2001

Induction and Comparison of Induced And Natural Polyploids of Momordica Dioica

Sikdar, Biswanth

University of Rajshahi

INDUCTION AND COMPARISON OF INDUCED AND NATURAL POLYPLOIDS OF MOMORDICA DIOICA.

A THESIS

SUBMITTED TO THE DEPARTMENT OF BOTANY, UNIVERSITY OF RAJSHAHI IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF

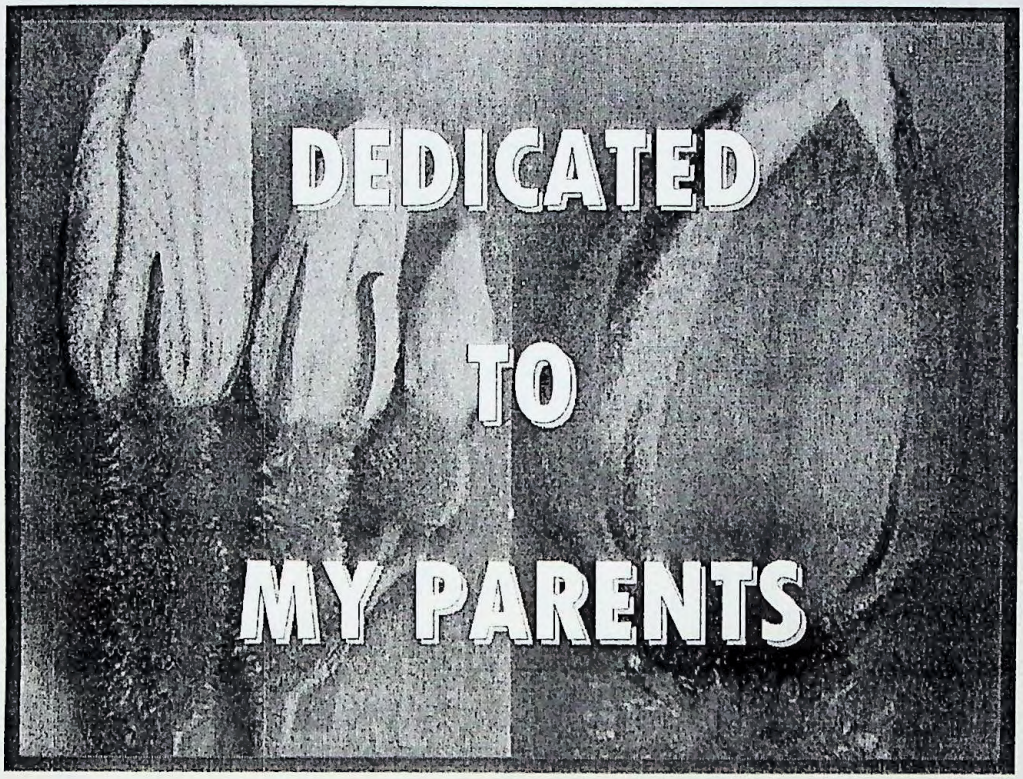
MASTER OF PHILOSOPHY

Submitted By

Biswanath Sikdar

June 2001

PLANT BREEDING LABORATORY
DEPARTMENT OF BOTANY
UNIVERSITY OF RAJSHAHI
RAJSHAHI 6205
BANGLADESH


DEDICATED
TO
MY PARENTS

DECLARATION

I hereby declare that the work embodied in this thesis has not already been submitted in substance for any degree and has not been concurrently submitted in candidature for any degree.

Biswanath Sikdar
28.6.2001

(Biswanth Sikdar)
Lecturer
Dept. of Genetics & Breeding
University of Rajshahi
Rajshahi 6205

CERTIFICATE

This is to certify that the work presented in this dissertation entitled 'INDUCTION AND COMPARISON OF INDUCED AND NATURAL POLYPLOIDS OF *Momordica dioica.*' is based on the work carried out under my supervision in the Department of Botany, University of Rajshahi and is suitable for submission as to the style and contents, for the partial fulfillment of the degree of Master of Philosophy in Botany.

28.6.2001

(Dr. O. J. Joarder)
Professor
&
Research Supervisor
Department of Genetics and Breeding
University of Rajshahi

ACKNOWLEDGEMENT

The author is extremely grateful to his respected teacher and supervisor Dr. O. I. Joarder, Professor, Department of Genetics and Breeding, University of Rajshahi for his kind permission to undertake the present work and for his patronage to dissemination of knowledge through experiments and research. With deepest sense of gratitude I acknowledge his valuable instructions, able guidance and keen interest taken by him in this assignment.

The encouragement provided by N. K. Paul, Chairman, Department of Botany and all respected teachers of the Department of Botany, is gratefully acknowledged.

The author is also indebted to Professor M. A. Khaleque, Chairman, Department of Genetics and Breeding, University of Rajshahi for his valuable suggestion. The continuous inspiration provided by my colleagues of the Department of Genetics and Breeding, University of Rajshahi deserve special thanks.

The author is grateful to Md. Amjad Hossain, senior Scientific officer (Ex- in-charge) and Mr. Proshanta Kumar Sardar, Abdul Alim, Scientific officer of Fruit Research Center, Shiamur, Rajshahi for giving facilities to use their field for this work and for their kind help during the entire period of this work. Thanks also due to the employees of the Research Center for their cordial assistance and unavoidable co-operation.

The author acknowledge the desirable help and unavoidable suggestions of Nasima Hossain joarder, Professor, Department of Botany.

I also acknowledge Mr. Rezual Islam, Ph. D. Fellow; Mr. Osman Goni, and Mr. Farad Ali, M. Phil. Fellow. Thanks are also due to M. Sc. students Department of Genetics and Breeding, for their kind help and co-operation.

I wish to express my sincere appreciation to Dr. M. Rezaul Karim and Dr. Ranajit Kumar Saha Chairman, Department of Biochemistry, R. U. to help during the biochemical analysis.

I am thankful to Mr. Mahbub Rahman and M. Masder for computer composing the thesis skillfully. Thanks also due to the employees of the Department of Genetics and Breeding for their sincere co-operation.

I would like to extend my gratefulness to my parents, brother, sister, wife, daughter for their continuous inspiration and encouragement during the research work.

The author

ABBREVIATIONS

cm	centimeters (s)
eg	exempli gratia = for example
et al.	et alii = other people
etc	et cetera = and others
Fig.	figure (s)
g	gram (s)
Ie.	Id est = that Is
kg	kilogram (s)
L	large
m	meter (s)
mm	millimeter
cm	centimeter
No.	number
ha	hector
in.	Inch!
mg	milligram (s)
mg/g	milligram per gram
s	small
sp.	species
viz	videlicet = Namely
♂	male
♀	female
⚥	bisexual
ml	millilter
NAA	naphthalene acetic acid
ppm	parts per million

pm ..... post meridian

am ..... anti meridian

µ ..... micron

µg ..... microgram

µm ..... micrometer

nm ..... manometer

2n ..... diploid

3n ..... triploid

4n ..... tetraploid

RCBD ..... randomized Complete Block Design

^{0}C ..... degree centigrate

Kg/h ..... killogram per hactre

MP ..... murate of potas

TSP ..... triple super phosphate

rmp ..... rotation per minutes

v/v ..... volume/volume

g/ml ..... gram per millilIter

$AgNO_3$ ..... silver nitrate

$Ca[NO_3]_2$ ..... calsium nitrate

$CuS\ O_4$ ..... copper sulphate

H_2O ..... water

(N) ..... normal

$H_2S\ O_4$ ..... sulfuric acid

$K_2S\ O_4$ ..... potasslum sulphate

N_2 ..... nitrogen

(M) ..... molar

HCL ..... hydrochloric acid

KOH ..... potasslum hydroxide

CONTENTS

ABSTRACT

In an attempt to develop seedless *Momordica dioica* Roxb., diploid, triploid and tetraploid genotypes having chromosome numbers 2n=28, 3n=42 and 4n=56 respectively were treated with aqueous colchicine solution of different concentrations namely, 0.1, 0.2, 0.3, 0.4, 0.5, 0.7 and 1.0% colchicine was applied with different methods viz., dipping, dropping and immersed on seeds and 2, 4 and 6 days old seedling for the induction of artificial polyploid type.

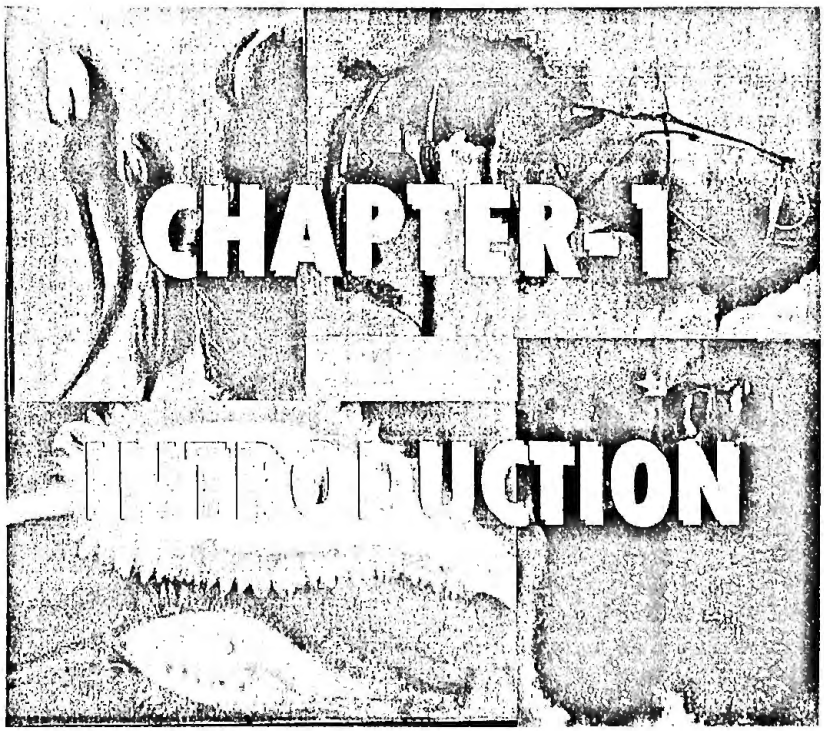
Abnormal seedling growth, reduction in germination and significant delay in germination time were recorded, when both duration of treatment time and concentration of colchicine were gradually increased. Affected percentage was very high in the dropping method in 4 and 6 days old seedling when higher concentration and long treatment time were given. Mortality percentage was very high when the shoot tips were immersed for long time, and no plant was found to survive when seedlings were dipped in any strength of colchicine solution for 24h.

From the affected seeds, 2 to 5 shoots were sprouted (initiated) from the base of the cotyledon and it was found to be normal as like as normal respective ploidy of kakrol plants. Some affected shoot tip of the seedling showed very typical form (like tumor) from normal respective tip of seedling. Growth of tumor like swelling tips were fully checked within 30 to 40 days after treatment. As a result some seedlings failed to survive and some initiated 1 to 6 branches from the swelling tip. Finally, induced polyploid plants were not obtained from the treated material. As all the cells of seeds or seedlings were not affected by colchicine treat

in chromosomes doubling level by, the unaffected normal cells dominated and started to produce normal shoots.

Different characteristics of normal ploidy of kakrol and their sex (♂ and ♀) were analyzed by standard biometrical methods. Within a ploidy level most of the characters showed significant difference at 5% and 1% levels. Some of the characters of both sexs (♂ and ♀) were found similar.

Artificial pollen germination was considered for triploid fruit setting. Four pollen cultural media were used regarding *in vitro* pollen germination. Brewbaker and Kwack's and Robert's 50% medium at 8 pH level were found to be suitable for pollen germination. Pollen germination was highest (87.14%) in tetraploid bisexual. No pollen germination of triploid kakrol were obtained in any culture medium. In the stigma of triploid kakrol, few pollen tube (2-5%) were found to penetrate for its inhibitory cells. So, inter and intra crosses with triploid kakrol was not possible and not a single fruit was developed from the ovary of the triploid kakrol.


CHAPTER-1
INTRODUCTION

INTRODUCTION

There are two species of teasle gourd (*Momordica*) viz., *M. cochinchinensis* spreng. and *M. dioica* Roxb. which are grown in a limited way in some parts of Bangladesh, but are often considered synonymous because of confusing vernacular names like kakoda, kaksa, kakrol etc., signifying either of the two species.

Teasle gourd is a vegetable common in the Indian subcontinent. In our country teasle gourd is known as kakrol. Soil condition and summer climates of this region are suitable for kakrol cultivation. Its introduction into Bangladesh is comparatively recent. No doubt, it was used in the wild state before the cultivated forms came in to use. There is a great variation among the cultivated types. Nearly all commercial genotypes, fruits are more or less ovoid in shape and have soft spines (Hussain and Rashid, 1974; Hoque, 1971).

Recently kakrol has become a major summer vegetable in this sub continent, because of its good nutritional value (Bhuiya *et al.* 1977; Fakir *et al.* 1992), medicinal value (Mishra and Sahu, 1983), high export potential (Hossain *et al.* 1992), high demand in local market (Hossain *et al.* 1996), it's available even upto seven months (mid April to mid November) (Sikdar, 1995) and lean period for vegetable also acts in favor of its high demand.

There are two cultiver types of kakrol found in this region, one is diploid and other is tetraploid. The somatic chromosome number of this diploid is 2n=28 and tetraploid having chromosome 4n=56. Fruit of tetraploid are larger than those of the diploid cultivers. Both of these two types of cultivers are called *Momordica dioica* Roxb. (Mishra and Sahu, 1983). Triploid *Momordica dioica* Roxb. having chromosome 3n=42 are available of the foot hills of Assam in India (Agarwal *et al.* 1974). A cross between diploid and tetraploid produces a triploid which has characters intermediate between these two cultivers (Sikdar, 1995).

Origin:

The center of origin of teasle gourd is unknown. But its wild types are available in Hilly region of south Asia (Mishra and Sahu, 1983; Rashid, 1976; Singh, 1990 and Agarwal *et al.* 1974). In Africa and south America the wild types of kakrol are also available (Shesadri, 1986). According to Hooker (1878) the center of origin of kakrol was one of the South Asian country such as Bangladesh, India, Srilanka, Burma, Malawi etc., (Kabir and Khanam, 1997).

Botany:

Systematic position of kakrol:

Class	:	Dicotyledoneae
Sub class	:	Metachlamydeae
Order	:	Cucurbitales
Family	:	Cucurbitaceae
Genus	:	*Momordica*
Species	:	*Momordica dioica* Roxb.

Habits: Kakrol is a shrub, annual and dioecious plant of slender climbing and trailing habit.

External morphology:

Root: Tuberous root, various size and shapes viz., tuberous type with both ends tapering, sound elliptical and sometimes very irregular in shape having smaller to large in size. Most of them are of moniliform in nature developing irregularly throughout the whole length of the root. Almost all parts of a tuberous root May give rise the new shoots although these new shoots are mostly concentrated to the basal part of the root.

Stem: The stem is solid climber with five sharply angular ridges. It possesses twisting habit with spirally arranged leaves and extra-axillary tendrils. Tendrils is unbranched. The twig is pulled either upwards or forwards on the support. In diploid hair is present.

Leaf: Deep green or light green, cordate shape with prominent palmately reticulated vine and midrib, 2-6in long and broad cordate, ovate, entire, acute denticulate, more or less 3-5 lobed.

Petiole: 2-3in long gland absent.

Flower: Axillary, imbricate aestivation, calyx 5 and Petal 5.

A) **Peduncle:** Male peduncle 1-2in long, female peduncle as long as the male.

B) **Flower colour:** Creamy, light yellow, yellow with or without black round spot at the base of the upper three petals.

C) **Bract** (male): Large, Cowl like, embracing the expanded flower.

D) **Bract** (Female): Small scale like.

E) **Calyx:** 5in long, creamy , light yellow, yellow in colour, black round sport are present at the base of upper petals of tetraploid and triploid kakrol.

Female: Epigynous, 0.7in long ovary, short style, 2 bilobate, 3 stigma.

Male: 5 stamens, synandrous (2+2+1) outer colour black with brown, The two anthered filaments two fib half way down. pollen round or oval shape, yellow or radish colour. Anthesis-after sunset or morning.

Fruit: Green to light yellow, 1.5-7in long, medium thick, densely covered with soft spines, ellipsid, ovoid, or cylindrical.

Seed: 0.25 -0.35in long small many compressed broad oblong, with a few well-marked corrugations on the margins, in tetraploid seeds are blackish with rough surface and margin irregular in diploid seeds are blackish and brown round surface, aril present (Prain, 1903).

Cultural practices of kakrol:

Climate: Kakrol are warm season plants but have a wide range of adaptability. High humid condition suit the crop best.

Soil: All sorts of soils of medium fertility are suitable but they respond to good fertilizer treatment. A well drained. sandy loam soil rich in organic matter, 6.5 pH and thoroughly prepared is desirable. Water logging and high pH are not suitable for kakrol cultivation.

Types: Diploid and Tetraploid cultivars types of kakrol are found in this region. Diploid cultiver are not cultivated on large scale in our country. Fruits of diploid are available in the market of Bihar in India (Mishra and sahu, 1983). Generally, unidentified local tetraploid varieties are grown in our country. There is no recognized variety of kakrol.

Sowing: Due to the peculiar sex behaviour of this plant, tuberous root cuttings are generally preferred to seeds, as it will be possible to propagate pistillate plants along with the limited number of staminate plants required for pollination. When seeds are sown more than 50 percent of the plants turn out to be staminate. The cutting are planted with irrigation facilities in March to April. 5 to 10cm long root cutting are used as seed. On the field, trenches are prepared about 30cm deep and filled with earth and farmyard manure to about 15cm. Then holes 2 to 3m apart are made. After the cutting has sprouted, the supports are needed. The shoots were allowed to trail over bamboo trailers approximately 80cm above the soil surface.

Manuring: The basal dose of about 30 tones farmyard manure will be sufficient for one hectare. In each basin 3kg cowdung, 200g urea, 1/2kg mustard oil cake, 250g TSP and 200g MP were applied as basal doses. In addition, during growth period, urea @ 50 kg/ha and MP @ 35 kg/ha were top dressed thrice on 10 May, 10 July and 10 September, 1996 and 1997 followed by mulching.

Irrigation: Normally rainfed crops, but when there are dry spells, irrigation may be required.

Weeding: As and when necessary, this operation will have to be attended to.

Harvesting: Young and tender fruits required. The harvested crop is kept under shade in a cool place. Fruit will keep well for 3 to 7 days.

Importance of vegetable cultivation:

Vegetables provide a good source of income to the growers and play an important part in human nutrition. They are quick growing and yield immediate returns to the growers. Their cultivation as such occupies an important place in the agricultural development and economy of the country. They have a vital role to play on the food front inasmuch as they reduce the demand on cereals and are one of the cheapest sources of natural protective foods contributing carbohydrates, vitamins and mineral salts in the human diet. Vegetables should find a place in the daily dietary because their food yield is three to four times more as compared to that obtained from cereal crops from a unit area because these are short duration crops and their yield is obtained in numerous pickings. Unlike cereals, they can be grown by everyone all through the year in one form or the other and the sowing and harvesting operations are carried out simultaneously.

Due to their perishable nature and huge demand in the cities, their cultivation is mostly concentrated near the outskirts and suburbs of big cities and towns. Vegetable growing, however, needs special skill, knowledge and hard labor throughout the year. It provides one of the most important sources of income from land as compared to any other type of farming. Vegetable growing finds place with nearly every tiller of the soil. Although area under vegetable crops has increased recently, the price trends have not gone down indicating considerable increase in their demand.

The yield per hectare is very low as compared to that of the developed countries. The soil and climatic conditions in our country are quite suitable for getting the maximum production per unit area. Research programmes pertaining to vegetable production indicate that adoption of all known improved agro-techiniques and existing improved varieties can result in doubling production very easily.

Successful vegetable growing is the intensive cultivation of vegetable crops that may be planted close together, that mature quickly, are grown successively and that offer large income from the unit area cultivated. As the vegetable gardens are generally located near the local markets where the land is usually of high value, the acreage is small and as such it is necessary for the grower to secure maximum returns from every inch of land, through intensive farming. Ordinarily the grower should plan to have continuous supply of a great variety of vegetables over as long a season as possible. This can be done by successive growing and inter-cropping. However, specialization in one crop or a few crops for the growing and timely marketing of vegetables should be followed in places where soil and climate are specially favorable. Before undertaking cultivation of vegetables on a commercial scale, it should be ascertained that proper transport facilities from the fields to the market are available.

Cultivation and consumption of vegetables are constantly increasing. There is general appreciation now of the benefits derived from variety and abundance of vegetables in the diet. It is, therefore, evident that the vegetable grower should not only produce vegetables in bulk but also compete in quality, grading and variety of the vegetables grown. Opportunities exist not only for the established growers in this trade but also for the prospective newcomers. Many fruit growers, dairy farmers, popultrymen can also increase their income by growing vegetables.

Vegetables are protective supplementary foods and are rich sources of vitamins and minerals which are so important for our health. Many of the vegetables contain large quantities of calcium and phosphorous. Although vitamins are found in small quantities, their effect on our health is indeed great.

Vitamin A is essential for normal growth, reproduction and maintenance of health and vigor, it protects against cool and influenza, helps in improving eyesight and can be had from lettuce, spinach, cabbage, peas, tomatoes, carrots, amaranth leaves and watercress.

Vitamin B-complex promotes the nerve condition and helps in proper functioning of the digestive tract. It is considered essential for promotion of appetite and prevention of beri-beri. Beans are rich source of this vitamin.

Vitamin C promotes general health and healthy gums, prevents scurvy and keeps the blood vessels in a good condition. Shortage of this vitamin affects the joints, which condition is sometimes mistaken for rheumatism. Raw or boiled vegetables are better sources of vitamin C than fried, as cooking destroys part of this vitamin, It can be obtained from green beans, cabbage, carrot, tomatoes, peas, potatoes, spinach and pepeer.

Vitamin D is necessary for building up bones, preventing rickets and diseases of teeth. It helps in the calcification of bones by proper utilization of calcium and phosphorous salts. All the green vegetables are particularly rich in this vitamin.

Vitamin E has an important effect on the generative functions and promotes fertility. Green lettuce and other green vegetables are good sources of this vitamin.

Vitamin K shortens blood-clotting time and green leafy vegetables are rich sources of this vitamin.

The vitamins are found in small or large quantities in most natural form in the vegetables.

Leafy vegetables are rich in many minerals such as calcium, iron, potassium and phosphorous. At least ten mineral elements are needed for our growth and development and proper functioning of the body. Phosphorous can be had from milk while vegetables can supply calcium and iron. Leafy vegetables like amaranth, fenugreek and spinach are rich in calcium while carrot, bitter gourd, onion and tomato are a good source of iron. Vegetables like onion, lads finger, summer squash, asparagus supply iodine, which also is needed by human body. Calcium, magnesium and potassium are the most important base elements for neutralizing the acid produced in the body.

The bulk of the fibrous framework of leaves, stems and even bulbs, tubers and roots of vegetables yield a spongy mass which not only helps satisfy our appetite, but also

assists in pushing the food through digestive canal, thus preventing constipation. Mineral salts and their mild acid juices and compounds further help the intestinal activity with their laxative effect.

By and large people in the world live on a vegetarian diet and it is, therefore, essential that production of vegetables is increased manifold to meet the demand of the people and to tide over the acute food shortage in the country. Vegetables hold a high potential for combating the food shortages as their yields per unit area are more than five times of any cereal crop and also they are abundantly rich in almost all ingredients of food, thus providing for not only an adequate but also a balanced diet (Katyal, 1977).

Nutritional value of kakrol as a vegetable: Importance of vegetables in human nutrition is well known. It plays an important role as balanced diet. It provides not only the energy-rich food but also promises supply of vital protective nutrients like minerals, vitamins and protein (Bose *et al.* 1993). Kakrol has excellent multinutrient value on the nutritional point of view, edible portion of these fruits shows 83% moisture, 2.65% protein, 0.63% β-carotene, 31mg/100g calcium, 0.612% lipid, 0.42% phosphorus, 45.2μg/100g thiamin, and 176μg/100g riboflavin (Mishra and Sahu, 1983). Kakrol is an important vegetable of the family Cucurbitaceae for its nutrition value, specially as a good source of high protein and lipid (Fakir *et al.* 1992). The mature seeds of the kakrol (Mahura local) have 39.6% and 4.665% lipid and fatty acid respectively (Dubey and Gaur, 1990).

Polyploids:

The majority of flowering plants have two genomes of which one is contributed by the male and the other by the female parent. By genome we mean the minimum set of chromosome complemet present in a species and which is inherited as a unit. There are however, quite a number of plant species in which the number of genomes is more than two. If there are three genomes, we say that the plant in question is triploid; one of the best examples of triploids is seedless banana. If there are four geneomes, the plant is called tetraploid. The Emmer wheat, *Triticum dicoccoides* affords a good example of a

tetraploid species. If there are 5, 6, 8 genomes, the species are respectively called pentaploid, hexaploid and octoploid. Some kinds of roses are pentaploids, the bread wheat *Triticum aestivum* is hexaploid and the cultivated strawberry is octoploid. There may be plant species in which the number of ploidy may be as high as 22 and *Morus nigra* is an example of a 22 ploidy plant having as many as 308 chromosomes in somatic cells. In some plant species like sugarcane, the number of chromosomes goes on increasing without detrimental effect on the development of the plant body. Further, increase in number is not only tolerated but is preserved on account of its vegetative mode of reproduction (Islam, 1973).

The characteristics of induced polyploids and their artificial induction:

Perhaps the tall vigorous tetraploid mutant of *Oenothera* of De Vries with larger flowers and fruits led the other plant scientists believe that artificial induction of polyploid would bring about similar increase in economic characters of plants. So when the effectiveness of colchicine as a strong polyploidizing agent was established in around 1939 or so, the different plant science laboratories all over the world undertook extensive programme of choromosome duplication for the purpose of producing better plant types with larger flower, larger fruit, larger grain size. After 10 years of work on this line the plant scientists realized that whether or not some economic characters of a plant would respond favourably to chromosome doublin largely depend upon the genotype of the plant in question and on its tolerance to chromosome doubling. In other words, some plant species respond favourably to chromosome doubling eg. rye, sugar-beet, others do not even thougth they are diploids, For instance, artificial maize and tomato tetraploids are poor in their performance. The effects of chromosome doubling on different plant characters are discussed below.

Plant height: It is rarely that the plant height undergoes increase through chromosome doubling. In fact, the height considerably decreases as a result of chromosome duplication.

The leaf: The leaf almost invariably becomes broader without any appreciable increase in length. This increase in one dimension raises the B/L ratio of the leaf. It also becomes thick, shows more pronounced venation and usually are darker green in colour. In some grasses eg. stipa the width in the leaf does not increase and the colour also does not change.

The size of guard cells almost invariably increases, so also the stomatal aperture. Simultaneously with the increase of the size of guard cells, the frequency of stomata decreases in the autopolyploid.

Number of branche: The number of branches usually undergoes reduction in afrificial polyploids eg. water-melon, bitter gourd. But in some cases, as in berseem , lucerne the number of branches increases.

Flower: If the treated plant species is a diploid and cross pollinated, the flower size usually increases. This fact of increase in size by chromosome doubling was made use of by horticulturists.

Pollen fertility: Almost invariably there is a great reduction in pollen fertility. In self-pollinated plants, the reduction in fertility is more pronounced than in cross pollinated ones.

Fruit: Fruit size may increase although the number of seeds may be reduced. This character of reduced number of seeds in tetraploid is of advantage in fruit plants; in these plants the smaller the number of seeds in their fruit, the greater is their demant. For instance, tetraploid apples, triploid banana, triploid water-melon are in higher demant than their diploid counterparts. On the other hand, if the grains are the edible, parts, such as, maize, rye, wheat, rice, the reduction in number of seeds is a great handicap because it reduces the yield considerably.

Natural polyploids of Cucurbitaceae family:

Natural polypliods are rare in the cucurbitaceae (Stebbins, 1950). So far, there are few well established cases of natural ploidy and that too limited to only few species (Nakajima, 1937; Rangaswamy, 1949; Whitaker, 1950; Kumar and Vishveshwaralah, 1951). Some of the cucurbits plant viz,. *Gomphohyne cissiforrmis, Momordica dioica, Melothria assamica, Trichosanthes palmata* and *Trichosanthes dioica* Roxb. are found in nature (Roy and Trivedr, 1966; Roy *et al.* 1966; Singh, 1972 and Islam *et al.* 1974).

Induction of bisexuality:

Kakrol is dioecious. In absence of male plant the female plant does not normally bear fruit. The male plants produce the first flower on the main stem 75 days after emergence from the soil. So the female flower buds develop quicker than the male ones (Hussain and Rashid, 1974). The male flower buds of tetraploid cultivar take 22-28 days from visible initiation to full flowering, whereas the female bud takes 19-22 days (Mishra and Sahu, 1983; Hussain and Rashid, 1974).

There is no insect pollinator available for efficient cross-pollination and normal fruit setting. So, farmers adopt manual hand-pollination to secure uniform cross-pollination, which is laborious and expensive. Fruits reach harvesting stage within 15-20 days after fruit set when they are tender and slightly green. An average vine yield is 42-60 fruits (Fakir *et al.* 1992).

Self fertilazation in dioicious plants has been made possible through changing flower sex by controlling ethylene action (McMurry and Miller, 1968; Iwahari *et al:* 1970; Beyer 1976, Kalloo and Franken, 1978; Nerson *et al.* 1987; Ali *et al.* 1991 and Sikdar *et al.* 2000) have successfully induced male sex in the female kakrol plant of different types of kakrol and suggested the possible way of selfing (sib) and crossing of breeding materials (female plants) for generation advancement. Thus there crossing between two female types of kakrol may facilitate recombination of desirable characters of parents in a single plant. Selection of high yielding individuals from female homo sexual hybrids may lead to establish a variety with a short period of time as kakrol is propagated vegetatively (Hosain *et al.* 1996).

In vitro Pollen grain germination:

In seed plants, pollen grains embody the male partners in sexual reproduction. Pollen grains develop in anthers, and the structural details of pollen development have been covered extensively by Maheshwari, (1950 and 1963), Davis (1966), Johri (1984), and Shivanna and Johri (1984). Pollen grains are generally shed in a desiccated condition and the moisture level is less than 20%. At the time of shedding, pollen grains are two-celled (ie., vegetative cell and two sperm cells formed by the division of the generative cell) (Brewbaker, 1959, 1967). Pollen grains are transferred to the stigma (pollination) by biotic or abiotic agents. After pollination, pollen grains issue pollen tubes, which grow through the pistil and discharge the male gametes in the vicinity of the egg cell for fertilization and eventual development of embryo and seed. Pollen biology thus encompasses pollen production, its transfer to the stigma, and details of pollen-pistil interaction leading to fertilization and seed set. Any break in these sequential events affects seed and fruit set. As seed is the economic product of most of our crop plants, studies on pollen biology are a prerequisite for attempts aimed at optimization and improvement of the yield (Knox *et al.* 1986, Mulcahy and Ottaviano, 1983). Several techniques are now being employed to study pollen biology.

Pollen biotechnology refers to the manipulation of various aspects of pollen biology for crop production and improvement. Pollen biotechnology is one of the most exciting areas of plant reproductive biology (Mulcahy, 1983; Mulcahy *et al.* 1986. Ottaviano and Mulcahy, 1989) and plays an important role in crop improvement programs. As most of our crop plants show poor adaptation to biotic and abiotic stresses such as diseases, pests, drought, and salinity, constant infiltration of new adaptive genes into the cultivars is necessary to sustain and further improve the yield. Most of these adaptive traits are controlled by multiple genes and are therefore not readily amenable for recombinant DNA technology. Crop improvement programs, therefore, have to depend largely on the conventional hybridization method, which is rather prolonged and cumbersome. Further, conventional hybridization cannot be carried out when the species

show strong cross ability barriers. This can well become a major limitation in the coming years, as in most of the crop species the genetic variability within the cultivars has been exhausted, and the breeder has to make use of the variability present in the wild relatives (Hawkes, 1977; Stalker, 1980; Goodman *et al.* 1987).

Application of pollen biotechnology to conventional breeding programs not only decreases the time and cost involved, but also greatly increases the efficacy of the conventional breeding methods (Mulcahy, 1983). The following are a few such applications.

Screening of a large number of plants for the presence of required genes and of recombinants is a basic step in any plant breeding program. Conventional screening is laborious, time-consuming, and expensive. Investigations during the past 10 years have shown that many of the adaptive genes, such as for resistance to diseases and tolerance to herbicides, salinity, temperature, and water stress, are expressed in pollen grains also (Mulcahy, 1983;). Therefore, plants resistant to a particular stress condition can be easily identified by studying the responses of pollen grains to the stress condition. This approach is convenient, rapid, and not expensive.

More importantly, the frequency of plants having desired genes/recombinants can be significantly improved by applying selection pressure to pollen. As pollen grains from a single heterozygous individual contain thousands of pollen types, the extent of genetic variability in a pollen population that can undergo selection is massive when compared to plant populations (Mulcahy, 1983; Ottaviano and Mulcahy, 1989). Many examples are already available on these lines. In tomato, selection pressure had been applied to achieve preferential fertilization by pollen genotypes tolerant to cold (Zamir and Gadish, 1987) and to salt (Sacher *et al.* 1983).

The presence of strong pre- and post-fertilization barriers is the major constraint in hybridization programs. Presently, many techniques such as *in vitro* fertilization, mentor pollen, and embryo rescue are available to overcome these barriers (Rangaswamy, 1963; 1977; Maheshwari and Rangaswamy, 1965; Shivanna and Johri, 1985). Application of

such techniques has enabled the production of a large number of interspecific and intergeneric hybrids in crop plants (Maheshwari and Rangaswamy, 1965; Raghavan, 1976).

***In vitro* germination methods:** *In vitro* germination is the most commonly used technique in pollen physiology (Heslop and Harrision, 1987; Steer and Steer, 1989). This technique provides a simple experimental method to study the physiology and biochemistry of pollen germination and pollen tube growth, as well as the responses of the pollen system to physical and chemical factors. As active growth of pollen tubes *in vitro* is generally accomplished within a few hours from not a dire necessity.

***In vivo* germination and pollen tube growth:** With controlled compatible pollination, pollen germination on the stigma and eventual pollen tube growth in the style constitute a pollen viability test. This test is less time-consuming than the fruit set and seed set test. Although not entirely quantitative, the number of pollen tubes growing in the style is a fair indication of the efficacy of the pollen sample in inducing fruit set and seed set. For example, in *Brassica oleracea*, pollen samples that result in at least 70 pollen tubes growing in the style are considered fully viable (Ockendon, 1974).

In this test the amount of pollen used for the various pollination should be nearly uniform. When fresh pollen samples are available, comparison of pollen performance in pistils pollinated with fresh pollen and in pistils pollinated with test pollen gives reasonably reliable information on the viability of the sample.

Anatomy of Stem: The following layers of cells are appear in cross section of kakrol plant:

Epidermis: The single outermost layer consist of compact barrel shaped cells having no intercellular spaces. The epidermis remains covered with a thin cuticle. Some of the epidermal cells possess multicellular epidermal hairs.

Cortex: This region consist of external collenchyma, chlorenchyma (photosynthetic tissue) and endodermis.

(a) **Collenchyma:** This lies immediately beneath the epidermis consisting of many layer of the cells in the ridges, where as in furrows it is only two or three layered or sometimes altogether absent.

(b) **Chlorenchyma:** Just below the collenchyma two or three layer of parenchyma containing chloroplast (collenchyma phloem tissue) present which help in the process of assimilation.

(c) **Endodermis:** It is inner most layer of the cortex, lying immediately out side the sclerenchymatous zone of pericycle. This layer is wavy and contains many starch grains.

(d) **Pericycle:** Just beneath the endodermis there is a multilayered zone of sclerenchymatous pericycle. The cells are lignified and appear polygonal in cross section.

(e) **Ground tissue:** The vascular bundles are found lying embedding in the thin walled parenchyma cells of ground tissue. The ground tissue extends from just below the sclerenchymatous pericycle to the central medullary cavity.

Vascular bundle: Generally vascular bundles are ten in number which are found to be arranged in two rows, those of the outer row corresponding to the ridges and those of the inner to the furrows. The vascular bundle are bicollateral each consisting of xylem, two strips (inner and outer) of cambium and two strands of phloem (inner and outer).

(a) Xylem: It occupies the central position of the vascular bundle, consisting of very wide, pitted vessels towards periphery of the metaxylem, and on the inner side of narrow vessels, wood fibrous and xylem parenchyma are also present. The xylem vessels are not arranged in radial rows.

(b) Cambium: In each vascular bundle two strips of cambium are found. The cambial activity remains confined within the vascular bundles. The cambium strip is fund between xylem and phloem on either side of the bundle. Of the two strips of cambium it is only the external one which divided and causes growth in thickness. The cells of cambium are thin walled, rectangular and arranged in radial rows. Usually the outer-cambium is many layered and flat while the inner cambium is few layered and somewhat curved. Only fascicular cambium is found. The stem is not woody, and therefore, the periderm and lenticell are not formed.

(c) Phloem: On the extreme ends of the vascular bundle the phloem occurs in two patches, towards the periphery the outer phloem, and towards pith, the inner phloem. Each strand of phloem consists of sieve tubes, companion cells and phloem parenchyma. Sieve tubes are very well developed. The sieve plates with perforations are also visible. Fibres and ray cells are absent.

Special Structure: The bicollateral open vascular bundles are found each consisting of xylem (central position), two strips of cambium (outer and inner) and two patches of phloem (outer and inner).

Past Research:

Normally, vegetable crops give high yield per unit area as compared to cereal crops. Presently the yield per unit area of most of the vegetables is very low in our country compared to many other countries. Thus, if the production and quality problems of vegetables quality will be high and yield of vegetable can nearly be doubled from the existing area.

In normal cultivation practices tuberous roots are cut into pieces and planted during warm climate (March / April). Sprouts grow very rapidly to form a 3-4 m branched vine at maturity. Male and female plants are grown together at a ratio of 1 male: 15 female (Rashid, 1976; Vijay, 1978). Flowers start to appear 1-2 months after planting

and continue until the apart of senescence of the vines (Hussain and Rashid, 1974). Fruits are harvested 15 - 20 days after anthesis of the respective female flowers, before the fruits turn yellow and the seeds become hard. Even in its native habitat, the vines die in October, as temperatures fall, but the plants perenate via the non growing tuberous roots in the following year. Its (*M. dioica* Roxb.) wild relative *Momordica cochinchinensis* is perennial has attributes like drought and cold tolerance (Islam *et al.* 1992).

There are some evidences of variation in flower and fruit morphotypes among the plants grown in native areas. In addition, there may be a scope to transfer useful characteristics such as, from its close relatives, eg. *M. charantia* L., *M. balsamina* L., *M subangulata* Blume, *M. tuberosa* Roxb. (Rashid, 1976; Vijay *et al.* 1977; Singh, 1978, 1990; Agarwal and Roy, 1974; Nakajima, 1937; Rangaswaya, 1949).

Morphological features of the indigenous types of kakrol are not well described (Rashid, 1976). Floral biology of *M. dioica* has been reported by Hussain and Rashid (1976) and of other species (*Momordica cochinchinensis*) by Vijay *et al.*, (1977). A general morphology of *M. dioica* and of other cucurbits have been studied by Hossain (1974) and Hoque (1971) respectively (Agarwal PK Roy RP, 1974; Nakajima, 1937; Rangaswaya, 1949).

Tarik and Reza (1992) collected twenty six female and seven male germplasms of *M. dioica* Roxb. from the extensive kakrol growing areas. One female germplasm of wild species was also collected from Jamalpur. The 26 female cultivarted germplasms collected were classified into 16 genotypes. Seven male germplasms were different from one another. Mandal (1996) collected wild and cultivated kakrol (from north western Bangladesh) for possibility of selection from hybrid population.

Seed germination of kakrol is difficult due to hard seed coat. One hundred percent germination from decoated seeds was reported by Ali *et al.* (1991) in *M. dioica.* Mishra and sahu (1983) reported that fresh seeds do not germinate but remain dormant for nine months. Hartmann and Kester (1968) reported scarification or gibberellin treatment of

seeds can improve seed germination. Hoque (1997) reported that the germination percentage was always higher in decoated seeds than with unpeeled seeds. Sikdar (1995) reported that germination of diploid kakrol seeds were always higher than triploid and tetraploid kakrol.

Induction of bisexual and staminate flowers on pistillate plant using $AgNO_3$. Is well established in cucumber and tomato (Beyer, 1976); in pickling cucumber (Kalloo and Franken, 1978); in *Momordica charantia* (Kabir *et al.* 1989) and in *Cucumis sativa* (More and Munger, 1986). Induction of bisexual flowers on pistillate *M. dioica* has been reported (Ali *et al.* 1991). Effect of silver nitrate on sex expression in kakrol (*Momordica dioica* Roxb) have been reported (Sikdar *et al.* 2000) Self pollination of breeding lines of dioecious plants has been made possible by changes in flower sex using stimulation or inhibition of ethylene action (McMurry and Miller, 1968; Iwahori *et al.* 1970; Beyer, 1876; Kalloo and Franken, 1978; More and Munger, 1986; More and Seshdri, 1988). Possibility of sexual crossing between two genetically female kakrol have been reported (Hossain *et al.* 1996)

Germination of pollen on the stigma was observed within 8h after pollination (Hussain and Rashid, 1974) with sucrose and glucose solution, a concentration of 15% gave the highest germination of pollen grain (38.2 & 33.3%, respectively) and longest pollen tube lengths (85.37 and 53.44µm, respectively). A 3ppm boric acid solution produce the highest germination percentage (33.1%). Among the grown plants, 21 were female and 25 were male ie. there was a 1:1 ratio (Ali *et al.* 1994). Hoque (1997) reported that through in vitro propagation true to type kakrol could be raised of different explants. Among the different media formulations tested $2.0mgl^{-1}$ BA+$0.2mgl^{-1}$ NAA in MS (Murashige and Skoog, 1962) salt was the best for the induction of multiple shoot from both nodal and shoot tip of kakrol.

Until recent past kakrol had been considered as a minor vegetable. However, its popularity in internal and for export market has been increased sharply during last

decade. As a result commercial cultivation of kakrol has also been expanded. Obviously the growers are now demanding information on improved varieties and production technology.

Hybridization between a tetraploid female and diploid male produced only male flowers while in the following season it also produced many female flowers, sex expression had changed from dioecious to monoecious (Jha and Roy, 1989).

Problems of kakrol cultivation:

As a crop, kakrol has a number of problems viz., its dioecious nature, low yield, small and d-shaped fruits, self, sib matting and cross incompatibility of induced bisexual flower, non synchoronous flowering, cumbersome hand pollination etc.

The main problems of large scale kakrol cultivation is the fact that it requires extensive cross pollination as the plant are dioicious.

Kakrol is preferable when it is tender. The fruits become inedible at maturity owing to the presence of a large number of hard seeds and market demand is very low for presence of large number of hard seeds in the fruit. Among the problems related to fruit quality, development of a high number of hard seeds after 15 days of pollination is the most important one. The fruit with hard seeds loose palatability and render them almost inedible after 18 days of anthesis. Harvesting of the fruit before the seeds become hard is not practised due to a significant yield loss which probably can not be compensated by the price. Thus the prospect of seedless kakrol demands attention.

The difficulties faced by the kakrol farmers as identified above may be overcome by induction of bisexuality, hybridization between different strains and selection for desirable varieties.

Rational and Objectives:

The present study was undertaken to:

i) Induction of polyploid of 2n, 3n and 4n kakrol.

ii) Development of seedless or less seeded high yielding variety.

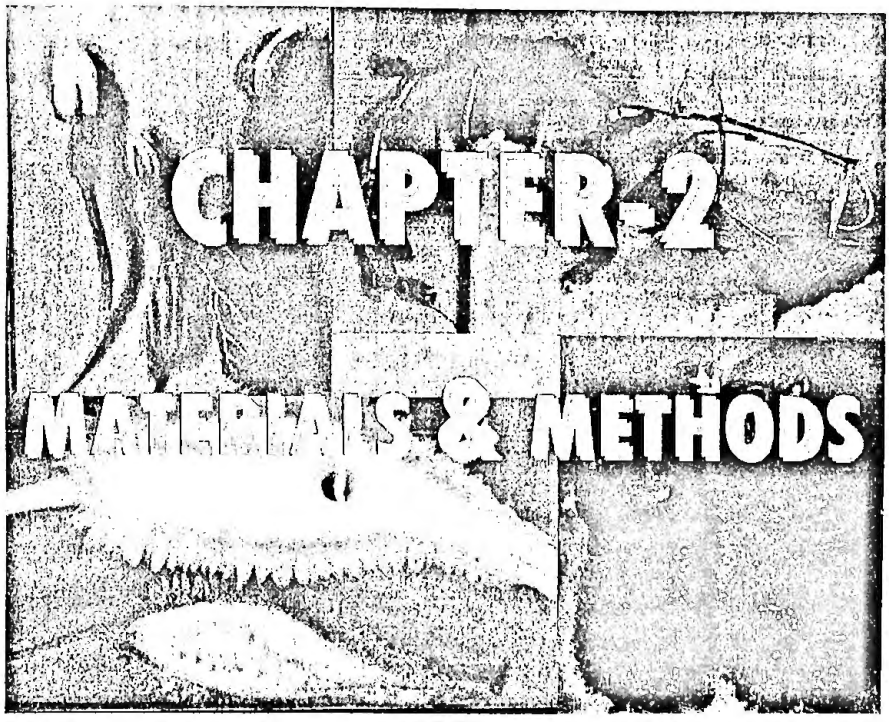
iii) Ascertain parthenocarpic plant.

iv) Identify suitable culture media for *in vitro* pollen germination.

v) Identification of self incompatibility alleles (S-alleles)

vi) Causes of failure of normal fruit setting of respective ovary.

vii) Compare the induced and natural ploidy and to determine their reproductive biology and genetical relationship.


CHAPTER-2
MATERIALS & METHODS

MATERIALS & METHODS

Materials:

Seeds and tuberous root of different types of kakrol such as diploid (2n), triploid (3n) (progeny of diploid × tetraploid), and tetraploid (4n) were available in the Genetics and Breeding laboratory of the Botany Department, University of Rajshahi. Kakrol is a dioicious and highly heterozygous plant. So we have selected seeds and tuberous roots one genotype from tetraploid kakrol, one from triploid and one from diploid kakrol.

Methods:

Field selection for experiment: The experiment was set up at Fruit Research Center, Shampur, Rajshahi during the period of 1996 to 1997. The selected field was sandy loamy and free from weeds and water logging. The soil pH was 6.5. Optimum cultural practice was made (Sikdar *et al.* 2000) which include weeding, fungicide spray, insecticide spray, application of organic fertilizer, timely irrigation etc.

Field design from transplanted plant: The experiment was laid out in randomized complete block design (RCBD) with three replications. The unit plot size was 12 × 2m having 0.75m footpath in each block. The plants were three for 4n male, three for 4n female, three for 3n male, three for 3n female, three for both male and female of 2n kakrol were randomly assignment in 3 unit plots.

Method used for germination of seed: Seeds of tetraploid, triploid and diploid were germinated in polyethene bags, petridish and in the seed bed.

Germination in polyethene bag: Different strength of colchicne treated seeds and control seeds (normal distilled water treated) of 4n, 3n and 2n kakrol were put into polyethene bags for germination with seed coat (unpeeled) and seed coat removed (decoated).

Polyethene bag preparation: Top soil, sand and organic compounds were throughly mixed and finely grounded. Top soil and sand were used in a proportion of 3:2 ratio. Organic compound in the form of cowdung was added at the rate of 10kg per 100kg of sand soil mixture. Perforated polyethene bags were filled with this soil mixture.

Cultural Practice: Polyethene bags were kept under a tree and were throughly, watered a day before the seeds were sown for germination. Regular watering were made to keep the soils sufficiently moist so that proper germination can take place.

Germination in petridish: Different strength of colchicne treated seeds and control seeds (normal distilled water treated) of 4n, 3n and 2n kakrol were put into Petridish for germination with seed coat (unpeeled) and seed coat removed (decoated).

Petridish preparation: Filter paper was laid into the petridishes with sufficient thickness. Then the petridishes were wetted by the distilled water, and peeled and unpeeled seeds were placed on the petridishe with covered. The petridish were kept into a dark condition at 28°c.

Preparation of seed beds: The experiment was carried out at the Fruit Research Center, Shampur, Rajshahi. Seed beds were prepared thoroughly and fertilized with cowdung and chemical fertilizer. the size of the seed bed was 5x1m.

Cultural practice of seed bed: Proper mulching and irrigation was made.

Field preparation for transplanted seedlings: The experimental field was ploughed several times and weeds were removed. The field was pulverized and cowdung was mixed thoroughly by repeated ploughing. Inorganic fertilizers were applied at the rate of 150kg/h urea, 75kg/h MP and 75kg/h TSP at the time of field preparation.

Raising of plants and seedlings: Seeds of all the types of kakrol were allowed to germinate on 15 February, 1996 and 30 February, 1997. After germination it was

treated by different strength of colchicine (except control seedling). Then it was transplanted in pits in the experimental field on 1st April to 30 April, 1996 and 1997.

Induction of polyploidy: Aqueous colchicine solution having concentration of 0.1, 0.2, 0.3, 0.4, 0.5, 0.7 and 1.0% were applied on germinating seeds (decoated and unpeeled) and seedling of 2, 4 and 6 days old to induce polyploidy in them.

Preparation of colchicine solution: 1g colchicine ($C_{22}H_{25}NO_6$) was weighed in a electronic balance. Then 100ml distilled water was taken in conical flax and weighed amount (1g) of colchicine was added to make the strength of the solution 1%. The solution was stored in the refrigerator at 4 to 5° C. Seven different concentration of colchicine viz., 0.1, 0.2, 0.3, 0.4, 0.5, 0.7 and 1.0% were used for induction of polyploidy of different types of kakrol.

Method of application of colchicine: Different concentration of colchicine were applied on seeds (decoated and unpeeled) and 2, 4 and 6 days old seedling on plumule length less than 4cm (before the first leaf had pierced) for different period of time viz., 3, 6, 9, 12, 18 and 24 hours. There were three methods of application of colchicine:

i) All seeds were immersed with colchicine of different strength for period as per treatment. The control seeds were also immersed in distilled water for same period of time.

ii) The tip of seedling were drown and immersed in different concentration of colchicine for said period of time as per the treatment.

iii) The seedling of the different ages were treated with colchicine of different concentration by glass dropper in such a way that the shoot apex remained wet for the said period of time as per the treatment. The treated and controlled seed and seedling were washed in running distilled water for 3 to 4 hours. The seed

were allowed to grow one month in petridishes, polyethene bags and seed bed, then it was transplanted to experimental plot. The seedlings were allowed to grow for 5 to 20 days in the petridishe, then transplanted into pots and polyethene bags. After one month the treated and controlled plants were transplanted to experimental plot.

Induction of bisexuality: Bisexuality was induced by treating shoot apex with 300, 400 and 500 ppm of silvernitrate ($AgNO_3$) solution of three types of female kakrol.

Method of application of $AgNO_3$ on twigs: Young twigs of female kakrol were drown immersed in the recommended solution of $AgNO_3$.

***In vitro* pollen germination:** Six different types of flower (normal male and induced bisexual) were used for *in vitro* germination of pollen grain.

Methods of pollen germination: Following method were used in this study: :

i) Brewbaker and kwack's medium,

ii) 50% Robert's medium,

iii) Sucrose 10% + boric acid 100mg/l,

iv) Sucrose 10% + boric acid 100mg / l + $Ca(NO_3)_2$ 300mg/l

The technique employed was to place a small drop of solution on a slide, sprinkle pollen from freshly collected and stored flowers on the drop and place the slide in an inverted petridish and kept different time (20 and 40 minutes) of interval at room temperature. Germination percentage was scored by a random count of 100 grains. At different period pollen tube length of randomly selected 50 tubes was determined microscopically by means of ocular micrometer and the average pollen tube length were calculated.

Anatomical work:

Six different types of kakrol vines were collected from field in the month of 15 May to 30 September, 1996 and 1997. The collected vines were preserved in the laboratory in transeau's solution. This solution has been prepared as follows:

Distilled water 60ml+Absolute alcohol 30ml+Formaldehyde 10ml=100ml solution. 10ml of pure glycerine was added to this solution.

Staining: The thin section was stained with 1% safranin in 50% alcohol and mounted in glaycerine.

Microscope calibration:

It is important to make exact calibration of the microscope to be used in the work before measurement of an object is taken. This is usually done by the ocular micrometer which consists of an eye piece micrometer and a stage micrometer.

The eye piece micrometer consists of a circular glass disc on the middle of which is situated a fine graduated scale. This disc is placed inside the eye-piece of the microscope when ever necessary. Sometimes it is placed Permanently in a 10x or 8x eye piéce which can be used after removing the original eye-piece of the microscope at all. The stage micrometer consists of a special kind of glass slide at the center of which is permanently situated a fine graduated scale and centered by a clean glass cover slip. The total length of ocully-meter scale is 1cm and is divided into 100 equal division. So each division cover a distance of 0.1mm or 100μ.

At the time of calibration of a microscope, the eye piece micrometer is placed in side the eye piece of the microscope and introduced on the draw tube. At the same time micrometer is placed on the stage of the microscope. When observed through the eye piece both the scales, will be distinctly visible after suitable adjustment.

By suitable ratting the eye piece both the scales are brought one above the other. It is clearly seen that certain number of division of the eye piece micrometer scale will exactly coincide with certain number of division on the stage micrometer scale. This is recorded. One should be careful in observing. The coincidence of the scales when one looks down the microscope directly vertically observation at a slightest angel will result in erroneous reading. Now, with the help of small arithmetic the calibration is determined. Let it be supposed that 16 divisions of the eye micrometer coincides with the 8 divisions of the stage micrometer then the length of one division of the eye piece micrometer will be as follows:

$$\frac{S}{E} \times 100$$

Where, S = number of division of the stage micrometer.

E = number of division of the eye-piece micrometer.

100 = Length of the division of the micrometer in μ. So the length of the division of the eye piece micrometer is (8/16) x 100 = 50μ.

In this way calibration under any magnification can be made suitably. The stage of micrometer is then removed from the stage of the microscope, but the eye piece micrometer is in position. Now a prepared slide is placed under the microscope and after suitably adjustment, the eye piece micrometer scale appears on the microscopic field. This time the scale is carefully placed over the objects to be measured. Now if a metaxylem of 4n female kakrol stem covers 10 divisions of the eye piece micrometer scale in length, the length of the cell stands at 10x5=50μ.

Determination of magnification of an object drawn by camera lucida:

It is very important to determine the exact magnification of an object drown under a microscope by a camera lucida. When a material is viewed through a 10x eye piece and 40x objective lenses, it is really magnified. 10x10=100 times. But when the object is drawn by camera lucida on a paper, the drawing becomes much more enlarged than the real magnification. Exact magnification of the drawing is generally

determined by constructing a scale by using a stage micrometer and a camera lucida using the same objective and eye piece lenses. The micrometer scale is focused in the microscopic field and its image is traced on the piece of paper containing the drawing by means of camera lucida. An image of the specimen can, with the aid of camera Lucida, then be super imposed on the drawn scale and the specimen was measured. Care must be taken so that the same magnification is used. The magnification of the draw figure can be simple arithmetic. For example, let us suppose that the drawn figure measures 50μ and the drawn scale is 5cm. by actual measurement with the metric scale. Knowing that 5cm. is equal to 50,000μ one divides 50,000 by and finds that the specimen has been enlarged 1000 times on the drawings sheet. This method has been followed in the present work.

Method of peeling collection for stomata: Thin peels from three different places of the lower surface of healthy leaves were collected and preserved in dry condition. Transparent nail polish was used to collect the peeling of kakrol.

Pollination technique:

Kakrol is a dioicious plant, so there was no need for emasculation. Only the selected flowers were labeled and bagged before the flower opening. In case of bisexual flower emasculation were made a day before anthesis to prevent self pollination. Male flowers were collected in sterilized petridish and pollen grain were dusted on the stigma of mature female flower. Proper labelling were made and lebels were kept till fruits were harvested.

Biochemical work:

Following biochemical traits were studied, procedure followed are described below:

1. Total chlorophyll of Kakro leaf
2. pH
3. Moisture
4. Dry-meter
5. Ash
6. Protein
7. Lipid
8. Starch
9. Vitamin C
10. β-Carotene

1. Total chlorophyll of kakrol leaf: Chlorophyll is the green pigments universely present in all photosynthetic tissue. ChlorophyII-b occurs in higher plants. A measurement of green pigments may indirectly denote the severity of disease especially in systematic diseases. Chlorophyll estimates may also be required to relate other biochemical changes in the plant tissue ChlorophyII content of the different types of kakrol leaf tissues was estimated following the method described by Mahadevan & Shidhar (1982).

Materials:

a) Mortar & pestle.

b) Buchner funnel.

c) Pipette, 5ml. 10ml.

d) Colorimeter.

Reagents:

a) Acetone. 80%

Procedure: One gram of kakrol leaf was cut into small pieces and homogenized well with excess acetone in a mortar pestle and then filtered the extract through a Buchner funnel using Whatmann No-24 filter paper. Then sufficient quantity of 80% acetone was added to the respective extraction. The content from the mortar was transferred to the Buchner funnel and washed with 80% acetone. The absorbance of this extract was measured at 645nm and 663nm for determination of chlorophyll-a and chlorophyll-b and total chlorophyll.

The chlorophyll contents were calculated on fresh weight basis employing the following formula as described by Mahadevan (1982). Using the specific absorption co-efficients for chlorophyll-a and chlorophyll-b at 663nm and 645nm in 80 percent acetone respectively.

$$\text{Total chlorophyll (mg/gm)} = \frac{20.2A_{645} + 8.02A_{663}}{1 \times 1000 \times w} \times V$$

$$\text{Chlorophyll-a (mg/gm)} = \frac{20.9A_{645} - 4.68A_{663}}{1 \times 1000 \times w} \times V$$

$$\text{Chlorophyll-b (mg/gm)} = \frac{20.7A_{663} - 2.69A_{645}}{1 \times 1000 \times w} \times V$$

Where, A = Optical density in each cases.

I = Length of light path in the cell (usually 1cm)

v = Volume of the extract in ml and

w = Fresh weight of the sample in gm.

2. pH:

Extraction of kakrol juice: About 50 to 60 g of Kakrol flesh for each types of kakrol were taken in a mortar. The Kakrol flesh was crushed thoroughly in the mortar with a pestle and then filter through two layers of Muslin cloth. The filtrate was then centrifuged for 15 minutes at 3000 rpm. and the clear supernatant was collected.

The pH of kakrol juice was determined by the conventional procedure using pH meter.

Standard buffer solution: pH7 buffer tablet (BDH chemicals Ltd. Poole. England) was dissolved in distilled water and made up to the mark of 100ml with distilled water.

Procedure: The electrode assembly of the pH meter was dipped in to the standard buffer solution of pH7 taken in a clean and dry beaker. The temperature correction knob was set to 28°C and the fine adjusted was made by asymmetry potentially knob to pH7. After washing with distilled water the electrode assembly was then dipped in to a solution of standard pH4 and adjusted to the required pH by fine asymmetry potentially knob. The electrode assembly was raised, washed twice with distilled water rinsed off with juice of the types and then dipped in to the juice of the kakrol. The pH of all the varieties of kakrol was determined by using the same procedure.

3. Moisture: Moisture content was by the conventional procedure.

Materials:

a) Porcelain crucible.
b) Electrical balance.
c) Oven.
d) Desiccator.

Procedure: Four to six grams of kakrol flesh are weighed in a porcelain crucible (which was previously cleaned, heated to 100°C, cooled and weighed). The crucible with the sample was heated in an electrical oven for about six hours at 100°C. It was then cooled in a desiccator and weighed again.

Calculation: Percent of moisture content (g per 100g of kakrol flesh)

$$= \frac{\text{Weight of the moisture}}{\text{Weight of the kakrol flesh}} \times 100$$

4. Dry-meter: Dry matter content was calculated from the data obtained for percent moisture content.

Calculation: % Dry-matter content=Total fresh weight - % moisture content

5. Ash: Ash content was determined by following the method of A.O.A.C. (1980).

Materials:

a) Porcelain crucible
b) Muffle furnace.
c) Electrical balance (Mattler H-18)
d) Desiccator

Procedure: Four to six grams of kakrol flesh were weighed in a porcelain crucible (which was previously clean and heated to about 100°C, cooled and weighed). The crucible was placed in a muffle furnace for about four hours at 600°C. It was then cooled in a desiccator and weighed. To ensure completion of ashing, the crucible was again heated in the muffle furnace for half an hour, cooled and weighed again. This was repeated till two consecutive weights were the same and the ash was almost white in color.

Calculation: Percent of ash content (g per 100g of kakrol flesh)

$$= \frac{\text{Weight of the obtained}}{\text{Weight of the kakrol flesh}} \times 100$$

6. Protein: Protein content of the different types of kakrol-flesh was determined according to Micro-Kjeldhal Methods (Wong, 1923).

Reagents:

a) Solid potassium sulphate

b) Concentrated sulphuric acid

c) 5% $CuSO_4$, $5H_2O$ in distilled water

d) 0.01(N) H_2SO_4 solution

e) Concentrated sodium hydroxide solution 5(N), approximately.

f) Boric acid solution containing bromocresol green: 10g of boric acid was dissolved in hot water (250ml.) and cooled. 1ml of 0.1% bromocresol green in alcohol was added and diluted up to 500 ml with distilled water.

g) Few quartz chips.

h) Nitrogen determination apparatus (micro model) according Paranas-Wagner, made of JENA Glass-all connections with interchangeable ground joints.

Procedure:

a) Digestion: 4 to 6ml. conc. H_2SO_4, 1.0g K_2SO_4, one to two drops 5% $CuSO_4$ solution (catalyst) and some quartz chips (to avoid bumping) were added in 1-2g of kakrol-flesh in a kjeldhal flask. The mixture was heated about 3-4 hours.

b) Collection of ammonia: The digestion was carried out in the steam distillation chamber of the nitrogen determination apparatus. After the completion of digestion the steam distillation chamber containing the digested mixture was filtered back to the nitrogen determination apparatus. Boric acid solution (15ml) in a small flask was so placed that the tip of the condenser outlet dipped below the surface of the boric acid solution. Sufficient amount of concentrated sodium hydroxide solution (approximately 30-40ml) was added to digest in the chamber to neutralize the amount of acid present. Steam was generated from the steam-generating flask and the sample in the chamber was steam distilled until 20ml of distillate was collected in the boric acid solution. The condenser outlet was then rinsed with little distilled water and the receiving flask was removed.

c) Titrimetric estimation of ammonia: The ammonia in the boric acid solution was titrated with 0.01(N) H_2SO_4 until the solution had been brought back to its original yellow-green colour. The titration was repeated with a control containing only 15 ml of boric acid solution diluted to approximately the final volume of the titrated sample. The volume of acid required was recorded.

Calculation: The total nitrogen was calculated using the formula given below:

i)100 ml of 1 N acid = 14g of nitrogen

ii) Xg of N_2 = 6.25×g of protein

Percentage of protein content (g per 100g of kakrol)

$$= \frac{\text{Weight of the obtained}}{\text{Weight of the kakrol flesh}} \times 100$$

7. Lipid:

Lipid content of the different types of kakrol-flesh was determined by the method of Bligh and Dyer 1959.

Reagent:

A mixture of chloroform and ethanol (2: 1 v/v)

Procedure: About one gm of flesh was first ground in a mortar and pestle with 10ml of distilled water. The grinded flesh was transferred to a separating funnel and 30ml of chloroform-ethanol mixture was added. The mixture was mixed well and kept overnight at room temperature in the dark. At the end of this period, 20ml chloroform and 20ml water was further added and mixed. A clear lower layer of chloroform containing all the lipid, a coloured aqueous layer of ethanol with all water soluble materiel, and a thick pasty interphase was seen.

The chloroform layer was carefully collected in a pre-weighed beaker (50ml) and discarded the other two layers. The beaker containing the chloroform layer was then placed on a steam bath for evaporation. After evaporation of the chloroform, the weight of the beaker was determined again. The difference in weight gives the amount of lipid.

Calculation: Percentage of lipid content (g per 100g of kakrol-flesh)

$$= \frac{\text{Weight of lipid obtained}}{\text{Weight of the kakrol flesh}} \times 100$$

8. Starch: The starch contont of the kakrol-flesh was determined by the Anthrone method (Jayaraman, 1981)

Reagents:

a) Anthrone reagent (0.2% in con. H_2SO_4)

b) Standard glucose solution (10g/100ml)

c) 1(M) HCI

Procedure: About five g of flesh were out into small pieces and homogenized well with 20ml of water. It was then filtered through double layer of muslin cloth. To the filtrate, twice the volume of ethanol was added to precipitate the starch, mainly starch. After kept it overnight in cold, the precipitate was collected by centrifugation in a clinical centrifuge at 3000rpm for 15 minutes. the precipitate was then dried over a steam bath. 40ml of 1(M) hydrochloric acid was added to the dried precipitate and heated to about 70°C. It was then transferred to a volumetric flask and diluted to 100 ml with 1(M)HCI. 1ml of diluted solution was taken in another 100ml with volumetric flask and diluted to 100ml with 1(M) HCI.

1ml aliquots of the flesh extract from each genotype were pipetted into test tubes. 4ml of the anthrone reagent were added to each test tubes and mixed well. Glass marbles were placed on top of each tube to prevent loss of water by evaporation. The tubes were placed in a boiling water bath for 10 minutes and then cooled. The absorbance of the blue-green solution was measured at 680 nm in a sprctrophotometre (Gallenkamp color spec.)

The standard curve of glucose was prepared by taking 0.0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1ml of standard glucose solution in different test tubes containing 0.0, 10µg, 40µg, 60µg, 80µg and 100µg of glucose, respectively and made the volume up to 1.0ml with distilled water. 4ml of anthrone reagent was added to each test tube and mixed well.

Calculation: The percentage of starch content $= \frac{\text{Weight of starch obtained}}{\text{Weight of kakrol flesh}} \times 100$

9. Vitamin C: Vitamin C content of kakrol-flesh was determined by the Bessey's titrimetric method (1933).

Reagents:

a) **Dye solution :** 200g of 206 dichlorophenol indophenol (BDH chemicals Ltd.) and 210g of sodium bicarbonate were dissolved in distilled water, made up to 1000ml and filtered the solution.

b) **3% metaphosphoric acid reagent:** 30g of mataphosphoric acid was dissolved in 80ml of acetic acid and made up to 1000ml with distilled water.

c) **Standard vitamin C solution (0.1g/ml) :** 10m of pure vitamin C (BDH) chemicals Ltd.) was dissolved in 3% metaphosphoric acid made up to 100ml with 3% metaphosphoric acid.

Procedure: 10ml of standard vitamin C solution was taken in a conical flask and titrated dye solution.

Four to five gms of kakrol-flesh were cut into small pieces and homogenized well with 3% metaphosphoric acid (20ml) and filtered it through double layer of muslin cloth. The filtrate was centrifuged at 3000rpm for 10 minutes and the clear supernatant was titrated with 2,6-dichlorophenolindophenol solution. The amount of vitamin C present in the extraction was determined by comparing with the titration result of standard vitamin C solution.

Calculation: Percentage of vitamin C content $= \frac{\text{Amount of vitamin C obtained}}{\text{Weight of sample (kakrol-flesh)}} \times 100$

10. β-Carotene: β-Carotene content of the different genotypes of kakrol was determined according to the procedure reported in the methods of vitamin assay (Anon, 1960) and methods of Biochemical analysis (Glick, 1957).

Reagents: Ammonium sulphate.

a) Acetone.
b) Petroleum ether (40°-60°C).
c) n-Hexane.
d) Activated alumina (BDH chemicals Ltd.)
e) Standard solution of β-carotene: A standard solution of β-carotene in 100 ml or petroleum ether.
f) 5.6% KOH. solution.

Column preparation: A column (400mm × 2.5mm) was prepared by using alumina as a packing materiel. 10% acetone in petroleum ether was used as a eluant buffer.

Procedure: Five g of fresh kakrol and about four g of ammonium sulphate were taken in a mortar, and rubed to an even paste with pastle. The extraction was carried out with acetone and small amount of hexane. Extraction was continued untill the acetone extract became colourless. 10ml of potassium hydroxide solution (5.6%) was added to the extract and it was kept in a dark place for half and hour. The mixture was then transferred to a separating funnel. 20ml of petroleum ether, a few ml of hexane and 10 ml of water were added to the separating funnel and shaking gently. The ether layer become colourless. The petroleum ether extract was concentrated by gentle heating. The concentrated extract (1-2) ml was applied on to the top of the alumina column and eluted with 10% acetone in petroleum ether. The absorbance of the elan was taken at 440nm in a coleman Junior II spectrophotometer.

Construction of standard curve of β-carotene: A standard curve was prepared by taking 0.0, 0.1, 0.2, 0.6, 0.8 and 1.0ml standard solution of β-carotene and the volume was made up to 5ml with petroleum ether and mixed well. The absorbance of the solutions were taken at 440nm in a Coleman Junior II spectrophotometer and a standard curve of β-carotene was prepared by plotting the data.

The amount of β-carotene content in each cultivar of kakrol was calculated by using the standard curve.

Calculation: Percentage of β-carotene (mg per 100g of kakrol)

$$= \frac{Amount\ of\ \beta\text{-carotene obtained}}{Weight\ \text{of kakrol flesh}} \times 100$$

Collection of data:

Data have been collected by the following method:

Germination of seeds: Total number of germinated seeds of different treatment were counted up to 50 days and expressed in percentage. Affected seedling and mortality number also recorded in percentage.

Morphological observation: From seedling different abnormality of morphological character such as leaf thickness, leaf colour etc. of morphological were recorded.

Sex conversion: Number of bisexual flower were counted.

Pollen germination: Total number of germination pollen were counted and recorded.

Pollen tube length: Pollen tube lengths were measured directly in microns.

Diameter of protoxylem: Diameter of protoxylem was measured directly in microns, which were converted in mm late.

Diameter of metaxylem: Diameter of metaxylem was measured directly in microns, which were converted in mm.

Length of phloem: The redial length (length parallel to periphery) was measured directly in microns, which were converted in mm.

Breadth of phloem: Breadth of phloem (breadth perpendicular to periphery) was measured directly in microns, which were converted in mm.

Length of vascular bundle: Length of vascular bundle (length perpendicular to periphery) was measured directly in microns, which were converted in mm.

Breadth of vascular bundle: Breadth of vascular bundle (breadth parallel to periphery) was measured directly in microns, which were converted in mm.

Area of the vascular bundle: Area of the vascular bundle measured in micron^2, which were converted into mm^2 later. A graphic scales drawn by the camera lucida by using micro scale. This graphic scale contains square units. Every square unit of graphic scale contains 2500μ^2(50μ+50μ) of original area. The area of vascular bundles were measured by counting the number of small square units of known area (2500 micron^2/small square unit area) covering a vascular bundle.

Micron were converted into mm by using a simple arithmetic:

$$1\text{Micron}^2 = \frac{1}{1000000}$$

Number of stomata: Total number of stomata per focus (15x40)were counted and recorded.

Guard cell length: Guard cell lengths were measured directly in microns

Guard cell width: Guard cell width were measured directly in microns

Leaf petiole length: The length of randomly selected petioles of each plant were measured by centimeter scale in cm.

Leaf petiole breadth: Breadth were measured in the middle of the petiole in cm.

Leaf blade length: From the leaf base to tip of the leaf were measured in cm.

Leaf blade breadth: Breadth were measured in the middle of the leaf in cm.

Tendril length: From the vine-node to tip of the tendril were measured in cm.

Internode length: Length of internode collected randomly of each types of plants were measured by using centimeter scale in cm.

Internode breadth: Breadth were measured in the middle of the vine-node in cm.

Bract length: From the base to tip of the bract were measured in cm.

Bract breadth: Breadth were measured in the middle of the bract in cm.

Calyx length: Calyx length was measured from base to apex in cm.

Calyx breadth: Calyx breadth was measured in cm through widest point.

Petal length: Petal length was measured from base to apex in cm.

Petal breadth: Petal breadth was measured in cm through widest point.

Flowering time: From the date of visible bud initiation to opening flower in days.

Flower diameter: Diameter of flower collected were randomly measured in cm.

Stamens length: Stamens length was measured from base to apex in cm.

Pollen diameter: Pollen diameter was measured directly in microns.

Pollen viability: Viable pollen were determine through acetocarmine staining. Those took stain were considered as viable pollen and those didn't considered as non-viable pollen. Number of viable pollen and non-viable pollen were counted and recorded.

Pollen viability (following morning): Viable pollen were determined through acetocarmine staining. Those took up stain were considered as viable pollen and those didn't considered as non-viable pollen. Number of viable pollen and non-viable pollen were counted and recorded.

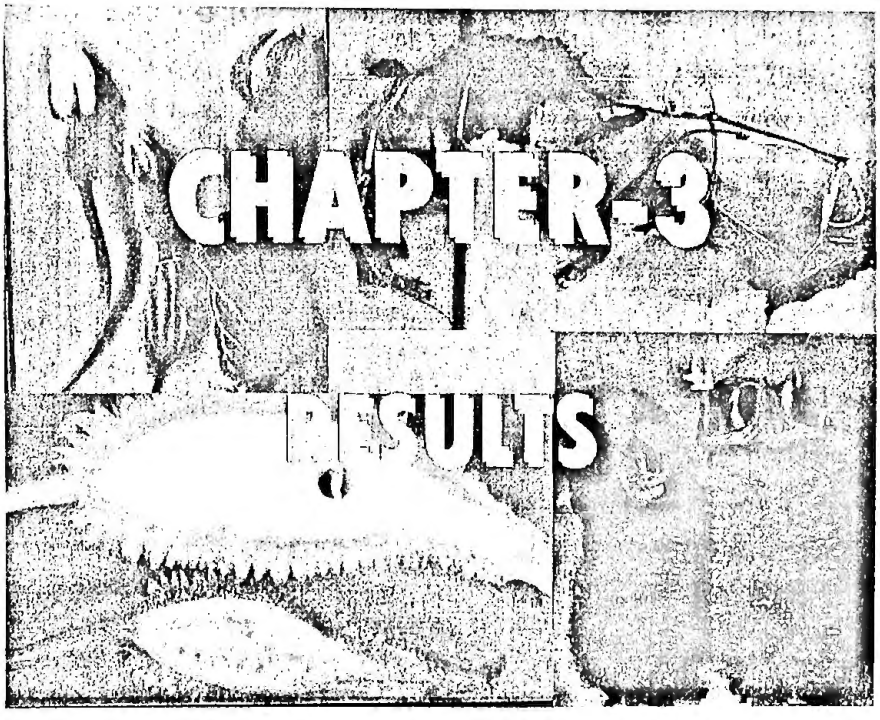
Fruit setting: Total number of fruit developed through hand pollination with different types of pollen source were recorded.

Fruit weight: Mature fruit were harvested from different type of pollination and weight was taken in g.

Seeds per fruit: Total number of seed per fruit were counted and recorded.

Seed weight per fruit: Weight of sun dried seed of a fruit were taken in g and recorded.

Analysis of data: Collected data were analysed by standard Biostatical methods (Mather, 1949; Allard, 1956c; Fisher *et al.* 1932 and Fisher, 1936).


CHAPTER-3
RESULTS

RESULTS

Comparative study of morphological characters of affected and naturally occurring *Momordica dioica* Roxb.:

The seeds and seedlings of diploid, triploid and tetraploid kakrol were treated with seven different concentrations of colchicine viz., 0.1, 0.2, 0.3, 0.4, 0.5, 0.7 and 1% for the induction of polyploid type. Through a seedling of the same season, the normal seedlings of kakrol are differed significantly from the treated seeds or seedlings in morphological characters. Different morphological characters were observed and recorded and the results are presented in **table 1.**

The treated and controlled seeds and seedlings were allowed to grow in petri dish, polyethene bag and seed bed. The normal and abnormal seedlings were recorded from these experiments. It was observed that the first 1 to 3 leaves of plants were thick and dark green in colour. Irregular shape of leaves were observed from the affected seedling. Scale like leaves were arranged on the hypocotyle of seedlings when the seed was treated with colchicine **(Plate I & II).**

A large number of seedlings were died with 5 to 40 days of treatment. All these seedlings were recorded as abnormal. Abnormal seedling growth, reduction of germination and significant delay in germination time were recorded. From affected seed 2 to 5 shoots were sprouted from the base of the cotyledon. Affected shoot tips of seedling showed very typical form as like as tumor from normal respective tip of seedling. The growth of the tips were fully checked within 30 to 40 days. Some treated shoot tips (tumor like tip) were initiated 1 to 6 branches.

There was no significant different observed in the morphological characters between the treated and naturally occurring three different genotypes of kakrol. The shoots when it were sprouted from cotyledons base and initiating from the swelling tips of seedlings were found to be normal as like as normal respective ploidy of *M. dioica.*

Table 1. Comparative study of morphological characters of naturally occurring diploid, triploid and tetraploid *M. dioica* with treated *M. dioica*.

Character	Genotypes	Normal	Treated
Seeds/Seedlings:			
Seedling height	Diploid	3.57±0.24cm (toller than treated seedling)	3.04±0.16cm
	Triploid	4.39±0.47cm	4.13±0.46cm
	Tetraploid	5.48±0.52cm	4.87±0.49cm
Leaf	Diploid	Thin	Thick
	Triploid	Thin	Thick
	Tetraploid	Thin	Thick
Leaf colour	Diploid	Light green	Dark green
	Triploid	Green	Dark green
	Tetraploid	Green	Dark green
Leaf shape	Diploid	Regular	Irregular
	Triploid	Regular	Irregular
	Tetraploid	Regular	Irregular
Scale like leaf	Diploid	Absent	Present on hypocotyle
	Triploid	Absent	Present on hypocotyle
	Tetraploid	Absent	Present on hypocotyle
Shoot tip	Diploid	Normal shoot tip	As like as tumor
	Triploid	Normal shoot tip	Tumor like
	Tetraploid	Normal shoot tip	Tumor like
Shoot tip colour	Diploid	light green	Some are deep green
	Triploid	light green	Some are deep green
	Tetraploid	light green	Deep green
No. of shoots initiate from the cotyledon base	Diploid	One	Two to five
	Triploid	One	Two to five
	Tetraploid	One	Two to five
Branching type from the seedling tip	Diploid	One	One to six
	Triploid	One	One to six
	Tetraploid	One	One to six
Mortality %	Diploid	Zero to 10%	70% to 90%
	Triploid	Zero to 5%	70% to 90%
	Tetraploid	Zero to 5%	70% to 85%
Growth (after 40 days)	Diploid	30-60cm	3-10cm
	Triploid	45-100cm	4-15cm
	Tetraploid	50-120cm	5-18cm
Behave	Diploid	Normal	Abnormal
	Triploid	Normal	Abnormal
	Tetraploid	Normal	Abnormal
Plants:			
Internode length	Diploid	3.81±0.48cm	36.77±0.51cm
	Triploid	10.09±1.21cm	10.24±1.36 cm
	Tetraploid	11.53±1.34cm	11.42±1.41 cm
Internode breadth	Diploid	0.38±0.10cm	0.37±0.11 cm
	Triploid	0.56±0.14cm	0.65±0.14 cm
	Tetraploid	0.80±0.23cm	0.81±0.22 cm

Table 1. Contd.

Character	Genotypes	Normal	Treated
Leaf blade length	Diploid	5.42±0.65cm	5.25±0.71 cm
	Triploid	7.85±0.88cm	7.81±1.00 cm
	Tetraploid	10.14±1.19cm	9.98±1.25 cm
Leaf blade breadth	Diploid	6.03±0.82cm	5.81±0.83 cm
	Triploid	9.14±1.36cm	8.95±1.33 cm
	Tetraploid	11.20±1.57cm	11.21±1.49 cm
Stomata number per focus	Diploid	14.32±2.15	13.93±1.80
	Triploid	16.55±2.64	16.44±2.53
	Tetraploid	14.25±2.38	14.50±2.31
Guard cell length	Diploid	20.32±3.04µm	19.87±3.35µm
	Triploid	32.07±4.22µm	31.62±4.40µm
	Tetraploid	34.84±4.99µm	34.11±5.26µm
Guard cell width	Diploid	7.50±1.06µm	7.46±1.039µm
	Triploid	9.65±1.24µm	9.92±2.15µm
	Tetraploid	12.75±2.00µm	12.73±2.29µm
Flower opening time (from visible bud to opening time)	Diploid	19.24±2.34(days)	20.02±1.67(days)
	Triploid	26.20±3.22(days)	25.93±2.98(days)
	Tetraploid	29.45±3.73(days)	29.08±3.77(days)
Flower diameter	Diploid	3.11±0.30cm	3.17±0.35 cm
	Triploid	6.85±0.56 cm	7.00±0.58 cm
	Tetraploid	8.90±0.94 cm	8.75±0.75 cm
Calyx length	Diploid	0.81±0.16 cm	0.76±0.13 cm
	Triploid	0.32±0.09 cm	0.34±0.11 cm
	Tetraploid	1.28±0.38 cm	1.30±0.42 cm
Calyx breadth	Diploid	0.20±0.02cm	0.21±0.02cm
	Triploid	0.27±0.04cm	0.27±0.03cm
	Tetraploid	0.28±0.06cm	0.27±0.04cm
Petal length	Diploid	2.41±0.35cm	2.35±0.25 cm
	Triploid	5.65±0.54 cm	5.51±0.48 cm
	Tetraploid	5.32±0.43 cm	5.40±0.53 cm
Petal breadth	Diploid	1.17±0.21 cm	1.16±0.18 cm
	Triploid	2.60±0.33 cm	2.55±0.26 cm
	Tetraploid	4.04±0.37 cm	3.85±0.34 cm
Ovary length	Diploid	1.12±0.22 cm	1.08±0.16 cm
	Triploid	1.34±0.17 cm	1.33±0.31 cm
	Tetraploid	1.72±0.28 cm	1.17±0.25 cm
Ovary diameter	Diploid	0.65±0.09 cm	0.66±0.09 cm
	Triploid	0.68±0.13 cm	0.70±0.14 cm
	Tetraploid	0.80±0.17 cm	0.79±0.16 cm
Fruit length	Diploid	5.40±0.75 cm	5.42±0.69 cm
	Triploid	-	-
	Tetraploid	9.54±1.08 cm	9.81±1.12 cm
Fruit weight	Diploid	17.81±1.97g	16.88±1.82g
	Triploid	-	-
	Tetraploid	75.29±2.67g	75.88±2.46g
Seed per fruit	Diploid	13.58±2.22	14.20±2.08
	Triploid	-	-
	Tetraploid	23.74±3.56	23.55±3.29
Weight of seed per fruit	Diploid	1.32±0.24g	1.40±0.031g
	Triploid	-	-
	Tetraploid	5.19±0.058g	5.12±0.60g

Effect of colchicine on germinating seeds:

Germination of seeds in kakrol usually require longtime compared to other cucurbitaceae. Decoated and unpeeled seeds of different genotype of kakrol were treated with different strength of colchicine viz., 0.1, 0.2, 0.3, 0.4, 0.5, 0.7 and 1% of different treatment time for the induction of artificial polyploid type **(Plate I Fig. A.)**. Three germination medium were used viz., petri dish, polyethne bag and seed bed. Detail of these three medium have been written in the chapter of method and materials.

Effect of colchicine on seeds of diploid kakrol after 3h treatment:

Germination percentage and germination time after 3h with colchicine in diploid kakrol is presented in **table 2.**

Petridish experiment: Seeds of S type of kakrol were germinated in petridish. The experiments were divided into two groups, in one group seeds were placed in petri dish with intact seed coat (unpeeled) and other group seed coat of seeds were removed (decoated seed).

One average, 58% seeds were germinated within 22 days with decoated seeds when it was treated with distill water and it was 10% when intact seed coat was present. With increase in colchicine concentrations there were decrease in germination in both of the decoated and unpeeled seeds. The lowest 30% seeds were germinated with decoated seeds in the petridish within 40 days when it was treated with 1% colchicine. When unpeeled seeds were treated with 0.4, 0.5, 0.7 and 1% colchicine there was no seed germination in the petri dish. 2 to 8% seeds were germinated with unpeeled seed. It is evident from this result that germination percentage is very high in decoated seeds.

Polyethene bag experiment: Thirty seven to 55% treated seeds were germinated with unpeeled seed. On the contrary the percentage of germination was higher in decoated

seeds and it was 40 to 65%. The germination of control seeds (treated with distill water) were 77 and 57%, respectively in decoated and unpeeled seeds.

Twenty to 45% reduction of germination were recorded in decoated seeds, where as unpeeled seeds with same concentration reduced only 10 to 25% germination in diploid kakrol.

Seed bed experiment: The germination percentage in seed bed experiments were similar to those of polyethene bag experiment. On average, 37 to 62% and 40 to 64%, respectively in decoated and unpeeled seeds were germinated. The rate of germination became gradually lower when the strength of colchicine were increased.

Significant delay in germination time was recorded after treatment with all the concentrations of colchicine in both of decoated and unpeeled seeds. On average, 25% germinated seeds were affected by the colchicine treatment in decoated seeds and it was 18% in unpeeled seeds.

Effect of colchicine on seeds of diploid kakrol after 6h treatment:

Germination percentage and germination time after 6h with colchicine in diploid kakrol is presented in **table 3.**

Petridish experiment: One average, 58% seeds were germinated within 20 days with decoated seeds when it was treated with distill water and it was 7% when intact seed coat was present. The lowest 15% seeds were germinated with decoated seeds in the petridish within 40 days when it was treated with 1% colchicine. When unpeeled seeds were treated with 0.3, 0.4, 0.5, 0.7 and 1% colchicine there was no seed germination in the petridish. 2 to 8% seeds were germinated with unpeeled seed. It is evident from this result that germination percentage is very high in decoated seeds.

Polyethene bag experiment: Twenty one to 56% treated seeds were germinated with unpeeled seed. On the contrary the percentage of germination was higher in decoated seeds and it was 30 to 59%. The germination of control seeds (treated with distill water) were 74 and 58%, respectively in decoated and unpeeled seeds.

Seed bed experiment: The germination percentage in seed bed experiment was similar to those of polyethene bag experiment. On average, 28 to 57% and 30 to 65% respectively in decoated and unpeeled seeds were germinated. The rate of germination became gradually lower when the strength of colchicine were increased.

Significant delay in germination time was recorded after treatment with all the concentrations of colchicine in both of decoated and unpeeled seeds. On average, 30% germinated seeds were affected by the colchicine treatment in decoated seeds and it was 23% in unpeeled seeds.

Effect of colchicine on seeds of diploid kakrol after 9h treatment:

Germination percentage and germination time after 9h with colchicine in diploid kakrol is presented in **table 4.**

Petridish experiment: One average, 52% seeds were germinated within 17 days with decoated seeds when it was treated with distill water and it was 8% when intact seed coat was present. With increase in colchicine concentrations there were decrease in germination in both of the decoated and unpeeled seeds. The lowest 11% seeds were germinated with decoated seeds in the petridish within 40 days when it was treated with 1% colchicine. When unpeeled seeds were treated with 0.2, 0.3, 0.4, 0.5, 0.7 and 1% colchicine there was no seed germination in the petri dish. 5% seeds were germinated with unpeeled seed, when it was treated with 0.1% colchicine solution. It is evident from this result that germination percentage is very high in decoated seeds.

Polyethene bag experiment: Eighteen to 53% treated seed were germinated with unpeeled seed and in decoated seeds it was 16 to 51%. The germination of control seeds (treated with distill water) were 70 and 57%, respectively in decoated and unpeeled seeds.

Seed bed experiment: On average, 15 to 55% and 20 to 57%, respectively in decoated and unpeeled seeds were germinated. 31% seeds were germinated when it was treated with 0.5% colchichine with decoated seed. and it was 29% in unpeeled seed. The lowest seeds were germinated when it was treated with 1% colchicine solution with decoated seeds of diploid kakrol. The rate of germination became gradually lower when the strength of colchicine were increased.

Effect of colchicine on seeds of diploid kakrol after 12h treatment:

Germination percentage and germination time after 12h with colchicine in diploid kakrol is presented in **table 5.**

Petridish experiment: One average, 43% seeds were germinated within 17 days with decoated seeds when it was treated with distill water and it was 10% when intact seed coat was present. The lowest 5% seeds were germinated with decoated seeds in the petri dish within 40 days when it was treated with 1% colchicine. The highest was 38% in decoated seeds when it was treated with 0.1% colchicine solution. When unpeeled seeds were treated with 0.2, 0.3, 0.4, 0.5, 0.7 and 1% colchicine there was no seed germination in the petri dish. 5% seeds were germinated with unpeeled seed when it was treated with 0.1% colchicine solution. It is evident from this result that germination percentage is very high in decoated seeds.

Polyethene bag experiment: Thirteen to 48% treated seed were germinated with unpeeled seed when it was treated with 0.1, 0.2, 0.3, 0.4, 0.5, 0.7 and 1% colchicine solution. On the contrary the percentage of germination was higher in decoated seeds

and it was 15 to 49%. The germination of control seeds (treated with distill water) were 61 and 57%, respectively in decoated and unpeeled seeds.

Seed bed experiment: On average, 17 to 51% and 18 to 52%, respectively in decoated and unpeeled seeds were germinated when it was treated with seven different strength of colchicine solution. The rate of germination became gradually lower when the strength of colchicine were increased.

Effect of colchicine on seeds of diploid kakrol after 18h treatment:

Germination percentage and germination time after 18h with colchicine in diploid kakrol is presented in **table 6.**

Petridish experiment: One average, 35% seeds were germinated within 16 days with decoated seeds when it was treated with distill water and it was 10% when intact seed coat was present. The lowest 2% seeds were germinated with decoated seeds in the petridish within 40 days when it was treated with 0.1% colchicine. In decoated seed 12 and 8% seeds were germinated when it was treated with 0.4 and 0.5% colchicine solution respectively. The highest was 22% when it was treated with 0.1% solution. When unpeeled seeds were treated with 0.3, 0.4, 0.5, 0.7 and 1% colchicine there was no seed germination in the petridish. 2 to 5% seeds were germinated with unpeeled seed.

Polyethene bag experiment: Ten to 41% treated seed were germinated with unpeeled seed and in decoated seeds and it was 4 to 27%. In decoated and unpeeled seed respectively 8 and 20% seeds were germinated when seeds were treated with 0.5% colchicine solution. The germination of control seeds (treated with distill water) were 40 and 52%, respectively in decoated and unpeeled seeds.

Seed bed experiment: On average, 4 to 31% and 12 to 47% respectively in decoated and unpeeled seeds were germinated. The highest 47% treated seeds were germinated

in unpeeled seed when it was treated with 0.1% colchicine solution and it was lowest (12%) with 1% colchicine treatment. The rate of germination became gradually lower when the strength of colchicine were increased.

Effect of colchicine on seeds of diploid kakrol after 24h treatment:

Germination percentage and germination time after 24h with colchicine in diploid kakrol is presented in **table 7.**

Petridish experiment: Fifteen percent seeds were germinated within 15 days with decoated seeds when it was treated with distill water and it was 14% when intact seed coat was present. With increase in colchicine concentrations there were decrease in germination in both of the decoated and unpeeled seeds. When unpeeled seeds were treated with 0.3, 0.4, 0.5, 0.7 and 1% colchicine there was no seed germination in the petridish. The lowest 5% seeds were germinated with unpeeled seed when it was treated with 0.2% colchicine solution and it was 2% in decoated seed when seeds were treated with 0.4% colchicine solution.

Polyethene bag experiment: Two to 31% treated seeds were germinated with unpeeled seed. The highest percentage of germination was 12 in decoated seed when it was treated with 0.1% colchicine solution. The percentage of germination was zero in decoated seed when it was treated with 0.7 and 1% colchicine solution. The germination of control seeds (treated with distill water) were 18 and 41%, respectively in decoated and unpeeled seeds.

Seed bed experiment: On average, 2 to 20% and 4 to 35%, respectively in decoated and unpeeled seeds were germinated when the seeds were treated with seven said strength of colchicine solution. The rate of germination became gradually lower when the strength of colchicine were increased. Only 2% seeds were germinated within 40 days in decoated seed when it was treated with 0.7% colchicine solution and in decoated seed it was 4%.

Table 2. Germination percentage and germinating time after 3h treatment with different concentration of colchicine in diploid kakrol.

Seed types	Concentration	Petri dish		Polyethene bag		Seed bed	
		Germination %	Germination time	Germination %	Germination time	Germination %	Germination time
Decoated	0.0 (Control)	58	22	77	13	70	12
	0.1	51	30	65	21	62	22
	0.2	47	32	60	23	58	24
	0.3	46	35	57	25	55	28
	0.4	45	36	54	28	51	28
	0.5	42	38	51	31	48	31
	0.7	35	40	46	25	44	36
	1.0	30	40	40	40	37	40
Unpeeled	0.0 (Control)	10	40	57	40	65	40
	0.1	8	40	55	40	64	40
	0.2	5	40	55	40	62	40
	0.3	2	40	51	40	58	40
	0.4	0	40	47	40	57	40
	0.5	0	40	42	40	52	40
	0.7	0	40	40	40	47	40
	1.0	0	40	37	40	40	40

Table 3. Germination percentage and germinating time after 6h treatment with different concentration of colchicine in diploid kakrol.

Seed types	Concentration %	Petri dish		Polyethene bag		Seed bed	
		Germination%	Germination time	Germination %	Germination time	Germination %	Germination time
Decoated	0.0 (Control)	58	20	74	11	71	12
	0.1	46	33	59	22	57	21
	0.2	42	40	55	26	53	25
	0.3	37	40	47	30	46	31
	0.4	31	40	44	33	42	35
	0.5	26	40	40	40	37	40
	0.7	22	40	33	40	30	40
	1.0	15	40	30	40	28	40
Unpeeled	0.0 (Control)	7	40	58	40	67	40
	0.1	6	40	56	40	65	40
	0.2	2	40	50	40	61	40
	0.3	0	40	47	40	52	40
	0.4	0	40	41	40	44	40
	0.5	0	40	33	40	39	40
	0.7	0	40	29	40	37	40
	1.0	0	40	21	40	30	40

Table 4. Germination percentage and germinating time after 9h treatment with different concentration of colchicine in diploid kakrol.

Seed types	Concentration %	Petridish		Polyethene bag		Seed bed	
		Germination %	Germination time	Germination %	Germination time	Germination %	Germination time
Decoated	0.0 (Control)	52	17	70	11	73	10
	0.1	43	22	51	22	55	22
	0.2	40	40	43	22	50	29
	0.3	35	40	37	31	44	33
	0.4	28	40	30	35	40	37
	0.5	22	40	22	40	31	38
	0.7	15	40	19	40	23	40
	1.0	11	40	16	40	15	40
Unpeeled	0.0 (Control)	8	40	57	40	64	40
	0.1	5	40	53	40	57	40
	0.2	0	40	42	40	46	40
	0.3	0	40	33	40	38	40
	0.4	0	40	28	40	33	40
	0.5	0	40	23	40	29	40
	0.7	0	40	20	40	24	40
	1.0	0	40	18	40	20	40

Table 5. Germination percentage and germinating time after 12h treatment with different concentration of colchicine in diploid kakrol.

Seed types	Concentration %	Petridish		Polyethene bag		Seed bed	
		Germination%	Germination time	Germination %	Germination time	Germination %	Germination time
Decoated	0.0 (Control)	43	17	61	10	63	9
	0.1	38	40	48	24	51	20
	0.2	33	40	40	28	47	29
	0.3	30	40	33	34	41	34
	0.4	21	40	29	35	34	38
	0.5	16	40	25	40	28	38
	0.7	10	40	19	40	24	40
	1.0	5	40	13	40	17	40
Unpeeled	0.0 (Control)	10	40	57	40	65	40
	0.1	5	40	49	40	52	40
	0.2	0	40	42	40	45	40
	0.3	0	40	35	40	38	40
	0.4	0	40	30	40	34	40
	0.5	0	40	24	40	27	40
	0.7	0	40	18	40	23	40
	1.0	0	40	15	40	18	40

Table 6. Germination percentage and germinating time after 18h treatment with different concentration of colchicine in diploid kakrol.

Seed types	Concentration %	Petridish		Polyethene bag		Seed bed	
		Germination%	Germination time	Germination %	Germination time	Germination %	Germination time
Decoated	0.0 (Control)	35	16	40	10	42	9
	0.1	22	23	27	22	31	29
	0.2	16	29	21	27	27	32
	0.3	14	34	15	32	25	35
	0.4	12	40	10	36	17	35
	0.5	8	40	8	40	12	37
	0.7	5	40	6	40	8	40
	1.0	2	40	4	40	4	40
Unpeeled	0.0 (Control)	10	40	52	40	61	40
	0.1	5	40	41	40	47	40
	0.2	2	40	35	40	40	40
	0.3	0	40	31	40	32	40
	0.4	0	40	24	40	26	40
	0.5	0	40	20	40	21	40
	0.7	0	40	13	40	15	40
	1.0	0	40	10	40	12	40

Table 7. Germination percentage and germinating time after 24h treatment with different concentration of colchicine in diploid kakrol.

Seed types	Concentration %	Petridish		Polyethene bag		Seed bed	
		Germination%	Germination time	Germination %	Germination time	Germination %	Germination time
Decoated	0.0 (Control)	15	15	18	8	20	9
	0.1	12	22	12	14	14	20
	0.2	7	40	10	40	12	29
	0.3	6	40	6	40	10	34
	0.4	2	40	4	40	7	38
	0.5	0	40	2	40	3	38
	0.7	0	40	0	40	2	40
	1.0	0	40	0	40	0	40
Unpeeled	0.0 (Control)	14	40	41	40	42	40
	0.1	8	40	31	40	35	40
	0.2	5	40	22	40	24	40
	0.3	0	40	17	40	18	40
	0.4	0	40	9	40	12	40
	0.5	0	40	5	40	8	40
	0.7	0	40	2	40	4	40
	1.0	0	40	2	40	4	40

Effect of colchicine on seeds of triploid kakrol after 3h treatment:

Germination percentage and germination time after 3h with colchicine in triploid kakrol is presented in **table 8.**

Petridish experiment: Seeds of triploid type of kakrol were germinated in petri dish. The experiments were divided into two groups, in one group seeds were placed in petridish with intact seed coat (unpeeled) and other group seed coat of seeds were removed (decoated seed).

One average, 52% seeds were germinated within 21 days with decoated seeds when it was treated with distill water and it was 9% when intact seed coat was present. With increase in colchicine concentrations there were decrease in germination in both of the decoated and unpeeled seeds. The lowest 32% seeds were germinated with decoated seeds in the petridish within 40 days when it was treated with 1% colchicine. When unpeeled seeds were treated with 0.4, 0.5, 0.7 and 1% colchicine there was no seed germination in the petridish. 4 to 8% seeds were germinated with unpeeled seed. It is evident from this result that germination percentage is very high in decoated seeds.

Polyethene bag experiment: Thirty to 60% treated seeds were germinated with unpeeled seed. On the contrary the percentage of germination was higher in decoated seeds and it was 46 to 72%. The germination of control seeds (treated with distill water) were 81 and 62%, respectively in decoated and unpeeled seeds.

Seed bed experiment: The germination percentage in seed bed experiment was similar to those of polyethene bag experiment. On average, 47 to 74% and 40 to 64%, respectively in decoated and unpeeled seeds were germinated. The rate of germination became gradually lower when the strength of colchicine were increased.

Significant delay in germination time were recorded after treatment with all the concentrations of colchicine in both of decoated and unpeeled seeds.

Effect of colchicine on seeds of triploid kakrol after 6h treatment:

Germination percentage and germination time after 6h with colchicine in triploid kakrol is presented in **table 9.**

Petridish experiment: One average, 51% seeds were germinated within 18 days with decoated seeds when it was treated with distill water and it was 11% when intact seed coat was present. The lowest 31% seeds were germinated with decoated seeds in the petridish within 40 days when it was treated with 1% colchicine. When unpeeled seeds were treated with 0.5, 0.7 and 1% colchicine there was no seed germination in the petridish. 2 to 10% seeds were germinated with unpeeled seed. It is evident from this result that germination percentage is very high in decoated seeds.

Polyethene bag experiment: Thirty to 70% treated seeds were germinated with decoated seed. On the contrary the percentage of germination was higher in unpeeled seeds and it was 36 to 73%. The germination of control seeds (treated with distill water) were 82 and 76%, respectively in decoated and unpeeled seeds.

Seed bed experiment: The germination percentage in seed bed experiment was similar to those of polyethene bag experiment. On average, 32 to 72% and 38 to 72%, respectively in decoated and unpeeled seeds were germinated. The rate of germination became gradually lower when the strength of colchicine were increased.

Significant delay in germination time were recorded after treatment with all the concentrations of colchicine in both of decoated and unpeeled seeds.

Effect of colchicine on seeds of triploid kakrol after 9h treatment:

Germination percentage and germination time after 9h with colchicine in triploid kakrol is presented in **table 10.**

Petridish experiment: One average, 53% seeds were germinated within 16 days with decoated seeds when it was treated with distill water and it was 19% when intact seed coat was present. With increase in colchicine concentrations there were decrease in germination in both of the decoated and unpeeled seeds. The lowest 25% seeds were germinated with decoated seeds in the petridish within 40 days when it was treated with 1% colchicine. When unpeeled seeds were treated with 0.5, 0.7 and 1% colchicine there was no seed germination in the petridish. 2% seeds were germinated with unpeeled seed, when it was treated with 0.4% colchicine solution. It is evident from this result that germination percentage is very high in decoated seeds.

Polyethene bag experiment: Twenty two to 62% treated seeds were germinated with unpeeled seed and in decoated seed it was 20 to 68% when it was treated with different strength of colclicine. The germination of control seeds (treated with distill water) were 77 and 72%, respectively in decoated and unpeeled seeds.

Seed bed experiment: On average, 18 to 66% and 20 to 65%, respectively in decoated and unpeeled seeds were germinated. 30% seed was germinated when it was treated with 0.5% colchichine with decoated seed and it was 32% in unpeeled seed. The lowest seeds were germinated when it was treated with 1% colchicine solution with decoated seeds of triploid kakrol. The rate of germination became gradually lower when the strength of colchicine were increased.

Effect of colchicine on seeds of triploid kakrol after 12h treatment:

Germination percentage and germination time after 12h with colchicine in triploid kakrol is presented in **table 11.**

Petridish experiment: One average, 45% seeds were germinated within 18 days with decoated seeds when it was treated with distill water and it was 12% when intact seed coat was present. The lowest 13% seeds were germinated with decoated seeds in the petridish within 40 days when it was treated with 1% colchicine. The highest was 41% in decoated seed when it was treated with 0.1% colchicine solution. When unpeeled seeds were treated with 0.4, 0.5, 0.7 and 1% colchicine there was no seed germination in the petridish. 2% seeds were germinated with unpeeled seed when it was treated with 0.3% colchicine solution. It is evident from this result that germination percentage is very high in decoated seeds.

Polyethene bag experiment: Sixteen to 50% treated seed were germinated with unpeeled seed when it was treated with 0.1, 0.2, 0.3, 0.4, 0.5, 0.7 and 1% colchicine solution. On the contrary the percentage of germination was higher in decoated seeds and it was 17 to 52%. The germination of control seeds (treated with distill water) were 68 and 65%, respectively in decoated and unpeeled seeds.

Seed bed experiment: On average, 18 to 54% and 17 to 53%, respectively in decoated and unpeeled seeds were germinated when it was treated with seven different strength of colchicine solution. The rate of germination became gradually lower when the strength of colchicine were increased.

Effect of colchicine on seeds of triploid kakrol after 18h treatment:

Germination percentage and germination time after 18h with colchicine in triploid kakrol is presented in **table 12.**

Petridish experiment: One average, 38% seeds were germinated within 17 days with decoated seeds when it was treated with distill water and it was 10% when intact seed coat was present. The lowest 2% seeds were germinated with decoated seeds in the petridish within 40 days when it was treated with 0.5% colchicine. In decoated seed 14

and 7% seeds were germinated when it was treated with 0.3 and 0.4% colchicine solution respectively. The highest was 20% when it was treated with 0.1% solution. When unpeeled seeds were treated with 0.3, 0.4, 0.5, 0.7 and 1% colchicine there was no seed germination in the petridish. 2 to 8% seeds were germinated with unpeeled seed.

Polyethene bag experiment: Twelve to 48% treated seeds were germinated with unpeeled seed and in decoated seeds and it was 0 (zero) to 32%. In decoated and unpeeled seed respectively 5 and 17% seeds were germinated when seeds were treated with 0.5% colchicine solution. The germination of control seeds (treated with distill water) were 44 and 50% respectively in decoated and unpeeled seeds.

Seed bed experiment: On average, 2 to 35% and 14 to 48%, respectively in decoated and unpeeled seeds were germinated. The highest 48% treated seeds were germinated in unpeeled seed when it was treated with 0.1% colchicine solution and it was lowest (14%) with 1% colchicine treatment. The rate of germination became gradually lower when the strength of colchicine were increased.

Effect of colchicine on seed of triploid kakrol after 24h treatment:

Germination percentage and germination time after 24h with colchicine in triploid kakrol is presented in **table 13.**

Petridish experiment: Eight percent seeds were germinated within 15 days with decoated seeds when it was treated with distill water and it was 14% when intact seed coat was present. With increase in colchicine concentrations there were decrease in germination in both of the decoated and unpeeled seeds. When unpeeled seeds were treated with 0.4, 0.5, 0.7 and 1% colchicine there was no seed germination in the petri dish. The lowest 2% seeds were germinated with unpeeled seed when it was treated

with 0.3% colchicine solution and it was 2% in decoated seed when seeds were treated with 0.2% colchicine solution..

Polyethene bag experiment: Ten to 45% treated seeds were germinated with unpeeled seed. The highest percentage of germination was 12 in decoated seed when it was treated with 0.1% colchicine solution. The percentage of germination was zero in decoated seed when it was treated with and 1% colchicine solution. The germination of control seeds (treated with distill water) were 15 and 53% respectively in decoated and unpeeled seeds.

Seed bed experiment: On average, 0 (zero) to 20% and 11 to 46%, respectively in decoated and unpeeled seeds were germinated when the seeds were treated with seven said strength of colchicine solution. The rate of germination became gradually lower when the strength of colchicine were increased. Only 2% seeds were germinated within 40 days in decoated seed when it was treated with 0.7% colchicine solution and in decoated seed it was 14%.

Table 8. Germination percentage and germinating time after 3h treatment with different concentration of colchicine in triploid kakrol.

Seed types	Concentration %	Petridish		Polyethene bag		Seed bed	
		Germination%	Germination time	Germination %	Germination time	Germination %	Germination time
Decoated	0.0 (Control)	52	21	81	14	82	13
	0.1	46	27	72	22	74	24
	0.2	43	31	67	25	70	27
	0.3	42	32	65	26	67	29
	0.4	39	37	59	30	62	31
	0.5	37	38	54	32	58	34
	0.7	34	40	51	37	53	38
	1.0	32	40	46	40	47	40
Unpeeled	0.0 (Control)	9	40	62	40	67	40
	0.1	8	40	60	40	64	40
	0.2	6	40	58	40	61	40
	0.3	4	40	52	40	57	40
	0.4	0	40	49	40	52	40
	0.5	0	40	46	40	49	40
	0.7	0	40	42	40	43	40
	1.0	0	40	38	40	40	40

Table 9. Germination percentage and germinating time after 6h treatment with different concentration of colchicine in triploid kakrol.

Seed types	Concentration %	Petridish		Polyethene bag		Seed bed	
		Germination%	Germination time	Germination %	Germination time	Germination %	Germination time
Decoated	0.0 (Control)	51	18	82	12	80	14
	0.1	48	36	70	20	72	24
	0.2	45	40	63	24	65	27
	0.3	43	40	59	28	60	34
	0.4	40	40	53	31	55	38
	0.5	38	40	44	35	46	40
	0.7	34	40	32	40	35	40
	1.0	31	40	30	40	32	40
Unpeeled	0.0 (Control)	11	40	76	40	77	40
	0.1	10	40	73	40	72	40
	0.2	8	40	67	40	68	40
	0.3	2	40	62	40	63	40
	0.4	2	40	56	40	59	40
	0.5	0	40	48	40	51	40
	0.7	0	40	43	40	46	40
	1.0	0	40	36	40	38	40

Table 10. Germination percentage and germinating time after 9h treatment with different concentration of colchicine in triploid kakrol.

Seed types	Concentration %	Petridish		Polyethene bag		Seed bed	
		Germination%	Germination time	Germination %	Germination time	Germination %	Germination time
Decoated	0.0 (Control)	53	16	77	13	79	13
	0.1	49	40	68	24	66	23
	0.2	42	40	61	26	58	28
	0.3	42	40	52	31	50	33
	0.4	35	40	40	35	42	34
	0.5	31	40	32	40	30	40
	0.7	28	40	24	40	25	40
	1.0	25	40	20	40	18	40
Unpeeled	0.0 (Control)	19	40	72	40	74	40
	0.1	12	40	62	40	65	40
	0.2	8	40	55	40	54	40
	0.3	5	40	47	40	49	40
	0.4	2	40	39	40	40	40
	0.5	0	40	30	40	32	40
	0.7	0	40	27	40	26	40
	1.0	0	40	22	40	20	40

Table 11. Germination percentage and germinating time after 12h treatment with different concentration of colchicine in triploid kakrol.

Seed types	Concentration %	Petridish		Polyethene bag		Seed bed	
		Germination %	Germination time	Germination %	Germination time	Germination %	Germination time
Decoated	0.0 (Control)	45	18	68	40	72	40
	0.1	41	40	52	40	54	40
	0.2	36	40	46	40	50	40
	0.3	31	40	38	40	41	40
	0.4	24	40	32	40	33	40
	0.5	20	40	26	40	29	40
	0.7	16	40	20	40	24	40
	1.0	13	40	17	40	18	40
Unpeeled	0.0 (Control)	12	40	65	40	66	40
	0.1	8	40	50	40	53	40
	0.2	6	40	41	40	45	40
	0.3	2	40	34	40	33	40
	0.4	0	40	28	40	28	40
	0.5	0	40	21	40	19	40
	0.7	0	40	17	40	19	40
	1.0	0	40	16	40	17	40

Table 12. Germination percentage and germinating time after 18h treatment with different concentration of colchicine in triploid kakrol.

Seed types	Concentration %	Petridish		Polyethene bag		Seed bed	
		Germination %	Germination time	Germination %	Germination time	Germination %	Germination time
Decoated	0.0 (Control)	38	17	44	40	48	40
	0.1	20	23	32	40	35	40
	0.2	16	27	28	40	31	40
	0.3	14	35	20	40	24	40
	0.4	7	40	12	40	16	40
	0.5	2	40	5	40	8	40
	0.7	0	40	2	40	4	40
	1.0	0	40	0	40	2	40
Unpeeled	0.0 (Control)	10	40	60	40	62	40
	0.1	8	40	48	40	48	40
	0.2	2	40	39	40	41	40
	0.3	0	40	30	40	33	40
	0.4	0	40	21	40	20	40
	0.5	0	40	17	40	19	40
	0.7	0	40	15	40	16	40
	1.0	0	40	12	40	14	40

Table 13. Germination percentage and germinating time after 24h treatment with different concentration of colchicine in triploid kakrol.

Seed types	Concentration %	Petridish		Polyethene bag		Seed bed	
		Germination%	Germination time	Germination %	Germination time	Germination %	Germination time
Decoated	0.0 (Control)	8	14	15	9	18	10
	0.1	5	20	12	22	14	20
	0.2	2	40	10	24	12	23
	0.3	0	40	7	36	10	30
	0.4	0	40	4	40	6	36
	0.5	0	40	2	40	4	40
	0.7	0	40	2	40	2	40
	1.0	0	40	0		0	40
Unpeeled	0.0 (Control)	10	40	53	40	56	40
	0.1	8	40	45	40	46	40
	0.2	5	40	34	40	35	40
	s0.3	2	40	27	40	30	40
	0.4	0	40	18	40	22	40
	0.5	0	40	16	40	18	40
	0.7	0	40	14	40	14	40
	1.0	0	40	10	40	11	40

Effect of colchicine on seeds of tetraploid kakrol after 3h treatment:

Germination percentage and germination time after 3h with colchicine in tetraploid kakrol is presented in **table 14.**

Petridish experiment: One average, 55% seeds were germinated within 20 days with decoated seeds when it was treated with distill water and it was 12% when intact seed coat was present. With increase in colchicine concentrations there were decrease in germination in both of the decoated and unpeeled seeds. The lowest 35% seeds were germinated with decoated seeds in the petridish within 40 days when it was treated with 1% colchicine. When unpeeled seeds were treated with 0.4 0.5, 0.7 and 1% colchicine there was no seed germination in the petridish. 5% to 10% seeds were germinated with unpeeled seed. It is evident from this result that germination percentage is very high in decoated seeds.

Polyethene bag experiment: 38 to 62% treated seed were germinated with unpeeled seed. On the contrary the percentage of germination was higher in decoated seeds and it was 47 to 75%. The germination of control seeds (treated with distill water) were 83 and 64%, respectively in decoated and unpeeled seeds.

Seed bed experiment: The germination percentage in seed bed experiment was similar to those of polyethene bag experiment. On average, 44 to 75% and 41 to 62%, respectively in decoated and unpeeled seeds were germinated. The rate of germination became gradually lower when the strength of colchicine were increased.

Significant delay in germination time were recorded after treatment with all the concentrations of colchicine in both of decoated and unpeeled seeds.

Effect of colchicine on seeds of tetraploid kakrol after 6h treatment:

Germination percentage and germination time after 6h with colchicine in tetraploid kakrol is presented in **table 15.**

Petridish experiment: One average, 54% seeds were germinated within 16 days with decoated seeds when it was treated with distill water and it was 12% when intact seed coat was present. The lowest 28% seeds were germinated with decoated seeds in the petridish within 40 days when it was treated with 1% colchicine. When unpeeled seeds were treated with 0.5, 0.7 and 1% colchicine there was no seed germination in the petridish. 2 to 8% seeds were germinated with unpeeled seed. It is evident from this result that germination percentage is very high in decoated seeds.

Polyethene bag experiment: Twenty eight to 72% treated seeds were germinated with decoated seed. On the contrary the percentage of germination was higher in unpeeled seeds and it was 36 to 73%. The germination of control seeds (treated with distill water) were 80 and 76% respectively in decoated and unpeeled seeds.

Seed bed experiment: The germination percentage in seed bed experiment was similar to those of polyethene bag experiment. On average, 31 to 74% and 36 to 78%, respectively in decoated and unpeeled seeds were germinated. The rate of germination became gradually lower when the strength of colchicine were increased.

Effect of colchicine on seeds of tetraploid kakrol after 9h treatment:

Germination percentage and germination time after 9h with colchicine in tetraploid kakrol is presented in **table 16.**

Petridish experiment: One average, 54% seeds were germinated within 18 days with decoated seeds when it was treated with distill water and it was 16% when intact seed coat was present. With increase in colchicine concentrations there were decrease in germination in both of the decoated and unpeeled seeds. The lowest 26% seeds were germinated with decoated seeds in the petridish within 40 days when it was treated with 1% colchicine. When unpeeled seeds were treated with 0.5, 0.7 and 1% colchicine there was no seed germination in the petridish. 2% seeds were germinated with unpeeled seed, when it was treated with 0.4% colchicine solution. It is evident from this result that germination percentage is very high in decoated seeds.

Polyethene bag experiment: Twenty three to 65% treated seeds were germinated with unpeeled seed and in decoated seeds it was 24 to 74% when it was treated with different strength of colclicine. The germination of control seeds (treated with distill water) were 82 and 78%, respectively in decoated and unpeeled seeds.

Seed bed experiment: On average, 21 to 72% and 20 to 65%, respectively in decoated and unpeeled seeds were germinated. 35% seeds were germinated when it was treated with 0.5% colchichine with decoated seed and it was 32% in unpeeled seed. The lowest seeds were germinated when it was treated with 1% colchicine solution with decoated seeds of triploid kakrol. The rate of germination became gradually lower when the strength of colchicine were increased.

Effect of colchicine on seeds of tetraploid kakrol after 12h treatment:

Germination percentage and germination time after 12h with colchicine in tetraploid kakrol is presented in **table 17.**

Petridish experiment: One average, 50% seeds were germinated within 16 days with decoated seeds when it was treated with distill water and it was 16% when intact seed coat was present. The lowest 22% seeds were germinated with decoated seeds in the petridish within 40 days when it was treated with 1% colchicine. The highest was 46% in decoated seed when it was treated with 0.1% colchicine solution. When unpeeled seeds were treated with 0.4, 0.5, 0.7 and 1% colchicine there was no seed germination in the petridish. 4% seeds were germinated with unpeeled seed when it was treated with 0.3% colchicine solution. It is evident from this result that germination percentage is very high in decoated seeds.

Polyethene bag experiment: Seventeen to 54% treated seeds were germinated with unpeeled seed when it was treated with 0.1, 0.2, 0.3, 0.4, 0.5, 0.7 and 1% colchicine solution. On the contrary the percentage of germination was higher in decoated seeds and it was 18 to 65%. The germination of control seeds (treated with distill water) were 74 and 72%, respectively in decoated and unpeeled seeds.

Seed bed experiment: On average, 16 to 66% and 18 to 56%, respectively in decoated and unpeeled seeds were germinated when it was treated with seven different strength of colchicine solution. The rate of germination became gradually lower when the strength of colchicine were increased.

Effect of colchicine on seeds of tetraploid kakrol after 18h treatment:

Germination percentage and germination time after 18h with colchicine in tetraploid kakrol is presented in **table 18.**

Petridish experiment: One average, 41% seeds were germinated within 18 days with decoated seeds when it was treated with distill water and it was 12% when intact seed coat was present. The lowest 4% seeds were germinated with decoated seeds in the petridish within 40 days when it was treated with 0.5% colchicine. In decoated seed 12 and 8% seeds were germinated when it was treated with 0.3% and 0.4% colchicine solution respectively. The highest was 22% when it was treated with 0.1% solution. When unpeeled seeds were treated with 0.4, 0.5, 0.7 and 1% colchicine there was no seed germination in the petridish. 2 to 8% seeds were germinated with unpeeled seed.

Polyethene bag experiment: Ten to 50% treated seeds were germinated with unpeeled seed and in decoated seeds and it was 6 to 44%. In decoated and unpeeled seed respectively 13 and 20% seeds were germinated when seeds were treated with 0.5% colchicine solution. The germination of control seeds (treated with distill water) were 60 and 62%, respectively in decoated and unpeeled seeds.

Seed bed experiment: On average, 8 to 42% and 12 to 50%, respectively in decoated and unpeeled seeds were germinated. The highest 50% treated seeds were germinated in unpeeled seed when it was treated with 0.1% colchicine solution and it was lowest (12%) with 1% colchicine treatment. The rate of germination became gradually lower when the strength of colchicine were increased.

Effect of colchicine on seeds of tetraploid kakrol after 24h treatment:

Germination percentage and germination time after 24h with colchicine in tetraploid kakrol is presented in **table 19.**

Petridish experiment: Twenty percent seeds were germinated within 12 days with decoated seeds when it was treated with distill water and it was 12% when intact seed coat was present. With increase in colchicine concentrations there were decrease in germination in both of the decoated and unpeeled seeds. When unpeeled seeds were treated with 0.5, 0.7 and 1% colchicine there was no seed germination in the petridish. The lowest 2% seeds were germinated with unpeeled seed when it was treated with 0.4% colchicine solution and it was 2% in decoated seed when seeds were treated with 0.4% colchicine solution..

Polyethene bag experiment: Eight to 46% treated seed were germinated with unpeeled sseed. The highest percentage of germination was 14 in decoated seed when it was treated with 0.1% colchicine solution. The percentage of germination was 2% in decoated seed when it was treated with and 1% colchicine solution. The germination of control seeds (treated with distill water) were 18 and 55%, respectively in decoated and unpeeled seeds.

Seed bed experiment: On average, 2 to 16% and 6 to 44%, respectively in decoated and unpeeled seeds were germinated when the seeds were treated with seven said strength of colchicine solution. The rate of germination became gradually lower when the strength of colchicine were increased. Only 2% seeds were germinated within 40 days in decoated seed when it was treated with 1% colchicine solution and in unpeeled seed it was 6%.

Table 14. Germination percentage and germinating time after 3h treatment with different concentration of colchicine in tetraploid kakrol.

Seed types	Concentration %	Petridish		Polyethene bag		Seed bed	
		Germination%	Germination time	Germination %	Germination time	Germination %	Germination time
Decoated	0.0 (Control)	55	20	83	13	85	12
	0.1	48s	25	75	20	75	22
	0.2	44	30	68	26	68	26
	0.3	40	33	66	30	69	30
	0.4	38	36	62	33	59	30
	0.5	38	40	55	38	55	34
	0.7	35	40	49	40	48	40
	1.0	35	40	47	40	44	40
Unpeeled	0.0 (Control)	12	40	64	40	66	40
	0.1	10	40	62	40	62	40
	0.2	8	40	56	40	61	40
	0.3	5	40	50	40	58	40
	0.4	0	40	50	40	50	40
	0.5	0	40	48	40	50	40
	0.7	0	40	40	40	44	40
	1.0	0	40	38	40	41	40

Table 15. Germination percentage and germinating time after 6h treatment with different concentration of colchicine in tetraploid kakrol.

Seed types	Concentration %	Petridish		Polyethene bag		Seed bed	
		Germination%	Germination time	Germination %	Germination time	Germination %	Germination time
Decoated	0.0 (Control)	54	16	80	12	83	12
	0.1	50	26	72	20	74	26
	0.2	45	35	65	24	68	28
	0.3	42	40	60	28	62	36
	0.4	38	40	54	31	54	40
	0.5	35	40	46	35	43	40
	0.7	32	40	31	40	36	40
	1.0	28	40	28	40	31	40
Unpeeled	0.0 (Control)	12	40	76	40	78	40
	0.1	8	40	73	40	70	40
	0.2	8	40	67	40	70	40
	0.3	4	40	62	40	61	40
	0.4	2	40	56	40	55	40
	0.5	0	40	48	40	51	40
	0.7	0	40	43	40	45	40
	1.0	0	40	36	40	36	40

Table 16. Germination percentage and germinating time after 9h treatment with different concentration of colchicine in tetraploid kakrol.

Seed types	Concentration %	Petridish		Polyethene bag		Seed bed	
		Germination %	Germination time	Germination %	Germination time	Germination %	Germination time
Decoated	0.0 (Control)	54	18	82	14	82	16
	0.1	47	26	74	26	72	24
	0.2	42	32	66	27	62	26
	0.3	40	40	55	35	54	35
	0.4	34	40	48	36	44	40
	0.5	27	40	35	40	35	40
	0.7	25	40	28	40	26	40
	1.0	26	40	24	40	21	40
Unpeeled	0.0 (Control)	16	40	78	40	74	40
	0.1	10	40	65	40	65	40
	0.2	8	40	56	40	54	40
	0.3	6	40	47	40	49	40
	0.4	2	40	42	40	40	40
	0.5	0	40	35	40	32	40
	0.7	0	40	28	40	26	40
	1.0	0	40	23	40	20	40

Table 17. Germination percentage and germinating time after 12h treatment with different concentration of colchicine in tetraploid kakrol.

Seed types	Concentration %	Petridish		Polyethene bag		Seed bed	
		Germination%	Germination time	Germination %	Germination time	Germination %	Germination time
Decoated	0.0 (Control)	50	16	74	40	75	40
	0.1	46	32	65	40	66	40
	0.2	41	40	48	40	55	40
	0.3	38	40	41	40	46	40
	0.4	34	40	36	40	36	40
	0.5	28	40	28	40	32	40
	0.7	24	40	21	40	23	40
	1.0	22	40	18	40	16	40
Unpeeled	0.0 (Control)	16	40	72	40	72	40
	0.1	8	40	54	40	56	40
	0.2	6	40	45	40	51	40
	0.3	4	40	38	40	42	40
	0.4	0	40	32	40	36	40
	0.5	0	40	25	40	28	40
	0.7	0	40	20	40	24	40
	1.0	0	40	17	40	18	40

Table 18. Germination percentage and germinating time after 18h treatment with different concentration of colchicine in tetraploid kakrol.

Seed types	Concentration %	Petridish		Polyethene bag		Seed bed	
		Germination%	Germination time	Germination %	Germination time	Germination %	Germination time
Decoated	0.0 (Control)	41	18	60	40	56	40
	0.1	22	26	44	40	42	40
	0.2	15	30	36	40	36	40
	0.3	12	35	28	40	28	40
	0.4	8	40	18	40	19	40
	0.5	4	40	13	40	14	40
	0.7	0	40	8	40	11	40
	1.0	0	40	6	40	8	40
Unpeeled	0.0 (Control)	12	40	62	40	62	40
	0.1	10	40	50	40	50	40
	0.2	6	40	43	40	46	40
	0.3	2	40	34	40	34	40
	0.4	0	40	25	40	26	40
	0.5	0	40	20	40	22	40
	0.7	0	40	18	40	18	40
	1.0	0	40	10	40	12	40

Table 19. Germination percentage and germinating time after 24h treatment with different concentration of colchicine in tetraploid kakrol.

Seed types	Concentration %	Petridish		Polyethene bag		Seed bed	
		Germination%	Germination time	Germination %	Germination time	Germination %	Germination time
Decoated	0.0 (Control)	20	12	18	12	20	10
	0.1	14	22	14	24	16	20
	0.2	8	40	12	26	13	23
	0.3	4	40	9	40	12	30
	0.4	2	40	6	40	8	36
	0.5	0	40	4	40	6	40
	0.7	0	40	4	40	4	40
	1.0	0	40	2		2	40
Unpeeled	0.0 (Control)	12	40	55	40	56	40
	0.1	10	40	46	40	44	40
	0.2	6	40	32	40	35	40
	0.3	4	40	26	40	28	40
	0.4	2	40	20	40	24	40
	0.5	0	40	18	40	20	40
	0.7	0	40	12	40	12	40
	1.0	0	40	8	40	6	40

Plate I

Fig. A. Effect of colchicine treatment on germinating seeds of kakrol.
a &b. Affected seeds (showing 2-3 shoots sprouted from cotyledon base), c. Untreated (normal seedling).

Effect of different concentration of colchicine on two days old seedlings:

The total number of seedling treated percentage of abnormal seedlings and the mortality percentage for different types of kakrol is presented in **table 20, 21, 22, 23, 24, 25 and 26 (Plate II. Fig. A & B).**

Colchicine solution of 0.1, 0.2, 0.3, 0.4, 0.5, 0.7 and 1.0 % were applied on the tip of 2 days old seedlings. There were two types of application viz., dropping method and dipping method were used for the induction of artificial polyploid. Two types of effect were noted. The affected shoot tip of 2 days old seedling to was be found very typical form as like as tumor. All such seedlings were scored as abnormal. Mortality percentage of treated seedling were recorded. The data show that there was a differential response of the genotypes of kakrol to colchicine treatment. An increase in treatment time, as 24, 18 and 12h lead to higher affected percentage of seedling in all the genotype of kakrol and the mortality percentage was also very high when shoot tips were dipping for long time in different strength of colchicine solution.

The appeared to be great sensitivity to colchicine when seedlings were treated with high concentration (0.7 and 1 %) as agents other strength of solution.

0.1% colchicine effect on two days old seedlings:

The result obtain from the treatment of 0.1% colchicine solution have been presented in **table 20.** Out of 50 seedlings for each treatment were applied with 0.1% colchicine. There was no effect observed from this treatment. But mortality percentage were 34 to 48% when treatment time was 18h for dipping method and it was 90 to 100% when treatment time was 24h. From dropping method, no affected seedling was to be found for different treatment time and mortality percentage was zero.

0.2% colchicine effect on two days old seedlings:

The result obtain from the treatment of 0.2% colchicine solution have been presented in **table 21.** There was no affected seedlings to be found for 3, 6 and 9h treatment time. In dropping method the highest affected seedling were recorded when treatment time was 24h and it were 8, 6 and 6%, respectively in diploid, triploid and tetraploid genotypes.

In dipping method, 6 to 8% affected seedlings were found when treatment time was 12h and it was 18 to 22% then treatment time was 24h. From 24h treatment in dipping method no seedlings were survive.

0.3% colchicine effect on two days old seedlings:

The result obtain from the treatment of 0.3% colchicine solution have been presented in **table 22.** The affected seedling were the highest (14%) in diploid kakrol for dropping method when treatment time was 24h and mortality percentage was 10.

The highest number of affected seedlings were obtained when seedling tips dipped 24h in 0.3% colchicine solution. Thirty to 34% seedlings were affected from this treatment. Affected seedlings were 14 to 20% and mortality percentage were 44 to 52% when treatment time was 18h. The control seedling mortality was 34%. Short treatment time as 3, 6 and 9h there were no colchicine effect to be observed.

0.4% colchicine effect on two days old seedlings:

Results obtained from 0.4% colchicine effect on 2 days old seedlings are shown in **table 23.** The highest number of affected seedlings were obtained when seedling tips sprayed (dropping) 24h in the strength of 0.4% colchicine and which was 24% in diploid kakrol. Mortality percentage was highest in tetraploid genotype of dropping method and which was 16%.

Out of 6 treatment time only 9, 12, 18 and 24h showed affected the seedlings. If period of dipping in this treatment below in there is no affected seedling to be found. When dipping period is 9 & 12h, diploid, triploid and tetraploid kakrol showed little success (6 to 14%). When dipping period increased affected seedlings percentage have increased gradually and after 24h percentage of affected seedling remained unchanged.

0.5% colchicine effect on two days old seedlings:

The result obtain from the treatment of 0.5% colchicine solution have been presented in **table 24.** In dipping method as may as 8 - 12% affected seedlings were obtained when the seedlings were dipped with 0.5%. colchicine solution for a period of 9h. As the dipping period increased from 9 to 24h percentage of affected seedling increased from 8 to 42.

In dropping method highest percentage of affected seedlings were obtained when all the genotypes sprayed with 0.5% colchicine for a period of 24h and the percentage of affected seedling were 38, 32 and 28%, respectively in diploid, triploid and tetraploid kakrol. Some percentage of affected seedling were obtained when the seedling sprayed with 0.5 colchicine for a period of 9h and it was 4 to 6%. In dipping method the seedlings dipped into colchicine solution at least 18h or less, other wise affected seedlings will die at any time duration.

0.7% colchicine effect on two days old seedlings:

Results obtained from 0.7% colchicine effect on 2 days old seedlings are shown in **table 25.** If dropping period is less there is no affected seedling to be observed. If dropping period in success starts, diploid shows 10%, triploid 12% and tetraploid 8% affected at 9h spray of colchicine. In 24h (for dropping method) 38 - 40% affected seedling were to be found and mortality percentage were 26 - 30%. For dipping

method no plant survive when the seedling tips were kept into 0.7% colchicine solution at 24h or more. The percentage of affected seedling were 40, 38 and 38%, respectively in diploid triploid and tetraploid kakrol when seedling tips were kept 0.7% colchicine solution at 18h.

Affected percentage and mortality percentage were zero when the seedling were treated with 0.7% colchicine for a period of 3h from both methods of application.

1% colchicine effect on two days old seedlings:

The result obtain from the treatment of 1% colchicine solution have been presented in **table 26.** Out of six treatment time (3, 6, 9, 12, 18 and 24h) only 9, 12, 18h showed higher affected seedling for dipping method. If period of treatment time was below 3 to 6h the affected seedling percentage were low. It was 2 to 10%. When dipping period increase percentage of affected seedling also increased gradually and after 12h the success of affected seedlings remained unchanged. 100% mortality was found when the seedling tips were dipped with 1% colchicine for 24h.

In dropping method out of 6 treatment time except 3h showed affected seedlings. When dropping period increased the percentage of affected seedlings also increased gradually. The highest affected seedlings were found when it was treated with 1% colchicine for 24h and which was 46, 42 and 40%, respectively in diploid, triploid and tetraploid seedlings of kakrol. Mortality percentage were 40, 34 and 32%, respectively in diploid, triploid and tetraploid seedling of kakrol when treatment time was 24h for dropping method. Mortality percentage was very high when treatment time increased (12 to 24h) for dipping method, which was 26 to 100%.

Table 20. Effect of 0.1% colchicine on two days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	3	2	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	6	2	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	9	2	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	12	2	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	34
			Diploid		50	0	48
			Triploid	Dipping	50	0	42
1	18	2	Tetraploid		50	0	36
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	90
			Diploid		50	0	100
			Triploid	Dipping	50	0	100
1	24	2	Tetraploid		50	0	100
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0

Table 21. Effect of 0.2% colchicine on two days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
I	3	2	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
I	6	2	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
I	9	2	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	8	4
			Triploid	Dipping	50	8	6
I	12	2	Tetraploid		50	6	2
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	34
			Diploid		50	14	50
			Triploid	Dipping	50	10	44
I	18	2	Tetraploid		50	10	42
			Control		10	0	0
			Diploid		50	2	0
			Triploid	Dropping	50	2	0
			Tetraploid		50	0	0
			Control		10	0	90
			Diploid		50	22	100
			Triploid	Dipping	50	18	100
I	24	2	Tetraploid		50	18	100
			Control		10	0	0
			Diploid		50	8	4
			Triploid	Dropping	50	6	4
			Tetraploid		50	6	2

Table 22. Effect of 0.3% colchicine on two days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	3	2	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	6	2	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	9	2	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	14	20
			Triploid	Dipping	50	10	22
1	12	2	Tetraploid		50	10	26
			Control		10	0	0
			Diploid		50	6	4
			Triploid	Dropping	50	4	2
			Tetraploid		50	2	0
			Control		10	0	34
			Diploid		50	20	52
			Triploid	Dipping	50	16	50
1	18	2	Tetraploid		50	14	44
			Control		10	0	0
			Diploid		50	8	4
			Triploid	Dropping	50	8	4
			Tetraploid		50	6	2
			Control		10	0	90
			Diploid		50	34	100
			Triploid	Dipping	50	30	100
1	24	2	Tetraploid		50	30	100
			Control		10	0	0
			Diploid		50	14	10
			Triploid	Dropping	50	12	6
			Tetraploid		50	10	8

Table 23. Effect of 0.4% colchicine on two days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	3	2	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	6	2	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	8	4
			Triploid	Dipping	50	8	4
1	9	2	Tetraploid		50	6	2
			Control		10	0	0
			Diploid		50	4	2
			Triploid	Dropping	50	2	0
			Tetraploid		50	2	0
			Control		10	0	0
			Diploid		50	14	26
			Triploid	Dipping	50	12	20
1	12	2	Tetraploid		50	14	22
			Control		10	0	0
			Diploid		50	12	6
			Triploid	Dropping	50	10	6
			Tetraploid		50	10	4
			Control		10	0	34
			Diploid		50	34	60
			Triploid	Dipping	50	30	60
1	18	2	Tetraploid		50	32	52
			Control		10	0	0
			Diploid		50	20	12
			Triploid	Dropping	50	18	14
			Tetraploid		50	16	10
			Control		10	0	90
			Diploid		50	40	100
			Triploid	Dipping	50	40	100
1	24	2	Tetraploid		50	36	100
			Control		10	0	0
			Diploid		50	24	14
			Triploid	Dropping	50	20	14
			Tetraploid		50	20	16

Table 24. Effect of 0.5% colchicine on two days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	3	2	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	6	2	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	12	8
			Triploid	Dipping	50	8	8
1	9	2	Tetraploid		50	8	8
			Control		10	0	0
			Diploid		50	6	4
			Triploid	Dropping	50	6	4
			Tetraploid		50	4	4
			Control		10	0	0
			Diploid		50	20	16
			Triploid	Dipping	50	18	14
1	12	2	Tetraploid		50	18	12
			Control		10	0	0
			Diploid		50	14	10
			Triploid	Dropping	50	14	10
			Tetraploid		50	14	12
			Control		10	0	34
			Diploid		50	38	64
			Triploid	Dipping	50	30	60
1	18	2	Tetraploid		50	32	54
			Control		10	0	0
			Diploid		50	26	20
			Triploid	Dropping	50	20	16
			Tetraploid		50	20	18
			Control		10	0	90
			Diploid		50	42	100
			Triploid	Dipping	50	44	100
1	24	2	Tetraploid		50	40	100
			Control		10	0	0
			Diploid		50	38	30
			Triploid	Dropping	50	32	26
			Tetraploid		50	28	24

Table 25. Effect of 0.7% colchicine on two days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	3	2	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	6	4
			Triploid	Dipping	50	4	4
1	6	2	Tetraploid		50	4	2
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	16	14
			Triploid	Dipping	50	16	12
1	9	2	Tetraploid		50	14	12
			Control		10	0	0
			Diploid		50	10	6
			Triploid	Dropping	50	12	6
			Tetraploid		50	8	4
			Control		10	0	0
			Diploid		50	26	20
			Triploid	Dipping	50	22	18
1	12	2	Tetraploid		50	24	20
			Control		10	0	0
			Diploid		50	20	18
			Triploid	Dropping	50	20	16
			Tetraploid		50	18	18
			Control		10	0	34
			Diploid		50	40	68
			Triploid	Dipping	50	38	62
1	18	2	Tetraploid		50	38	60
			Control		10	0	0
			Diploid		50	32	30
			Triploid	Dropping	50	28	26
			Tetraploid		50	30	26
			Control		10	0	90
			Diploid		50	46	100
			Triploid	Dipping	50	46	100
1	24	2	Tetraploid		50	44	100
			Control		10	0	0
			Diploid		50	40	30
			Triploid	Dropping	50	40	26
			Tetraploid		50	38	28

Table 26. Effect of 1% colchicine on two days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	4	0
			Triploid	Dipping	50	2	0
1	3	2	Tetraploid		50	2	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	10	6
			Triploid	Dipping	50	10	4
1	6	2	Tetraploid		50	6	2
			Control		10	0	0
			Diploid		50	4	2
			Triploid	Dropping	50	4	0
			Tetraploid		50	2	0
			Control		10	0	0
			Diploid		50	20	22
			Triploid	Dipping	50	20	24
1	9	2	Tetraploid		50	16	20
			Control		10	0	0
			Diploid		50	16	12
			Triploid	Dropping	50	14	10
			Tetraploid		50	16	14
			Control		10	0	0
			Diploid		50	30	28
			Triploid	Dipping	50	30	26
1	12	2	Tetraploid		50	32	30
			Control		10	0	0
			Diploid		50	26	20
			Triploid	Dropping	50	26	20
			Tetraploid		50	28	24
			Control		10	0	34
			Diploid		50	42	70
			Triploid	Dipping	50	42	70
1	18	2	Tetraploid		50	40	68
			Control		10	0	0
			Diploid		50	38	30
			Triploid	Dropping	50	38	32
			Tetraploid		50	30	26
			Control		10	0	90
			Diploid		50	48	100
			Triploid	Dipping	50	48	100
1	24	2	Tetraploid		50	46	100
			Control		10	0	0
			Diploid		50	46	40
			Triploid	Dropping	50	42	34
			Tetraploid		50	40	32

Effect of different concentration of colchicine on four days old seedling:

The total number of seedling treated percentage of abnormal seedlings and the mortality percentage for different types of kakrol is presented in **table 27, 28, 29, 30, 31, 32 and 33 (Plate II. Fig. A & B).**

Colchicine solution of 0.1, 0.2, 0.3, 0.4, 0.5, 0.7 and 1.0 % were applied on the tip of 4 days old seedlings. There were two types of application viz., dropping method and dipping method were used for the induction of artificial polyploid. Two types of effect were noted. The affected shoot tip of 4 days old seedling to was be found very typical form as like as tumor. All such seedlings were scored as abnormal. Mortality percentage of treated seedlings were recorded. The data show that there was a differential response of the genotypes of kakrol to colchicine treatment. An increase in treatment time, as 24, 18 and 12h lead to higher affected percentage of seedling in all the genotype of kakrol and the mortality percentage was also very high when shoot tips were dipping for long time in different strength of colchicine solution.

The appeared to be great sensitivity to colchicine when seedlings were treated with high concentration (0.7 and 1 %) as agents other strength of solution.

0.1% colchicine effect on four days old seedlings:

The result obtain from the treatment of 0.1% colchicine solution have been presented in **table 27.** Out of 50 seedlings for each treatment were applied with 0.1% colchicine. There was no effect observed from this treatment. But mortality percentage were 26 to 40% when treatment time was 18h for dipping method and it was 82 to 100% when treatment time was 24h. From dropping method, no affected seedling was found for different treatment time and mortality percentage was zero.

0.2% colchicine effect on four days old seedlings:

The result obtain from the treatment of 0.2% colchicine solution have been presented in **table 28.** There was no affected seedlings to be found when treatment time was 3h and 6h. In dropping method the highest affected seedling were recorded when treatment time was 24h and it were 12, 10 and 10%, respectively in diploid, triploid and tetraploid genotypes.

In dipping method, 2 to 4% and 12 to 18% affected seedlings were found when treatment time was 9h and 18h respectively. It was 18 to 24% then treatment time was 24h. From 24h treatment in dipping method no seedlings were survive.

0.3% colchicine effect on four days old seedlings:

The result obtain from the treatment of 0.3% colchicine solution have been presented in **table 29.** The affected seedlings were the highest (16%) in diploid kakrol. From dropping method, mortality percentage was found to be ranged from 8 to 10% in 24h treatment and it was 82 to 100% for dipping method.

The highest number of affected seedlings were obtained when seedling tips dipped 24h in 0.3% colchicine solution. 30 to 36% seedlings were affected from this treatment. Affected seedling were 16 to 22% and mortality percentage were 42 to 48% when treatment time was 18h. The control seedlings mortality was 26%. Short treatment time as 3h and 6h there were no colchicine effect to be observed.

0.4% colchicine effect on four days old seedlings:

Results obtained from 0.4% colchicine effect on 4 days old seedlings are shown in **table 30.** Out of 6 treatment time only 9, 12, 18 and 24h showed affected seedlings in dropping method. If period of dropping in this treatment below in there is no affected seedling to be found. The highest number of affected seedlings were obtained

when seedling tips sprayed (dropping) 24h in the strength of 0.4% colchicine and which was 28% in tetraploid kakrol. Mortality percentage was highest in diploid genotype of dropping method and which was 20%.

When dipping period is 6 & 9h, diploid, triploid and tetraploid kakrol showed little success (2 to 14%). When dipping period increased affected seedlings percentage have increased gradually and after 24h percentage of affected seedlings remained unchanged.

0.5% colchicine effect on four days old seedlings:

The result obtain from the treatment of 0.5% colchicine solution have been presented in **table 31.** In dipping method as may as 8 -10% affected seedlings were obtained when the seedlings were dipped with 0.5%. colchicine solution for a period of 6h. As the dipping period increased from 9 to 24h percentage of affected seedlings increased from 16 to 46.

In dropping method highest percentage of affected seedlings were obtained when all the genotypes sprayed with 0.5% colchicine for a period of 24h and the percentage of affected seedlings were 40, 40 and 32%, respectively in diploid, triploid and tetraploid kakrol. Some percentage of affected seedlings were obtained when the seedling sprayed with 0.5% colchicine for a period of 6h and it was 2 to 6%. In dipping method the seedlings dipped into colchicine solution at least 18h or less, other wise affected seedling will die at any time duration.

0.7% colchicine effect on four days old seedlings:

Results obtained from 0.7% colchicine effect on 4 days old seedlings are shown in **table 32.** If dropping period is less there is no affected seedling to be observed. If dropping period in success starts, diploid shows 10%, triploid 10% and tetraploid 8% affected at 6h spray of colchicine. In 24h (for dropping method) 42 - 48% affected

seedlings were to be found and mortality percentage were 34 - 38%. For dipping method no plant survive when the seedling tips were kept into 0.7% colchicine solution at 24h or more. The percentage of affected seedlings were 42, 40 and 40%, respectively in diploid triploid and tetraploid kakrol when seedling tips were kept 0.7% colchicine solution at 18h.

Affected percentage and mortality percentage were zero when the seedlings were treated with 0.7% colchicine for a period of 3h from both methods of application.

1% colchicine effect on four days old seedlings:

The result obtain from the treatment of 1% colchicine solution have been presented in **table 33.** Out of six treatment time (3, 6, 9, 12, 18 and 24h) only 9, 12, 18h showed higher affected seedling for dipping method. If period of treatment time was below 3 to 6h the affected seedlings percentage were very low. It was 6 to 10%. When dipping period increase percentage of affected seedlings also increased gradually and after 12h the success of affected seedlings remained unchanged. 100% mortality was found when the seedling tips were dipped with 1% colchicine for 24h.

In dropping method out of 6 treatment time except 3h showed affected seedlings. When dropping period increased the percentage of affected seedlings also increased gradually. The highest affected seedlings were found when it was treated with 1% colchicine for 24h and which was 52, 50 and 50%, respectively in diploid, triploid and tetraploid seedling of kakrol. Mortality percentage were 46, 40 and 42%, respectively in diploid, triploid and tetraploid seedlings of kakrol when treatment time was 24h for dropping method. Mortality percentage was very high when treatment time increased (12 to 24h) for dipping method, which was 26 to 100%.

Table 27. Effect of 0.1% colchicine on four days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Deeping	50	0	0
1	3	4	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Deeping	50	0	0
1	6	4	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Deeping	50	0	0
1	9	4	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Deeping	50	0	0
1	12	4	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	26
			Diploid		50	0	40
			Triploid	Deeping	50	0	38
1	18	4	Tetraploid		50	0	32
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	82
			Diploid		50	0	100
			Triploid	Deeping	50	0	100
1	24	4	Tetraploid		50	0	100
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0

Table 28. Effect of 0.2% colchicine on four days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Deeping	50	0	0
1	3	4	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Deeping	50	0	0
1	6	4	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	4	2
			Triploid	Deeping	50	2	2
1	9	4	Tetraploid		50	2	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	16	12
			Triploid	Deeping	50	12	10
1	12	4	Tetraploid		50	10	10
			Control		10	0	0
			Diploid		50	2	2
			Triploid	Dropping	50	2	0
			Tetraploid		50	0	0
			Control		10	0	26
			Diploid		50	18	40
			Triploid	Deeping	50	12	38
1	18	4	Tetraploid		50	14	32
			Control		10	0	0
			Diploid		50	4	2
			Triploid	Dropping	50	4	2
			Tetraploid		50	4	0
			Control		10	0	82
			Diploid		50	24	100
			Triploid	Deeping	50	24	100
1	24	4	Tetraploid		50	18	100
			Control		10	0	0
			Diploid		50	12	8
			Triploid	Dropping	50	10	8
			Tetraploid		50	10	6

Table 29. Effect of 0.3% colchicine on four days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Deeping	50	0	0
1	3	4	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Deeping	50	0	0
1	6	4	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	6	10
			Triploid	Deeping	50	2	10
1	9	4	Tetraploid		50	4	8
			Control		10	0	0
			Diploid		50	4	2
			Triploid	Dropping	50	4	0
			Tetraploid		50	4	2
			Control		10	0	0
			Diploid		50	14	20
			Triploid	Deeping	50	10	16
1	12	4	Tetraploid		50	12	18
			Control		10	0	0
			Diploid		50	6	4
			Triploid	Dropping	50	8	4
			Tetraploid		50	6	2
			Control		10	0	26
			Diploid		50	22	48
			Triploid	Deeping	50	22	46
1	18	4	Tetraploid		50	16	42
			Control		10	0	0
			Diploid		50	10	6
			Triploid	Dropping	50	10	8
			Tetraploid		50	10	6
			Control		10	0	82
			Diploid		50	36	100
			Triploid	Deeping	50	36	100
1	24	4	Tetraploid		50	30	100
			Control		10	0	0
			Diploid		50	16	10
			Triploid	Dropping	50	10	8
			Tetraploid		50	12	8

Table 30. Effect of 0.4% colchicine on four days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Deeping	50	0	0
1	3	4	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	4	2
			Triploid	Deeping	50	4	2
1	6	4	Tetraploid		50	2	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	14	12
			Triploid	Deeping	50	8	12
1	9	4	Tetraploid		50	10	10
			Control		10	0	0
			Diploid		50	10	8
			Triploid	Dropping	50	12	6
			Tetraploid		50	10	6
			Control		10	0	0
			Diploid		50	18	14
			Triploid	Deeping	50	14	14
1	12	4	Tetraploid		50	16	12
			Control		10	0	0
			Diploid		50	14	10
			Triploid	Dropping	50	12	6
			Tetraploid		50	14	6
			Control		10	0	26
			Diploid		50	38	52
			Triploid	Deeping	50	38	46
1	18	4	Tetraploid		50	32	42
			Control		10	0	0
			Diploid		50	24	16
			Triploid	Dropping	50	20	14
			Tetraploid		50	20	16
			Control		10	0	82
			Diploid		50	42	100
			Triploid	Deeping	50	40	100
1	24	4	Tetraploid		50	42	100
			Control		10	0	0
			Diploid		50	26	20
			Triploid	Dropping	50	28	14
			Tetraploid		50	26	18

Table 31. Effect of 0.5% colchicine on four days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Deeping	50	0	0
1	3	4	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	8	6
			Triploid	Deeping	50	10	6
1	6	4	Tetraploid		50	10	4
			Control		10	0	0
			Diploid		50	6	4
			Triploid	Dropping	50	6	4
			Tetraploid		50	2	0
			Control		10	0	0
			Diploid		50	16	14
			Triploid	Deeping	50	16	14
1	9	4	Tetraploid		50	16	12
			Control		10	0	0
			Diploid		50	16	12
			Triploid	Dropping	50	16	10
			Tetraploid		50	14	10
			Control		10	0	0
			Diploid		50	26	20
			Triploid	Deeping	50	28	18
1	12	4	Tetraploid		50	26	20
			Control		10	0	0
			Diploid		50	20	16
			Triploid	Dropping	50	18	12
			Tetraploid		50	14	10
			Control		10	0	26
			Diploid		50	40	54
			Triploid	Deeping	50	38	52
1	18	4	Tetraploid		50	42	52
			Control		10	0	0
			Diploid		50	32	24
			Triploid	Dropping	50	28	22
			Tetraploid		50	32	22
			Control		10	0	82
			Diploid		50	44	100
			Triploid	Deeping	50	44	100
1	24	4	Tetraploid		50	46	100
			Control		10	0	0
			Diploid		50	40	28
			Triploid	Dropping	50	40	30
			Tetraploid		50	32	24

Table 32. Effect of 0.7% colchicine on four days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Deeping	50	0	0
1	3	4	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	12	8
			Triploid	Deeping	50	8	6
1	6	4	Tetraploid		50	12	4
			Control		10	0	0
			Diploid		50	10	6
			Triploid	Dropping	50	10	4
			Tetraploid		50	8	4
			Control		10	0	0
			Diploid		50	22	16
			Triploid	Deeping	50	18	12
1	9	4	Tetraploid		50	20	12
			Control		10	0	0
			Diploid		50	20	18
			Triploid	Dropping	50	22	18
			Tetraploid		50	22	14
			Control		10	0	0
			Diploid		50	36	32
			Triploid	Deeping	50	34	30
1	12	4	Tetraploid		50	34	30
			Control		10	0	0
			Diploid		50	28	28
			Triploid	Dropping	50	28	20
			Tetraploid		50	32	24
			Control		10	0	26
			Diploid		50	44	60
			Triploid	Deeping	50	38	60
1	18	4	Tetraploid		50	42	54
			Control		10	0	0
			Diploid		50	42	32
			Triploid	Dropping	50	40	30
			Tetraploid		50	40	30
			Control		10	0	82
			Diploid		50	48	100
			Triploid	Deeping	50	48	100
1	24	4	Tetraploid		50	44	100
			Control		10	0	0
			Diploid		50	48	38
			Triploid	Dropping	50	42	34
			Tetraploid		50	48	34

Table 33. Effect of 1% colchicine on four days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	10	4
			Triploid	Deeping	50	6	4
I	3	4	Tetraploid		50	6	2
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	18	14
			Triploid	Deeping	50	18	10
I	6	4	Tetraploid		50	12	8
			Control		10	0	0
			Diploid		50	12	6
			Triploid	Dropping	50	8	4
			Tetraploid		50	10	4
			Control		10	0	0
			Diploid		50	30	20
			Triploid	Deeping	50	30	22
I	9	4	Tetraploid		50	24	18
			Control		10	0	0
			Diploid		50	26	20
			Triploid	Dropping	50	26	18
			Tetraploid		50	20	18
			Control		10	0	0
			Diploid		50	40	36
			Triploid	Deeping	50	32	26
I	12	4	Tetraploid		50	36	30
			Control		10	0	0
			Diploid		50	38	30
			Triploid	Dropping	50	36	28
			Tetraploid		50	40	26
			Control		10	0	26
			Diploid		50	46	66
			Triploid	Deeping	50	42	64
I	18	4	Tetraploid		50	44	66
			Control		10	0	0
			Diploid		50	44	38
			Triploid	Dropping	50	46	34
			Tetraploid		50	40	36
			Control		10	0	82
			Diploid		50	48	100
			Triploid	Deeping	50	46	100
I	24	4	Tetraploid		50	48	100
			Control		10	0	0
			Diploid		50	52	46
			Triploid	Dropping	50	50	40
			Tetraploid		50	50	42

Effect of different concentration of colchicine on six days old seedlings:

The total number of seedling treated percentage of abnormal seedlings and the mortality percentage for different types of kakrol is presented in **table 34, 35, 36, 37, 38, 39 and 40 (Plate II. Fig. A & B).**

Colchicine solution of 0.1, 0.2, 0.3, 0.4, 0.5, 0.7 and 1.0 % were applied on the tip of 6 days old seedlings. There were two types of application viz., dropping method and dipping method were used for the induction of artificial polyploid. Two types of effect were noted. The affected shoot tip of 6 days old seedling to was be found very typical form as like as tumor. All such seedlings were scored as abnormal. Mortality percentage of treated seedlings were recorded. The data show that there was a differential response of the genotypes of kakrol to colchicine treatment. An increase in treatment time, as 24, 18 and 12h lead to higher affected percentage of seedlings in all the genotype of kakrol and the mortality percentage was also very high when shoot tips were dipping for long time in different strength of colchicine solution.

The appeared to be great sensitivity to colchicine when seedlings were treated with high concentration (0.7 and 1 %) as agents other strength of solution.

0.1% colchicine effect on six days old seedlings:

The result obtain from the treatment of 0.1% colchicine solution have been presented in **table 34.** Out of 50 seedlings for each treatment were applied with 0.1% colchicine. There was no effect observed from this treatment. But mortality percentage were 24 to 30% when treatment time was 18h for dipping method and it was 78 to 100% when treatment time was 24h. From dropping method, no affected seedlings was found for different treatment time and mortality percentage was zero.

0.2% colchicine effect on six days old seedlings:

The result obtain from the treatment of 0.2% colchicine solution have been presented in **table 35.** There was no affected seedlings to be found when treatment time was 3h and 6h treatment time. In dropping method the highest affected seedlings were recorded when treatment time was 24h and it were 14, 12 and 10%, respectively in diploid, triploid and tetraploid genotypes.

In dipping method, 2 to 6% affected seedlings were found when treatment time was 9h and it was 22 to 24% then treatment time was 24h. From 24h treatment in dipping method no seedlings were survive.

0.3% colchicine effect on 6 days old seedlings:

The result obtain from the treatment of 0.3% colchicine solution have been presented in **table 36.** The affected seedlings were the highest (16%) in tetraploid kakrol for dropping method when treatment time was 24h and the highest mortality percentage was 12 in diploid types of kakrol.

The highest number of affected seedlings were obtained when seedling tips dipped 24h in 0.3% colchicine solution. Thirty two percent to 36% seedlings were affected from this treatment. Affected seedlings were 20 to 24% and mortality percentage were 40 to 44% when treatment time was 18h. The control seedlings mortality was 24%. Short treatment time as 3 and 6h there were no colchicine effect to be observed for any method of colchicine application.

0.4% colchicine effect on 6 days old seedlings:

Results obtained from 0.4% colchicine effect on 6 days old seedlings are shown in **table 37.** The highest number of affected seedlings were obtained when seedling tips sprayed (dropping) 24h in the strength of 0.4% colchicine and which was 26% in

diploid kakrol. Mortality percentage was highest in diploid genotype of dropping method and which was 22%.

Out of 6 treatment time except 3h showed affected seedlings. If period of dipping in this treatment below in there is no affected seedlings found. When dipping period is 6 & 9h, diploid, triploid and tetraploid kakrol showed little success (2 to 14%). When dipping period increased affected seedlings percentage have increased gradually and after 24h percentage of affected seedlings remained unchanged.

0.5% colchicine effect on six days old seedlings:

The result obtain from the treatment of 0.5% colchicine solution have been presented in **table 38.** In dipping method as may as 4 - 6% affected seedlings were obtained when the seedlings were dipped with 0.5%. colchicine solution for a period of 3h. As the dipping period increased from 6 to 24h percentage of affected seedlings increased from 10 to 46.

In dropping method highest percentage of affected seedlings were obtained when all the genotypes sprayed with 0.5% colchicine for a period of 24h and the percentage of affected seedling were 42, 40 and 40%, respectively in diploid, triploid and tetraploid kakrol. Some percentage of affected seedlings were obtained when the seedlings sprayed with 0.5% colchicine for a period of 6h and it was 4 to 6%. In dipping method the seedlings dipped into colchicine solution at least 18h or less, other wise affected seedling will die at any time duration.

0.7% colchicine effect on six days old seedlings:

Results obtained from 0.7% colchicine effect on 6 days old seedlings are shown in **table 39.** If dropping period is less 3h is no affected seedling to be observed. If dropping period in success starts, diploid shows 2%, triploid 2% and tetraploid 0% affected at 3h spray of colchicine. In 24h (for dropping method) 46 - 48% affected

seedlings were to be found and mortality percentage were 32 - 40%. For dipping method no plant survive when the seedling tips were kept into 0.7% colchicine solution at 24h or more. The percentage of affected seedlings were 40, 38 and 38%, respectively in diploid triploid and tetraploid kakrol when seedling tips were kept 0.7% colchicine solution at 18h.

1% colchicine effect on six days old seedlings:

The result obtain from the treatment of 1% colchicine solution have been presented in **table 40.** If period of treatment time was below 3h to 6h the affected seedlings percentage were low of both types of colchicine application. It was 2 to 20%. When dipping period increase percentage of affected seedlings also increased gradually and after 12h the success of affected seedlings remained unchanged. 100% mortality was found when the seedling tip were dipped with 1% colchicine for 24h.

In dropping method affected seedlings were found in all the treatment time. When dropping period increased the percentage of affected seedlings also increased gradually. The highest affected seedlings were found when it was treated with 1% colchicine for 24h and which was 54, 52 and 50%, respectively in diploid, triploid and tetraploid seedling of kakrol. Mortality percentage were 48, 44 and 40%, respectively in diploid, triploid and tetraploid seedlings of kakrol when treatment time was 24h for dropping method. Mortality percentage was very high when treatment time increased (12 to 24h) for dipping method, which was 26 to 100%.

Table 34. Effect of 0.1% colchicine on six days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	3	6	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	6	6	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	9	6	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	12	6	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	24
			Diploid		50	0	30
			Triploid	Dipping	50	0	30
1	18	6	Tetraploid		50	0	28
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	78
			Diploid		50	0	100
			Triploid	Dipping	50	0	100
1	24	6	Tetraploid		50	0	100
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0

Table 35. Effect of 0.2% colchicine on six days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	3	6	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	6	6	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	4	2
			Triploid	Dipping	50	2	0
1	9	6	Tetraploid		50	4	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	16	14
			Triploid	Dipping	50	16	12
1	12	6	Tetraploid		50	14	12
			Control		10	0	0
			Diploid		50	4	0
			Triploid	Dropping	50	4	2
			Tetraploid		50	2	0
			Control		10	0	24
			Diploid		50	18	42
			Triploid	Dipping	50	18	40
1	18	6	Tetraploid		50	16	40
			Control		10	0	0
			Diploid		50	6	2
			Triploid	Dropping	50	6	2
			Tetraploid		50	4	0
			Control		10	0	78
			Diploid		50	24	100
			Triploid	Dipping	50	22	100
1	24	6	Tetraploid		50	22	100
			Control		10	0	0
			Diploid		50	14	10
			Triploid	Dropping	50	12	8
			Tetraploid		50	10	6

Table 36. Effect of 0.3% colchicine on six days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	3	6	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	6	6	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	6	10
			Triploid	Dipping	50	8	12
1	9	6	Tetraploid		50	4	8
			Control		10	0	0
			Diploid		50	6	4
			Triploid	Dropping	50	4	2
			Tetraploid		50	6	2
			Control		10	0	0
			Diploid		50	16	22
			Triploid	Dipping	50	10	18
1	12	6	Tetraploid		50	12	14
			Control		10	0	0
			Diploid		50	8	4
			Triploid	Dropping	50	8	4
			Tetraploid		50	6	4
			Control		10	0	24
			Diploid		50	22	44
			Triploid	Dipping	50	24	40
1	18	6	Tetraploid		50	20	40
			Control		10	0	0
			Diploid		50	12	6
			Triploid	Dropping	50	10	4
			Tetraploid		50	10	4
			Control		10	0	78
			Diploid		50	34	100
			Triploid	Dipping	50	36	100
1	24	6	Tetraploid		50	32	100
			Control		10	0	0
			Diploid		50	14	12
			Triploid	Dropping	50	14	10
			Tetraploid		50	16	10

Table 37. Effect of 0.4% colchicine on six days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dipping	50	0	0
1	3	6	Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	2	0
			Triploid	Dipping	50	2	2
1	6	6	Tetraploid		50	4	2
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	12	12
			Triploid	Dipping	50	14	16
1	9	6	Tetraploid		50	10	12
			Control		10	0	0
			Diploid		50	8	6
			Triploid	Dropping	50	10	8
			Tetraploid		50	8	6
			Control		10	0	0
			Diploid		50	20	16
			Triploid	Dipping	50	16	12
1	12	6	Tetraploid		50	10	14
			Control		10	0	0
			Diploid		50	16	10
			Triploid	Dropping	50	10	12
			Tetraploid		50	12	6
			Control		10	0	24
			Diploid		50	38	48
			Triploid	Dipping	50	36	44
1	18	6	Tetraploid		50	38	42
			Control		10	0	0
			Diploid		50	20	14
			Triploid	Dropping	50	22	18
			Tetraploid		50	22	16
			Control		10	0	78
			Diploid		50	44	100
			Triploid	Dipping	50	42	100
1	24	6	Tetraploid		50	38	100
			Control		10	0	0
			Diploid		50	26	22
			Triploid	Dropping	50	24	20
			Tetraploid		50	24	18

Table 38. Effect of 0.5% colchicine on six days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	6	2
			Triploid	Dipping	50	4	2
1	3	6	Tetraploid		50	4	0
			Control		10	0	0
			Diploid		50	0	0
			Triploid	Dropping	50	0	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	12	6
			Triploid	Dipping	50	12	8
1	6	6	Tetraploid		50	10	4
			Control		10	0	0
			Diploid		50	6	4
			Triploid	Dropping	50	4	4
			Tetraploid		50	6	0
			Control		10	0	0
			Diploid		50	18	16
			Triploid	Dipping	50	14	12
1	9	6	Tetraploid		50	16	14
			Control		10	0	0
			Diploid		50	14	10
			Triploid	Dropping	50	16	12
			Tetraploid		50	12	8
			Control		10	0	0
			Diploid		50	28	18
			Triploid	Dipping	50	24	20
1	12	6	Tetraploid		50	26	22
			Control		10	0	0
			Diploid		50	18	14
			Triploid	Dropping	50	16	14
			Tetraploid		50	20	16
			Control		10	0	24
			Diploid		50	40	56
			Triploid	Dipping	50	42	50
1	18	6	Tetraploid		50	42	48
			Control		10	0	0
			Diploid		50	30	22
			Triploid	Dropping	50	32	22
			Tetraploid		50	34	26
			Control		10	0	78
			Diploid		50	46	100
			Triploid	Dipping	50	42	100
1	24	6	Tetraploid		50	44	100
			Control		10	0	0
			Diploid		50	42	36
			Triploid	Dropping	50	40	32
			Tetraploid		50	40	30

Table 39. Effect of 0.7% colchicine on six days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	6	2
			Triploid	Dipping	50	4	2
1	3	6	Tetraploid		50	4	0
			Control		10	0	0
			Diploid		50	2	0
			Triploid	Dropping	50	2	0
			Tetraploid		50	0	0
			Control		10	0	0
			Diploid		50	10	8
			Triploid	Dipping	50	10	6
1	6	6	Tetraploid		50	12	6
			Control		10	0	0
			Diploid		50	10	6
			Triploid	Dropping	50	8	4
			Tetraploid		50	8	6
			Control		10	0	0
			Diploid		50	26	24
			Triploid	Dipping	50	22	22
1	9	6	Tetraploid		50	20	24
			Control		10	0	0
			Diploid		50	22	18
			Triploid	Dropping	50	18	16
			Tetraploid		50	20	14
			Control		10	0	0
			Diploid		50	38	34
			Triploid	Dipping	50	36	30
1	12	6	Tetraploid		50	28	28
			Control		10	0	0
			Diploid		50	38	26
			Triploid	Dropping	50	36	22
			Tetraploid		50	32	22
			Control		10	0	24
			Diploid		50	40	62
			Triploid	Dipping	50	36	60
1	18	6	Tetraploid		50	38	56
			Control		10	0	0
			Diploid		50	40	28
			Triploid	Dropping	50	38	30
			Tetraploid		50	38	32
			Control		10	0	78
			Diploid		50	48	100
			Triploid	Dipping	50	42	100
1	24	6	Tetraploid		50	44	100
			Control		10	0	0
			Diploid		50	46	40
			Triploid	Dropping	50	46	32
			Tetraploid		50	48	34

Table 40. Effect of 1% colchicine on six days old seedlings of different genotypes of kakrol.

Treatment No.	Treatment Time (in hours)	Age of Seedlings in days	Types of Kakrol	Method of Treatment	No of seedlings Treated	Percentage affected	Mortality Percentage
			Control		10	0	0
			Diploid		50	8	4
			Triploid	Dipping	50	6	4
1	3	6	Tetraploid		50	6	2
			Control		10	0	0
			Diploid		50	2	0
			Triploid	Dropping	50	2	0
			Tetraploid		50	2	0
			Control		10	0	0
			Diploid		50	20	12
			Triploid	Dipping	50	18	8
1	6	6	Tetraploid		50	14	6
			Control		10	0	0
			Diploid		50	10	6
			Triploid	Dropping	50	12	4
			Tetraploid		50	12	4
			Control		10	0	0
			Diploid		50	32	20
			Triploid	Dipping	50	28	22
1	9	6	Tetraploid		50	30	22
			Control		10	0	0
			Diploid		50	24	20
			Triploid	Dropping	50	24	18
			Tetraploid		50	22	20
			Control		10	0	0
			Diploid		50	40	38
			Triploid	Dipping	50	38	28
1	12	6	Tetraploid		50	38	26
			Control		10	0	0
			Diploid		50	40	32
			Triploid	Dropping	50	36	30
			Tetraploid		50	32	26
			Control		10	0	24
			Diploid		50	44	64
			Triploid	Dipping	50	42	64
1	18	6	Tetraploid		50	44	62
			Control		10	0	0
			Diploid		50	46	40
			Triploid	Dropping	50	46	36
			Tetraploid		50	42	32
			Control		10	0	78
			Diploid		50	52	100
			Triploid	Dipping	50	48	100
1	24	6	Tetraploid		50	44	1000
			Control		10	0	0
			Diploid		50	54	48
			Triploid	Dropping	50	52	44
			Tetraploid		50	52	40

Plate II

Fig. A. Effect of colchicine on six days old seedlings of kakrol.
a. Affected diploid seedling, b. Affected tetraploid seedling and c. Untreated seedling.
B. Transplanted seedlings (affected) on the petri dish.

Morphological and anatomical studies:

Comparative studies on morphological and anatomical of different organs of kakrol plant were done. Results are described under different heads.

Internode length: The internode length of different types of kakrol have been shown in **table 41.** The internode length was highest in tetraploid male plant and it was 12.63cm. The lowest internode length was 3.676cm in diploid male. The mean internode length was 4.04, 10.83, 10.346 and 12.123cm, respectively in diploid female, triploid male and triplosid female and tetraploid female.

Result of analysis of variance are shown in **table 41.1.** The item ploidy was highly significant at 1% level indicated significant differences existing among the type included in the study. In this analysis, the item sex was not significant. It reflected no differences exist relation in different in respect of internode length. Item Interaction between ploidy and sex was significant at 5% level. It show that one past of the experiment differ from the other past.

Internode breadth: The inernode breadth of different types of kakrol flower have been shown in **table 42.** The inernode breadth was the highest in tetraploid female plant and it was 0.806cm. The lowest inernode breadth was 0.38 cm in diploid male. The mean inernode breadth was 0.393, 0.673, 0.646 and 0.803cm respectively in diploid female, triploid male, triploid female, and tetraploid male.

Result of analysis of variance are shown in **table 42.1.** The item ploidy was not significant indicated no differences existing among the type included in the study. In this analysis, the item sex was not significant. It reflected that no differences exist relation in different in respect of inernode breadth. Item Interaction between ploidy and sex was significant at 5% level. It show that one past of the experiment differ from the other past.

Table 41. Measurement of mean internode length (cm) of different type of kakrol.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	3.676	10.83	12.63
Female (♀)	4.04	10.346	12.123
	LSD Values		
	5%	1%	
Ploidy	0.3523	0.501	
Sex	0.288	0.409	
Ploidy x Sex	0.51	0.7086	

Table 41.1. Analysis of variance of interned length means data in table 41.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^h	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	242.05	121.019	1613.586***	4.10	7.56
Sex	1	0.1968	0.1968	2.624^{NS}	4.96	10.04
Ploidy x Sex	2	0.75		5.0**	4.10	7.56
Error	10		0.075			
Total	17					

^{ns}= not significant, ^{h}**= Significant at 5% level and ^{h}***= Significant at 1% level.

Table 42. Measurement of mean internode breadth (cm) of different types of kakrol.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	0.38	0.673	0.803
Female (♀)	0.393	0.646	0.806
	LSD Values		
	5%	1%	
Ploidy	0.10077	0.143334	
Sex	0.06718	0.09556	
Ploidy x Sex	0.20155	0.28668	

Table 42.1. Analysis of variance of internode breadth means data in table 42.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^h	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	0.5415	0.27	1.992^{NS}	4.10	7.56
Sex	1	0.000009	0.0	0.0^{NS}	4.96	10.04
Ploidy x Sex	2	1.357	0.678	5.0**	4.10	7.56
Error	10		0.1357			
Total	17					

^{ns}= not significant, ^{h}**= Significant at 5% level and ^{h}***= Significant at 1% level.

Petiole length: Mean length of petiole of different types of kakrol studied are shown in **table 43.** The lowest length was recorded in male plant of diploid kakrol which was 3.36cm: The petiole length of tetraploid male was the highest among all the types studied and it was 7.34cm. The mean petiole length of diploid ♀, triploid ♂, triploid ♀ and tetraploid ♀ were 3.48, 5.53, 5.34 and 7.213cm, respectively.

Analysis of variance of leaf petiole length of different types of kakrol are shown in **table 43.1.** From the analysis of variance within a ploidy level the petiole length showed significant difference at 1% level. Within a ploidy level the LSD values were 0.1151 and 0.1636cm at 5% and 1% level respectively. Between sex of respective types of kakrol were found not significant. Interaction between ploidy and sex were to be found significant difference at 5% level and LSD value was 0.1627cm. It show that one past of the experiment differ from the other past.

Petiole breadth: The petiole breadth of different types of kakrol have been shown in **table 44.** The breadth of petiole was highest in tetraploid male and it was 0.5533cm. The lowest petiole breadth was 0.3567cm in diploid male. The mean petiole breadth was 0.35, 0.41, 0.43 and 0.5067cm, respectively in diploid female triploid male and female and tetraploid male.

Result of analysis of variance are shown in **table 44.1.** The item ploidy was highly significant at 1% level indicated significant differences existing among the type included in the study. In this analysis, the item sex was not significant. It reflected that no differences exist relation in different in respect of petiole breadth. Item Interaction between ploidy ands sex was significant at 5% level. It show that one past of the experiment differ from the other past.

Table 43. Measurement of mean petiole length (cm) of different types of kakrol.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	3.36	5.53	7.34
Female (♀)	3.48	5.34	7.213
	LSD Values		
	5%	1%	
Ploidy	0.1151	0.1636	
Sex	0.0939	0.1336	
Ploidy x Sex	0.1627	0.2336	

Table 43.1. Analysis of variance of petiole length means data in table 43.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	44.651	22.325	22790.74***	4.10	7.56
Sex	1	0.01938	0.01938	2.422^{NS}	4.96	10.04
Ploidy x Sex	2	0.0804	0.0402	5.00**	4.10	7.56
Error	10		0.0080			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Table 44. Measurement of mean petiole breadth (cm) of different type of kakrol.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	0.3567	0.41	0.5533
Female (♀)	0.35	0.43	0.5067
	LSD Values		
	5%	1%	
Ploidy	0.047	0.0669	
Sex	0.0077	0.0546	
Ploidy x Sex	0.06679	0.0947	

Table 44.1. Analysis of variance of petiole breadth means data in table 44.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	0.0955	0.04774	35.6343***	4.10	7.56
Sex	1	0.0006	0.0006	0.44776^{NS}	4.96	10.04
Ploidy x Sex	2	0.0134	0.0069	5.00**	4.10	7.56
Error	10		0.00134			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Leaf blade length: Mean length of leaf blade of different types of kakrol studied are shown in **table 45.** The lowest leaf blade length was recorded in male plant of diploid kakrol which was 5.15cm The leaf blade length of tetraploid female was the highest among all the types studied and it was 10.4033cm. The mean length of diploid ♀, triploid ♂, triploid ♀ and tetraploid male were 5.3933, 7.99, 7.92 and 9.83cm, respectively (**Plate III. Fig. A**).

Analysis of variance of leaf blade length of different types of kakrol are shown in **table 45.1.** From the analysis of variance within a ploidy level the leaf blade length showed significant difference at 1% level. Within a ploidy level the LSD values were 0.02329cm and 0.0331cm at 5% and 1% level respectively. Between sex of respective types of kakrol were to be found significant at 5% level. Interaction between ploidy and sex were to be found significant difference at 5% level and LSD value was 0.04659cm. It show that one past of the experiment differ from the other past.

Leaf blade breadth: The leaf blade breadth of different types of kakrol flowers have been shown in **table 46.** The breadth of leaf blade was highest in tetraploid female and it was 11.2433cm. The lowest leaf blade breadth length was 5.783cm in diploid male. The mean breadth was 6.22, 9.543, 9.393 and 11.1167cm respectively in diploid female triploid male and female and tetraploid male (**Plate III. Fig A**).

Result of analysis of variance are shown in **table 46.1.** The item ploidy was highly significant at 1% level indicated significant differences existing among the type included in the study. In this analysis, the item sex was not significant. It reflected that no differences exist relation in different in respect of leaf blade breadth. Item Interaction between ploidy and sex was significant at 5% level and LSD value was 0.2928. It show that one past of the experiment differ from the other past.

Table 45. Measurement of mean leaf blade length (cm) of different types of kakrol.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	5.15	7.99	9.83
Female (♀)	5.3933	7.92	10.4033

	LSD Values	
	5%	1%
Ploidy	0.02329	0.0331
Sex	0.0155	0.0220
Ploidy x Sex	0.04659	0.0662

Table 45.1. Analysis of variance of leaf blade length means data in table 45.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^h	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	70.6941	35.3471	1126.78***	4.10	7.56
Sex	1	0.2787	0.2787	8.8842**	4.96	10.04
Ploidy x Sex	2	0.3137		5.00**	4.10	7.56
Error	10		0.03137			
Total	17					

^{ns}= not significant, ^{h}**= Significant at 5% level and ^{h}***= Significant at 1% level.

Table 46. Measurement of mean leaf blade breadth length (cm) of different types of kakrol.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	5.783	9.543	11.1167
Female (♀)	6.22	9.393	11.2433

	LSD Values	
	5%	1%
Ploidy	0.2070	0.2944
Sex	0.1690	0.2404
Ploidy x Sex	0.2928	0.4164

Table 46.1. Analysis of variance of stamen length means data in table 46.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^h	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	83.5254	41.7627	1612.459***	4.10	7.56
Sex	1	0.0854	0.0854	3.297^{NS}	4.96	10.04
Ploidy x Sex	2	0.2585	0.12925	5.00**	4.10	7.56
Error	10		0.0259			
Total	17					

ns= not significant, ^{h}**= Significant at 5% level and ^{h}***= Significant at 1% level.

Tendril length: Mean length of tendril of different types of kakrol studied are shown in **table 47.** The lowest tendril length was recorded in male plant of diploid types of kakrol which was 19.3767cm. The tendril length of tetraploid female was the highest among all the types studied and it was 30.8467cm. The mean length of diploid ♀, triploid ♂, triploid ♀ and tetraploid male were 20.50, 27.49, 27.7667 and 28.91cm, respectively.

Analysis of variance of tendril length of different types of kakrol are shown in **table 47.1.** From the analysis of variance within a ploidy level the tendril length showed significant difference at 1% level. Within a ploidy level the LSD values were 0.5848cm and 0.8318cm at 5% and 1% level respectively. Between sex of respective types of kakrol were to be found highly significant at 1% level. Interaction between ploidy and sex were to be found significant difference at level and LSD value was 0.8271cm. It show that one past of the experiment differ from the other past.

Flower diameter length: Mean length of flower diameter of different types of kakrol studied are shown in **table 48.** The lowest flower diameter was recorded in male flower of diploid types of kakrol which was 2.9466cm. The flower diameter of tetraploid female was the highest among all the types studied and it was 9.13cm. The mean flower diameter of diploid ♀, triploid ♂, triploid ♀ and tetraploid male were 3.2033, 6.9866, 7.2333 and 8.86cm, respectively.

Analysis of variance of flower diameter of different types of kakrol are shown in **table 48.1.** From the analysis of variance within a ploidy level the flower diameter showed significant difference at 1% level. Between sex of respective types of kakrol were to be found highly significant. Interaction between ploidy and sex were to be found significant difference at 5% level and LSD value was 0.01523cm. It show that one past of the experiment differ from the other past.

Table 47. Measurement of mean tendril length (cm) of different types of kakrol.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	19.3767	27.49	28.91
Female (♀)	20.50	27.7667	30.8467
	LSD Values		
	5%	1%	
Ploidy	0.5848	0.8318	
Sex	0.4775	0.6792	
Ploidy x Sex	0.8271	1.1764	

Table 47.1. Analysis of variance of tendril length means data in table 47.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	326.0044	163.0022	788.5931***	4.10	7.56
Sex	1	5.5667	5.5667	26.9313***	4.96	10.04
Ploidy x Sex	2	2.067	1.0335	5.00**	4.10	7.56
Error	10		0.2067			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Table 48. Measurement of mean flower diameter (cm) of different types of kakrol.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	2.9466	6.9866	8.86
Female (♀)	3.2033	7.2333	9.13
	LSD Values		
	5%	1%	
Ploidy	0.01076	0.01531	
Sex	0.00879	0.01250	
Ploidy x Sex	0.01523	0.02166	

Table 48.1. Analysis of variance of flower diameter means data in table 48.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	109.873	54.933	78.3634***	4.10	7.56
Sex	1	0.3041	0.3041	433.945***	4.96	10.04
Ploidy x Sex	2	0.0007011		5.00**	4.10	7.56
Error	10		0.0000701			
Total	17					

ns= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Bract length: Mean bract length of different genotypes of kakrol flower studied are shown in **table 49 and plate III. fig B.** The lowest bract length was recorded in female flower of diploid kakrol which was 0.2467cm. The bract length of tetraploid male was the highest among all the types studied and it was 2.68cm. The mean bract length of diploid ♂, triploid ♂, triploid ♀ and tetraploid ♀ were 0.8967, 2.0267, 0.3733 and 0.432cm, respectively.

Analysis of variance of bract length of different types of kakrol flower are shown in **table 49.1.** From the analysis of variance within a ploidy level the bract length showed significant difference at 1% level. Within a ploidy level the LSD values were 0.32cm and 0.45cm at 5% and 1% level, respectively. Between sex of respective types of kakrol were to be found highly significant. Interaction between ploidy and sex were to be found significant difference at 5% level and LSD value was 0.45cm.

Bract breadth: The bract breadth of different types of kakrol flower have been shown in **table 50.** The bract breadth was the highest in tetraploid male plant and it was 3.733cm. The lowest bract breadth was 0.263cm in triploid female. The mean bract breadth was 1.61, 0.77, 2.87 and 0.35cm, respectively in diploid male, diploid female, triploid male and tetraploid female **(Plate III. Fig. B)**.

Result of analysis of variance are shown in **table 50.1.** The item ploidy was significant at 5% indicated differences existing among the type included in the study. In this analysis, the item sex was highly significant. It reflected that differences exist relation in different in respect of bract breadth. Item Interaction between ploidy and sex was significant at 5% level. It show that one past of the experiment differ from the other past.

Table 49. Measurement of mean bract length (cm) of different type of kakrol flowers.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	0.8967	2.0267	2.68
Female (♀)	0.2467	0.3733	0.423

	LSD Values	
	5%	1%
Ploidy	0.32	0.45
Sex	0.26	0.38
Ploidy x Sex	0.45	0.64

Table 49.1. Analysis of variance of bract length means data in table 49.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	2.96	1.48	24.26***	4.10	7.56
Sex	1	11.76	11.76	192.79***	4.96	10.04
Ploidy x Sex	2	0.61	0.35	5.0**	4.10	7.56
Error	10		0.061			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Table 50. Measurement of mean bract breadth (cm) of different type of kakrol flowers.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	1.61	2.87	3.733
Female (♀)	0.77	0.263	0.35

	LSD Values	
	5%	1%
Ploidy	0.72	1.03
Sex	0.59	0.84
Ploidy x Sex	1.02	1.45

Table 50.1. Analysis of variance of bract breadth means data in table 50.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	3.71	1.86	5.92**	4.10	7.56
Sex	1	26.97	26.97	85.89**	4.96	10.04
Ploidy x Sex	2	3.14	1.57	5.0*	4.10	7.56
Error	10		0.314			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Calyx length: The calyx length of different types of kakrol flower have been shown in **table 51.** The calyx length was the highest in tetraploid male plant and it was 1.563cm. The lowest calyx length area was 0.83cm in diploid female. The mean calyx length was 0.856, 1.296, 0.333 and 1.323cm, respectively in diploid male, triploid male, triploid female and tetraploid female (**Plate III. Fig C**).

Result of analysis of variance are shown in **table 51.1.** The item ploidy was highly significant at 1% indicated differences existing among the type included in the study. In this analysis, the item sex was highly significant. It reflected that differences exist relation in diffcrent in respect of calyx length. Item Interaction between ploidy and sex was significant at 5% level. It show that one past of the experiment differ from the other past.

Calyx diameter: The calyx diameter of different types of kakrol flowers have been shown in **table 52.** The diameter of calyx was highest in tetraploid male and it was 0.63cm. The lowest calyx diameter was 0.21cm in diploid male. The mean diameter was 0.22, 0.49, 0.30 and 0.30cm respectively in diploid female triploid male and female and tetraploid female.

Result of analysis of variance are shown in **table 52.1.** The item ploidy was highly significant at 1% level indicated significant differences existing among the type included in the study. In this analysis, the item sex was highly significant. It reflected differences exist relation in different in respect of calyx diameter. Item Interaction between ploidy and sex was significant at 5% level.

Table 51. Measurement of mean calyx length (cm) of different type of kakrol flowers.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	0.856	1.296	1.563
Female (♀)	0.83	0.333	1.323

	LSD Values	
	5%	1%
Ploidy	0.1199	0.1706
Sex	0.0979	0.1393
Ploidy x Sex	0.1696	0.2413

Table 51.1. Analysis of variance of calyx length means data in table 51.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	1.0832	0.5416	62.2528***	4.10	7.56
Sex	1	0.1985	0.1985	22.281***	4.96	10.04
Ploidy x Sex	2	0.087	0.0435	5.0**	4.10	7.56
Error	10		0.0087			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Table 52. Measurement of mean calyx diameter (cm) of different type of kakrol flowers.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	0.21	0.49	0.63
Female (♀)	0.22	0.30	0.30

	LSD Values	
	5%	1%
Ploidy	0.1196	0.1701
Sex	0.0976	0.1389
Ploidy x Sex	0.1691	0.2406

Table 52.1. Analysis of variance of calyx diameter means data in table 52.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	0.1975	0.0987	11.41***	4.10	7.56
Sex	1	0.1318	0.1318	15.24***	4.96	10.04
Ploidy x Sex	2	0.0865		5.00**	4.10	7.56
Error	10		0.00865			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Petal length: The petal length of different types of kakrol flower have been shown in **table 53.** The petal length was the highest in tetraploid male plant and it was 6.51cm. The lowest petal length was 2.33cm in diploid female. The mean petal length was 2.42, 5.226, 5.643 and 5.313cm, respectively in diploid male, triploid male and triploid female and tetraploid female.

Result of analysis of variance are shown in **table 53.1.** The item ploidy was significant at 1% indicated differences existing among the type included in the study. In this analysis, the item sex was not significant. It reflected that no differences exist relation in different in respect of petal lengths. Item Interaction between ploidy and sex was significant at 5% level.

Petal breadth: Mean petal breadth of different genotypes of kakrol flower studied are shown in **table 54.** The lowest petal breadth was recorded in female flower of diploid kakrol which was 1.176cm. The petal breadth of tetraploid female was the highest among all the types studied and it was 3.953cm. The mean petal breadth of diploid ♂, triploid ♂, triploid ♀ and tetraploid ♂ were 1.20, 3.306, 2.683 and 3.613cm, respectively.

Analysis of variance of petal breadth of different types of kakrol flower are shown in table **54.1.** From the analysis of variance within a ploidy level the petal breadth showed significant difference at 1% level. Within a ploidy level the LSD values were 0.3426cm and 0.4875cm at 5% and 1% level respectively. Between sex of respective types of kakrol were to be found not significant. Interaction between ploidy and sex were to be found significant difference at 5% level and LSD value was 0.4847cm. It show that one past of the experiment differ from the other past.

Table 53. Measurement of mean petal length (cm) of different type of kakrol flowers.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	2.42	5.226	6.51
Female (♀)	2.33	5.643	5.313
	LSD Values		
	5%	1%	
Ploidy	0.5815	0.8271	
Sex	0.4748	0.6753	
Ploidy x Sex	0.8224	1.1698	

Table 53.1. Analysis of variance of petal length means data in table 53.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	44.1537	22.076	108.0078***	4.10	7.56
Sex	1	0.3756	0.3756	1.837^{NS}	4.96	10.04
Ploidy x Sex	2	2.044	1.022	5.0**	4.10	7.56
Error	10		0.2044			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Table 54. Measurement of mean petal breadth (cm) of different type of kakrol flowers.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	1.20	3.306	3.613
Female (♀)	1.176	2.683	3.953
	LSD Values		
	5%	1%	
Ploidy	0.3427	0.4875	
Sex	0.2798	0.3980	
Ploidy x Sex	0.4847	0.6894	

Table 54.1. Analysis of variance of petal breadth means data in table 54.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	21.2391	10.6195	149.57***	4.10	7.56
Sex	1	0.047	0.047	0.662^{NS}	4.96	10.04
Ploidy x Sex	2	0.71	0.355	5.0**	4.10	7.56
Error	10		0.071			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

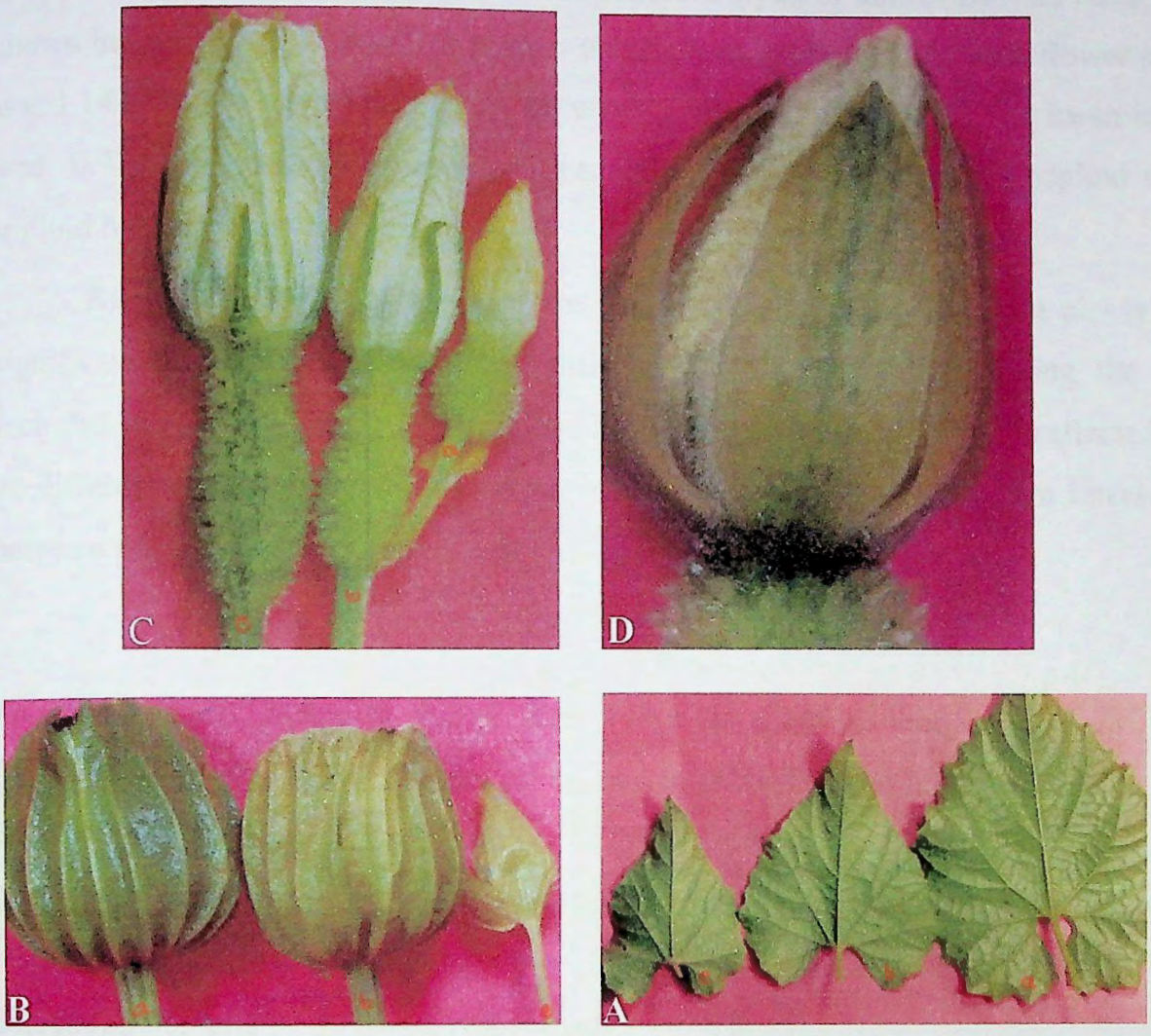


Plate III

Fig. A. Leaves of different genotypes of kakrol: a. Diploid, b. Triploid and c. Tetraploid.
B. Different types of male bract showing: a. Tetraploid, b. Triploid and c. Diploid.
C. Showing different types of calyx of female flowers: a. Tetraploid, b. Triploid and c. Diploid.
D. Calyx of tetraploid bisexual flower.

Stamen length: The length of stamen of different types of kakrol flowers have been shown in **table 55.** The length of stamen was highest in induced bisexual flower and it was 1.143cm. The lowest stamen length was 0.84cm in diploid male. The mean length was 0.9133, 0.9933, 1.06 and 1.1cm, respectively in diploid bisexual triploid male, triploid bisexual and tetraploid male.

Result of analysis of variance are shown in **table 55.1.** The item ploidy was significant at 1% level indicated significant differences existing among the type included in the study. In this analysis, the item sex was not significant. It reflected that no differences exist relation in different in respect of stamen length. Item Interaction between ploidy and sex was significant at 5% level.

Flowering time: Mean flowering time (duration from visible bud) of different genotypes of kakrol flower studied are shown in **table 56.** The lowest flowering time was recorded in female and male flower of diploid kakrol which was 19.666 days. The flowering time of tetraploid female was the highest among all the types studied and it was 30 days. The mean flowering time of triploid ♂, triploid ♀ and tetraploid ♂ were 24.333, 25.333 and 29.333days, respectively.

Analysis of variance of flowering time of different types of kakrol flower are shown in **table 56.1.** From the analysis of variance within a ploidy level the flowering time showed significant difference at 1% level. Within a ploidy level the LSD values were 0.36days and 0.51days at 5% and 1% level, respectively. Between sex of respective types of kakrol were to be found significant at 1% level. Interaction between ploidy and sex were to be found significant difference at 5% level and LSD value was 0.51days.

Table 55. Measurement of mean stamen length (cm) of different type of kakrol flowers.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	0.84	0.9933	1.1
Female ♂ (♀)	0.9133	1.06	1.1433
	LSD Values		
	5%	1%	
Ploidy	0.01109	0.01578	
Sex	0.00905	0.01288	
Ploidy x Sex	0.01569	0.02231	

Table 55.1. Analysis of variance of stamen length means data in table 55.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	0.1831	0.0915	1230.51***	4.10	7.56
Sex	1	0.0168	0.0168	225.88***	4.96	10.04
Ploidy x Sex	2	0.000744	0.000372	5.00	4.10	7.56
Error	10		0.0000744			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Table 56. Measurement of mean flowering time (in days) of different type of kakrol flowers.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	19.666	24.333	29.333
Female (♀)	19.666	25.333	30.0
	LSD Values		
	5%	1%	
Ploidy	0.36	0.51	
Sex	0.30	0.41	
Ploidy x Sex	0.51	0.73	

Table 56.1. Analysis of variance of flowering time means data in table 56.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	300.11	150.06	1923.85***	4.10	7.56
Sex	1	1.39	1.39	17.82***	4.96	10.04
Ploidy x Sex	2	0.78	0.39	5.0**	4.10	7.56
Error	10		0.078			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Pollen diameter: Mean pollen diameter of different types of kakrol studied are shown in **table 57.** The lowest pollen diameter was recorded in male flower of diploid kakrol which was 63.23μ. The pollen diameter of tetraploid bisexual (♀) flower was the highest among all the types studied and it was 85.91μ. The mean length of diploid bisexual (♀), triploid ♂, triploid bisexual (♀) and tetraploid ♂ were 66.77, 72.24, 73.22 and 85.62μ, respectively.

Analysis of variance of pollen diameter of different types of kakrol flower are shown in **table 57.1.** From the analysis of variance within a ploidy level the pollen diameter showed significant difference at 1% level. Within a ploidy level the LSD values were 1.21μ and 1.71μ at 5% and 1% level respectively. Between sex of respective types of kakrol were to be found significant at 1% level. Interaction between ploidy and sex were to be found significant difference at 5% level and LSD value was 1.71μ. It show that one past of the experiment differ from the other past.

Pollen viability: The pollen viability of different types of kakrol flowers have been shown in **table 58.** The pollen viability percentage was highest in tetraploid female bisexual flower and it was 97.07%. The lowest pollen viability percentage was 61.54% in diploid male. The mean pollen viability percentage was 65.94, 3.46, 6.03 and 94.46%, respectively in diploid bisexual (♀), triploid ♂ and triploid bisexual (♀) and tetraploid ♂.

Result of analysis of variance are shown in **table 58.1.** The item ploidy was highly significant at 1% level indicated significant differences existing among the type included in the study. In this analysis, the item sex was highly significant at 1% level. It reflected differences exist relation in different in respect of pollen viability. Item Interaction between ploidy and sex was significant at 5% level.

Table 57. Measurement of mean pollen diameter (μ) of different types of kakrol.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	63.23	72.24	85.62
Female ♂ (♀)	66.77	73.22	85.91

	LSD Values	
	5%	1%
Ploidy	1.21	1.71
Sex	0.99	1.39
Ploidy x Sex	1.71	2.43

Table 57.1. Analysis of variance of pollen diameter means data in table 57.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	1321	660.675	750.767***	4.10	7.56
Sex	1	11.58	11.58	13.159***	4.96	10.04
Ploidy x Sex	2	8.8		5.0**	4.10	7.56
Error	10		0.88			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Table 58. Measurement of mean pollen viability of different type of kakrol flowers.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	61.54	3.46	94.46
Female ♂ (♀)	65.94	6.03	97.07

	LSD Values	
	5%	1%
Ploidy	0.73	1.05
Sex	0.60	0.86
Ploidy x Sex	1.04	1.49

Table 58.1. Analysis of variance of pollen viability means data in table 58.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	25580.30	12790.15	39233.589***	4.10	7.56
Sex	1	45.79	45.79	140.4601***	4.96	10.04
Ploidy x Sex	2	3.26		5.0**	4.10	7.56
Error	10		0.326			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Pollen viability of the following morning: Mean pollen viability percentage of the following morning of different types of kakrol flower studied are shown in **table 59.** The lowest pollen viability of the following morning was recorded in male flower of diploid kakrol which was 55.86%. The pollen viability percentage (following morning) of tetraploid bisexual flower was the highest among all the types studied and it was 95.04%. The mean pollen viability of following morning of diploid ♂ (♀), triploid ♂, triploid ♂ (♀) and tetraploid ♂ were 63.92, 3.46, 5.58 and 93.32%, respectively.

Analysis of variance of pollen viability of following morning of different types of kakrol flower are shown in **table 59.1.** From the analysis of variance within a ploidy level the pollen viability of following morning showed significant difference at 1% level. Within a ploidy level the LSD values were 1.98 and 2.82% at 5% and 1% level, respectively. Between sex of respective types of kakrol were to be found significant at 1% level. Interaction between ploidy and sex were to be found significant difference at 5% level and LSD value was 3.54%.

Vascular bundle area: The vascular bundle area of different types of kakrol have been shown in **table 60 (Plate IV).** The vascular bundle area was the highest in tetraploid male plant and it was 0.10851mm^2. The lowest vascular bundle area was 0.0517mm^2 in diploid female. The mean vascular bundle area was 0.0521, 0.0723, 0.0711 and 0.10722mm^2 respectively in diploid male, triploid male and triploid female and tetraploid female.

Result of analysis of variance are shown in **table 60.1.** The item ploidy was highly significant at 1% indicated differences existing among the type included in the study. In this analysis, the item sex was not significant. It reflected that no differences exist relation in different in respect of vascular bundle area. Item Interaction between ploidy and sex was significant at 5% level. It show that one past of the experiment differ from the other past.

Table 59. Measurement of mean pollen viability of the following morning of different types of kakrol.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	55.86	3.46	93.32
Female ♂ (♀)	63.92	5.58	95.04
	LSD Values		
	5%	1%	
Ploidy	1.98	2.82	
Sex	2.05	2.92	
Ploidy x Sex	3.54	5.04	

Table 59.1. Analysis of variance of pollen viability following morning means data in table 59.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	24563.05	12281.53	3237.092***	4.10	7.56
Sex	1	70.81	70.81	18.6637***	4.96	10.04
Ploidy x Sex	2	37.94		5.0**	4.10	7.56
Error	10		3.794			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Table 60. Measurement of mean vascular bundle area (mm^2) of different types of kakrol.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	0.0521	0.0723	0.10851
Female (♀)	0.0517	0.0711	0.10722
	LSD Values		
	5%	1%	
Ploidy	0.004249	0.0060436	
Sex	0.003469	0.0049347	
Ploidy x Sex	0.006009	0.008574	

Table 60.1. Analysis of variance of vascular bundle area means data in table 0.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	0.0096243	0.008412	440.99***	4.10	7.56
Sex	1	0.000118	0.0000118	1.0813^{NS}	4.96	10.04
Ploidy x Sex	2	0.00010912	0.004556	5.0**	4.10	7.56
Error	10		0.00010912			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Vascular bundle breadth: Mean vascular bundle breadth of different genotypes of kakrol studied are shown in **table 61.** The lowest vascular bundle breadth was recorded in female plant of diploid kakrol which was 0.1892mm. The vascular bundle breadth of tetraploid male was the highest among all the types studied and it was 0.3041mm. The mean vascular bundle breadth of diploid ♂, triploid ♂, triploid ♀ and tetraploid female were 0.1972, 0.2514, 0.2522 and 0.3003mm, respectively (**Plate IV.**).

Analysis of variance of vascular bundle breadth of different types of kakrol are shown in **table 61.1.** From the analysis of variance within a ploidy level the vascular bundle breadth showed significant difference at 1% level. Within a ploidy level the LSD values were 0.01571mm and 0.02234mm at 5% and 1% level respectively. Between sex of respective types of kakrol were to be found not significant. Interaction Between ploidy and sex were to be found significant difference at 5% level and LSD value was 0.02222mm. It show that one past of the experiment differ from the other past.

Protoxylem diameter: Mean protoxylem diameter of different genotypes of kakrol studied are shown in **table 62.** The protoxylem diameter was recorded in female plant of diploid kakrol which was 0.0123mm. The protoxylem diameter of tetraploid female was the highest among all the types studied and it was 0.0154mm. The mean protoxylem diameter of diploid ♂. triploid ♂, triploid ♀ and tetraploid male were 0.0312, 0.017, 0.0171 and 0.0153mm respectively.

Analysis of variance of protoxylem diameter of different types of kakrol are shown in **table 62.1.** From the analysis of variance within a ploidy level the protoxylem diameter showed significant difference at 5% level. Between sex of respective types of kakrol were to be found not significant. Interaction between ploidy and sex were to be found significant difference at 5% level and LSD value was 0.000002577mm.

Table 61. Measurement of mean breadth of vascular bundle (mm) of different types of kakrol.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	0.1972	0.2514	0.3041
Female (♀)	0.1892	0.2522	0.3003

	LSD Values	
	5%	1%
Ploidy	0.01571	0.02234
Sex	0.012829	0.018247
Ploidy x Sex	0.02222	0.031606

Table 61.1. Analysis of variance of vascular bundle breadth means data in table 61.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^h	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	0.035892	0.01794	120.2734***	4.10	7.56
Sex	1	0.0004903	0.0004903	3.28599^{NS}	4.96	10.04
Ploidy x Sex	2	0.0014921	0.000746	5.05**	4.10	7.56
Error	10		0.00014921			
Total	17					

^{ns}= not significant, ^{h}**= Significant at 5% level and ^{h}***= Significant at 1% level.

Table 62. Measurement of mean protoxylem diameter for interned no. 4 of different types of kakrol in mm.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	0.0312	0.017	0.0153
Female (♀)	0.0128	0.0171	0.0154

	LSD Values	
	5%	1%
Ploidy	0.000001288	0.000001832
Sex	0.000000859	0.000001221
Ploidy x Sex	0.000002577	0.000003665

Table 62.1. Analysis of variance of protoxylem diameter for interned no. 4 means data in table 62.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^h	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	0.0000371	0.0000185	10.662***	4.10	7.56
Sex	1	0.00000012	0.00000012	0.06916^{NS}	4.96	10.04
Ploidy x Sex	2	0.00001735	0.000008675	5.0**	4.10	7.56
Error	10		0.000001735			
Total	17					

^{ns}= not significant, ^{h}**= Significant at 5% level and ^{h}***= Significant at 1% level.

Metaxylem diameter: The metaxylem diameter of different types of kakrol have been shown in **table 63.** The metaxylem diameter was the highest in tetraploid male plant and it was 0.0581mm. The lowest metaxylem diameter was 0.0451mm in diploid female. The mean metaxylem diameter was 0.0455, 0.0523, 0.0515 and 0.0575mm, respectively in diploid male, triploid male and triploid female and tetraploid female.

Result of analysis of variance are shown in **table 63.1.** The item ploidy was highly significant at 1% level indicated significant differences existing among the type included in the study. In this analysis, the item sex was highly significant. It reflected the differences exist relation in different in respect of metaxylem diameter. Item Interaction between ploidy and sex was significant at 5% level.

Phloem length: The phloem length of different types of kakrol have been shown in **table 64.** The phloem length was the highest in tetraploid male plant and it was 0.20032mm. The lowest phloem length was 0.1574mm in diploid female. The mean phloem length was 0.1592, 0.1825, 0.1808 and 0.1982mm respectively in diploid male, triploid male and triploid female and tetraploid female.

Result of analysis of variance are shown in **table 64.1.** The item ploidy was not significant indicated no differences existing among the type included in the study. In this analysis, the item sex was not significant. It reflected that no differences exist relation in different in respect of phloem length. Item Interaction between ploidy and sex was significant at 5% level. It show that one past of the experiment differ from the other past.

Table 63. Measurement of mean metaxylem diameter (mm) for interned no. 4 of different type of kakrol.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	0.0455	0.0523	0.0581
Female (♀)	0.0451	0.0515	0.0575

	LSD Values	
	5%	1%
Ploidy	0.001716	0.002441
Sex	0.001401	0.00199
Ploidy x Sex	0.002427	0.003452

Table 63.1. Analysis of variance of metaxylem diameter for interned no. 4 means data in table 63.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	0.0002805	0.0001402	78.792***	4.10	7.56
Sex	1	0.0000269	0.0000269	15.134***	4.96	10.04
Ploidy x Sex	2	0.0000178	0.0000089	5.0**	4.10	7.56
Error	10		0.00000178			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Table 64. Measurement of mean phloem length (mm) of different types of kakrol.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	0.1592	0.1825	0.20032
Female (♀)	0.1574	0.1808	0.1982

	LSD Values	
	5%	1%
Ploidy	0.02288	0.03255
Sex	0.018687	0.02658016
Ploidy x Sex	0.032367	0.046038

Table 64.1. Analysis of variance of phloem length means data in table 64.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	0.002171	0.001085	3.4288^{NS}	4.10	7.56
Sex	1	0.0000302	0.000302	0.0953^{NS}	4.96	10.04
Ploidy x Sex	2	0.0031658	0.0015829	5.0**	4.10	7.56
Error	10		0.00031658			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Phloem breadth: Mean phloem breadth of different genotypes of kakrol studied are shown in **table 65.** The lowest phloem breadth was recorded in female plant of diploid kakrol which was 0.0711mm. The phloem breadth of tetraploid male was the highest among all the types studied and it was 0.1135mm. The mean phloem breadth of diploid ♂, triploid ♂, triploid ♀ and tetraploid female were 0.0836, 0.0802, 0.0811 and 0.1081mm, respectively.

Analysis of variance of phloem breadth of different types of kakrol are shown in **table 65.1.** From the analysis of variance within a ploidy level the phloem breadth showed significant difference at 1% level. Within a ploidy level the LSD values were 0.01051mm and 0.0149mm at 5% and 1% level, respectively. Between sex of respective types of kakrol were to be found not significant. Interaction between ploidy and sex were to be found significant difference at 5% level and LSD value was 0.01486mm. It show that one past of the experiment differ from the other past.

Guard cell length: Mean guard cell length of different genotypes of kakrol studied are shown in **table 66.** The lowest guard cell length was recorded in male plant of diploid kakrol which was 20.05µm. The guard cell length of tetraploid female was the highest among all the types studied and it was 35.8967µm. The mean guard cell length of diploid ♀, triploid ♂, triploid ♀ and tetraploid male were 21.466, 30.993, 31.5667 and 35.4567µm, respectively (**Plate V**).

Analysis of variance of guard cell length of different types of kakrol are shown in **table 66.1.** From the analysis of variance within a ploidy level the guard cell length showed significant difference at 1% level. Within a ploidy level the LSD values were 0.373µm and 0.530µm at 5% and 1% level, respectively. Between sex of respective types of kakrol were to be found highly significant. Interaction between ploidy and sex were to be found significant difference at 5% level and LSD value was 0.5278µm.

Table 65. Measurement of mean phloem breadth (mm) of different types of kakrol.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	0.0836	0.0802	0.1135
Female (♀)	0.0711	0.0811	0.1081

	LSD Values	
	5%	1%
Ploidy	0.01051	0.0149
Sex	0.008584	0.01221
Ploidy x Sex	0.01486	0.02114

Table 65.1. Analysis of variance of phloem breadth means data in table 65.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	0.003623	0.0018116	27.116***	4.10	7.56
Sex	1	0.0001392	0.000334	2.083^{NS}	4.96	10.04
Ploidy x Sex	2	0.0006681	0.000334	5.0**	4.10	7.56
Error	10		0.00006681			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Table 66. Measurement of mean guard cell length (μm) of different types of kakrol.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	20.05	30.993	35.4567
Female (♀)	21.466	31.5667	35.8967

	LSD Values	
	5%	1%
Ploidy	0.373	0.530
Sex	0.3047	0.433
Ploidy x Sex	0.5278	0.75

Table 66.1. Analysis of variance of guard cell length means data in table 66.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	705.186	352.593	4187.5635***	4.10	7.56
Sex	1	2.952	2.952	35.0594***	4.96	10.04
Ploidy x Sex	2	0.842	0.421	5.0**	4.10	7.56
Error	10		0.0842			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

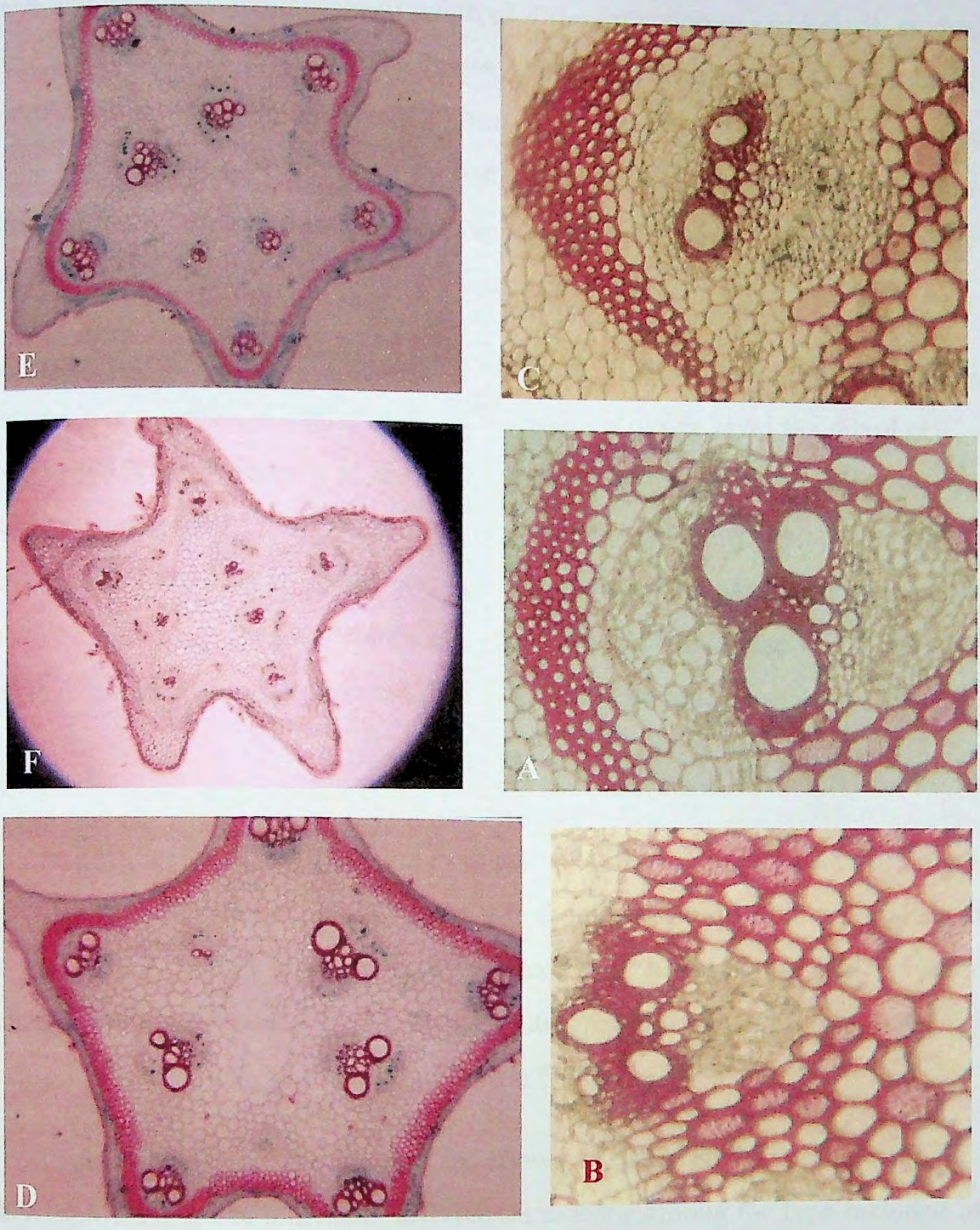


Plate IV

Fig. T. S. of different types of kakrol shoots: **A.** Vascular bundle of tetraploid kakrol (15 x 10), **B.** Vascular bundle of triploid kakrol (15 x 10), **C.** Vascular bundle of diploid kakrol (15 x 10), **D**. T. S. of tetraploid kakrol shoots (internode no. 4; 5 x 10), **E.** T. S. of triploid kakrol shoots (internode no. 4; 5 x 10), **F.** T. S. of diploid kakrol shoots (internode no. 4; 5 x 10).

Guard cell width: Mean guard cell width of different genotypes of kakrol studied are shown in **table 67 & plate V.** The lowest guard cell width was recorded in female plant of diploid kakrol which was 7.103µm. The guard cell width of tetraploid male was the highest among all the types studied and it was 12.923µm. The mean guard cell width of diploid ♂, triploid ♂, triploid ♀ and tetraploid ♀ were 7.56, 9.766, 10.20 and 12.922µm, respectively.

Analysis of variance of guard cell width of different types of kakrol are shown in **table 67.1.** From the analysis of variance within a ploidy level the guard cell width showed significant difference at 1% level. Within a ploidy level the LSD values were 0.3135µm and 0.4459µm at 5% and 1% level respectively. Between sex of respective types of kakrol were to be found not significant. Interaction between ploidy and sex were to be found significant difference at 5% level and LSD value was 0.08825µm. It show that one past of the experiment differ from the other past.

Stomata number: The mean stomata number of different types of kakrol have been shown in **table 68.** The stomata number was the highest in triploid male plant and it was 17.67. The lowest stomata number was 13.33 in tetraploid male. The mean stomata number was 14.33, 15.33, 17 and 13.67, respectively in diploid male, diploid female and triploid male and tetraploid female (**Plate V**).

Result of analysis of variance are shown in **table 68.1.** The item ploidy was highly significant at 1% indicated differences existing among the type included in the study. In this analysis, the item sex was not significant. It reflected that no differences exist relation in different in respect of stomata number. Item Interaction between ploidy and sex was significant at 5% level.

Table 67. Measurement of mean guard cell width (µm) of different types of kakrol.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	7.56	9.766	12.923
Female (♀)	7.103	10.20	12.922

	LSD Values	
	5%	1%
Ploidy	0.3135	0.4459
Sex	0.1149	0.3641
Ploidy x Sex	0.08825	0.1255

Table 67.1. Analysis of variance of guard cell width means data in table 67.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	93.8833	46.941	789.991***	4.10	7.56
Sex	1	0.000277	0.000277	0.0047^{NS}	4.96	10.04
Ploidy x Sex	2	0.5942	0.2971	5.0**	4.10	7.56
Error	10		0.05942			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Table 68. Measurement of mean stomata number of different types of kakrol.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Male (♂)	14.33	17.67	13.33
Female (♀)	15.33	17	13.67

	LSD Values	
	5%	1%
Ploidy	0.62135	0.883789
Sex	0.50733	0.721611
Ploidy x Sex	0.878732	1.249867

Table 68.1. Analysis of variance of stomata number means data in table 68.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	45.444	22.7222	97.3731***	4.10	7.56
Sex	1	0.2222	0.2222	0.9523^{NS}	4.96	10.04
Ploidy x Sex	2	2.3333	1.1666	5.00**	4.10	7.56
Error	10		0.2333			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

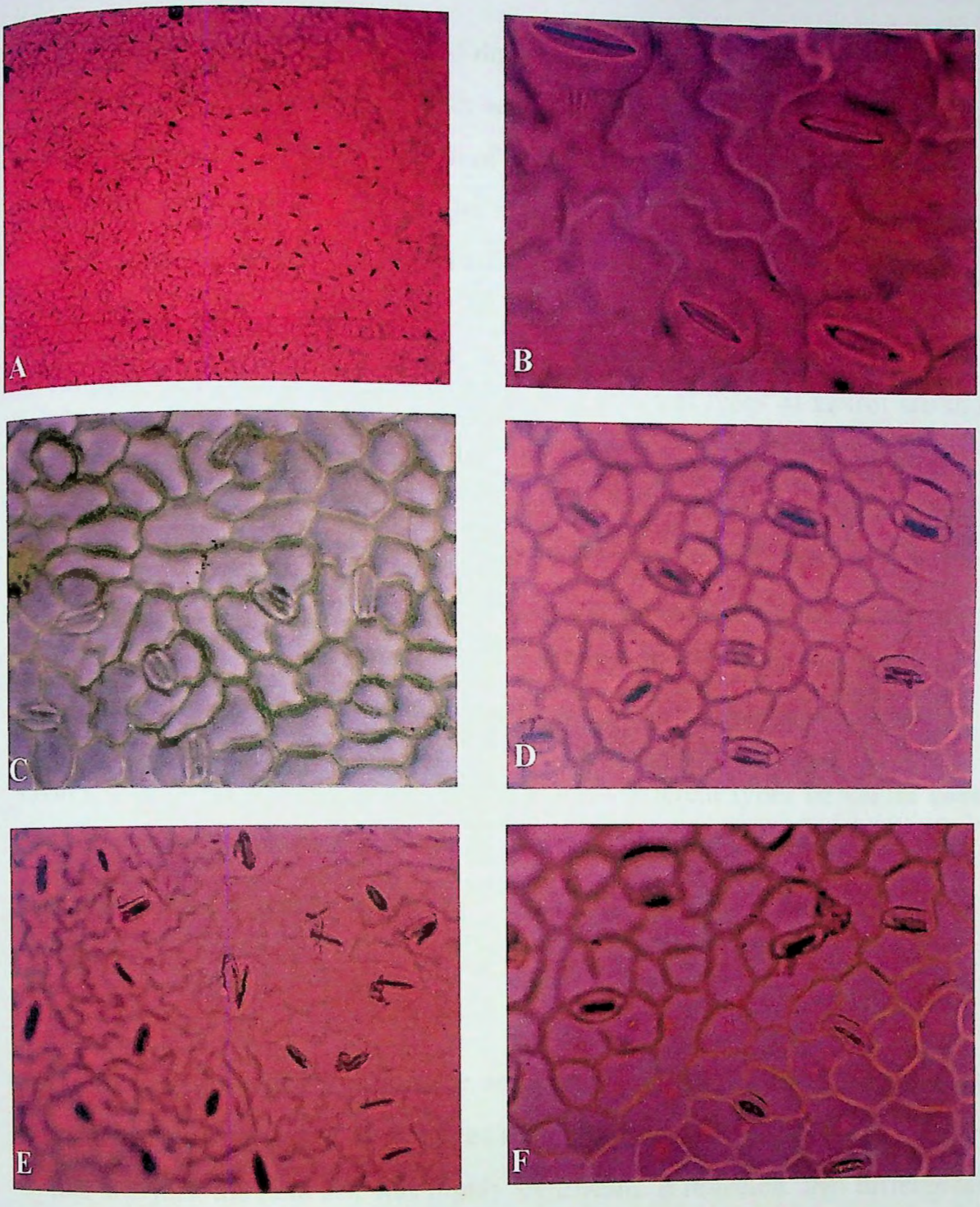


Plate V

Fig. Comparative shape and size of stomata: A. Stomata of diploid kakrol (18 x 10), B. Tetraploid (18 x 100), C. Diploid (18 x 40), D. Tetraploid (18 x 400, E. Triploid (18 x 40), F. Tetraploid (upper)

Ovary length: Mean ovary length of different genotypes of kakrol studied are shown in **table 69.** The lowest ovary length was recorded in female plant of diploid kakrol which was 1.04cm. The ovary length of tetraploid ♂(☿) was the highest among all the types studied and it was 2.41cm. The mean ovary length of diploid ♂(☿), triploid ♀, triploid ♂(☿) and tetraploid ♀ were 1.6733, 1.4066, 2.0433 and 1.8233cm, respectively (**Plate III**).

Analysis of variance of ovary length of different types of kakrol are shown in **table 69.1.** From the analysis of variance within a ploidy level the ovary length showed significant difference at 1% level. Within a ploidy level the LSD values were 0.2425cm and 0.34492cm at 5% and 1% level, respectively. Between sex of respective types of kakrol were to be found significant at 1% level. Interaction between ploidy and sex were to be found significant difference at 5% level and LSD value was 0.34294cm. It show that one past of the experiment differ from the other past.

Ovary diameter: The mean ovary diameter of different types of kakrol have been shown in **table 70.** The ovary diameter was the highest in tetraploid ♂(☿) plant and it was 1.276cm. The lowest ovary diameter was 0.62cm in diploid female. The mean ovary diameter was 1.12, 0.7, 0.74 and 0.883cm respectively in diploid ♂(☿), triploid ♀ and triploid ♂(☿) and tetraploid ♀.

Result of analysis of variance are shown in **table 68.1.** The item ploidy was significant at 1% indicated differences existing among the type included in the study. In this analysis, the item sex was highly significant. It reflected that differences exist relation in different in respect of ovary diameter. Item Interaction between ploidy and sex was significant at 5% level.

Table 69. Measurement of mean ovary length (cm) of different types of kakrol.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Female (♀)	1.04	1.406	1.823
Female ♂ (♀)	1.673	2.043	2.41

	LSD Values	
	5%	1%
Ploidy	0.2425	0.3449
Sex	0.198	0.2816
Ploidy x Sex	0.3429	0.4877

Table 67.1. Analysis of variance of ovary length means data in table 69.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	1.6073	0.80363	22.6119***	4.10	7.56
Sex	1	1.4966	1.4966	42.1105***	4.96	10.04
Ploidy x Sex	2	0.35541	0.1777	5.0**	4.10	7.56
Error	10		0.03554			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Table 70. Measurement of mean ovary diameter of different types of kakrol.

Sex	Ploidy		
	Diploid (2n)	Triploid (3n)	Tetraploid (4n)
Female (♀)	0.62	0.7	0.883
Female ♂ (♀)	1.12	0.74	1.278

	LSD Values	
	5%	1%
Ploidy	0.16966	0.24132
Sex	0.13852	0.19703
Ploidy x Sex	0.2399	0.3427

Table 70.1. Analysis of variance of ovary diameter means data in table 70.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F^b	Tabular F	
					5%	1%
Replication	2					
Ploidy	2	0.3925	0.1962	11.278***	4.10	7.56
Sex	1	0.4355	0.4355	25.0336***	4.96	10.04
Ploidy x Sex	2	0173966	0.86983	5.0**	4.10	7.56
Error	10		0.0173966			
Total	17					

^{ns}= not significant, ^{b}**= Significant at 5% level and ^{b}***= Significant at 1% level.

Biochemical characters:

Premature and mature leaves and fruits were collected from diploid, triploid and tetraploid plant for biochemical studies. Chlorophyll a, Chlorophyll b, total Chlorophyll, pH, moisture, dry-matter, ash, protein, lipid, starch, vitamin C and β-Carotene were analysed by standard biomhemical technique. The results are obtained in **table 71.**

Chlorophyll: The highest amount of Chlorophyll a, Chlorophyll b and total Chlorophyll were obtained in mature stage of tetraploid kakrol, which was 2.08%, 0.81 and 2.89%, respectively. The lowest Chlorophyll a, Chlorophyll b and total Chlorophyll were 1.35, 0.52 and 1.87%, respectively in premature stage of diploid kakrol leaf.

pH: The highest pH value 5.12 in mature stage of tetraploid kakrol was recorded. The lowest value of pH was 4.52 in premature stage of diploid kakrol.

Moisture: The maximum moisture was found in premature stage of tetraploid kakrol which was 87.20%. The lowest moisture was recorded in mature stage of diploid kakrol and it was 79.33%.

Dry-matter: The lowest 12.81% dry-matter was found when the fruit of tetraploid kakrol were premature. The highest percentage of dry-matter was 20.73 in mature stage of diploid kakrol fruit.

Ash: The highest ash content was recorded in mature stage of diploid of kakrol and it was 1.08%. The lowest was 0.86% in premature stage of tetraploid kakrol.

Protein: Protein percentage were 1.69 and 1.93, respectively in premature and mature stage of tetraploid kakrol. On the contrary, the amount of protein percentage was always higher in diploid kakrol and it was 1.80 and 2.14% respectively in premature and mature stage of fruit.

Lipid: The lipid percentage of kakrol fruits increased gradually with the advancement of maturity levels. The highest lipid percentage (0.91%) were recorded in mature stage of tetraploid kakrol and the lowest was 0.41% in premature stage of diploid kakrol fruit.

Starch: Within increase of fruit age there was decrease in amount of starch content. The highest starch percentage (8.32%) was recorded in premature stage of tetraploid fruit and it was lowest (5.18%) in premature stage of diploid kakrol.

Vitamin C: The premature stage of tetraploid fruits exhibited a higher (0.71%) content of vitamin C than other type of kakrol at both stages of fruit maturity. The lowest amount of vitamin C was recorded in mature stage of tetraploid fruit.

β-Carotene: The amount of β-Carotene became gradually higher when fruits age were increased. The highest percentage of β-Carotene was 0.73% in mature stage of tetraploid fruit.

Induction of bisexuality:

The results of the application of $AgNO_3$ on female twigs of three types of kakrol are presented in **table1 72 and plate VIII.** Two different effects of $AgNO_3$ treatment were observed, one was induction of large bract and another was induction of large calyx.

The highest numbers of unisexual flowers converted to bisexual flowers per twig were recorded in vines of diploid, triploid and tetraploid types where 300, 400 and 500ppm $AgNO_3$ were spread and it were 8.55, 7.58 and 4.75 per twig, respectively in diploid, triploid and tetraploid types of kakrol. The highest number of female flowers with large bract (13.44) were recorded in diploid kakrol when the female twigs were sprayed with 300ppm $AgNO_3$. In tetraploid kakrol it was 10.20 when the female twigs were treated with 500ppm $AgNO_3$.

Table 71. Biochemical characteristics of different types of kakrol at two stages of development.

Characters	Diploid		Triploid		Tetraploid	
	Premature	Mature	Premature	Mature	Premature	Mature
Chlorophyll a %	1.35	1.52	1.55	1.73	1.94	2.08
Chlorophyll b %	0.52	0.67	0.60	0.76	0.76	0.81
Total chlorophyll %	1.87	2.19	2.15	2.49	2.70	2.89
pH	4.52	4.76	-	-	4.85	5.12
Moisture %	83.44	79.33	-	-	87.20	82.25
Dry matter %	16.57	20.72	-	-	12.81	17.74
Ash %	0.91	1.08	-	-	0.86	1.05
Protein %	1.80	2.14	-	-	1.69	1.93
Lipid %	0.41	0.84	-	-	0.49	0.91
Starch %	7.97	5.18	-	-	8.32	6.00
Vitamin C %	0.64	0.51	-	-	0.71	0.49
β-Carotene %	0.45	0.66	-	-	0.50	0.73

Table 72. Effect of silver nitrate on the induction of bisexual flower of different types of kakrol.

$AgNO_3$ (ppm)	Diploid		Triploid		Tetraploid	
	Number of female flowers with large bract	Number of normal bisexual flower	Number of female flowers with large bract	Number of normal bisexual flower	Number of female flowers with large bract	Number of normal bisexual flower
300	13.47	8.55	12.43	5.08	9.59	2.73
400	10.68	7.32	10.84	7.58	8.46	3.98
500	9.12	4.58	8.85	6.05	10.28	4.75

In vitro pollen germination ability:

Four pollen cultural medium viz., Brewbaker and kwack medium, 50% Robert medium, sucrose 10% + boric acid 100mg/L medium and sucrose 10% + boric acid 100mg / l + $CaNO_3$ 300mg/l were used for pollen germination. Pollen grain from normal male and bisexual female flowers of diploid, triploid and tetraploid types were collected and mixed them separately in different pollen germination medium and percentage of pollen germination and corresponding pollen tube length were recorded at two different time (20 and 40 minutes) intervals. Result obtained are sown in **table 73 and plate VI.**

The highest percentage of pollen grain germination was 87.14 in tetraploid bisexual when it was treated with Brewbaker and kwack medium after 40 minutes treatment and 22.08% germinated pollen were recorded in tetraploid kakrol when treatment time was 20 minutes.

Where as the lowest percentage were 9.64 and 45.82 in diploid kakrol when sucrose 10% + boric acid 100mg/L medium were used for 20 minutes and 40 minutes respectively. In 50% medium 81.40%, 86.05%, 72.25% and 81.15% pollen were germinated respectively in tetraploid male, tetraploid bisexual, diploid male and diploid bisexual when treatment time was 40 minutes. Where as 15.87, 17.52, 15.70 and 17.03% pollen grains were germinated for 20 minutes. There was not a single pollen was germinated of any pollen germinating medium for any treatment time.

Results on the average pollen table length in different medium was shown in **table 73 and plate VI.**

The highest pollen tube length (2575μ) was recorded in tetraploid bisexual when treatment time was 40 minutes in 50% Robert medium. The lowest pollen tube length (1980μ) was observed in diploid male in sucrose 10% + boric acid 100mg/L medium after 40 minutes treatment.

Table 73. Percentage of *in vitro* pollen germination in different germination media and average pollen tube length of pollen grain of different types of kakrol.

Pollen sources	Germination medium	Time require for pollen germination and pollen tube growth			
		20 minute		40 minute	
		% of germination	Tube length μ	% of germination	Tube length μ
Tetraploid ♂	Brewbaker and kwack's medium	15.47	1675	85.45	2510
Tetraploid ♀		22.08	1750	87.14	2540
Triploid ♂		-	-	-	-
Triploid ♀		-	-	-	-
Diploid ♂		14.51	1460	73.24	2155
Diploid ♀		18.32	1550	83.43	2200
Tetraploid ♂	50% Robert medium	15.87	1630	81.40	2475
Tetraploid ♀		17.52	1655	86.05	2575
Triploid ♂		-	-	-	-
Triploid ♀		-	-	-	-
Diploid ♂		15.75	1550	72.25	2090
Diploid ♀		17.03	1410	81.15	2225
Tetraploid ♂	Sucrose 10% + boric acid 100mg/L	10.07	1310	52.45	2070
Tetraploid ♀		11.14	1475	54.73	2135
Triploid ♂		-	-	-	-
Triploid ♀		-	-	-	-
Diploid ♂		9.64	1140	45.82	1980
Diploid ♀		10.53	1275	50.09	2040
Tetraploid ♂	Sucrose 10% + boric acid 100mg / l + $CaNO_3$ 300mg/l	11.75	1380	60.12	2310
Tetraploid ♀		14.51	1600	62.19	2375
Triploid ♂		-	-	-	-
Triploid ♀		-	-	-	-
Diploid ♂		10.33	1220	51.22	2200
Diploid ♀		12.86	1285	52.43	2210

In vitro pollen germination with varied pH:

The pollen grain allowed to germinate in four different pollen germinating medium with four different pH viz., 6, 7, 8 and 9, data was presented in **table 74.** In Brewbaker and kwack's pollen germinating medium 85.75% and 72.92% pollen were germinated respectively in tetraploid and diploid for 40 minute treatment when the pH was just 8. In Brewbaker and kwack's pollen germinating medium when pH was 6, 7 and 9 there was no pollen germination at 40 minutes treatment. 81.32%, 52.82% and 60.43% pollen were germinated in tetraploid kakrol respectively in 50% Robert medium, Sucrose 10% +boric acid 100mg/L and Sucrose 10% + boric acid 100mg / l + $CaNO_3$ 300mg/l and, it was 72.08%, 46.05% and 51.85% in diploid type of kakrol. There was no germinating pollen to be found when pH was 6, 7 and 9.

Table 74. Percentage of pollen germination in different medium of varied pH.

Medium	Time require for pollen germination (minute)	pH	Pollen source	% of germination
Brewbaker and kwack's medium	40	6	Tetraploid ♂	-
	40		Diploid ♂	-
	40	7	Tetraploid ♂	-
	40		Diploid ♂	-
	40	8	Tetraploid ♂	85.75
	40		Diploid ♂	72.92
	40	9	Tetraploid ♂	-
	40		Diploid ♂	-
50% Robert medium	40	6	Tetraploid ♂	-
	40		Diploid ♂	-
	40	7	Tetraploid ♂	-
	40		Diploid ♂	-
	40	8	Tetraploid ♂	81.32
	40		Diploid ♂	72.08
	40	9	Tetraploid ♂	-
	40		Diploid ♂	-
Sucrose 10% + boric acid 100mg/L	40	6	Tetraploid ♂	-
	40		Diploid ♂	-
	40	7	Tetraploid ♂	-
	40		Diploid ♂	-
	40	8	Tetraploid ♂	52.48
	40		Diploid ♂	46.05
	40	9	Tetraploid ♂	-
	40		Diploid ♂	-
Sucrose 10% + boric acid 100mg / l + $CaNO_3$ 300mg/l	40	6	Tetraploid ♂	-
	40		Diploid ♂	-
	40	7	Tetraploid ♂	-
	40		Diploid ♂	-
	40	8	Tetraploid ♂	60.43
	40		Diploid ♂	51.85
	40	9	Tetraploid ♂	-
			Diploid ♂	-

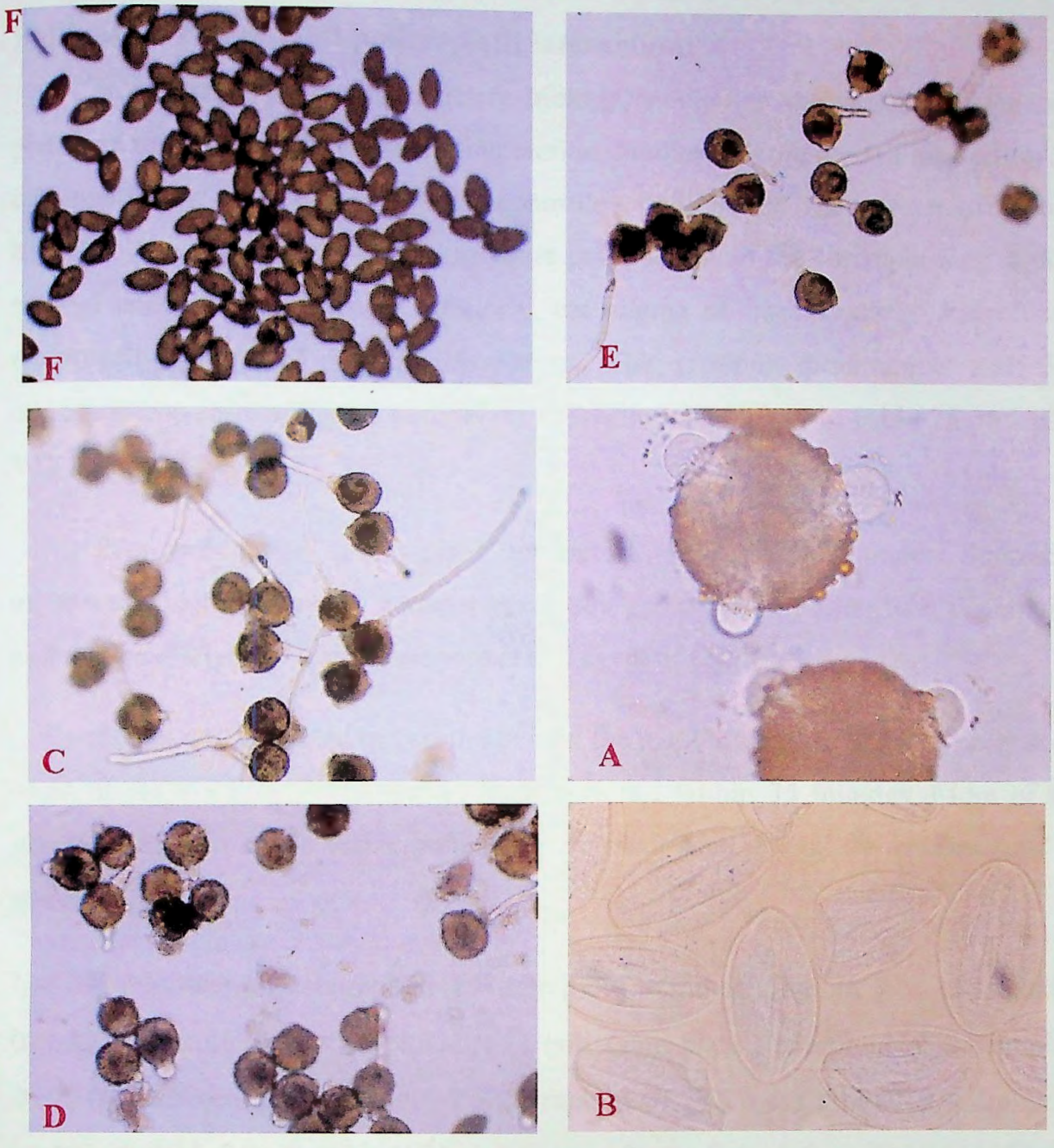


Plate VI

Fig. *In vitro* pollen grains germination of different types of kakrol flowers:
A. Tetraploid pollen grains, **B.** Triploid pollen grains, **C.** *In vitro* pollen (tetraploid) germination on Brewbaker and Kwack medium, **D.** Bursting of tetraploid pollen grains, **E.** Germinating diploid pollen grains on pH 8 Brewbaker and Kwack medium, **F.** Triploid pollen grains on Brewbaker and Kwack medium.

Pollination success and pollen-pistil interaction:

In investigation on reproductive biology, studies on the structural aspects of pistil and the details of pollen-pistil interaction. Studies on pollen-pistil interaction and incompatibility require carrying out controlled pollination. The stigma of diploid, triploid and tetraploid types of kakrol were pollinated with the corresponding diploid, triploid and tetraploid flower. Similarly, the stigma of three types of kakrol were reciprocally pollinated within themselves. The crossing programme and their corresponding results of post pollination interaction are shown in **table 75 and plate VII.**

Post pollination pollen-pistil interaction were studied under fluorescent microscope using following parameters: pollen germination, pollen tube penetration, pollen tube elongation, callose responses and event of fertilization.

Pollen grains started to germination on the pistil after 20 minutes of pollination. Most of the viable pollen found to be germinated within 35 minutes. More of less germination time of the viable pollens on the pistil same for all the crosses (self sib and cross).

Out of 39 crosses, only tetraploid ♀ × tetraploid ♂ (cross), diploid ♀ × tetraploid ♂ (cross), tetraploid ♀ × tetraploid ♂(⚥) (sib same plant), tetraploid ♀ × tetraploid ♂(⚥) (sib different plant), diploid ♀ × tetraploid ♂(⚥) (cross), diploid ♀ × diploid ♂ (cross), diploid ♀ × diploid ♂(⚥) (sib same plant), diploid ♀ × diploid ♂(⚥) (sib different plant) showed success of the crossing programme.

The percentage of fruit setting were 82 and 88 when respectively tetraploid ♀ × tetraploid ♂ (cont. cross) and diploid ♀ × tetraploid ♂ (cross) crosses were made.

Fluorescent microscope study reveals that most of the viable pollen grains (96%) were germinated on the stigma surface when tetraploid ♀ was crossed with

tetraploid ♂. In diploid ♀ × tetraploid ♂ crosses 93% pollen were germinated on the stigma of diploid kakrol. In these cross combinations pollen tubes continued to grow through stigmatic papillae, Most of the pollen tube were found to be advancing towards the ovary **(Plate VII).** The growth of pollen tube within the stylar tissue was monitored by the conspicuous callose wall of the developing pollen tubes **(Plate VII).**

The pollen of bisexual was as effective as that of the male flowers on the normal female flowers for sib and cross matting. The pollen of the same or other bisexual flowers, even when these flowers were crossed by the pollen of the normal male flowers. The success of crosses were found to be nil.

In tetraploid ♀ × tetraploid ♂(⚥) (sib some plant), tetraploid ♀ × tetraploid ♂ (⚥) (sib different plant) and diploid ♀ × tetraploid ♂(⚥) (cross) crosses the success of fruit setting were 72 , 88 and 86%, respectively.

Pollen germination on the stigma of the induced bisexual flower (tetraploid and diploid) was normal as like as normal respective female flower. More than 85% pollen were germinated on the stigma of diploid and tetraploid bisexual flower and 80% viable pollen began to develop pollen tube. At initial stage the growth of the pollen tube was normal **(Plate VII).** But later stage the growth of pollen tube into the style of bisexual flower was very slow. In the sib self and cross matting pollinated pistil of bisexual flower the growth of pollen tube stop in the upper part of the style and they do not reach the embryo sac to effect fertilization. Instead the normal growth, the pollen tube were started to deform and virtually develop tumor. In this case the pollen tube did not traverse even one third of the length of style.

In diploid ♀ × diploid ♂ (cross), diploid ♀ × diploid ♂(⚥)(sib same plant), diploid ♀ × diploid ♂(⚥)(sib different plant), crosses success of fruit setting was 72, 90 and 80%, respectively.

Between tetraploid ♀ and diploid ♂ crosses were made but all failed. The pollen tube of diploid kakrol do not reach in to the ovary. But the pollen grains were germinated on the tetraploid stigma as like as normal pollen.

Tetraploid × triploid, diploid × triploid, and triploid × triploid (with all possible) about1000 attempts of crossing failed to give any fruit. Triploid kakrol plant was found to be sterile. The germination percentage of triploid pollen was nil. The triploid stigma when it was pollinated with different pollen source viz., diploid, tiploid, and tetraploid, The pollen germination was observed on the stigma and was found that only a few (20-25%) pollen grains started germination 4h after pollination and growth of pollen tube was very slow. Normally the pollen tubes do not penetrate the stigma of triploid kakrol. Only 2-5% pollen tubes were penetrated the stigma cells. Both localized and diffused callose production was observed on the stigmatic papillae in these cross combination. The ovary of these crosses shrivelled and became yellow 4 to 7 days after pollination.

Fruit weight of the crosses tetraploid ♀ x tetraploid ♂ (cross), tetraploid ♀ x tetraploid ♂ (♀) (sib different plant) and tetraploid ♀ x tetraploid ♂ (♀) (same plant) were 75.24±4.2, 76.29±3.92 and 50.36±3.87, respectively. All appears that the fruit weight have reduced through sib matting of same plant. The fruit weight as obtained from diploid ♀ x diploid ♂ (♀), (same and different plant) were significantly greater than those obtained from diploid ♀ x diploid ♂ (cross). Different types of flowers and fruits of kakrol are shown in **plate VIII**.

Table 75. Results of crosses from different pollen source.

Pistillate flower	Pollen Source	Mode of pollination	Total no. of crosses	No. of. fruits set	Fruit weight (g)	Seed number/fruit
Tetraploid female		Cross (control)	50	41	75.24±4.12	24.28±1.33
Diploid female		Cross	50	44	22.55±2.80	15.22±1.51
Tetraploid female ♀	Tetraploid ♂	Cross	50	-	-	-
Diploid female ♀		Cross	50	-	-	-
Triploid female		Cross	50	-	-	-
Triploid ♀		Cross	50	-	-	-
Tetraplid female		Sib (same plant)	50	36	50.36±3.87	18.64±2.03
Tetraploid female		Sib (different plant)	50	44	76.29±3.92	23.94±1.05
Tetraploid female ♀		Sib (same flower)	50	-	-	-
Diploid Female	Tetraploid ♀	Cross	50	43	21.73±1.42	17.61±1.30
Diploid female ♀		Cross	50	-	-	-
Triploid female		Cross	50	-	-	-
Triploid ♀		Cross	50	-	-	-
Diploid female		Cross(control)	50	38	17.25±1.56	14.64±1.20
Tetraploid female		Cross	50	-	-	-
Diploid female ♀	Diploid ♂	Cross	50	-	-	-
Tetraploid female ♀		Cross	50	-	-	-
Triploid female		Cross	50	-	-	-
Triploid ♀		Cross	50	-	-	-
Diploid Female		Sib (same plant)	50	45	24.88±2.19	22.38±2.67
Diploid Female		Sib (different plant)	50	40	25.28±2.47	22.45±2.08
Diploid ♀		Sib (same flower)	50	-	-	-
Tetraploid female	Diploid ♀	Cross	50	-	-	-
Tetraploid ♀		Cross	50	-	-	-
Triploid female		Cross	50	-	-	-
Triploid ♀		Cross	50	-	-	-
Tetraploid female		Cross	50	-	-	-
Tetraploid ♀		Cross	50	-	-	-
Diploid female	Tetraploid ♂	Cross	50	-	-	-
Diploid ♀		Cross	50	-	-	-
Triploid female		Cross	50	-	-	-
Triploid ♀		Cross	50	-	-	-
Tetraploid female		Cross	50	-	-	-
Tetraploid ♀		Cross	50	-	-	-
Diploid female	Tetraploid ♀	Cross	50	-	-	-
Diploid female ♀		Cross	50	-	-	-
Triploid female		Sib (same plant)	50	-	-	-
Triploid female		Sib (different plant)	50	-	-	-
Triploid ♀		Sib (same flower)	50	-	-	-

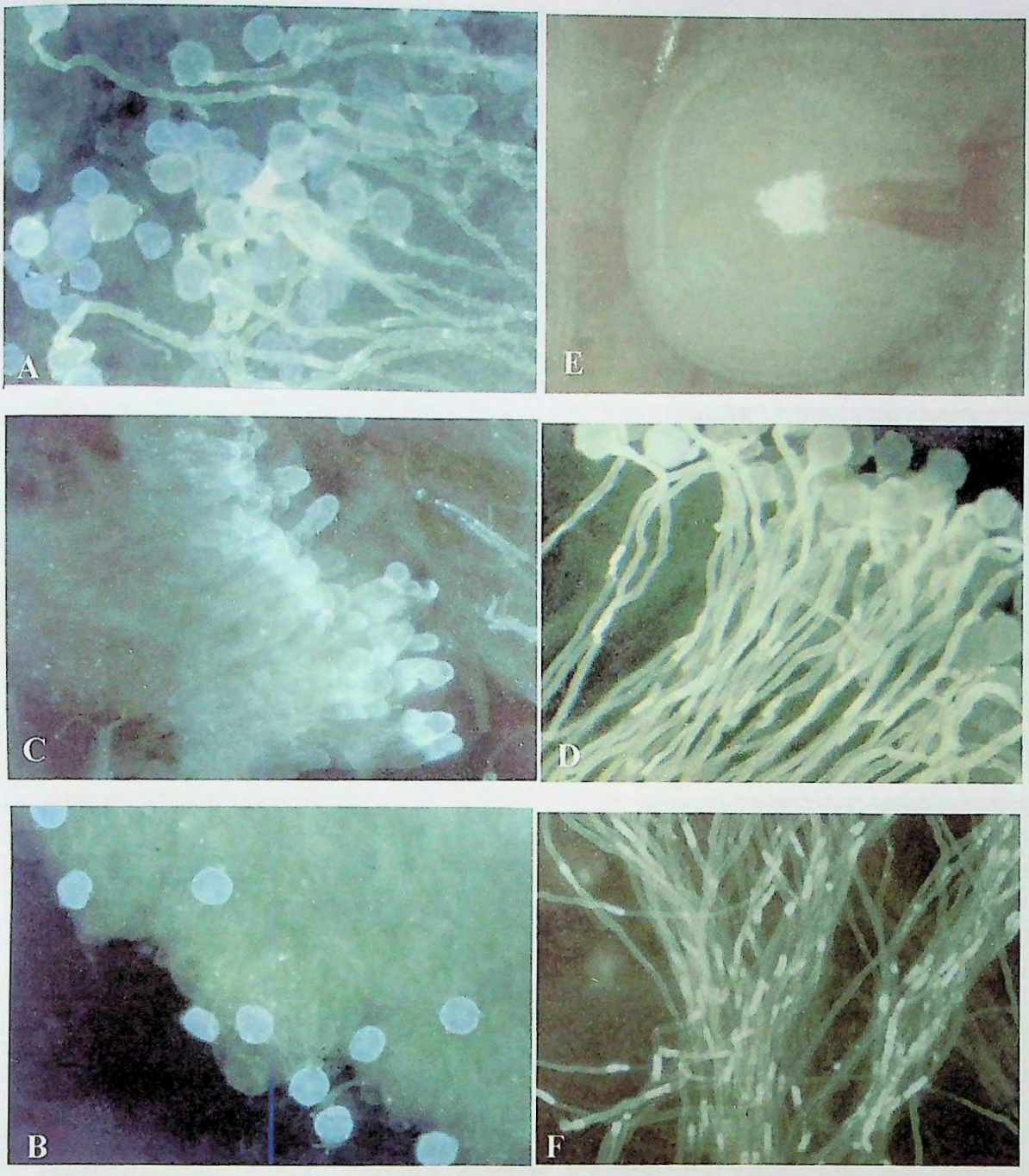


Plate VII

Fig. Fluorescent micrographs showing some of the post pollination responses: **A.** portion of the self-pollinated pistil of tetraploid (tetraploid x tetraploid) kakrol showing development of pollen tubes (4 x 10, after 24h pollination), **B.** Pollen grains of the postils of triploid (triploid X triploid) kakrol showing sent percent pollen grains failed to germinate on the stigmatic surface (4 x 10, after 24h pollination), **C.** Same as fig. b. in higher magnification (10 x 10), but formation of bright callose on entire stigmatic papillae (arrows), **D.** A portion of the pistil of diploid (diploid x diploid) kakrol showing development of pollen tubes (10 x 10, after 10h pollination), **E.** Unfertilized ovule of tetraploid bisexual flower. **F.** Callose plague in stigma.

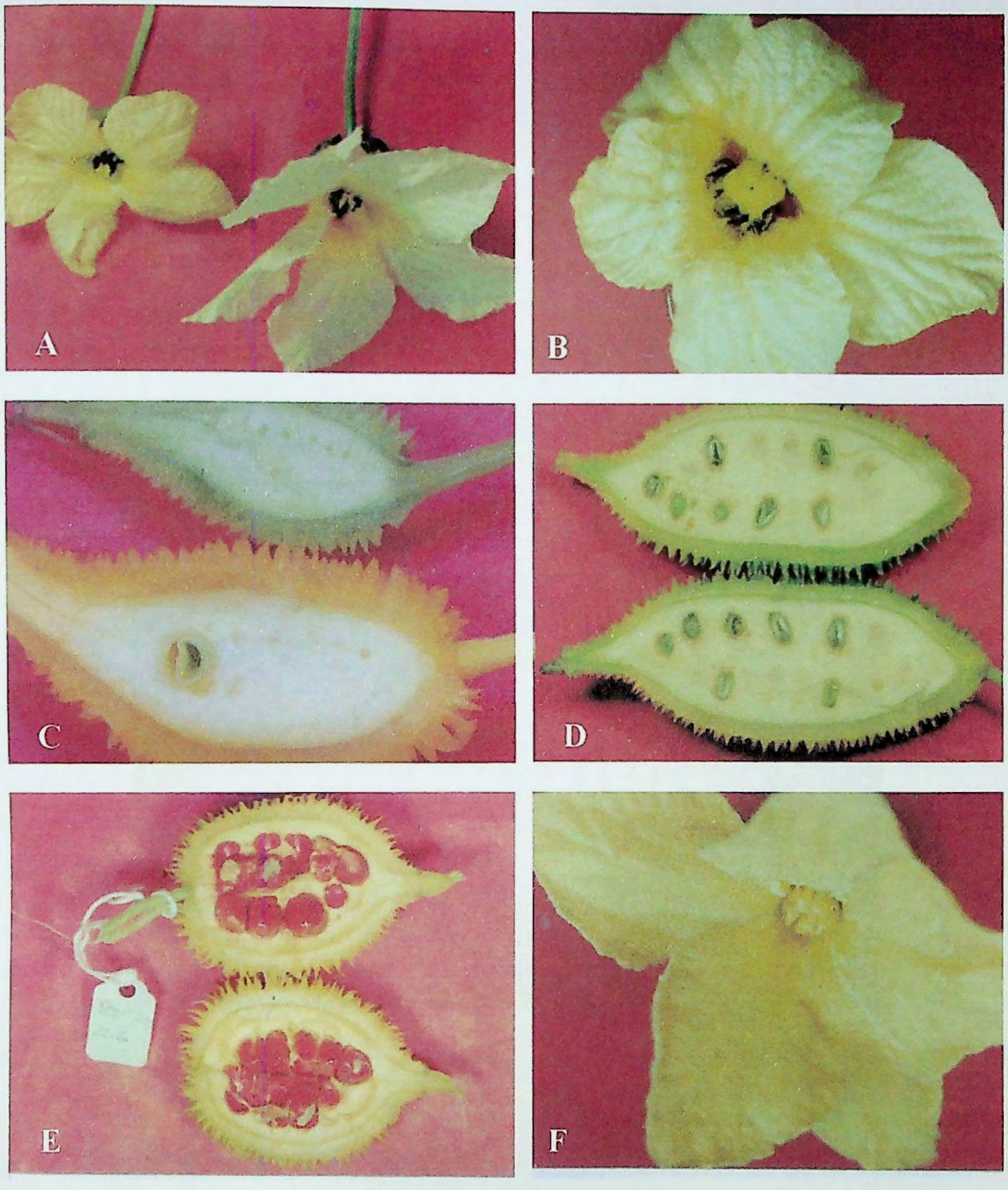
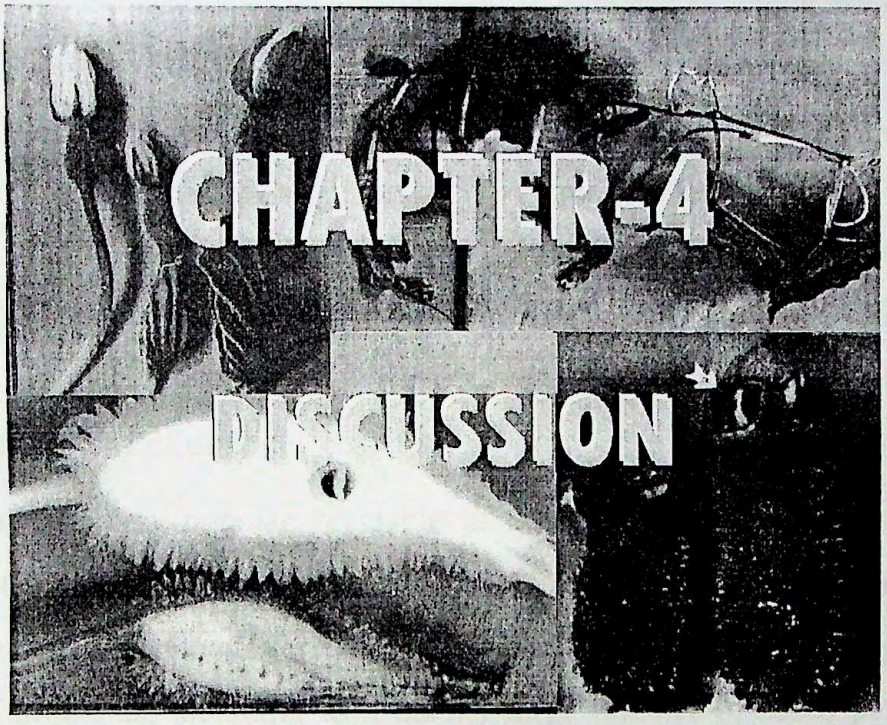


Plate VIII

Fig. Different types of fruits andflowers: **A.** Triploid male flowers, **B.** Tetraoloid bisexual flowers, **C.** Triploid fruits, **D.** Triploid fruits, **E.** Dploid fruits, **F.** Diploid fruits.


CHAPTER-4
DISCUSSION

DISCUSSION

The species *Momordica dioica* of the family Cucurbitaceae is distributed all over the tropics and sub-tropics but the largest concentration of species is in tropical Africa, (Shesadri, 1986; Agarwal and Roy, 1974). The species is known as kakrol. Kakrol is shrub, dioecious and an annual vegetable crops. Seed grown population is highly heterozygous because of unavoidable cross pollination. There are two cultivar types of kakrol found in this Sub-continent, one is diploid and other is tetraploid, having chromosome number 2n=28 and 4n=56, respectively. The diploid one is small in size and the tetraploid one is large in size (Siddique and Rahman, 1987). There is a sterile type of triploid kakrol (3n=42), which has characters intermediate between these two cultivars. Most of the farmers in Bangladesh cultivate tetraploid kakrol due to its genetic resources. Diploid is efficient for researcher. In this investigation diploid, tetraploid and progeny of diploid × tetraploid kakrol were used as genetic material.

Kakrol is a very popular vegetable in our country. Its production has been increased in the recent years as compared to those of 1970's due to the introduction of a hand pollination techniques suggested by Hossain *et al.* (1987). As a crop kakrol has a number of problems, including fruit quality large number of seeds per fruit, hard seed and dioecious nature. Any varietal improvement in kakrol is handicapped by its dioecious nature. Harvesting of the fruit before the seeds become hard is not practiced due to a significant yield loss which probably can not be compensated by the price. Therefore, production of seedless or less seeded fruit with palatability is necessary.

Effect of colchicine has been studied in different plant materials and mode of action has been interpreted variously. Radiosensitivity is now known to depend on both nuclear factors like, interphase chromosome volume (ICV), chromosome number, chromosome size, nucleolus and heterochromatin, centromere number and position, degree of polyploidy and nuclear DNA content (Dutta, 1978; Sparrow and Miksche,

1960; Sparrow *et al.* 1967; Nirula, 1963; Sparrow & Sparrow, 1965; Bostrack and Sparrow, 1970). Here we considered the ploidy level for an experiment and tried to produce seedless, less seed and parthenocarpic fruit in kakrol.

Induction of polyploidy using colchicine is well established in water- melon (Kihara, 1951); in *Trichosanthesis dioica* Roxb. (Islam, 1974); in *Glycine max* and *Pisum sativum* (Tang and Loo, 1941); in *Trifolium alexandrinum* (Islam, 1973); in ryegrass (Ahloowalia, 1967); in sorghum (Franzke and Ross, 1952) and in *Ricinus communis* (Khan, 1960). Induction of polyploidy in cucurbitaceae family has been reported (Kihara, 1950; Islam, 1974). Colchicine has also been reported to induce mutations in several crop plants (Datta, 1976; Scatt and Patterson, 1965; Datta and Gupta, 1984). So far report goes no such research study has been undertaken in kakrol. In the present study polyploidy was induced in diploid, triploid and tetraploid kakrol.

Effect of different strengths of colchicine on seeds would be identified by germination time, percentage of germination and seedling behavior, with increased colchicine concentrations there was decrease in germination of each type of kakrol. The germination percentage was always lower in the petri dish than the polyethene bag and seed bed experiment. In petri dish, germination percentages of unpeeled seed were very low (2 to 20%) when it was treated with lower strength of colchicine. In higher concentration it was zero.

The reduction of germination was very high in decoated seed whereas unpeeled seeds with same concentration reduced only 10 to 25% germination. Datta (1978) reported that in *Luffa acutangula,* reduction of germination was maximum of 60%, when it was treated with 1% colchicine solution, but in *Lagenaria siceraria* only 40% germination. Here, the thickness of seed coat influenced the germination rate of seed. The thickness of seed coat of *Luffa acutangula* was significantly less than that of *Lagenaria siceraria.* The germination percentage of kakrol in seed bed experiment was similar to those of polyethene bag experiment. In higher strength of colchicine, the rate of germination in decoated seed was lower than that of the unpeeled seed. On

average, germination rate was always high in unpeeled seed in both seed bed and polyethene bag experiments.

Significant delay of germination time was also observed after treatment with all the concentrations of colchicine in both materials (unpeeled and decoated seed). Thicker wall may always be less colchicine penetration than thinner walls. Similar results were also found in *Lagenaria siceraria* and in *Luffa acutangula* as reported by Datta (1978). Of the two types of seeds of the different genotypes of kakrol tested, decoated seeds were found to be more effective than the coated seeds. Better response was observed in 0.5% to 1% colchicine solution for below 18h treatment.

The affected seeds initiated two to five shoots from the cotyledon base and scale like leaves were also found on the shoot. Normally, one seed initiated one shoot and no leaf was found on hypocotyle. There was no significant difference within the sprouted shoots of the treated seeds with normal seedling of respective types of kakrol. In dicot plant such as *Citrus* sp. and *Mangifera* sp. multiple shoots were developed from cotyledon base as reported by Bose (1986).

Lower concentration of colchicine had little effect on 2, 4 and 6 days old seedlings. However, higher strength of colchicine affected the seedling. Two types of effect was to be observed; one is typical form of shoot tip and the other is mortality rate of the seedlings.

In the dipping method, the rate of affected seedlings and mortality was significantly higher than that of the dropping method. Most of the seedlings did not survive for long duration of treatment time in the dipping method.

Effect of colchicine on seedlings was observed in the dipping method for all the treatment time. However, use of higher strength of colchicine for longer time showed significant effect (20-50%) on seedlings. Among the six concentrations of colchicine, the highest effect (50%) was observed in 0.7% and 1% colchicine solutions.

The abnormal seedlings, surviving the colchicine treatment, had thicker leaves, dark green leaves, tumor like shoot tip and shoot tip was completely checked in growth for 30 to 40 days. Then these seedlings were initiated 1 to 6 branches or failed to survive. The morphological characters of initiating branch from swelling tip were as normal as the respective types of seedlings. There was no significant difference in between initiating branches from the swelling tips and normal tips from respective genotypes. So, the induced polyploid plants were not obtained from the treated seeds and seedling. Here mentionable, all the cells of seeds (embryo) and seedling (tip) were not induced to ploidy level by colchicine treatment, later on the unaffected cells of embryo and shoot tip initiated (sprouted) normal shoots.

In ryegrass, a large number of selected artificial tetraploid plants turned to be diploid and mixoploids when examined cytologically. Some of the plants which were analysed from the microsporocytes showed diploid and tetraploid spikes on the same plant (Ahloowalia, 1966).

In the present study, it was proved that the presence of higher concentration and long treatment time of colchicine affected the seeds or seedlings. But the present investigation of artificial induced polyploidy was not succeded, due to the peculiar growth characters of the seedlings.

To determine the ploidy differences, morphological and anatomical characters were investigated. The morphological characters of the three genotypes of kakrol (diploid, triploid, tetraploid and their respective male and female or bisexual types), such as internode length, internode breadth, tendril length, petiole length, petiole breadth, leaf blade length, leaf blade breadth, flower diameter, bract length, bract breadth, calyx length, calyx breadth, petal length, petal breadth, stamen length, pollen viability, pollen viability in the following morning, pollen diameter, ovary length and ovary diameter were collected. Analysis of variance showed significant variations among the ploidy levels for all the characters except internode breadth. This gives an

idea of morphological difference of these three types of kakrol (ploidy level) and all the morphological characters exhibited a wide and pronounced rang of variation in different ploidy levels, which shows that the characters included in the analysis are quantative in nature. The quantative nature of the characters studied was reported in mustard by Chandhori and Proshed (1968) and Joarder and Eunus (1969). In the present study, the sex items were significant except petiole length, leaf blade breadth, petiole breadth, internode length, petal breadth, petal length and internode breadth. Here, most of the cases the reproductive organs were highly significant for the item sex. The item between ploidy and sex was also significant at 5% level.

The size of the morphological organs of kakrol depends on the degree of ploidy. In general, all the morphological organs were bigger in size in tetraploids than in the triploids and diploids. The triploid plants were gigantic in nature and the various morphological features were intermediate between the diploid and naturally occurring tetraploid kakrol plants. Increasing ploidy level enhanced the viguor of size of the organs have been reported in several plants (Islam, 1973, 1974; Zeilinga and Schouten, 1968).

Nine different anatomical characters were studied. The tetraploid kakrol has well developed vascular tissues with five vascular bundles in the ridge and five vascular stands in the central region of the stem. Thus the tetraploid genotype has one stand on each ridge and five central stands thus ten bundles altogether. Diploid and triploid genotypes have eight to nine strands, five on the ridges and three in the center.

The vascular bundles are very weakly developed in the diploid and triploid ploidy level. The progenies of hybrids (F_1) show maternal types of stem anatomical features in the triploid genotypes.

A large metaxylem diameter is a diagnostic features of the different genotypes of kakrol. Large diameter of metaxylem and large phloem area are the indicator of

large scale water transport and large scale food transport (Hanif and Langer, 1972). Bhandry and Mukhopadhyay (1997) reported that the large diameter of metaxylem was transported much water than smaller diameter of metaxylem. The growth of tetraploid showed the highest performance specially in the metaxylem diameter, phloem length, phloem breadth and vascular bundle area. The vascular bundles of male species are somewhat less wide and contains comparatively smaller area of vascular bundle. It indicates that the large scale fruiting in the female species of tetraploid genotypes. Metaxylem diameter increased gradually from diploid to tetraploid genotypes. Phloem length and breadth also increased when the genotypes increased from diploid to tetraploid.

Among the genotypes, the highest vascular bundle area was to be obtained in the tetraploid female plants and the lowest vascular bundle area was to be found in diploid male plants.

The guard cell length and breadth was to be found highly significant among the ploidy levels. Guard cell length was the highest in tetraploid female and lowest in diploid male plants. But the stomata number per unit area was highest in the triploid male plant and lowest in tetraploid male genotypes of kakrol. As is usually the case, the dimention of guard cells was larger in the tetraploid with a consequent reduction of the frequency of stomata per unit area. Similar results were scored by Agarwal and Roy (1974) and such a reduction of the frequency of stomata have been noted in tetraploid *Trichosanthes dioica* Roxb. (Islam *et al.* 1974).

To compare nutritional values of the three genotypes of kakrol at two maturity stages characters such as chlorophyll a, b, and total chlorophyll, pH, moisture, drymatter, ash, protein, lipid starch, vitamin C and β- carotene were analysed. In all the genotypes, the values of all the measured variables except moisture, starch and

vitamin C increased with increasing fruit age. The contents (%) of dry matter, ash and protein were highest in the mature stage of diploid kakrol.

Chlorophyll a, b, and total chlorophyll, pH, lipid and β-carotene were highest in the mature stage of fruit development of tetraploid genotypes. Moisture was highest in premature stage of diploid kakrol genotypes. Similar results as reported by Mishra and Sahu (1983) and Bose *et al.* (1983). Fakir *et al* (1982) found high ash content in kakrol Narikeli at the green stage. Goni *et al.* (2000) reported that the low ash content was recorded in KK003 in green stage. Awasthi *et al.* (1988) reported that starch, vitamin C and β- carotene were higher in some genotypes and also found greater variation among the traits. From the biochemical investigation, diploid genotypes might be considered to be superior as it contained higher amount of protein, ash and dry matter. But chlorophyll a, b, and total chlorophyll were highest in tetraploid kakrol.

The application of $AgNO_3$ to female plants produced bisexual flowers. Each of the stamen developed from the base of the style and anther reached just beneath the stigma. From the base of style, three rudimentary structures appeared which developed into androecium. Among the different concentrations of $AgNO_3$ treated, 300 produced the highest number of bisexual flowers per vine in the diploid kakrol whereas 400 and 500ppm $AgNO_3$ produced the highest bisexual flower per vine in triploid and tetraploid types of kakrol, respectively. Ali *et al.* (1991) reported that 400 to 600ppm of $AgNO_3$ produced the highest number of bisexual flowers per vine in the tetraploid genotypes. The reproductive organs of the bisexual flowers induced from the diploid, triploid and tetraploid female plants showed more vigour than the corresponding normal types. Diploid, triploid and tetraploid female plants induced bisexual flowers upon the application of $AgNO_3$. Application of $AgNO_3$ may bring some sorts of physiological change in the growing shoots of kakrol which eventually influence in the increase of vigour of the reproductive organs.

For pollen germination, four different pollen cultural media viz., Brewbaker and Kwack's medium, 50% Robert medium, sucrose 10% +boric acid 100mg/l medium and sucrose 10% + boric acid 100mg / l + $CaNO_3$ 300mg/l were used for pollen germination. The highest percentage of pollen grain germination was 87.14% in tetraploid bisexual when Brewbaker and Kwack medim was used for 40 minutes. Wereas the lowest percentage of 9.64 was found in diploid kakrol when the diploid pollen grains were treated with Sucrose 10% + boric acid 100mg / l + $CaNO_3$ 300mg/l. For *in vitro* germination the best medium was Brewbaker and Kwack medium.

The highest pollen tube length was recorded in the tetraploid bisexual when 50%. Robert medium was used for 40 minutes. Pollen grains were also germinated in these media with varied pH. The results indicated that the highest pollen grain germination of 85.77% was obtained in the tetraploid male. When pH was 8 in different cultural media then the maximum pollen grains germinated. But when pH values were 6, 7 and 9 then no pollen germinated. Carbohydrate, sucrose, boron and calcium play an important role in the *in vitro* pollen grain germination and pollen tube growth (Brewbaker and Kwack, 1963; Kwack, 1967 and Steer and Steer, 1989) which corroborates the results of the present investigation.

Several investigators have noted upsets of varying degrees in seed setting, seed development and in germination capacity following intercrossing between diploid and their corresponding autotetraploids (Griffiths *et al.,* 1971). In some instances the triploid embryos abort and none or very few triploid individuals are developed e.g. in rye (Hakansson and Ellerstrom, 1950; Muntzing, 1951) and in maize (Cooper, 1951; Cavanagh and Alexander, 1963). In other cases the triploids may be fully vigorous but sterile, e.g. beet, where triploids are freely formed.

The pollens of induced bisexuals were as effective as that of normal flowers. In diploid and tetraploid, the percentage of success in crossing with pollens from bisexual

flowers was as high as that of control. The pollen of the bisexual flower was not effective on the same flower or other bisexual flowers. Moreover, when these bisexual flowers were pollinated with the pollens of normal male flowers, no fruit development was observed. Microscopic observations revealed that 85% pollen germinated normally but after a certain growth of pollen tube, tumor developed at the tip that formed barrier for tube penetration into the ovule. As a result, the pollen tubes failed to elongate and the ovary remained unfertilized.

The stigma of normal tetraploid female received pollens only from normal tetraploid male and induced tetraploid bisexual. In contrast Muntzing (1951) reported that in the styles of tetraploid rye, the pollen tubes of haploid pollen grains grow faster than the pollen tubes of 2n pollen grains (Hagberg and Ellerstrom, 1959). Randolph (1935, 1941) found pollen of diploid maize to be more effective than that of tetraploids in bringing about fertilization in both diploid and tetraploid maize under conditions of open pollination. However, normal diploid female accepted pollen from normal diploid, tetraploid male as well as from induced bisexuals. In both cases, numerous pollen tubes were found to develop from the germinated pollen grains and elongated through the stigmatic papillae. The study revealed that numerous developing pollen tubes were able to enter the sigma. This study further indicated that maximum number of ovules received pollen tubes to perform fertilization. The stigmas of triploids did not accept pollens from any source.

The pollen grains started germination on the stigmatic papillae after 20 minutes from the time of pollination and the phases of germination completed within 35 minuets. Pollens from all sources except from triploid germinated uniformly in case of all the pollinated stigmas studied. Cent percent pollen grains of triploid type failed to germinate on the stigmatic surface. In most cases both localized and diffused callose production was observed on the stigmata in papillae which might prevent pollen

germination. However, stigmatic papillae from unpollinated pistils did not produce any callose.

In exceptional cases, cross between tetraploid female and tetraploid male a, few germinating pollen grains produced tubes to grow within the stigmatic papillar cells. Careful examination showed that on an average only one or two ovules per ovary received pollen tubes and set one or two seeds. Jha and Roy (1989) reported that crosses between diploid and tetraploid male produced only male flowering plants. They also reported that in the following season one branch of the hybrid produced hermophrodite flowers but no fruit setting occurred.

Cytological studies (*Roy et al.* 1966) in a naturally occurring tetraploid *M. dioica* did not show the presence of any heteromorphy in chromosomes, all of which are nearly of the same size. Earlier attempts (Roy and Travedi, 1966) to cross the sex mechanism did not succeed. The discovery of a natural triploid has made it possible to undertake further crossing experiments to determine the heterogametic sex in this species.

The natural triploid of kakrol showed a general enlargement of different vegetative and flower parts. However, cytological irregularities like the presence of laggards, unequal separation of chromosomes at anaphase I and II and the presence of micronuclei etc. led to a high degree of pollen sterility. Out of 39 crosses made with diploids only few fruits have been obtained with two to three seeds in each (Agarwal and Roy, 1976).

The fruits of successful crosses between and within tetraploid and diploid types were characterized using three parameters, like fruit weight, seed number and seed weight per fruit. The fruits produced through crossing of the flowers of same plant with pollen from bisexual flowers were greater in size in both tetraploid and diploid types of kakrol, and the seed number and seed weight were more or less same as

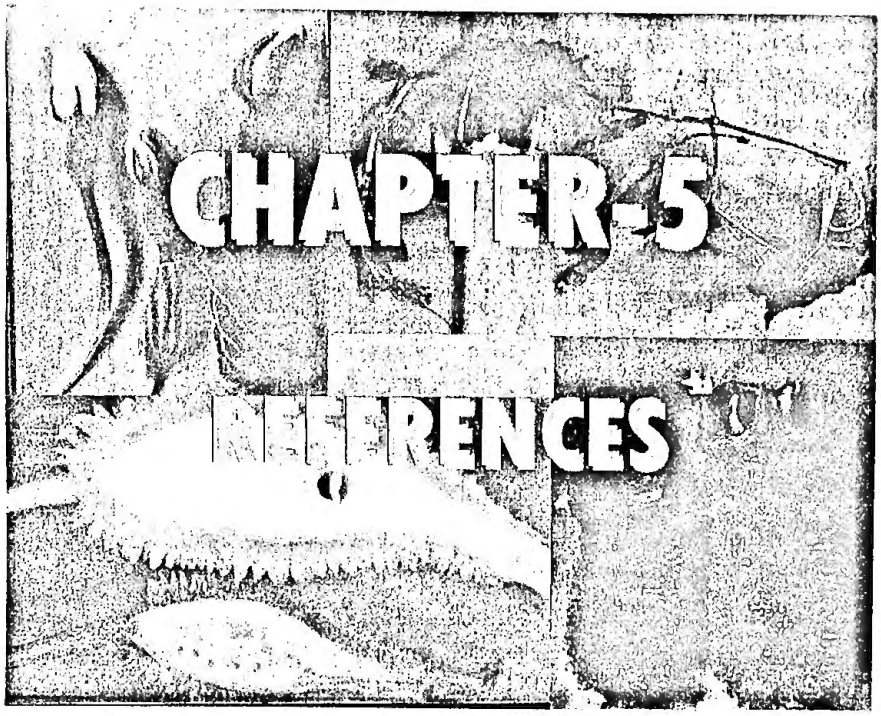
compared to fruits of normal crosses. The fruit weight was high in crosses like tetraploid normal female x tetraploid bisexual, diploid female x diploid bisexual.

The most interesting results obtained in the present investigation was that the diploid flower when pollinated with diploid bisexual pollen (sib same plant and different plant). Large size of fruit and a greater number of seeds seem merely an effect of hormons which are usually released by pollen grains upon their contact with stigmatic tissue (Maheshwari, 1950).

Conclusions:

The results of the present study indicate that it is not possible to induce chromosome doubling in kakrol by conventional methods of colchicine application. The intercrosses between naturally occurring diploid, triploid and tetraploid has very low potential in improving the crop. But female homo sexual crossing through the induction of male sex in genetically female plant is completely a new approach for kakrol improvement. The study shows that crossing between two female plants is possible through the conversion of sex. This technique will enable us to perform crosses between precisely evaluated genotypes and should foster the recombination of important characters of female kakrol.

Pollen grains do not germinate on the stigma of triploid flowers. Moreover, triploid plants do not produce any functional ovules as a result fruit setting does not occur. If hexaploid can be induced, fertile plant may be expected. As parthenocarpy appeared not to exist. Parthenocarpic fruit failed to develop in the triploid plant. Diploid and tetraploid plants also do not produce any parthenocarpic fruit.


CHAPTER-5
REFERENCES

REFERENCES

A.O, A.C 1980. Official Methods of Analysis, 13th Edn. Association of official Analytical Chemists, Washington. D.C.

Agarwal PK and Roy RP 1974. Natural polyploids in cucerbitacea 1, cytogenetical studies in Triploid *Momordica* Rox.**29**: 8-13.

Ahloowalia BS 1967. Colchicine induced polyploids in ryehrass. Euphytica. **16:** 49-60.

Ali M, Okubo HT and Fujieda K 1991. Techniques for propagation and breeding of kakrol (*Momordica dioica* Roxb.). *Scientia Hort.* **47**: 337-343.

Bandari JB and Mukhopadhyay R 1997. Morphological and Anatomical studies on (*Antrophyum callipholium* BL.) Phytomorphology. **42(2):** 155-160.

Bessey OA and King CG 1933. The distribution of vitamin-C in plant tissues and its determination. *J. Biol. Chem.* **103:** 687.

Beyer JE 1976. Silver ion, a potent antiethylene agent in cucumber and tomato. *Hort. Sci.* **43**: 806-810.

Bhuiya. MRH, Habib AKMA and Rashid MM 1977. Content and Loss of vitamin C in Vegetables during storage and cooking. Bangladesh Hort. **5:** 1-6.

Bligh EG and Dyer WJ 1959. Total lipid extraction and purification. *Can. J. Biochem. Physiol.*, **37:** 911-915.

Bose TK, Som MG and Kabir J 1993. *Vegetable Crops*. Naya Prokash, Calcutta. pp. 40-50.

Bostrack JM and Sparrow AH 1970. The radiosensitivity in Gymnosperm. II. On the nature of radiation injury and cause of death of *Pinus rigida* and *P. strobus* after chronic gamma irradiation, Red. Bot. **10:** 131-143.

Brewbaker JL 1959. Biology of the angiosperm pollen grain, *Indian J. Genet. Plant Breed.* **19:** 121-133.

Brewbaker JL 1967. The distribution and phylogenetic Significance of binucleate and trinucleate pollen grains in angiosperms. *Am J. Bot.* **54:** 1069-1083.

Brewbaker JL and kwack BH 1963. The essential role of calcium iron in pollen germination and pollen tube growth. Am. J. Bot. **50:** 859-865.

Cavanagh JA and Alexander DE 1963. Survival of tetraploid maize in mixed 2n-4n plantings. Crop. Sci. **3:** 329-331.

Chandhari LB and Prashad 1968. Genetic variation and heritability of quantitative characters in India mustard (*Brassica juncea* L.). Czern. and Cross. II. Indian J. Agric. Sci. **38(5):** 820-825.

Cooper DC 1951. Caryopsis development following matings between diploid and tetraploid strains of Zeamay. Am. J. Bot. **38:** 702-708.

Datta SK 1976. Cytogenetical studies in T*richosanthes anguina* Linn. Ph. D. thesis submitted to Calcutta University.

Datta SK 1978. Study of effect of colchicine on *Luffa acutangula* Roxb. and *Lagenaria siceraria* in relation to germination and polyploidy induction. Bangladesh J. Bot. **7(1):** 59-64.

Datta SK and Gupta MN 1986. Colchicine induced somatic flower colour mutation in Chrysanthemus. Bangladesh J. Bot. **16(1):** 107-108.

Davis GL 1966. *Systematic embryology of angiosperms.* John Wiley, New York.

Dubey AK and Gaur GS 1990. Biochemical studies of four strains of kakrol (*Momordica dioica Roxb*). Vegetable Sci. **17:** 31-37.

Dubey AK and Gaur GS 1989. Studies on pollen germination and storage in kakrol (Momordica dioica Roxb.). *Haryana. J. Hart. Sci.* **18:** 119-122.

Fakir MSA, Prodhan AKMA, Hossain MA, Akabar and Rahaman MA 1992. Yield, nutritional value and keeping quality of tasle gourd (*Momordica dioica*). *Intl. J. Trop.* Agric. **10**: 233-238.

Fisher RA, Immer RR and Tedin O 1932. The genetic interpretation of statistics on he third degree in the inheritance of quantitative inheritance. Genetics **17:** 107-124

Fisher RA 1936. The use of multiple measurements in taxonomic problems. Ann. Eugen. 7: 179-189.

Franzke CJ and Ross JG 1952. Colchicine-induced variants in sorghum. J. Hered. **43:** 107-115.

Glick 1957. Methods of Biochemical analysis. Edited by Glick, **(4):** 2.

Goni MO, Sikdar B and Joarder OI 2000. Biochemical variation of four genotypes of kakrol (*Momordica dioica* Roxb). Bangladesh J. Genet. Biotech. **1(1):** 105-108.

Goodman RM. Hauptli H, Crossway A and Knauf VC 1987. Gene transfer in crop improvement. *Science.* **236:** 48-54.

Griffiths DF, Pegler RAD and Toguthaisri T 1971. Crossing compatibility between diploid and tetraploid perennial ryegrass (*Loliuro perenne* L.) Euphytica. **20:** 102-112.

Hagberg A and Ellerstrom S 1959. The competition between diploid, tetraploid and aneuploid rye. Hereditas. **45:** 3690416.

Hanif M and Langer RHM 1972. The vascular system of the spiklet in wheat (Triticum aestivum). Ann. Bot. **36:** 721-727.

Hartmann HT and Kester DE (edt.) 1968. Plant propagation principles and practice. Prentice-Hall. Englewood Cliffs.

Hawkes JG 1977. The importance of wild germplasm in plant breeding. *Euphytica.* **26**: 615-621.

Heslop Harrison J 1987. Pollen germination and pollen tube growth. *Int. Rev. Cytol.* **107:** 1-78.

Hoque MA 1997. Propagation and Breeding of kakrol (*Momordica dioica* Roxb). Ph.D. Thesis . Dept. of Botany , Rajshahi University. Bangladesh.

Hoque ME 1971. *A comparative study on the external morphology and growth habit of ten cucurbitaceous vegetables.* M. Sc. (Ag): Dissertation, Bangladesh Agric. Univ. Mymensingh.

Hossain MA, Fakir MSA, Rahman MM and Chowdhury MSH 1987. Fruit setting by artificial and natural pollination in teasle gourd. Bangladesh hert., **15(2):** 40-45.

Hossain MA, Islam M and Ali M 1996. Sexual crossing between two genetically female plants and sex genetics of kakrol (*Momordica dioica* Roxb). Euphytic. **90:** 121-125.

Hossain SMM, Ntarik and AKM. Sr. Mollik 1992. Evaluation of teaslle gowrd genotypes of Bengladesh.

Hussain, MA and Rashid MM 1974. Floral biology of kakrol. Bangladesh Hort. **2(1):** 1-4.

Islam AS 1973. Fundamentals of genetics (Garden pea to gene synthesis). Bangla academy. Dhaka. Bangladesh. pp. 228-263.

Islam AS, Haque M and Khanam Z 1974. Colchiploidy in *Trichosanthes dioica* Roxb. in an attempt to produce seedless fruits. *Bangladesh J. Bot.* **3(2):** 59-65.

Islam MO, Monowar H, Islam HSM and Chadha ML 1992. Interspecific hybridization in kakrol (*Momordica dioica* Roxb.) for wider adaptibility (Unpublished).

Iwahori S, Lyons JM and Smith OE 1970. Sex expression in cucumber plants as affected by 2-chloroethylphosphonic acid, ethylene, and growth regulators, plant. Physiol. **46:** 412-415.

Jayaraman J 1981. Laboratory Manual in Biochemistry. Wiley Eastern Ltd. New Delhi, India.

Jha UC & Roy RP 1989. Hermophrodite flowers in dioecious *Momordica dioica* Roxb. *Curr. Sci.* **58:** 1249-1250.

Joarder OI and Eunus 1969. Genetic studies on yield and yield components of *Brassica campestris* L. Pak. J. Bot. **1:** 39-58.

Johri BM (ed.) 1984. *Embryology of angiosperm.* Berlin Heidelberg, New York Tokyo.

Kabir MH and Khanam NN 1997. *Botany of crop plants*. Bangla Academy. Dhaka. pp. 266-268.

Kalloo and Franken S 1978. Chemical induction of staminate flowers in four determinate gynoecium lines of pickling cucumber. *Gartenbauwissenschaft.* **43**: 280-286.

Katyal SL 1977. Vegetable growing in India. Mohan Primlani,Oxford & IBH Pub. Co. New Delhi-110 001. pp. 1-4.

Khan AA 1959. Studies on colchicine-induced tetraploids in *Ricinus communis* L. Pak. J. Sci. Indus. Res. **3:** 83-86.

Kihara H 1951. Triploid water melons. Proc. Amer, Soc. Hort. Sci. **58:** 217-230.

Knox RB, Williams EG and Dumas C 1986. Pollen, Pistil and reproductive function in Crop Plants. *Plant. Breed. Rev.* **4:** 9-79.

Kumar LSS and Vishveshwaralah S 1951. Chromosome number of some wild cucurbits. *Curr. Sci.* **20:** 211.

Kwack BH 1967. Studies on Cellular Size of Calcium acition in promoting pollen tube grow the. physiol plant **20:** 825-833.

Mahadevan A and Sridhar R 1982. Method in Physiological Plant Pathology. Sivakami Publications, Madras. pp. 316.

Maheshwari P (ed.) 1950. *An introduction to the embryology of angiosperms.* Mc Graw Hill, New York.

Maheshwari P (ed.) 1963. *Recent advances in the embryology of angiosperms.* Int. Soc. Plant Morphol. University Delhi.

Maheshwari P and Rangaswamy NS 1965. Embryology in relation to physiology and genetics. In: Preston RD (ed.) *Advances in botanical research* (vol. 2) Academic Press, New York. pp. 219-321.

Mather K 1959. Biometrical genetics (1st eds.) Methuen, London.

Mather K 1959. Biometrical genetics. Dover Publication. Inc. New York.

McMurry AL and Miller CH 1968. Cucumber sex expression modified by 2-chloroethanephosphonic acid. Science, **162:** 1397-1398.

Mishra KC and Sahu RP 1983. Large-scale cultivation of small bittergourd: problems and possibilities. *Indian Hort.* **1**: 5-8.

Mondal A 1996. Study of reproductive biology and improvement of teasle gourd (*Momordica cochinchinensis spreng.*) using hybridization and tissue culture. M.Phil Thesis. Institute of Bio. Sci. R.U. Bangladesh.

More TA and Munger HM 1986. Gynoecious sex expression and stability in cucumber (*Cucumis sativus* L.). *Euphytica* **35**: 899-903.

Mulcahy DL 1983. Manipulation of gametophytic populations. In: Lange W Zeven AC, Hogenboom NG (ed.) *Efficiency in plant Breeding.* PUDOC. Wageningen. pp. 114-119.

Mulcahy DL, Mulcahy GB and Ottaviano E (ed.) 1986. *Biotechnology and ecology of pollen.* Springer. Berlin Heidelberg New York. Tokyo.

Mulchy DL and Ottaviano E (ed.) 1983. *Pollen: Biology and implication for plant breeding.* Elsevier Biomedical. New York.

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

Nakajima G 1937. Cytological studies in some dioecious plants. *Cytologia, Fujii Jubl.* Vol. 282-292.

Nerson H, Cohen H, Paris HS, Edelstein M, Burger Y, Dehari T, Zelikovich G and Demri O 1987. Alteration of growth habit and sex expression in cucumber (*Cucumis sativus*) by paclobutrazol. Hassadeh **68(1):** 68-70, 72, (Cited from Hort. Abst. **58(3):** 1469, 1988).

Nirula S 1963. Studies on some nuclear factors controlling radiation sensitivity and the induced mutation rate in Eu-and para - sorghum species. Red. Bot. **3:** 1449-1451.

Ockendon DJ 1974. The value of stored pollen in incompatibility studies is *Brassica. Incompatibility. Newslett.* **4:** 17-19.

Ottaviano E and Mulcahy DL 1989. Genetics of angiosperm pollen. *Adv. Genet.* **26:** 1-64.

Prain D 1903. *Bengal plants.* Vol. I and II. Calcutta. India (Reprinted in 1963).

Raghavan V 1976. *Experimental embryology of vascular plants.* Academic Press. London.

Randolph LF 1935. Cytogenetics of tetraploid maize. J. Agric. Res. **50:** 591-605.

Rangaswamy NS 1963. Control of fertilization and embryo development. In: Maheshwari P (ed.). *Recent advances in the embryology of angiosperms.* Int. Soc. Plant Morphol. University Delhi. pp. 327-335.

Rangaswanmy K 1949. Proc. 36th Ind. *Sci Congr.* **3:** 137.

Rashid MM 1976. Vegetables in Bangladesh (in Bengali) ist edition Bangla Academy, Dhaka, Bangladesh, pp,494.

Roy RP and Trivedi RN 1966. Cytology of Gomphogyne cissiformis. *Curr. Sci.* **35:** 420-421.

Roy RP, Tharur V and Trivedi RN 1966. Cytological studies in *Momordica* L. *Jour. Cytol. Genet.* **1:** 1-5.

Sacher R, Mulcahy DL and Staples R 1983. Developmental Selection during self pollination of *Lycopersicon* x *Solanum* F_1 for salt tolerance in F_2. In: Mulcahy DL and Ottaviano E (ed.) *Pollen: biology and implications for plant breeding.* Elsevier Biomedical. New York, pp. 329-334.

Scott KG and Paterson FL 1965. Colchicine induced mutants in decatus barley. Crop. Sci. **5:** 44-47.

Shesahdri VS 1986. Cucurbit vegetable crops in India. Naya Prakash, Bidhan.Sarani, India. pp. 91-164.

Shivanna KR 1985. Some applied aspects of pollination biology. *Biol Mem.* **11:** 113-119.

Shivanna KR Johir BM 1985. *The angiosperm pollen: Structure and function.* Wiley Eastsern, New Delhi.

Siddique B and Rahman 1987. Krishi Katha. **5:** 249-256.

Sikdar B, Roy AK, Sardar PK and Joarder OI 2000. Effect of Silver nitrate on sex expression in kakrol (*Momordica dioica* Roxb.) Bangladesh I. Genet. Biotech, **1(1):** 135-143.

Sikdar B. 1995. Induction of bisexually and comparative study of normal unisexual and induced bisexual flowers of Kakrol M. Sc. Thesis, Department of Botany, Rajshahi University.

Singh AK 1990. Cytogenetics and evolution in the Cucurbitaceae in: D. M. Bates, RW Robinson and C Jaffrey (Editors). *Biology and Utilization of cucurbitaceae.* Comstock publishing Associates Cornell University Press. Ithaca NY and London. pp. 10-28.

Singh AK 1990. Cytogenetics and lution in the cacurbitaceae, In:D.M.

Singh H, 1978. Parthenocarpy in *Trichosanthes dioica* Roxb. and *Momordica dioica* Roxb. Curr. Sci. **47:** 735.

Sparrow AH and Sparrow RC 1965. Relative radiosensitivities of woody and herbaceous spermatophytes. Science. **147:** 1449-1451.

Sparrow AH and Miksche JP 1960. The relationship between chromosomal, nuclear, or cellular size and the radiosensitivity of different plant taxa. Red. Res. **12:** 474.

Sparrow AH, Underbrink AG and Sparrow RC 1967. Chromosomes and cellular radiosensitivit y. I. The relationship of Do to chromosome volume and complexot y in 79 different organisms. Red. Res. **32:** 915-945.

Stalker HT 1980. Utilization of wild species for crop improvement. *Adv. Agron.* **33:** 111-147.

Stebbins GL Jr. 1950. *Variation and evolution and plants*. Columbia Univ. Press. New York.

Steer MW and steer JM 1989. Pollen tube tip growth. *New Phytol.* **111:** 323-358.

Tang PS and Loo SW 1941. Polyploidy in soybean, pea, wheat and rice induced by colchicine treatment. Science. **91:** 66.

Tarik M and Reza S 1992. *Germplasm collection and evaluation of kakrol*, Project Report. BARI. Bangladesh.

Vijay OP 1978. Note on sex percentage in dioecious kakrol. *Curr. Res.* **7:** 174.

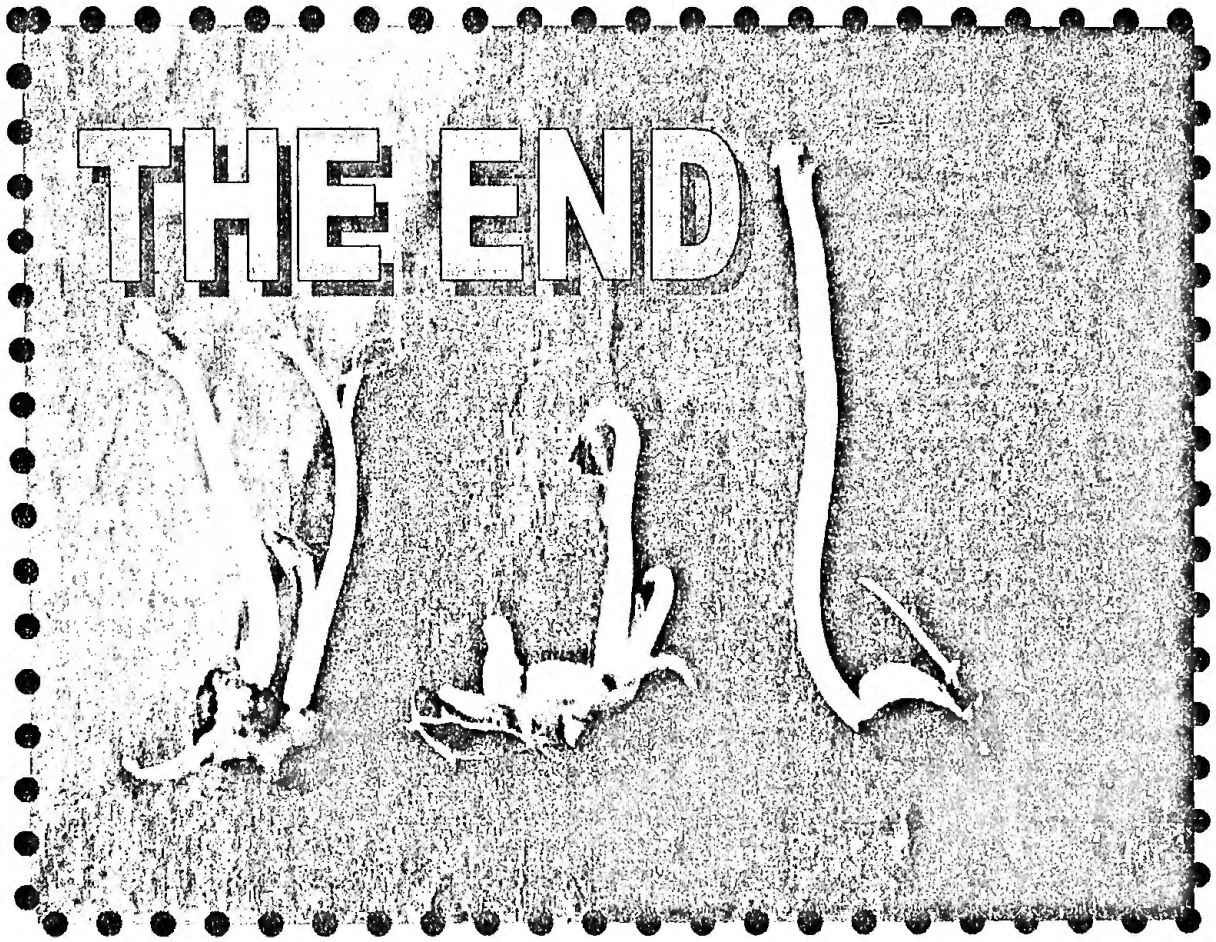
Vijay OP, Jalikop Stt. and Nath P 1977. Studies on floral biology of kakrol (*Momordica dioica* Roxb.). *Ind. J. Hort.* **34:** 284-288.

Whitaker TW 1950. Polyploidy in Echinocystis. *Madrono.* **10**: 209-211.

Wong T 1923. J. Biol. Chem; 55, 427.

Zamir D and Gadish 1987. Pollen selection for low temperature adaptation in tomato. Theor. Appl. Genet. **74:** 545-548.

Zeilinga AE and Schouten HP 1968. I Polyploidy in garden tulips. II. The production of tetraploids. Euphytica. **17:** 303-310.


THE END