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SOME OBSERVATIONS ON THE INDUCTION OF COLCHIPLOIDY IN *SOLANUM MELONGENA* LINN. (BRINJAL)

R. M. DATTA AND A. K. NEOGY

Calcutta University, Calcutta

ABSTRACT

The present investigation deals with the morphological, cytological and cytohistological studies between the normal and colchicine induced polyploids of *Solanum melongena* Linn. (variety Long Green).

Treatments given to the growing tips of the young seedlings having two cotyledonary leaves with 0.2 percent aqueous colchicine solution for 12 hours gave best results from the point of view of survival of seedlings and the induction of polyploidy.

The induced polyploid plants show thick, deep green and broad leaves with larger stomata and epidermal cells. The polyploid was characterised by larger flowers, pollen grains and seeds. The pollen fertility, fruit size and fruit setting in the polyploid plants were less than those of the control.

The chromosome numbers of *Solanum melongena* variety long green (diploid) and the induced polyploid were determined as n = 12 and n = 24, respectively. Most of the possible chromosomal associations ranging from 12 quadrivalents to 24 bivalents at diakinesis and metaphase I were recorded in the polyploid. Abnormalities such as unequal distribution of chromosomes at anaphase, presence of lagging chromosomes were also noted in the polyploid.

INTRODUCTION

Janaki Ammal (1931) first recorded a triploid brinjal and later wrote on cytological details. Induction of colchiploid may produce *gigas* characters. As this is an important economic vegetable crop of India, it is worthwhile to get its colchiploids which may help in our agriculture.

MATERIALS AND METHODS

Brinjal seeds of long green variety were obtained from the Horticulturist, State Horticultural Research Station, Krishnagar, Nadia and were sown in the month of November, 1962 in earthen pots in the Department.

As soon as the first two leaves emerged out, colchicine solution was applied on the corpus region with a cotton swab. Swabs were kept always moist by regular application of the desired doses of colchicine with the help of a fine pointed glass dropper. Treatments employed were 0.2% colchicine applied for 12, 24 and 30 hours. 20 seedlings were utilized for each treatment. The best treatment from the point of view of survival of seedlings and the induction of polyploidy was found to be 0.2% colchicine, applied for 12 hours.

After the scheduled time of application of the drugs as over, cotton swabs were removed and seedlings were left overnight for growth. Next morning stem-tips of 10 treated seedlings together with portions of plumule were fixed in F.A.A. for 24 hours. Stemtips of control seedlings were also fixed for comparison. The paraffin schedule was followed. Sections were cut 9μ thick. Slides were stained in Haidenhain's haematoxylin.

Other treated seedlings and control were allowed to grow. After a month seedlings were carefully transplanted in separate bigger pots. Morphological data regarding height, thickness of stem, number of leaves etc. were collected and tabulated at fortnight intervals.

Flower buds of different sizes from the treated and the control plants were fixed in acetic-alcohol (1:3) between 9 and 11 A.M. I.S.T. Squashes of p.m.c.'s were stained in aceto-carmine for cytological analysis.

Fruit and seed characters of treated and normal plants were also studied.

OBSERVATIONS

Morphological: In the cotyledonary stage when 0.2% colchicine was applied, swelling in the hypocotyl region is observed after 6 hours. Cotyledons became thick and broadened.

Mortality percentages show that treatments with longer hours result in death of more plants due to the poisonous action of the drug. In 36 hours' treatment not shown in Table I overleaf, all plants died.

All the treated seedlings show the effects of different treatments such as abnormally shaped cotyledonary leaves and stunted growth at early stage. Later *gigas* characters become evident.

TABLE I

Mortality percentage of treated seedlings

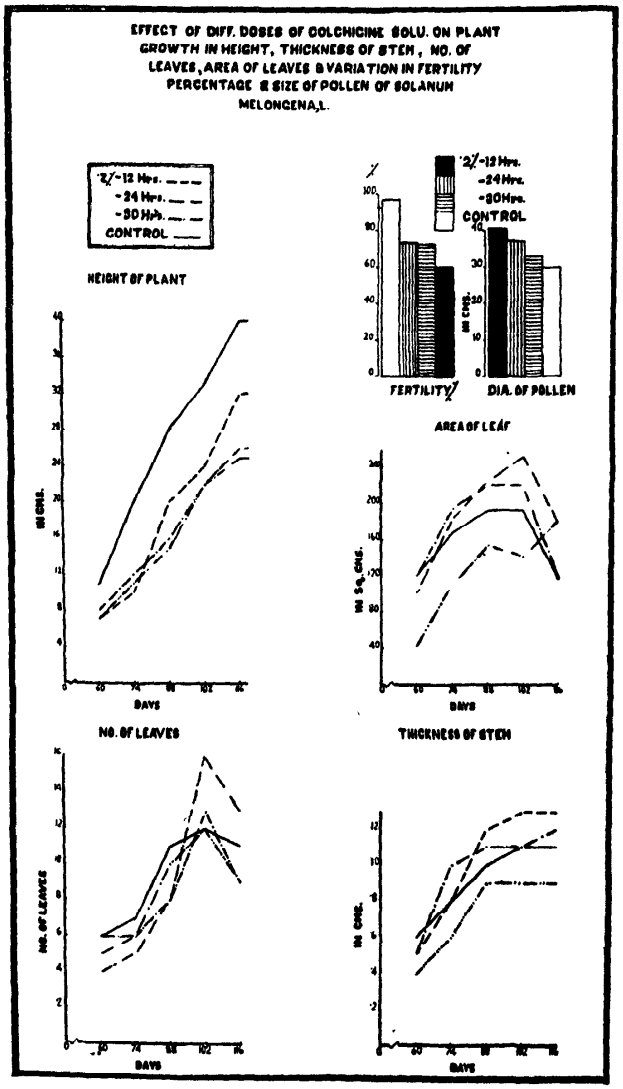
2% colchicine solution treatments in hours	No. of plants treated	No. of plants died	Mortality percentage
12 hours	10	2	20
24 ,,	10	4	40
30 ,,	10	4	40
Control	10	Nil	Nil

Table II shows growth in height of the main shoot in cm at fortnight intervals (60 days after sowing). Growth is recorded to be less in affected plants when compared to control. (*vide* Graph I).

Table III shows that increase in the thickness of stem takes place in 0.2% colchicine treated for 12 and 24 hours. In case of treatment with 30 hours there is decrease in thickness, when compared to control and other treatments. Maximum thickness is found in 12 hours' treatment (*vide* Graph I).

Table IV shows the number of leaves emerging from the main shoot, which are more in affected plants treated with 0.2% colchicine solution for 12 and 24 hours; maximum being in 12 hours' treatment (*vide* Graph I).

Table V shows the increase in the leaf area of plants treated with 0.2% colchicine for 12 and 24 hours. Maximum leaf area was obtained in plants treated for 12 hours (*vide* Graph I).



Graph I

TABLE II

Growth in height of the main shoot in cm at fortnight intervals
(Mean of 5 plants)

2% colchicine treatments in hours	Days after sowing (Date within brackets)				
	60 (6.1.63)	74 (20.1.63)	88 (3.2.63)	102 (17.2.63)	116 (3.3.63)
12 hours	7±0.53	10±0.64	20±0.51	24±0.51	32±0.71
24 ,,	8±0.50	12±0.71	16±0.61	22±0.60	26±0.61
30 ,,	7±0.62	11±0.72	15±0.54	22±0.61	25±0.61
Control	11±0.71	20±0.71	28±0.50	33±0.53	40±0.73

TABLE III

Increase or decrease in thickness of the main stem in cm at fortnight intervals (Mean of 5 plants)

2% colchicine treatments in hours	Days after sowing (Dates within brackets).				
	60 (6.1.63)	74 (20.1.63)	88 (3.2.63)	102 (17.2.63)	116 (3.3.63)
12 hours	0.5±0.03	0.5±0.03	1.2±0.02	1.3±0.01	1.3±0.01
24 ,,	0.5±0.02	1.0±0.01	1.1±0.03	1.1±0.02	1.2±0.02
30 ,,	0.4±0.01	0.6±0.01	0.9±0.02	0.9±0.03	0.9±0.01
Control	0.6±0.02	0.8±0.02	1.0±0.02	1.1±0.01	1.1±0.01

TABLE IV

Number of leaves emerged out from the main shoot at fortnight intervals (Mean of 5 plants)

2% colchicine treatments in hours	Days after sowing (Date within brackets)				
	60 (6.1.63)	74 (20.1.63)	88 (8.2.63)	102 (17.2.63)	116 (3.3.63)
12 hours	4±0.13	5±0.22	8±0.31	16±0.32	13±0.36
24 ,,	6±0.12	6±0.12	8±0.11	13±0.31	9±0.32
30 ,,	5±0.21	6±0.22	10±0.31	12±0.21	9±0.31
Control	6±0.15	7±0.21	11±0.22	12±0.30	11±0.33

TABLE V

Area of the largest leaf in square cm at fortnight intervals (Mean of 5 plants)

2% colchicine treatments for hours	Days after sowing (Date within brackets)				
	60 (6.1.63)	74 (20.1.63)	88 (3.2.63)	102 (17.2.63)	116 (3.3.63)
12 hours	99±12.01	182±15.61	224±17.21	252±12.01	180±16.03
24 ,,	120±13.06	195±13.21	221±14.36	221±18.19	120±11.04
30 ,,	42±12.1	110±11.31	154±11.92	143±13.21	180±13.1
Control	120±14.5	168±11.21	192±13.20	192±14.66	117±11.32

TABLE VI

Fertility percentages and sizes of pollen grains

2% colchicine solution treatments in hours	Total no. of pollen examined	No. of stained grains*	No. of non-stained grains	Fertility percentage	Range of diameters in μ	Average diameters of 500 pollen in μ
12 hours	532	322	210	60.52	8.4-50.4	40.53
24 ,,	500	366	134	73.20	12.6-42.0	36.96
30 ,,	512	372	140	72.65	12.5-42.0	33.39
Control	544	528	16	97.05	29.4-33.60	30.45

*Stained grains are regarded as potentially viable,

Leaves of Polyploid plants at maturity show various abnormalities in shape (Plate I) and deep green in colour. Length and breadth of leaf, guard cells, stomatal apertures, sizes of pollen, thickness of leaf and stem are found to be increased in polyploid plants as compared to diploids (Table VII). But height of plants (Table II) and pollen fertility percentages (Table VI) are less in polyploid plants.

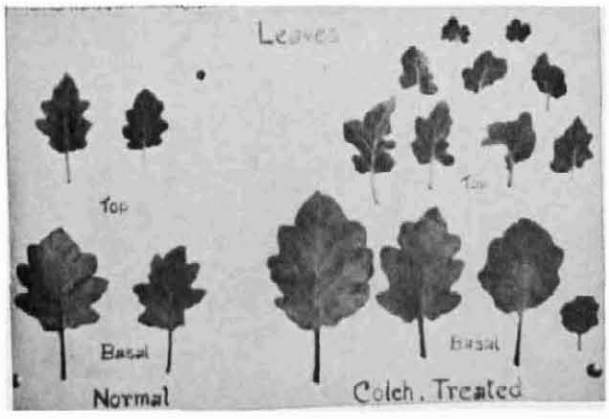


PLATE I : Leaves of normal and colchicine treated plants

TABLE VII

Measurements of vegetative parts, stomata and pollen grains

Character	Control	Polyploid
Height of plants in cm (Mean height)	40±0.73	32±0.71
Thickness of stem in cm (Mean thickness)	1.1±0.01	1.3±0.01
Length of leaf in cm	16	18
Breadth of leaf in cm	12	14
Thickness of leaf in μ	157.3	357.3
Length of guard cell of stomata in μ	31.25	56.25
Breadth of guard cell of stomata in μ	9.37	18.75
Length of stomatal aperture in μ	18.75	37.50
Breadth of stomatal aperture in μ	9.37	18.75
Pollen size in μ (Mean diameter)	30.45	40.53
Pollen fertility percentage	97.05	60.52

Flowering is delayed and extended in polyploids as the number of fruit set is less in them. The flowers on the polyploid plants are large; the pedicel, sepals, petals, stamens and pistil of these show a general enlargement over those of the control (Table VIII and Plate II). Number of sepals, petals and stamens are however, increased in polyploid plants and were found to be 7, as compared to 5 in diploids. Fruits of the polyploid plants are, however, smaller than the control. Full seed size is however, bigger in polyploid fruits.

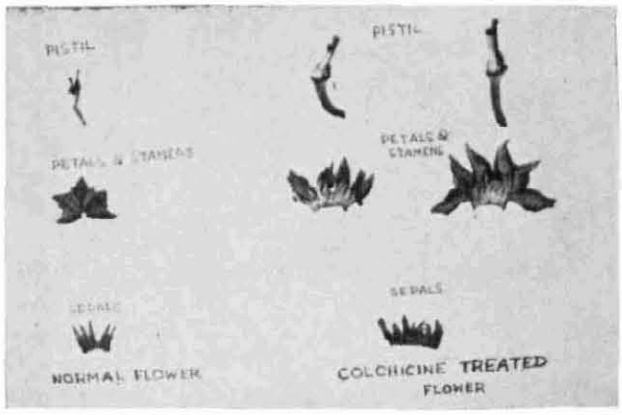


PLATE II : Flower parts of diploid and tetraploid plants

Table VIII shows increased size of all floral parts except fruits.

TABLE VIII

Measurements of floral organs, fruits and seed in cm

Character	Control	Polyploid
Length of pedicel	1.1	2.0
Length of clayx	1.7	2.2
Breadth of calyx	1.0	1.1
Length of corolla	2.3	4.3
Breadth of corolla	1.4	2.3
Length of stamen	0.7	1.3
Breadth of stamen	0.2	0.4
Length of style	0.4	0.8
Length of ovary	0.3	0.5
Breadth of ovary	0.2	0.4
Length of fruit	16.2	9.5
Breadth of fruit	4.5	3.3
Length of seed	0.3	0.4
Breadth of seed	0.2	0.3

Cytohistological: In the affected corpus regions increase in the number of nuclei and nucleoli and cell size is noticed. Details are given in tables IX and X.

TABLE IX

Increase in the number of nuclei and nucleoli in the corpus-cells of Solanum melongena Linn. by the action of different doses of colchicine solutions treated under different hours

0.2% *colchicine solution treated for* 12 *hours*

Total No. of cells observed	No. of Cells with 2 nuclei	No. of cells with 3 nuclei	No. of cells with 4 nuclei	No. of nuclei with 2 nucleoli	No. of nuclei with 3 nucleoli	No. of nuclei with 5 nucleoli	No. of nuclei with 7 nucleoli
128	18	2	1	23	7	3	1

0.2% *colchicine solution treated for* 24 *hours*

Total No. of cells observed	No. of cells with 2 nuclei	No. of cells with 3 nuclei	No. of cells with 5 nuclei	No. of nuclei with 2 nucleoli	No. of nuclei with 9 nucleoli
143	46	9	1	56	2

0.2% *colchicine solution treated for* 30 *hours*

Total No. of cells observed	No. of cells with 2 nuclei	No. of cells with 3 nuclei	No. of cells with 6 nuclei	No. of nuclei with 2 nucleoli	No. of nuclei with 3 nucleoli	No. of nuclei with 4 nucleoli	No. of nuclei with 5 nucleoli	No. of nuclei with 9 nucleoli	No. of nuclei with 12 nucleoli	No. of nuclei with 13 nucleoli	No. of nuclei with 14 nucleoli	No. of nuclei with 18 nucleoli
73	21	3	1	29	6	8	3	2	1	1	1	1

TABLE X

Measurements of normal and colchicine treated cells

2% colchicine solution	Mean length in μ	Mean breadth in μ
12 hours	27.24	15.89
24 hours	22.70	13.62
30 hours	18.16	11.35
Control	11.35	6.71

It is evident from Table IX that the number of nucleoli within a nucleus and that of nuclei within individual cell increase with the increase of the dose during the durations of treatment. On the contrary it is observed that the increase in cell size (Table X) was greater in the corpus region treated for shorter duration (12 hours).

Cytological: 12 II's are observed in the p.m.c.'s in diploid (Figs. 1 and 2). There is found no abnormality in meiosis in normal plants.

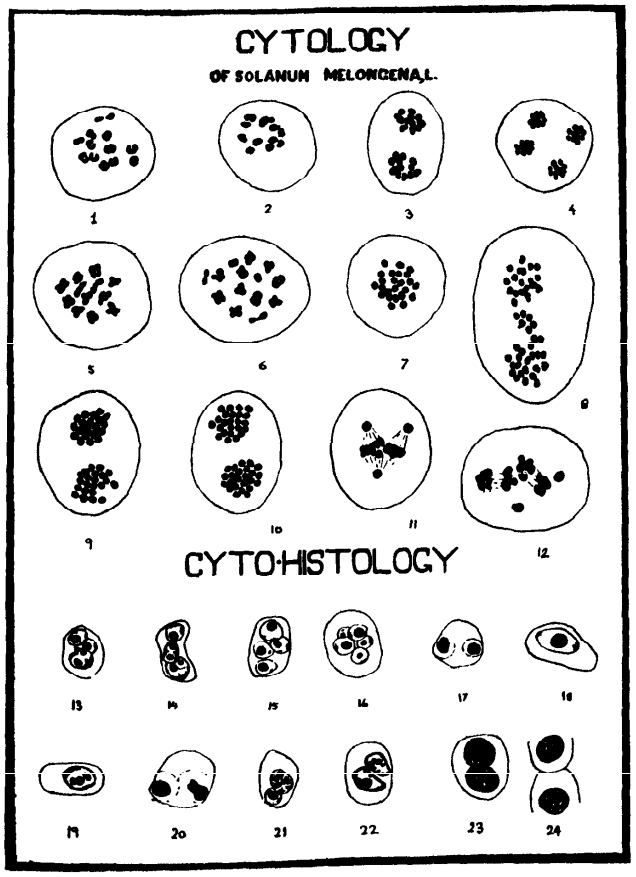
In the colchiploid 12 p.m.c.'s in diakinesis were analysed. They are enumerated as below:

(*a*) Figs. 5-12 IV = 48 (8)*
(*b*) Figs. 6-10 IV+4¶I = 48 (3)*
(*c*) Figs. 7-24 II = 48 (1)*

*No. of times observed

P.M.C. showing perfect normal pairing (24 II) was observed only once, though maximum possible number of quadrivalents (12 IV) were observed eight times. Majority of quadrivalents are of ring type and a few are of chain type. This variation in shape of quadrivalent points out that there are fundamental differences in the groups of chromosomes. In anaphase I separation of chromosomes is not regular. Some laggards are also noted (Fig. 8). Fig. 9 shows irregular distribution of chromosomes (20-22). It appears that some 6 chromosomes are cast out and eliminated.

The possibility of aneuploid gametes is expected. Normally in telophase I all the chromosomes reach the poles and nuclei are organised,



Figs. 1-4 : Meiosis in diploid. x 300 : 1. Diakinesis showing 12 II. 2. Metaphase I showing 12 II. 3. Metaphase II showing 12 chromosomes in each pole. 4. Telophase II showing 12 chromosomes in each group. Figs. 5-12 : Meiosis in tetraploid. x 300 : 5. Diakinesis showing 12 IV. 6. Diakinesis showing 10 IV & 4 II. 7. Metaphase I with 24 II. 8. Anaphase I showing lagging chromosomes. 9. Metaphase II showing 22 chromosomes in one group and 20 in other. 10. Metaphase II showing 24 chromosomes in each group. 11. Early Anaphase II shows four bivalents separated out earlier. 12. Late anaphase II shows some bivalents cast out. Figs. 13-15 : Cells from the corpus treated with colchicine for 12 hours. x 300 : 13. 3 nucleate cell with one 2-nucleolate nucleus. 14. 3 nucleate cell with two 2-nucleolate nuclus. 15. 4 nucleate cell with uninucleolate nucleus. Figs. 16-20 : Cells from the corpus treated with colchicine for 24 hours. x 300: 16. 5 nucleate cell with uninucleolate nucleus. 17. 2 nucleate cell with clumping chromosomes. 18. Cell showing big nucleus with increased nucleolus. 19. Uninucleate cell with 3 nucleoli in the nucleus. 20. 2 nucleate cell with clumping chromosomes. Figs. 21-24 : Cells from the corpus treated with colchicine for 30 hours. x 300 : 21. 2 nucleate cell with 6 nucleoli in one nucleus. 22. 2 nucleate cell with 7 nucleoli in one cell and 4 in the other. 23. 2 nucleate cell with multinucleolate nucleus. 24. Uninucleate cell with multinucleolate nucleus.

Metaphase II, usually regular (Fig. 10) shows some irregularities also. In anaphase II, some chromosomes separate out earlier and reach the poles and some are cast out (Figs. 11 & 12).

DISCUSSION

Increase in chromosome number may bring out *gigas* characters. Increase in the size of flower and floral parts are noted here as observed in many other plants *e.g., Cosmos* (Newcomer, 1941), *Fagaria* (Islam, 1954), *Cucumis* (Shifriss, 1942), *Iberis* (Bali and Tandon, 1959), *Corchorus* (Datta, 1963) etc. However, 4n *Delphinium* (Mehlquist, Blodgett and Bruscia, 1943) and 4n *Portulaca* (Blakeslee, 1941) flowers are indistinguishable from 2n. In 4n *Fagaria vesca* (Islam, 1954) flowers formed earlier were larger than those formed later. In 16-ploid *Fragaria grandiflora* the subsequently formed flowers were larger than those formed earlier, although the octoploid flowers were found much larger than the largest 16-ploid ones.

Corolla length is increased. But chromosome doubling has also a reverse effect. Length decreases, when the chromosome of a polyploid is doubled *e.g., Chrysanthemum* (Emsweller and Ruttle, 1941) and *Anona squamosa* (Islam, 1960).

Larger stomata and pollen grains are found. Many workers *e.g.,* Lindstorm and Koos (1931) in tomato, Dermen (1947) in pears, Kerns and Collins (1947) in pine apple, Rife (1948) in *Coleus*, Datta (1963) in jute species etc. recorded such observations. On the contrary, Sears (1939) found no significant difference in the stomatal size in 2n and 4n wheat. Size difference in pollen grains has not been noted in *Cosmos* (Newcomer, 1941) and Pears (Dermen, 1947).

Leaves have been formed to be thicker and greener. In *Anona squamosa* (Islam, 1953), *Fragaria vesca* (Islam, 1954) and lucerne (Islam and Multani, 1953) leaves were found to be thicker contrary to what Stebbins (1942) recorded in *Stipa* tetraploids. Further, Kostoff (1938) observed proportional increase in the intensity of the green colour in the leaves of tetraploids and octoploids. He noted that this change was associated with increased depth of leaf tissue in the polyploids with a proportionate increase in the number of chloroplasts. Incidentally he did not find any appreciable change in the size of the chloroplasts in the three types of plants (2n, 4n and 8n). He also noted that the extract of chlorophyll from 8n most concentrated and that of 4n more than that of 2n. Dermen (1947) also recorded progressive intensity of green colour in the leaves of successive autoploid *Fragaria vesca* plants but according to Islam (1954) tetraploid strains of two varieties of *Fragaria vesca*

show less green colour. Progressive green contents suggest presence of more chlorophyll in these tissues.

Flower colour in 2n is pinkish but it has become much deeper in 4n. Morrison (1939) noted intensification of flower colour and also of fragrance in marigold flower following chromosome doubling.

Growth rate in height has been found to be slower in 4n. Causes may be due to altered cell volume surface ratio, slower rate of cell division or a lower rate of respiration as suggested by Noggle (1946). Smaller plant height due to slower growth rate was reported in a number of crops, *e.g.*, Jute species (Datta, 1963), tomato (Kostoff and Kendal, 1934), *Alyssium* (Jaretzky, 1928), *Nicotina* (Desmukh and Pal, 1950; Noguti, Oka and Otuka, 1940) and cabbage (Newcomer, 1941). Eigsti (1947) found a lower mitotic rate in autotetraploids as compared to the diploids. On the contrary, Levan (1942) and Kuckuck and Levan (1951) found that the tetraploid linseed types exhibited a marked increase in height over the corresponding diploids whereas the amount of increase in height was significantly lower or absent in case of flax types.

Flowering is delayed and extended in these polyploids. Cain (1944) gave a list of plants which flower later.

In *Ananas* (Kerns and Collins, 1947) and potato (Swaminathan and Howard, 1953) flowering is noted to be delayed. Datta (1963) reported that though no difference was observed in the time of flowering between 2n and 4n jute species, the flowering period of 4n's was longer by about three weeks, which may very likely be due to the poor pod setting in their cases. Such a behaviour was noted in 4n rye (Muntzing, 1951), 4n jute (Islam, 1955) and 4n *Linaria* (Bali and Tandon, 1957). Some tetraploids even flower earlier *e.g. Ribes* (Vaarama, 1947 and 1953) and Berseem (Islam and Multani, 1958).

Pollen fertility is less as observed by Datta (1963) and Bali and Tandon (1959 a, b) etc.

Fruit set is delayed and much less. Only two fruits of smaller size were obtained whereas in control 20 fruits of bigger size were found. Less fruit set may be due to cytological abnormalities. Bali and Tandon (1958 a, b) recorded cytological abnormalities and suggested as the probable causes for poor fruit and seed set in 4n *Alyssum* and 4n *Iberis*.

Fruit size is less and seed size is bigger in 4n water melon (Kihara, 1952). Such a phenomenon is noted here.

Normally 12 II's are observed here as recorded by Kojima (1925) and Janaki Ammal (1931, 1934) in diploid egg-plant. In tetraploid 12 IV to 24 II are noted. Such variations are observed by Datta (1963) in jute species. Occurrence of cytological abnormalities like unequal distribution of chromosome at anaphase, presence of laggards, formation of varying number of multivalents etc. are reported in many other induced tetraploids.

In the present cytohistological study cells become enlarged, clumping of chromosomes and formation of multinucleate and multinucleolate cells are observed. Lobing of nuclei takes place, which causes frequent increase in their number showing thereby that induction of colchiploidy has occurred. Sass and Green (1945) observed various deformities in the affected root tips and plumules of maize seedlings, which include formation of multipolar spindles, clumping of chromosomes, multinucleate cells having varying degrees of lobing, prominent specially in the marginal meristem of leaf primordia, epidermis and mesophyll of young leaves and in the plerome and periblem of root tips. Promeristem was found to be relatively unresponsive. Chromosome clumping and enlargement of cells were also noted by Dermen and Bain (1944) in cranberry. Datta (1963) observed increase in the number of nucleoli in the plumule corpus of jute species.

A certain degree of lateral expansion is observed. The condition of marked lateral cell expansion in the promoristem, plerome and periblem was in accord with observations of Hawkes (1942) who ascribed transverse expansion and lack of extensive linear elongation of cells to a loss of cell polarity.

Further work is being continued.

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