INFLUENCE OF DIFFERENT FACTORS ON PLANT REGENERATION SYSTEM OF JUTE SPECIES

By

HASINA BEGUM

REGISTRATION NO. 25245/ 00360

A Thesis

Submitted to the Faculty of Agriculture,
Sher-e-Bangla Agricultural University, Dhaka,
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

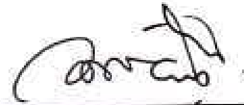
IN

GENETICS AND PLANT BREEDING

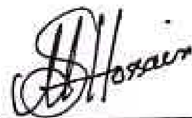
SEMESTER: JANUARY-JUNE, 2006

Approved by:

(Dr. Asma Khatun)
Chief Scientific Officer
Bangladesh Jute Research Institute
Supervisor



(Dr. Md. Shahidur Rashid Bhuiyan)
Professor
Dept. of Genetics and Plant Breeding
Sher-e-Bangla Agricultural University
Dhaka-1207
Co-Supervisor

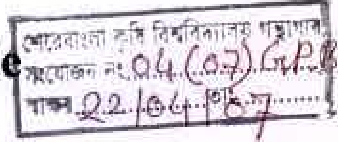


(Dr. Md. Sarowar Hossain)
Chairman
Examination Committee

Government of the Peoples Republic of Bangladesh

Bangladesh Jute Research Institute

Manik Mia Avenue, Dhaka-1270.



Phone : 9110868, 8121931-2
Fax : 8802-9118415
E-mail: bjri@bdmail. net

CERTIFICATE

This is to certify that thesis entitled ***"INFLUENCE OF DIFFERENT FACTORS ON PLANT REGENERATION SYSTEM OF JUTE SPECIES"*** *submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE in GENETICS AND PLANT BREEDING,*** *embodies the result of a piece of bona fide research work carried out by* ***HASINA BEGUM,*** *Registration No. 25245/00360 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated : 11.02.2007
Dhaka, Bangladesh

(Dr. Asma Khatun)
Supervisor

DEDICATED TO
MY
BELOVED PARENTS

ACKNOWLEDGEMENTS

All praises gratitude are due to the Almighty God, the great, the gracious, merciful and supreme ruler the universe to complete the research work and thesis successfully for the degree of Masters of Science in Genetics and Plant Breeding.

The author expresses the deepest sense of gratitude, sincere appreciation and heartfelt indebtedness to his reverend research supervisor Dr. Asma Khatun, Chief Scientific Officer, Bangladesh Jute Research Institute, Dhaka for his scholastic guidance, innovative suggestion, constant supervision and inspiration, valuable advice and helpful criticism in carrying out the research work and preparation of this manuscript.

The author deem it a proud privilege to acknowledge his gratefulness, boundless gratitude and best regards to his respectable co-supervisor Professor Dr. Shahidur Rashid Bhuiyan, Department of Genetics & Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka for his valuable advice, constructive criticism and factual comments in upgrading the research work.

It is a great pleasure and privilege to express his profound gratitude and sincere regards to Dr. Md. Sarowar Hossain, Associate Professor & Chairman of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka, for his help, criticism, suggestions and provisions of facilities and supports needed to undertake this research work.

Special appreciation and warmest gratitude are extended to Ir. Zabun Nahar, Senior Scientific Officer, BJRI, Dhaka who provided creative suggestions, guidance and constant inspiration from the beginning to the completion of the work. Her contribution, love and affection would persist in the memory of the researcher for countless days.

Heartful thanks and appreciation are due to Md. Golam Mostafa, Senior Scientific Officer, Bangladesh Jute Research Institute, Dhaka for his kind cooperation, important suggestions and precious comments on the study, which smother the way of conducting the research work.

The author humbly desires to acknowledge her heartiest appreciation and cordial thanks to Chandan Kumar Saha, Principal Scientific Officer and Md. Joynul Abedin, Scientific Officer, BJRI, Dhaka who helped so much in conducting the research work.

The author feel much pleasure to convey the profound thanks to her friends Shamal Shuvra, Tutul, Nirmal and other well wishers for their cooperation, cheerfulness and help during the on going of the research. She particularly thankful to Hasem Bhai and Shafic Bhai who heavily encourage her to undertake and complete this research and thesis work.

Last but not least the author expresses her deepest sense of gratitude and heatful thanks to her beloved parents, elder sister, and all the relatives for their blessings ,enduring sacrifice, inspiration ,cooperation forbearance and endless love throughout her life to complete her higher study at Sher-e-Bangla Agricultural University , Dhaka.

Dated:11.02.2007
Dhaka, Bangladesh *The Author*

LIST OF ABBREVIATIONS

Symbols		Acronyms
%	:	Percentage
0.1 N	:	0.1 Normal
BAP	:	6-benzyl amino purine
BBS	:	Bangladesh Bureau of Statistics
CaMV	:	Cauliflower Mosaic Virus
CIP	:	International Potato Centre
DMRT	:	Duncan's Multiple Range Test
dw	:	Distilled Water
e.g.	:	Exempli gratia (by way of example)
et al.	:	et alu=other people
etc.	:	et cetera (means and the rest)
FAO	:	Food and Agriculture Organization
Fig.	:	Figure
g	:	Gram
gl^{-1}	:	Gram per litre
GDP	:	Gross Domestic Product
HCl	:	Hydrocloric acid
$HgCl_2$	:	Mercuric Cloride
hrs.	:	Hours
i.e.	:	ed est (means That is)
IARI	:	Indian Agricultural Research Institute
ICRISAT	:	International Crop Research Institute for the Semi-arid Tropics
IRRI	:	International Rice Research Institute
j.	:	Journal
mgl^{-1}	:	Milligram per litre
ml	:	Mili litre
MS	:	Murashige and Skoog

LIST OF ABBREVIATIONS (Contd.)

Symbols		Acronyms
Na_2-EDTA	:	Sodium salt of ferric ethylene diamine tetraacetate
NAA	:	α-napthelene acetic acid
NaCl	:	Sodium chloride
NaOH	:	Sodium hydroxide
No.	:	Number
nptII	:	Neomycin phosphotransferase II
NS	:	Non-significant
pH	:	Negative logarithm of hydrogen ion concentration (-log $[H^+]$)
req.	:	Required
T-DNA	:	Transfer DNA
TK	:	Taka
UK	:	United Kingdom
USDA	:	United States Department of Agriculture
UV	:	Ultra violet
var.	:	Variety
via	:	By way of
viz.	:	Namely
μg	:	Microgram

INFLUENCE OF DIFFERENT FACTORS ON PLANT REGENERATION SYSTEM OF JUTE SPECIES

ABSTRACT

Experiments were conducted during the period from August 2005 to May 2006 in the Genetic Engineering Laboratory, Department of Cytogenetics, Bangladesh Jute Research Institute (BJRI), Dhaka. In the first experiment, five varieties of *Corchorus* sp were used to investigate their *in vitro* germination percentage. And in the second experiment, five varieties of *Corchorus* sp. were used to investigate their *in vitro* regeneration performance. In the third experiment, varieties of *Corchorus* sp were used to investigate their *in vitro* shoot regeneration from the explants (cotyledons) of *C. capsularis* and *C. oiltorius* with different hormonal concentrations. Fourth to ninth experiments were conducted to show the different factors on plant regeneration from cotyledons of *C. capsularis* and *C. olitorius*. In the first experiment, seed germination percentage was found highest in CVE-3 in cotton-based medium (98.88%) compared to agar-based medium (87.17%). Among the phytohormone combination, MS+ 2 mg/l BAP + 0.5 mg/l IAA showed the highest shoot regeneration (60.66%). Among the varieties, CVE-3 was highly responsive to shoot regeneration (67.99%). MS media without hormone (MSO) was used for root formation. The variety CVE-3 response better than O-72 towards shoot regeneration at different pH level. Among the sucrose concentration 3% (30mg/l) showed the highest shoot regeneration (87.49%). Among the different concentration of vitamins x2 times (3.0mg/l) showed the highest shoot regeneration. Using different concentration of surfactant 0.1% surfactant showed the highest shoot regeneration (91.663%). In the NaCl experiment, among the three varieties CVE-3 was found highly responsive to shoot regeneration (97.22%) and 0.125, 0.25 and 0.50 (%) shows the better responsive to shoot regeneration. Using different concentration of $FeSO_4$, x2 times (3.0mg/l) showed the highest shoot regeneration. The variety CVE-3 response better than O-72 towards shoot regeneration at different concentration of $FeSO_4$.

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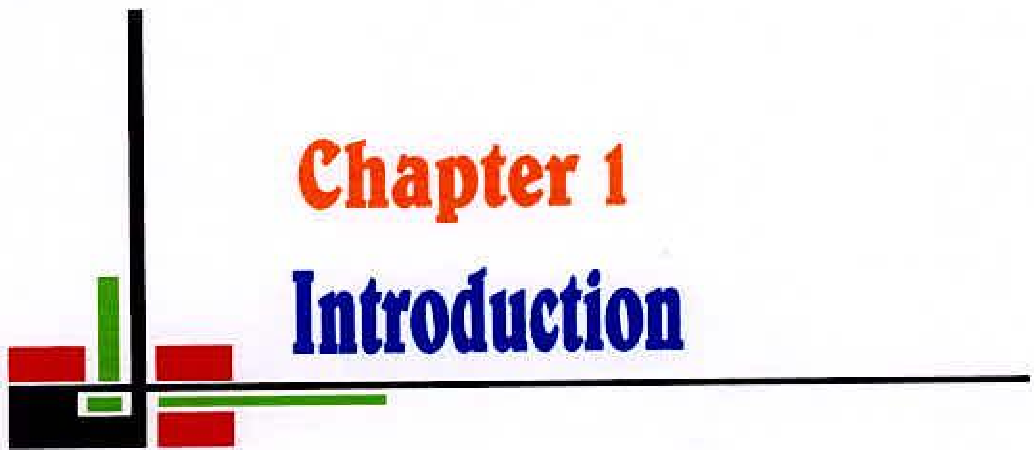

Chapter 1
Introduction

Jute is a fibre yielding crop obtained from the bast of two cultivated species of genus *Corchorus* namely *Corchorus capsularis* and *Crochorus olitorius* of the Tiliaceae family. The genus *Corchorus* has around 50-60 species, but over 170 *Corchorus* names are given in index kewensis(Bhajwani 1988). These species are found throughout the tropics and subtropics. *Corchorus capsularis* is called "Deshi pat", "Tita pat" or "White pat" whereas *Crochorus olitorius* is called Tosa pat, produce better quality fibres, i.e. finer, softer, stronger and lustrous than those of *Corchrous capsularis* (Islam, 1981). The fibres of *Corchorus capsularis* are ordinarily whitish and in *Corchorus olitorius* the fibres are either yellowish golden or grayish in colour. Jute is the most important bast fibre crop next to cotton. Jute is not only a major foreign currency earner, but also a major source of employment. It is of prime importance in the rural economics of the regions in which it is grown.

Once upon a time jute was referred to as the Golden Fibre of Bangladesh, because of its immense contribution for to the economy of this country. Considerable number of our population is engaged directly or indirectly in production and processing of jute. Jute exports constitute a major source of foreign exchange (12-13%) earning in Bangladesh and contribute 8% of total GDP (BBS, 2003). During the year of 2004-2005 total supply of raw jute was 48.24 lakh bales, total domestic requirements was 31.21 lakh bales and Bangladesh exports 17.04 lakh bales of raw jute and jute goods and earned about 18000 million Tk. (FAO, 2005).

Jute consitutes a major world crop and it is one of the most important cash crops of Bangladesh. It occupies 5th position after rice, pulses, oil seeds and wheat in respect of cultivated area (BBS, 2003). Bangladesh is second largest producer of jute followed by India and produces the best quality jute and leads the export market. In the year of 2004-2005 the acreage, and production of jute was 450.0 thousand ha. and 810 thousand tones respectively (FAO, 2005). It is extensively used in agricultural and industrial products. Jute is mainly used for manufacturing products for the packaging of grains, sugar, cocoa, coffee and other food crops as well as packaging for cement, fertilizer, salt, cotton etc, which currently account for the consumption of 80% of jute products i.e. hasseian (burlap) and sacks. The use of jute products and accounts for about 15% of the world jute consumption (BBS, 2003). Jute fibre is also used to produce carpet, yarns, cordage, felts and padding decorative fabrics and other items of industrial use.

Despite high socio-economic importance of the jute, cultivation of jute in Bangladesh is increasingly shifting to less productive land with marginal care. Thus creating challenges in dealing with new emerging production constraints. In every year about 7 lakh 67 thousand bales of jute are damaged by insect-pest (Ahmed and Jalil, 1993). Diseases have also an adverse effect on yield (about 5 lakh bales damaged by diseases). Abiotic stresses like drought, flood, low temperature etc. are detrimental to this crop. With the lauching of global compaign for environmental awareness international opinion is being created on jute for its expanded production and use, as it is biodegradable and friendly to the environment. Jute is a plant, all parts of which have extensive uses. A sustainable improvement in jute productivity under less favourable environment can only be achieved with a constant flow of new genetic materials. The existing variability for constraints, like insect-pest and diseases, poor

soil fertility, water stress, fiber quality, photo-insensivity etc. is a serious issue that needs to be addressed (Aggarwal, 2000). One of the major constraints to increase jute productivity is the non-availability of modern varieties with improved plant types.

At present not much success was achieved for jute improvement through conventional breeding methods. New genotypes are, therefore, very much needed to be introduced for jute breeding. Although a number high yielding variety of jute have been released from the Bangladesh Jute Research Institute (BJRI) through conventional breeding techniques, but these techniques have some limitations. It is therefore, very important to explore other means of modern scientific techniques for example, tissue culture or genetic engineering to accelerate the pace of varietal improvement.

Tissue culture techniques can be applied conveniently to overcome the incompatibility barrier through fusion of protoplasts from vegetative cells of interspecific, intergeneric and interfamilial group. But in several instances, callus derived from fused proplasts could not be induced to regenerate plantlets (Rao 1985). From these informations, it is evident that all the tissue culture techniques play a vital role in the enrichment of genetic variability, this particular technique contributed a little in the production of disease and pest resistant plants as well as plants of better agronomic characters in jute.

The chances for availability of new genotype of jute with disease resistance in nature are very remote unless new techniques are launched to create variability. Biotechnology is a recently flourished novel approach and therefore it is very important to explore the science for varietal improvement of jute.

The pre-requisite for the genetic transformation in jute is to establish an efficient regeneration system from different explants to matured fertile plants. Plant regeneration from the cotyledonary petioles were reported earlier from *C. capsularis* (Khatun *et al.*, 1992) and from shoot apices of *C. olitorius* (Khatun, 1993).

Research on plant biotechnology in bast fibre crops especially on jute has been conducted in a few laboratory of Bangladesh and in some other countries like India, China, and in a few laboratories in England. Some achievements have been made in the laboratories of Bangladesh and India using tissue culture techniques. Biotechnological approaches for crop improvement is a new research thurst in Bangladesh.

The history of plant tissue culture begins from 1902, when Haberlandt made the first attempt in *in vitro* isolated cell culture without any success. The first success in tissue culture was reported in carrot and tobacco, when isolated cambial tissue was cultured for long period of time. During the last few decades, the technique of plant tissue culture has been introduced as new and powerful tools for crop improvement and it received high attention of biologists.

The regeneration of plant from tissue or explants is an important and essential genetic manipulation of plants. The totipotency of cell or tissue open up several new contingencies in plant breeding programmes that provided gene manipulation and selection of desirable character. Tissue culture techniques have several advantages over traditional propagation methods: cultures have to start with small plant parts; therefore, only a small amount of space is required to multiply large number of plants.

Cultures are conducted in aseptic environment with the assurance of the production of disease-free materials and without risk of reinfection. High frequency regeneration of plants from *in vitro* cultured tissues is a pre requisite for successful application of tissue culture technique. Cell and tissue culture provides the engineering of this crop to supplement conventional breeding. Thus any improvement in the growth of such cultures could be beneficial in both basic research and applied plant biotechnology. Establishment of an efficient plant regeneration system for the explants of jute (*Corchorus capsularis* and *C. olitorius*) is therefore the prerequisite for introducing foreign gene through genetic transformation.

Based on the above information, the present study was undertaken to study the *in vitro* regeneration performance of the varieties of two cultivated *Corchorus* species.

The specific objectives of the present research work were therefore:

i. *To optimize the in vitro regeneration performance of five varieties of Corchorus capasularis and C. olitorius.*

ii. To see the effect of different levels of pH on plant regeneration of *C. capsularis and C. olitorius.*

iii. To standardize a protocol for shoot regeneration with different concentration of sucrose.

The present study has been divided into nine separate experiments. These are

Experiment-i *In vitro* regeneration of *Corchorus* spp.

Experiment-ii *In vitro* regeneration performance of five varieties of *Corchorus* species

Experiment-iii Optimization of shoot regeneration from the explants of *C. capsularis* with different hormonal concentrations.

Experiment-iv Effect of different levels of pH on plant regeneration from cotyledons of *Corchorus* species

Experiment-v Influence of different concentration of sucrose on plant regeneration from cotyledons of *C. capsularis* and *C. olitorius*

Experiment-vi Influence of different concentration of vitamins on plant regeneration from cotyledons of *C. capsularis* and *C. olitorius*

Experiment-vii Influence of surfactants (pluronic F-68) on plant regeneration from cotyledons of *C. capsularis* and *C. olitorius*

Experiment-viii Influence of NaCl on plant regeneration from cotyledons of of *C. capsularis* and *C. olitorius*

Experiment- ix Influence of $FeSO_4$ on plant regeneration from cotyledons of *C. capsularis* and *C. olitorius*

Chapter 2
Review of literature

Jute is the most important fibre crop of Bangladesh. The crop received much attention by a large number of researchers on various aspects of production and utilization. Improvements of crop plants like jute through conventional method require long time. Plant biotechnology now a day offers many opportunities for breeders with chances to solve certain breeding problems at cellular level. Biotechnological research on jute has been initiated in early sixties (Islam, 1964). However, output is still very limited. Recent advances in tissue culture and recombinant DNA technology have opened new avenues in transformation of higher plants, which consequently produced many transgenic plants with new genetic properties. Establishment of an efficient plant regeneration system from the explants of jute is a prerequisite to create variability and to introduce foreign genes into this crop through genetic transformation (Khatun, 1998). Brief review of works done on plant regeneration of jute is summarized bellow.

2.1 Experiment-i *In vitro* regeneration of *Corchorus* species

2.1.1 Concept of tissue culture

Conventional techniques are lengthy processes and take more time for crop improvement. The techniques of plant tissue culture have been developed as a new and powerful tool for crop improvement (Carlson, 1975, Razdan and Cocking, 1981) and received wide attention of modern scientists (D' Aamato, 1978, Skirvin, 1978, Larkin and Scowcroft, 1982).

Regeneration from explants like cotyledon, hypocotyle, leaf, shoot apex on defined nutrient media under sterile conditions is the basis of plant tissue culture. When explants of a plant are grown in a defined medium, an undifferentiated collection of

cells arise which then developed into whole plants from this undifferentiated callus, this process is known as regeneration.

Nowadays, plant tissue culture techniques have been emerged as a world wide accepted concept (Haque, 2001) and opened up several new avenues for manipulation of crop plants to induce genetic changes and selection of desirable traits (Nath, 2001). Besides, plant regeneration from *in vitro* cultures is a prerequisite of many plant genetic transformation techniques (Akter, 2001).

Tissue culture technique is now used extensively in many national and international organizations, such as CIP, IARI, ICRISAT, USDA, where programmes of crop improvement are in progress for development of different crops.

2.1.2 Tissue culture of jute

In vitro regeneration has been quite difficult among the species *Corchorus* through tissue culture technique. It appears that jute is a difficult recalcitrant tissue and regeneration from totally differentiated tissue, like callus. Where there are reports of regeneration from cotyledon or hypocotyle derived callus, there are usually portions of meristematic tissue left from where regeneration actually occurs.

2.1.3 Need for tissue culture in jute

Tissue culture provides the opportunities for the genetic engineering of this crop to supplement conventional breeding. In 1925, Laibach conducted immature embryo culture in interspecific hybridization of flax and obtained the interspecific hybrid plant. This is the first study on tissue in bast fibre crops as well as one of earliest success in plant tissue culture.

Tissue culture research in jute was started in 1964, when Islam cultured interspecific hybrid embryo (*Corchorus capsularis* x *Corchorus olitorius*) and hybrid plants were obtained. *In vitro* regeneration is quite difficult among the species of *Corchorus* through tissue culture technique (Khatun, 1993).

Plant regeneration of jute from meristem (Rahman *et al.*, 1985), cotyledon (Rahman *et al.*, 1985; Khatun *et al.*, 1992; Ali, 1992), leaf (Islam, 1981), plumule (Das *et al.*, 1986), hypocotyle (Khatun *et al.*, 1992; Ghosh and Chatterjee, 1990; Seraj, 1992), apical meristems (Rahman, 1985) and anther culture [(IBFCAAS), 1974; Islam, 1981] have been reported.

Islam (1981) obtained callus initiation and root formation from explants of *Corchorus olitorius* and claimed to have obtained a few shoots directly from leaf explants of *Corchorus olitorius* on MS medium.

To overcome the problems of the viral infection, virus-free plantlet have been produced through shoot tip culture of *Corchorus capsularis* (Das, 1983).

Experiment –i: *In vitro* seed germination of *Corchorus spp.* on agar-based and clinical cotton supported medium

2.1.4 *In vitro* seed germination

Healthy seedling production was one of the major criteria for plant regeneration. But very few little and attention has been paid so far on *in vitro* seed germination of jute. Some literatures related to *in vitro* seed germination are cited below:

Khatun (2001) conducted an experiment to study the germination percentage of different varieties of *Corchorus* species namely O-9897, O-72, CVL-1, CVE-3, and Tricap-2 on hormone free agar-solidified MS basal media and cotton based MS liquid

media. She observed that the germination percentage of the varieties was higher on cotton based medium than the agar medium.

Khatun (2001) also noted that the highest germination percentage was found in the variety Tricap-2 (98.66%) on cotton based medium and the lowest germination percentage was found in the variety O-4 (38.66%) on agar solidified medium.

Experiment-ii. *In vitro* regeneration performance of five varieties of *Corchorus* *species*

2.1.5 Callus induction

A callus is an amorphous mass of loosely arranged thin walled parenchyma cells arising from the proliferating cells of parent tissue (Dodds and Robert, 1990). Callus induction from different explants of various jute (*Corchorus capsularis*) varieties in the combinations of growth regulators were reported by several workers. The most relevant literatures related to callus induction have been reviewed here:

2.1.6 Varietal difference

Murashige and Skoog (1962) reported that nutritional requirements for optimum growth of tissue in may vary with varieties. Even tissue culture of different parts of plant may show different requirements for satisfactory growth.

Khatun (2001) conducted an experiment on six varieties of jute (CVL-1, CVE-3, D-154, CC-45, BJC and Tricap-2) and observed that the frequency of shoot production varied greatly among the varieties. She reported that Tricap-2 showed best performance in shoot regeneration.

Two species of *Corchorus* was tested for plant regeneration (Khatun, 2001) and she observed that plant regeneration from the explants of *Corchorus olitorius* was very

different in culture condition than *Corchorus capsularis*. Several attempts were made by using various hormone concentration and media combinations *in vitro* to obtain plant regeneration from various explants of *Corchorus olitorius*.

Saha *et al.* (1999) reported that JRC-312 showed the best shoot regeneration ability followed by JRC-212 and D-154.

Tewari *et al.* (1999) conducted an experiment using three cultivars JRC-212, JRC-321 and JRC-7447 for plant regeneration.

2.1.7 Effect of explants

Khatun (2001) reported that plant regeneration from the explants (Cotyledon segments, hypocotyl segments and root segments) of *Corchorus olitorius* (Var. O-9898, OM-1, O-72 and O-4) was difficult, but the explants of cotyledonary petiole of all these varieties produce shoots from the cut ends.

Tewari *et al.* (1999) reported that 2, 4-D induced callusing in 100% of explants when cotyledons, segments of hypocotyl and roots of white Jute (*Corchorus capsularis*) were cultured on MS medium supplemented with 16 adjuvant individually and/or in combination.

Rahman *et al.* (1985) showed that callus initiated from both apical meristems and cotyledon of var. D-154 of *Corchorus capsularis*, when culture on BAP and tyrosine fortified MS media forms shoot.

Khatun *et al.* (1992) reported plant regeneration from cotyledon derived callus in D-154. They used phytohormones BAP and IAA supplemented with MS medium to set

multiple shoots from cotyledon derived calli. Ali (1992) also reported similar observation.

Seraj *et al.* (1992) reported that callus initiation from hypocotyls of D-154 and CVL-1 of *Corchorus capsularis* when cultured on BAP and tyrosine fortified MS medium. They also used antioxidant NDGA (nordihydroguaiaretic acid).

Ghosh and Chatterjee (1990) reported plant regeneration from hypocotyle derived callus on MS medium in *Corchorus capsularis.*

Islam (1981) reported that callus initiation from explants of both *Corchorus capsularis* and *Corchorus olitorius* and claimed that have obtained a few shoots from leaf explants of *Corchorus olitorius.*

2.1.8 Maintenance of callus

Very little work and attention has been paid so far on maintenance of callus of jute. However some of them are reviewed below.

A long-term regeneration system for garlic clones was developed by Myers and Simon (1990) where callus was initiated on modified Gamborg's B5 medium supplemented with 4.5μM 2, 4-D and maintained on the same basal medium with 4.7 μM piclorum and 0.4μM 2ip (isopentenyladenine). Regeneration potential of callus after 5, 12 and 16 months on maintenance medium was measured using several plant growth regulator treatment.

The organogenic callus of *Corchorus capsularis* (Var. D-154 and CVL-1) when rich in large starch granules, was transferred to MS basal medium and differentiated into single or multiple shoots (Seraj *et al.*, 1992).

2.1.9 Somatic embryogenesis

Somatic embryogenesis has a tremendous potential for large scale production of plant material (Amirato and Styer, 1985) and is considered as an effective aid to genetic transformation study. It represents an alternative for massive clonal propagation and appears to be a potential solution to the problem of field propagation, especially in areas with frequent disease transmission and maintenance of cultivars that have been selected for their important genetic characteristics (De Garcia and Martinez, 1995). There have been very few reports of somatic embryogenesis in jute.

Somatic embryogenesis was induced from cotyledon and protoplast derived callus of *Corchorus capsularis* in the presence of 2, 4-D and BAP (Khatun *et al.*, 1993).

Wang *et al.* (1992) reported that basic MS medium with B5 vitamins and different concentration of Br (brassinolied) at 0.01 ppm + 2, 4-D at 5.0 ppm iduced callus formation in all cultivars except Coker 201 and coker 312 when he cultured the hypocotyls explants from 8 cotton cultivars. He also reported that after removal of 2, 4-D embryogenic callus and embryoids were formed.

2.1.10 Organogenesis

The totipotency of somatic cells has been explained in vegetative propagation of plant species. *In vitro* studies have revealed that most plants would differentiate shoot ends and roots from somatic as well as reproductive tissues. Whole plant regeneration from cultured cells may occur either through shoot-end differentiation of plant from callus has been reported by different workers. The literatures closely related to *in vitro* regeneration of jute are cited below:

Khatun (2000) reported that the cotyledonary explants of *C. olitorius* produced multiple shoot when cultured in MS medium with 0.5 mg IAA/L and 3.0 mg BAP/L. She also reported that the best *in vitro* response for shoot regeneration was obtained from O-9897.

Khatun (2001) observed multiple shoots from cotyledons with attached petioles explants of *C. capsularis* on MS medium supplemented with 2 mg BAP and 0.5 mg IAA/L.

Das *et al.* (1986) showed that when plumules of var. D-154 of *Corchorus capsularis* were cultured on BAP and tyrosine fortified MS media, the tissue developed into multiple shoots.

2.1.11 Induction of root

In vitro root induction of jute was reported by several researchers. The information, which are closely related information are reviewed here:

Saha *et al.* (1999) reported that the best root formation induced in the MS medium with 2.5μM IBA and 1.5% sucrose.

Ahmed *et al.* (1989) reported that the *in vitro* regenerated shoots of *C. olitorius* (var. O-4) produced roots most successfully on MS medium with 3.0 mg nirdihydrogniaretic acid + 0.3 mg IBA/L.

Bigaria (1998) conducted a field experiment to study the influence of IBA, environmental factors and planting position on the regeneration of stem cutting and leaves of *Hibiscus cannabinus*. Stem cutting and leaves were treated with IBA at 10, 25, 50 100, 150 and 200 ppm. Through their early differentiation of adventitious roots and stimulate the sprouting of buds on the stem cutting.

Khatun (2001) reported that the regenerated plantlets of *C. capsularis* produced rooting on MS medium without hormone within seven days.

Root formation was induced in the *in vitro* regenerated shoots by culturing them on half strength of MS medium with 0.1-1.0 mg/l either of NAA, IBA and IAA. Among the three types of auxin, NAA was found to be most effective at different concentrations tested for producing roots on the cut margin of the shoot and 0.1 mg/l NAA found to be the best concentration of auxin for proper rooting in which 100% shoots showed rooting within six weeks of culture.

2.3 Experiment-iii. Optimization of shoot regeneration from the explants of *C. capsularis* with different hormonal concentrations

2.3.1 Callus induction

Callus induction from different explants of jute (*Corchorus capsularis*) varieties in the combination of growth regulators were reported by several workers. The most important literatures have been reviewed here.

2.3.2 Effects of growth regulators

Tewari *et al.* (1999) reported that MS medium supplemented with 2, 4-D induced callusing in 100% explants of jute.

Khatun (2001) cultured *in vitro* grown cotyledons (with attached petioles) of *C. capsularis* in agar solidified MS medium supplemented with 0.5 mg/L IAA and different concentration of BAP (2, 3, 4 or 5 mg/L) and noted least performance in callus induction and shoot regeneration on the combination of MS + 0.5 mg/L IAA and 2 mg/L BAP.

Li *et al.* (1989) conducted an experiment of *Gossypium hirsutum* and reported that hypocotyls and cotyledons of 15 upland cultivars (*Gossypium hirsutum*) were cultured in MS medium supplemented with various phytohomones at 16 hours light and 8 hrs dark with fluorescent illumination at 1500-2000 lux. Calli were formed at 6-10 days, with hypocotyls forming calli 3-4 days earlier than cotyledons. Morphological traits of the calli depended on the cultivar and the hormones added to the medium. Grey or yellowish-grey friable calli cultured for 50-60 days produced embryos, while green, white or green and white dense calli did not. Calluses of cv. ASJ2 on medium supplemented with 0.1 mg kinetin per liter multiplied most rapidly, producing almost double the weight of calli of the other cultivars, but ASJ2 produced the fewest calli. Zhongmian 12 produced the worst calli, followed by Zhong 13, Stoneville 213 and Coker 312.

2.3.3 Shoot regeneration

Khatun (2001) cultured *in vitro* grown cotyledons (with attached petioles) of *C. capsularis* in agar solidified MS medium supplemented with 0.5 mg/L IAA and

different concentration of BAP (2, 3, 4 or 5 mg/L) and noted best performance in shoot regeneration on the combination of MS + 0.5 mg/L IAA and 2 mg/L BAP.

The highest frequency of callus induction (99.2) was observed on MS medium containing (2.0mg/l) of NAA and (0.5mg/l) of Kn. This kind of auxin alone or in combination with callus induction has been reported in the past. (Rao *et al.*, 1973) (Groenwald *et al.*, 1977) and (Lupi *et al.*, 1985).

The combination of auxin in high concentration and cytokinin in low was more effective for callus formation (Rao, *et al.*, 1973).

Nidhi Prabhakar (1998) reported that the effect of thidiazuron on a comparative study of the effect of BAP and thidiazuron (TDZ) on shoot bud differentiation from excised cotyledons of *Brassica juncea* cv. PR-45 showed that the latter is ten times more effective than the former and in combination produces synergistic effect. At its optimum concentration (5×10^{-4} mM) TDZ induced 100% regeneration with 7.4 shoots per cotyledon.

Khalekuzzaman *et al.* (2000) reported that an efficient tissue culture method for a high rate of shoot regeneration was developed for *Corchorus capsularis* L. var. D-154 (jute). Cotyledonary explants with and without petiole were cultured in MS medium supplemented with auxin and cytokinin. Explants with the petiole showed a better response than those without it in respect of shoot initiation. The best shoot proliferation was observed when cotyledon-derived calli was subcultured in MS medium fortified with a combination of 2.0 mg/l BA and 0.5 mg/l IAA. *In vitro* elongated shoots were rooted with 100% success by treating them with a 0.3 mg/l

IBA. Rooted plantlets were successfully established into soil more than 80% plants survived. The regenerants matured as normal and produced fertile seeds.

2.4 Experiments-iv: Effect of different levels of pH on plant regeneration from cotyledons of *Corchorus* species

Naher and Khatun (2004) reported that two varieties of *C. capsularis* (Vars. CVL-1 and D-154) and *C. olitorius* (O-72) performed differently on shoot regeneration in different pH levels (e.g. 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0) in association with MS plant regeneration medium. They found that O-72 responded for maximum shoot regeneration at pH 5.0 (65.00%) and CVL-1 at pH 7.0, CVE-3 at pH 7.0 (63.33 %.). They also reported that shoot regeneration percentage of O-72 gradually decreased as pH levels were increased and shoot regeneration of CVL-1 and CVE-3 gradually increased as the pH levels were increased.

Modarres Sanavy and Jami Moeini(2003) conducted an experiment and reported that solid MS medium with 0.25 mg/l GA_3, 0.01 mg/l NAA 2.0 mg/l calcium pantothenate, 30 g/l sucrose and 7g/l showed significant differences between different pH levels in respect of its ability to induction of rooting and shooting in plantlets produced from the single nodes of two cultivated potato (*Solanum tuberosum L.*) varieties after subjecting them with thermotherapy. Overall pH 5.5 was the best for all the traits. Low and high levels of pH from 5.5 were found to reduce the growth and rooting of single nodes. The reduction was more pronounced at low levels than high levels of pH.

Pierick (1997) reported that there is insufficient information about the effect of pH on explants growth *in vitro*. It seems that pH in the range of 5-6.5 supports growth, because lower pH (less than 4.5) and higher pH (more than 7.0) generally stop growth and development of plantlets.

Gulsen and Domanoglu (1991) studied the effect of different levels of sucrose, agar and pH on multiplication rate and shoot quality of Quince Tree on modified MS medium with 3.0 mg/l BA, 0.1 mg/l IAA and 0.1 mg/l GA_3. They obtained best shoot multiplication and development in the medium containing 30 g/l sucrose and 5 g/l agar at pH 5.5.

2.5 Experiment-v: Influence of different concentration of sucrose on plant regeneration from cotyledons of *C. capsularis* and *C. olitorius*

Akhond *et al.* (1997) reported that plantlets of taro *(Colcasia escuienta* var. *antiquorum* cv. Bilashi) were regenerated trom shoot-tip-derived calli cultured on MS medium supplemented with 1.0 mg/l α-napthaleneacetic acid (NAA) and 5.0 mg/l 6-benzyladenine (BA). *In vitro* storage at low temperature was tested by transferring well developed plantlets to growth regulator free MS media containing different sucrose levels. The cultures were kept at 8°C in complete darkness while the control treatment was maintained on MS medium at 2 ± 1°C under light conditions. After 18 months of storage without subculture at 8°C in the dark, the plantlet survival rate was 92% for cultures containing 30 g/l sucrose. The highest number of auxiliary shoot multiplication and the highest number of plantlets were obtained from medium containing 40 g/l sucrose. Plantlet survival was 100% when transferred to soil. No

morphological variation was observed among the plants of the population raised from *in vitro* long-term cultures.

Westcott *et al.* (1977) reported that high levels of sucrose may help to prolong the interval between subcultures of materials in an *in vitro* repository.

Staritsky *et al.* (1986), Pathirana (1991) reported that use of different sucrose levels in the storage medium for *Colocasia has been* raising the osmolarity of the storage medium in combination with reduced temperature might help prolong storage periods (Staritsky *et al.*, 1986).

Bessembinder *et al.* (1993) reported that although all the shoots of the plantlets under normal conditions died within eight months, small corms remained. After 12 months, regrowth of the auxiliary shoot was observed from these corms in 67% of cultures. Regrowth of plantlets from 40% of the cultures from this treatment survived and remained healthy for 16 months. Death of taro plants in storage for eight months at 28/24°C and 12 hrs photoperiod was also observed.

Pathirana(1991) reported that the number of auxiliary shoots per plant was highest in the treatment with 40 g/l sucrose after 18 months of storage at 8°C . The highest number of plantlets was obtained from the 40 g/l sucrose treatment following the resumption of regrowth. Without any growth regulators, sufficient shoot multiplication was achieved after regrowth in the treatments with 40 and 45 g/l sucrose in the media. The survival percentage of the cultures with 40 g/l sucrose was sufficient considering the number of plantlets obtained after resumption of growth.

2.6 Experiment-vii Influence of surfactants (pluronic F-68) on plant regeneration from cotyledons of *C. capsularis* and *C. olitorius*

Khatun *et al.* (1992) conducted an experiment of stimulation of differentiation in jute cotyledon cultured with pluronic F-68. They reported that the addition to MS-based medium of 0.1 or 0.5% (w/v) of either commercial grade Pluronic F-68 or a purified fraction obtained by passage through silica gel, stimulated shoot production from the petiole of cotyledon of *Corchorus capsularis* vars. D154 and C134. This effect was pronounced with C134, because of the failure of control cotyledon to differentiate into shoots in MS medium without Pluronic. The implications of these results are discussed in relation to the potential value of non-ionic surfactants as additives to plant culture media for stimulating growth and differentiation.

Lowe *et al.*(1993) showed that a novel approach to the growth of cultured plant cells, tissues and organs by supplementation of culture media with low concentrations (<1.0% w/v) of surfactants is discussed. Studies using *Arabidopsis thaliana, Solanum dulcamara* and *Corchorus capsularis* demonstrated the considerable growth-stimulating effects of pluronic (Poloxamer) co-polymers in both liquid and semi-solid systems. The possible mechnism (s) involved and their implications are considered in relation to the application of such compounds in plant biotechnology.

Kumar *et al.* (1991) reported that the non-ionic, copolymer surfactant, Pluronic F-68 (Poloxamer 188), is a valuable growth-promoting supplement in plant culture systems. For example, addition of low concentrations of Pluronic F-68 to culture media stimulated growth of callus, isolated protoplants and *Agrobacterium rhizogens*-transformed roots of *Solanum duleamara*

King *et al.* (1991) reported that related studies with animal cells have shown that Pluronic F-68 stimulates increased 2-deoxyglucose uptake and amino acid incorporation into protein perhaps by increasing cytoplasmic membrane permeability. This is supported by patch-clamp experiments using artificial lipid bilayers in which pluronic F-68 caused the formation of short-lived, trans membrane pores. The inclusion of surfactants in plant culture media could prove beneficial, not only for stimulating tissue growth, but also in promoting differentiation.

2.7 Experiment-viii Influence of NaCl on plant regeneration from cotyledons of *C. capsularis* and *C. olitorius*

Chaudhuri and Choudhuri(1993) reported that 30-day-old seedlings of two jute species (*Corchorus capsularis* cv. JRC 212 and *C. olitorius* cv. JRO 632) grown in terracotta pots but removed and washed with distilled water were subjected to short-term salinity stress (160 and 200 mM NaCl for 1 and 2 d). Relative water content, leaf water potential, water uptake, transpiration rate, water retention, stomatal conductance, not photosynthetic rate and water use efficiency of both jute species decreased due to salinity stress. The decrease was greater in *C. olitorius* than in *C. capsularis* and as stress increased. Greater accumulation of Na^+ and Cl^- and a lower ratio of K^+:Na^+ in the root and in the shoot of *C. olitorius* compared with *C. capsularis* were also recorded. Pretreatment of seedlings with kinetin (0.09 mM), glutamic acid (4 mM) and calcium nitrate (5 mM) for 24 h significantly improved net photosynthesis, transpiration and water use efficiency of salinity stressed plants, the effect being more marked in *C. olitorius*. Among the pretreatment chemicals, calcium nitrate was most affective.

Chapter 3

Materials and Methods

3.1 Location

The experiments were conducted during the period of July 2005 to May 2006 in the Genetic Engineering Laboratory of Cytognetics Department, Genetic resources and seed Division, Bangladesh Jute Research Institute, Dhaka.

3.2 Experimental materials

The following genetic materials of Jute (*Corchorus capsularis* and *Corchorus olitorius*) were used in the present investigation:

a. *Corchorus capsularis* L. var CVE-3
b. *Corchorus capsularis* L. var CVL-1
c. *Corchorus capsularis* L. var Tricap-2
d. *Corchrus olitorius* L. var O-9897
e. *Corchrus olitorius* L. var O-72

3.3 Sources of the materials

The materials used in the experiment were obtained by the courtesy of Bangladesh Jute Research Institute (BJRI), Dhaka.

3.4 Methods

The following culture media were used in the present investigation depending on specific purposes as mentioned below:

3.4.1 For seed germination

MS (Murashige and Skoog, 1962) medium.

3.4.2 For callus induction and shoot differentiation

MS (Murashige and Skoog, 1962) medium supplemented with hormone, p^{H}, vitamin, surfactant, NaCl, $FeSO_4$.

3.4.3 For root induction

Half strength MS (Murashige and Skoog, 1962) medium

3.4.4 Preparation of culture media

For the induction of callus and shoot regeneration in jute a number of culture media have been advocated by different scientists of which MS (Murashige and Skoog, 1962) medium was used for investigating the present piece of work. A nutrient medium consists of organic and inorganic salts, irons, a carbon source, some vitamins and growth regulators were used. Based on the types of explants different media along with different concentration were used. Compositions of MS medium formulated by Murashige and Skoog, 1962, is presented below :

Constituents of stock solution for MS (Murashige and Skoog, 1962) medium

Constituents		
a) Macronutrients	**Concentration (mg/l)**	**Concentration (g/l) 10X**
KNO_3	1900.00	19.00
NH_4NO_3	1650.00	16.50
KH_2PO_4	170.00	1.7
$CaCl_2.2H_2O$	440.00	4.4
$MgSO_4.7H_2O$	370.00	3.7
b) Micronutrients		**100X**
$MnSO_4.4H_2O$	22.30	2.23
H_3BO_3	6.20	0.62
$ZnSO_4.7H_2O$	8.60	0.86
KI	0.83	0.083
$Na_2MoO_4.2H_2O$	0.25	0.025
$CuSO_4.5H_2O$	0.025	0.0025
$CoCl_2.6H_2O$	0.025	0.0025
C) Iron sources		10X
$FeSO_4.7H_2O$	27.80	0.278
Na_2EDTA	37.30	0.373
d) Organic nutrients		(mg/l) 100x
Glycine	2.00	200
Nicotinic acid	0.50	50
Pyridoxin-HCl	0.50	50
Thimine-HCl	0.10	10
Myo-inositol	100.00	0.10
Agar	8000.00	8.00 g/l
Sugar	30000.00	30.00 g/l

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15.3.15

Different steps of media preparation have been briefly presented here

3.4.5 Preparation of stock solutions

The first requisite for preparation of medium was the preparation of stock solutions. Stock solution of growth regulators were prepared separately by dissolving the desired quantity of ingredients in appropriate solvent and the required final volume was made with water for ready use to expedite the preparation of the medium wherever needed. Separate stock solutions for macronutrients, micronutrients, iron, vitamins and growth regulators were prepared and stored appropriately for use.

3.4.5.1 Stock solution A (macronutrients)

The stock solution of macronutrients was made up to 10 folds (10x) the final strength of medium in 1000 ml of distilled water. Ten times the weight of salts required per liter of the medium were weighted accurately and dissolved in 750 ml of distilled water and volume was made up to 1000 ml by further addition of distilled water. This stock solution was filtered and poured into a clean brown bottle, labeled with marker and stored in a refrigerator at 4°C for use.

3.4.5.2 Stock solution B (micro-nutrients)

This was made up to 100 folds (100x) the final strength of the medium in 1000 ml distilled water (DW). The stock solution was filtered labeled and stored in a refrigerator 4°C for later use.

3.4.5.3 Stock solution C (Iron source)

$FeSO_4$ (0.028gm) added directly into solution. This was prepared at 10 folds (10x) the final strength of $FeS0_4$ and Na_2-EDTA in 100 ml distilled water and chelated by heating on a heater cum magnetic stirrer. Then the volume was made up to 1000 ml

by further addition of distilled water. Finally, the stock solution was filtered, labeled and stored in a refrigerator at 4°C for later use.

3.4.5.4 Stock solution D (Vitamins)

Each of the desired ingredients except myo-inositol were taken at 100 folds (100x) of their final strength in a measuring cylinder and dissolved in 750 ml of distilled water. The final volume was made up to 1000 ml by further addition of distilled water. The solution was dispensed into 10 ml aliquots and stored at – 20°C. Myo-inositol was used directly at the time of media preparation.

3.4.5.5 Stock solution for hormones

Stock solution of hormones was prepared separately at 100 ppm by dissolving the desired quantity of ingredients in appropriate solvent and the required volume was made with distilled water and stored in a refrigerator at 4°C for later use. The following growth regulators (phytohormone supplements) were used in the present investigation

Auxin: 3-indole acetic acid (IAA) was disolved in ethanol

Cytokinins: 6-benzyl amino purine (BAP) was disolved in 0.1 NaOH.

The growth regulators were dissolved in appropriate solvents as IAA in ethanol and BAP in 0.1N NaOH.

For the preparation of stock solution of any of these hormones, 0.02g of each of the hormone powder was taken on a clean beaker and dissolved in 1 ml of the particular solvent. The mixture was then collected in a 50 ml measuring cylinder and volume was made up to 20 ml by the further addition of distilled water. The solution was then poured into a clean glass container and stored at -4°C and used for maximum period of two weeks.

3.4.5.6 Steps followed for the preparation of culture media

In the course of present investigation, the following steps were followed for preparation of different culture media.

3.4.5.7 Preparation of MS medium

To prepare one liter (1000 ml) of MS medium, the following steps were followed:

i) 100 ml of macro-nutrients, 10 ml of micro-nutrients, 100 ml of iron and 10 ml of vitamins were taken from each of these stock solutions into a 2 liter Erlenmeyer flask on a magnetic stirred stirrer.

ii) Distilled water was added in the flask to dissolve all ingredients and made the total volume 400 ml.

iii) 100 mg of myo-inositol was added directly to the solution and dissolved well.

iv) Thirty grams of sucrose was added to this solution and agitated gently to dissolve completely.

v) Different concentrations of hormone supplements were added to the solution either in single or in combinations as required and mixed well.

vi) The whole mixture was then made up to 500 ml with further addition of distilled water.

vii) pH of the medium was adjusted to 5.8 with a digital pH meter with help of 0.1 N NaOH or 0.1 N HCL, whichever was necessary.

viii) Seven gram agar was added in 500 ml of water. The mixture was then heated gently with continuous stirring till complete dissolution of agar. Hot agar (500 ml) was then mixed with 500 ml of medium. The mixture was thoroughly mixed by shaking.

ix) Required volume of hot medium was dispensed into culture vessels or conical flasks. After dispensing the medium the culture vessels were plugged with cork and/or non-absorbent cotton and marked with different codes with the help of a glass marker to indicate specific hormonal combinations

3.4.6 Sterilization

To ensure aseptic condition *in vitro,* all instruments, glassware and culture media were sterilized properly by autoclaving.

3.4.6.1 Sterilization of culture media

The conical flasks containing prepared media were autoclaved at 1.16 kg cm^{-2} pressure and 121°C temperature for 20 minutes. For bacteria culture, YMB medium was then poured into sterile petri dishes in a laminar air flow cabinet and were allowed to cool before use.

3.4.6.2 Sterilization of glasswares and instruments

Beakers, test tubes, conical flasks, pipettes, instruments like forceps, scalpels, were wrapped with brown paper packets. Empty flasks were capped with cotton plug and then sterilized in an autoclave at a temperature 121°C for 20 minutes at 1.16 kg cm^{-2} pressure.

3.4.6.3 Sterilization of culture room

The culture room was initially cleaned by gently washing all floors and walls with a detergent followed by wiping with 70% ethyl alcohol. The process of sterilization was repeated at regular intervals. Generally, laminar airflow cabinet was sterilized by wiping the working surface area with 70% ethyl alcohol.

3.4.6.4 Precautions to ensure aseptic condition

Aseptic manipulations were carried out in a laminar airflow cabinet. The cabinet was switched on for at least half an hour before use and cleaned with 70% ethyl alcohol to overcome the surface contaminants. During the entire period of inoculation the autoclaved scalpels, forceps were kept immersed into 70% alcohol contained in a glass jar inside the cabinet. At the time transfer these were again sterilized by flaming method inside the cabinet. Both of the hands were rinsed with 70% alcohol. All measures were taken to obtain maximum contamination free condition during the surgical operation of the explants.

3.4.7 Culture techniques

3.4.7.1 Experiment-i. *In vitro* regeneration of *Corchorus* species

a) Axenic culture

Seeds of *C. capsularis* (vars. Tri cap-2, CVE-3, CVL-1) and *C. olitorius (*vars. O-9897, O-72) were surface sterilized by immersing in absolute alcohol for 1 minute and then in 0.1% (w/v) Mercuric Chloride for 20 minutes. Seeds were thoroughly washed with autoclaved distilled water for 6 times. The sterilized seeds were transferred into a 100 ml conical flask containing 50 ml of hormone free MS agar-solidified (0.8%, w/v) medium. Twenty seeds were inoculated in each flask.

In another set of experiment, clinical cotton was used instead of agar as a supporting material for seed germination in association with MS basal medium. Clinical cotton was placed at the bottom of 100 ml flasks. Each flask contained 20 ml of hormone free MS liquid medium. Seeds of *C. capsularis* varieties were surface sterilized by immersing in absolute alcohol for 1 minute and then in 0.1% (w/v) Mercuric Chloride for 20 minute followed by 6 washes and placed on the surface of cotton-based MS

liquid medium. Cultures were placed in a growth room with 28°C temperature under 1.0 Wm^{-2} of daylight fluorescent tubes with 12 hour photoperiod. Twenty seeds were inoculated in each flask. Seven days old seedlings were used for further research work and data collection.

b) Explants culture

The seedlings raised in axenic culture were used as the source of explants. The cotyledon with attached petiole were used as explants. Cotyledon with attached petioles of *Corchorus* spp. were taken from *in vitro* grown seedlings for this study. In this case, seedlings were allowed to develop for 7 days to make sure that the undeveloped apical shoot buds were not attached with the petioles. Therefore, the optimum explants source, cotyledons with their attached petioles were excised from 7 days old seedlings and were cultured in 250 ml conical flasks containing 50 ml of agar-solidified MS medium supplemented by IAA (0.5 mg/l) and BAP (2.0 mg/l). Ten explants were placed in each culture flask. The culture flasks containing explants were placed in a growth room at 28°C under a 1.0 Wm^{-2} of daylight fluorescent tubes with a 12 h photoperiod. The cultured flasks were checked daily to note the development of contamination. Data were recorded 6 weeks after culture.

c) Transfer of regenerated shoot-buds for root induction

When the shoots were 2-3 cm in length, they were rescued aseptically from the flasks and cultured in 250 ml conical flask containing freshly prepared MSO (hormone free MS medium) medium for root production. The conical flasks containing plantlets were incubated at 28°C under 1.0 Wm^{-2} of daylight fluorescent tubes with 12 h photoperiod. Observations were carried out to note the responses.

3.4.7.2 Exeriment-ii. *In vitro* regeneration performance of five varieties of *Corchorus capsularis and Corchorus olitorius*

The following culture methods were employed in the present investigation:

a) Axenic culture

b) Explants culture

c) Subculture

a) Axenic culture

Sterilized seeds were placed into sterilized seed germination medium in culture vessels. Twenty five seeds were placed in each flask. The culture was then incubated in dark till the germination of seeds. These were then transferred to 12 hours light for normal seedling growth. Seven to ten days old seedlings were used as source of contamination-free explants.

b) Explants culture

The seedlings raised in axenic culture were used as the source of explants. The cotyledons with attached petioles were used as explants. Cotyledons with attached petioles of *C. capsularis* were taken from *in vitro* grown seedlings for this study. In this case, seedlings were allowed to develop for 7-10 days to make sure that the undeveloped apical shoot buds were not attached with the petioles. Therefore, the optimum explants source, cotyledons with their attached petioles were excised from 7-10 days old seedlings and were cultured in 250ml conical flasks containing 50ml of agar-solidified MS medium with IAA (0.5 mg /l) and BAP (2.0mg/l).Ten explants were inoculated in each culture flask. The culture flasks containing explants were placed in growth room and were maintained at 28^0 C under a $1.0Wm^{-2}$ of daylight fluorescent tubes with a 12 h photoperiod. The culture flasks were checked daily to

note the response the development of contamination. Data were recorded 4 weeks after culture.

c) Subculture or transfer

i) Subculture of the callus for shoot regeneration

Two weeks after inoculation of explants, the calli attained convenient size. Then they were removed aseptically from the cultured flask on a sterilized glass plate inside the laminar airflow cabinet and were placed again on freshly prepared sterilized medium containing appropriate hormonal supplements for shoot induction from the cells. The culture flasks showing signs of contamination were discarded.

ii) Transfer of regenerated shoot-buds for root induction

When the shoots were 2-3 cm in length, they were rescued aseptically from the cultured flasks and were separated from each other and again cultured individually on 250ml conical flask with freshly prepared MSO (hormone free MS medium) medium for root production. The conical flasks containing plantlets were incubated at 28^0 C under a 1.0 Wm^{-2} of daylight fluorescent tubes with a 12 h photoperiod. Day to day observations was carried out to note the responses.

3.4.7.3 Experiment-iii. Optimization of shoot regeneration from the explants of *C. capsularis* under different hormone concentration

Seeds were germinated on cotton supported liquid medium following the techniques described in section 3.4.7.1 and cotyledons with attached petiole were used as explants. Ten explants were placed in each culture flask containing different treatments of BAP (0 mg/l, 1 mg/l, 2 mg/l, 3 mg/l and 4 mg/l) and IAA (0.0 mg/l, 0.5 mg/l, 1.0 mg/l, 1.5 mg/l and 2 mg/l). The culture flasks containing explants were

placed under fluorescent light in growth room with controlled temperature (28°C). The flasks were checked daily to note the appearance of shoot regeneration.

3.4.7.4 Experiment-iv. Effect of different levels of pH on shoot regeneration in *Corchorus capsularis* and *C. olitorius*

The following culture techniques were employed in the present study:

a) Axenic culture

b) Explant culture

a) Axenic culture

Discussed in axenic culture above.

b) Explants culture

The seedling raised in axenic culture were used as the source of explants. Here cotyledon(with attached petioles) were used as explants. Ten explants were placed in each culture flask containing different levels of pH (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5) supplemented with 2mg/l BAP and 0.5mg/l IAA. The culture flasks containing explants were placed under fluorescent light in room with controlled temperature (28^0 C) until callus initiation. The flasks were checked daily to note the appearance of callus.

3.4.7.5 Experiment-v Influence of different concentration of sucrose on plant regeneration from cotyledons of *C. capsularis* and *C. olitorius*

The following culture techniques were employed in the present study:

a) Axenic culture

b) Explants culture

a) Axenic culture

Discussed in axenic culture above.

b) Explants culture

The seedlings raised in axenic culture and explants culture were used as the source of explants. Twenty four explants were placed in three replications of containing different concentration of Sucrose (0, 1%, 2%, 3%,) supplemented with 2 mg/l BAP and 0.5 mg/l IAA. In three replication containing explants were placed under fluorescent light in a growth room with controlled temperature (28^{0}C). The flasks were checked daily to note the appearance of callus and shoot regeneration.

3.4.7.6 Experiment-vi Influence of different concentration of vitamins on plant regeneration from cotyledons of *C. capsularis* and *C. olitorius*

The following culture techniques were employed in the present study:

a) Axenic culture

b) Explants culture

a) Axenic culture

Discussed in axenic culture above.

b) Explants culture

The seedlings raised in axenic culture and explants culture were used as the source of explants. Twenty four explants were placed in three replications of containing different concentration of vitamin (0, x 1 times, x 2 times, x 3 times, x 4 times) supplemented with 2 mg/l BAP and 0.5 mg/l IAA. [0(control), x 1 times (1.5 mg/l) x

2 times (3.0 mg/l), x 3 times(4.5 mg/l) x 4 times(6.0mg/l)]. In three replication containing explants were placed under fluorescent light in a growth room with controlled temperature (28^0C). The flasks were checked daily to note the appearance of callus and shoot regeneration.

3.4.7.7 Experiment-vii Influence of surfactants (pluronic F-68) on plant regeneration from cotyledon of *C. capsularis* and *C. olitorius*

The following culture techniques were employed in the present study:

a) Axenic culture

b) Explants culture

a) Axenic culture

Discussed in axenic culture above.

b) Explants culture

The seedlings raised in axenic culture and explants culture were used as the source of explants. Twenty four explants were inoculated in three replications of containing different concentration of surfactant %(0, 0.001, 0.01, 0.1, 0.5) supplemented with 2.0 mg/l BAP and 0.5 mg/l IAA. In three replication containing explants were placed under fluorescent light in a growth room with controlled temperature (28^0C). The flasks were checked daily to note the appearance of callus and shoot regeneration.

3.4.7.8 Experiment-viii Influence of NaCl on plant regeneration from cotyledons of *C. capsularis* and *C. olitorius*

The following culture techniques were employed in the present study:

a) Axenic culture

b) Explants culture

a) Axenic culture

Discussed in axenic culture above.

b) Explant culture

The seedlings raised in axenic culture and explants culture were used as the source of explants. Twenty four explants were placed in three replications containing different concentration of NaCl %(0, 0.0625, 0.125, 0.250, 0.5, 1.0) supplemented with 2 mg/l BAP and 0.5 mg/l IAA. In three replication containing explants were placed under fluorescent light in a growth room with controlled temperature (28^0C). The flasks were checked daily to note the appearance of callus and shoot regeneration.

3.4.7.9 Experiment- ix Influence of $FeSO_4$ on plant regeneration of *C. capsularis* and *C. olitorius*

The following culture techniques were employed in the present study:

a) Axenic culture

b) Explants culture

a) Axenic culture

Discussed in axenic culture above.

b) Explants culture

The seedlings raised in axenic culture and explants culture were used as the source of explants. Twenty four explants were placed in three replications containing different concentration of $FeSO_4$ (0, x 1 times, x 2 times, x 3 times, x 4 times) supplemented with 2 mg/l BAP and 0.5 mg/l IAA. 0(control), x 1 times(1.5 mg/l) x 2 times(3.0 mg/l), x 3 times(4.5 mg/l) x 4 times(6.0mg/l). In three replication containing explants were placed under fluorescent light in a growth room with controlled temperature (28^0C). The flasks were checked daily to note the appearance of callus and shoot regeneration.

Data collection

Experiment-i

Germination percentage

The germination percentage was estimated as the ratio of the number of seed germinated to the number of seed placed in the germination medium.

$$\textbf{Germination percentage} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds placed in the medium}} \times 100$$

Experiment –ii-ix

To investigate the effect of different treatments of the experiment, data were collected from the different parameter as given here

a) Per cent callus induction

Percentage callus induction was calculated on the basis of the number of explants placed and the number of explants induced callus.

$$\textbf{Percent callus induction} = \frac{\text{No. of explants induced calli}}{\text{No. of explants placed}} \times 100$$

b) Percent plant regeneration

The percentage of plant regeneration was calculated based on the number calli transferred to regeneration medium and the number of calli produced plantlets.

$$\textbf{Percent plant regeneration :} \frac{\text{No. of calli producted shoot}}{\text{No. of explants cultured}} \times 100$$

Statistical analysis of data

The data for the characters under present study were statistically analyzed wherever applicable. The experiments were conducted in growth room and arranged in completely randomized Design (CRD). The analysis of variance for different characters was performed and means were compared by the Duncan's Multiple Range Test (DMRT).

Chapter 4
Results and Discussion

Considerable attention is being focused on the large scale culture of pant materials by improving the media components *in vitro*. The rate of plant regeneration is limited in some species including jute. Therefore, there is necessity for an additional stimulant to increase the rate of multiplication of regenerated shoots. Supplementation of culture media with non-ionic surfactants is a novel approach for the improvement of growth of cultured plant tissues and organs.

Varietal differences were observed among the five varieties of *C. capsularis* and *C. olitorius* for plant regeneration from the cotyledon explants. In a preliminary experiment on explants culture of jute it has been observed that different pH levels have effect on plant regeneration of different varieties.

Jute has been pushed to the marginal lands these days due to the emerging pressure of food crops. Coastal areas are usually not suitable for jute cultivation because of the presence high concentration of NaCI. However, a preliminary research was initiated in the biotechnological laboratory to see if there was any effect of NaCI on jute varieties *in vitro* so that the salt tolerant variety could be grown in the coastal lands. Noticeable results were observed for jute seeds, explants and shoot culture on different concentrations of salt. If the salinity tolerance level of jute could be established in the laboratory, jute can be grown in the known level of saline condition of the lands. Therefore, the objective of this programme is to identify the tolerance level of jute in the laboratory.

4.1 Influence of different factors on plant regeneration system of jute species

4.1.1. Experiment-i. *In vitro* regeneration of *Corchorus* spp.

Healthy seedling production is one of the major criteria for plant regeneration from jute explants. Seeds of five C. *capsularis and C. olitorius* varieties (Tricap-2 CVE-3, CVL-1, O-9897, O-72) were germinated on both agar solidified medium and surgical cotton-based liquid medium. In present study, number of seeds germinated and percentage of seed germination were observed.

4.1.1.1. Percent seed germination

4.1.1.1.1. Effect of variety

A significant variation in percent seed germination was observed among the test varieties. The highest percentage of seed germination was found in variety CVE-3 (92.72%) and lowest was found in O-9897 (67.22%) (Table. 1).

4.1.1.1.2. Effect of media

Percent seed germination from *C. capsularis and C. olitorius* varieties was found to be higher on cotton-based liquid MS medium (95.81%) compared to agar solidified MS medium (69.81%). The result was shown in Table 2. Germination of jute seed and seedling growth in cotton-based liquid medium was found to be comparatively higher than agar solidified medium (Plate 1 & 2). This finding was similar to the findings of Khatun (2001) who reported that germination percentage was higher in cotton-based medium than the agar-based medium.

4.1.1.1.3. Combined effect of variety and media

The combined effect of variety and media on percent seed germination has been presented in Table 3. The percent seed germination was found highest in cotton-based medium CVE-3 (98.88%) and lowest in agar-based medium O-9897(43.10%).

Table 1: Main effect of different varieties and optimization of seed germination from the cultivars of *C. capsularis and C. olitorius* on agar-based and clinical cotton supported medium

Varieties	Number of seed germinated	Percentage of seed germination
Varieties		
CVL-1	46.00 a	92.00 a
CVE-3	76.44 a	92.72 a
Tricap-2	44.77 b	89.55 b
O-9897	33.79 d	67.22 d
O-72	35.39 c	70.79 c

Figures followed by same letter in a column do not differ significantly by DMRT.

Table 2: Main effect of seed germination from the cultivars of *C. capsularis* and *C. olitorius* on agar-based and clinical cotton supported medium

Number of seed	Number of seed germination	Percentage of seed germination
Agar - 50	34.94 b	69.81 b
Cotton- 50	47.62 a	95.10 a
CV (%)	**1.21**	

Figures followed by same letter in a column do not differ significantly by DMRT.

Table 3: Combined effect of optimization of seed germination from the cultivars of *C. capsularis* and *C. olitorius* on agar-based and clinical cotton supported medium

Varieties	Number of seed used	Number of seed germinated	Seed germination (%)
CVL-1	Agar	44.55 d	89.11 d
	Cotton	47.44 b	94.88 b
CVE-3	Agar	43.58 e	87.17 e
	Cotton	49.44 a	98.88 a
Tricap-2	Agar	43.44 e	86.55 e
	Cotton	45.96 c	91.92 c
O-9897	Agar	21.56 f	43.12 f
	Cotton	46.033 c	91.333 c
O-72	Agar	21.553 f	43.107 f
	Cotton	49.23 a	98.43 a

Figures followed by same letter in a column do not differ significantly by DMRT.

Plate 1. Seed germination of variety CVE-3 on clinical cotton and agar based media.

Plate 2. Seed germination of variety O-72 on clinical cotton and agar based media.

4.1.2.Experiment-ii. *In vitro* regeneration performance of five varieties of *C. capsularis* and *C. olitorius*

Plant regeneration through callus induction offers unique facilities of reproducible protocol as well as recovery of somaclonal variants, which can be utilized for the future crop improvement programs. Therefore, induction of calli from cotyledonary explants and subsequent regeneration of complete plantlets is very important. The varieties viz. Tricap-2, CVE-3, CVL-1, O-9897, O-72 were used to see their regeneration performance.

4.1.2.1 Callus induction

Cotyledons (with attached petiole) of five varieties of C. *capsularis* and *C. olitorius* were cultured on MS medium supplemented with 2 mg/l BAP and 0.5 mg/l IAA. Callus induction performances of all the varieties were evaluated and results are presented in Table 4.

The test varieties differed significantly for both number of explants showing callus and callus induction. Cotyledons (with attached petiole) started callus initiation with a change in their shape within 6 days of incubation in all the test varieties. The initial response of callus induction was exhibited by swelling of the cut ends of the cotyledons. Callus formation was completed within 8 days. Calli induced from cotyledons were mostly compact, greenish and small in size. The percentage of callus induction was highest in CVE-3 (94%) followed by Tricap-2 (88%) (Plate 3 & 4) (Table-4).

4.1.2.2 Days required for callus initiation

The varieties Tricap-2, CVE-3 requires shorter time than O-9897, O-72 to initiate callus induction. It was observed that days required for callus initiation was minimum in CVE-3 (5.6) and maximum in O-9897 (8.20) (Table-4).

4.1.2.3 Shoot regeneration

The ultimate goal of *in vitro* technique is production of free-living plantlets via shoot and root formation from callus. The responses of different varieties towards shoot regeneration are presented in Table 5.

Shoot regeneration was the highest in CVE-3(67.99%) followed by Tricap-2 (55.66%) and CVL-1 (59.49%) (Plate 5, 6 and 7). High responsive genotype CVE-3 for callus induction had high regeneration capacity, indicating that callus induction capacity is related to regeneration of shoot. This result correlated with the findings of Khatun (2001) who reported that CVE-3 had high shoot regeneration capacity.

Table 4: Genotypic effect on number of explants showing callus, percent callus induction and days to callus initiation

Variety	Explants showing callus	Callus induction (%)	Days to callus initiation
CVE-3	9.40 a	94.00 a	6.00 b
Tricap-2	8.80 ab	88.00 ab	5.60 b
CVL-1	8.00 bc	80.00 bc	6.20 b
O-72	7.40 cd	74.00 cd	8.00 a
O-9897	6.80 d	68.00 d	8.20 a

Figures followed by same letter in a column do not differ significantly by DMRT.

Table 5: Genotypic effect on number of explants showing shoot regeneration and percent shoot regeneration

Variety	Explants showing shoot regeneration (%)	Shoot regeneration (%)
CVE- 3	6.40 a	67.99 a
Tricap- 2	5.80 ab	65.66 a
CVL- 1	4.80 bc	59.40 ab
O-72	3.80 cd	50.71 bc
O-9897	3.00 d	45.57 c

Figures followed by same letter in a column do not differ significantly by DMRT.

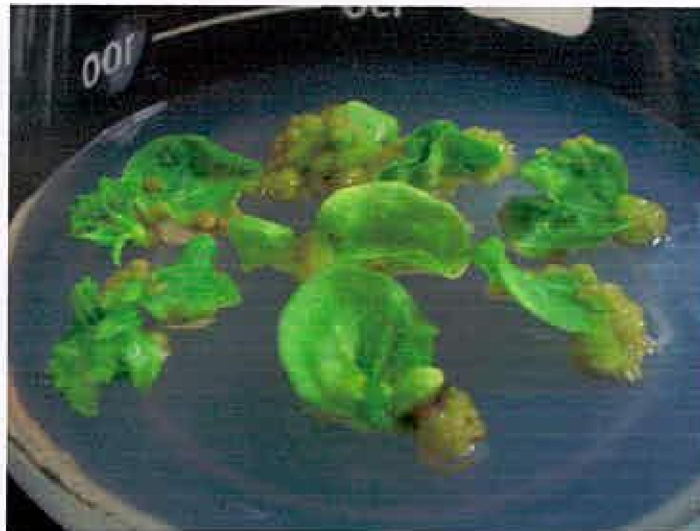


Plate 3. Callus initiation of Tricap-2 on MS +2 mg/l BAP +0.5 mg/l IAA

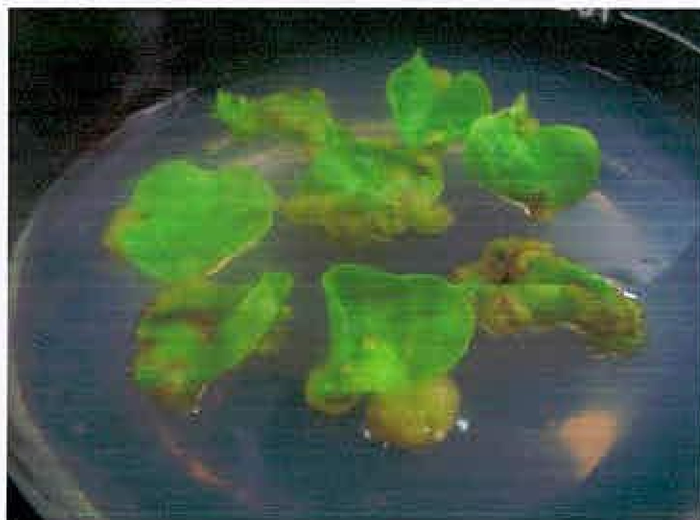


Plate 4. Callus initiation of CVE-3 on MS +2 mg/l BAP +0.5 mg/l IAA

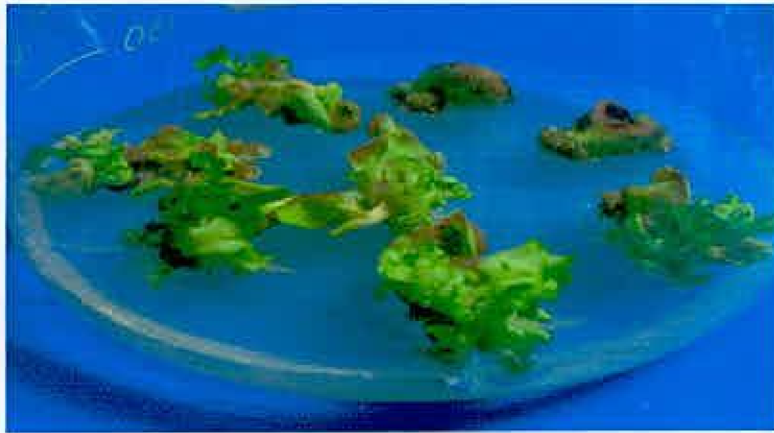


Plate 5. Shoot regeneration of Tricap-2 on MS +2 mg/l BAP +0.5 mg/l IAA

Plate 6. Shoot regeneration of CVL-1 on MS +2 mg/l BAP +0.5 mg/l IAA

Plate 7. Shoot regeneration of CVE-3 on MS +2 mg/l BAP +0.5 mg/l IA

4.1.2.4 Regeneration of root

Shoots regeneration from cultured cotyledons were transferred to MSO (MS medium without hormone) medium in order to induce root from the differentiated shoots. The results are presented in the Table 6.

Different genotype showed significant variation in producing root. CVE-3 showed highest percent of root initiation (95%) followed by Tricap-2 (85%) (Plate 8 and 9). Days required for root initiation was minimum in O-72 (5.60) and maximum in CVE-3 (7.60). CVE-3 showed superiority in rooting relative to other varieties

Table 6: Effect of different variety on number of shoot to which root induced, percent root initiation and days to root initiation

Variety	No. of shoot to which root induced	Percent root initiation	Days to root initiation
CVE- 3	19.00 a	95.00 a	7.60 a
Tricap- 2	17.00 b	85.00 b	7.20 a
CVL-1	15.20 c	76.00 c	5.80 bc
O-72	13.20 d	66.00 d	5.60 c
O-9897	12.00 e	60.00 e	7.40 a

Figures followed by same letter in a column do not differ significantly by DMRT.

Plate 8. Initiation of roots from regenerated shoot of Tricap-2 on MSO medium

Plate 9. Initiation of roots from regenerated shoot of CVE-3 on MSO medium

4.1.3 Experiment -iii.Optimization of plant regeneration from the explants of *C. capsularis* with different concentration of BAP and IAA.

In this experiment different combination of BAP and IAA were used for callus induction and shoot regeneration using cotyledonary petioles as explants of var.CVE-3.

4.1.3.1 Callus Induction

In vitro callus induction depends on a number of factors including proper concentration of growth regulators.

4.1.3.1.1 Effect of phytohormone (BAP)

Different concentration of BAP levels showed significant variation for number of explants showing callus, percent callus induction and days required for callus induction, indicating significant differences among the concentrations of the BAP on these characters. BAP at 2 mg/l was found to be the best for all the characters (Table- 7).

4.1.3.1.2 Effect of phytohormone (IAA)

Mean value due to different concentration of IAA for number of explants showing callus, percent callus induction and days required for callus induction were significant, indicating the presence of variation among the concentrations used for this study. IAA at 0.5 mg/l concentration was found to be the best. Maximum number of explants showing callus (8.44) with the highest percentage of callus induction (84.40%) was found at this concentration. It was also observed that the above concentration requires shorter days to initiate callus (Table-7).

4.1.3.1.3 Combined effect of BAP and IAA

Combined effect of BAP and IAA on number of explants showing callus, percent callus induction and days required for callus induction are presented in Table 8.

The highest percentage of callus induction was found in the combination of MS+2 mg/l BAP+0.5 mg/l IAA (96%) followed by MS+ 2mg/l BAP+ 1.5 mg/l IAA (94%). Minimum days (6.0) required for callus induction was observed in the combination of MS+ 0.0 mg/l BAP+ 1.0 mg/l IAA.

Table 7: Main effect of different hormone concentration of BAP and IAA on number of explants showing callus and percent callus induction and days required for callus induction in *C. capsularis* var. CVE-3

Treatment	No. of explants showing callus	Percent callus Induction	Days required for callus induction
BAP (mg/L)			
0.0	5.36 c	53.60 c	7.16 a
1.0	8.32 ab	83.20 ab	8.00 a
2.0	8.96 a	89.60 a	5.72 b
3.0	8.04 ab	80.40 ab	7.44 a
4.0	7.56 b	75.60 b	7.52 a
IAA (mg/L)			
0.0	5.84 b	58.40 b	6.72 bc
0.5	8.44 a	84.40 a	6.12 c
1.0	8.12 a	81.20 a	6.88 bc
1.5	7.92 a	79.20 a	7.76 ab
2.0	7.92 a	79.20 a	8.36 a

Figures followed by same letter in a column do not differ significantly by DMRT.

Table 8: Combined effect of BAP and IAA on number of explants showing callus, percent callus induction and days required for callus induction *C. capsularis* var. CVE-3

Treatment		No. of explants showing callus	Percent callus induction	Days required for callus induction
BAP (mg/L)	IAA (mg/L)			
0.0	0.0	-	-	-
	0.5	6.80 gh	68.00 gh	6.20 f
	1.0	7.00 fgh	70.00 fgh	6.00 f
	1.5	6.40 h	64.00 h	7.80 bcd
	2.0	6.60 gh	66.00 gh	8.60 ab
1.0	0.0	7.00 fgh	70.00 fgh	9.20 a
	0.5	9.00 abcd	90.00 abcd	6.40 ef
	1.0	8.80 abcde	88.00 abcde	7.00 cdef
	1.5	8.40 abcdef	84.00 abcdef	8.20 abc
	2.0	8.40 abcdef	84.00 abcdef	9.20 a
2.0	0.0	7.80 cdefg	78.00 cdefgh	6.60 def
	0.5	9.60 a	96.00 a	6.80 def
	1.0	9.20 abc	92.00 abc	6.40 f
	1.5	9.40 ab	94.00 ab	7.80 bcd
	2.0	8.80 abcde	88.00 abcde	8.20 abc
3.0	0.0	7.40 efgh	74.00 efgh	6.00 f
	0.5	8.40 abcdef	84.00 abcdef	6.60 def
	1.0	8.00 bcdefg	80.00 bcdefg	8.20 abc
	1.5	8.40 abcdef	84.00 abcdef	7.80 bcd
	2.0	8.00 bcdefg	80.00 bcdfgh	8.60 ab
4.0	0.0	7.00 fgh	70.00 fgh	8.80 ab
	0.5	8.40 abcdef	84.00 abcdef	7.60 bcde
	1.0	7.60 defgh	76.00 defgh	6.80 def
	1.5	7.00 fgh	70.00 fgh	7.20 cdef
	2.0	7.80 cdefgh	78.00 cdefgh	7.20 cdef

Figures followed by same letter in a column do not differ significantly by DMRT.

4.1.3.2 Shoot regeneration

4.1.3.2.1 Effect of phytohormone (BAP)

Different concentration of BAP levels showed significant variations for number of explants showing shoot, percent shoot regeneration and days required for shoot regeneration. The responses of calli to different concentrations of BAP towards shoot regeneration are presented in Table 9. Shoot regeneration was found highest at 2 mg/l BAP (42.23%) followed by 3 mg/l BAP (30.12%). Days required for shoot regeneration was also minimum (18.72) at 2 mg/l BAP concentration.

Number of explants showing shoot and percent shoot regeneration gradually increased with the increasing level of BAP up to 2 mg/l. Further increasing of BAP level did not show any improvement of number of explants showing shoot and percent shoot regeneration. The minimum regeneration (14.85%) was observed 0 BAP treatment level.

4.1.3.2.2 Effect of phytohormone (IAA)

The highest percentage of shoot regeneration was found at 0.5 mg/l IAA (35.94%) than the other concentrations. Days required for shoot regeneration was also found minimum (19.74) at this concentration. Shoot regeneration was minimum (19.75%) at control level.

4.1.3.2.3 Combined effect of BAP and IAA

The combined effect of BAP and IAA showed that highest shoot regeneration percentage (60.66%) was recorded in MS medium supplemented with 2 mg/l BAP and 0.5 mg/l IAA followed by MS+2mg/l BAP+ 1.0 mg/l IAA (47.28%). This finding

is similar to the finding of Khatun (2001) who found best performance in shoot regeneration on the combination of MS+2 mg/l BAP+0.5 mg/l IAA. No shoot regeneration ability was found without IAA and BAP (Table 8 & Appendix-x). Days required of shoot regeneration was also found minimum in the combination of MS+2 mg/l BAP + 0.5 mg/l IAA. It might be concluded that MS+2mg/l BAP+0.5 mg/l IAA combination was favorable for higher percentage of shoot regeneration; on the other hand extreme lower and higher combinations of IAA and BAP did not favoured shoot regeneration. The results have been given in Appendix-X

Table 9:Effect of different hormone concentration on number of explants showing shoot, percent shoot regeneration and days required for shoot regeneration

Treatment	No. of explants showing shoot	Percent shoot regeneration	Days required for shoot regeneration
BAP (mg/L)			
0.0	1.00 c	14.85 c	22.64 b
1.0	2.20 b	25.13 bc	24.12 a
2.0	3.84 a	42.23 a	18.72 c
3.0	2.44 b	30.12 ab	22.12 b
4.0	1.76 bc	23.36 ab	22.52 b
IAA (mg/L)			
0.0	1.44 c	19.75 b	19.76 b
0.5	3.16 a	35.94 a	19.76 b
1.0	2.64 ab	31.39 ab	22.56 a
1.5	2.16 abc	23.49 ab	22.44 a
2.0	1.84 bc	25.12 ab	22.88 a

Figures followed by same letter in a column do not differ significantly by DMRT.

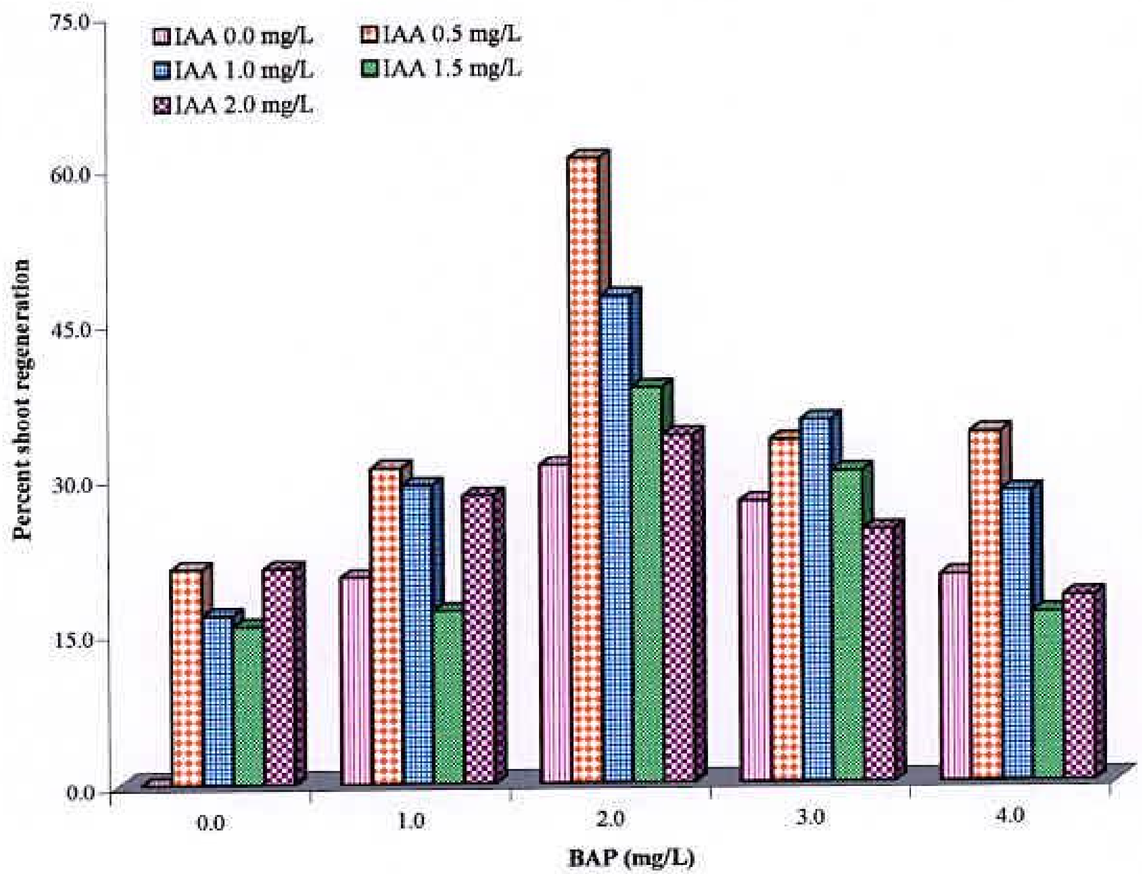


Figure-1: Combined effect of different concentrations of BAP and IAA on shoot regeneration

4.1.4 Experiment –iv. Effects of different levels of pH on plant regeneration from cotyledons of *C. capsularis* and *C. olitorius*

Two varieties of *C. capsularis* and *C. olitorius* (vars. CVE-3 and O-72) were cultured in the presence of a range of pH levels (e.g. 4.0, 4.5, 5.5, 6.0, 6.5, 7.0, 7.5) in association with MS plant regeneration medium. Two varieties responded differently on various levels of pH (Table-11).

4.1.4.1 Percent shoot regeneration

Mean square values of two different varieties were found statistically significant for percent shoot regeneration and number of shoot per cotyledon, indicating significant differences between the varieties. It was observed that CVE-3 showed relatively better response (52.95%) towards shoot regeneration than that of O-72 (35.08%) (Table-10).

The highest percentage of shoot regeneration was found at pH 5.5 (58.50%) and lowest at pH 4.0 (35.80%) (Table-10). It may be concluded that pH has significant effect on shoot regeneration.

The results the combined effect of variety and pH level on percent shoot regeneration and number of shoot per cotyledon are presented in the Table 11. In case of O-72, highest shoot regeneration percentage was found at pH 5.5 and lowest at pH 7.5 (Plate.10 &11). It was observed that percentage of shoot regeneration gradually decreased at pH levels were increased in var. O-72 (Table -11). On the other hand a contradictory result was observed from the cultured cotyledons of CVE-3. In case of CVE-3, highest percentage of shoot regeneration was found at pH 7.5 which was 71% and lowest (35%) at pH 4.0 (Plate. 12&13). Shoot regeneration from cultured cotyledons of CVE-3 gradually increases as the pH levels were increased. However,

observing the result of the number of cotyledons responded for shoot production of both of the varieties might suggest that CVE-3 was more capable to grow at various range of pH containing soil compared to O-72. The results showed conformity with the findings of Naher and Khatun (2004) who also found the similar result.

4.1.4.2 Number of shoot /cotyledon

The highest number of shoot regeneration per cotyledon was recorded in variety CVE-3 (8.57) than that of O-72 (6.8) (Table-10). The combined effect of variety and pH for number of shoot per cotyledon was highest in CVE-3 (13) at pH 6.0 and lowest in O-72 (2.8) at pH 7.5 (Table-11).

Table 10: Main effect of different pH level on percent shoots regeneration and number of shoot/cotyledon

Variety	Percent shoots regeneration	No. of shoot/cotyledon
O-72	35.08 b	6.80 b
CVE-3	52.95 a	8.57 a

Table 11: Combined effect of variety and pH level on percent shoot regeneration and number of shoot/cotyledon

Variety	pH level	Percent shoot regeneration	No. of shoot/cotyledon
O-72	4.0	36.60 ef	5.80fg
	4.5	41.80 def	8.20 de
	5.0	48.60 cd	9.00 cd
	5.5	67.00 ab	9.80 c
	6.0	37.60 ef	8.20 de
	6.5	24.00 g	5.80 fg
	7.0	17.60 g	4.80 g
	7.5	7.40 h	2.80 h
CVE-3	4.0	35.00 f	5.40 fg
	4.5	41.00 def	7.20 e
	5.0	45.00 cde	9.80 c
	5.5	50.00 cd	11.00 b
	6.0	52.00 c	13.00 a
	6.5	62.00 b	9.00 cd
	7.0	67.00 ab	7.20 e
	7.5	71.00 a	6.00 f

Figures followed by same letter in a column do not differ significantly by DMRT.

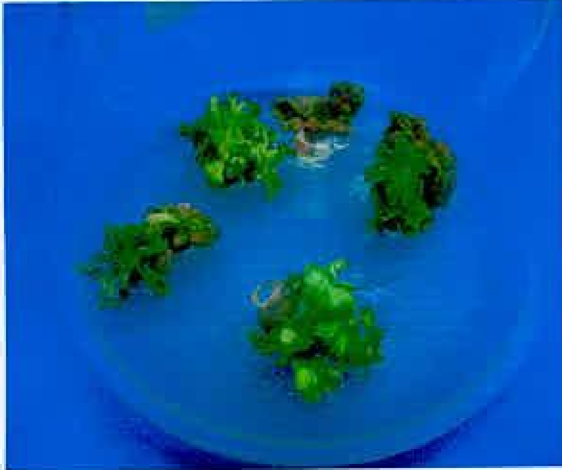


Plate 10. Shoot regeneration of var. O-72 at pH 5.5

Plate 11. Shoot regeneration of var. O-72 at pH 7.0

Plate12. Shoot regeneration of var. CVE-3 at pH 7.0

Plate 13. Shoot regeneration of var. CVE-3 at pH 4.0

4.1.5 Experiment-v. Influence of different concentration of sucrose on plant regeneration from cotyledons of *C. capsularis* and *C. olitorius*

4.1.5.1 Callus induction

In vitro callus induction depends on a number of factors including proper concentration of sucrose.

4.1.5.2 Effect of variety

There was no significant effect of varieties in percent shoot regeneration. But different variety showed significant difference in shoot/cotyledon (Table-12).

4.1.5.3 Effect of Sucrose concentration

The significant mean square due to different concentration of sucrose reverted the presence of adequate differences among the concentration for all the characters under study (Appendix-IX). It was noted from the present result that 3% (30gm/l) sucrose concentration was found the best for all the characters. (Table-12)

Table 12: Main effect of varieties and Sucrose concentration on no. of explants showing shoot, percent of shoot regeneration and number of shoot per cotyledon

Treatment	Number of explants showing shoot regeneration	Percent shoot regeneration	Number of shoot/cotyledon
CVE-3	13.17	54.86	6.08
O-72	12.16	50.69	5.75
Sucrose (gm)			
0 (Control)	0.00 d	0.00 d	0.00 d
10	12.66 c	52.77 c	7.33 b
20	17.00 b	70.83 b	7.50 b
30	21.00 a	87.49 a	8.83 a
CV (%)	**11.16**	**13.68**	**4.88**

Figures followed by same letter in a column do not differ significantly by DMRT.

4.1.5.4 Number of shoot/cotyledon

The highest number of shoot per cotyledon was recorded in variety CVE-3 (6.083) than that of O-72 (5.75) (Table-12) At 3% (30gm/l) sucrose concentration, number of shoot per cotyledon was found the highest (9.00) in var. CVE-3. Number of shoot per cotyledon was found 8.66 in var. O-72 at the same sucrose concentration (Table-13). It was observed that the number of shoot per cotyledon increased as the sucrose concentration increased.

4.1.5.5 Combined effect of variety and sucrose

The combined effect of variety and sucrose (Table-13) showed that highest shoot regeneration percentage in var. CVE-3 (91.66%) was recorded in MS media supplemented with 3% (30gm/l) sucrose followed by MS+2% (20gm/l) sucrose concentration. The highest shoot regeneration percentage (83.33%) was recorded in var. O-72 in MS media supplemented with 3% (30gm/l) sucrose followed by MS+ 2% (20gm/l) sucrose concentration (69.44%). The finding is similar to the findings to Khatun (2001) who found the best performance in shoot regeneration on the combination of MS medium with 3% sucrose concentration. No shoot regeneration ability was found without sucrose. It might be concluded that MS media with 3% sucrose combination was favourable for higher percentage of shoot regeneration; on the other hand lower combination of sucrose inhibited shoot regeneration. (Appendix-IX)

Table 13: Combined effect of different concentration sucrose on number of explants showing shoot regeneration, percent shoots regeneration and number of shoot produced per cotyledon

Variety	Sucrose concentration (gm/l)	Number of explants showing shoot regeneration	Percent shoot regeneration	Number of shoot/cotyledon
CVE-3	0 (Control)	0.00 d	0.00 e	0.00 d
	10	13.33 c	55.55 d	7.00 c
	20	17.33 b	72.22 bc	7.00 c
	30	22.00 a	91.66 a	9.00 a
O-72	0 (Control)	0.000 d	0.0 c	0.00 d
	10	12.00 c	49.99 d	7.66 b
	20	16.66 b	69.44 c	8.00 b
	30	20.00 a	83.30 ab	8.66 a
CV (%)		**11.16**	**13.68**	**4.88**

Figures followed by same letter in a column do not differ significantly by DMRT.

Plate 14. Shoot regeneration in jute var. CVE-3 on 3 % (30gm/l) sucrose concentration

Plate 15. Shoot regeneration in jute var. O-72 on 3 % (30gm/l) sucrose concentration

4.1.6 Experiment-vi. Influence of different concentration of vitamins on plant regeneration from cotyledons of *C. capsularis* and *C. olitorius*

4.1.6.1 Shoot regeneration

In the present investigation, contyledon as explants of the test varieties (CVE-3,O-72) were cultured on MS medium supplemented with 2mg/l BAP and 0.5mg/l IAA. Using different concentration of vitamins, CVE-3 produced more shoot (44.44%) than O-72. (Table-14).

4.1.6.2 Effect of variety

There was significant effect of varieties in shoot regeneration, percent shoot regeneration and number of shoot per cotyledon. CVE-3 showed superiority over O-72 for all the characters (Table-14). This result has conformity with the findings of Khatun *et al.*, (1992).

Table 14: Main effect of varieties and vitamin concentration on number of explants showing shoot, percent shoot regeneration and number of shoot produced per cotyledon

Treatment	Number of explants showing shoot regeneration	Percent shoot regeneration	Number of shoot/cotyledon
CVE-3	5.33	44.44	6.00
O-72	4.93	41.10	5.40

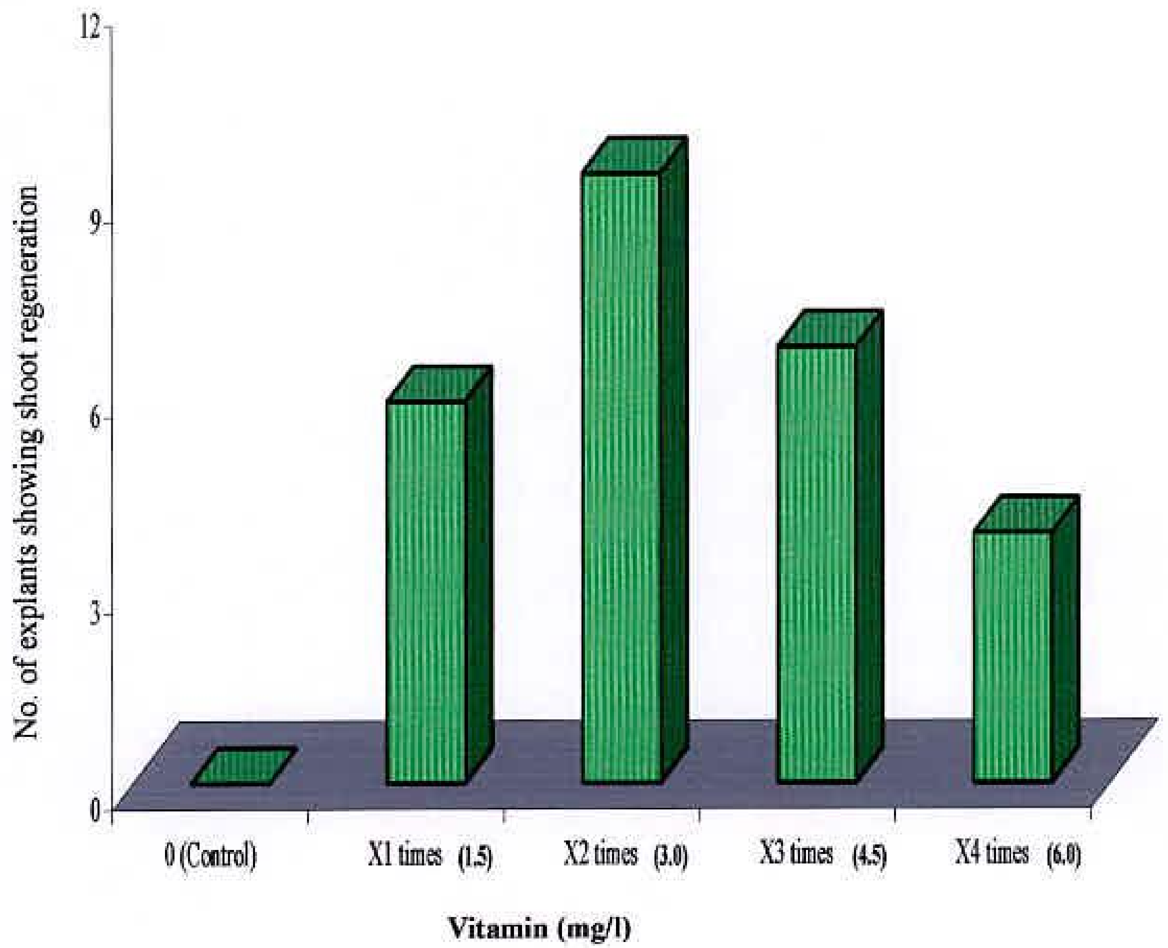


Figure-2: **Main effect of different concentration of Vitamin on number of explants showing shoot regeneration**

4.1.6.3 Effect of different concentration of vitamins

Mean value due to different concentration of vitamins for number of explants showing shoot, percent shoot regeneration and number of shoot produce per cotyledon were significant, indicating the presence of variation among the vitamin concentration used for the study, x 2 times (3.0mg/l) concentration was found to be the best. Maximum numbers of explants showing shoot was 19.33 and the highest percent of shoot regeneration was 80.85% found at this concentration. The variety CVE-3 perform better than the other variety and number of shoot produced per cotyledon was 9.00.

Number of explants showing shoot and percent shoot regeneration gradually increased with the increase of vitamin level up to x 2 times. Further increase of vitamin level did not show any improvement of shoot regeneration and percent shoot regeneration (Table- 15)

4.1.6.4 Number of shoot/cotyledon

The highest number of shoot per cotyledon was recorded in variety CVE-3 (6.00) than that of O-72 (5.4) (Table-15). At x2 times vitamin concentration number of shoot per cotyledon was found the highest (9.00) in var. CVE-3. The highest number of shoot per cotyledon was (7.66) found var. O-72 at same concentration (Table-15). It was observed that the number of shoot per cotyledon decreased as the vitamin concentration increased. The combined effect of variety and vitamins for number of shoot per cotyledon was the highest in CVE-3 (9.00) at x2 times vitamin concentration and the lowest in O-72 (7.00) both at x3 & x4 times vitamin concentration.

4.1.6.5 Combined effect of variety and vitamin

The combined effect of variety and vitamins (Table -15) showed that the highest shoot regeneration percentage (80.55%) was recorded in MS media supplemented with x2 times vitamin concentration followed by MS media with x3times vitamin concentration (58.33%). The highest shoot regeneration percentage (75.00%) was recorded var. O-72 in MS media supplemented with x2 times vitamin concentration followed by MS media with x3 times vitamin concentration (52.77%). This finding is similar to the findings of Khatun (2001) who found the best performance in shoot regeneration on the combination of MS media with x2 times vitamin concentration. No shoot regeneration ability was found without vitamin. It might be concluded that MS media with x2 times vitamins combination was favorable for higher percentage of shoot regeneration (Plate 16 & 17). On the other hand extreme lower and higher combination of vitamin inhibited shoot regeneration. The result have been given in Table-15.

Table 15: Combined effect of different concentration of vitamins on number of explants showing shoot, percent shoot regeneration and number of shoot produce per cotyledon

Variety	Vitamin concentration (mg/l)	Number of explants showing shoot	Percent shoot regeneration	Number of shoot/cotyledon
CVE-3	0 (Control)	0.00 f	0.00 f	0.000 e
	X1 times (1.5)	12.00 cd	49.99 cd	7.00 c
	X2 times (3.0)	19.33 a	80.55 a	9.00 a
	X3 times (4.5)	14.00 bc	58.33 bc	7.00 c
	X4 times (6.0)	8.00 df	33.33 de	6.00 d
O-72	0 (Control)	0.00 f	0.00 f	0.00 e
	X1 times (1.5)	11.33cde	47.22 cde	7.00 c
	X2 times (3.0)	18.00 ab	74.99 ab	7.66 b
	X3 times (4.5)	12.66 c	52.77 c	7.00 c
	X4 times (6.0)	7.33 e	30.55 e	7.00 c
CV (%)		**23.32**	**23.33**	**4.53**

Figures followed by same letter in a column do not differ significantly by DMRT.

Plate 16. Shoot regeneration in jute var. CVE-3 on x 2 times (3.0mg/l) vitamin concentration

Plate 17. Shoot regeneration in jute var. O-72 on x 2 times (3.0mg/l) vitamin concentration

4.1.7 Experiment-vii. Influence of surfactants (Pluronic F-68) on plant regeneration from cotyledons of *C. capsularis* and *C. olitorius*

4.1.7.1 Effect of variety

There was significant effect of varieties in shoot regeneration and percent shoot regeneration. But there was no significant effect of number of shoot produced per cotyledon. The variety CVE-3 produced more cotyledon than other variety (Table-16)

4.1.7.2 Effect of surfactant concentration:

Mean value due to different concentration of surfactant for number of explants showing shoot, percent shoot regeneration and number of shoot, produced per cotyledon were significant (Appendix-viii), indicating the presence of variation among the surfactant concentration used for the study, 0.1 % was found to be the best. Maximum number of explants showing shoot regeneration was (23.33) and percent shoot regeneration was (97.22) (Table-16). Shoot growth was increased in lower concentration (0.1%) of surfactant and further growth was inhibited as the concentration was increased at (0.5%). This result was conformity with the findings of Khatun *et al.*, (1992).

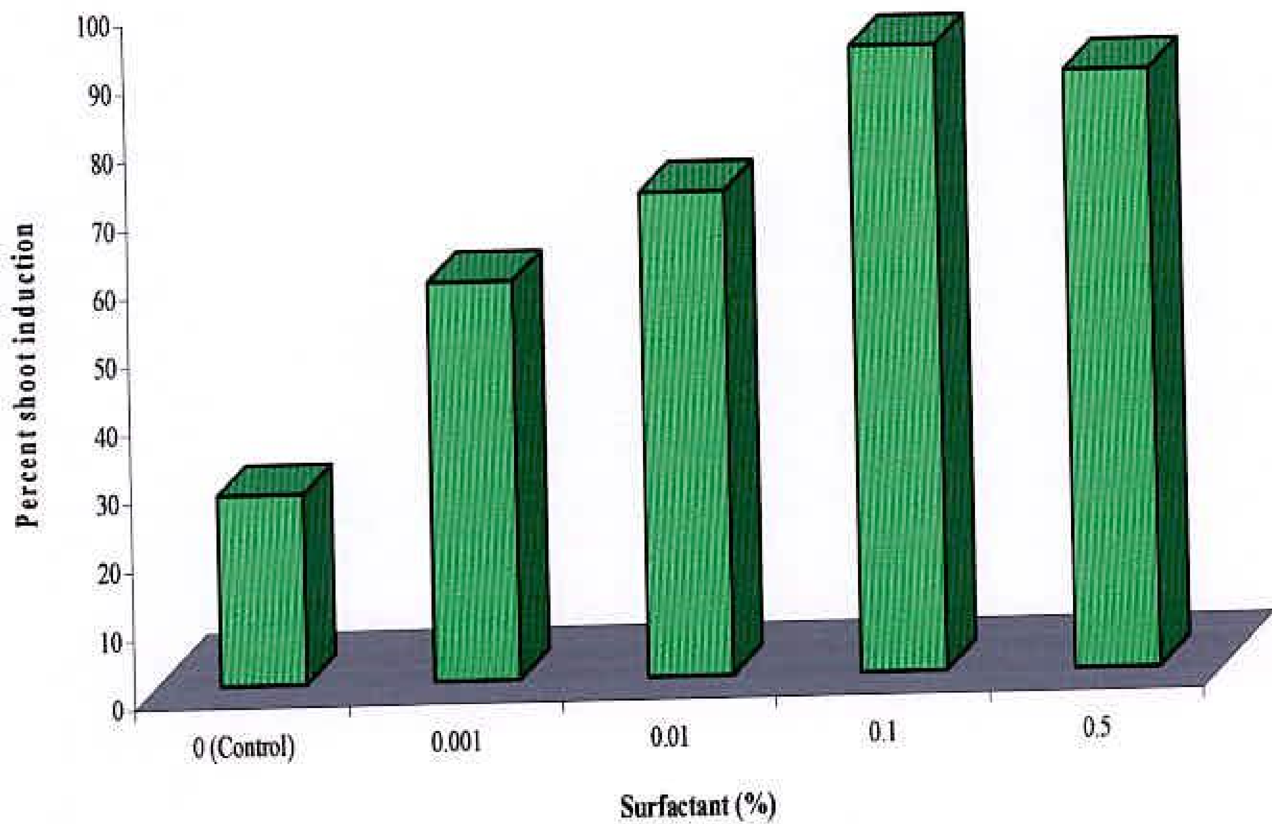


Figure-3: Main effect of different concentration of surfactant on percent shoot regeneration

4.1.7.3 **Number of shoot per cotyledon**

The combined effect of variety and surfactant for number of shoot per cotyledon was highest in CVE-3 (8.66) at 0.1% surfactant and lowest in O-72 (6.88) at 0.001% surfactant. A lower shoot per cotyledon was noticed both at lower or higher surfactant concentration (Table -16).

4.1.7.4 Combined effect of variety x surfactant

The combined effect of variety and surfactant (Table-16) showed that highest shoot regeneration percentage in var. CVE-3 (97.22%) was recorded in MS media supplemented with 0.1% surfactant followed by MS media with 0.5% surfactant (91.66%). Highest shoot regeneration percentage (86.10%) was recorded (var. O-72) in MS media supplemented with 0.1% surfactant followed by MS media with 0.5% surfactant (83.33%) (Plate 18 & 19) .This findings is similar to the findings of Khatun (1992) who found best performance in shoot regeneration on the combination of MS media with 0.1% surfactant. It might be concluded that MS media with 0.1% surfactant combination is favourable for higher percentage of shoot regeneration; on the other hand both lower and higher combination of surfactant reduced shoot regeneration. The result have been given in Table-16.

Table 16: Combined effect of different concentration of surfactant (F-68) on number of explants showing shoot, percent shoot regeneration and number of shoot produced per cotyledon

Variety	Surfactant Concentration (%)	Number of explants showing shoot	Percent shoot regeneration	Number of shoot/cotyledon
CVE-3	0 (Control)	7.334 c	30.55 e	7.00 b
	0.001	14.00 d	58.33 d	7.00 b
	0.01	18.00 bc	74.99 bc	7.33 ab
	0.1	23.33 a	97.22 a	8.66 a
	0.5	22.00 a	91.66 a	7.00 b
O-72	0 (Control)	6.00 e	24.99 e	7.00 b
	0.01	14.00 d	58.33 d	6.88 ab
	0.01	16.00 cd	66.66 cd	7.00 b
	0.1	20.66 ab	86.10 ab	8.55 a
	0.5	20.00 ab	83.33 ab	7.00 b
CV (%)		**12.40**	**12.40**	**9.26**

Figures followed by same letter in a column do not differ significantly by DMRT.

Plate 18. Shoot regeneration in jute var. CVE-3 on 0.1% surfactant

Plate 19. Shoot regeneration in jute var. O-72 on 0.1% surfactant

4.1.8 **Experiment-viii. Influence of NaCl on plant regeneration from cotyledons of *C. capsularis* and *C. olitorius***

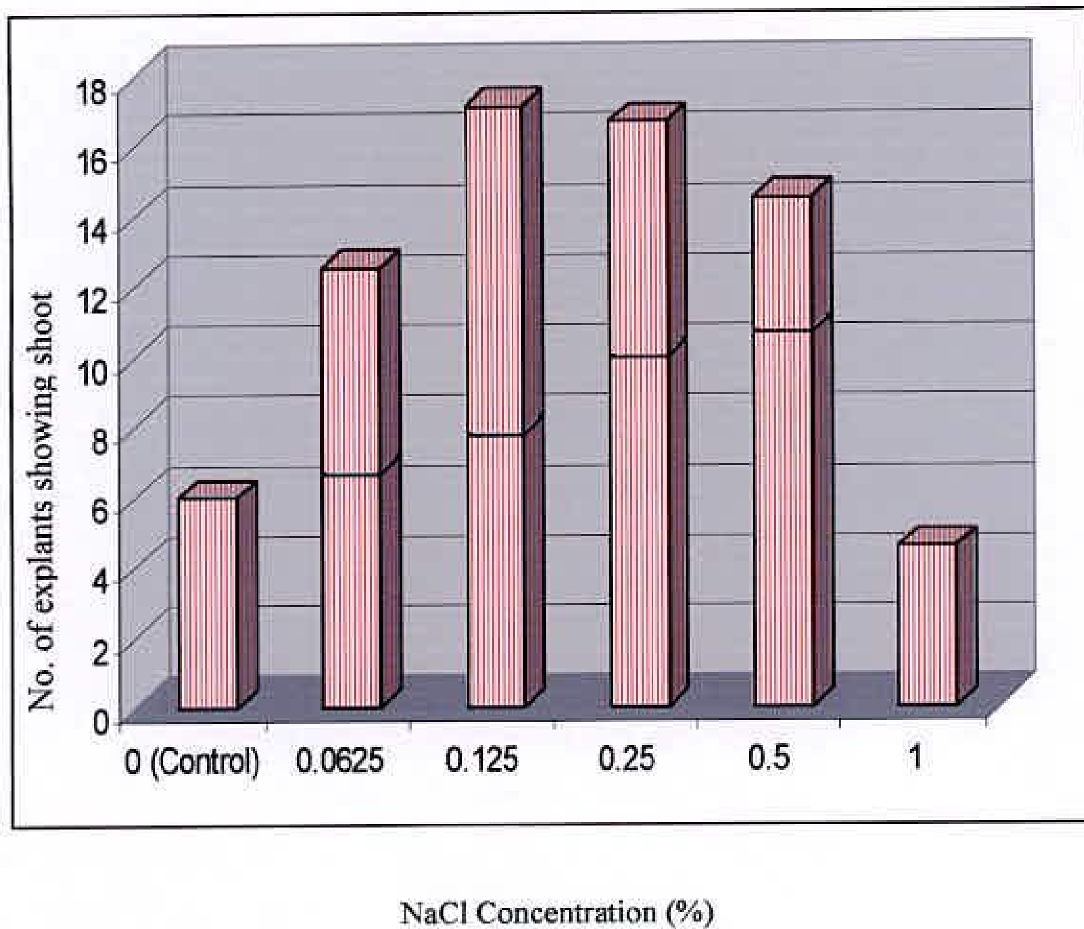
In vitro callus induction and regeneration depends on a number of factors including proper concentration of NaCl.

4.1.8.1 Effect of variety:

There was no significant effect of varieties in shoot regeneration and percent shoot regeneration. But a significant effect of number of shoot produced per each cotyledon was observed (Appendix-v).

4.1.8.2 Effect of NaCl concentration:

Mean value due to different concentration of NaCl for number of explants showing shoot, percent shoot regeneration and number of shoot produce per each cotyledon were significant, (Appendix-v) indications the effect of variation among the NaCl concentration 0.125 mg/l was found to be the best. Number of explants showing shoot and percent shoot regeneration gradually increased with the increase of NaCl level up to 0.5 mg/l. Further increase of NaCl level did not show any improvement of shoot regeneration and percent shoot regeneration (Table-17, Figure-4). This result has conformity with the findings of Khatun and Naher (2005).



NaCl Concentration (%)

Figure -4: Main effect of different concentration of NaCl on number of explants showing shoot

4.1.8.3 Number of shoot per cotyledon

The combined effect of variety and NaCl for number of shoot per cotyledon was highest in CVE-3 (8.33) at 0.5%.NaCl and lowest in O-72 (6.00) at 1.0% NaCl. It was observed that the number of shoot per cotyledon decreased as the NaCl concentration increased (Table-17).

4.1.8.4 Combined effect of variety and NaCl

The combined effect of variety and NaCl (Table-17) showed that highest shoot regeneration percentage in var. CVE-3 (97.22%) and Tricap-2 (94.44%) was recorded in MS media supplemented with 0.5% NaCl followed by MS media with 0.25% NaCl (91.66%) in variety CVE-3. In variety Tricap -2 highest shoot regeneration percentage was 94.440% at 0.5% NaCl followed by MS media with 0.25% NaCl (86.12%) And variety O-72, highest shoot regeneration percentage (74.99%) at 0.5% NaCl followed by Ms media with 0.25% NaCl (72.22%).This findings is similar to the findings of Khatun (2005) who found best performance in shoot regeneration on the combination of MS media with 0.5% NaCl. It might be concluded that MS media with 0.5% NaCl combination was favourable for higher percentage of shoot regeneration (Plate 20 & 21). On the other hand extreme lower and higher combination of NaCl inhibited shoot regeneration (Table-17).

Table 17: Combined effect of different concentration of NaCl (%) on number of explants showing shoot, percent shoot regeneration and number of shoot produced per cotyledon

Variety	NaCl concentration (%)	Number of explants showing shoot	Percent shoot regeneration	Number of shoot/cotyledon
CVE-3	0 (Control)	12.00 g	56.30 h	5.00 d
	0.0625	14.00 d	58.33 ef	6.33 cd
	0.125	12.66 d	74.99 bc	6.667 cd
	0.250	22.00 ab	91.66 a	7.000 bc
	0.500	23.33 a	97.22 a	8.33 a
	1.0	6.66 ef	27.77 g	6.66 cd
Tricap-2	0 (Control)	12.00 g	56.30 h	5.00 d
	0.0625	14.00 d	58.33 def	6.33 cd
	0.125	18.00 abcd	74.99 bc	6.66 cd
	0.250	20.66 abc	86.12 ab	7.00 bc
	0.500	22.66 ab	94.44 a	8.33 a
	1.0	6.66 f	27.77 g	6.66 cd
O-72	0 (Control)	10.04 g	48.00 h	6.00 d
	0.0625	12.000 de	49.997 f	6.33 cd
	0.125	16.00 cd	66.66 cde	6.66 cd
	0.250	17.33 bcd	72.220	7.667 ab
	0.500	18.00 abcd	74.99 bcd	8.333 a
	1.0	14.00 d	58.33 ef	6.00 d
CV (%)		**23.74**	**13.51**	**7.71**

Figures followed by same letter in a column do not differ significantly by DMRT.

Plate 20. Shoot regeneration in jute var. CVE-3 on 0.5% (0.5 ml/l) NaCl oncentration

Plate 21. Shoot regeneration in jute var.O-72 on 0.5% (0.5ml/l) NaCl concentration

4.1.9 Experiment-ix. Influence of $FeSO_4$ on plant regeneration from cotyledons of *Corchorus capsularis* and *C. olitorius*

In vitro shoot regeneration depends on a number of factors including proper concentration of $FeSO_4$.

4.1.9.1 Effect of variety

There was no significant effect of varieties in shoot regeneration and percent shoot regeneration. But there was significant effect of number of shoot produce per each cotyledon (Appendix-VI).

4.1.9.2 Effect of $FeSO_4$ concentration:

Mean value due to different concentration of $FeSO_4$ for number of explants showings shoot, percent shoot regeneration and number of shoot produced per cotyledon were significant, (Appendix-vi) indicating the effect in variation among the $FeSO_4$ concentration used for the study, $FeSO_4$ concentration x 2 times (3.0mg/l) was found to be the best. Number of explants showing shoot and percent shoot regeneration gradually increase with the increase of $FeSO_4$ level up to x 2 times. Further increase of $FeSO_4$ level did not show any improvement of shoot regeneration and percent shoot regeneration (Table-18). The results have conformity with the finding of Khatun (1996) who reported that x2 times (3.0ml/l) $FeSO_4$ showed the best performance in shoot regeneration.

4.1.9.3 Number of shoot per cotyledon:

The combined effect of variety and $FeSO_4$ for number of shoot per cotyledon was highest in CVE-3 (8.00) at x2 times (3.0ml/l) $FeSO_4$ and lowest in O-72 (7.00) at x4times (6.0ml/l) $FeSO_4$. It was observed that the number of shoot per cotyledon decreased as the $FeSO_4$ concentration increased (Table-18).

Table 18: Main effect of varieties and $FeSO_4$ concentration on number of explants showing shoot, percent shoot regeneration and number of shoot produced per cotyledon

Treatment	Number of explants showing shoot	Percent shoot regeneration	Number of shoot/cotyledon
CVE-3	10.66	44.44	6.00
O-72	10.66	44.44	5.40
$FeSO_4$ (mg/l)			
0 (Control)	0.00 d	0.00 d	0.00 c
X1 Times (1.5)	13.00 b	54.16 b	6.83 b
X2 Times (3.0)	18.00 a	74.99 a	7.50 a
X3 Times (4.5)	14.33 b	59.72 b	7.00 b
X4 Times (6.0)	8.00 c	33.33 c	7.00 b
CV (%)	**18.75**	**18.75**	**3.22**

Figures followed by same letter in a column do not differ significantly by DMRT.

4.1.9.4 Combined effect varieties x $FeSO_4$

The combined effect of variety and $FeSO_4$ (Table -19) showed that the highest shoot regeneration percentage (80.55%) was recorded in MS media supplemented with x2 times $FeSO_4$ concentration followed by MS media with x3 times $FeSO_4$ concentration (61.11%). Highest shoot regeneration percentage (69.44%) was recorded (var. O-72) in MS media supplemented with x2 times $FeSO_4$ concentration followed by MS media with x3 times $FeSO_4$ concentration (58.33%). This finding was similar to the findings of Khatun (1996) who found best performance in shoot regeneration on the combination of MS media with x2 times $FeSO_4$concentration. No shoot regeneration ability was found without $FeSO_4$.It might be concluded that MS media with x2times $FeSO_4$ combination was favourable for higher percentage of shoot regeneration (Plate 22 & 23). On the other hand extreme lower and higher combination of $FeSO_4$ inhibited shoot regeneration. The result have been given in Table-19.

Table 19: Combined effect of different concentration of $FeSO_4$ on number of explants showing shoot, percent shoot regeneration and number of shoot produced per cotyledon

Variety	$FeSO_4$ concentration (mg/l)	Number of explants showing shoot	Percent shoot regeneration	Number of shoot/cotyledon
CVE-3	0 (Control)	0.00 e	0.00 e	0.00 e
	X1 times (1.5)	14.00 bc	58.33 bc	6.00 d
	X2 times (3.0)	19.33 a	80.55 a	8.00 a
	X3 times (4.5)	14.66 bc	61.11 bc	7.66 b
	X4 times (6.0)	8.00 d	33.33 d	7.00 c
O-72	0 (Control)	0.00 e	0.00 e	0.00 e
	X1 times (1.5)	12.00 c	49.99 c	7.00 c
	X2 times (3.0)	16.66 ab	69.44 ab	7.66 b
	X3 times (4.5)	14.00 bc	58.33 bc	7.00 c
	X4 times (6.0)	8.00 d	33.33 d	7.00 c
CV (%)		**18.75**	**18.75**	**3.22**

Figures followed by same letter in a column do not differ significantly by DMRT.

Plate 22. Shoot regeneration in jute var. CVE-3 on x 2 times (3.0 mg/l) $FeSO_4$ concentration

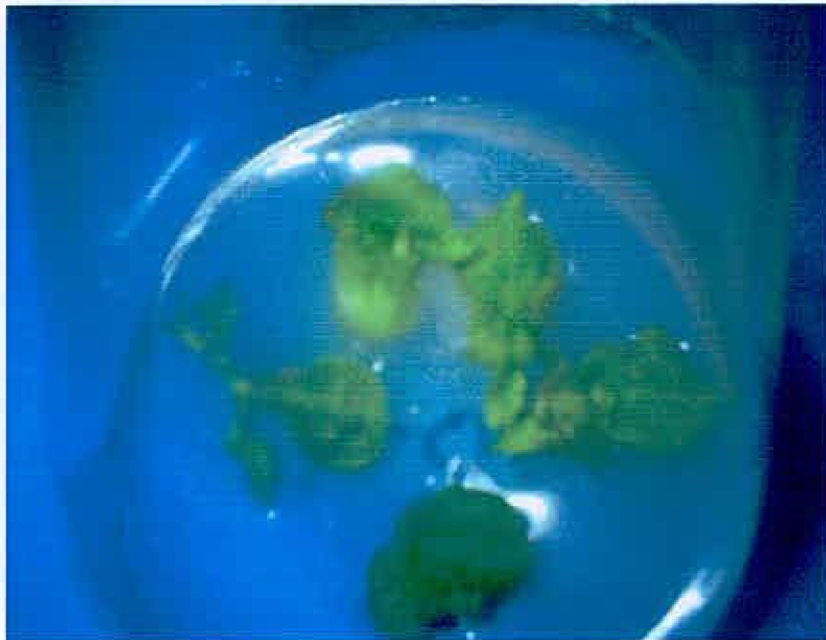


Plate 23. Shoot regeneration in jute var. O-72 on x 2 times (3.0 mg/l) $FeSO_4$ concentration

Chapter 5
Summary and conclusion

Experiments were carried out in the Genetic Engineering Laboratory, Cytogenetic Department, Bangladesh Jute Research Institute (BJRI), Dhaka during the period from August 2005 to may 2006.

In the first experiment, a detailed investigation was carried out to study the seed germination. A significant variation among the test varieties was found for percent seed germination. The variety CVE-3 showed the highest germination percentage (92.72%) and the lowest was found in O-9897 (67.22%). In cotton-based media, the germination percentage was found higher (95.10%) compared to agar solidified media (69.81%). From the results of the present study it was found that cotton-based media was better than agar-solidified media for seed germination.

In the second experiment, a detailed investigation was carried out to study callus induction ability and subsequent plant regeneration of five *Corchorus* genotypes using cotyledons (with attach petioles) as explants.

Cotyledons (with attached petiole) of five *Corchorus* varieties were cultured on MS medium supplemented with 2 mg/l BAP and 0.5 mg/l IAA to observe their callus induction and shoot regeneration capacity. A wide range of variation in callus induction was exhibited by the varieties. The range of callus induction varied from 68% to 94%. The highest callusing was found in CVE-3 (94%) followed by Tricap-2 (88%). The highest percentage of shoot regeneration was found in CVE-3 (67.99%) and the lowest in O-9897 (45.57%). MSO (MS medium without hormone) was used to observe the rooting responses of regenerated shoots by the varieties. CVE-3

showed the highest percent of root initiation (95%) followed by Tricap-2 (85%). Regenerated plantlets of all the varieties were rooted on MSO medium within 7 days.

Different combinations and concentrations of BAP and IAA were used to observe callus induction and shoot regeneration. The highest percentage of callus induction (96%) and shoot regeneration (60.66%) was found in the combination of MS+2 mg/l BAP + 0.5 mg/l IAA. No callus induction and shoot regeneration was found without BAP and IAA. It was worth noting that percent shoot regeneration gradually increased with the increasing level of BAP up to 2 mg/l. Further increase of BAP level did not show any improvement to shooting.

In varietal experiment, it was found that the germination percentage of CVE-3 was highest and *C. olitorius* (O-72) response batter. So, select these two variety for next experiment. So, for the next experiment these two varieties were selected.

Two varieties of *C. capsularis* and *C.olitorius* (vars. CVE-3 and O-70) were cultured in the presence of a range of pH level (e.g. 4.0, 4.5, 5.5, 6.0, 6.5, 7.0, 7.5) in association with MS plant regeneration medium. Two varieties responded differently on various levels of pH. It was observed that CVE-3 showed relatively better shoot regeneration (52.95%) than that of O-72 (35.07%).

Two varieties of *C. capsularis C. olitorius* (vars. CVE-3 and O-70) were cultured in different concentration of sucrose (0, 1%, 2%, 3%) in association with MS plant regeneration medium. Two varieties responded differently on different concentration of sucrose. It was observed that CVE-3 showed relatively better response shoot regeneration (91.66%) than that of O-72 (83.33%). And number of shoot/cotyledon was the highest in CVE-3 (9.00).

In different concentration of sucrose, significant mean square for different characters indicated the presence of sufficient variation among the sucrose concentration for shoot regeneration and other character. 3% (30 gm/l.) sucrose concentration was found best. The result indicates that 3% sucrose concentration was suitable for shoot regeneration.

Two varieties of *C. capsularis* and *C. olitorius* (vars. CVE-3 and O-72) were cultured in different concentration of vitamins (0,1.5ml/l, 3.0ml/l, 4.5ml/l, 6.0ml/l) in association with MS plant regeneration medium. Two varieties responded differently on different concentration of vitamins. It was observed that CVE-3 showed relatively better response (80.55%) towards shoot regeneration than that of O-72 (74.99%). Vitamin x2 times concentration showed best performance. Maximum number of explants showed shoot regeneration in this concentration and number of shoot per cotyledon was 6.00.

Two varieties of *C. capsularis* and *C. oiltorius* (vars. CVE-3 and O-72) were cultured in different concentration of surfactants (0, 0.001, 0.01, 0.1, 0.5%) in association with MS plant regeneration medium. The varieties responded differently on different concentration of surfactant. It was observed that CVE-3 showed relatively better shoot regeneration (97.22%) than that of O-72 (86.107%). Surfactant concentration at 0.1% was found to be best. Maximum number of explants showed shoot regeneration in this concentration and in the combined effect of variety and surfactant, maximum number of shoot per cotyledon was 8.67.

The hormone combinations IAA 0.5 mg/l and BAP 2.0 mg/l were used in association with pluronic F-68 with different concentrations. In most of the levels of pluronic F-68 gave higher percentage of shoot regeneration than the control. Shoot growth was

increased in the lower concentration of pluronic F-68 and the growth was inhibited as the concentration was further increased.

Cotyledon with attached petioles of *C. Capsularis* and *C. olitorius* (CVE-3, Tricap-2 and O-72) were excised from those seedlings and placed on a agar solidified MS medium containing 0.5mg/l AA and 2.0 mg/l BAP in association with different concentrations of NaCl(0, 0.0625, 0.125, 0.250, 0.50, 1.0)%. It was found that initially callus was formed in presence of NaCl irrespective of all concentration. Shoot regeneration was also obtained in presence of all concentrations of NaCl. Regeneration percentage decreased with the increasing concentration of NaCl and also regeneration plantlets were found to be more elongated when it was grown in lower concentration of NaCl containing medium at 0.5 mg/l concentration. In the combined effect of variety and NaCl, maximum number of shoot per cotyledon was 8.333 in var. CVE-3.

Two varieties of *C. capsularis* and *C. olitorius* (vars. CVE-3 and O-72) were cultured in different concentration of $FeSO_4$ (0, x1times, x 2 times, x 3 times, x 4 times) in association with MS plant regeneration medium. Two varieties responded differently on different concentration of $FeSO_4$. It was observed that CVE-3 showed relatively better shoot regeneration (80.55%) compared to O-72 (69.447%). $FeSO_4$ x 2 times concentration (3.0mg/l) showed best performance and maximum number of explants showed shoot regeneration in this concentration. The number of shoot per cotyledon was highest in var. CVE-3 (6.00)

In the first experiment, seed germination percentage was found highest in CVE-3 in cotton-based medium (98.88%) than agar-based medium (87.17%). Among the phytohormone combination, MS+ 2 mg/l BAP + 0.5 mg/l IAA showed the highest shoot regeneration (60.66%). Among the varieties, CVE-3 was highly responsive to shoot regeneration (67.99%). MS media without hormone (MSO) was used for root formation. The variety CVE-3 response better than O-72 towards shoot regeneration at different pH level. Among the sucrose concentration 3% (30mg/l) showed the highest shoot regeneration (87.49%). Among the different concentration of vitamins, x 2 times (3.0mg/l) showed the highest shoot regeneration. Using different concentration of surfactant 0.1% surfactant showed the highest shoot regeneration (91.66%). In the NaCl experiment, among the three varieties CVE-3 was found highly response to shoot regeneration (97.22%) and 0.125, 0.25 and 0.50 (%) shows the better responsive to shoot regeneration. Using different concentration of $FeSO_4$, x2 times (3.0mg/l) showed the highest shoot regeneration. The variety CVE-3 response better than O-72 towards shoot regeneration at different concentration of $FeSO_4$.

If any one want to develop the regeneration protocol of jute species he should take the following research programme

- He should used cotton-based medium compared to agar-based medium for seed germination.
- Among the varieties, CVE-3 was highly responsive to shoot regeneration.
- Among the phytohormone combination, MS+ 2 mg/l BAP + 0.5 mg/l IAA showed the highest shoot regeneration.
- Among the sucrose concentration 3% (30mg/l) showed the highest shoot regeneration.
- Among the different concentration of vitamins, x2 times (3.0mg/l) showed the highest shoot regeneration.
- Using different concentration of surfactant, 0.1% surfactant showed the highest shoot regeneration.
- Among the different concentration of NaCl, 0.125%, 0.25% and 0.5% shows the better responsive to shoot regeneration.
- Using different concentration of $FeSO_4$, x2 times (3.0mg/l) showed the highest shoot regeneration.

References

Ahad, M. A. and Debnath, N. C. (1989). phosphorous availability and pH changes in the rhizophere of rice, maize, soyabean and Jute. J. of the Andaman- sci.-Assoc. **5**(1): 27-30.

Ahmed, G. Hossain, A. B. M. and Islam, M. S. (1989). Regeneration of multiple shoots in jute *Corchorus olitorius* (var. O-4) from cotyledon and hypocotyls explants of germinating seeds. Indian J. Exp. Bio. **27**: 334-337.

Ahmed, T. U., Jalil, A. F. M. and Abdul, M. (1993). Bangladesher Krishir Onostakari Pokamakar:Ziban Britranta O Nyantran. Life history and control of insects harmful to Bangladesh Agriculture. **1** : pp. 131-135

Ahmed, G., Hossian, A. B. M., Mamun, A. N. K., Roy, P. K. and Hossian, M. (1999). Approaches to develop genetic transformation system in jute, *Corchorus capsularis* L. var. D-154. Proc. of the workshop on "Application of biotechnol. in the improvement of jute, kenaf and allied fibres-ogaseII, Gaujiym Cguba. pp.28-38.

Aggarwal, V. K. (2000). Breeding Technique of Jute, Kenaf and Mesta. Consultancy Report, ARMR(Winrock international), BARC, Dhaka.

Akter, N. (2001). Effects of different explants and concentrations of NAA on callus induction and plant regeneration of brinjal cv.Uttara. A Thesis of Master of Science. Department of Horticulture. Bangladesh Agricultural University, Mymensingh.

Ali, M. A. (1992). *In vitro* studies in *corchorus* and *Raphanus* Ph. D. Thesis, Reading University, UK.

Amirto, P. V. and Styer, D. J. (1985). Strategies for large-scale manipulation of somatic Embryos in suspention cultures. **In:** Milton Z., Peter, D. and Alexander H (eds.), Biotechnol. in Plant Sci. Academic Press, New York. 161-178.

Bankole, S. A., Eseighe, D. A. and Enikuomehin, O. A. (1996). Mycoflora and aflatoxin production in pigeon pea stored in jute sacks and iron binds. Mycopathologia. **132**(3): 155-160.

BBS. (2003). Statistical Pocket Book of Bangladesh. Bangladesh Bureau of Statistics. Planning Division, Ministry of Planning. Govt. Peoples Republic of Bangladesh.

Bessembinder, J. J. E., Staritsky, G. and Zandvoort E. A. (1993) Long-term *in vitro* storage of *Colocasia esculenta* under minimum growth conditions. Plant Cell Tissue Org. Cult. **33**: 121-127.

Bhajwani, S. S. and Razdan, M. K. (1983). Cell Culture. In: Developments in Crop Sci. Plant Tiss. Cul.: Theor. And Practice. **5**: 25-42.

Bigaria, A. K. (1998). Influence of indilc-3-butyric acid, environmental factors and posture on the regeneration of stem cuttings and leaves of *Hibiscus cannabinus*. Advances in plant Sci.**1**(1):54-66.

Bhajwani, S. S., Banerhjee, M. and Mukhopadhyay, A. (1988). In plant breeding and genetic Engineering.(Ed. Zakri, A.H.).Malaysia ISBN-983-99500-0-4.233-268.

Carlson, P. S. (1975). Crop improvement through techniques of plant cells and tissue cultures. Biol. Sci. **25**: 747-749.

Chaudhuri, K. and Choudhuri, M. A. (1993). Differential tolarance of Seedlings of two jute species to NaCl Salinity Stress. Indian J. of Exp. Botany. **31**:1, 90-92.

Das, B., Haque, M. M., Islam, A. S., Ranman, M. H. and Hoque, N. I. (1986). *In vitro* plantlet development in jute. In: Roa. A. N., Mohan Ram H. Y. (eds) proc. Nayudamma Mem Symp. On Agricultural applications in biotechnol. Madeas, India, 106-114.

Das, L. (1983). Recovery of virus free plantlets of cultivated jute species (*Corchorus olitorius, C. capsularis*) meristem culture techniques. **In:** plant cell culture in crop improvement (ed. by Sen. A. K. et al. New York. Plenum Press 1983. pp. 459-464.

D' Aamato, F. (1978). Chromosome number variation on cultured cells regenerated plants. **In:** T. A. Thorpe (ed). Frontiers of plant Tissue Culture. Canada. pp. 247-295.

De Garcia, E. and Martinez, S. (1995). Somatic embryogenesis in *Solanum tuberosum* L.cv. Desiree from stem nodal sections. J. Plant Physiol. **145**: 526-530.

Dodds, J. H. and Roberts, L. W. (1990). Anther and Pollen culture. **In:** J. H. Dodds and L. W. Roberts (Editors), Exp. in Plant Tiss. Cult. Cambrige University Press, New York, NY. pp. 156-171.

FAO, (2005). Production year book for 2004. FAO. UN. Italy. Rome. P. 118.

Ghosh, P. K. and Chatterjee (1990). Regeneration of plants from hypocotyl derived callus tissue of jute (*Corchorus capsularis L.)* Cell Chrom. Res. **13**: 26-29.

Groenwald, E. G., Wessels, D. C. J., and Koelman, K. (1997) Callus formation and subsequent plant regeneration from seed tissue of *Guava* species. Z. P. flanzen Physiol. **81**:369-393.

Gulsen, Y. and Demanoglu, H. (1991). The effect of sucrose, agar and pH on shoot multiplication and quality in quince. A micropropagation. Acta Horticulture **289**: 115-116.

Haque, M. A. (2001). Individual chromosome identification through karyotype analysis and production of tissue culture regenerants in garlic. A Thesis of Master of Science. Department of Genetics and plant breeding. Bangladesh Agricultural University, Mymensingh.

IBFC, CAAS.(1974). Science and Technique of Fibre Crops (Hunan), (2): 22.

Islam, A. S. (1964). A rare hybrid combination through application of hormone through embryo culture. Nature, 210, 310.

Islam, A. S. (1981). Production of desirable jute plants through tissue culture. Rao, A. N. *|(Ed.) Symposium on Tissue culture of Economically Important Plants, Singapore, pp. 159-161.

Khatun, A., Davey, M.R.,Laouar L.,Mulligan, B. J, Power, J. B. and Lowe, K.C. (1992). Stimulation of differentiation in Jute cotyledons cultured with pluronic F-68. plant tiss. cult. **2** (2):75-80.

Khatun, A. (1993). Genetic manipulation in Jute (*Corchorus spp.*). Ph D Thesis. The Univ. of Nottingham, UK.

Khatun, A., Laour, L., Davey, M. R., Power, J. B. and Lowe, K. C. (1993). Stimulation of shoot regeneration from jute cotyledons cultured with non-ionic surfactants and relationship to physico-chemical properties. Plant cell Reports. 1993, **13**(1):49-53.

Khatun, A. (1996). Effect of different levels of $FeSO_4$ on shoot regeneration from the cotyledon of *C. capsularis.* Annual report. Bangladesh Jute Research Institute. Manik Mia Avenue, Dhaka pp. 6-7.

Khatun, A. (1998). Biotechnological Approaches for Varietal Important of Jute and Allied Fibres. Proc. of the Workshop on Application of Biotechnology in the Important of Jute, Kenaf and Allied Fibres – Phase I, IJO, Beifing, Chaina.

Khatun, A. (2001). Varietal improvement of jute through *Agrobacterium mediated* transformation. Bangladesh Jute Research Institute, Manik Mia Avenue, Dhaka-1207.

Khatun, A., Saha, C. K., Naher, Z., Mahbub, S., Siddique, S. and Bilkis, S. (2003). Plant regeneration from the cotyledons of tossa jute (*Corchorus olitorius L.*). Biotechnol. **3** (3): 206-213.

Khatun, A. and Naher, Z. (2005). Effect of different levels of NaCl on seed germination, shoot culture and plant regeneration from the cotyledon of *C. capsularis* and *Corchorus olitorius*. Annual report. Bangladesh Jute Research Institute. Manik Mia Avenue, Dhaka p. 7.

Khalekuzzaman, M., Takagi, Y., Islam, R., Alam, M. F. and Hossain, M. (2000). Establishment of a high frequency shoot regeneration method from cotyledon explants in Jute *Corchorus capsularis L.var*. D-154. Plant tiss. cult. **10** (2): 143-148.

King, A.T., Davey, M. R., Mellor, I. R., Mulligan, B. J. and Lowe, K.C.(1991). Surfactant effects on yeast cells. Enzyme Microb. Technol. **13**: 148-153.

Kumar, V., Laouar, L., Davey, M. R., Mellor, I. R., Mulligan, B. J and Lowe, K. C.(1991). Effects of Pluronic F-68 on callus growth and protoplast planting efficiency of *solanum dulcamara* .plant cell Rep. **10**: 52-54.

Larkin, P. J. and Scowcroft, W. R. (1982). Somaclonal variatin: A new option for plant improvement. In: Vasil, I. K., W. R. Scowcroft and K.

Li, X.L., Wen, L. Y., Guo, X. M., Li, E. L. and Shi, C. M. (1989). Study on regeneration of cotton plants through somatic culture. China cottons 1989, No. 6, 13-15.

Lowe, K.C., Davey, M. R., power, J. B. and Mulligan, B. J. (1993). Surfactant supplements systems in plant culture. Agro-food-Industry-Hi- Tech. **4**(1): 9-13.

Lupi, C., Bennici, A. and Crennal, D. (1985). *In vitro* culture of *Bellavalai romana* (L) RCHB. In plant regeneration through adventitious shoots and somatic embryos. Protoplasmes **125** :135-189.

Modarres Sanavy,S.A.M. and Jami Moeini, M. (2003). Effects of different pH levels of medium on growth and rooting of single nods resulted from potato meristem culture. Plant tiss. cult. **13**(2): 151-154.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. **15**: 473-493.

Myers, J. M. and Simon, P. W. (1999). Regeneration of garlic callus as affected by clonal variation, plant growth regulators and culture condition over time. Plant cell Report. **19** (1): 32.

Naher, Z. and Khatun, A. (2004). Effect of different levels of pH on plant regeneration from cotyledons of *C. capsularis*. Annual Report. Bangladesh-Jute Research Institute, Manik Mia Avenue, Dhaka. pp. 11-13.

Naher, Z., Khatun, A., Mahbub, M. A. and Siddique, A. B. (2003). Influence of genotypes on plant regeneration from cotyledons of *Corchorus capsularis L.* Biotechnol. **2** (1): 44-51.

Nath.U.K. (2001). Study on genetic diversity and *in vitro* regeneration potentiality of groundnut. A Thesis of Master of Science. Department of Genetics and plant breeding. Bangladesh Agricultural University, Mymensingh.

Nidhi Prabhakar.(1998). Effect of Thidiazuron and BAP on shoot buds differentiation from excised cotyledons of *Brassica juncea* (L) Czern. **8**(1): 55-60.

Pathirana, R. (1991) Conservation of plant genetic resources through *in vitro* methods. In: Zakir, A. H.; Normah, M. N.; Karim, A. G. A. and Senawi, M. T. (eds.) proceedings of the MNCPGR/CSC international workshop on tissue culture for the conservation of biodiversity and plant genetic resources. FRIM/MNCPGR. Kuala Lumpur Malaysia. pp. 212-230.

Pierick, R.L.M. (1997) *In vitro* culture of higher plants Dordrecht, Kluwer Academic, 348p.

Rahman, S. M. Z., Hadiuzzaman, S., Haque, M. M. and Islam, A. S. (1985). Shoot formation in *Corchorus capsularis* var. D-154 from unorganized callus. Bangladesh J. Bot. **14: 141-145**. With *Agrobacterium rhizogens* and a binary vector plasmid. Plant cell Rep. **9:** 88-92.

Rao, P.S.; Handro, N. and Harada, H. (1973) Hormonal control of differentiation of shoots, roots and embryos in leaf and stem culture of *Petunia inflata* and *Petunia hybrida*. Physic. Plant **28**: 458-463.

Rao, P.S. (1985). Plant protoplast: a new tool in plant biotechnology. Current science.**54** (7): 335-336

Razdan, M. K. and Cocking, E. C. (1981). Improvement of legumes by exploiting extra specific genetic variations. Euphytica **30**: 819-833.

Saha, M. N. (1996). *In vitro* culture of jute (*corchorus capsularis* CVL 1) and its Transformation. M. Sc. Thesis, Dept of Biochemistry, University of Dnaka.

Saha, T., Ghosh, M. and Sen, S. K. (1999). Plant regeneration from cotyledonary explants of jute, *Corchorus capsularis L.* Plant cell Report **18**: 544-548.

Seraj, Z. I., Sarkar, S. B. and Islam, A. S. (1992). Plant regeneration in a jute species (*Corchorus capsularis*) and its possible relationship with glyoxalase-1. Plant Cell Reports **12**: 29-33.

Skirvin, R. M. (1978). Natural and induced variation in tissue culture Euphytica **27**: 241-266.

Staritsky, G.; Dekkers, A. J.; Louwaars, N. P. and Zandvoort, E. A. (1986). *In vitro* conservation of aroid germplasm at reduced temperatures and under osmotic stress. In: Withers, L.A.; Alderson, P. G. (eds.) Plant Tiss. Cult. and its Agric. Application. Butterworths, London, pp. 277-283.

Tewari, A. S., Minakshi, S.; Rangaswamy, N. S. and Sethi, M. (1999). Somatic embryogenesis and plant regeneration in three cultivars of white jute. Phytomorphology. **49**(3):303-308.

Wang, W., Zhang, X.L. and Lin, J. L. (1992). Effect of brassinolide on somatic embryogenesis of *Gossypim hirsutum*. Plant Physic. Communication.1992, **28**(1): 15-18.

Westcott, R. J.; Henshaw, G. G. and Roa, W. M. (1997). Tissue culture of potato germ plasm: culture intiation and plant regeneration. Plant Sci. Lett. 9.309-315. cited in Bhojwani SS and Razdan MK 1983 plant tiss. cult. Theory and practices, Elsevier, The Netherlands, p. 383.

Yousuf, A. M. A., Islam, M. O. and Ali, M. (1997). *In vitro* conservation of taro (*Colocasia esculenta var. antiguorum*) under different sucrose levels plant tissue culture 7 (2): 81-88.

Appendices

Appendix I: Mean square of number of seed germination and percentage of seed germination

Source	Degrees of freedom	Mean square of	
		Number of seed germination	Percentage of seed germination
Factor A (Variety)	4	227.706**	922.186**
Factor B (Media)	1	1206.502**	4796.122**
Factor A × Factor B (Variety × Media)	4	229.094**	900.231**
Error	20	0.251	0.935

** = 1% significant level of probability

Appendix II: Mean square of number of cotyledon producing shoot, percentage of cotyledon producing shoot and number of shoot produced by each cotyledon

Source	Degrees of freedom	Mean square of		
		Number of cotyledon producing shoot	Percentage of cotyledon producing shoot	Number of shoot/cotyledon
Varieties	4	410.933**	642.083**	410.933**
Error	8	0.783	1.224	0.783

** = 1% significant level of probability

Appendix III: Mean square of number of explants growing shoot, percentage shoot regeneration number of shoot produced per cotyledon

Source	Degrees of freedom	Mean square of		
		Number of explants growing shoot	Percentage shoot regeneration	Number of shoot/cotyledon
Varieties	4	25.733**	198.514**	1.067**
Error	8	0.733	5.654	0.517

** = 1% significant level of probability

Appendix IV: Mean square of number of explants showing shoot regeneration, percent shoot regeneration and number of shoot produced per cotyledon

Source	Degrees of freedom	Mean square of		
		Number of explants showing shoot regeneration	Percent of shoot regeneration	Number of shoot/cotyledon
Varieties	4	3.100*	203.652NS	3.733**
Error	8	0.600	54.416	0.133

** = 1% significant level of probability

* = 5% significant level of probability

NS = Non-significant

Appendix V: Mean square of NaCl effect on number of explants showing shoot, percent of shoot regeneration and number of shoot produced per cotyledon

Source	Degrees of freedom	Mean square of		
		Number of explants showing shoot	Percent of shoot regeneration	Number of shoot/cotyledon
Factor A (Variety)	2	0.722NS	101.580NS	2.167**
Factor B (NaCl)	5	139.011**	9994.487**	80.622**
Factor A × Factor B (Variety × NaCl)	10	5.967*	342.345**	0.789**
Error	30	2.463	57.873	0.185

** = 1% significant level of probability

* = 5% significant level of probability

NS = Non-significant

Appendix VI: Mean square of $FeSO_4$ effect on number of explants showing shoot, percent shoot regeneration and number of shoot produced per cotyledon

Source	Degrees of freedom	Mean square of		
		Number of explants showing shoot	Percent of shoot regeneration	Number of shoot/cotyledon
Factor A (Variety)	1	0.000NS	0.000NS	2.133**
Factor B ($FeSO_4$)	4	72.583**	5039.963**	60.583**
Factor A × Factor B (Variety × $FeSO_4$)	4	1.083*	75.227NS	0.883**
Error	20	1.000	69.431	0.033

** = 1% significant level of probability

* = 5% significant level of probability

NS = Non-significant

Appendix VII: Mean square of vitamin effect on number of explants showing shoot, percent shoot regeneration and number of shoot produced per cotyledon

Source	Degrees of freedom	Mean square of		
		Number of explants showing shoot	Percent of shoot regeneration	Number of shoot/cotyledon
Factor A (Variety)	1	1.200NS	83.300NS	2.700**
Factor B (Vitamin)	4	72.783**	5053.824**	63.033**
Factor A × Factor B (Variety × Vitamin)	4	0.117NS	8.101NS	1.533**
Error	20	1.433	99.546	0.067

** = 1% significant level of probability

NS = Non-significant

Appendix VIII: Mean square of surfactant effect on number of explants showing shoot, percent shoot regeneration and number of shoot produced per cotyledon

Source	Degrees of freedom	Mean square of		
		Number explants showing shoot	Percent shoot regeneration	Number of shoot/cotyledon
Factor A (Variety)	1	4.800*	333.333*	0.033NS
Factor B (Surfactant)	4	57.383**	3985.085**	1.700*
Factor A × Factor B (Variety × Surfactant)	4	0.383NS	26.630NS	0.533NS
Error	20	1.000	69.442	0.500

** = 1% significant level of probability

* = 5% significant level of probability

NS = Non-significant

Appendix IX: Mean square of sucrose effect on number of explants showing shoot, percent shoot regeneration and number of shoot produced per cotyledon

Source	Degrees of freedom	Mean square of		
		Number of explants showing shoot	Percent of shoot regeneration	Number of shoot/cotyledon
Factor A (Variety)	1	1.500NS	104.208NS	0.667**
Factor B (Sucrose)	3	124.333**	8633.556**	96.056**
Factor A × Factor B (Variety × Sucrose)	3	0.278NS	19.289NS	0.556**
Error	16	0.500	52.087	0.083

** = 1% significant level of probability

NS = Non-significant

Appendix-X: Combined effect of BAP and IAA on number of explants producing shoot, percent regeneration and days required for shoot regeneration

Treatment		Number of explants producing shoot	Percent shoot regeneration	Days required for shoot regeneration
BAP (mg/L)	IAA (mg/L)			
0.0	0.0	-	-	-
	0.5	-	-	-
	1.0	-	-	-
	1.5	-	-	-
	2.0	-	-	-
1.0	0.0	1.40 hi	14.28 i	25.00 b
	0.5	2.40 def	26.77 defgh	23.80 bc
	1.0	2.60 def	25.11 efgh	22.80 cd
	1.5	2.00 fgh	23.88 fgh	24.40 de
	2.0	2.00 fgh	24.15 efgh	24.80 b
2.0	0.0	2.00e fgh	28.09 defg	26.20 a
	0.5	5.80 a	60.66 a	19.20 g
	1.0	4.40 b	47.27 b	21.20ef
	1.5	3.60 c	38.38 c	21.80 de
	2.0	3.00 cd	33.83 cd	20.20 fg
3.0	0.0	2.00 fgh	27.38 defgh	23.80 bc
	0.5	2.80 de	33.23 cd	20.00 fg
	1.0	2.60 def	33.18 cd	24.00 bc
	1.5	2.60 def	30.88 cdef	22.80 cd
	2.0	2.00 fgh	24.57 efgh	24.60 b
4.0	0.0	1.60ghi	22.68 gh	26.60 a
	0.5	2.40def	31.95 cde	24.00 bc
	1.0	2.20efg	29.04 defg	22.00 de
	1.5	1.40	19.75 hi	20.40 fg
	2.0	1.00i	12.93i	19.60 g

Appendix-XI: Main effect of different variety on percent shoots regeneration and number of shoot/cotyledon

Variety	Percent shoots regeneration	Number of shoot/cotyledon
O-72	35.075 b	6.800 b
CVE-3	52.950 a	8.575 a

Appendix XII: Main effect of different pH level on percent shoots regeneration and number of shoot/cotyledon

pH level	Percent shoots regeneration	Number of shoot/cotyledon
4.0	35.80 c	5.60 d
4.5	41.40 bc	7.70 c
5.0	46.80 b	9.40 b
5.5	58.50 a	10.40 ab
6.0	44.80 bc	10.60 a
6.5	43.00 bc	7.40 cd
7.0	42.30 bc	6.00 d

Appendix XIII: Analysis of variance (mean squares) for number of shoot to which root induced and percent shoot regeneration

Source of variation	Degrees of freedom	Characters		
		Number of shoot to which root induced	Percent root initiated	Days of root initiation
Factor A (BAP)	4	47.308**	4730.800**	18.668**
Factor B (IAA)	4	26.668**	2666.800**	19.708**
Factor A x B (BAP x IAA)	16	6.663**	666.300**	14.398**
Error	100	0.880	88.000	0.650

** = 1% significant level of probability

Appendix XIV: Analysis of variance (mean squares) for number of explants showing shoot, percent shoot regeneration and days required for shoot regeneration

Source of variation	Degrees of freedom	Characters		
		Number of explants showing shoot	Percent shoot regeneration	Days required for shoot regeneration
Factor A (BAP)	4	45.888*	5765.633**	2599.348**
Factor B (IAA)	4	6.988**	665.829**	13.388**
Factor A x B (BAP x IAA)	16	1.693**	155.841**	22.728**
Error	80	0.282	28.549	0.895

** = 1% significant level of probability

Appendix XV: Analysis of variance (mean squares) for percent shoots regeneration and number of shoot/cotyledon

Source of variation	Degrees of freedom	Characters	
		Percent shoot regeneration	Number of shoot/cotyledon
Factor A (Variety)	1	6390.313**	63.012**
Factor B (pH)	7	452.670**	52.670**
Factor A x B (Variety x pH)	7	2129.427**	9.755**
Error	64	42.250	0.644

** = 1% significant level of probability

Appendix XVI: Analysis of variance (mean squares) for number of explants growing shoot, percent of shoot regeneration and days to shoot regeneration

Source of variation	Degrees of freedom	Characters		
		Number of explants showing shoot	Percent of shoot regeneration	Number of shoot/cotyledon
Factor A (Variety)	4	4.453**	445.333**	6.673**
Error	8	0.537	53.667	0.895

** = 1% significant level of probability

Appendix XVII: Analysis of variance (mean squares) for number of explants showing shoot and percent shoot regeneration

Source of variation	Degrees of freedom	Characters	
		Number of shoot	Percent of shoot
Factor A (Variety)	4	8.133**	401.214**
Error	8	0.983	59.094

** = 1% significant level of probability

Appendix XVIII: Analysis of variance (mean squares) for number of shoots to which root induced, percent root initiation and days of root initiation

Source of variation	Degrees of freedom	Characters		
		Number of shoot to which root induced	Percent of initiated	Days of root initiation
Factor A (Variety)	4	32.253**	831.333**	3.653**
Error	8	0.637	15.917	0.320

** = 1% significant level of probability

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