INDUCTION OF AUTOTETRAPLOIDS IN LEMONGRASS

BY

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "Induction of autotetraploids in lemongrass" is a bonafide record of work done by me during the course of research and that 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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20th August, 1983.

CERTIFICATE

Certified that this thesis, entitled "Induction of autotetraploids in lemongrass" is a record of research work done independently by Smt. RAJESWARI MEENATTOOR, J., under my guidance and supervision and that it has not previously formed the basis for the award of any degrees, fellowship or associateship to her.

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INTRODUCTION

INTRODUCTION

Polyploidy indicates a condition in which individuals have more than two sets of chromosomes or genomes in their somatic cells. Polyploid plants arise in nature either by the multiplication of sets of chromosomes within the limits of a species which is referred to as autopolyploidy or by combination of chromosome sets from two or more species referred to as allopolyploidy. Many commonly cultivated crop species such as wheat, oats, tobacco, cotton, coffee, potato and sugarcane are natural polyploids.

The production of artificial polyploids gained acceptance and rapid momentum with the discovery that the plant alkaloid colchicine extracted from the seeds and corms of the autumn crocus Colchicum autumnale could double the chromosome number. Of the several agents which have been tried for the induction of polyploids, colchicine alone gave consistent results. Colchicine affects cell division by inhibiting spindle formation and thereby preventing the separation of the daughter chromosomes at anaphase. Following the failure of anaphase separation, a tetraploid restitution nucleus is formed which during the later divisions in the absence of the alkaloid undergoes normal mitosis and gives rise to a tetraploid tissue. Since the discovery of this alkaloid by Blakeslee and Avery (1937) large numbers of crop plants of different species have been subjected to colchicine treatment and the effect has been shown to be general in all higher plants (Williams, 1964).

One of the most consistent consequences of autopolyploidy is an increase in cell size which is frequently reflected in thicker and leathery leaves, thicker stems, increased flower size and larger fruits. Increase in cell size in polyploids is most readily observed in the epidermal cells and the stomatal guard cells. Measurements on these provide an indication of whether an increase in chromosome number has occurred. Increase in cell size in the autopolyploids is invariably associated with slower growth so that most artificial polyploids are late in maturity. Because of frequent failure of chromosome pairing and unbalance, fertility is always reduced in artificial autopolyploids. The induction of autopolyploid forms of diploid crop plants is considered as a means of increasing crop production, since it has been realised that some autopolyploids are larger than the respective diploid forms.

Indian lemongrass, Cymbopogon flexuosus is the chief source of lemongrass oil of commerce. The oil extracted from the leaves is extensively used in the manufacture of cosmetics and in the synthesis of Vitamin-A. The form of Cymbopogon flexuosus grown in the state is a diploid and thus provides scope for induction and development of autotetraploids. It is expected that the quantity of the oil could be increased and its quality improved by induction of autotetraploidy. In the present investigation an attempt is made to induce autotetraploidy in lemongrass, variety OD-19 by seed treatment with colchicine.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Polyploidy refers to a condition in which there are more than two sets of chromosomes in a plant or animal cell. The classical polyploid Oenothera lammerckiana var. gigas, discovered by Hugo de-Vries at the dawn of this century proved to have twice the number of chromosomes found in the standard form (Stomps, 1942). The success of any polyploid in nature or in agriculture depends upon how closely it meets the requirements for each situation. 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.

Estimates reveal that more than half of the angiosperms are polyploids. Autopolyploids can be either natural or artificial. Natural autopolyploids arise as a result of doubling of chromosomes in the somatic tissues or doubling of chromosomes in the intervarietal hybrids. Artificial induction of polyploidy is possible through several methods.

I. Methods of Induction of Autopolyploids

1. Decapitation and Callus method

This involves decapitation of apical buds followed by callus formation. Lindstrom tried this method in tomato (Eigsti and Dustin, 1955). When plants are injured the wounds heal rapidly. In the course of rapid cell division to form callus and thus heal the wound, mis-steps in cell

division occur through failure of cell wall formation leading to a doubling of the somatic chromosome compliment.

1. Heat Shock Method

High temperature treatment of inflorescence during the time of division of the zygote brings about abnormalities in cytotic activities. Treatments of germinated tissue at high temperatures for short periods were also employed by several workers. Dorsey (1936) has produced chromosome doubling by this method in different species of Triticum such as Triticum durum, Triticum polonicum and Triticum vulgare.

3. Twin method

This was one of the earliest methods. In a low frequency of germinating seedlings, twin embryos will be found which will give rise to tetraploid plants. Muntzing (1937) was the first to utilize this method to obtain polyploids in rye.

4. Application of Chemicals

Several chemicals such as acenaphthene, chloral hydrate, benzene, sulfanilamide and mercuric chloride were used in the earlier periods for the induction of polyploids. In due course colchicine came into use and has become the most popular polyploidising agent.

5. The Colchicine technique

A promising avenue in the realm of polyploidy research was opened with the introduction of colchicine in 1937. The modern period of research with colchicine began in 1889 when Pernice described metaphasic arrest produced by this drug

(Eigeti and Dustin, 1955). 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 extracted from the plant Colchicum autumnale was very effective in doubling the chromosome number. This discovery paved the way for future involvement of colchicine on a large scale for the production of polyploids of different crops.

It replaced all the conventional methods of chromosome doubling in plants. Blakeslee and Avery (1937) have stressed the success of colchicine as a polyploidising agent over other chemicals. By heat shock method one polyploid plant was obtained out of 600 cotton plants tried while colchicine treatment produced fifty polyploids out of 100 plants (Beasly, 1940). Several workers at the chromosome laboratory, Svalof also showed the superiority of this chemical over heat shock treatment (Levan, 1945). Then a rapid change over to colchicine took place, the reason being attributed to its two specific advantages namely high effectiveness for making polyploids with many different species and its very little damage to the treated young plants. High solubility in water, non toxicity to plants even at higher doses, etc. adds to its advantages (Eigeti and Dustin, 1955).

Scientists and breeders have tried the induction of polyploidy in different plant groups such as ornamentals, forage crops, cereals, vegetables and fruits. The studies made on these induced polyploids help us to gather information

regarding the diversified effects of induced polyploidy on various plant characters.

II. Morphological Effects of Autopolyploidy

The effects of autopolyploidy on different plant parts have already been reported. They can be grouped into growth habit, plant height, number of shoots, size of different plant parts, nature of stomatal guard cells, cell constituents, flowering, pollen fertility and yield (Eigsti and Dustin, 1955).

1. Growth Habit

Autotetraploids of different crops have exhibited varying degrees of changes in vigour of the plants and leaf and stem characters (Ramanujam and Deshmukh, 1945; Huntzing, 1951; Mehta et al., 1965 b; Mehta and Swaminathan, 1965). Kostoff (1938 a) found that colchicine treatment in different crop plants have caused highly suppressed germination and enormously swollen seedlings. The tetraploid of rape was found to be dwarf with gigas characters. The leaves and stems were thick (Morinaga and Kuriyama, 1937). Ramanujam and Deshmukh (1945) obtained autotetraploids in different species of Oleiferous Brassicae. These were uniformly more vigorous and had sturdier stems and leaves and were late in maturity. Polyploidy induction in Brassica campestris (Chowdhury et al. 1967) gave plants which were slower in growth at the earlier stages but exhibited greater vigour at the later stages.

According to Tyagi and Das (1970), autotetraploid plants of brown sarson had slower growth rate and were late in maturity. Colchicine induced tetraploids of toria were more vigorous and later in maturity as recorded by Singh (1976 b).

In Muntzing's experiment (1951), the tetraploids of rye had thicker and stouter stems; larger but shorter and broader leaves which were dark green in colour. They were remarkably vigorous. Bremer-Reinders and Bremer (1952) recorded that the reaction of wheat and rye to colchicine treatment was a markedly retarded growth, the plantlets being thickest and darkish green in colour. The tetraploids of westerwolths Ryegrass had thicker, longer and darker green leaves than the diploids (Wit and Speckmen, 1955). Wit (1958) reported that the tetraploid rye grass (Multiflorum LAM) showed a more compact habit of growth and darker colour of stems and leaves.

Induced tetraploids of Trifolium foenum graceum (methra) showed greater and quicker growth and more succulent stems. Leaves were broader, thicker, dark green and more succulent (Mehta et al., 1965 a). Studies on induced polyploids of Senji showed that they were with poor growth and vegetative vigour and it was found to be unfit for polyploidy breeding by Mehta et al. (1965 b). Autotetraploids of the pasture legume glycine showed slower growth rate at the initial stages and they were having thicker, softer and more succulent stems (Amarsingh, 1968). In different strains of Medicago

viz., M. sativa, M. media, M. lupulina and M. denticulata polyploidy resulted in many vigorous plants (Atwood and Grun, 1951).

In Antirrhinum, the polyploids were hardier, more vigorous, stouter and stockier than the corresponding diploids (Mahal et al. 1968). Biswas and Bhattacharya (1971) reported that the induced tetraploids of Cyamopsis psoraloides were dwarfs having smaller leaves which were darker, thicker and fleshy. Growth of the seedlings was found to be stunted. The tetraploid plants of Trichosanthes showed increased vigour after prolonged stunted growth of 10 to 15 days over diploid and the stem was robust with dark green leaf lamina (Singh and Roy, 1975 a).

Colchicine treatment in cotton hybrids resulted in retardation of growth, broader leaf lobes, prominent leaf veins and roughoid appearance with intensification of hairiness (Amin, 1940). Harland (1940) produced polyploids in cotton by using colchicine which produced coarser, thicker, larger and hardier leaves compared to their diploids. Polyploids induced in cotton by Stephens (1940) had broader, rough and distorted leaves and coarser stem hairs than the diploid. Polyploids of Nicotiana species produced by colchicine treatment were reported to have longer vegetative period and thicker and broader leaves (Kostoff 1938 b). The tetraploid of Nicotiana glauca had thicker stem and lesser number of leaves as recorded by Deshmukh and Pal (1950).

Tetraploid til was obtained by Richaria and Persai (1940). These plants were shorter, stiffer, thicker stemmed with coarser, dark green, broad and thick leaves. Polyploid branches of Linum species were reported to have a lower rate of vegetative development, thicker stems and larger leaves which were undulated and scabrous with a bluish hue over their normal green colour (Yermanos and Gill, 1967). Madhusoodanan and Arora (1979) reported that the induced autotetraploid seedlings of Matricaria chamomilla were slow growing resulting in compact plants.

In Chilli, induced polyploids exhibited a slower growth, thicker, darker, broader leaves and stouter branches (Pal et al. 1941). A strikingly vigorous plant in the brinjal variety Muktakeshi was found to be a natural tetraploid. This plant was found to be characterised by stouter branches, broader and thicker leaves, petioles and midribs (Singh, 1941). Colchicine treated plants of Abelmoschus esculentus showed stunted and slow growth (Rajasekharan and Ganesan, 1968). According to Pushpa et al. (1974) the induced autotetraploids of Indian spinach were characterised by larger, thicker and succulent leaves.

Induction of polyploidy in Phaseolus radiatus (mung) resulted in stunted growth of the plants in the first generation and in the second generation more hardier and vigorous growth (Kumar and Abraham, 1942). The induced autotetraploids of blackgram were reported to be slow growing by Goswami (1979). Studies on autotetraploids of

Arachis duranensis have revealed that (Raman and Kesavan, 1963) they had a lower rate of growth, dark green and thicker leaves compared to diploids.

In sugarbeet tetraploids, leaves were thicker, shrivelled, shorter, broader and coarser and they were robust in appearance (Rasmusson and Levan, 1939). In tetraploid beets, Kloen and Speckmann (1953) observed thicker, dark green leaves with irregular margins in the treated plants. The growth was slowed down to a comparable extent for the first fortnight after which affected plants had a luxuriant growth with renewed vigour in Daucus carota (Sinha and Sinha, 1975).

Das and Mukherjee (1967) produced eleven autotetraploids out of thirteen varieties tried, in grapes. They showed diagnostic features like vigorous growth, thicker and deeper coloured leaves. In Achras sapota colchicine treated plants had bigger and thicker leaves than the original material (D'cruz and Jadhav, 1972). Colchicine treated apple seedlings had vigorous habit and large dark green leaves (Bavtuto, 1974). Levan (1939) reported a resting period for the colchicine treated Petunia plants for a duration of 1 to 2 months. The tetraploids were more robust, leaves were thicker with a frail consistency, wrinkled and irregularly split, shoots were often wrinkled and twisted, the whole plant developing into a dwarf.

In Alyssum maritinum, an ornamental plant, the induced polyploids produced leaves which were thicker, longer, broader and deep green in colour compared to control (Bali and Tandon,

1958). Srivastava (1965) tried colchiploidy in Zinnia which resulted in stunted and slower growth of the seedlings. Bose and Panigrahi (1969) induced polyploidy in two varieties of Zinnia linearis in which the leaves became thicker, leathery, crinkled and dark green in colour. Kartel (1967) reported that the tetraploid plants of Betula verrucosa were having an altered leaf shape with slower initial and faster subsequent growth.

According to Arora (1975) the induced tetraploids of verbena species were slow growing resulting in compactness and a bushy, less spreading habit. Slower growth rate and deep green thick rough foliage were observed in the induced polyploids of Meoardonia dianthera by Kaul (1975).

Kondo (1942) reported that the artificial tetraploids developed from a number of rice varieties were distinctly more robust and vigorous than the diploids. An autotetraploid of rice was reported to have thicker and coarser culms and leaves and the duration of the crop extended by 30 days (Sambamurthy, 1973). An autotetraploid in pearl-millet was found to have thick crinkled leaves with slow emergence (Krishnaswamy et al. 1950). The induced tetraploids in Sorghum (Magoon and Tayyab, 1968) exhibited thick extensive and dark green foliage, slower growth and bushy habit. In grain sorghum, induction of tetraploidy was tried by Murty et al. (1978) who reported that it was not possible to distinguish the tetraploids from the diploids morphologically though they had a slower growth rate.

2. Plant Height

There are varying reports on increase and decrease in plant height as a result of colchicine treatment. William and Douglas (1966) reported that the tetraploids of crested wheat grass were taller than the diploids. In brown Sarson the tetraploid was taller than the diploid (Choudhury et al. 1967). The tetraploid plants of Brassica tourne forties were also taller than the diploid (Singh, 1970). In Brassica campestris and Brassica nigra, also the tetraploids were taller than their diploids as reported by Singh (1976 a). According to Katare and Nerkar (1976), the tetraploids of Alfalfa were taller. The autotetraploid maize was taller than the diploid (Dudley and Alexander, 1969). Increased plant height was recorded in the autotetraploid rice by Sambamurthy (1973). In Petunia, the tetraploids were almost giants compared to diploids (Levan, 1939).

During a study on colchicine induced tetraploids of five varieties of blackgram, Sen and Chheda (1958) found tetraploidy to be associated with an increase in plant height. The difference in height between the tetraploid and diploid of Phaseolus aureus was in favour of the tetraploid as recorded by Kabi and Bhaduri (1978).

No difference in plant height was observed by Schank and Knowles (1961) in the colchicine induced polyploids of safflower. Induced tetraploids of Solanum indicum did not show any increase in plant height as reported by Rajasekharan (1970). Morinaga and Kuriyama (1937) found the autotetraploid

rape to be dwarf in nature. Decrease in plant height was reported by Siddiq (1967) in the induced autotetraploids of Sorghum vulgare. Magoon and Tayyab (1968) reported that the average height of the tetraploid of Sorghum nitens has decreased significantly. Rajasekharan and Ganesan (1968) reported decreased plant height in the tetraploid of bhindi. Reduction in plant height was observed in the induced polyploids of Amaranthus by Behera and Patnaik (1975). The autotetraploids of cluster bean had reduced height compared to diploid (Lobana and Verma, 1972).

Plant height was drastically reduced in the tetraploid of sunflower (Saini and Dhesi, 1972). Gupta and Roy (1980) reported that the colchicine treated sunflower plants exhibited distinct dwarf characters. According to Nair and Ratnambal (1974) the induced tetraploids in Areca catechu were shorter than the diploids. Reduced plant height in the tetraploids of Nicotiana glauca was recorded by Deshmukh and Pal (1950). The tetraploids of Moghania macrophylla were shorter than their diploids (Sinha and Kumar, 1979).

3. Number of Shoots

Reports on the effect of colchicine on the number of shoots produced are both positive and negative. Mehta et al. (1963) reported that the tetraploids of Berseem were capable of producing larger number of tillers than their diploids.

by Katare and Nerker (1976).

Mehta et al. (1965 a) observed that many tetraploids of Trifolium foenum graceum produced double the number of branches as compared to the diploids. Larger number of branches were reported in the polyploid of Glycine javenica by Amarsingh (1968). The autotetraploid plants of brown sarson possessed larger number of sturdier branches (Tyagi and Das, 1970). As reported by Kabi and Bhaduri (1978) the number of branches in the tetraploid of Phaseolus aureus was larger compared to its diploid. Heavy branching was reported in the tetraploid of Pachyrhizus angulatus by Prasad and Deepesh (1969). Increased number of branches was one of the characters in the polyploid of Portulaca (Singh, 1979 b). Sinha and Sinha (1975) reported profuse branching of colchicine treated plants of Daucus carota and Foeniculum vulgare.

Reduction in the number of shoots was reported in several crops as a result of colchicine treatment. Tillering was found to be slower from the beginning in the tetraploid westerwoltho ryegrass which produced only fewer tillers as observed by Wit and Speckmann (1955). A reduction in the number of primary and secondary branches occurred in the tetraploid plants of Brassica tournifortics as evidenced by the reports of Singh (1970). In toria, the colchicine induced tetraploids had lesser number of branches (Singh, 1976 b). According to Sen and Chheda (1958), the number of branches in the tetraploids of blackgram was reduced. The number of branches in the tetraploid okra var. Pusa sawani was smaller

than that of the diploid as recorded by Rajesekharan and Ganesan (1968). Reduced branching was associated with the colchiploid of Aplude mutica according to Murty and Satyavati (1978). A decrease in the number of branches was reported by Sinha and Kumar (1979) in the colchiploid of Moghonia.

4. Size of Different Plant Parts

Induction of polyploidy in different crop plants have resulted in varying degrees of changes in the size of flowers, fruits and seeds.

Gigas nature was evident in the tetraploids of rape with larger flowers (Morinaga and Kuriyama, 1937). The autotetraploids of Oleiferous brassicae obtained by Ramanujam and Deshmukh (1945) were having bigger flowers, fruits and seeds. Induced tetraploidy in toria gave rise to bigger flowers, pollen grains, siliqua and seeds (Singh, 1976 b). In Nicotiana species and Phlox, polyploid plants had larger floralb buds, broader corolla tubes, larger trichomes, ovules and seeds (Kostoff, 1938 b). Larger flowers were produced by the tetraploid of Nicotiana glauca according to Deshmukh and Pal (1950). Bigger flowers, seeds, glands and pollens were observed in the colchicine treated plants of cotton by Amin (1940).

Wit and Speckmann (1955) observed that the tetraploids, of ryegrass had larger ears, spikelets, florets and seeds than the diploids. Mehta et al. (1965 a) recorded larger flowers, floral parts, pollens and seeds in the induced polyploids of Methra. Floral characters showed gigantism in

their shape, size and texture in the induced tetraploids of Sorghum nitens. In Sorghum nitidum the tetraploids had considerable gigantism in respect of vegetative characters but the panicle size and number of panicle branches and spikelets were greatly reduced (Magoon and Tayyab, 1968).

Larger flowers, floral parts and seeds were noticed in the autotetraploids of glycine by Amarsingh (1968). In Antirrhinum the polyploids had larger flowers than the diploids as reported by Mahal et al. (1968). Singh and Roy (1971) reported morphological gigantism in the colchiploids of Trigonella cretica while those of T. corniculata had smaller vegetative parts than the diploids. Flowers and pollens of the polyploid of Trichosanthes were enlarged as revealed by the studies of Singh and Roy (1975 a).

Larger flowers and seeds were noticed in the induced polyploids of chilli by Pal et al. (1941). The naturally occurring tetraploid brinjal had bigger flowers and floral parts as reported by Singh (1941). Rajasekharan (1970) recorded larger flowers in the autotetraploid Solanum indicum. Experimental polyploids in two varieties of Cucumis melo (Singh and Roy, 1975 b) were studied in which there was no increase in fruit size in the variety momordica whereas the variety ugrestis showed increase in fruit size to some extent. The colchicine induced tetraploids of Solanum nigrum bore large, globose, deep purplish black fruits (Rao, 1979). Singh (1979 a) suggested that induction of autopolyploidy in the family Cucurbitaceae brings about gigantism in all

features. Kabi and Bhaduri (1978) observed that the tetraploids of greengram were distinctly gigas with larger leaflets, flowers, pods and seeds. In blackgram, larger flowers, floral parts and seeds had been reported by Son and Chheda (1958).

The tetraploids of safflower (Schank and Knowles, 1961) had larger flowers, pollens and seeds. According to Yermanos and Gill (1967), tetraploids in Linum had larger flowers, capsules and seeds. Levan (1939) reported that the triploid petunias were almost giants compared to the diploids with larger organs such as leaves and flowers. The number of flowers and number of leaflets increased in the colchicine treated zinnia (Srivastava, 1965). Tetraploid plants of Atropa belladonna showed an increase in the size of the flowers, berries and seeds (Glazova and Shugaeva, 1970). Increase in size of leaf, flower, anther and pollen was reported by Singh (1979 b) in the tetraploids of portulaca.

5. Nature of stomatal guardcells

In experimental polyploids cell size is usually greater than in the corresponding diploids. This has been the case in several species of Solanum, Cleome, Oenothera, Datura and Crepis. The enlarged cell size is easily evident in the guard cells of the stomata. An evaluation of morphological effects of induced polyploidy in different crop species has brought out that increased size of stomata generally indicate the occurrence of polyploidy. Larger stomata were observed in the polyploids of Nicotiana and Phlox by Kostoff (1938 b).

Deshmukh and Pal (1950) recorded the stomatal size of tetraploid Nicotiana glauca to be 45.6 x 29.1 μ as against 35.7 x 24.9 μ in the corresponding diploid.

Mehta et al. (1963) found tetraploids of Berseem to have larger stomata. Increased stomatal size was observed in the autotetraploid of glycine by Anarsingh (1968). Striking increase in stomatal size was reported in induced tetraploids of Sorghum nitens, the length being 45.83 μ and 35.1 μ in tetraploids and diploids respectively (Magoon and Tayyab, 1968). Biswas and Battacharya (1971) reported that in the induced polyploids of Cyamopsis psoraloides, stomatal frequency per unit area was less while length and width were more than that of the diploids. Hans (1971) observed larger stomata in the polyploids of Trema.

Singh and Roy (1975 a) during their studies on polyploids of Trichosanthes anguina detected increased size of stomatal cells. Larger stomata were detected in alfalfa by Mohammed (1979). Difference in size of stomata is mentioned by Rasmusson and Levan (1939) in the tetraploid sugar beets. Reduced frequency of stomata was observed in the colchicine treated plants of carrot by Sinha and Sinha (1975).

According to Schank and Knowles (1961) the tetraploids of safflower had larger stomata compared to the diploids. The stomatal area of tetraploid and diploid plants of Ocimum kilimandscharium was recorded as 475 sq μ and 366 sq μ respectively. The number of guardcells per unit area was 7.5 and 8.3 in the upper surface and 15.7 and 18.2 in

the lower surface in the case of tetraploids and diploids (Bose and Choudhury, 1962). Enlarged size of stomata was observed in Linum species (Yermanos and Gill, 1967) in which the size was recorded as 33.8 μ in the diploid and 43.0 μ in the tetraploid. Reduction in the frequency of stomata was noticed in the colchicine treated groundnut plants as evidenced by the studies of Lal and Mehrotra (1968). In sunflower the induced polyploids had a stomatal size of 34.01 x 23.98 μ while it was 28.28 x 18.97 μ in the diploid (Saini and Dhesi, 1972). Number of stomata per unit area decreased and size increased in the colchiploid sunflower (Gupta and Roy, 1980).

There was drastic reduction in the number of stomata per unit area along with increase in size in the induced tetraploids of Brassica (Singh, 1970). Larger stomata were observed in the autopolyploids of Brown sarson by Tyagi and Das (1970). In a comparison between the induced tetraploids and diploids of Brassica nigra and B. campestris, the stomatal frequency was 71.6 per cent in the former and 63.8 per cent in the latter. The stomatal length was 72 per cent more than that in B. nigra. Mean stomatal length was 49 μ in the autotetraploid pearlmillet whereas in the diploid, it was 52 μ (Krishnaswamy et al. 1950). Enlarged size of stomata was observed by Siddiq (1967) in the colchicine induced autotetraploids of Sorghum vulgare.

Nair and Ratnambal (1974) reported fewer stomatal cells per unit area in the induced tetraploid of Areca catechu.

Larger stomata were reported in the induced polyploids of Crotalaria by Gupta and Sinha (1978). Bose and Banerjee (1968) recorded abnormally large stomata in the colchicine treated tomato plants. In bhindi, Rajasekharan and Ganesan (1968) reported the length of stomata in the tetraploid to be 29.05 μ whereas in the diploid it was 19.25 μ. In the induced polyploids of Indian spinach, the frequency of stomata was 12.86 per unit area, while in the diploid it was 17.16 per unit area. The size was 4.03 x 2.25 μ and 3.83 x 2.23 μ in the tetraploid and diploid respectively (Pushpa et al. 1974).

In the polyploid of Alyssum, stomatal size was 44.59 x 11.75 μ against 21.28 x 5.40 μ in the diploid as observed by Bali and Tandon (1958). Srivastava (1965) reported that the number of stomata was 19.2 in the colchicine treated plants of zinnia and 36.61 in untreated plants. The size was 45.84 μ x 31.26 μ and 33.22 μ x 24.53 μ in the treated and untreated plants respectively. Bose and Panigrahi (1969) also observed significant decrease in the number of stomata per unit area in the induced polyploid.

Arora (1975) in a study of the differential response of verbena species to colchitetraploidy detected difference in the stomatal size of diploids and tetraploids. Increased stomatal size and frequency was reported by Kaul (1975) in Mecardonia dianthera as a result of colchicine treatment. In the acquatic ornamental plant Nymphaea, induced colchiploidy (Gupta, 1979) resulted in larger stomatal cells. Increase in the size of stomata was reported by Singh (1979 b)

in the polyploids of Portulaca. The size of stomata increased in the colchicine treated plants of Moghenia macrophylla as recorded by Sinha and Kumar (1979).

6. Cell Constituents

Reports show that doubling of chromosomes in some crop plants has brought about changes in cell constituents such as proteins, vitamins and oil. Randolph and Hand (1938) reported an increase of 43 per cent in the total carotinoid content per unit weight of corn as a result of xchromosome doubling. In the tetraploids of the rice variety Dubovka - 129, Petibskaya et al. (1976) recorded 1.4 to 1.5 times more crude protein and 1.3 to 1.4 times more lysine per unit grain weight than in the diploids. Higher protein content in mulberry tetraploids was reported by Dzhafarov and Alekperova (1978). In the autotetraploid of barley, 2.5 to 6.3 per cent higher protein content was recorded (Shulyndin, 1978). Tiwari et al. (1980) suggested that a 52 per cent increase in protein could be achieved by inducing autotetraploidy in barley.

The autotetraploid of safflower was low in oil content (Schank and Knowles, 1961). Lower oil content was reported in the tetraploids of Linum species by Yermanos and Gill (1967). According to Shpota and Bochkarev (1972), polypolidisation in winter rape and B. nigra causes a sharp deterioration in the oil content of seeds.

7. Flowering

Autotetraploids are found to show delayed flowering. The tetraploids of Methra produced by Mehta et al. (1965 a)

were three to four weeks later in flowering than their diploids. Delayed and prolonged flowering was noticed by Prasad and Deepesh (1969) in the colchiploids of Pachyrhizus angulatus. A delay of 8 to 45 days in flowering was recorded in the induced polyploid legume Cyamopsis by Biswas and Battacharya (1971). They also observed that flowering was not only delayed but the duration of flowering was prolonged. Mohammed (1979) reported that polyploid plants of alfalfa failed to flower over two successive seasons.

Delayed and prolonged flowering was observed in Phlox by Raghuvanshi and Pathak (1975). Singh and Roy (1975 a) reported delayed flowering in the polyploids of Trichosanthes. In crotalaria species delayed flowering in the polyploids was reported by Gupta and Sinha (1978). The tetraploids of Nicotiana glauca were shy flowering as observed by Deshmukh and Pal (1950). This was detected in the tetraploid ryegrass by Wit and Speckmann (1955). The tetraploids of Italian ryegrass came to ear one to two weeks later than the diploids as recorded by Wit (1958). Flowering was late by twenty days in the tetraploid of sorghum (Siddiq, 1967). In sorghum tetraploids there was a delay in flowering by 45 to 60 days in pot grown plants (Murty et al. 1978).

Tyagi and Das (1970) observed delayed flowering in the autotetraploids of brown sarson. In Brassica nigra, the tetraploids flowered 7.7 days and in B. campestris, 15.3 days later than their corresponding diploids (Singh, 1976 a).

Ten days delay was noticed in the polyploid of portulaca by Singh (1979 b). According to Lobana and Verma (1972), flowering in the tetraploids of clusterbean was a fortnight late. Colchicine induced tetraploids of greengram flowered earlier than the diploids as reported by Kabi and Bhaduri (1978).

Pal et al. (1941) detected later flowering in the colchicine induced polyploids of chilli. The colchiploids of Bhindi came to flowering five days later than the diploids. In Amaranthus species, flowering was considerably delayed (Behera and Patnaik, 1975). Delayed and extended flowering was detected in the polyploids of Cucurbitaceae by Singh (1979 a).

Only a few days delay in flowering was noticed in the safflower tetraploids as recorded by Schank and Knowles (1961). Bose and Choudhury (1962) found that the polyploids of Ocimum were late in flowering by 30 to 45 days and the flowering period was prolonged by 75 to 90 days over the diploid. In the induced autotetraploid of Matricaria, flowering was delayed by one week and the duration of flowering extended by fifteen days. Flowering was delayed and extended in the colchicine induced polyploids of Alyssum (Bali and Tandon, 1958). As Srivastava (1965) recorded, in the tetraploids of zinnia, flowerbuds appeared 10 to 16 days later and the flower opening was two weeks later than in the diploids. Extended flowering was observed in the polyploid of Mecardonia by Kaul (1975).

8. Pollen Fertility

In chilli, varying percentages of pollen sterility was exhibited by the induced polyploids (Pal et al., 1941). 40 to 50 per cent pollen sterility was noticed in the tetraploid brinjal (Singh, 1941). In the autotetraploid of Solanum indicum, pollen fertility was 70.2 per cent (Rajasekharan, 1970). According to Rao (1979), the colchicine induced tetraploids of Solanum nigrum had 59.97 per cent pollen fertility. In different varieties of blackgram, varying degrees of pollen sterility was reported by Sen and Chheda (1958). Mital (1967) reported high percentage of pollen sterility in the induced polyploids of mung. Percentage of normal pollen in the tetraploid of Bhindi was 18 as against 98 in the diploid (Rajasekharan and Ganesan, 1968). In the tetraploid cluster bean, pollen sterility was 89.9 per cent as against 19.5 per cent in diploids (Lobana and Verma, 1972). Pushpa et al. (1974) reported pollen sterility in the induced tetraploid of Indian spinach as 11.7 per cent and in its diploid as 5.0 per cent. Goswamy (1979) reported 13.6 to 21.4 per cent pollen sterility in the tetraploid blackgram as against 3.2 to 7.2 per cent in its diploid.

In Brassica, 5 to 10 per cent pollen sterility was detected by Ramanujam and Deshmukh (1945). High pollen sterility was observed in the autotetraploid brown sarson (Tyagi and Das, 1970). According to Singh (1976 b), the percentage of pollen sterility was more in the tetraploids of toria than in their diploids. In the tetraploid of Brassica nigra, there was no difference in the percentage of fertile

pollens from the diploid whereas in B. campestris the percentage of fertile pollen was reduced from 97.4 to 82.0 per cent (Singh, 1976 b). In ocimum pollen sterility was 46 to 53 per cent as against 19 to 29 per cent in its diploid (Bose and Choudhury, 1962). In groundnut polyploids, increased sterility was observed by Lal and Mehrotra (1968). The sunflower tetraploid had 86.71 per cent pollen fertility whereas it was 96.66 per cent in its diploid (Saini and Dhesi, 1972).

85.3 per cent sterility was observed in the tetraploids of crested wheat grass (William and Douglas, 1966). Tetraploid (Sorghum nitens recorded a low fertility of 12.34 per cent against 91.02 per cent in the diploid. In S. nitidum fertility was 43 and 95 per cent in the tetraploid and diploid respectively (Magoon and Tayyab, 1968). According to Prasad and Deepesh (1969), pollen sterility was very high in the tetraploid of Pachyrhizus. In the autotetraploid rice, sterility of pollen was 65.72 per cent as recorded by Sambamurthy (1973).

Low pollen fertility of 76.06 per cent was noticed in the tetraploids of Alyssum as against 95.58 per cent in the diploid(Bali and Tandon, 1958). In the tetraploid zinnia, Srivastava (1965) reported increased pollen sterility (36.2 to 69.8%) compared to its diploid (6.4 to 38.5%). Decreased pollen fertility was recorded by Arora (1975) in the colchiploids of Verbena species. Pollen fertility was found to be very low in the polyploids of crotalaria (Gupta and

Sinha, 1978). The tetraploids of portulaca recorded a pollen sterility of 25.6 per cent against 5.2 per cent in its diploid counterpart (Singh, 1979 b).

9. Yield

An induced polyploid of peppermint (krasnodar-2) produced 24.65 kg menthal per hectare compared to 22.04 kg in the diploid. In Salvia solorea, the yield of essential oil was 90 per cent higher in the tetraploid and in Lilium candidum also the oil content was higher in the polyploids (Roznikova et al. 1972). Two tetraploid varieties of radish had 25 to 50 per cent increase in their root yield (Rud and Lutkov, 1972).

Tetraploid alsike clover (Trifolium hybridum) gave consistent increase in forage from 15 to 25 per cent (Turesson, 1946). According to Thomas (1969) the tetraploids of red clover were higher in forage yield than the diploids. The tetraploids of pearlmillet (Minocha et al. 1972) were inferior to their diploids in green matter yield. The ocimum polyploid, gave nearly double the quantity of leaves in comparison with that of the diploids, which led to increased out turn of distillate (Bose and Choudhury, 1962).

MATERIALS AND METHODS

MATERIALS AND METHODS

An investigation was undertaken in the Department of Plant Breeding, College of Agriculture, Vellayani, Trivandrum during 1979 to 1981 with a view to induce autotetraploids in lemongrass.

A. Materials.

1. Seeds

Seeds of the lemongrass variety OD-19 was utilized for the experiment. OD-19 is the variety released from the Lemongrass Research Station at Odakkali of the Kerala Agricultural University. It is an improved variety yielding more oil than local varieties. The oil is of better quality containing 87 per cent citrol. Seeds obtained from the Research Station were tested for germinability and used for the experiment.

2. Chemicals

Colchicine was the chemical applied for the induction of autotetraploidy. This alkaloid is extracted from Colchicum autumnale. It is a cream coloured powder soluble in water. For seed treatment, stock solution of 0.25 per cent concentration was prepared in distilled water and diluted to the different required concentrations.

B. Methods.

1. Standardisation of doses

The concentrations of colchicine for the treatment were fixed based on the results of preliminary trials. Two trials

were conducted using different concentration of colchicine, the duration of presoaking and the period of treatment. In the first trial, seeds were presoaked for 12 hours and treated with colchicine for eight hours using the concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 per cent. Seeds in none of the treatments germinated indicating that the treatment effects were very drastic. In the second trial, presoaking and treatment durations were reduced to four hours. Concentrations of colchicine were reduced to 0, 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 per cent. Based on the germination percentage, the doses for the experiment were fixed in the range of 0 to 0.25 per cent.

2. Seed treatment

Two series of treatments A and B were conducted. In series A, presoaking was followed by an interval of four hours before colchicine treatment whereas in series B, no interval of time was given between presoaking and treatment.

Series A	Presoaking	- 4 hours
	Interval	- 4 hours
	Treatment	- 4 hours
Series B	Presoaking	- 4 hours
	Treatment	- 4 hours

The concentrations employed for the treatment ranged from 0 to 0.25 per cent as follows:

A_0	-	0.00	B_0	-	0.00
A_1	-	0.05%	B_1	-	0.05%
A_2	-	0.10%	B_2	-	0.10%

A_3	-	0.15%	B_3	-	0.15%
A_4	-	0.20%	B_4	-	0.20%
A_5	-	0.25%	B_5	-	0.25%

A stock solution (0.25 per cent) was prepared by dissolving 0.6 g colchicine first in a drop of alcohol and then in 240 ml distilled water. This solution was diluted to the different concentrations as follows:

Concentration (%)	Volume of stock solution (ml)	Volume of water (ml)	Volume of treatment solution (ml)
0	0	40	40
0.05	8	32	40
0.10	16	24	40
0.15	24	16	40
0.20	32	8	40
0.25	40	0	40

In each treatment one gram of seed was treated with 40 ml of the solution. Presoaked seeds were transferred to the conical flasks containing the colchicine solution. During the entire period of treatment, the solution was shaken intermittently. On completion of the treatment the seeds were washed thoroughly and sown in pots filled with potting mixture. 60 days old seedlings were transplanted in singles in the mainfield at a spacing of 50 x 15 cm.

3. Observations

The following observations were taken on the plants

resulting from the treatment.

(i) Survival

(ii) Early deformity

(iii) Height of plants

(iv) Number of tillers

(v) Stomatal observations

(vi) Days to flowering

(vii) Pollen fertility

(i) Survival: The number of seedlings surviving in each treatment wase recorded on the 10th, 20th, 30th and 60th days after sowing and the survival percentage was worked out in relation to the control.

(ii) Early deformity: The standard and treated populations were critically observed to detect abnormalities such as reduced growth, size and malformations of different plant parts. These were recorded.

(iii) Height of plants: Forty plants were selected at random from each treatment for height measurement in the nursery as well as the mainfield. In the nursery seedling height was measured on the 30th and 60th days after sowing. Height measurements in the mainfield were recorded on the 90th, 120th, 150th and 180th days after sowing. Plant height was measured from soil surface to the tip of the longest leaf. The mean height of plants in each treatment was estimated and expressed as porcentage of the control.

(iv) Number of tillers: Forty plants were selected at random

from each treatment. The number of tillers in each plant was recorded on the 120th, 150th and 180th days after sowing. The mean number of tillers per plant in each treatment was calculated and the percentage estimated.

(v) Stomatal observations: Ten vigorous plants were selected from each treatment for stomatal observations. In each plant, the first fully opened and mature leaf from the top was selected. Leaf impressions were taken using natural colour nail polish from three portions on the lower surface of each leaf, that is, from the tip, middle and base. The impressions were taken by giving a thin coat of nail polish on the leaf surface and peeling it off after drying. From each of these impressions, ten microscopic fields were scored for number of stomata, and the mean number per microscopic field estimated for each plant.

The length and breadth of stomata were also recorded from the three portions of each leaf. It was measured from ten cells selected at random in each microscopic field using a standardised ocular micrometer. The length and breadth of stomata in each plant was estimated as the mean of 300 (3 x 10 x 10) measurements.

(vi) Days to flowering: The date of flowering of plants in each treatment was noted and the delay in flowering recorded in comparison with the control.

(vii) Pollen fertility: Mature spikelets were selected from all plants which flowered to assess pollen fertility. Anthers from the spikelets were dissected out and stained with glycerine

acetocarmine. The well stained and properly filled pollen grains were scored as fertile and others as sterile. In each plant, thirty microscopic fields were scored. Mean fertility of each plant and treatment was estimated and expressed as percentage.

4. Identification of polyploids

Plants suspected as polyploids based on increased vigour, larger size of stomata and reduced pollen fertility were subjected to a detailed morphological study in respect of the following characters and data recorded.

(i) Height of plant
(ii) Number of tillers
(iii) Vigour of plant
(iv) Colour of leaves
(v) Nature of leaves
(vi) Stomatal observations
(vii) Delay in flowering
(viii) Pollen fertility

The plants which exceeded the standard in respect of these characters were marked as suspected polyploids. The morphological characters, number and size of stomata, delayed flowering and pollen fertility were studied in detail in these selected plants.

RESULTS

RESULTS

Induction of autotetraploids in lemongrass was tried by seed treatment with colchicine. The application of colchicine brings about doubling of chromosomes which results in morphological and cytological changes. These changes may be either favourable or unfavourable. In the present investigation an attempt was made to study the various effects induced by colchicine in lemongrass. The results are presented below.

1. Survival

The survival counts of plants recorded at different stages of growth in each concentration estimated as mean percentages are given in table 1. Survival of plants was lower than that of the control in all treatments at all the stages of observation. Ten days after sowing, the survival percentage in relation to the control decreased with increasing concentrations in both the series, indicating early death of seedlings following the treatment. Lowest value of 29.6 per cent was recorded at the concentration of 0.15 per cent in series B and the highest value of 77.4 per cent was observed in concentration of 0.05 per cent in series A. Survival percentages in series B were generally lower than those in series A particularly at the higher doses. The lowest values were 52.7 per cent in series A and 29.6 per cent in series B.

Observations on survival at the subsequent stages also revealed a similar trend of higher lethality at the higher

Table 1. Survival counts and mean percentages.

Concentration (%)	10th day		20th day		30th day		60th day	
	No.	%	No.	%	No.	%	No.	%
Series A	(4 hours interval after presoaking)							
0	186	100	151	100	147	100	144	100
0.05	144	77.4	107	70.9	106	72.1	101	70.1
0.10	120	64.5	102	67.6	98	66.6	82	56.9
0.15	105	56.5	83	55.0	75	51.0	70	48.6
0.20	111	59.7	92	60.9	79	53.7	70	48.6
0.25	98	52.7	94	62.3	71	48.3	60	41.7
Series B	(No interval after presoaking)							
0	206	100	165	100	154	100	130	100
0.05	140	68.0	122	73.9	112	72.7	96	73.8
0.10	135	65.5	125	75.8	119	77.3	99	76.2
0.15	61	29.6	54	32.7	48	31.2	50	38.5
0.20	76	36.9	63	38.2	55	35.7	45	34.6
0.25	70	34.0	62	37.6	57	37.1	44	33.9

doses in both the series. Thus a reduction in survival was found with increase in concentrations of colchicine, the reduction being more drastic at the higher concentration in series B. The survival percentages on the 60th day were not appreciably different from those on the 10th day at all concentrations in both the series. This indicates that the survival data on the 10th day can be taken as a reliable index of lethality, consequent to colchicine treatment in lemongrass.

2. Early deformity

The population resulting from colchicine treatment was studied critically to assess the nature and extent of deformity induced by colchicine. Out of the whole population of treated plants one plant of series A, at 0.2 per cent and two plants of series B at 0.25 per cent concentration were found to have stunted growth and reduced leaf size. The leaves were light coloured, narrow and dried off gradually. The plants had 3 to 4 leaves and at three months age, they died off.

3. Height of plants

Observations on height of plants were made at intervals of thirty days upto the 180th day and mean height and percentage of control in each treatment worked out. The data are presented in table 2.

Between the two series of treatments no difference in plant height was observed. In series A, 30 days old seedlings recorded lower values for height when compared to the control in all treatments except 0.1 and 0.15 per cent. They differed

Table 2. Height of plants

Concentration (%)	30th day Height (cm)	%	60th day Height (cm)	%	90th day Height (cm)	%	120th day Height (cm)	%	150th day Height (cm)	%	180th day Height (cm)	%
Series A (4 hours interval after presoaking)												
0	6.7	100	15.3	100	34.4	100	59.2	100	93.3	100	110.5	100
0.05	6.5	96.9	15.0	97.7	39.3	114.2	80.3	135.6	135.6	145.3	152.5	138.0
0.10	7.2	107.3	17.8	115.7	35.8	104.3	62.5	105.6	85.2	91.3	102.7	92.9
0.15	8.5	127.3	21.4	139.5	50.8	147.7	79.5	134.3	126.4	135.5	136.3	123.4
0.20	6.4	95.4	20.7	134.8	30.5	88.7	68.5	115.7	128.2	137.4	147.1	133.1
0.25	5.8	86.4	15.0	97.7	29.8	86.5	69.0	116.5	145.8	156.3	168.3	152.3
Series B (No interval after presoaking)												
0	7.8	100	16.0	100	36.6	100	44.0	100	82.9	100	105.9	100
0.05	7.4	94.9	19.2	120.0	38.2	104.4	64.2	145.3	106.3	128.3	147.2	139.0
0.10	8.4	107.6	17.7	110.6	50.3	138.8	76.4	172.9	123.5	149.0	150.3	141.9
0.15	5.1	65.4	13.3	83.1	30.1	82.2	77.4	175.1	123.1	148.5	155.0	146.4
0.20	5.0	65.1	13.9	86.9	26.6	73.0	60.1	136.0	99.4	119.9	137.0	129.4
0.25	5.7	73.1	24.0	150.0	41.9	114.5	84.7	191.6	140.2	169.1	155.3	146.7

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from the control with an increase of 7.3 and 27.3 per cent respectively. In series B, seedlings at 0.1 per cent concentration had an increase of 7.6 per cent over the control. The lowest percentage of 64.1 was recorded at 0.2 per cent of series B. Height of plants increased steadily from two months onwards, the extent of increase varying among the different treatments. The highest increase was at 0.25 per cent of series B. When the plants reached 120 days of age, they recorded a steady increase in height in all the treatments in both the series. 0.25 per cent of series B recorded an increase in height of 91.6 per cent. Later stages also recorded steady increase in plant height. All the treatments in series A and B recorded higher percentage at the last observation except 0.1 per cent of series A. The plant height observations at the different stages reveal that the colchicine treated plants have initial slower growth and subsequent rapid growth in terms of plant height.

4. Number of tillers

The number of tillers recorded on 120th, 150th and 180th days after sowing are presented in table 3. With increase in the age of plants, the number of tillers also increased in all the treatments. Early tillering was a conspicuous feature observed in the treated plants as compared to the control. At the age of 120 days, the number of tillers in all the treatments in both the series were 2 to 4 times that of the control. The tiller counts at 0.15 and 0.2 per cent of series A were four times that of the control. 0.25 per cent

Table 3. Number of tillers.

Concentration (%)	120th day		150th day		180th day	
	No.	%	No.	%	No.	%
Series A	(4 hours interval after presoaking)					
0	1.3	100	7.7	100	15.3	100
0.05	3.6	276.9	7.7	100	13.8	90.2
0.10	3.0	230.8	10.9	141.6	12.4	81.1
0.15	5.6	430.8	12.2	158.4	15.0	98.1
0.20	5.5	423.1	12.1	157.1	14.3	93.5
0.25	4.9	369.2	11.7	151.9	16.6	108.5
Series B	(No interval after presoaking)					
0 0	1.4	100	5.2	100	12.8	100
0.05	3.8	271.4	10.3	198.1	12.3	96.1
0.10	3.6	257.4	11.5	221.2	15.0	117.2
0.15	3.7	264.3	11.7	225.0	15.5	121.7
0.20	2.5	178.6	8.9	171.2	14.0	109.4
0.25	4.4	314.3	15.0	288.5	17.6	137.5

in both the series recorded values more than three times of the control. Nearly double the number of tillers in relation to the control was recorded at 0.05 and 0.10 per cent in series A and 0.05, 0.10 and 0.2 per cent in series B. Similar trend of increase in the number of tillers was observed on 150 days old plants, the higher concentrations of 0.15, 0.20 and 0.25 per cent recording higher percentages of 158.4, 157.1 and 151.9 per cent respectively of the control. In series B, all the treatment concentrations recorded more than 150 per cent of the control value and 0.10, 0.15 and 0.25 per cent recorded values of 221.2, 225 and 288.5 per cent respectively. Tiller counts on 180th day revealed that the treatments were not much different from the control in the number of tillers. In series A, all the treatments were on par with the control while in series B, the values were slightly higher to the control.

5. Number of stomata

Ten plants from each treatment were selected at random and the number of stomata per microscopic field recorded. In each plant, thirty microscopic fields were counted and the mean values for each plant and treatment were calculated and presented in table 4.

In both the series and at all the concentrations, the mean number of stomata per microscopic field was on par with the control, the mean number ranging from 34.3 to 41.9 in the different concentrations. There were individual plants with reduced number of stomata in both the series. Plant number 5

Table 4. Number of stomata (per microscopic field).

Concentration (%)	Plant-1	Plant-2	Plant-3	Plant-4	Plant-5	Plant-6	Plant-7	Plant-8	Plant-9	Plant-10	Mean
Series A	(4 hours interval after presoaking)										
0	30.1	33.4	31.0	36.7	34.5	41.1	41.5	41.3	36.8	36.9	36.3
0.05	32.1	37.2	30.2	36.7	36.4	32.8	38.0	37.9	32.9	37.1	35.1
0.10	34.2	33.8	31.2	41.7	30.0	32.2	33.6	36.5	36.1	39.9	34.9
0.15	41.0	32.2	40.8	32.1	39.6	38.1	36.7	33.6	33.7	38.1	36.6
0.20	30.5	39.5	35.3	35.6	36.6	32.5	31.3	30.0	33.2	38.1	34.3
0.25	33.4	32.7	31.4	32.8	35.1	36.9	33.7	38.5	37.0	38.0	35.0
Series B	(No interval after presoaking)										
0	43.1	47.4	41.1	40.9	42.3	44.0	40.0	42.0	43.2	45.1	41.9
0.05	42.1	35.9	34.9	40.3	36.8	39.4	36.2	42.7	36.6	37.1	38.2
0.10	37.4	40.5	35.4	34.8	38.7	39.0	38.3	38.4	38.2	36.6	37.7
0.15	41.9	45.1	30.3	37.6	29.5	34.0	35.3	35.1	33.9	38.7	36.1
0.20	36.1	35.8	30.0	37.8	39.3	34.3	31.5	30.0	36.2	38.0	34.9
0.25	36.3	36.8	35.3	37.8	35.7	36.4	30.7	36.7	37.8	36.9	35.8

of 0.15 per cent in series B recorded a stomatal number of 29.5 per microscopic field, the lowest value recorded. In series B, 0.2 per cent, plant number 3 and 8 had 30 stomata per microscopic field. A mean value of 30.2 was observed in plant number 3 of 0.05 per cent (series A) and plant number 1 of 0.2 per cent (series B) had the mean value as 30.5.

6. Size of stomata

The length and width of stomata were measured and recorded. Ten cells in each microscopic field, constituting a total of 30 microscopic fields in each treatment were subjected to stomatal size observations. The mean values for each plant and treatment for the length and width of stomata were calculated and are presented in tables 5 and 6.

Between the two series, the mean length of stomata was not different. The mean values ranged from 72.4 to 80.2 μ in series A and 66.1 to 77 μ in series B. In both the series, the mean values were higher than their control in all the treatments except in 0.25 per cent of series A. The mean values were on par among the different treatments. Conspicuously higher values were obtained in individual plants in series A and B. The highest value of 93.8 μ was recorded in plant number 3 of 0.05 per cent in series A. Plant number 6 of 0.1 per cent of series A gave 91.6 μ and plant number 5 of the same series had a mean length of 86.8 μ. Mean values of 85.4 μ and 84.0 μ were recorded by plant number 9 and plant number 6 of 0.05 per cent in series A. Two plants of series A,

Table 5. Length of stomata (microns).

Concentration (%)	Plant-1	Plant-2	Plant-3	Plant-4	Plant-5	Plant-6	Plant-7	Plant-8	Plant-9	Plant-10	Mean
Series A	(4 hours interval after presoaking)										
0	78.4	79.8	75.6	74.2	77.0	65.8	67.2	68.6	70.0	67.2	72.4
0.05	75.6	78.4	93.8	78.4	78.4	84.0	79.8	77.0	85.4	71.4	80.2
0.10	72.8	79.8	81.2	70.0	86.8	91.6	74.2	71.4	70.0	67.2	76.5
0.15	70.0	84.0	75.6	82.6	72.8	81.2	78.4	74.2	77.0	70.0	76.6
0.20	85.4	75.6	78.4	82.6	78.4	82.6	78.4	85.4	84.0	70.0	80.1
0.25	77.0	78.4	81.4	74.2	70.0	65.8	71.4	68.6	67.6	70.0	72.4
Series B	(No interval after presoaking)										
0	72.8	71.4	70.0	71.4	72.8	67.6	56.0	57.4	58.8	63.0	66.1
0.05	68.6	70.0	78.4	74.2	72.8	72.8	75.6	71.4	70.0	72.8	72.7
0.10	74.2	84.8	78.2	80.8	74.2	70.0	70.0	74.2	75.6	70.0	75.2
0.15	70.0	67.2	86.8	77.0	83.2	79.8	78.4	77.0	74.2	71.4	77.0
0.20	81.2	74.2	84.0	70.0	71.4	68.6	70.0	86.8	68.6	70.0	74.5
0.25	81.2	79.8	74.2	74.6	77.0	75.6	84.0	79.8	71.4	68.6	76.7

Table 6. Width of stomata (microns).

Concentration (%)	Plant-1	Plant-2	Plant-3	Plant-4	Plant-5	Plant-6	Plant-7	Plant-8	Plant-9	Plant-10	Mean
Series A	(4 hours interval after presoaking)										
0	53.2	61.6	56.0	54.6	56.0	50.4	53.2	57.4	53.2	50.4	54.6
0.05	53.2	53.2	64.4	57.4	54.6	51.8	53.2	58.8	57.4	53.2	55.7
0.10	54.6	56.0	53.2	54.6	63.0	64.4	56.0	53.2	56.0	54.6	56.6
0.15	56.0	58.8	54.6	60.2	56.0	53.2	51.8	49.0	53.2	51.8	54.5
0.20	60.2	56.0	53.2	54.6	53.2	49.0	50.4	58.8	57.4	54.6	54.8
0.25	53.2	42.0	57.4	53.2	51.8	56.0	56.0	51.8	56.0	54.6	53.2
Series B	(No interval after presoaking)										
0	44.8	57.4	51.8	58.8	56.0	58.8	56.0	54.6	61.6	61.6	56.1
0.05	54.6	56.0	56.0	53.2	58.8	56.0	51.8	54.6	58.8	59.2	55.9
0.10	53.2	50.4	54.6	54.6	56.0	56.0	53.2	56.0	53.2	56.0	54.3
0.15	56.0	56.0	63.0	56.0	64.4	56.0	54.6	58.8	54.5	54.6	57.4
0.20	54.6	53.2	63.0	56.0	56.0	54.6	56.0	64.4	54.6	56.0	56.8
0.25	54.6	53.2	49.0	50.4	51.8	54.6	53.2	51.8	53.2	53.2	52.5

plant number 2 and 4 had mean values of 84.0 and 82.6 μ respectively. In series B, plant number 2 of 0.1 per cent recorded mean stomatal length to be 84.8 μ. Mean width of stomata in the two series showed no significant difference. The mean values of control and treatments were also on par. But there were individual plants with wider stomata. The values of individual plants ranged from 50.4 to 64.4 μ in series A and 49.0 to 64.4 μ in series B. Plant number 3 of series A, 0.05 per cent and plant number 6 of 0.10 per cent recorded a mean stomatal width of 64.4 μ. In series B, two plants of 0.15 per cent, plant number 3 and 5 had mean values of 63.0 μ and 64.4 μ respectively. 64.4 μ was recorded in plant number 8 of 0.2 per cent in series B.

There were individual plants with higher values for length and width of stomata and reduced values for number of stomata in both the series, which could be categorised as suspected polyploids. In series A, plant number 3 of 0.05 per cent, plant number 5 and 6 of 0.10 per cent and plant number 1 and 8 of 0.2 per cent were selected. In series B, two plants of 0.15 per cent, plant number 3 and 5 and one plant of 0.2 per cent, plant number 8 were the outstanding plants with respect to the above mentioned characters.

7. Days to flowering

The colchicine treated population was critically studied with respect to flowering. The number of days to flowering was recorded in comparison with the control and presented in table 7.

Table 7. Number of days to flower.

Concentration (%)	Number of days to flower										
	Plant-1	Plant-2	Plant-3	Plant-4	Plant-5	Plant-6	Plant-7	Plant-8	Plant-9	Plant-10	Mean
Series A	(4 hours interval after presoaking)										
0	135	129	140	141	139	137	135	140	139	141	137.6
0.05	146	154	165	156	146	148	147	150	-	-	151.5
0.10	148	158	157	153	167	158	159	154	-	-	156.8
0.15	148	158	156	158	158	165	171	156	-	-	158.8
0.20	165	175	173	-	-	-	-	-	-	-	167.7
0.25	165	168	-	-	-	-	-	-	-	-	166.5
Series B	(No interval after presoaking)										
0	140	141	135	136	142	145	137	135	131	138	138.0
0.05	145	147	150	147	-	-	-	-	-	-	147.3
0.10	152	148	155	150	-	-	-	-	-	-	151.3
0.15	155	158	156	167	166	173	-	-	-	-	162.5
0.20	168	176	-	-	-	-	-	-	-	-	172.0
0.25	167	169	-	-	-	-	-	-	-	-	168.0

Flowering started very late in the colchicine treated plants. In series A, eight plants at 0.05, 0.10 and 0.15 per cent flowered. In 0.2 per cent, three plants and 0.25 per cent only two plants come to flowering. In series B, the plants which came to flowering were lesser than that in series A. Only four plants flowered in 0.05 and 0.10 per cent in series B, whereas six plants flowered in the 0.15 per cent treatment. In all the treatments the number of days to flowering was more than that of the control. The number of days required to flower increased with the concentration indicating that the treatments had significant effect on delaying flowering. The mean number of days to flowering ranged from 147.3 to 172.0 in the different treatments.

8. Pollen fertility

The mean pollen fertility percentage of each treatment was estimated and presented in table 8. The pollen fertility percentage of the control of the two series were 83.1 and 82.4 respectively. Between the two series there was not much difference in pollen fertility values. All the treatments gave lower values compared to the control. The mean fertility value ranged from 76.3 to 81.6 per cent in series A and 76.0 to 81.4 per cent in series B. In both the series, 0.2 per cent gave the lowest values of pollen fertility and 0.25 per cent gave the highest values. Lowest fertility of 73.1 per cent was recorded in plant number 2 of 0.2 per cent in series B. 73.2 per cent fertility was recorded in plant number 6 of 0.15 per cent, series B.

Table 8. Pollen fertility.

Concentration (%)	Pollen fertility (%)										
	Plant-1	Plant-2	Plant-3	Plant-4	Plant-5	Plant-6	Plant-7	Plant-8	Plant-9	Plant-10	Mean
Series A	(4 hours interval after presoaking)										
0	83.1	85.1	82.1	84.0	84.1	84.5	83.3	81.5	83.3	80.0	83.1
0.05	80.2	79.5	76.5	80.0	83.8	77.0	77.2	78.2	-	-	78.4
0.10	80.0	81.5	81.0	79.5	78.0	75.8	74.5	81.5	-	-	78.7
0.15	81.6	81.5	82.1	82.0	81.8	80.0	81.8	81.2	-	-	81.5
0.20	80.8	74.2	74.0	-	-	-	-	-	-	-	76.3
0.25	81.2	82.1	-	-	-	-	-	-	-	-	81.6
Series B	(No interval after presoaking)										
0	83.0	83.1	82.3	81.7	83.8	82.2	84.6	82.1	82.7	81.0	82.4
0.05	80.8	80.2	80.0	80.3	-	-	-	-	-	-	80.3
0.10	81.5	80.7	80.7	81.1	-	-	-	-	-	-	81.0
0.15	80.0	84.6	82.3	74.0	81.7	73.2	-	-	-	-	79.3
0.20	78.9	73.1	-	-	-	-	-	-	-	-	76.0
0.25	81.6	81.2	-	-	-	-	-	-	-	-	81.4

9. Suspected polyploids

Based on increased vigour, larger size of stomata and pollen fertility, eight plants were selected as suspected polyploids. These plants exhibited increased vigour in respect of height, number of tillers, nature of leaves and stomatal characters. They were subjected to a detailed morphological study and the characters studied with those of the control are given in table 9. A standard plant is represented in Figure 1.

Plant No.1.

This plant was identified in series A, 0.05 per cent concentration (Fig.2). It had a tall plant height of 200 cm and was more vigorous than the control plants. The leaves were dark green, broader and thicker with prominent midrib. Stem was thick, sturdy and dark coloured. The number of tillers was estimated as 58. This plant was robust with early profuse tillering habit. It had a bushy appearance due to thick growth of leaves. The stomatal frequency was much less with a mean number of 30.2 per microscopic field. But the size was large with a mean length of 93.8μ and width of 64.4μ. The plant took 165 days for flowering and the pollen fertility recorded was 76.5 per cent.

Plant No.2.

One of the two plants identified in series A, 0.10 per cent concentration is shown in Fig.3. The height recorded was 190 cm. This vigorous plant had sixty thick sturdy tillers.

Table 9. Morphological characters of suspected polyploids.

Plant No.	Concentration (%)	Height (cm)	Number of tillers	Mean number of stomata per microscopic field	Mean length of stomata	Mean width of stomata	Days to flowering	Pollen fertility (%)
0	0	110.5	44	39.1	69.3	55.4	157.8	82.8
Series A								
1	0.05	200	58	30.2	93.8	64.4	165	76.5
2	0.10	190	60	30.0	86.8	63.0	167	75.8
3	0.15	195	65	32.2	91.6	64.4	171	74.5
4	0.20	200	56	30.5	85.4	60.2	173	74.0
5	0.20	188	58	30.0	85.4	58.8	175	74.2
Series B								
6	0.15	194	62	30.3	86.8	63.0	173	74.0
7	0.15	205	60	29.5	88.2	64.4	167	73.2
8	0.20	198	58	30.0	86.8	64.4	176	73.1

Figure 1. A standard plant of lemongrass - Variety - OD-19.

Figure 1.

Figure 2. Suspected polyploid - Plant No.1.
(Identified in series A, 0.05% concentration)

Figure 3. Suspected polyploid - Plant No.2.
(Identified in series A, 0.10% concentration)

Figure 2.

Figure 3.

Leaves were dark green, thicker with slight crinkling on the leaf margins at early stages. Stomatal frequency was low with a mean number of 30 per microscopic field. Mean stomatal length of 86.8 μ and mean width of 63.0 μ were recorded. The plant is robust and flowering took place 167 days after planting. The pollen fertility estimated was 75.8 per cent.

Plant No.3.

Figure 4 shows a plant suspected as polyploid in series A, 0.15 per cent concentration. It was conspicuous with bushy nature and larger number of tillers. Plant height was 195 cm and the number of tillers was 65. Leaves were broader and thicker. Large sized stomata with reduced frequency was observed in this plant too. The mean number of stomata per microscopic field was 32.2 with mean length of 91.6 μ and width 64.4 μ. Days to flowering was 171 and pollen fertility 74.5 per cent.

Plant No.4.

In series A, 0.2 per cent concentration, two plants were identified of which one is shown in Figure 5. This had dark green broad and thick leaves which were crinkled at the margins. Plant height was 200 cm. Number of tillers estimated was 56. The mean stomatal length was 85.4 μ and width 60.2 μ. Frequency of stomata was lower with a mean of 30.5 per microscopic field. The plant had a tall appearance. It took 173 days to flower. 74 per cent pollen fertility was recorded.

Figure 4. Suspected polyploid - Plant No.3. (Identified in series A, 0.15% concentration)

Figure 5. Suspected polyploid - Plant No.4. (Identified in series A, 0.20% concentration)

Figure 4.

Figure 5.

Plant No.5.

Figure 6 shows the second plant in series A, 0.2 per cent concentration. It had conspicuously very vigorous nature with dark, thick and broad leaves. Plant height was 188 cm and the number of tillers, 58. The stem was stouter, hardier and leaves were brittle too. The mean number of stomata per microscopic field was 30, the mean stomatal length and width being 85.4 μ and 58.8 μ respectively. It took 175 days to flower and pollen fertility was 74.2 per cent.

Plant No.6.

In series B, 0.15 per cent concentration, two plants were identified of which one is shown in Figure 7. The plant was vigorous with broader, thicker and dark green leaves. Height was 194 cm and the number of tillers recorded comes to 62. Stomatal frequency was 30.2 per microscopic field. Length and width of stomata were more than the control, the mean being 86.8 μ and 63.0 μ respectively. Number of days to flowering was 173 and pollen fertility 74 per cent. The plant was vigorous with profuse tillering.

Plant No.7.

Figure 8 shows the second plant in series B, 0.15 per cent concentration. It was tall with 205 cm height and 60 tillers which were darker, thicker and hardier. Leaves were also thick, dark green and broad. Stomatal observations showed a mean stomatal number of 29.5 per microscopic field. The mean length and width were 88.2 and 64.4 μ respectively.

Figure 6. Suspected polyploid - Plant No.5. (Identified in series A, 0.20% concentration)

Figure 7. Suspected polyploid - Plant No.6. (Identified in series B, 0.15% concentration)

Figure 6.

Figure 7.

Figure 8. Suspected polyploid - Plant No.7. (Identified in series B, 0.15% concentration)

Figure 9. Suspected polyploid - Plant No.8. (Identified in series B, 0.20% concentration)

Figure 8.

Figure 9.

This plant took 167 days to flower. The pollen fertility was 73.2 per cent.

Plant No.8.

This belongs to series B, 0.2 per cent concentration. In terms of vigour, this plant exceeded the control. It had a height of 198 cm with 58 tillers. Leaves were dark green, broad and thick with slight crinkling in early stages (Fig.9). The mean stomatal length was 86.8 μ and width, 64.4 μ. The mean stomatal number per microscopic field was 30.0. This plant took 176 days to flower and pollen fertility was 73.1 per cent.

Generally, the plants selected as suspected polyploids were far more vigorous and robust than the control plants. They were taller, the stems were darker and the leaves were conspicuously thicker and broader in comparison with the control. In plant No.5, the leaves were found to be brittle. Early crinkling of leaves was a feature observed in some of the suspected polyploids. The stomata were reduced in number per unit area and the size enlarged which is a criteria for selecting polyploid plants. The stomatal size in terms of length and width were found to be greater than the control in these selected plants. These plants showed a tendency of profuse tillering at the early stages. They were late in flowering compared to the control plants. Reduced fertility in these plants was an indication of their polyploid nature.

DISCUSSION

DISCUSSION

Polyploidy is a condition in which the individuals have more than two sets of chromosomes in their somatic cell. This offers the breeder an opportunity to bring about changes in plant characters by altering the chromosome number. Polyploids are either naturally occurring or artificially induced. Polyploids are classified broadly into two groups - the autoploids and alloploids. Autoploids are produced by doubling of the individual chromosomes and 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. The role of colchicine as an agent to induce autopolyploidy was conclusively demonstrated as early as 1937. The present investigation was an attempt to study the various effects induced in lemongrass by colchicine treatment. The results of the investigation are discussed below.

Effects of colchicine treatment:

The survival of plants in comparison to the control was lower in the treatments. Similar results were obtained by Pal and Ramanujam (1939) in chilli polyploids. The reduced survival in the treated seeds could be due to the toxic effect of colchicine. The survival percentage in all the treatments was found to decrease as the concentrations increased. Smith (1939) reported similar results in Nicotiana. A very reduced survival in colchicine treated seedlings was reported in cotton

by Stephens (1940). It was observed by Wit and Speckmann (1955) that the number of surviving seedlings decreased with increasing concentrations and durations of treatment. Similar results were obtained by Sen and Chhedâ (1958) in blackgram. Mortality of seedlings at different stages of growth was reported in zinnia by Bose and Panigrahi (1969) and Srivastava (1965). In crested wheat grass the germination and survival were drastically reduced as the concentration increased (William and Douglas, 1966). Thus reduction in survival of plants could be an indication of the toxic effect of colchicine.

Observations on growth of plants at different stages revealed that the plants in general had an initial slower but subsequent faster rate of growth. Drastically stunted growth resulted in seedling mortality of three plants, at the age of three months. Seedling mortality as a result of colchicine treatment was recorded by several workers. Krishnaswamy et al. (1950) reported high seedling mortality in pearl millet due to colchicine treatment. Such early mortality could be attributed to the arrest of growth by colchicine. Initial slower growth was recorded in the autotetraploids of Glycine by Anarsingh (1968) and in sorghum tetraploids by Mogoon and Tayyab (1968). In colchicine treated bhindi, stunted growth was reported by Rajasekharan and Ganesan (1968). Tetraploids with stunted growth in Cyamopsis psoraloides were obtained during the studies conducted by Biswas and Battacharya (1971). Those evidences reveal that stunted growth and seedling mortality could be the treatment effects of colchicine.

Observations on plant height made at regular intervals indicated that colchicine had significant effect on height of plants. The height increase was maximum at higher concentrations and minimum at lower concentrations. During a study in blackgram, Sen and Chhedá (1958) found tetraploidy to be associated with increase in plant height. Similar results were reported in crested wheat grass by William and Douglas (1966). Increased plant height was observed in the advanced generations of autotetraploid maize (Dudley and Alexander, 1969). Sambamurthy (1973) recorded an increase by 25 cm in rice autotetraploid. Increased height was reported in Alfalfa by Kataro and Narkar (1976), in Brassica by Singh (1976 b) and in Phaseolus by Kabi and Bhaduri (1978). The increased height in treated plants may be the manifestation of gigas expression as a result of colchicine treatment.

The treatments had significant effect on number of tillers. Early tillering was observed in both the series. This may be due to the lesser competition because of the reduced survival in treated population. With increase in age of plants, the number of tillers also increased in all the treatments. Increase in the number of tillers was two to four times that of the control plants. This may be due to the inactivation of apical meristem by the toxic effect of colchicine resulting in axillary bud activation leading to the increased number of axillary tillers. There are several reports on increased branching resulting from colchicine in different plants. Higher number of tillers was reported in

Berseem and Trifolium by Mehta et al. (1963, 1965 a). Heavy branching was recorded by Prased and Deepesh (1969) in Pachyrizus tetraploid. In Alfalfa polyploids, Katare and Nerkar (1976) reported better tillering than control plants. Higher number of sturdy branches resulted in brown sarson autotetraploids (Tyagi and Das, 1970). Thus polyploidy in plants could result in increased tillering or branching.

In both the series and at all concentrations, the mean number of stomata per microscopic field was on par with the control. There were individual plants with reduced number of stomata. Reduction in the number of stomata is pointed out as an indication of polyploid nature of the plant by several workers. Plants with reduced number of stomata in this investigation are taken as suspected polyploids. Polyploids with reduced number of stomata were reported in sorghum by Siddiq (1967) and in Bajra by Gill et al. (1970). Reduction in Stomatal frequency was reported in Brassica by Singh (1976 a) and in Sunflower by Gupta and Roy (1980).

Individual plants with larger stomata in terms of length and width were identified. Increased size of stomata generally indicate the occurrence of polyploidy. Larger stomata were observed in polyploids of different crops such as Alyssum (Bali and Tandon, 1958), Safflower (Schank and Knowles, 1961), Ocimum (Bose and Chowdhury, 1962), Berseem (Mehta et al., 1963) and Sorghum (Siddiq, 1967). The stomatal length as well as width were higher than those of control in the plants selected as suspected polyploids. In the polyploids of Cyamopsis,

Biswas and Bhattacharya (1971) reported increased length and width compared to the diploids. Significant increase in the stomatal length and width were observed in the induced tetraploid of Sorghum nitens by Magoon and Tayyab (1968).

The number of days to flowering was more in all the treatments. Flowering in colchicine treated plants was delayed by 18 to 51 days in the different treatments. Several workers have obtained similar results in different crop species. Wit (1958) observed two weeks delay in flowering in Italian ryegrass. In ocimum, 30 to 45 days delay was recorded in the polyploids by Bose and Chowdhury (1962). Twenty days delay in flowering occurred in the tetraploid sorghum as recorded by Siddiq (1967) and Murty et al. (1978). The delay in flowering in the colchicine treated population may be due to the prolonged vegetative growth period ending in later flowering. Mohammed (1979) reported the polyploid plants of Medicago sativa failed to flower over two successive seasons. Delayed and extended flowering were noticed in the polyploids of Cucurbitaceae and Portulaca (Singh, 1979 b).

The mean fertility percentages in all the concentrations in the two series were lower than the control. The fertility percentage varied from 76 to 81.6 per cent in the different treatment concentrations. Low fertility has been attributed as one of the effects of polyploidy in several crop species by various workers. Deenuldi and Pal (1950) recorded high sterility in Nicotiana. Similar observations were made in Ocimum, (Bose and Chowdhury, 1962), crested wheat grass (William and

Douglas., 1966), mung (Mital, 1967), groundnut (Lal and Mehrotra, 1968) and Sorghum (Magoon and Tayyab, 1968). The reduction in pollen fertility may be due to meiotic irregularities (Bose and Sharma, 1970). Chin (1946) suggested that high pollen sterility in tetraploids is due to irregular segregation of some of the multivalents at meiosis. Genetically controlled physiological factors may cause sterility as opined by Stebbins (1947, 1950). In the present study, the reduced pollen fertility might be due to any of the above reasons.

Identification of Polyploids:

Those plants which showed expressions characteristic of polyploidy such as robust vegetative growth, reduced number of stomata per unit area, increased stomatal size, delayed flowering and reduced pollen fertility were selected as suspected polyploids. All the eight plants so identified were taller than the standard. The increase in height was maximum in plant number 7 (Series B, 0.15%). Increased height was reported in the tetraploids of brown sarson (Chowdhury et al., 1967), maize (Dudley and Alexander, 1969) and Brassica (Singh, 1970). In the autotetraploid rice, Sambamurthy (1973) observed significant increase in plant height. Katare and Nerkar (1976) in alfalfa, Levan (1939) in petunia and Sen and Chheda (1958) in blackgram reported increased plant height to be associated with tetraploidy.

The third plant (Series A, 0.15%) had the largest number of tillers. Similar observations on increased number of tillers were made by Mehta et al. (1965 a) in trifolium

tetraploids which gave double the number of tillers as that of their control. In berseem also Mehta et al. (1963) reported similar results. The induced polyploids of Sorghum nitidum exhibited profuse tillering (Magoon and Tayyab, 1968) and the tetraploid of pachyrhizus recorded heavy branching as reported by Prasad and Deepesh (1969).

In the selected plants, the leaves were characteristically thicker and broader with dark green colour. Eigsti and Dustin (1955) suggested that an increase in thickness in the leaves may be used as a criterion to distinguish plants that have responded to colchicine treatment. Mehta et al. (1965 a) in trifolium, Mehta and Swaminathan (1965) in berseem, Bali and Tandon (1958) in rye grass recorded similar observations.

Reduced number and increased size of stomata is a criteria for identifying polyploids. The selected plants had significantly reduced number and enlarged size of stomata. The number of stomata ranged from 29.5 to 32.2 while in the control plant it was 39.1 per microscopic field. Of the selected plants, numbers two, five and eight had a reduced number of 30 stomata per microscopic field. The mean length and width of stomata in the standard plant was 69.3 μ and 55.4 μ respectively whereas in the selected plants the length varied from 85.4 to 93.8 μ and breadth from 58.8 to 64.4 μ. The biggest stomata was observed in plant number one with 93.8 μ length and 64.4 μ width. Increase in the size of stomata generally indicate polyploidy. Similar results were

recorded in Nicotiana by Deshmukh and Pal (1950), in Alyssum by Bali and Tandon (1958), in broun sarson by Tyagi and Das (1970) in pearl millet by Krishnaswamy et al. (1950) and in brassica by Singh (1970).

In all selected plants flowering was delayed by 31 to 42 days compared to the control. In sorghum tetraploids, a delay in flowering by 45 to 60 days was reported by Murty et al. (1978). Biswas and Battacharya (1971) recorded a delay of 8 to 45 days in flowering in the induced polyploids of cyamopsis. Similar results were obtained in chilli by Pal et al. (1941), in Nicotiana by Deshmukh and Pal (1950) and in rye-grass by Wit and Speckmann (1955). The delayed flowering may be attributed to the prolonged vegetative growth effected by colchicine treatment.

Reduced pollen fertility was used as a criteria for identifying polyploids. In the standard plants the pollen fertility was 82.8 per cent whereas in the selected plants, the values ranged from 73.1 to 76.5 per cent. Varying degrees of pollen sterility were reported in the polyploids of chilli by Pal et al. (1941) and in blackgram by Sen and Chheda (1958). Singh (1941) recorded 40 to 51 per cent pollen sterility in tetraploid brinjal. In Brassica, 5 to 10 per cent pollen sterility was detected by Ramanujam and Deshmukh (1945). In groundnut polyploids, increased sterility was observed by Lal and Mehrotra (1968). High pollen sterility in tetraploids was reported by Rajasekharan and Ganesan (1968) in bhindi and by Lobana and Verma (1972) in cluster bean.

The eight plants selected as suspected polyploids in the present investigation were identified by eliminating those which have not responded to colchicine treatment. Further utilization of these suspected polyploids, is possible after cytological confirmation as genuine polyploids. Since the phenotypic response to changes in chromosome number varies widely from species to species and even from plant to plant within a species, cytological confirmation is required to establish polyploidy. They could later be evaluated for their economic advantages and possible utilization in crop improvement programmes.

SUMMARY

SUMMARY

The present study was conducted in the College of Agriculture, Vellayani during the years 1979 to 1981 with a view to induce autotetraploids in lemongrass. Seeds of lemongrass variety OD-19 were obtained from the Lemongrass Research Station at Odakkali. Colchicine was used for seed treatment. The concentrations of colchicine were fixed in the range of 0 to 0.25 per cent based on the results of preliminary trials. Two series of treatments (A and B) were conducted. In both the series the treatment duration and presoaking period was 4 hours. But in Series A, presoaking was followed by an interval of 4 hours before treatment whereas no interval of time was given between presoaking and treatment in Series B.

Observations on survival, early deformity, height of plants, number of tillers, number, length and width of stomata, days to flowering and pollen fertility were made. The results obtained at different stages of growth of the treated plants and conclusions drawn are summarised below.

Survival of treated plants was reduced with increase in concentrations of colchicine. In both the series, higher lethality resulted at higher doses of colchicine. At all stages of observation, the survival of treated plants was lower than that of the control in all treatments.

In the treated population, early deformity was observed in three plants which were stunted with reduced leaf size and they died off at the age of three months.

Between the two series of treatments no difference in plant height was observed. The plant height observations at different stages revealed that the colchicine treated plants have initial slower growth and subsequent rapid growth in terms of plant height.

With increase in the age of plants, the number of tillers also increased in all the treatments. Early tillering was a conspicuous feature observed in the treated plants as compared to the control. There was no difference between the two series of treatments in the number of tillers.

The mean number, length and width of stomata per microscopic field was on par with the control. There were individual plants in both the series with lower number and increased length and width of stomata which are taken as indication of polyploidy.

Flowering started very late in the colchicine treated plants. The number of days required to flower increased with the concentration indicating that the treatments had significant effect on delaying flowering.

The pollen fertility was reduced in all the treatments compared to the control plants. Between the two series of treatments there was no difference in pollen fertility values.

Based on increased vigour, larger size of stomata and reduced pollen fertility, eight plants were selected as suspected polyploids. They exhibited increased vigour in terms of height, tillers, nature of leaves and stomatal characters. The plants selected as suspected polyploids were more vigorous

and robust than the control plants. They were taller, the stems were darker and the leaves were conspicuously thicker and broader in comparison to the control. Stomata were reduced in number per unit area and the size enlarged in terms of length and width. These plants exhibited profuse tillering at the early stages. They were very late in flowering with reduced pollen fertility indicating polyploid nature. These plants suspected as polyploids could be confirmed based on further detailed cytological studies.

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*Original not seen

INDUCTION OF AUTOTETRAPLOIDS IN LEMONGRASS

BY

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ABSTRACT OF A THESIS

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ABSTRACT

Indian lemongrass (Cymbopogon flexuousus) is the chief source of lemongrass oil of commerce. The form of C. Flexuousus grown in the State being a diploid offers scope for induction of autotetraploids. In the present investigation an attempt was made to induce autotetraploidy in lemongrass by seed treatment with colchicine.

Two series of treatments with and without interval between presoaking and treatment were carried out, in which the treatment, presoaking and interval duration were 4 hours. The concentrations employed ranged from 0 to 0.25 per cent.

In the population resulting from colchicine treatment, observations on survival, early deformity, plant height, number of tillers, stomatal observations, days to flowering and pollen fertility were made. Based on these observations, eight plants suspected as polyploids were identified.

The survival of colchicine treated plants was lower than their control in all the treatments. In colchicine treated plants the height, number of tillers, length and width of stomata and number of days to flower increased whereas there was a reduction in the number of stomata and pollen fertility.

The plants selected as suspected polyploids after rigorous screening of the population were far more vigorous and robust than their control. They were taller with darker stems and thicker leaves. They had reduced number of stomata per unit area and enlarged size. They were late in flowering with reduced pollen fertility. These eight plants provide scope for further cytological studies and development of autotetraploids in lemongrass.