IMPROVEMENT OF CULTIVAR JUTE THROUGH TISSUE CULTURE

BY
BITHEE DAS

A
DISSERTATION
SUBMITTED TO THE
UNIVERSITY OF DHAKA
IN FULFILMENT
OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF PHILOSOPHY
IN BOTANY

Plant Breeding and Tissue Culture Laboratory
Department of Botany
University of Dhaka
Dhaka

DHAKA 1990

CERTIFICATE

This is to certify that the research work embodying the results reported in this thesis has been carried out in the Plant Breeding and Tissue Culture Laboratory and research field, Department of Botany, University of Dhaka, under my supervision and guidance. It is further certified that the work presented here is original and suitable for submission for the fulfilment of the degree of Master of Philosophy in Botany.

30/12/90

Dr. M. Mozammel Haque
Professor
Department of Botany
University of Dhaka.

ACKNOWLEDGEMENT

The author expresses her sincere and heartiest gratitude to Dr. M. Mozammel Haque, Professor of Botany, University of Dhaka, under whose constant guidance and supervision the present investigation was undertaken.

The author expresses her sincere and heartiest gratitude to Professor Ahmad Shamsul Islam, Department of Botany, University of Dhaka for constant inspiration, kind help and suggestions during the study.

The author is grateful to Dhaka University authority for awarding her university scholarship for her present study. She is also grateful to the Chairman, Department of Botany for providing facilities in the laboratory and in the fields.

Sincere thanks are due to Dr. Syed Hadiuzzaman, Dr. M. Imdadul Haque, Dr. Rakha Hari Sarkar, Dr. Azibor Rahman, Mr. Hafizur Rahman, Mr. Abdus Samad, Department of Botany for their encouragement, kind help and suggestions during the study.

The author is grateful to all the teachers, fellow, M. Phil and Ph.D. research workers and the staff members of Botany Department for their help and encouragement during the period of this work.

Sincere thanks are due to Mrs. Mitali Banik, S.O. of BRRI for her constant company, help and inspiration during the course of the study.

Special thanks are due to Mr. Sukumar Chandra Singh, Data Encoder of BARC, for his untiring efforts in getting this thesis typed.

Finally the author expresses appreciation to her family members and friends for their inspiration and encouragement throughout the study.

The author

ABBREVIATIONS

BAP	6 - benzyl amino purine
BJRI	Bangladesh Jute Research Institute
C	Centigrade
C	Control
Cm	Centimeter (s)
CH	Casein hydrolysate
2,4-D	2,4-dichlorophenoxy acetic acid
e.g.	*exempli gratia*, for example
et al.	*et alii*, and others
etc.	*et cetera*, and the rest
F_1	First filial generation
F_2	Second filial generation
F_3	Third filiaI generation
F_4	Fourth filial generation
F_5	Fifth filial generation
Fig	Figure (s)
gm	Gram (s)
gln	Glutamine
IAA	indole - 3 - acetic acid
IBA	indole -3 - butyric acid
lb/Ibs	pound/pounds
i.e.	*id est*, that is
2-ip	2 - isopentanyl adenine
Kn/Kinetin	6 - furfuryl amino purine

l.c.	loco citalo, before cited
L/B	Length/Breadth
m	meter (s)
mm	milimeter (s)
mg	miligram (s)
mg/l	miligram/litre
ml	mililitre
MS	Murashige and Skoog
MMS	Modified Murashige and Skoog
NAA	α- napthalene acetic acid
NDGA	nordihydroguiaretic acid
No.	Number
0.1N	0.1 Normal
ppm	parts per million
R_o	First generation of plants regenerated from cell culture
R_1	Second generation of plants regenerated from cell culture
R_2	Third generation of plants regenerated from cell culture
SH	Schenk and Hildebrandt
TCL	Tissue Culture Laboratory
TCL 1-24	Plants regenerated from cell culture in Tissue Culture Laboratory
tyr	Tyrosine
Var	Variety

Viz	Videlect, namely
%	Percentage
VS	Versus

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Fig. 14 a -14m) comparing the colour, size and shape of TCL - seeds from their control

INTRODUCTION

Jute, the golden fibre is still number one cash crop for Bangladesh; and in India, Nepal, Thailand, Burma jute is considered to be an important fibre crop. Jute is regarded as the foremost bast fibre and the second most important textile fibre after cotton (Kundu 1956; Kirby 1963). The well-being of a large section of the people of Bangladesh is dependent on the fair price of this crop which fluctuates a great deal from year to year.

Jute is a common term for both the plant and fibre obtained from the bark of the two cultivated species of the genus *Corchorus*, namely, *C. olitorius L.* and *C. capsularis L.* belonging to the family *Tiliaceae*. *C. olitorius* is usually called 'Tossa' and *C. capsularis* as 'White' jute. Both the species are annual short-day plant. These two species possess characteristics which are more or less complementary to each other. In case of *C. olitorius* distribution and growth of fibres are more even along the stem while in case of *C. capsularis* it is more concentrated in the lower portion of the stem. The internodal length, ultimate fibre and flowers of *C. capsularis* are comparatively smaller. 'Tossa' produces better quality fibres with higher tensile strength than 'White'. In spite of its superior quality it cannot be grown in more than 30% of the area under jute cultivation. This is because 70% of the land mass of Bangladesh is flooded almost every year causing heavy damage to crops which are

susceptible to water logging. The other species *C. capsularis* is grown in low lying areas due to its quality to withstand flood water. It occupies 70% of the jute acreage (Sarma 1969; Cobley and Steele 1976).

However, due to genetic improvement of some cultivars, more and more land is now being occupied by some cultivars of *C. olitorius*. The fibre colour of *C. capsularis* ranges from white to snow white. It is used to produce a kind of cloth called 'juton' in which cotton and jute fibres are mixed in the proportion of 70:30 or 80:20. If the fibre strength of *C. olitorius* and the white colour character of *C. capsularis* can be combined together, the resulting hybrid will give a superior quality 'juton' at a comparatively low cost.

Both the species vary in their disease resistance capability. *C. olitorius* is resistant to mosaic virus (Alim 1978), anthracnose caused by *Colletotrichum corchori* and partial resistant to nematodes but susceptible to dieback disease caused by *Gleosporium singulatum* and mites. As against this *C. capsularis* is resistant to dieback disease, partially resistant to mites but susceptible to mosaic virus, anthracnose and nematodes.

Therefore, it is important to produce sexual, parasexual and somaclonal variants in which the flood and disease resistance and other desirable characters mentioned above could be combined.

However, jute is fast losing its market in the face of its competition with synthetic man-made fibre. In order to restore

its place, new and diversified use of jute needs to be found out. This may be accomplished by creating varieties of jute with more than 2 mm long ultimate fibres with narrower lumen and with less lignin (7-8 instead of 12%), having stem with more or less equal diameter from base to apex, varieties capable of retting in two instead of three weeks, photoinsensitive to day length, tolerant to cold temperature of February. It is also necessary to breed varieties having all the above qualities combined with a shortened 100-day life cycle so that jute varieties could be fitted in a three crop a year pattern. In Bangladesh jute crop is being pushed more and more to marginal land because of the country's need to produce more food grain for feeding her ever increasing population.

Hence the urgent necessity to create new jute varieties which could not only grow in marginal land but also compete with synthetic fibres.

Some of the desirable characters may arise *de novo* through mutation or somaclonal variation. Changes in the colour of fibre from white to snow white have been observed in population of *C. capsularis* in an experimental farm of Bangladesh Jute Research Institute (BJRI) (Ahmed 1983) at Kustia. When mutation breeding proceed to be of limited value in this (jute) crop, a number of recent reviews have treated the subject of somaclonal variation from the standpoint of its ubiquity, the likely causes and origins and its potential impact on plant improvement (Larkin and Scowcroft 1981, 1983; Orton 1984; Evans *et al.* 1984; Larkin *et*

al. 1985; Scowcroft *et al.* 1985; Karp and Bright 1985; Scowcroft 1985). Larkin *et al.* (1984) observed somaclonal variations in wheat through tissue culture. The production of somaclonal variants in rice through anther culture was reported by Schaeffer *et al.* (1984). Therefore, it was considered possible that jute plants derived from somaclonal variants may give us strains with qualities we have been looking for. Some of these qualities may be flood tolerant or strains with snow white fibres or strains resistant to root rot or dieback.

The combination of the above mentioned characters through sexual hybridization of the two species is an important way to produce desired cultivars. This has been tried since 1907. The interspecific hybridization was first attempted by Finlow (1917, 1921, 1923). But he failed to get any hybrid. Later on similar results were obtained by Howard (1924), Patel *et al.* (1944), Patel and Datta (1960), Srinath and Kundu (1952), Bhadury and Chakravarti (1948), Qureshi (1964) and others.

In spite of the success in obtaining hybrids between the two incompatible species, *C. olitorius* X *C. capsularis* they could not be bred to produce any commercial variety (Mia and Shaikh 1967).

As the sexual hybridization techniques have not yielded desired results it is necessary to look for some other means such as somatic fusion in combining the desirable characters of the two jute species.

The discovery of enzymatic isolation of plant protoplast (Cocking 1960) and their fusion (Kao *et al.* 1974), Scheider and

Vasil (1980) has opened the alternative possibility of producing desirable cultivars by somatic hybridization. Protoplast fusion or somatic hybridization brings the total genome of two species in one event.

One of the obvious novelties of protoplast fusion is that it permits genomes of sexually incompatible species and genera to be brought together. There are some successful reports on the production of somatic hybrids resulting from fusion of protoplasts between species which are very difficult or impossible to hybridize sexually e.g. *Lycopersicon esculentum* + *Solanum tuberosum* (Melcher *et al.* (1978); *Datura innoxia* + *Atropa belladona* (Krumbiegel and Schieder 1979); *Arabidopsis thaliana* + *Brassica campestris* (Gleba and Hoffman 1980); and more recently between horticulturally important sexually incompatible species *Petunia parodii* + *P. parviflora* (Power *et al.* 1980).

Not much work has been done on tissue culture of jute. Only a few laboratories in Bangladesh and India are undertaking tissue culture work on this crop. Iyer and Raina (1972) for the first time reported the production of diploid callus from the connectives of the jute anthers which did not differentiate. Islam *et al.* (1978) succeeded in isolating protoplasts from leaf mesophyll cells of *C. olitorius* var. 04 and *C. capsularis* var.D_{154} and Lalnaris, but the isolated protoplasts failed to divide. Das *et al.* (1981) reported success of shoot regeneration from protoplast derived callus of *C. capsularis* and *C. olitorius*, but it was not confirmed by any other subsequent publication.

For the production of haploid jute plants attempts so far

failed and have not proceeded other than the callus stage and their differentiation only into roots (Islam *et al.* 1981). Jahan and Chowdhury (1980) have also reported the production of diploid callus only from the connectives of anthers of *C. olitorius* similar to that reported by Iyer and Raina (1972).

In such a condition effort was directed to obtain differentiation through callus derived from shoot tips, leaf, petiole etc. Shoot differentiation was found in shoot tip derived callus (Islam *et al.* 1982). But it could not be ascertained whether the shoots were differentiated from the callus itself through organogenesis or by direct regeneration of the preexisting meristematic tissue. Next the success of organogenesis from shoot tip derived callus after repeated subculture was reported. The shoot regeneration from callus after repeated subculture ascertained the organogenesis from the callus itself. Observation of this view has also been obtained from the observation of serial sections of callus (Rahman *et al.* 1985). However, the production of shoots from the shoot tip derived callus was only a few. Earlier Vasil (1983) reported that shoot regeneration could be induced easily in wheat if scutellum is used instead of other explants. In the light of this report the object of the present investigation was to find out whether different segments of germinating seeds of the two fibre yielding species would be a more favourable material compared to shoot tip, leaf, petiole and anthers.

In brief, the objective of the present investigation was:

(1) to find out which explant would be more favourable for regeneration;

(2) to standardize the media composition for differentiation; and

(3) to evaluate the regenerated plants in respect of morphological characters.

REVIEW OF LITERATURE

Not much work has been done on tissue culture of jute. Only a few laboratories in Bangladesh and India are engaged in tissue culture work on this crop. Various attempts have been made in the past to develop a recombinant line combining the characters of *C. olitorius* and *C. capsularis*.

Islam and Rashid (1960) reported the first successful hybrid of the two cultivated species following cross pollination by smearing the pedicel of the flowers with indole-3-acetic acid to prevent abscission of pollinated flower and enhance the growth of the hybrid embryo and endosperm.

Patel and Datta (1960) carried out hybridization on an extensive scale for three consecutive years between different cultivated as well as wild types of these two species. Pod setting was observed particularly in all the crosses and with a few nonviable seeds.

Patel and Datta (1960) and Banerjee and Datta (1960) observed that the rate of pollen tube growth down the style is comparatively slower in the cross, *C. olitorius* (δ) and *C. Capsularis* (φ) than when they are self pollinated.

From the embryological studies they concluded that the inefficiency of most of the pollen tubes to grow repidly and fertilize the majority of ovules, delayed fertilization, non-synchronization between the development of endosperm and embryo and failure of the hybrid seed to germinate are the main barriers for a successful interspecific hybridization.

Islam *et al.* (1970) reported successful hybridization between cultivar *C. olitorius* and *C. depressus* a photoneutral wild species. But in the F_3 generation they were being doubled and became tetraploid (Islam *et al.* 1973). Although most of the resulting amphiploids were photoneutral, they were all branched and shorter in height, the characters which made them inferior to their diploid counterparts. When fully developed, the leaves of these amphiploids dropped (Islam *et al.* 1973). Neither of the F_4 bred true nor did F_5 progeny raised from the seeds of the photoneutral plants of the selected F_4 (Islam *et al.* 1975).

Since embryo rescue/culture is regarded to be a specific area of tissue culture, a brief account of the work involving embryo rescue has been included in this review.

For the first time, Islam (1964) showed that through embryo rescue, F_1 hybrid between *C. capsularis* and *C. olitorius* using former as mother could be obtained. However, the F_1 plant died before its transfer to the pot.

One good example of the use of embryo rescue was demonstrated by Islam and Abbasi (1966) in the cross between colchicin induced *C. olitorius* var C.G and a natural new world wild tetraploid *Corchorus* species viz. *C. siliquosus.* Following the cross in which 300 ppm IAA in lanolin paste was applied around the stalk of the pollinated flower, embryo was rescued and grown in White medium. F_1 hybrid was successfully raised in pot. It proved to be a completely sterile hybrid having high pollen sterility.

Through embryo culture Haque and Islam (1967) confirmed the earlier report that Tossa-Daise jute can be successfully crossed.

For the first time Iyer and Raina (1972) reported the production of diploid callus from the connectives of the jute anthers, their callus did not differentiate.

Islam *et al.* (1978) succeeded in isolating protoplasts from leaf mesophyll cells of *C. olitorius* var O-4 and *C. capsularis* var D-154 and Lalnaris, but the isolated protoplasts failed to divide.

Jahan and Chowdhury (1980) reported the production of diploid callus only from the connectives of anthers of *C. olitorius* similar to that reported by Iyer and Raina (1972).

Das *et al.* (1981) reported success of shoot regeneration from protoplast derived callus of *C. capsularis* and *C. olitorius*, but no subsequent publication is available from that laboratory on the performance of their regenerants.

Attempts to obtain haploid jute plants through anther culture have so far failed, however no difficulty was encountered to obtain profuse callus and roots from the callus (Islam *et al.* 1981).

Some success of shoot regeneration from shoot tip and leaf derived callus was obtained by Islam (1981). He reported shoot regeneration at a very low frequency from leaf derived callus of *C. olitorius* in MS (Murashige and Skoog, 1962) medium supplemented with 0.2 mg/l IAA and 2 mg/l zeatin.

Islam *et al.* (1981) tried to produce dihaploids through anther culture from a spontaneous amphidiploid of the hybrid, *C. olitorius* x *C. depressus*. They obtained only callus and profuse

rooting but no plantlets.

Sen *et al.* (1981) observed that IBA with Kn stimulated growth of shoot explants without callus formation in some *Corchorus* species whereas NAA with Kn induced only callus. According to them (Sen *et al.* 1981) when these callii were transferred to IBA and Kn supplemented MS medium, a large number of shoots regenerated.

Islam *et al.* (1982) reported single and multiple shoot regeneration from shoot tip derived callus in MS, MMS, SH media when they were supplemented with more or less equal proportion of auxin and cytokinin. They later observed that the best material in respect of shoot regeneration from shoot tip was an exotic tetraploid *Corchorus* species, viz. *C. siliquosus.*

Vasil (1983) reported that shoot regeneration could be induced in wheat if scutellum is used instead of other explants.

Rahman (1985) reported shoot regeneration in IBA and Kn supplemented MS medium from shoot tip explants. He reported that wild race of *C. capsularis* was the best material. Rahman (l.c) also reported multiple shoot regeneration from shoot tip explants in MS + 0.5 mg/l BAP + 25 mg/l tyrosine and 0.1 mg/l IBA + 50 mg/l glutamine. He observed as many as six shoots/explant in *C. capsularis* var D-154. Rahman (l.c.) reported the success of root initiation in White medium + 1.0 mg/l IAA.

Hoque (1987) reported multiple shoot regeneration from the root decapitated germinating seeds of the male parent, *C. capsularis* var D-154 and the female parent, *C. olitorius* var O-4 and also from their F_1 and F_2 hybrids in MS + 0.5 mg/l BAP + 25

mg/l tyrosine. He however, noticed that the regenerants from 0-4 performed poorly and died before they could be transplanted.

Ahmed *et al.* (1989) reported that it is possible to save the regenerants of *C. olitorius* var 0-4 from degeneration with an antioxidant, namely, nordihydroguiaretic acid (NDGA). The above authors added NDGA in the rooting medium in the first subculture.

Master (1989) used NDGA with BAP and tyrosine supplemented MS medium. The explants used were plumule with cotyledons, obtained from 20 hours old germinating seeds. From the date of inoculation, it took about 12 days for the explant to produce a number of swollen buds and by subculturing them in the same medium, shoots developed from each of these isolated buds. These shoots became rooted by transferring them into rooting medium containing MS + 0.2 mg/l IBA.

MATERIALS

Three cultivars of *Corchorus capsularis* L. (2n = 14), namely, D-154, CVL-1 and CC-45 and two cultivars of *C. olitorius* L. (2n = 14), namely, C.G. and O_4 were used in the present investigation. The origin, source and morphological characteristics of each of these cultivars are described under following heads.

***C. capsularis* L. (2n = 14 var. D-154**

Origin: A pure line selection from 'Kakya Bombai' was made in 1919 at Dhaka. It is less susceptible to chlorosis and possessed a green coppery red pigmentation. Comparatively resistant to stem rot and gives a high yield but requires 130-135 days to mature. This is the dominant variety in Bangladesh and grown widely in spite of a few varieties released since 1940s.

Source : BJRI Germplasm Bank.

Morphological characters

Habit and habitat : Herbaceous annual shrub.

Stem : Cylindrical, unbranched, tall up to 315-340 cm, base diameter 1.78±0.14, green but in mature plants the base of the branches becomes pigmented.

Leaves : Lanceolate, serrate, basal serration usually prolonged, petiole slender, length of the leaves 13.4-16.0 cm and breadth 6.0-8.0 cm, length breadth ratio 1.77:1, stipule free lateral, pigmented at the tips only.

Flowers : Small, yellow, sepals 5, light coppery red in colour, petals 5 in number, ovate acuminate, stamens 18-23, pollen

After collecting base line data and psychometric measures researcher used in depth interview to explore the nature and intensity of specific psychological problems of the participants after being diagnosed of having breast cancer. This will be followed by case conceptualization.

After taking proper assessment formulation sharing and treatment goal was set collaboratively. According to data collected from assessment session researcher was administered psychotherapy like cognitive behavior therapy, interpersonal therapy, marital therapy and family therapy.

In the last session researcher was perform post measures using same quantitative measures administered in the first session.

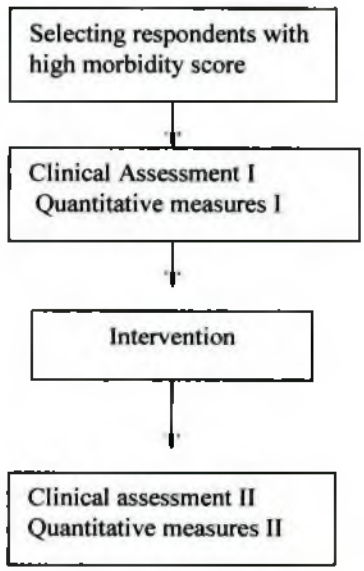

Figure: 2: Flow chart regarding entering the whole research process

3.5 Data processing and analysis

Data were analyzed using both qualitative and quantitative data analysis method. Quantitative data from self rating of patient's problems, functional level assessment

stainability 96-98%, ovary 5-celled, style small, stigmas capitate.

Fruits : Capsule, spherical with irregular projections and depressions all over the surface. Number of seeds/capsule 30-40, seed colour chocolate brown, pyramidal in shape, weight of 1000 seeds 3.1gm.

Flowering habit : Late type, flowers in between 105 and 120 days.

***C. capsularis* L. (2n = 14) var. CVL-1**

Origin : A full green variety of *C. capsularis* was evolved through single plant selection from a local collection grown at Kishorganj substation of BJRI in 1954 and was released in 1977.

Source : BJRI Germplasm Bank.

Morphological characters

Habit and habitat : Herbaceous annual shrub.

Stem : Cylindrical, smooth with full green pigmentation, height 315.92-320.1 cm, feebly branched.

Leaves : Ovate-lanceolate in shape, serrate, stipules free lateral, length breadth ratio 1.76:1.

Flowers : Medium, sepals 5, light green in colour, petals 5, light yellow, pollen stainability 95-98%, style small.

Fruits : Mostly in clusters of 2-3, globular, no. of seeds/ capsule 30-40, seed colour chocolate brown, 1000 seeds weight about 3.0 gm.

Flowering habit : Flowers in between 105 and 120 days depending on the date of sowing and soil condition. Less photosensitive than *C. olitorius*.

C. capsularis L. (2n = 14) var. CC-45

Origin : BJRI collected the seeds from Cairo, Egypt with a collection of *C. capsularis* germplasm. From the collection BJRI released CC-45 as a variety by pure line selection. This variety is photoinsensitive and does not show early flowering even if sown in the month of February. It has shown better cropping in low lands which become flooded in early rainy season.

Source : BJRI Germplasm Bank.

Morphological characters

Habit and habitat : Herbaceous annual shrub.

Stem : Cylindrical, smooth with full-green pigmentation, unbranched, tall up to 312.8 - 320 cm.

Leaves : Lanceolate, serrate, stipules free lateral, length breadth ratio of leaves 2:1.

Flowers : Medium, sepals 5, light green in colour, petals 5, yellow in colour.

Fruits : Mostly in cluster, globular, seed colour chocolate brown, number of seeds/capsule 30-40.

C. olitorius L. (2n = 14) var. C.G.

Origin : It was developed in BJRI, Dhaka as early as in 1915 under the name D_{38}. Since the seeds of this variety were collected from Chunchura of India and the colour of the plant was completely green, the name D_{38} was subsequently changed to Chinchura Green (C.G.).

Source : BJRI Germplasm Bank.

Morphological characters

Habit and habitat : Herbaceous shrub.

Stem : Cylindrical, unbranched, height 450-500 cm, base diameter 1.75±0.15 cm.

Leaves : Lanceolate, serrate, basal serration often prolonged into a tail like appendage called serature. A pair of free lateral stipule present. Length breadth ratio of leaves is 2.3:1.

Flowers : Large, yellow, axillary, sepals 5, green, petals 5, yellow in colour, petals comparatively larger than those of *C. capsularis*, anthers globose, percentage of pollen grain stainability 97.5, style oblong, short constricted.

Fruits : Cylindrical capsule, green in colour when unrippen, surface smooth, ten ribbed, ribs parallel. Colour of the seeds black with greenish tinge, number of seeds/capsule 200-250, weight of 1000 seeds about 2.0 gm, percentage of seed germination is 98.

Flowering habit : Late type, generally flowers in between 104 and 112 days.

C. olitorius **L. (2n = 14) var. O-4**

Origin : A pure line selection from germplasm collection of Bangladesh Jute Research Institute (BJRI), released as the commercial variety in 1964 and now grown in over 50 percent of the area under 'Tossa' jute.

Source : BJRI Germplasm Bank.

Morphological characters

Habit and habitat : Herbaceous annual shrub.

Stem : Cylindrical, 334-370 cm tall green, unbranched under normal spacing (30 cm in between rows and 7.5 cm between plants).

Leaves : Lanceolate, serrate, basal serration prolonged into tail like appendage called serature, length breadth ratio 2.2:1. Light green in colour. A pair of free lateral stipule present at the base of the petiole. The crown of leaves at the top of stem loosely arranged.

Flower : Large, yellow, sepals 5, light green in colour, petals 5, yellow in colour, axillary, in cluster of 1-2, entire, pollen grain stainability 98%, style oblong, short, constricted five locular ovary.

Fruit : Cylindrical capsule, green in colour, ten ribbed, ribs distinct, parallel, upon ripening the capsule dehisces at the union of the carpels and shatter their seeds. Seed colour black with greenish tinge.

Flowering habit : Late type, generally flowers in between 118 and 132 days.

METHODS

Experiments were carried out in Plant Breeding and Tissue Culture Laboratory of the Department of Botany, University of Dhaka and its adjoining Botanical garden and net house.

The different procedures followed in the experiment are described below under separate heads.

Tissue Culture

Different steps of tissue culture are as follows :

Preparation of culture media:

The following media were used in the present investigation:

1) MS, medium (Murashige and Skoog, 1962);

2) MMS, medium (Murashige and Skoog, 1981);

3) White's medium (White, 1943);

4) R-2 medium, (Chaleff, 1980);

5) SH medium (Schenk and Hildebrandt, 1972)

Preparation of stock solution:

The different constituents of the media were prepared into stock solution for ready use during the preparation of the media. As different constituents are required in different concentrations, separate stock solutions for macro, micro, vitamins, hormones etc. were used.

Stock solution A (major constituents or macronutrients):

It was made up to ten times the final strength of the medium in one litre of distilled water. Ten times the weight of the salts present per litre of the medium were weighed accurately, dissolved one at a time and sequentially in 750 ml of distilled

water and then made up to one litre. The stock solution was filtered through a Whatman No. 1 filter paper, if it contained any solid contaminants like cellulose, dust, cotton etc. The stock solution was stored in a refrigerator at 4°C.

Stock solution B (micronutrients):

It was made at ten times the final strength of the medium in 100 ml of distilled water as described for the stock solution A. In this case the two constituents $FeSO_4$ and Na_2EDTA were separately dissolved in 100 ml distilled water in a 250 ml conical flask and kept for 24 hrs at 58°C by placing it in an incubator. $CoCl_2$ was separately dissolved in 100 ml of distilled water. All stock solutions were filtered and stored separately in a refrigerator at 4°C.

Stock solution C (organic constituents):

This was also made at ten times the final strength of the medium in 100 ml of distilled water as described for stock solution, A and B. Inositol was directly added during the preparation of medium in desired concentration.

Hormonal stock solution:

Phytohormones namely, napthalene acetic acid (NAA), indole - 3-acetic acid (IAA), idole-3-butyric acid (IBA), 2,4 - dichloro-phenoxy-acetic acid (2,4-D), 6-furfuryl aminopurine (Kinetin/Kn), benzyl-amino purine (BAP) were dissolved in appropriate solvent. The solvents used for dissolving these salts were shown as against each hormone:

Hormone	Solvent
NAA	: 0.1 N NaOH
IAA	: 70% ethyl alcohol
IBA	: 70% ethyl alcohol
2,4-D	: 70% ethyl alcohol
Kinetin	: 0.1 N HCl
BAP	: 0.1 N NaOH
Tyrosine	: 0.1 N NaOH
2-ip	: 70% ethyl alcohol.

To prepare a stock solution of any of the above hormones 10 mg of the hormone were placed on a clean watch glass and then dissolved in 1 ml of the particular solvent. The mixture was then washed off with distilled water and poured into a 100 ml measuring cylinder. Sufficient distilled water was added to make the volume up to the desired level. Thus stock solution of all the hormones were made and stored in deep freeze at 0°C.

Preparation of working medium:

To make one litre of any of the above mentioned media the following steps were followed :

(i) 100 ml of stock solution A and 10 ml each of stock solution B and C were poured in a conical flask.

(ii) Desired amount of sucrose, generally 3% (30 gms), were dissolved in 500 ml of distilled water and added to the stock solution that was taken in the conical flask.

(iii) Different concentration of hormonal supplements as required was added singly or in different combination to this solution and thoroughly mixed.

Since each hormonal stock solution contained 10 mg of the hormone in 100 ml of solution, the addition of 10 ml of any hormonal stock solution for one litre of medium resulted in 1 mg/l concentration of that hormonal supplement similarly for different concentration of hormones, the amount of hormonal stock solutions was added to the medium.

(iv) The whole mixture was then poured into a one litre measuring cylinder and made up to the desired volume with the addition of distilled water. Then the mixture was poured into a two litre flask and again mixed well.

(v) The pH of the medium was adjusted to 5.8 using a digital pH meter by means of 0.1 N NaOH and 0.1 N HCl, whichever necessary.

(vi) 0.8% of 'Difco' or 'Bacto' agar was added to the medium and heated to melt the agar completely. Proper care was taken so as not to boil the solution while melting agar.

In case of liquid medium, agar was not added to the medium.

(vii) The medium was then poured into test tubes/conical flasks while still hot and the culture vessels were plugged tightly with cotton wool. The different hormone supplemented medium containing test tubes/conical flasks were properly marked by a glass marker to indicate the specific supplementation.

(viii) The medium was then autoclaved at 15 lbs per square inch pressure at the temperature of 120°C for 20 minutes to ensure sterilization.

Collection of explants:

In the present investigation the explants used for culturing

were collected from different sources, namely,

(i) Field or pot growing plants,

(ii) Seedlings germinated and grown aseptically in MS medium and

(iii) Seeds soaked in sterile distilled water for 17-18 hours.

(i) Explants collected from field or pot growing plants:

For this purpose, plants were grown in ten inches diameter earthen pots and in well ploughed plots. Different explants like shoot tip, leaf, petiole and anthers etc were collected from field and pot grown plants.

(ii) Explants collected from seedlings germinated and grown in MS medium:

For this purpose, seedlings were grown in conical flasks containing MS medium with 2% sucrose without any hormonal supplementation. pH of the medium was adjusted at 5.8 and 0.8% agar was added to the medium. A 50 ml of sterilized medium was taken in each 150 ml conical flask. Seeds of different jute varieties were surface sterilized with 0.1% $HgCl_2$ for 20 min. The seeds were then washed well with sterile distilled water for four times and then inoculated in aseptic culture media for their germination and growth. From the seedlings shoot tip, epicotyl and hypocotyl were collected for inoculation.

(iii) Explants collected from seeds soaked in sterile distilled water for about 17-18 hours:

A small amount of seeds was sterilized in 0.1% $HgCl_2$ for 20 mins and after thorough washing placed in sterile test tubes containing enough sterile water to just cover the seeds with it.

On the following morning i.e. after 17-18 hours, most of the seed were found to germinate. The embryos at this stage were about 1.5 to 2 mm long.

Since intact embryos in MS medium developed into seedlings without callusing and somatic embryogenesis, the embryo was divided into different parts namely, (i) plumule with cotyledon (ii) plumule without cotyledon, (iii) cotyledon, (iv) embryo without cotyledon; (v) root-tip etc and these segments were inoculated in the culture medium.

Culture technique

The following techniques were followed in course of culturing the plant parts:

(i) Explant culture : Different explants namely, shoot tip, leaf, petiole, epicotyl, hypocotyl, plumule with/without cotyledon, cotyledon, embryo without cotyledon, root-tip etc were cultured. Shoot tips were collected from field grown as well as from the aseptically grown seedlings. The shoot tips which were collected from field grown plants were surface sterilized with 0.1% $HgCl_2$ for 20 minutes and then washed with sterile distilled water for four times. The outer whorl of leaves were then removed from shoot-tip (approximately 0.3 to 0.5 mm). The apical region was isolated aseptically under a binocular microscope in the microflow cabinet with the help of a sharp razor. The dome of the apical end was cut in a V-shaped fashion. The dissected shoot-tips were transferred onto semisolid medium contained in test tubes or in conical flask.

Other explants like leaf, petiole etc were collected from field as well as aseptically grown plants. The leaf, petiole which were collected from field grown plants were surface sterilized as stated above. Cotyledon, epicotyl, hypocotyl were collected from aseptically grown seedlings. Plumule with/without cotyledon, cotyledon, embryo without cotyledon, root-tip etc. were collected from germinating seeds soaked in sterile distilled water for about 17-18 hours. All these explants were inoculated onto semisolid medium in test tubes/ conical flasks.

(ii) Anther culture: Since it is well known that anthers containing uninucleate microspores are most suitable for androgenesis, it is of prime importance to determine the uninucleate microspores stage of anthers before inoculation. For this purpose anthers were first cytologically examined. The flower buds two days prior to opening and anthers having a light yellow colour were found to contain the microspores of this stage. It may be mentioned here that the size of the flower buds at this stage vary from variety to variety and also due to seasonal variation in the same variety.

The flower buds containing anthers of appropriate stage were collected from healthy growing plants. The excised flower buds were washed in distilled water, dipped in absolute alcohol for one minute and thereafter immediately transferred to 0.1% $HgCl_2$ solution where the buds were kept for 15 minutes. The buds were then rinsed with sterile distilled water for four times to remove all traces of, $HgCl_2$ solution. For inoculation a shallow incision was made along the joint of sepals with the help of a fine needle

holding the bud with forceps. The sepals and petals were placed back and turned off with the help of a pair of forceps. The anthers were then removed by cutting at the base of the filament and collected in a sterile Petri dish. The filaments from anthers were carefully removed and about five anthers were plated in each test tube.

Precautions: During culturing all the dissecting instruments (which were autoclaved earlier) namely, razor, knives, needles and forceps were dipped in 70% alcohol and flammed over a spirit lamp before each time of use. The floor of the cabinet and hands were washed with 70% alcohol before starting the procedure.

Incubation: The culture tube and conical flasks containing inocula were incubated in a dark cupboard shelf in a temperature controlled room. With the appearance of callus, the test tubes were transferred to another shelf with light intensity 20,000 lux from fluorescent light. The light period of the culture room was maintained at 12 hours and temperature was 25±2°C.

Subculturing process:

In case of explants producing callus, when the callii attained a size of about 20-25 mm in diameter they were subcultured on to fresh media for further proliferation or regeneration. For this purpose the calli were removed aseptically from the test tube on a sterile Petri dish, cut into 0.4 to 0.5 cm long pieces by a sterile scalpel and placed on suitable media in tubes or conical flasks.

In case of explants showing somatic embryogenesis, when just initiated, they were subcultured in the fresh media contained in the conical flasks. When shoots were produced in higher number, bigger shoots were separated and cultured in the rooting medium for root formation. The rest explants containing small shoots were cut into 2-3 pieces and were subcultured in the same freshly prepared medium for further development.

Transfer of plantlets from test tubes to pot

After one and a half month plantlets attained a height of 5-8 cm. They were carefully removed from the test tubes and after washing the roots in sterile distilled water, they were transferred to 6" dia pots containing soil and covered with polythene bag. It was observed that it made no difference whether the plantlets were transferred to sterile or ordinary soil prepared with garden soil, organic manure and sand in the proportion of 2:1:1. On the 6/7th day the polythene bag was perforated to allow good ventilation. The polythene bag was finally removed on the 15th day. After a month, the plantlets were transferred to bigger pots where they grew to maturity. The plants were numbered serially as TCL-1, TCL-2 etc.

When the plants grew to maturity, different morphological characters namely, L/B ratio, petiole length, serrature length, stipule length, time of flowering, pollen stainability, number of total fruits/plant, number of seeds/fruit, germination percentage of seeds etc. of R_0, R_1 and R_2 generation of tissue culture derived plants (TCL) were recorded. Different morphological characters were recorded by measuring them with scale and noting

with pencils. Pollen grains from freshly collected flowers were stained in aceto carmine to have a rough estimate of the percentage of fertile pollen grains and also to study their differences in respect of shape and size. Germination percentage was observed by sowing seeds in pots.

RESULTS

Experiments were conducted to obtain plantlet regeneration from explants derived from seedlings grown in White's and MS media (shoot tips, epicotyl and hypocotyle); from plants grown in pot and field (shoot tip, leaf, petiole and anthers) and from germinating seeds (plumule with or without cotyledon, embryo without cotyledon, root tip, cotyledon etc Fig.1) in distilled water. It was observed that the age, nature of the explants, media composition and supplementation in the media played effective role for producing regeneration.

In the present investigation standard MS (Murashige and Skoog, 1962) as well as modified MS, (MMS 1981), R-2 (Chaleff, 1980), SH (Schenk and Hildebrandt, 1972) with different hormonal supplements were used for plantlet regeneration from different explants. Besides different hormonal supplements, one amino acid namely, tyrosine in different concentrations was added to MS medium.

A. Effect of different media on shoot tip explants: Shoot tips were cultured in MS, MMS and SH media (Tables 1,2,3,4,5 and 6) for regeneration along with different hormonal supplements.

1. Effect of IBA and Kn in MS medium: IBA and Kn were used in 16 different combinations in MS medium to produce callus, shoot and root from the shoot tip of five jute cultivars (Table 1). In table it is shown that the best callus formation (92.50%) was found (Fig.2) in O-4 of *Corchorus olitorius* when shoot tips were cultured in MS medium supplemented with 0.5 mg/l IBA and 1.0 mg/l

Fig 1. Showing different steps of collecting explants from germinating seeds

Fig. 2 Vigorously growing callus of shoot tip in 0-4 of *C. olitorius* in MS + 0.5 mg/l IBA + 1.0 mg/l Kn (which produced highest frequency, 92.50% of callus).

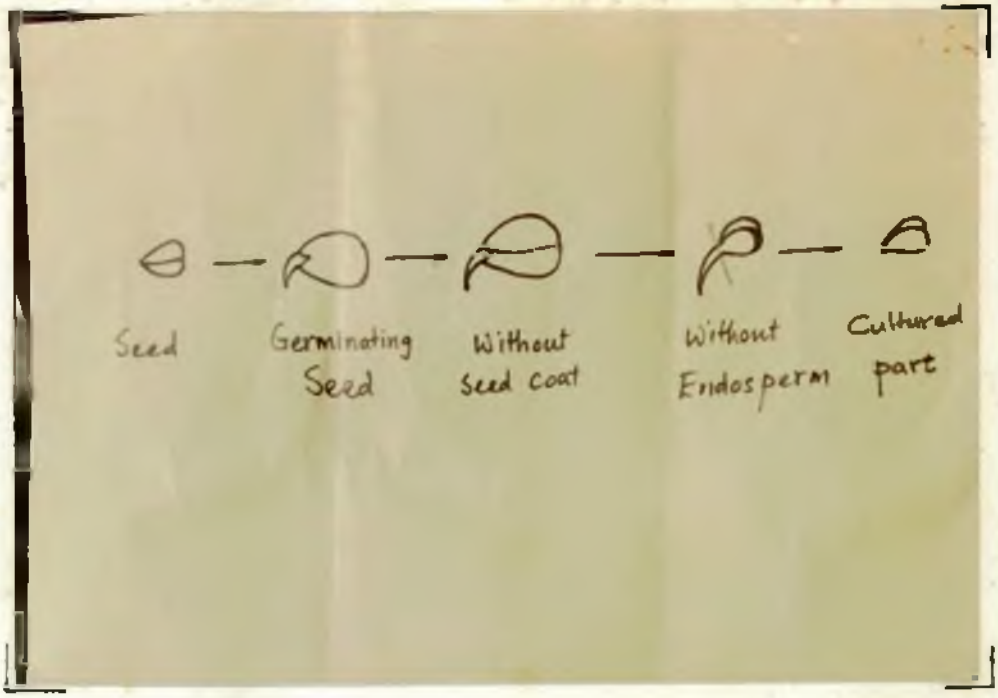

Fig.1

Fig.2

Kn followed by 90% callusing in C.G. of *C. olitorius* in the same combination. The lowest percentage (16%) of callusing was found in CVL-1 of *C. capsularis* in 5.0 mg/l IBA + 0.5 mg/l Kn supplemented MS medium. Out of sixteen different hormonal combinations used, there was no callus formation from shoot tips of five cultivars (*C. olitorius* and *C. capsularis*) in 1.0 mg/l IBA + 5.0 mg/l Kn and 5.0 mg/l IBA + 5.0 mg/l Kn supplemented medium.

In Table 1 it is shown that in D-154 highest percent (17.86%) of callii showed shoot regeneration in 0.5 mg/l IBA + 0.5 mg/l Kn producing 3.4±1.14 shoots/explant. It was found that although frequency of callusing (92.50%) was best in 0.5 mg/l IBA + 1.0 mg/l Kn but highest frequency of shoot regeneration (17.86%) was found in 0.5 mg/l IBA + 0.5 mg/l Kn. Among five cultivars jute, only D-154 and CVL-1 showed shoot regeneration in the two of the 16 hormonal combinations. These combinations were e.g. 0.5 mg/l IBA + 0.5 mg/l Kn and 1.0 mg/l IBA + 1.0 mg/l Kn. In the first combination (0.5 mg/l IBA + 0.5 mg/l Kn) the frequency of shoots in D-154 and CVL-1 was 3.4±1.14 and 3 ± 0.5 shoots/explant and in the second (1.0 mg/l IBA + 1.0 mg/l Kn) the corresponding number of shoots was2.75±0.5 and 2.67±0.58 shoots/explant.

Best rooting (97.67%) was found in the callii of CC-45 followed by 95.65% and 95.42% rooting in D-154 and CVL-1, respectively when MS medium was supplemented with 5.0 mg/l IBA + 0.1 mg/l Kn. The lowest frequency of rooting (6.06%) was found in the callii of O-4 in 1.0 mg/l IBA + 0.5 mg/l Kn supplemented

medium.

II. Effect of IBA and Kn in SH medium: IBA and Kn were used in different combinations in SH medium to see their effect in the production of callus, shoot and root from shoot tip of jute cultivars (Table 2). In table it is shown that the highest frequency of callusing (62.86%) was found in D-154 when SH medium was supplemented with 0.5 mg/l IBA + 1.0 mg/l Kn. The poorest percentage of callusing (35.71%) observed in C.G., of C. olitorius when cultured in 5.0 mg/l IBA + 1.0 mg/l Kn.

In D-154 highest percentage of shoot regeneration (13.64%) was observed in MS fortified with 0.5 mg/l IBA + 0.5 mg/l Kn followed by 9.51% in CC-45. The number of shoots per explant was 4 $\pm$ 1 and 4.5$\pm$0.71 in D-154 and CC-45 respectively. However, callus derived from SH medium supplemented with 1.0 mg/l IBA + 1.0 mg/l Kn showed lowest percentage of shoot regeneration (8.11%). The number of shoots/explant was recorded 3.67 $\pm$ 1.15 in D-154.

The best root formation (54.54%) was observed in the callii of CC-45, in 5.0 mg/l IBA + 0.5 mg/l Kn. Poorest rooting (2%) was found in O-4 in 1.0 mg/l IBA + 5.0 mg/l Kn.

III. Effect of various hormonal supplements on callus, shoot and root formation in MS medium: In the present investigation various hormonal supplements, namely, BAP, 2-ip (2-isopentanyl adenine), IBA, CH, 2,4-D, IAA, Urea, tyrosine, NH_4NO_3, inositol, YE and $Ca(NO_3)_2$ etc were used in MS medium for callus, shoot and root induction. Twelve different combinations and concentration

of the above supplements were used for shoot tip culture (Table 3). Table shows that the highest callusing (57.14%) was found in D-154 in MS medium supplemented with 1.0 mg/l BAP+1.0 mg/l 2-ip + 0.5 mg/l 2,4-D + 1000 mg/l CH while 54.17% the second highest frequency was found in C.G. in MS containing 0.25 mg/l BAP + o.1 mg/l IBA + 0.1 mg/l IAA + 100 mg/l tyrosine + 100 mg/l urea as supplements. The lowest frequency of callusing (29.17%) was found in C.G. in 1.0 mg/l 2-ip + 1.0 mg/l IAA + 400 mg/l YE.

None of the twelve different combinations was found to show shoot regeneration in five different jute cultivars (Table 3).

Root regeneration was found in only one combination, namely, in the MS medium containing 0.25 mg/l BAP + 0.1 mg/l IBA + 0.1 mg/l IAA + 100 tyrosine + 100 mg/l urea. The callus of CVL-1 gave best response (36.36%) for rooting in the last mentioned supplements followed by 20% in D-154 and 0-4.

IV. Effect of BAP, IAA and NAA in MMS medium on callus, shoot and root regeneration: BAP, IAA and NAA were used in three different combinations for shoot tip culture (Table 4). In table it is shown that the highest percentage of callus formation (58.33%) was observed in C.G. and the poorest (41.67%) in CC-45 in 0.5 mg/l BAP + 1.0 mg/l NAA while 57.14% in D-154 in 0.25 mg/l BAP + 0.1 mg/l NAA.

No shoot regeneration was observed from the callus derived from the above combinations.

Root formation was observed when MMS medium was supplemented with 0.5 mg/l BAP + 1.0 mg/l IAA. The best rooting (23.53%) in D-154 followed by 20% in CVL-1 and 0-4, and 21.43% in CC-45.

V. Effect of 0.5 mg/l BAP + 25 mg/l tyrosine in MS medium:

For shoot tip culture 0.5 mg/l BAP + 25 mg/l tyrosine were used in MS medium (Table 5). In table it is shown that the best callusing (40%) was observed in D-154 followed by 36, 30, 22 and 20% in CVL-1, CC-45, O-4 and C.G, respectively.

No root regeneration from callus was observed in this combination.

Highest frequency of shoots (10±2 shoots/explant Fig. 3a) was found in D-154 following 9±1.6 (Fig.3b.) and 7±2 shoots/ explants in CVL-1 and CC-45, respectively. Explants of C.G. and O-4 did not show any shoot regeneration.

VI. Effect of different combinations of BAP and IBA in MS medium in combination with 25 mg/l tyrosine: BAP and IBA were used in MS medium in nine combinations with 25 mg/l tyrosine to see their response on callus, shoot and root regeneration from shoot tip explants (Table 6). Table shows that the highest callus (54.54%) formed in D-154 was observed in 1.0 mg/l IBA + 1.0 mg/l BAP + 25 mg/l tyrosine supplemented MS medium. The next one was also D-154 producing 53.57% callus in 0.5 mg/l IBA + I.0 mg/l BAP + 25 mg/l tyrosine while the poorest one being 30% in O-4 found in 0.1 mg/l IBA + 1.0 mg/l BAP + 25 mg/l tyrosine.

The best shoot regeneration 9±1.58 shoots /explant was observed in D-154 while 8±0.82 shoots/explant found in CVL-1 and 5±1.00 shoots/explant in CC-45 in 0.5 mg/l IBA + 0.5 mg/l BAP + 25 mg/l tyrosine. The lowest frequency of shoot regeneration (4 shoots/explant in D-154 and 3 shoots/explant in

Fig.3(a) 10±2 shoots / explant in D-154 of *C. capsularis* and

b) 9±2 shoots / explant in CVL-1 of *C. capsularis* regenerated from *shoot tip* in MS + 0.5 mg/l BAP + 25 mg/l tyrosine

Fig.3a

Fig.3b

CVL-1) was observed in 0.1 mg/l IBA + 0.5 mg/l BAP + 25 mg/l tyrosine. However, it was not possible to produce shoots from the shoot tips of C.G. and 0-4 of *C. olitorius* in the above nine combinations.

In the Table 6 it is revealed that the highest frequency (58.33%) of root formation was observed in the callii of CVL-1 in 1.0 mg/l IBA + 0.1 mg/l BAP + 25 mg/l tyrosine. Poorest rooting (18.18%) was observed in the callii of CVL-1 in 0.1mg/l IBA + 0.5 mg/l BAP + 25 mg/l tyrosine.

It has been observed that 0.5 mg/l IBA + 0.5 mg/l BAP + 25 mg/l tyrosine was required for best shoot regeneration i.e. half strength of both IBA and BAP showed best response for shoot regeneration.

From the above results using different media and hormonal combinations for culturing shoot tip explants it has been found that the highest percentage (92.50%) of callus (Fig.2, Table 1) formation was found from shoot tip of 0-4 when cultured in MS medium supplemented with 0.5 mg/l IBA + 1.0 mg/l Kn following 90% callus in C.G. in the same medium. The lowest frequency of callusing,(16%) was found in CVL-1 in MS + 5.0 mg/l IBA + 0.5 mg/l Kn. From the result it has been observed that shoot tips of *C. olitorius* varieties are very much efficient for callus formation in MS medium supplemented with IBA and Kn.

The best shoot regeneration from shoot tip was observed in D-154 (10±2 shoots/explant) when MS medium was supplemented with 0.5 mg/l BAP + 25 mg/l tyrosine (Table 5, Fig.3a). Lowest frequency of shoot regeneration was observed in CVL-1 (2.67 ±

0.58 shoots/explant) in MS + 1.0 mg/l IBA + 1.0 mg/l Kn.

Among different hormonal combination best rooting was found in the callii of CC-45 of 97.67% in MS + 5.0 mg/l IBA + 0.1 mg/l Kn. Poorest rooting (2%) was observed in the callii of 0-4 in SH + 1.0 mg/l IBA + 5.0 mg/l Kn.

Different media, namely, MS, MMS and SH were tried in the present investigation for shoot regeneration. But best response was observed in MS medium while a small number of shoots were also observed in SH medium. From the results it has been observed that among different supplements used in the basal media, namely, IBA, Kn, BAP and tyrosine were found essential for shoot regeneration.

B. Effect of different media and hormonal supplements on anther culture: Anthers were cultured in MS, MMS and R-2 media for plantlet regeneration. Results of these observations are shown in Tables 7, 8 and 4.

1. Effect of different hormonal supplements in MS medium: Different supplements e.g. IAA, Kn, IBA, BAP, NAA and 2, 4-D were used for anther culture to regenerate callus, shoot and root (Table 7). In table it is shown that the best callus formation (52.17%) was recorded in 0-4 when MS medium was supplemented with 2 mg/l 2,4-D + 2 mg/l Kn. The poorest one was 24.51% in CC-45 in the combination of 2 mg/l IAA + 2 mg/l Kn.

There was no shoot formation from anthers in MS medium.

Highest percentage of rooting (57.89%) directly from the callii was observed in 0-4 When MS medium was supplemented with

Table 1. Effect of different supplements (IBA and Kn) in MS medium on callus, shoot and root formation from shoot tip explant of Jute cultivar.

Supplements (mg/l)	Material	No.of explants inoculated	No.of explants producing callus	% of callus	No.of callii producing shoot	% of callii producing shoot	No.of shoots/ explant	No.of root formed	% of root
1	2	3	4	5	6	7	8	9	10
0.1 IBA	D-154*	58	42	72.41	-	-	-	38	90.48
+0.1 Kn	CVL-I*	58	41	70.68	-	-	-	37	88.09
	CC-45*	60	42	70.00	-	-	-	34	80.95
	C.G.**	60	46	76.67	-	-	-	4	8.69
	04**	60	48	80.00	-	-	-	4	8.33
0.1 IBA	D-154	54	44	81.48	-	-	-	16	36.36
+0.5 Kn	CVL-1	54	42	77.78	-	-	-	16	38.09
	CC-45	54	40	74.07	-	-	-	12	30.00
	C.G.	54	32	59.26	-	-	-	4	12.50
	04	54	32	59.26	-	-	-	2	6.25

*Varieties of *C. capsularis*
**Varieties of *C. olitorius*.

(Contd.)

1	2	3	4	5	6	7	8	9	10
0.1 IBA	D-154	50	41	82.00	-	-	-	-	-
+1.0 Kn	CVL-1	50	40	80.00	-	-	-	-	-
	CC-45	50	39	78.00	-	-	-	-	-
	C.G	50	38	76.00	-	-	-	-	-
	04	50	40	80.00	-	-	-	-	-
0.1 IBA	D-154	50	40	80.00	-	-	-	-	-
+5.0 Kn	CVL-1	50	40	80.00	-	-	-	-	-
	CC-45	50	38	76.00	-	-	-	-	-
	C.G.	50	41	82.00	-	-	-	-	-
	04	50	42	84.00	-	-	-	-	-
0.5 IBA	D-154	50	24	48.00	-	-	-	20	83.33
+0.1 Kn	CVL-1	50	23	46.00	-	-	-	21	91.30
	CC-45	50	20	40.00	-	-	-	18	81.82
	C.G.	50	21	42.00	-	-	-	3	14.28
	04	50	22	44.00	-	-	-	4	18.18

(Contd.)

1	2	3	4	5	6	7	8	9	10
0.5 IBA	D-154	48	28	58.33	5	17.86	3.4±1.14	18	64.28
+0.5 Kn	CVL-1	48	28	58.33	3	10.71	3±1	16	57.14
	CC-45	48	24	50.00	-	-	-	15	62.50
	C.G.	50	31	62.00	-	-	-	12	38.71
	04	52	36	69.23	-	-	-	10	27.78
0.5 IBA	D-154	40	28	70.00	-	-	-	-	-
+1.0 Kn	CVL-1	40	26	65.00	-	-	-	-	-
	CC-45	40	25	62.50	-	-	-	-	-
	C.G.	40	36	90.00	-	-	-	10	27.77
	04	40	37	92.50	-	-	-	7	18.92
0.5 IBA	D-154	40	32	80.00	-	-	-	-	-
+5.0 Kn	CVL-1	40	30	75.00	-	-	-	-	-
	CC-45	40	26	65.00	-	-	-	-	-
	C.G.	40	24	60.00	-	-	-	-	-
	04	40	25	62.50	-	-	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9	10
1.0 IBA	D-154	70	58	82.86	-	-	-	28	48.27
+0.1 Kn	CVL-1	70	56	80.00	-	-	-	26	44.83
	CC-45	70	53	75.71	-	-	-	25	44.64
	C.G.	68	52	76.47	-	-	-	4	7.69
	04	68	54	79.41	-	-	-	8	14.81
1.0 IBA	D-154	52	38	73.08	-	-	-	14	36.84
+0.5 Kn	CVL-1	52	35	67.31	-	-	-	12	34.28
	CC-45	52	35	67.31	-	-	-	10	28.57
	C.G.	50	30	60.00	-	-	-	2	6.67
	04	50	33	66.00	-	-	-	2	6.06
1.0 IBA	D-154	60	45	75.00	4	8.89	2.75±0.5	38	84.44
+1.0 Kn	CVL-1	60	43	71.66	3	6.98	2.67±0.58	36	83.72
	CC-45	60	42	70.00	-	-	-	37	88.09
	C.G.	60	44	73.33	-	-	-	4	9.09
	04	60	46	76.67	-	-	-	4	8.69

(Contd.)

1	2	3	4	5	6	7	8	9	10
1.0 IBA	D-154	60	-	-	-	-	-	-	-
+5.0 Kn	CVL-1	60	-	-	-	-	-	-	-
	CC-45	50	-	-	-	-	-	-	-
	C.G.	50	-	-	-	-	-	-	-
	04	50	-	-	-	-	-	-	-
5.0 IBA	D-154	58	46	79.31	-	-	-	44	95.65
+0.1 Kn	CVL-1	58	44	75.86	-	-	-	42	95.42
	CC-45	58	43	74.14	-	-	-	42	97.67
	C.G.	58	34	58.62	-	-	-	12	35.29
	04	58	36	62.07	-	-	-	12	33.33
5.0 IBA	D-154	50	10	20.00	-	-	-	8	80.00
+0.5 Kn	CVL-1	50	8	16.00	-	-	-	6	75.00
	CC-45	50	9	18.00	-	-	-	6	66.67
	C.G.	50	25	50.00	-	-	-	20	80.00
	04	50	16	32.00	-	-	-	8	50.00

(Contd.)

1	2	3	4	5	6	7	8	9	10
5.0 IBA	D-154	32	28	87.50	-	-	-	12	42.86
+1.0 Kn	CVL-1	32	26	81.25	-	-	-	11	38.46
	CC-45	32	24	75.00	-	-	-	10	41.67
	C.G.	32	24	75.00	-	-	-	4	16.67
	04	32	20	62.50	-	-	-	4	20.00
5.0 IBA	D-154	32	-	-	-	-	-	-	-
+5.0 Kn	CVL-1	40	-	-	-	-	-	-	-
	CC-45	40	-	-	-	-	-	-	-
	C.G.	40	-	-	-	-	-	-	-
	04	40	-	-	-	-	-	-	-

Table 2. Effect of auxin and cytokinin (IBA and Kn) in SH medium on callus, shoot and root formation from shoot tip explants of jute cultivar.

Supplements (mg/l)	Material	No.of explants inoculated	No.of explants producing callus	% of callus	No.of callii producing shoot	% of callii producing shoot	No.of shoots/ explant	No.of root formed	% of root
1	2	3	4	5	6	7	8	9	10
0.1 IBA	D-154	50	26	52.00	-	-	-	6	23.08
+0.1 Kn	CVL-1	50	25	50.00	-	-	-	-	-
	CC-45	50	22	44.00	-	-	-	-	-
	C.G.	50	24	48.00	-	-	-	-	-
	04	50	23	46.00	-	-	-	4	17.39
0.1 IBA	D-154	35	20	57.14	-	-	-	-	-
+0.5 Kn	CVL-1	35	18	51.42	-	-	-	-	-
	CC-45	35	17	48.57	-	-	-	-	-
	C.G.	35	16	45.71	-	-	-	2	12.50
	04	35	17	48.57	-	-	-	1	5.88

(Contd.)

1	2	3	4	5	6	7	8	9	10
0.1 IBA	D-154	35	22	62.86	-	-	-	-	-
+1.0 Kn	CVL-1	35	21	60.00	-	-	-	-	-
	CC-45	35	20	57.14	-	-	-	-	-
	C.G.	35	18	51.43	-	-	-	-	-
	04	35	17	48.57	-	-	-	-	-
0.1 IBA	D-154	40	25	62.50	-	-	-	-	-
+5.0 Kn	CVL-1	40	23	57.50	-	-	-	-	-
	CC-45	40	22	55.00	-	-	-	-	-
	C.G.	40	20	50.00	-	-	-	-	-
	04	40	21	52.50	-	-	-	-	-
0.5 IBA	D-154	42	20	47.62	-	-	-	10	50.00
+0.1 Kn	CVL-1	42	19	45.24	-	-	-	8	42.10
	CC-45	42	18	42.86	-	-	-	7	44.44
	C.G.	42	17	40.48	-	-	-	2	11.76
	04	42	18	42.46	-	-	-	3	16.67

(Contd.)

1	2	3	4	5	6	7	8	9	10
0.5 IBA	D-154	42	22	52.38	3	13.64	4±1	4	18.18
+0.5 Kn	CVL-1	42	20	47.61	-	-	-	4	20.00
	CC-45	42	21	50.00	2	9.51	4.5±0.71	3	15.00
	C.G.	42	18	42.86	-	-	-	-	-
	04	42	20	47.61	-	-	-	-	-
0.5 IBA	D-154	80	47	58.75	-	-	-	24	51.06
+1.0 Kn	CVL-1	80	46	57.50	-	-	-	24	52.17
	CC-45	80	42	52.50	-	-	-	22	52.38
	C.G.	80	44	55.00	-	-	-	20	45.45
	04	80	45	56.25	-	-	-	20	44.44
0.5 IBA	D-154	45	24	53.33	-	-	-	2	8.33
+5.0 Kn	CVL-1	45	23	51.11	-	-	-	-	-
	CC-45	45	22	48.89	-	-	-		-
	C.G.	45	21	46.67	-	-	-	4	19.05
	04	45	21	46.67	-	-	-	2	9.52

(Contd.)

1	2	3	4	5	6	7	8	9	10
1.0 IBA	D-154	42	24	57.14	-	-	-	5	20.83
+0.1 Kn	CVL-1	42	23	54.76	-	-	-	6	26.09
	CC-45	42	22	52.38	-	-	-	5	22.73
	C.G.	40	21	52.50	-	-	-	4	19.04
	04	40	20	50.00	-	-	-	5	25.00
1.0 IBA	D-154	50	27	54.00	-	-	-	9	33.33
+0.5 Kn	CVL-1	50	28	56.00	-	-	-	7	25.00
	CC-45	50	26	52.00	-	-	-	8	30.77
	C.G.	50	22	44.00	-	-	-	2	9.09
	04	50	23	46.00	-	-	-	3	13.04
1.0 IBA	D-154	70	37	52.85	3	8.11	3.67±1.15	12	32.43
+1.0 Kn	CVL-1	70	35	50.00	-	-	-	11	31.42
	CC-45	70	34	48.57	-	-	-	11	32.35
	C.G.	70	33	47.14	-	-	-	13	39.39
	04	70	34	48.57	-	-	-	11	32.35

(Contd.)

1	2	3	4	5	6	7	8	9	10
1.0 IBA	D-154	42	22	52.38	-	-	-	5	22.73
+5.0 Kn	CVL-1	42	21	50.00	-	-	-	7	33.33
	CC-45	42	20	47.62	-	-	-	7	35.00
	C.G.	40	18	45.00	-	-	-	6	15.00
	04	40	20	50.00	-	-	-	4	2.00
5.0 IBA	D-154	48	20	41.67	-	-	-	10	50.00
+0.1 Kn	CVL-1	48	22	45.83	-	-	-	10	45.45
	CC-45	48	20	41.67	-	-	-	8	40.00
	C.G.	48	19	39.58	-	-	-	7	36.84
	04	48	18	37.50	-	-	-	9	50.00
5.0 IBA	D-154	50	25	50.00	-	-	-	13	52.00
+0.5 Kn	CVL-1	50	22	44.00	-	-	-	11	50.00
	CC-45	50	22	40.00	-	-	-	12	54.54
	C.G.	52	20	38.46	-	-	-	9	45.00
	04	52	20	38.46	-	-	-	9	45.00

Table 3. Effect of various supplements in MS medium on callus, shoot and root formation from shoot tip explants.

Supplements (mg/l)	Material	No.of explants inoculated	No.of explants producing callus	% of callus	No.of callii producing shoot	% of callii producing Shoot	No.of shoots/ explant	No.of root formed	% of root
1	2	3	4	5	6	7	8	9	10
1.0 BAP	D-154	32	15	46.87	-	-	-	-	-
+1.0 2-ip	CVL-1	32	13	40.68	-	-	-	-	-
+0.5 IBA	CC-45	30	10	33.33	-	-	-	-	-
+1000 CH	C.G.	30	11	36.67	-	-	-	-	-
	04	30	9	30.00	-	-	-	-	-
0.25 BAP+	D-154	28	16	57.14	-	-	-	-	-
1.0 2-ip+	CVL-1	28	12	42.86	-	-	-	-	-
0.5 2,4-D	CC-45	28	13	46.43	-	-	-	-	-
+1000 CH	C.G.	28	11	39.28	-	-	-	-	-
	04	28	12	42.86	-	-	-	-	-
0.25 BAP	D-154	28	10	35.71	-	-	-	2	20.00
+0.1 IBA	CVL-1	28	11	39.28	-	-	-	4	36.36
+0.1 IAA	CC-45	26	10	38.46	-	-	-	-	-
+100 tyrosine	C.G.	24	13	54.17	-	-	-	-	-
+100 Urea	04	24	10	41.67	-	-	-	2	20.00

(Contd.)

1	2	3	4	5	6	7	8	9	10
1.0 2-ip	D-154	30	13	43.33	-	-	-	-	-
+1.0 IAA	CVL-1	30	13	43.33	-	-	-	-	-
+2000	CC-45	30	14	46.67	-	-	-	-	-
$NH_4 NO_3$	C.G.	30	11	36.67	-	-	-	-	
	04	30	12	40.00	-	-	-	-	-
1.0 2-ip	D-154	24	12	50.00	-	-	-	-	-
+1.0 IAA	CVL-1	24	10	41.67	-	-	-	-	-
3000 NH_4NO_3	CC-45	24	9	37.50	-	-	-	-	-
	C.G.	24	11	45.83	-	-	-	-	-
	04	24	11	45.83	-	-	-	-	-
1.0 2-ip	D-154	28	13	46.43	-	-	-	-	-
+1.0 IAA	CVL-1	28	10	35.71	-	-	-	-	-
+500 inositol	CC-45	28	11	39.28	-	-	-	-	-
	C.G.	28	14	50.00	-	-	-	-	-
	04	28	11	39.28	-	-	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9	10
1.0 2-ip	D-154	25	10	40.00	-	-	-	-	-
+1.0 IAA	CVL-1	25	8	32.00	-	-	-	-	-
+1000 inositol	CC-45	25	9	36.00	-	-	-	-	-
	C.G.	25	12	48.00	-	-	-	-	-
	04	25	11	44.00	-	-	-	-	-
1.0 2-ip	D-154	30	14	46.67	-	-	-	-	-
+1.0 IAA	CVL-1	30	12	40.00	-	-	-	-	-
+100 YE	CC-45	30	10	33.33	-	-	-	-	-
	C.G.	30	9	30.00	-	-	-	-	-
	04	30	11	44.00	-	-	-	-	-
1.0 2-ip	D-154	24	12	50.00	-	-	-	-	-
+1.0 IAA	CVL-1	24	10	41.67	-	-	-	-	-
+400 YE	CC-45	24	8	33.33	-	-	-	-	-
	C.G.	24	7	29.17	-	-	-	-	-
	04	24	9	37.50	-	-	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9	10
1.0 2-ip	D-154	22	9	40.91	-	-	-	-	-
+1.0 IAA	CVL-1	20	10	50.00	-	-	-	-	-
+500$Ca(No_3)_2$	CC-45	22	7	31.82	-	-	-	-	
	C.G.	22	10	45.45	-	-	-	-	-
	04	20	8	40.00	-	-	-	-	-
1.0 2-ip	D-154	30	14	46.67					
+1.0 IAA	CVL-1	30	11	36.67	-	-	-	-	-
1000 $Ca(No_3)_2$	CC-45	30	11	36.67	-	-	-	-	-
	C.G.	32	12	37.50	-	-	-	-	-
	04	32	10	31.25	-	-	-	-	-
1.0 2-ip	D-154	26	11	42.31	-	-	-	-	-
+1.0 IAA	CVL-1	26	9	38.46	-	-	-	-	-
+500 NH_4NO_3	CC-45	26	9	34.61	-	-	-	-	-
+500 $Ca(No_3)_2$	C.G.	26	8	30.77	-	-	-	-	-
500 inositol	04	28	11	29.28					

Table 4. Effect of various supplements in MMS medium on callus, shoot and root formation from various explants of jute.

Explants	supplements (mg/l)	Material	No.of explants inoculated	No.of callii formed	%of callus	No.of callii producing shoot	No.of shoots/ explant	No.of callii producing root	% of root
1	2	3	4	5	6	7	8	9	10
Shoot tip	0.5 BAP	D-154	30	17	56.67	-	-	4	23.53
	+1.0 IAA	CVL-1	30	15	50.00	-	-	3	20.00
		CC-45	32	14	43.75	-	-	3	21.43
		C.G.	30	16	53.33	-	-	-	-
		O4	30	15	50.00	-	-	3	20.00
	0.5 BAP	D-154	24	12	50.00	-	-	-	-
	+1.0 NAA	CVL-1	24	11	45.83	-	-	-	-
		CC-45	24	10	41.67	-	-	-	-
		C.G	24	14	58.33	-	-	-	-
		O4	24	11	45.83	-	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9	10
	0.25 BAP	D-154	28	16	57.14	-	-	-	-
	+0.1 NAA	CVL-1	28	15	53.57	-	-	-	-
		CC-45	28	15	53.57	-	-	-	-
		C.G.	28	14	50.00	-	-	-	-
		04	28	13	46.43	-	-	-	-
Leaf	0.5 BAP	D-146	25	13	52.00				
	+2.0 IAA	CVL-1	25	10	40.00	-	-	-	-
		CC-45	24	11	45.83	-	-	-	-
		C.G.	24	12	50.00	-	-	-	-
		04	24	12	50.00	-	-	2	16.67
	0.5 BAP	D-154	20	10	50.00	-	-	-	-
	+1.0 NAA	CVL-1	20	8	40.00	-	-	-	-
		CC-45	20	8	40.00	-	-	-	-
		C.G.	20	11	55.00	-	-	-	-
		04	20	12	60.00	-	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9	10
	0.5 BAP	D-154	25	12	48.00	-	-	-	-
	+0.1 NAA	CVL-1	26	10	38.46	-	-	-	-
		CC-45	25	11	44.00	-	-	-	-
		C.G.	25	14	56.00	-	-	-	-
		04	25	13	52.00	-	-	-	-
Petiole	0.5 BAP	D-154	24	12	50.00	-	-	4	33.33
	+1.0 IAA	CVL-	22	10	45.45	-	-	2	20.00
		CC-45	24	10	41.67	-	-	-	-
		C.G.	22	11	50.00	-	-	-	-
		04	22	10	45.45	-	-	3	30.00
	0.5 BAP	D-154	22	12	54.54	-	-	-	-
	+1.0 NAA	CVL-1	22	11	50.00	-	-	-	-
		CC-45	20	9	45.00	-	-	-	-
		C.G.	20	8	40.00	-	-	-	-
		04	20	10	50.00	-	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9	10
	0.25 BAP	D-154	24	11	45.83	-	-	-	-
	+0.1 NAA	OVL-1	24	13	54.17	-	-	-	-
		CC-45	24	10	41.67	-	-	-	-
		C.G.	24	11	45.83	-	-	-	-
		04	20	12	60.00	-	-	-	-
Anther	Liqud medium Solid medium	D-154	32	10	31.25	-	-	-	-
		OVL-1	32	13	40.62	-	-	-	-
	0.5 BAP	CC-45	32	11	34.37	-	-	-	-
	+1.0 IAA	C.G.	32	9	28.12	-	-	-	-
		04	32	11	34.37	-	-	-	-
	0.5 BAP	D-154	30	13	43.33	-	-	-	-
	+1.0 NAA	OVL-1	30	14	46.67	-	-	-	-
		CC-45	30	8	26.67	-	-	-	-
		04	30	10	33.33	-	-	-	-
	0.25 BAP	D-154	22	9	40.91	-	-	-	-
	+0.1 NAA	OVL-1	22	8	36.36	-	-	-	-
		CC-45	20	9	45.00	-	-	-	-
		C.G.	20	6	30.00	-	-	-	-
		04	20	7	35.00	-	-	-	-

Table 5. Effect of 0.5 mg/l BAP + 25 mg/l tyrosine in MS on callus formation and shoot regeneration in different explants obtained from five days old seedlings.

Nature of explant	Material	No.of explants inoculated	No.of explants producing callus	% of callus	No.of explants producing shoot	% of explant producing shoot	No.of shoots/ explant
1	2	3	4	5	6	7	8
shoot tip	D-154	50	20	40.00	8	16.00	10±2
	CVL-1	50	18	36.00	8	16.00	9±1.60
	CC-45	50	15	30.00	6	12.00	7±2
	C.G.	50	10	20.00	-	-	-
	04	50	11	22.00	-	-	-
Epicotyl	D-154	50	15	30.00	-	-	-
	CVL-1	50	15	30.00	-	-	-
	CC-45	50	12	24.00	-	-	-
	C.G.	50	13	26.00	-	-	-
	04	50	13	26.00	-	-	-
Hypocotyl	D-154	30	15	50.00	-	-	-
	CVL-1	28	12	42.86	-	-	-
	CC-45	28	11	39.28	-	-	-
	C.G.	28	10	35.71	-	-	-
	04	26	11	42.31	-	-	-

Table 6. Effect of different combinations of auxin and cytokinin (BAP and IBA) in MS medium in combinations with 25 mg/l tyrosine on callus formation and shoot regeneration from different explants obtained from five days old seedlings.

Supplements (mg/l)	Nature of explant	Material	No.of explants inoculated	No.of explants producing callus	% of callus	No.of calli producing root	% of root	No.of explants producing shoot	% of explant producing shoot	No.of shoots/ explant
1	2	3	4	5	6	7	8	9	10	11
0.5 IBA	Shoot tip	D-154	48	20	41.67	8	40.00	5	10.42	9±1.58
+0.5 BAP		CVL-1	48	20	41.67	7	35.00	4	4.33	8±0.82
+25 tyrosine		CC-45	46	22	47.83	8	36.36	5	10.87	5±1.00
		C.G.	40	18	45.00	6	33.33	-	-	-
		04	42	20	47.62	9	45.00	-	-	-
	Epicotyl	D-154	40	22	55.00	7	31.81	-	-	-
		CVL-1	42	20	47.62	8	40.00	-	-	-
		CC-45	42	22	52.38	7	31.81	-	-	-
		C.G.	40	20	50.00	6	30.00	-	-	-
		04	40	18	45.00	4	22.22	-	-	-
	Hypocotyl	D-154	32	18	56.25	7	38.89	-	-	-
		CVL-1	32	15	46.87	7	46.67	-	-	-
		CC-45	32	16	50.00	6	37.50	-	-	-
		C.G.	32	12	37.50	4	33.33	-	-	-
		04	32	12	37.50	4	33.33	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9	10	11
1.0 IBA	Shoot tip	D-154	30	12	40.00	5	41.67	3	10.00	7±1
+0.5 BAP		CVL-1	32	12	37.50	4	33.33	2	6.25	7±0
+25 tyrosine		CC-45	32	14	43.75	6	42.86	2	6.25	6±0
		C.G.	30	13	43.33	5	38.46	-	-	-
		04	32	11	34.37	5	45.45	-	-	-
	Epicotyl	D-154	30	15	50.00	7	46.67	-	-	-
		CVL-1	30	14	46.67	6	42.86	-	-	-
		CC-45	30	15	50.00	6	40.00	-	-	-
		C.G.	30	12	40.00	5	41.67	-	-	-
		04	30	11	36.67	5	45.45	-	-	-
	Hypocotyl	D-154	28	14	50.00	8	57.14	-	-	-
		CVL-1	28	14	50.00	6	42.86	-	-	-
		CC-45	28	12	42.86	5	41.67	-	-	-
		C.G.	25	10	40.00	5	50.00	-	-	-
		04	25	10	40.00	4	40.00	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9	10	11
0.5 IBA	Shoot tip	D-154	28	15	53.57	7	46.67	-	-	-
+1.0 BAP		CVL-1	28	10	35.71	4	40.00	-	-	-
+25 tyrosine		CC-45	26	12	46.15	5	41.67	-	-	-
		C.G.	25	10	40.00	4	40.00	-	-	-
		04	25	11	44.00	5	45.45	-	-	-
	Epicotyl	D-154	30	15	50.00	6	40.00	-	-	-
		CVL-1	25	12	48.00	4	33.33	-	-	-
		CC-45	25	12	48.00	5	41.67	-	-	-
		C.G.	22	10	40.00	4	40.00	-	-	-
		04	22	10	40.00	3	30.00	-	-	-
	Hypocotyl	D-154	25	12	48.00	4	33.33	-	-	-
		CVL-1	25	10	40.00	4	40.00	-	-	-
		CC-45	25	12	48.00	5	41.67	-	-	-
		C.G.	25	9	36.00	4	44.44	-	-	-
		04	25	11	44.00	5	45.45	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9	10	11
0.5 IBA	Shoot tip	D-154	30	12	40.00	4	33.33	-	-	-
+0.1 BAP		CVL-1	30	10	33.33	4	40.00	-	-	-
+25 tyrosine		CC-45	30	11	36.67	5	45.45	-	-	-
		C.G.	30	11	36.67	4	36.36	-	-	-
		04	30	10	33.33	3	30.00	-	-	-
	Epicotyl	D-154	30	12	40.00	5	41.67	-	-	-
		CVL-1	30	11	36.67	5	45.45	-	-	-
		CC-45	25	10	40.00	4	40.00	-	-	-
		C.G.	25	11	44.00	5	45.45	-	-	-
		04	25	8	32.00	4	50.00	-	-	-
	Hypocotyl	D-154	30	11	36.67	5	45.45	-	-	-
		CVL-1	30	10	33.33	4	40.00	-	-	-
		CC-45	30	9	30.00	2	22.22	-	-	-
		C.G.	28	10	35.71	4	40.00	-	-	-
		04	24	8	33.33	3	37.50	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9	10	11
0.1 IBA	Shoot tip	D-154	25	10	40.00	3	30.00	2	8.00	4
+0.5 BAP		CVL-1	25	11	44.00	2	18.18	1	4.00	3
+25 tyrosine		CC-45	25	10	40.00	-	-	-	-	-
		C.G.	25	8	32.00	-	-	-	-	-
		04	25	8	32.00	-	-	-	-	-
	Epicotyl	D-154	28	13	15.38	2	15.00	-	-	-
		CVL-1	28	14	50.00	4	28.57	-	-	-
		CC-45	24	11	45.83	1	9.09	-	-	-
		C.G.	22	10	45.45	-	-	-	-	-
		04	22	8	36.36	-	-	-	-	-
	Hypocotyl	D-154	30	14	46.67	4	28.57	-	-	-
		CVL-1	30	12	40.00	2	16.67	-	-	-
		CC-45	30	11	36.67	-	-	-	-	-
		C.G.	30	8	26.67	-	-	-	-	-
		04	30	9	30.00	-	-	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9	10	11
1.0 IBA	Shoot tip	D-154	24	10	41.67	5	50.00	-	-	-
+0.1 BAP		CVL-1	24	12	50.00	7	58.33	-	-	-
+25 tyrosine		CC-45	24	11	45.83	5	45.45	-	-	-
		C.G.	24	10	41.67	2	20.00	-	-	-
		04	24	11	45.83	3	27.27	-	-	-
	Epicotyl	D-154	26	11	42.31	6	54.54	-	-	-
		CVL-1	24	12	50.00	5	41.67	-	-	-
		CC-45	24	10	41.67	5	50.00	-	-	-
		C.G.	20	9	45.00	4	44.44	-	-	-
		04	20	8	40.00	2	25.00	-	-	-
	Hypocotyl	D-154	22	10	45.45	4	40.00	-	-	-
		CVL-1	22	10	45.45	3	30.00	-	-	-
		CC-45	24	8	33.33	3	37.50	-	-	-
		C.G.	20	9	45.00	3	33.33	-	-	-
		04	20	8	40.00	2	25.00	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9	10	11
0.1 IBA	Shoot tip	D-154	20	9	45.00	-	-	-	-	-
+1.0 BAP		CVL-1	22	10	45.45	-	-	-	-	-
+25 tyrosine		CC-45	20	9	45.00	-	-	-	-	-
		C.G.	20	8	40.00	-	-	-	-	-
		04	20	6	30.00	-	-	-	-	-
	Epicotyl	D-154	20	10	50.00	-	-	-	-	-
		CVL-1	20	11	55.00	-	-	-	-	-
		CC-45	20	9	45.00	-	-	-	-	-
		C.G.	20	7	35.00	-	-	-	-	-
		04	20	8	40.00	-	-	-	-	-
	Hypocotyl	D-154	25	12	48.00	-	-	-	-	-
		CVL-1	25	11	44.00	-	-	-	-	-
		CC-45	25	12	48.00	-	-	-	-	-
		C.G.	25	10	40.00	-	-	-	-	-
		04	25	10	40.00	-	-	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9	10	11
1.0 IBA Shoot tip		D-154	22	12	54.54	5	41.67	-	-	-
+1.0 BAP		CVL-1	22	10	45.45	3	30.00	-	-	-
+25 tyrosine		CC-45	20	8	40.00	-	-	-	-	-
		C.G.	20	7	35.00	-	-	-	-	-
		04	20	9	45.00	2	22.22	-	-	-
	Epicotyl	D-154	30	14	46.67	6	42.86	-	-	-
		CVL-1	30	11	36.67	4	36.36	-	-	-
		CC-45	28	11	39.28	-	-	-	-	-
		C.G.	28	10	35.71	2	20.00	-	-	-
		04	24	9	37.50	-	-	-	-	-
	Hypocotyl	D-154	20	9	45.00	3	33.33	-	-	-
		CVL-1	20	10	50.00	-	-	-	-	-
		CC-45	20	7	35.00	-	-	-	-	-
		C.G.	20	8	40.00	2	25.00	-	-	-
		04	20	6	30.00	-	-	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9	10	11
0.1 IBA	Shoot tip	D-154	24	10	41.67	-	-	-	-	-
+0.1 BAP		CVL-1	24	10	41.67	-	-	-	-	-
+25 tyrosine		CC-45	24	8	33.33	-	-	-	-	-
		C.G.	24	9	37.50	-	-	-	-	-
		04	24	8	33.33	-	-	-	-	-
	Epicotyl	D-154	20	10	50.00	-	-	-	-	-
		CVL-1	20	8	40.00	-	-	-	-	-
		CC-45	22	10	45.45	-	-	-	-	-
		C.G.	20	9	45.00	-	-	-	-	-
		04	22	10	45.45	-	-	-	-	-
	Hypocotyl	D-154	25	11	44.00	-	-	-	-	-
		CVL-1	25	12	48.00	-	-	-	-	-
		CC-45	28	12	42.86	-	-	-	-	-
		C.G.	26	10	38.46	-	-	-	-	-
		04	25	11	44.00	-	-	-	-	-

2mg/l IAA + 2mg/l Kn. Poorest rooting (2.73%) was observed in C.G. in 0.5 mg/l IBA + 0.5 mg/l BAP + 0.1 mg/l 2,4-D.

II. Effect of different supplements in R-2 medium: Different supplements like, IAA, IBA, Kn, BAP, NAA and 2,4-D were used in different concentrations and combinations in R-2 medium to produce callus, shoot and root from anthers of different jute cultivars (Table 8). It is evident from the Table that highest percentage of callusing, 50% was found in O-4 cultivars of *C. olitorius* in 2 mg/l IAA + 1 mg/l Kn. The lowest callusing, 36% was observed from anthers of CVL-1 in o.5 mg/l IBA + 0.5 mg/l BAP + 0.1 mg/l 2,4-D.

However, it was not possible to regenerate any shoot from the above combinations.

Best rooting (45.45%) was found directly from the callii of D-154 in the combination of 2.0 mg/l NAA + 2.0 mg/l Kn. Lowest frequency of rooting, 21.05% was observed in CC-45 in 2 mg/l IAA + 1 mg/l Kn.

III. Effect of various supplements in MMS medium: Various supplements, namely, BAP, IAA and NAA were used in MMS for culturing of anthers (Table 4). From the result it has been found that the highest frequency of callusing (46.67%) was found in CVL-1 in the combination 0.5 mg/l BAP + 1.0 mg/l NAA. While the poorest one was 26.67% observed from the anthers of C.G. in 0.5 mg/l BAP + 1.0 mg/l NAA.

However, there was no shoot or root regeneration from the above callii.

From the above results it has been observed that the highest

Table 7. Effect of various supplements in MS medium on callus, shoot and root differentiation from anthers of jute cultivar.

Supplements (mg/l)	Material	No.of explant inoculated	No.of explants producing callus	% of callus	No.fo callii producing shoot	No.of shoots /explant	No.of callii producing root	% of root
1	2	3	4	5	6	7	8	9
2 IAA	D-154	102	28	27.45	-	-	12	42.86
+2 Kn	CVL-1	102	26	25.49	-	-	8	30.77
	CC-45	102	25	24.51	-	-	10	40.00
	C.G.	102	35	34.31	-	-	20	57.14
	04	102	38	37.25	-	-	22	57.89
0.5 IBA	D-154	45	21	46.66	-	-	6	28.57
+1 Kn	CVL-1	45	18	40.00	-	-	7	38.89
	CC-45	45	18	40.00	-	-	6	33.33
	C.G.	50	17	34.00	-	-	8	47.05
	04	50	23	46.00	-	-	10	43.47
1.0 IBA	D-154	43	16	37.21	-	-	-	-
+1.0 Kn	CVL-1	42	15	35.71	-	-	-	-
	CC-45	43	14	32.56	-	-	-	-
	C.G.	48	19	39.58	-	-	-	-
	04	48	21	43.75	-	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9
0.5 IBA	D-154	50	16	32.00	-	-	-	-
+0.5 BAP	CVL-1	50	14	28.00	-	-	-	-
+0.1,2,4-D	CC-45	50	15	30.00	-	-	-	-
	C.G.	50	20	40.00	-	-	5	25.00
	04	50	22	44.00	-	-	5	22.73
2.0 NAA	D-154	40	20	40.00	-	-	8	40.00
+2.0 Kn	CVL-1	50	18	36.00	-	-	8	44.44
	CC-45	50	16	32.00	-	-	7	43.75
	C.G.	50	20	40.00	-	-	10	50.00
	04	50	21	42.00	-	-	10	45.45
2,2,4-D	D-154	48	22	45.83	-	-	-	-
+2 Kn	CVL-1	48	20	41.67	-	-	-	-
	CC-45	45	20	44.44	-	-	-	-
	C.G.	46	23	50.00	-	-	-	-
	04	46	24	52.17	-	-	-	-

Table 8. Effect of different supplements in R-2 medium on callus, shoot and root formation from anthers of cultivar jute

1	2	3	4	5	6	7	8	9
2 IAA	D-154	42	20	47.62	-	-	9	45.00
+1 Kn	CVL-1	42	18	42.86	-	-	5	27.78
	CC-45	42	19	45.24	-	-	4	21.05
	C.G.	42	16	38.09	-	-	4	25.00
	04	42	21	50.00	-	-	9	42.86
0.5 IBA	D-154	45	22	48.89	-	-	8	36.36
+1 Kn	CVL-1	40	18	45.00	-	-	7	38.89
	CC-45	40	17	42.50	-	-	7	41.18
	C.G.	42	19	45.24	-	-	5	26.31
	04	42	20	47.62	-	-	6	30.00
1.0 IBA	D-154	62	28	45.16	-	-	10	35.71
+1.0 Kn	CVL-1	62	26	41.93	-	-	11	42.31
	CC-45	60	25	41.67	-	-	9	36.00
	C.G.	60	23	38.33	-	-	7	30.43
	04	60	26	43.33	-	-	9	34.61

(Contd.)

1	2	3	4	5	6	7	8	9
0.5 IBA	D-154	50	20	40.00	-	-	-	-
+0.5 BAP	CVL-1	50	18	36.00	-	-	-	-
+0.1,24-D	CC-45	50	19	38.00	-	-	-	-
	C.G.	48	20	41.67	-	-	-	-
	04	48	21	43.75	-	-	-	-
2.0 NAA	D-154	48	22	45.83	-	-	10	45.45
+2.0 Kn	CC-45	48	20	41.67	-	-	8	40.00
	CC-45	48	23	47.92	-	-	10	43.48
	C.G.	48	20	41.67	-	-	8	40.00
	04	48	21	43.75	-	-	6	28.57
2,2,4-D	D-154	50	20	40.00	-	-	-	-
+2.0 Kn	CVL-1	52	22	42.31	-	-	-	-
	CC-45	50	21	42.00	-	-	-	-
	C.G.	50	23	46.00	-	-	-	-
	04	50	24	48.00	-	-	-	-

percentage of callusing (52.17%) from the anthers of O-4 observed when MS medium was supplemented with 2 mg/l 2,4-D + 2 mg/l Kn but there was no shoot regeneration.

C. Effect of various supplements in MMS medium on callus, shoot and root regeneration from leaf explants of different jute cultivars: Various supplements, namely, BAP, IAA and NAA were used in three different combinations and concentrations in MMS medium to regenerate plantlet (Table 4). It was observed from the Table that the highest callusing, 60% from the leaf was found in O-4 in MMS medium supplemented with 0.5 mg/l BAP + 1.0 mg/l NAA. Lowest frequency of callusing, 38.46% was observed in CVL-1 in MMS + 0.25 mg/l BAP + 0.1 mg/l NAA.

However, none of the above three different combinations was able to produce any shoot.

Root regeneration from the callii was observed only in 0.5 mg/l BAP + 1.0 mg/l IAA. The highest rooting was 30% in the callii of CVL-1 followed by 23% and 16.67% in D-154 and O-4, respectively.

D. Effect of different supplements in MMS medium on callus, shoot and root formation from petiole explants of different jute cultivar: Different hormonal supplements, like, BAP, IAA and NAA were used in three different combinations and concentrations in MMS medium to regenerate plantlet (Table 4). In Table it is shown that the best callusing (60%) was observed in MMS + 0.25 mg/l BAP + 0.1 mg/l NAA while the lowest frequency of callusing (40%) was found in C.G. in 0.5 mg/l BAP + 1.0 mg/l NAA.

Among these different combinations, no shoot regeneration

was observed.

The best root formation (33.33%) was observed in D-154 followed by 30% and 20% in O-4 and CVL-1, respectively. Rooting was observed only in 0.5 mg/l BAP + 1.0 mg/l IAA supplemented MMS medium.

E. Effect of various supplements in MS medium on epicotyl and hypocotyl culture: Various supplements, namely, BAP, IBA, tyrosine were used in MS medium for culturing epicotyl and hypocotyl . Results of these observations have been given in Table 5 and 6. From the Table it has been observed that the highest frequency of callusing, 55% from epicotyl was observed in CVL-1 when cultured in MS + 0.1 mg/l IBA + 1.0 mg/l BAP + 25 mg/l tyrosine while poorest callusing (24%) was observed in CC-45 in 0.5 mg/l BAP + 25 mg/l tyrosine.

There was no shoot regeneration from epicotyl in none of the above combinations.

Best rooting (54.54%) was observed in D-154 callii when callii sub-cultured in 1.0 mg/l IBA + 0.1 mg/l BAP + 25 mg/l tyrosine. Lowest percentage (9.09%) of rooting was observed from the callii of CC-45 in 0.1 mg/l IBA + 0.5 mg/l BAP + 25 mg/l tyrosine.

Hypocotyl of five different cultivars of jute were cultured in the above combinations. From the Table 5 and 6 it has been observed that the best callusing from hypocotyl (56.25%) was observed in D-154 in 0.5 mg/l IBA + 0.5 mg/l BAP+ 25 mg/l tyrosine. From this explant no shoot regeneration was found from

Fig.4 Multiple shoots regenerated from the explant *Plumule with cotyledon* in MS + 0.5 mg/l BAP + 25 mg/l tyrosine.

a) 35 ± 4.92 shoots / explant in D-154

b) 31 ± 2.94 shoots / explant in CVL-1

Fig. 4a

Fig.4b

Fig.4 c) 28 ± 3.26 shoots / explant in CC-45

d) 15 ± 2.9 shoots / explant in O-4

Fig. 4c

Fig. 4d

in cotyledon (6±1.56, 4±1.15, 3±0.71 in D-154, CVL-1 and CC-45, respectively, (Figs.6a-6c). Root tips, embryo without cotyledon and seed of five different varieties produced callii in different frequency but there was no shoot regeneration from the above explants. Among five different varieties, C.G. did not show any shoot regeneration in the above combinations.

In the Table-10 (where IBA and BAP were used in nine different combinations with 25mg/l tyrosine) it is shown that the highest frequency of callusing (60%) was observed in the cotyledon of CVL-1 when MS medium was supplemented with 0.5mg/l IBA + 1.0 mg/l BAP + 25mg/l tyrosine while the lowest frequency (5%) was observed in the cotyledon explant of 0-4.

Highest frequency of shoot regeneration (15±2.49 shoots/ explant,Fig.7a) was observed in D-154 when plumule with cotyledon was cultured in MS ± 0.5 mg/l IBA + 0.5 mg/l BAP + 25 mg/l tyrosine following 10±.1.05, 8±0.94, 3±0.71, 2±0.71 in CVL-1, CC-45, C.G. and 0-4, respectively (Figs.7b-7e).

Among six explant of the five different cultivars used in the present investigation, three explants, namely, plumule with cotyledon, plumule without cotyledon and cotyledon showed shoot regeneration except other three explant, and plumule with cotyledon gave best response among three showing 15±2.49, 10±1.05, 8±0.94, 3±0.71, 2±0.71, shoots/explant in D-154, CVL-1, CC-45, C.G. and 0-4, respectively. Out of nine combinations used, shoot regeneration was observed in most of the combinations except one i.e. in 0.1 mg/l IBA + 1 mg/l BAP + 25 mg/l tyrosine. Among the eight combinations, MS + 0.5 mg/l IBA+0.5 mg/l BAP + 25

Table 9. Effect of 0.5 mg/l BAP + 25 mg/l tyrosine in MS on callus formation and shoot regeneration from different explants obtained from 17-18 hours old germinating seeds.

Nature of explant	Material	No.of explants inoculated	No.of explants producing callus	% of callus	No.of explants producing shoot	% of explant producing shoot	No.of Shoots/ explant
1	2	3	4	5	6	7	8
Plumulewith	D-154	952	108	11.34	728	76.47	35±4.92
cotyledon	CVL-1	952	250	26.26	560	58.82	31 ± 2.94
	CC-45	952	241	25.31	560	58.82	28 ± 3.26
	C.G.	700	250	35.71	-	-	-
	04	700	290	41.43	12	1.71	15 ± 2.90
Plumulewithout	D-154	980	190	19.39	644	65.71	31 ± 2.45
cotyledon	CVL-45	980	241	24.59	504	51.43	30 ± 2.67
	CC-45	896	208	23.21	480	53.57	26 ± 3.40
	C.G.	700	220	31.43	-	-	-
	04	700	249	35.57	7	1.00	9 ± 1.41
Cotyledon	D-154	260	155	59.61	15	5.77	6 ± 1.56
	CVL-1	240	135	56.25	12	5.00	4 ± 1.15
	CC-45	200	150	75.00	5	2.50	3 ± 0.71
	C.G.	140	115	82.14	-	-	-
	04	160	108	67.50	-	-	-

(Contd.)

1	2	3	4	5	6	7	8
Root tip	D-154	545	212	38.90	-	-	-
	CVL-1	500	215	43.00	-	-	-
	CC-45	500	215	43.00	-	-	-
	C.G.	298	110	36.91	-	-	-
	04	298	115	38.59	-	-	-
Embryowithout	D-154	600	282	47.00	-	-	-
cotyledon	CVL-1	600	265	44.17	-	-	-
	CC-45	545	265	48.62	-	-	-
	C.G.	500	212	42.40	-	-	-
	04	500	210	42.00	-	-	-
Seed	D-154	760	292	38.42	-	-	-
	CVL-1	748	300	40.11	-	-	-
	CC-45	650	250	38.46	-	-	-
	C.G.	550	248	45.09	-	-	-
	04	550	240	43.64	-	-	-

Table 10. Effect of different combinations of auxin and cytokinin (IBA and BAP)in MS in combination with tyrosine on callus formation and shoot regeneration from different explants obtained from 17-18 hours old germinating seeds.

Supplements (mg/l)	Nature of explant	Material	No.of explants inoculated	No.of explants producing callus	% of callus	No.of explants producing shoot	% of explants producing shoot	No.of shoots/ explant
1	2	3	4	5	6	7	8	9
0.5 IBA	Plumulewith	D-154	55	20	36.36	25	45.45	15 ± 2.49
+0.5 BAP	cotyledon	CVL-1	55	25	45.45	25	45.45	10 ± 1.05
+25 tyrosine		CC-45	55	20	36.36	21	38.18	8 ± 0.94
		C.G.	35	16	45.71	5	14.18	3±0.71
		04	35	18	51.43	5	14.18	2±0.71
	Plumulewithout	D-154	40	16	40.00	15	37.50	10 ± 1.41
	cotyledon	CVL-1	40	16	40.00	16	40.00	8±1.25
		CC-45	40	18	45.00	15	37.50	8±1.05
		C.G.	40	14	35.00	3	7.50	2±1
		04	40	12	30.00	2	5.00	2±0
	Cotyledon	D-154	40	20	50.00	5	12.50	3±0.71
		CVL-1	40	20	50.00	5	12.50	3±1
		CC-45	42	18	42.86	-	-	-
		C.G.	38	8	21.05	-	-	-
		04	38	9	23.68	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9
	Root tip	D-154	35	18	51.43	-	-	-
		CVL-1	35	15	42.86	-	-	-
		CC-45	30	15	50.00	-	-	-
		C.G.	30	12	40.00	-	-	-
		04	30	11	36.67	-	-	-
	Embryowithout	D-154	40	20	50.00	-	-	-
	cotyledon	CVL-1	40	22	55.00	-	-	-
		CC-45	40	18	45.00	-	-	-
		C.G.	40	16	40.00	-	-	-
		04	40	16	40.00	-	-	-
	Seed	D-154	40	18	45.00	-	-	-
		CVL-1	40	18	45.00	-	-	-
		CC-45	40	17	42.50	-	-	-
		C.G.	40	20	50.00	-	-	-
		04	40	18	45.00	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9
1.0 I8A	Plumulewith	D-154	60	22	36.67	22	36.67	12 ± 1.15
+0.5 BAP	cotyledon	CVL-1	60	18	30.00	20	33.33	10 ± 1.49
+25 tyrosine		CC-45	60	20	33.33	20	33.33	9 ± 1.33
		C.G.	40	14	35.00	3	7.50	3±1
		04	35	12	34.28	4	11.43	2±0.82
	Plumulewithout	D-154	50	20	40.00	16	32.00	10 ± 2
	cotyledon	CVL-1	48	20	41.67	15	31.25	8±1.15
		CC-45	50	16	32.00	15	30.00	5 ±1.15
		C.G.	40	18	45.00	2	5.00	3±0
		04	40	14	35.00	4	10.00	3±0.82
	Cotyledon	D-154	40	16	40.00	4	10.00	2±0
		CVL-1	40	10	25.00	3	7.50	3±1
		CC-45	40	12	30.00	3	7.50	2±1
		C.G.	40	4	10.00	-	-	-
		04	40	2	5.00	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9
	Root tip	B-154	32	14	43.75	-	-	-
		CVL-1	30	14	46.67	-	-	-
		CC-45	32	17	53.12	-	-	-
		C.G.	30	14	46.67	-	-	-
		04	30	12	40.00	-	-	-
	Embryowithout	B-154	24	13	54.17	-	-	-
	cotyledon	CVL-1	24	11	45.83	-	-	-
		CC-45	24	12	50.00	-	-	-
		G.C.	20	8	40.00	-	-	-
		04	20	8	40.00	-	-	-
	Seed	B-154	22	10	45.45	-	-	-
		CVL-1	24	10	41.67	-	-	-
		CC-45	22	8	36.36	-	-	-
		C.G.	20	5	25.00	-	-	-
		0.4	20	5	25.00	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9
0.5 IBA	Plumule with	D-154	45	20	44.44	16	35.55	5 ± 1.49
+1.0 BAP	cotyledon	CVL-1	45	20	44.44	10	22.22	5 ± 1.25
+25 tyrosine		CC-45	35	18	51.43	12	34.28	6 ± 1.76
		C.G.	40	20	50.00	-	-	-
		04	40	22	55.00	5	12.0	3±1
	Plumule without	D-154	40	20	50.00	12	30.00	4 ± 1.41
	cotyledon	CVL-1	42	21	50.00	10	23.81	5±1.41
		CC-45	40	17	42.50	10	25.00	3±1.15
		C.G.	35	19	54.28	-	-	-
		04	35	18	51.43	-	-	-
	Cotyledon	D-154	35	20	57.14	4	11.43	2±0.82
		CVL-1	35	21	60.00	3	8.57	2±1
		CC-45	35	20	57.14	3	8.57	3±1
		C.G.	35	18	51.43	-	-	-
		04	35	18	51.43	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9
	Root tip	D-154	30	15	50.00	-	-	-
		CVL-1	28	15	53.57	-	-	-
		CC-45	28	14	50.00	-	-	-
		C.G.	30	13	43.33	-	-	-
		04	24	10	41.67	-	-	-
	Embrycwithout	D-154	24	12	50.00			
	cotyledon	CVL-1	24	10	41.67	-	-	-
		CC-45	24	11	45.83	-	-	-
		C.G.	24	9	37.50	-	-	-
		04	24	10	41.67	-	-	-
	Seed	D-154	34	13	38.23	-	-	-
		CVL-1	34	15	44.12	-	-	-
		CC-45	34	11	32.35	-	-	-
		C.G.	32	10	31.25	-	-	-
		04	32	8	25.00	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9
0.5 IBA	Plumule with	D-154	30	10	33.33	11	36.37	6 ± 1.82
+0.1 BAP	cotyledon	CVL-1	30	10	33.33	10	33.33	3 ± 1.15
+25 tyrosine		CC-45	30	11	36.67	10	33.33	3 ± 0.67
		C.G.	30	9	30.00	2	6.67	2 ± 0
		04	30	8	26.67	2	6.67	2 ± 0
	Plumule without	D-154	32	14	43.75	10	31.25	5 ± 1.56
	cotyledon	CVL-1	35	18	51.43	10	28.57	6 ± 1.41
		CC-45	32	15	46.87	10	31.25	4 ± 1.15
		C.G.	30	12	40.00	1	3.33	3 ± 0
		04	30	12	40.00	2	6.67	2 ± 0
	Cotyledon	D-154	26	13	50.00	2	7.69	2 ± 0
		CVL-1	24	11	45.83	1	4.17	2 ± 0
		CC-45	24	11	45.83	-	-	-
		C.G.	20	11	55.00	-	-	-
		04	22	12	54.54	-	-	-

(Contd.

1	2	3	4	5	6	7	8	9
	Root tip	D-154	25	10	40.00	-	-	-
		CVL-1	25	12	48.00	-	-	-
		CC-45	25	10	40.00	-	-	-
		C.G.	25	11	44.00	-	-	-
		04	25	12	48.00	-	-	-
	Embryo without	D-154	22	10	45.45	-	-	-
	cotyledon	CVL-1	24	9	37.50	-	-	-
		CC-45	22	10	45.45	-	-	-
		C.G.	20	8	40.00	-	-	-
		04	22	9	40.91	-	-	-
	Seed	D-154	20	8	40.00	-	-	-
		CVL-1	20	7	35.00	-	-	-
		CC-45	20	8	40.00	-	-	-
		C.G.	20	5	25.00	-	-	-
		04	20	7	35.00	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9
0.1 IBA	Plumule with	D-154	30	10	33.33	11	36.67	5 ± 1.15
+0.5 BAP	cotyledon	CVL-1	32	12	37.50	11	34.37	5 ± 2.21
+25 tyrosine		CC-45	32	11	34.37	7	21.87	3 ± 2.21
		C.G.	30	11	36.67	-	-	-
		04	30	13	43.33	-	-	-
	Plumule without	D-154	36	13	36.11	11	30.55	4 ± 1.41
	cotyledon	CVL-1	36	12	33.33	12	33.33	6 ± 1.49
		CC-45	32	10	31.25	9	28.12	3 ± 0.87
		C.G.	34	11	32.35	-	-	-
		04	30	10	33.33	-	-	-
	Cotyledon	D-154	22	10	45.45	-	-	-
		CVL-1	22	11	50.00	-	-	-
		CC-45	20	8	40.00	-	-	-
		C.G.	20	9	45.00	-	-	-
		04	20	7	35.00	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9
	Root tip	D-154	30	12	40.00	-	-	-
		CVL-1	30	12	40.00	-	-	-
		CC-45	25	10	40.00	-	-	-
		C.G.	25	11	44.00	-	-	-
		04	25	13	52.00	-	-	-
	Embryo without	D-154	25	10	40.00	-	-	-
	cotyledon	CVL-1	25	11	44.00	-	-	-
		CC-45	25	10	40.00	-	-	-
		C.G.	25	10	40.00	-	-	-
		04	25	8	32.00	-	-	-
	Seed	D-154	25	12	48.00	-	-	-
		CVL-1	30	13	43.33	-	-	-
		CC-45	28	12	42.86	-	-	-
		C.G.	20	10	50.00	-	-	-
		04	20	9	45.00	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9
1.0 IBA	Plumule with	D-154	40	13	32.50	2	5.00	2 ± 0
+0.1 BAP	cotyledon	CVL-1	38	12	31.58	2	5.26	3 ±1.41
+25 tyrosine		CC-45	38	14	36.84	2	5.26	2 ± 0
		C.G.	35	11	31.43	-	-	-
		04	30	11	36.67	-	-	-
	Plumule without	D-154	38	15	39.47	1	2.63	2 ± 0
	cotyledon	CVL-1	32	13	40.62	2	6.25	2 ± 0
		CC-45	40	15	37.50	1	2.50	2 ± 0
		C.G.	35	14	40.00	1	2.86	2 ± 0
		04	35	11	31.43	-	-	-
	Cotyledon	D-154	25	11	44.00	-	-	-
		CVL-1	25	11	44.00	-	-	-
		CC-45	25	9	36.00	-	-	-
		C.G.	22	7	31.81	-	-	-
		04	22	9	40.91	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9
	Root tip	D-154	20	8	40.00	-	-	-
		CVL-1	20	7	35.00	-	-	-
		CC-45	20	8	40.00	-	-	-
		C.G.	25	9	36.00	-	-	-
		04	25	10	40.00	-	-	-
	Embryo without	D-154	25	11	44.00	-	-	-
	cotyledon	CVL-1	25	10	40.00	-	-	-
		CC-45	25	12	48.00	-	-	-
		C.G.	20	8	40.00	-	-	-
		04	24	7	29.17	-	-	-
	Seed	D-154	20	8	40.00	-	-	-
		CVL-1	22	10	45.45	-	-	-
		CC-45	20	9	45.00	-	-	-
		C.G.	21	9	42.86	-	-	-
		04	24	10	41.67	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9
0.1 IBA	Plumule with	D-154	30	12	40.00	8	26.67	3 ± 0.75
+1.0 BAP	cotyledon	CVL-1	28	13	46.43	7	25.00	2 ± 0.82
+25 tyrosine		CC-45	27	12	44.44	7	25.92	3 ± 0.82
		C.G.	24	11	45.83	3	12.50	2 ± 1
		04	24	10	41.67	4	16.67	2 ± 0
	Plumule without	D-154	30	12	40.00	6	20.00	4 ± 1.41
	cotyledon	CVL-1	30	14	46.67	7	23.33	4 ± 1.29
		CC-45	28	11	39.28	5	17.86	3 ± 0.71
		C.G.	25	10	40.00	2	8.0	2 ± 0
		04	26	11	42.31	3	11.54	3 ± 1
	Cotyledon	D-154	25	10	40.00	2	8.00	2 ± 0
		CVL-1	25	11	44.00	1	4.00	3 ± 0
		CC-45	25	11	44.00	1	4.00	2 ± 0
		C.G.	25	10	40.00	-	-	-
		04	25	9	36.00	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9
	Root tip	B-154	24	10	41.67	-	-	-
		CVL-1	24	10	41.67	-	-	-
		CC-45	28	11	39.28	-	-	-
		C.G.	26	11	42.31	-	-	-
		04	24	9	37.50	-	-	-
	Embryo without	B-154	24	11	45.83	-	-	-
	cotyledon	CVL-1	24	10	41.67	-	-	-
		CC-45	24	8	33.33	-	-	-
		C.G.	24	9	37.50	-	-	-
		04	24	10	41.67	-	-	-
	Seed	B-154	25	12	48.00	-	-	-
		CVL-1	25	10	40.00	-	-	-
		CC-45	22	11	50.00	-	-	-
		C.G.	20	9	45.00	-	-	-
		04	22	10	45.45	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9
1.0 IBA	Plumule with	D-154	30	12	40.00	9	30.00	3 ± 0.71
+1.0 BAP	cotyledon	CVL-1	30	13	43.33	8	26.67	5 ± 1.31
+25 tyrosine		CC-45	28	10	35.71	6	21.43	4 ± 1.41
		C.G.	26	11	42.31	2	7.69	2 ± 0
		04	26	11	42.31	2	7.69	2 ± 0
	Plumule without	D-154	30	14	46.67	6	20.00	4 ± 1.79
	cotyledon	CVL-1	30	14	46.67	5	16.67	3 ± 0.71
		CC-45	30	12	40.00	7	23.33	3 ± 0.82
		C.G.	30	11	36.67	-	-	-
		04	30	12	40.00	-	-	-
	Cotyledon	D-154	28	11	39.28	-	-	-
		CVL-1	26	10	38.46	-	-	-
		CC-45	28	11	39.28	-	-	-
		C.G.	29	9	31.03	-	-	-
		04	25	10	40.00	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9
	Root tip	D-154	24	11	45.83	-	-	-
		CVL-1	24	10	41.67	-	-	-
		CC-45	24	11	45.83	-	-	-
		C.G.	24	10	41.67	-	-	-
		04	24	10	41.67	-	-	-
	Embryo without	D-154	25	12	48.00	-	-	-
	cotyledon	CVL-1	25	10	40.00	-	-	-
		CC-45	25	11	44.00	-	-	-
		C.G.	25	12	48.00	-	-	-
		04	25	10	40.00	-	-	-
	Seed	D-154	20	9	45.00	-	-	-
		CVL-1	20	10	50.00	-	-	-
		CC-45	20	8	40.00	-	-	-
		C.G.	20	7	35.00	-	-	-
		04	20	5	25.00	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9
0.1 IBA	Plumule with	D-154	28	12	42.86	-	-	-
+0.1 BAP	cotyledon	CVL-1	28	13	46.43	-	-	-
+25 tyrosine		CC-45	28	11	39.28	-	-	-
		C.G.	26	10	38.46	-	-	-
		04	26	10	38.46	-	-	-
	Plumule without	D-154	25	11	44.00	-	-	-
	cotyledon	CVL-1	25	11	44.00	-	-	-
		CC-45	22	10	45.45	-	-	-
		C.G.	24	11	45.83	-	-	-
		04	22	10	45.45	-	-	-
	Root tip	D-154	20	9	45.00	-	-	-
		CVL-1	24	11	45.83	-	-	-
		CC-45	24	10	41.67	-	-	-
		C.G.	20	8	40.00	-	-	-
		04	22	10	45.45	-	-	-

(Contd.)

1	2	3	4	5	6	7	8	9
	Cotyledon	D-154	20	9	45.00	-	-	-
		CVL-1	20	9	45.00	-	-	-
		CC-45	20	8	40.00	-	-	-
		C.G.	20	7	35.00	-	-	-
		04	20	5	25.00	-	-	-
	Embryo without	D-154	20	9	45.00	-	-	-
	cotyledon	CVL-1	20	8	40.00	-	-	-
		CC-45	20	7	35.00	-	-	-
		C.G.	20	8	40.00	-	-	-
		04	20	7	35.00	-	-	-
	Seed	D-154	25	12	48.00	-	-	-
		CVL-1	24	10	41.67	-	-	-
		CC-45	24	11	45.83	-	-	-
		C.G.	20	9	45.00	-	-	-
		04	20	9	45.00	-	-	-

Fig.5 Multiple shoots regenerated from the explant *plumule without cotyledon* in 0.5 mg/l BAP + 25 mg/l tyrosine

a) 31 ± 2.45 shoots / explant in D-154.

b) 30 ± 2.69 shoots / explant in CVL-1.

Fig. 5a

Fig.5b

Fig.5 c) 26 ± 6.4 shoots / explant in CC-45 and

d) 9 ± 1.41 shoots / explant in O-4.

Fig. 5c

Fig. 5d

Fig.6 Regeneration of multiple shoots from cotyledon in MS + 0.5 mg/l BAP + 25 mg/l tyrosine.

a) 6 ± 1.56 shoots / explant in D-154

b) 4 ± 1.15 shoots / explant in CVL-1 and

Fig.6a

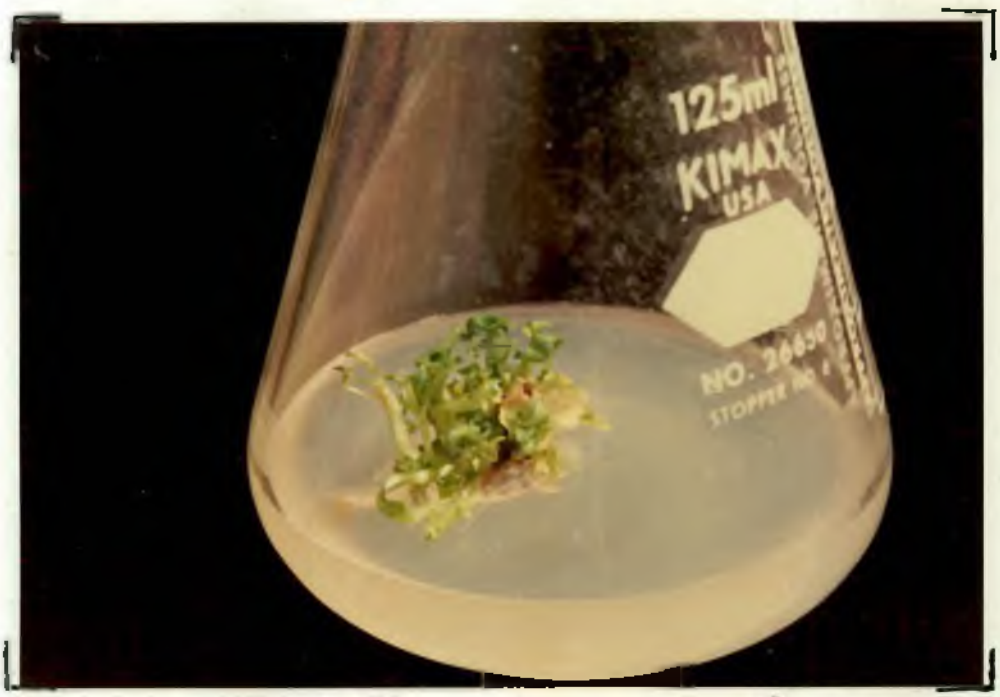

Fig. 6b

Fig.6 c) 3 ± 0.71 shoots / explant in CC-45

Fig.7 Regeneration of multiple shoots from the explant plumule with cotyledon in MS + 0.5 mg/l IBA + 0.5 mg/l BAP + 25 mg/l tyrosine.

a) 15 ± 2.49 shoots / explant in D-154

Fig. 6c

Fig.7a

Fig.7 b) 10 ± 1.05 shoots / explant in CVL-1.

c) 8 ± 0.94 shoots / explant in CC-45.

Fig. 7b

Fig.7c

Fig.7 d) 2 ± 0.71 shoots / explant in C.G. and

e) 2 ± 0.71 shoots / explant in 0-4.

Fig.7d

Fig. 7e

mg/l tyrosine was found best while MS + 1.0 mg/l IBA + 0.5 mg/l BAP + 25 mg/l tyrosine gave poorest response for shoot regeneration.

When BAP and tyrosine supplemented medium, and BAP, IBA and tyrosine supplemented medium compared (Table 11a and 11b), it was found that BAP, tyrosine supplemented medium was the best one, when explants used either from five days old seedlings or from 17-18 hours. old germinating seeds. In the Table 11c it is also shown that explants obtained from 17-18 hours old germinating seeds (plumule with cotyledon, plumule without cotyledon and cotyledon) showed higher frequency of shoot regeneration than explants (shoot tip) obtained from five days old seedlings.

G. Separation of multiple shoots and induction of roots: At the next stage of the experiment the multiple shoots were carefully separated aseptically from each other and transferred to the solidified agar medium containing different concentrations of auxins. For rooting MS medium having two strengths e.g. full MS and half MS were used. Half MS was found best for root induction and formation of strong root.

The following seven hormonal combinations, three in full MS and four in half MS were used for root induction,

1. MS + 0.1 mg/l IAA + 0.1 mg/l IBA;
2. MS + 0.2 mg/l IAA;
3. MS + 0.2 mg/l IBA;
4. Half MS + 0.1 mg/l IAA + 0.1 mg/l IBA;
5. Half MS + 0.2 mg/l IBA;
6. Half MS + 0.4 mg/l IBA;

Table 11a. Comparison of shoot regenerating capability between BAP + tyrosine supplemented MS medium and BAP + IBA + tyrosine supplemented MS medium when explants were inoculated from five days old seedlings.

Supplements	No.of explants inoculated	No.of explant producing shoot	% of explant producing shoot	No.of shoots/ explant
BAP + tyrosine	640	22	3.44	8.67
BAP + IBA + tyrosine	3601	24	0.67	6.125

Table 11 b. Comparison of shoot regenerating capacity between BAP + tyrosine supplemented MS medium and BAP + IBA + tyrosine supplemented MS medium used for culturing the explants obtained from 17-18 hours old germinating seeds.

Supplements	No of explants inoculated	No of explants producing shoot	% of explant producing shoot
BAP + tyrosine	17656	3527	19.98
BAP + IBA + tyrosine	7838	602	7.68

Table 11 c. Comparison of shoot regenerating frequency of explants obtained from 17-18 hours old germinating seeds and five days old seedlings.

Nature of explant	Collected from which type	No.of explants inoculated	No.of explants producing shoot	% of explant producing shoot
Plumule with	17-18 hours	4256 (BAP+tyrosine)	1860	37.28
cotyledon	old germinating	+1589 (BAP+IBA+tyr.)	+319	
	seeds	= 5845	=2179	
Plumule without	"	4256 (BAP+tyr)	1635	32.49
cotyledon		+1534 (BAP+IBA+tyr.)	+246	
		= 5790	=1881	
Cotyledon	"	1000 (BAP+tyr.)	32 +	3.03
		+1273 (BAP+IBA+tyr.)	37	
		= 2273	=69	
Shoot tip	Five days old seedlings	250 (BAP+tyr.)	22	3.10
		+1233 (BAP+IBA+tyr.)	+24	
		=1483	=46	

7. Half MS + 0.2 mg/l IAA + 0.2 mg/l IBA + 0.4 mg/l Kn.

The last one contained only kinetin.

In Table 12 it is shown that out of seven hormonal combinations half MS + 0.2 mg/l IBA was found to be the best for giving 50% rooting (Figs.8ai,aii,aiii) in D-154 followed by 44.67, 41.67 and 20% in CVL-1, CC-45 and O-4, respectively (Figs.8b-8d), while MS + 0.2 mg/l IBA was the second one produced 42.86, 42.31, 34.15 and 25% rooting in D-154, CVL-1, CC-45 and O-4 respectively. Poorest response was found in half MS + 0.2 mg/l IAA + 0.2 mg/l IBA + 0.4 mg/l Kn. In this combination when the shoots were transplanted for rooting, in some cases callus formation occurred from the cut portion of shoots and in three tubes 2-4 shoots were formed from such callus (Figs.9). Among the shoots of five materials given for root induction, shoots of D-154, CVL-1, CC-45 and O-4 became rooted in 37.60, 32.91, 32.03 and 15%, respectively but shoots of C.G. did not show root induction (Table 12a). It has also been observed that root induction took place within 2-3 weeks. It may be mentioned here that root formation was poor in callus derived shoots when they were transferred to rooting medium in off season i.e. in September and October.

H. Transfer of plantlets from test tube to pot and their establishment: After one and a half month, plantlets were carefully removed from the test tubes and after washing the roots in sterile distilled water, they were transferred to pots of 6" diameter and covered with polythene bags. It was observed that it made no difference whether the seedlings were transferred to

Fig.8 Regeneration of roots from shoots when MS medium was supplemented with 0.2 mg/l IBA.

aI), aII), and aIII) comparing the frequency of root formation in shoots of D-154 in different combinations, namely, $\frac{1}{2}$MS + 0.2 mg/l IBA, MS + 0.2 mg/l IBA and $\frac{1}{2}$MS + 0.1 mg/l IBA.

Fig.8 b) Regeneration of roots in CVL-1 shoot in $\frac{1}{2}$MS + 0.2 mg/l IBA

Table 12. Frequency of root formation in MS medium using different hormonal combinations.

Supplements	Name of the variety	No. of shoots inoculated	No.of shoots rooted	% of root formation
1	2	3	4	5
MS + 0.1 mg/l IAA +0.1 mg/l IBA	D-154	56	23	41.07
	CVL-1	40	13	32.50
	CC-45	42	14	33.33
	C.G.	4	-	-
	04	4	1	25.00
MS + 0.2 mg/l IAA	D-154	50	18	36.00
	CVL-1	40	13	32.50
	CC-45	40	14	35.00
	C.G.	3	-	-
	04	6	1	16.67
MS + 0.2 mg/ +IBA	D-154	56	24	42.86
	CVL-1	52	22	42.31
	CC-45	41	14	34.15
	C.G.	4	-	-
	04	8	2	25.00
½MS+0.1 mg/l IAA +0.1 mg/l IBA	D-154	52	22	42.31
	CVL-1	42	14	33.33
	CC-45	38	13	34.21
	C.G.	2	-	-
	04	6	1	16.67

1	2	3	4	5
½MS+0.2 mg/l IBA	D-154	64	32	50.00
	CVL-1	52	23	44.23
	CC-45	48	20	41.67
	C.G.	1	-	-
	04	5	1	20.00
½MS+0.4 Mg/l IBA	D-154	50	14	28.00
	CVL-1	42	11	26.19
	CC-45	40	10	25.00
	C.G.	-	-	-
	04	7	-	-
½MS+0.2 mg/l IAA	D-154	47	8	17.02
+0.2 Mg/l IBA	CVL-1	45	7	15.55
+0.4 mg/l Kn	CC-45	32	5	15.62
	C.G.	-	-	-
	04	4	-	-

Table 12a. Varietal difference in root formation in MS using different hormonal combinations.

Materials	No.of shoots inoculated	No.of shoots rooted	% of root formation
D-154	375	141	37.60
CVL-1	313	103	32.91
CC-45	281	90	32.03
C.G.	14	-	-
04	40	6	15.00

Fig. 8a1

Fig. 8a2

Fig.8a III

Fig.8b

Fig.8 c) Root regereration in CC-45 and

d) 0-4 shoots in $\frac{1}{2}$MS + 0.2 mg/l IBA

Fig. 8c

Fig. 8d

Fig.9 Shoots regenerated from the callus produced from the cut portion of the shoot transplanted for rooting in ½MS + 0.2 mg/l IAA + 0.2 mg/l IBA + 0.4 mg/l Kn (three tubes).

9a

9b

9c

Figs.9

sterile or ordinary soil prepared with garden soil, organic manure and sand in the proportion of 2:1:1. On the 6/7th day, polythene bags were perforated to allow good ventilation. Polythene bag was finally removed on the 15th day. After a month they were transferred to a bigger pot where they grew to maturity.

In total one hundred and nine (109) plantlets were transferred to the soil to establish them as mature plants. Results of these observations were given in Table 13. It was observed that out of 109 plantlets, seventeen plantlets established themselves (Figs.10a-10d) of which sixteen plants were from D-154, and one from CVL-1, Among five materials used, 57 plants of D-154, 28 plants of CVL-1, 22 of CC-45 and two of O-4 were transferred of which 16 of D-154 and one of CVL-1 were established, respectively. No shoot of CC-45 and O-4 was established (Table 13).

Established plants belonged to the species of *C. capsularis*. Among these, thirteen plantlets of D-154 were established in the 1st year of the experiment done, the other three of D-154 and one of CVL-1 were established in the next year. The plantlets were serially numbered with the prefix TCL in 1,2,3 and so on at the time of transfer to the soil.

The characteristic features of the TCL plants are given below:

a. Observation of different morphological characters:

1. Different morphological characters (Fig.11) namely, (i) length and breadth of leaf and their ratio; (ii) petiole length;

Table 13. Frequency of plantlet establishment in different varieties of *corchorus species*

Nature of explants	Material	No.of plantlets transferred into the soil	No.of plantlets established
Plumule with cotyledon	D-154	33	12(36.36%)
	CVL-1	13	1(7.69%)
	CC-45	12	-
	C.G.	-	-
	04	2	-
Plumule without cotyledon	D-154	19	3(15.79%)
	CVL-1	14	-
	CC-45	9	-
	C.G.	-	-
	04	-	-
Shoot tip	D-154	5	1(20%)
	CVL-1	1	-
	CC-45	1	-
	C.G.	-	-
	04	-	-
		109	17

Fig.10 Established plantlets after transferring to the soil

a) D-154 plantlets of different ages.

b) D-154 plantlets (15 days after transferring to the soil).

Fig. 10a

Fig. 10b

Fig.10 c) CVL-1 plantlets (24 days after transferring to the soil).

d) D-154 plantlets (One month and ten days after transferring to the soil).

Fig.10c

Fig.10d

(iii) serrature length; (iv) stipule length; (v) plant height and (vi) base diameter; 2. Pollen viability; 3. Fruit and seed productivity and 4. Germination percentage of seeds were observed in different field of experiment. Results of different morphological characters were given in Table 14a, b and c.

Observation of Ro TCL plants: From the Table 14a the results of Ro TCL plants were observed.

i. Of the seventeen tissue culture derived individuals, five named, TCL-3,5,6,11 and 15 were very weak and three named 5,6 and 15 did not flower at all.

ii. Length/breadth ratio of R_o TCL plants of D-154 was 2.06:0.87 in TCL-3 which was the minimum and 3.57 : 1.21 in TCL-2 which was the maximum while the length breadth ratio of control D-154 was found to be 2:1. Minimum petiole length 1.33±0.24 was observed in TCL-5 and maximum petiole length 5.68±0.55 in TCL-21. When length of serrature and stipule observed, it was found that maximum serrature length (1.15±0.11 cm) and stipule length (1.21±0.19 cm) observed in TCL-11 and TCL-2 and minimum serrature length 0.86±0.21 cm and stipule length 0.97±0.18 cm observed in TCL-6 and TCL-3, respectively. Highest plant height (319.9 cm) was found in TCL-1 while the smallest one, 316 cm was in TCL-6. Maximum base diameter, 1.9 cm, was observed in TCL-1 and 12 and minimum 1.4 cm in TCL-5,6 and 15. When different morphological characters of TCL-20 i.e. *C. capsularis* var. CVL-1 were experimented it was found that L/B ratio, petiole, serrature, stipule length, plant height and plant base diameter 2.81:11, 5.48±0.82, 0.92±0.15, 0.94±0.19, 316.9 and 1.8 cm, respectively.

Observation of R_1 TCL plants:

In R_1 generation twelve TCL plants of D-154 and one of CVL-1 were adapted i.e. out of seventeen R_o TCL plants thirteen were survived to the next generation. From the Table 14b it was observed that among twelve TCL plants of D-154 highest length/breadth ratio 3.23:1.02 cm in TCL-9 and the lowest L/B ratio 2.14:1.25 in TCL-1. Minimum petiole, serrature and stipule length 2.87±0.05, 0.88±0.24, 0.99±0.19 cm in TCL-9, TCL-13 and TCL-7 and maximum 7.05±0.89, 1.17±0.23, 1.17±0.19 in TCL-10, TCL-10 and TCL-4, respectively. Highest plant height 320.33±2.67 cm was found in TCL-24 while the lowest one 313.96±3.12 cm in TCL-4. When plant base diameter observed, it was found that maximum plant base diameter 1.94±0.25 cm in TCL-24 and minimum 1.21±0.29 cm in TCL-4. In case of observing different morphological characters of TCL-20 i.e. CVL-1 it was found that L/B ratio, petiole, serrature, stipule length, plant height and base diameter were 2.7:1.06 cm, 5.22±0.88, 0.9±0.25, 0.93± 0.16, 317.67±3.89 and 1.73±0.33, respectively.

Observation of R_2 TCL plants:

Eight TCL plants of D-154 were raised to the R_2 generation. Different morphological characters of these lines were observed and results of these observations were given in Table 14c. From the Table it was found that maximum L/B ratio 3.01:1.2 cm in TCL-1 and minimum 2.29:1.1 cm in TCL-6. When petiole, serrature and stipule length were measured, the maximum length 7.06±0.35, 1.26±0.16, 1.33±0.11 cm in TCL-2, TCL-9, TCL-2 and the minimum

length 5.1±0.89, 0.82±0.24, 0.96±0.22 cm in TCL-16, TCL-2 and TCL-4 observed, respectively. Highest plant height 316.77±3.9 cm was observed in TCL-11 and the lowest 313.59±2.43 cm in TCL-4. Maximum base diameter 1.67±0.34 cm was found in TCL-11 and minimum 1.15±0.36 cm in TCL-4.

b. Observation of pollen stainability:

From the Table 15 it was observed that pollen stainability ranged from 84.40-100% compared to 96-99% in *C. capsularis*. The highest pollen stainability (100%) was observed in TCL-2, D-154 variety of *C. capsularis* while the lowest pollen stainability (84.40%) found in TCL-12.

C. Observation of germination percentage of R_2 (Table 16a) and R_1 (Table 16b) TCL plants seed grown for the production of R_1 and R_2 generations: From the Table 16a it was found that highest germination percentage 85% in TCL-1 while the lowest 5% in TCL-10.TCL-20,CVL-1 variety of *C. capsularis* showed 64.54% germination. From the Table 16b it was observed that the highest germination of seeds (76.80%) in TCL-4 and the lowest, 34.31% was found in TCL-9. While observing the germination percentage of R_0 and R_1 seeds, it was found that the R_0 seeds have possessed more germinability than R_1 seeds.

d. Observation of fruit and seed productivity in different generations:

Fruit (Fig.12a) and seed productivity of thirteen TCL-plants were observed in three different generations(e.g. R_0,R_1,R_2 etc). From the Table 17a it was observed that the highest number of

Table 14a. Study of morphological characteristics of tissue culture derived (designated by TCL) R_0 plants (in cm)

Name of the TCL plants	L/B ratio (in cm) of leaf.	Petiole length	Serrature length	Stipule length	Plant height	Plant base diameter
TCL-1(D-154)	2.71:0.9	1.69±0.35	0.94±0.19	1.04±0.19	319.9	1.9
TCL-2(")	3.57:1.21	1.54±0.35	0.93±0.25	1.21±0.19	319.8	1.8
TCL-3(")	2.06:0.87	2.4±0.36	0.88±0.15	0.97±0.18	316.9	1.8
TCL-4(")	2.63:0.90	1.54±0.33	0.88±0.15	1.01±0.19	319.2	1.7
TCL-5(")	2.52:1.07	1.33±0.24	0.89±0.17	1.06±0.17	317.9	1.4
TCL-6(")	2.46:1.08	1.33±0.26	0.86±0.21	1.08±0.15	316.0	1.4
TCL-7(")	2.49:1.05	1.57±0.19	1.02±0.14	1.04±0.14	318.7	1.6
TCL-9(")	2.34:0.96	1.93±0.32	1.12±0.12	1.11±0.12	319.5	1.8
TCL-10(")	2.55:1.03	2.04±0.34	0.97±0.15	1.04±0.13	319.7	1.8
TCL-11(")	2.62:1.26	2.75±0.32	1.15±0.11	1.07±0.15	317.9	1.6
TCL-13(")	2.74:1.15	2.4±0.56	1±0.17	1.02±0.17	318.0	1.5
TCL-15(")	2.66:1.14	1.43±0.29	1.04±0.19	1.05±0.13	316.9	1.4
TCL-16(")	2.65:0.93	1.73±0.25	1.04±0.14	1.01±0.1	319.8	1.8
TCL-18(")	2.81:1.12	5.05±1.55	1.01±0.14	1.02±0.08	319.3	1.9
TCL-21(")	2.81:1.19	5.68±0.55	0.99±0.55	1.06±0.15	318.9	1.6
TCL-24(")	2.85:1.23	5.52±0.47	1.1±0.18	1.08±0.17	318.7	1.7
TCL-20(BVL-1)	2.81:1.1	5.48±0.82	0.92±0.15	0.94±0.19	316.9	1.8

Table 14b. Study of morphological characteristics of tissue culture derived R_1 plants (in cm).

Name of TCL plants	L/B ratio of leaf	Petiole length	Serrature length	Stipule length	Plant height	Plant base diameter
TCL-1(D-154)	12.14:1.25	6.03±0.75	0.89±0.21	1.08±0.12	316.73±1.28	1.55±0.4
TCL-2(")	2.68:1	3.73±1.23	0.93±0.27	1.2±0.18	314.97±3.39	1.6±0.42
TCL-4(")	2.19:0.86	4.04±0.76	1.06±0.3	1.17±0.19	313.96±3.12	1.21±0.29
TCL-7(")	3.02:1.31	3.49±0.95	0.91±0.14	0.99±0.19	315.47±2.93	1.45±0.39
TCL-9(")	3.23:1.02	2.87±0.05	1.14±0.22	1.07±0.14	315.17±3.11	1.48±0.31
TCL-10(")	2.47:1.07	7.05±0.89	1.17±0.23	1.13±0.13	314.88±3.72	1.64±0.32
TCL-13(")	2.52:1.29	5.28±1.06	0.88±0.24	1.0±0.12	314.94±3.61	1.59±0.37
TCL-11(")	2.62:0.88	3.93±1.17	1.12±0.18	1.11±0.11	315.02±3.64	1.53±0.32
TCL-16(")	2.65:1.15	5.01±1.22	1.04±0.18	1.1±0.09	314.56±3.49	1.44±0.33
TCL-12(")	2.5:1.04	6.44±1.46	1.08±0.23	1.09±0.16	317.62±3.29	1.68±0.3
TCL-21(")	3.1:1.25	6.52±1.34	0.99±0.24	1.08±0.15	316.2±3.95	1.5±0.51
TCL-24(")	2.92:1.15	6.38±0.92	1.09±0.17	1.08±0.19	320.33±2.67	1.94±0.25
TCL-20(CVL-1)	2.7:1.06	5.22±0.88	0.9±0.15	0.93±0.16	317.67±3.89	1.73±0.33

Table 14c. Study of morphological characteristics of tissue culture derived R_2 plants (in cm).

Name of TCL plants	L/B ratio of leaf	Petiole length	Serrature length	Stipule length	Plant height	Plant base diameter
TCL-1(D-154)	3.01:1.2	5.8±0.26	0.95±0.24	1.12±0.12	316.23±3.6	1.53±0.42
TCL-2(")	2.93:1.18	6.06±0.35	0.82±0.24	1.33±0.11	314.3±3.14	1.57±0.32
TCL-4(")	2.67:0.99	4.85±0.73	0.88±0.24	0.96±0.22	313.59±2.43	1.15±0.36
TCL-7(")	2.29:1.1	5.25±0.64	1.04±0.18	1.03±0.13	314.66±3.51	1.33±0.38
TCL-9(")	2.54:0.99	5.74±0.42	1.26±0.16	1.17±0.08	315.33±4.82	1.64±0.51
TCL-11(")	2.97:1.05	5.22±0.52	1.22±0.25	1.11±0.11	316.77±0.3.9	1.67±0.34
TCL-13(")	2.83:1.05	5.24±1.06	0.98±0.23	1.01±0.14	314.76±4.48	1.38±0.37
TCL-16(")	2.9:1.12	5.1±0.89	1.19±0.23	1.06±0.08	315.75±4.4	1.59±0.37

Table 15. Study of pollen stainability of different tissue culture derived plants.

Variety of *C. capsularis*	Total no of pollen	No of stainable pollen	% of stainability
TCL-1(D-154)	300	282	94.00
TCL-2(")	300	300	100.00
TCL-4(")	500	475	95.00
TCL-7(")	400	374	93.50
TCL-9(")	500	494	98.80
TCL-11(")	200	187	93.50
TCL-13(")	300	270	90.00
TCL-16(")	200	185	92.50
TCL-12(")	500	422	84.40
TCL-21(")	500	488	97.60
TCL-22(")	500	483	96.60
TCL-20(CVL-1)	500	487	97.40

fruits (72 fruits/plant) found in TCL-1 and the lowest (2 fruits/plant) was found in TCL-13 in case of R_0 generation. While observing R_1 generation, highest number of fruits (57.5±10.61 fruits/plants) were obtained from TCL-13 and the lowest (21.9±15.39 fruits/plant) in TCL-16. TCL-20 i.e. CVL-1 produced 39.2±7.67 fruits/plant (Table 17a) in Ro generation and 59.3±27.75 fruits/plant (Table 17b) in R_1 generation. From the Table 17c it was found that TCL-4 produced highest number of fruits e.g. 66.5±25.23 fruits/plant and the lowest (49.7±23.24 fruits/plant) in TCL-11 when R_2 generation observed. Highest number of seeds, 40±1.41 seeds/fruit, were observed in TCL-13 and the lowest, 24.6±4.03 seeds/fruit, in TCL-10 in case of R_0 generation (Table 17a). In R_1 generation (Table 17b) TCL-4 was found to bear highest number of seeds (38.6±7.6 seeds/fruit) and the lowest, 29±2.47 seeds/fruit in TCL-1. While observing, Table 17c showed that the highest number of seeds, 39.4±6.04 seeds/fruit were produced in TCL-4 and the poorest one being TCL-4 (30.5±3.41 seeds/fruit). It was observed that in R_1 and R_2 generation TCL-4 produced highest number of seeds and TCL-1 produced lowest number of seeds. But in compared to control D-154, tissue culture derived plants produced lower number of seeds than control. In CVL-1 (TCL-20), the number of seeds were 39.2±7.67 seeds/fruit and 37.3±8.19 seeds/fruit in R_0 and R_1 generation, respectively (Table 17a,b) i.e.nearer to control CVL-1 (37.3±8.19 seeds/fruit) (Table 18). The colour, shape, size and weight of seeds varied from each other and also from their control (Figs.14a-14m).

Table 16a. Percentage of seed germination of tissue culture derived Ro plants.

Name of the TCL plants	No.of seeds sown	No.of seeds germinated	Germination%
TCL-1(D-154)	120	102	85.00
TCL-2 "	200	99	49.5
TCL-4 "	100	20	20.00
TCL-7 "	128	100	78.12
TCL-9 "	100	15	15.00
TCL-10 "	20	1	5.00
TCL-11 "	120	66	55.00
TCL-12 "	80	8	10.00
TCL-13 "	129	65	50.38
TCL-16 "	228	176	77.19
TCL-21 "	88	70	79.54
TCL-24 "	127	100	78.74
TCL-20(CVL-1)	110	71	64.54

Table 16b. Percentage of seed germination of tissue culture derived R_1 plants

Name of the TCL plants	No.of seeds sown	No.of seeds germinated	Germination%
TCL-1(D-154)	115	82	71.30
TCL-2 "	92	60	65.22
TCL-4 "	125	96	76.80
TCL-7 "	128	79	61.72
TCL-9 "	192	66	34.31
TCL-11 "	100	71	71.00
TCL-13 "	150	109	72.67
TCL-16 "	138	92	66.67

Fig.11 Showing stem, leaves, branching habit and flowered branch.

Fig.12 Showing fruit productivity in clusters
a) 2-3 fruits/cluster

Fig.11

Fig.12a

In respect of no. of fruits/cluster it was observed that in R_0 generation TCL-4,7,11,12 and 21 were found to bear four fruits/bunch in 5,3,1,6 and 2 plants, respectively (Table 17a). In R_1 generation four fruits/ bunch were obtained in 2,3,4,1,26, and 4 plants of TCL-4,7,11,16,12 and 21 respectively (Table 17b). In R_2 generation 90,38, 16 and 22 plants of TCL-4,7,11 and 16 were found to bear four - five fruits/bunch, respectively (Table 17c) (Figs. 12b,12c).

It was also observed from the table 17a, b,c,that TCL-4,16 and 12 produced smooth surfaced and brownish coloured fruits in one, one and 12 plants respectively in case of R_1 generation, and TCL-4 and 16 showed smooth surfaced fruits in 33 and 23 plants of R_2 generation.(Figs. 13a, 13b). There was no smooth surfaced fruits in R_0 generation.

Therefore, a good deal of variation has been observed among the somaclones in respect of different morphological and genetical characters.

Table 17a. Fruit and seed productivity in the Ro progeny of TCL plants.

Name of the TCL plants	Total no of fruits/plant	No.of bunch bearing four/ more fruits	No.of seeds/ fruit
TCL-1 (D-154)	72	-	28.7±2.06
TCL-2 "	68	-	30.6±4.79
TCL-4 "	40	5	39.4±6.33
TCL-7 "	20	3	33.7±5.58
TCL-9 "	20	-	36.9±5.0
TCL-10 "	28	-	24.6±4.03
TCL-11 "	6	1	32.33±5.82
TCL-13 "	2	-	40 ± 1.41
TCL-16 "	7	-	31.57±2.99
TCL-12 "	58	6	37.5±3.89
TCL-21 "	20	2	29.3±3.37
TCL-24 "	28	-	37.9±2.68
TCL-20(CVL-1)	25	-	39.2±7.67

Table 17b. Fruit and seed productivity in the R_1 progeny of TCL plants.

Name of the tissue culture derived plant	Total no of plants	No.of plants bearing four or more fruits/ cluster	No.of plants bearing smooth surfaced fruits	No.of fruits/ plant	No of seeds/ fruit
TCL-1(D-154)	17	-	-	28.4±15.87	29±2.49
TCL-2 "	11	-	-	23.5±14.55	30.2±3.94
TCL-4 "	4	2	1	35.5±11.56	38.6±7.6
TCL-7 "	17	3	-	24.8±14.57	36±4.69
TCL-9 "	3	-	-	50.67±9.07	36.5±5.1
TCL-10 "	1	-	-	-	-
TCL-11 "	11	4	-	24.9±15.54	30.7±4.55
TCL-13 "	2	-	-	57.5±10.61	36.4±4.4
TCL-16 "	10	1	1	21.9±15.39	21.9±2.92
TCL-12 "	92	26	12	49.8±21.24	38±3.02
TCL-21 "	16	4	-	48.4±25.33	30.3±3.71
TCL-24 "	20	-	-	46.3±22.37	37.7±2.91
TCL-20(CVL-1)	123	-	-	59.3±27.75	37.3±8.19

Table 17c. Fruit and seed productivity in the R_2 progeny of TCL plants.

Name of the TCL plants	Total No. of plants	No.of plants bearing four or more fruits/ cluster	No.of plants bearing smooth surfaced fruits	No.of fruits/ Plant	No of seeds/ fruit	Total weitht of 1000 seeds (ingm).
TCL-1 "	65	-	-	57±26.85	30.5±3.41	3.99
TCL-2 "	20	-	-	59.8±26.15	31.5±6.43	3.85
TCL-4 "	188	90	33	60.5±25.23	39.4±6.04	3.56
TCL-7 "	190	38	-	52.7±25.21	34.4±5.06	3.65
TCL-9 "	30	-	-	52.8±26.1	38.6±5.54	3.39
TCL-11 "	35	16	-	49.7±23.24	36.5±4.5	3.10
TCL-13 "	116	-	-	52.2±4.16	37±4.16	3.7
TCL-16 " 157	22	23	57.9±20.26	32.8±2.39	3.52	

N.B. Table 18. Furit and seed productivity of the parental (control) type of the TCL plants.

C. Capsularis var. D-154	125	-	-	66±22.22	42±12.46	3.1
C. Capsularis Var. CVL-1	123	-	-	63.4±18.84	37.3±8.19	3.0

Fig.12 b) 4 fruits/cluster and

c) 5 fruits/cluster.

Fig.12b

Fig.12c

Fig.13 a) Showing smooth surfaced fruit

b) Showing smooth surfaced and brownish coloured fruits.

Fig. 13a

Fig. 13b

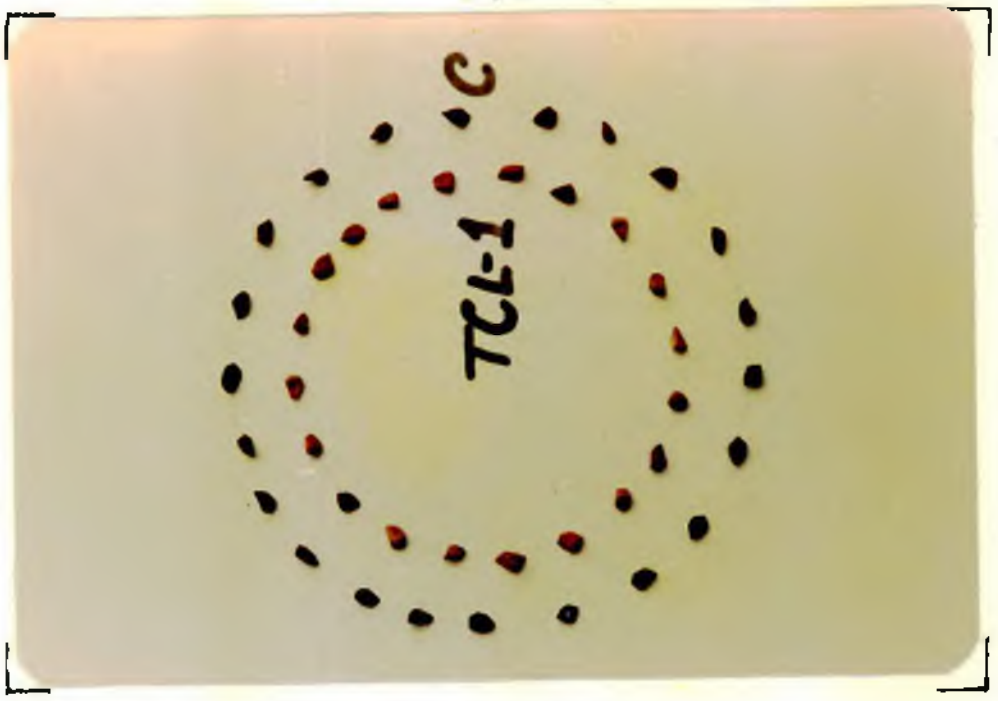

Fig. 14a

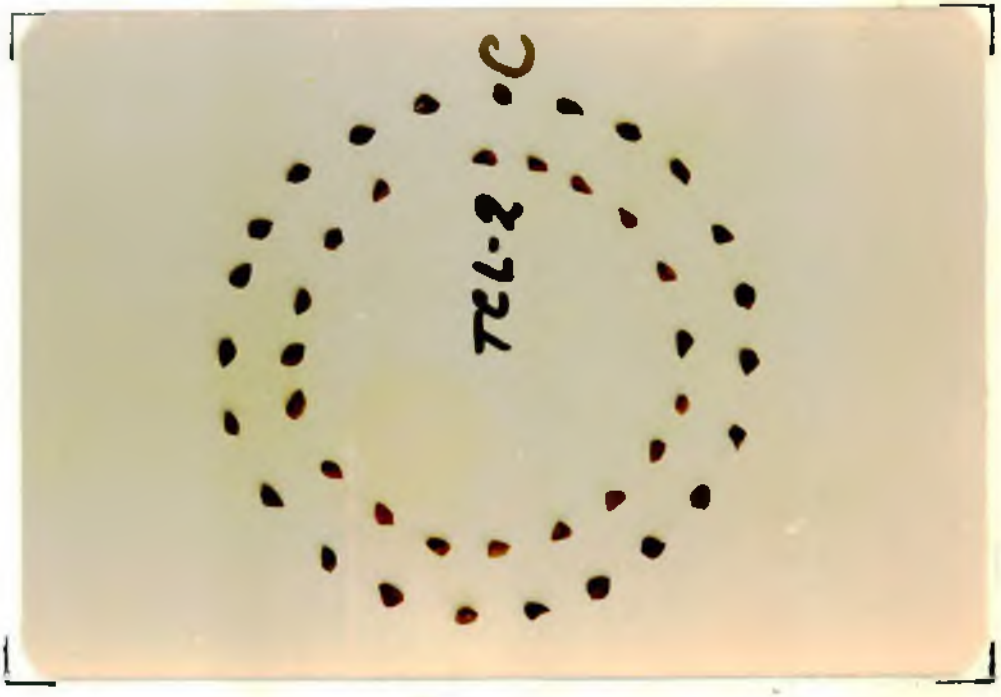

Fig. 14b

Fig.14 c) TCL - 4 vs control (D-154)

d) TCL - 7 vs control (D-154)

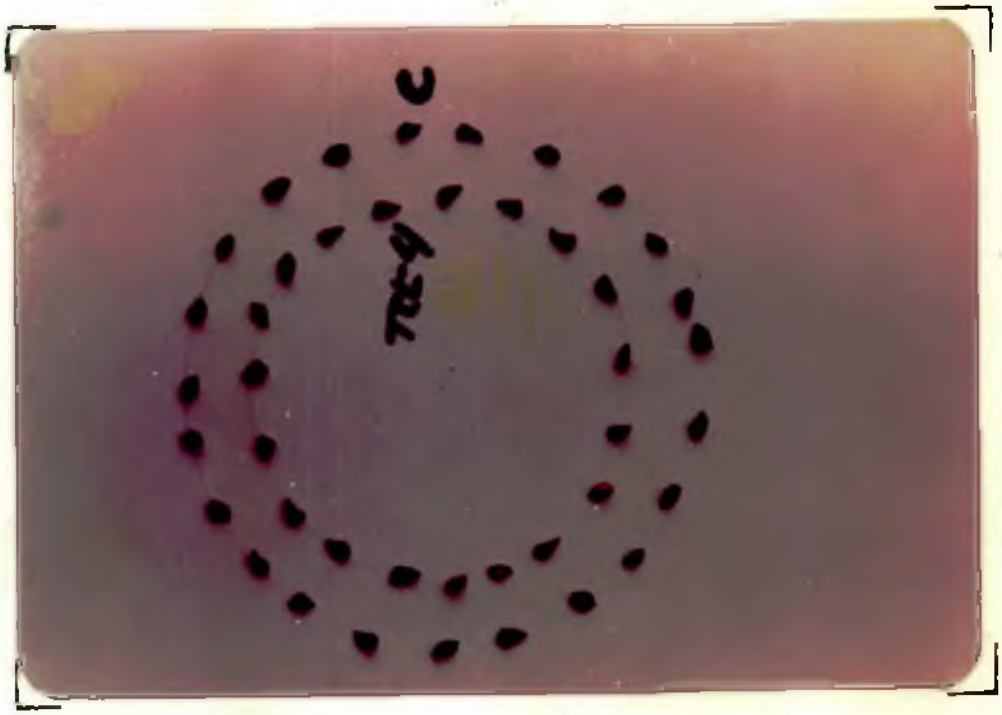

Fig.14c

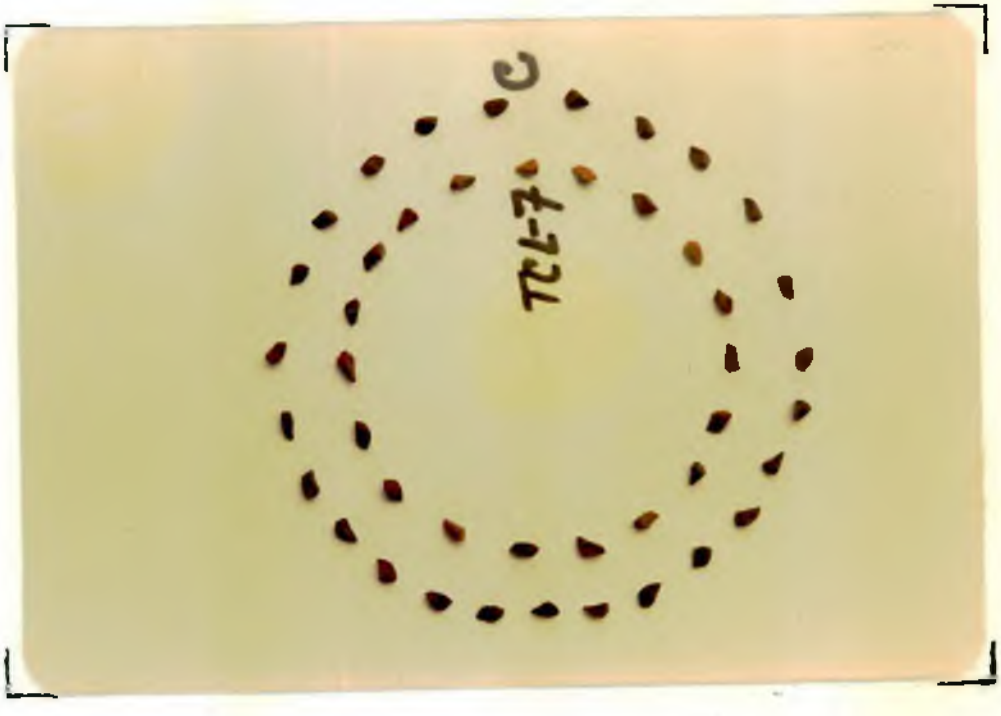

Fig.14d

Fig.14 e) TCL - 9 vs control (D-154)

f) TCL -10 vs control (D-154)

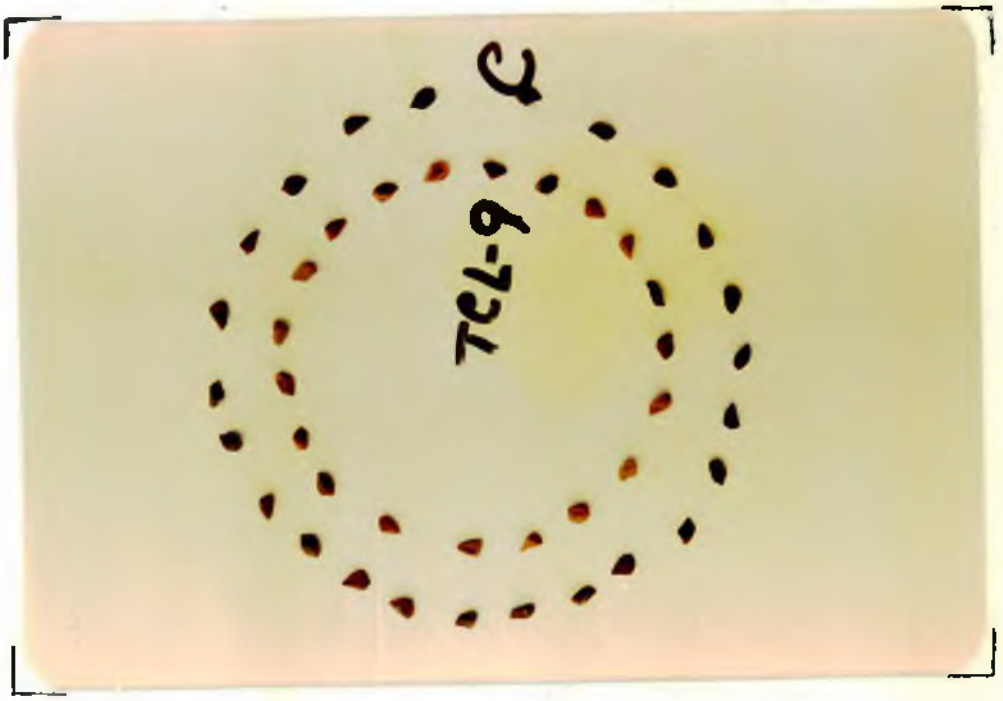

Fig. 14e

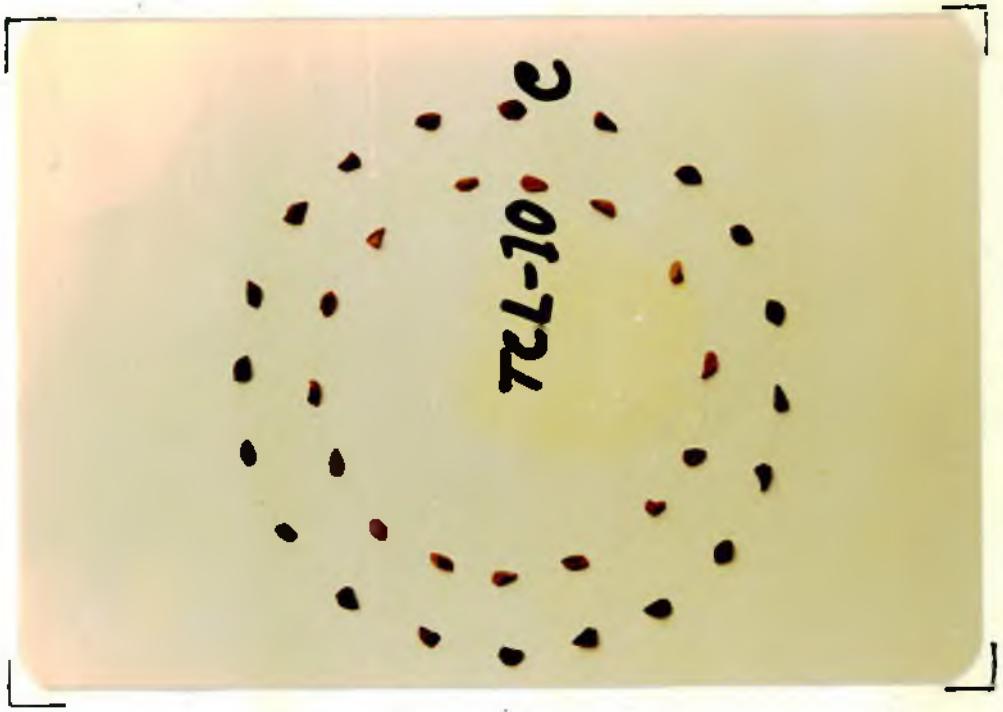

Fig .14f

Fig.14 g) TCL -11 vs control (D-154)

h) TCL - 12 vs control (D-154)

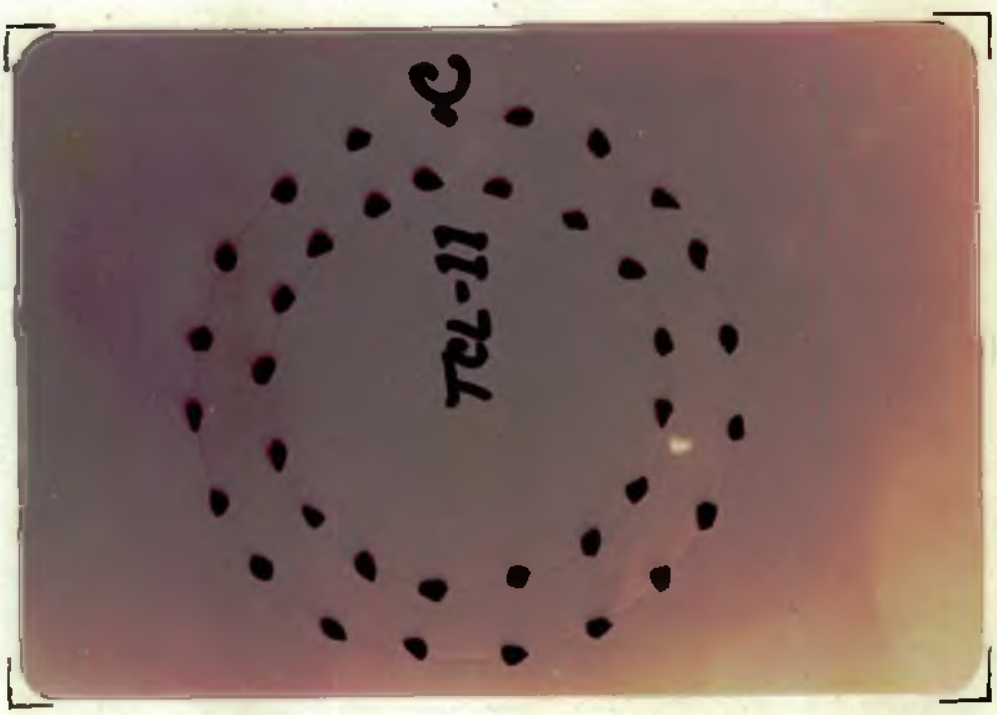

Fig. 14g

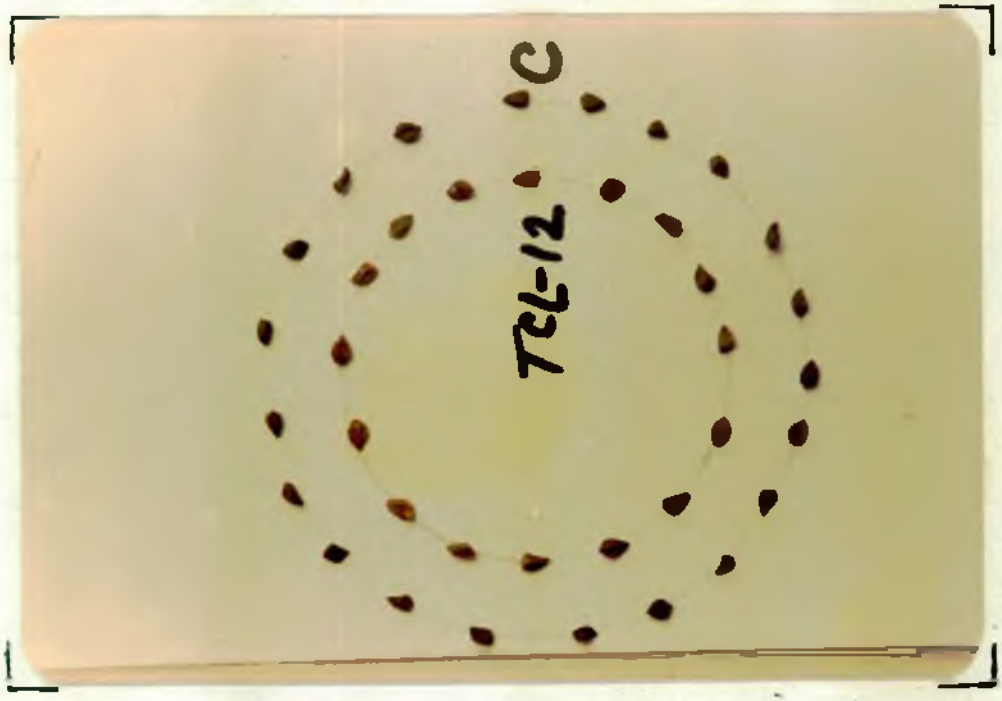

Fig.14h

Fig.14 i) TCL - 13 vs control (D-154)

j) TCL - 16 vs control (D-154)

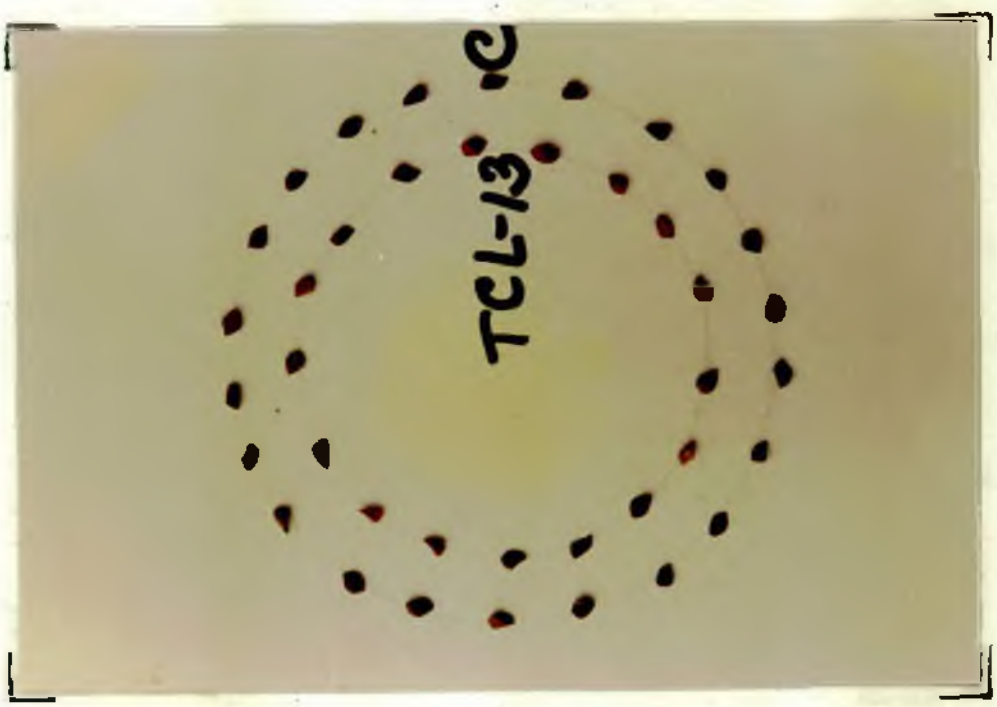

Fig. 14i

Fig. 14j

Fig.14 k) TCL -20 vs control (CVL-1)

l) TCL - 21 vs control (D-154)

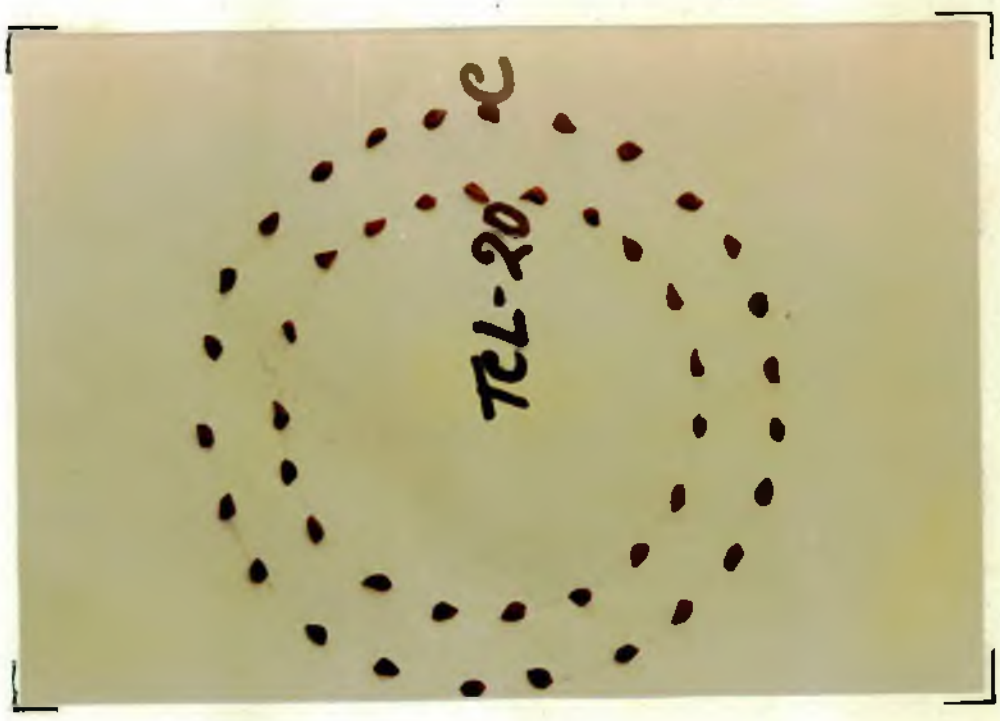

Fig.14k

Fig 14l

Fig. 14 m) TCL - 24 vs control (D-154)

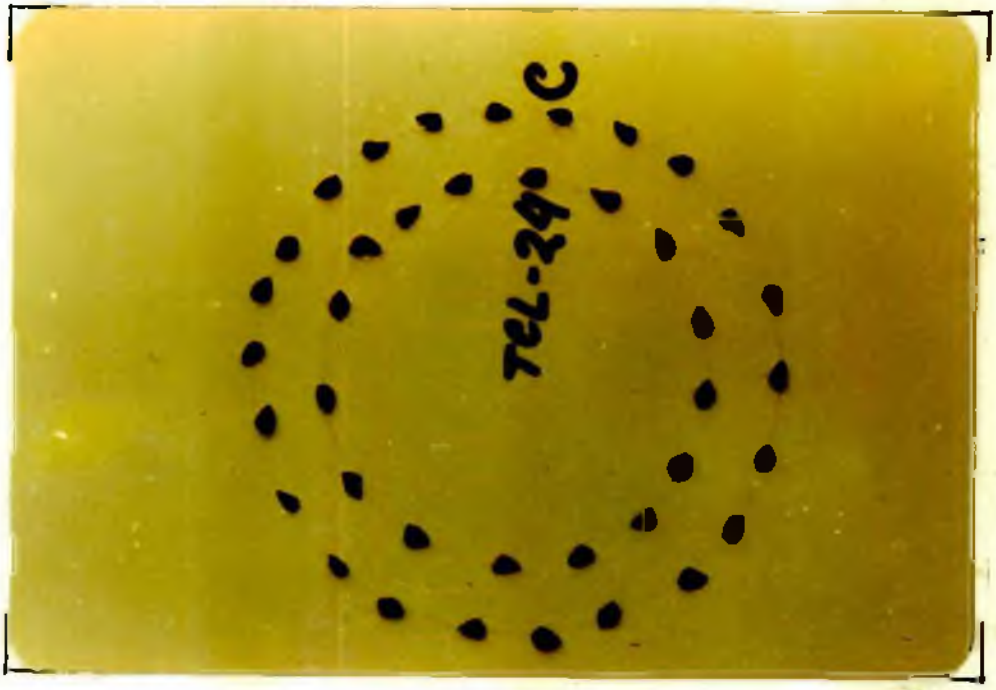

Fig. 14m

DISCUSSION

This work of present investigation was undertaken to obtain differentiation through callus derived from shoot tips, leaf, petiole, epicotyl, hypocotyl, anthers and different segments of germinating seeds.

In the present investigation BAP, IAA and NAA were used in three different combinations and concentrations in MMS (Murashige and Skoog, 1981) to regenerate plantlets from leaf explants. But there was no shoot regeneration except callusing (60%). On the other hand, Islam (1981) reported some success of shoot regeneration from leaf derived callus at a very low frequency (25%) in *C. olitorius* when MS (Murashige and Skoog, 1962) medium was supplemented with 0.2 mg/l IAA and 2 mg/l zeatin. Since the frequency of regeneration was very low this was not tried.

By using equal proportion of auxin (IBA) and cytokinin (Kn), shoot regeneration was observed in the present research work from the shoot tip explants in MS and SH (Schenk and Hildebrandt, 1972) media. Sen *et al.* (1981) observed that a combination of IBA and Kn stimulated the growth of shoot explants without callus formation in some *Corchorus* species whereas NAA and Kn together induced callus only. According to them when these callii were transferred to IBA and Kn supplemented MS medium, a large number of shoots regenerated from shoot tip explants. Islam *et al.*(1982) reported single and multiple shoot formation in the shoot tip derived callus of *C. siliquosus*, D-154 and O/D in MS, MMS and SH media only when more or less equal proportion of auxin

and cytokinin (2mg/l Kn + 2mg/l IBA and 0.4 mg/l Kn + 0.4 mg/l IBA) were used. Rahman (1984) on the other hand reported success with the same species of *Corchorus* only when he tried MS medium. In other words he failed to obtain any regeneration of shoots in SH and MMS. In the present work, MMS was tried but the same proportion of constituents (0.5 mg/l IBA + 1.0 mg/l Kn) was not tried. In here the best material in this respect was the variety D-154 of *C. capsularis*. Islam *et al.* (1982) reported that the best material in respect of shoot regeneration was *C. siliquosus* while according to Rahman (l.c.) wild race of *C. capsularis* was the best material. No investigation was undertaken in the present study to determine which of the two material gives best regeneration frequency. Therefore no comment can be made as to the regenerating ability of the above two materials.

In the present investigation 10 $\pm$ 2 shoots/explant was obtained in 16% of the explants of D-154 as well as 9 $\pm$ 1.6 shoots/explant in CVL-1 and 7 $\pm$ 2 shoots/explant in CC-45 when MS medium was supplemented with 0.5 mg/l BAP + 25 mg/l tyrosine (Table 5). Rahman (1984) reported multiple shoot regeneration from shoot tip explant in MS + 0.5 mg/l BAP + 25 mg/l tyrosine and 0.1 mg/l IBA + 50 mg/l gln. He observed as many as 6 shoots/explant in *C. capsularis* var D-154 which was the poorest result in respect of present investigation.

In the present work (9 $\pm$ 1.58, 8 $\pm$ 0.82 and 5 $\pm$ 1 shoots/explant, Table 6) regenerants were obtained in D-154, CVL-1 and CC-45 from shoot tip explants when MS medium was supplemented

with 0.5 mg/l IBA, 0.5 mg/l BAP and 25 mg/l tyrosine, the above result, namely, inclusion of IBA (0.5 mg/l) in the medium as a supplement for better regeneration was not reported earlier.

The present work was done with segments of the root decapitated germinating seeds. These germinating seeds were 17-18 hours. old. Among different explants plumule with/without cotyledon, and cotyledon showed shoot regeneration in BAP (0.5 mg/l) and tyrosine (25 mg/l) supplemented MS medium. Plumule with cotyledon gave highest frequency of shoot regeneration in MS + 0.5 mg/l BAP + 25 mg/l tyrosine i.e. 35 $\pm$ 4.92, 31 $\pm$ 2.94, 28 $\pm$ 3.26, and 15 $\pm$2.9 shoots/explant in D-154, CVL-1, CC-45 and O-4, respectively. Unlike the present results Rahman *et al.* (1985) obtained only 6 shoots/explant in D-154 from shoot tip explants excised from 10-day old seedlings, as against seven times more i.e. 35 regenerants in the germinating seed derived callus.

Rahman (1984) reported multiple shoot regeneration through callus formation. In the present work, multiple shoot regeneration was obtained either througth the intervention of callus or directly from the explant, the latter developing into green swollen structure.

Among the regenerating capability of different segments it was found that the number of shoots was more when cotyledons remained attached to the plumule. Isolated cotyledons were also found to produce shoots but in a highly reduced frequency.

Rahman (1984) reported successful root initiation in 1.0 mg/l IAA supplemented White's medium. In the present work best rooting was found in the medium containing 0.2 mg/l IBA in half

MS strength. In the latter rooting frequency was 50, 42.86, 42.31, 34.15 and 25% in D-154, CVL-1, CC-45 and O-4,respectively.

It may be mentioned here that root formation was poor in callus derived shoots when they were transferred to rooting medium in off season - i.e. in September and October which was not reported by the earlier worker.

Rahman (1984), in his work reported that the plants were gradually adapted by transferring them into liquid medium on cotton pad and later to Hoagland's solution soaked vermiculite. In this way the plantlets were successfully transferred into the soil. In the present work, after following root development, plantlets were carefully removed from the test tubes and after washing the roots in sterile distilled water, they were transferred to pots of 6" diameter and covered with polythene bags. It was observed that it made no difference whether the seedlings were transferred to sterile soil or to the one prepared with garden soil, organic manure and sand in the proportion of 2:1:1. On the 6/7th day, polythene bags were perforated to allow good ventilation. Polythene bag was removed every day gradually increasing the exposure time till at last the bag was finally removed on the 15th day. After a month they were transferred to a bigger pot where they grew to maturity.

Out of one hundred and nine plantlets transferred only , seventeen were established, sixteen belonging to D-154 and the remaining one being CVL-1 plant. Established plants varied from their control type in different character, namely morphological

(e.g. L/B ratio of leaf, petiole, serrature and stipule length of leaf;), pollen stainability; germination percentage; and fruit and seed productivity and on the basis of their variation, they were referred as somaclonal variants as reported by different workers in different crops e.g. tomato (Evans *et al.*, 1984), wheat (Larkin *et al.*, 1984) and rice (Schaeffer *et al*, 1984).

Somaclonal Variants:

Eight lines of somaclonal variants representing R_2 generation of tissue culture derived plants of D-154 of *Corchorus capsularis* were grown in the field along with the control. In addition three R_1 generation of the same variety of *C. capsularis* and one line of CVL-1 variety of the same species were also raised along with the parents. The number of plants obtained in each of the 14 lines have been shown in the accompanying table (Table 19).

A good deal of variation has been observed among the somaclones in respect of the following characters (i) plant height (ii) base diameter (iii) number of plants bearing four or more fruits / cluster (iv) number of individuals showing fruits of smooth surface (v) number of fruits / plant and (vi) number of seed and (vii) shape, size and colour of seeds.

An analysis of the accompanying Table (Table 19) shows that in line no. 4 out of 199, 33 plants were found to bear fruits of smooth surface in an approximate ratio of 6 : 1. In line no. 16, 23 out of 157 plants showed the same characteristic of the fruit. But here the ratio was 7 (rough surfaced fruit) : 1 (smooth fruit).

In respect of no. of fruits/cluster in the same line namely, TCL-4 out of 199 plants 90 showed 4 or more fruits/cluster. vs 3-4 fruits/cluster. In line no. 7 this ratio was different namely, it was 6 : 1.

In addition 27 plants have been observed with more or less equal base diameter from base to apex. All the three characters described above namely, smooth surface of the fruit, no. of fruits/cluster and plants with more or less uniform stem diameter which appeared in the R_2 generation behaved as a recessive character. Until more experiments are carried out it would not be proper to characterise the mode of inheritance i.e. whether this is a mono - or digenic character. That recessive mutations quite frequently in somaclonal variants is already well known in a number of crop plants such as in wheat, rice, potato, tomato and a number of medicinal and aromatic plants. The three new characters which were obtained have significant agronomic importance. For instance, the individuals with less tapering stem is expected to ret uniformly and as such will reduce the wastage of fibre resulting from the over retting/under retting of jute stem. The increase of fruits/cluster is also desirable character because the same quantity of seeds may be obtained by growing smaller number of plants. Smooth surfaced fruit character may be used as a good marker to keep the seeds of a desirable variety distinct from an undesirable one by transferring this character to any commercially important line.

A good deal of variation in respect of no. of fruits/plant

and no. of seeds/fruit have been shown among the somaclones. Small no. of seeds/fruit is certainly an indication of partial sterility. As far as possible the lines with smaller no. of fruits/plant and smaller no. of seeds/fruit will not be used in future breeding work unless they have some special characters of agronomic importance. Fortunately the individuals obtained with desirable characters have been found to be as fertile as the control.

Table 19. Summary of the data recorded from the R_1 and R_2 of the tissue culture derived plants along with their parents.

Plant material	Total no. of plants	Plant height (in cm)	Base diameter (in cm)	No. of plants bearing four or more fruits /cluster	No. of plants bearing smooth surfaced fruits	No. of fruits /Plant	No. of seeds /fruit
D-154 (c)	125	330.51 ± 4.2	1.78 ± 0.14	-	-	66 ± 22.22	42 ± 12.46
TCL-1 (R_2)	65	316.23 ± 3.6	1.53 ± 0.42	-	-	57 ± 26.85	30.5 ± 3.41
TCL-2 (")	20	314.3 ± 3.14	1.57 ± 0.32	-	-	59.6 ± 26.15	21.5 ± 6.43
TCL-4 (")	188	313.59 ± 2.43	1.16 ± 0.36	90	33	60.5 ± 25.23	39.4 ± 6.04
TCL-7 (")	190	314.66 ± 3.51	1.33 ± 0.38	38	-	52.7 ± 25.21	34.4 ± 5.06
TCL-9 (")	30	315.33 ± 4.82	1.64 ± 0.51	-	-	52.8 ± 26.1	38.6 ± 5.54
TCL-11(")	35	316.77 ± 3.9	1.67 ± 0.34	16	-	49.7 ± 23.24	36.5 ± 4.5
TCL-13(")	116	314.76 ± 4.48	1.38 ± 0.37	-	-	52.2 ± 34.65	37 ± 4.16
TCL-16(")	157	315.75 ± 4.4	1.59 ± 0.37	22	23	57.9 ± 20.26	32.8 ± 2.39
TCL-12(R_1)	92	317.62 ± 3.29	1.68 ± 0.3	26	12	49.8 ± 21.24	38 ± 3.02
TCL-21(")	16	316.2 ± 3.95	1.5 ± 0.51	4	-	48.4 ± 25.33	30.3 ± 3.71
TCL-24(")	20	320.33 ± 2.67	1.95 ± 0.25	-	-	46.3 ± 22.37	37.7 ± 2.91
CVL-1 (c)	128	326.61 ± 4.66	1.94 ± 0.34	-	-	63.4 ± 18.84	41 ± 9.28
TCL-20(R_1)	123	317.65 ± 3.89	1.73 ± 0.33	-	-	59.3 ± 27.75	37.3 ± 8.19

D-154 and CVL-1 are the varieties of *C. capsularis*. R_1 and R_2 indicate first and second generations respectively.

SUMMARY

The objective of the present work was to find out improved type of cultivar jute through tissue culture.

This aim was performed:

i) by finding which explant would be more favourable material for regeneration;

ii) by standardizing the media composition for differentiation; and

iii) by evaluating the regenerated plants in respect of morphological characters.

In the present investigation experiments were conducted to obtain plantlet regeneration by culturing fifteen different types of explants of five jute cultivars (D-154, CVL-1 and CC-45 varieties of *Corchorus capsularis*, and C.G. and O-4 varieties of *C. olitorius*) in four different types of media (MS, MMS, SH and R-2) containing fifty seven different combinations of hormones.

Among five different jute cultivars *C. capsularis* var D-154 was found best (35±4.29 shoots/explant) and *C. capsularis* var CVL-1 was the second one (31±2.94 shoots/explant) in respect of shoot regeneration.

Out of fifteen different explants of five jute cultivars used, highest frequency of callus (92.50%) was observed in shoot tip explant of O-4 variety of *C. olitorius* when cultured in MS + 0.5 mg/l IBA + 1.0 mg/l Kn.

Among fifteen different explants - plumule with cotyledon of D-154 showed highest frequency of shoot regeneration while plumule without cotyledon of the same variety was the next

explant producing 31 ± 2.45 shoots/explant. Shoot tip explant produced 10 ± 2 shoots/explant and cotyledon showed 6 ± 1.56 shoots/explant.

Four different media (MS, MMS, SH and R-2) were tried among which MS was found to be the best for regeneration.

Among fifty seven different hormonal combinations, MS + 0.5 mg/l BAP + 25 mg/l tyrosine was the best one for regenerating shoot (35 ± 4.92 shoots/explant) and MS + 0.5 mg/l IBA +0.5 mg/l BAP + 25 mg/l tyrosine was the next one (15 ± 2.49 shoots/explant).

Out of seven different hormonal combinations used for root diffrentiation $\frac{1}{2}$MS + 0.2 mg/l IBA was found to be the best for giving highest frequency (50%) and healthy rooting.

In total one hundred and nine (109) plantlets were transferred to the soil, among which sixteen plantlets were from D-154 and one from CVL-1 i.e. seventeen plantlets were established themselves.

Established plants belonged to the species *C. capsularis*. The plantlets were serially numbered with the prefix TCL in 1,2,3 and so on at the time of transfer to the soil.

Eight lines of somaclonal variants representing R_2 generation of tissue culture derived plants of D-154 variety of *C. capsularis* were grown in the field along with the control. In addition three R1 generation of the same variety of *C. capsularis* and one line of CVL-1 variety of the same species were also raised along with the control to analyse the range of

variability. A good deal of variation has been observed among the somaclones in respect of the following chracters: (i) length and breadth of leaf and their ratio; (ii) petiole length; (iii) serrature length; (iv) stipule length; (v) plant height and base diameter; (vi) pollen viability; (vii) fruit and seed productivity and germination percentage of seeds.

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APPENDIX

a) **MURASHIGE AND SKOOG (MS) MEDIUM, 1962.**

Macronutrients	mg/l
KNO_3	1900.00
NH_4NO_3	1650.00
KH_2PO_4	170.00
$CaCl_2$, $2H_2O$	440.00
$MgSO_4$, $7H_2O$	370.00
Micronutrients	
$FeSO_4$, $7H_2O$	27.80
Na_2EDTA	37.30
$MnSO_4$, $4H_2O$	22.30
H_3BO_3	6.20
$ZnSO_4$, $4H_2O$	8.60
KI	0.83
Na_2MoO_4, $4H_2O$	0.25
$CuSO_4$, $5H_2O$	0.025
$CoCl_2$, $6H_2O$	0.025
Organic nutrients	
Glycine	2.00
Inositol	100.00
Nicotinic acid	0.50
Pyrodoxin HCl	0.50
Thiamine HCl	0.10

pH adjusted to 5.8 before autoclaving.

b) **MODIFIED MURASHIGE AND SKOOG (MMS) MEDIUM, 1981**

Macronutrients	mg/l
$NH_4 NO_3$	1650.00
KNO_3	1900.00
KH_2PO_4	170.00
$CaCl_2, 2H_2O$	440.00
$Mg SO_4, 7H_2O$	370.00
Micronutrients	
H_3BO_3	6.20
$Mn SO_4, 4H_2O$	22.30
$Zn SO_4, 7H_2O$	8.60
KI	0.83
$Na_2MoO_4, 2H_2O$	0.25
$Cu SO_4, 5H_2O$	0.025
$CoCl_2, 6H_2O$	0.025
Na_2EDTA	37.30
$FeSO_4, 7H_2O$	27.80
Organic nutrients	
Myo-inositol	100.00
Thiamine-HCl	1.00
Casein hydrolysate	400.00

pH adjusted to 5.8 before autoclaving.

c) **SCHENK AND HILDEBRANDT (SH) MEDIUM, 1972**

Macronutrients	mg/l
$MgSO_4$, $7H_2O$	400.00
$CaCl_2$, $2H_2$	200.00
KNO_3	2500.00
$NH_4H_2PO_4$	300.00
Micronutrients	
$FeSO_4$, $7H_2O$	15.00
Na_2EDTA	20.00
$MnSO_4$, $4H_2O$	10.00
$ZnSO_4$, $7H_2O$	1.00
$CuSO_4$, $5H_2O$	0.20
$CoCl_2$, $6H_2O$	0.10
H_3BO_3	5.10
$NaMoO_4$, $2H_2O$	0.10
Organic nutrients	
Inositol	1000.00
Nicotinic acid	0.50
Pyridoxin HCl	0.50
Thiamine HCl	5.00

pH adjusted to 5.8 before autoclaving.

d) **CHALEFF'S R-2 MEDIUM, 1980**

Macronutrients	mg/l
NH_4NO_3	400.00
KNO_3	2530.00
KH_2PO_4	170.00
$MgSO_4$, $7H_2O$	350.00
$CaCl_2$, $2H_2O$	440.00
Micronutrients	
$FeSO_4$, $7H_2O$	27.80
Na_2EDTA	37.30
H_3BO_3	6.20
$MnSO_4$, $4H_2O$	16.80
$ZnSO_4$, $7H_2O$	6.62
KI	0.83
$NaMoO_4$, $2H_2O$	0.25
$CuSO_4$, $5H_2O$	0.025
$CoCl_2$, $6H_2O$	0.025
Organic nutrients	
Thiamine HCl	1.00
Inositol	100.00

pH adjusted to 6.0 before autoclaving.