-15	40	ws	12-15	35	ws
BAP + NAA						
0.5 + 0.5	10-15	30	lgc	10-15	30	lgc
0.5 + 1.0	10-15	45	gc	10-15	60	lgc
0.5 + 2.0	10-15	30	dgc	10-15	40	gc
1.0 + 0.5	10-15	20	lgc	10-15	35	lgc
1.0 + 1.0	10-15	45	gc	10-15	50	gc
1.0 + 2.0	10-15	20	dgc	10-15	25	dgc
2.0 + 0.5	-	-	-	-	-	-
2.0 + 1.0	-	-	-	-	-	-
2.0 + 2.0	10-15	20	dgc	10-15	20	gc
Kn+NAA						
0.5 + 0.5	-	-	-	-	-	-
0.5 + 1.0	12-15	25	lgc	12-15	35	lgc
0.5 + 2.0	12-15	40	lgc	12-15	45	lgc
1.0 + 0.5	12-15	20	lgf	12-15	20	lgf
1.0 + 1.0	12-15	35	gc	12-15	45	lgc
1.0 + 2.0	12-15	40	gc	12-15	35	gc
2.0 + 0.5	-	-	-	-	-	-
2.0 + 1.0	-	-	-	-	-	-
2.0 + 2.0	12-15	15	gc	12-15	20	gc

* **cwf:** creamy white friable, **wf:** white friable, **wn:** white nodular, **ws:** white soft, **lgc:** light green compact, **gc:** green compact, **dgc:** dark green compact, **lgf:** light green friable

C. Adventitious shoot regeneration from different types of callus in *V. negundo*.

Calli produced in different plant growth regulators supplemented on MS medium did not differentiate into shoot buds in the same medium after subsequent subcultures. For the development of adventitious shoot buds from various types of calli, different media formulations were needed. A wide range of cytokinin (BAP and Kn) and auxin (IAA and NAA) supplemented media were used to scrutinize the organogenic response of calli. Among the different types of calli light green friable and green compact found to regenerative on different plant growth regulators supplemented media. Internode derived callus showed better performance than leaf derived callus. The results are presented according to types of explants under separate heads.

(i) Plant regeneration from leaf derived callus

Different experiments were conducted to investigate plant regeneration ability through callus culture from leaf explants. Data on percentage of organogenic calli induced, average number of shoot per callus and average length of shoot per callus were collected after 7 weeks of culture.

Experiment 1: Effect of different concentrations and combinations of BAP and Kn and in combinations with IAA or NAA on adventitious shoot regeneration from leaf derived callus of *V. negundo*.

Under this experiment leaf derived calli of *V. negundo* were used to investigate the effect of different hormonal concentrations and combinations. For shoot differentiation, light green compact calli were subcultured on MS medium supplemented with four concentrations (0.5, 1.0, 2.0 and 3.0 mg/l) of BAP and Kn used alone and in combinations of each other or three concentrations (0.5, 1.0 and 2.0 mg/l) of BAP and Kn with two concentrations (0.2 and 0.5 mg/l) of IAA or NAA to scrutinize the organogenic response. Data were recorded after 7 weeks of culture and presented in **Table 3.3.35**. Adventitious shoot regeneration was not noticed in all media formulations. The percentage of calli produced shoots ranged from 15.00 – 55.00%. Highest percentage 55.00% of adventitious shoots regeneration occurred in MS medium containing 0.5 mg/l BAP+0.5 mg/l IAA followed by 50.00% in MS medium containing 1.0 mg/l BAP+0.5 mg/l IAA. The lowest percentage (15.00%) of adventitious shoots regeneration was observed in media having 2.0 mg/l Kn alone. The highest number of adventitious shoots regeneration per callus was 3.20 ± 0.35 in media having 0.5 mg/l BAP+0.5 mg/l IAA followed by 3.06 ± 0.15 in 1.0 mg/l BAP+0.5 mg/l IAA. The lowest number of adventitious shoots regeneration per callus was 1.20 ± 0.11 in media having 2.0 mg/l BAP+0.2 mg/l IAA. Highest length of shoot 3.34 ± 0.27 cm was recorded in 0.5 mg/l BAP+0.5 mg/l IAA and the lowest length of shoots 1.90 ± 0.13 cm was recorded in media having 1.0 mg/l BAP+0.2 mg/l NAA.

(ii) Plant regeneration from internode derived callus

Different experiments were conducted to investigate plant regeneration ability through callus culture from internode explants. Data on percentage of organogenic calli induced shoots, average number of shoots per callus and average length of shoots per callus were collected after 7 weeks of culture.

Experiment 1: Effect of different concentrations and combinations of BAP and Kn and in combinations with IAA or NAA on adventitious shoot regeneration from internode derived callus of *V. negundo*.

Under this experiment internode derived calli of *V. negundo* were used to investigate the effect of different hormonal concentrations and combinations. For shoot differentiation, light green compact calli were subcultured on MS medium supplemented with four concentrations (0.5, 1.0, 2.0 and 3.0 mg/l) of BAP and Kn used alone and in combinations of each other or three concentrations (0.5, 1.0 and 2.0 mg/l) of BAP and Kn with two concentrations (0.2 and 0.5 mg/l) of IAA or NAA to scrutinize the organogenic response. Data were recorded after 7 weeks of culture and presented in **Table 3.3.35**. Adventitious shoot regeneration was not noticed in all media formulations. The percentage of calli produced shoots ranged from 15.00 – 70.00%. Highest percentage 70.00% of adventitious shoots regeneration occurred in MS medium containing 0.5 mg/l BAP+0.5 mg/l IAA followed by 55.00% in MS medium containing 1.0 mg/l BAP+0.5 mg/l IAA. The lowest percentage (15.00%) of adventitious shoots regeneration was observed in media having 2.0 mg/l Kn + 0.2 mg/l IAA. The highest number of adventitious shoots regeneration per callus was 4.30 ± 0.18 in media having 0.5 mg/l BAP+0.5 mg/l IAA followed by 3.70 ± 0.15 in 1.0 mg/l BAP+0.5 mg/l IAA. The lowest number of adventitious shoots regeneration per callus was 1.88 ± 0.18 in media having 0.5 mg/l BAP+1.0 mg/l Kn. Highest length of shoot 4.16 ± 0.25 cm was recorded in 0.5 mg/l BAP+0.5 mg/l IAA and the lowest length of shoots 1.88 ± 0.18 cm was recorded in media having 0.5 mg/l BAP+1.0 mg/l Kn.

Table 3.3.35. Effect of BAP and Kn alone and in combinations with NAA or IAA on adventitious shoot regeneration from leaf and internode derived callus in *V. negundo*. Data were collected after 7 weeks of inoculation.

Hormonal combination (mg/l)	Leaf derived callus			Internode derived callus		
	% of regenerable calli	*Average number of shoots/callus (mean±SE)	*Average length (cm) of shoots/callus (mean±SE)	% of regenerable calli	*Average number of shoots/callus (mean±SE)	*Average length (cm) of shoots/callus (mean±SE)
BAP						
0.5	-	-	-	20	1.82 ± 0.06	1.98 ± 0.13
1.0	30	2.10 ± 0.17	2.60 ± 0.17	35	2.62 ± 0.15	2.74 ± 0.11
2.0	20	1.90 ± 0.13	2.32 ± 0.14	20	2.00 ± 0.11	2.22 ± 0.14
3.0	-	-	-	-	-	-
Kn						
0.5	-	-	-	-	-	-
1.0	20	1.50 ± 0.15	2.14 ± 0.17	20	1.61 ± 0.15	2.18 ± 0.17
2.0	15	1.25 ± 0.08	2.26 ± 0.14	20	1.33 ± 0.07	2.10 ± 0.11
3.0	-	-	-	-	-	-
BAP+Kn						
0.5 + 0.5	35	1.80 ± 0.15	1.94 ± 0.10	30	1.78 ± 0.24	2.06 ± 0.12
0.5 + 1.0	25	1.96 ± 0.18	2.13 ± 0.09	35	1.80 ± 0.16	1.88 ± 0.18
1.0 + 0.5	40	2.28 ± 0.25	2.31 ± 0.27	40	2.47 ± 0.29	2.74 ± 0.21
1.0 + 1.0	30	1.90 ± 0.14	2.30 ± 0.19	25	2.10 ± 0.17	2.49 ± 0.13
2.0 + 0.5	45	2.30 ± 0.33	2.50 ± 0.14	50	3.52 ± 0.20	3.13 ± 0.20
2.0 + 1.0	30	2.17 ± 0.12	2.36 ± 0.11	30	2.85 ± 0.17	2.68 ± 0.12
BAP + NAA						
0.5 + 0.2	-	-	-	-	-	-
0.5 + 0.5	25	1.86 ± 0.09	2.20 ± 0.11	40	2.11 ± 0.05	2.04 ± 0.12
1.0 + 0.2	30	2.05 ± 0.17	1.90 ± 0.13	30	2.34 ± 0.17	2.68 ± 0.11
1.0 + 0.5	40	2.20 ± 0.13	2.40 ± 0.17	45	2.84 ± 0.23	3.05 ± 0.36
2.0 + 0.2	30	1.20 ± 0.11	1.93 ± 0.13	35	2.26 ± 0.17	2.10 ± 0.17
2.0 + 0.5	35	1.70 ± 0.28	2.32 ± 0.26	35	2.40 ± 0.11	2.32 ± 0.22
Kn + NAA						
0.5 + 0.2	-	-	-	-	-	-
0.5 + 0.5	-	-	-	-	-	-
1.0 + 0.2	20	2.02 ± 0.11	2.61 ± 0.12	30	2.10 ± 0.17	2.70 ± 0.18
1.0 + 0.5	35	2.14 ± 0.17	2.75 ± 0.21	35	2.16 ± 0.12	3.11 ± 0.24
2.0 + 0.2	-	-	-	-	-	-
2.0 + 0.5	-	-	-	-	-	-
BAP + IAA						
0.5 + 0.2	45	2.81 ± 0.28	2.64 ± 0.15	45	2.72 ± 0.11	3.35 ± 0.17
0.5 + 0.5	55	3.20 ± 0.35	3.34 ± 0.27	70	4.30 ± 0.18	4.16 ± 0.25
1.0 + 0.2	45	2.70 ± 0.11	2.87 ± 0.11	40	3.43 ± 0.11	3.20 ± 0.11
1.0 + 0.5	50	3.06 ± 0.15	3.20 ± 0.17	55	3.70 ± 0.15	3.36 ± 0.20
2.0 + 0.2	20	2.16 ± 0.10	2.24 ± 0.10	20	2.22 ± 0.12	2.34 ± 0.15
2.0 + 0.5	30	2.29 ± 0.17	2.42 ± 0.13	30	2.34 ± 0.16	2.35 ± 0.11
Kn + IAA						
0.5 + 0.2	-	-	-	-	-	-
0.5 + 0.5	-	-	-	-	-	-
1.0 + 0.2	-	-	-	20	2.08 ± 0.17	2.60 ± 0.14
1.0 + 0.5	25	2.50 ± 0.17	2.54 ± 0.10	30	2.61 ± 0.15	2.88 ± 0.17
2.0 + 0.2	20	2.12 ± 0.11	2.30 ± 0.11	15	2.27 ± 0.11	2.20 ± 0.11
2.0 + 0.5	-	-	-	-	-	-

- : Failed to differentiate any shoots. *Values are the mean of three replicates with 20 explants.

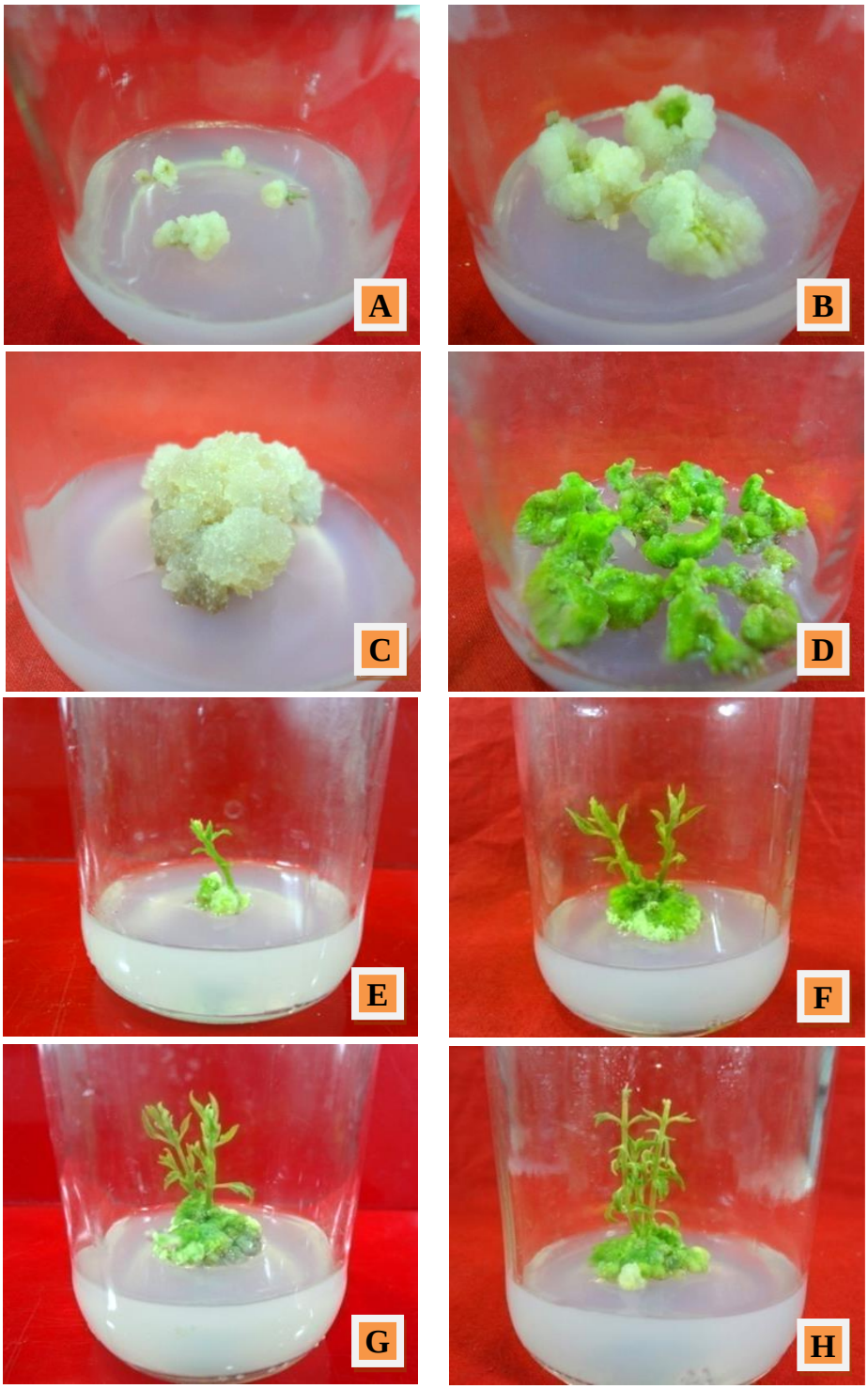


Plate 3.3.13. Callus induction from internode and leaf explants with plant regeneration from callus of *V. negundo.*

A-C: Callus induction from internode explant in MS+3mg/l 2,4-D; **D:** Callus induction from leaf explant in MS+2mg/l 2,4-D +1.0 mg/l BAP; **E**: Initiation of callus regeneration in MS+0.5 mg/l BAP+0.5mg/l IAA after 4 weeks of culture from leaf derived callus and; **F-I**: proliferation and elongation of shoot buds from leaf derived callus in the same medium.

D. Rooting of *in vitro* grown shoots in *V. negundo*.

Shoot cuttings 5– 7 cm long *in vitro* grown shoots were separated and transferred to rooting media. Half strength MS medium fortified with different concentrations of NAA, IAA and IBA alone were used for rooting experiment. Rooting started within 8 to 15 days of culture. Among the tested concentrations MS medium having 0.5 – 2.0 mg/l IAA found highest percentage of root formation. Data on days to root initiation, frequency of root formation (%), average number of root per culture and average length of root per culture were recorded after 5 weeks of culture. The results are described experiment wise:

Experiment 1: Effect of different concentrations of NAA, IAA, IBA and MS=0 on root induction from shoots derived from *in vitro* grown shoots of *V. negundo.*

For adventitious root formation, *in vitro* grown shoots obtained from two types of explants were cultured on MS medium with six concentrations (0.1, 0.2, 0.5, 1.0, 1.5, 2.0 and 3.0 mg/l) of NAA, IAA, IBA and MS=0 (without hormones). Results obtained for the root induction, percentage of root formation, morphology, average number and length of roots are shown in **Table 3.3.36**. Irrespective concentrations of different auxins and MS=0 medium failed to induce any regeneration of roots. Percentage range of cultures produced roots varied from 20.00 – 93.33%. Highest 93.33% of root regeneration was recorded in 1.0 mg/l IAA. The lowest 20.00% of root regeneration was recorded in media having 0.1 mg/l IAA. The highest average number of roots per shoot was recorded 10.63 ± 0.16 in media having 1.0 mg/l IAA (**Plate 3.3.14**) followed by 8.54 ± 0.30 in 1.5 mg/l IAA. The lowest number of root per shoot was recorded 2.40 ± 0.20 in media having 0.2 mg/l NAA. Highest average length of roots 6.60 ± 0.33 cm was recorded in 0.5 mg/l IBA and the lowest length of roots 2.08 ± 0.28 cm was recorded in 0.2 mg/l NAA. In most cases morphology of roots was thin, thick and long.

Table 3.3.36. Effect of half strength MS medium with different concentrations of auxins used singly on root induction in *V. negundo*. Data were recorded after 6 weeks of culture.

Growth regulators (mg/l)	Days taken for root initiation	% of explants induced root development	* Average number of root per culture (mean ± SE)	* Average length (cm) of root per culture (mean ± SE)
MS=0	-	-	-	-
NAA				
0.1	-	-	-	-
0.2	10-15	36.67	2.40 ± 0.20	2.08 ± 0.28
0.5	10-15	63.33	4.21 ± 0.43	4.10 ± 0.35
1.0	10-15	56.67	4.10 ± 0.00	4.30 ± 0.25
1.5	10-15	50.00	3.36 ± 0.25	2.32 ± 0.16
2.0	10-15	30.00	3.12 ± 0.19	2.10 ± 0.50
3.0	-	-	-	-
IAA				
0.1	10-15	20.00	3.32 ± 0.11	2.62 ± 0.09
0.2	8-12	27.67	3.35 ± 0.22	3.41 ± 0.13
0.5	8-12	83.33	7.65 ± 0.16	4.47 ± 0.11
1.0	8-12	93.33	10.63 ± 0.16	4.63 ± 0.25
1.5	8-12	86.67	8.54 ± 0.33	4.75 ± 0.29
2.0	8-12	90.00	8.40 ± 0.37	3.90 ± 0.38
3.0	8-12	63.33	4.64 ± 0.17	3.25± 0.23
IBA				
0.1	-	-	-	-
0.2	10-15	43.33	2.86 ± 0.25	3.45 ± 0.19
0.5	10-15	80.00	5.20 ± 0.49	6.60 ± 0.33
1.0	10-15	83.33	4.60 ± 0.27	5.20 ± 0.39
1.5	10-15	70.00	4.02 ± 0.18	4.92 ± 0.18
2.0	10-15	60.00	3.90 ± 0.30	3.50 ± 0.31
3.0	10-15	50.00	2.92 ± 0.18	3.13 ± 0.14

-: Failed to any initiation of root
* Values are the mean of three replicates with 10 explants.

Plate 3.3.14. Induction of adventitious roots and establishment in soil of *V. negundo*. **A:** Induction and elongation of adventitious roots on *in vitro* grown shoots in half strength MS medium containing 1.0 mg/l IAA after 5 weeks of culture; **B:** Picture of *V. negundo* with *in vitro* regenerated roots outside of culture bottle; **C:** Acclimatized regenerated plants in soil after 50 days.

3.3.2. Artificial Seed Production

Centella asiatica and ***Rauvolfia serpentina*** plants were selected for the study of artificial seed production. *In vitro* grown plants were used as the source for providing necessary explants. Different concentrations of auxins and cytokinins were used alone or in combinations to investigate the induction of shoot regeneration. These hormonal concentrations were tested in MS basal media (Murashige and Skoog 1962). Nodes and shoot tips were used as explants for artificial seed production. Details of the results so far obtained from each of the experiments are described under following heads:

3.3.2.1. *Encapsulated artificial seed regenerations from different explants of C. asiatica*

Different concentrations of auxins and cytokinins were used alone or in combinations to investigate the initiation of shoot and its subsequent regeneration in *C. asiatica*. Encapsulated shoot tips and encapsulated nodal explants were cultured for shoot regeneration. Encapsulated artificial seeds were cultured on MS agar media supplemented with different concentrations of two cytokinins (BAP and Kn) used alone or in combination with IAA. Data on days of germination, percentage of germination, average number of shoot per culture and average length of shoot per culture were collected after 6 weeks of culture. The results are presented according to types of explants used under separate heads:

A. Encapsulated shoot tip explants

Experiment 1: Effect of different concentrations and combinations of plant growth regulators on artificial seed proliferation from shoot tip explants of *C. asiatica*

Under present investigation four concentrations (0.5, 1.0, 2.0 and 3.0 mg/l) of BAP and Kn used alone and in combination of BAP+Kn and Kn+IAA in different concentrations were treated in MS medium for the purpose of multiple shoot induction from encapsulated shoot tip explants of *C. asiatica*. Data were taken after 6 weeks of inoculation and days of germination, percentage of germination, number of shoot/culture and length of shoot/culture were measured. The results are presented in **Table 3.3.37**. Morphogenic responses of the explants were found to vary with hormonal formulations present in the culture media. Shoot germination started within 15-20 days. Shoot germination ranged from 40 – 76.67%. From artificial seed highest percentage (76.67%) of multiple shoot formation was observed in MS medium containing 2.0 mg/l Kn+ 0.5 mg/l followed by 70.00% in MS medium containing 2.0 mg/l Kn + 1.0 mg/l IAA. The lowest percentage (40.00%) of multiple shoot formation was observed in media having 0.5 mg/l Kn alone and in 1.0 mg/l BAP+ 0.5 mg/l Kn.

Highest mean number of shoots was 3.74 ± 0.14 in MS medium having 2.0 mg/l Kn+ 0.5 mg/l IAA (**Plate 3.3.15.I**) followed by 3.50 ± 0.27 in 2.0 mg/l Kn+ 1.0 mg/l IAA. Lowest mean number of shoot was 1.56 ± 0.14 in media containing 1.0 mg/l BAP+ 0.5 mg/l Kn. Average length of shoots gradually increased after induction of shoot. Highest average length was recorded 2.10 ± 0.19 cm in 2.0 mg/l Kn followed by 2.06 ± 0.13 cm in 2.0 mg/l Kn+ 0.5 mg/l IAA. The lowest average length was 1.25 ± 0.11 cm in 0.5 mg/l BAP. Experiment results revealed that in combination of 2.0 mg/l Kn+ 0.5 mg/l IAA, 2.0 mg/l Kn + 1.0 mg/l IAA and 2.0 mg/l Kn alone were found most effective concentrations for multiple shoot induction.

B. Encapsulated nodal explants

Experiment 1: Effect of different concentrations and combinations of plant growth regulators on artificial seed proliferation from nodal explants of *C. asiatica*

In this present investigation four concentrations (0.5, 1.0, 2.0 and 3.0 mg/l) of BAP and Kn used alone and in combination of BAP+Kn and Kn+IAA in different concentrations were treated in MS medium for the purpose of multiple shoot induction from encapsulated nodal explants of *C. asiatica*. Data were taken after 6 weeks of inoculation and days of germination, percentage of germination, number of shoots per culture and length of shoots per culture were measured. The results are presented in **Table 3.3.38**. Morphogenic responses of the explants were found to vary with hormonal formulations present in the culture media. Shoot germination started within 15-20 days. Shoot germination ranged from 40.00–90.00%. The percentage of artificial seeds showing shoot germination was the highest 90% and number of shoots per culture was the highest 4.20 ± 0.19 at 2.0 mg/l Kn in combination with 0.5 mg/l IAA (**Plate 3.3.15. D-E**) followed by 83.33% germination and 4.08 ± 0.22 shoots per culture was at 2.0 mg/l Kn in combination with 1.0 mg/l IAA. The lowest percentage (40%) of shoot formation and lowest mean number of shoots 1.79 ± 0.09 was at BAP 0.5 mg/l. Highest average length was recorded 2.14 ± 0.14 cm in 2.0 mg/l Kn followed by 2.06 ± 0.06 cm in 1.0 mg/l Kn. The lowest average length was 1.18 ± 0.12 cm in 0.5 mg/l BAP. Experimental results revealed that in combination of 2.0 mg/l Kn+ 0.5 mg/l IAA, 2.0 mg/l Kn + 1.0 mg/l IAA and 2.0 mg/l Kn alone were found most effective concentrations for multiple shoot induction.

Table 3.3.37. Effect of different concentrations and combinations of plant growth regulators of artificial seed proliferation in shoot tip explants of *C. asiatica*. Data were recorded after 6 weeks of culture.

Plant growth regulators (mg/l)	Days of germination	Percentage (%) of germination	* Average number of shoots per culture (mean ± SE)	* Average length (cm) of shoots per culture (mean ± SE)
BAP				
0.5	15-20	46.00	1.83 ± 0.14	1.25 ± 0.11
1.0	15-20	60.00	2.60 ± 0.17	1.95 ± 0.10
2.0	15-20	50.00	2.06 ± 0.15	1.67 ± 0.18
3.0	15-20	46.67	1.90 ± 0.12	1.66 ± 0.09
Kn				
0.5	15-20	40.00	2.09 ± 0.08	1.63 ± 0.16
1.0	15-20	60.00	2.57 ± 0.16	1.90 ± 0.10
2.0	15-20	66.67	3.13 ± 0.14	2.10 ± 0.19
3.0	15-20	60.00	2.50 ± 0.22	1.81 ± 0.17
BAP + Kn				
1.0 + 0.5	15-20	40.00	1.56 ± 0.14	1.42 ± 0.08
1.0 + 1.0	15-20	46.67	2.17 ± 0.19	1.70 ± 0.16
1.0 + 2.0	15-20	60.00	2.79 ± 0.30	1.83 ± 0.21
1.0 + 3.0	15-20	53.33	2.43 ± 0.26	1.80 ± 0.06
2.0 + 0.5	15-20	43.33	1.84 ± 0.07	1.53 ± 0.13
2.0 + 1.0	15-20	56.67	2.55 ± 0.10	1.86 ± 0.18
2.0 + 2.0	15-20	56.67	2.60 ± 0.17	1.76 ± 0.15
2.0 + 3.0	15-20	50.00	2.28 ± 0.14	1.57 ± 0.08
Kn + IAA				
2.0 + 0.2	15-20	60.00	3.21 ± 0.20	1.71 ± 0.16
2.0 + 0.5	15-20	76.67	3.74 ± 0.14	2.06 ± 0.13
2.0 + 1.0	15-20	70.00	3.50 ± 0.27	1.93 ± 0.11
2.0 + 2.0	15-20	60.00	2.92 ± 0.12	1.59 ± 0.15
3.0 + 0.2	15-20	50.00	2.42 ± 0.13	1.74 ± 0.18
3.0 + 0.5	15-20	66.67	3.17 ± 0.25	1.88 ± 0.05
3.0 + 1.0	15-20	56.67	2.36 ± 0.17	1.61 ± 0.13
3.0 + 2.0	15-20	50.00	2.14 ± 0.17	1.32 ± 0.16

* Values are the mean of three replicates with 10 explants.

Table 3.3.38. Effect of different concentrations and combinations of plant growth regulators of artificial seed proliferation in nodal explants of *C. asiatica*. Data were recorded after 6 weeks of culture.

Plant growth regulators (mg/l)	Days of germination	Percentage (%) of germination	* Average number of shoots per culture (mean ± SE)	* Average length (cm) of shoots per culture (mean ± SE)
BAP				
0.5	15-20	40.00	1.79 ± 0.09	1.18 ± 0.12
1.0	15-20	63.33	2.84 ± 0.19	1.86 ± 0.17
2.0	15-20	56.67	2.20 ± 0.12	1.55 ± 0.10
3.0	15-20	50.00	2.07 ± 0.11	1.60 ± 0.16
Kn				
0.5	15-20	50.00	2.27 ± 0.06	1.86 ± 0.11
1.0	15-20	70.00	2.75 ± 0.10	2.06 ± 0.06
2.0	15-20	70.00	3.30 ± 0.21	2.14 ± 0.14
3.0	15-20	73.33	2.98 ± 0.14	1.66 ± 0.09
BAP + Kn				
1.0 + 0.5	15-20	43.33	1.86 ± 0.11	1.52 ± 0.08
1.0 + 1.0	15-20	50.00	2.65 ± 0.16	1.88 ± 0.11
1.0 + 2.0	15-20	66.67	3.12 ± 0.21	2.05 ± 0.18
1.0 + 3.0	15-20	56.67	2.70 ± 0.26	1.94 ± 0.07
2.0 + 0.5	15-20	50.00	2.18 ± 0.12	1.58 ± 0.10
2.0 + 1.0	15-20	66.67	2.70 ± 0.15	1.94 ± 0.14
2.0 + 2.0	15-20	70.00	2.87 ± 0.23	1.80 ± 0.09
2.0 + 3.0	15-20	50.00	1.91 ± 0.14	1.65 ± 0.05
Kn + IAA				
2.0 + 0.2	15-20	76.67	3.37 ± 0.16	1.56 ± 0.08
2.0 + 0.5	15-20	90.00	4.20 ± 0.19	1.92 ± 0.13
2.0 + 1.0	15-20	83.33	4.08 ± 0.22	1.85 ± 0.11
2.0 + 2.0	15-20	70.00	3.12 ± 0.08	1.90 ± 0.12
3.0 + 0.2	15-20	73.33	3.03 ± 0.14	1.69 ± 0.16
3.0 + 0.5	15-20	80.00	3.25 ± 0.18	1.77 ± 0.10
3.0 + 1.0	15-20	60.00	2.73 ± 0.21	1.44 ± 0.13
3.0 + 2.0	15-20	60.00	2.65 ± 0.17	1.18 ± 0.06

* Values are the mean of three replicates with 10 explants.

Plate 3.3.15. Artificial seeds of *C. asiatica* and different stages of germination.

A: Artificial seeds (nodal explants) encapsulated by sodium alginate; **B-C:** Germinated artificial seeds (nodal explants) in MS + 2.0 mg/l Kn + 0.5 mg/l IAA after 2 weeks of culture; **D-E**: Artificial seed derived plant after 3-4 week of culture. **F:** Artificial seeds (shoot tip explants) encapsulated by sodium alginate; **G-H:** Germinated artificial seeds (shoot tip explants) in MS + 2.0 mg/l Kn + 0.5 mg/l IAA after 2 weeks of culture; **I**: Artificial seed derived plant after 3-4 week of culture.

C. Survival test of artificial seeds in *C. asiatica* under different storage temperatures.

In the present investigation, artificial seeds of *C. asiatica* were kept in storage under growth chamber at 20 ± 2ºC, in refrigerator at 4 ± 1ºC and at -1 ± 1ºC for survival test. Both shoot tip and nodal encapsulated artificial seeds were used for this survival test. At the end of each storage period, artificial seeds were immediately transferred to fresh germination medium and placed for the recovery of plantlets. After storage period the artificial seeds were again grown under *in vitro* conditions on nutrient media for shoot development and root induction.

The survival percentage of encapsulated shoot tips and nodal explants decreased significantly with the increased storage periods and temperature. However, the reduction in viability was recorded more at 20 ± 2ºC in contrast to storage at 4 ± 1ºC and -1 ± 1ºC. Under the storage of artificial seeds, the mean number of encapsulated shoot tip and nodal explants that survived after 7 days at 20 ± 2ºC storage temperature was 50-65 percent, whereas the survival percentage of encapsulated explants at the storage of 4 ± 1ºC was 80-85 percent. After 15 days of storage at 20 ± 2ºC, 15-35 percent was found while it was 70-75 percent at 4 ± 1ºC. After 30 days of storage at 20 ± 2ºC, 0-15 percent was found while it was 60-65 percent at 4 ± 1ºC. After 45 days of storage at 20 ± 2ºC, no survival encapsulated explants was found, while it was 50-55 percent at 4 ± 1ºC. But after 60 days of storage at 20 ± 2ºC, no survived encapsulated explants were found while it was 25-30 percent at 4 ± 1ºC. On the other hand, storage at 0ºC temperature no survived encapsulated explants was found. After 60 days and above no survived encapsulated explants was found at 20 ± 2ºC. On the other hand, survivability found extended around 25–30% under storage temperature 4 ± 1ºC (**Table 3.3.39**).

Table 3.3.39. Effect of storage on the viability of artificial seed of *C. asiatica* (For each treatment 20 explants were used).

Sl. No.	Storage period (days)	Survival percentage (shoot tip explants)			Survival percentage (nodal explants)		
		Storage temperature			Storage temperature		
		Storage at 20 ± 2ºC	Storage at 4 ± 1ºC	Storage at -1 ± 1ºC	Storage at 20 ± 2ºC	Storage at 4 ± 1ºC	Storage at -1 ± 1ºC
1	7	50	80	-	65	85	-
2	15	15	70	-	35	75	-
3	30	-	60	-	15	65	-
4	45	-	55	-	-	50	-
5	60	-	25	-	-	30	-
6	75	-	-	-	-	-	-

3.3.2.2. *Encapsulated artificial seed regenerations from different explants of R. serpentina*

Encapsulated shoot tips and nodal explants were cultured for shoot regeneration. Encapsulated artificial seeds were cultured on MS agar gelled media supplemented with different concentrations of BAP and Kn alone or combined with NAA to investigate the initiation of shoot and its subsequent regeneration. Data on days of germination, percentage of germination, average number of shoot per culture and average length of shoot per culture were collected after 6 weeks of culture. The results are presented according to types of explants used under separate heads:

A. Encapsulated shoot tip explants

Experiment 1: Effect of different concentrations and combinations of plant growth regulators on artificial seed proliferation from shoot tip explants of *R. serpentina*

In the present investigation four concentrations (0.5, 1.0, 2.0 and 3.0 mg/l) of BAP and Kn used alone and in combination of two concentrations (1.0 and 2.0 mg/l) of BAP or Kn with four concentrations (0.1, 0.2, 0.5 and 1.0 mg/l) of NAA were treated in MS medium for the purpose of multiple shoot induction from encapsulated shoot tip explants of *R. serpentina*. Data were taken after 6 weeks of inoculation and days of germination, percentage of germination, number of shoot per culture and length of shoot per culture were measured. The results are presented in **Table 3.3.40.** Morphogenic responses of the explants were found to vary with hormonal formulations present in the culture media. Shoot germination started within 12-16 days. Shoot germination ranged from 30.00 – 76.67%. The percentage of artificial seeds showing shoot germination was the highest 76.67% and number of shoots per culture was the highest 4.13 ± 0.33 at 1.0 mg/l BAP in combination with 0.2 mg/l NAA (**Plate 3.3.16. E**) followed by 66.67% germination and 3.72 ± 0.16 shoots per culture was at 1.0 mg/l Kn in combination with 0.2 mg/l NAA. The lowest percentage (30.00%) of multiple shoot formation and lowest average number of shoot per culture 1.58 ± 0.13 was observed in media having 0.5 mg/l Kn alone. Average length of shoots gradually increased after induction of shoot. Highest average length was recorded 3.56 ± 0.19 cm in 1.0 mg/l Kn in combination with 0.2 mg/l NAA followed by 3.48 ± 0.19 cm in 1.0 mg/l BAP+0.2 mg/l NAA. The lowest average length was 2.28 ± 0.11 cm in 0.5 mg/l BAP. Experiment result revealed that in combination of 1.0 mg/l BAP+ 0.2 mg/l NAA and 1.0 mg/l Kn + 0.2 mg/l NAA were found most effective concentrations for multiple shoot induction.

B. Encapsulated nodal explants

Experiment 1: Effect of different concentrations and combinations of plant growth regulators on artificial seed proliferation from nodal explants of *R. serpentina*

In this investigation four concentrations (0.5, 1.0, 2.0 and 3.0 mg/l) of BAP and Kn used alone and in combination of two concentrations (1.0 and 2.0 mg/l) of BAP or Kn with four concentrations (0.1, 0.2, 0.5 and 1.0 mg/l) of NAA were treated in MS medium for the purpose of multiple shoot induction from encapsulated nodal explants of *R. serpentina*. Data were taken after 6 weeks of inoculation and days of germination, percentage of germination, number of shoot per culture and length of shoot per culture were measured. The results are presented in **Table 3.3.41.** Morphogenic responses of the explants were found to vary with hormonal formulations present in the culture media. Shoot germination started within 12-16 days. Shoot germination ranged from 20.00 – 63.33%. The percentage of artificial seeds showing shoot germination was the highest 63.33% and number of shoots per culture was the highest 3.86 ± 0.17 at 1.0 mg/l BAP in combination with 0.2 mg/l NAA followed by 60.00% germination and 3.61 ± 0.25 shoots per culture was at 1.0 mg/l BAP in combination with 0.5 mg/l NAA. The lowest percentage (20.00%) of multiple shoot formation and lowest average number of shoot per culture 1.34 ± 0.09 was observed in media having 0.5 mg/l Kn alone. Average length of shoots gradually increased after induction of shoot. Highest average length was recorded 3.42 ± 0.17 cm in 1.0 mg/l Kn in combination with 0.2 mg/l NAA followed by 3.38 ± 0.23 cm in 2.0 mg/l BAP alone. The lowest average length was 2.35 ± 0.15 cm in 2.0 mg/l BAP. Experiment result revealed that in combination of 1.0 mg/l BAP+ 0.2 mg/l NAA, 1.0 mg/l BAP+ 0.5 mg/l NAA and 1.0 mg/l Kn + 0.2 mg/l NAA were found most effective concentrations for multiple shoot induction.

Table 3.3.40. Effect of different concentrations and combinations of plant growth regulators of artificial seed proliferation in shoot tip explants of *R. serpentina*. Data were recorded after 6 weeks of culture.

Plant growth regulators (mg/l)	Days of germination	Percentage (%) of germination	* Average number of shoots per culture (mean ± SE)	* Average length (cm) of shoots per culture (mean ± SE)
BAP				
0.5	12-16	33.33	1.90 ± 0.08	2.28 ± 0.11
1.0	12-16	46.67	2.40 ± 0.11	3.33 ± 0.18
2.0	12-16	60.00	2.95 ± 0.24	3.42 ± 0.17
3.0	12-16	36.67	1.97 ± 0.23	3.00 ± 0.17
Kn				
0.5	12-16	30.00	1.58 ± 0.13	2.93 ± 0.16
1.0	12-16	43.33	1.99 ± 0.13	3.29 ± 0.27
2.0	12-16	56.67	2.61 ± 0.10	3.33± 0.16
3.0	12-16	40.00	1.87 ± 0.15	3.06 ± 0.18
BAP + NAA				
1.0 + 0.1	12-16	50.00	3.28 ± 0.10	3.16 ± 0.14
1.0 + 0.2	12-16	76.67	4.13 ± 0.33	3.48 ± 0.19
1.0 + 0.5	12-16	53.33	3.23 ± 0.16	3.10 ± 0.15
1.0 + 1.0	12-16	50.00	2.90 ± 0.13	2.86 ± 0.14
2.0 + 0.1	12-16	56.67	2.79 ± 0.15	3.04 ± 0.17
2.0 + 0.2	12-16	60.00	3.35 ± 0.25	3.17 ± 0.12
2.0 + 0.5	12-16	53.33	3.17 ± 0.12	3.22 ± 0.15
2.0 + 1.0	12-16	33.33	2.11 ± 0.09	2.77 ± 0.13
Kn + NAA				
1.0 + 0.1	12-16	36.67	2.30 ± 0.12	2.76 ± 0.15
1.0 + 0.2	12-16	66.67	3.72 ± 0.16	3.56 ± 0.19
1.0 + 0.5	12-16	56.67	3.10 ± 0.19	3.14 ± 0.26
1.0 + 1.0	12-16	40.00	2.43 ± 0.20	2.70 ± 0.14
2.0 + 0.1	12-16	46.67	2.42 ± 0.12	2.69 ± 0.08
2.0 + 0.2	12-16	60.00	3.15 ± 0.14	3.06 ± 0.24
2.0 + 0.5	12-16	50.00	3.07 ± 0.21	2.90 ± 0.22
2.0 + 1.0	12-16	36.67	2.30 ± 0.25	2.78 ± 0.11

* Values are the mean of three replicates with 10 explants.

Table 3.3.41. Effect of different concentrations and combinations of plant growth regulators of artificial seed proliferation in nodal explants of *R. serpentina*. Data were recorded after 6 weeks of culture.

Plant growth regulators (mg/l)	Days of germination	Percentage (%) of germination	* Average number of shoots per culture (mean ± SE)	* Average length (cm) of shoots per culture (mean ± SE)
BAP				
0.5	12-16	30.00	1.83 ± 0.13	2.35 ± 0.15
1.0	12-16	40.00	2.37 ± 0.20	3.21 ± 0.15
2.0	12-16	56.67	2.85 ± 0.30	3.38 ± 0.23
3.0	12-16	36.67	1.90 ± 0.27	2.90 ± 0.14
Kn				
0.5	15-20	20.00	1.34 ± 0.09	2.70 ± 0.15
1.0	15-20	36.67	1.65 ± 0.12	3.06 ± 0.21
2.0	15-20	50.00	2.34 ± 0.17	3.25± 0.17
3.0	15-20	33.33	1.36 ± 0.11	2.97 ± 0.12
BAP + NAA				
1.0 + 0.1	12-16	50.00	3.18 ± 0.12	2.70 ± 0.23
1.0 + 0.2	12-16	63.33	3.86 ± 0.17	3.23 ± 0.16
1.0 + 0.5	12-16	60.00	3.61 ± 0.25	3.08 ± 0.13
1.0 + 1.0	12-16	46.67	3.05 ± 0.09	2.66 ± 0.20
2.0 + 0.1	12-16	50.00	2.79 ± 0.23	3.16 ± 0.14
2.0 + 0.2	12-16	56.67	3.21 ± 0.10	3.34 ± 0.20
2.0 + 0.5	12-16	50.00	3.02 ± 0.10	3.20 ± 0.19
2.0 + 1.0	12-16	30.00	1.87 ± 0.18	2.64 ± 0.21
Kn + NAA				
1.0 + 0.1	12-16	40.00	2.45 ± 0.15	3.10 ± 0.11
1.0 + 0.2	12-16	56.67	3.48 ± 0.13	3.42 ± 0.17
1.0 + 0.5	12-16	50.00	3.14 ± 0.17	3.28 ± 0.14
1.0 + 1.0	12-16	40.00	2.39 ± 0.12	2.80 ± 0.21
2.0 + 0.1	12-16	50.00	2.46 ± 0.24	2.67 ± 0.10
2.0 + 0.2	12-16	53.33	3.13 ± 0.16	2.90 ± 0.15
2.0 + 0.5	12-16	46.67	2.95 ± 0.11	2.81 ± 0.24
2.0 + 1.0	12-16	40.00	2.26 ± 0.17	2.73 ± 0.19

* Values are the mean of three replicates with 10 explants.

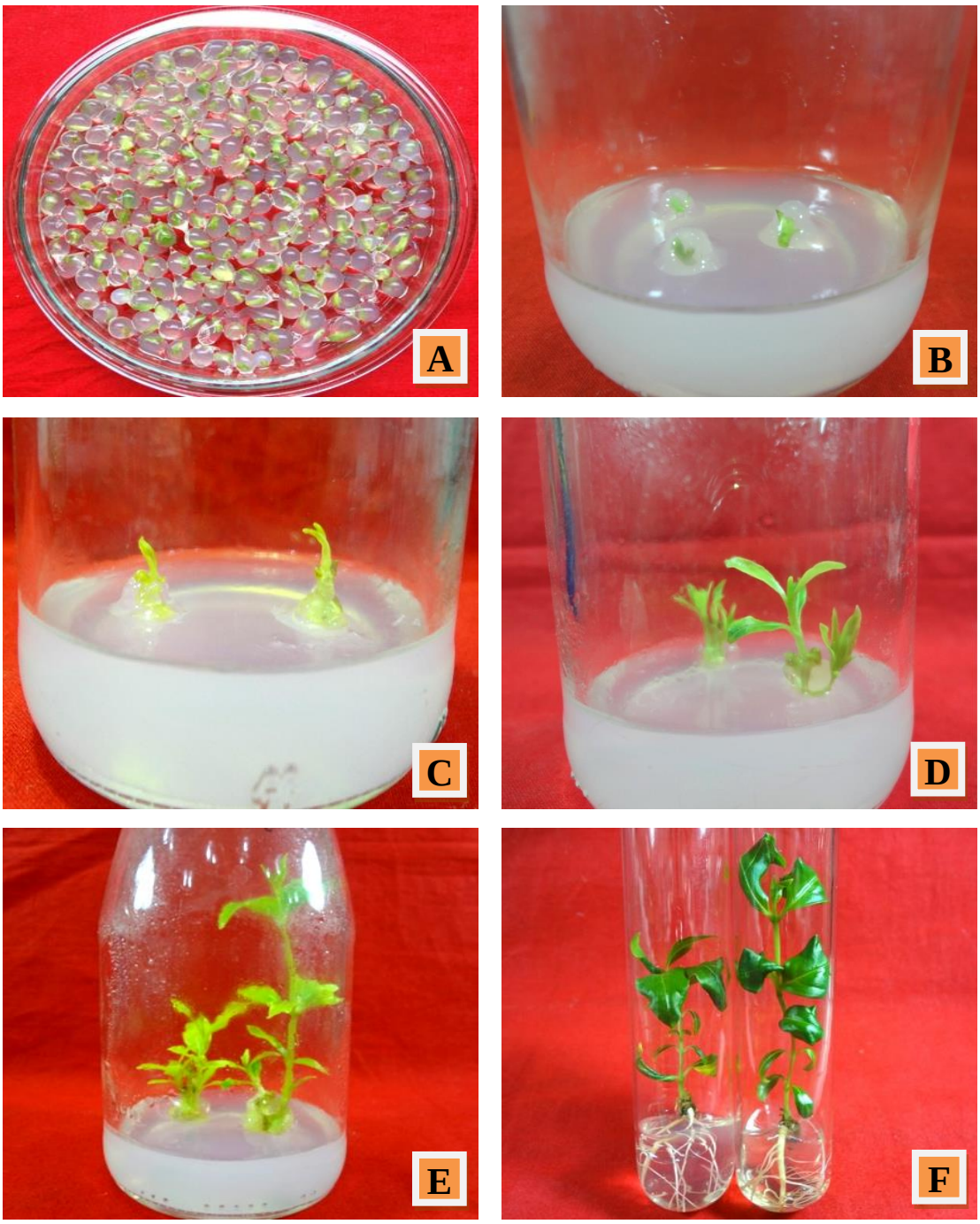


Plate 3.3.16. Artificial seeds and different stages of plant growth from artificial seeds of *R. serpentina.*

A: Artificial seeds (nodal and shoot tip explants) encapsulated by sodium alginate; **B:** Artificial seeds inoculated in the medium after 30 days of storage at 4ºC; **C-D:** Sprouting of artificial seeds in MS + 1.0 mg/l BAP + 0.2 mg/l NAA after 2-3 weeks of culture; **E**: Artificial seed derived plant after 4-5 week of culture; **F:** Rooted artificial seed derived plantlets.

C. Survival test of artificial seeds in *R. serpentina* under different storage temperature regime.

Artificial seeds of *R. serpentina* were kept in storage under growth chamber at 20 ± 2ºC, in refrigerator at 4 ± 1ºC and at -1 ± 1ºC for survival test. Both shoot tip and nodal encapsulated artificial seeds were used for this survival test. At the end of each storage period, artificial seeds were immediately transferred to fresh germination medium and placed for the recovery of plantlets. After storage period the artificial seeds were grown under *in vitro* conditions on nutrient media for shoot development and root induction.

The survival percentage of encapsulated shoot tips and nodal explants decreased significantly with the increase of storage periods and temperature. However, the reduction in viability was recorded more at 20 ± 2ºC in contrast to storage at 4 ± 1ºC and -1 ± 1ºC. Under the storage of artificial seeds, the mean number of encapsulated shoot tip and nodal explants that survived after 7 days at 20 ± 2ºC storage temperature was 40-50 percent, whereas the survival percentage of encapsulated explants at the storage of 4 ± 1ºC was 60-75 percent. After 15 days of storage at 20 ± 2ºC, 20-25 percent was found while it was 55-65 percent at 4 ± 1ºC. After 30 days of storage at 20 ± 2ºC, 10 percent was found while it was 50-60 percent at 4 ± 1ºC. After 45 days of storage at 20 ± 2ºC, no survived encapsulated explants was found, while it was 40-45 percent at 4 ± 1ºC. But after 60 days of storage at 20 ± 2ºC, no survived encapsulated explants were found while it was 20-25 percent at 4 ± 1ºC. On the other hand, storage at 0ºC temperature no survived encapsulated explants was found. After 60 days and above no survived encapsulated explants was found at 20 ± 2ºC. On the other hand, survivability found extended around 20–25% under storage temperature 4 ± 1ºC (**Table 3.3.42**).

Table 3.3.42. Effect of storage on the viability of artificial seed of *R. serpentina* (For each treatment 20 explants were used).

Sl. No.	Storage period (days)	Survival percentage (shoot tip explants)			Survival percentage (nodal explants)		
		Storage temperature			Storage temperature		
		Storage at 20 ± 2ºC	Storage at 4 ± 1ºC	Storage at -1 ± 1ºC	Storage at 20 ± 2ºC	Storage at 4 ± 1ºC	Storage at -1 ± 1ºC
1	7	50	75	-	40	60	-
2	15	25	65	-	20	55	-
3	30	10	60	-	10	50	-
4	45	-	45	-	-	40	-
5	60	-	25	-	-	20	-
6	75	-	-	-	-	-	-

3.3.3. Cell Suspension Culture

The present investigation was carried out for cell suspension culture of four selected plant species *viz.* *Centella asiatica*, *Coccinia cordifolia*, *Mucuna pruriens and Rauvolfia serpentina*. The objective of this investigation was to establish a protocol for the cell suspension culture in order to generate the avenue for isolation of secondary metabolite from cell suspension culture. Details of the results obtained from each of the species and experiments are described below-

In the present investigation, free cells isolated from friable calli containing rapid dividing cells of four selected plant species *C. asiatica* (**Plate 3.3.17.A**), *C. cordifolia* (**Plate 3.3.18.A**), *M. pruriens* (**Plate 3.3.19.A**) *and R. serpentina* (**Plate 3.3.20.A**) were used as explants and the effects of different concentrations and combinations of different auxins and cytokinin (NAA, IAA, 2,4-D and BAP) on cell suspension culture were investigated. The filtrated cells and cell aggregates were allowed to settle for 20-25 minutes and finally poured off the supernatant. Using sterilized pipette, 5 ml residue cells were re-suspended into the fresh liquid medium and dispensed the cells equally in several sterilized flasks of 250 ml containing 70 ml liquid medium. The flasks containing cells were placed on the rotary shaker with 200 rpm and allowed the free cells to grow. Under agitated state the cells were grown in different sizes and growth stages. Every after 4 days of culture the liquid medium was filtrated through the sieve with pore having diameter of 500µ. This time, the filtrate contained only the free growing cell masses. The growing plants cells were confirmed by examining the drop of filtrate under microscope examination (**Plate 3.3.17.D, 3.3.18.D, 3.3.19.D and 3.3.20.D**). This cell suspension culture was maintained in the lab and served as the stock for starting any experiments with cell culture. Data (weight in gram) were recorded for cell weight per 10 ml cell suspension for 9 times in case of *C. asiatica* and *C. cordifolia*; for 7 times for *M. pruriens* and for 8 times for *R. serpentina* with the interval of every 4 days of culture. The experimental observation revealed that the cells grew gradually in the suspension culture of all hormonal concentrations and combinations. But the cell growth rate found to differ from different hormonal concentrations and a growth curve was drawn computing the highest value in each case of the species (**Fig. 3.3.1**). The effects so far obtained from each of the experiments of different concentrations and combinations of different hormones on cell suspension culture of selected species are described below:

3.3.3.1. *Cell suspension culture of C. asiatica*

Cell suspension culture was established from friable calli derived from leaf in *C. asiatica* with two experiments:

Experiment 1: Effect of different concentrations of NAA with BAP, 1.0 mg/l biotin and 1.0 mg/l glutamine on cell growth of *C. asiatica*.

The experiment was conducted to find out the combined effect of three concentrations (0.5, 1.0 and 2.0 mg/l) of NAA with two concentrations (0.5 or 1.0 mg/l) of BAP, 1.0 mg/l biotin and 1.0 mg/l glutamine on MS medium cell suspension culture. The cultures were maintained under total dark regime at 25±2°C temperature. Results obtained on cell weight and the cell aggregates were found to vary with variations in hormonal formulation present in the culture media that are presented in **Table 3.3.43.**

The cells and the cell aggregates of all cultivars responded in all the media formulations. The peak period for growth was observed within 12^{th}–24^{th} day of suspension culture (**Fig.3.3.1**). The highest weight of cells and cell aggregates were obtained on 32^{nd} day of cell suspension culture in MS medium supplemented with 2.0 mg/l NAA + 0.5 mg/l BAP and it was 0.144 ± 0.013 gm. The minimum weight of cells was 0.132 ± 0.014 gm in MS medium having 0.5 mg/l NAA + 0.5 mg/l BAP on the 32^{nd} day of culture. Lower concentrations of NAA with BAP were found less effective for cell growth. The growth of cells was continued until 32 days of culture but it was ceased down after 32 days of culture.

Experiment 2: Effect of different concentrations of 2,4-D with BAP, 1.0 mg/l biotin and 1.0 mg/l glutamine on cell growth of *C. asiatica*.

This experiment was conducted to find out the effect of different concentrations of 2,4-D with BAP, biotin and glutamine on cells suspension culture of *C. asiatica*. The cells and the cell aggregates were cultured on MS medium supplemented with three different concentrations (0.5, 1.0 and 2.0 mg/l) of 2,4-D with two concentrations (0.5 or 1.0 mg/l) of BAP, and the growth of the cell suspension was assessed by measuring the weight of these cells and clusters and the results are shown in **Table 3.3.44.**

Among the combinations 1.0 mg/l 2,4-D in combination with 0.5 mg/l BAP was found to be the most effective formulation on cell suspension culture of *C. asiatica*. The maximum cell weight was 0.138 ± 0.012 gm in the same hormonal concentration on 32^{nd} day of culture.

The lower and higher concentrations of 2,4-D with BAP were found less effective on cell suspension culture. The minimum weight of cells and clusters was 0.125 ± 0.011 gm in MS medium having 0.5 mg/l 2,4-D + 0.5 mg/l BAP on the 32^{nd} day of culture. The growth of cells was continued until 32 days of culture but it was ceased down after 32 days of culture.

Table 3.3.43. Effect of different concentrations of NAA with BAP, 1.0 mg/l biotin and 1.0 mg/l glutamine on cell growth of *C. asiatica*.

Concentrations of BAP+NAA (mg/l)	Days after incubation	Weight of cells (gm) (mean ± SE)
0.5+0.5	4th	0.078 ± 0.009
	8th	0.089 ± 0.010
	12th	0.110 ± 0.012
	16th	0.118 ± 0.012
	20th	0.123 ± 0.011
	24th	0.127 ± 0.012
	28th	0.130 ± 0.013
	32nd	0.132 ± 0.014
	36th	0.132 ± 0.013
0.5+1.0	4th	0.084 ± 0.008
	8th	0.098 ± 0.008
	12th	0.118 ± 0.010
	16th	0.125 ± 0.012
	20th	0.131 ± 0.012
	24th	0.136 ± 0.013
	28th	0.138 ± 0.011
	32nd	0.140 ± 0.013
	36th	0.140 ± 0.014
0.5+2.0	4th	0.085 ± 0.008
	8th	0.088 ± 0.009
	12th	0.095 ± 0.011
	16th	0.104 ± 0.010
	20th	0.118 ± 0.012
	24th	0.137 ± 0.013
	28th	0.141 ± 0.011
	32nd	0.144 ± 0.013
	36th	0.144 ± 0.014
1.0+1.0	4th	0.082 ± 0.008
	8th	0.098 ± 0.009
	12th	0.116 ± 0.010
	16th	0.125 ± 0.012
	20th	0.130 ± 0.011
	24th	0.134 ± 0.013
	28th	0.136 ± 0.012
	32nd	0.138 ± 0.014
	36th	0.138 ± 0.014
1.0+2.0	4th	0.082 ± 0.010
	8th	0.096 ± 0.010
	12th	0.117 ± 0.012
	16th	0.125 ± 0.011
	20th	0.132 ± 0.013
	24th	0.137 ± 0.012
	28th	0.138 ± 0.013
	32nd	0.140 ± 0.014
	36th	0.140 ± 0.014

Table 3.3.44. Effect of different concentrations of 2,4-D with BAP, 1.0 mg/l biotin and 1.0 mg/l glutamine on cell growth of *C. asiatica*.

Concentrations of BAP+2,4-D (mg/l)	Days after incubation	Weight of cells (gm)($\bar{x}$ ± SE)
0.5+0.5	4th	0.072 ± 0.006
	8th	0.085 ± 0.008
	12th	0.106 ± 0.008
	16th	0.113 ± 0.009
	20th	0.118 ± 0.010
	24th	0.120 ± 0.009
	28th	0.123 ± 0.010
	32nd	0.125 ± 0.011
	36th	0.125 ± 0.012
0.5+1.0	4th	0.081 ± 0.006
	8th	0.096 ± 0.009
	12th	0.114 ± 0.007
	16th	0.124 ± 0.011
	20th	0.130 ± 0.010
	24th	0.133 ± 0.011
	28th	0.136 ± 0.012
	32nd	0.138 ± 0.012
	36th	0.138 ± 0.012
0.5+2.0	4th	0.080 ± 0.006
	8th	0.094 ± 0.008
	12th	0.113 ± 0.010
	16th	0.122 ± 0.011
	20th	0.128 ± 0.010
	24th	0.131 ± 0.011
	28th	0.134 ± 0.012
	32nd	0.137 ± 0.012
	36th	0.136 ± 0.014
1.0+1.0	4th	0.076 ± 0.008
	8th	0.092 ± 0.008
	12th	0.112 ± 0.009
	16th	0.118 ± 0.010
	20th	0.126 ± 0.009
	24th	0.130 ± 0.010
	28th	0.134 ± 0.010
	32nd	0.136 ± 0.011
	36th	0.136 ± 0.012
1.0+2.0	4th	0.074 ± 0.008
	8th	0.090 ± 0.009
	12th	0.111 ± 0.010
	16th	0.118 ± 0.012
	20th	0.123 ± 0.011
	24th	0.128 ± 0.012
	28th	0.132 ± 0.010
	32nd	0.134 ± 0.014
	36th	0.134 ± 0.014

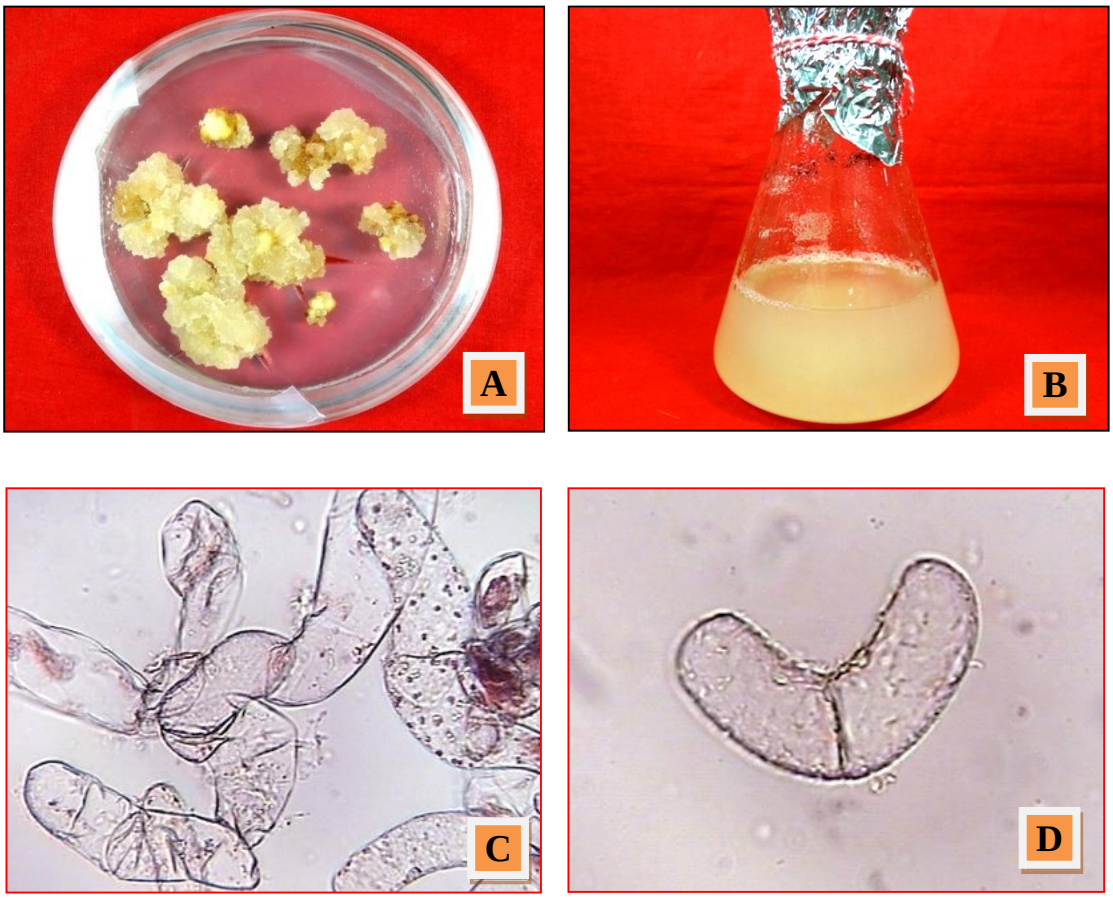


Plate 3.3.17. A-D: Photographs showing the different stages of cell culture of *C. asiatica*; **A:** Embryogenic calli; **B:** Cell suspension culture; **C:** Isolated cells; **D:** Dividing Single cell.

3.3.3.2. *Cell suspension culture of C. cordifolia*

Cell suspension culture was established from friable calli derived from leaf in *C. cordifolia* with two experiments:

Experiment 1: Effect of different concentrations of NAA with BAP on cell growth of *C. cordifolia*

This experiment was conducted using three concentrations (0.2, 0.5 and 1.0 mg/l) of NAA in combination with two concentrations (1.0 and 2.0 mg/l) of BAP accompanied with 1.0 mg/l biotin and 1.0 mg/l glutamine to understand their combined effect on cell suspension culture in *C. cordifolia*. The cultures were maintained under total dark regime at 25±2°C temperature. Data were collected per 10 ml cell suspension culture for 9 times with the interval of every after 4 days of culture. The experimental observation revealed that the cell grew gradually with the increasing of time of all

hormonal concentrations and combinations. The collected data were summarized in **Table 3.3.45.**

Among the five culture media formulations, 1.0 mg/l BAP in combination with 0.5 mg/l NAA, 1.0 mg/l biotin and 1.0 mg/l glutamine was found to be most effective on cell suspension culture for *C. cordifolia*. The optimum period of cell growth was observed within 8^{th}–28^{th} day of cell suspension culture (**Fig 3.3.1**) and the maximum cell weight was 0.136 ± 0.012 gm on the 32^{nd} day of culture in the same medium. The lowest weight of cells was 0.122 ± 0.014 gm in MS medium having 2.0 mg/l NAA + 0.2 mg/l NAA on the 32^{nd} day of culture. Lower and higher concentrations of NAA with BAP were found less effective for cell growth. The growth of cells was continued until 32 days of culture but it was ceased down after 32 days of culture.

Experiment 2: Effect of different concentrations of 2,4-D with BAP on cell suspension culture of *C. cordifolia*

This experiment was conducted to find out the effect of different concentrations of 2,4-D with BAP on cells suspension culture of *C. cordifolia*. The cells and the cell aggregates were cultured on MS medium supplemented with three different concentrations (0.2, 0.5 and 1.0 mg/l) of 2,4-D with two concentrations (1.0 and 2.0 mg/l) of BAP, 1.0 mg/l biotin and 1.0 mg/l glutamine. The growth of the cell suspension was assessed by measuring the weight of these cells and clusters and the results are shown in **Table 3.3.46.**

Among the combinations 1.0 mg/l 2,4-D in combination with 1.0 mg/l BAP was found to be the most effective formulation on cell suspension culture. The maximum cell weight was 0.126 ± 0.012 gm in the same hormonal concentration on 32^{nd} day of culture. The lower and higher concentrations of 2,4-D with BAP were found less effective on cell suspension culture. The minimum weight of cells and clusters was 0.118 ± 0.011 gm in MS medium having 0.2 mg/l 2,4-D + 1.0 mg/l BAP on the 32^{nd} day of culture. The growth of cells was continued until 32 days of culture but it was ceased down after 32 days of culture.

Table 3.3.45. Effect of different concentrations of NAA with 1.0 or 2.0 mg/l BAP, 1.0 mg/l biotin and 1.0 mg/l glutamine on cell growth of *C. cordifolia*.

Concentrations of BAP+NAA (mg/l)	Days after incubation	Weight of cells (gm) (mean ± SE)
1.0 + 0.2	4th	0.058 ± 0.006
	8th	0.072 ± 0.007
	12th	0.090 ± 0.007
	16th	0.104 ± 0.009
	20th	0.110 ± 0.010
	24th	0.116 ± 0.011
	28th	0.120 ± 0.011
	32nd	0.124 ± 0.012
	36th	0.124 ± 0.012
1.0 + 0.5	4th	0.062 ± 0.004
	8th	0.072 ± 0.006
	12th	0.092 ± 0.010
	16th	0.108 ± 0.014
	20th	0.116 ± 0.013
	24th	0.124 ± 0.010
	28th	0.132 ± 0.012
	32nd	0.136 ± 0.012
	36th	0.136 ± 0.014
1.0 + 1.0	4th	0.056 ± 0.006
	8th	0.068 ± 0.006
	12th	0.102 ± 0.008
	16th	0.110 ± 0.010
	20th	0.114 ± 0.010
	24th	0.120 ± 0.011
	28th	0.124 ± 0.011
	32nd	0.126 ± 0.012
	36th	0.126 ± 0.010
2.0 + 0.2	4th	0.056 ± 0.008
	8th	0.068 ± 0.009
	12th	0.086 ± 0.010
	16th	0.098 ± 0.011
	20th	0.110 ± 0.013
	24th	0.114 ± 0.012
	28th	0.117 ± 0.013
	32nd	0.122 ± 0.014
	36th	0.122 ± 0.013
2.0 + 0.5	4th	0.060 ± 0.008
	8th	0.074 ± 0.006
	12th	0.092 ± 0.010
	16th	0.108 ± 0.010
	20th	0.116 ± 0.012
	24th	0.120 ± 0.011
	28th	0.126 ± 0.012
	32nd	0.130 ± 0.013
	36th	0.130 ± 0.014

Table 3.3.46. Effect of different concentrations of 2,4-D with 0.5 or 1.0 mg/l BAP, 1.0 mg/l biotin and 1.0 mg/l glutamine on cell growth of *C. cordifolia*.

Concentrations of BAP+2,4-D (mg/l)	Days after incubation	Weight of cells (gm)($\bar{x}$ ± SE)
1.0 + 0.2	4th	0.052 ± 0.006
	8th	0.060 ± 0.006
	12th	0.082 ± 0.008
	16th	0.094 ± 0.007
	20th	0.105 ± 0.008
	24th	0.112 ± 0.008
	28th	0.116 ± 0.010
	32nd	0.118 ± 0.011
	36th	0.118 ± 0.012
1.0 + 0.5	4th	0.056 ± 0.008
	8th	0.070 ± 0.006
	12th	0.090 ± 0.010
	16th	0.100 ± 0.009
	20th	0.108 ± 0.011
	24th	0.114 ± 0.011
	28th	0.118 ± 0.012
	32nd	0.120 ± 0.013
	36th	0.120 ± 0.012
1.0 + 1.0	4th	0.058 ± 0.006
	8th	0.073 ± 0.007
	12th	0.094 ± 0.008
	16th	0.106 ± 0.009
	20th	0.115 ± 0.011
	24th	0.119 ± 0.010
	28th	0.124 ± 0.011
	32nd	0.126 ± 0.012
	36th	0.126 ± 0.012
2.0 + 0.2	4th	0.054 ± 0.004
	8th	0.066 ± 0.006
	12th	0.084 ± 0.008
	16th	0.096 ± 0.008
	20th	0.108 ± 0.009
	24th	0.114 ± 0.010
	28th	0.118 ± 0.012
	32nd	0.122 ± 0.011
	36th	0.122 ± 0.013
2.0 + 0.5	4th	0.056 ± 0.006
	8th	0.070 ± 0.007
	12th	0.092 ± 0.008
	16th	0.100 ± 0.009
	20th	0.112 ± 0.010
	24th	0.116 ± 0.011
	28th	0.119 ± 0.008
	32nd	0.122 ± 0.012
	36th	0.122 ± 0.010

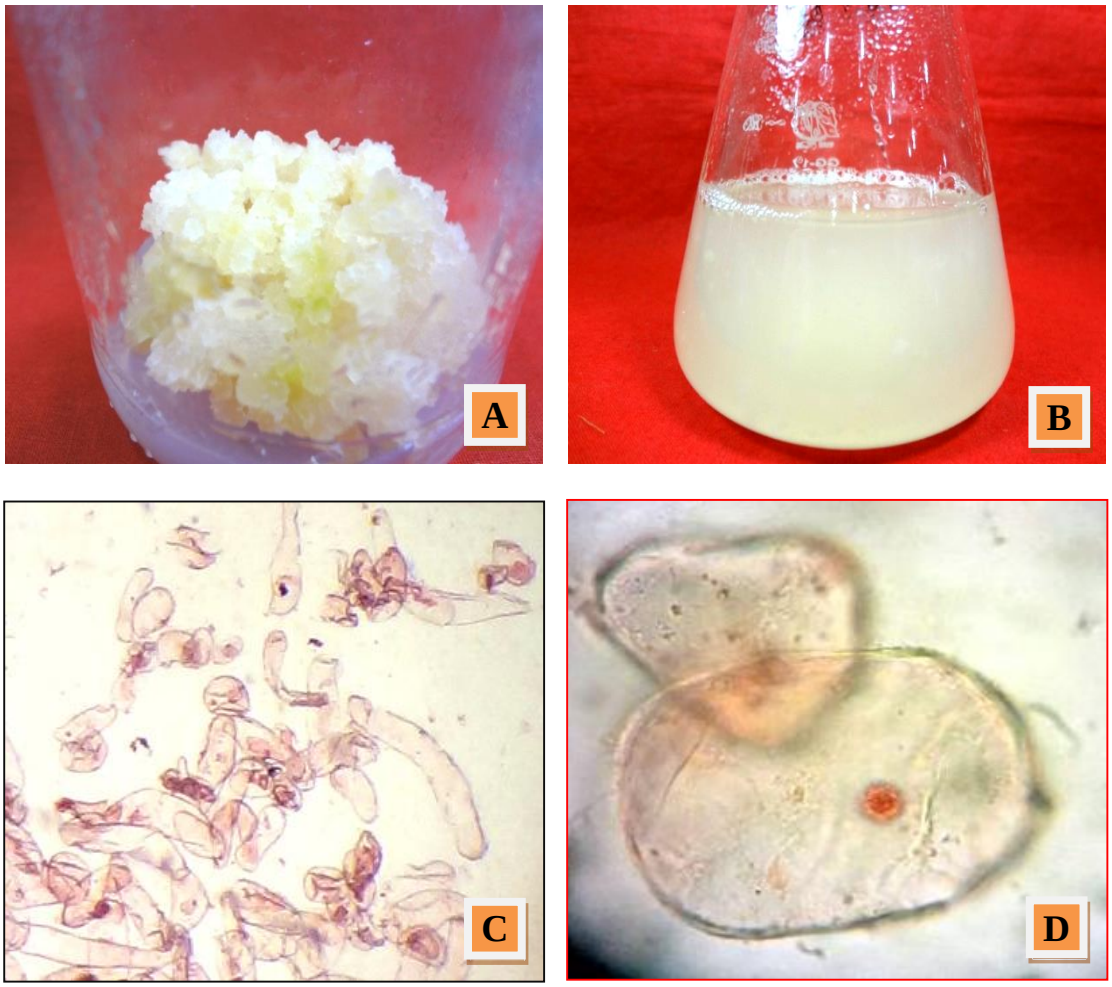


Plate 3.3.18. A-D: Photographs showing the different stages of cell culture of *C. cordifolia*; **A:** Embryogenic calli; **B:** Cell suspension culture; **C:** Isolated cells. **D:** Single cells.

3.3.3.3. *Cell suspension culture of M. pruriens*

Cell suspension culture was established from friable calli derived from leaf in *M. pruriens* with two experiments:

Experiment 1: Effect of different concentrations of BAP with IAA on cell suspension culture of *M. pruriens*

This experiment was conducted using three concentrations (0.5, 1.0 and 2.0 mg/l) of IAA in combination with two concentrations (2.0 and 3.0 mg/l) of BAP accompanied with 1.0 mg/l biotin and 1.0 mg/l glutamine to understand their combined effect on cell suspension culture in *M. pruriens*. The cultures were maintained under controlled conditions at 25±2°C temperature and 16h/8h day/night regime. Data were collected per 10 ml cell suspension culture for 7 times with the interval of every after 4 days of

culture. Results obtained on cell weight of cells and the cell aggregates were found to vary with variations in hormonal formulation present in the culture media that are presented in **Table 3.3.47.**

The cells and the cell aggregates of all cultivars responded in all the media formulations. The peak period was observed within 12^{th}–20^{th} day of suspension culture (**Fig.3.3.1**). The highest weight of cells and cell aggregates were obtained on 24^{nd} day of cell suspension culture in MS medium supplemented with 3.0 mg/l BAP + 0.5 mg/l IAA and it was 0.128 ± 0.011 gm. The minimum weight of cells was 0.114 ± 0.012 gm in MS medium having 3.0 mg/l BAP + 0.5 mg/l IAA on the 24^{th} day of culture. Higher concentrations of BAP with lower concentration of IAA were found to be most effective for cell growth. The growth of cells was continued until 24 days of culture but it was ceased down after 24 days of culture.

Experiment 2: Effect of different concentrations of 2,4-D alone and in combination with BAP on cell growth of *M. pruriens*

In this present investigation three concentrations (0.5, 1.0 and 2.0 mg/l) of 2,4-D used alone and in combination of 0.5 mg/l BAP, 1.0 mg/l biotin and 1.0 mg/l glutamine to understand their combined effect on cell suspension culture in *M. pruriens*. Data were collected per 10 ml cell suspension culture for 7 times with the interval of every after 4 days of culture. The experimental observation revealed that the cells grew gradually with the increasing to time of all hormonal concentrations and combinations. The collected data were summarized in **Table 3.3.48.**

Among the combinations 1.0 mg/l 2,4-D was found to be the most effective formulation on cell suspension culture. The maximum cell weight was 0.125 ± 0.013 gm in the same hormonal concentration on 24^{th} day of culture. The lower and higher concentrations of 2,4-D were found less effective on cell suspension culture. The minimum weight of cells and clusters was 0.113 ± 0.012 gm in MS medium having 0.5 mg/l 2,4-D on the 24^{th} day of culture. The growth of cells was continued until 24 days of culture but it was ceased down after 24 days of culture.

Table 3.3.47. Effect of different concentrations of BAP with IAA, 1.0 mg/l biotin and 1.0 mg/l glutamine on cell suspension culture of *M. pruriens.*

Concentrations of BAP+IAA (mg/L)	Days after incubation	Weight of cells (gm) (mean ± SE)
2.0 + 0.5	4th	0.043 ± 0.004
	8th	0.047 ± 0.006
	12th	0.058 ± 0.009
	16th	0.094 ± 0.010
	20th	0.108 ± 0.010
	24th	0.116 ± 0.011
	28th	0.116 ± 0.012
2.0 + 1.0	4th	0.042 ± 0.006
	8th	0.048 ± 0.007
	12th	0.060 ± 0.008
	16th	0.100 ± 0.010
	20th	0.114 ± 0.010
	24th	0.122 ± 0.012
	28th	0.122 ± 0.011
3.0 + 0.5	4th	0.044 ± 0.007
	8th	0.050 ± 0.008
	12th	0.062 ± 0.008
	16th	0.105 ± 0.010
	20th	0.122 ± 0.011
	24th	0.128 ± 0.011
	28th	0.128 ± 0.012
3.0 + 1.0	4th	0.044 ± 0.006
	8th	0.049 ± 0.006
	12th	0.060 ± 0.008
	16th	0.102 ± 0.011
	20th	0.120 ± 0.011
	24th	0.126 ± 0.012
	28th	0.126 ± 0.012
3.0 + 2.0	4th	0.040 ± 0.006
	8th	0.046 ± 0.008
	12th	0.058 ± 0.010
	16th	0.096 ± 0.011
	20th	0.108 ± 0.011
	24th	0.114 ± 0.012
	28th	0.114 ± 0.012

Table 3.3.48. Effect of different concentrations of 2,4-D alone and in combination with BAP, 1.0 mg/l biotin and 1.0 mg/l glutamine on cell suspension culture of *M. pruriens.*

Concentrations of BAP+2,4-D (mg/L)	Days after incubation	Weight of cells (gm)($\bar{X}$ ± SE)
0.0 + 0.5	4th	0.040 ± 0.004
	8th	0.047 ± 0.008
	12th	0.056 ± 0.010
	16th	0.090 ± 0.011
	20th	0.108 ± 0.011
	24th	0.113 ± 0.012
	28th	0.113 ± 0.012
0.0 + 1.0	4th	0.044 ± 0.008
	8th	0.048 ± 0.007
	12th	0.060 ± 0.009
	16th	0.099 ± 0.010
	20th	0.122 ± 0.010
	24th	0.125 ± 0.012
	28th	0.125 ± 0.013
0.0 + 2.0	4th	0.042 ± 0.006
	8th	0.048 ± 0.008
	12th	0.158 ± 0.008
	16th	0.092 ± 0.010
	20th	0.109 ± 0.010
	24th	0.116 ± 0.012
	28th	0.116 ± 0.013
0.5 + 1.0	4th	0.041 ± 0.008
	8th	0.047 ± 0.008
	12th	0.156 ± 0.006
	16th	0.094 ± 0.009
	20th	0.108 ± 0.010
	24th	0.115 ± 0.011
	28th	0.115 ± 0.012
0.5 + 2.0	4th	0.042 ± 0.006
	8th	0.048 ± 0.006
	12th	0.059 ± 0.008
	16th	0.097 ± 0.009
	20th	0.113 ± 0.010
	24th	0.118 ± 0.011
	28th	0.118 ± 0.012

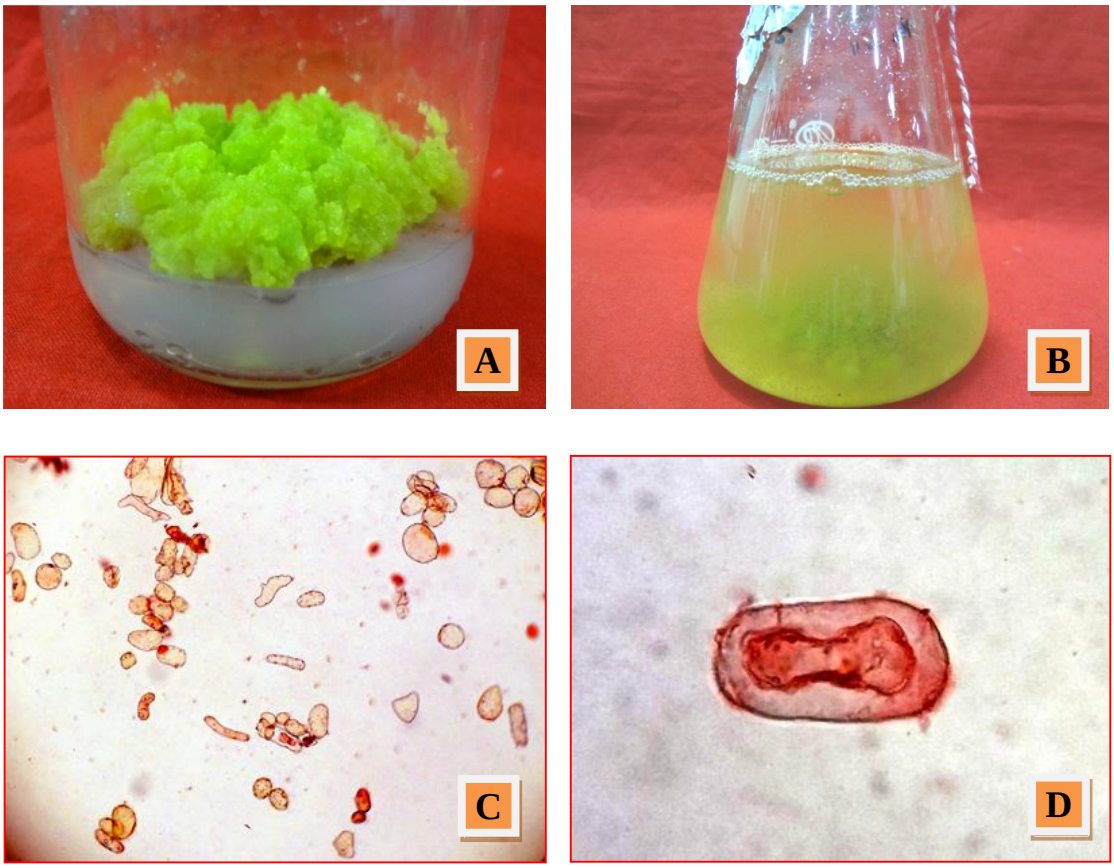


Plate 3.3.19. A-D: Photographs showing the different stages of cell culture of *M. pruriens*; **A:** Embryogenic calli; **B:** Cell suspension culture; **C:** Isolated cells; **D:** Dividing single cell.

3.3.3.4. *Cell suspension culture of R. serpentina*

Cell suspension culture was established from friable calli derived from leaf in *R. serpentina* with two experiments:

Experiment 1: Effect of different concentrations of 2,4-D on cell growth of *R. serpentina*

The cell and the cell aggregates were cultured on MS medium supplemented with different concentrations (0.5, 1.0, 2.0, 2.5 and 3.0 mg/l) of 2,4-D with 1.0 mg/l biotin and 1.0 mg/l glutamine and cultures were maintained under total dark regime at 25±2°C temperature. Data were collected per 10 ml cell suspension culture for 8 times with the interval of every after 4 days of culture. Results obtained on cell weight of cells and the aggregates were found to vary with hormonal formulation present in the culture media that are shown in **Table 3.3.49.**

The cells and the cell aggregates responded in all the media formulations. The highest weight of cells and cell aggregates was obtained on the 28th day of cell suspension culture in MS medium supplemented with 2.5 mg/l 2,4-D. The maximum cell weight was 0.124 ± 0.013 gm on the 28th day of culture in the same medium. The minimum weight of cells and cell aggregates was 0.108 ± 0.012 gm in MS medium having 0.5 mg/l 2,4-D on the 28th day of culture. Lower and higher concentrations of 2,4-D were found less effective for cell growth. The growth of cells was continued until 28 days of culture but it was ceased down after 28 days of culture.

Experiment 2: Effect of different concentrations of 2,4-D with BAP on cell suspension culture of *R. serpentina*

This experiment was conducted using three concentrations (1.0, 2.0 and 3.0 mg/l) of 2,4-D in combination with two concentrations (0.5 and 1.0 mg/l) of BAP accompanied with 1.0 mg/l biotin and 1.0 mg/l glutamine to understand their combined effect on cell suspension culture in *R. serpentina*. The cultures were maintained under total dark regime at 25±2°C temperature. Results obtained on cell weight of cells and the cell aggregates were found to vary with variations in hormonal formulation present in the culture media that are presented in **Table 3.3.50.**

The cells and the cell aggregates responded in all the media formulations. The peak period of cell growth was observed within 12th–20th day of cell suspension culture (**Fig 3.3.1**). Among the combinations 2.0 mg/l 2,4-D in combination with 0.5 mg/l BAP was found to be the most effective formulation on cell suspension culture. The maximum cell weight was 0.132 ± 0.013 gm in the same hormonal concentration on 28th day of culture. The lower and higher concentrations of 2,4-D with BAP were found less effective on cell suspension culture. The minimum weight of cells and clusters was 0.122 ± 0.013 gm in MS medium having 1.0 mg/l 2,4-D + 0.5 mg/l BAP on the 28th day of culture. The growth of cells was continued until 28 days of culture but it was ceased down after 28 days of culture.

Table 3.3.49. Effect of different concentrations of 2,4-D with 1.0 mg/l biotin and 1.0 mg/l glutamine on cell growth of *R. serpentina.*

Concentrations of 2,4-D (mg/l)	Days after incubation	Weight of cells (gm) (mean ± SE)
0.5	4th	0.058 ± 0.006
	8th	0.065 ± 0.007
	12th	0.076 ± 0.008
	16th	0.094 ± 0.009
	20th	0.100 ± 0.010
	24th	0.106 ± 0.010
	28th	0.108 ± 0.012
	32nd	0.108 ± 0.013
1.0	4th	0.060 ± 0.006
	8th	0.068 ± 0.007
	12th	0.077 ± 0.008
	16th	0.095 ± 0.008
	20th	0.104 ± 0.010
	24th	0.108 ± 0.009
	28th	0.110 ± 0.011
	32nd	0.110 ± 0.012
2.0	4th	0.064 ± 0.006
	8th	0.071 ± 0.007
	12th	0.078 ± 0.008
	16th	0.100 ± 0.010
	20th	0.112 ± 0.011
	24th	0.117 ± 0.012
	28th	0.120 ± 0.013
	32nd	0.120 ± 0.013
2.5	4th	0.065 ± 0.008
	8th	0.072 ± 0.007
	12th	0.080 ± 0.009
	16th	0.102 ± 0.010
	20th	0.115 ± 0.011
	24th	0.119 ± 0.012
	28th	0.124 ± 0.013
	32nd	0.124 ± 0.013
3.0	4th	0.061 ± 0.007
	8th	0.070 ± 0.007
	12th	0.078 ± 0.008
	16th	0.098 ± 0.010
	20th	0.106 ± 0.010
	24th	0.111 ± 0.012
	28th	0.114 ± 0.012
	32nd	0.114 ± 0.014

Table 3.3.50. Effect of different concentrations of 2,4-D with 0.5 or 1.0 mg/l BAP, 1.0 mg/l biotin and 1.0 mg/l glutamine on cell growth of *R. serpentina*.

Concentrations of BAP+2,4-D (mg/l)	Days after incubation	Weight of cells (gm)($\bar{x}$ ± SE)
0.5+1.0	4th	0.062 ± 0.005
	8th	0.070 ± 0.007
	12th	0.082 ± 0.010
	16th	0.104 ± 0.011
	20th	0.114 ± 0.010
	24th	0.118 ± 0.010
	28th	0.122 ± 0.012
	32nd	0.122 ± 0.013
1.0+1.0	4th	0.066 ± 0.007
	8th	0.076 ± 0.007
	12th	0.086 ± 0.008
	16th	0.110 ± 0.009
	20th	0.120 ± 0.010
	24th	0.124 ± 0.010
	28th	0.126 ± 0.012
	32nd	0.126 ± 0.012
0.5+2.0	4th	0.070 ± 0.006
	8th	0.076 ± 0.007
	12th	0.088 ± 0.009
	16th	0.114 ± 0.008
	20th	0.125 ± 0.010
	24th	0.130 ± 0.011
	28th	0.132 ± 0.013
	32nd	0.131 ± 0.014
1.0+2.0	4th	0.066 ± 0.008
	8th	0.078 ± 0.008
	12th	0.088 ± 0.009
	16th	0.110 ± 0.009
	20th	0.119 ± 0.011
	24th	0.122 ± 0.012
	28th	0.127 ± 0.013
	32nd	0.126 ± 0.013
0.5+3.0	4th	0.064 ± 0.006
	8th	0.072 ± 0.007
	12th	0.085 ± 0.008
	16th	0.105 ± 0.010
	20th	0.116 ± 0.012
	24th	0.120 ± 0.011
	28th	0.124 ± 0.012
	32nd	0.124 ± 0.013

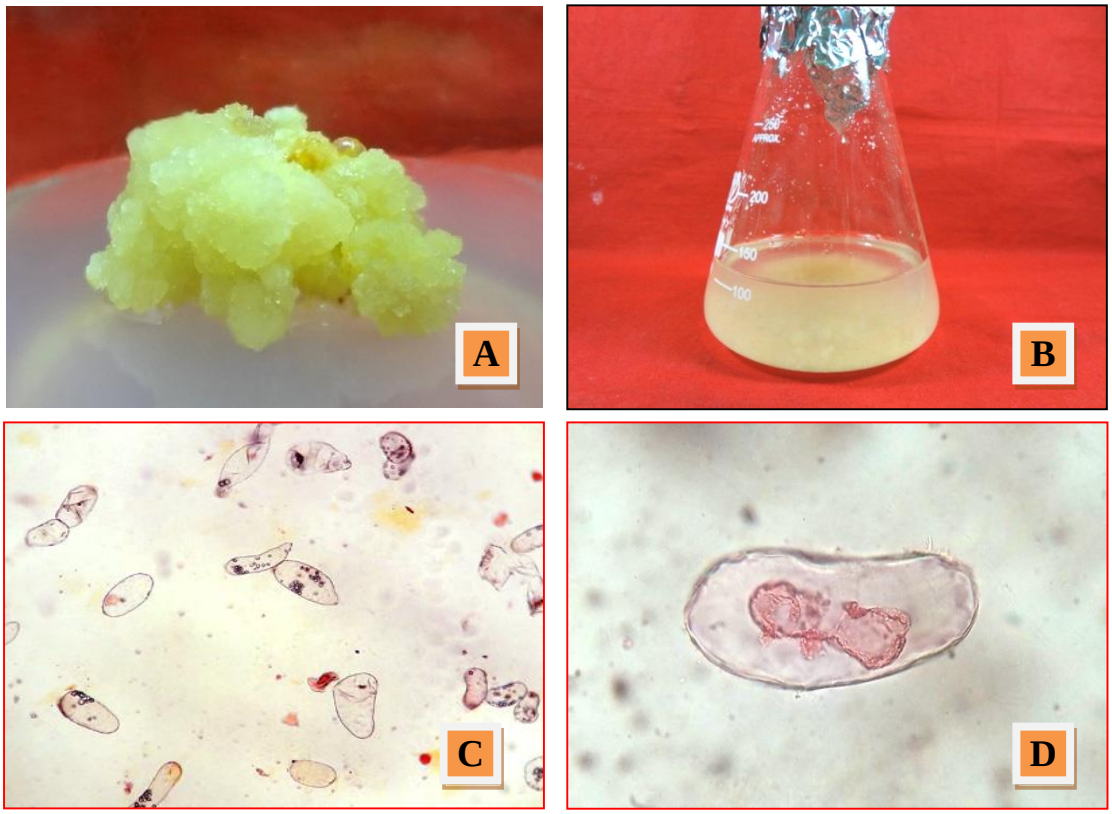


Plate 3.3.20. A-D: Photographs showing the different stages of cell culture of *R. serpentina;* **A:** Embryogenic calli; **B:** Cell suspension culture; **C:** Micrograph of 12-days-old cells cultured; **D:** Dividing single cell.

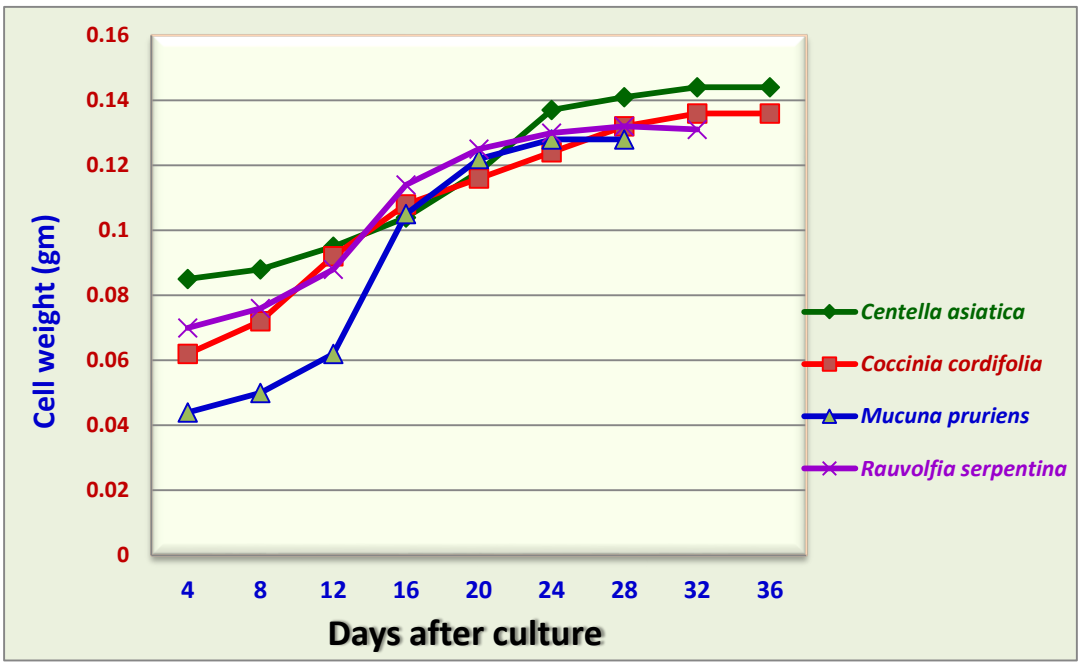


Fig. 3.3.1. Growth curves of four selected species by plotting the cell growth under different time periods in MS liquid medium.

3.4. DISCUSSION

3.4.1. Micropropagation

Medicinal plants are the most important source of life saving drugs for the majority of the world's population. The World Health Organization (WHO) has estimated that the present demand for medicinal plants is approximately US $14 billion per year. The demand for medicinal plant-based raw materials is growing at the rate of 15 to 25% annually, and according to an estimate of WHO, the demand for medicinal plants is likely to increase more than US $5 trillion in 2050. The biotechnological tools are important to select, multiply and conserve the critical genotypes of medicinal plants. *In vitro* regeneration holds tremendous potential for the production of high quality plant based medicine. Plant tissue culture studies were carried out for the preservation of medicinal plant resources and efficient production of pharmaceutically important secondary metabolites. Plant tissue culture technology is being widely used for large scale plant multiplication. Apart from their use as a tool of research, plant tissue culture techniques have in recent years, become of major industrial importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites. Small pieces of tissue (named explants) can be used to produce hundreds and thousands of plants in the continuous process. A single explant can be multiplied into several thousand plants in relatively short time period and space under controlled conditions, irrespective of the season and weather on a year round basis. Endangered, threatened and rare species have successfully been grown and conserved by micropropagation because of high coefficient of multiplication and small demands on number of initial plants and space.

In plant cell culture, plant tissues and organs are grown *in vitro* on artificial media, under aseptic and controlled environment. The technique depends mainly on the concept of totipotentiality of plant cells which refers to the ability of a single cell to express the full genome by cell division. Along with the totipotent potential of plant cell, the capacity of cells to alter their metabolism, growth and development is also equally important and crucial to regenerate the entire plant. Plant tissue culture medium contains all the nutrients required for the normal growth and development of plants. It is mainly composed of macronutrients, micronutrients, vitamins, other organic components, plant growth regulators, carbon source and some gelling agents in case of solid medium Murashige and Skoog medium (MS medium) is most extensively used for the vegetative propagation of many plant species *in vitro*. The pH of the media is also important that affects both the growth of plants and activity of plant growth regulators. It is adjusted to the value between 5.4 – 5.8. Both the solid and liquid medium can be used for culturing. The composition of the medium,

particularly the plant hormones and the nitrogen source has profound effects on the response of the initial explant. Plant growth regulators (PGR's) play an essential role in determining the development pathway of plant cells and tissues in culture medium. The auxins, cytokinins and gibberellins are most commonly used plant growth regulators. The type and the concentration of hormones use depend mainly on the species of the plant, the tissue or organ cultured and the objective of the experiment. Auxins and cytokinins are most widely used plant growth regulators in pant tissue culture and their amount determined the type of culture established or regenerated. The high concentration of auxins generally favors root formation, whereas the high concentration of cytokinins promotes shoot regeneration. A balance of both auxin and cytokinin leads to the development of mass of undifferentiated cells known as callus.

In some medicinal plant species seed propagation has not been successful. Moreover, most of the plant raised through seeds are highly heterozygous and show great variations in growth, habit and yield and may have to be discarded because of poor quality of products for their commercial release. Likewise, majority of plants are not amenable to vegetative propagation through cutting and grafting, thus limiting multiplication of desired cultivars. However, *in vivo* vegetative propagation techniques are time consuming and expensive and the propagules carry the diseases and pest form the mother plant to the seedlings.

Tissue culture has now become a well established technique for culturing and studying the physiological behavior of isolated plants organs, tissues, cells, protoplasts and even organelles under precisely controlled physical and chemical conditions. Most of the medicinal plants either do not produce seeds or seeds are too small and do not germinate in soils. Thus mass multiplication of disease free planting material is a general problem. In this regard the micropropagation holds significant promise for true to type, rapid and mass multiplication under disease free conditions. Besides, the callus derived plants exhibit huge genetic variation that could be exploited for developing superior clones/varieties particularly in vegetatively propagated plant species.

In this investigation effort has been made to collect the ethnobotanical information about 192 medicinal plants growing in Madhupur Tract. Injudicious use of these medicinal plant species together with habitat destruction has threatened the species, especially in the Madhupur tract with intense agricultural activities. *In vitro* culture techniques provide an alternative means of propagation and a tool for plant improvement. It was necessary to develop the tissue culture protocols for all of these identified medicinal plants experiencing propagation barrier, but considering volume of work and time limitation, five important medicinal plants (*Centella asiatica*,

Commelina benghalensis, *Curcuma zedoaria*, *Mucuna pruriens* and *Vitex negundo*) was selected for tissue culture study under the present investigation because of their medicinal value and market potentiality. The present study was undertaken to examine the potential of different explants, to respond under *in vitro* condition with the possibility of developing a protocol for *in vitro* regeneration and multiplication. As for conservation strategy artificial seed production has been successfully developed in case of *C. asiatica* and *R. serpentina.* At the advent of plant biotechnology, since 1980, efforts has been made to develop tissue culture protocol for the commercially important plants, including medicinal plants for their large scale production, but in this investigation, emphasis was given particularly on plant species that has been reported very important for the herbal doctors of Madhupur tract. Tissue culture not only give the opportunity of cloning plants at a higher rate but also gives many other opportunities such as conservation (Engelmann 1991) including a somaclonal variation (Camerson *et al*. 1989); Drew and Smith 1990), facilitated desirable gene transfer (David *et al*. 1984) and production of important secondary metabolites (Nigra *et al*. 1987; Jha *et al*. 1988; Pilli and Oliveira 2000) by callus and cell culture.

The nature of explants and age of the source plant is an important factor related to success of *in vitro* culture. Generally explants for juvenile plants give better response than those of aged plant (Sommer and Claderas 1981). Direct and indirect organogenesis have been successfully established in many medicinal plant species using shoot tips (Huang *et al.* 2000; Soniya and Das 2002; Baksha *et al.* 2005; Binita *et al*. 2005; Banu and Bari 2007; Karuppusamy and Pullaiah 2007), node (Handique and Bora 1999; Chetia and Handique 2000; Banu *et al*. 2001; Barua and Handique 2002; Das and Handique 2002; Chandramu *et al*. 2003; Faisal *et al*. 2006; Sinha *et al*. 2005; Karuppusamy and Pullaiah 2007), internode (Azad and Amin 1998; Chen *et al.* 2001; Martin 2002) and leaf (Das and Rout 2002; Banu and Bari 2007; Senthikumar e*t al*. 2007) as explants. However, for all the five plant species five kinds of explants namely shoot tip, node, axillary bud, internode and leaf explants of about 2-4 months old plants were used for culture. The explants were taken from 1-2 months old juvenile tissue so that phenolics and other inhibitory compounds secreted by the tissue could not inhibit the growth of tissue in culture.

Surface sterilization of explants is an important step for *in vitro* propagation of any plants because the nutrient culture media, which are generally used in tissue culture techniques, are most suitable for the growth of microbes and pathogens. For this purpose, a well known surface sterilizing agent $HgCl_2$ was employed at 0.1% for sterilization of explants but treatment duration was different according to explants and

plant species. In case of *C. asiatica* shoot apex, node and leaf explants took 4 min; internode segments took 5 min for getting contamination free culture. On the other hand, effective surface sterilization was done at 4-5 min for shoot tip and nodal explants of *C. benghalensis*. In *C. zedoaria* shoot tip and axillary bud explants took 5-6 min for contamination free culture. In *M. pruriens* shoot tip, node, internode and leaf segments took 4-5 min. In case of *V. negundo* axillary bud and shoot tip explant took 6 min and leaf 4-5 min for contamination free culture. In our study all the five selected plant species gave response to the PGR supplemented media and the kind of response was dependent on species, explant and hormone supplement in the media.

MS (Murashige and Skoog 1962) medium is a recognized basal medium for tissue culture techniques and extensively used for *in vitro* propagation of various plant species including medicinal plants (Sudha *et al*. 1998; Rout *et al*. 2000; Selvakumar *et al*. 2001; Chandramu *et al*. 2003; Hassan and Roy 2004; Soniya and Das 2002; Baksha *et al*. 2005; Binita *et al*. 2005; Sinha *et al*. 2005; Siddique *et al*. 2006, Senthikumar *et al*. 2007; Banu and Bari 2007; Biswas 2006). Throughout the investigation MS medium was used as the basal medium for direct and indirect organogenesis of five important medicinal plants (*C. asiatica*, *C. benghalensis*, *C. zedoaria*, *M. pruriens* and *V. negundo*). Growth regulators, especially cytokinins and auxins played significant role in *in vitro* culture of higher plants and it can be said that *in vitro* culture is often impossible without growth regulators (Pierik 1987). Auxins are generally responsible for elongation and swelling of tissues, cell division and formation of adventitious roots, the inhibition of adventitious and axillary shoot formation and often embryogenesis in suspension culture. With low auxin concentrations adventitious root formation predominates, whereas with high auxin concentration root formation fails to occur and callus formation takes place (Pierik 1987). Cytokinins usually promote cell division, especially if added together with an auxin. In higher concentration they can induce adventitious shoot formation, but root formation is generally inhibited. They promote axillary shoot formation by decreasing apical dominance (Pierik 1987). During the present investigation BAP and Kin were applied as cytokinins and NAA, IAA, IBA, and 2,4-D used as auxin. L-glutamine and GA3 were used as additives.

3.4.1.1. *In vitro culture of Centella asiatica*

C. asiatica is an important medicinal plants, which is locally known as “Thankuni” and the plant growing as one of the important plants in Madhupur Tract. It also grows wild in forest and is planted in homestead and home garden in Bangladesh. It is commonly used to treat dysentery and some people have it as salad (Rahman 2008). The plant enjoys considerable reputation in Indian system of medicine as diuretic,

alterative and tonic (Kirtikar and Basu 1975). *C. asiatica* contains a variety of biologically active chemical constituents like asiaticoside, madecassoside, centelloside, kaempferol, betulinic and centellic acids (Prajapati *et. al.* 2006). The whole plant possesses antibacterial, anti-inflammatory, anti-febrile and galactogogic activities. It is used in the therapy of fever, measles, haematemesis, epistaxis, diarrhoea, dysentery and jaundice (Hanida & Kapoor 1988).

In recent years, there has been an increased interest in *in vitro* culture techniques which offer a viable tool for mass multiplication and germplasm conservation of rare, endangered and threatened medicinal plants (Ajithkumar and Seeni 1998, Prakash *et. al*. 1999). Considering its medicinal properties and over exploitation from natural population the requirement for application of tissue culture techniques in the rapid multiplication of elite clones and germplasm conservation is a crucial prerequisite. Moreover, a stable supply of the bioactive secondary products has become an utmost priority.

There have been a few reports to date on micropropagation of *C. asiatica* using shoot tips and nodal explants. However, in Bangladesh, there is no report on the establishment of a micropropagation protocol for *C. asiatica* (Rahman 2008). The present study was carried out to establish a simple, reliable and reproducible protocol for large scale plantlets production through tissue culture using shoot tips and nodal segments to meet the requirement of the pharmaceutical industries.

In the present study BAP and Kn were used alone in different concentrations or in combination with BAP + Kn, BAP + IAA, Kn + IAA. and BAP + Kn + IAA for direct organogenesis. Among the different types of explants (shoot apex, node), nodal explants was proved to be the best and most effective explant for tissue culture of *C. asiatica* as it responded better than shoot apex for direct organogenesis in all media compositions. When BAP and Kn applied singly proliferation response was found to be poor with only 2-7 shoots. On the other hand, multiple shoot induction enhanced remarkably when low concentration of auxins was used in combination with higher concentration of BAP and Kn. Similar synergistic effect of BAP or Kn in combination with an auxin has been demonstrated in many medicinal plants (Casado *et al*. 2002; Fraternale *et al*. 2002; Salvi *et al*. 2002; Martin 2003). Result of the present study proved that combination of Kn + IAA was very efficient for multiple shoot induction from shoot apex and nodal explant of *C. asiatica*. The highest percentage of (100%) of multiple shoot induction, highest number of shoot per culture (8.20 ± 0.25) and highest average length of shoot per culture (2.20 ± 0.06 cm) was noted from nodal explant in the media having 2.0 mg/l Kn + 0.5 mg/l IAA. On the other hand, maximum (100%) shoot proliferation was noted from shoot apex explant in the same

media but highest number of shoot per explant was only 5.80 ± 0.33. Highest average length of shoot per culture (2.10 ± 0.18 cm) was noted in the media having 2.0 mg/l Kn + 0.5 mg/l IAA. In contrast, the potential of BAP in combination with IAA was demonstrated in the same species (Raghu *et al*. 2007) and also in other medicinal plants such as in *Plumbago rosea* (Harikrishnan and Hariharan 1996) and *Alpinia calcarata* (Agretious *et al*. 1996). George *et al*. (2004) reported BA alone showed good shoot induction in *C. asiatica*. BA with IAA combination showed superiority to NAA combinations reported by Tiwari *et al*. (2000) in the same plant.

Indirect organogenesis of *C. asiatica* was also carried out using leaf and internode segments and cultured them on MS medium supplemented with different concentrations of 2,4-D and NAA alone and in combinations of BAP+2,4-D, Kn+2,4-D, BAP+NAA and Kn+NAA in different concentrations. Both leaf and internode explants developed callus at cut surfaces and subsequently covered the entire surface of explant within 10-20 days. Here, internode explants performed better than leaf explants. Callogenic response was observed on all media except low concentrations of auxins. In most cases colour of calli was whitish, whitish yellow, green, light green, pinkish and creamy and texture of calli were compact and friable. Among all tested concentrations and combinations highest percentage (93.33%) of callus formation occurred in MS medium containing 0.5mg/l BAP+2.0 mg/l NAA from leaf explant followed by 90% in MS medium containing 0.5mg/l BAP+1.0 mg/l NAA and 1.0mg/l BAP+2.0 mg/l NAA. Similar results were reported in the same species (Bibi *et al*. 2011; Joshi *et al*. 2013). Our results contradict Patra *et al*. (1998) who reported that addition of NAA in MS medium containing BAP or Kn decreased callus formation response. While the best callogenic response from leaf explants was observed on MS medium supplemented with BAP or Kn alone with 2,4-D, good callus formation was not observed when media was supplemented BAP and NAA (Martin 2004). Tan *et al*. (2010) and Joshee *et al*. (2007) observed high frequency of callus induction in 2,4-D supplemented MS medium.

For callus regeneration, leaf and internode derived callus were cultured on MS medium supplement with different concentrations of BAP and Kn alone or in combination with NAA or IAA. Results showed that BAP+NAA combination was better than that of other combinations. Among the tested media combinations the leaf derived callus gave better performance than internode derived callus to regeneration of shoot. However, the best regeneration of shoots was obtained from MS medium containing 1.0 mg/l BAP+0.5 mg/l NAA. In this combination 70% callus produced adventitious shoots from leaf derived callus with a highest average 4.20±0.15 numbers shoot per culture and average 2.06 ± 0.22 cm lengths of shoots per culture.

Similar results were reported in *C. asiatica* (Bibi *et al*. 2011; Deshpande *et al*. 2010), in *Rauvolfia serpentina* (Sarker *et al*. 1996), in *Adhatoda vasica* (Azad and Amin 1998) in *Mimosa pudica* (Munshi *et al*. 2001), in *Physalis pubescens* (Rao *et al*. 2004) and in *Citrullus lanatus* (Sultana *et al*. 2004). Patra *et al*. (1998) reported shoot bud was the highest on 4.0mg/l BAP+2.0mg/l Kn+0.25mg/l NAA+20.0 mg/l adenine sulfate.

Rooting experiments were conducted on MS basal medium with 3% sucrose supplemented and different concentrations (0.2–4.0 mg/l) of auxins (NAA, IAA and IBA) used alone and in combination of BAP and Kn with IAA or IBA. Among the different hormonal concentrations and combinations the highest frequency (100%) with highest root number (16.53±0.24) was obtained on MS medium containing 2.0 mg/l IAA. Highest average length of roots 6.78 ± 0.10 cm was recorded in the same medium. Combination of auxin (IAA and IBA) and cytokinin (BAP or Kn) was less effective than auxin alone. Similar results were reported in the same plant (Shashikala *et al*. 2005) in *V. negundo* (Jamal *et al*. 2010) in *Cleistanthus collinus* (Quarishi and Mishra 1998). Sahoo and Chand (1998) obtained the best rooting response in IAA (1.0 mg/l) combined with IBA(1.0 mg/l) in *Vitex negundo*. In contrast, Panathula *et al*. (2014), Karthikeyan *et al*. (2009), Mohapatra *et al*. (2008), Nath *et al*. (2003), Tiwari *et al*. (2000) and Patra *et al*. (1998) obtained best rooting using half strength MS media supplemented with different concentration of IBA. On the other hand, Bibi *et al*. (2011) and Sivakumar *et al*. (2006) were reported that best rooting response was in the medium containing NAA.

3.4.1.2. *In vitro culture of Commelina benghalensis*

C. benghalensis L. (family Commelinaceae), locally known as Dholpata, is a perennial herb. In Bangladesh the herb is used for otitis media, suppurative sores, burns, conjunctivitis and skin diseases (eczema, abscesses, acne, scabies and warts) (Khan *et al.*2011). It is used in the Indian subcontinent as a folk medicine for the treatment of variety of ailments. The plant is used for mouth thrush (Ssenyonga and Brehony, 1993), inflammation of the conjunctiva, psychosis, epilepsy, nose blockage in children (Okello and Ssegawa 2007), insanity (Tabuti *et al*. 2003) and exophthalmia. In China, *C. benghalensis* L. is used medicinally as a diuretic, febrifuge and antiinflammatory (Deyuan and Robert 2000). It is used as an animal fodder, eaten by humans as a vegetable in Pakistan, also used there medicinally, but with different purported effects, including as a laxative and to cure inflammations of the skin as well as leprosy (Qaiser and Jafri 1975). Medicinal plants are of great interest to the researches in the field of biotechnology as most of the drug industries depend, in part, on plants for the production of pharmaceutical compounds (Chand *et*

al. 1997). *In vitro* culture techniques which offer a viable tool for mass multiplication and germplasm conservation of rare, endangered, aromatic and medicinal plants (Arora and Bhojwani 1989; Sharma *et al*. 1991; Sudha and Seeni 1994; Sahoo and Chand 1998; Karuppusamy and Pullaiah 2007; Jawahar *et al*. 2008). Unfortunately, there is no such report available for clonal propagation of *C. benghalensis* L. In this investigation, we established a protocol for rapid *in vitro* clonal multiplication of this important medicinal plant species followed by successful outdoor establishment of regenerated plants.

In the present investigation, shoot tip and nodal segment were cultured in MS medium with different hormonal concentrations and combinations for multiple shoot proliferation. BAP and Kn were used alone in different concentrations or in combinations with NAA or IAA. Between the two types of explants (shoot tip and nodal segment) nodal explant was proved to be the best and most efficient explant for tissue culture of *C. benghalensis* as it responded better than shoot tip for direct organogenesis in all media compositions. When BAP and Kn applied singly proliferation response was found to be poor with only 2–3 shoots per explant. But multiple shoot induction enhanced remarkably when high concentration of cytokinins (BAP and Kn) were used in combination with low concentration of auxin (IAA or NAA) with 3-8 shoots per explant. The synergistic effect of BAP in combination with an auxin has been demonstrated in many medicinal plants (Mohapatra *et al*. 2008; Ahmad and Anis, 2011; Casado *et al*. 2002; Fraternale *et al*. 2002). Tripepi (1997) reported that combination of BAP with NAA was found most effective in shoot organogenesis. Results of the present study proved that combination of BAP with NAA was very efficient for multiple shoot induction from nodal explant of *C. benghalensis*. The highest percentage (96.67%) of multiple shoot induction was noted from the nodal explant in the media having 1.0 mg/l BAP+0.5 mg/l NAA. The highest mean number of shoot per culture 8.12 ± 0.41 was observed in the same medium. On the other hand, 90% shoot proliferation and 5.45 ± 0.39 shoots was noted from shoot tip explants in the media having 1.0 mg/l BAP+0.5 mg/l NAA. The present findings are in agreement with the results obtained in other medicinal plant species such as *Vitex negundo* (Ahmad and Anis, 2011), *Centella asiatica* (Mohapatra *et al*. 2008), *Bupleurum distichophyllum* (Karuppusamy and Pullaiah 2007) and *Erigeron breviscapus* (Liu *et al*. 2008).

For root induction, *in vitro* derived shoots were transferred to half MS media augmented with NAA, IAA and IBA. Among the auxins employed for rhizogenesis, growth hormone IBA facilitates maximum rooting efficiency. Root induction was accomplished by adding IBA, at different concentrations. Roots were developed after

8-12 days from the date of transfer of shoots into rooting medium. Maximum numbers of roots (14.56±0.52) and highest average length (5.20 ± 0.19) of roots were achieved on half strength MS media augmented with 1.5 mg/l IBA. However, IBA was found to be more effective and induced higher root initiation and establishment. IBA was more effective than NAA and IAA in promoting rooting of a wide variety of plants, and it is used commercially for rooting of many plant species worldwide. In case of *Commelina ensifolia*, high efficiency of root induction was achieved when MS medium was supplemented with 3.0mg/l IBA+ 1.0 mg/l NAA (Mahesh *et al*. 2012). Similar pattern of root induction was achieved in *Plumbago zeylanica* when the *in vitro* developed shoots were transferred into MS medium fortified with 4.92 mg/l of IBA (Selvakumar *et al*. 2001). The results of this present study find good agreement with these earlier reports in *Salvadora persica*, *in* half MS media supplemented with 3.0 mg/l IBA. Similarly, MS medium fortified with 0.5 mg/l IBA, 0.1 mg/l NAA and 0.1 mg/l IAA was found to be suitable for higher root induction in *Spilanthes paniculata* (Mahendran *et al.* 2006). The effectiveness of IBA in rooting is well documented in a number of medicinal plants like *Liquidambar styraciflua* (Kim *et al*. 1997), *Anemopaegma arvense* (Pereira *et al*. 2003) and *Pongamia pinnata* (Sugla *et al.* 2007). Hence, the results of this present study could provide a vital piece of information for the establishment of a rapid protocol for large scale multiplication of medicinally important plant *C. benghalensis* with good level of genetic fidelity in a short period.

3.4.1.3. *In vitro culture of Curcuma zedoaria*

C. zedoaria is another important herbaceous medicinal plant of Madhupur tract and to the best of our knowledge very limited report was available regarding *in vitro* mass propagation of this plant. Loc *et al*. (2005) reported that MS medium supplemented with 20% (v/v) coconut water, 3.0 mg/l BAP and 0.5 mg/l IBA could induce the formation of 5.6 shoots per explant for *C. zedoaria*. Bharalee *et al*. (2005) found that MS medium supplemented with 4.0 mg/l BAP and 1.5 mg/l NAA was the best medium for shoot multiplication of *C. caesia* (3.5 shoots per explant) and MS medium having 1.0 mg/l BAP + 0.5 mg/l NAA for *C. zedoaria* (4.5 shoots per explant). Balachandran *et al*. (1990) reported that *C. domestica* could produce 3.4 shoots per explant, *C. caesia* produced 2.8 shoots per explant and *C. aeruginosa* produced 2.7 shoots per explant using MS medium supplemented with 3 mg/l BAP. Nayak (2000) reported MS medium supplemented with 5.0 mg/l BAP was most effective for shoot multiplication of *C. aromatica* producing an average of 3.3 shoots per explant. Our results indicated that BA and IBA as 2.0 mg/l and 1.0 mg/l

supplemented into the MS medium was sufficient for the induction of multiple shoots from the buds and shoots of *C. zedoaria*.

In the present study single medium based efficient and large scale micropropagation protocol was established using two types of explants *viz.* shoot tip and axillary bud through direct organogenesis. Here, direct shoot multiplication was achieved from shoot tip and axillary bud explants. For direct organogenesis BAP and Kn were used singly or in combination with NAA or IBA. Among the two types of explants shoot apex proved most efficient explant for tissue culture of *C. zedoaria*. BAP is considered to be one of the most useful cytokinin for achieving the micropropagation and showed highest effect with respect to the axillary buds (Joshi and Dhar 2003; Martin 2002). Present investigation revealed that between two cytokinins tested, BAP was more effective than Kn in terms of shoot induction in *C. zedoaria*. Of the various levels of BAP supplemented to MS, 2.0 mg/l BAP developed 90% multiple shoots with a mean of 5.62 ± 0.29 shoot per culture and average 6.10 ± 0.25 cm length of shoot per culture. Application of lower concentration of auxins (NAA, IBA) along with BAP and Kn increased multiple shoots induction and elongation of shoots. The best response was achieved using 2.0 mg/l BAP+1.0 mg/l IBA from shoot apex explants. In this combination 93.33% explants showed proliferation and highest average number of shoots and length (cm) of shoots per culture was 6.20±0.54 and 6.48 ±0.49 respectively. Similar experiment with the use of BAP and IBA were also carried out for induction of multiple shoot by Banisalam *et al*. (2011). Comparison between *in vitro* and *in vivo* antibacterial activity of *Curcuma zedoaria* was also reported from Malaysia (Miachir *et al*. 2004).

The aseptic shoots of *C. zedoaria* were trimmed of new leaves and roots and were cultured in liquid MS medium supplemented with three concentrations (1.0, 2.0 and 3.0 mg/l) of BAP or Kn with four concentrations NAA (0.2, 0.5, 1.0 and 2.0 mg/l) or three concentrations of IBA (0.5, 1.0 and 2.0 mg/l) and 20% cocoanut water in 100 ml conical flasks and a single shoot was inoculated in each flask. The aseptic shoots of *C. zedoaria* were also cultured on MS solid medium with the same formulation. The number shoots produced from each shoot from the liquid medium and the solid medium was recorded after 6 weeks of culture. The aseptic shoots explants of *C. zedoaria* cultured in liquid MS medium supplemented with 2.0 mg/l BAP and 1.0 mg/l IBA and 20% cocoanut water produced significantly higher number of multiple shoots than those of cultured on the solid medium with the same formulation. *C. zedoaria* formed 20.43 ± 0.62 shoots per explant in liquid medium and 6.20 ± 0.54 shoots per explant in solid medium. Nalawaade *et al*. (2003) also reported that shoot multiplication rate of *Zingiber zerumbet* in liquid medium was higher than the semi

solid medium. Improved rate of shoot multiplication in liquid medium had also been reported in other plant species such as the *Curcuma* species (Chan and Thong 2004), *Artemisia* species (Liu *et al*. 2004) and *Centaurium* species (Piatczak *et al*. 2004). Piatczak *et al*. (2004) explained that the fast proliferation of shoots in liquid medium was due to the tissues were completely submerged in the liquid medium presenting a large surface area for the uptake of nutrients and plant growth regulators. However, we observed some of the plantlets of *C. zedoaria* became hyperhydric when cultured in the liquid proliferation medium. But the hyperhydricity of the plants disappeared after acclimatization and transferred to the soil.

3.4.1.4. *In vitro culture of Mucuna pruriens*

M. pruriens (L.) DC. (Fabaceae), commonly known as velvet bean, is an important tropical legume found in bushes and hedges of Madhupur tract. *M. pruriens* is recognized as an aphrodisiac in Ayurvedic medicine, and it has been shown to increase the testosterone levels leading to deposition of protein in the muscles, as well as increased muscle mass and strength (Anonymous 2003). All the parts of the plant possess valuable medicinal properties (Caius 1989). The report on the occurrence of the catecholic amino acid 3-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA) attracted attention for utilization of the plant for L-dopa production (Bell and Janzen 1971; Daxenbichler *et al*. 1971). L-DOPA, a neurotransmitter precursor, has found wide application for symptomatic relief of Parkinson's disease and mental disorder. After the discovery that *Mucuna* seeds contain L-DOPA, its demand in international markets has increased many fold (Farooqi *et al*. 1999). The objective of the study reported here was to develop an efficient method for rapid *in vitro* propagation of *M. pruriens* using cotyledonary node explants followed by successful outdoor establishment of regenerated plants. There are few reports available regarding *in vitro* mass propagation of this plant. Faisal *et al*. (2006) developed a protocol on *in vitro* rapid regeneration of plantlets from nodal explants of *M. pruriens*. They obtained high frequency multiple shoots in half strength MS media having different concentrations of BAP individually and in combination with NAA (5.0µM BAP +0.5µM NAA was best). Another report from Chattopadhyay *et al*. (1995) developed a protocol for rapid micropropagation for *M. pruriens*. They found maximum shoot regeneration in Revised Tobacco (RT) media supplemented with 9.8µM 2-iP +2.7µM NAA. Sathyanarayana *et al*. (2008) reported a rapid and reliable method for high fidelity micropropagation from axillary bud explants of *M. pruriens* and they found highest number of multiple shoot on MS supplemented with 3.5 µM BAP.

In the present study BAP and Kn were used alone in different concentrations or in combination with BAP + Kn, BAP + NAA, Kn + NAA and BAP + Kn + IAA for

direct organogenesis. Among the different types of explants (shoot apex, node), nodal explants was proved to be the best and most effective explant for tissue culture of *M. pruriens* as it responded better than shoot apex for direct organogenesis in all media compositions. BAP was found to be most effective cytokinin when compared to Kn. BAP along with NAA exhibited a synergistic effect on multiple shoot formation. Combination of BAP+Kn and Kn+ NAA were less effective. But combination of BAP+Kn+IAA at low concentrations produced highest number of multiple shoots. 96.67% shoot formation frequency with 8.05 ± 0.33 shoots per explant and the average shoot length was 5.21 ± 0.25 cm was observed on medium containing 0.2 mg/l BAP+0.2 mg/l Kn+0.2 mg/l IAA. The synergistic effect of BAP and Kn in combination with an auxin has been demonstrated in many medicinal plants (Casado *et al*. 2002; Fraternale *et al*. 2002; Salvi *et al*. 2002; Martin 2003). Faisal *et al*. (2006) reported that highest number of multiple shoots and maximum average length were standardised on half strength MS medium supplemented with 5.0µM BAP +0.5µM NAA.

Indirect organogenesis of *M. pruriens* was also carried out using leaf and internode segments and cultured them on MS medium supplemented with different concentrations of 2,4-D and NAA alone and in combinations of BAP+2,4-D, Kn+2,4-D, BAP+IAA and Kn+IAA in different concentrations. Both leaf and internode explants developed callus at cut surfaces and subsequently covered the entire surface of explant within 10-15 days. Here, internode explants performed better than leaf explants. Callogenic response was observed on all media tested except low concentrations of auxins. Synergistic effect of high concentrations of BAP and low concentrations of IAA was showed high frequency of callus induction. In contrast, Chawla and Arora (2005) observed that media containing high auxin and low cytokinin concentrations promote cell proliferation resulting in callus formation in *Cicer arietinum L*. In my study most cases colour of calli was whitish, yellow green, light green, green and creamy and texture of calli were compact, nodular and friable. Among all tested concentrations and combinations highest percentage (95.00%) of callus formation occurred in MS medium containing 3.0mg/l BAP+0.5 mg/l IAA from leaf explant followed by 75% in MS medium containing 3.0 mg/l BAP+1.0 mg/l IAA. The result obtained were agreement with the previous reports by other investigators in *Physalis minima* (Sheeba *et al*. 2013), *Solanum tuberosum* (Mutasim *et al*. 2010; Nasrin *et al.* 2003). In contrast to present study, 2, 4 –D alone (Harini and Sathyanarayana 2009), NAA alone (Patel *et al*. 2007), and combination of 2,4-D+NAA+BAP (Lahiri *et al*. 2012) were effective for callus induction from *M. pruriens.*

For callus regeneration, leaf and internode derived callus were cultured on MS medium supplement with different concentrations of BAP and Kn separately or in combination with NAA. Results showed that low concentrations of BAP+NAA combinations were better than that of other combinations. BAP at low concentrations also favour to shoot induction. High concentrations of BAP failed to callus induction. Among the tested media combinations the internode derived callus gave better performance than leaf derived callus to regeneration of shoot. However, the best regeneration of shoots was obtained from MS medium containing 0.5 mg/l BAP+0.2 mg/l NAA. In this combination 60% callus produced adventitious shoots from internode derived callus with a highest average 4.76±0.34 numbers shoot per culture and average 3.80 ± 0.27 cm lengths of shoots per culture. Effect of BAP+NAA combination on callus regeneration was suitable in many medicinal plant species such as *Centella asiatica* (Bibi *et al*. 2011), *Vitex trifolia* (Arulanandam and Ghanthikumar 2011), *Rauvolfia serpentina* (Sarker *et al*. 1996), *Physalis pubescen* (Rao *et al*. 2004). Effect of BAP on callus regeneration was similar to previous record obtained by Lahiri *et al*. (2012) in *Mucuna pruriens*.

Rooting experiments were conducted on half MS basal medium with 3% sucrose supplemented and different concentrations (0.2–3.0 mg/l) of auxins (NAA and IBA) used alone and in combination of BAP and IBA with NAA. Among the different hormonal concentrations and combinations the highest frequency (100%) with highest root number (12.08±0.53) was obtained half MS medium containing 2.0 mg/l IBA+0.5 mg/l NAA. Highest average length of roots 7.29 ± 0.51 cm was recorded in 2.0 mg/l IBA. Combinations of IBA and NAA were most effective than other combinations. The results of this present study find good agreement with observed in other medicinal plants such as *Plumbago zylanica* (Kumar *et al*. 2014). Similarly, MS medium fortified with 0.5 mg/l IBA, 0.1 mg/l NAA and 0.1 mg/l IAA was found to be suitable for higher root induction in in *Spilanthes paniculata* (Mahendran *et al.* 2006). Rooting medium consisted of half MS supplemented with NAA was reported in *Mucuna pruriens* (Lahiri *et al*. 2012; Chattopadhyay *et al*. 1995; Sathyanarayana *et al*. 2008). The favourable response of IBA in root induction was also reported for this plant (Faisal *et al*. 2006; Patel *et al*. 2007) as well as for other medicinal plant species (Panathula *et al*. 2014, Ahmad and Anis 2011).

3.4.1.5. *In vitro culture of Vitex negundo*

V. negundo L. locally termed as Nishinda is a deciduous, woody aromatic and multipurpose medicinal shrub belonging to the Verbenaceae family. *V. negundo* have diverse medicinal uses in the folk medicinal system of Bangladesh (Rahmatullah *et al.* 2010; Khan and Rashid 2006). In traditionally use, generally leaf is used for

medicinal purpose but root, flower and fruit also have the medicinal values (Hasan 1982). Leaf of the plant contains essential oil, an alkaloid, nishindin. Stem and bark contain flavonoid glycosides. Leaves of nishinda very effectively reduce the inflammatory swellings of joints in rheumatic attacks. Juice of fresh leaf removes fetid discharges and worms from ulcers. Flower oil is applied to sinuses and scrofulous sores. Root juice is tonic, expectorant and diuretic (Ghani 1998). *In vitro* propagation has a number of advantages over the sexual one in a large scale propagation programme (Abbott 1978). Considering the medicinal and aromatic values of *V. negundo*, very few attempts were made to standardize micropropagation technique for cloning this plant. Few attempts for direct *in vitro* regeneration of this plant have been made earlier (Sahoo and Chand 1998; Chandramu *et al*. 2003; Rani and Nair 2006; Jawahar *et al*. 2008; Ahmad and Anis 2011; Rathore and Shekhawat 2011). Sahoo and Chand (1998) reported that MS containing 2.0mg/l BAP was most effective in inducting bud break. Multiple-shoot formation, faster bud break coupled with an enhanced frequency of shoot development (92%) and internode elongation were dependent on the synergistic influence of gibberellic acid (GA_3) when used at an optimal concentration (0.4 mg/l) along with BAP (2.0 mg/l). Rathore and Shekhawat (2011) reported MS medium with 1.0mg/l+additives (50.0 mg/l ascorbic acid+25.0mg/l citric acid) and 0.1mg/l IAA was suitable for shoot multiplication and elongation. MS medium supplemented with Kinetin was also produced less number of shoots. Ahmad and Anis (2011) reported MS medium supplemented with BAP (5.0µM) with NAA (0.5µM) was found to be the best for multiple shoot formation in *V. negundo*.

The present study was undertaken to establish a protocol for *in vitro* clonal propagation of *V. negundo* through shoot tip and nodal explant both direct and indirect proliferation. Here single medium based micropropagation protocol was established using four types of explants *viz.* shoot tip, node, leaf and internode segment through direct and indirect organogenesis. For direct shoot regeneration shoot tip and nodal segments were cultured on MS medium with BAP and Kn individually or in combination with NAA, IAA and IBA. Nodal explants was proved to be the best and most efficient explant for tissue culture of this plant as it responded better than shoot for direct organogenesis in all media compositions. When BAP and Kn applied singly proliferation response was found to be moderate with only 2–4 shoots but shoot induction remarkably enhanced when low concentrations of NAA or IAA were used in combination with BAP and Kn. The synergistic effect of BAP in combination with an auxin (IAA, NAA) has been demonstrated in many other medicinal plants (Casado *et. al*. 2002; Fraternale *et al*. 2002; Salvi *et al*. 2002; Martin

2003), *Smilax zeylanica* (Thirugnanasampandan *et al*. 2009), *Plumbago indica* L and *Boerhaavia diffusa* (Biswas *et al*. 2011). Results of the present study proved that combination BAP or Kn with IAA was very efficient for multiple shoot induction from shoot tip and nodal explants of *V. negundo*. The highest percentage (93.33%) of multiple shoot induction was noted from nodal explants in the media having 2.0 mg/l Kn + 0.5 mg/l IAA. The highest average number of shoots was 5.56 ± 0.32 in media having 2.0 mg/l Kn + 0.2 mg/l NAA followed by 5.25 ± 0.18 in 2.0 mg/l BAP + 0.5 mg/l IAA. These findings are in agreement with the results obtained in *V. negundo* (Ahmad and Anis 2011). Similar culture response on a medium containing Kn and NAA has also been observed in *Chlorophytum arundinaceum* (Lattoo *et al*. 2006). Siddique *et al*. (2006) reported that combination of BAP with IAA is most often used for shoot organogenesis in *Aristolochia indica* and *Hemidesmus indicus*. Finally, GA_3 was used alone and in combination with BAP or Kn for elongation and shoot multiplication. Here the highest 96.67% shoot induction and elongation was noted from nodal explants in media having 2.0 mg/l Kn + 0.5 mg/l GA_3. Highest average number of shoots 7.76 ± 0.39 per culture and highest average length of shoots 6.32 ± 0.27 cm were found in the same media. Similar results have been reported by Sahoo and Chand (1998) in *Vitex negundo* L., Ghanti *et al*. (2004) in *Mentha piperita* L., Chinnamadasamy *et al*. (2010) in *Plumbago zeylanica*. Sathyanarayana *et al*. (2008) observed adequate shoot elongation using GA_3 in *Mucuna pruriens*.

Plant regeneration was achieved from leaf and internode derived callus cultures of *Vitex negundo*. Explants were cultured on MS medium supplemented with different concentrations of 2,4–D alone or in combinations of BAP+2,4–D, Kn+2,4–D, BAP+NAA and Kn+NAA. Results showed that leaf and internode explants produced creamy white friable, white friable, white nodular, white soft, light green compact, green compact, dark green compact, light green friable callus. Among all tested concentrations and combinations Kn+NAA and BAP+NAA produced poor percentage of callus induction. In another experiment, when 2,4–D alone or 2,4–D with BAP or Kn used together better callus formation was observed in both explants. Here, internode explants performed better than leaf explants. The highest 70% white friable callus induction was obtained from internode explants in MS medium having 3.0mg/l 2,4–D. within 6 weeks of culture. Highest degree of light green callus was found in 1.0 mg/l NAA + 0.5 mg/l BAP which is very important for shoot regeneration. Similar reports were reported in *V. negundo* (Jawahar *et al*. 2008; Thiruvengadam and Jayabalan 2000).

Regeneration of shoots from different types of calli was carried out using BAP and Kn alone or each other and in combination with NAA or IAA. Among different

concentrations and combinations used BAP along with IAA exhibited a synergistic effect on shoot induction from both internode and leaf explants. Almost similar effect was also found on BAP with NAA. Effect of Kn along with either IAA or NAA were very poor to induce shoot initiation. Maximum combinations failed to shoot regeneration. The best regeneration of shoots was obtained from MS medium containing 0.5 mg/l BAP+0.5 mg/l IAA. In this combination 70% callus produced adventitious shoots from internode derived callus with a highest average number 4.30±0.18 shoot per culture and average 4.16 ± 0.25 cm lengths of shoots. On the other hand highest 55% of callus produced adventitious shoots from leaf derived callus and highest 3.20 ± 0.35 shoot per culture and average 3.34 ± 0.27 cm lengths of shoots per culture found in the same medium. Similar reports were reported in *V. negundo* (Jawahar *et al*. 2008) in *Rauwolfia serpentina* (Sarker *et al*. 1996). NAA combined with BAP has been reported the best shoot proliferating combination in *V. negundo* (Thiruvengadam and Jayabalan 2000), *Vitex trifolia* (Arulanandam and Ghanthikumar 2011) *Centella asiatica* (Shashikala *et al.* 2005) and *Cardiospermum halicacabum* (Jawahar *et al*. 2008).

Rooting experiments were conducted on half strength MS basal medium with 3% sucrose supplemented and different concentrations (0.1–3.0 mg/l) of auxins (NAA, IAA and IBA) used alone. Among the different hormonal concentrations the highest frequency (93.33%) with highest root number (10.63±0.16) was obtained in half strength MS medium containing 1.0 mg/l IAA. IBA was also effective for rooting. Highest average length of roots 6.60 ± 0.33 cm was recorded in 0.5 mg/l IBA. Similar results were reported in the same plant (Jamal *et al*. 2010; Jawahar *et al*. 2008), in *Cleistanthus collinus* (Quarishi and Mishra (1998). Sahoo and Chand (1998) obtained the best rooting response in IAA (1.0 mg/l) combined with IBA (1.0 mg/l) in *V. negundo* .

3.4.2. Artificial Seed Production

Synthetic seed technology is the most significant applications of plant tissue culture, have great potential for large scale production of different types of plants at low cost as an alternative to true seeds (Roy and Mandal 2008). An artificial seed is often described as artificially encapsulated somatic embryos, shoot tips, shoot buds, cell aggregate or any other tissue that possess the ability to convert into a plant under *in vitro* or *ex vitro* condition, that can be used for sowing as a seed, that retain this potential also after storage (Capuano 1998), which is at most equivalent to an immature zygotic embryos, possibly at post-heart stage or early cotyledonary stage (Bekheet 2006). Development of synthetic seeds by the encapsulation of various explants (somatic embryos, axillary buds, nodal segment, protocorms and bulblets) from ornamental and medicinal species etc. have been tried in a number of plant

species (Reddy *et al.*, 2012). In recent years, the alginate-encapsulated *in vitro*-derived shoot tips and nodal segments have been employed as an alternative to somatic embryos method of artificial seed production (Kikowska and Thiem 2011). There are several advantages of artificial seeds such as ease of handling, low production cost, ease of exchange of plant materials, genetic uniformity of plantlets, direct delivery to the soil, shorten the breeding cycle and reduction of the storage space (Ganapathi *et al.* 1992). In addition large scale propagation method, rapid multiplication of plants (Bekheet 2006), used in advanced procedures of cryopreservation for the long term preservation of plant germplasm are other benefits. Medicinal plants are of great interest to the researchers in the field of biotechnology as most of the drug industries depend in part on plants like fruit, root, leaves, bark, whole plant, tubers, dry tubers, rhizome, rasine, gum, stem for the production of pharmaceutical compounds (Chand *et al.* 1997) for example Gentianine and Xanthones which act as Anticarcinogenic, Antihepatotoxic; centellin, Podophyllotoxin which act as Antimicrobial (Bharat *et al*. 2012; Chaurasia *et al*. 2012), there are other various examples of pharmaceutical compounds which are produced by medicinal plants.

A number of encapsulating agents have been tried out of which agar, agarose, alginate, carragenan, gelrite and polyacrylamide are important (Kitto and Janick 1985). However, it has been suggested that most suitable encapsulating agent is sodium alginate (Bapat *et al.* 1987) due to its solubility at room temperature and its ability to form completely permeable gel with calcium chloride ($CaCl_2 + 2H_2O$). Our findings have revealed that this method provides an efficient mechanism for encapsulating the shoot tip and nodal segment in *Centella asiatica* and *Rauvolfia serpentina*. Alginate is one of the most commonly used polymers for immobilization of plant cells and production of manufactured seeds because it is available in large quantities, is inert, non-toxic, cheap and easily handled (Endress 1994). However, studies on *in vitro* germplasm conservation using alginate encapsulation techniques have been reported for only a few species. Embryogenic tissue of *Samtalum album* (Bapat and Rao 1988), axillary buds of *Morus indica* (Bapat *et al*. 1987), nodal segments of *Rauvolfia serpentina* (Faisal *et al*. 2012) and shoot tip of *Gypsophila paniculata* (Rady and Hanafy 2004) have been encapsulated in alginate beads. In the present investigation *in vitro* shoot tip and nodal segment of *Centella asiatica* and *Rauvolfia serpentina* were encapsulated in sodium alginate prepared using MS basal medium. Different concentrations and combinations of BAP, Kn, NAA and IAA were used in alginate bead.

In case of *Centella asiatica*, MS basal medium supplemented with different concentrations of BAP and Kn individually or combined with each other or IAA was used in artificial seed beads. These encapsulated synthetic seeds were cultured on MS

medium containing same growth regulators. Among the different concentrations of phytohormones, highest 90% of shoot formation was observed in MS medium containing 2.0 mg/l Kn+ 0.5 mg/l IAA from nodal segments. Highest average number of shoot 4.20 ± 0.19 was observed in the same medium and average highest length of shoot 2.14 ± 0.14 cm was found in the medium having 2.0 mg/l Kn. Joshee *et al*. (2007) produced artificial seed or synthetic seeds using somatic embryo and germinated them in MS basal medium in the same plant. Micropropagation through encapsulation of vegetative propagules has also been reported in a few medicinally important plant species like *Ocimum* sp. (Mandal *et al*. 2000), *Phyllanthus amarus* (Singh *et al*. 2006a), *Withania somnifera* (Singh *et al*. 2006 b), *Tylophora indica* (Faisal and Anis 2007), *Rauvolfia serpentina* (Ray and Bhattacharya 2008), *Spilanthus acmella* (Singh *et al*. 2009) and *Eclipta alba* (Singh *et al*. 2010).

In case of *Rauvolfia serpentina*, sodium alginate-encapsulated beads were cultured on MS medium supplemented with various PGRs such as 0.5, 1.0, 2.0 and 3.0 mg/L BAP and Kn, alone and in combination with NAA for germination experiments. Of the various concentrations used, 1.0 mg/l BAP with combination of 0.2 mg/L NAA was found to be the most effective (76.67%) in terms of frequency of germination. Highest average number of shoot 4.13 ± 0.33 formations was observed in the same medium. Similar results have been reported in other medicinal plants such as *Clitoria ternatea* (Kumar and Thomas 2012) in *Swertia chirayita* (Kumar and Chandra 2014). Same phytohormones were also best in seed bead of Mulberry (Machii 1992). Nodal segments showed comparatively low germination percentage. Kumar *et al*. (2010) reported that best response for shoot sprouting from encapsulated shoot tips of jojoba (*Simmondsia chinensis*) was recorded on 0.8% agar-solidified full-strength MS medium.

The influence of storage at 20 ± 2ºC, 4±1ºC and –1±1ºC temperature on germination rate was also examined for the both experimental plants, *Centella asiatica* and *Rauvolfia serpentina*. This investigation indicates that synthetic seed of *Centella asiatica* obtained from nodal explants could be stored at 20 ± 2ºC for 30 days and the germination rate was 15% and after 30 days at 20 ± 2ºC no response of artificial seeds. And at 4ºC for 60 days the germination rate was 30% after 60 days synthetic seed did not give any response being stored at 4ºC. Ipekci and Gozukirmizi (2003) also observed that the encapsulated embryos of *Paulownia elongate* was survived when the synthetic seeds were stored at 4ºC for 60 days and the germination rate was 32.40%. This type of result was also supported the result of Alfalfa seeds (Redenbaugh *et al.* 1987), *Asparagagus cooperi* (Ghosh and Sen 1994), *Eucalyptus citrisdora* (Muralidharan and Mascarenhas 1995), *Camellina* (Janeiro *et al*. 1995), Mulberry (Machii 1992; Bapat *et al*. 1987). But no result found at -1 ± 1ºC temperature. In case of *Rauvolfia serpentina*, synthetic seed obtained from shoot tip

explants could be stored at 20 ± 2ºC for 30 days and the germination rate was 10% and after 30 days at 20 ± 2ºC no response of artificial seeds. And at 4ºC for 60 days the germination rate was 25% after 60 days synthetic seed did not give any response being stored at 4ºC. No result found at -1 ± 1ºC temperature. Faisal *et al*. (2012) also observed that the encapsulated nodal segment of *R. serpentina* was survived when the synthetic seeds were stored at 4ºC for 8 weeks and the germination rate was 50±1.8%. The results from our experiment with cold-stored encapsulated seeds were in agreement with the study of Faisal *et al.* (2006) concerning the rates of encapsulated segments with axillary buds in *Rauvolfia tetraphylla* and in *Eclipta alba* (Singh *et al*. 2010) stored at 4 °C. In contrast, Srivastava *et al*. (2009) reported that after 6 months of storage at 25 ± 2ºC in moist condition, the encapsulated microshoots of *Cineraria maritima* were capable of regrowth within 2 weeks following culture. The frequency of regrowth was 82% after 4 weeks of culture on PGR-free MS medium. Muthiah *et al*. (2013) also found similar results in *Bacopa nonnieri* after 6 months of storage at 4ºC. Conversion response of encapsulated seeds decreased gradually after storage at different temperature when the storage duration was increased. The decline in the conversion of encapsulated propagules stored at low temperature may be due to inhibited respiration of plant tissues because of alginate cover (Redenbaugh *et al*. 1987; Naik and Chand 2006).

3.4.3. Cell Suspension Culture

Plants are the tremendous source for the discovery of new products with medicinal importance in drug development. Today several distinct chemicals derived from plants are important drugs, which are currently used in one or more countries in the world. Secondary metabolites are economically important as drugs, flavor and fragrances, dye and pigments, pesticides, and food additives. Many of the drugs sold today are simple synthetic modifications or copies of the naturally obtained substances. The evolving commercial importance of secondary metabolites has in recent years resulted in a great interest in secondary metabolism, particularly in the possibility of altering the production of bioactive plant metabolites by means of tissue culture technology. Plant cell and tissue culture technologies can be established routinely under sterile conditions from explants, such as plant leaves, stems, roots, and meristems for both the ways for multiplication and extraction of secondary metabolites. *In vitro* production of secondary metabolite in plant cell suspension cultures has been reported from various medicinal plants. Plant cell suspension cultures are widely used in plant biology as a convenient tool for the investigation of a wide range of phenomena, bypassing the structural complexity of the plant organism in toto. The homogeneity of an *in vitro* cell population, the large availability of material, the high rate of cell growth and the good reproducibility of conditions make suspension-cultured cells suitable for the analysis of complex physiological processes

at the cellular and molecular levels. Moreover, plant cell cultures provide a valuable platform for the production of high value secondary metabolites and other substances of commercial interest.

Growth conditions for plant culture must provide it with all essential nutrients, which are normally provided by the plant itself. Furthermore, these conditions must be optimized to promote the growth of the particular cell type. The medium, in which the cells are grown, must contain a carbon source, vitamins, salts and other organic supplements. The organic nutrients come in different concentrations of macronutrients and micronutrients. Macronutrients include nitrogen, potassium, calcium and magnesium, which are always found in a concentration greater than 0.5 mM. The micronutrients include iron, copper, zinc and cobalt and can be adjusted for maximum growth of each culture type. In addition, medium must provide vitamins which are usually synthesized by plants. In addition to vitamins and nutrients, growth hormones can also influence the growth rate of plants growth in artificial environments. Many plants grown in a conventional manner produce their own growth hormones, however, in culture, artificial hormones are supplied to ensure optimal growth of the plants.

Besides having the appropriate nutrients in the media, the maintenance of sterile conditions is essential for the success of the cell culture allowing it to be free of microorganisms. This requires that all equipment used in creating a cell culture must be sterilized to ensure contamination free environment.

Cell suspension cultures are rapidly dividing homogenous suspensions of cells grown in liquid nutrient media from which samples can be taken. In a cell suspension, a mass of cells, called a callus, must first be collected. The callus can then be suspended in a liquid callus induction media containing all the required nutrients and elements to allow for optimal growth which acts to turn all cells into undifferentiated cells. The cell suspension is then placed on a shaker to allow the cell aggregates to disperse to form smaller clumps and single cells that are homogenously distributed throughout the liquid media. The cells will continuously grow until one of the factors limiting causing cell growth to slow.

The suspensions contained both free cells and cell aggregates, in varying proportions between different cultures. Cell clusters up to 2 mm diameter not uncommon and formed by repeated divisions, held together by internal cross walls (plasmadesmata). Soon after the cell division phase, the cells enlarge and separated as free cells from the aggregates. Plant cells in suspension possess a thin unspecialized cell wall (0.2 to 0.6 µ in diameter) and show a wide variety of shapes and sizes, ranging from nearly spherical to approximately cylindrical, and of linear dimensions in the range 20 to 40 µ diameter and 100 to 200 µ long.

In the present investigation, embryogenic calli obtained from MS semisolid medium were used to initiate cell suspension. For the purpose of cell suspension culture the cells were cultured on to MS liquid medium supplemented with different auxins like 2,4-D, NAA and IAA alone or in combinations with BAP. After 4 days of culture under shaking resulted that, the production of some individual cells with small cell clusters in the liquid medium. Microscopic observation were made to characterize the individual cells isolated from the culture medium and in respect of their shape they may be regarded as spherical, elliptical and elongated shape. These cells were with dense cytoplasm and part of them possessed high embryogenic potential. The embryogenic single cells and cell clusters were capable to divide symmetrically and asymmetrically by transverse division which resulted in two cell stages. Iantcheva *et al.* (2006) observed similar asymmetrically cell division in *Medicago truncatula* in cell suspension culture.

In the present investigation a number of experiments were performed to study the effect of hormone on cell growth and the optimum cell division period in culture medium of four selected species.

In case of *C. asiatica*, among the different concentrations and combinations of different hormones, 0.5 mg/l BAP + 2.0mg/l NAA found to be most effective on cell culture. In this media formulation the highest weight of cells and cell aggregates was 0.144 ± 0.013 gm on 32nd day of cell suspension culture. Depending on cell growth a growth curve was developed (**Fig: 3.3.1.)** to address the time range for rapid growth period. Experimental results indicated that the peak period of cell growth runs from 12 – 24 days and after 32 days their growth seemed to be ceased down. It means that growth period of *C. asiatica* plant runs up to 4 weeks and a peak time stands for 12 – 24 days. The results revealed that 4 week period appeared as typical period for the cell culture of *C. asiatica* plant and it is necessary to transfer the cell to the fresh medium after each 4 weeks of culture for its maintenance in the lab. Similar peak period of cell growth was obtained in *C. asiatica* where cell biomass increased with culture time from the start to day 24 with a maximum value of fresh weight (Loc and Tam 2010).

In *Coccinia cordifolia*, among the different concentrations and combinations of different hormones, 1.0 mg/l BAP+0.5 mg/l NAA found to be most effective on cell culture. In this media formulation the highest weight of cells and cell aggregates was 0.136 ± 0.012 gm on the 32nd day of culture. The **Fig. 3.3.1** indicates that the peak period of cell growth runs from 8 – 28 days and after 32 days their growth seemed to be ceased down. It means that growth period of *C. cordifolia* plant runs up to 4 weeks and a peak time stands for 8 – 28 days. The results revealed that 4 week period appeared as typical period for the cell culture of *C. cordifolia* plant and it is necessary to transfer the cell to the fresh medium after each four and a half weeks of culture for

its maintenance in the lab. Rahman and Bari (2013) reported that *Ricinus communis* L. cv. Roktima show the highest cell growth on MS medium having 2.0 mg/l BAP+0.2mg/l NAA and the cell continued to grow until 14 days but the peak of cell growth was 4th day to 6th day.

In *M. pruriens*, the best response was found in MS supplemented with 3.0 mg/l BAP + 0.5 mg/l IAA on cell suspension culture. In this media formulation the highest weight of cells and cell aggregates was 0.128 ± 0.011 gm on the 24th day of cell suspension culture. According to **Fig. 3.1.1**, the peak period of cell growth runs from 12– 20 days and after 24 days their growth seemed to be ceased down. It means that growth period of *M. pruriens* plant runs up to 3 weeks and a peak time stands for 12–20 days. The results revealed that 3 week period appeared as typical period for the cell culture of *M. pruriens* plant and it is necessary to transfer the cell to the fresh medium after each 3 weeks of culture for its maintenance in the lab. Chattopadhyay *et al*. (1994) reported that the effect of cytokinins e.g. kinetin and BAP and auxins e.g. NAA, IAA and 2,4-D at different concentrations were no significant variations in cell growth and L-DOPA production on cell suspension culture in *M. pruriens*.

Finally, in case of *R. serpentina*, among the different concentrations and combinations of different hormones, 2.0 mg/l 2,4–D+0.5 mg/l BAP found to be most effective on cell culture. In this media formulation the highest weight of cells and cell aggregates was 0.132 ± 0.013 gm on 28th day of cell suspension culture. According to **Fig. 3.1.1**, the peak period of cell growth runs from 12 – 20 days and after 28 days their growth seemed to be ceased down. It means that growth period of *R. serpentina* plant runs up to 4 weeks and a peak time stands for 12 – 24 days. The results revealed that 4 week period appeared as typical period for the cell culture of *R. serpentina* plant and it is necessary to transfer the cell to the fresh medium after each 4 weeks of culture for its maintenance in the lab. These cultures can be maintained for long time without loss of embryogenic capacity by subculture at the end of the experimental growth phase to fresh medium (Iantcheva *et al*. 2006).

The peak period of cell growth was observed from 8th day to 24th day for all the investigated species. In contrast, Hossain *et al*. (2007) reported that cell growth show the highest peak within 4 – 6 days of incubation in brinjal. Bari *et al*. (2009) reported in *Abrus precatorius* where cells attained their highest peak within 6 – 8 days of growth. In sugar beets, using different concentrations and combinations of BAP and 2,4-D, the growth patterns of cell suspension cultures were examined during a range of culture periods (0, 3, 5, 7, 9, 11, 13 and 15 days). In all lines, the growth rates of cells were initially slow but as the culture proceeded, they increased significantly and accumulated great amount of biomass over a period of 15 days in Sugar Beet (Song *et al*. 2002). Plant cell growth and their growth measurement procedure were also very

clearly described in PROTCOL (Mustafa *et al*. 2010). In some countries endeavours are being made to design the cell culture bioreactor for commercial production of cell product in laboratory (Ozlem *et al*. 2010). But in banana cell suspension growth is absolutely different from our investigated species, in banana, most of the cultivars showed the highest performance within 60-70 days of culture in liquid medium and the cell growth became stationary after 80 days of culture (Hossain and Bari 2011).The time required to establish the cell suspension culture varies greatly and depends on the tissue of the plant species and the medium composition. The use of fine suspension culture offers the opportunity to confirm single cell origin of somatic embryos and the asymmetry of the first cell division, which starts the process. The other advantage of cell suspension culture is the use of single embryogenic cells and somatic embryos as a source for direct gene transfer via particle bombardment, transit gene expression and confocal microscopy observation. Cell suspension cultures are particularly suitable for physiological, biochemical and molecular studies of the process of somatic embryogenesis and its different stages – induction development, maturation and conversion. The development of a genome and proteome database of model *Medicago truncatula* species together with the presence of protocol of cell suspension provided the opportunity to identify and characterized genes involved in the whole process of somatic embryogenesis (Iantcheva *et al*. 2006).

From the foregoing discussion it may be concluded that the auxins NAA, 2,4-D, and IAA have great role in cell suspension culture. Under this investigation NAA in combination with BAP proved to be suitable media composition for enhancing cell growth of *C. asiatica*, *C. cordifolia* and *R. serpentina*. On the other hand IAA in combination with BAP proved to be suitable media composition for enhancing cell growth of *M. pruriens* in artificial medium. The present experiments clearly demonstrated that in our investigated species, cell begins to grow only within 8-12 days of culture proving their potential for developing cell culture industry for the production of important alkaloid/secondary metabolites like Asiaticoside, Cucurbitacin, Mucunine, Prurieninine, L-DOPA, Serpentine ajmaline, Sarpagine, Yohimbine, Heteroyohimbine as a secondary metabolite. In regards of other medicinal plants *C. asiatica*, *C. cordifolia* and *R. serpentina* proved suitable plants species for developing cell suspension culture in artificial medium and the present findings open up a new potential venture for obtaining important secondary metabolites as a source from their cell suspension culture.

3.5. SUMMARY

3.5.1. Micropropagation

The present investigation was undertaken with the objective to standardize techniques for *in vitro* culture of five important medicinal plant species of Madhupur Tract namely, *Centella asiatica, Commelina benghalensis, Curcuma zedoaria, Mucuna pruriens* and *Vitex negundo*. It was considered important that if such protocol could be developed that would be of great use in supporting the future demand as well as conservation of such uncared medicinal plant species. The experiments were conducted with the use of different kinds of explants *viz*. nodal, shoot tip, axillary bud, internodal and leaf segments cultured in MS medium fortified with different kinds of auxins (NAA, IAA, IBA and 2,4–D) and cytokinins (BAP and Kn) at various concentrations either at individual or combination levels.

Both direct and indirect organogenesis took place in three plant species and only direct organogenesis took place in two plant species and the response was dependent on the nature of explants and hormonal supplements as applied in the medium. In case of *C. asiatica* best result was obtained in direct organogenesis via shoot bud formation in nodal segments and 2.0 mg/l Kn+0.5 mg/l IAA combination of growth regulators induced maximum buds per explant. In case of indirect organogenesis, with the development of shoot buds from light green compact calli that developed best in leaf segments on 1.0 mg/l BAP+0.5 mg/l NAA supplemented media. In many cases cultured explants produced white and friable callus that usually failed to differentiate.

In *C. benghalensis*, nodal segments also gave the best response compared to the shoot tip explants in direct organogenesis and 1.0 mg/l BAP+0.5 mg/l NAA combination of growth regulators induced maximum number of multiple shoots per explant.

Among the two kinds of explants in *C. zedoaria*, shoot tip explants was proved best for generating shoot buds trough direct organogenesis. The plant growth regulators supplements play a great role in the process of organogenesis. In case of *C. zedoaria* liquid MS medium produced significantly higher number of multiple shoots than those of cultured on the solid medium with the same formulation. Combination of 2.0 mg/l BAP+1.0 mg/l IBA with 20% cocoanut water in liquid MS medium gave the best direct regeneration frequency.

In *M. pruriens* nodal segments gave the best response compared to the shoot tip explants in direct organogenesis and 0.2 mg/l BAP+ 0.2 mg/l Kn + 0.2 mg/l IAA combination of growth regulators induced maximum shoot buds per explant. In case of indirect organogenesis, better shoot buds initiated from internode derived light green compact callus in MS media supplemented with 0.5 mg/l BAP +0.2 mg/l NAA.

Finally, in case of *V. negundo*, nodal segments also gave the best response compared to the shoot tip explants in direct organogenesis and 2.0 mg/l Kn + 0.5 mg/l GA_3 combination of growth regulators induced maximum buds per explant. In case of indirect organogenesis, best response was obtained in the media supplemented with 0.5 mg/l BAP +0.5mg/l IAA.

Considering overall performance, among the five species nodal segments was found best as a source of explants in four species for *in vitro* mass propagation. Application of plant growth regulators played an important role in *in vitro* regeneration in all medicinal plants and combination of auxins and cytokinins were proved better than their individual use. When the shoot buds developed either through direct or indirect organogenesis attained a height of 3-5cm. They were individually grown on full or half strength MS medium supplemented with auxins but in some cases auxins was used in combinations with BAP or Kn where rooting was found to grow at the base of bud. MS+2.0mg/l IAA was best for producing root system in *C. asiatica*. Half strength MS+1.5mg/l IBA was best for producing root system in *C. benghalensis*. On the other hand half strength MS+2.0mg/l IBA + 0.5mg/l BAP was best for producing root system in *C. zedoaria*, half strength MS+2.0mg/l IBA + 0.5mg/l NAA was best for producing root system in *M. pruriens* and finally half strength MS+1.0mg/l IAA was best for producing root system in *V. negundo.* Through successive phages of acclimatization the *in vitro* plantlets in five plant species were transferred finally to earthen pots in outside environment.

The protocols developed here can reliably be used in mass scale production of these important medicinal plants. It also offers the opportunities of selecting desirable somaclones if originate in the course of indirect organogenesis. The findings also indicate that it is possible to develop similar protocols for *in vitro* propagation of other medicinal plants of Madhupur tract and can be used in mass propagation and conservation programmes.

3.5.2. Artificial Seed Production

Artificial seed technology finds application in large scale clonal propagation and conservation of clonal germplasm of elite and endangered plants in near future with the development of appropriate storage techniques. They offer several advantages as easy handling, storability, reduced size of propagules and transportability. For conservation of important medicinal plants attempt was taken to produce artificial seed by encapsulating the shoot tip and nodal segments of two important medicinal plants *viz. Centella asiatica* and *Rauvolfia serpentina*. For shoot growth of artificial seeds, encapsulated shoot tips and encapsulated nodal segment were cultured in MS basal media containing different concentrations and combinations of BAP, Kn and

NAA or IAA. In *C. asiatica* the highest 90% of shoot formation was observed in MS medium containing 2.0 mg/l Kn + 0.5 mg/l IAA from nodal segments which was followed by 83.33% in MS medium amended with 2.0 mg/l Kn + 1.0 mg/l IAA. On the other hand, highest percentage (76.67%) of shoot proliferation was observed from shoot tip in MS medium containing 2.0 mg/l Kn + 0.5 mg/l IAA followed by the growth (70%) in MS medium containing 2.0 mg/l Kn + 1.0 mg/l IAA. In *R. serpentina* the highest 63.33% of shoot formation was observed in MS medium containing 1.0 mg/l BAP + 0.2 mg/l NAA from nodal segments which was followed by 60% in MS medium amended with 1.0 mg/l BAP + 0.5 mg/l NAA. On the other hand highest percentage (76.67%) of shoot proliferation was observed from shoot tip in MS medium containing 1.0 mg/l BAP + 0.2 mg/l NAA followed by the growth (66.67%) in MS medium containing 1.0 mg/l Kn + 0.2 mg/l NAA. The experimental results indicated that cytokinins play an important role in germination of artificial seeds, being encapsulated by sodium alginate.

Another experiment was laid out on the survival (viability) test of encapsulated shoot tip and nodal segments of *C. asiatica* and *R. serpentina* under different storage temperature regime. Under this experiment three storage temperatures were maintained for storing the artificial seed *viz.* 20±2ºC, 4±1ºC and -1±1ºC for different periods. In case of *C. asiatica* highest survival percentage was noted after 60 days of storage at 4±1ºC, when 25-30% of survivality was observed in encapsulated explants. Similarly highest survival percentage was noted after 60 days of storage at 4±1ºC in *R. serpentina*, when 20-25% of survivality was observed in encapsulated explants. But after 60 days of storage under three storage temperature regimes the encapsulated explants failed to show any survivability.

3.5.3. Cell Suspension Culture

In the present investigation a number of experiments were performed to study the effect of hormone on cell growth and the optimum cell division period in culture medium of four selected medicinal plant species.

In case of *C. asiatica*, among the different concentrations and combinations of different hormones, 0.5 mg/l BAP + 2.0mg/l NAA found to be most effective on cell culture. In this media formulation the highest weight of cells and cell aggregates was 0.144 ± 0.013 gm on 32nd day of cell suspension culture. The peak period of cell growth runs from 12 – 24 days and after 32 days their growth seemed to be ceased down. Cell growth in *C. asiatica* plant runs up to 4 weeks and a peak time stands for 12 – 24 days. The results revealed that 4 week period appeared as typical period for the cell culture of *C. asiatica* plant and it is necessary to transfer the cell to the fresh medium after each 4 weeks of culture for its maintenance in the lab. In *C. cordifolia*,

among the different concentrations and combinations of different hormones, 1.0 mg/l BAP+0.5 mg/l NAA found to be most effective on cell culture. In this media formulation the highest weight of cells and cell aggregates was 0.136 ± 0.012 gm on the 32nd day of culture. The peak period of cell growth runs from 8 – 28 days and after 32 days their growth seemed to be ceased down. Growth period of *C. cordifolia* plant runs up to 4 weeks and a peak time stands for 8 – 28 days and it is necessary to transfer the cell to the fresh medium after each four and a half weeks of culture for its maintenance in the lab. In *M. pruriens*, the best response was found in MS supplemented with 3.0 mg/l BAP + 0.5 mg/l IAA on cell suspension culture. In this media formulation the highest weight of cells and cell aggregates was 0.128 ± 0.011 gm on the 24th day of cell suspension culture. The peak period of cell growth runs from 12– 20 days and after 24 days their growth seemed to be ceased down. Three weeks period appeared as typical period for the cell culture of *M. pruriens* plant and it is necessary to transfer the cell to the fresh medium after each 3 weeks of culture for its maintenance. Finally, in case of *R. serpentina*, among the different concentrations and combinations of different hormones, 2.0 mg/l 2,4–D+0.5 mg/l BAP found to be most effective on cell culture. In this media formulation the highest weight of cells and cell aggregates was 0.132 ± 0.013 gm on 28th day of cell suspension culture. The peak period of cell growth runs from 12 – 20 days and after 28 days their growth seemed to be ceased down. It means that growth period of *R. serpentina* plant runs up to 4 weeks and a peak time stands for 12 – 24 days. Thus the typical period for this plant is 4 week and it is necessary to transfer the cell to the fresh medium after each 4 weeks of culture for its maintenance.

Under this investigation the auxins NAA, 2,4-D, and IAA have great role in cell suspension culture. NAA in combination with BAP proved to be suitable media composition for enhancing cell growth of *C. asiatica*, *C. cordifolia* and *R. serpentina*. In case of *M. pruriens*, IAA in combination with BAP proved to be suitable media composition for enhancing cell growth in artificial medium. The present experiments clearly demonstrated that in our investigated species, cell begins to grow only within 8-12 days of culture proving their potential for developing cell culture industry for the production of important alkaloid/secondary metabolites. The investigated species proved that they are suitable for developing cell suspension culture in artificial medium and the present findings open up a new potential venture for obtaining important secondary metabolites as a source from their cell suspension culture.

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REFERENCES

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REFERENCES

Abbott AJ. 1978. Practice and promise of micropropagation of woody species. Acta Hort. 79: 113 – 127.

Agretious TK, Martin KP, Hariharan M. 1996. *In vitro* clonal multiplication of *Alpinia calcarata* Roscoe. Phytomorphology. 46: 133 – 138.

Ahmad N, Anis M. 2011. An efficient *in vitro* process for recurrent production of cloned plants of *Vitex negundo* L. Eur J Forest Res. 130: 135–144.

Ajithkumar D, 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.

Alam MK, Chowdhury JU, Hasan MA. 1996. Some folk formularies from Bangladesh. Bangladesh J. Life Sci. 8(1): 49 – 63.

Alam MK. 1992. Medicinal Ethnobotany of the Marma tribe of Bangladesh. Economic Botany. 46(3): 330 – 335.

Amo-Marco JB, Ibancz MR. 1998. Micropropagation of *Lilium cavanillesii* Erben, a threatened statice, from inflorescence stems. Plant Growth Regul. 24: 49-54.

Anonymous. 2003. The wealth of India: a dictionary of Indian raw materials and industrial products, vol. 4. Publications and Information Directorate, CSIR, New Delhi: 166–167.

Arora R, Bhojwani SS. 1989. *In vitro* propagation and low temperature storage of *Saussurea lappa* C.B. Clarke – an endangered medicinal plant. Plant Cell Rep. 8: 44–47.

Arrilaga MCB, Segura J. 1987. Somatic embryogenesis from hypocolyls Callus of Digitalis obscura L. Plant Cell Rep 6: 223-226.

Arulanandam L. JP, Ghanthikumar S. 2011. Indirect organogenesis of *Vitex trifolia* Linn.-An important medicinal plant. Indian Journal of Natural Products and Resources. 2(2): 261 – 264.

Azad MAK, Amin MN. 1998. *In vitro* regeneration of plantlets from internode explants of *Adhatoda vasica* Nees. Plant Tissue Cult. 8(1): 27–34.

Baksha R, Jahan MAA, Khatun R, Munshi JL. 2005. Micropropagation of *Aloe barbadensis* Mill. through *in vitro* culture of shoot tip explants. Plant Tiss. Cult. Biotech. 15(2): 121 – 126.

Balachandran SM, Bhat SR, Chandel KPS. 1990. *In vitro* clonal multiplication of turmeric (*Curcuma* spp.) and ginger (*Zingiber officinale* Rosc.). Plant Cell Rep. 8: 521 – 524.

Banisalam B, Sani W, Philip K, Imdadul H, Khorasani A. 2011. Comparison between *in vitro* and *in vivo* antibacterial activity of *Curcuma zedoaria* from Malaysia. Afrian J. of Biotechnol. 10(55): 11676 – 11684.

Banu LA, Bari MA, Haque E. 2001. *In vitro* propagation of *Ocimum sanctum* L. through nodal explants. Bangladesh J. Genet. Biotech. 2(1&2): 143 – 146.

Banu LA, Bari MA. 2007. Protocol establishment for multiplication and regeneration of *Ocimum sanctum* Linn. An important medicinal plant with high religious value in Bangladesh. Journal of Plant Sci. 2(5.): 530 – 537.

Bapat VA, Mhatre M, Rao PS. 1987. Propagation of *Morus indica* L. (Mulberry) by encapsulated shoot buds. Plant Cell Rep. 6: 393–395.

Bapat VA, Rao PS. 1988. Sandalwood plantlets from synthetic seeds. Plant Cell– A valuable medicinal plant. Indian J. Applied & Pure Bio. 26(2): 193–198.

Bari MA, Banu LA, Hossain MJ. 2009. Cell suspension culture and somatic embryogenesis in *Abrus precatorius* Intl. J. BioRes. 7(4): 19 – 24.

Barua P, Handique PJ. 2002. *In vitro* propagation of *Phlogacanthus thyrsiflorus* Ness. A rare medicinal plant of Assam (India). J Curr. Sci. 2(2): 275 – 278.

Bekheet SA. 2006. A synthetic seed method through encapsulation of *in vitro* proliferated bulblets of garlic (*Allium sativum*). Arab J Biotech. 9:415–426.

Bell EA, Janzen DH. 1971. Medicinal and ecological consideration of L-Dopa and 5-HTP in seeds. Nature. 229:136–137.

Bennet SSR. 1987. Name changes in flowering plants of India and adjacent regions. Triseas publishers, Dehra Dun. India. 1 – 772.

Bhagyalakshmi N, Singh NS. 1988. Meristem culture and micropropagation of a variety of ginger (*Zingiber officinale* Rosc.) with a high yield of Oleoresin. J Horticult Sci. 63: 321 – 327.

Bharalee R, Das A, Kalita MC. 2005. *In vitro* clonal propagation of *Curcuma ceasia* Roxb. and *Curcuma zedoaria* Rosc. from rhizome bud explants. J. Plant Biochem. Biotechnol. 14: 61 – 63.

Bharat K, Pradhan, Hemant K, Badola. 2012. Effects of microhabitat, light and temperature on seed germination of a critically endangered Himalayan

medicinal herb, *Swertia chirayita*: Conservation implications. Plant Biosystems.146(2): 345–351.

Bhojwani SS. 1980. *In vitro* propagation of garlic by shoot proliferation. Sci Hortic. 13: 47-52.

Biawas A, Bari MA, Mohashweta Roy, SK Bhadra SK. 2011. *In vitro* propagation of *Stemona tuberosa* Lour- A rare medicinal plant through high frequency shoot multiplication using nodal explants. Plant Tissue Cult. & Botech. 21(2): 151 – 159.

Bibi Y, Zia M, Nisa S, Habib D, Waheed A, Chaudhary MF. 2011. Regeneration of *Centella asiatica* plants from non-embryogenic cell lines and evaluation of antibacterial and antifungal properties of regenerated calli and plants. Journal of Biological Engineering. 5: 13.

Binita BC, Ashok MD, Yogesh TJ. 2005. *Bacopa monnieri* (L.) Pannell: A rapid, efficient and cost effective micropropagation. Plant Tiss. Cult. Biotech. 15(2): 167 – 175.

Biswas A. 2006. Indigenous knowledge of herbal medicine and *in vitro* propagation of some rare medicinal plants in Chittagong Hill Tracts. Ph.D thesis, Institute of Biological Sciences, University of Rajshahi, Bangladesh.

Biswas K, Ghos SA. 1973. Indian Banoushadi. Vol. I – V. Sibendranath Kanjilal, Superintendent Calcutta University Press, 48, Hazra Road, Bullygange, Calcutta.

Bonfill M, Cusido RM, Palazon J, Pinol MT, Morales C. 2002. Influence of auxins on organogenesis and ginsenoside production in Panax ginseng calluses. Plant Cell Tiss. Org. Cult. 68: 73-78.

Caius JF. 1989. The Medicinal and Poisonous Legumes of India, Jodhpur, India: Scientific Publishers. pp. 70–71.

Camerson JS, Hancock JF, Flore JA. 1989. The influence of micropropagation on yield components, dry matter partitioning and gas exchange characteristics of Strawberry. Scientia Horticulture. 38: 61 – 67.

Capuano G, Piccioni E, Standardi A. 1998. Effect of different treatments on the conversion of M.26 apple rootstock synthetic seeds obtained from encapsulated apical and axillary micropropagated buds. J Hort Sci Biotechnol. 73: 299–305.

Cardellina JH. 2002. Challenges and opportunities confronting the botanical dietary supplement industry. J Nat Prod. 65:1073–1084.

Casado JP, Navarro MC, Utrilla MP, Martinez A, Jimenez J. 2002. Micropropagation of *Santholina canescens* Lagasca and *in vitro* volatiles production by shoot explants. Plant Cell Tissue Org. Cult. 69: 147 – 153.

Chan LK, Thong WH. 2004. *In vitro* propagation of Zingiberaceae species with medicinal properties. J. Plant Biotechnol. 6: 181 – 188.

Chand S, Sahrawat AK, Prakash DVSSR. 1997. *In vitro* culture of *Pimpinella anisum* L (anise). J Plant Biochem. Biotechnol. 6: 1–5.

Chandramu C, Rao DM, Reddy VD. 2003. High frequency induction of multiple shoots from nodal explants of *Vitex negundo* L. using sodium sulphate. J. Plant Biotech. 5(2): 107 – 113.

Chattopadhyay S, Datta SK, Mahato SB 1994. Production of L-DOPA from cell suspension culture of *Mucuna pruriens f. pruriens*. Plant Cell Reports. 13: 519 – 522.

Chattopadhyaya S, Dutta SK. 1995. Rapid Micropropagation Mucuna pruriens f. pruriens L, Plant Cell Reports. 15(3-4): 271.

Chaurasia OP, Ballabh B, Tayade A, Kumar R, Kumar GP, Singh SB. 2012. *Podophyllum* L: An endergered and anticancerous medicinal plant– An overview. Indian J Traditional Knowledge. 11(2): 234–241.

Chawla HS, Arora A. 2005. Organogenic plant regeneration via callus induction in chick pea (*Cicer arietinum L.*) - Role of genotypes, growth regulators and explants. Indian Journal of Biotechnology. 4: 251 – 256.

Cheetham PSJ. 1995. Biotransformation: new routes to food ingredients. Chem Ind.: 265 – 268.

Chen CC, Chen SJ, Sagare AP, Tsay HS. 2001. Adventitious shoot regeneration from stem, internode explants of *Adenophora triphylla* (thumb.) A.DC. (Campanulaceae)- an important medicinal herb. Bot. Bull. Acad. Sin. 42: 1–7.

Chetia S, Handique PJ. 2000. High frequency *In vitro* shoot multiplication of *Plumbago indica*, a rare medicinal plant. Curr. Sci. 78(10): 1187 – 1188.

Chinnamadasamy K, Arjunan D, Ramasamy MV. 2010. Rapid Micropropagation of *Plumbago zeylanica* L. An Important Medicinal Plant. J. of American Sciences. 6(10): 1027 – 1031

Das G, Rout GR. 2002. Plant regeneration through somatic embryogenesis in leaf derived callus of *Plumbago indica*. Biologia Plantarum. 45(2): 299 – 302.

Das J, Handique PJ. 2002. Micropropagation of a rare medicinal plant species *Polygonum microcephallum* Don. through high frequency shoot multiplication. J. Phytol. Res. 15: 197 – 200.

David C, Chilton MD, Tempe J. 1984. Conservation of T-DNA in plants regenerated from hairy root cultures. Biotechnol. 2: 73 – 76.

Daxenbichler ME, Etten CH, Van Hallman EA, Earle FR, Barclay AS. 1971. Seeds as sources of L-Dopa. Med. Chem. 14: 463–465.

Deshpande HA, Chalse MN, Bhalsing SR. 2010. *Centella asiatica* Linn. Plant regeneration through leaf derived callus. J. of Herbal Medicine and Toxicology. 4(2): 119 – 122.

Dey TK. 2006. Useful plants of Bangladesh. 2nd ed. The Ad. Communication, Chittagong, Bangladesh: 747.

Deyuan H, Robert AD. 2000. In: Flora of China, Z.Y. Wu, P.H. Raven, D.Y. Hong (Ed.) *Commelina diffusa*. Botanical Garden Press, Beijing.pp. 36.

Dicosmo F, Misawa M. 1995. Plant cell and tissue culture: Alternatives for metabolite production. *Biotechnology Advances*, 13, 3: 425 – 453.

Dörnenburg H, Knorr D. 1995. Strategies for the improvement of secondary metabolite production in plant cell cultures. Enzym Microb Technol. 17: 674– 684.

Drew RA, Smith MK. 1990. Field evaluation of tissue cultured bananas in southeastern Queensland. Aust. J. Expt. Agri. 30: 569 – 574.

Endress R. 1994. Plant Cell Biotechnology. Springer–verlag, Berlin: 256–269.

Engelmann F. 1991. *In vitro* conservation of tropical plant germplasm. A review. Euphytica. 57: 227 – 243.

Fabre J, Dereuddre J. 1990. Encapsulation – dehydration: a new approach to cryopreservation of *Solanum* shoot tips, Cryo-Letters. 11: 413-426.

Facchini PJ, Bird DA, St. Pierre B. 2004. Can *Arabidopsis* make complex alkaloid? Trend Plant Sci. 9: 116 -122.

Faisal M, Ahmad N, Anis M. 2006. *In vitro* plant regeneration from alginate-encapsulated microcuttings of *Rauvofia tetraphylla* L. American-Eurasina J Agric Environ Sci. 1: 1–6.

Faisal M, Alatar AAA, Ahmad N, Anis M, Hegazy AK, 2012. Assessment of Genetic Fidelity in *Rauvolfia serpentina* Plantlets Grown from Synthetic (Encapsulated) Seeds Following *in vitro* Storage at 4 °C. Molecules. 17: 5050–5061.

Faisal M, Anis M. 2007. Regeneration of plants from alginate encapsulated shoots of *Tylophora indica* (Burm.f.) Merrill, an endangered medicinal plant. J Hort Sci Biotechnol. 82:351–354.

Faisal M, Siddique I, Anis M. 2006. *In vitro* rapid regeneration of plantlets from nodal explants of *Mucuna pruriens* - a valuable medicinal plant. Ann. Appl. Biol. 148: 1–6.

Farooqi AA, Khan MM, Asundhara M. 1999. Production Technology of Medicinal and Aromatic Crops, Bangalore, India: Natural Remedies Pvt. Ltd. pp. 26–28.

Fischer R, Liao YC, Hoffmann K, Schillberg S, Emans N. 1999. Molecular farming of recombinant antibodies in Plants. Biologicval Chemistry, 380: 825 – 839.

Fraternale D, Giamperi L, Ricci D, Rocchi MBL. 2002. Micropropagation of *Bupleurum fruticosum* : The effect of triacontanol. Plant Cell Tiss. Org. Cult. 69: 135 – 140.

Fukai S, Togashi M, Goi M. 1994. Cryopreservation of *in vitro*-grown Dianthus by encapsulation dehydration. Technl bull Facult Agr Kagawa. Univ. 46: 101–107.

Ganapathi TR, Suprasanna P, Bapat VA, Rao PS. 1992. Propagation of Banana through encapsulated shoot tips. Plant Cell Rep 11: 571–575.

George EF and Serrington PD. 1984. In: Plant propagation by tissue culture, Exegetics Ltd., Eversley, England: 39-71.

George S, Remashree AB, Sebastian D, Hariharan M. 2004. Micropropagation of *Centella asiatica* L. through axillary bud multiplication. Phytomorphology 54: 31 – 34.

Ghani A. 1990. Traditional medicine (origin, practice and state of the art). In: Traditional medicine. Jahangirnagor University, Savar, Dhaka.

Ghani A. 1998. Medicinal Plants of Bangladesh. Asiatic Society of Bangladesh, Dhaka, Bangladesh. pp. 319 – 320.

Ghani A. 2003. Medicinal Plants of Bangladesh with Chemical Constituents and Uses. 2nd edn. Asiatic Society of Bangladesh, Dhaka, Ramna: 161; 197; 177–178; 308 – 309; 364 – 365; 426.

Ghanti K, Koviraj CP, Venugopal RB, Jabeen FTZ, Rao. 2004. Rapid regeneration of *Mentha piperita*. Ind. J. of Biotechnology. 3: 594 – 598.

Ghosh B, Sen S. 1994. Plant regeneration from alginate encapsulated somatic embryos of *Asparagus cooperi* Baker. Plant Cell Rep. 13:381–385.

Gilani SS, Abbas SQ, Shinwari ZK, Hussain F, Nargis K. 2003. Ethnobotanical Studies of Kurram Agency, Pakistan Through Rural Community Participation. *Pak. J. Biol. Sci.* 6(15): 1368 – 1375.

Gona KK, Omid K. 2008. Karami2picloram-induced somatic emberyogenesis in leaves of strawberry (*Fragaria ananassa* L.). Acta Biologica Cracoviensia Series Botanica. 50(1): 69–72.

Grotewold E. 2004. The challenges of moving chemicals within and out of cells: insights into the transport of plant nature products. Planta.219: 906-909.

Halmagyi A, Fischer-Kluver G, Mixwagner G, Shcumacher HM. 2004. Cryopresevation of *Chrysanthemum morifolium* (*Dendranthema grandiflora* Ramat.) using different approaches. Plant Cell Rep. 22: 371–375.

Handique PJ, Bora P. 1999. *In vitro* regeneration of a medicinal plant – *Houttuynia cordata* T. from nodal explants. Curr. Sci. 76(9): 1245 – 1247.

Hanida SS, Kapoor VK. 1988. Pharmacognosy. Vallabh Prakashhan Educational Publishers, Delhi.

Haradhan B. 2004. Present status of medicinal plants in different forests of Bangladesh and their management strategies. Aronno, Management Plan Division, Chittagong, Bangladesh: 55 – 71.

Harikrishnan KN, Hariharan M. 1996. Direct Shoot regeneration from nodal explants of *Plumbago rosea* L. – A Medicinal plant. Phytomorphology 46: 53–58.

Harini SS, Sathyanarayana N. 2009. Somatic embryogenesis in *Mucuna pruriens*. African J. of Biotech. 8(22): 6175 – 6180.

Harun-Er-Rashid (ed.). 2011. Madhupur Tract. Banglapedia: National Encyclopedia of Bangladesh, Asiatic Society of Bangladesh, Dhaka.

Hasan A. 1982. Medicinal Plants in Bangladesh. 1st Ed. Hasan Book House, 65, Paridas Rd. Banglabazar, Dhaka-1. pp. 10 – 12.

Hassan AKMS, Roy SK. 2004. Micropropagation of *Smilex zeylanica* L. A perennial climbing medicinal shrub, through axillary shoot proliferation. Bangladesh J. Life Sci. 16: 33 – 39.

Hooker JD. 1865 – 1885: Flora of British India. Vol. I – VII., L. Reeve & Co. Ltd. London.

Hooker JD. 1875. Flora of British India. Vols. I – VII

Horinouchi S. 2009. Combinatorial biosynthesis of plant medicinal polyketide by microorganisms. Curr. Opin. Chem. Biol. 13 (2): 197 – 204.

Hossain MJ, Bari MA. 2011. Shoot tip culture, somatic embryogenesis and cell suspension culture in banana [Ph.D. Thesis]. Institute of Biological Sciences. University of Rajshahi, Bangladesh.

Hossain MJ, Rahman M, Bari MA. 2007. Establishment of Cell Suspension Culture and Plantlet Regeneration of Brinjal (*Solanum melongena* L.). Journal of Plant Science. 2(4): 407 – 415.

Huang CL, Hsieh MT, Hsieh WC, Sagare AP, Tsay HS. 2000. *In vitro* propagation of *Limonium wrightii* (Hance) Ktze. (Plumbaginaceae), an ethnomedicinal plant, from shoot – tip, leaf – and inflorescence.

Huq AM. 1986. Plant Names of Bangladesh (Native and Scientific). Bangladesh National Herbarium, BARC. Dhaka, Bangladesh.

Iantcheva A, Vlahova M, Atanassov A, Duque AS, Araújo S, Santos DF dos, Fevereiro P. 2006. *Medicago truncatula* handbook, Version-12: 1 – 12.

Ipekci Z, Gozukirmizi N. 2003. Direct somatic embryogenesis and synthetic seed production from *Paulownia elongata*. Plant Cell Rep. 22: 16–23.

Jamal MAHM, Rahman ANMRB, Paul DK, Islam MR. 2010. *In vitro* regeneration for mass propagation in commercial scale of medicinal plant *Vitex negundo* L. J. bio-sci. 18: 140 – 145.

Janeiro LV, Ballester A, Vieitez AM. 1995. Effect of cold storage on somatic embryogenesis systems of *Camellia*. J Hort Sci. 70: 665–672.

Jawahar M, Karthikeyan AVP, Vijai D, Maharajan M, Ravipaul S, Jeyaseelan M. 2008. *In vitro* plant regeneration from different explants of *Cardiospermum helicacabum* L. International Journal of Biology and Chemical Sciences. 2(1): 14 – 20.

Jha S, Sahu NP, Mahato SB. 1988. Production of the alkaloids emetine and cephacline in callus cultures of *Cephaelis ipecacuanha*. Planta Medica. 54: 504 – 506.

Joshee N, Biswas BK, Yadav AK. 2007. Somatic Embryogenesis and Plant Development in *Centella asiatica* L., a High Prized Medicinal Plant of the Tropics. HortScience. 42(3): 633-637.

Joshi K, Chaturvedi P, Shubhpriya. 2013. Efficient *in vitro* regeneration protocol of *Centella asiatica* (L.) Urban: An endemic and underutilized nutraceutical herb. Afr. J. Biotechnol. 12(33): 5164 – 5172.

Joshi M, Dhar U. 2003. *In vitro* propagation of *Saussurea aobvallata* (DC.) Edgew. – an endangered ethnoreligious medicinal herb of Himalaya. Plant Cell Rep. 21: 933–939.

Kamada H. 1985. Artificial seeds. In: Practical Technology on the Mass Production of Clonal Plants. Tokyo: CMC Publisher: 48.

Karthikeyan K, Chandran C, Kulothungan S. 2009. Rapid clonal multiplication through *in vitro* axillary shoot proliferation of *Centella asiatica* L. Indian J. Biotechnol. 8: 232 – 235.

Karuppusamy S, Pullaiah T. 2007. *In vitro* shoot multiplication of *Bupleurum distichophyllum* Wight. A native medicinal plant of southern India. Plant Tissue Culture and Biotechnology 17(2): 115 – 124.

Khan MAA, Islam MT, Rahman MA, Ahsan Q. 2011. Antibacterial activity of different fractions of *Commelina benghalensis* L. *Der Pharmacia Sinica Pelagia*; 2(2): 320 – 326.

Khan MY, Aliabbas S, Kumar V, Rajkumar S. 2009a. Recent advances in medicinal plant biotechnology. Indian J. Biotechnol. 8: 9 – 22.

Khan S, Mirja KJ, Tayyab Md, Abedin MZ. 2009b. RAPD profile for authentication of medicinal plant *Glycirrhiza glabra* L. MAPSB 3 (1): 48-51.

Khan SA, Huq AM. 1975. Medicinal Plants of Bangladesh (A preliminary list) BARC, Dhaka: 1 – 25.

Khan, N. and Rashid, A, 2006. A study on the indigenous medicinal plants and healing practices in Chittagong Hill tracts (Bangladesh). African Journal of Traditional, Complementary and Alternative Medicines. 3: 37 – 47.

Kikowska M, Thiem B. 2011. *Eryngium maritimum* L. – *in vitro* cultures of a rare medicinal plant. Business Meets Science to cooperate in current topics. Bioconnect Conference, Poznań 15.05.2011–16.05.2011.

Kim MK, Soomer HE, Bongarten BC, Merkle SA. 1997. High frequency induction of adventitious shoots from hypocotyls segments of *Liquidamber styraciflua* L. by thidiazuron. Plant Cell Rep 16: 536–540.

Kim YH, Janick J. 1987. Production of synthetic seeds of celery. Hort Sci 22: 89 (Abstr.)

Kinoshita I, Saito A. 1990. Propagation of Japanese white birch by encapsulated axillary buds. (1) Regeneration of plantlets under aseptic conditions J. Jpn. For. Soc. 72: 166 – 170.

Kirtikar KR, Basu BD. 1975. Indian medicinal plants. Vol. II, Joyyed Press, New Delhi.

Kirtikar KR, Basu BD. 1987. Indian Medicinal Plants. (Vol. i-iv) Lalit Mohan Basu MB. 49, Leader Road, Allahbad, India.

Kirtikar KR, Basu BD. 1995. Indian Medicinal Plants, Plates (Vol. i-iv) Sundhindra Nath Basu MB., At the Panini Office, Bahaduraganj, Allahbad, India.

Kitto S, Janick J. 1985. Production of synthetic seeds by encapsulating asexual embryos of carrot. J Amer Soc Hort Sci. 110: 277–288.

Kumar GK, Thomas TD. 2012. High frequency somatic embryogenesis and synthetic seed production in *Clitoria ternatea* Linn. Plant Cell Tiss Org Cult. 110: 141–151.

Kumar S, Rai MK, Singh N, Mangal M. 2010. Alginate-encapsulation of shoot tips of jojoba [*Simmondsia chinensis* (Link) Schneider] for germplasm exchange and distribution. Physiol Mol Biol Plants. 16(4): 379–382.

Kumar V, Chandra S. 2014. High frequency somatic embryogenesis and synthetic seed production of the endangered species *Swertia chirayita*. Biologia Versita. 69(2): 186–192.

Lahiri K, Mukhopadhyay MJ, Desjardins Y, Mukhopadhyay S. 2012. Rapid and stable *in vitro* regeneration of plants through callus morphogenesis in two varieties of *Mucuna pruriens* L. – an anti Parkinson's drug yielding plant. Nucleus. 55: 37–43.

Lattoo SK, Bamotra S, Dhar RS, Khan S, Dhar AK. 2006. Rapid plant regeneration and analysis of genetic fidelity of *in vitro* derived plants of *Chlorophytum arundinaceum* Baker–an endangered medicinal herb. Plant Cell Rep 25: 499–506.

Liu CZ, Gao M, Gua B. 2008. Plant regeneration of *Erigeron breviscapus* (vant.) Hand. Mazz. and its chromatographic fingerprint analysis for quality control. Plant Cell Rep. 27: 39 – 45.

Liu CZ, Murch SJ, El-Demerdash M, Saxena PK. 2004. *Artemisia judaica* L. micropropagation and antioxidant activity. J. Biotechnol. 110: 63 – 71.

Loc NH, Duc DT, Kwon TH, Yang MS. 2005. Micropropagation of zedoary (*Curcuma zedoaria* Roscoe) - a valuable medicinal plant, Plant Cell Tiss. Organ Cult. 81: 119 – 122.

Loc NH, Tam An NT. 2010. Asiaticoside Production from *Centella* (*Centella asiatica* L. Urban) Cell Culture. Biotechnology and Bioprocess Engineering. 15: 1065 – 1070.

Ma XM, Wu CF, Wang GR. 2011. Application of artificial seeds in rapid multiplication of *Pseudostellaria heterophylla*. Afri J Biotechnol. 10(70): 15744–15748.

Machii H. 1992. *In vitro* growth of encapsulated adventitious buds in mulberry *Morus alba* L. Japanese J Breed. 42: 553–559.

Mahendran G, Devi K Lavanya, Bai V Narmatha. 2006. Micropropagation of *Spilanthes paniculata* Wall ex. DC. *Plant Cell Biotech. Molec. Biol*. 7(1-2): 85 – 88.

Mahesh R, Muthuchelian K, Maridass M, Raju G. 2012. *In vitro* propagation of *Commelina ensifolia* R.Br. *Botanical Report*. 1(1):10 – 13.

Maitra MK, Akhter SH. 2011. Neotectonics in Madhupur Tract and its surrounding floodplains. Dhaka University Journal of Earth and Environmental Sciences. 1(2): 83-89.

Mandal J, Patnaik S, Chand PK. 2000. Alginate encapsulation of axillary buds of *Ocimum americanum* L. (hoary basil), *O. basilicum* L. (sweet basil), *O.gratissimum* L. (shrubby basil), and *O. sanctum* L. (sacred basil). In Vitro Cell Dev Biol Plant. 36:287–292.

Martin KP. 2002. Rapid propagation of *Holostema ada* – Kodien schult. A rare medicinal plant, through axillary bud multiplication and indirect organogenesis. Plant Cell Rep. 21: 112 – 117.

Martin KP. 2003. Plant regeneration through somatic embryogenesis on *Holostemma ada* – kodien, a rare medicinal plant. Plant Cell Tiss. Org. Cult. 72: 79 – 82.

Martin KP. 2004. Plant regeneration through somatic embryogenesis in medicinally important *Centella asiatica* L. In Vitro Cell Dev Biology- Plant. 40: 586 – 591.

Maruyama E. 1996. Micropropagation of bolaina blanca (*Guazuma crinita* Mart.), a fast – growing tree in the Amazon region. J For Res. 1: 211–217.

Matsubara S, Chen D. 1989. *In vitro* production of garlic plants and field acclimatization. Hort Sci. 24: 676–679.

Mia MMK, Kadir MF, Hossan MS, Rahmatullah M. 2009. Medicinal plants of the Garo tribe inhabiting the Madhupur forest region of Bangladesh. *Am.-Eurasian J. Sustain. Agric., 3(2)*: 165 – 171.

Miachir JI, Romani VLM, Amaral AFDC, Mello MO, Crocomo OJ, Melo M. 2004. Micropropagation and callogenesis of *Curcuma zedoaria* Roscoe, Sci. Agric. (Piracicaba, Braz.). 61: 422 – 432.

Mishra B, Sangwan RS, Mishra S, Jadaun JS, Sabir F, *et al*. 2014. Effect of cadmium stress on inductive enzymatic and nonenzymatic responses of ROS and sugar metabolism in multiple shoot cultures of Ashwagandha (*Withania somnifera* Dunal). Protoplasma (in press, doi: DOI 10.1007/s00709-014-0613-4)

Mohanraj R, Ananthan R, Bai VN. 2009. Production and storage of synthetic seeds in *Coelogynus breviscapa* lindl. Asian J Biotech. 1: 124–128.

Mohapatra H, Barik DP, Rath SP. 2008. *In vitro* regeneration of medicinal plant *Centella asiatica*. Biol. Plantarum.52(2): 339 – 342.

Muhammad I, Ishfaq AH, Maurizio M, Touqeer A, Nadeem AA, Alvaro S. 2010. *In vitro* storage of synthetic seeds: Effect of different storage conditions and intervals on their conversion ability. Afri J Biotechnol. 9(35): 5712–5721.

Mulabagal V, Tsay HS. 2004. Plant cell culture – an alternative and efficient source for the production of biologically important secondary metabolites. In J. Appl. Sci. Eng. 2(1): 29-48.

Munshi MK, Hossain SN, Islam R, Hakim L, Hossain M. 2001. Micropropagation of *Mimosa pudica* L. through *in vitro* culture and using gama irradiation. Plant Tissue Cult. 11(2): 151 – 157.

Muralidharan EM, Mascarenhas AF. 1995. Somatic embryogenesis in *Eucalyptus citrisdora*. In: Jain S. Gupta P, Newton R (eds). In: somatic embryogenesis in woody plants. Angiosperms Kluwer Dordrecht. 2: 23–40.

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

Murashige T. 1978. The impact of Plant Tissue Culture on Agriculture. In: Frontiers Plant Tissue Cultures, Thrope T (Ed.) International Association for Plant Tissue Culture: University of Calgary, Alberta:15–26

Mustafa Natali R, Ward de Winter, Frank van Iren, Robert Verpoorte. 2010. Initiation, growth and cryopreservation of plant cell suspension cultures. PROTOCOL: 1 – 28.

Mutasim MK, Khadiga G, Elaleem A, Rasheid SM. 2010. Callus formation and organogenesis of potato (*Solanum tuberosum L.*) cultivar almera. Journal of Phytology. 2(5): 40 – 46.

Muthiah JVL, Shunmugiah KP, Manikandan R. 2013. Genetic fidelity assessment of encapsulated *in vitro* tissues of *Bacopa monnieri* after 6 months of storage by using ISSR and RAPD markers. Turk J Bot 37: 1008–1017.

Nagakubo T, Nagasawa A, Ohkawa H. 1993. Micropropagation of garlic through *in vitro* bulblet formation. Plant Cell Org Cult. 32: 175–183.

Naik SK, Chand PK. 2006. Nutrient–alginate encapsulation of *in vitro* nodal segments of pomegranate (*Punica granatum* L.) for germplasm distribution and exchange. Sci Hort. 108:247–252.

Nalawaade SM, Sagare AP, Lee CY, Kao CL, Tsay HS. 2003. Studies on tissue culture of Chinese medicinal plant resources in Taiwan and their sustainable utilization. Bot. Bull. Acad. Sin. 44: 79 – 98.

Nasrin S, Hossain MM, Khatun A, Alam MF, Mondal RK. 2003. Inductin and evaluation of somaclonal variation in potato (*Solanum tuberosum L.*). Online Journal of Biological Sciences. 3(2): 183 – 190.

Nath S, Alak KB. 2003. *In vitro* method for propagation of *Centella asiatica* (L) Ur Lucas ban by shoot tip culture. J. Plant Biochem Biotechnol. 12: 167 – 169.

Nayak, S., 2000. *In vitro* multiplication and microrhizome induction in *Curcuma aromatica salisb*. J. Plant Growth Regul. 32: 41 – 47.

Nhut DT, Thuy-Tien TN, Ngoc-Huong MT, Thanh-Hien NT, Huyen PX, Luan VQ, Jaime A. 2005. Teixeira da Silva. Artificial seeds for propagation and preservation of *Cymbidium* spp. Propag Ornam Plants. 5: 67–73.

Nigra HM, Caso OH, Guilietti AM. 1987. Production of solasodine by calli from different parts of *Solanum elaeagnifolium* Cav. Plants. Plant Cell Rep. 6: 535 – 537.

Nin S, Schiff S, Bennici A, Magherini R. 1994. *In vitro* propagation of *Artemisia absinthium* L. Adv Hortic Sci. 8: 145-147.

Okello J, Ssegawa P. 2007. Medicinal plants used by communities of Ngai Subcounty, Apac District, northern Uganda. *African Journal of Ecology*. 45: 76 – 83.

Ozlem YC, Aynur G, Fazilet VS. 2010. Large Scale Cultivation of Plant Cell and Tissue Culture in Bioreactors. Transworld Research Network. 1, 54.

Padua de LS, Bunyapraphatsara N, Lemmens RHMJ. 1999. *Medicinal and Poisonous Plants*, Vol. 1. Plant Resources of South East Asia 12/1, Backhuys Publishers, Leiden, Netherlands.

Panathula CS, Mahadev MD, Naidu CV. 2014. Silver thiosulphate enhance *in vitro* regeneration of *Centella asiatica* (L.) –An important antijaundice medicinal plant. Int. J. Med. Arom. Plants. 4(2): 82 – 87.

Pasha MK, Zaman MB. 1988. Name Changes in Plants of Bangladesh III. Chittagong University Studies. Part II. Sci. Vol 12(1): 107 – 112.

Patel S, Mehta A, Pandey RK. 2007. *In vitro* regeneration and callus formation from different parts of seedling of Mucuna pruriens BAK. A valuable medicinal plant. The Biosean. An Int. Quarterly J. of Life Sci. 2(1): 63 – 66.

Patra A, Rai B, Rout GR, Das P. 1998. Successful regeneration from callus cultures of *Centella asiatica* (L.) Urban. Plant Growth Regul. 24: 13 – 16.

Pereira AMS, Amui SF, Bertoni BW, Moraes RM, France SC. 2003. Micropropagation of *Anemopaegma arvense*: Conservation of an endangered medical plant. Planta Med 69: 571–573.

Piatczak E, Weilanek M, Wysokinska H. 2004. Liquid culture system for shoot multiplication and secoiridoid production of micropropagated plants of *Centaurium erythreae* Rafn. Plant Sci. 168: 431 – 437.

Pierik RLM. 1987. *In vitro* culture of higher plants. Martinus Nijhoff Publishers, Dordrecht/ Boston/ Lancaster: 80 – 81.

Pilli RA, de Oliveira MCF. 2000. Recent progress in the chemistry of the Stemona alkaloids. Nt. Prod. Rep. 17: 117 – 127.

Prajapati ND, Purohit SS, Sharma AK, Kumar T. 2006. A handbook of medicinal plants. Hindustan Press, Agrobios, India. pp.129.

Prakash ES, Valli K, Sairam PS, Reddy P, Rao KR. 1999. Regeneration of Plants from seed-derived callus of *Hybanthus enneaspermus* L. Muell. a rare enthnobotanical herb. Plant Cell Rep. 18: 873 – 878.

Qaiser M, Jafri SMH. 1975. *Commelina benghalensis*, in Flora of Pakistan, Ali SI, Qaiser M (eds.), St. Louis: University of Karachi and Missouri botanical garden: 10.

Quarishi A, Mishra SK. 1998. Micropropagation of nodal explant from adult trees of *Cleistanthus collinus*. Plant cell Rep. 17: 430 – 433.

Rady MR, Hanafy MS. 2004. Synthetic seed technology for encapsulation and regrowth of *in vitro*-derived *Gypsophila paniculata* L. shoot-tips. Arab J Biotech. 7(2): 251–264.

Raghu AV, Martin G, Priya V Geetha SP, Balachandran I. 2007. Low Cost Alternatives for the Micropropagation of *Centella asiatica*. J. Plant Sci. 6: 592 – 599.

Rahman MA, Bari MA. 2013. Antibacterial Activity of Cell Suspension Cultures of Castor (*Ricinus communis* L. cv. Roktima). European Journal of Medicinal Plants. 3(1): 65 – 77.

Rahman MM. 2008. Encyclopedia of Flora and Fauna of Bangladesh, Angiosperm, Dicotyledones. Vl: 155 – 156.

Rahmatullah M, Tajbilur Kabir AAB, Rahman MM, Hossan MS, Khatun Z, Khatun MA, Jahan R. 2010. Ethnomedicinal Practices among a Minority Group of Christians Residing in Mirzapur Village of Dinajpur District, Bangladesh. Advances in Natural and Applied Science. 4(1): 45 – 51.

Rani DN, Nair GM. 2006. Effects of plant growth regulators of high frequency shoot multiplication and callus regeneration of an important Indian medicinal plant, Nirgundi (*Vitex negundo L.*). *In Vitro* Cell Dev Biol Plant 42: 69–73.

Rao RS, Ravishankar GA. 2002. Plant cell cultures; Chemical factories of secondary metabolites. Biotechnol. Adv. 20: 101 – 153.

Rao YV, Shankar AR, Lakshmi TVR, Rao KGR. 2004. Plant regeneration in *Physalis pubescens* L. and its induced mutant. Plant Tissue Cul. 14(1): 9 –15.

Rathore MS, Shekhawat NS. 2011. Micropropagation of *Vitex negundo* through *in vitro* bud proliferation. Aric. Sci. Res. J. Vol. 1(8): 191 – 198.

Ravishanker GA, Ramachandra Rao. 2000. Biotechnological production of phyto-pharmaceuticals. J. Biochem. Mol. Biol. Biophys. 4: 73 – 102.

Ray A, Bhattacharyaa S. 2008. Storage and plant regeneration from encapsulated shoot tips of *Rauvolfia serpentina*-an effective way of conservation and mass propagation. S Afr J Bot. 74: 776–779.

Reddy MC, Murthy KSR, Pullaiah T. 2012. Synthetic seeds: A review in agriculture and forestry. African J Biotechnol. 11(78): 14254–14275.

Redenbaugh K, Slade D, Viss P, Fujii J. 1987. Encapsulation of somatic embryos in synthetic seed coats. Hort Sci. 22: 803–809.

Rout GR, Samantaray S, Das P. 2000. *In vitro* multiplication and propagation of medicinal plants. Biotechnology Advances. 18: 91 – 120.

Roy B, Mandal AB. 2008. Development of synthetic seed involving androgenic and pro-embryos in elite indica rice. Ind J Biotechnol. 7: 515–519.

Sabir F, Sangwan RS, Kumar R, Sangwan NS. 2012. Salt stress-induced responses in growth and metabolism in callus cultures and differentiating *in vitro* shoots of Indian ginseng (*Withania somnifera* Dunal). J Plant Growth Regul. 31: 537–548

Sahoo Y, Chand PK. 1998. Micropropagation of *Vitex negundo* L. a woody aromatic medicinal shrub, through high frequency axillary shoot proliferation. Plant Cell Rep 18: 301–307.

Said HM. 1995. Development of Herbal Medicines in Modern Medical Therapy. Hamdard Foundation. Dhaka.

Saiprasad GVS. 2001. Artificial seed and their application. Resonance. 5: 39–47

Sakai A, Kobayashi S, Oiyama I. 1990. Cryopreservation of nucellar cells of navel orange (*Citrus sinensis* Osb. var. *brasiliensis* Tanaka) by vitrification, Plant Cell Reports. 9: 30 – 33.

Salvi ND, George L, Eapen S. 2002. Micropropagation and field evaluation of micropropagated plants of termaric. Plant Cell Tiss. Org. Cult. 68: 143 – 151.

Sarker KP, Islam R, Islam A, Hoque A, Joarder OI. 1996. Plant regeneration of *Rouwolfia serpentina* by organogenesis from callus cultures. Plant Tissue Cult. 6(1): 63 – 65.

Sathyanarayana N, Bharathkumar TN, Vikas PB and Rajesha R. 2008. *In vitro* clonal propagation of *Mucuna pruriens* var. *utilis* and its evaluation of genetic stability through RAPD markers. African J. Biotech. 7(8): 973 – 980.

Scragg AH, Alan EJ. 1997. Production of triterpenoid quassin and cell suspension cultures of *Picrasana quassioides* Bennett. Plant Cell Rep. 5:356 – 359.

SEDF (South Asia Enterprise Development Facility). 2003. Medicinal Plants marketing in Bangladesh. A market study report. SEDF-Intercooperation, Dhaka.

Selvakumar V, Anbudurai PR, Balakumar T. 2001. *In Vitro* Propagation of the medicinal plant *Plumbago zeylanica* L. through nodal explants. *In Vitro Cell. Dev. Biol. Plant.* 37: 280 – 284.

Senthikumar P, Paulsamy S, Vijayakumar KK, Kalimuthu K. 2007. *In vitro* regeneration of the medicinal herb of Nilgiri Shola, *Acmella calva* L. from leaf derived callus. Plant Tissue Cult & Biotech. 17(2): 109 – 114.

Sharma N, Chandel KPS, Srivastava VK. 1991. *In vitro* propagation of *Coleus forskohlii* Briq. a threatened medicinal plant. Plant Cell Rep. 10: 67 – 70.

Shashikala CM, Shashidhara S, Rajeshekharan PE. 2005. *In vitro* regeneration of *Centella asiatica* L. Plant Cell Biotech. and Mol. Biol. 6: 53 – 56.

Shawky AB. 2006. A synthetic seed method through encapsulation of *in vitro* proliferated bulblets of garlic (*Allium sativum* L.). Arab J. Biotech. 9(3): 415–426.

Sheeba E, Palanivel S, Parvathi S. 2013. Effect of plant growth regulators on callus induction in *Physalis minima* Linn. IJIRSET. 2(9): 4847 – 4851.

Siddique NA, Kabir MM, Bari MA. 2006. Comparative *in vitro* study of plant regeneration from nodal segments derived callus in *Aristolochia indica* Linn and *Hemidesmus indicus* (L.) R. Br. Endangered medicinal plants in Bangladesh. Journal of Plant Sciences. 1(2): 106 – 118.

Sin WLTT, Teng MC. 2002. Explant preparation affects culture initiation and regeneration of *Panax ginseng* and *Panax quinquefolius*. Plant Cell Tiss. Org. Cult. 68: 233 – 239.

Singh AK, Sharma M, Varshney R, Agarwal SS, Bansal KC. 2006a. Plant regeneration from alginate to encapsulated shoot tips of *Phyllanthus amarus* Schum and Thonn, a medicinally important plant species. In Vitro Cell Dev Biol Plant. 42:109–113.

Singh AK, Varshney R, Sharma M, Agarwal SS, Bansal KC. 2006b. Regeneration of plants from alginate-encapsulated shoot tips of *Withania somnifera* (L.) Dunal, a medicinally important plant species. J Plant Physiol. 163:220–223.

Singh M, Chaturvedi R. 2012. Evaluation of nutrient uptake and physical parameters on cell biomass growth and production of spilanthol in suspension cultures of *Spilanthes acmella* Murr. Bioprocess Biosyst Eng. 35: 943–951.

Singh SK, Rai MK, Asthana P, Pandey S, Jaiswal VS, Jaiswal U. 2009. Plant regeneration from alginate-encapsulated shoot tips of *Spilanthes acmella* (L.) Murr., a medicinally important and herbal pesticidal plant species. Acta Physiol Plant. 31: 649–653.

Singh SK, Rai MK, Asthana P, Sahoo L. 2010. Alginate-encapsulation of nodal segments for propagation, short-term conservation and germplasm exchange and distribution of *Eclipta alba* (L.). Acta Physiol Plant. 32: 607–610.

Sinha S, Hassan AKMS, Roy SK. 2005. Regeneration of *Hydnocarpus kurzi* (King.) Warb. – A red listed medicinal plant. Plant Tissue Cult. Biotech. 15(2): 113 – 119.

Sivakumar G, Alagumanian S, Rao. 2006. High Frequency *in vitro* Multiplication of *Centella asiatica*: An Important Industrial Medicinal Herb. Eng. Life Sci. 6 No. 6: 597–601.

Sivakumar G. 2006. Bioreactor technology: a novel industrial tool for high-tech production of bioactive molecules and biopharmaceuticals from plant roots. Biotechnol. J. 1 (12): 1419 – 1427.

Slade D, Fu JA, Redenbaugh K. 1989. Artificial seeds: A method for the encapsulation of somatic embryos. J Tiss Cult Method. 12: 179–182.

Sommer HE, Claderas LS. 1981. *In vitro* methods applied to forest trees. In: Thorp TA (ed.) Plant Tissue culture – Methods and Applications in Agriculture. Acad. Press. New York: 349 – 358.

Song IG REL, Ekrem G REL, Zeki KAYA. 2002. Establishment of Cell Suspension Cultures and Plant Regeneration in Sugar Beet (*Beta vulgaris* L.). Turk J. Bot. 26. 197 – 205.

Soniya EV, Das MR. 2002. *In vitro* micropropagation of *Piper longum* – an important medicinal plant. Plant Cell, Tissue and Organ Cult. 70: 325- 327.

Srivastava S, Srivastava AK. 2007. Hairy root culture for mass-production of high-value secondary metabolites. Cr. Rev. Biotechn. 27 (1): 29 – 43.

Srivastava V, Khan SA, Banerjee S. 2009. An evaluation of genetic fidelity of encapsulated microshoots of the medicinal plant: *Cineraria maritima* following six months of storage. Plant Cell Tiss Org Cult. 99:193–198.

Ssenyonga M, Brehony E. 1993. Herbal medicine-its use in treating some symptoms of AIDS. International Conference on AIDS, 6-11 Jun 1993, CONCERN, Masaka, Uganda, 9, 75.

Stanly C, Keng CL. 2007. Micropropagation of *Curcuma zedoaria* Roscoe and *Zingiber zerumbet* Smith. Biotechnology. 6: 555 – 560.

Stephen R, Jayabalan N. 2000. Artificial seed production in coriander (*Coriandrum sativum* L.). Plant Tiss Cult. 10: 45–49.

Sudha CG, Krishnan PM, Pushpangadan P. 1998. *In vitro* propagation of *Holostemma annulare* (Roxb.) K. Schum; a rare medicinal plant. In Vitro Cell. Dev. Biol. Plant. 33: 57 – 63.

Sudha GC, Seeni S. 1994. *In vitro* multiplication and field establishment of *Adhatoda beddomii* CB Clarke, a rare medicinal plant. Plant Cell Rep. 10: 67–70.

Sugla T, Purkayastha J, Singh SK. 2007. Micropropagation of *Pongamia pinnata* through enhanced axillary branching. In Vitro Cell Dev Biol Plant 43: 409–414.

Sultana RS, Bari MA, Rahman MH, Rahman MM, Siddique NA, Khatun N. 2004. *In vitro* rapid regeneration of plantlets from leaf explant of watermelon (*Citrullus lanatus* Thumb). Biotechnology. 3(2): 131 – 135.

Tabassum B, Ahmad IA, Farooq AM, Rehman Z, Latif Z, Husnain T. 2010. Viability assessment of *in vitro* produced synthetic seeds of cucumber. African J Biotechnol. 9(42): 7026–7032.

Tabuti JR, Lye KA, Dhillion SS. 2003. Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration, *Journal of Ethnopharmacology*. 88: 19 – 44.

Tan SH, Musa R, Ariff A, Maziah M. 2010. Effect of plant growth regulators on callus, cell suspension and cell line selection for flavonoid production from pegaga (*Centella asiatica* L. urban). Am. J. Biochem. & Biotech. 64: 284 – 299.

Thirugnanasampandan R, Mutharaian VN, Narmatha BV. 2009. *In vitro* propagation and free radical studies of *Smilax zeylanica* Vent. African Journal of biotechnology. 8 (3): 395 – 400.

Thiruvengadam M, Jayabalan N. 2000. *In vitro* regeneration of plantlets from internode-derived callus of *Vitex negundo* L. In Vitro J. Plant Biotech. 2: 151 – 155.

Thobunluepop P, Pawelzik E, Vearasilp S. 2009. Possibility of sweet corn synthetic seed production. Pak J Biol Sci. 12: 1085–1089.

Ticktin T, Dalle SP. 2005. Medicinal plant use in the practice of midwifery in rural Honduras. J. Ethnopharmacol. 96(1-2): 233 – 248.

Tiwari KN, Sharma NC, Tiwari V, Singh BD. 2000. Micropropagation of *Centella asiatica* (L.), a valuable medicinal herb. Plant Cell Tiss Org. 63:179 – 185.

Tripepi RR. 1997. Adventitious shoot regeneration. In: Geneve RL, Preece JE, Merkle SA ed. Biotechnology of ornamental plants. Wallingford, UK: CAB International: 55 – 71.

Wang GR, Qi NM. 2010. Influence of mist Intervals and aeration rate on growth and secondary metabolite production of *Pseudostellaria heterophylla* adventitious roots in a Siphon-mist Bioreactor. J. Biotechnol Bioeng. 15: 1–5. coordinated behavior of cell as an embryogenic group. Annals of Botany. 57: 443–462.

Wawrosch C, Malla RR, Kopp B. 2001. Clonal propagation of *Lilium nepalense* D. Don, a threatened medicinal plant of Nepal. Plant Cell Rep. 10: 457–460.

WHO (World Health Organization). 1976. African Traditional Medicine. AFRO Technical Report Series, No. 1.

Wink M, Alfermann AW, Franke R, Wetterauer B, Distl M, Windhovel J, Krohn O, Fuss E, Garden H, Mohagheghzaden A, Wildi E, Ripplinger P. 2005. Sustainable bioproduction of phytochemicals by plant *in vitro* cultures: anticancer agents. Plant Genetic Resources. 3: 90 – 100.

Yusuf M, Begum J, Hoque MN, Chowdhury JU. 2009. Medicinal Plants of Bangladesh, 2nd ed. BCSIR Laboratories Chittagong. 340.

Yusuf M, Chowdhury JU, Wahab MA, Begum J. 1994. Medicinal plants of Bangladesh. Bangladesh council of Scientific and Industrial Research, Dhaka-1205, Bangladesh: 1 – 540.

Zhang W, Curtin C, Franco C. 2002. Towards manipulation of postbiosynthetic events in secondary metabolism of plant cell cultures. Enzyme Microb Technol. 30: 688–696.

CHAPTER - V

APPENDIX

APPENDIX – I

Appendix 1. Questionnaire to collect of Socioeconomic and Ethnobotanical information from the respondents of Madhupur Tract.

A. Socioeconomic information:

Name:……. Fathers/husband's Name:……....

Village:.....Union:.....Thana/Upazila:.....

District:…….Education:…….....Religion:.....

Sex:..... Age..... Profession:…… Family member: .....

Male:..... Female:…….. Total Land:..... Medicinal plants Land: .....

Number of Patients (Per week)…..... Income(yearly)…….....

B. Botanical and Ethnobotanical information:

Types of diseases used to treat	Types of plant specific to disease used	Local name of the plant	Monocot/ dicot	Lifespan	Habit	Mode of propagation	Usable parts of plants	Mode of administer action

APPENDIX - II

Appendix 2. Compositions of MS (Murashige and Skoog. 1962) Medium used in the Present Study.

Constituents	Concentrations (mg/l)
Macro-nutrients	
KNO_3	1900.00
NH_4NO_3	1650.00
$CaCl_2.2H_2O$	440.00
$MgSO_4.7H_2O$	370.00
KH_2PO_4	170.00
Micro-nutrients	
KI	0.83
H_3BO_3	6.20
$MnSO_4.\ 4H_2O$	22.30
$ZnSO_4.\ 7H_2O$	8.60
$Na_2MoO_4\ .\ 2H_2O$	0.25
$CuSO_4.\ 5\ H_2O$	0.025
$CoCl_2.\ 6H_2O$	0.025
$Na_2EDTA.\ 2H_2O$	37.30
$FeSO_4.\ 7H_2O$	27.80
Organic-nutrients	
Myo-inositol	100.00
Nicotinic acid	0.50
Pyridoxinic HCl	0.50
Thiamine HCl	0.50
Glycine	2.00
Sucrose	30000.00
Agar powder	8000.00
pH	5.8