ANALYSIS OF PROGENIES OF THE CROSS, CORCHORUS OLITORIUS x C. CAPSULARIS THROUGH TISSUE CULTURE AND BIOCHEMICAL METHODS

BY

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A THESIS

SUBMITTED TO THE UNIVERSITY OF DHAKA

IN FULFILMENT OF THE REQUIREMENTS OF THE DEGREE

OF

DOCTOR OF PHILOSOPHY

IN

BOTANY

DHAKA

1987

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C E R T I F I C A T E

This is to certify that the research work embodying the results reported in this thesis has been carried out under my guidance in the experimental field and Plant Breeding & Tissue Culture Laboratory of Department of Botany, University of Dhaka, Department of Pharmacy, University of Dhaka and BCSIR Laboratory, Dhaka. It is further certified that the research work presented here is original and suitable for submission for the award of Ph.D.

(Professor A.S. Islam)
Ph.D., D.Sc.

A C K N O W L E D G E M E N T

The author expresses his sincere and heartiest gratitude to Professor Ahmad Shamsul Islam of Department of Botany, University of Dhaka under whose constant guidance and supervision the present investigation was undertaken.

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

The author is grateful to the authority of Bangladesh Jute Research Institute for having kindly provided some of the seed material for the present investigation.

Sincere thanks are due to Dr. M. Mozammel Haque, Associate Professor of Botany, Dhaka University for constant inspiration, helpful criticism and suggestions during the course of study.

The author is indebted to the Director, IFST, BCSIR Laboratories, Dhaka and Dr. Munir Uddin Ahmed, Chairman, Department of Pharmacy, Dhaka University for giving him

kind permission to carry out biochemical tests.

Sincere thanks are due to Dr. Joseph Gomes, Dr. N,M. Mia, Mrs. Laila A. Banu, Mr. Uttam Kumar of BCSIR Laboratory, Dhaka, Mr. A. Rashid, Lecturer of Pharmacy and Mrs. Nilufar Sadeque, Assistant Professor of Botany, Dhaka University for their kind help in carrying biochemical tests.

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.

Sincere thanks are also due to Dr. K.M. Shah Newaz, Mr. Azibor Rahman, Abdus Samad, Dr. Shamsuddin Ahmed, Chand Sultana and Bithee Das for technical help and encouragement.

Finally the author expresses appreciation to his parents, family members, friends specially to Mr. & Mrs. Shahidul Haque, Mr. Mosharraf Hossain for their constant inspiration and encouragement throughout the study.

The author

A B B R E V I A T I O N

ADH	Alcohol dehydrogenase
&	and
BAP	benzyl amino purine
B/L	Breadth / Length
C	Centigrade
c.f.	*Confer*. compare
cm	Centimeter(s)
cv	Cultivar(s)
e.g.	*exempli gratia*. for example
et al.	*et alii* and others
etc.	*et cetera*. and the rest
FAA	Formalin Acetic Alcohol
F_1	First filial generation
F_2	Second filial generation
F_3	Third filial generation
Fig.	Figure(s)
g	gram(s)
hr	hour(s)
IAA	indole-3-acetic acid
IBA	indole-3-butyric acid
i.e.	*id est*, that is

l.c.	(L. loco citalo) in the place before cited
m	meter(s)
mg	miligram(s)
mg/l	miligram / litre
/u	micron
ml	mililitre
mm	milimeter(s)
MS	Murashige and Skoog
No.	Number
%	Percentage
PG	Pollen grain(s)
TLC	Thin Layer Chromatography

C O N T E N T S

L I S T O F T A B L E S

INTRODUCTION

I N T R O D U C T I O N

In the genus Corchorus the two economically important jute fibre yielding species are : C. capsularis and C. olitorius commercially known as ' White ' and ' Tossa ' respectively. Both species are distinguished by their morphological differences. C. capsularis is characterised by its branching habit, being usually shorter in height than C. olitorius, having smaller flowers with a smaller number of stamens compared to C. olitorius and producing globose fruits in bunches of 3-4. In contrast, C. olitorius is tall, comparatively unbranched, having comparatively larger flowers, producing cylindrical fruits in pairs or singles. Of the two, C. olitorius produces superior fibre with greater strength, length and lustre. The white fibre of C. capsularis is weaker, shorter without any lustre. However, C. capsularis is more adaptive ; it can withstand flood , salinity and drought conditions, less sensitive to photoperiods and shows better response to fertilizer application than C. olitorius. Furthermore the same disease does not affect the two species to the same degree ; while some varieties of C. capsularis are susceptible to mosaic

virus, all varieties of C. olitorius are immune to it. It was felt that if the desirable characters of the two species could be combined through hybridization, this would result in an ideal jute plant.

To obtain commercial hybrids through interspecific crosses is an extremely difficult task because of the sterility and other physiological and genetical factors that accompany such hybrids. Among flowering plants getting fertile hybrids is much more difficult in dicotyledons compared to monocotyledons. In spite of such formidable difficulties breeders attempted from time to time to obtain hybrids through interspecific crosses when all their avenues to get genes for resistance to diseases and pests from intraspecific crosses get exhausted. Encouraged by the success of Sears (1956) who obtained leaf rust resistant wheat and of Saunders (1969) who produced jassid resistant Egyptian cotton, Gossypium barbadense, a number of work in this line covering various crops was initiated. Some of the recent work which deserve mention is production of mildew resistant Avena by Aung and Thomas, rust resistant wheat variety 'Kite' (c.f. Islam 1984) and a number of useful hybrids involving G. hirsutum and G. barbadense (Singh and Raut 1983).

Although a number of desirable varieties of jute have

been released from time to time in both the species through intervarietal crosses, none has come up to the expectation of the breeders. This being the situation the need to combine the genome of the two species was keenly felt in order to breed an ideal jute variety. It is with this end in view that Kundu (1956) attempted to cross, C. olitorius with C. capsularis but was unsuccessful. Ganesan et al. (1957) demonstrated that the primary cause for failure of the cross, C. olitorius x C. capsularis was not due to the lack of fertilization but due to premature arrest of growth in the hybrid embryo and endosperm. Three years later using C. olitorius as mother Islam and Rashid (1960) reported the first successful hybrid of the two species by applying around the pedicel a high concentration of indole-3-acetic acid (300 ppm) to prevent abscission of pollinated flower and enhance the growth of the hybrid embryo and endosperm. A year later using C. olitorius as female Swaminathan et al. (1961) succeeded in obtaining two F_1 plants. Their female plant was grafted on to the stock of C. capsularis and pollination was carried out by means of x-ray irradiated pollen of C. capsularis. Unfortunately in spite of the initial success, breeders did not succeed to market any variety out of this interspecific hybrid neither in India nor in Bangladesh. This is because in

the advanced generation no recombinants could be recovered and what was left finally in the F_3 and F_4 population resembled the female parent C. olitorius almost in totality. Islam (1964) was able to obtain a single F_1 reciprocal hybrid through hormone application and embryo culture. Unfortunately this lone F_1 hybrid plant died before it produced any flower.

Das (1968) made interspecific cross through reciprocal grafting and observed that the cross in which both male and female parents were grafted, gave higher frequency of globular embryos and endosperm compared to partially cellular embryos in the cross in which only one parent namely, C. olitorius was grafted and a few nucleate (4-8) stage in the nongrafted crosses. Iyer et al.(1961) and Sacher et al. (1964) observed that pollen from grafted plants promotes the growth of the hybrid embryo. Das (l.c.) remarked that although grafting does neither alter the genotypes nor the phenotype of the scion, it does help in the formation of hybrid embryo. Harland (1955) suggested that the two grafted species apparently possess in common some factors of a biochemical nature. Furthermore pollen from grafted parents is able to improve fruit-set and

seed formation in the interspecific crosses by delaying abscission process. Probably this is the reason why both Swaminathan et al. (1961) and Das (1968) had more success in obtaining hybrid plants in this combination.

Bhaduri and Bairagi (1968) also succeeded in crossing the two jute species but unlike the previous workers (Islam and Rashid 1960 ; Swaminathan et al. 1961) they achieved success using C. capsularis as female parent. The variety of C. capsularis which they used was sweet-leaved like the taste of C. olitorius. This closer relationship may have contributed to the success of the cross. The above authors have not mentioned in their report whether their reciprocal cross in which previous workers reported success in the past, yielded any F_1 hybrids.

In the present investigation different improved techniques as mentioned below were combined in an attempt to maximise yield of F_1 hybrids between the cultivars of C. olitorius and those of C. capsularis. The combined techniques consisted of a) using C. olitorius scion grafted on to C. capsularis stock as mother and vice versa,b) smearing the stalk of the pollinated flowers with different concentrations of indole-3-acetic acid in lanolin paste, c) growing the excised hybrid embryos in suitable artificial medium and d) saving the transplanted seedlings after transfer to pots from test tubes by covering the pots with perforated

polythene bag.

Although F_1 hybrids in the above combination have been produced by different workers from time to time many breeders still doubt whether at all it is possible to get an F_1 between the two jute species. Their doubt is based on the fact that in F_3 and advanced generations, only the maternal type of plants is obtained i.e. if C. olitorius is the female parent, the F_3 population resemble the mother in almost all respects ; in the reciprocal cross in which C. capsularis is the mother plant the resemblance is very close between the individuals of F_3 and female (Bhaduri and Bairagi 1968). It was therefore felt keenly that more parameters should be used to establish the hybrid nature of the product of the cross, C. olitorius x C. capsularis beyond any doubt whatsoever. It was therefore decided to use more parameters for this purpose, the most important one being the use of regenerating ability of the male parent as a marker. The other parameters used were : comparative thin layer chromatography (TLC), isoenzyme and protein studies.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Various attempts have been made in the past to develop a recombinant line combining the characters of C. olitorius and C. capsularis.

Finlow and Burkill (1912) reported that jute is a self pollinated crop and chances of cross pollination are only 1-2 per cent.

Howard et al. (1919) reported that cross fertilization is very rare in jute as the anthers burst and shed pollen in the buds.

Ghose and Das Gupta (1945) reported that the extent of cross fertilization in C. olitorius was found to be about 9-13 per cent and in C. capsularis it is less than C. olitorius.

Patel et al. (1944) attempted to cross C. olitorius and C. capsularis and each of the two with C. aestuans, C. fascicularis and C. trilocularis, but there was no success.

Srinath and Kundu (1952) first attempted to find out the basic causes of incompatibility between the two species and came to the conclusion that the rate of pollen tube growth was the same in both self-pollinated C. olitorius and in crosses with C. capsularis. Similar was the case when C. capsularis was used as the female parent but there was a

slight difference in the rate of pollen tube growth between the reciprocal matings. Their conclusion which was supported later by embryological studies of Banerji and Datta (1960) indicates that the incompatibility was not due to any retardation in the growth of pollen tubes along the stylar tissues regardless of the direction of the cross and the shortness of the style between the species offers no advantage in overcoming interspecific incompatibility.

Ganesan et al. (1957) reported that fertilization takes place normally between the two species but premature abortion of the hybrid embryos leads to the failure of seed-setting.

Through cytogenetical studies Datta and Sen (1959) determined the causes of seed failure or seed setting in c-4x wild C. capsularis L. x its progenitor wild C. capsularis L. and their reciprocals. Seeds from the cross 2x ♀ x c-4x ♂ olitorius germinated, but in the cross 2x ♀ x c-4x ♂ capsularis the two hybrid seeds did not germinate.

Sulbha and Swaminathan (1959) recommended the use of reciprocal grafting, pollen irradiation and hormone application to overcome the incompatibility barrier in the above cross combination.

Datta and Dana (1960) made interspecific hybridization between Corchorus olitorius and Australian C. sidoides and cultivars of C. capsularis and C. sidoides. There was pod

setting in both the crosses, in some with no seeds and in others with seeds of various sizes. However no seeds germinated. Later Islam (personal communication) found that the material used by Datta and Dana was C. aestuans and not C. sidoides as reported by them. From the point of view their results were not different from those reported by Islam and Haque (1967).

Islam and Rashid (1960) have reported for the first time a successful hybrid between two cultivated jute species by the application of 300 ppm indole-3-acetic acid around the pedicel of the pollinated flower.

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 in 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 (♀) and C. capsularis (♂) than when they are self-pollinated.

To find out the main barriers of interspecific hybridization embryological studies were done by Banerji (1932), Banerjee and Datta(1960), Cooper and Brink (1940) and Patel and Datta (1960). They concluded that the inefficiency of

most of the pollen tubes to grow rapidly 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.

Patel and Datta (1960) crossed C. siliquosus with C. olitorius and C. siliquosus with C. capsularis but they were not able to get any pod in either of the cross combination.

Swaminathan et al. (1961) have discussed the morphology, cytology and breeding behaviour of hybrids between C. olitorius and C. capsularis and showed that the F_2 exhibits the phenomena of cryptic structural hybridity.

Datta and Sen (1961) crossed C. sidoides (possibly C. aestuans as latter determined by Islam by raising plants from the seeds labelled as C. sidoides) with C. siliquosus but they did not get any hybrid.

With the help of embryological studies Iyer et al.(1961) showed a detailed comparative study of fertilization and seed development in some varieties of C. olitorius and C. capsularis and their hybrids. They observed that grafting of the female parent as scion on to the male parent helped in the development of hybrid embryo.

Islam (1964) obtained F_1 hybrids from the cross, C. capsularis x C. olitorius with the former as female through the

application of hormone in lanolin paste and embryo culture. He used Nitsch and White's media for culturing the embryo prior to their transfer into the soil. Out of 15 seedlings two were semi-albino and died soon after their transfer and the remaining 13 died at different times between 3 and 8 months before flowering. This slow growth, albino character of some seedlings and their premature death indicated that they were all true hybrid.

Sachar et al. (1964) reported with the help of embryological studies that the incompatibility barriers can be overcome if the female parent is grafted onto the stock of the male parent and pollen is used from the grafted male parent.

Datta and Choudhury (1965) reported their failure to obtain interspecific hybrids between the colchicine induced autotetraploids of the above two species.

In a detailed review paper Islam (1965) summarised the results of crosses made by his students between C. capsularis and C. olitorius with species from different continents including those locally available.

Datta and Choudhury (1966) reported failure of seed setting between the tetraploid C. capsularis and 3x C.olitorius.

Islam and Abbasi (1966) obtained sterile hybrid from the cross of C. siliquosus and colchicine induced tetraploid

C. olitorius.

Mughal and Islam (1966) obtained polyhaploids following interspecific crosses between c-4x C. olitorius and C. pascuorum (2n=28), a natural tetraploid from Australia.

Frost and Bose (1966) investigated the phenolic compounds in the two species of jute, C. olitorius and C. capsularis and product of their cross by means of thin layer chromatography in the patterns of spots. They found that the two species differ distinctly in the presence of spots detected in the thin layer plate. Spots were identical in the suspected hybrid and the female parent indicating that the former was the result of self pollination.

Islam and Ali (1966) investigated the causes of failure of the reciprocal cross, C. olitorius x C. trilocularis. They reported that the primary causes of failure of the cross was due to the inability of the pollen tube of one species to grow along the whole length of the style of the other species. It was also reported that the establishment of contact between the pollen tube of one species with the ovule of the other by means of IBA did not bring about fertilization in one direction and in the other did not produce embryo. They concluded that the above two species form a cenospecies and that no gene flow is possible between them.

Using gamma irradiated male parent with bilobed and crinkled leaf characters, Mia and Sheikh (1967) obtained

some interspecific hybrids from the cross, C. olitorius var. C.G. (0 kR) x C. capsularis var. D-154 (80 kR). The hybrids attained a greater height than the parents and were highly fertile. Because of the presence of bilobed leaves in the progeny of their cross, they thought that they were all genuine hybrids. However, from their fertility data it seems that the effects on their F_1 progeny might be due to factors other than fusion of male and female gamets.

Mughal (1967) reported reciprocal hybrids between C. hirtus, a natural tetraploid species from South America and c-4x C. trilocularis.

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

Islam and Haque (1967) reported the occurrence of a natural C. olitorius - C. aestuans hybrid which they did not succeed to produce artificially even by applying hormone around the pedicel of pollinated flowers combined with embryo culture.

Mughal and Khan (1967) reported the causes of failure of the cross, c-4x C. olitorius x C. hirtus, a natural wild tetraploid from South America. They observed that the failure of the cross was not due to the inability of the pollen tube to enter the ovule of the female parent; rather it was due

to the formation of an abscission layer around the stalk of the pollinated flower.

Using C. capsularis var. Tripura as female, Bhaduri and Bairagi (1968) reported viable hybrids for the first time between the two jute species. Being sweet leaved variety this strain of C. capsularis resembles C. olitorius in its leaf taste and may have contributed to their success. Although their F_2 population consisted of 2,349 plants only one individual plants appeared almost like the male donor, C. olitorius var. JRO 620 in respect of unbranched habit and pigmentation. This plant could not be saved from the attack of stem rot. They suggested that a larger F_2 population be raised to recover individuals with male characters and recombinant types.

Reciprocal interspecific crosses between C. capsularis and C. olitorius and also reciprocal crosses between the grafted C. capsularis were made by Das (1968). Altogether he obtained 83 fruits, highest percentage of fruit setting being found from the cross, grafted C. olitorius x grafted C. capsularis. In the straight crosses he failed to obtain any fruit between C. capsularis and C. olitorius. He also observed that crosses involving C. capsularis as female parent gave lower fruit set percentage.Number of fertile seeds were low in all the crosses irrespective of the direction.

Haque and Islam (1969) reported that the backcross derivatives of their selected F_2's of a natural cross, C. aestuans x C. olitorius could be developed to give rise to partially stem rot resistant variety of C. olitorius with more or less uniform stem diameter. Some of the promising lines of B_3F_6 out of this selected material are in the advanced stage of trial (personal communication). It may be mentioned here that C. aestuans is resistant to MS-3 of Macrophomina phaseolina.

Through the application of improved techniques Islam et al. (1970) showed genomic relationship of C. depressus with both C. capsularis and C. olitorius. According to their cytological data C. depressus is more closely related to C. capsularis compared to C. olitorius.

Islam et al. (1973) crossed C. olitorius var. C.G. with a photoneutral species C. depressus. They obtained 26 F_3 plant populations among which five were photoneutral. They also reported that among the five F_3 plants two were amphi-diploid.

Later Islam et al. (1975) raised F_4 and F_5 plant populations from amphidiploids and observed that the photoneutral character segregates as a recessive trait. They observed paracentric inversions during meiosis which according to these workers may be the cause for the non-fixation of the photo-neutral character in the progeny.

Bhaduri et al. (1975) described some of the recombinant types isolated from the advanced generations of the cross between C. capsularis and C. olitorius. They also observed that a characteristic petal notch of the male parent was present as a dominant character in the F_1 and in the recombinant types. Subsequently Bhaduri et al. (1981) reported the performance of the non-segregating recombinants (F-10) of the interspecific hybrids (C. capsularis var. Tripura x C. olitorius var. JRO-620) against the best C. capsularis control. They observed that the recombinant T-24 type was the best fibre yielder, both at 100-day and small pod (150 days) stage.

To evolve a stem-rot resistant strain of C. olitorius Haque et al. (1979) studied four different cross combinations namely, B_3F_6 of C. olitorius var. C.G. x C. aestuans ; B_1F_6 of C. olitorius x C. depressus; B_1F_1 of C. olitorius var. O-4 x (C. olitorius var. O-4 x C. olitorius var. Assam red) and F_1 of C. olitorius var. O-4 x C. capsularis var. Lalnaris for their degree of resistance to the attack of 2 virulent races, MS-3 and MS -9 causing stem-rot and observed that the wild parent C. aestuans was resistant to both races of Macrophomina phaseolina and B_3F_6 of C. olitorius x C. aestuans were moderately so while other hybrids and their parents were susceptible.

Haque et al. (1980) studied in vivo pollen tube growth in the cross between C. olitorius var. Assam red and C. aestuans to investigate the causes of failure of fruit-set resulting from interspecific hybridization. They observed that in self-pollinated flowers of C. olitorius the pollen tubes took 9 hrs to reach their ovules, but in the cross with C. aestuans the pollen of the latter covered the same distance in 7-8 hrs i.e. in less time. They concluded that the cause of lack of fruit-set lies in the factors other than the failure of pollen tube to reach the ovule of the female parent.

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.

Kumar et al. (1981) reported that it is possible to fuse the protoplasts of the two jute yielding species.

Using x-ray irradiation Rohman (1982) for the first time reported the development of male sterile mutant in C. capsularis var. JRC 212 at a dose of 70 kR. Unfortunately his mutant had no practical utility in breeding work because of its association with undesirable ribbon leaf character not considered suitable for any breeding work in jute.

Raut and Naik (1983) succeeded in obtaining F_1 and F_2 generations from the cross between C. olitorius and C. capsularis with the help of some improved techniques like reciprocal grafting, hormone application and ovary culture.

Aranzeb and Khatun (1983) reviewed the work done on interspecific hybridization in Bangladesh Jute Research Institute. They reported that they were not very successful in obtaining interspecific hybrids at tetraploid as well as diploid level and concluded that an increase in the number of genomes offers no advantage in the case of jute to reduce the incompatibility barrier. They also suggested that techniques such as embryo culture, protoplast culture or somatic hybridization and bridge cross be resorted to for getting interspecific hybrids.

Zhou (1984) reported the wide variation in peroxidase activity of C. capsularis and C. olitorius by using electrophoretic method. He observed that in C. olitorius 1st, 3rd and 23rd bands were wide darker staining and 2nd band was absent while in C. capsularis the 6th, 7th, 20th, 21st and 23rd bands were dark staining. He also concluded that the electrograms were suitable for cultivar identification and also could be used for distinction between certain combination of cultivars in interspecific crosses.

Sarker and Bhaduri (1986) carried out a seven parent

diallel cross between six selected lines of C. capsularis var. Tripura x C. olitorius var. JRO-620 and the C. capsularis parent for finding out suitable parents ensuing adequate seed and fibre yield per plant. They observed that T-17 was the best general combiner for plant height and base diameter and T-2 for fibre yield and seed production. The combination T-2 x T-6 showed best specific combining ability in respect of fibre yield whereas in respect of height and base diameter T-7 x T-8 was the best specific combiner.

M A T E R I A L S

M A T E R I A L S

The following cultivars of Corchorus olitorius L. and C. capsularis L. were used in the present investigation.

3.1.1. C. olitorius L. var. O-4 :

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

Source : BJRI germplasm.

3.1.2. C. olitorius L. var. C.G. :

Origin : It was developed in BJRI, Dhaka in as early as 1915 under the name D-38. Since the seeds of this variety was collected from Chuchura 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.

3.1.3. C. capsularis L. var. D-154 :

Origin : A moderately virus resistant pure line selection of the then Bengal Department of Agriculture from " Kakya Bombai " released in 1919 at Dhaka with high yield; it was the principal variety predominant until early 1950's

when new varieties became available.

Source : BJRI germplasm.

3.1.4. C. capsularis L. var. CVL-1 :

Origin : A full green variety of C. capsularis L. was evolved through single plant selection from a local collection grown at Kishorgang sub-station of BJRI in 1954 and was released in 1977.

Source : BJRI germplasm.

3.2. Morphological characters of parents :

3.2.1. C. olitorius L. var. O-4 :

Stem : Tall (335-370), erect, cylindrical, green, does not develop adventitious roots in humid condition.

Branch : Unbranched under normal spacing (30 cm in between rows and 7.5 cm between plants).

Leaf : Lanceolate, serrate, basal serration prolonged into tail like appendage called serrature. Length Breadth ratio 2.2 : 1. Light green in colour, leaf surface non-glossy. A pair of free lateral stipules present at the base of petiole. The crown of leaves at the top of stem loosely arranged.

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

Fruit : Capsule, ten-ribbed, ribs distinct, parallel,

forms an imperfect beak with 5 teeth like structure at the terminal end of fruit. Upon ripening the capsule dehisces at the union of the carpels and shatter their seeds. Seed colour black with greenish tinge(Fig. 1)

Weight of 1000 seeds : 2.3 g

Germination percentage of fresh seeds : In Petri dish 98 to 100.

Late maturing, short day and takes 110-120 days to flower if sown in mid-April. Under short day i.e. before mid-April it flowers in 40 days.

3.2.2. C. olitorius L. var. C.G. :

Stem : Tall (330-360), erect, cylindrical, base diameter 1.75 ± 0.15 cm.

Branch : Unbranched.

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

Flower : Large, yellow, axillary, entire, petals comparatively larger than C. capsularis. anthers globose, percentage of pollen grain stainability 97.5. Style oblong, short constricted.

Fruit : Capsule, ten ribbed, surface smooth, ribs are parallel.

Seeds : Colour of the seeds black with greenish tinge, weight of 100 seeds 0.2 g , no. of seeds/capsule is 200-250.

Fig. 1

Percentage of seed germination is about 98.

Vegetative and Reproductive phase : Late maturing generally flowers in between 104 and 112 days.

3.2.3. C. capsularis L. var. D-154 :

Stem : Tall(315-340), erect, cylindrical, base diameter 2.9 ±0.2 cm.

Branch : Unbranched.

Leaves : Lanceolate, serrate, basal serration usually prolonged, petiole slender, stipule free lateral. Length of the leaves 13.4 cm to 16.0 cm and breadth 6.0 to 8.0 cm. Length breadth ratio 1.77 : 1.

Flower : Flowers small, yellow, sepals 4 to 5 in number, petals ovate, acuminate. Percentage of pollen grain stainability 96 to 98. Ovary generally five celled, style small.

Fruit : Capsule, spherical with irregular projections and depressions all over the surface (Fig. 2). No. of seeds/ capsule is 30 to 40, seed colour chocolate brown, weight of 1000 seeds is 3.1 g.

Vegetative and Reproductive phase : Late type, flowers in between 105 and 120 days.

3.2.4. C. capsularis L. var. CVL-1 :

Stem : Tall (315.92 - 320.1 cm), erect, cylindrical.

Branch :Feebly branched.

Leaves : Large, nearly ovate, lanceolate, serrate. Length breadth ratio of leaves is 1.76 : 1.

Fig. 2

Flowers : Medium, colour of the sepals light green, colour of the petals light yellow, percentage of pollen stainability is 95 to 98, style small.

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

Vegetative and Reproductive phase : Late maturing and takes 110-120 days if sown in mid-late March. Less photosensitive than C. olitorius.

METHODS

M E T H O D S

Experiments were carried out in Plant Breeding and Tissue Culture Laboratory of Department of Botany, University of Dhaka, its adjoining Botanical Garden, net house. Biochemical part of this thesis has been carried out in Plant Protein and Plant Enzymology Laboratory of BCSIR Laboratories and in the Department of Pharmacy, Dhaka University.

Seeds were sown in earthen pots as well as in the experimental fields. Seeds were dried in the sun for a few days before they were stored in a desiccator. Seeds were treated with a suitable fungicide just before their sowing either in the field or pot.

3.3.1. Grafting techniques :

Six-week old young plants were used for simple cleft graft. About 5 cm long strip of each parent was removed exposing pith with the help of a fine razor blade. The two exposed parts were brought firmly in contact with each other and securely tied with a piece of strong jute string. Because of the abundant mucilage following the cut in the stem, it was not necessary to provide additional moisture to the graft. When the wound of the graft completely healed up, the unwanted

top of the parent contributing stock was removed. After 3 days the stock of the scion-contributing parent was discarded.

3.3.2. Crossing techniques :

Conventional methods for crossing were followed. Every possible precaution was taken to prevent self pollination. To avoid contamination, emasculation was done in the evening preceding the day of pollination. The pedicel of each pollinated flower was smeared with 300 mg/l of indole-3-acetic acid (IAA) in lanolin paste. Thirty mg of IAA were first dissolved in 3-4 drops of absolute alcohol and then mixed thoroughly in lanolin till it turned into a fine paste.

3.3.3. Pollen grain study :

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.

3.3.4. Estimation of leaf and seed moisture :

For the estimation of leaf moisture, fresh leaves from middle region of the growing plants were used. The leaves collected were first blotted with fresh blotting paper to remove superficial water (if any) and then the leaf blades were cut into small pieces. Two gms of leaf sample was taken

in a porcelein crucible. Weight of the empty crucible and that with the sample was recorded. Two such leaf samples were taken for each specimen. The crucible with the sample were then kept into an oven, the temperature of which was maintained at 110°C. After 12 hrs of hot treatment the crucible with specimen was taken out from the oven and cooled in a desiccator. After cooling, the dry weight was taken. Heating and cooling was repeated till a constant dry weight was obtained.

Estimates of moisture content expressed in percentage is given below :

$$\frac{\text{Weight lost by the sample}}{\text{Total weight of the fresh sample}} \times 100$$

For the estimation of moisture content of the seed, same procedure was followed. Properly dried seed samples were first crushed with a morter and pestle and two gms of powdered seed sample was taken in each crucible. Each sample was taken in two replications.

3.3.5. Estimation of total protein :

Total protein was estimated following Kjeldahl method (Sher, 1955) with few modifications.

For the estimation of total protein of leaves, fresh leaf samples were used. The fresh leaves collected from the middle

portion of the growing plants were cut into small pieces and 2 gms of the sample was taken in Kjeldahl's flask. Ten gms of sodium sulphate, 2 gms of copper sulphate and 20 ml of concentrated H_2SO_4 was added to the leaf specimen. The flasks were then fitted to fuming tunnel and heated till the sample became a clear solution. It was then cooled and collected in a volumetric flask and increased the volume up to 100 ml by adding distilled water.

10 ml of the solution was distilled with 5 ml 50 % NaOH and 5 ml $Na_2S_2O_3$. The distillate was collected in 10 ml of 2 % boric acid with 2 drops of indicator. Indicator was prepared by mixing 0.2 % napthalene blue and 0.2 % napthalene red in 1 : 2 v/v ratio. When the volume of the distillate was about 20 ml, it was then taken and used for titration with 0.02 N HCl.

From the titration results, the amount of Nitrogen was calculated by using the formula :

$$\text{Amount of } N_2 = \frac{\text{Amount of HCl x strength of HCl x Mol. wt. of } N_2 \text{ x } 100}{\text{Amount of sample distilled}}$$

Amount of of protein was calculated by the formula :

$$\text{Amount of protein} = \text{Amount of } N_2 \text{ x } 6.25$$

3.3.6. Extraction of protein and enzyme :

Alcohol dehydrogenase (ADH) : 12-15 hrs presoaked seeds were decoated and used for extraction , the seeds were thereafter washed with distilled water and gently blotted dry with filter papers. Extraction was done by homogenizing 1 g of decoated seeds for five minutes in a chilled morter with 5 ml 0.05 M Tris-HCl buffer (pH 7.6) containing 10 mM β-mercaptoethanol (BME) and 12.5 % sucrose. The slurry was centrifuged at 10,000 g for 30 minutes at $4^{o}C$. The supernatant was filtered through Whatman No. 1 filter paper. This filtration removed most of the lipid material found floating on top of the supernatant. The resultant preparation was used directly for protein estimation, assay of enzyme activity and polyacrylamide gel electrophoresis. Generally fresh extracts were used. However, sometimes the extract was stored at $4^{0}C$ in a stoppered test tube for 24 hrs. This storage caused precipitation of some of the carbohydrate material which was then centrifuged off and the clear supernatant was used. But there was always some drop in enzyme activity due to storage.

3.3.7. Determination of protein :

The protein concentration of crude extracts was determined according to the method of Lovery et al. (1951) using bovine serum albumin as standard.

3.3.8. Measurement of enzyme activity :

ADH assay : ADH activity was measured to the method of Bonnichsen and Brink (1955) with a slight modification. The enzyme reaction mixture consisted of 2.75 ml of 0.05 M Tris-HCl buffer (pH 8.8), 0.1 ml of 1.13 M ethanol and 0.05 ml of the enzyme extract. This solution was then used to set the machine at zero. Absorbance was measured at 340 nm on a LKB digital spectrophotometer (Model, Ultroscope, 4050). The increase in absorbance of the reduced form of NAD^+ was recorded continuously for 5 minutes at 30 seconds intervals after the addition of 0.1 ml of 0.4 mM NAD^+. One enzyme unit is defined as the amount of enzyme necessary to produce a change of 0.001 O.D. per minute per mg of protein. The change in O.D. was linear with time for at least one minute and the velocity was proportional to the amount of enzyme added over the range of enzyme activity used in these experiments. Whenever necessary the enzyme extract was diluted suitably.

3.3.9. Electrophoretic procedure :

Protein separation was carried out by polyacrylamide disc electrophoresis according to the method of Bloemendaal (1963). Power supply and electrophoretic set manufactured by E.C. Apparatus Corporation, Florida, U.S.A., Model E.C. 454 were used.

3.3.9.1. Preparation of stock Tris-glycine buffer :

3 g of Tris (hydroxymethyl) methylamine (0.247 M) and 14.4 g of glycine (0.191 M) were dissolved in 100 ml redistilled water.

3.3.9.2. Working buffer :

The stock buffer solution was diluted ten times with redistilled water before use. The pH of this buffer was 8.5. For each electrophoretic run 800 ml of this buffer was needed, 600 ml for the lower and 200 ml for the upper buffer reservoirs.

3.3.9.3. Preparation of stock gel solution :

a) 30 g of acrylamide and 0.8 g of N-N-methylene bisacrylamide were dissolved in 100 ml redistilled water and filtered through Whatman No. 1 filter paper.

b) 1.6 g of β-dimethyl aminopropionitrile added to 40 ml stock Tris-glycine buffer which was then diluted 2.5 times to make the final volume 100 ml (for polymerization).

c) 30 mg % potassium ferricyanide solution was made in redistilled water (for delaying polymerization).

d) 480 mg % ammonium persulphate solution was made in redistilled water (for enhancing polymerization).

The above solution remained stable for at least one month if stored in a brown bottle at $4^{o}C$.

3.3.9.4. Polymerization of gel :

For polymerization of gels and electrophoresis, 18 glass tubes, 8 cm long and inside diameter 5 mm were placed in a vertical position on a plastic rack with suitable holes and screws. The lower end of the tube was closed by rubber caps. Ten ml each of solution a, b, c and d (sec. 3.3.9.3.) were pipetted sequentially in a conical flask. The gel mixture was immediately poured into the gel tubes up to 5 mm from the upper rim. The gels were then allowed to polymerize at room temperature. The polymerization occurred within 40-50 minutes to form a colourless gel. The completion of polymerization was indicated by a sharp boundary line between the polymerized and unpolymerized portions. The unpolymerized solution was discarded and the gel surface was washed with the working buffer.

3.3.9.5. Pre-electrophoresis :

The tubes with polymerized gels were removed from the plastic rack and the rubber caps were removed carefully. The tubes were immediately inserted into the rubber grommetlined bores of the upper electrode buffer reservoir that the rims projected about 2 cm above the bores. Pre-cooled ($4^{o}C$) electrode buffer (Tris-glycine working buffer) was slowly poured into the upper and lower electrode reservoirs and special care was taken to prevent the formation of air bubbles

at the tube rims. The electrodes were connected with the power supply and pre-electrophoresis was carried out for 15-20 minutes at 100 V i.e. 2-3 mA/gel to purify the gels from unreacted materials of polymerization process and ionic impurities of the gel components. In addition to this, pre-electrophoresis set up a constant resistance inside the gel.

3.3.9.6. Application of sample :

After pre-electrophoresis the upper reservoir was removed and seed extracts containing equal amounts of proteins were applied on top of individual gel to form a layer under the buffer layer. A 0.05 ml 20 % sucrose solution was mixed with each individual seed extract before addition to increase the density of the extract. For comparative studies, the addition of the same amount of proteins was essential.

In most analysis, a small amount of 0.001 % bromophenol blue with 10 / 1 of 20 % bovine serum albumin was added on top of one gel without sample. Bromophenol blue formed complex with albumin and the dye served as a marker of the migrating boundary during electrophoresis.

After application of the samples, the upper reservoir was replaced on the lower reservoir and the apparatus was connected to the power supply. Electrophoresis was done at 100 V (2.5-3.0 mA/gel) for 15 minutes and then for 50-60 minutes at 200 V (3-4 mA/gel) for satisfactory separation

of the bands.Electrophoresis was always carried out at room temperature under continuous water circulation around the buffer reservoirs.

3.3.9.7. Treatment of gels and detection of isoenzymes :

Immediately after electrophoresis the gels were removed from the tubes with the help of ejecting water from a hypodermic syringe and washed in distilled water. The enzyme bands were located by incubating the gels in appropriate enzyme substrate mixture. A control gel for each individual enzyme was incubated in the reaction mixture without the substrate. In the control gels no band was detected.

3.3.9.8. Staining method for dehydrogenases :

The method for staining dehydrogenases (NAD-dependent) was standardised on the basis of the technique developed for quantitative determination of LDH activity by Babbson and Phillips (1965). The staining method is based on the fact that the protons transferred from the substrates (proton donors) to nicotinamide adenine dinucleotide (NAD^+) by dehydrogenases to form $NADH_2$ is taken up by phenazine methosulfate (PMS) and then transferred to 3-(4-Iodophenyl)-2-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT) which is thereby reduced to an insoluble formazon which becomes visible as a purple red colour.

The colour reagent for visualizing dehydrogenases was

prepared by dissolving INT (20 mg), PMS (5 mg) and NAD (20 mg) in 10 ml Tris-glycine buffer pH 8.8. The reagent was prepared freshly just before use.

3.3.9.9. Staining mixture for protein :

Freshly prepared 0.06 % amido-black solution in 2 % acetic acid was used for staining the gels for protein bands. The gels were detached from their tubes and incubated for 30 minutes. They were then destained by repeated rinses with 7 % acetic acid. Blue bands become visible against faint or almost clear background. Three replications were prepared for each plant sample.

3.3.9.10. Staining mixture for ADH :

For each gel the reaction mixture consisted of 3.7 ml Tris-glycine buffer, pH 8.5, 0.1 ml 99.8 % ethanol and 0.3 ml colour reagent. The zymograms were stained in a water bath at 37^{o}C in the dark for 15-25 minutes until the bands had reached a desired intensity.

3.3.9.11. Storage of stained gels :

Following staining the gels were washed with distilled water and placed in stoppered test tubes in 2 % acetic acid. In this solution separated fractions of the isoenzyme remained stable without loss of intensity for several months if stored in the dark at 4^{o}C.

3.3.9.12. Processing and representation of electrophoretic results :

The distance of each zone of enzyme activity on the gel was measured from the starting line (line of application of sample) and on the basis of this measurement the mobilities of the isoenzymes have been expressed.

The isoenzymes in the investigated species have been shown schematically and whenever possible original photographs of zymograms have been presented. In each schematic representation positive and negative poles have been marked by (+) and (-) signs respectively. The line of application of sample is always shown on the upper end of the gel. The electrophoretically separated bands have been numbered according to the recommendation of Standard Committee of Enzyme (Webb 1979).

3.3.10. Study of methanol soluble compounds in the seeds of C. olitorius, C. capsularis and product of their cross, with the help of thin layer chromatography :

3.3.10.1. Extraction :

The seeds (250 g) of C. olitorius var. O-4, C. capsularis var. D-154 and their F_1 and F_2 hybrids were powdered with the help morter and pestle. The powdered seeds were then taken into a clean conical flask and methanol was added just to sufficiently cover the powdered seeds. Extraction

was carried out over a period of 48 hrs with occasional shaking and stirring. The methanol extract was then filtered off with Whatman No. 1 filter paper. The filtrate was kept in refrigerator for use.

3.3.10.2. Analysis of methanol extract :

The methanol extract was analysed by ascending one dimensional thin-layer chromatography using different solvent systems in order to determine the number of components present in the extract.

Stationary phase : Silica gel-G.

Solvent systems :

a) Chloroform : methanol (5 : 5 ; 6 : 4 ; 7 : 3 ; 7.5 : 2.5 ; 8 : 2)

b) Ethyl acetate : methanol (6 : 4 ; 7 : 3 ; 7.5 : 2.5 ; 8 : 2)

c) Acetone : methanol (5 : 5 ; 6 : 4 ; 6.5 : 3.5 ; 7 : 3; 7.5 : 2.5 ; 8 : 2)

3.3.10.3. Preparation of silica gel-G plates :

Glass plates measuring 20 x 5 cm were washed meticulously with detergent solution and then dried in an oven. After drying the plates were cooled and placed on the aligning tray. The plates were again ringed with acetone. A slurry was made by shaking silica gel-G (1.5 g / plate) with distilled water (2 ml / g of silica gel-G) in a stoppered conical

flask of suitable size. The slurry was then spread uniformly over the glass plates, using a spreader to have a uniform layer making thickness of 0.5 mm.

3.3.10.4. Activation of plates :

The coated plates were first air dried for about 15 minutes and then activated by heating in an oven at 110°C for 1 hr.

3.3.10.5. Saturation of solvent chamber :

Rectangular glass chamber with air tight glass lid was used for running of the plates containing extracts. The selected solvent system was poured into the glass chamber and a filter paper was placed in the tank to promote evaporation of solvents. The lid of the tank was replaced and a time lapse of 30 minutes was allowed to saturate the internal atmosphere of the tank with vapours of the solvent.

3.3.10.6. Resolution of total and nitrogenous compounds :

A small spot from the extract was applied at 2 cm above the lower edge of the activated silica gel-G plate with a fine capillary tube. The spot diameter was kept below 0.5 cm. The spots were finally dried and was ready for running.

A straight line was cut 2 cm below the upper edge of the activated plate which marked the limit of solvent flow. The spotted plate was then placed in the chromatographic tank containing the selected solvent system system. When the deve-

loping mobile phase reached the premarked point, the plate was taken out and dried in the air.

3.3.10.7. Detection of components :

The properly developed and dried plate was treated with Vanillin-sulfuric acid and heated at 110°C for 15 minutes to detect the number of component(s) separated.

3.3.10.8. Detection of nitrogenous compounds :

For detection of nitrogenous compound(s) the properly developed and dried plate was sprayed with modified Dragendroff's reagent and heated at 110°Cfor 15 minutes. The separated zones indicating the presence of nitrogenous compounds probably alkaloids.

The compounds were compared by calculating the Rf values of the individual compound.

3.3.10.9. Calculation of Rf value :

The Rf values of the separated compounds were determined using the following formula :

$$\text{Rf value} = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent}}$$

3.3.10.10. Preparation of spray reagent :

a) Preparation of Vanilin-sulfuric acid :

Vanilin-sulfuric acid spray reagent was prepared by dissolving Vanillin (1 g) in concentrated sulfuric acid (100 ml).

b) Preparation of modified Dragendroff's reagent (Munier and Machebouf modification) :

Solution (i) : Basic bismuth subnitrate (0.85 g) was dissolved in a solution of acetic acid (10 ml) and distilled water (50 ml).

Solution (ii) : Potassium iodide (8 g) was dissolved in distilled water (20 ml).

Stock solution : It was prepared by mixing equal volumes of solution (i) and (ii).

Spray reagent : Modified Dragendroff's reagent was prepared by mixing stock solution, acetic acid and distilled water in a proportion of 1 : 2 : 10.

3.3.11. Tissue culture :

3.3.11.1. Preparation of stock solution :

The first step in the preparation of the medium was the preparation of stock solution of the various constituents of the medium.

i) Stock solution A (major constituent / macronutrient) :

It was made up to 10 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 $4^{o}C$.

ii) Stock solution B (Micronutrients) :

It was made at 10 times the final strength of the medium in 100 ml of distilled water as described for the stock solution A. In this case $FeSO_4$ and Na_2EDTA were separately dissolved in 100 ml distilled water in a 250 ml conical flask and kept for 24 hrs at $58^{o}C$ 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 $4^{o}C$.

iii) Stock solution C (organic constituents) :

This was also made at 10 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 preparation of medium in desired concentrations.

iv) Hormonal stock solution :

Phytohormones namely, napthalene acetic acid (NAA), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 6-furfuryl amino purine (kinetin/kn), benzyl amino purine (BAP) were dissolved in appropriate solvent. Solvent used against each hormone has been shown below :

Hormone	Solvent
NAA	: 0.1 N NaOH
IAA	: 70 % ethyl alcohol
kinetin	: 0.1 N HCl
BAP	: 0.1 N NaOH
IBA	: 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 $0^{o}C$.

3.3.11.2. Preparation of medium :

Final medium was prepared with distilled water using the above stocks. Supplements to the basal medium were added prior to final adjustment of the volume. Using 0.5 M NaOH or 0.5 M HCl pH was adjusted to 5.8 with a digital pH meter. Medium was sterilized following the solidified with 0.8 % bactoagar.

Fixed volumes of hot medium were transferred to culture tubes / flasks. The culture vessels were plugged with non-absorbent cotton wool. The vessels were then autoclaved at 15 lbs per inch pressure at $120^{o}C$ for 20 minutes.

All inoculations and aseptic manipulations were carried out in a laminar flow cabinet.

3.3.11.3. Explant culture :

For study the regenerating ability of the explants namely, plumule with / without cotyledon and cotyledons of 17-hr old germinating seeds of the F_1, F_2 and their parents of the cross, Grafted C. olitorius var. O-4 x Grafted C. capsularis var. D-154 were used. The explants were inoculated aseptically in MS medium supplemented with different combinations and concentrations of hormone.

3.3.11.4. Preparation of explants :

A few seeds of each material were sterilized in 0.1 %

$HgCl_2$ for 20 minutes and after thorough washing they were placed in sterile test tubes containing enough sterile distilled water to just cover the seeds. On the following morning i.e. after 17-18 hrs most of the seeds were found to germinate. Since the seedlings with intact roots produced plantlets without any callusing in MS medium, root apices (0.5 mm) were removed from the seedlings ; such seedlings without root-tip were cut into different parts namely, plumule with or without cotyledon, cotyledons, roots etc. These 0.5-1.0 mm long segments were then used as explants in the MS medium supplemented with BAP, IBA and tyrosine.

3.3.11.5. Embryo culture :

Embryos were aseptically dissected out from 12-15 hr presoaked hybrid seeds and their parents. Using a laminar flow cabinet dissected embryos were carefully transferred to different nutrient media namely, White's (1943), ½ MS and Murashige and Skoog (1962) medium solidified with 0.8 % agar.

R E S U L T S

R E S U L T S

4.1 Production of hybrids :

For the production of viable hybrids different cross combinations and techniques namely, straight cross, cross between reciprocally grafted plants, cross using different concentrations of IAA in lanolin paste around the pedicel of the pollinated flowers and embryo culture etc. were attempted. The results of these cross combinations are described below in detail under different heads.

4.1.1 Straight cross :

Crosses were made between the ungrafted parents of C. olitorius and C. capsularis without application of hormone around the pedicel of the pollinated flowers. There was altogether 8 cross combinations including reciprocals. Two cultivars of C. olitorius namely, O-4 and C.G. and two cultivars of C. capsularis (D-154 and CVL-1) were used in this cross combination. Using C. olitorius as the female parent 77 crosses were made, in the reciprocal direction 69 crosses were made. Fruit set was obtained only when C. olitorius was used as female. Fruit set percentage varied among the different cross combinations.

Table 1. Summary of the reciprocal crosses, C. olitorius x C. capsularis without application of hormone.

Cross combination	No. of flowers pollinated	No. of fruit set, % in parenthesis	No. of fruits harvested	Total no. of seeds	No. of full seeds
1. O-4 x D-154	18	2 (11.11)	2	52	-
2. Reciprocal	16	-	-	-	-
3. C.G. x D-154	19	-	-	-	-
4. Reciprocal	18	-	-	-	-
5. O-4 x CVL-1	20	4 (20)	2	74	-
6. Reciprocal	18	-	-	-	-
7. C.G. x CVL-1	20	2 (10)	2	58	-
8. Reciprocal	17	-	-	-	-

O-4 and C .G. are cultivars of C. olitorius while D-154 and CVL-1 are cultivars of C. capsularis.

Maximum fruit set was recorded in the combination of O-4 x CVL-1 (20 %) while in the combinations of O-4 x D-154 and C.G. x CVL-1 the percentage of fruit set was 11.11 and 10 respectively. There was no fruit set in the combination of C.G. x D-154 (Table 1). Shape and size of the hybrid fruits varied from 2.9 to 4.3 (Fig. 3). Only 6 fruits were harvested from this cross combination and seeds per fruit varied from 21 to 48. A total of 184 hybrid seeds were obtained from the above combinations but all of the seeds were shrivelled and did not germinate even in nutrient media. On dissection the shrivelled seeds were found to be devoid of any embryo.

4.1.2 Cross between grafted parents without hormone :

Production of grafted plants : Using the following combinations a total of 211 grafts including reciprocals were made in 1984. The first named parent in the combination is the root stock and the latter is the scion.

i) C. olitorius var. O-4 + C. capsularis var. D-154

ii) C. olitorius var. O-4 + C. capsularis var. CVL-1

iii) C. olitorius var. C.G. + C. capsularis var. D-154

iv) C. olitorius var. C.G. + C. capsularis var. CVL-1

Fig. 3

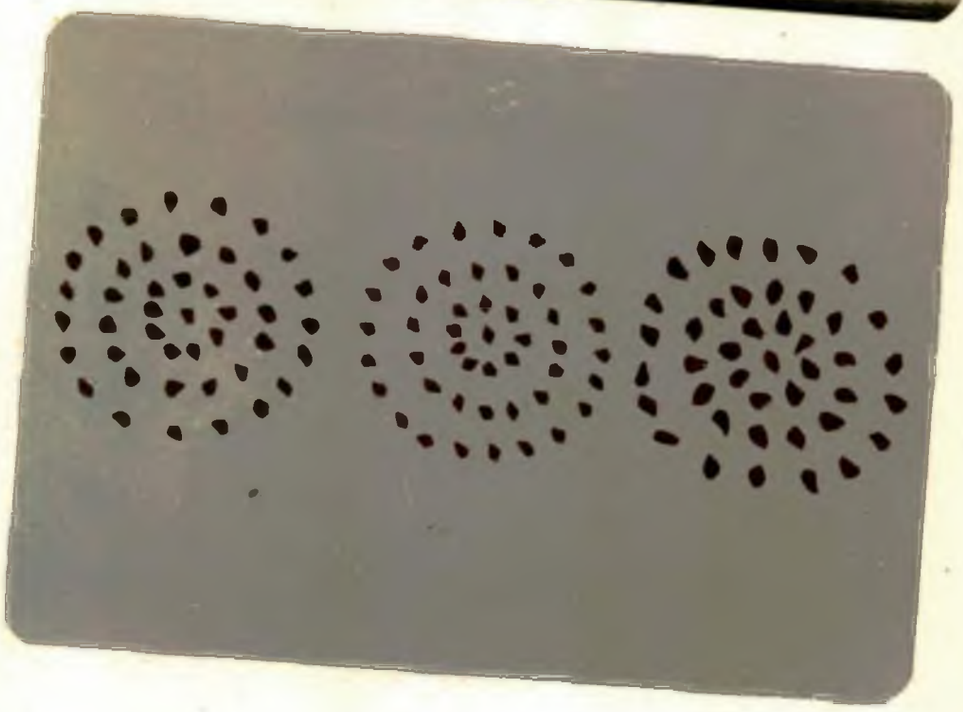


Fig. 4

Out of 211 grafts, a total of 130 grafts survived up to maturity (61.61 %). Details of these graft combinations and their survival percentage is given in table 2.

Survival was found to be highest (73.33 %) in which C. capsularis var. CVL-1 was grafted on to the stock of C. olitorius var. O-4 and lowest (52 %) in the combination in which C. olitorius var. C.G. was grafted on to the stock of C. capsularis var. D-154. It has been observed that C. olitorius var. O-4 was more efficient as the root stock than all of C. capsularis.

Results of the cross between grafted parents without application of hormone around the pedicel of the pollinated flowers have been summarised in table 3. The table shows that crosses were made in six combinations including reciprocals. Using C. olitorius as female, 75 crosses were made while in the reciprocal the number of crosses were 35. The results clearly indicate that fruit set increased when reciprocally grafted plants were used. However, the number of fruit set varied from cultivar to cultivar within the same species, highest percentage (45 %) having been observed in the combination of CVL-1 + O-4 x

Table 2. Survival percentage of the grafted plants.

Graft combination	No. of grafts made	No. of surviving grafts	% of survival
1. C. olitorius var. O-4 + C. capsularis var. D-154	30	21	70.0
2. Reciprocal	30	18	60.0
3. C. olitorius var. C.G. + C. capsularis var. D-154	25	14	56.0
4. Reciprocal	25	13	52.0
5. C. olitorius var.O-4 + C. capsularis var. CVL-1	30	22	73.33
6. Reciprocal	24	15	62.5
7. C. olitorius var. C.G. + C. capsularis var. CVL-1	24	14	58.0
8. Reciprocal	22	12	54.54

The first mentioned parent is root stock and the one following is scion. O-4, C.G. are cultivars of C. olitorius while D-154 and CVL-1 are cultivars of C. capsularis.

O-4 + CVL-1. In other combinations namely, D-154 + O-4 x O-4 + D-154 ; D-154 + C.G. x C.G. + D-154 and CVL-1 + C.G. x C.G. + CVL-1 fruit set percentage was observed to be 38.89 ; 22.22 and 26.31 respectively (Table 3).

Fruit set was relatively lower when C. capsularis was grafted on to the stock of C. olitorius and used as female. Reciprocal crosses were made only in two combinations namely, O-4 + D-154 x D-154 + O-4 and O-4 + CVL-1 x CVL-1 + O-4. Fruit set was 18.75 % in the former while 15.78 % in the latter combination.

Fruit size from the above crosses varied a good deal from large to small. The no. of seeds/fruit ranged from 35 to 89 when reciprocally grafted C. olitorius was the female while in the reciprocal direction it was 15 to 32.

Like those from the straight cross all the hybrid seeds in the grafted material were shrivelled. Seed germination was tried in the soil, vermiculite and nutrient media. When none of the hybrid seeds germinated in soil, vermiculite and nutrient media a few from the remaining portion were dissected and found to be empty (devoid of any embryo).

Table 3. Summary of the reciprocal crosses between reciprocally grafted C. olitorius and C. capsularis without application of hormone.

Cross combination	No. of flowers pollinated	No. of fruit set % in parenthesis	No. of fruits harvested	Total no. of seeds	No. of full seeds
1. D-154 + O-4 x O-4 + D-154	18	7 (38.89)	5	205	-
2. Reciprocal	16	3 (18.75)	2	56	-
3. D-154 + C.G. x C.G. + D-154	18	4 (22.22)	3	125	-
4. CVL-1 + O-4 x O-4 + CVL-1	20	9 (45.0)	6	286	-
5. Reciprocal	19	3 (15.78)	3	56	-
6. CVL-1 + C.G. x C.G. + CVL-1	19	5 (26.31)	3	172	-

Each combination is a graft in which the first mentioned parent is root stock and the one following is scion. O-4, C.G. are cultivars of C. olitorius while D-154 and CVL-1 are cultivars of C. capsularis.

4.1.3 Cross between ungrafted parents with different concentrations of IAA in lanolin paste :

Following the report by Islam and Rashid (1961), Islam (1964), Ghosal and Bajaj (1983), Raut and Naik (1983) in their successful application of hormone for enhancing fruit set, reciprocal crosses were made between the two species using different concentration of IAA namely, 200, 300 and 400 mg/l in lanolin paste around the pedicel of the pollinated flowers.

4.1.3.1 Effect of 200 mg/l IAA in fruit set and viable seed production :

Reciprocal crosses were made in two combinations using 200 mg/l IAA in lanolin paste around the pedicel of the pollinated flower. Fruit set was observed in both the combinations in variable frequency. Fruit set was more or less similar in the cross combination when C. olitorius was used as female. In the O-4 x D-154 fruit set percentage was 26.31 while in the combination O-4 x CVL-1 it was 25 %. In the reciprocal direction , fruit set percentage was 11.76 (D-154 x O-4) and 15(CVL-1 x O-4) (Table 4).

Fruits obtained from this combination were variable in size. Number of seeds/fruit ranged from 16 to 46 when C. olitorius was female but in reciprocal direction it was

Table 4. Summary of the reciprocal crosses between C. olitorius and C. capsularis with the application of 200 mg/l IAA.

Cross combination	No. of flowers pollinated	No. of fruit set % in parenthesis	No. of fruits harvested	Total no. of seeds	No. of full seeds
1. O-4 x D-154	19	5 (26.31)	2	82	-
2. Reciprocal	17	2 (11.76)	2	46	-
3. O-4 x CVL-1	16	4 (25.0)	1	16	-
4. Reciprocal	20	3 (15.0)	2	68	-

CVL-1 and D-154 are cultivars of C. capsularis while O-4 is the cultivar of C. olitorius.

found to be 21 to 38.

Like the seeds of the straight cross none of the seeds were fully developed and did not produce any hybrid seedling.

4.1.3.2 Effect of 300 mg/l IAA in viable seed production :

Reciprocal crosses were made in 8 combinations between the two cultivars of both the species using 300 mg/l IAA in lanolin paste around the pedicel of the pollinated flowers. Fruit set was observed in all the combinations. A total of 73 crosses were made using C. olitorius as female while in the reciprocal direction 55 crosses were made.

Maximum fruit set (42 %) was observed in the combination of O-4 x CVL-1, while in the other three combinations where C. olitorius was female namely, O-4 x D-154 ; C.G. x D-154 and C.G. x CVL-1 percentage of fruit set was 35, 30 and 31.25 respectively.

In the reciprocal direction highest percentage of fruit set was recorded in the combination of CVL-1 x O-4 (27.78 %) and in the other combinations viz. D-154 x O-4; D-154 x C.G. and CVL-1 x C.G. it was 16.67 ; 23.52 and 14.28 respectively (Table 5)

Table 5. Summary of the reciprocal crosses, C. olitorius x C. capsularis with the application of 300 mg/l IAA.

Cross combination	No. of flowers pollinated	No. of fruit set % in parenthesis	No. of fruits harvested	Total no. of seeds	No. of full seeds
1. O-4 x D-154	20	7 (35.0)	3	165	12
2. Reciprocal	18	3 (16.67)	2	38	-
3. C.G. x D-154	18	6 (33.33)	4	196	-
4. Reciprocal	17	4 (23.52)	3	58	-
5. O-4 x CVL-1	19	8 (42.0)	5	274	7
6. Reciprocal	18	5 (27.78)	1	26	-
7. C.G. x CVL-1	16	7 (31.25)	4	179	14
8. Reciprocal	14	2 (14.28)	-	-	-

O-4 and C.G. are cultivars of C. olitorius while D-154 and CVL-1 are cultivars of C. capsularis.

Seed size resulted from both the combinations varied (Figs. 4 & 5). Number of seeds/fruit ranged from 38 to 96 where C. olitorius was the female. In the reciprocal direction number of seeds/fruit ranged from 15 to 26.

A few fully developed seeds were obtained from three combinations namely, O-4 x D-154 (12); O-4 x CVL-1 (7) and C.G. x CVL-1 (14).

When germination of hybrid seeds did not take place in sterilized soil and sterilized vermiculite, two seeds from each of the above combinations were cultured in White's (1943) nutrient medium. Out of six seeds cultured only two , one from each of the combination of O-4 x D-154 and C.G. x CVL-1 germinated. Both plantlets died one after two and the other after 4 days following transplantation (Table 8).

4.1.3.3 Effect of 400 mg/l IAA in viable seed production:

Crosses were made between the ungrafted parents of C. olitorius and C. capsularis with the application of 400 mg/l IAA around the pedicel of the pollinated flowers. There was altogether four cross combinations including reciprocals. O-4 (C. olitorius) and D-154, CVL-1 (C. capsularis) were used in the cross. Using C. olitorius as female 39 crosses were made while in the reciprocal

Table 6. Summary of the reciprocal crosses between C. olitorius and C. capsularis with the application of 400 mg/1 IAA.

Cross combination	No. of flowers pollinated	No. of fruit set % in parenthesis	No. of fruits harvested	Total no. of seeds	No. of full seeds
1. O-4 x D-154	20	3 (15.0)	2	29	-
2. Reciprocal	17	2 (11.76)	2	21	-
3. O-4 x CVL-1	19	3 (15.78)	2	42	-
4. Reciprocal	18	2 (11.76)	2	27	-

O-4 is the cultivar of C. olitorius while D-154 and CVL-1 are cultivars of C. capsularis.

Fig. 5

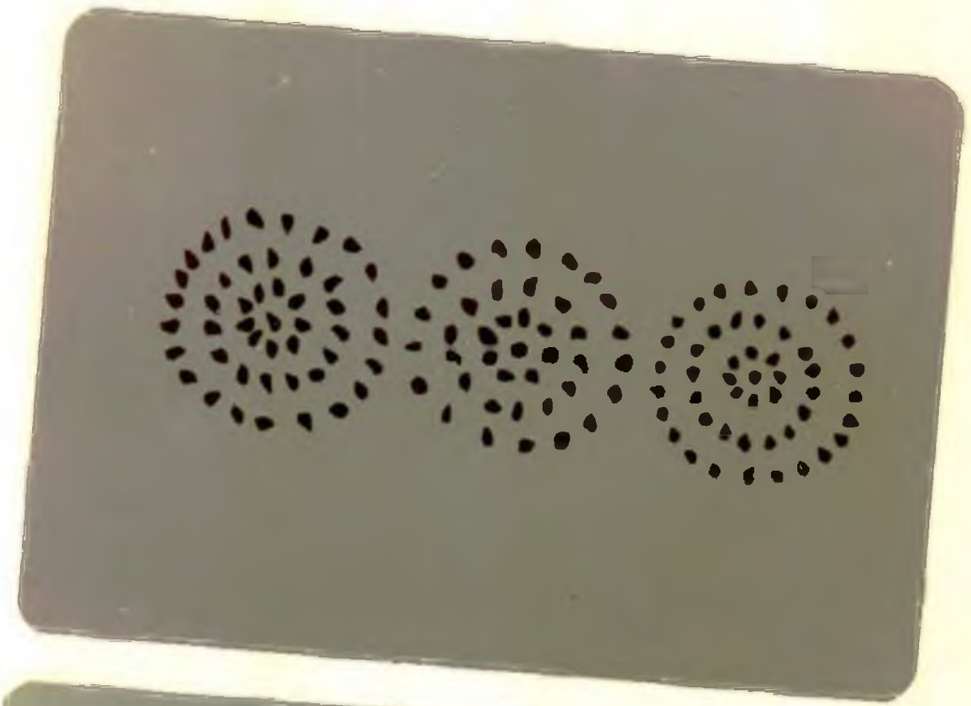


Fig. 6

direction 35 crosses were made. Fruit set was observed from the three combinations namely, O-4 x D-154 ; D-154 x O-4 and O-4 x CVL-1. Fruit set percentage was more or less same in all the combinations (Table 6). Unlike in lower concentration of IAA, not only the fruit set but seeds/fruit were greatly reduced (Fig. 6).

A total of 71 seeds were obtained from the cross in which C. olitorius was the female parent. Only 48 seeds were obtained by using C. capsularis as female. Seeds obtained from all the cross combinations were shrivelled and on dissection the seeds were found to be devoid of any embryo.

The results show clearly that of the three concentrations of IAA used that of 300 mg/l was found to be the best to stimulate fruit and seed development in the cross.

4.1.4 Combined effect of grafting and hormonal treatment on hybrid seed development :

The results of the above cross combinations have been summarised in table 7. The table shows that crosses were made altogether in six combinations including reciprocals. Using C. olitorius as female (scion) a total of 56 crosses were made while in the reciprocal direction the number of crosses were 51.

Table 7. Summary of the reciprocal crosses between reciprocally grafted C. olitorius and C. capsularis with the application of 300 mg/1 IAA.

Cross combination	No. of flowers pollinated	No. of fruit set % in parenthesis	No. of fruits harvested	Total no. of seeds	No. of full seeds
1. D-154 + O-4 x O-4 + D-154	19	10 (52.63)	6	262	45
2. Reciprocal	17	4 (23.52)	2	38	-
3. D-154 + C.G. x C.G. + D-154	18	5 (27.78)	4	191	21
4. Reciprocal	18	3 (16.67)	2	47	-
5. CVL-1 + O-4 x O-4 + CVL-1	19	11 (57.89)	5	215	29
6. Reciprocal	16	4 (25.0)	2	61	-

Each combination is a graft in which the first mentioned parent is root stock and the one following is scion. O-4 , C.G. are cultivars of C. olitorius while D-154 and CVL-1 are cultivars of C. capsularis.

The combined use of grafted material and hormone enhanced both fruit set and seed development compared to when crossing was done with only one treatment (Figs. 7 & 8). Fruit set was obtained in all the cross combinations. Maximum fruit set was observed when reciprocally grafted C. olitorius was used as female. Percentage of fruit set varied within the same species, highest percentage (57.59) of fruit set having been observed from the cross, CVL-1 + O-4 x O-4 + CVL-1. In the other combinations namely, D-154 + O-4 x O-4 +D-154 and D-154 + C.G. x C.G. + D-154 fruit set percentage was 52.63 and 27.78 respectively.

In the reciprocal direction viz. O-4 + D-154 x D-154 + O-4 ; C.G. + D-154 x D-154 + C.G. and O-4 + CVL-1 x CVL-1 + O-4, fruit set percentage was 23.52 ; 16.67 and 25 respectively.

Full seeds were obtained only from three combinations where reciprocally grafted C. olitorius was the female parent. The three combinations are D-154 + O-4 x O-4 + D-154 ; D-154 + C.G. x C.G. + D-154 and CVL-1 + O-4 x O-4 + CVL-1 and number of full seeds obtained from each combination are 45 ; 21 and 29 respectively.

As reported earlier by Islam and Rashid (1960, 61),

Fig.7. Two hybrid fruits resulting from the cross, reciprocally grafted C. olitorius var. O-4 (♀) x C. capsularis var. D-154 (♂) with the application of 300 mg/l IAA. x .8

Fig.8. Two hybrid fruits resulting from the cross, reciprocally grafted C. capsularis var. D-154 (♀) x C. olitorius var. O-4 (♂) with the application of 300 mg/l IAA around the pedicel of the pollinated flowers. x .8

Fig. 7

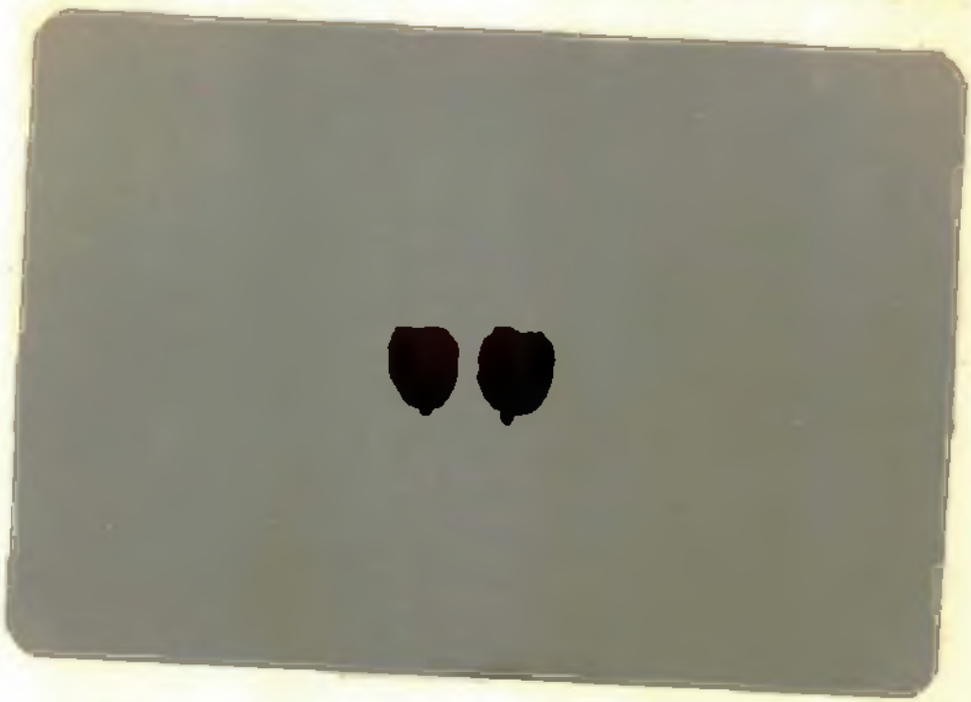


Fig. 8

Swaminathan and Iyer (1961), Haque (1970), Raut and Naik (1983) no full seed was obtained from reciprocal direction (Table 7). Bhaduri and Bairagi's report (1968) of success in the reciprocal direction deserve mention here. There was no doubt that their success could be attributed to their choice of capsularis variety which was different from the cultivars used in the present investigation.

Germination of seeds was tried in sterilized soil, sterilized vermiculite and White's medium. Out of 11 seeds sown in the sterilized vermiculite only two germinated from the combination of CVL-1 + O-4 x O-4 + CVL-1. The seedlings were very weak and died during transplantation.

In White's medium out of 11 seeds cultured three germinated from the combination of D-154 + O-4 x O-4 + D-154 ; two from the combination D-154 + C.G. x C.G. + D-154 and only one from the combination CVL-1 + O-4 x O-4 + CVL-1. It has not been possible to obtain any hybrid plants from these germinated seedlings. Unlike the normal selfed, seed coat of the hybrid seeds did not open easily and it remained stuck with cotyledons for a longer period. Out of six seeds which germinated in the White's medium two were contaminated and other four died within three to seven days after their transplantation into the soil.

Table 8. Germination of hybrid seeds in different germinating media.

Cross combination	Germinating media								
	Soil			Vermiculite			White madium		
	No. of seeds sown	No. of seeds germinated	No. of adult plants	No. of seeds sown	No. of seeds germinated	No. of adult plants	No. of seeds sown	No. of seeds germinated	No. of adult plants
1. O-4 x D-154 (+)	-	-	-	2	-	-	2	1	-
2. O-4 x CVL-1 (+)	-	-	-	-	-	-	2	-	-
3. C.G. x CVL-1(+)	2	-	-	2	-	-	2	1	-
4. D-154 + O-4 (+) x O-4 + D-154	5	-	-	5	2	-	5	3	-
5. D-154 + C.G.(+) x C.G. + D-154	3	-	-	3	-	-	3	2	-
6. CVL-1 + O-4 (+) x O-4 + CVL-1	3	-	-	3	1	-	3	1	-

(+) in parenthesis means 300 mg/l IAA in lanolin paste was applied around the pedicel of the pollinated flowers. O-4, C.G. are cultivars of C. olitorius while CVL-1 and D-154 are cultivars of C. capsularis. Combinations 4,5 and 6 are grafts in which first mentioned parent is root stock and the one following is scion.

4.1.5 Embryo culture :

Encouraged by the reports of Islam (1964), Gosal and Bajaj (1983), Raut and Naik (1983) of successful development of embryo into mature plantlets from some difficult interspecific crosses, the present experiment was undertaken.

Embryos were dissected out from the 12-hrs presoaked sterilized hybrid seeds obtained from different combinations of reciprocally grafted crosses with 300 mg/l IAA. Embryos were cultured in ½ MS , MS and White's nutrient media. Supplements namely, 100 mg/l adenine sulphate (AdS) and 0.2 mg/l IBA were added in ½ MS and MS media. No supplement was added to White's medium.

Germination percentage of the embryos was more or less same in all the nutrient media. But development of the embryos in different media varied. Half strength MS medium containing 100 mg/l AdS and 0.2 mg/l IBA was comparatively better than in the other two media (Fig. 9). In MS and White's media plantlet development was rapid but the plantlets were relatively weaker than the plantlets developed in ½ MS medium. None of the plantlets developed up to maturity except two from the combination of D-154 + O-4 x O-4 + D-154 in ½ MS medium (Table 9) and they were serially numbered as E/84-1 and E/84-2.

Table 9. Response of different nutrient media on germination of hybrid embryo. Supplements 100 mg/lAdS and 0.2 mg/l IBA were common in ½ MS and MS media.

. Cross combination	Medium	No. of embryos inoculated	No. of embryos germinated	No. of adult plants
1. O-4 x D-154 (+)	½ MS	2	2	-
	MS	2	1	-
	White	2	1	-
2. O-4 x CVL-1 (+)	½ MS	2	1	-
	MS	-	-	-
	White	2	1	-
3. C.G. x CVL-1 (+)	½ MS	2	2	-
	MS	2	1	-
	White	2	-	-
4. D-154 + O-4 (+)	½ MS	5	4	2
x	MS	5	3	-
O-4 + D-154	White	5	4	-
5. CVL-1 + O-4 (+)	½MS	4	4	-
x	MS	4	2	-
O-4 + CVL-1	White	4	3	-
6. D-154 + C.G. (+)	½ MS	3	2	-
x	MS	3	1	-
C.G. + D-154	White	3	2	-

(+) in parenthesis means 300 mg/l IAA in lanolin paste was applied around the pedicel of the pollinated flowers. O-4, C.G. are cultivars of C. oliterius while CVL-1 and D-154 are cultivars of C. cansularis. Combinations 4.5,and 6 are grafts in which the first mentioned parent is root stock and the one following is scion.

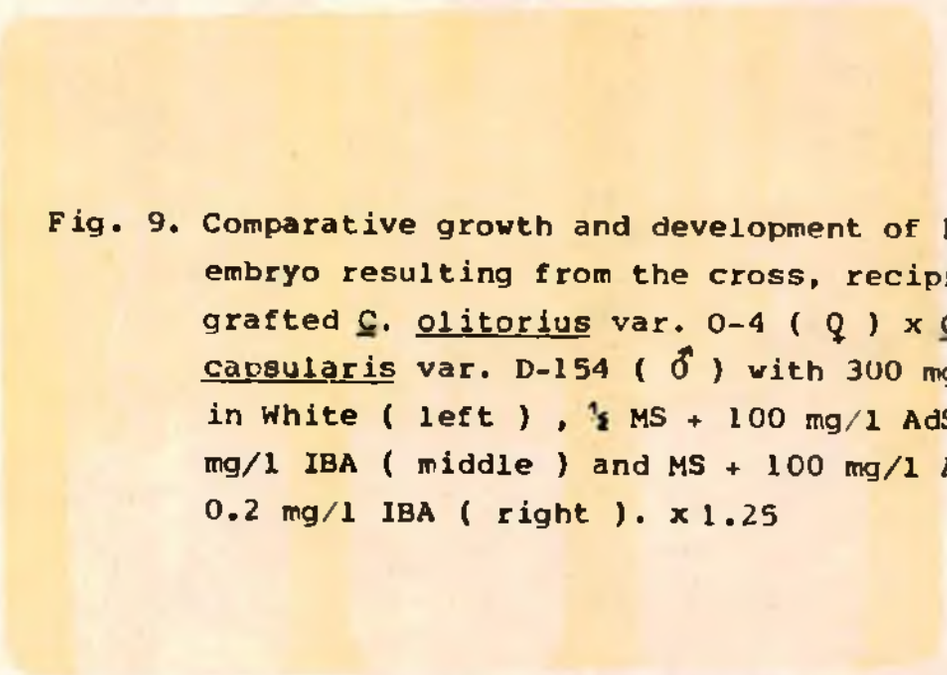


Fig. 9. Comparative growth and development of hybrid embryo resulting from the cross, reciprocally grafted C. olitorius var. O-4 (♀) x C. capsularis var. D-154 (♂) with 300 mg/l IAA in White (left) , ½ MS + 100 mg/l AdS + 0.2 mg/l IBA (middle) and MS + 100 mg/l AdS + 0.2 mg/l IBA (right). x 1.25

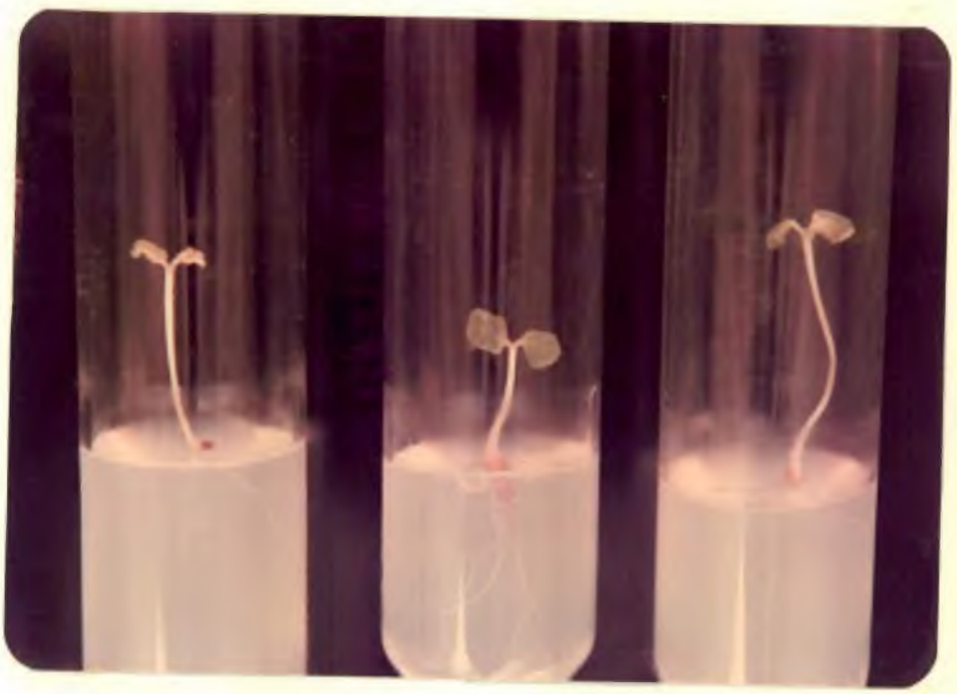


Fig. 9

4.1.6 Transfer of seedlings from test tube to pot :

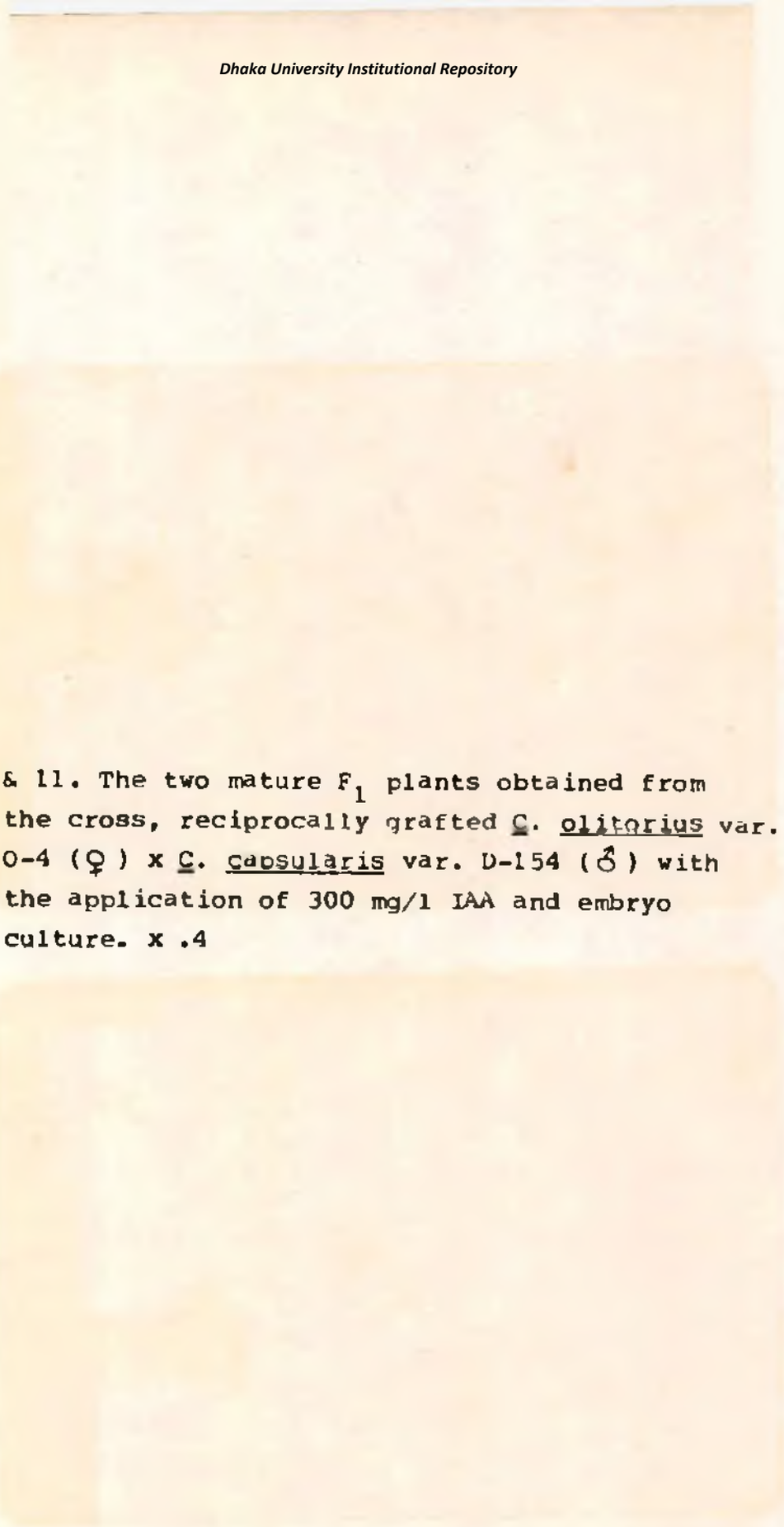
After one months the seedlings were carefully removed from the test tubes and after washing the roots in sterile distilled water, they were transferred to pots of 6" dia and covered with polythene bag . On the 6/7 th day the polythene bag were perforated to allow good ventilation. The polythene bag was removed every day gradually increasing the exposure time till at last the bag was finally removed on the 15 th day. After a month they were transferred to a bigger pot where they grew to maturity.

4.2 Morphological characters of the F_1 progeny :

Morphological characters of the F_1 plants namely, plant habit, B/L ratio and serration of leaf, flower, fruits, seed were recorded alongwith their parents. Results of these observations have been summarised and presented in table 10. Brief description of the above characters is given below under different heads.

Plant habit : It has been observed in table that unlike both the parents the F_1s showed branching habit (Figs. 10 & 11).

Leaf : B/L ratio of the leaf of 0-4 (C. olitorius)



Figs.10 & 11. The two mature F_1 plants obtained from the cross, reciprocally grafted C. olitorius var. O-4 (♀) x C. capsularis var. D-154 (♂) with the application of 300 mg/l IAA and embryo culture. x .4

Fig. 11
(E/84-2)

Fig. 10
(E/84-1)

Table 10. Comparison of morphological characters of the F_1 hybrid with the parents, C. olitorius and C. capsularis. (All measurements in cm unless otherwise mentioned)

Characters	C. olitorius	F_1 hybrid	C. capsularis
1. Plant height	335-378	165-170	315-340
2. Base diameter	2.6 ± 0.3	2.2	2.9 ± 0.2
3. Leaf			
a) Stipule	comparatively large	Intermediate	small
b) B/L ratio	0.46	0.52	0.56
c) Serration	Serrated	Serrated	Dentate
4. Flower			
a) Bud size	Large	Intermediate	Small
b) Petal shape and size	Large, entire	Intermediate	Smaller than C. olitorius
c) Colour	Deep yellow	Pale yellow	Yellow
d) Pollen dia	37.0 ± 2.4	33.2 ± 2.8	32.0 ± 2.4
e) PG stainability %	98-100	64-78	96-99

(cont...)

Table 10 continued.

Characters	C. olitorius	F_1 hybrid	C. capsularis
5. Fruit			
a) No. at each node	1-2, mostly one	1-2	2-3 or more
b) Shape & size (range)	Elongated, 6.85-7.50	Elongated but variable in size, 3.2-6.20	Spherical,1.12
c) No. of ribs	Ten	Ten like C. olitorius, ridge irregular	Irregular
d) No. of seeds/capsule	180-260	32-180	30-40
e) Wt. of 100 seeds in g (average of 10 lots)	0.23	0.26	0.31
f) Colour of the seeds	Black with greenish tinge	Pale chocolate	Chocolate

was found to be 0.46 and that of D-154 (C. capsularis) 0.56. In the F_1 this ratio was 0.52 showing near dominance of the male parent. In the hybrid the serration was like that of the female parent.

Flower : Different characters of flower namely, bud size, petal shape and size, colour of flowers, pollen dia and pollen grain stainability were recorded. Bud size, petal shape and size and colour of the flowers were found to be intermediate between the two parents and were closer to the male parent, C. capsularis.

Pollen grain : The pollen grains of F_1s were variable in size and their stainability ranged from 64-78 % compared to 98-100 % in C. olitorius and 96-99 % in C. capsularis (Fig. 12).

Fruit : A good deal of variation was observed not only among the two F_1s but within each F_1 individual in respect of fruit shape and size, no. of seeds/capsule, shape of the ribs. Dominance of the female parent was observed in respect of fruit shape, no. of ribs. However, the ribs were not as regular as in the female parent suggesting influence of male character. As regards the no. of seeds in the two F_1 hybrids, the number was mostly intermediate. It is to be noted that reduction in number

Fig.12. Pollen grains of one of the F_1 hybrids shown in Fig. 10 & 11 showing stained and non-stained pollen as well as their variation in shape and size. x ca 1500

Fig. 12

of seeds is also due to pollen sterility. Weight of 100 seeds was recorded to be 0.23 g in C. olitorius and 0.31 g in C. capsularis while in F_1 it was 0.26 g.

Seed colour : Compared to greenish black seeds of the female parent and chocolate seed of the male, both F_1s contained pale chocolate seeds indicating near dominance of the latter.

4.3 Morphological characters of the F_2 progeny :

In table 11 a comparative account of main morphological characters of the F_2 population together with their parents has been presented. Some of the salient points shown in the table have been discussed below :

A casual observation of the F_2s suggests that the plants are maternal type but a close look reveals that in a number of characters they are different from the female parent.

Plant height : In the F_2 plant height was 340-370 cm while in the female it was 335-378 cm and 315-340 cm in the male. Unlike the two F_1s which grew only to a height of 165 and 170 cm, none of the F_2s were stunted in growth.

Leaf : In B/L ratio of the leaf dominance of the female was observed. However, some of the F_2s showed

Table 11. Comparison of morphological characters of the F_2 with the parents, C. olitorius and C. capsularis (measurements in cm unless otherwise mentioned).

Characters	C. olitorius	O/C F_2*	C. capsularis
1. Plant height	335-378	340-370	315-340
2. Base dia	2.5 ± 0.3	2.6 ±0.2	2.8 ±0.2
3. Leaf			
a) Stipule	Large	Intermediate	Small
b) B/L ratio	0.46	0.48	0.54
4. Flower			
a) Bud size	Large	Smaller than C. olitorius	Small
b) Petal shape & size	Large	Smaller than C. olitorius	Small
c) Pollen dia	37.6 ±2.4	33.8 ±2.3	32.0 ± 2.4
d) PG stainability	98 - 100	86 - 91	96 - 99

(cont...)

Table 11 continued .

Characters	C. olitorius	O/C F_2*	C. capsularis
5. Fruit			
a) Shape & size (range)	Elongate, 6.85-7.50	Elongate, variable in size, 2.3-7.9	Spherical, 1.2
b) No. of ribs	Ten ribbed	Mostly ten ribbed, ribs irregular	Irregular
c) No. of seeds / capsule	180-260	72-206	30-40
d) Colour of seeds	Black with greenish tinge	Pale chocolate to light black	Chocolate
e) Wt. of 100 seeds in g (average of 10 lots)	0.23	0.25	0.31

* O/C F_2 = C. olitorius x C. capsularis, F_2.

abnormal leaves not conforming to B/L ratio of either parent. Abnormal leaves were observed in 12 out of 179 F_2 plants. These abnormalities included dissected, bifurcated, characteristically small sized leaves (Fig. 13). These abnormal leaves were observed only at the third / fourth leaf stage. Moreover leaves of various shape were also found in some of the adult plants.

Flower : The flowers and petals of the F_2s were larger than the male parent indicating near dominance of the female parent. Pollen dia was found intermediate between the two parents. Pollen grain stainability considerably increased in the F_2 and the percentage ranged from 86-91 compared to 64-78 % in the two F_1s.

Fruit : The fruit shape which showed dominance of the female parent in the F_1 did not segregate in the F_2 to produce any individual with the recessive spherical fruit shape character of the male parent. However, a good deal of variation in the size and shape of fruits was noticed. There were individuals with as elongated near the female, some characterised by the fruit shape of the F_1s and some still smaller with constriction appearing in the middle and in the top portion of the fruit. The shape and

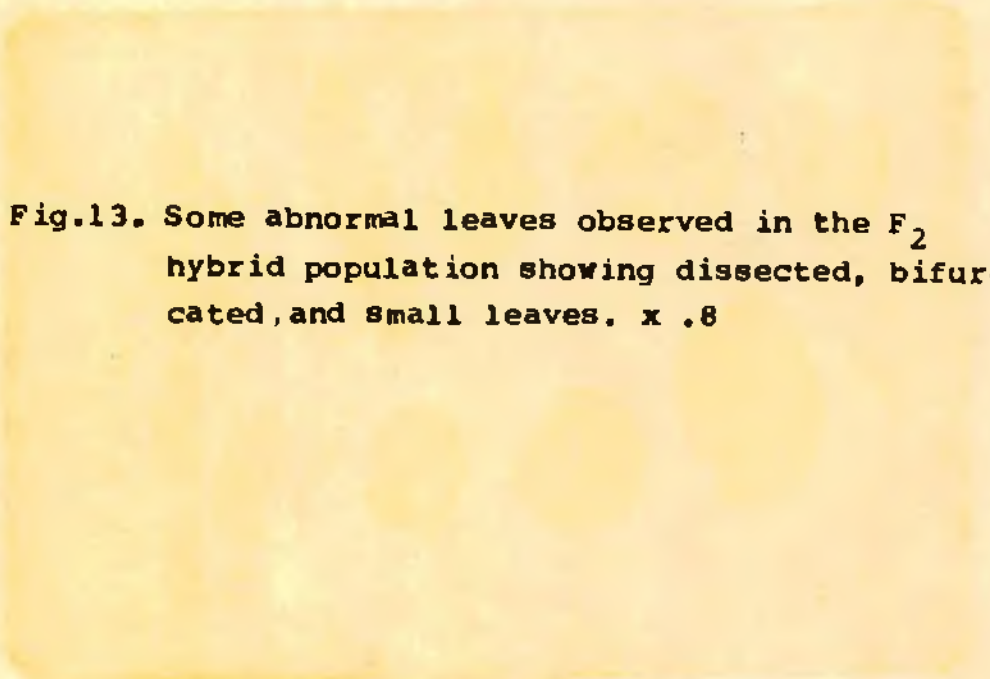


Fig.13. Some abnormal leaves observed in the F_2 hybrid population showing dissected, bifurcated, and small leaves. x .8

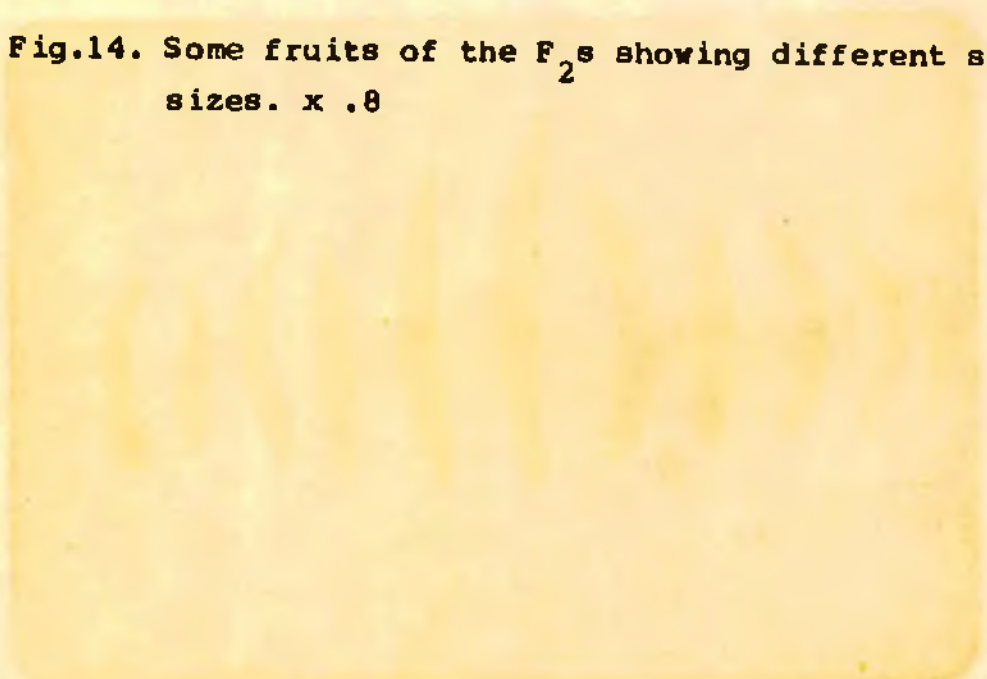


Fig.14. Some fruits of the F_2s showing different shape sizes. x .8

Fig. 13

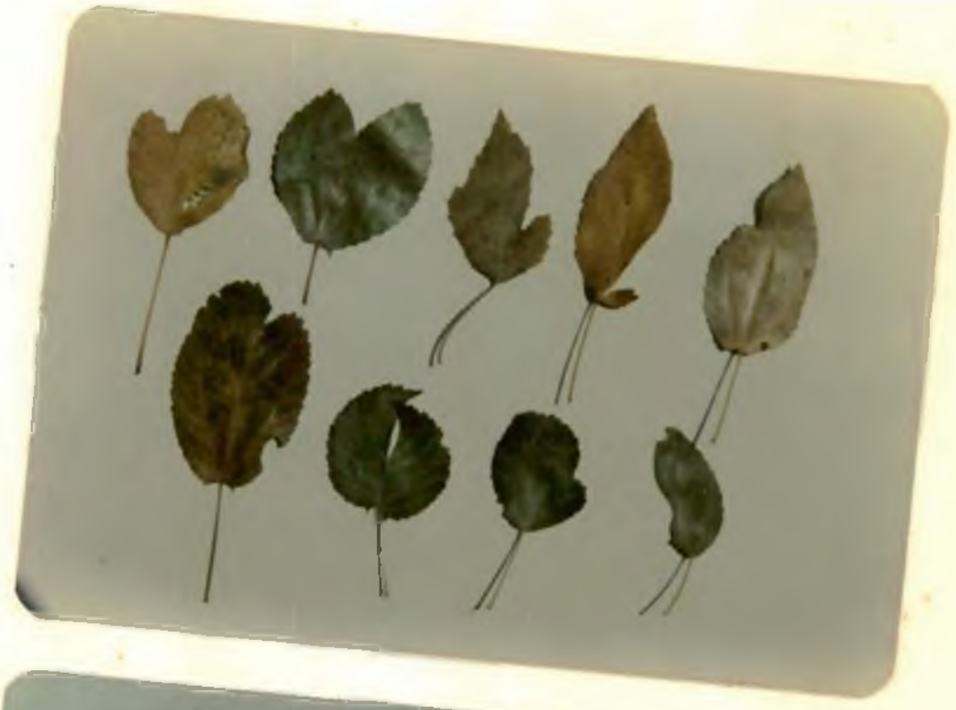


Fig. 14

size of the fruits as observed in the F_2 hybrid population were more or less similar with those reported earlier by Islam and Rashid (1961) (Fig. 14).

Seed : For determining seed weight 100 seeds were selected at random and weighed. Compared to the F_1 (0.26g) the 100 seed weight of F_2s was recorded to be 0.25 g. Number of seeds/fruit in the F_1s was recorded to be 32-180 while in the F_2 this number has been increased and was recorded to be 72-206. In none of the F_2 individuals the number of seeds were as many as the female parent. Sterility of pollen grain and possibly of embryosac in certain F_2s may have a contributory factor in reducing the number of seeds other than the genetical determinants.

Seed colour did not segregate into a clear cut ratio and no individual appeared in the F_2 with identical seed colour of the female parent. On the basis of colour, F_2 seeds could be divided into two groups : pale chocolate : light black, with majority of seeds falling into the first group.

4.4 Application of some biochemical tests for confirmation of hybrid nature of the product of the cross, C. olitorius x C. capsularis :

Since different closely related species differ in their phenolic compounds, protein contents, patterns in protein bands, isoenzymes and other chemicals of various groups, a comparative analysis of the parents and their progeny reveal whether the latter have resulted from self pollination or from actual hybridization.

4.4.1 Moisture content in leaves and seeds :

The present work was undertaken to determine the difference / variation if any in the moisture content of the leaves and seeds of the F_1 progeny of the cross, C. olitorius x C. capsularis and their parents. Results have been summarised in table 12. It has been observed that there was variation in the moisture content in both leaves and seeds. In case of seeds, moisture content was recorded to be 9.25 % in the F_1 whereas in the female parent it was 9.93 % and in the male it was 8.41 %. But in case of leaves, moisture content was recorded to be 29.98 % in the F_1 while in the female it was 36.74 % and in the male 30.41 %.

From the results it is evident that dominance of the

Table 12. Determination of moisture content in the seeds and leaves of C. olitorius var. O-4, C. capsularis var. D-154 and their F_1 hybrids (2g of samples were taken. Calculation is based on the average of three readings)

Plant material	% of moisture (seed)	% of moisture (leaf)
C. olitorius var. O-4	9.93	36.74
F_1	9.25	30.40
C. capsularis var. D-154	8.41	29.98

female parent was observed in case of moisture of seed. This was opposite in case of moisture of leaves i.e. the dominance of male was seen when leaves were used instead of seed for determination of moisture content.

4.4.2 Determination of protein content in the leaves and seeds of F_1 progeny and their parents :

Protein content (%) in seeds and leaves were estimated in the F_1 progeny along with their parents and the results have been plotted in table 13.

From the table it was observed that in the seed, the F_1 contained 25.81 % of total protein whereas in the female parent it was 24.28 % and in the male 27.15 %. In case of leaves protein content was recorded to be 6.12 % in the F_1 and 7.10 % and 5.78 % in the female and male respectively.

In respect of protein content (%) in both leaves and seeds the F_1 showed more or less intermediate between both the parents.

4.4.3 Thin layer chromatography :

Following the report of Frost and Bose (1966) the present experiment was undertaken to find out the difference

Table 13. Estimation of protein content in the seeds and leaves of C. olitorius var. O-4, C. capsularis var. D-154 and their F_1 hybrids. (calculation is based on the average of three readings[1])

Plant material	% of protein (seed)	% of protein (leaf)
C. olitorius var. O-4	24.28	7.10
F_1	25.81	6.12
C. capsularis var. D-154	27.13	5.78

if any in methanol soluble total compounds and nitrogenous compounds in the seeds of the progenies of the cross, C. olitorius x C. capsularis and their parents with the help of thin layer chromatographic (TLC) method.

Two chromatograms were run. In the first, solvent system was acetone and methanol in the proportion of 6 : 4 in the second only methanol. The purpose of using the first solvent was to detect the overall difference of compounds in the two parental species and their F_1 and F_2 progeny by comparing their Rf values (Table 14).

The object of running the second chromatogram was to detect whether the two species and their F_1 and F_2 hybrid derivatives differed in respect of the nitrogenous compounds.

4.4.3.1 First chromatogram :

In this chromatogram the number of compounds confined to four in the male parent and five in the female parent (Fig. 15). It has been observed that in the male parent D-154 and the female O-4 spot number A-3, A-5 are common. As regards spot number A-6 , both the parents are characterised by the spots but they differ in respect of the length and intensity of the spot. The shorter length of the spot in the female parent indicates that some compound(s)

Table 14. Calculation of Rf values of methanol soluble total and nitrogenous compounds as observed in the thin layer chromatographic plates.

Compounds	Distance travelled by the compound(A)	Distance travelled by the solvent (B)	Rf values A/B
First chromatogram (total compound)			
A_1	13.8	15.5	0.89
A_2	13.2	15.5	0.85
A_3	12.3	15.5	0.79
A_4	11.3	15.5	0.73
A_5	10.1	15.5	0.65
A_6	5.2	15.5	0.33
Second chromatogram (nitrogenous compound)			
B_1	2.4	12.3	0.20
B_2	1.5	12.3	0.13

Fig.15. Schematic diagram of the chromatogram showing presence of methanol soluble total compounds ; C. olitorius var. O-4 (extreme left) ; C. capsularis var. D-154 (extreme right) their F_1 (left to C. capsularis) and F_2 (right to C. olitorius). x 1.0

Note that the F_1 has inherited a compound (A-5) from the male parent. The compound A-1 and A-2 of C. olitorius and A-4 of C. capsularis are missing in F_2.

which is tagged to spot A-6 in the male is missing in it. Probably two way chromatograms will separate the compounds in the two parents giving a definite clue whether the greater length of the spot A-6 in the male parent is due to the presence of an extra compound(s). While spot number A-4 is present only in the male, the female parent is characterised by spot number A-1 and A-2. The presence in the F_1 of (a) as big a spot of number A-6 as the male parent, (b) diluted form of spot number A-4 as in the male plus the absence of spot number A-1 and A-2 present an unmistakable evidence that the plants raised from the seeds of crosses were true hybrids. Practically no difference was however observed between the spots of the female parent and those noted in F_2. Complete disappearance of the spots in the F_2 as found in the male and F_1 is difficult to explain.

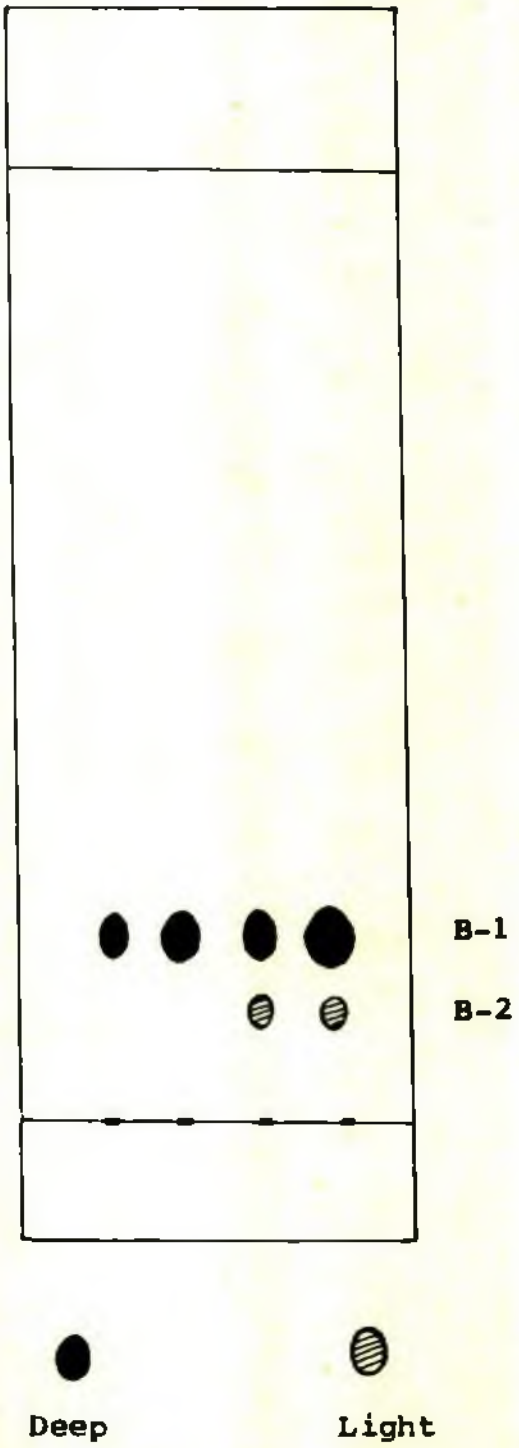
4.4.3.2 Second chromatogram :

In this chromatogram the number of spots was confined to only one in the female parent and two in the male (Fig. 1(The spots in the male parent are numbered B-1 and B-2 while the one in the female is numbered B-1. The F_1 showed both number B-1 and B-2 spots in lower intensity as exhibited by the male. The presence of spot number B-2 in the F_1 leaves no doubt that the plants raised from the seeds of

Fig. 16. Schematic diagram of the chromatogram showing the presence of methanol soluble nitrogenous compounds in the seeds of C. olitorius var. O-4 (extreme left); C. capsularis var. D-154 (extreme right) and their F_1 (left to C. capsularis) and F_2 hybrids (right to C. olitorius). x .75

Note : In the F_1 the spot B-2 characteristic of the male parent is present. In F_2 this spot is missing.

Fig. 16



the interspecific cross were indeed genuine hybrid. It is however difficult to explain as to why spot number B-2 completely disappeared from the F_2.

4.4.4 Protein band as an aid to identify hybrid :

The application of disc electrophoresis as a method for solving taxonomic and physiological problems was emphasised by Fox et al. (1964) and Vaughan and Waite (1967a) who investigated the seed proteins of legumes and Brassica.

Since genetic differences are often reflected in shifts of protein patterns, more refined separation methods, such as disc electrophoresis are of special interest for research on taxonomy, genetics and cultivation of plants (Ericksson 1968 ; Davis et al. 1973).

Fox et al. (1.c.), Vaughan and Waite (1.c.) pointed out that morphological classification could be supplemented by biochemical categories. It is even possible to identify different genotypes of one species by their protein patterns. It is with this end in view, in the present investigation protein band patterns were compared from the suitably prepared extracts of germinating seeds of the F_1s of the cross, C. olitorius x C. capsularis with their parents.

According to the Rf values the maximum number of protein bands were observed in the D-154 variety of C. capsularis (Fig. 17). Starting from below (furthest from the point of origin) they have been serially numbered from 1-9. The intensity of the bands have been indicated by solid black (highest intensity), oblique lines (light intensity) and vertical lines (faint intensity).

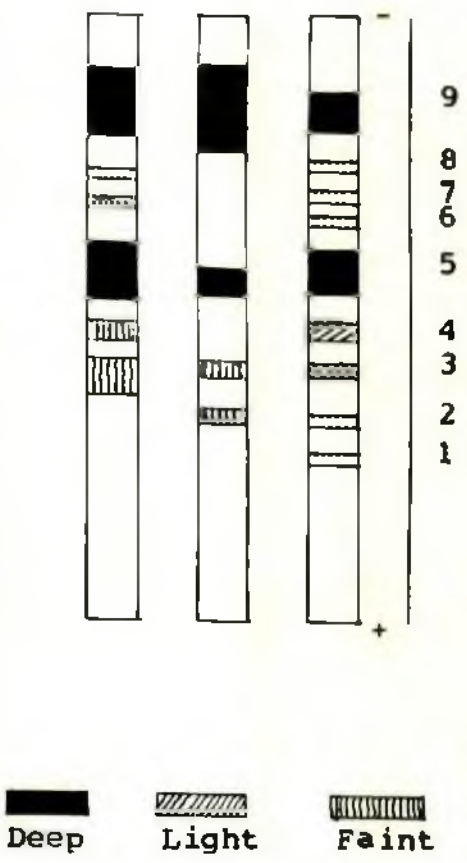
A comparison of the bands of the two parents shows that six bands namely, 3, 4, 5, 8 and 9 are common. Band number 1, 2, 6 which are present in the male parent are altogether missing in the female. Furthermore it may be mentioned that the length of band of 3, 5 and 9 and their intensity are different in the two parents.

In the putative hybrid, band no. 2 which is characteristic of the male parent is present. The band no. 9 in the above plants is conspicuously greater in length compared to that in the two parents. Band no. 1, 4, 6, 7 and 8 are absent in the hybrid.

Thus the results clearly show that the protein bands of the hybrid is different from the two parents in respect of the number of bands, their length and intensity. The presence of band no. 2 provides sufficient proof

Fig.17. Schematic diagram of electrophoretogram showing the patterns of protein bands in C. olitorius var. O-4 (left) ; C. capsularis var. D-154 (right) and their F_1 hybrid (middle). x 1.0

Fig. 17



that the plants in question have inherited the male character as far as this band is concerned. Judging from this additional piece of evidence it may be concluded that the plants in question arising from the crossing of C. olitorius and C. capsularis are true hybrid.

4.4.5 Isoenzyme study as a supplementary evidence to confirm hybrid nature :

Isoenzymes may be defined as multimolecular forms of enzymes which are derived from the same organ or tissue and have at least one substrate as common.

During the last twenty five years, the field of isoenzymology has expanded greatly. It is now a well known fact that many enzymes exist in multiple molecular forms in different tissues or organs of various organisms of both plant and animal origin. The heterogeniety seems to exist in different enzymes for different structural forms.

Isoenzymes are being used by geneticists because of a number of advantages over the conventional morphological markers. They are being used for estimating degree of genetic polymorphism present in populations, genetic differences between species, differential gene

action and expression during development, gene dosage effects on enzyme structure and function, intragenic complementation and heterosis and the evolution of genes and organism (Gomes 1978). Isoenzymes are also effectively being employed in studies to ascertain phylogenetic relationship (Lewontin and Hubby 1966 ; Hart 1969, 70 ; Allard and Kahler 1971; Whitt et al. 1973; Scandalios 1974).

Plant isoenzymes are also being used as marker for selecting and identifying cultivars of different crops. Plant Breeders supplement their morphological, cytological and reproductive data with the help of isoenzymes (Brewer 1970).

Using electrophoretic method, Meister (1950), Vesell and Bearn (1957) separated different fractions of lactate dehydrogenase from various human and animal tissues. Markert and Moller (1959) were able to separate the lactate dehydrogenase enzyme into five distinct forms.

Menzel and Hancock (1984) compared electrophoretic patterns of alcohol dehydrogenase (ADH) and malate dehydrogenase (MDH) isoenzyme in five high polyploids of Hibiscus genus with those of selected diploid, tetraploid and hexaploid species and aided by these data formulated

putative genomic constitutions of these species.

Encouraged by the reports of the above workers the present investigation was undertaken to find out the patterns in the bands of isoenzyme, ADH in C. olitorius var. O-4, C. capsularis var. D-154 and their hybrid.

The male and female parent share band number 4. However this band is different in the two parents both in length and intensity, being smaller and less intense in the male (Figs. 18 & 19). The three bands namely, 1-3 are absent in the female. Band 4 in both putative hybrid and the male parent is identical in intensity and length thereby providing adequate proof that the plants arising out of the cross, C. olitorius x C. capsularis are indeed hybrids. If they were self pollinated the length of band and its colour intensity would have been the same as the female.

4.4.6 Differential shoot regenerating ability of the progeny to prove convincingly that they are true hybrids :

A technique namely, identifying the hybrid in its ability to regenerate half as efficinetly as the male parent has been developed. Islam et al. (1986) reported

Fig.18. Zymogram showing comparative band patterns of isoenzyme ADH in C. olitorius var. O-4 (left); C. capsularis var. D-154 (right) and their F_1 hybrid (middle). x 1.0

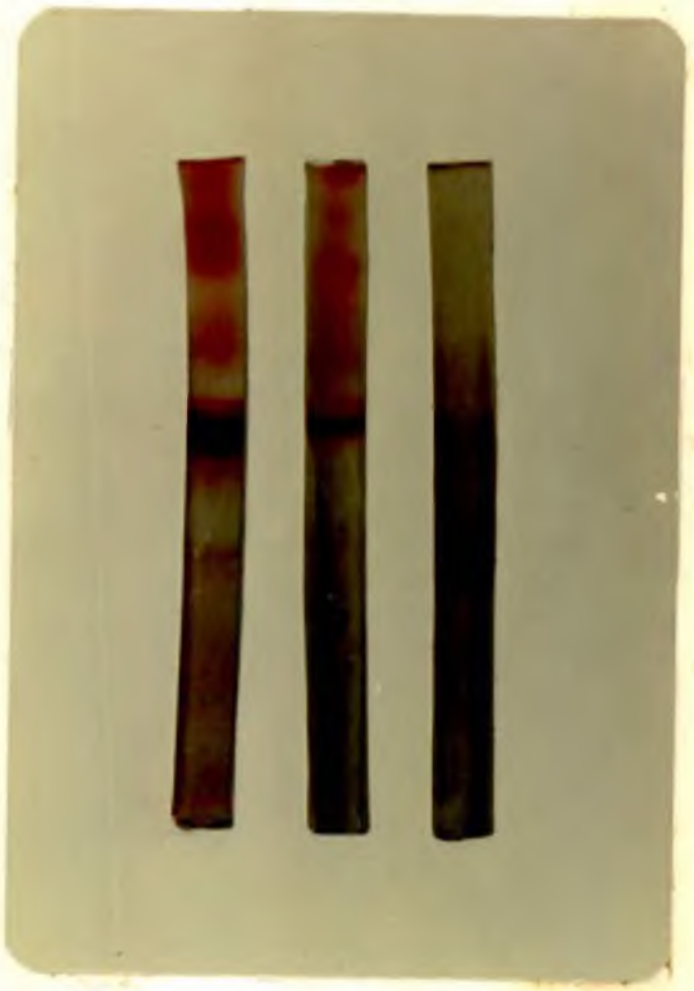
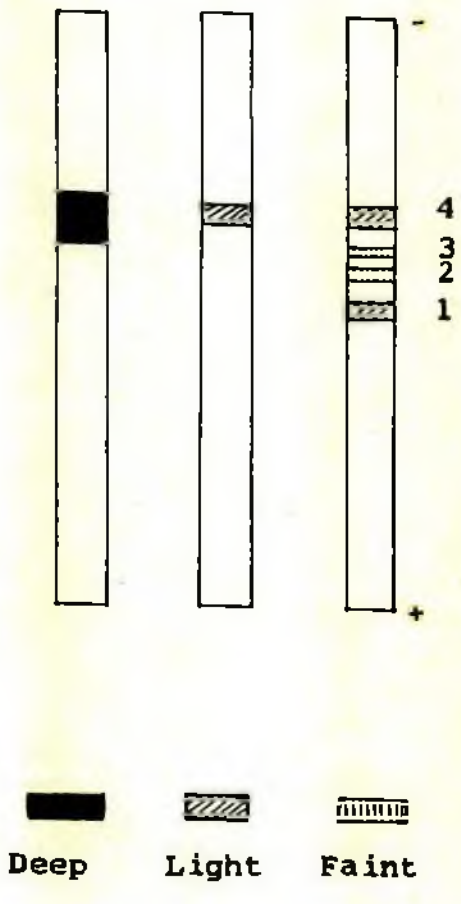


Fig. 18

Fig.19. Schematic diagram of the zymogram showing the ADH isoenzyme band patterns in C. olitorius var. O-4 (left); C. capsularis var. D-154 (right) and their F_1 hybrid (middle). x 1.0

Fig. 19



that in MS medium supplemented with 25 mg/l tyrosine and 0.5 mg/l BAP freshly germinated seedlings of some cultivars of C. capsularis with decapitated roots produced multiple shoots in varying numbers from 25-35 (Fig. 20a). As against this, of the two cultivars namely, C.G. and O-4 of the second species (C. olitorius), only the latter produced 3-4 weak shoots which degenerated before their subculture (Fig. 20b). No shoots were observed in the cultivar C.G. (Table 15).

In the present crossing programme, two cultivars of C. olitorius described above with hardly any regenerating capacity was used as female and two cultivars namely, D-154 and CVL-1 of C. capsularis having multiple shoot forming ability as male. In the F_1s multiple shoots varying from 15-20 (which roughly the average of the two parents) were observed (Fig. 20c). Since regenerating ability was only the characteristic of the varieties of the male parent, it can be safely concluded that the F_1s in question are all true hybrids. Furthermore, this technique can be easily used to multiply F_1s of this interspecific cross which are very difficult to produce through hybridization. In addition, the multiplied F_1 plants obtained through tissue culture may be used

Table 15. Differential response of regeneration of multiple shoots in two species of jute and their hybrids on MS medium supplemented with 0.5 mg/l BAP + 25 mg/l tyrosine. Results have been computed from the mean of 3 replications with 50 in each experiment. Explants used were plumule from 17-hr old freshly germinated seeds with (+) cotyledon.

Species and varieties	No. of explants inoculated	No. of explants produced shoots	Range of shoot production					
			No. of explants produced 2-5 shoots	No. of explants produced 6-10 shoots	No. of explants produced 11-15 shoots	No. of explants produced 16-20 shoots	No. of explants produced 21-25 shoots	No. of explants produced 26-35 shoots
C. capsularis var. D-154(+)	150	93	-	-	-	-	15	78
C. olitorius var. O_4(+)	150	32	32	-	-	-	-	-
C. olitorius var. O_4 x *C. capsularis* var. D-154								
F_1 (+)	150	58	-	-	44	14	-	-
F_2 (+)	150	54	7	9	24	14	-	-

to produce somaclonal variants, The latter can be exploited for selection of desirable individuals in the population.

In the F_2, segregation of this character namely shoot forming ability from a few as in the female parent to as many shoots as in the male together with a varying number of shoots in the intermediate group was observed (Figs. 20 d,e,f).

4.4.7 Study of F_3 population :

Comparative study of 1225 F_3 plants and the female parent O-4 was made. The results of such study are summarised in table 16.

The study shows that in stem colour, height, leaf shape, flower size,shape and characters of fruit there was hardly any difference between the F_3 and the female parent. However in fruit size there was significant difference, the largest fruit of the F_3 being smaller than the smallest fruit of the female plant. One very distinct male trait was seen in the F_3 namely more or less the same short distance between the serrature and the base of leaf base as is seen in the male parent.

Fig. 20 a) Multiple shoots (25-35) arising from the root decapitated 17-hr old germinating seeds of the male parent, C. capsularis var. D-154 in tyrosine and BAP supplemented MS medium in 3-4 weeks.

b) A few shoots arising from the female parent, C. olitorius var. O-4 in the same medium within the same period.

c) Intermediate number of shoots (15-20) in the F_1s in the same medium within the same period.

d-f) Range of variation in the number of multiple shoots from 5-20 in the F_2.

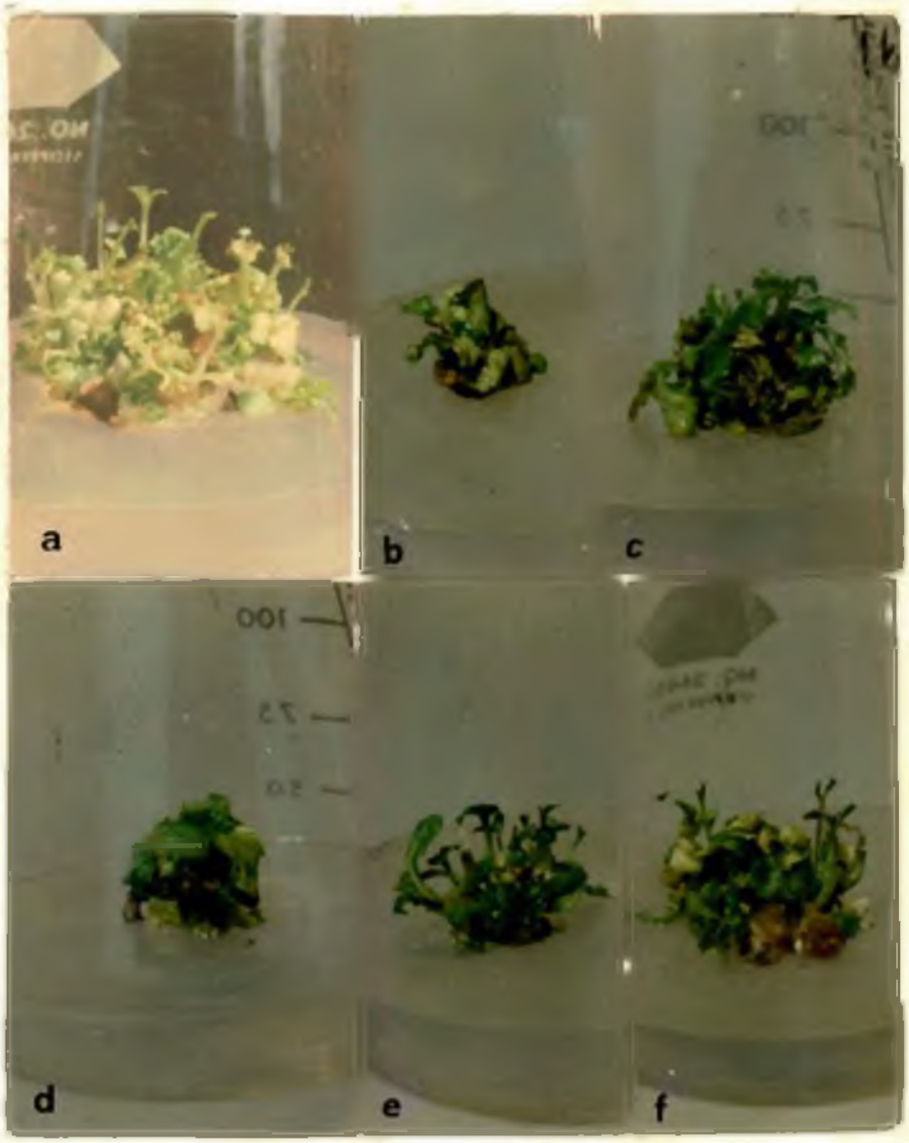


Fig.20

Table 16. Comparison of fruit length and serrature distance in F_3 and O-4.

Plant material studied	% of infected plants	Fruit length	Distance between the origin of two serratures
F_3	39.84	4.744 ± 0.913 cm	0.891 ± 0.169 cm
O-4	58.49	6.612 ± 0.666 "	1.566 ± 0.232 "
D-154	-	-	0.717 ± 0.169 "

O-4 and D-154 are the cultivars of C. olitorius and C. capsularis respectively.

D I S C U S S I O N

D I S C U S S I O N

This work of interspecific hybridization was undertaken in spite of the fact that a number of workers carried out the same cross combination. This is because hybrid derivatives in the advanced generations resembled so much their maternal parent that doubt was expressed in the minds of practical jute breeders whether at all such population representated true hybrid progeny. The present set of experiments were therefore designed to prove that the genomes of the two species can be combined beyond any shade of doubt and that the resemblance of advanced progeny of this cross to the maternal parent is due to elimination of intermediate and male type of progeny. This confirmation was obtained from three different kinds of evidence (a) morphological (b) biochemical and (c) tissue culture in addition to the precautions and techniques adopted to produce the F_1 hybrid.

In the present investigation the techniques namely, reciprocal grafting of the two parents, application of hormone around the pedicel of the pollinated flowers and embryo culture were combined reported to promote

hybrid seed formation by the previous workers.

When these techniques were employed both fruit set and number of fully developed seeds increased. In addition to the techniques used by the previous workers namely, cross between reciprocally grafted parents without application of hormone, cross between ungrafted parents with/without the application of different concentrations of hormone were also repeated.

When crosses were made between the ungrafted/grafted parents of the two species without the application of hormone, the number of fruit set and number of fully developed seeds were more or less same as reported by Subha and Swaminathan (1959), Sachar et al. (1964), Das (1968), Haque (1970). But the result greatly differed with that of Patel and Datta (1960). As against the report of 10-40 % fruit set of the above workers they obtained 14.2-90 %. However, in respect of the results in the fruit set of reciprocal crosses and development of full seeds their results showed more or less similar with the reports of the above workers as well as those reported here. This greater number of fruit set may be due to the genotypic variances from the material used in the present investigation as well as by the previous

workers. But none of the above workers succeeded in obtaining any hybrid plant from the reciprocal crosses, i.e. by using C. capsularis as female parent. On the other hand Bhaduri and Bairagi (1968) have been the only one to succeed in obtaining hybrids in the reciprocal direction i.e. using C. capsularis as the female parent, while they had no success when they used C. olitorius as mother. The variety of C. capsularis which they used was sweet-leaved like the taste of C. olitorius and had abnormal leaves indicating that they were genotypically different from the C. capsularis cultivars used in the present investigation and by earlier workers. This difference in genotypes may have contributed to the success of the cross of the above workers in which C. capsularis was the female parent. The above authors however, have not mentioned in their report whether their reciprocal cross which yielded semifertile hybrids in the past was successful. Most probably they did not confirm that the different genotype of their C. capsularis parent might be the cause of their failure to derive hybrid in the direction where others succeeded.

Others (Raut and Naik 1983) obtained only seeds

using C. capsularis as female and did not report whether hybrid seeds germinated to produce F_1 progeny.

In the present investigation when hormone was applied around the pedicel of the pollinated flower, the number of fruit set and number of fully developed seeds were more or less same as reported by Islam and Rashid (1960,61), Haque (1970). But the result of fruit set in the cross between C. olitorius and C. capsularis differed with the result of Raut and Naik (1983). This difference in fruit set may be due to the genotypic variation of their parental material and those used in the present investigation. One more factor contributing to the difference in the results is that they used GA_3 whereas in the present investigation IAA was used.

Different concentrations of IAA namely, 200, 300 and 400 mg/1 IAA were used to find out the optimum concentration for maximum fruit setting. Like the reports of Islam and Rashid (1960), Islam (1964), Haque (1970) 300 mg/1 IAA gave maximum fruit setting.

By applying 300 mg/1 IAA around the pedicel of the pollinated flower Islam and Rashid (1960) succeeded in preventing the formation of abscission layer. Through

anatomical studies of the IBA treated pedicels with their control in interspecific crosses of Gossypium. Islam and Bhutto (1969) confirmrd their earlier findings that treatment with auxins inhibits formation of abscission layer.

The results reported here on the combined effect of reciprocal grafting and hormone application (300 mg/1) around the pedicel of the pollinated flower on the number of fruit set and development of full seeds were more or less similar with the previous report of Raut and Naik (1983). However, the latter workers did not publish any further information as to whether the hybrid seeds germinated to produce F_1 progeny.

The previous reports of the above workers and results of the present investigation suggest that auxins play double role by promoting the growth of pollen tube and suppress abscission layer thereby allowing free flow of food material from the mother plant to the developing embryo.

With the help of embryological studies Das (1968) showed that the two sets of crosses, one made with reciprocally grafted parents gave a higher frequency of glo-

bular embryos compared to the situation in which grafting was confined to the C. capsularis parent only. In addition in the former endosperm was completely cellular as against its partial development in the latter. Moreover Das (l.c.) observed three significant developments in the crosses involving reciprocally grafted parents : (i) following the prevention of abscission, division of the zygote producing globular pro-embryos of various sizes (ii) development of endosperm in straight crosses up to only 4-8 nucleate stages rarely becoming cellular in the micropylar half almost within the same period as in a selfed C. capsularis flower and (iii) reduction in the accumulation of starch and tannin-like material in the integuments and chalaza.

Harland (1955) suggested that if two species can be grafted, apparently they possess some common factors of biochemical nature. Similar results were also reported by Addison and Travares (1952) in their experiments on Theobroma species. They concluded that success of the crosses and the outcome of grafting have a common physiological basis.

Seed germination : In the present investigation seed

germination was tried in sterilized soil, sterilized vermiculite and White's medium. None of the seeds germinated in soil. However, only a few germinated in sterilized vermiculite watered with full strength Hoagland solution and in White's medium but none of the seedlings attained maturity.

Following the report of Islam (1964), Haque (1970) further attempts were made to grow the hybrid embryos in different nutrient media. In the present investigation only two plantlets raised through embryo culture (½ MS + 100 mg/l AdS + 0.2 mg/l IBA) grew to maturity.

The two plants raised through embryo culture proved to be hybrid from different morphological observations.

Morphologically the hybrid plants closely resembled the F_1s reported earlier by Islam and Rashid (1960) and Haque (1970). However, some of the male characters such as presence of notch in the F_1 hybrid reported by Islam and Rashid (l.c.) were absent in the two F_1 hybrids. The two hybrids of the present investigation did not also show any white patches characterised the hybrids of the above workers. The differences in some of the characters in F_1 may be ascribed to the difference in the female

cultivars used.

Furthermore in B/L ratio of leaf, bud size, petal shape and size, colour of the flower, pollen dia, fruit shape, weight of the seeds were found to be intermediate. But the ridges of the fruits and seed colour showed near dominance of the male parent.

Pollen fertility ranged from 64-78 % as a rule only hybrids of distantly related species would show such a large percentage of sterility.

Although the F_2s reported here were largely maternal in appearance like those reported earlier. But unlike the previous workers some of the F_2s were quite different from the rest of the population. For instance the leaves of some F_2s were dissected, bifurcated and very small in size.

Haque (1970) reported three fruits in a cluster in some of his F_2 and F_3 plants, this character was also observed in the present investigation.

The skewed type of recombination in the direction of C. olitorius in F_2 and F_3 as reported by Swaminathan and Iyer (1961), Islam and Rashid (1960), Haque (1970) was also observed in the present study. The skewed type

of recombination is the characteristic of the progeny of many interspecific crosses, e.g. Gossypium arboreum x G. herbaceum ; G. hirsutum x G. barbadense (c.f. Haque 1970). The reason for the appearance of the maternal type of characters in the F_2 and F_3 of the hybrid reported here may be on account of zygotic lethality or semilethality arising out of unsuitable recombinations. The poor germination of F_1 and F_2 seeds recorded in the present study may be on account of this undesirable recombination.

Some biochemical parameters namely, moisture content in the leaves and seeds; protein content in the leaves and seeds; patterns of protein banding; patterns of ADH isozyme and study of methanol soluble compounds using TLC methods were carried out in the present investigation as an aid to prove that the progeny of the cross, C. olitorius x C. capsularis were true hybrids.

The results showed clear cut difference between the cultivars of the two species of jute in leaf and seed moisture and their protein content. In both these characters the F_1s gave intermediate values.

It may be worthwhile to determine both moisture and

protein content in similar situation when it is difficult to distinguish F_1s from their parents merely by comparative study of morphological characters.

Protein study : The present electrophoretic study of protein patterns showed significant difference in the number, length and intensity of protein bands between the two species and their progeny. Presence of band number 2 of male in the F_1 which is absent in the female and difference with female parent in respect of length and intensity of some bands irrevocally proves that the seeds from the cross were indeed of hybrid origin.

McDaniel (1970) studied the Hordeum proteins with the help of electrophoretic method. Johnson and Hall (1965) used this technique to analyse the phylogenetic affinities in the tribe Triticinae. Using similar techniques Islam (1965) gave probable explanation on the mode of gene action effecting the floral primordia development in Atlas and Atsel varieties of barley.

Isozyme study : In the present study, band patterns of isoenzyme ADH of F_1 progeny were compared with those

of their parents. Morphologically one may confuse the F_1 with the female parent because of their close resemblance ; analysis of band pattern dispels this doubt. As has been shown in the results that the F_1s possess one band characteristically different from the female in terms of intensity and length.

The explanation of absense of some of the bands characterizing the male can be found in the observation of Kato and Tokumasu (1979). These authors observed that the hybrid bands originated mostly from either of the parents or from both. They also observed that not all the bands detected in the parents contributed to the amphidiploid e.g. 5 % of the esterase bands of B. japonica and 22 % of those of B. sativus were not detected in Brassicoraphanus. Further, in Brassicoraphanus there were a few bands which were not present in the parents. They concluded that the isoenzyme bands of Brassicoraphanus may be regarded as a summation of parent-derived bands with the substraction of some parental bands and also with the addition of some hybrid bands if any.

Using electrophoretic method, Zhou (1984) analysed

the isoenzyme, peroxidase band patterns in C. olitorius and C. capsularis and concluded that the electrophoretic method could be suitably used to obtain confirmation about the progeny as to whether they represent true F_1s.

Electrophoretic study provides another unmistakable evidence that the progeny of the cross in the present study were genuine F_1 hybrids.

TLC study : As against the TLC study of Frost and Bose (1966) who found identical spots in both hybrid and the female parent, clear cut difference was observed in the present study between the female cultivar and the hybrid. This results of TLC furnished one more strong evidence supporting that the progeny could not be other than F_1 hybrid.

That paper chromatography is an invaluable aid in determining phylogenetic relationship and the nature of the progeny of a cross has been amply demonstrated by Iwasa et al. (1978). They studied the methanol-soluble leaf extracts of Raphanobrassica and observed that some substances of one of its parents (B. oleracea) were lacking though all substances of another parent

(R. sativus) were present. Moreover they also detected some spots characteristics of hybrid not present in either of the parents or other related species of Brassica and Sinapis they studied.

Variation was also observed in respect of nitrogenous compounds between the two species and their progeny. According to Rf values two compounds (B_1 and B_2) were present in the male and in the female there was only one (B_1). Compound B_1 of the same intensity which characterised the two parents was also present in F_1. In addition, F_1 showed a faint spot of B_2 specific of the male indicating that the spots in the progeny were not the same as the maternal parent. This study provided one more good evidence signifying that the progeny were derived from the actual cross.

Tissue culture : Results of tissue culture experiments in determining the hybridity of the progeny of the cross, C. olitorius x C. capsularis have already been presented in page 96, It has been shown there that, of the two parents only cultivars of C. capsularis produced multiple shoots in a defined medium containing tyrosine (25 mg/l) and BAP (0.5 mg/l) as against 3-4 shoots

in the cultivars of the other parent C. olitorius.

Freshly germinated F_1 seeds in the MS medium supplemented with 25 mg/l tyrosine and 0.5 mg/l BAP differentiated multiple shoots. Their number of multiple shoots was found to be intermediate compared to the sum total of shoots that arose under similar situation.

Using this technique it may be possible also to determine the F_3 individuals appearing identical with the female parent in morphological characters contain any genes for regenerating ability from the male. If their regenerating ability is found more than the female, that would obviously indicate the presence of male characters and would establish the hybrid nature of the F_3 population which are otherwise mistakably considered as maternal.

From the foregoing discussion it may be concluded without any fear of contradiction that the production of interspecific hybrids between the cultivars of the two specis is not necessary any more because it will amount to repeatation of as many as six techniques have been employed to prove the hybrid nature of the progeny and every time the results confirm the genuineness of the hybridity.

Except for the short distance between the serrature and the base of the leaf blade and smaller fruit length the F_3 individuals were similar to the female parent in all other morphological characters.

The absence of some spots in various biochemical test of F_2 individuals points out to the same conclusion that in the F_3 generation intermediate type disappears as is seen in most of the morphological characters of the F_3 population. In the face of such difficulties some other criteria, such as physiological and other characters viz. resistance to flood, pest and diseases should be looked into in the F_3 and F_4 individuals in order to detect whether there is introgression of any visually undetectable characters in the individuals which look morphologically identical with the maternal plant. The characters which may serve as a guide to distinguish F_3 and F_4 individuals should be : i) non-shattering habit ii) resistance to flood iii) immunity to dieback disease (caused by Gleosporium species) and susceptibility to mosaic iv) less photosynsitivity and v) white fibre colour of the male parent, C. capsularis.

It is therefore suggested that F_3 (from the stock) and F_4 population be screened for the above physiological charac-

ters and their reaction to diseases. Those individuals which will react differently in their response to the above characters compared to the female would represent the hybrid derivatives. Breeding programme should therefore continue with these selected individuals.

If after completion of all kinds of tests - physiological, biochemical etc. the F_4 individuals still prove to be maternal in all respects, the future breeding programme should be diverted to raising somaclonal variation right from the F_1 generation. There is every likelihood that such a strategy may yield individuals with some desirable characters from the male. In addition to reducing the number of breeding years it may generate a large number of variants which can be exploited for the improvement of jute crop which is so vital in maintaining the economy of Bangladesh.

S U M M A R Y

S U M M A R Y

In order to prove beyond any shadow of doubt that it is possible to obtain C. olitorius-C. capsularis hybrid,crosses were made employing all the techniques known to date i.e. use of grafted male and female parent, treating flower stalk of the crossed flowers with hormone and rearing the hybrid embryos in aseptic nutrient media. The progeny were examined more critically by physiological and biochemical tests to leave no room for doubt they were genuine hybrids. The results are enumerated briefly as follows.

1. The application of the above combined techniques yielded two semifertile F_1s in only one direction namely, when grafted O_4 variety of C. olitorius was used as mother and the dissected embryos were grown in nutrient medium.
2. The two F_1s were maternal in general appearance but they were genuine hybrids which were indisputably proved by the following facts :

a) Pollen stainability ranged from 64 to 78 % in the two F_1s compared to almost that of a 100 % in C. olitorius

and 96 to 99 % in C. capsularis. In addition, pollen grains were of variable shape and size.

b) B/L ratio of the leaf, bud size, petal shape and size of the F_1s were found intermediate between the two parents.

c) Fruit shape of the F_1s were like that of the female parent, C. olitorius but the size of the fruits was variable and ranged from 3.2 to 6.20 cm compared to 6.85 to 7.50 cm in the female and 0.7 to 1.3 in the male parent. Number of seeds/fruit varied from 32 to 180 compared to 180 to 260 in O_4.

d) Seed fertility was found to be 68.73 % compared to 95 to 99 % in the parents.

e) Seed colour of the F_1s was pale chocolate compared to chocolate in the male and greenish black in the female parent.

f) Slow and stunted growth of both the F_1s.

3. In addition to the morphological characters a number of physiological and biochemical tests were carried out to analyse the progeny of the cross. These consisted of : i) determination of moisture and protein contents of the leaves and seeds ii) comparative electro-

phoretic study of protein band patterns iii) zymogram study of alcohol dehydrogenase (ADH) isoenzymes and iv) thin layer chromatographic (TLC) study of methanol soluble compounds of the seed. All the biochemical tests proved unequivocally that the progeny were genuine F_1 hybrids. The results of the above tests are given below in brief :

i) Moisture content of the seeds of the F_1s was found closer to the female parent but it was opposite in case of the moisture of the leaves. In the percentage of protein content of both leaves and seeds, the F_1s gave approximately intermediate value between the two parents.

ii) The F_1s differed from their parents in respect of the number, length and intensity of protein bands.

iii) Thin layer chromatographic study showed that methanol soluble total and nitrogenous compounds of the F_1 and F_2 progeny differed with those of their parents in spot patterns.

4. Finally one more test was carried out to establish that the two plants out of the product of the cross were genuine hybrids. This test consisted of comparing

the regenerating ability of the plumule of freshly germinated seeds of the male and female parent with that of the plants of the first and second generation in 25 mg/l tyrosine and 0.5 mg/l BAP supplemented MS medium. The cotyledon attached plumules of cultivars of C. capsularis var. D-154, CVL-1 produced multiple shoots in varying numbers from 25 to 35 whereas the female parent O_4, a variety of C. olitorius was found to produce only 3 to 4 weak shoots. In the F_1s multiple shoots varying from 15-20 (which were roughly the average of the two parents) and in the F_2s, a wide range of variation in their number was observed. Since regenerating ability was only the characteristic of the varieties of the male parent, it can be safely concluded that the F_1s in question were all true hybrids.

5. All the 179 F_2s reported in the present investigation were maternal in general appearance like those reported earlier. But unlike the findings of the previous workers the leaves of some of the F_2s were quite different from the rest of the population namely they were dissected, bifurcated and rather small in size; so

also their fruit shape, size and the total number of seeds per fruit.

In addition, some of the F_2s showed distinct male characters, such as smaller distance between the serrature and the petiole at the point of its insertion with the leaf blade; chocolate seed colour of lighter shade; clusters of three fruits and greater regenerating ability of its plumule.

6. The F_3 consisting of 1225 individuals were maternal in morphological appearance but could be distinguished by a male character namely shorter distance between the serrature and the petiole at the point of its insertion with the leaf blade. Their fruits also differed a good deal in shape and size unlike the control female, O_4 in which the fruits are always uniform.

R E F E R E N C E S

R E F E R E N C E S

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