INDUCTION OF POLYPLOIDY IN HAPLOID OF *G hirsutum* L. AND DIPLOID OF *G. arboreum* L.

By

HEMANTKUMAR NIVRUTTI SHINDE
B.Sc. (Horti.)
Regd. No. 96208

A thesis submitted to the
MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI-413 722, DIST.AHMEDNAGAR
Maharashtra State (India)

In partial fulfilment of the requirements for the degree
of
MASTER OF SCIENCE (AGRICULTURE)
in
AGRICULTURAL BOTANY
(CYTOGENETICS AND PLANT BREEDING)

DEPARTMENT OF BOTANY
POST GRADUATE INSTITUTE
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Approved by

(Dr. S.S. Mehetre)
Chairman & Research Guide

)r.S.D. Ugale)
ommittee Member

(Dr.N.D. Jambhale)
Committee Member

DEPARTMENT OF BOTANY
POST GRADUATE INSTITUTE
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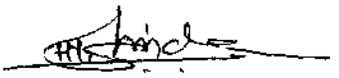
CANDIDATE'S DECLARATION

I hereby declare that this thesis or part thereof has not been submitted by me or any other person to any other University or Institute for a Degree or Diploma.

Place : MPKV, Rahuri

Dated : 24/5/1999



(Shinde Hemantkumar N.)

Dr. S.S. Mehetre,
Cotton Breeder,
All India Co-ordinated Cotton Improvement Project
Mahatma Phule Krishi Vidyapeeth,
Rahuri - 413 722, Dist. Ahmednagar,
Maharashtra (India).

CERTIFICATE

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Place : MPKV, Rahuri
Dated :25/5/1999

(S.S. Mehetre)
Research Guide

Dr. S.S. Kadam,
Associate Dean,
Post Graduate Institute,
Mahatma Phule Krishi Vidyapeeth,
Rahuri - 413 722, Dist. Ahmednagar,
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Place : MPKV, Rahuri

Dated : / /1999

(S.S. Kadam)

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Dated : 24/5 /1999

(Shinde Hemantkumar N.)

CONTENTS

Contents contd...

Contents contd..

Contents contd...

Contents contd...

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LIST OF TABLES

Contd...

Contd..

Contd...

Contd....

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LIST OF ABBREVIATIONS

Abbreviation		Description
Av.	...	Average
°C	...	Degree celcius
c.v.	...	Coefficient of variation
$	...	Dollar
et al.	...	et alli (and others)
Fig.	...	Figure
G.	...	*Gossypium*
gm	...	gram
ha	...	hectare
hr.	...	hour
I.C.A.R.	...	Indian Council of Agril. Research
IKI	...	Potassium iodide
kg	...	kilo gram
mg	...	Milli gram
mm	...	Millimeter
No.	...	Number
%	...	Per cent
PMC	...	Pollen mother cell
spp	...	Species
sq.cm.	...	Square centimeter
var.	...	Variety
viz.	...	Videlicet (namely)
$\chi^{ta/II}$	...	Chiama per biavalent
µ	...	Micron

ABSTRACT

INDUCTION OF POLYPLOIDY IN HAPLOID OF *G. hirsutum* L. AND DIPLOID OF *G. arboreum* L.

By

HEMANTKUMAR NIVRUTTI SHINDE

A candidate for M.Sc. (Agri.) degree

Mahatma Phule Krishi Vidyapeeth, Rahuri-413 722

1999

Research Guide : Dr. S.S. Mehetre
Department : Agricultural Botany
Major field : Cytogenetics and Plant Breeding

The present investigation entitled, "Induction of polyploidy in haploid of *Gossypium hirsutum* L. and diploid of *Gossypium arboreum* L." was conducted at the "All India Co-ordinated Cotton Improvement Project", Mahatma Phule Krishi Vidyapeeth, Rahuri during 1996-97 with the objectives

1) To doubled the chromosome number of the haploids of *G. hirsutum* L. 2n=2x=26
2) To study the cytomorphology and pollen fertility in induced polyploid C_1 plants
3) To obtain F_1 interspecific cross between doubled *G. arboreum* L. x *G. hirsutum* L. cotton.

Out of three techniques of haploid induction tried, one haploid each in gamma rays irradition and the emasculated

buds with 0.2% and 0.4% toleudine blue was induced in RHCr003, JLH 168, LRA-5166 varieties respectively. Thus toleudine blue treatment, is more efficient for haploid induction in *Gossypium hirsutum*.

The comparative studies for morphological characters between haploid and their tetraploid parent indicated that haploids have typical miniature shape with small and wrinkled leaves slow in growth, delayed flowering with nondehiscent anthers, less or no bolls, reduced size of stomata, its numbers per unit area and chloroplasts per stomata. All quantitative studies in haploid showed significant reduction in the ratio of 1:2 when compared with their respective tetraploid parents.

The meiotic studies in *G. hirsutum* haploids indicated that there was rare bivalents formation, unequal chromosome distribution in the microscope thus resulting in considerable pollen sterility. The rare fertile pollens found due to monads and dyads observed in second division of meiosis.

Average number of bivalents varied considerably, lower frequency of bivalent (1.5 to 3.5) was observed in *G. hirsutum* haploids resulting in complete male and female sterility.

Treatment of colchicine at various concentrations and treatments to various plant part. The polyploidy has been

induced in the haploids of *G. hirsutum* L. variety RHCr003 by treating axilary bud with 0.5% concentration was colchicine for 12 hrs. One branch with doubled chromosome number had been obtained.

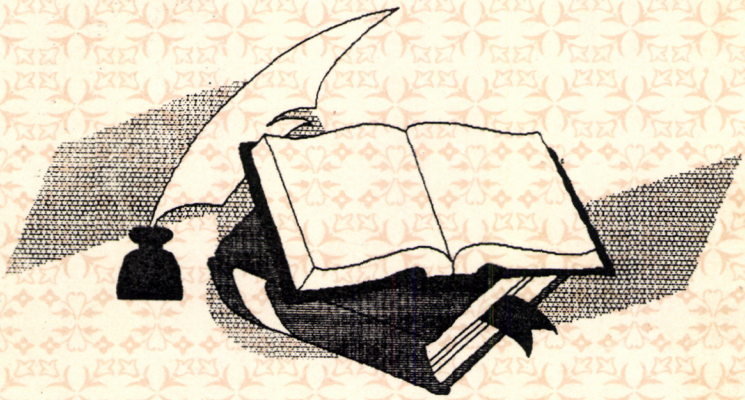
The observations on morphological characters indicated increase in the values of different character like leaf area, stomata size, number of chloroplast, petal length, style length, number of bracteoles, which is certainly due to effect of duplication of chromosomes.

Cytological studies in colchiploid branch indicated that PMC's of flower buds contained 2n=4x=52 chromosomes. On an average of 7 univalent, 16 bivalent, 3 trivalents and 1 quadrivalent were noticed at metaphase. Second meiotic division was highly irregular. Chromosome separation was unequal and due to presence of lagards and bridges abnormal tetrads and sporad containing unequal pollen grains were formed. All these abnormalities resulted in sterility. Normal pollen grains with 5-6% fertility were found in very few PMC's.

Chromose doubling was successful in one branch each of *G. arboreum* varieties Y-1 and Sawata by treating them with 0.1% and 0.2% colchicine respectively. The comparative study of morphological character indicated that colchiploid branches had shorter internodes and petiole, increased leaf

area, stomatal size, chloroplast / stoma, reduced number of anthers, increased petal length, style length, bracteole teeth, reduced boll diameter, boll / branch, boll weight, lint weight, seed weight, seeds/boll, increased motes/boll.

Cytological studies in *G. arboreum* in varieties indicated that normal sporad and fertile pollen were formed due to regular pairing of homologus chromosome in meiotic prophase, their seperation in first division and chromatid seperation in 2nd division. On analysis of PMCs in colchiploid branches of *G. arboreum* varieties Y-1 and Sawata indicated that, the chromosome configurations in these PMC's were 1 univalent, 9 bivalent, 3 trivalent, 6 quadrivalent. Unequal separation of chromosomes laggard, univalents bridges leading to pollen sterility were also noticed in these branches. It was not become possible to study the crossability of colchiploid by crossing them with other varieties and species of their origin because only a single branch has been induced. On selfing flower buds dropped down. Therefore for their further utilization method of air-layering has been used.



INTRODUCTION

1. INTRODUCTION

Cotton is one of the most ancient and important commercial crop next only to foodgrains. It constitutes nearly 70 per cent of the raw material for the textile industry which earned a little over $ 10 billions in foreign exchange during 1996-97. In addition, it provides directly or indirectly huge employment in rural as well as urban sectors. Apart from its value as a fibre, the potential of cotton in use such as edible oil (seed oil) and other byproducts like particle board, paper, corrugated boxes is enormous. Only a part of this potential is now realised at present.

Till independence India used to be a major cotton producer with a surplus for export. However, after partition (at the time of independence) a major cotton area (nearly 40 per cent) producing long and medium staple cotton, went to Pakistan. To sustain the mill industry which remained in India, import of raw cotton became necessary. This scenario continued for two decades or more.

At the time of independence, cotton production was around 23 lakh bales as against the requirement of 44 lakh bales. From 1947 to 1967, the cotton research and developmental activities were looked after by the Indian Central Cotton Committee (ICCC) through PIRRCOM Centres.

The major emphasis during this period was on enhancing input use including that of fertilizers and pesticides alongwith some varietal improvement efforts. These intensive efforts led to a considerable increase in cotton area (78 lakh ha) and production (53 lakh bales). Productivity however showed only marginal improvement (114 kg/ha).

Research and development efforts received a major impetus with the establishment of the All India Coordinated Cotton Improvement Project in 1967 followed by the establishment of Central Institute for Cotton Research at Nagpur in 1976. The efforts initiated under this project with emphasis on multilocation and multidisciplinary approaches involving the State Agriculture Universities and ICAR Institutes led to a phenomenal improvement including the development and commercial cultivation of hybrids, development of production and protection technologies suitable to the varied agro-climatic cotton growing zones.

There has also been a manifold improvement in production, productivity and quality with no virtual increase in area excepting in the last 2-3 years. India now produces around 160 lakh bales of cotton ranging from short staple to extra long staple from an area of 83 lakh ha with a productivity of 324 kg/ha (Anonymous 1997a).

Cotton is cultivated in three distinctly different agroecological zones through four different species of *Gossypium* and F1 hybrids. The species composition has shifted from the predominance of diploid (*G. arboreum* and *G. herbaceum*) till the early sixties to one with dominance of *G. hirsutum* and tetraploid hybrids beyond the seventies. The productivity ranges from 177 to 538 kg/ha in different states as the crop is grown predominantly as a rainfed crop constituting about 64 per cent of total cultivated area. The central zone comprising Maharashtra, Gujarat and Madhya Pradesh covers 56 per cent of the whole cotton area and contributes 47 per cent production with an average production of 270 kg/ha (Anonymous 1997a). Of the three states, Maharashtra with 33 per cent of total cotton area has the lowest productivity of 177 kg/ha with hardly three per cent of the area under irrigation. The position in respect of Gujarat and Madhya Pradesh is better in terms of productivity and irrigated area.

India is perhaps the only country where out of the 36 species of the genus *Gossypium* L., all the four cultivated species are grown on a commercial scale. However, the major area (about 50 per cent) is under *G. hirsutum* L. (American cotton), 29 per cent under *G. arboreum* L. and 21 per cent under *G. herbaceum* L. The area under *G. barbadense* L. (Egyptian cotton) is limited only to a few thousand hectares.

The New World cottons (*G. hirsutum* and *G. barbadense*) are tetraploids with chromosome number 2n=4x=52 and the *desi* (Asiatic or local) cotton (*G. arboreum* and *G. herbaceum*) are diploid with 2n=2x=26.

Several morphological characteristics rendering cotton plants more tolerant to cotton bollworms have been identified and ascertained. It is believed that the glandless cotton varieties could be made more tolerant by incorporating these morphological characters without affecting their yield and quality.

Hybridization between species is restored for securing genes or gene combinations that are not normally available within the limits of species. Further improvement in certain characters through transgressive breeding is also possible. The work on this line reported by several workers is mostly on the hybridization between tetraploid (2n=4x=52) x diploid (2n=2x=26) species. Such transfer is possible with certain difficulties and breeding programme is comparatively lengthy because most F_{1s} being triploid and sterile. Only after doubling its chromosome complement it can be back crossed to cultivated tetraploid to obtain plants with 2n = 4x = 52 chromosomes having desired character combinations (Sikka and Joshi, 1960).

The evolution of the genus *Gossypium* (cotton) has included a very successful experiment in polyploid formation. World cotton commerce of about $20 billion annually is dominated by improved forms of two "AD" tetraploid (2n=4x=52) species, *Gossypium hirsutum* L. and *Gossypium barbadense* L. Tetraploid cottons are thought to have formed about 1-2 million years ago, in the New World, by hybridization between a maternal Old World "A" genome taxon resembling *Gossypium herbaceum* (2n=2x=26) and paternal New World "D" genome taxon resembling *Gossypium raimondii* (Wendel, 1989) or *Gossypium gossypioides* (Wendel *et al.*, 1995) (both 2n=2x=26). The antiquity of this New World event precludes human involvement in polyploid formation.

Wild "A" genome diploid and AD tetraploid *Gossypium* taxa produce spinnable fibers were a likely impetus for domestication (Stephens, 1967 and Fryxell, 1979). Domestication of tetraploid cottons existed in the New World by 3500-2300 B.C., (Stephens, 1947) and have been widely distributed by humans throughout the World's Warmer latitudes. Domesticated "A" genome diploids existed in the Old World by 2700 B.C., (Chaudhari, 1971) and one of only two extent species, *Gossypium arboreum*, remains intensively bred and cultivated in Asia. Its close relative and possible progenitor, the other "A" genome diploid species *G. herbaceum* also produces spinnable fiber.

Although the seeds of "D" genome diploids are pubscent, none produce spinnable fibers, Lee (1984). There is no evidence that domestication of D genome *Gossypium* taxa has ever been attempted, although their geographic distribution overlaps that of several wild tetraploids. No taxa from the other recognized diploid *Gossypium* genomes (B,C,E,F and G) have been domesticated.

The genus (*Gossypium* L.) has since long caught the interest of cytogeneticists all over the world for various reasons. First chromosome counts in *Gossypium* species were reported by Zaitzev (1923) and Nikolajeva (1923). Later on, extensive cytological studies have been made in *Gossypium* on various cytological aspects like morphology of meiotic and somatic chromosome, pairing of meiotic chromosomes, behaviour of different genomic combinations and chromosomal aberrations etc. The Old World cultivated Asiatic cotton form a n=13 chromosome group, while the New World cottons belong to n=26 chromosome group. The wild species of this genus belong to n=13 chromosome group except *G. tomentosum* Nutt.

Four different major genomes B,C,D and E have been identified in wild species of *Gossypium*. Cultivated old world *G. arboreum* and *G. herbaceum* cottons belong to `A' genome, while the New World cottons *G. hirsutum* L. and *G.barbadense* L. and wild species belongs to $(AD)_1$, $(AD)_2$ and $(AD)_3$ genomes respectively. The genome symbols assigned to different

L. Kimber (1961 a and b) has made comparison between (Poly) haploids n=26 and normal tetraploids (2n=52) of *G. barbadense* and has inferred that there is genetic control of the pairing in the polyploid and studied cytogenetics of haploids in *Gossypium*.

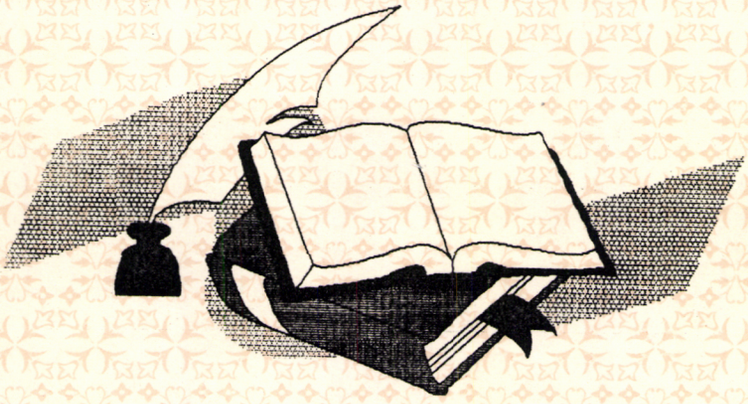
The improved varieties developed so far are the result of traditional breeding methods only. Haploids (Hougus *et al.*, 1958) in potato are found to be very useful tool to develop new varieties by chromosome doubling. Similar attempts were made in cotton by Turecotte and Feaster (1973 and 1974) and the doubled pure lines were found to be comparable with their parents. Hence, present studies have been proposed with a view to developing *G. hirsutum* varieties by doubling chromosome number of available haploids. Diploidization of heterozygous loci in doubled haploids may result in restoring the heterozygosity in the colchiploids. These lines are further likely to yield superior over inbreds/varieties.

The present investigation is undertaken with the following objectives.

1) To doubled the chromosome number of the haploids of *G. hirsutum* L. 2n = 2x = 26.

2) To study the cytomorphology and pollen fertility in induced polyploid C_1 plants.

3) To obtain F_1 interspecific cross between doubled *G. arboreum* L. x *G. hirsutum* L. cotton.



REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Cytological studies in the genus *Gossypium* L.

Extensive cytological studies have been made in the genus *Gossypium* L. by several workers, on various aspect like the chromosome number, chromosome morphology and their characteristics in different *Gossypium* species. Relationship among *Gossypium* species have been investigated from the study of meiotic chromosome behaviours of interspecific hybrids, polyploids and numerous hybrid combinations (Beasley, 1942). Large number of references, are also available on different cytological aspect of this genus. Use of all these aspects and chromosome variants in genetic analysis have been studied and reviewed extensively by a number of workers and hence these are excluded from the present studies. The review of cytological aspects of doubled haploids and polyploids is being presented in this chapter.

2.1.1 Doubled haploid in genus *Gossypium*

The availability of methods for obtaining large numbers of doubled haploids will have its greatest impact on the development phase of a breeding programme because the methods will permit the breeder to fix the genetic systems of individual gamet intestable, reproducible form at any stage in the breeding process. Thus utilization of doubled haploids

will probably reduce the development phase. The likely effects on the evaluation phase will be an increase in the number of genotypes that will have to be tested with standard breeding methods, considerable testing is done during the development phases and only a few lines require more evaluation. In contrast, effective utilization of doubled haploid methods will eliminate resting during the development phase.

In any plant improvement project the initial step is to identify or develop a genetically variable population. Such a population can be an open pollinated variety, a random-intercrossed population, or a segregating generation of a cross or crosses involving two or more lines. Selection experiments such a those reported by Manning (1963) provide ample evidence that old "land" varieties are variable and useful source populations. However, the exact genetic composition of recently released cultivars in the allopolyploid species is not clearly established. Some scientists claimed that most cultivars in the self pollinated species never become completely homozygous, but maintain a degree of heterozygosity through out many generations of self pollination. In the mid-thirties, Harland (1936) stated "It is at present admitted that no pure line of cotton has ever been produced, although some Sea Island strains maintained by self fertilization since 1917 constitute fair approximations to homozygosity".

Feaster and Turecotte (1973) found three doubled haploids of Pima cotton from three relatively heterogenous cultivars to be comparable in yield to the parental cultivars. While these authors did not specify the exact nature of the doubled haploids, it can be reasonably assumed that they represented unselected plants from the cultivars. Thus the plant to plant variation within the three original cultivars must have been within limited range.

In upland cotton, Meredith et al. (1970) found at least one significant difference between doubled haploids and their three parental cultivars for all characters studied except lint yield.

Zhang *et al.* (1996) reported that, haploid cotton (*Gossypium hirsutum*) chromosome numbers were efficiently doubled by immersing lateral buds in 0.3 - 0.5% colchicine solution under shaded and moist conditions for 24 hours. Alternatively, the basal part of lateral buds were microinjected with 20 f_1 of colchicine, a more inexpensive and convenient procedure. Rates of chromosome doubling obtained were 40%.

2.2 Colchicine induced polyploidy

Besides the naturally occuring allotetraploid species in *Gossypium*, several other auto-and allopolyploids have been obtained and used in many different cross

combinations. Cytogenetical investigations on this material have given valuable information regarding its utility in cotton breeding and also additional evidence for the genome inter-relationships in the genus.

The term polyploidy is, an organism having more than two sets of homologus chromosome. The chromosome number for each species or organism is fixed and the body cells contain 2n number of chromosome such organism is called diploid. Some of the polyploids in cotton have originated spontaneously, others have been produced artificially. A number of techniques have been used to produce polyploids by colchicine treatment.

Amin (1940) treated *G. herbaceum*, *G. arboreum* and *G. hirsutum* with colchicine; but when they were doubled they were sterile showing very defective bursting of anthers. Fertile types were however obtained by treating the sterile hybrids, *G. arboreum* x *G. anomalum*, *G. davidsonii* x *G. anomalum* and *G. hirsutum* x *G. herbaceum*. Beasley (1940a) produced polyploids from eleven types of *Gossypium* by immersion of apical meristem in 0.2% aqueous solution of colchicine for 24 hours. This treatment caused 10-55% of treated plants to produce polyploid branches. He outlined a system of formulae for making the composition of polyploids which were obtained by him, auto and allotetraploids, pentaploid, octaploids and heptaploids. Further he found that

sterile hybrids of distantly related species were usually fertile with one exception.

Stephens (1940) gave colchicine treatment to newly germinated seeds; plumules in the cotyledons; apical buds of the main stem; flower buds and young bolls. He obtained success with (1) using the seeds after second day after germination, immersing it for 1-2 days in aqueous solution of 0.025% colchicine, washing and planting out treatment (2) the best concentration was found to be 0.5 - 1.0% colchicine, applied with the small brush to the plumule just as the first leaf was on the point of unfolding 10-25 drops were applied singly allowing time to dry between each crop. He obtained tetraploid and hexaploid of *G.arboreum* and hybrid *G. barbadense* x *G. raimondii*, respectively.

Nakatomi (1940) gave detailed account of experiments of colchicine treatment of seeds and seedlings of Asiatic and upland varieties of cotton to produce polyploids. He described the cytological behaviour of one polyploid plant (n=26).

Yamashita (1940) reported polyploid cotton plants 2n = 104 from a Sea Island 2n = 52 by treating them with colchicine. He observed few well developed pollen grains in this plant which were sterile.

According to Mendes (1942) the immersion of 4 delinted seeds in a 0.15% solution of colchicine for 16 hours was the most effective treatment. Sometime it failed in *G. hirsutum* but all *G. harbaceum* plants reacted equally. Roots of abnormal looking plants of *G. hirsutum* often contained a mixture of 2n = 52 and 2n = 104 tissues. Flowers with mixed tetraploid and octaploid tissue was also encountered.

Stephens (1942) produced a tetraploid from *G. arboreum* var. neglectum (4n = 52) by colchicine treatment. Its meiotic studies gave further support to the hypothesis that diploid *Gossypium* species are secondary polyploids.

Douwes (1952) described techniques both involving soaking the seeds in water for 24 hours and removing the testa to eliminate discrepancies arising from the differential permeability of the seeds coat in various *Gossypium* spp. In the first method the whole embryo was placed between sheets of blotting paper soaked in 0.025 and 0.05% colchicine. Tetraploid configurations were seen in pollen mother cells in individuals treated with 0.025% for 4-12 hours or 0.05% for 1-8 hours, but the roots of many seedling were severely damaged by exposure to colchicine. In order to avoid the stunting caused by root injuries, the second method was devised. Colchicine treatment was delayed until four days after germination when the roots were sufficiently differentiated to allow the shoots only to be

inverted in the solution. Polyploidy thus induced with little interference in the rate of growth by a two hours treatment with 0.05% at 23-26°C. Although exposure for 10 hours failed to produce lethal effects, it is thought that a 6 hour treatment may prove most efficient for the gen us as a whole.

Menzel and Brown (1952) reported that chromosome doubling by colchicine treatment failed to influence the degree of mosaic formation.

Kamra (1958) reported slow development and considerable alterations in morphological characters of colchicine induced polyploids of inbred strains of Old and New World Cottons and of an interspecific hybrids. An increase in pollen grain diameter and reduction in fertility was also observed. Increased boll size was observed in autotetraploids of *G. herbaceum* race *percicum* and *G. arboreum* strain *Sangainum* and their F_1 hybrid. The octaploids of *G. hirsutum* strain Cocker - 100 and *G. barbadense* strain Pima - 32 was more variable but the means were lower. Fibre parameter determined by means of a 10 meters were significantly increased in tetraploids of *G. arboreum* strain *Sangainum* and *G. herbaceum* race *percicum* compared to diploids. In octaploid of *G. hirsutum* strain Cocker 100 no corresponding increase was noted this may have been due to the Pima strain showed a considerable increase in fibre parameter over that of the tetraploids.

According to Arutjunova (1959) reported chromosome doubling by colchicine proved to be effective in overcoming the compactability between species hybrids.

Mehbub and Muhammad (1964) reported good germination of two deshi varieties, 231 R and 124 F, treated with 0.03 - 0.1% colchicine, polyploidy was induced within these concentration. At higher concentration there was considerable mortality. American cotton gave better germination at lower concentration but showed higher mortality than deshi varieties at higher concentration.

Zaid *et al.* (1965) reported optimum results with 0.3% colchicine for 24 hours on seedling. Of 100 metaphases I plates 72 were too difficult to examine owing to the high degree of association. In the remained the average configurations were 0.1 VIII + 0.3 VI + 1.17 V + 3.32 IV + 2.71 III + 12.3 II + 1.6 I; the maximum number of bivalents per cell was 72 and the minimum 2, which result did not confirm those of Stephens.

Salanlei and Parameswarappa (1968) reported naked seed mutant in 0.002% colchicine treated *G. hirsutum* variety Laxmi which bred true for mutant character. It's seed had hair like structures at the micropilar end. Plants progenies selected two plants were found vigorous, increased yield and resistance to *X. malvacerum.* Cytological studies indicates no chromosomal variations in number or structure.

Salanlei and Parameshwarappa (1968) reported a fuzzy variety Laxmi of naked seed mutant in a *G. hirsutum* cotton after treatment with 0.002% colchicine. It bred true in subsequent generation. Two progenies gave increased typed yield, better resistance to *xanthomonous malvaceus*. Cytological studies indicated no variation in the either chromosome number or structure.

According to the Sargava (1970) treatment of germinating seeds with 0.01% colchicine was not successful, but from 55 seedling of *G. hirsutum* and *G. arboreum* treated with 1% colchicine in lanoline, eight proved to be polyploids further data showed their pollen dimensions and fertility. The plants had 2n = 52 chromosomes at meiosis usually separated regularly.

Kul'baeva and Golosov (1968) reported that doubling the chromosome number in the *Gossypium arboreum* and *G. herbaceum* improved fertility noteably in the former on pollination by tetraploid varieties S-4727 and 108F. The autotetraploid had good fertility, producing 4.5 - 34.7% viable seed.

Krishnaswami and Kothandaraman (1977) treated sterile hybrids between varieties of cultivated cotton as female and six wild species as males with colchicine. Halo length was high in hexaploids. Leaf morphology was similar to that of tetraploids, flower characteristic were

intermediate between parents. Pollen and seed fertility were variable within and between hexaploid.

Arutjunova (1979) reported cases of somatic reduction in the progeny of 4x *G. barbaceum* x *G. arboreum* leading to formation of n = 26 and 13 gamates and on this basis 4x = 2n = 52 and 2x = 2n = 26 progenies arised.

Lazarevo et al (1979) reported somatic reduction by treating growing point of F_1 hybrid *G. harknessii* x *G. raimondii* (2n=26) with 0.1% solution of colchicine the chromosome number of 2n=42. The loss of five pairs of chromosomes was due to the somatic reduction caused by colchicine treatment. Average chromosome/PMC at metaphase-I was 0.4 IV + 1.5 III + 8.03 II + 18.47I. Anomalies led to pollen sterility in aneuploid.

Zhang et al (1985) reported a technique of chromosome doubling which involves the dipping shoot tips of sterile F_1 plants in 0.05% colchicine + 0.5% DMSO.

Meshram and Tayyab (1990), reported that, sterile hybrids were produced by crossing *G. anomalum* x *G. thurberi* (genome 2 (B_1D_1), *G. davidsonii x G. anomalum* (2 (D_3-dB_1)) and *G. hirsutum x G. anomalum* (2 (AD) 1 B_1). Stem buds treated with 0.2% colchicine produced shoots which were fertile. The *G. anomalum x G. thurberi* and *G. davidsonii x G. anomalum* plants were allotetraploids while the other was

hexaploid. The morphology and fertility of these allopolyploids showed that their vegetative characteristics were similar to one of the parents while the floral characteristics were similar to one of the parents while the floral characteristics were intermediate. Average number of anthers/flower were very low in *G. anomalum x G. thurberi* compared with the others, while 100-seed weight was higher and pollen sterility lower in *G. hirsutum x G. anomalum* than in the others. Seeds of *G. anomalum x G. thurberi* were without lint. Deshpande *et al.* (1991) reported that polyploidy was induced in the diploid cotton species *G. arboreum* (2n=26), cultivar PA-85/85, by immersing seedlings in 0.5% colchicine solution. The tetrapaloid (4n=52) was crossed with *G. hirsutum* (2n=4x=52) cultivar Purnima and the new hybrid produced had 27.6% fertile pollen. The F_1 plants had similar leaf shape, plant habit, petal spot, and petal and pollen colour to *G. arboreum* and were also tolerant to the sucking pest complex. Bud and flower size resembled those of *G. hirsutum* F_1 males with 2n=52, crossed with male sterile *G. hirsutum* lines, gave some seed set.

Wang et al. (1992) reported that, seeds of cotton when treated with colchicine concentration of 0.05 - 0.3%, emergence rate and seedling survival rate decreased with increasing concentration of colchicine. Seedling survival rate was lowest using colchicine concentration of 0.2 - 0.3%

and seedlings showed abnormal growth; however, at this concentration chromosome doubling rate was highest (2.5 to 22.4%), under low concentrations (0.05%), seedlings grew normally but often failed to develop into chromosome - doubled plants. Twenty days after sowing, none of the seedlings with a plant height of 31 mm and above was chromosome doubled whilst 67.50% of seedling with a plant height below 10 mm were doubled. Thus, plant height may be used as an index for the early identification of chromosome doubled plants. Within 15 days of emergence, none of the seedlings with a first true leaf was chromosome doubled while 64.5% of seedlings growing 25 days after emergence and having a first true leaf were chromosome doubled.

2.2.1 Polyploidy breeding

Amin (1937) could not succeed in producing polyploidy in American x Asiatic hybrids by callus. Further, Beasley and Richmond (1939) produced polyploids from hybrids of *G. hirsutum x G. anomalum* and of *G. arboreum* var. *neglectum* x *G. anomalum*. Beasley (1940a) produced polyploids of pure *Gossypium* species or hybrids between closely related species were fertile as female parents but were almost or completely male sterile. Fibers produced on polyploids usually have greater length and diameter than those of the types from which they came. Beasely (1940 and 1941) also obtained purelines of *G. hirsutum* and *G. barbadense* by

doubling the chromosome numbers of haploids of these species. Harland (1940) recorded successful induction of polyploidy in *G. arboreum*, *G. thurberi*, *G. barbadense*, *G. hirsutum*, *G. arboreum* x *G. thurberi*, *G. barbadense* x *G. thurberi*, *G. barbadense* x *G.armourianum*, *G. barbadense* x *G. aridum*. He crossed the *G. arboreum* x *G. thurberi* hybrid (n=26) with many *G. hirsutum* and *G. barbadense* types and emphasized its importance in view of its bollworm immunity.

Brown (1948) reported that fertility in the synthetic polyploids from species hybrids is inversely correlated with the taxogenetic relationship between parent species. Trisomics derived from hexaploids have given the partially stable lines with an extra pair of chromosomes from diploid species.

Krishnaswami and Andal (1978) their studies with a range of species (including *G. herbaceum*, *G. arboreum*, *G. hirsutum* and *G. barbadense* and several wild types) hybrids between them and natural induced polyploids revealed that differences in the chloroplast number of the stomatal guard cells were directly related to ploidy. Taking the chloroplast numbers in diploids was a base, the percentage increase in this number in triploids, tetraploids was 25, 72 and 102, respectively. It was considered that stomatal chloroplast count can enable the rapid identification of polyploids in *Gossypium*.

2.2.2. Autopolyploids breeding in cotton

When same sets of chromosome of a genome are increased in number, autopolyploids are obtained. Genetical and morphological characters expressed by the autoploids depend on the genic constitution of the parent. They are less fertile than diploids.

Beasley (1942) reported that in meiosis of autopolyploids about 2/3 chromosomes formed quadrivalents and remaining as bivalents.

Stephens (1944) crossed an autotetraploid *G. arboreum* (AAAA) with *G. sturtii* and a triploid was produced. This triploid was treated with colchicine and the pollens of the resultant flowers were applied to *G. barbadense* (AADD). Out of this he obtained one completely sterile 52 chromosome hybrid which was believed to have received the AC genome from the pollen parent. Its, chromosome complement was presumed to be AACD. At meiosis the hybrid exhibited an average of 11.8 $\pm$ 0.8 univalent per PMC. Thus, on the basis of chromosome pairing it was concluded that A genome pairs but at very low degree with C and D genomes. Thus, he concluded that *G. sturtii* is not the American ancestor of the New World tetraploid cotton.

Anonymous (1964) reported autotetraploids of variety 23/R and octaploid of variety 124f.

Oakes (1966 a and b) studied pre and post fertilization phenomenon, respectively associated with sterility in ten tetraploids prefertilization phenomenon included failure of embryo sac formation, indehiscence of anther sacs, pollen inviability and failure of pollen tube to travel the style. Aberrations were observed in meagasporogensis of synthesized tetraploids and their combinations, the degree of abnormal sporogenesis appearing greatest for microsporogensis. The synthesized tetraploid combinations exhibited maximum aberrant pollens while in post fertilization phenomenon associated with sterility were ovule abortion, including somatoplastic protoplast of nucleus, single fertilization and malfunction of endosperm embryo or both. Gametophytic sterility was observed, the later type playing a major role in fertility.

Golosov (1968) crossed colchicine induced autotetraploids 2n = 52 of *G. arboreum* and *G. herbaceum* and were crossed with other upland tetraploid species. Crosses by pollination by upland cottons gave seed set of 18.0 - 34.6% and F_1 plants from some of the crosses developed normally and grew into tall plants with intermediate characters. All F_1S except between *G. arboreum* autotetraploid 02650 abd tetraploid *G. hirsutum* var S-4727 were sterile, which yielded few bolls containing seeds on being backcrossed to its parent forms.

Gulamova (1971) reported autotetraploid of *G. arboreum* induced by the treatment with 0.1% colchicine. In the autotetraploid significant increases were observed in size of general cells and pollen grains, as compared with diploid, but pollen viability was lower. Tetraploids were taller and slightly later in the maturity than diploids. Meng and Sun (1984) studied embryo development in colchicine induced autotetraploid *G. arboreum* x *G. hirsutum* embryo in comparison with control *G. arboreum* x *G. hirsutum*. They found that in colchicine induced autotetraploid *G. arboreum* x *G. hirsutum* embryo and endosperm development was very slow after fertilization. The embryo and proembryo cell were larger and showed the characteristics of the female parent. After normal differentiation most embryos progressed no further than the torpedo stage and only about 6% developed to fully maturity.

2.2.3 Allopolyploids breeding in cotton

In *Gossypium* alloploid plants containing genomal sets from two, three or four species have been obtained. These plants form a series with triploid, tetraploid, pentaploid, hexaploid and octoploid multiples of the basic chromosome number and have been obtained by chromosome duplication before or after hybridisation.

Gerstel (1953) studied segregation in allopolyploids and reported that *G. arboreum* - *G. herbaceum*,

gave tetrasomic allels for pollen colour, anthocyanin pigmentation, leaf shape and character lead to lethal. The 4n *G. anomalum* x *G. arboreum* produced very rare segregates while *G. arboreum x G. thruberi* did not gave any segregation for anthocyanin pigmentation pollen colour, corolla colour or leaf shape.

Sarvella (1958) reported behaviour of the chromosome of a series of 6n and 4n allopolyploid hybrids. The average number of multivalents PMC were a) 6n (*G. hirsutum* x *G. anomalum*) 1.7 ± 0.18; 4n (*G. arboreum* x *G. herbaceum* x *G. anomalum*) 1.90 ± 0.10; 6n (*G. hirsutum* x *G. thurberi*) 3.61 ± 0.28; 4n (*G. thurberi* x *G. raimondii*) 3.94 ± 0.19; 6n (*G. hirsutum* x *G. raimondii*) 6.16 ± 0.19 and 4n (*G. arboreum* x *G. herbaceum*); 9.42 ± 0.69. Averages chiasmata were 1/2 chiasma / bivalent.

Phillips (1962) reported segregation in a new allopolyploids of New World x Asiatic and New World x Wild American hexaploids.

2.2.3.1 Tetraploids breeding in cotton

Tetraploids possessing varying doses of one, two or three different major genomes of *Gossypium* have been synthesized.

Beasely (1940) produced a synthetic allotetraploid by doubling of the hybrid of *G. thurberi* x *G. arboreum*, which

was female fertile, but usually male sterile. In crosses with American (n=26) and Asiatic cotton the hybrids so formed were fertile.

Further, Iyengar (1942) reported a synthetic tetraploid of *G. anomalum* x *G. arboreum* cross by treating young F_1 plant having 4-5 leaves with 0.05% colchicine by wetting the shoot tips at interval of 12 hours and the shoot was then washed with water. The treated shoot grew 12 feet before flowering. Its seed bred true. In crosses with *G. hirsutum*, *G. barbadense* and *G. religiosum (G. hirsutum* var. *Punctatum*) seed setting was good and was improved when synthetic tetraploid was used as the female. Synthetic tetraploid had seed weight of 0.7 gms, lint index 19 gm, halo length 26.2 mm. fibre diameter 18.9 μ and fibre weight of 1.22 (compared with 1.85 for parental *arboreum*).

Stephens (1942) reported that the female gametes of the tetraploid were 40-50% fertile. Pollen fertility was lower because of poor germination or slow pollentube growth of many apparently viable grains. The tetraploid when used as female parent crossed readily with several wild diploid and New World (n = 26) species. He reported hybrids between tetraploid x *G.armourianum*; *G. raimondii*; *G. sturtii*. The metaphase I of these triploid hybrids indicates on an average less than one trivalent / PMC. It was therefore concluded that homologus between Asiatic and wild diploid species are

therefore very low. Further he noted that *G. sturtii* chromosomes are not closely homologus with either Asiatic or American diploid chromosomes.

Iyengar (1944,a) reported observations on the meiotic behaviour of autotetraploids of *G. herbaceum* and *G. arboreum* (two strains each) and allotetraploid of three *G. arboreum* x *G. herbaceum* hybrids. A high proportion of quadrivalents was seen in all the tetraploids but the proportion was slightly lower in the allotetraploids. Maximum association leading to 13 quadrivalent was rare. Meiosis in later stage was in general regular. Normal spores were formed but the pollens were largely defective in autotetraploids.

These autotetraploids were sterile when they were crossed with Asiatics, but some success was obtained when they were crossed with cultivated Americans. Autotetraploids were self fertile and most of their progenies had 52 somatic chromosomes. Meiosis in sterile hybrid plant (2n = 52) was obtained from autotetraploid *G. herbaceum* x *G. hirsutum* showed that many of the chromosomes of the three `A' sets are associated with trivalents and the chromosomes of the `D' set are left as univalents.

Jagannatha et al. (1952) crossed a tetraploid hybrid *G. arboreum x G. thruberi to G. hirsutum* and studied its F_9 generation.

Gerstel and Phillips (1957) studied segregation in series of synthetic allotetraploid. The gametic ratio obtained for five loci in 4n (*G. arboreum* x *G. herbaceum*) was 5:1 or narrower. The chromosomes in this allotetraploid therefore, showed tendency to associate preferentially. When tested as female parent in cross with 6n (*G. hirsutum* x *G. thurberi*); 4n (*G. thurberi* x *G. raimondii*) gave a ratio of approximately 13:1 for the segregation at locus R_1^{RAI}. Pairing of the chromosomes in the meiosis was normal in both of the above allotetraploids. The difference in segregation ratios of 4A and 4D combinations therefore, indicated greater sensitivity of allotetraploid segregation compared with F_1 pairing to small differences in chromosome homology. Data obtained for 4n (*G. arboreum* x *G. anomalum*) and 4n (*G. anomalum* x *G. herbaceum*) revealed very wide ratios for the five loci tested. While 4n (*G. arboreum* x *G. thurberi*) showed no segregation for two charactes and hardly any for the third characater studied. Pa yellow pollen; R_2 Red plant, Ya petal colour and L leaf shape were inherited independently as indicated by data from the combinations of Old World species and *G. anomalum*.

Kul'baeva and Valikhodrhaeva (1976) reported that chromosome doubling leads to disturbances in both megasporogenesis and microsporogenesis involving uneven movement of chromosomes to the poles and formation of

secondary associations between non homologous chromosomes in the autotetraploids forms.

Mirakhmedov et al (1985) obtained a synthetic fertile allotetraploid (2n=52) by colchicine treatment of the F_1 diploid hybrid *G. thurberi* x *G. raimondii*. It behaved like a diploid and was easily crossable with wild and cultivated forms of natural *G. hirsutum* and *G. barbadense* allotetraploid. Its meiosis was normal following crosses of *G. thurberi* x *G. raimondii* to *G. hirsutum* and *G. barbadense* cultivars. Subsequent backcrosses and selection in the F_3 - F_4 plants with high verticillium wilt resistant were obtained receiving their resistance from two wild species.

2.2.3.2 Hexaploids breeding in cotton

Hexaploids with two to four different major genomes have been reported in *Gossypium*.

Beasely and Richmond (1941) reported hexaploid of *G. hirsutum* x *G. arboreum* by doubling its chromosomes, which had regular meiotic chromosome behaviour and about 85% pollen viability.

Its fibers were finer than upland with finer convolutions. It was back crossed twice to upland. Further seeds produced from plants of doubled *G. arboreum* and *G. thurberi* cross with *G. hirsutum* (Cocker-100) and backcrossed to *G. hirsutum* gave an extremely variable population. Some

plants were prolific but the ginning out turn was low. The fibers of some plants were finer than those of upland parent had fewer convolutions and were stronger.

About 30 plants from hexaploid of upland x asiatic back-crossed twice to upland gave a range in fertility from 0 to over 50%. All the plants examined had over 26 pairs of chromosomes and some had 4-5 extra Asiatic ones. As many as possible, the plants were self pollinated and also back crossed again to the upland parent. Some of the plants had fibers coarser than upland and others showed some resistance to leaf spot possessed by Asiatic parent plant selected for high fertility from the F_2 of hexaploid of *G. hirsutum* x *G. arboreum* gave progenies with approximately the same percentage of sterile plants as the F_2 progeny. Doubling the chromosome number in a plant in which about half of the homologus chromosomes failed to pair did not increase the percentage of chromosomes capable of pairing.

Iyengar (1944, b) reported chromosomes conjugation in (a) four hexaploids of cultivated Asiatics x cultivated Americans (b) two hexaploids of wild x cultivated Americans (c) two hexaploids involving wild African x cultivated Americans. Chromosome conjugation was also studied in their respective triploids. Though the triploids showed marked variations in conjugations the hexaploids showed only slight differences.

Crosses between hexaploids and suitable diploid gave fertile tetraploids with 52 chromosomes. During meiosis, the chromosomes paired mostly as bivalents. These facts indirectly showed that cultivated American cottons with 52 chromosomes are allopolyploids having two sets of Asiatic and two sets of wild American chromosomes.

Gerstel (1956) studied segregation in colchicine induced hexaploids of (*G. hirsutum* x *G. raimondii*) x *G. hirsutum* with and without petal spot it segregated in 8.68:1 while colchicine induced hexaploid (*G. hirsutum* x *G. thurberi*) x hexaploid (*G. hirsutum* x *G. raimondii*) gave corresponding ratio 67%. Evidence was obtained that these deviates from 5:1 ratio expected from random chromosome segregation were partly attributable to post meiotic selection. The segregation ratios indicate that New World cottons are more closely related to *G. raimondii* than *G. thurberi*.

Menon (1956) observed 30-36% fertility in hexaploids of hybrids involving *G. arboreum*, *G. hirsutum* and *G. harknessii*. The general dearrangement caused by complex genomic composition might result in several types of a typical chromosome behaviour in the same individual, thus explaining the occurrence of both mosaic formation and somatic reduction in different parts of single hexaploid hybrid.

Santhanam (1958) reported a colchicine induced hexaploid form *G. hirsutum* x *G. raimondii*. He backcrossed it twice to Cambodia strain MCUI. Selection upto F_8 has resulted in progenies giving equal yield of seed cotton superior halo and ginning percentage.

Phillips (1962) observed average segregation for several loci in AD x A hexaploids was close to autopolyploid 5:1 ratio indicating that *G. herbaceum* and *G. arboreum* are closely related to New World species. Average segregation of loci for each D species in AD x D hexaploids was as follows; *G. raimondii* 9.3:1; *G. harknessii*, 16.4:1; *G.armourianum* 17.4:1; *G. aridum* 20.3:1; *G. lobatum* 21.4:1; *G. thurberi* 32.1:1; *G. gossyploides* 66.5:1; *G. raimondii* is assumed to be the wild species mostly closely related to New World species.

Phillips and strickland (1966) reported colchicine induced hexaploid of *G. hirsutum x G. longicalyx*. They reported 6.78 and 2.00 bivalents in the triploid and hexaploid, respectively. Quintanilha and Cabral (1966) reported produced hexaploids and tetraploid of the crosses *G. hirsutum* x *G.armourianum* and *G. herbaceum* var *africanum* x *G. arboreum*, respectively.

Arutjunova and Volkova (1971) reported hexaploid between *G. hirsutum* x *G. stocksii* forming 39II, 30% normal tetrad and 75% pollen viability. It produced pentaploids in crosses with tetraploid.

Marappan and Bahawandoss (1972) synthesized a hexaploid from a cross between *G. hirsutum* and *G. raimondii*, it was partly fertile probably because of multivalent formation. They reported one less hairy and less vigorous plant with smaller leaves and flowers resulting *G. hirsutum* but pigmentation in petals and filaments indicates the influence of *G. raimondii* genes, it had 2n = 52 chromosomes.

Malik and Shaikh (1974) obtained hexaploid plants from colchicine treatment to the triploid en = 39 (*G. hirsutum* x *G. sturtianum*). It showed 2-9 I, 38-35 II and 0-5 III and 0-3 IV.

Lemeshev et al (1986) reported that unlike hybrids the hexaploid plants were highly fertile and set seed from open pollination and selfing. They studied meiosis in PMCs tetrad formation and pollen fertility in the material involving wild species *G. harkenssii* and *G.armourianum*.

2.2.3.3 Octqploid breeding in cotton

Mendes (1940) reported that the most effective treatment for doubling the chromosome consists soaking of delinted seed in a 0.15% colchicine solution for about 16 hours. Octaploid plants 2n = 104 of *G. hirsutum* formed quadrivalent at division I. The anaphase I was regular seperating 52 chromosomes at either poles. Anaphase II was also regular.

Mendes (1942) reported that at anaphase-I in octaploid 52 chromosome generally went to each pole, but deviations were occassionally observed. Secondary association was frequent. Pollen formation was more or less normal but the anthers mostly failed to dehisce. The pollen were larger and variable in size than normal plants. No seeds were obtained either from self pollination or with either octaploids or tetraploids. Occasional fruits were formed from open pollination. The seeds obtained were usually abnormally large, could often be induced to germinate by incision of the testa. The plants produced frequently having 2n = 52 chromosomes.

2.2.4 Amphidiploids breeding in cotton

When two species which are genetically not related are crossed. They show sterility due to formation of univalents. In such cases the difficulty is overcomed by doubling the chromosome hybrid. So that each chromosome get homologous chromosome and perfect paring. Such organism or hybrid in which the chromosome number is doubled are called amphidiploids.

Zhebrak and Rzaev (1940) reported amphidiploids plants / sectors produced by colchicine treatment of the shoots apices or seeds in the following crosses :

G. hirsutum x G. sturtianum

G. hirsutum x *G.armourianum* ;

G. herbaceum x *G. anomalum*;

G. hirsutum x G. barbadense

G. hirsutum x G. arboreum and G. hirsutum x G. stocksii.

They observed inverse relationship fertility of F_1 and of the amphidiploidy.

Harland (1941) resynthesised the New World amphidiploid which was effected by doubling of the chromosome complement of the hybrid *G. thurberi x G. arboreum* with colchicine which was male sterile, but was completely fertile as female parent in crosses with both *G. barbadense* and *G. hirsutum*. Zhurbin (1941) obtained a male sterile but female fertile amphidiploid shoot by treating a shoot of *G. arboreum* x *G. thurberi* with colchicine. Amphidiploids (2n = 104) have also been obtained by treating of germinating seeds of *G. hirsutum* x *G. barbadense*. These were partly fertile. A triploid (hexaploid) plant (2n = 78) was also obtained but it was suggested that this was due to the union of an unreduced gamete with a reduce one and not due to the colchicine treatment.

Brown (1948) fertile amphidiploids have been made from the following hybrids *G. davidonii* x *G. anomalum* (this doubled spontaneously) *G. arboreum* x *G. anomalum*, *G. arboreum* x *G. stocksii*, *G. arboreum* x *G. thruberi*. The last has been used to introduce genes for strength in cultivated cotton.

Ansari (1958) reported a colchicine induced amphidiploid of *G. stocksii* x *G. arboreum*.

Rhyne (1965) reported anomalous behaviour of host spot of *G. anomalum* in amphidiploid *G. hirsutum*.

Arutjunova (1968) produced triploid hybrid plants by mix pollination by pollinating *G. hirsutum* with pollen mixtures of *G. herbaceum* x *G. arboreum*; *G. harknessii* and *G. thurberi* a treatment of 0.05% colchicine have given rise to amphidiploids with 2n = 78 they were fertile and backcrossed with *G. hirsutum* and *G. barbadense*. Another amphidiploid reported by him was *G. herbaceum x G. arboreum* by treating F_1 with 0.1% colchicine, it displayed heterosis. He backcrossed the tetraploid with *G. hirsutum* and *G. barbadense*.

Volkova (1972) reported hexaploid amphidiploids by crossing *G. hirsutum with diploid G. sturtii, G. stockssii* and *G. anomalum* and subsequent doubling the chromosomes. During M-I in the hexaploids, chromosome were seen lying beyond the equatorial plate. At anaphase I lagging chromosome and bridges occurred. At M-II and anaphase II disturbances hook form of lagging chromosomes. In PMCs with normal pairing there were 39 bivalents with 1.2 chiasmata. Univalents and associations of 3-4 chromosomes were also observed. The highest yielding most fertile hexaploid was *G. hirsutum* x *G. stocksii*.

Kul'baeva et al (1973) presented result of the study of amphidiploids obtained by crossing *G. arboreum* with *G. herbaceum* and induced autotetraploid of *G. arboreum* together with their interspecific hybrids from the crosses with *G. hirsutum.* The amphidiploids some what exceeded the autotetraploids in number of pollen grains germination on the style and in boll set after pollination with own and foreign pollen.

Arutjunova (1979) reported that the aneuploidy was observed by him in amphidiploid *G. hirsutum* x *G. herbaceum* x *G. harknessii* on the basis of somatic segregation and in 6x *G. hirsutum x G. sturtii*, pollen of *G. sturtii* was formed as a result of genome segregation by crossing 2x *G. raimondii* with 6x *G. hirsutum x G. sturtii.*

Egarmberdiev and Falatova (1979) reported longer pollen grains than their parents with greater variations in pollen grain size in amphidiploids *G. hirsutum* x *G. trilobum* and *G. hirsutum* x *G. raimondii.*

Semenikhina and Egarmberdiev (1979) reported that in *G. hirsutum* x *G. raimondii* amphidiploids besides the characteristics of initial species, new traits not found in the parents were observed such as greatly increased seed size and weight and very thick soft fibre, when pollen was 80% viable the amphidiploid set 3-6 bolls/season with 1-12 seeds/boll.

Egarmberdiev and Falatova (1981) obtained fertile amphidiploid (2n=38) by treating *G. hirsutum x G. trilobum* F_1 sterile hybrid with 0.1% colchicine.

Shakhmedova et. al (1981) polyploidized the F_1 hybrids between *G. hirsutum* x *G. sturtti G. raimondii* and *G. trilobum* and found that amphidiploids differed in protein banding pattern although the natural forms were same in each case. Two of the amphidiploids had patterns qualitatively different from those of the parents, but *G. hirsutum* x *G. raimondii* had a pattern similar to that of *G. hirsutum*. Confirming that *G. raimondii* was involved in the evolution of 4x cotton species.

Egarmberdiev and Falatove (1982) reported a wilt resistant cotton amphidiploid by treating triploid *G. hirsutum* S-4727 x *G. trilobum* with 0.1% colchicine.

He and Chen (1982) reported F_1 and F_2 plants of amphidiploid *G. hirsutum* x *G. anomalum*.

Makhudov and Eminebeili (1982) reported amphidiploids of *G. hirsutum* x *G. trilobum* and fertile tetraploids of *G. trilobum* x *G. harknessii* and *G. trilobum* x *G. anomalum*. After 3-4 backcrosses of the 78 chromosomes amphidiploids to *G. hirsutum*, promising sequidiploid material (2n=65) AADD was selected. Backcrossing the sequidiploid to *G. hirsutum* gave 52 chromosomes forms some of which were economical useful. The inter-specific tetraploids hybrids of

G. trilobum with *G. harknessii* and *G. anomalum* crossed readily with *G. hirsutum* to give fertile 52 chromosome form with wilt resistance (<1.3% infection).

Rizaeva and Larareva (1982) achieved chromosome doubling by means of 0.1% colchicine and produced hexaploid amphidiploids of the interspecific crosses within section intergrifolia. These hexaploids were backcrossed with *G. hirsutum* and *G. barbadense*. The amphidiploids had such useful characters as cold resistance, hairy leaves and strong fibre enabling them to be used as promising breeding material.

Mirakhmedov et al (1985) obtained 2n=78 plants with treating the growing points of 2n=39 F_1 hybrid *G. hirsutum* x *G. anomalum* with colchicine. Its progeny was drought resistant. Fruiting plants were selected with fine white fibre pollen viability in the amphidiploid was 80-92% fertile seaudiploid (2n=65) was obtained only by crossing the amphidiploids with *G. hirsutum* varieties several cycles of back crossing of seaudiploid to *G. hirsutum* led to improvements in the hybrid and gradual elimination of some of *G. anomalum* characters. Wilt resistant was retained while fibre quality was improved, fertile progeny was obtained by crossing *G. hirsutum* x *G. anomalum* with *G. hirsutum x G. arboreum*.

Sanam'yan (1986) reported spontaneous alloploidy and greatest percentage of unreduced gamates in amphidiploids *G. raimondii* x *G. thurberi*; *G. herbaceum* x *G. thurberi*, *G. arboreum* x *G.armourianum*.

2.3 Interspecific hybridization in cotton

Anonymous (1942), treated *G. taitense* x *G. hirsutum* and *G.armourianum* x *G. hirsutum* plants with colchicine and obtained seeds from them as well as reported 4n plant of *G. arboreum* x *G. anomalum*.

Amin (1941) discussed on interspecific hybridization and colchicine induced polyploidy in cotton further he (1943) described the application of colchicine to cotton its action and results obtained. Anonymous (1944) obtained 22 bolls by crossing doubled (*G. arboreum* x *G. thurberi*) with *G. hirsutum*. The bolls contained were brown, short but strong and with silky lint. The self sterile hybrid between *G. hirsutum x G. raimondii* become partly fertile after doubling its chromosome number. However it could not gave seeds when it was crossed with *G. hirsutum* L.

Beasley and Brown (1942) could not restore the fertility in the asynaptic plant of cross *G. hirsutum* x *G. barbadense* by doubling its chromosomes. Further in 1943 they produced plants having additional chromosome pair to *G. hirsutum* by doubling chromosomes of triploid hybrid *G.*

hirsutum x *G. arboreum* and *G. hirsutum* x *G. harknessii* by colchicine and repeated backcrossing with tetraploid cotton.

Kasparyan (1945) reported doubling of *G. arboreum* by means of colchicine. The tetraploid *G. arboreum* plants crossed successfully with *G. hirsutum* but failed to cross with diploid plants.

Anonymous (1945) obtained few seeds from a cross between the doubled hybrid *G. arboreum* x *G. thurberi* and *G. hirsutum*. The lint of these seeds was over 25 mm long, fine strong and slightly brown in colour, the seeds were either fully fuzzy or naked according to the *G. hirsutum* parents used. Selection for increased seed number/boll was also produced. The triple was crossed reciprocally with two parents. The cross with *G. hirsutum* was only successful when the triple hybrid was used as the female parent. The cross with the other parent was successfull in both directions but more successfull when the doubled hybrid was used as female parent. The original double hybrid showed seasonal rhythm in fertility. A triple hybrid showed susceptibility to bollworm. Stephens (1945) reported the compatibility of tetraploid *G. arboreum* with other Old World species and the cytology of two resulting triploid hybrids. Based on metaphase I, pairing found in series of polyploids. Stephens (1945) suggested that gradual quantitative change shown by Silow (1944) is responsible for speciation in the *G.*

arboreum - *herbaceum* - *anomalum* group is a process which has been continued through out the genes and the gross structural changes have been superimposed on the basic mechanism. Stephens and Cassidy (1946) reported partially fertile synthetic allotetraploids from *G. arboreum* x *G. anomalum*; *G. herbaceum* x *G. anomalum*, *G. herbaceum* x *G. stocksii* and *G. herbaceum* x *G. stocksii*.

Varuntsyan (1946) reported failure of crosses even with the application of colchicine between different species of cotton with either the same or different chromosome numbers. Ganeshan (1947) produced *G. thurboreum* by doubling the chromosomes of *G. arboreum* x *G. thurberi* hybrid which was male sterile except monsoon (July to October) when self pollinated set bolls. It crossed well as a female parent with both *G. hirsutum* ; *G. barbadense* and F_1^s were self sterile. Thurboreum showed some degree of bollworm resistance especially in the shoot tips, flower buds and flowers. It is suggested that a more fruitfull line to attack would transfer the immunity from *G. thurberi* to *G. arboreum* then cross this form with *G. thurberi* and double with colchicine. *G. hirsutum* was crossed with *G. raimondii* and the hybrid was treated with colchicine. The hexaploid was self fertile, but did not backcross to *G. hirsutum*. The C_2 generation was crossed successfully with *G. hirsutum* and back crossed to the latter. The strong and fine lint of *G.*

raimondii is also found in the hybrid. Further, he doubled *G. arboreum* x *G. anomalum* hybrid and crossed it with *G. hirsutum* to transfer fine lint of *G. arboreum* to cultivated cottons. Out of 300 plants, only one survived and others died due to wilt.

Bernado (1963) reported numerical imbalance resulting because of univalent formation and elimination of female gamete and zygote in the 6n hybrids viz., *G. barbadense x G. gossyploides; G. hirsutum* x *G. raimondii* and *G. barbadense* x *G. arboreum.*

Pandya et al (1963) employed polyploidy to produce new fertile varieties from interspecific hybrids at Surat. Type - 6 - 6 was derived from hexaploid ($CO._2$ x 1027 ALF) - F_1 doubled. It expressed bushy growth, good bearing capacity and early maturity. Its staple length was barley one inch but its ginning out turn was 38.0%. HH-8 and HH-25 had good staple length but have low in yielding capacity and ginning out turn were selected from the crosses between the two hexaploids of ($CO._2$ x 1027 ALF) F_1D x ($CO._2$ x *G. anomalum*), it gave CO - any varieties when backgrossed to BC-68 was obtained from (28-1) D x *G.armourianum* the resultant tetraploid being backcrossed to $CO._2$ and cocker wilds. At Indore TH-144 was bred from the cross (*G. arboreum* x *G. thurberi*) F_1 doubled x *G. hirsutum.*

Krishnamurthi and Pannaiya (1964) reported that doubling o chromosome of hybrid *G. hirsutum x G. raimondii* number was associated with thicker coarser leaves and increase in the size of other plants parts. Saunders (1964) transferred hairiness by crossing *G. anomalum* and *G. barbadense* and backcrossing *G. barbadense* after doubling the chromosomes of the hybrid.

Bernado (1965) reported the effect of chromosome elimination segregation frequencies in the hexaploid hybrid of *G. barbadense x G. arboreum; G. hirsutum x G. raimondii and G. barbadense x G. gossyploides* and their test cross progenies. He reported univalent and distribution of chromosome number in progenies and extend of chromosome loss. Saunders et al. (1965) produced eight hexaploid stocks of hybrid material. Kammacher and Poisson (1964) reported hexaploids of cross *G. hirsutum* x *G. anomalum.*

Poission (1970) studied synthetic tetraploid and pentaploid of crosses *G. herbaceum x G. anomalum and G. hirsutum x G. anomolum* followed by chromosome doubling of the F_1 and Crossing with *G. hirsutum.*

Mursal (1975) reported detailed cytological analysis in doubled F_1 *G. arboreum* x *G. raimondii.*

Louant and Marechal (1975) reported trispecies hybrids between doubled F_1 (*G. therberi* x *G. anomalum*) *G.*

hirsutum and doubled *G. hirsutum* x *G. anomalum* x *G. harknessii.*

Demol et al (1976) studied material comprised of *G. hirsutum* and six allohexaploids derived from crossing *G. anomalum, G. thurberi, G. harknessii, G. raimondii, G. axygiaum.*

Shakhmedova and Lemeshev (1979) obtained fertile hexaploids by treating sterile *G. hirsutum* x *G. anomalum* F_1S with colchicine which were 120-150 cms tall and very hairy with large leaves. All had 78 chromosomes with 34-39% pollen fertility. Seed set after selfing was 26% and after crossing with *G. hirsutum.* The 6x forms were all late 123 days from sowing to anthesis.

Shakhmedova and Voblaya (1981) selected lines combining earliness boll weight, fibre length and yield in the cross *G. hirsutum* x *G. anomalum* followed by colchicine and backcrossing to the *G. hirsutum* var 108 F.

Dilday (1986) reported a hexaploid 2n=78 plant from interspecific cross of tetraploid 2n=52 *G. hirsutum* x *G. sturtianum* showed desirable phenotype and had flower bud and seed gossypol % of 0.29 and 0.02 respectively. Fertile pentaploid from crosses between hexaploid x tetraploid *G. hirsutum* texas market was also reported.

Niyazov and Ruban (1987) produced hexaploid of *G. hirsutum* var Acala 1517-70 and Deltapine 80 were crossed respectively with *G. thurberi and G. aridum* to give triploids which were intermediate between the parents in morphology. Hexaploids were produced by colchicine treatment and crossed out the *G. hirsutum* varieties Tashkent 1 and Paymaster 266.

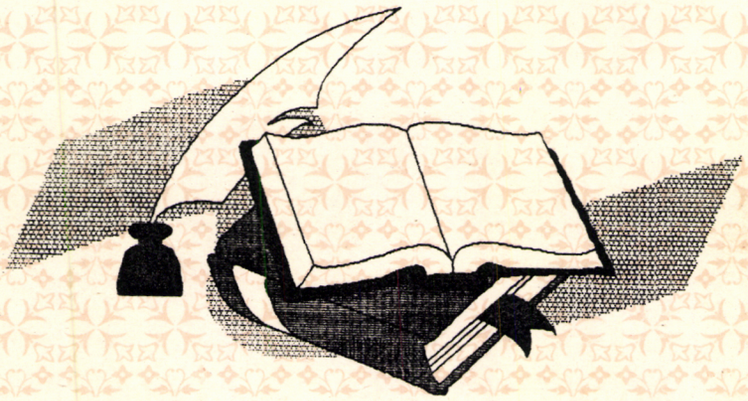
Qian et al (1988) reported that chromosome doubling of the F_1 hybrid from the cross *G. hirsutum* x *G. raimondii* to overcome its infertility.

Lu Huangjan (1995) observed the pollen mother cells (PMCs) of interspecific cotton hybrid TM-1 (Texas Marker 1, *G. hirsutum*) x Shixiya 1 (*G. arboreum*), TM-1 x *G. armourianum* and TM-1 x *G. thurberi* during meiosis. The chromosome configuration of most PMCs at metaphase - I in these hybrids was 13_{II} + 13 I. Bivalents synapsized into ring, rod or v-shape. The synapsis of bivalents in TM-1 x *G. armourianum* was more close than in the other two hybrids. Hybrids produced anomalous synapsis of AD chromosomes and multivalents as well as normal synapsis of AA and DD chromosme. Most univalents at metaphase-I scattered on the two sides of the equatorial on the two sides of the equatorial plate and moved earlier to the poles. One or two bivalents were not on the equatorial plate. Chromosome bridges were frequently formed when bivalents moved towards the poles, which slowed down the the chromosomes and resulted

in non synchronization of cell division. Lagging chromosomes, scattered chromosomes far from most other chromosomes and lonely chromosome groups were found during the first and second divisions. PMCs yielded more nucleoli at telophase-II. At the tetraspore stage, the three hybrids produced not only tetrads but also polyads (2-10 microspores) with the tetrad percentages being 70.2%, 63.1% and 35.1%, respectively in TM-1 x Shixiya 1, TM-1 x *G. armourianum* and TM-1 x *G. thurberi*.

Intense directional selection by humans has consistently produced AD tetraploid cottons that have superior yield and or quality characteristics compared to the A genome diploid cultivars Lee (1984). Selective breeding of *G. hirsutum* (AADD) has emphasized maximum yield, whereas *G. barbadense* (AADD) is prized for its fibers of superior length, strength and fineness. Side-by-side trials of 13 elite *G. hirsutum* genotypes and 21 *G. arboreum* diploids (AA) adapted to a common production region (India) show average seed cotton yield of 1.135 $\pm$ 90 kg/ha for the tetraploids, a 30% advantage over the 903 $\pm$ 78 kg/ha of the diploids, at similar quality levels. Anonymous (1997b), such an equitable comparison can not be made for *G. barbadense* and *G. arboreum* because they are bred for adaptation to different production regions. However, the fiber of "extralong-staple" *G. barbadense* tetraploids, representing 25% of the World's

cotton, commands a premium price due to 40% higher fiber length (~35 mm), strength (~ 30 g per tex or more), and fineness over leading A genome cultivars, Anonymous (1997b), at similar yield levels obsolete *G. barbadense* cultivars reported by had up to 100% longer fibers (50.8 mm, Niles and Feaster (1984) than modern *G. arboreum* (25.5 $\pm$ 1.6 mm, Anonymous (1997b).



MATERIAL AND METHODS

3. MATERIALS AND METHODS

The present research work entitled "Induction of polyploidy in haploid of *G. hirsutum* L. and diploid of *G. arboreum* L." was undertaken at "All India Coordinated Cotton Improvement Project", Mahatma Phule Krishi Vidyapeeth, Rahuri, District-Ahmednagar, which is located at an altitude of 532 meters between 19° 47' N to 19° 57'N latitude and 74° 82' E to 74° 91' E longitude. The investigation was conducted during the period 1996-98.

The details of materials used and methods followed during the course of these studies are given below.

3.1 Experimental material

The seed of *G. hirsutum* L. varieties RHCr003, JLH-168 LRA-5166 and *G. arboreum* L. varieties Y-1, PA-304, Namdeo, Sawta, PA-333 were included in present investigation.

3.1.1 Method-I

Methods used to induce three haploids are shown below :

The haploids induced by Chikhale (1998) in *G. hirsutum* variety RHCr003 through gamma ray treatment (25 kR) and in JLH-168 and LRA-5166 by toleudine blue (0.2% and 0.4%) treatment were used for the present study.

3.1.1.1 Method-I (Gamma ray irradiation)

RHCr003 varieties seeds were irradiated with gamma ray with 15 KR, 20 KR, 25 KR doses and were sown with their respective controls (unirrigated).

3.1.1.2 Method-II (Toleudine - blue treatment)

Pollen grains treated with 0.2%, 0.4%, 0.8% and 1% toleudine blue solutions were used to pollinate the flower buds emasculated on previous day. The pollination was done during 8 to 11 a.m. and boll shedding percentage were monitored after every day.

3.1.2 *G. arboreum* varieties are treated with colchicine to doubled their chromosome number

1) Y-1
2) PA-304
3) Namdeo
4) Sawta
5) PA-333

3.1.3 Doubling of chromosomes

For the purpose of doubling of chromosome following treatments are given (Plate 1 and Plate 2).

1) Colchicine treatment : *G. hirsutum* the concentration of colchicine used was 0.5%, 0.2%, 0.1%, 0.05%. The distilled water was used as control. The treatment was given for the duration of 12, 18 and 24 hrs. The auxilary

Plate 1 Methods of treating seedling different plant part with different colchicine

Fig. 1

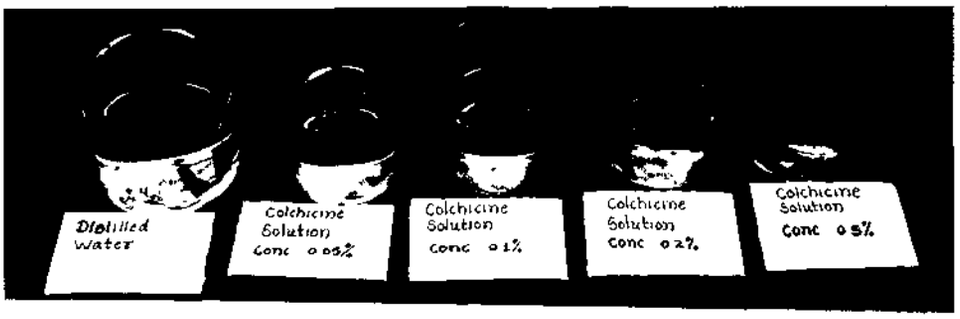


Fig. 2

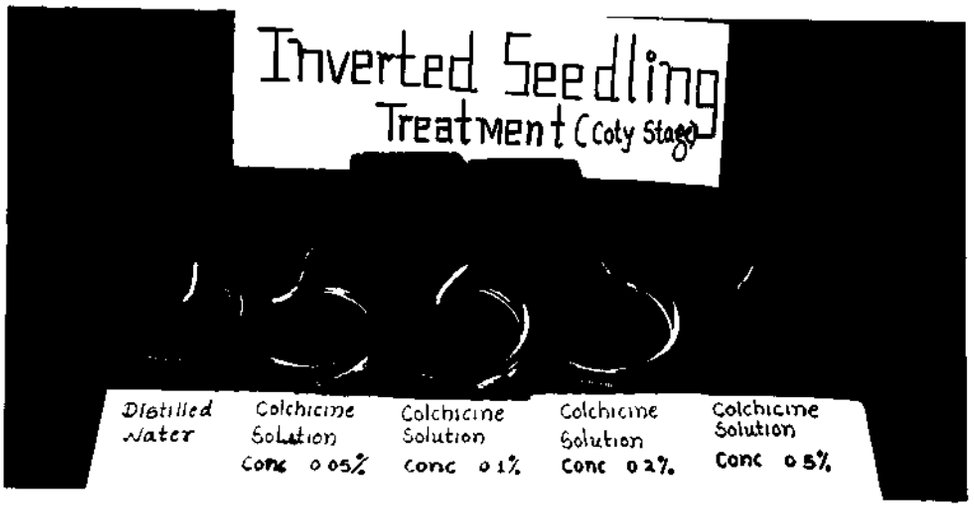


Fig. 3

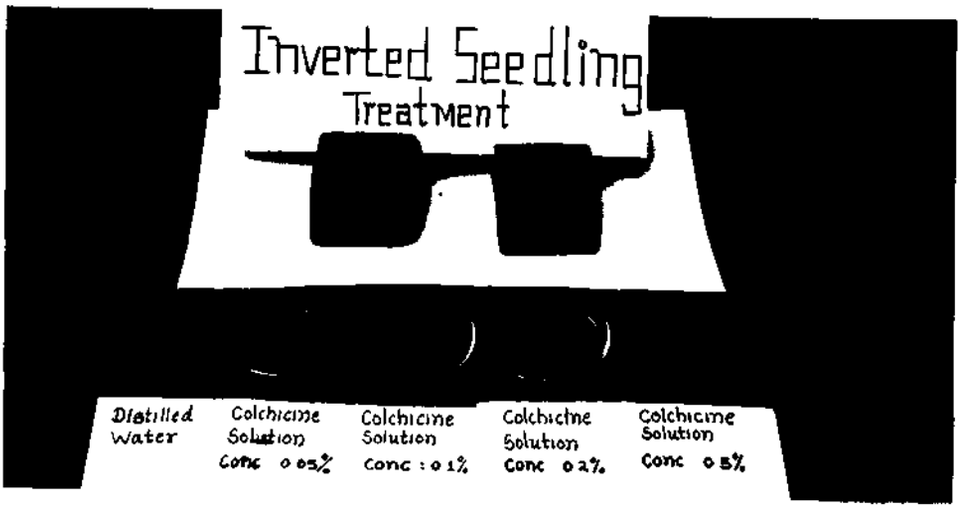


Fig. 4

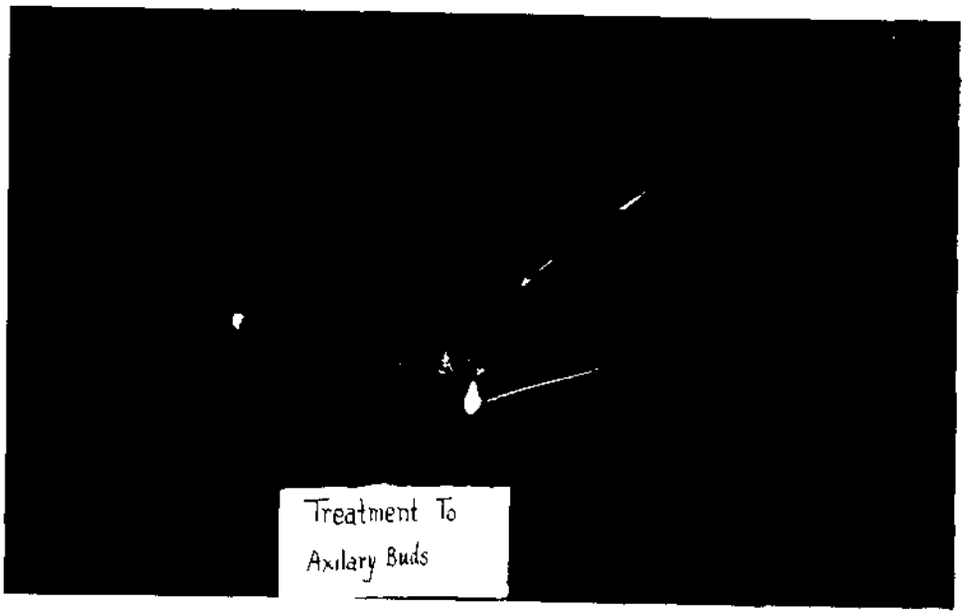


PLATE -1

Plate 2 Methods of treating seedling different plant part with different colchicine

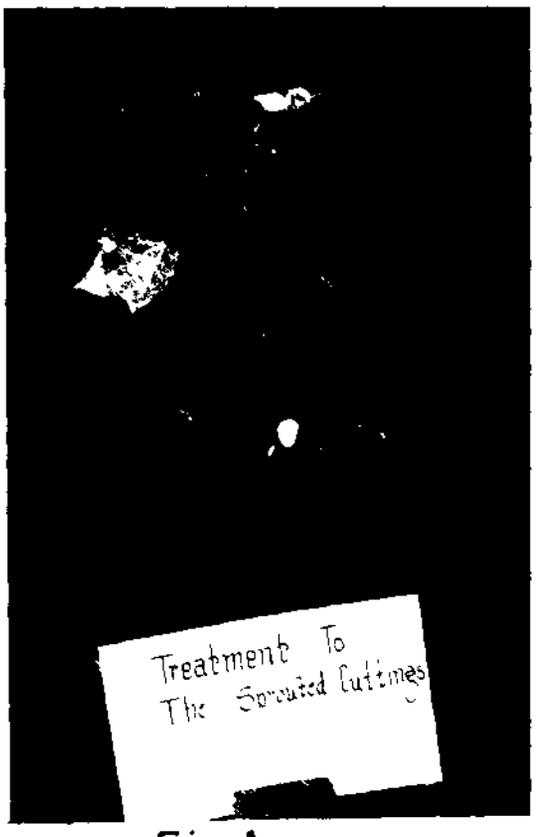


Fig. 1

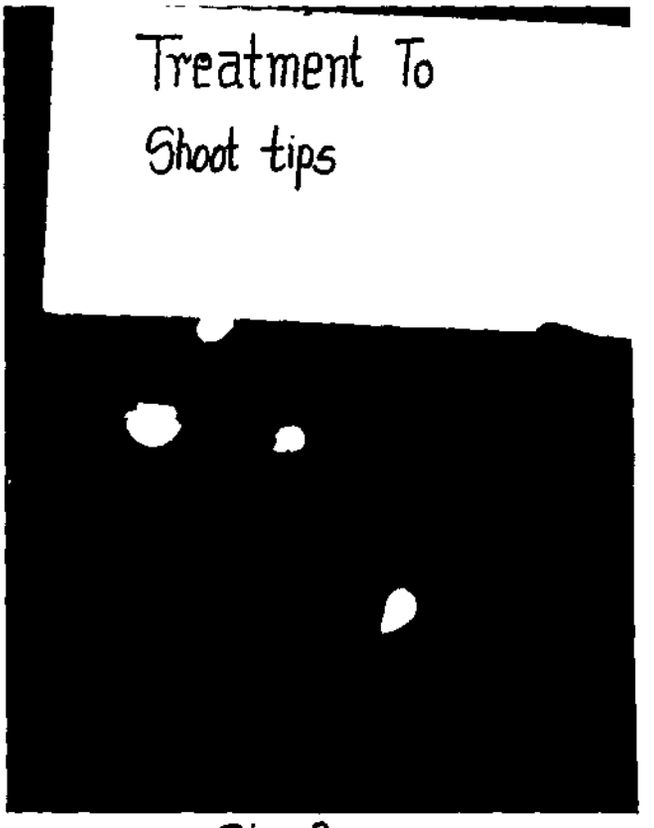


Fig. 2

Fig. 3

PLATE - 2

Plate 3 Comparison morphological leaves, flowers and floral parts of a haploid and colchicine doubled colchiploid of *G. hirsutum* L. cotton.

Fig. 1

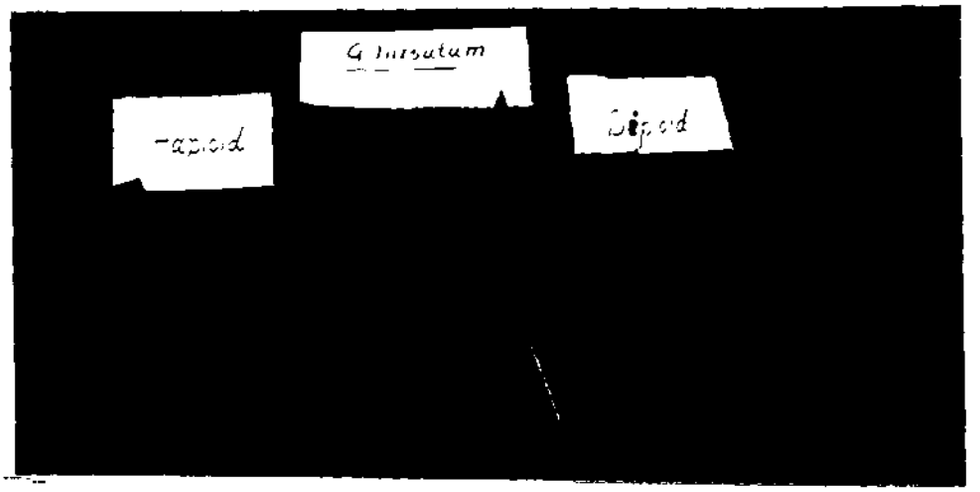


Fig. 2

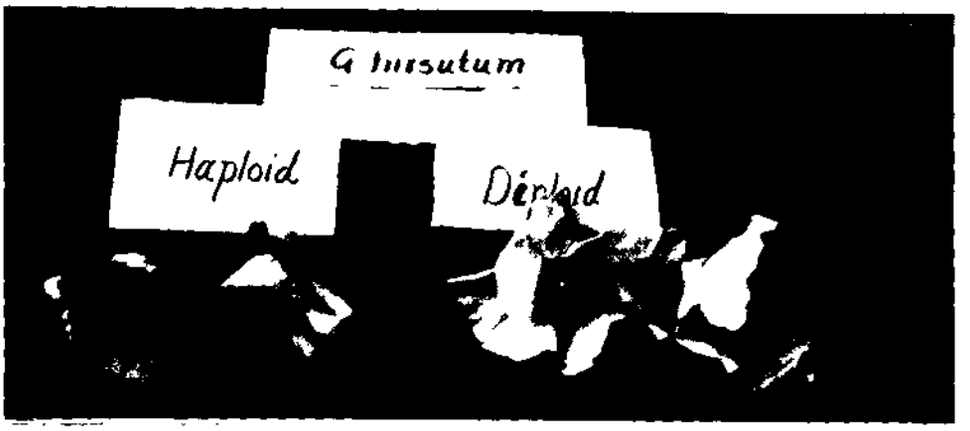


Fig. 3

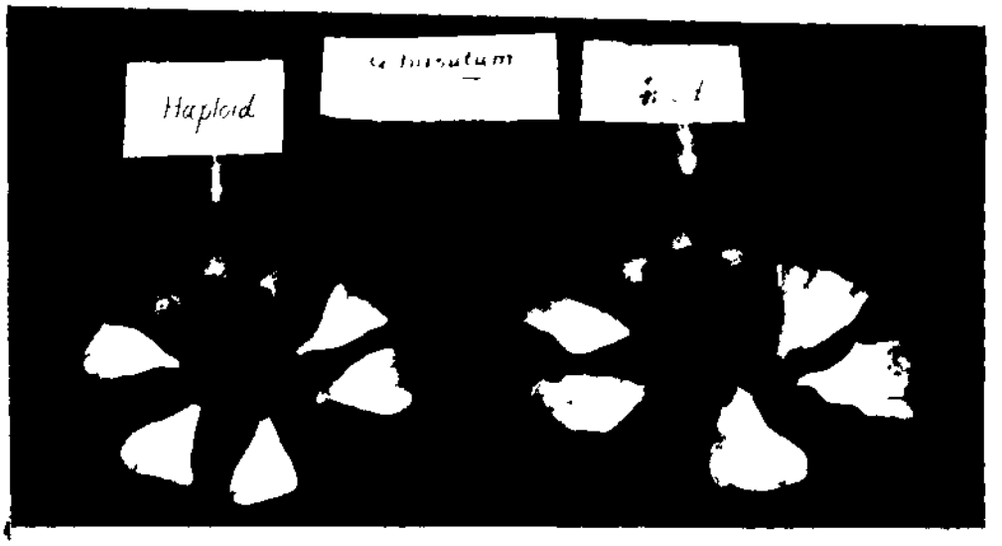


PLATE - 3

buds and shoot tips of haploid plants were treated with colchicine.

G. arboreum : by dipping shoots of 10 days old seedling in colchicine concentration of 0.05%, 0.1%, 0.2%, 0.5%.

2) Treatment of Colchicine + lanoline paste to apical and axillary buds of haploids.

a) Lanoline paste - 0.2% colchicine + lanoline homogenious mixture was prepared on weight/weight basis and applied uniformatly to the auxillary and apical sprouting buds during 7.30 a.m. to 8.30 a.m. The paste was retained for 40 hrs and then removed. The residues of paste was removed.

3) Exposure of sprouted cuttings to extreme low and high temperature shocks.

Standardization of high temperature above 40°C each exposure to high temperature of 1°C. Beyond 44°C there was scortchining and deating shoot therefore all treatments are limited to the temperature 44°C.

3.2 Methods

3.2.1 Cytological technique for study of meiosis

To study the chromosome number of all haploids and colchicine treated plants and branches, the flower buds were fixed in cornoy's fluid. (6 parts of absolute ethyl alcohol : 3 parts chloroform : 1 part glacial acetic acid). This formula was modified from time to time for thick and non-

dehiscent anthers. The buds of 4-6 mm size were fixed during period from 7.45 to 8.30 a.m. in screw caped 10 ml corning vials, labelled and stored in the refrigerator for 24 hours. Then the antehrs were squeezed in 1% aceto-carmine or 2% propionocarmine stains.

The slide with cover slips were then pressed firmly and heated gently and then observed under microscope. The cells were observed from pachytene to pollen formation stage. The selected slides were made permanent by destaining with 45 per cent propionic acid replaced with n-butyl alcohol and finally mounted in Canada balsam (Johnson, 1940).

3.2.2 Preparation of permanent slide for meiotic study

The slides having good plates at metaphase-I and anaphase-I were temporarily sealed with parafin. The slides were made permanent on the next day by the procedure given by Celarier (1956) and Jadhav (1961) as follows.

The temporary seal was removed. The slide were kept facing the coverslip downwards in the ridged porcelain dish containing 45 per cent propionic acid. As soon as the coverslip was detached from slide, the sides of the slide were changed. After five minutes the slide and coverslip were passed through following series, keeping in each for three minutes.

i) Equal part of propionic acid and n-butyl alcohol.

ii) One part of propionic acid and three parts of n-butyl alcohol.

iii) n-butyl alcohol

The slide was removed and placed on a piece of blotting paper, so as to remove the excess of n-butyl alcohol. A small drop of canada balsm was placed on the squashed material and the coverslip was placed in its original position. For quick and better results the slides were fixed in liquid nitrogen to about -160 to -190°C by immersing slides into cylinder containing liquid nitrogen.

From such preparations, plates showing well separated chromosomes were selected for actual observations on chromosome behaviour during meiosis studies in the *G. hirsutum* and *G. arboreum* plants and different meiotic stages by examining number of PMC with except to chromosome associations, such as univalents, bivalents. Also the distribution of chromosomes at anaphase-I were recorded in different haploid plants.

3.2.3 Micro-photography

Microphotographs were taken with the help of automatic 35 mm camera fixed to Leitz research microscope at the magnification of 1000 x. ORWO Panchromatic - 35 mm film of 125 ASA was used for taking photographs. In order to get

good contrast green and yellow filters were used. Photographs were taken at varied intensities of light. The film was developed by photographer in the University Photographic Laboratory.

3.2.4 Camera lucida drawings

The camera lucida drawings were made at stage level, using oil immersion lens 95 x and eye piece of lox, Hard Bristol paper, 5HB pencil with fine point and Reeves India ink.

3.2.5 Pollen sterility tests

For the pollen viability flowers were collected during 8-10 a.m. which were selfed on previous day and used for sterility test by following tests.

i) Iodine test

The solution was prepared by adding one gram potassium iodide (IKI) and one gram Iodine crystal in 70 per cent alcohol. The pollen grains were dusted on slide in a drop of IKI solution, then coverslip was placed and drop of one per cent IKI solution was passed from the corner of coverslip, slides were labelled and allowed to stain and pollen grains were classified as dark stained, medium stained and unstained. Dark stained pollens were identified as fertile, unstained as sterile pollens and medium stain pollens are identified as partial sterile.

ii) Propiono - carmine test

One per cent carmine solution prepared is 45 per cent propionic acid was used. The slides were prepared by staining pollen grains with 1 per cent propino carmine and slides were labelled.

iii) Differential staining test

To classify the pollens as sterile and fertile, which was the limitaion of carmine and iodine test, the differential stain as suggested by Alexander (1969) was used to overcome the difficulty. The strain was prepared by dissolving the ingredients in the following order.

1) Malachite green - 10 mg (1 ml of 1% in 95% of ethanol)
2) Distilled water - 50 ml
3) Glycerol - 25 ml
4) Phenol - 5 ml
5) Chloral hydrate - 5 gm
6) Acid fuschin - 0.5 mg (5ml of 1% solution in water)
7) Orange `G' - 5 mg (0.5ml of 1% solution in water)
8) Glacial acetic acid- 5 - 10 ml

The stain was stored in coloured bottles in the refrigerator. To get quick results pH of the stain was maintained at 3.2. It can be acidified by adding a small quantity of propionic acid. The slides were observed under 100 x magnification. The fertile pollens stained red whereas the nonviable sterile pollens were stained green.

3.2.6 Pollen germination test

The method adopted for study of *in vitro* germination of pollen was as follows (Iyengar, 1939 and Sharipov, 1987).

Pollens were collected at the time of anthesis and placed in sucrose solution of different concentration in cavity slides and coverslip. Germination of pollen grain was completed within an hour after their sowing. Glycerine - aceto carmine (1:1) was added for clarity in counting the number of germinated pollen grains.

3.2.7 Determination of pollen grain size

Pollen grain size was estimated with the help of stage and occular micrometers by adopting following procedure.

1) Place occular micrometer, draw tube of microscope, see whether the divisions of occular micrometer are distinctive or otherewise.
2) Then stage micrometer was mounted on stage microscope and observed.
3) Callibration

The division of the occular micrometer which coincide the divisions on stage micrometer under 40 x were counted. The calculated value of occular division as :

$$\frac{\text{No. of occular division coincide with stage}}{\text{No. of stage division coincide with occular}} \times \text{value of one stage division } (\mu)$$

4) Remove the stage micrometer

5) Mount the slide containing pollen grains/stomata

6) Measure the diameter of pollen grains in terms of number of occular divisions.

Sr.No.	Size of Pollen grain	Average
1.		
2.		
!		
100		

$$\text{Size of pollen in microns} = \text{Average size of pollen in occular division} \times \text{Value of occular division in microns } (\mu)$$

3.2.8 Number of stomata per unit area

The number of stomata in one microscopic field was measured and average of 10 counts were worked out. Area of one microscopic field was calculated. All observations were recorded under 45 x magnification.

3.3 Observations

3.3.1 Morphological observations

Observations on the following morphological characters were recorded. The mean and coefficient variation were calculated as per (Mehetre and Thombre, 1978). Ten

random samples were selected for measurement in these haploids and their diploid counter parts.

1) Plant height (cm)
2) No. of sympodia
3) No.of monopodia
4) Internodal length (cm)
5) Leaf area (Sq.cm)
6) Petiole length (cm)
7) Stomata size (μ)
8) Chloroplasts/stomata
9) No.of anthers/flower
10) Length of style (cm)
11) Petal length (cm)
12) Bracteole/teeth
13) Boll diameter (cm)
14) No.of boll / plant
15) Boll weight (g/boll)
16) Lint weight (g/boll)
17) Seed weight (g/boll)
18) No.of seed/boll
19) No. of motes/boll
20) Seed cotton yield/boll

For all the above observations the difference between the haploids and parental means were compared and the coefficient of variation for the mutant characters were calculated.

3.3.2 Cytological observations

3.3.2.1 Observations on meiosis and pollen development

Description of plants	Meiotic stage	PMCs examined (No.)	Chromosome Associations			
			I	II	III	IV
parents						
hybrids						
colchiploid						

3.3.2.2 The distribution of chromosomes at anaphase-I

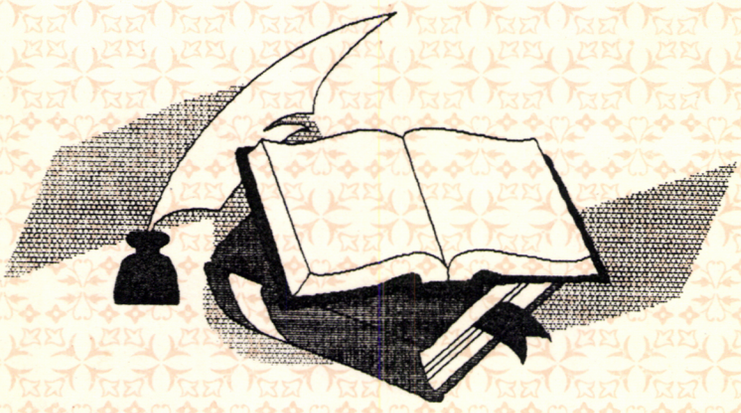
Haploid	No. of PMCs examined	13/13	12/14	11/15	10/16	9/17	8/18	Others
Parents								
hybrids								
colchiploid								

3.3.2.3 Studies on pollen

Plants	Pollen fertility/sterility	Pollen germination	Pollen size
Haploids			
Parents			
hybrids			
colchiploid			

3.3.2.4 Statistical analysis

The effect of reduction in chromosome number / hybridization doubling of chromosome numbers on different morphological characters of the treatments are compared with their parents by studying significance of difference of means and coefficients of variation calculated as per Snydecor and Cochran (1967).



RESULTS

4. RESULTS

The present work entitled " Induction of polyploidy in haploid of *G. hirsutum* and diploid of *G. arboreum* L." was undertaken with a view to obtain doubled chromosome number in the haploids of *G. hirsutum*, study the cytomorphology and pollen fertility in induced polyploid C_1 plants and to obtain F_1 interspecific cross between doubled *G. arboreum* x *G. hirsutum* L. cotton.

The haploids induced by Chikhale (1998) in *G. hirsutum* variety RHCr003 through gamma ray treatment (25 kR) and in JLH-168 and LRA-5166 by toleudine blue (0.2% and 0.4%) treatment were used for the present study.

The results obtained during present investigation are presented below.

4.1 Studies on morphological characters of haploid (n=2x=26) in G. hirsutum variety RHCr003

The observations were recorded for different morphological characters in the haploid of G. hirsutum var. RHCr003 induced by gamma ray irradiation with 25 kR dose, along with its parent and coefficient of variation (c.v.) for different characters in haploid and its parent are presented in Table 1.

1. **Plant height (cm)/plant :**

Average plant height in parent RHCr003 variety was 143.24 cm while in its haploid plant height was 74.18 cm showing reduction in about 2:1 ratio.

2. **Number of sympodia/plant :**

Sympodial branches were observed in both haploid and parental variety. In haploid there was 4 sympodial branches while in parent there were 6 branches. C.V. was not worked out for haploids and parent.

3. **Number of monopodia/plant :**

In haploid, number of monopodial branches reduced, however, this was not in 1:2 proportion as compared to diploid parent. The *G. hirsutum* haploid had one monopodia where as its parent had three monopodia. In haploid and parent C.V. was not worked out.

4. **Internodal length (cm)/internode :**

The haploid showed reduced internodal length. The internodal length was reduced to about one half of its parent. The mean of internodal length in haploid was 3.60 cm while in its parent it was 6.40 cm. While c.v. was 12.40 and 19.40 in haploid and parent, respectively.

5. **Leaf area (sq.cm/leaf) :**

Leaf area in haploid was found to be reduced. The reduction was about in 2:1 ratio. The leaf area of haploid was 69.38 sq.cm and its c.v. was 18.68 while that of parent was 121.72 sq.cm with c.v. 26.36.

Table 1. Morphological characters studied in Haploid & parent of G. hirsutum var. RHCroo3 haploid induced by gamma ray irradiation 25 kR alongwith coefficient of variation for different characters

Sr. No.	Characters	Haploid		Parent	
		Mean	c.v.%	Mean	c.v.%
1.	Plant height (cm)/plant	74.18	-	143.24	-
2.	Number of sympodia/plant	4.00	-	6.00	-
3.	Number of monopodia/plant	1.00	-	3.00	-
4.	Internodal length (cm)/internode	3.60	12.40	6.40	19.40
5.	Leaf area (sq.cm)/leaf	69.38	18.68	121.72	26.36
6.	Petiole length (cm)/petiole	5.22	11.50	11.32	8.42
7.	Stomata size (μ)/stomata	22.62	18.40	38.24	16.32
8.	No.of chloroplasts/stoma	18.24	16.20	18.32	7.30
9.	No. of anthers/flower	85.4	17.70	152.54	8.30
10.	Petal length (cm)/petal	2.45	9.90	4.25	5.40
11.	Style length (cm)/style	1.98	5.44	3.40	2.08
12.	No.of bracteoles teeth/bracteole	6.49	8.80	8.45	7.35
13.	Boll diameter (cm)/boll	-	-	5.54	8.35
14.	No. of bolls/plant	-	-	56.00	13.40
15.	Boll weight (g/boll)	-	-	4.22	7.82
16.	Lint weight (g/boll)	-	-	2.08	6.80
17.	Seed weight (g/boll)	-	-	2.24	8.32
18.	No. of seeds/boll	-	-	20.38	6.60
19.	No. of motes/boll	-	-	0.60	-
20.	Seed cotton yield/plant	-	-	170.80	11.52

6. Petiole length (cm)/petiole :

Petiole length of haploid was 5.22 cm while in parent it was 11.32 cm which indicated the reduction in petiole length was about 2:1 ratio between parent and haploid respectively. The c.v. was 11.50 and 8.42 for haploid and parent, respectively.

7. Stomata size (μ/stoma) :

The stomata size in haploid was 22.62 μ with c.v. 18.40 while its parent had 38.24 μ with c.v. 16.32 indicated significant reduction in stomata size in haploid, though significant was not close to 1:2 ratio.

8. Number of chloroplast/stoma :

Number of chloroplast in guard cells of stomata in haploid and its parent indicated that ratio of number of chloroplasts was approximately equal to 1:2. The difference in the number of chloroplast between these two ploidy levels was significant and similar trend of variation was observed. The mean number of chloroplast per stoma in haploid was 18.24 with c.v. 16.20 while its parent was 18.32 chloroplast with c.v. 7.30.

9. Number of anthers/flower :

A significant reduction in anther number was observed in haploid as compared to their parents. The number of anthers/flowers in haploid was 85.40 with c.v. 17.70 while its diploid counterpart had 152.54 anthers per flower with

c.v. 8.30. The reduction in number was not, however, in 2:1 ratio of parent and haploid respectively.

10. Petal length (cm/petal) :

Length of petal studied at both the ploidy levels indicated that there was a significant reduction at haploid level than that of its respective parent. The mean petal length in haploid was 2.45 cm while its parent had 4.25 cm. The c.v. for petal length was 9.90 in haploid while its was 5.40 in parent.

11. Style length (cm)/style :

The length of style measured in haploid of *G. hirsutum* was 1.98 cm and its parent had 3.40 cm indicated that length of style was reduced and highly variable in haploid as compared to its parent. The c.v. was 5.44 in haploid and in parent it was 2.08.

12. Number of bracteoles teeth/bracteole :

No significant difference was found between haploid and parent bracteole teeth number. The variation in bracteole teeth number was almost similar at both ploidy levels. The c.v. was 8.80 and 7.35 in haploid and parent, respectively.

The haploid plant of RHCroo3 variety of *G. hirsutum* was highly male and female sterile hence did not set fruits. Hence, the observations for boll, lint, seed and yield was not recorded in haploid plant.

4.2 Studies on morphological characters of haploids in *G. hirsutum* variety JLH-168 0.2% toleudine blue treatment

The data on the observations were recorded for different morphological characters in the haploid of *G. hirsutum* var. JLH-168 induced by 0.2% toleudine blue treatment and its parent are presented in Table 2.

1. Plant height (cm/plant) :

The haploid was 64.23 cm in height while average height in parental variety was 132.40 cm. Thus height in haploid plant was reduced considerably.

2. Number of sympodia/plant :

The mean no. of sympodia in parent was 6.00 as against 2.00 in haploid. Also sympodia in haploid was very short than its parent.

3. Number of monopodia/plant :

The number of monopodia in haploid was 1.00 while in parent it was 5.00.

4. Internodal length (cm/internode) :

Mean internodal length was 2.75 cm in the haploid plant while its parent was 5.80 cm. The c.v. for this character shows similar trend at both ploidy levels.

5. Leaf area (sq.cm/leaf) :

The leaf area in haploid and parent was 61.30 sq.cm. and 123.20 sq.cm respectively with c.v. 16.32 and 21.40 for haploid and parent respectively.

Table 2. Morphological characters studied in *G. hirsutum* var. JLH-168 induced by 0.2% toleudine blue treatment alongwith coefficient of variation for different characters

Sr. No.	Characters	Haploid		Parent	
		Mean	c.v.%	Mean	c.v.%
1.	Plant height (cm)/plant	64.23	-	132.40	-
2.	Number of sympodia/plant	2.00	-	6.00	-
3.	Number of monopodia/plant	1.00	-	5.00	-
4.	Internodal length (cm)/internode	2.75	13.30	5.80	15.30
5.	Leaf area (sq.cm)/leaf	61.30	16.32	123.20	21.40
6.	Petiole length (cm)/petiole	4.30	11.40	9.30	7.50
7.	Stomata size (μ)/stomata	17.20	15.20	33.70	14.40
8.	No.of chloroplasts/stoma	6.60	14.30	16.32	8.20
9.	No. of anthers/flower	76.30	19.40	134.00	9.32
10.	Petal length (cm)/petal	3.40	6.36	5.36	6.30
11.	Style length (cm)/style	2.32	7.50	4.50	2.50
12.	No.of bracteoles teeth/bracteole	6.50	7.30	8.80	5.50
13.	Boll diameter (cm)/boll	-	-	4.30	10.50
14.	No. of bolls/plant	-	-	46.0	8.50
15.	Boll weight (g/boll)	-	-	3.80	8.50
16.	Lint weight (g/boll)	-	-	2.40	6.68
17.	Seed weight (g/boll)	-	-	2.80	5.80
18.	No. of seeds/boll	-	-	21.30	7.84
19.	No. of motes/boll	-	-	0.30	-
20.	Seed cotton yield/plant	-	-	130.00	13.30

6. Petiole length (cm/petole) :

Average petiole length in haploid plant was 4.30 cm while its parent has 9.30 cm. The c.v. was 11.40 and 7.50 in haploid and parent, respectively shows similar trend at both ploidy levels.

7. Stomata size (μ/stomata) :

The mean stomata size in haploid leaf cells was 17.20 μ and 33.70 μ/stomata respectively indicating significant reduction in stoma size in haploids. The c.v. in haploid was 15.20 and 14.40 in its parent.

8. Number of chloroplast/stoma :

The number of chloroplast/cell in guard cells stomata in haploid and parent averaged 6.60 and 16.32, respectively. The coefficient of variation was 14.30 and 8.20, respectively in haploid and parent.

9. Number of anthers/flower :

The mean number of anthers/flower was reduced to 76.30 in haploid as compared to parent anthers 134.00/flower. Thus there was a significant difference between haploid and parent anther number. In haploid there was a greater variation from flower to flower for this character than in its parents.

10. Petal length (cm/petal) :

The average petal length in haploid was 3.40 cm with c.v. 6.36 and in parent 5.36 with c.v. 6.30. Thus the observed difference was significant.

11. Style length (cm/style) :

The style length in haploid was 2.32 cm while in parent it was 4.50 cm.

12. Number of bracteole teeth/bracteole :

No significant difference was found for no. of bracteole teeth/bracteole between haploid and parent. The trend of variation in bracteole teeth number was almost similar at both the ploidy levels in haploids studied.

The haploid plant of JLH-168 variety of *G. hirsutum* was highly male and female sterile hence did not set fruits. Hence, observations for boll, lint, seed and yield was not recorded.

4.3 Studies on morphological characters of haploids (n=2x=26) in *G. hirsutum* var. LRA-5166 0.4% toleudine blue treatment

The observations were recorded for different morphological characters in the haploid of *G. hirsutum* var. LRA-5166 induced by toleudine blue treatment of 0.4% to pollen grains. Also coefficient of variation (c.v.) for different characters in haploid and its parent are presented in Table 3.

1. Plant height (cm/plant) :

The average plant height in parent was 148.30 cm while in its haploid it was 72.20 cm showing reduction was about 2:1 ratio.

2. Number of sympodia/plant :

Sympodial branches were observed in both haploid and parental variety. In haploid there was 2.00 sympodial branches while in parent there was 5.00 branches.

3. Number of monopodia/plant :

In haploid number of monopodial branches were reduced. The haploid had 2.00 monopodia whereas parent had 4.00 monopodia / plant. It shows that reduction was about 1:2 ratio.

4. Internodal length (cm/internode) :

The haploid showed reduced internodal length. The reduction was about one half in haploid. The mean internodal length in haploids was 2.00 cm while in its parent it was 6.90 cm while the c.v. was 8.90 and 12.10 in haploid and its parent respectively.

5. Leaf area (Sq.cm/leaf) :

Leaf area in haploid was found to be reduced. The reduction was about one half in haploid. Leaf area in haploid was 68.40 sq.cm. and its c.v. was 16.20 while that of parent was 145.30 sq.cm. with c.v. 10.30.

6. Petiole length (cm\petiole) :

Petiole length of haploid was 3.20 cm while in parent it was 8.40 cm which indicate that the reduction in petiole length was about 2:1 ratio in parent and haploid respectively. The c.v. was 14.50 and 23.40 in haploid and parent respectively.

Table 3. Morphological characters studied in *G. hirsutum* var. LRA-5166 induced by using 0.4% toleudine blue treatment along with coefficient of variation for different characters

Sr. No.	Characters	Haploid		Parent	
		Mean	c.v.%	Mean	c.v.%
1.	Plant height (cm)/plant	72.20	-	148.30	-
2.	Number of sympodia/plant	2.00	-	5.00	-
3.	Number of monopodia/plant	2.00	-	4.00	-
4.	Internodal length (cm)/internode	2.00	8.90	6.90	12.10
5.	Leaf area (sq.cm)/leaf	68.40	16.20	145.30	10.30
6.	Petiole length (cm)/petiole	3.20	14.50	8.40	23.40
7.	Stomata size (μ)/stomata	15.16	15.12	34.30	12.20
8.	No.of chloroplasts/stoma	18.20	19.60	13.40	6.70
9.	No. of anthers/flower	8.20	12.10	166.30	8.40
10.	Petal length (cm)/petal	2.10	8.68	6.30	5.90
11.	Style length (cm)/style	2.22	11.20	4.10	3.50
12.	No. of bracteoles teeth/bracteole	3.23	12.40	7.30	7.10
13.	Boll diameter (cm)/boll	-	-	3.80	12.50
14.	No. of bolls/plant	-	-	43.00	9.10
15.	Boll weight (g/boll)	-	-	3.85	10.20
16.	Lint weight (g/boll)	-	-	2.12	5.60
17.	Seed weight (g/boll)	-	-	2.30	4.30
18.	No. of seed/boll	-	-	19.40	8.70
19.	No. of motes/boll	-	-	0.20	-
20.	Seed cotton yield/g/plant	-	-	98.20	15.20

7. Stomata size (μ/stomata) :

The stomata size in haploid was 15.16 μ with c.v. 15.12 and in parent was 34.30 μ with c.v. 12.20 indicated significant reduction in stomata size in haploid, though significance was not close to 1:2 ratio.

8. Number of chloroplast / stoma :

The number of chloroplast in guard cells of stomata in haploid and its parent indicated that ratio of number of chloroplast was approximately equal. The mean of number of chloroplast in haploid was 18.20 with c.v. 19.60 and its parent was 19.40 and c.v. 6.70.

9. Number of anthers / flower :

Number of anthers/flower in haploid was 8.20 with c.v. 12.10 and its parent was 166.30 with c.v. 8.40. The reduction was not, however, in 2:1 ratio between parent and haploid but it was significant reduction.

10. Petal length (cm/petal) :

The length of petal in haploid was 2.10 cm and its parent counterpart was 6.30 cm. The c.v. was 8.68 and 5.90 in haploid and parent, respectively indicated that reduction was highly variable in haploid as compared to its parent.

11. Style length (cm/style) :

The length of style in haploid was 2.22 cm while its parent was 4.10 cm with c.v. 11.20 and 3.50 in haploid and parent respectively.

12. Number of bracteole teeth/bracteole :

No significant difference was found for bracteole teeth/bracteole in haploid and parent. The mean of haploid was 3.23/bracteole and its parent was 7.30 / bracteole with c.v. 12.40 and 7.10 in haploid and parent respectively.

The haploid plant of LRA-5166 was highly male and female sterile hence did not set fruits. Hence the observations for boll, lint, seed and yield were not recorded in haploid.

4.4 Cytological studies of haploids

4.4.1 Cytological studies of gamma rays induced haploid (n=2x=26) in *G. hirsutum* var. RHCr003

The data on meiotic studies in gamma rays induced *G. hirsutum* cotton variety RHCroo3 haploid and its parent is presented in Table 4 and 5.

4.4.1.1 Chromosome association at meiosis in haploid and its parent

The data on meiotic studies is presented in table 4 which indicate that in haploid on an average 1.0, 2.10 and 2.00 bivalents were formed with 0.07, 0.11 and 0.22, chiasma per bivalent from pachytene to metaphase-I. However, there are no trivalents and tetravalents in haploid.

In parent there was 26.00 bivalent formed at each stage of meiosis from pachytene to metaphase-I. While there was 0.21, 0.33 and 0.47 chiasma per bivalent from pachytene to Metaphase-I, respectively. There was no trivalents and tetravalents in parents.

Plate 4 Meiosis in normal and colchicine doubled plants of *G. arboreum* cotton.

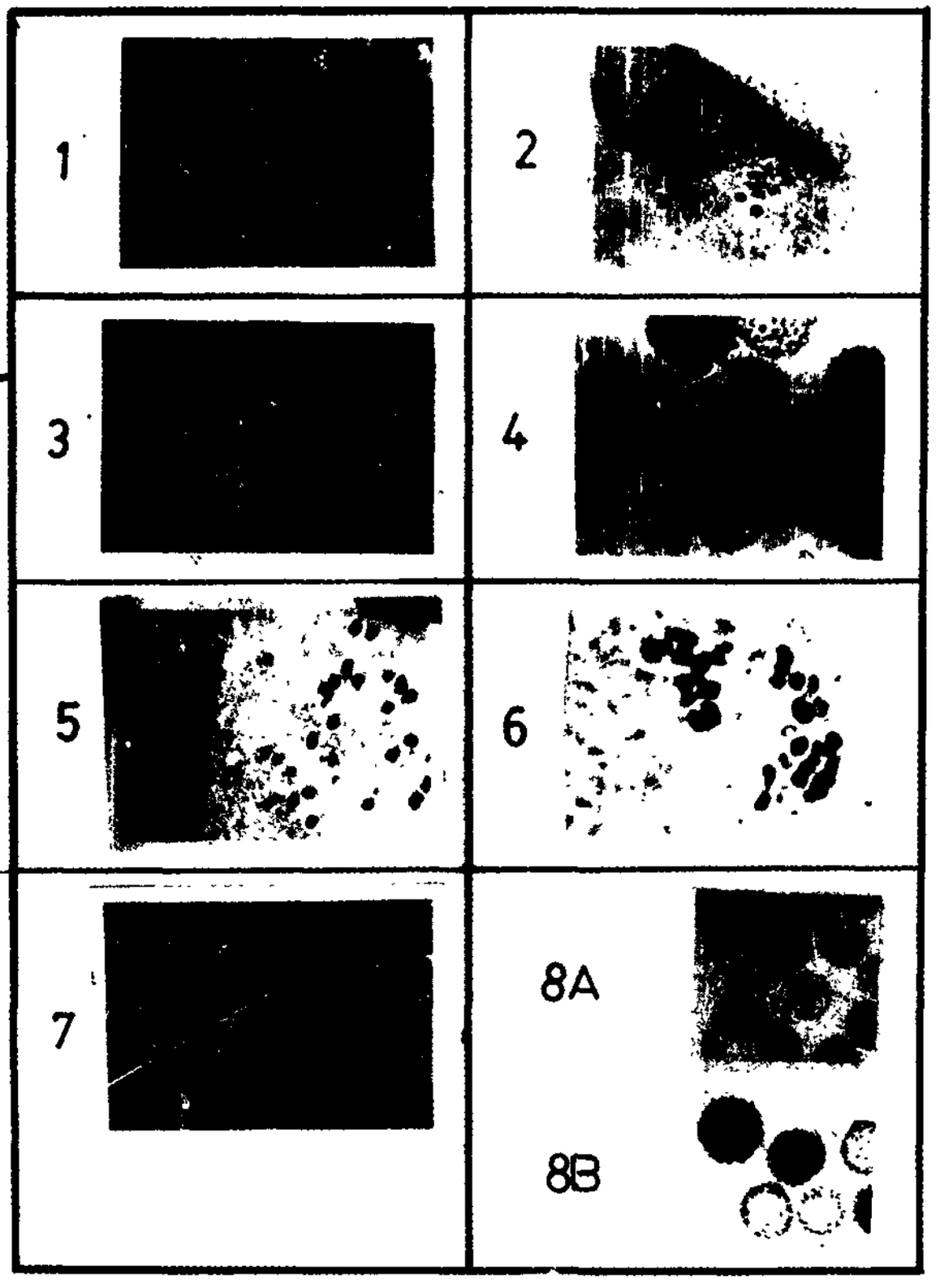


PLATE-4

Description of figures included in Plate-5

Plate 5

1. Normal meiosis and normal bivalents formed in *G. hirsutum* var. JLH-168 (950 x)

2. Normal fertile pollen with uniform size in *G. arboreum* Var Y-1(450 x)

3. Bivalents, quadrivalents and occasional octavalent observed in chromosome doubled *G. arboreum* var. Y-1.

4. Unequal distribution of chromatids at different poles at anaphase-II (750 x) in colchiploid *G. arboreum* var Y-1.

5. Unequal furrowing of cytoplasm and unequal separation of chromatids at late anaphase-II in doubled chromosome plant of *G. arboreum* L. var. Sawta

6. Sporads with one micronuclei (450 x) in haploid of LRA-5166.

7. Unequal separation of univalents at anaphase-I and in haploid of *G. hirsutum* var. JLH-168 (750 x).

8(A). Sporad with one micronuclei in haploid of *G. hirsutum* variety JLH-168 and

8(B) Pollen showing differential staining intensity in doubled haploid of *G. hirsusutm* var RHCr003.

Table 4. The meiotic behaviour observed in gamma rays induced *G.hirsutum* cotton haploid and its parent var.RHCr003.

Sr. No.	Plant description	Meiotic stage	No.of PMCs examined	Chromosome association I	II	III	IV	X'ta/II
1.	Haploid	Pachytene	10	24.0	1.00	-	-	0.07
		Diakinesis	12	21.8	2.10	-	-	0.11
		Metaphase-I	16	22.00	2.00	-	-	0.22
2.	Parent	Pachytene	20	-	26.00	-	-	0.21
		Diakinesis	24	-	26.00	-	-	0.33
		Metaphase-I	18	-	26.00	-	-	0.47

4.4.1.2 Distribution of chromosome at anaphase in haploid and parent in *G. hirsutum* var. RHCr003

Table 5. The observations of chromosome at anaphase-I in gamma ray induced haploid in *G. hirsutum* cotton variety - RHCr003

Species	No.of PMCs examined	Chromosome distribution of anaphase - I in haploid						Chromosome distribution in parent at anaphase-I
		13/13	14/12	15/11	16/10	17/9	18/8	26/26
Haploid	30	4	8	7	5	4	2	-
Parent RHCroo3	30	-	-	-	-	-	-	30

It was observed that the separation of chromosome at each pole varied from 13/13 to 18/8 (Table 5). In haploid 4 PMCs showed 13/13, 8 showed, 14/12, 7 showed 15/11, 5 showed 16/10, 4 PMCs showed 17/9 and 2 PMCs showed 18/8 distribution of chromosome at anaphase-I. However parent showed 26/26 chromosomes in all 30 PMCs.

4.4.1.3 Study of pollen grain size in haploid and its parent

Pollen grains formed in this haploid were variable in size. The average size being 69.29 micron (Table 6). At least 4 per cent normal sized pollens were formed in haploid. The average size of pollens in parent was 114.36 micron.

Table 6. Pollen grain size in gamma ray induced haploid of *G. hirsutum* var RHCr003 alongwith its parent

Plant	Pollen grain size in occular unit (1 occular unit-5.10 µ)												
	5	6	7	8	9	10	11	12	13	14	15	16	17
Haploid	2	5	6	-	15	5	13	16	12	-	-	13	7
Parent	-	-	-	-	-	-	-	-	2	-	2	-	3

	Pollen grain size in occular unit (1 occular unit = 5.10 µ)										
	18	19	20	21	22	23	24	25	26	Av.size of pollen	Total pollen
Haploid	-	-	4	-	4	-	-	-	-	69.29	100
Parent	2	7	11	13	12	14	14	11	9	114.36	100

4.4.1.4 Pollen fertility and germination study in gamma ray induced haploid and its parent

Pollen sterility test with IKI propiono carmine and differential stain was carried out in haploid and its parent (Table 7).

Table 7. Study of pollen fertility, pollen germination per cent in haploid and its parent in RHCroo3 haploid

Species	Pollen fertility (%)	Pollen germination (%)	Pollen size (μ)
Haploid	0.50	3.40	63.29
Parent	89.40	82.30	114.36

The pollen fertility in haploid was 0.50 per cent while that of its parent was 89.40 per cent. Pollen germination in haploid was only 3.40 per cent and that of parent 82.30 per cent. The pollen size in haploid was 63.29 μ while that of its parent was 114.36 μ.

4.4.2 Cytological studies in haploid induced by 0.2% toleudine blue in JLH-168 variety of *G. hirsutum*

4.4.2.1 Chromosome association at meiosis in haploid and its parent in JLH-168 variety of *G. hirsutum*

Table 8. The meiotic behaviour observed in 0.2% toleudine blue induced *G. hirsutum* haploid and its parent var. JLH-168

Sr. No.	Plant Description	Meiotic stage	No.of PMCs examined	Chromosome association I	II	III	IV	X'ta/II
1.	Haploid-1	Pachytene	10	20.00	3.00	-	-	0.02
		Diakinesis	15	23.00	1.50	-	-	0.16
		Metaphase-I	14	22.00	2.00	-	-	0.08
2.	Parent	Pachytene	10	-	26.00	-	-	0.32
		Diakinesis	18	-	26.00	-	-	0.18
		Metaphase-I	12	-	26.00	-	-	0.40

The data on meiotic studies are presented in Table 8. The haploid showed on an average of 3.00, 1.50 and 2.00 bivalents with 0.02, 0.16 and 0.08 chiasma per bivalent from pachytene to metaphase-I, respectively in haploid (Plate 5, Fig.1).

In parent there was 26, bivalents formed at each stage of meiosis from pachytene to metaphase-I, while there was 0.32, 0.18 and 0.40 chiasma per bivalent from pachytene to metaphase-I. There were no trivalents and tetravalent both in haploid and parents.

4.4.2.2 Distribution of chromosome at anaphase in diploid and parent in *G. hirsutum* var JLH-168

The observation of chromosome at anaphase-I in 0.2% toleudine blue induced haploid in *G. hirsutum* variety JLH-168, is presented table 9.

Table 9. Distribution of chromosome at anaphase-I in 0.2% toleudine blue induced haploid in JLH-168 variety

Species	No.of PMCs examined	Chromosome distribution of anaphase-I in haploid						Chromosome distribution in parent at anaphase-I
		13/13	14/12	15/11	16/10	17/9	18/8	26/26
Haploid	30	8	7	6	4	3	2	-
Parent JLH-168	30	-	-	-	-	-	-	30

Chromosome distribution at each pole given in *G. hirsutum* haploid ranged from 13/13 to 18/8. In haploid 8 PMCs showed 13/13, 7 showed 14/12, 6 showed, 15/11, 4 showed 16/10, 3 showed 17/9 and 2 showed 18/8 distribution of chromosome at anaphase-I. However, the parent showed 26/26 in all 30 PMCs (Plate 5, Fig.7).

4.4.2.3 Study of pollen grain size in haploid and its parent

Table 10. Pollen grain size in 0.2% toleudine blue induced haploid in JLH-168 haploid

Plant	Pollen grain size in occular unit (1 occular unit-5.10 μ)											
	8	9	10	11	12	13	14	15	16	17	18	19
Haploid	8	8	5	12	9	8	9	7	7	6	2	7
Parent	-	-	-	-	-	-	-	-	4	-	1	5

Pollen grain size in occular unit (1 occular unit=5.10μ)

	20	21	22	23	24	25	26	27	28	29	Av.size of pollen	Total pollen
Haploid	2	3	4	-	3	-	-	-	-	-	69.29	100
Parent	8	12	6	8	5	15	11	7	8	5	120.70	100

From Table 10, it is revealed that in *G. hirsutum* var. JLH-168 cotton haploid variable size pollen grains were formed. The average size being 69.29 micron. The average size of pollen in the parent was 120.70 micron.

4.4.2.4 Pollen fertility and germination study in toleudine blue induced haploid along with its parent

Pollen sterility test with IKI, propiono carmine and differential stain was carried out in haploid and its parent.

Table 11. Study of pollen fertility, pollen germination per cent in haploid and its parent

Species	Pollen fertility (%)	Pollen germination (%)	Pollen size (μ)
Haploid	0.90	2.20	69.30
Parent	92.30	88.40	120.70

From Table 11 it is revealed that the pollen fertility in haploid was 0.9 per cent while that of its parent was 92.30 per cent. Pollen germination in haploid was only 2.20 per cent and that of its parent 88.40 per cent.

4.4.3 Cytological studies in haploid induced by 0.4 per cent toleudine blue in *G. hirsutum* var. LRA-5166

4.4.3.1 Chromosome association at meiosis in haploid and its parent in LRA-5166 variety of *G. hirsutum*

The data on meiotic studies in 0.4 per cent toleudine blue induced haploid in LRA-5166 variety and its parent is presented in Table 12 and 13.

Table 12. The meiotic behaviour observed in 0.4% toleudine blue induced *G hirsutum* haploid and its parent var. LRA-5166

Sr. No.	Plant description	Meiotic stage	No.of PMCs examined	Chromosome association				X'ta/II
				I	II	III	IV	
1.	Haploid	Pachytene	11	24.00	1.00	-	-	0.04
		Diakinesis	16	23.00	1.50	-	-	0.12
		Metaphase-I	13	19.00	3.50	-	-	0.14
2.	Parent	Pachytene	14	-	26.00	-	-	0.31
		Diakinesis	12	-	26.00	-	-	0.24
		Metaphase-I	15	-	26.00	-	-	0.30

The data on meiotic studies is presented in Table 12 indicate that in haploid on an average 1.00, 1.50 and 3.50 bivalents was formed with 0.04, 0.12 and 0.14 chiasma per bivalent from pachytene to metaphase-I. However, there are no trivalents and tetravalents in haploid.

In parent there was 26 bivalents formed at each stage of meiosis from pachytene to metaphase-I while there was 0.31, 0.24 and 0.30 chiasma per bivalent from pachytene to metaphase-I respectively. There was no trivalent and tetravalent in parent (Plate 4, Fig.1).

4.4.3.2 Distribution of chromosome at anaphase in haploid and its parent in *G. hirsutum* var. LRA-5166

Table 13. Observation of chromosome at anaphase-I in 0.4 per cent toleudine blue induced haploid in *G. hirsutum* variety LRA-5166

Species	No.of PMCs examined	Chromosome distribution of anaphase - I in haploid						Chromosome distribution in parent at anaphase-I 26/26
		13/13	14/12	15/11	16/10	17/9	18/8	
Haploid	25	7	6	4	3	2	3	-
Parent LRA-5166	25	-	-	-	-	-	-	25

Chromosome distribution at each pole is given in table 13, indicatesthat in *G. hirsutum* var. LRA-5166 haploid it ranges from 13/13 to 18/ 8. In haploid 7 PMCs showed 13/13, 6 showed 14/12, 4 showed 15/11, 3 showed 16/10, 2 showed 17/9 and 3 showed 18/8 distribution of chromosome at anaphase-I. However parent shows 26/26 distribution in all 25 PMCs (Plate 4, Fig.2).

4.4.3.3 Study of pollen grain size in haploid and its parent

Table 14. Pollen grain size in haploid and its parent in *G. hirsutum* var. LRA-5166 induce by 0.4 % toleudine blue treatment

Plant	Pollen grain size in occular unit (1 occular unit-5.10 µ)														
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Haploid	5	7	6	8	10	4	81	9	6	13	7	3	-	-	
Parent	-	-	-	-	-	-	2	-	-	-	7	8	9	8	
	Pollen grain size in occular unit (1 occular unit=5.10µ)														
	21	22	23	24	25	26	27	28	29	30	31	32	Av. size of pollen	Total pollen	
Haploid	7	-	3	5	2	-	-	-	-	-	-	-	68.90	100	
Parent	6	8	6	13	7	9	4	6	3	3	3	2	118.60	100	

From Table-14, it is observed that pollen grain formed in the LRA-5166 variety haploid pollen were of variable in size. The average size of pollen being 68.90 micron. At least 3 per cent normal sized pollens were formed in haploid. The average size of pollens in parent in 118.60 micron.

4.4.3.4 Pollen fertility and germination study in 0.4% toleudine blue induced haploid in LRA-5166 variety along with its parent

Pollen sterility test with IKI, propiono carmic and differential stain was carried out in haploid and its parent. The results are presented in Table 15.

Table 15. Study of pollen fertility, pollen germination per cent in haploid and its parent

Species	Pollen fertility (%)	Pollen germination (%)	Pollen size (µ)
Haploid	0.50	3.00	68.90
Parent	94.30	89.20	118.80

The pollen fertility in haploid was 0.50 per cent while that of its parent was 94.30 per cent. Pollen germination in haploid was only 3.00 per cent and that of its parent it was 89.20 per cent.

4.4.3.5 Chromosome association at meiosis in haploid plant in variety $RHCrOO_3$, LRA-5166.

Table 16. Chromosome association in different haploids

Sr. No.	Meiotic stage	Haploids	Chromosome association				Xta/II
			I	II	III	IV	
I.	Pachytene stage	a) Gamma-ray induced	24.00	1.00	-	-	0.07
		b) 0.2% toleudine blue induced	20.00	3.00	-	-	0.02
		c) 0.4% toleudine blue induced	24.00	1.00	-	-	0.04
		Average	22.66	1.33	-	-	0.043
II.	Diakinesis stage	a) Gamma-ray induced	21.80	2.10	-	-	0.11
		b) 0.2% toleudine blue induced	23.00	1.50	-	-	0.16
		c) 0.4% toleudine blue induced	23.00	1.50	-	-	0.12
		Average	22.60	1.70	-	-	0.13
III.	Metaphase-I	a) Gamma-ray induced	22.00	2.00	-	-	0.22
		b) 0.2% toleudine blue induced	22.00	2.00	-	-	0.08
		c) 0.4% toleudine blue induced	19.00	3.50	-	-	0.14
		Average	21.00	2.50	-	-	0.14
Mean chromosome association			22.08	1.84	-	-	0.104

During pachytene number of bivalents ranged from 1-3/PMC with an average of 1.33 II per PMC with Xta 0.043 II. Further, at diakinesis II/PMC were ranged from 1.50-2.10 with Xta 0.13/II while at metaphase-I number of bivalents/PMC ranged from 2.00-3.50 with an average 2.50 and Xta 0.14/II. On an average 22.08^{I} + 1.84^{II} chromosome configuration were observed in haploids induced in present work.

Table 17. Pollen fertility, germination and pollen grain size studies in haploids in *G. hirsutum*

Sr. No.	Plants Species (Haploids)	Pollen fertility	Pollen germination	Pollen grain size (μ)
1.	RHCroo3	0.50	3.40	63.29
2.	JLH-168	0.90	2.20	69.30
3.	LRA-5166	0.50	3.00	68.90
	Average	0.633	2.866	67.163

Pollen studies in *G. hirsutum* haploid indicated that pollen fertility ranged from 0.50 - 0.90 per cent while pollen germination ranged from 2.20 - 3.40 per cent and pollen grain size ranged from 63.29 μ - 69.30 μ in *G. hirsutum* haploids. The average pollen fertility was 0.63 per cent while the average pollen germination was 2.86 per cent and the average pollen grain size was 67.16 per cent.

4.5 Studies on morphological characters of haploid (2x = 26) and colchiploid (2n=4x=52) in *G. hirsutum* variety RHCroo3 0.5% colchicine treatment

The observations were recorded for different morphological characters in the haploid of *G. hirsutum* var. RHCroo3 along with its colchiploid and coefficient of variation (c.v.) for different characters which are presented in Table 18 (Plate 3).

1. Plant height (cm/plant) :

Average plant height in haploid plant was 74.18 cm.

2. Number of sympodia/plant :

Sympodial branches was observed in haploid and it was 4.00 sympodial branches.

3. Number of monopodia/plant :

In haploid plant was 1 monopodial branch was observed.

4. Internodal length (cm/internode) :

Colchiploid plant showed reduced internodal length as compared to haploid plant. In colchiploid plant internodal length was 3.51 cm and in haploid it was 3.60 cm/internode, while c.v. was 9.14 and 12.40 in colchiploid and haploid respectively.

5. Petiole length (cm/petiole) :

Petiole length of haploid was 5.22 cm while in colchiploid it was 5.16 cm. Which indicated that slight reduction in petiole length in colchicine plant.

Table 18. Morphological character studied in haploid *G. hirsutum* L. var. RHCroo3 and its colchicine treated colchiploid plant (C1) in 0.5% colchicine treatment

Sr. No.	Characters	Haploid		Colchiploid	
		Mean	c.v.%	Mean	c.v.%
1.	Plant height (cm)/plant	74.18	-	-	-
2.	Number of sympodia/plant	4.00	-	-	-
3.	Number of monopodia/plant	1.00	-	-	-
4.	Internodal length (cm)/internode	3.60	12.40	3.51	9.14
5.	Petiole length (cm)/petiole	5.22	18.68	5.16	12.26
6.	Leaf area (Sq.cm)/leaf	69.38	11.50	78.42	13.56
7.	Stomata size (µ)/stomata	22.62	18.40	24.37	12.27
8.	No.of chloroplasts/stoma	18.24	16.20	31.48	13.83
9.	No. of anthers/flower	85.40	17.70	64.67	14.10
10.	Petal length (cm)/petal	2.45	9.90	4.71	8.38
11.	Style length (cm)/style	1.98	5.44	2.14	7.42
12.	No. of bracteoles teeth/bracteole	6.49	8.80	6.72	7.59
13.	Boll diameter (cm)/boll	-	-	-	-
14.	No. of bolls/plant	-	-	-	-
15.	Boll weight (g/boll)	-	-	-	-
16.	Lint weight (g/boll)	-	-	-	-
17.	Seed weight (g/boll)	-	-	-	-
18.	No. of seeds/boll	-	-	-	-
19.	No. of motes/boll	-	-	-	-
20.	Seed cotton yield/g/plant	-	-	-	-

6. Leaf area (sq.cm/leaf) :

Leaf area in haploid was found to be slightly reduced as compared to colchiploid plant. In haploid plant leaf area was 69.38 sq.cm/leaf and in colchiploid plant it was 78.42 sq.cm/leaf.

7. Stomata size (µ/stoma) :

The stomata size in haploid was 22.62 µ with c.v. 18.40 while in colchiploid was 24.37 µ with c.v. 12.27. It indicated that the stomata size was more in colchiploid plant than into haploid plant.

8. Number of chloroplasts/stoma :

The number of chloroplast in guard cells of stomata in haploid was 18.24/stoma and in colchiploid was 31.48/stoma, and its c.v. was 16.20 and in colchiploid 13.83. It indicated that there was an increase in chloroplast number in colchiploid plant.

9. Number of anthers/flower :

A significant reduction in anther number was observed in colchiploid as compared to haploid plant. The number of anthers/flower in haploid was 85.40/flower with c.v. 17.70 and in colchiploid was 64.67/flower with c.v. 14.10.

10. Petal length (cm/petal) :

Length of petal studied at both the ploidy levels indicated that there was a significant reduction at haploid level than that in colchiploid plant. The mean of petal

length in haploid was 2.45 cm while in colchiploid was 4.71. The c.v. for petal length was 9.90 in haploid while in colchiploid was 8.38.

11. Length of style (cm/style) :

The length of style measured in haploid of *G. hirsutum* was 1.98 cm and in colchiploid was 2.14 cm. It indicated that there was an increase in style length in colchiploid as compared to haploid plant.

12. Number of bracteoles teeth/bracteole :

No significant difference was found between haploid and colchiploid plant. The variation in bracteole teeth number was almost similar at both ploidy level. The no. of bracteoles teeth/bracteole in haploid was 6.49 and in colchiploid was 6.72.

The haploid plant of RHCroo3 variety in *G. hirsutum* was highly male and female sterile hence did not set fruits. Hence, observations for boll, lint seed and yield was not recorded.

4.5.1 Chromosome association at meiosis in colchiploid var. RHCroo3

Table 19. Observation of behaviour of chromosome during meiosis of *G. hirsutum* var. RHCroo3 colchiploid

Sr. No.	Meiotic Stage	Chromosome association				Total No.of PMCs examined
		I	II	III	IV	
1.	Pachytene	6.70	16.30	2.90	1.00	20
2.	Diakinesis	7.20	15.90	3.00	1.00	35
3.	Metaphase-I	7.00	16.00	3.00	1.00	45

The mean number of chromosome associations observed from pachytene to metaphase-I are given in Table 19.

It was observed that there was a pairing of chromosomes from pachytene to metaphase-I stage of meiosis. On an average 1.00, 1.00 and 1.00 quadrivalents were observed at pachytene, diakinesis, metaphase-I respectively of the meiosis. The chromosome number was confirmed as 2n=4x=52 at each stage without any fragments.

4.5.2 Distribution of chromosomes at anaphase-I in colchiploid var. RHCroo3

Table 20. Distribution of chromosome to the poles at anaphase-I of meiosis in colchiploid *G. hirsutum* variety RHCroo3

Segregation of univalent	Chromosome distribution at each pole.							Total no. of PMCs observed
	19/7	18/8	17/9	16/10	15/11	14/12	13/13	
PMCs observed	3	5	7	14	8	6	2	45

It was observed in table 20, that the separation of chromosome at each pole varied from 19/7 to 13/13. In colchiploid 3 showed 19/7, 5 showed 18/8, 7 showed 17/9 14 showed 16/10, 8 showed 15/11, 6 showed 14/12, 2 showed 13/13, in distribution of chromosome at anaphase-I.

Table 21. Frequency of number of microspores produced by PMCs in the sporad stage in colchiploid of *G. hirsutum* variety RHCroo3

	Number of microspores formed per PMC								Total	Avr. micro-spore per PMC
	1	2	3	4	5	6	7	8		
PMC's	4	8	14	21	28	17	5	3	100	
Total microspores	4	16	42	84	140	102	35	24	447	4.47

In the case of colchiploid during meiosis spindle formation was observed they did not reached the equatorial plate. The chromosomes being irregularly arranged in the metaphase plate, did not pass to either poles and formed micronuclei. Whole chromosomes as such or after division reached the poles. This resulted in the formation of upto eight nuclei. The various types of sporads and the frequencies are given in Table 21. The average number of microspores produced/PMC was 4.47. The pollen grains formed in this plant were found highly variable in size.

Table 22. The frequency of various sizes of pollen grains observed in *G. hirsutum* and also in average pollen grain size, pollen fertility and pollen germination in haploid and colchiploid plant variety RHCroo3

Species	Size of pollens in occular units (one occular units=5.10 μ)										Average size of pollen (μ)	Pollen fertility	Pollen germi-nation
	7	8	9	10	11	12	13	14	15	16			
G. hirsutum	-	-	-	16	27	26	17	14	-	-	76.81	94.70	89.80
Colchiploid	-	-	-	-	-	19	23	30	28	-	69.71	24.30	19.30

Table 23. Treated varieties of cotton with colchicine in various concentration

Species (Variety)	Colchicine Treatment																											
	0.05%				0.1%				0.2%				0.5%				2% colchicin + Lanolin paste											
	Apical bud		Auxilary bud		Apical bud		Auxilary bud		Apical bud		Auxilary bud		Apical bud		Auxilary bud		Apical bud		Auxilary bud		Exposure cutting Low Temp.		Sprouted cutting High Temp.		Colc-hicin Frequ.		C+L		Temp.
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B				
G.hirsutum																												
1) RHCr003	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	1	25	0	25	0	25	0	25	0	0.005		0	0
2) JLH-168	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	0		0	0
3) LRA-5166	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	0		0	0
G.arboreum																												
1) Y-1	25	0	25	0	25	0	25	1	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	0.005		0	0
2) PA-304	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	0		0	0
3) Nandeo	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	0		0	0
4) Sawta	25	0	25	0	25	0	25	0	25	1	25	0.	25	0	25	0	25	0	25	0	25	0	25	0	0.005		0	0
5) PA-333	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	0		0	0

A = No.of plant treated, B=No.of plant in which polyploidy induced, C+L = Cochicine + Lanolin paste
Low temperature = 0°C for 6 hours, High temperature = 44°C for 3 hours

Treatment of 0.5% colchicine to the auxilary buds in *G. hirsutum* variety RHCr003 and in 0.1% colchicine treatment to the auxilary bud in *G. arboreum* variety Y-1 also in 0.2% colchicine treatment to the apical bud of *G. arboreum* variety Sawta has resulted in successful doubling chromosomes with frequency was 0.005 in these varieties.

The smallest, largest and average pollen grain sizes observed (Table 22) in *G. hirsutum* var. RHCroo3 plant were 51.00 µ, 71.40 µ and 76.81 µ respectively while in its colchiploid counterparts they were 61.20 µ, 76.50 µ and 68.71 µ respectively. Fertile pollen observed in the normal plant was 94.70 per cent and pollen germination was 89.80 per cent. However, the fertility of pollen in its colchiploid counterparts reduced to 24.30 per cent and pollen germination test indicated that only 19.30 per cent pollen were germinated (Plate 5, Fig.8B).

4.6 Different morphological character in *G.arboreum* L. var. Y-1 induced 0.1% colchicine treatment

The observation were recorded for different morphological characters in the diploid and colchiploid of *G. arboreum* L. variety Y-1 induced 0.1% colchicine concentration along with it's coefficient of variation (c.v.) for different characters are presented in Table 24 (Plate 4).

1. Plant height (cm/plant) :

The average height of diploid plant was 92.10 cm.

2. Number of sympodia/plant :

The mean number of sympodia in diploid was 9.15. The sympodia observed in diploid plant were quite long with 5-7 internodes.

3. Number of monopodia/plant :

The number of monopodias in diploid was 3.80.

4. Internodal length (cm)/internode :

Average internodal length was 4.92 cm in the diploid plant, while it was 3.85 cm in the colchiploid plant.

5. Petiole length (cm)/petiole :

Average petiole length calculated in diploid plant was 9.76 cm while in colchiploid plant it was 7.28 cm/petiole The difference between the mean of diploid and colchiploid plant for this character were significant. The pattern of variation was also same in both the ploidy levels.

6. Leaf area (sq.cm)/leaf :

Leaf area calculated in diploid and colchiploid plants were 96.74 sq.cm and 110.12 sq.cm, respectively. There was significant difference between diploid and colchiploid means. The coefficients of variation for leaf area of diploid and colchiploid indicated that leaf area was found more variable in colchiploid as compared to its diploid counterparts.

7. Stomata size (μ/stoma) :

The mean stomata size in diploid and colchiploid leaf cells was 28.76 μ and 32.73 μ per stoma, respectively indicating significant increase in stoma size in colchiploids. Stomata size found more variable in colchiploid as compared to their respective diploids.

Table 24. Morphological characters studied in *G. arboreum* L. var. Y-1 and its colchicine in treated 0.1% colchicine concentration colchiploid plant (C1)

Sr. No.	Characters	Diploid 2n=2x=26		Colchiploid 2n=4x=52	
		Mean	c.v.%	Mean	c.v.%
1.	Plant height (cm)/plant	92.10	8.32	-	-
2.	Number of sympodia/plant	9.15	8.27	-	-
3.	Number of monopodia/plant	3.80	10.11	-	-
4.	Internodal length (cm)/ internode	4.92	11.23	3.85	11.16
5.	Petiole length (cm)/petiole	9.76	7.29	7.28	9.23
6.	Leaf area (Sq.cm)/leaf	96.74	7.58	110.12	13.41
7.	Stomata size (μ)/stomata	28.76	8.22	32.73	10.22
8.	No.of chloroplasts/stoma	15.28	9.26	17.82	12.44
9.	No. of anthers/flower	47.12	8.13	27.59	13.10
10.	Petal length (cm)/petal	5.26	8.29	7.14	9.26
11.	Style length (cm)/style	4.55	9.17	6.15	12.19
12.	No. of bracteoles teeth/ bracteole	8.60	7.16	9.70	8.27
13.	Boll diameter (cm)/boll	3.60	8.63	2.76	17.34
14.	No. of bolls/plant	35.00	8.57	1.00	-
15.	Boll weight (g/boll/branch)	2.60	7.37	1.90	-
16.	Lint weight (g/boll)	1.20	8.23	0.42	-
17.	Seed weight (g/boll)	1.20	9.87	1.48	-
18.	No. of seeds/boll	21.80	5.12	7.20	-
19.	No. of motes/boll	0.18	4.14	11.20	-
20.	Seed cotton yield/g/plant	91.00	6.87	-	-

8. Chloroplasts number/stoma :

The number of chloroplast per cell in guard cells of stomata in diploid and colchiploids averaged 15.28 and 17.82/stoma, respectively. The observed means showed significant differences between diploid and colchiploid. The variation for this character was much higher in colchiploids as compared to diploid.

9. Number of anthers/flower :

The mean number of anthers/flower was reduced to 27.59 in colchiploid as compared to diploid anther 47.12/flower. Thus there was a significant difference between colchiploid and diploid anther number. Higher degree of variation was noticed for anther number in colchiploids as compared to its diploids.

10. Petal length (cm/petal) :

The diploid had small flowers, in which the petal length averaged to 5.26 cm as compared to their colchiploid counterpart with 7.14 cm petal length. The observed difference was significant. The variability observed for petal length was similar in diploid and its colchiploid counterpart.

11. Length of style (cm/style) :

Style length in diploid 4.55 cm was found to be highly variable than in colchiploid 6.15 cm. There was

a significant difference between diploid and colchiploid means. The style length averaged 4.55 cm and 6.15 cm in diploid and colchiploid, respectively.

12. Number of bracteoles teeth/bracteole :

In colchiploid plant bracteole teeth were 9.70/bracteole while there were 8.60/bracteole in diploid.

No significant difference was found between colchiploid and diploid. The variation for bracteole teeth was almost similar at both the ploidy levels.

13. Boll diameter (cm/boll) :

The average boll diameter of bolls in diploid plants was 3.60 cm and colchiploid plant were 2.76 cm per boll respectively. The colchiploid produced significantly. Smaller bolls than the diploid. The bolls of colchiploid varied highly significant as compared to diploid.

14. Number of bolls/plant :

The average number of bolls/plant in diploid and in colchiploid plants were 35.00 and 1.00. Significantly lower boll number was recorded in colchiploid as compared to the diploid.

15. Boll weight (g/boll) :

The average boll weight in diploid plant was 2.60 g/boll and in colchiploid plant was 1.90 g/boll. Significantly lower boll weight was recorded in colchiploid as compared to the diploid.

16. Lint weight (g/boll) :

The average lint weight calculated in diploid plant was 1.20 g/boll while in colchiploid plant it was 0.42 g/boll. Lint weight in colchiploid plant was reduced significantly as compare to diploid.

17. Seed weight (g/boll) :

The average seed weight calculated in diploid plant was 1.20 g/boll, while in colchiploid plant it was 1.48 g/boll. Seed weight in diploid reduced significantly as compared to colchiploid.

18. Number of seeds/boll :

The average number of seed in diploid plant was 21.80/boll and in colchiploid plant it was 7.20/boll. Significantly reduced number of seed/boll in colchiploid plant as compared to diploid plant was observed.

19. Number of motes/boll :

The average number of motes in diploid plant was 0.18/boll and in colchiploid plant it was 11.20/boll. Colchiploid bolls produced highly significantly higher number of motes 11.20/boll as compared to diploid 0.18/boll. Further in colchiploid the motes number was found highly variable as compared to diploids.

20. Seed cotton yield g/boll :

The seed cotton yield was more in diploid plant than in the other plant. Seed cotton yield in diploid plant 91.00 gm/boll.

4.6.1 Chromosome association at meiosis in colchiploid var Y-1

Table 25. Observation of behaviour of chromosome during meiosis in *G. arboreum* var. Y-1 colchiploid

Sr. No.	Meiotic Stages	Chromosome association				Total No.of PMCs examined
		I	II	III	IV	
1.	Pachytene	6.6	19.2	1.0	1.0	15
2.	Diakinesis	8.6	18.2	1.0	1.0	25
3.	Metaphase-I	6.5	19.5	1.1	0.8	70

The mean number of chromosomes associations observed from pachytene to metaphase-I are given in Table 25.

In the above table, it is revealed that there was pairing of chromosomes from pachytene stage of meiosis. On an average 1.0, 1.0 and 0.8 quadrivalents were observed at pachytene, diakinesis and metaphase-I respectively of the meiosis. The chromosome number was confirmed as 2n=4x=52 at each stage without any fragments (Plate 4, Fig.6).

4.6.2 Distribution of chromosome at anaphase-I in *G. arboreum* colchiploid plant var. Y-1

Table 26. Distribution of chromosome to the poles at anaphase-I of meiosis in colchiploid *G. arboreum* var Y-1

Segregation of univalent	Chromosome distribution at each pole.							Total no. of PMCs observed
	20/32	21/31	22/30	23/29	24/28	25/27	26/26	
PMCs observed	8	9	16	7	4	3	1	48

It was observed from Table 26, that the separation of chromosome at each pole varied from 20/32 to 26/26. In colchiploid 8 PMCs showed 20/32, 9 showed 21/31, 16 showed 22/30, 7 showed 23/29, 4 showed 24/28, 3 showed 25/27, 1 showed 26/26, in distribution of chromosome at anaphase-I.

Table 27. Frequency of number of microspores produced by PMC's in the sporad stage in colchiploid of *G. arboreum* var. Y-1

	Number of microspores formed per PMC								Total	Average micro-spore per PMC
	1	2	3	4	5	6	7	8		
PMC's	3	9	13	19	24	13	10	9	100	
Total microspores	3	18	39	76	120	78	70	72	476	4.76

In the case of colchiploid during meiosis spindle formation was observed they did not reached the equatorial plate. The chromosomes being irregularly in the metaphase plate, did not pass to either poles and formed micronuclei. (Whole chromosomes as such or after division reached the poles). This resulted in the formation of upto eight nuclei. The various types of sporads and the frequencies are given in Table 27. The average number of microspores produced/PMC was 4.76. The pollen grains formed in this plant were found highly variable in size.

Table 28. The frequency of various sizes of pollen grains observed in *G. arboreum* and also in average pollen grain size, pollen fertility and pollen germination in diploid and colchiploid plant var. Y-1

Species	Size of pollen grain in occular units (one occular units=5.10 μ)										Average size of pollen (μ)	Pollen fertility	Pollen germination
	7	8	9	10	11	12	13	14	15	16			
G. arboreum 2n=26	-	-	20	25	25	20	10	-	-	-	54.825	92.80	87.40
Colchiploid plant 2n=4x=52	-	-	-	-	10	30	50	2	3	5	64.926	23.40	18.30

The smallest, largest and average pollen grain sizes observed (Table 28) in *G. arboreum* var Y-1 plant were 45.90 μ, 66.30 μ and 54.825 μ respectively while in its colchiploid counterparts they were 56.10 μ, 81.60 μ and 64.926 μ respectively. Fertile pollen observed in the normal plant was 92.80 per cent and pollen germination was 87.40%. However, the fertility of pollen in its colchiploid counter parts reduced to 23.40 per cent and pollen germination test indicated that only 18.30 per cent pollen grains were germinated (Plate 4, Fig.8).

4.7 Different morphological character in *G.arboreum* L. var. Sawta

The observations were recorded for different morphological characters in the diploid and colchiploid of *G. arboreum* L. variety Sawta induced 0.2% colchicine

concentration along with its coefficient of variation for different characters are presented in Table 29.

1. Plant height (cm/plant) :

The average height of diploid plant was 91.48 cm.

2. Number of sympodia/plant :

The mean number of sympodia in diploid was 8.95 The sympodia observed in diploid plant were considerably quite long having 5-6 short internodes.

3. Number of monopodia/plant :

The number of monopodias in diploid plant was 3.32 and its c.v. was 11.13.

4. Internodal length (cm/internodes) :

The average internodal length was 4.35 cm in the diploid plant, while 3.45 cm in the colchiploid plant. Significantly reduction in internodal length in colchiploid plant as compared to diploid plant.

5. Petiole length (cm/petiole) :

The average petiole length calculated in diploid plant was 9.48 cm while in colchiploid plant it was 7.15 cm/petiole. The difference between the mean of diploid and colchiploid plant for this character were significant. The pattern of variation was also same in both the ploidy levels.

Table 29. Morphological characters studied in *G. arboreum* L. var. Sawta and its colchicine treated 0.2% concentration colchiploid plant (C1)

Sr. No.	Characters	Diploid 2n=2x=26		Colchiploid 2n=4x=52	
		Mean	c.v.%	Mean	c.v.%
1.	Plant height (cm)/plant	91.48	9.27	-	-
2.	Number of sympodia/plant	8.95	9.16	-	-
3.	Number of monopodia/plant	3.32	11.13	-	-
4.	Internodal length (cm)/internode	4.35	10.22	3.45	12.89
5.	Petiole length (cm/petiole)	9.48	8.17	7.15	7.16
6.	Leaf area (Sq cm/leaf)	96.14	7.10	110.01	8.48
7.	Stomata size (µ)/stomata	28.22	8.12	32.13	10.47
8.	No.of chloroplasts/stoma	15.12	9.15	17.28	7.16
9.	No. of anthers/flower	46.89	9.26	27.21	8.13
10.	Petal length (cm)/petal	5.11	10.85	7.12	10.33
11.	Style length (cm)/style	4.32	11.26	5.89	12.10
12.	No. of bracteole teeth/bracteole	8.28	13.47	9.43	10.29
13.	Boll diameter (cm)/boll	3.48	8.42	2.35	7.53
14.	No. of bolls/plant	34.57	7.35	1.00	-
15.	Boll weight (g/boll)	2.33	7.28	1.49	-
16.	Lint weight (g/boll)	1.12	8.43	0.38	-
17.	Seed weight (g/boll)	1.13	8.73	1.39	-
18.	No. of seeds/boll	21.32	9.21	7.14	-
19.	No. of motes/boll	0.17	13.12	11.15	-
20.	Seed cotton yield/plant	80.38	7.31	-	-

6. Leaf area (sq.cm/leaf) :

Leaf area calculated in diploid and colchiploid plants were 96.14 sq.cm and 110.01 sq.cm, respectively. There was significant difference between diploid and colchiploid means. The coefficient of variation for leaf area of diploid and colchiploid indicated that leaf area was found more variable in colchiploid as compared to its diploid counter parts.

7. Stomata size (μ/stoma) :

The mean stomata size in diploid and colchiploid leaf cells was 28.22 μ and 32.13 μ/stoma, respectively indicating significant increase in stoma size in colchiploids. Stomata size found more variable in colchiploid as compared to their diploids.

8. Number of chloroplast/stoma :

The number of chloroplast/cell in guard cells of stomata in diploid and colchiploids averaged 15.12 and 17.28/stoma respectively. The means observed showed significant differences between diploid and colchiploid. The variation for this character was much higher in colchiploids as compared to diploid.

9. Number of anthers/flower :

The mean number of anthers/flower was reduced to 27.21 in colchiploid as compared to diploid anther 46.89/flower. Thus there was significant difference between

colchiploid and diploid anther number. Higher degree of variation was noticed for anther number in colchiploids as compared to its diploids.

10. Petal length (cm/petal) :

The diploid had small flower, in which the petal length averaged to 5.11 cm as compared to their colchiploid counter part with 7.12 cm petal length. The observed difference was significant. The variability observed for petal length was similar in diploid and its colchiploid counter part.

11. Length of style (cm/style) :

The style length in diploid 4.32 cm was found to be highly variable than in colchiploid 5.89 cm. There was a significant difference between diploid and colchiploid means.

12. Number of bracteole teeth/bracteole :

In colchiploid plant bracteole teeth were 9.43/bracteole while they were 8.28/bracteole in diploid. No significant difference was found between colchiploid and diploid. The variation for bracteole teeth was almost similar at both the ploidy levels.

13. Boll diameter (cm/boll) :

The average boll diameter of bolls in diploid plant was 3.48 cm and colchiploid plant was 2.35 cm/boll respectively. The colchiploid produced significantly smaller bolls than the diploid. The bolls of colchiploid varied significantly as high as compared to diploid.

14. Number of boll/plant :

The average number of bolls/plant in diploid and in colchiploid plants were 34.57 and 1.00. Significant lower boll number was recorded in colchiploid as compared to the diploid.

15. Boll weight (g/boll) :

The average boll weight in diploid plant was 2.33 g/boll and in colchiploid plant was 1.49 g/boll. Significantly lower boll weight was recorded in colchiploid as compared to the diploid.

16. Lint weight (g/boll) :

The average lint weight calculated in diploid plant was 1.12 g/boll while in colchiploid plant it was 0.38 g/boll. Lint weight in colchiploid plant was reduced significantly as compared to diploid.

17. Seed weight (g/boll) :

The average seed weight calculated in diploid plant was 1.13 g/boll, while in colchiploid plant it was 1.39

g/boll. Seed weight in diploid was reduced significantly as compared to colchiploid.

18. Number of seed/boll :

The average number of seed in diploid plant was 21.32/ boll and in colchiploid plant was 7.14/boll. Significantly reduced number of seed/boll in colchiploid plant as compared to diploid plant was observed.

19. Number of motes/boll :

Average number of motes in diploid plant was 0.17/boll and in colchiploid plant was 11.15/boll. Colchiploid bolls produced highly significantly higher number of motes 11.15/boll as compared to diploid 0.17/boll further in colchiploid the motes number was found highly variable as compare to diploids.

20. Seed cotton yield :

The seed cotton yield/plant of diploid was 80.38gm.

4.7.1 Chromosome association of meiosis in colchiploid var. sawta

Table 30. Observation of behaviour of chromosome during meiosis in *G. arboreum* var. Sawta

Sr. No.	Meiotic stages	Chromosome association				Total No.of PMCs examined
		I	II	III	IV	
1.	Pachytene	7.3	18.95	1.20	0.8	20
2.	Diakinesis	8.4	18.30	1.0	1.0	25
3.	Metaphase-I	6.9	18.90	1.1	1.0	55

The mean number of chromosomes associations observed from pachytene to metaphase-I are given in table 30.

From the table, it is revealed that there was a pairing of chromosomes from pachytene stage of meiosis on an average 0.8, 1.0 and 1.0 quadrivalents were observed at pachytene, diakinesis and metaphase-I respectively of the meiosis. The chromosome number was confirmed as 2n=4x=52 at each stage without any fragments.

4.7.2 Distribution of chromosome at anaphase-I in *G. arboreum* colchiploid plant var. Sawta

Table 31. Distribution of chromosome to the poles at anaphase-I of meiosis in colchiploid *G. arboreum* var. Sawta

Segregation of univalent	Chromosome distribution at each pole.							Total no. of PMCs observed
	20/32	21/31	22/30	23/29	24/28	25/27	26/26	
PMCs observed	6	7	13	8	5	4	3	46

It was observed in table 31, that the separation of chromosome at each pole varied from 20/32 to 26/26. In colchiploid 6 PMCs showed 20/32, 7 showed 21/31, 13 showed 22/30, 8 showed 23/29, 5 showed 24/28, 4 showed 25/27, 3 showed 26/26, in distribution of chromosomes at anaphase-I.

Table 32. Frequency of number of microspores produced by PMCs in the sporad stage in colchiploid of *G. arboreum* var. Sawta

	Number of microspores formed per PMC								Total	Averase micro-spores per PMC
	1	2	3	4	5	6	7	8		
PMC's	2	7	11	22	26	16	10	6	100	
Total microspores	2	14	33	88	130	96	70	48	481	4.81

In the case of colchiploid during meiosis spindle formation was observed they did not reached the equatorial plate. The chromosomes being irregularly in the metaphase plate, did not pass to either poles and formation micronuclei whole chromosomes as such or after division reached the poles. The resulted in the formation of upto eight nuclei. The various types of sporads and the frequencies are given in Table 32. The average number of microspores produced/PMC was 4.81. The pollen grains formed in this plant were found highly variable in size.

Table 33. The frequency of various sizes of pollen grains observed in *G. arboreum* pollen grains size, pollen fertility and pollen germination in diploid and colchiploid plant var.Sawta

Species	Size of pollen grains in occular units (one occular units = 5.10 u)										Average size of pollen (u)	Pollen fertility	Pollen germi-nation
	7	8	9	10	11	12	13	14	15	16			
G. arboreum 2n = 26	-	-	10	15	50	-	20	5	-	-	57.12	94.30	89.60
Colchiploid plant 2n=4x=52	-	-	-	-	25	30	17	15	9	4	64.515	25.70	20.50

The smallest, largest and average pollen grain sizes observed (Table 33) in *G. arboreum* var. Sawta plant were 45.90 µ, 71.40 µ, 57.12 µ respectively. While in its colchiploid counterparts they were 56.10 µ, 81.60 µ and 64.515 µ respectively. Fertile pollen observed in the normal plant was 94.30 per cent pollen germination was 89.60 per cent. However, the fertility of pollen in its colchiploid counterparts reduced to 25.70 per cent and pollen germination test indicated that only 20.50 per cent pollen were germinated.

Table 34. Chromosome association in different colchiploid

Sr. No.	Meiotic stage	Colchiploid	Chromosome association			
			I	II	III	IV
I.	Pachytene	Y1	6.60	19.20	1.00	1.00
		Sawta	7.30	18.95	1.20	0.80
		Average	6.95	19.075	1.10	0.90
II.	Diakinesis	Y1	8.60	18.20	1.00	1.00
		Sawta	8.40	18.30	1.00	1.00
		Average	8.50	18.25	1.00	1.00
III.	Metaphase-I	Y1	6.50	19.50	1.10	0.80
		Sawta	6.90	18.90	1.10	1.00
		Average	6.70	19.20	1.10	0.90
Mean chromosome association			7.38	18.84	1.066	0.933

During pachytene the number of bivalents ranged from 18.95 - 19.20/PMC, while trivalents ranged from 1.00 - 1.20 with average 1.10 and qudrivalents ranged from 0.80 - 1.00 with an average 0.90. Further, at diakinesis II/PMC were ranged from 18.20 - 18.30/PMC with average 18.25. While trivalent ranged from 1.00 - 1.00 with average 1.00 and in qudrivalent ranged from 1.00 - 1.00 with an average 1.00, while at in metaphase-I number of II/PMC were ranged from 18.90 - 19.50/PMC with average 19.20, while trivalents ranged from 1.10 - 1.10 with an average 1.10 and in qudrivalent ranged from 0.80 - 1.00 with an average 0.90.

The overall chromosome configuration were observed in different colchiploid of *G. arboreum* was 7.38 I + 18.84 II + 1.06 III + 0.93 IV.

Table 35. Pollen fertility, pollen germination and pollen grain size studies in colchiploid in *G. arboreum*

Sr. No.	Plants (Colchiploid)	Pollen fertility	Pollen germination	Pollen grain size (µ)
1.	Y-1	23.40	18.30	64.92
2.	Sawta	25.70	20.50	64.51
	Average	24.55	19.40	64.71

Pollen studies in *G. arboreum* colchiploid indicated (Table 35) that pollen fertility ranged from 23.40

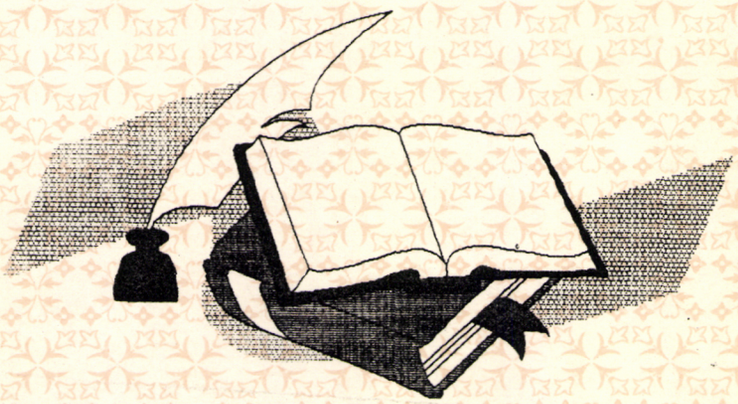
- 25.70 per cent while pollen germination ranged from 18.30 to 20.50 per cent while pollen grain size ranged from 64.51 µ to 64.92 µ in *G. arboreum* colchiploid. The average pollen fertility was 24.55%, pollen germination was 19.40 per cent and pollen grain size was 64.71 µ.

Table 36. Chromosome behaviour during meiotic stages

Sr. No.	*G. arboreum* varieties colchiploid 2n=4x=52	Chromosome behaviour during meiotic stages							
		Diakensis				Metaphase-I			
		I	II	III	IV	I	II	III	IV
1.	Y-1	8.6	18.20	1.0	1.0	6.5	19.5	1.1	0.8
2.	Sawta	8.4	18.30	1.0	1.0	6.9	18.90	1.1	1.0
Average		8.5	18.25	1.0	1.0	6.7	19.20	1.10	0.9
Mean chromosome association		7.6	18.72	1.05	0.95				

During diakensis number of bivalents/PMC were ranged from 18.2 - 18.3/PMC (Table 36), while trivalent ranged from 1.00 - 1.00 and in quadrivalent ranged from 1.00 - 1.00. At metaphase-I number of bivalents/PMC ranged from 18.90 - 19.50/PMC, while trivalent ranged from 1.10 - 1.10 and in qudrivalent ranged from 0.80 - 1.00.

The overall chromosome configuration were observed in different colchiploid of *G. arboreum* was 7.60 I + 18.72 II + 1.05 III + 0.95 IV.



DISCUSSION

5. DISCUSSION

Since the polyploidy has played a significant role in evolution of tetraploid *Gossypium* spp. a lot of basic work has been done in this field. Further, polyploidy is an important advent tool for hybridization between species. Which is resorted for securing genes or gene combinations that are not available within the limits of species. Improvement in these certain characters through transgressive breeding is possible. The work on this line has been reported by several workers in mostly on the hybridization between tetraploid and the diploid spp. Such transfer is possible with difficulties and breeding programme is comparatively lengthy because most F1's being triploid are sterile. Only after doubling its chromosome complement it can be back crossed to cultivated tetraploids to obtain plants with 2n = 4x = 52 having desired character combinations (Sikka and Joshi, 1960).

Most quantitative trait locis influencing fiber quality and yield are located on the "D" subgenome derived from an ancestor that does not produce spinnable fibers. D subgenome quantitative trait locus may partly account for the fact that domestication and breeding of tetraploid cottons has resulted in fiber yield and quality levels superior to

those achieved by parallel improvement of "A" genome diploid cottons. The merger of two genomes with different evolutionary histories in a common nucleus appears to offer unique avenues for phenotypic response to selection. This may partly compensate for reduction in quantitative variation associated with polyploids among extant angiosperms. These findings impel molecular dissection of the roles of divergent subgenomes in quantitative inheritance in many other polyploids and further exploration of both "Synthetic" polyploids and exotic diploid genotypes for agriculturally useful variation.

The utilization of haploids (n=2x=26) of tetraploid (2n=4x=52) to cultivated cotton spp. in interspecific hybridization with wild diploids offers scope for minimizing period for interspecific transfer as reported in potato (Hougas *et al.*, 1958) Harland (1955) discussed a potential of haploids in interspecific cotton breeding. Further, haploids have significance in genetics and cytological research (Sadasiviah, 1974). Looking to the significance and importance of haploids and their doubled forms and autotetraploids of *arboreum* in cotton breeding, present investigations were ventured and the result obtained are discussed in this chapter.

The haploids induced by Chikhale (1998) in *G. hirsutum* variety RHCr003 through gamma ray treatment (25 kR)

and in JLH-168 and LRA-5166 by toleudine blue (0.2% and 0.4%) treatment were used for the present study.

5.1 Studies on comparative morphology of haploids and their diploid parents :

The results of the various morphological observations have indicated reduction in expression of all the morphological characters in haploid as compared to diploid parent. This reduction was almost in the proportion of 1:2. The values of c.v. indicated that there was a more or less similar trend of variation at both the ploidy level. Slightly higher variations were observed for certain characters. Which might be due to the sampling error. These results on the morphological characters of haploids are similar to those obtained by Dergach (1970), Harland (1955), Lee (1970), Owing (1964), Roux (1958), Silow and Stephens (1944), Tiranti (1965) and Mehetre (1978). Chaudhari (1976) had studied the quantitative relationship between the haploid and diploid plant with a view to identify haploid by visual observations from field populations of commercial varieties. The results obtained here are similar to those of Chaudhari (1976).

5.2 Fertility in the haploids :

G. hirsutum cotton haploids are generally found to be completely sterile, while *G. barbadense* haploids are partially sterile (Chaudhari, 1976). Webber (1938) obtained three open pollinated bolls from *G. barbadense* haploids.

While Harland (1936) observed these haploids fertile as a female parent. Endrizzi (1959) was not able to obtain seeds from *G. hirsutum* haploids even after pollinating them over a long period. Different haploids studied here were found partially to complete sterile thus the results obtained here are in conformity with those reported earlier workers.

5.3 Induction of polyploidy :

The results on the induction of polyploidy by different treatments in haploids of *G. hirsutum* cotton and *G. arboreum* diploid cultivars are presented in Table 23. The earlier workers Stephens (1944), Kasparyan (1945), Stephens (1945), Silow (1944), Stephens and Cassidy (1946), Varuntsyan (1946), Brown (1948) reported colchicine induced polyploids in *Gossypium* spp. Menzel (1952) reported chromosome doubling in cotton by colchicine thus taking into account of successful utilization of colchicine for inducing polyploids in cotton by earlier workers. Present attempt has been made to double the chromosomes of haploids of *G. hirsutum* and cultivars of *G. arboreum*. Since phylogenetic relationships are very important in breeding of polyploid spp. if a good breeder attempts to transfer genetic trait from wild spp. to a specific cultivar, doubled haploids will have their greatest value if they represents random gametes from base material. Such randomness permits the scientists to use doubled haploids for inheritance studies. The ultimate objective of the breeder is to produce strain or hybrid that

are superior to those already in commercial production. The availability of methods for obtaining large number of doubled haploids will have its greatest impact on development phase of breeding programme because the methods will permit the breeder to fix genetic system of individual gamete in testable, reproducible form at any stage in the breeding process. Thus utilization of doubled haploids will reduce the time and efforts required to complete the development phase.

In upland cotton Meredith *et al.* (1970) found at least one significant difference between doubled haploid and their three parental cultivars for all characters studied except lint yield. Further Feaster and Turecotte (1973) found three doubled haploid of Pima cotton from three relatively heterogeneous cultivars to be comparable in yield to the parental cultivars.

Although a limited amount of data are available on the commercial use of doubled haploids the review of earlier work indicate that they will be utilized in the same manner as standard inbred lines. Doubled haploids of cotton have been found to be similar to their parent varieties in yield stability, productivity of their crosses, interaction with locations and type of gene action, (Feaster and Turecotte 1973, Kohel, 1969 and Meredith *et al.* 1970). Further data by Kohel and White (1963) and Kohel (1969) indicate that completely homozygous lines will not be at disadvantage due

to developmental homeostasis associated with heterozygosity. Thus, utilization of haploids has potential of saving breeder from 2 to 5 years in cultivar development.

Looking to the significance of doubled haploids in cotton breeding as discussed above the induction of polyploidy or doubling of chromosome was undertaken and it has been possible to obtain one branch with doubled chromosomes by treating axilary bud with 0.5% colchicine for 12 hrs. The comparative observations on the morphological characters indicated increase in the values of different characters (Table 18) which is certainly due to the effect of duplication of chromosomes. These results are confirmatory to the earlier workers (Meredith *et al.*, 1970).

5.4 Polyploidy in *G. arboreum* varieties

Treatment of 0.1 per cent colchicine to the axillary buds in *G. arboreum* variety Y-1 and 0.2 per cent colchicine treatment to the apical buds of *G. arboreum* variety Sawta has resulted in successful doubling of chromosomes with frequency 0.005 in both varieties. Thus the results reported by earlier workers are in confirmatory with present research, (Feaster and Turecotte 1973, Kohel, 1969, Meredith *et al.* 1970).

Colchiploid branch in Y-1 showed shorter internodes (3.85) and petiole (7.28 cm) increased leaf area (110.12 cm2/leaf) stomatal size (32.73), chloroplast/stoma (17.32),

reduced number of anthers (27.59) increased petal length (7.14 cm) style length (6.15 cm), bracteole teeth (7.70) reduced boll diameter (2.76 cm), bolls/branch (1.00), boll weight (1.90) lint weight (0.42) seed weight (1.48). Seeds/boll (7.20) increased motes/boll (11.20). These observations are in confirmatory with earlier workers.

Colchiploid branch in Sawata showed shorter internodes (3.45) and petiole (7.15 cm), increased leaf area (110.01 cm^2/leaf), stomatal size (32.13 µ), chloroplast/stoma (17.28), reduced no. of anthers (27.21) increased petal length (7.12 cm) style length (5.89 cm), bracteole teeth (9.43) reduced boll diameter (2.35 cm), bolls/branch (1.00) boll weight (1.49). Seeds/boll (7.14) increased motes/boll (11.15). These observations are in confirmatory with earlier workers.

5.5 Cytological studies

5.5.1 Haploids

The details of chromosome behaviour during meiosis in various haploids presented in Table 16 and detail information on sporad formation (tetrads), pollen morphology (size), fertility etc. are presented in Table 17.

The data on chromosome association in different haploids during diakinesis and metaphase-I is presented in Table 16. The average bivalent formation has ranged from 1.5

to 2.1/PMC with an average of 1.7 bivalents (II) per PMC. The bivalents (II) formed were true bivalents as at least single chiasmata was observed in majority of the bivalents.

The meiotic analysis indicated that bivalent formation was frequent and synapsis of chromosome was at random than preferential. The secondary associations of chromosomes were also observed in certain PMCs which indicated residual homology between A and D chromosomes.

The results obtained here are in conformatory with those reported by earlier workers. Beasley (1942), Endrizzi (1959) and Brown (1961) reported 5.16 and 7 to 9 bivalents per PMC's. Catcheside (1937) considered that occurrance of chromosome associations in haploids as evidence form duplicated segments.

5.5.1.1 Chiasma formation in haploids

The observations made in PMC's of different groups of cotton haploids indicated chiasma from pachytene to metaphase-I. According to Barrow (1971) bivalents in haploids showed clear visible chiasmata though there frequency was low (0.005%). While other workers did not observe such chiasma formation, this is attributed to the differential rate of coiling of A and D genome chromosomes (Endrizzi, 1962). The results reported in the present investigations are in similar to those reported by earlier workers.

5.5.1.2 Sterility in haploids

High degree of sterility observed in haploids was due to chromosomal abnormalities and no. of univalents observed during meiotic process. According to Swanson *et al.* (1967) production of normal viable and fertile pollen by normal meiosis requires regular pairing of homologus chromosomes in meiotic prophase. Separation in first division and chromatid separation in second division to provide a full haploid compliment of chromosomes in each gamete.

In case of haploids during the first division of meiosis comparatively higher number of univalents were observed in the present studies. During the second meiotic division in the haploids, spindle fibers were formed, bivalents found to lie on spindles, univalents were also found to lie on spindles. Chromosomes were often observed not to respond to the spindles and remained as 'laggards' leading to unequal separation of chromosomes. The term meta-anaphase' coined by Tometrop (1939) for the meiotic stage of *Hordeum distichum* haploid can also be applied to cotton haploids, where rare bivalents and large number of univalents were observed. Similar observations were also reported by Barrow (1971) who studied meiosis and pollen development in *G. barbadense* haploid plants.

In the haploids at the end of second division chromosomes got arranged in two to three groups in different

planes. The microspores produced by these groups varied in size. The occasional normal looking pollen in these haploids might be formed from dyads observed. Such microspores developed spines on their exine while smaller pollens failed to develop such normal spines. The rarely fertile pollens observed in the haploids might be those containing twenty - six chromosomes formed from monads. Thus, the observations made by Barrow (1971) are confirmed in the present studies.

5.5.2 Colchiploids

It was become possible to double the chromosomes of one branch of haploid. The data on morphological characters indicated that significant increase in the characters in the colchiploid as compared to haploid branch in the same plant (Table 18). From the cytological observations recorded in the PMC's of the flower buds of this branch it is concluded that the PMC's contain 2n=4x=52 chromosomes. At metaphase univalents (I), bivalents (II), trivalents (III) and quadrivalents (IV) were observed. On an average seven univalents, sixteen bivalents, three trivalent one quadrivalent (Table 19) were noticed. All these chromosome configurations form 3 to 4 distinct groups due to secondary associations and auto syndesis. A second meiotic division was highly irregular. Unequal seperation of chromosomes, lagards and bridges were also noticed in few PMC's which laid to formation of abnormal tetrads and sporad containing unequal chromosome pollen grains with highly

variable in size. All these abnormalities resulted in sterility. Very few PMC's showed equal separation of chromosomes at anaphase-I and chromatids at anaphase-II laid to formation of normal pollens with 5 to 6 per cent fertility (Table 20). These results are in confirmatory with earlier workers.

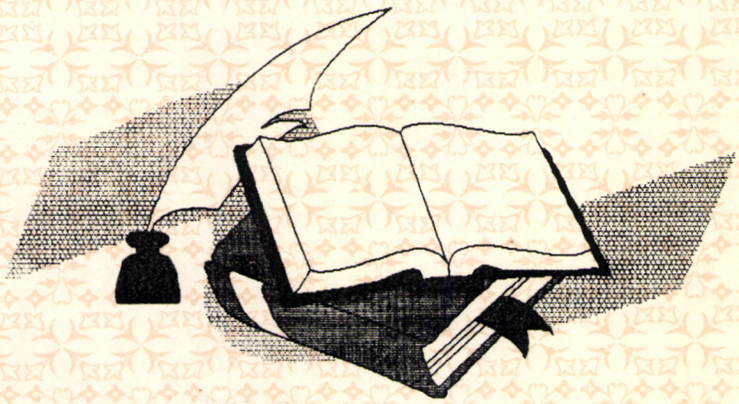
5.5.3 Cytological studies in *G. arboreum* varieties

Completely regular pairing of homologus chromosome in meiotic prophase and their seperation in 1st division and chromatid seperation in 2nd division was observed in all the varieties leading to formation of normal sporad and fertile pollens (Tables 28 to 33). These results are in confirmatory with those reported by earlier workers (Davie, 1933 and Beasley, 1942). It has been possible to double the chromosomes of one branch in *G. arboreum* variety. The significant differences between treated and remainder branches suspected the induction of chromosome doubling. The induction of chromosome doubling in this plant which was further confirmed by cytological observations in PMC's of this branch. It was become possible to analyse only to 2 PMCs. The average chromosome configuration in these PMCs was 1 univalent, 9 bivalent, 3 trivalents, 6 quadrivalent. These results are in confirmatory to Patel *et al.* (1947) Stephens (1942) showed cells with total number of associations less than 13 and individual association with more than four chromosomes at first metaphase in 4n *G. arboreum*. Thus, the

result reported here are partly confirmatory with earlier workers. The 2nd meiotic division was highly irregular. Unequal separation of chromosomes laggard, univalents, bridges were also noticed which was the cause of the pollen sterility in this branch.

5.6 The crossability of colchiploid

The colchiploid could not be crossed with other varieties and species of their origin as only a single branch was induced in each variety (Table 23) on selfing the flower buds were dropped and hence could not become possible to obtain selfed/open pollinated bolls. The doubled/colchiploid branches were air-layered as per Mehetre and Thombre (1977) for propogating them vegetatively, for further utilization.



SUMMARY AND CONCLUSION

6. SUMMARY AND CONCLUSION

The present investigation entitled "Induction of polyploidy in haploid of *G. hirsutum* L. and diploid of *G. arboreum* L." were undertaken following objectives.

1. To doubled the chromosome number of the haploids cotton 2n=2x=26 of *G. hirsutum* L.
2. To study the cytomorphology and pollen fertility in C_1 generation in induced polyploid.
3. To obtain F_1 interspecific cross between doubled *G. arboreum x G. hirsutum* L. cotton.

6.1 Different method of haploid induction

It has been possible to induce haploids by following methods.

1. Gamma ray irradiated *G. hirsutum* variety RHCr003 with 25 kR (Frequency - 0.002).
2. Pollination of emasculated flowers with 0.2% Toleudine - blue treated pollens in *G. hirsutum* Var. JLH-168 (Frequency - 0.0625) and 0.4% Toleudine - blue treated pollens in *G.hirsutum* var. LRA-5166 (Frequency - 0.0833).

The frequency of induction was more in toleudine blue treatment i.e. it was 0.036 in *G. hirsutum* cotton than any other method. Hence, this method is more efficient than other methods used in present studies.

6.2 Morphological studies in different haploids and their parents

The comparison of morphological characters in haploid (2n=2x=26) and their tetraploid parent (2n=4x=52) was studied. It was observed that haploids were characterised by their typical miniature shape. In general haploids were slow in growth, with small and wrinkled leaves, delayed but prolonged flowering habit, smaller flowers with non-dehiscent anthers and no one of few boll number. The observations on stomata indicated that there was significant reduction in stomata size, number of stomata per unit area and chloroplasts per stoma. However, chloroplast count technique is found more suitable in screening of large number of populations with fair amount of accuracy.

The *G. hirsutum* haploids (n=2x=26) of varieties obtained and investigated in present studies were found to be highly male and female sterile.

6.3 Cytological studies in different haploids

The meiotic studies in PMC's of *G. hirsutum* haploids (n=2x=26) indicated that there was rare bivalents formation. Unpaired chromosomes were distributed unequally at 1-7 poles and they were included unequally in the microspores thus, resulting in pollens with high size variations and considerable pollen sterility. Exceptionally rare fertile pollens observed might be due to the monads and

dyads observed in the second division of meiosis. Monads and dyads had more or less than 26 chromosomes due to division of univalnt and unequal distribution, thus complete male and female sterility observed in *G. hirsutum* variety RHCr003, JLH-168, LRA-5166 haploids were due to highly irregular meiosis.

6.4 Polyploidy in haploids of *G. hirsutum*

One branch with doubled chromosome has been obtained in the haploids of *G. hirsutum* by treating auxilary bud with 0.5% colchicine. The comparative observations on the morphological character indicated increasing the value of leaf area, stomata size, number of chloroplast, petal length, style length, number of bracteoles which is certainly due to the effect of duplication of chromosomes.

From the cytological observations recorded in the PMCs of the flower buds of this branch. It can be concluded that PMCs contain 2n=4x=52 chromosome. On an average at metaphase 7 univalent (I), 16 bivalents (II), 3 trivalent (III), 1 qudrivalent (IV), were observed. All these chromosome configuration from 3-4 distinct groups due to secondary association and autosyndesis. Second metiotic division was highly irregular there is unequal separation of chromosome and due to presence of laggards and bridges present in few PMC's abnormal tetrades and sporades containing unequal chromosomes, pollen grain with highly

variable in size, it resulted in sterility in very few PMCs normal pollen grains with 5-6 % fertility were observed.

6.5 Polyploidy in *G. arboreum*

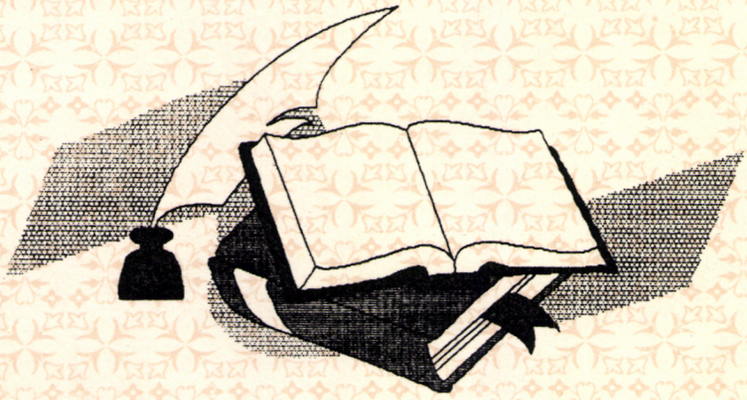
Treatment of 0.1 % colchicine to the axiliary bud of *G. arboreum* variety Y-1 and 0.2 % colchicine treatment to the apical bud of *G. arboreum* variety Sawta resulted in doubling of chromosome with frequency 0.005 in single branch of both the variety.

The comparative study of morphological characters indicated that colchiploid branches showed shorter internodes and petiole, increased leaf area, stomatal size, chloroplasts/stoma, reduced number of anthers, increased petal length, style length, bracteole teeth, reduced boll diameter, boll/branch, boll weight, lint weight, seed weight, seeds/boll, increased motes/boll.

Cytological studied in *G. arboreum* varieties indicated that normal sporad and fertile pollen were form due to regular pair of homologous chromosome in meiotic prophase their seperation in first division and chromatid sepration in 2nd division. On analysis of PMC's in colchiploid branches of *G. arboreum* varieties Y-1 and Sawta indicated that the chromosome configuration in these PMCs was 1 univalent, 9 bivalent, 3 trivalent, 7 qudrivalent. Unequal separation of chromosomes lagard, univalents bridges leading to pollen sterility were also noticed in these branches.

6.6 Crossability of colchiploid

It was not become possible to cross the colchiploid with other varieties and species of their origin because only a single branch was induced in each variety. On selfing the flower buds were dropped down and hence could not become possible to obtain selfed / open pollinated buds. Therefore, the method of air-layering was used for propogation them vegetatively for their further utilization.



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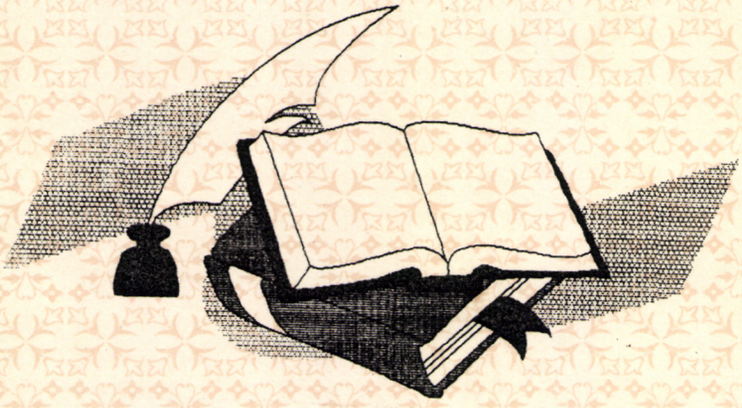

VITA

8. V I T A

SHINDE HEMANTKUMAR NIVRUTTI

A Candidate for the degree
of
MASTER OF SCIENCE (AGRICULTURE)
in
CYTOGENETICS AND PLANT BREEDING
1999.

Title of thesis : "Induction of Polyploidy in haploid of *G. hirsutum* L. and diploid of *G. arboreum* L.

Major field : Agricultural Botany (Cytogenetics and Plant Breeding)

Biographical Information

Personal data : Born at Rahuri Dist. Ahmednagar, on 23rd May, 1973, son of Dr. Nivrutti Vithal Shinde.

Education : Attended primary school at Savitribai Phule Prathamik Vidyalya, Mahatma Phule Krishi Vidyapeeth, Rahuri, Tal. Rahuri. Completed secondary school at Savitribai Phule Madhyamik Vidyalya, Mahatma Phule Krishi Vidyapeeth, Rahuri, Tal. Rahuri. Passed S.S.C. examination in 1988. Completed Higher secondary education at Padmasri Vikhe Patil College Pravarnagar, Tal. Shrirampur, Dist. Ahmednagar, passed H.S.C. examination in 1990. Received Bachelor of Science (Horticulture) degree in second class from College of Horticulture, Pune in 1996. Completed the requirement for Master of Science (Agri.) degree specializing in "Cytogenetics and Plant Breeding" at MPKV, Rahuri, Dist. Ahmednagar (M.S.) in 1999.

Other : N.C.C. `C' certificate passed in `B' grade in 1995.
