INDUCTION OF POLYPLOIDY IN KACHOLAM, (*Kaempferia galanga* L.)

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "**Induction of polyploidy in Kacholam (*Kaempferia galanga* L.)**" is a bonafide record of work done by me during the course of research work and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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Certified that this thesis entitled "**Induction of polyploidy in Kacholam (*Kaempferia galanga* L.)**" is a record of research work done independently by **Sri.Ajithmohan,N.K.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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AJITHMOHAN, N.K.

Dedicated
to my Teachers

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INTRODUCTION

1. INTRODUCTION

Kaempferia galanga L., popularly known as 'kacholam', is an important medicinal plant belonging to the family Zingiberaceae. The genus *Kaempferia* is believed to have originated in South East Asia, probably in Burma, and is distributed in the tropics and subtropics of Asia and Africa (Holtum, 1950). Its medicinal property has been exploited in the indigenous systems of medicine of many countries such as Malaysia, Philippines, Thailand and also India. Of the 55 species reported in the genus, only 10 are known to occur in India among which, *K. galanga* L. and *K. rotunda* L. are of economic importance.

Kacholam is an attractive rhizomatous herb where the economic part is, the underground rhizomes. The rhizomes possess stimulant, expectorant, diuretic and carminative properties. Powdered rhizomes mixed with honey is administered against coughs. A decoction made using the rhizome is prescribed for dyspepsia, head ache, rheumatism and malaria. The rhizome boiled in oil, is externally applied to remove nasal obstructions. Kacholam can cure skin disorders, piles, oedema, fever, epilepsy, splenic disorders and asthma. The rhizomes can also promote or improve digestion. They are also used for curing inflammatory wounds. Apart from rhizomes, extract of whole plant is also used in some tonics (Burkill, 1935; Kirthikar and Basu, 1935; Brown, 1941; Quisumbing, 1951; Aiyer and Kolammal, 1964).

In Ayurveda, kacholam is reported to be effective against

diseases caused by the morbidity of 'Vaatha' and 'Kapha', the major two among the 'Thridoshas'. Kacholam is an ingredient of several ayurvedic preparations such as *'Kachuraadi choornam'*, *'Kachuraadi thailam'*, *'Kachuraadi vaattu'*, *'Asanelaadi thailam'*, *'Kalyaanakaghritham'*, *'Dasamoolaarishtam'* and *'Chyavanapraasam'* (Sivarajan and Balachandran, 1994).

Besides medicinal uses, kacholam finds use in cosmetic industry too. It is used as a perfume in hairwashes, powders and other cosmetics and in the manufacture of agarbathis. Its anti-dandruff property make it an essential ingredient in hair washes. The rhizomes yield 2.4-4.0 per cent volatile oil which is utilized in the manufacture of perfumes and curry flavourings. Antifungal and larvicidal properties are also reported for Kacholam. Recently some Japanese scientists have identified some anticancerous principles in *Kaempferia galanga* L. thus the uses of the crop are very many and may extend further.

The humid tropical climate of Kerala is suited for the luxuriant growth of kacholam. The cultivation practices are simple and the crop is adapted to partially shaded conditions. Hence there is immense scope for cultivation in the homesteads of the state and as an intercrop in the coconut and other perennial gardens. But so far, no specific variety of this crop has been identified for Kerala. At present only local cultivars are available with the farmers. Of late, the cultivation of the crop is fast spreading due to high demand and better market price. As in any other crop, better varieties in yield, quality and other agronomic attributes is a pre-requisite for making this crop further popular and its cultivation more economic. This need added emphasis due to

the fact that no improved cultivar or variety has so far been recognised in kacholam.

Crop improvement programme in kacholam has not been attempted in a serious manner, mainly due to the fact that it has gained importance only in recent years. The crop is propagtated through vegetative means i.e., through rhizomes. Recently, tissue culture method for rapid multiplication has also been standarised for the crop (Vincent *et al.*, 1992). The genetic resources available with the crop exhibits little variability. Lack of natural genetic diversity and absence of seed setting are major constraints for improvement through selection and combination breeding. However, genetic variability is to be created for attempting crop improvement in kacholam as the basic requirement. Alternate methods for inducing genetic variability for crop improvement in this crop include induced mutagenesis and polyploidy. Efforts are underway for creating genetic variability in the crop through induced mutagenesis in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara.

The present investigation is aimed at induction of genetic variability in *Kaempferia galanga* L. through polyploidy so as to select desirable variants for direct commercial exploitation or as base material for further genetic improvement. The cytogenetics of the parent material and the variants so isolated will also be studied so as to utilize the materials effectively.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Origin and Distribution

The genus *Kaempferia* is supposed to have been originated in South East Asia, probably in Burma. It was evolved as an evergreen herb in the shady forest conditions of Burma and from there, it appears to have migrated across most of the tropical Asia, and right across Africa (Holtum, 1950). The genus is widely distributed in the tropics and subtropics of Asia and Africa (Synge, 1956). Willis (1960) suggested that family Zingiberaceae is chiefly Indo-Malayan in distribution. Hooker (1892) reported that *Kaempferia galanga* L. is distributed throughout the plains of India. According to Gamble (1926), it is distributed at low elevations along the West Coast. It has been recorded as occuring throughout the plains of India, especially in Bengal, Deccan, South Konkan and Kerala (Aiyer and Kolammal, 1964).

2.2 Economic Importance

Medicinal properties of *Kaempferia galanga* L. have been described by many workers. As early as in the 17th century, Rheede (1678-1703) in *Hortus Malabaricus* described the morphology of the crop and its medicinal value for curing Asthma. The herb is used as a flavouring for rice. Rhizome and leaves are employed as a perfume in hairwashes, powders and other cosmetics. They are worn by women for fragrance and also used for protecting clothes against insects. Rhizomes are also eaten along with betel as a masticatory (Burkill, 1935, Quisumbing, 1951).

The rhizome is used as a stimulant, expectorant, carminative and diuretic. They are used in the preparation of gargles. They are administered with honey in coughs and pectoral affections. In Philippines, a decoction of rhizome is used for dyspepsia, head ache and malaria. Boiled in oil, the rhizomes are applied externally to remove nasal obstructions. Roasted rhizomes are applied hot for rheumatism and for hastening the opening of inflammatory tumours. They are also used as a wash in dandruff and for relieving irritation produced by stinging caterpillars. Mixed with oil, rhizomes are used as a cicatrizant. In Malaya, they are used for chills in elephants. The juice of the plant is an ingredient of some tonic preparations. The leaves are used in lotions and poultices for sore eye, sore throat, swellings, rheumatism and fevers (Burkill, 1935; Kirthikar and Basu, 1935; Brown, 1941; Quisumbing, 1951). It is also reported to promote or improve digestion and digestive powers and cures or allays skin or cutaneous disorders, piles, oedema, epilepsy, splenic disorders, difficult breathing (asthma) and diseases caused by the morbidity of 'Vaatha' and 'Kapha' (Aiyer and Kolammal, 1964). It removes bad odour of mouth. Recently larvicidal and anticancerous principles have been obtained from the rhizome extract of *K. galanga* (Kosuge *et al*., 1985; Kiuchi *et al.*, 1988). The hot water extract of *Kaempferia* rhizomes showed strong larvicidal activity against the larvae of dog round worm, *Toxocara canis* (Kiuchi *et al.*, 1988). Mangaly and Sabu (1991) reported the use of *K. galanga* as an ingredient of ayurvedic preparations for skin disorders, rheumatism etc. Toxicity against neonate larvae of *Spodoptera littoralis* was also reported in a contact residual bioassay (Pandit *et al.*, 1993).

2.3 Taxonomy

Kaempferia galanga L. is a monocotyledonous plant, belonging to the

family Zingiberaceae of the order Zingiberales, under the series Epigynae (Bentham and Hooker, 1894). According to Hutchinson (1934), there are four tribes in the family Zingiberaceae viz., Costea, Hedycheae, Globeae and Zingibereae. Willis (1960) suggested that family Zingiberaceae contains 45 genera and 800 species, while Panchaksharappa (1966) opined that among the families of Scitaminales, Zingiberaceae consists of 47 genera and 1400 species.

Schumann (1904) divided the family Zingiberaceae into two subfamilies viz., Zingiberoideae and Costoideae. The Zingiberoideae is further divided into three tribes viz., Globeae, Hedycheae and Zingibereae. The genus Kaempferia comes under the tribe Hedycheae. Hooker (1892) recognised four sub-genera under the genus *Kaempferia* namely Sincorus, Protanthium, Monolophus and Stachyanthesis. Among this, *K. galanga* comes under the sub-genera Sincorus. Sincorus includes eleven other species apart from *K. galanga* viz., *Kaempferia marginata, K. speciosa, K. angustifolia, K. ovalifolia, K. pandurata, K. prainiana, K. roscoeana, K. parviflora, K. involucrata, K. andersoni* and *K. cocinna.*

2.4 Morphology

Kaempferia galanga L. is an annual herb grown for its aromatic rhizomes. According to Hooker (1892) *Kaempferia* is a plant with tuberous root stock. Leaves are 3-6 inches long, spreading flat on the ground, deep green in colour with deltoid tip. Petioles are short and channelled. Kirthikar and Basu (1935) described *K. galanga* L. as a stemless herb with tuberous, aromatic rootstock which possess fleshy, cylindric, non-aromatic root fibres. Leaves are a few in number, horizontally spreading and lying flat on the surface of the ground and having a length of approximately 6.3-12.5 cm and breadth of 4.5-9.0 cm. They are deep green in colour, rotund, ovate, deltoid and acuminate. Leaf margins are

neither thickened nor coloured. Flowers arise from the centre of the plant from between the leaves.

Aiyer and Kolammal (1964) described the leaves of kacholam as deep green, orbicular, suborbicular, orbiculate-ovate or ovate-cordate with thin membraneous blade, 6.2 to 15.0 cm long and 5.0 to 15.0 cm wide, smooth above, deltoid acuminate at tip, somewhat woody towards the base and 10-12 ribbed with the margin wavy but not thickened or coloured. The tuberous root stock is vertically oriented having several smaller secondary tubers and a cluster of roots, most of which are long, narrow and white, a few are shorter and tuberous at their tips. The main tuber is conical in form, wider below, narrower at the tip and distinctly marked with a number of transverse or horizontal or annular scars of scale leaves spaced 3 to 5 mm apart. Directly attached to the nodes are a limited number of smaller tubers which are also vertically oriented. Surface of the tubers are fairly smooth and greyish or light brown in colour.

Drury (1978) described *K. galanga* as a plant with biennial tuberous rhizome, stemless, stalked leaves, spreading flat on the surface of the earth which are either ovate, rotund or cordate in shape. Leaf margins are membraneous and wavy. Biswas and Chopra (1982) noted *K. rotunda* L. as a perennial stemless herb, about one foot high. Rootstock is tuberous with very thick root fibres. Leaves are oblong and erect with short, channelled petiole.

2.5 Cytogenetics

The chromosome number of *Kaempferia* has been studied only by a few workers and the reports available are conflicting. Raghavan and Venkatasubban (1943) studied the cytology of four species of *Kaempferia* namely *K. gibsonii*, *K.*

gilbertii, *K. rotunda* and *K. galanga* and reported that they showed a regular polyploid series having chromosome number 24, 36, 54 and 54 respectively. The chromosomes in all the species were fairly alike with more or less the same size. Since the chromosome numbers were all multiples of six, six was ascertained as the basic chromosome number of the genus. They also reported that the increase in chromosome number in the genus is not by the diminution of the size of chromosomes. They found two chromosomes with subterminal constriction in *K. gibsonii* and a pair of SAT chromosomes in *K. rotunda* and concluded that the speciation in this genera is due to polyploidy, aneuploidy and structural changes.

Chakravorthi (1948) studied the cytology of five species of *Kaempferia* viz., *K. gibsonii*, *K. gilbertii*, *K. cienkowskya*, *K. atrovirens* and *K. rotunda* and opined that there is clear evidence of polyploidy. He described *K. atrovirens* as a diploid species showing normal chromosome pairing with eleven bivalents. According to him, *K. rotunda* and *K. gilbertii* are triploids with a chromosome number of 33. Further, he found the chromosome number to be 2n = 28 for *K. cienkowskya* and concluded that the genus is having two distinct polyploid series based on x = 11 and x = 14. Raghavan and Arora (1958) assigned a chromosome number of 2n = 54 for *K. galanga* L. but according to Sharma and Bhattacharya (1959) the chromosome number of *K. galanga* L. is 22.

Twenty seven species belonging to eleven genera of the family Zingiberaceae were studied by Ramachandran (1969) and he reported a chromosome number of 2n = 44 for *K. rotunda* L. and 2n = 54 for *K. galanga* L. According to him, *K. galanga* L. is presumably an aneuploid pentaploid.

Mahanthy (1970) studied chromosome numbers of 14 species of *Kaempferia*. According to him, 2n number of *K. angustifolia* is 22. In *K.*

brachystemon, the somatic number was found to be 2n = 24. A similar species *K. ethelae* is also having a chromosome number of 2n = 26. The species *K. elegans* and *K. rosea* were assigned a chromosome number of 2n = 22 and for *K. gilbertii* and *K. rotunda* he reported a chromosome number of 2n = 33. A chromosome number of 2n = 42 was assigned for another species of *Kaempferia*. Based on his studies on floral morphology, geographical distribution and cytology the author suggested that the basic chromosome number x = 11 can be considered as the original one for Zingiberales as a whole. He also proposed that African representatives of *Kaempferia* may be assigned the status of a separate genus, *Cienkowskya*.

Beltran and Kam (1984) studied the cytotaxonomy of Zingiberaceae and found that Asiatic *Kaempferias* had a basic chromosome number of x = 11 while the African ones have x = 14.

Quite recently, Rekha (1993) reported the results of cytological studies in *Kaempferia galanga* L. For the first time she reported a chromosome number of 2n = 55 for the crop. She also studied the karyomorphology and presented for the first time the karyotype of kacholam. She found that the 55 chromosomes could be classified into 11 sets of five each and she proposed the species to be a pentaploid with the basic chromosome number x = 11.

2.6 Polyploidy Breeding

Polyploidy is a widespread phenomenon in plants. It refers to a condition in which there are more than two sets of chromosomes in the cells of an organism. It has been estimated by Muntzing (1936) that more than 50 per cent of the angiosperms are polyploids. The first known example of polyploidy in plants

was the gigas mutant of *Oenothera lamarckiana* discovered by Hugo de Vries in 1901 which was later found to be a tetraploid, containing double the number of chromosomes than the normal plants. (Lutz, 1907 and Gates, 1909). Later studies revealed that polyploidy is of two different kinds involving either duplication of the same genome or summation of different genomes, referred to as autopolyploidy and allopolyploidy respectively.

Polyploidy has played a very important role in evolution of plants, allopolyploidy being the major contributor. Autopolyploidy has contributed to a limited extent in evolution of plant species. Some of our present day crops such as potato, coffee, sweet-potato, groundnut, alfalfa, banana etc. are autopolyploids. In nature, allopolyploids are produced by chromosome doubling of interspecific hybrids and since they combine some characteristics of each parental species, are often hardier than their diploid progenitors. The chromosome doubling enable them to overcome sterility to a great extent, that they behave like normal diploids and hence they carry the name amphidiploids. Some of the important allopolyploid crops are wheat, oats, cotton, tobacco, some *Brassica* species etc.

Polyploids find several applications in crop improvement. Overcoming self incompatibility, making distant crosses possible, use as a bridging species in gene transfer between two specie, as a source of new genetic variation or use directly as crop varieties are some, to list a few. The significant achievement in allopolyploidy breeding is creation of a new crop species viz., *Triticale*.

Polyploidization can be achieved artificially in two ways; mitotic or meiotic. The former involves deregulation of mitosis at any stage in the vegetative cycle, resulting in doubling of chromosomes with two identical gene complements. The meiotic mechanism involves fusion of unreduced gametes with somatic

chromosome number (2n). Several chemicals such as acenaphthene, chloral hydrate, benzene, nitrous oxide, 8 hydroxy quinoline, sulfanilamide, mercuric chloride etc. were used in the earlier periods to bring about mitotic arrest, for the induction of polyploidy. In 1937, Blakeslee *et al.* discovered the polyploidizing property of an alkaloid, colchicine, extracted from the bulbs and seeds of the plant *Colchicum autumnale*. It was far more effective than other chemicals and in due course, colchicine replaced all the conventional methods of chromosome doubling in plants. Blakeslee and Avery (1937) working on *Portulaca, Datura* and *Cucurbita* and Nebel and Ruttle (1938) working on *Tradescantia, Petunia,* Snapdragons and Marigolds noticed that this alkaloid was very effective in doubling the chromosome number. These pioneering works paved the way for future use of colchicine on a large scale for production of polyploids in different crops. The reasons for the quick acceptance of colchicine, as a chromosome doubling agent, can be attributed to its two specific advantages namely high effectiveness for making polyploids in many different species and with very little damage to the treated plants. High solubility in water, non-toxicity to plants even at higher doses etc. adds to its merits (Eigsti and Dustin, 1955).

2.7 Methods of Colchicine application

For inducing polyploidy, the meristematic region is to be treated with colchicine solution i.e., actively dividing cells are to be exposed to colchicine. Treating germinating seeds, treating whole seedlings or apical bud treatment of young seedlings using cotton swab are commonly followed for obtaining polyploids. Rajendran *et al.* (1977) reported tetraploid production in *Amorphophallus companulatus* by treating sprouting seeds with 0.2 per cent colchicine. Out of 124 treated seeds they obtained one tetraploid. Sudharsan (1989) reported production of

polyploids in *Elettaria cardamomum* by soaking the seeds in 0.5 per cent colchicine for 90 minutes. Dhawan and Tyagi (1989) developed two autooctaploids in *Hyoscyamus muticus* by soaking the seeds in 0.5 per cent solution of colchicine for 72 hours. Nair and Ravindran (1992) induced polyploidy in black pepper (*Piper nigrum* L.) by treating seeds with 0.5 per cent colchicine solution. Autotetraploidy was induced in *Plantago ovata* by seed treatment with 0.4 per cent colchicine for 48 hours (Kumar and Srivastava, 1994).

Pushparajan (1988) produced tetraploid *Sida rhombifolia* ssp. *retusa* by treatment of seedlings with 0.3 per cent colchicine for 16 hours and 8 hours each on successive days. Tetraploids were obtained in *Raphanus sativus* by treating diploid seedlings with 0.3 per cent colchicine solution for 2 hours (Kumar and Chaurasia, 1990).

Kulkarny *et al.* (1984) induced autotetraploidy in *Catharanthus roseus* by immersing apical buds of seven day old seedlings in 0.5 per cent colchicine for 19 hours. Cotton swab method using 0.15 per cent colchicine was found to be successful in producing autotetraploids in *Trigonella foenum-graecum* (Arya *et al.*, 1988). Gaonkar and Torne (1991) reported successful induction of polyploidy in *Ageratum conyzoides* by treating apical buds of seedlings with 0.25 per cent colchicine for three consecutive days, four hours on each day, from 9 am to 1 pm. Verma and Raina (1991) reported induction of tetraploidy in *Phlox drummondi* by cotton swab method using 0.15 per cent colchicine for 12 hours, spread equally over two days. Autotetraploid was induced in *Petunia hybrida* by treating the apical portions of seedlings with 0.25 per cent colchicine solution (Singh, 1992).

Jos *et al.* (1986) produced autotetraploids in taro by treating tubers

with 0.5 per cent aqueous solution of colchicine for 48 hours. Ramachandran and Nair (1992) induced autotetraploidy in ginger, *Zingiber officinale*, by treating buds on rhizomes with 0.25 per cent aqueous solution of colchicine. A hole about 2 mm in diameter and 4 mm in depth was made close to a sprouting bud, with a cork borer, and was kept filled for four hours, from 10-14 hours. The rhizomes were washed in running water for one hour and stored in moist soil overnight. The treatment was repeated for two consecutive days after which the rhizomes were planted in separate pots. Out of 90 treated rhizomes, 4 true tetraploids were obtained.

2.8 Economic characters of some polyploids

In this section, the important economic characters of autopolyploids developed in some related crops as well as some other crop plants are reviewed.

The features associated with autopolyploidy include increased vigour and increased size of various plant parts - larger cells and hence larger vegetative and floral parts. Yield will also be higher, if the economically important part is vegetative. Other features are delayed germination, slow rate of growth, delayed flowering, larger flowers but fewer in number and sterility (Singh, 1990). Thus autopolyploidy breeding is attempted with the objective of obtaining enhanced vigour and higher vegetative yield. Enhancement in some other characters such as alkaloid content, resistance to pests and diseases etc. are also expected. However, there are several reports in which the autopolyploid developed being weak and inferior to the original diploid. According to Stebbins (1971), the increase of cell size, which generally characterises polyploids, does not necessarily lead to increased size of the plant as a whole or even of its individual organs, because of the reduction in the number of cell divisions in these plants.

Induced polyploidy has not been reported in kacholam, so far, by any workers. Other important members of the kacholam family Zingiberaceae are ginger, turmeric, cardamom and *Costus* sp. Among these, in ginger, cardamom and *Costus* sp., autoployploids have been developed. Ramachandran (1982) reported successful development of tetraploids in a local clone of ginger called 'Maaran' by treating the eyes on the rhizomes with 0.25 per cent colchicine solution. The tetraploid was more vigorous in growth with increased plant height, had larger and thicker rhizomes and larger, thicker and darker green leaves. Flowering was in the second year, just like normal ginger. Flowers and floral parts were bigger. The tetraploid nature of plants was confirmed by cytological studies. While the chromosome number of ginger was 2n = 22, the tetraploid had 44 chromosomes in root tip cells. Ramachandran and Nair (1992) further reported the superiority of the tetraploid over diploid ginger. The paper covers studies on morphology, cytology, pollen fertility, yield and oil content of rhizomes in diploids as well as induced tetraploids of ginger. The tetraploid had higher yield and the rhizomes were more regular in shape with reduced branching tendency. The tetraploid rhizomes showed a marginal decrease in oil content (2.3%) as compared to the original diploid cultivar (2.8%). Ratnambal and Nair (1988) had reported that flowering absent in tetraploid ginger. In cardamom, autopolyploids were produced by seed treatment with 0.5 per cent colchicine solution (Sudharsan, 1989). The induced tetraploids were characterised by thicker leaves with a thick waxy coating on the abaxial surface. The stomata were larger and number of stomata per cm^2 of leaf lamina was lower. Ammal and Prasad (1984) observed that in *Costus speciosus,* diosgenin content was maximum in the diploid followed by triploid and tetraploid.

Bai *et al.* (1976) while describing autotetraploids of *Ipomoea obscura* reported polyploids had thicker, broader, coarser and darker green leaves. Length

and breadth of stomata increased significantly and their number per unit leaf area decreased. Rajendran *et al.* (1977) reported in elephant foot yam, the autotetraploid developed through colchicine treatment of seeds was more vigorous compared to diploid. In induced tetraploids of taro, the tuber development was found much restricted (Jos *et al.*, 1986). They found that there was a decrease in the number of stomata as the level of ploidy increased. Bahl and Tyagi (1988) while describing autotetraploids in *Coleus forskohlii*, reported that though the tetraploids exhibited increase in plant height, the number of primary branches as well as number of leaves per plant decreased. But leaf area per plant was found to be increased. Number of stomata per mm^2 and size of stomata increased in the autotetraploids. Srivastava and Raina (1992) observed that initial growth was slow in autotetraploids of *Clitoria*.

The colchicine induced polyploid of *Abelmoschus esculentus* had smaller, thicker, darker leaves than the diploids and showed inhibited growth and flowering. The fruits formed were small and irregular (Patil, 1963; Rajasekharan and Ganesan, 1968). Sinha and Sinha (1975) reported profuse branching in colchicine treated plants of *Dacus carota* and *Foeniculum vulgare*. In *Zinnia elegans*, the tetraploid induced by Gupta and Koak (1976) was stunted in growth and was smaller in size compared with diploids. The plant was male sterile also. Colchicine induced sunflower plants exhibited distinct dwarf characters (Gupta and Roy, 1980). Verzea *et al.* (1983) reported that autotetraploid induced in *Datura innoxia* were taller than diploids and more vigorous with greater height of branching. They surpassed diploids by upto 40 per cent in total alkaloid yield. Zhang and Zhao (1984) reported that radish tetraploids, induced with 0.1 per cent colchicine, bolted 5-7 days later than the diploids and had larger flowers and petals. The autotetraploid induced in *Catharanthus roseus* by Kulkarni *et al.* (1984) has less

number of leaves per plant compared to diploids. The alkaloid content was same in diploids as well as in the tetraploids. Kulkarni and Ravindra (1988) reported that tetraploid lines of *Catharanthus roseus* yielded about 4 and 5 times more leaf and root total alkaloid respectively than the diploid lines. The induced tetraploids in *Hyoscyamus muticus* (Lavania, 1986) were vigorous with increased plant height. There was also increase in leaf size and thickness. The colchicine induced autooctaploids of *Hyoscyamus muticus*, produced by seed treatment in 0.5 per cent colchicine for 72 hours, exhibited a reduction in plant height as well as the number of branches. The leaves and floral parts were larger and the main shoot was thicker (Dhawan and Tyagi, 1989). Srivastava and Lavania (1990) revealed that in *Hyoscyamus albus,* induced autotetraploids showed a 19.61 per cent lower biomass yield. Lavania and Srivastava (1991) observed that tetraploids of *Hyoscyamus niger* were more vigorous than the diploids. Gaonkar and Torne (1991) reported that autotetraploids in *Ageratum conyzoides* were dwarf with increased number of branches, smaller and thicker leaves and larger stomata and pollen grains. Pollen sterility and seed sterility were found to be increased. Colchiploid developed in *Phlox drummondii* had a slower growth rate and the flowering was delayed by about 20 days compared to the diploid. The colchiploids showed significant gigantism as they were about 1.5 times bigger than the diploids. They had thicker stem and dark green, fleshy, broader leaves. The flowers also were conspicuously larger in size. There was pronounced increase in the size of stomata and pollen grains. The number of stomata per unit area in the tetraploid was 40 per cent less (Verma and Raina, 1991). The autotetraploid strain of vetiver named as 'Sugandha', exhibited 60 per cent improvement in oil productivity (Lavania, 1991). In *Plantago ovata,* autotetraploids induced by seed treatment with 0.4 per cent colchicine for 48 hours, were larger and more vigorous than diploids. The flowers stayed longer resulting in improvement in keeping quality and exten-

sion of blooming period to some extent. However, there was increased pollen sterility. The gigas character of C_1 tended to lessen in C_2 generation but was more, in comparison with the diploids.

2.9 Scope of polyploidy breeding in kacholam

The general breeding methodology that can be adopted for crop improvement include introduction, selection, hybridization, combination breeding, mutation breeding and polyploidy breeding. Introduction aims at increasing variability available in a crop, by bringing in new genotypes from a different geographical area. Selection exploits natural variability present in a crop or variability brought about by introduction. For selection to be effective there should be considerable amount of genetic variability existing in the base population. Hybrid production as well as combination breeding creates new genotypes/genic combinations. For this, there should be variability in the population with hybridization feasibility. More improvement will be brought about, when the divergence between the parents is more. Mutation breeding and polyploidy breeding aim at creating and exploiting new variability that does not exist naturally. Induced mutation create new alleles by altering existing alleles of genes. Polyploidy breeding alter the ploidy level of plants. Differences in the genome level changes the gene dosage leading to genetic variability. The scope of introduction, selection and combination breeding for the genetic improvement of kacholam is very limited as natural variability is quite meagre and seed setting is absent.

Polyploidy breeding is worth trying in kacholam since the economically important part is rhizome and the crop is clonally propagated. Any improvement in yield or quality that may be induced by polyploidy can readily be maintained as the associated sterility does not hinder the propagation.

Cytological investigation by Rekha (1993) had revealed that the chromosome number of the crop is 55 and it is suggested to be a pentaploid. Even then polyploidy is worth trying because of the following reasons. Natural variability in the crop is very less and also since seed set is absent, hybridization and recombination breeding are very difficult. The chromosome number in this crop is not too high as compared to crops such as okra, *Abelmoschus esculentus* (2n = 72, 120, 130 or 132), pineapple, *Ananas comosus* (2n = 50 or 100), *Agave sisalana* (2n = 138) etc. (Chaudhari, 1978). Also there is an optimum level of ploidy for each species below and above which the plant will be at a disadvantage. For example, for sugarbeet (*Beta vulgaris*) the optimum level is 3x i.e., triploid, while for Timothy grass (*Phleum pratense*) it is between 8-10x (Singh, 1990). There are commercially important clonally propagated crops which are high level polyploids. Sweet potato, *Ipomea batatas*, which is a stem tuber crop, is a hexaploid with 2n = 6x = 90 (Sundarraj and Thulasidas, 1976). Sugarcane, belonging to the genus *Saccharum*, has the basic chromosome number x = 6, 8 or 10 and shows very high ploidy levels. Noble cane, *S. officinarum* L. has a somatic chromosome number 2n = 80. Some other species show still higher chromosome numbers viz., *S. robustum* 2n = 60-194, *S. spontaneum* 2n = 40-128 and *S. barberi* 2n = 82-124 (Purseglove, 1975). Moreover the kacholam plant as a whole is relatively small so that an enhancement in the overall size of the plant and in its performance can be expected by inducing polyploidy.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

With the objective of creating genetic variability in kacholam, through induction of polyploidy, studies were undertaken in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara during 1994-'95.

3.1 MATERIALS

3.1.1 Rhizomes

Rhizomes of kacholam selection *Vellanikkara* available with the genetic stock of AICRP on Medicinal and Aromatic Plants, Vellanikkara were utilized for the experiment.

3.1.2 Chemicals

Colchicine was used to induce polyploidy in the present investigation. It is the most effective and widely used chemical for induction of polyploidy. Though several other chemicals are reported to have polyploidizing effect, they are much less effective than colchicine and are not commonly used (Singh, 1990). Colchicine is an alkaloid extracted from the plant autumn crocus (*Colchicum autumnale*). It is commercially available as a cream coloured powder soluble in water. It can bring about chromosome doubling at lower concentrations. Colchicine supplied by S.D. FINE-CHEM LTD., Mumbai was used in the present experiment.

3.2 METHODS

3.2.1 Fixing doses of colchicine

To find out the optimum dose of colchicine, first a preliminary trial was conducted by treating the rhizomes with different doses of colchicine and observing the germination or sprouting of the treated rhizomes, under

laboratory conditions.

First healthy rhizomes were selected, washed and cut into small pieces of around 5 g weight and with 2 or 3 viable buds. Then 20 each of the rhizome bits were kept immersed in aqueous solutions of colchicine with concentrations 0, 0.25, 0.50, 0.75, 1.00, 1.50 and 2.00 per cent for 4 hours, from 7-11 a.m., on two consecutive days. For this, first a stock solution was prepared by dissolving 3 g of colchicine in a small quantity of glycerol (15 ml) and then making up the volume to 150 ml. This solution was diluted to different concentrations as follows. In the case of control treatment, 5 ml of glycerol was dissolved in water and the volume was made upto 50 ml.

Concentration (%)	Volume of stock solution (ml)	Volume of water (ml)	Volume of the treatment solution (ml)
0.00	0.00	50.00	50
0.25	6.25	43.75	50
0.50	12.50	37.50	50
0.75	18.75	31.25	50
1.00	25.00	25.00	50
1.50	37.50	12.50	50
2.00	50.00	00.00	50

After the first day's treatment, rhizomes were taken out and washed thoroughly in running water and kept on a moist sand bed. On the second day also, the same treatment was repeated. After the treatment, rhizomes were washed thoroughly and sown in a sand bed which was then kept moistened by regular sprays of water. The sprouting was observed. Based on the germination percentage,

the doses for the treatments were fixed.

3.2.2 Colchicine treatment

3.2.2.1 Preparation of rhizomes

Fully matured quality rhizomes were first cleaned by washing with water for removing the soil and then the scales were scraped off using a scalpel, so that the buds are clearly visible. Rhizomes were then cut into small pieces weighing about 5 g each and with 2 or 3 viable buds of which only one healthy bud was retained for treating with colchicine, the rest being removed with the scalpel. Buds at two stages of sprouting were selected viz., just emerging bud (S_1) and bud at an advanced stage of sprouting (S_2). The prepared rhizomes were then grouped into two according to the stage of bud growth. In half of the rhizomes in both the groups, a hole about 2 mm diameter and 4 mm depth was drilled close to the bud using a cork borer.

3.2.2.2 Preparation of colchicine solution

A weighed quantity of the commercially available colchicine powder was mixed with a small quantity of glycerol (10% of the final volume) and dissolved in distilled water and then the volume was made up to achieve the required concentration. The concentrations tried were 0, 0.05, 0.15, 0.25, 0.35 and 0.45 per cent and accordingly 0, 25 mg, 75 mg, 125 mg, 175 mg and 225 mg colchicine were weighed and dissolved in 5 ml glycerol each and volume made up to 50 ml with distilled water.

3.2.2.3 Colchicine application

Buds at two stages of sprouting were selected for colchicine treat-

ment. The application was done by two methods and at two durations of treatment. The same treatment was repeated on two consecutive days.

Stage of sprouting

1. Stage I (S_1)

   just emerging buds

2. Stage II (S_2)

   buds at an advanced stage of sprouting

Methods of colchicine application

1. Method I (M_1) - hole method

   Here, colchicine was applied in holes drilled close to the bud. The hole was kept filled with the solution for the specified time (Plate 1).

2. Method II (M_2) - Cotton swab method

   Here the bud was kept covered with a piece of cotton wool soaked in colchicine solution for the specified time (Plate 2).

Duration of treatment

1. T_1

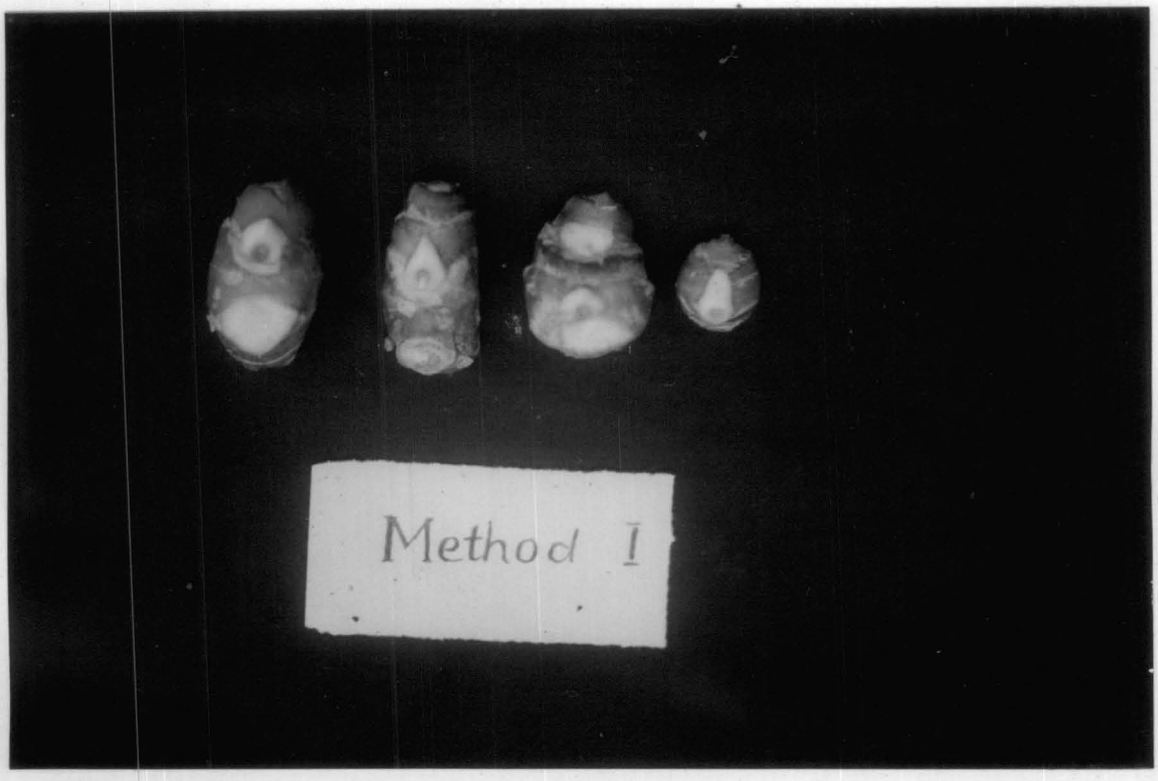
   4 hours on two consecutive days, from 7 a.m. to 11 a.m.

2. T_2

   6 hours on two consecutive days, from 7 a.m. to 1 p.m.

Plate 1 Method 1 of colchicine application: hole method

Plate 2 Method 2 of colchicine application: cotton swab method


Method I


Method II

In the experiment, two types of controls were maintained - absolute control and treatment controls. In absolute control, the rhizomes were planted as such without any treatment. In treatment controls, the rhizomes were prepared by scraping off the scales and removing the buds, retaining a single one and they were subjected to the same treatment except colchicine i.e., either the hole made near the bud was kept filled with distilled water mixed with glycerol (10% of final volume) or the buds were kept covered with a piece of cotton wool soaked in distilled water mixed with glycerol (10% of final volume); for the specified time.

Thus there are totally 48 treatment combinations viz., 2 stages of sprouting (S_1 and S_2) x 2 methods of application (M_1 and M_2) x 2 time of treatment (T_1 and T_2) x 6 doses of colchicine (C_1 to C_6) including the treatment controls. Twenty rhizome bits were subjected to each of the treatment.

After the treatments, the rhizomes were washed in running water for one hour and stored in moist soil overnight. On the next day also the same treatment was repeated, after which the rhizomes were planted in sand trays. Untreated controls were also planted. They were kept in the laboratory for two weeks, i.e., till the sprout emerges. Then the young plants were lifted undisturbed and planted in the main field at a spacing of 15 x 25 cms. Planting and after care was done according to Package of Practices Recommendations (KAU, 1993), except the application of chemical fertilizers.

The field was inspected and plants were observed regularly. Plants showing marked variation from the controls were marked and observations on establishment, growth, morphological features and biometrical characters

were recorded.

3.2.3 Observations

3.2.3.1 Germination

The number of rhizomes sprouted per plot was recorded at fort-nightly interval and the germination percentage was worked out. The data will be statistically analysed.

3.2.3.2 Number of leaves

Number of leaves produced per plant was recorded at monthly interval.

3.2.3.3 Leaf shape

The shape of leaves of each treated plant was observed visually and recorded as described by Lawrence (1951).

3.2.3.4 Length and width of leaves

Five fully opened leaves were selected at random from each of the treatment plant for recording the length and width of leaves. The length was measured as the distance between the base and tip of leaf blade. The average length and width of leaves were then computed.

3.2.3.5 Number of side shoots

The number of side shoots/suckers produced by each treatment plant was recorded and the average worked out.

3.2.3.6 Flowering

Number of plants flowered in each treatment was recorded and the mean percentage worked out.

3.2.3.7 Variations

Variation from control noted in any of the characters were also

recorded.

3.2.4 Identification of polyploids

Plants showing marked phenotypic difference from the control plants were harvested separately during the end of January, after all the leaves had dried up. There were totally 18 plants identified. Rhizomes were then dried in shade after dipping in cowdung slurry and stored in polythene covers. During May 2nd week, they were sown in sand trays along with the rhizome of untreated plants and watered regularly. When roots were formed i.e., after 1½-2 weeks, the root tips were collected and the mitotic stages were critically observed to ascertain the chromosome number and also to study cytological abnormalities. The morphological characters, number and size of stomata and flowering were studied in detail, in the selected plants in comparison to the standard.

3.2.4.1 Mitotic studies

In order to find out the chromosome number of variants, mitotic studies were carried out using root tip squash method. The procedure standardised and first reported by Rekha (1993) was adopted for this.

3.2.4.1.1 Time of collection of roots for mitotic studies

Expecting a high rate of cell division in the morning hours, roots were collected at an interval of 30 minutes starting from 5.30 a.m. upto 10.00 a.m. and slides were prepared and observed for dividing cells.

3.2.4.1.2 Procedure for mitotic studies

Young and actively growing roots were excised and washed

to remove adhering soil particles. They were first pretreated in saturated aqueous solution of α bromonaphthalene for a period of 4 hours at 4°C. The pretreated roots were washed thoroughly in 4-5 changes of distilled water and fixed in Carnoy's B fluid to which a little ferric acetate had been added.

Carnoy's B fixative

1 part acetic acid + 3 parts chloroform + 6 parts ethyl alcohol

After 24 hours the roots were washed thoroughly and drained on a filter paper and stained in Snow's carmine for 24 hours.

Snow's Carmine stain

Components		Preparation
Carmine	4 g	Four grams of carmine powder was dissolved in 15 ml boiling distilled water to which 1 ml of concentrated HCl had been added. Then 95 ml of 85% alcohol was added to it. The stain was then cooled, filtered and stored in tightly stoppered bottles (Snow, 1963)
85% alcohol	95 ml	
Concentrated HCl	1 ml	
Distilled water	15 ml	

A large quantity of the stain i.e., about 2 to 3 times as much as needed to cover the root rips was used for getting efficient penetration. To improve the stainability of chromosomes, a drop of ferric acetate was added to the stain also. The stained roots were squashed on a glass slide, in 45 per cent acetic acid. After keeping the coverglass, the slide was warmed slightly and pressed under the folds of a filter paper. The slides were sealed

with nail polish and screened for mitotic chromosomes. Camera lucida drawings of the good plates were made and photomicrographs were taken using a Leitz Biomed Research Microscope with Automatic Camera Unit.

3.2.4.2 Stomatal Study

Stomatal size is found to bear a direct correlation with the ploidy level of plants. An increase in the size of stomata is an indication of increased cell size which results from increase in the number of chromosomes.

For observing stomata, peeling of the lower epidermis of the leaf was taken and mounted in glycerol and observed under a light microscope. The length and width were measured with the help of Ocular and Stage Micrometers. Photographs of good preparations were taken.

In order to ascertain the stomatal index, peelings of the lower epidermis were taken from the base, middle and tip of the leaf lamina and number of stomatal openings, as well as number of other cells were counted per microscopic field. The stomatal index was worked out using the formula,

$$\text{Stomatal Index} = \frac{\text{Number of stomata}}{\text{Number of stomata} + \text{Number of other epidermal cells}} \times 100$$

Five mature leaves selected at random were observed per plant and mean stomatal index was determined.

The stomatal density was also worked out by counting number of stomatal openings per 1 mm^2 of the lower epidermis and expressed as number

per cm^2 of the leaf lamina. Here also 5 mature leaves were observed per plant, with 3 measurements from each leaf and average was worked out.

3.2.4.3 Meiotic Studies

Meiotic studies were carried out following the fixation and staining technique as described below. The procedure was first reported by Rekha (1993).

Inflorescences of variant as well as control plants were collected between 11 am and 12 noon and fixed in acetic alcohol (1:3) for 24 hours.

The single anther dissected out from flower bud of proper maturity was kept on a clean slide and smeared well in a drop of 1 per cent acetocarmine to which a little ferric acetate had been added. Cover glass was then placed over it and the slide was gently warmed and pressed under folds of a filter paper. The prepared slides were examined under a microscope and those slides with meiotic stages were sealed well ~~used~~ using nail polish. They were examined in detail for meiotic chromosomes, for their characteristics and behaviour.

RESULTS

4. RESULTS

An attempt was made for inducing polyploidy in *Kaempferia galanga* L. at the College of Horticulture, Vellanikkara during 1994-95, by treating the rhizomes with colchicine. The various effects of colchicine on kacholam as observed in the present study are presented below.

4.1 Preliminary trial to fix the doses of colchicine

The germination observed in the preliminary trial is presented in Table 1. From the table, it is clear that the LD_{50} value of colchicine for kacholam is somewhere around 0.5 per cent. Thus the doses for the experiment was fixed in the range of 0-0.5 per cent, at an interval of 0.1 per cent, starting from 0.05 per cent.

4.2 The experiment

In the present experiment, there were totally 48 treatment combinations i.e., 2 stages of bud sprouting x 2 methods of colchicine application x 2 treatment durations x 6 concentrations of the chemical. In each of the treatments, 20 rhizome pieces were used. After the treatment, rhizomes were planted temporarily in sand trays and later transplanted to the mainfield. The growth of the treated plants were observed critically in comparison with untreated control plants.

4.3 Survival of plants

There were death of plants resulting from the treatment in the initial stages. After two months no further mortality was noticed. The survival counts of plants recorded in various treatment combinations estimated as mean values are given in Table 2(a) and 2(b).

The survival of plants treated in the two methods M_1 and M_2 at both the treatment durations T_1 and T_2 are presented in Table 2(a). The mean

Table 1.

Sprouting in the preliminary trial

Days after sowing	Number of rhizomes sprouted						
	Concentration of colchicine (%)						
	0	0.25	0.5	0.75	1.0	1.5	2.0
5th	19	14	7	2	1	-	-
10th	19	16	8	4	1	-	-
15th	19	16	8	5	0	-	-

Table 2(a).

Survival of colchicine treated plants: Method of treatment vs Duration of treatment

Concentration of colchicine	Mean number of plants survived in					
	M_1	M_2	M_1T_1	M_1T_2	M_2T_1	M_2T_2
C_1 (Control)	18.25	18.00	18.5	18.0	19.0	17.0
C_2	12.50	17.50	15.5	9.5	17.0	18.0
C_3	12.00	17.50	13.5	11.0	18.0	17.0
C_4	11.50	17.25	13.5	9.5	16.0	18.5
C_5	9.00	17.00	11.5	6.5	16.5	17.5
C_6	8.00	16.50	8.5	7.5	15.5	17.5
Average of C_2-C_6	10.60	17.20	12.4	8.8	16.6	17.7

(all values expressed out of 20)

C_x indicate the concentration of colchicine

C_1 - 0% C_2 - 0.05% C_3 - 0.15%

C_4 - 0.25% C_5 - 0.35% C_6 - 0.45%

M_x indicate the method of treatment

M_1 - hole method M_2 - cotton swab method

T_x indicate the time of treatment

T_1 - 4 hours on two consecutive days T_2 - 6 hours on two consecutive days

Table 2(b).

Survival of colchicine treated plants: Stage of bud vs Method of treatment

Concentration of colchicine	Mean number of plants survived in					
	S_1	S_2	S_1M_1	S_1M_2	S_2M_1	S_2M_2
C_1	18.50	17.75	19.5	18.5	17.5	18.0
C_2	14.25	15.75	12.5	16.0	12.5	19.0
C_3	16.00	13.50	12.5	20.0	12.5	15.0
C_4	15.25	13.50	7.5	18.0	10.5	16.5
C_5	12.75	13.25	8.5	18.0	9.5	16.0
C_6	13.00	11.50	8.5	17.5	12.5	15.5
Average of C_2-C_6	14.30	13.50	9.8	17.9	11.4	16.4

(all values expressed out of 20)

S_x indicate the stage of bud growth

S_1 - just activated buds S_2 - advanced stage of sprouting

For M_1, $X^2_{15} = 5.49$; for M_2, $X^2_{15} = 3.66$

For M_1, $X^2_5 = 38957320^{**}$; for M_2, $X^2_5 = 35216470^{**}$

number of plants survived in M_1 was 10.6 while that of M_2 was 17.2. On the other hand, the mean survival of controls (0%) were 18.25 and 18.00 respectively. This clearly indicates that mortality of the plants is not due to the mechanical injury that may be caused while drilling the hole near the bud, but due to the effect of colchicine. Taking into consideration the duration of treatment, in M_1 a higher treatment duration tended to reduce the survival rate. The average of M_1T_1 was 12.4 and that of M_1T_2 was 8.8. In M_2, reverse trend was observed and the average values of M_2T_1 was 16.6 and that of M_2T_2 was 17.7.

Table 2(b) presents the survival of treated plants comparing the stages of bud growth and methods of treatment. A higher mortality was observed in method 1 compared to method 2 in both the stages of bud growth. The mean survival count in S_1M_1 was 9.8 and that of S_1M_2 17.9 while the values in S_2M_1 and S_2M_2 were 11.4 and 16.4 respectively. For M_1, a slightly higher mortality was observed in S_1 (mean survival count 9.8) compared to S_2 (mean survival count 11.4) whereas for M_2 the trend was reverse (the mean survival counts for S_1 and S_2 were 17.9 and 16.4 respectively). In general, plant mortality was found slightly higher in the advanced stage of bud sprouting (mean survival count in S_2 - 13.5) compared to buds that have just begun to sprout (mean survival count in S_1 - 14.3).

However, though the difference between M_1 and M_2 was found statistically significant, X^2_{15} for M_1 as well as M_2 was not significant. Thus it must be concluded that stage of bud growth and time of treatment have no significant effect on M_1 and M_2. Some of the treatments indicate a trend that as the concentration of colchicine increases, the number of plants survived decreases. But this was not found statistically significant, since X^2_5 for M_1 and M_2 were highly significant.

4.4 Growth of plants

In the main field, the growth of the plants emerged from the treated rhizomes were observed in comparison with untreated control plants.

In general, the plants resulting from the method of treatment M_1, wherein colchicine was applied in holes drilled close to the sprouting bud were smaller in size than the control plants. Initial growth rate was slow, number of suckers and total number of leaves per plant were less, and size of leaves were smaller. The mean number of suckers per plant in M_1 was 2.28, two months after planting and 4.45, four months after planting. The corresponding values in controls were 4.10 and 7.17 respectively [Table 3(a) and 3(b)]. Total number of leaves per plant was 3.46, two months after planting and 8.79, four months after planting in the case of M_1 where as the controls recorded the values 8.68 and 19.78 respectively [Table 4(a) and 4(b)]. The leaf size recorded three months after planting is furnished in Table 5 and it can be clearly seen that mean leaf length and breadth in M_1 were less than the controls. Many of the plants had an unhealthy appearance because of fewer number of leaves while in the case of control plants there was profuse suckering and clustering of leaves. But several of the plants were found to possess normal rate of growth towards the end of the growing season so that at maturity they were more or less like the untreated plants. In other words the deleterious effects noted in the early stages of growth were compensated towards the later stage of growth.

The plants resulting from the method of treatment M_2 wherein cotton swab techniqe was adopted, were very much like the control plants with no appreciable difference. There were neither gigas plants nor plants with reduced vigour.

Normally in kacholam, plants start flowering about 2 months after planting. The flowering season is from June to September and peak flowering is noticed during July and August. The percentage of plants flowered in various treatments is presented in Table 6.

Table 3(a).

Mean number of suckers per plant, 2 months after planting

	M_1				M_2			
	S_1T_1	S_2T_1	S_1T_2	S_2T_2	S_1T_1	S_2T_1	S_1T_2	S_2T_2
C_1	4.32	3.91	4.41	3.77	4.47	3.90	4.30	3.78
C_2	2.62	2.17	2.34	2.72	5.02	4.12	4.03	3.91
C_3	2.51	2.23	2.14	2.91	4.11	3.74	3.65	3.17
C_4	2.32	2.14	2.13	2.31	3.64	3.71	3.61	3.12
C_5	2.16	2.90	2.09	2.01	3.55	3.69	3.53	3.09
C_6	1.96	2.02	1.97	1.86	3.69	3.44	3.12	3.11
	Grand mean of treatments = 2.28 Grand mean of controls = 4.10				Grand mean of treatments = 3.65 Grand mean of controls = 4.11			

Table 3(b).

Mean number of suckers per plant, 4 months after planting

	M_1				M_2			
	S_1T_1	S_2T_1	S_1T_2	S_2T_2	S_1T_1	S_2T_1	S_1T_2	S_2T_2
C_1	7.86	8.12	6.69	6.01	7.40	6.93	7.48	6.99
C_2	4.96	5.16	5.07	4.97	8.77	8.14	8.08	7.50
C_3	4.84	4.64	4.36	4.78	8.55	6.91	7.18	6.98
C_4	4.36	4.41	4.28	4.37	7.69	6.66	6.52	6.63
C_5	4.16	4.23	4.26	4.17	7.34	6.39	6.49	6.30
C_6	3.94	4.18	3.78	4.01	6.94	6.22	6.48	6.26
	Grand mean of treatments = 4.45 Grand mean of controls = 7.17				Grand mean of treatments = 7.10 Grand mean of controls = 7.20			

Table 4(a).

Mean number of leaves per plant, 2 months after planting

	M_1				M_2			
	S_1T_1	S_2T_1	S_1T_2	S_2T_2	S_1T_1	S_2T_1	S_1T_2	S_2T_2
C_1	8.75	8.02	9.05	8.92	8.85	9.42	8.28	8.91
C_2	3.75	4.62	5.50	5.66	9.35	8.35	9.07	9.00
C_3	4.64	4.22	3.95	4.65	9.14	8.57	8.71	8.35
C_4	2.34	3.16	3.35	3.32	8.78	9.21	9.28	8.50
C_5	2.75	2.86	2.99	2.52	8.71	8.92	8.42	9.50
C_6	2.00	2.57	2.14	2.11	9.28	8.85	8.21	8.64
	Grand mean of treatments = 3.46 Grand mean of controls = 8.68				Grand mean of treatments = 8.84 Grand mean of controls = 8.86			

Table 4(b).

Mean number of leaves per plant, 4 months after planting

	M_1				M_2			
	S_1T_1	S_2T_1	S_1T_2	S_2T_2	S_1T_1	S_2T_1	S_1T_2	S_2T_2
C_1	20.22	18.69	21.32	18.91	21.28	20.42	18.96	19.98
C_2	9.93	9.12	12.32	14.75	21.63	18.25	22.32	22.70
C_3	9.64	9.09	10.44	10.36	22.74	21.12	19.66	18.20
C_4	7.90	8.94	9.62	8.62	19.75	20.56	21.35	20.92
C_5	6.22	6.21	8.33	8.65	19.85	18.91	19.78	20.20
C_6	5.57	5.22	6.92	7.99	20.91	20.38	18.52	18.35
	Grand mean of treatments = 8.79				Grand mean of treatments = 20.30			
	Grand mean of controls = 19.78				Grand mean of controls = 20.16			

Table 5.

Average leaf size per plant, 3 months after planting

	M_1								M_2							
	S_1T_1		S_2T_1		S_1T_2		S_2T_2		S_1T_1		S_2T_1		S_1T_2		S_2T_2	
	l	b	l	b	l	b	l	b	l	b	l	b	l	b	l	b
C_1	10.12	7.52	9.29	7.16	11.62	8.17	9.34	6.92	13.41	8.91	12.78	9.22	13.64	9.00	12.99	8.53
C_2	8.96	6.86	8.83	6.98	7.37	5.62	7.86	5.78	12.28	8.24	12.56	9.45	11.74	8.28	11.94	8.38
C_3	8.66	5.73	8.25	5.79	7.02	5.46	7.14	5.62	11.67	8.45	11.62	8.37	13.33	9.23	12.13	8.93
C_4	7.71	5.55	7.31	5.26	6.63	5.35	6.43	5.55	11.52	8.50	11.55	8.26	12.13	9.13	12.04	8.77
C_5	6.97	5.21	6.96	5.05	6.61	4.83	6.49	4.85	11.61	8.30	11.42	8.08	11.66	8.26	11.71	8.34
C_6	6.58	4.81	6.90	5.03	6.45	4.61	6.16	5.13	10.99	7.94	11.11	8.03	11.23	7.88	11.28	7.90

Mean leaf length of treatments	= 7.26	Mean leaf length of treatments	= 11.78
Mean leaf length of controls	= 10.09	Mean leaf length of controls	= 13.2
Mean leaf breadth of treatments	= 5.45	Mean leaf breadth of treatments	= 8.44
Mean leaf breadth of controls	= 7.44	Mean leaf breadth of controls	= 8.92

Table 6.

Mean percentage of flowering in treated plants

	$S_1M_1T_1$	$S_1M_1T_2$	$S_2M_1T_1$	$S_2M_1T_2$	$S_1M_2T_1$	$S_1M_2T_2$	$S_2M_2T_1$	$S_2M_2T_2$
C_1	100.00	94.44	94.74	100.00	95.00	100.00	100.00	94.44
C_2	90.00	100.00	80.00	81.25	93.75	95.00	93.75	100.00
C_3	54.54	72.73	84.62	69.23	100.00	100.00	100.00	100.00
C_4	63.63	75.00	71.43	53.85	94.44	94.74	100.00	92.86
C_5	50.00	28.57	81.82	41.67	100.00	100.00	94.12	87.50
C_6	33.33	33.33	27.27	16.67	95.00	100.00	93.33	100.00

Grand mean of controls in M_1 = 97.3% Grand mean of control in M_2 = 97.36%

Grand mean of treatments in M_1 = 60.45% Grand mean of treatments in M_2 = 96.72%

From the table it is evident that in M_1 a lower proportion of plants flowered and in M_2 number of plants flowered were on par with the control. Flowering was noticed in all the concentrations of colchicine treatment. And no delayed flowering was observed i.e., no flowering was noticed after September.

Towards the end of the growing season 18 plants showing marked phenotypic difference from the normal plants were identified as suspected polyploids. The pattern of selection of variants from among the various treatment combinations are presented in Table 7.

The selected variants include 14 plants from M_1 and 4 from M_2. Thirteen plants selected from M_1 were characterised by reduction in vigour and they didn't flower during the crop period. The 14th plant was the largest/most vigorous plant from M_1. Of the 4 plants selected from M_2, one was the largest plant and one was the smallest. Two other variants were also selected from M_2. The morphological features of the selected plants as compared to normal plants are furnished in Table 8.

4.5 Morphology of the variants selected

From Table 8, it is clear that most of the variants selected were characterised by reduced vigour and plant size. No increased plant size was noted as a result of the treatment. Some variants had thicker and darker green leaves which is a character associated with polyploids. Other variations noted include round shape and pale green colour of leaves. One variant plant appeared chimeric (plant No.5) with prominent striations on the leaves in one of the side shoots. Purple colouration of the leaf tip on the under surface was another characteristic noted and in one variant, the young leaves had yellowish tip. Some variants had

Table 7.

Number of variants identified from different treatment combinations

	$S_1M_1T_1$	$S_1M_1T_2$	$S_2M_1T_1$	$S_2M_1T_2$	$S_1M_2T_1$	$S_1M_2T_2$	$S_2M_2T_1$	$S_1M_2T_2$
C_1								
C_2	1		2					
C_3				1			1	
C_4	2		1		1			1
C_5	2	1				1		
C_6	2	1		1				

Morphological features of selected variants

Plant Number	Treatment combination from which the plant has been identified	Number of suckers	Number of leaves	Leaf size		Flowering	Other characters
				Length (cm)	Width (cm)		
1	$S_2M_1T_1C_2$	2	5	6.42	2.31	x	Small plant, less vigorous Leaves smaller
2	$S_2M_1T_1C_4$	3	6	7.25	6.15	x	Small plant, less vigorous Leaves small, dark green in colour, with wavy margins
3	$S_2M_1T_1C_5$	4	11	9.5	7.0	x	Leaves thick and roundish
4	$S_2M_1T_2C_4$	3	7	8.5	4.5	x	Small plant Leaves small, pale green in colour Leaf tip has a purple colouration on the undersurface
5	$S_2M_1T_2C_5$	2	8	3.75	1.80	x	The smallest plant in M_1 Leaves very small, pale and elongated Petiole long and visible above ground Leaves stand separate without any clustering
6	$S_2M_2T_1C_4$	3	6	6.5	3.75	x	Small plant, less vigorous Leaves small, pale
7	$S_2M_2T_1C_5$	1	4	6.5	4.5	x	Small plant, less vigorous Leaves thick and darker green but small in size
8	$S_2M_2T_2C_4$	3	7	8.2	5.2	x	Small plant Leaves pale and thin
9	$S_2M_2T_2C_5$	2	5	7.5	4.25	x	Small plant Young leaves had a yellow colouration in the tip
10	$S_3M_1T_1C_2$	2	8	7.0	4.0	x	Plant small and less vigorous Leaves small with prominent tip
11	$S_3M_1T_1C_4$	3	8	9.25	4.60	x	Small plant Leaves narrow and pointed In one side shoot the leaves appear striped with horizontal dark green and light green bands
12	$S_3M_1T_1C_5$	3	7	8.0	3.0	x	Smaller leaves with wavy margins Plant less vigorous Purple colouration of leaf tip on the undersurface of young leaves
13	$S_3M_1T_2C_4$	3	8	9.5	5.5	x	Leaves thick Plant less vigorous
14	$S_3M_1T_2C_5$	5	14	12.0	8.25		The largest plant in M_1
15	$S_3M_2T_1C_4$	5	11	9.5	6.5	x	Leaves dark green with round tip Leaf veins prominent
16	$S_3M_2T_1C_5$	5	12	12.0	7.0		Leaves thick and pale
17	$S_3M_2T_2C_4$	9	26	13.0	9.0		The larges plant in M_2
18	$S_3M_2T_2C_5$	4	9	9.5	6.5		The smallest plant in M_2 Less vigorous Purple colouration of leaf tip on the undersurface
Control		8	22	13.50	9.20		

S_2 and S_3 are stages of bud sprouting, S_1 being just emerging buds and S_3 an advanced stage of sprouting

M_1 and M_2 are methods of colchicine application. In M_1 colchicine was applied in a hole drilled close to the bud and in M_2 a cotton piece soaked in the chemical was placed over the bud

C_2 indicate concentration of colchicine - C_2 - 0.05%, C_3 - 0.15%, C_4 - 0.25%, C_5 - 0.35% and C_6 - 0.45%

T_1 and T_2 are time of treatment - T_1 - 4 hours and T_2 - 6 hours, repeated on two consecutive days

wavy leaf margins.

The selected plants were marked in the field using pegs and harvested separately in the last week of January, after all the leaves had dried up. There were no morphological difference for the rhizomes, except in size between the variants and the control plants (Plate 3). All the variants except one had one or more mother rhizomes and a few finger rhizomes. One variant (plant No.6) had a single mother rhizome. The rhizomes of another variant (plant No.5) were very small and globular with closely spaced leaf scars or nodes. The fresh weight of rhizomes ranged from 1.38 g to 48.00 g. Another characteristic of kacholam rhizomes is the presence of 'waterbags' i.e., swellings on the roots which were present in the variants as well.

The rhizomes after drying in shade were stored in polythene bags for detailed cytological and morphological studies in the next growing season.

4.6 Study of the variants selected for detection of polyploids

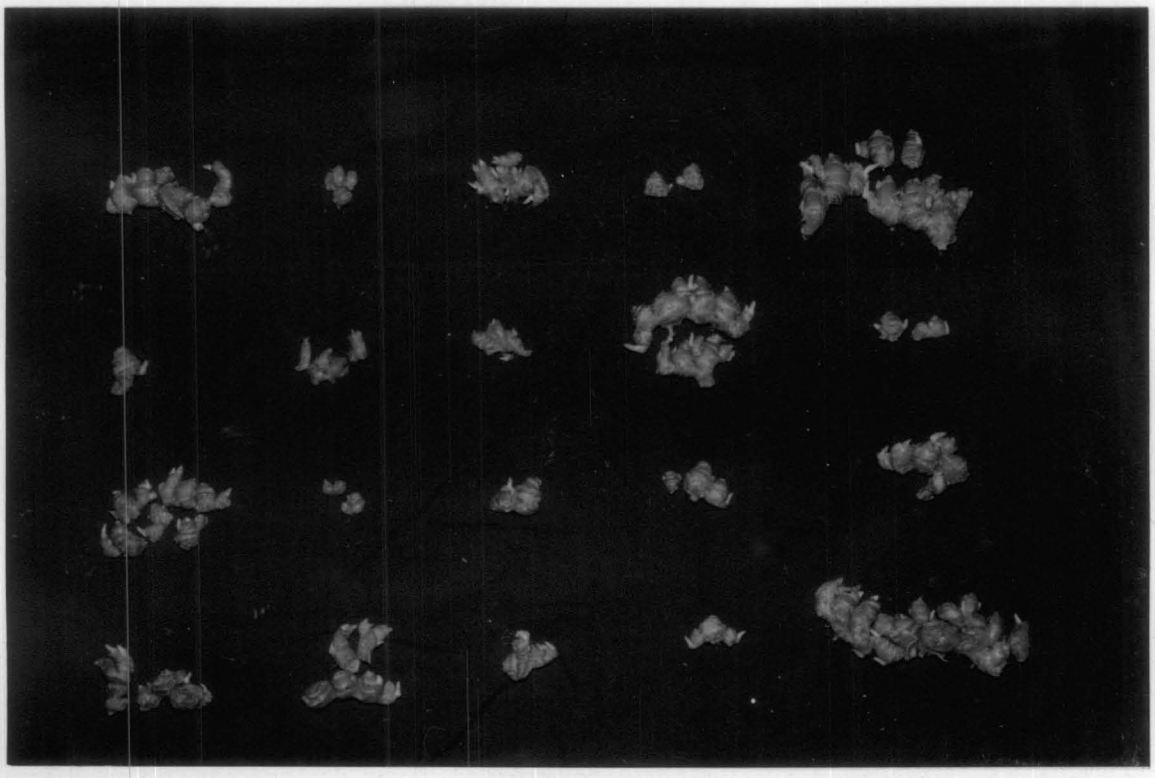
The usual morphological features associated with polyploidy include increased cell size and size of vegetative parts, extended vegetative phase, delayed flowering, increase in the size of stomata and decrease in its density etc However, these indications are only secondary and hence can be used for preliminary screening for the presence of polyploids. The final proof to confirm a plant to be a polyploid is the increased number of chromosomes in the somatic cells which can be confirmed by observing the mitotic division. Meiotic irregularities are also associated with polyploids and there will be various chromosome associations.

For detailed study of the variants isolated, in the following crop season, the rhizomes were planted in sand trays in the lab and watered regularly

Plate 3 Rhizomes of variants identified and control plants

LAY–OUT

Control	Variant 1	Variant 2	Variant 5	Control
Variant 7 (Polyploid)	Variant 4	Variant 6	Variant 14	Variant 9
Variant 15	Variant 8	Variant 3	Variant 13	Variant 18
Variant 12	Variant 16	Variant 10	Variant 11	Variant 17



Two weeks later the root tips were excised and mitosis was observed. The plants were then replanted in earthen pots filled with potting mixture and their growth was critically examined and observations on biometrical characters were recorded.

4.6.1 Mitotic studies

In order to find out the chromosome number of the parent material as well as the variants, root tip squash preparations were made and examined. The results are presented below.

The ideal time of collection of roots for mitotic studies was found to be 7.30 a.m., both for the parent material and for the variants. Cytological preparations from the roots collected at 7.30 a.m. had maximum cells at early metaphase stage. Pre-treatment of roots in saturated aqueous solution of α bromonaphthalene at 4°C for 4 hours followed by 24 hour fixation in Carnoy's B fluid followed by staining in Snow's Carmine for 24 hours was found to give good cytological preparation with proper condensation of chromosomes and good spread. The stained roots were squashed in 45 per cent acetic acid. Addition of a little ferric acetate to the fixative and also to the stain improved the stainability of chromosomes.

4.6.1.1 Mitotic study of the parent material

Root tip squash studies in *Kaempferia galanga* L. using Snow's carmine stain revealed that the somatic chromosome number in this crop is 55 (Plate 4).

4.6.1.2 Mitotic studies in the variants

Root tip squash studies were conducted in all the variants iden-

tified. Out of 18 variants selected, one had 110 chromosomes in their somatic cells. It is an induced polyploid with 2n = 110 (Plate 7). All the remaining ones had 55 chromosomes in the root tip cells.

4.6.2 Morphological studies

For studying morphology of aerial parts, the rhizomes were divided into sections about 4-5 g in weight, and planted temporarily in sand trays. Two weeks after, the root tips were excised for mitotic studies. Kacholam produce thin fibrous roots as well as fairly thick roots. The colour of the roots is white with creamy yellow tip. There were no difference in colour, thickness and texture among the roots of the polyploid as well as other variants and the control plants.

After excising roots for the mitotic studies the plants were allowed to grow in pots and morphology of the polyploid as well as other variants was studied in detail. Table 9 presents observations on biometrical characters.

From the table, it is evident that observations on 14 variant plants are on par with the control plants. None of the aberrant leaf characteristics were expressed. However the polyploid as well as three other plants differ substantially from normal plants. Those four may be stable variations in kacholam brought about by colchicine, one being a higher level polyploid. The morphology of a standard plant, the polyploid and three other stable variants are described below.

4.6.2.1 Morphology of a standard plant

A typical plant of *Kaempferia galanga* L. (Plate 4) is having a bushy appearance with 15 or more leaves in 5-10 suckers. Leaves spread

Table 9.

Biometrical characters of variants, the polyploid and control plants in the second generation

Plant No.	Total number of leaves						Average leaf size		Number of suckers	Days to flowering	Yield (g)
	1 map	2 map	3 map	4 map	5 map	6 map	Length (cm)	Width (cm)			
Control	3	8	13	18	24	25	11.25	8.3	8	49	45.38
Control	4	8	12	17	23	23	13.75	8.25	7	53	46.53
1	4	7	13	18	24	26	13.17	9.45	9	53	52.16
2	2	4	7	8	9	9	10.25	6.36	2	—	12.96
3	4	9	14	21	26	26	10.50	8.15	8	54	48.03
4	3	6	10	15	22	23	11.95	9.40	6	50	42.56
5	3	4	5	8	9	9	4.82	2.34	2	—	7.44
6	4	8	14	20	24	25	12.08	9.30	7	53	43.00
7 (Polyploid)	2	3	4	5	7	7	6.75	4.20	3	—	11.32
8	3	8	11	16	21	22	13.03	9.56	8	54	42.17
9	2	4	6	8	9	10	8.93	7.24	4	—	15.20
10	5	8	14	22	28	27	11.52	9.38	10	50	48.81
11	4	8	18	28	33	33	12.56	8.80	8	51	73.42
12	3	9	16	27	32	31	11.52	9.33	8	49	68.19
13	3	8	17	25	29	28	13.23	9.87	10	49	59.12
14	3	7	16	23	26	25	10.30	8.60	9	50	53.38
15	3	7	14	19	23	25	11.65	7.65	8	52	52.67
16	4	8	13	18	24	23	12.36	9.63	7	52	55.79
17	3	7	14	21	26	27	10.48	8.91	8	51	57.98
18	4	8	15	23	27	27	12.27	8.58	8	50	58.86

horizontally on the ground and the usual plant spread will be around 25-30 cm. The plant is stemless and only leaves are visible above the ground. The plant height is usually less than 15 cm. The average length and breadth of leaves of the plant under study was 12.13 cm and 9.03 cm respectively. The mean length of stomata was 48 μ and the stomatal index was 10.7 (mean stomatal density 5,600/cm² of the leaf lamina) (Plate 7).

The growing season is from June to January and the rhizome is having a dormancy from December to May. By the end of the growing season the leaves dry up. Kacholam flowers during July to September. The flowers appear in the centre of a sucker and the inflorescence is enclosed in the sheathy leaf petiole. Flower opening is in succession and the outermost one opens first. A period of 8-10 days is required for the completion of blooming in a cluster.

The rhizomes are aromatic, consist of a few mother rhizomes and several finger rhizomes. Rhizomes contain a number of buds covered by leathery scales. Per plant yield of rhizomes will be around 50-70 g.

4.6.2.2 Morphology of the polyploid

The polyploid was obtained from the treatment combination $S_1M_1T_1C_0$ i.e., from a rhizome with first stage of bud sprouting (S_1) treated with 0.45 per cent colchicine (C_0) in a hole drilled close to the bud (M_1) for a time of four hours (T_1), the treatment being repeated on two consecutive days.

The plant was less vigorous and slow in growth and it had a robust appearance with dark green and thicker leaves (Plate 8). Number of suckers was reduced. There was a single shoot in the first year and three in the second generation. The total number of leaves produced during the entire growing season was

12. There was no difference in leaf shape from normal plant. The length and breadth of the largest leaf was 7.08 cm and 4.23 cm respectively. The mean length of stomata was 64 μ, which is 33.3 per cent higher than the normal plants. The index was high with a value of 12.6 (mean stomatal density - 4400/cm² of leaf lamina) (Plate 9). The plant did not flower neither in the first year nor in the second year.

The mitotic study of root tip cells revealed that in somatic cells the plant contains 110 chromosomes. Meiotic study could not be conducted since the plant did not flower.

The rhizomes morphology was similar to the normal plants except the size. In the first generation there was a single mother rhizome weighing 4 g and no finger rhizomes. It yielded one mother rhizome and three finger rhizomes in the second generation which weighed 11.32 g.

4.6.2.3 Morphology of the variants

Of the 17 other plants identified as variants, 14 behaved like normal plants in the second generation (see Table 9). There was no sign of growth retardation and there was no reduction in the size of plants. All of them flowered in the normal flowering season. So it must be concluded that the variations observed in them in the first generation were not permanent. But three of the variants, viz., plants numbered 2, 5 and 9 (see Table 9) maintained their characteristics in the second generation also. The morphology of these stable variants are described below.

4.6.2.3.1 Plant number 2

This was obtained from the treatment $S_1M_1T_1C_4$ i.e., from a

Plate 4 A normal plant of kacholam

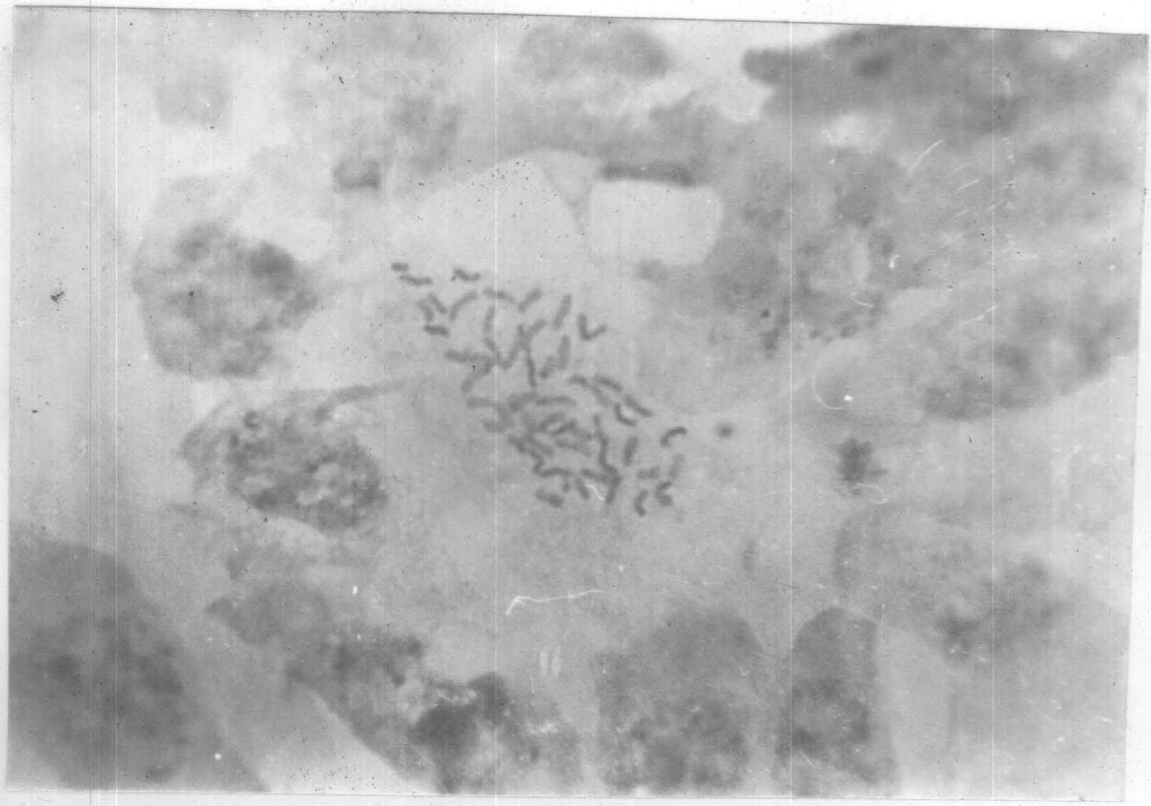
Plate 5 Induced polyploid in kacholam


8·4·14


1·3·9

Plate 6 Somatic chromosomes of normal kacholam plant (X 4000)

Plate 7 Somatic chromosomes of the polyploid (X 6000)



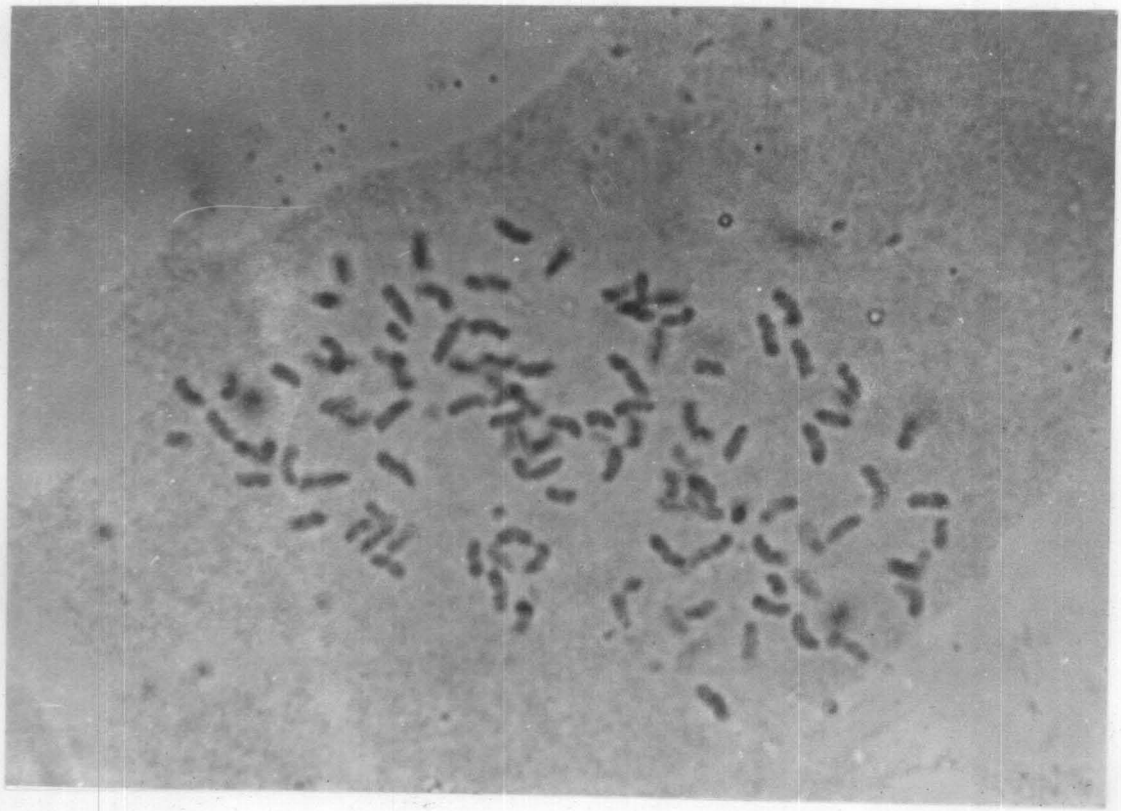
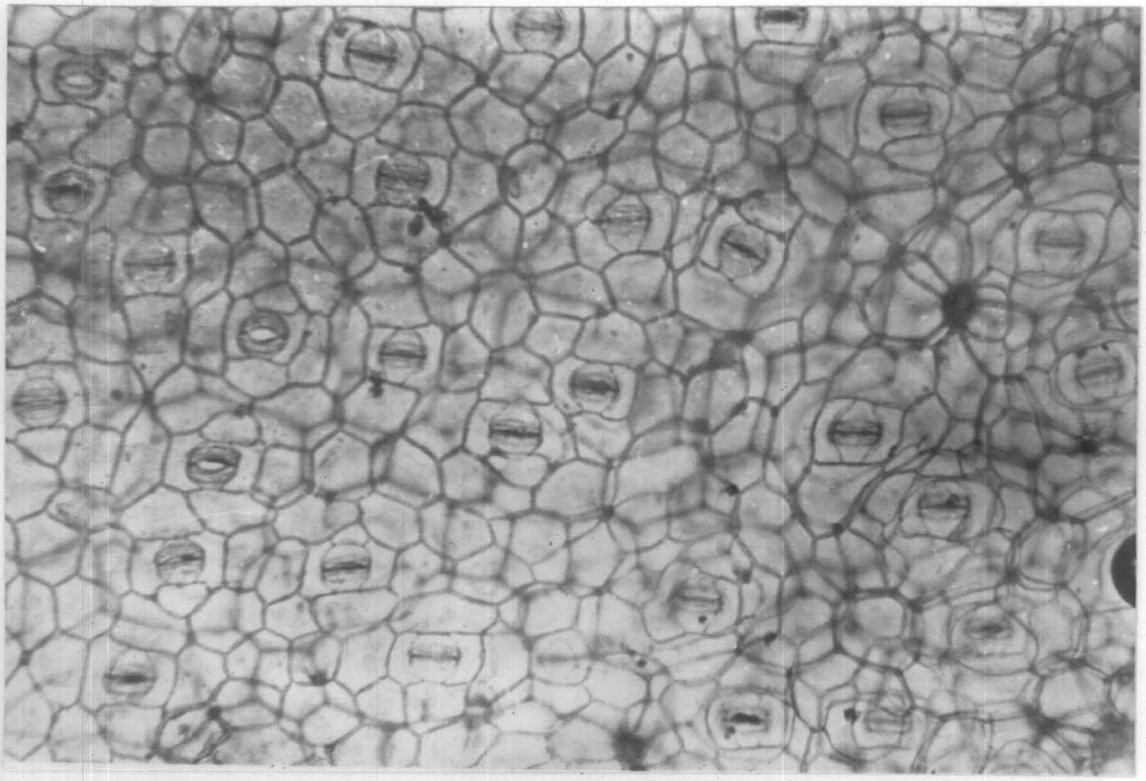


Plate 8 Stomata of normal kacholam plant
(X 400)

Plate 9 Stomata of the polyploid
(X 400)



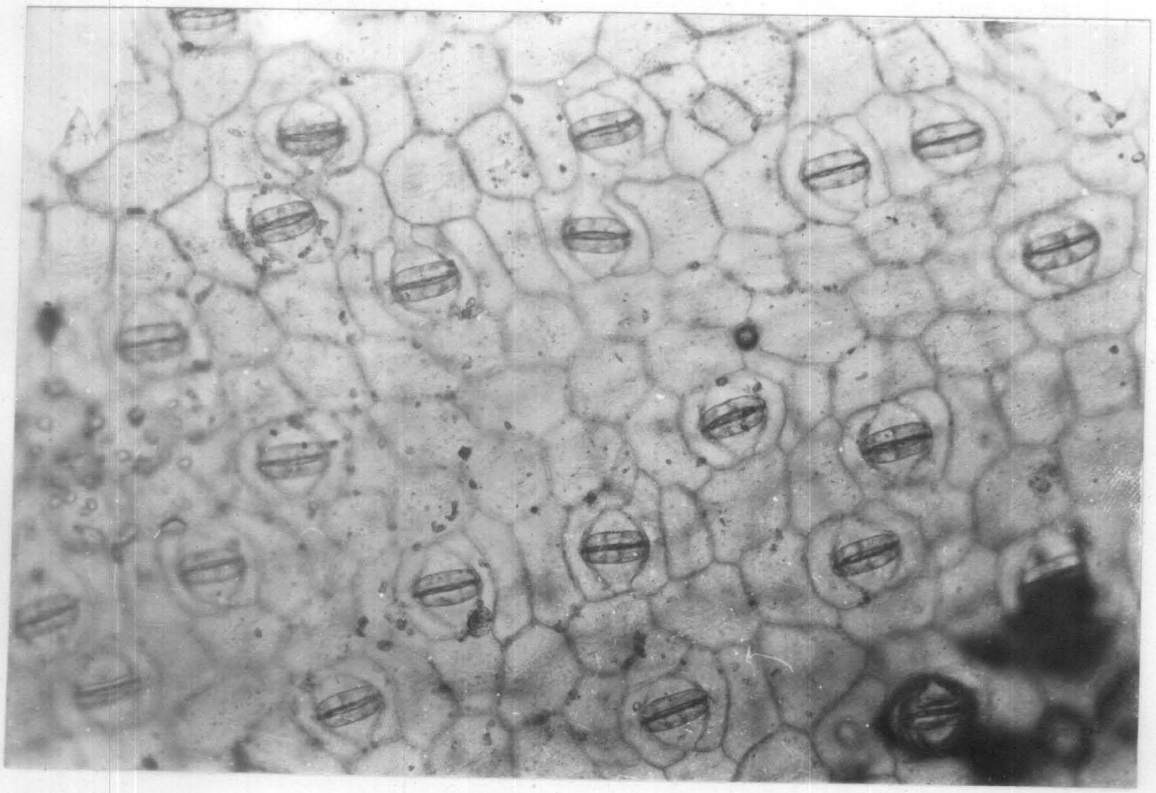


Plate 10 Variant plant No. 2

Plate 11 Variant plant No. 5

rhizome with the first stage of bud sprouting (S_1) treated with 0.25 per cent colchicine (C_4) in a hole drilled close to the bud (M_1) for a time duration of four hours on two consecutive days (T_1).

The plant was small with lesser number of suckers and leaves (Plate 10). The number of suckers was two and number of leaves six months after planting was nine. The leaf size was almost the same as the control plants. The margins of the leaf lamina were wavy. The size of the largest leaf was 11 cm x 7 cm. The leaf colour was normal. The stomatal size and density was on par with the control plants. The plant did not flower.

The yield was also less. In the first year the rhizomes weighed 3.14 g and in the second year the yield was 12.92 g.

4.6.2.3.2 Plant number 5

This was obtained from the treatment $S_1M_1T_1C_5$ i.e., from a rhizome with the first stage of bud sprouting (S_1) treated with 0.35 per cent colchicine (C_5) in method M_1 for a duration of four hours on two consecutive days (T_1).

The plant was the smallest among the treated plants (Plate 11). The leaves were very small and narrow and their number was also less. The average leaf size was 4.82 cm x 2.34 cm. Leaf petiole was longer and leaf lamina was pale green in colour. The side shoots are not distinguishable as the leaves stand separate. Above the ground, a portion of leaf petiole and leaf lamina were visible. The leaves were not clustered and the stomatal size was identical to the control plants. The plant did not flower.

The plant yielded two very small rhizomes which were globular


465


187

in shape with closely spaced leaf scars weighing 1.38 g in the first year. In the second year, four such rhizomes, a little bigger in size, were produced. The yield was 7.44 grams.

4.6.2.3.3 Plant number 9

This plant was from the treatment $S_1M_1T_2C_6$, i.e., from a rhizome with the first stage of bud sprouting (S_1) treated in method M_1 with 0.45 percent colchicine (C_6) for a time of six hours on two consecutive days (T_2).

The number and size of leaves were reduced (Plate 12). The average size of leaves was 8.93 cm x 7.24 cm. In the first year the young leaves exhibited a yellow colouration in the tip which did not appear in the second year.

The rhizome growth was also found retarded. The yield recorded in the first year was 5.06 g and in the second year it was 15.20 g.

4.6.3 Meiotic studies

As the polyploid did not flower, meiotic study could not be conducted. In case of normal kacholam plant the procedure followed by found to give good preparations. Univalents, bivalents and multivalents could be detected in pollen mother cells during metaphase I.

Plate 12 Variant plant No. 9

DISCUSSION

5. DISCUSSION

Kacholam, *Kaempferia galanga* L. is an important medicinal plant belonging to the family Zingiberaceae. It is useful for treating a variety of ailments and it forms an essential ingredient in several ayurvedic preparations. At present production of this crop is not able to cope up with the demand. Improvement of the crop by conventional methods of breeding is not possible due to seedless nature of the plant. Existing variability is also much limited for want of seed production. In this context the present investigation is carried out to induce polyploidy in kacholam. Colchicine was used to bring about chromosome doubling. Autopolyploidy, produced by doubling of chromosomes, have been found to be of much value in the case of plants in which economic parts are vegetative, due to their tendency to have vigorous vegetative growth. Kacholam is ideal for polyploidy breeding, since rhizomes are the economic part as well as the propagation material. The present investigation is an attempt to induce polyploidy in kacholam and to study the morphological features of the induced polyploid and also other variants obtained through colchicine treatment.

5.1 Survival of colchicine treated plants

The plants resulting from colchicine treatment exhibited a lower survival rate in comparison to control. The survival rate decreased with increase in concentration of colchicine, in general. In other words a higher lethality was found associated with a higher dose. Similar trends were reported in several crops by

several workers. Gupta and Koak (1976) in *Zinnia elegans*, Schifino and Moraes-Fernandes (1987) in *Trifolium*, Kumar *et al.* (1988) in *Capsicum annuum* and Dhawan and Tyagi (1989) in *Hyoscyamus muticus* reported similar trends. Kumar *et al.* (1988) opined that the germination depression and low survival rate can be attributed either to the toxic effect of colchicine or to irregular cell division that results in death of seedlings.

Among the two methods of colchicine application tried in the present investigation, M_1, in which colchicine was applied in a hole drilled close to the bud, exhibited a lower survival rate compared to M_2, the cotton swab method. The mean percentage of plants survived in M_1 was 53.0 per cent, while that of M_2 was 86.0 per cent. Considering the fact that mean survival in treatment controls were almost equal, the difference in survival rate can very well be attributed to the effect of colchicine. In M_1 colchicine was introduced deep into the tissue and hence the effect might have been more pronounced. This is further strengthened by the fact that the stable variants including the polyploid were obtained from M_1. It is to be noted that higher mortality of treated plants was observed in M_1 compared to M_2 in both the stages of bud growth. The survival of S_1M_1 was 49 per cent and S_1M_2 was 89.5 per cent whereas for S_2S_1 and S_2M_2 the corresponding values were 57 and 82 per cent.

When we compare the two methods of colchicine application, M_1 and M_2, taking into consideration to duration of treatment, it can be found that the effect of treatment duration is more pronounced in M_1. In other words a higher treatment duration declined the survival rate. The mean percentage for M_1T_1 was 62 while that of M_1T_2 was 44. In M_2 such a trend was not observed. The respective values of T_1 and T_2 for M_2 were 83 per cent and 88.5 per cent. The reason for this behaviour may be the fact that inner tissues were put in contact

with the chemical in M_1.

Plant mortality was found slightly higher in advanced stage of bud sprouting (S_2) compared to buds that have just begun to sprout (S_1). The mean survival for S_1 was 71.5 per cent and that for S_2 was 67.5 per cent. Also none of the stable variants could be isolated from S_2. Hence it can be concluded that just activated buds are ideal for colchicine treatment for polyploidy induction in kacholam.

5.2 Growth of plants

The effect of M_1 which reduced the survival of treated plants was also reflected in the growth of the plants. In general the plants in M_1 were smaller in size than the control plants. The plants in M_1 were less vigorous while in the case of M_2 the plants were more or less like the control plants with no appreciable difference. In M_1 a lower proportion of plants flowered (60.45%) while in M_2 almost all the plants flowered in the normal flowering season (96.72%). Out of 18 variant plants selected from among the treated plants, 14 were from M_1 and four from M_2. Thus M_1 was more successful in inducing variability compared to M_2

5.3 Methods of colchicine application

In the present investigation, two methods of colchicine application were tried, viz., colchicine application in a hole drilled close to the sprouting bud (M_1) and cotton swab method (M_2). Among these two, the polyploid as well as three stable variants were obtained from M_1 and no stable variation could be spotted in M_2. Thus M_1 is proved to be an ideal technique for induction of polyploidy in kacholam. Ramachandran (1982) and Ramachandran and Nair (1992) reported the success of this technique for polyploidy induction in ginger, a similar

rhizomatous crop of the same family. In the present study also since this technique was successful in inducing polyploidy, it can be proposed as an ideal technique for polyploidy induction in rhizomatous crops, in general.

5.4 Chromosome number of kacholam

Root tip cells of *Kaempferia galanga* L. when observed under a microscope as a squash preparation using Snow's carmine stain revealed that they contain 55 chromosomes. Thus the chromosome number of this crop is confirmed to be 2n = 55. This result is in agreement with earlier report by Rekha (1993). She further reported classification of the 55 chromosomes into eleven sets of five structurally similar chromosomes and this indicates that *Kaempferia galanga* L. is a pentaploid with the basic number x = 11. The present investigation fully supports this. Mahanathy (1970) reported a basic chromosome number of x = 11 for members of the family Zingiberaceae. Beltran and Kam (1984) also reported the basic number as 11 in Asiatic *Kaempferia*. The present study also supports these findings on the basic number. The results of the present investigation disprove the reports of Raghavan and Venkatasubban (1943) and Ramachandran (1969), that the chromosome number of *Kaempferia galanga* L. is 2n = 54, since all the 55 chromosomes could be clearly counted in good preparations. Further, Ramachandran (1969) also suspected aneuploid pentaploidy in his report of 54 chromosomes.

5.5 Mitotic studies

In the present investigation the ideal time of collection of roots for mitotic studies was found to be 7.30 am. Though Rekha (1993) reported the occurrence of mitotic division throughout the day time and the peak at 6.00 am, in

our study peak division was observed to be around 7.30 am, because cytological preparations from the roots collected at 7.30 am had maximum cells at early metaphase stage. The procedure of staining standardised by Rekha (1993) was adopted in the present study and it was found to give very good cytological preparation. Staining as well as chromosome spread was satisfactory. Addition of a little ferric acetate to the fixative as well as to the stain was found to improve the stainability of chromosomes.

5.5.1 Mitotic study of the polyploid

While observing the mitosis of variants selected, a single plant was proved to be a polyploid. It had 110 chromosomes in the somatic cells, while the chromosome number of kacholam is 55. As kacholam is proposed to be a pentatploid with the basic number x = 11 (Rekha, 1993), the variant now got induced must be a still higher level polyploid ie., a decaploid with 10 sets of chromosomes (Plate 5).

5.6 Morphology of the polyploid

The polyploid obtained was poor in general, as compared to normal plants. The plant size was small because of retarded growth rate. The size as well as number of leaves was found reduced. Number of suckers was also reduced. The leaves were thicker and darker green. In several other crops also, the polyploids induced were found to have thicker, coarser and darker green leaves as reported by Sudharsan (1989) in cardamom, Bai *et al.* (1976) in *Ipomoea obscura*, Patil (1963) and Rajasekharan and Ganesan (1968) in *Abelmoschus esculentus*, Ramachandran (1982) in ginger, Lavania (1986) in *Hyoscyamus muticus*, Gaonkar and Torne (1991) in *Ageratum conyzoides* and Verma and Raina (1991) in *Phlox*

drummondii. Slow growth rate was reported in colchiploids of *Phlox drummondii* by Verma and Raina (1991). Initial slow growth rate was reported in induced autotetraploids of *Clitoria* by Srivastava and Raina in 1992. Stunted growth and small plant size was reported in the tetraploid induced in *Zinnia elegans* by Gupta and Koak (1976). Colchicine induced sunflower plants exhibited distinct dwarf characters (Gupta and Roy, 1980). Reduced plant height was reported in induced autooctaploids of *Hyoscyamus muticus* by Dhawan and Tyagi (1989). The number of branches were also reduced In *Ageratum conyzoides*, Gaonkar and Torne (1991) also reported reduction in plant height. Number of leaves per plant was found to be decreased in induced autotetraploids of *Coleus forskohlii*, as reported by Bahl and Tyagi (1988). The autotetraploid induced in *Catharanthus roseus* by Kulkarni *et al.* (1984) also had less number of leaves per plant compared to diploids.

The polyploid obtained in the present investigation had larger stomata with reduced stomatal density compared to the normal plants. There was 33.3 per cent increase in the size of stomata, in case of the polyploid, and the number per unit leaf area decreased by 21.5 per cent. Sudharsan (1989) reported that in induced polyploids of cardamom, the stomata were larger and the number of stomata per unit leaf area was lower. According to Bai *et al.* (1976), in autotetraploids of *Ipomea obscura*, length and breadth of stomata increased significantly and the number decreased per unit area. Jos *et al.* (1986) also reported decrease in the number of stomata as the levels of ploidy increased in taro. In the report of Bahl and Tyagi (1988), in autotetraploids of *Coleus forskohlii*, the stomatal size as well as number per mm^2 increased. But in the present study, the size of stomata increased but their number per unit leaf area decreased. In *Phlox drummondii*, Verma and Raina (1991) reported that colchicine induced tetraploid exhibited pronounced increase in size of stomata but the number per unit area was 40 per cent less.

The isolated polyploid in kacholam gave a lower yield than control plants. But in ginger, which is a very close relative of the crop, induced polyploid recorded a higher yield (Ramachandran, 1982 and Ramachandran and Nair, 1992). The rhizomes of the tetraploid ginger was larger, thicker, with increased nodal length and less branching. The reasons for this difference in response to polyploidization of both these crops may be the fact that while cultivated ginger is diploid, kacholam is pentaploid. Though the polyploid induced in ginger is superior to the diploid, same may not be the case when a higher level polyploid is induced from a pentaploid. Because each crop is having an optimum level of ploidy, it can a very well be deduced that pentaploidy is optimum for kacholam and hence a higher level polyploid may not perform well.

Jos *et al.* (1986) reported that the tuber development was much restricted in induced tetraploids of taro. In induced polyploid of kacholam also, rhizome development was found to be much restricted.

Flowering was not noticed in the induced polyploid of kacholam for two crop seasons. In ginger tetraploids Ratnambal and Nair (1982) reported similar behaviour. But Ramachandran and Nair (1992) observed flowering in tetraploids in the second year, just like normal ginger.

5.7 Morphology of other variants

Three stable variants were obtained in the present investigation which were also characterised by reduced vigour. The number of suckers as well as number of leaves were less. In two of the variants the leaf size was found to be much reduced. The chromosome numbers were not increased in these plants which was also evident from the presence of normal sized stomata. The rhizome growth was also found to be retarded. The reasons for these variations may be some deleterious mutations brought about by colchicine.

SUMMARY AND CONCLUSION

6. SUMMARY AND CONCLUSION

A trial on inducing polyploidy in kacholam, *Kaempferia galanga* L. was carried out in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara during 1994-95. The major objectives were to find a suitable technique of colchicine treatment for polyploidy induction, develop a higher level polyploid in kacholam with the intention of increasing the variability in the crop and also to verify the existing reports on chromosome numbers in kacholam. The salient findings of the study are summarised below.

1. Treatment of rhizomes with 0.45 per cent colchicine yielded the polyploid. Here the method followed was colchicine application in a hole of about 2 mm diameter and 4 mm depth, drilled close to the sprouting bud, the hole being kept filled for four hours on two consecutive days.

2. The above mentioned method yielded, in addition to the polyploid, three other stable variants while the cotton swab method was not successful in inducing variability. Thus colchicine application in a hole drilled close to the sprouting bud is much superior to cotton swab method, in kacholam.

3. Immediately activated buds are the best for treating with colchicine for inducing polyploidy.

4. Root tip squash studies revealed that the number of chromosomes in the

somatic cells of *Kaempferia galanga* L. was 55. This number is being reported for the second time in this species.

5. For mitotic studies roots were collected from plants grown under indoor conditions. 7.30 am was found to be the ideal time for collection of roots since they gave maximum number of cells in early metaphase.

6. The procedure adopted for mitotic study was pretreatment of roots in α Bromonaphthalene for 4 hours at 4°C followed by 24 hours fixation in carnoys B fluid which is followed by staining in Snow's carmine for 24 hours after which the roots were squashed in 45 per cent acetic acid. This procedure gave very good cytological preparation with condensed chromosomes which were properly stained. Addition of a little ferric acetate to the fixative as well as to the stain yielded better stained chromosomes.

7. Mitotic study of the polyploid showed that somatic chromosome number of the plant was 110. As kacholam is proposed to be a pentaploid, this one must be a decaploid.

8. The polyploid was characterised by small plant size and reduced vigour. Rhizome development was found retarded and per plant yield was less.

9. The leaves were thicker with reduced size. Stomata was conspicuously larger with reduced density.

10. Flowering was not observed in the induced polyploid for two growing seasons.

CONCLUSION

Being a preliminary investigation, conclusive results cannot be drawn regarding the suitability of the crop for polyploidy breeding. Production and study of more number of polyploids are necessary for this. The present investigation was successful in evolving an appropriate technique of rhizome treatment of colchicine for inducing polyploidy. The polyploid developed in this study was poor with regard to yield compared to normal plants. But further multiplication of the polyploid obtained will facilitate the estimation of oil, oleoresin and also other bio- chemical ingredients.

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* Originals not seen

APPENDIX

APPENDIX-I

Meteorological parameters at Vellanikkara, Thrissur during the two crop seasons

Month & year		Mean sunshine (hrs)	Mean temp. (°C)		Mean RH (%)	Total rainfall (mm)	No of rainy days	Cumulative pan evapo-ration (mm)
			Max.	Min.				
I CROP SEASON								
1994	May	8.0	33.6	24.7	75	124.2	7	137.0
	June	2.1	28.9	22.9	90	955.1	27	84.2
	July	1.4	28.6	22.4	91	1002.1	29	86.1
	August	3.0	30.0	22.8	85	509.2	20	91.4
	September	7.3	31.8	23.2	78	240.5	8	113.9
	October	6.7	32.3	22.7	80	358.2	20	97.1
	November	8.1	31.8	23.3	68	125.3	5	137.9
	December	10.6	32.2	22.2	58	0	0	169.6
1995	January	9.6	32.9	22.4	59	0	0	178.5
II CROP SEASON								
1995	May	6.5	33.5	23.9	78	370.5	13	129.3
	June	3.7	31.6	23.1	86	500.4	19	103.7
	July	6.1	29.9	23.2	89	884.7	26	88.5
	August	3.7	30.6	23.7	86	448.7	22	96.4
	September	6.1	30.1	23.5	82	282.5	13	97.7
	October	8.3	33.2	23.2	78	110.4	8	113.8
	November	6.5	31.3	22.5	80	88.4	5	89.1
	December	10.3	32.5	21.3	57	0	0	195.9
1996	January	9.4	33.1	22.4	53	0	0	208.6

INDUCTION OF POLYPLOIDY IN KACHOLAM, (*Kaempferia galanga* L.)

By

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ABSTRACT OF A THESIS

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ABSTRACT

A trial on induction of polyploidy in *Kaempferia galanga* L. was undertaken at the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara, during the period 1994-'95. A local selection *Vellanikkara* collected from the genetic stock maintained at AICRP on M & AP was used for the study. The objective was to create variability in kacholam by developing polyploids.

The rhizomes of the crop were treated with colchicine for inducing polyploidy. Five concentrations of colchicine viz., 0.05, 0.15, 0.25, 0.35 and 0.45 per cent (C_2 to C_6, C_1 being control i.e., 0%) was tried for two time durations viz., four hours and six hours (T_1 and T_2), the same treatment being repeated on two consecutive days. Two methods of treatment were tried the first (M_1) being chemical application in a hole drilled close to the sprouting bud and the second (M_2) being the ordinary cotton swab method. Rhizomes with two different stages of bud sprouting were used: (1) with just emerging buds (S_1) and (2) buds at an advanced stage of sprouting (S_2).

The treated rhizomes were planted in the field and their growth was examined. In the end of the crop season, 18 variant plants were identified and they were put to detailed morphological and cytological study in the next growing season. Mitotic study of the parent material confirmed that the chromosome number of kacholam is 55. Of the 18 variants identified one was found to contain

110 chromosomes in the somatic cells. As kacholam is proposed to be a pentaploid the induced polyploid must be a decaploid.

The polyploid was obtained from the treatment combination $S_1M_1T_1C_6$. It was characterised by small plant size and reduced vigour. The leaves were thick with reduced size. Stomatal size increased conspicuously with their numbers reduced. The rhizome development was found retarded and the per plant yield was also less. The rhizome morphology was on par with the normal plants. The plant flowered neither in the first season nor in the second.

In addition to the polyploid, three other stable variants were obtained from the experiment. They were also characterised by reduced vigour and yield.