Chapter V

EFFECT OF

A. COLCHICINE
B. CHEMICAL MUTAGENS
(Diethyl Sulphate and N-Methyl -N'-Nitro-N-nitrosoguanidine)

ON *Passiflora incarnata* L.

EFFECT OF COLCHICINE ON PASSIFLORA INCARNATA

INTRODUCTION

Artificial production of polyploids by treatment with colchicine was discovered by Blakeslee and Avery (1937). Among the important publications exclusively devoted to the ornamental plants of our country, mention may be made of the contributions of Blatter and Millard (1954), Bor and Raizada (1954), Santapau (1966), Randhawa (1967) and Pal (1973). Ornamentals were remained more mobile and now appear to be cosmopolitan and not indigenous to any particular region (Khoshoo, 1976).

The present investigation deals with the study of effect of colchicine on P. incarnata.

MATERIALS AND METHODS

Passiflora incarnata cuttings of 7" length were taken from the mother plant having 2n = 18. These are treated with 0.2% aq. colchicine prepared in distilled water. Out of 25 treated cuttings, 7 of them showed visible changes and were planted in separate pots. Photographs of the morphology of diploid and tetraploid plants were taken. The number of chromosomes was counted after leaf tip mitosis, following the procedure of Kaur and Nizam (1971).

Drawings were made with the aid of Camera lucida at magnifications of X 450 to measure the number of stomata per

unit area and their size.

In the present investigation the characters studied were presented in Table 5.1.

RESULTS

Results were presented in Table 5.1. The observed morphological changes were :

1) Reduced growth (fig. 5.1).
2) Curling of the leaves (fig. 5.2).
3) Thickness of leaf increased (fig. 5.3).
4) Stomatal frequency in diploid and induced tetraploids (Fig. 5.4).
5) Morphological anomalies in treated plants (with coloured contrast) (fig. 5.5) & 5.6).

DISCUSSION

An examination of the Table 5.1, brings out clearly the morphological changes induced as a result of colchiploidy. The tetraploid is dwarf (88 cms) as compared to normal diploid (167 cms.) possessing thicker, stouter stems and are more vigorous (fig. 5.1). They have larger and thicker roots. The reduction in height of tetraploids in early stages of growth and development may be probably due to increase in the cell size with doubling of chromatin material which ultimately reduces the rate of cell division (Khostoff, 1940) as well as decrease the rate of metabolic activities. Number of branches and

Table 5.1 showing the morphological changes induced by colchicine (0.2% aq.) treatment in *P. incarnata*

Character	Diploid	Tetraploid
Height in cms.	167	88
Branches per vine	7	5
Leaves per branch	24	22
Total leaves on the plant	169	52
Leaf area (LXB) $(cm)^2$	9.42x6.8	12.2x 8.41
Number of stomata/unit area	12	10
Size of stomata (LxB)μ	15.86x7.49	21.67x12.50
Number of tendrills/branch	15	13
Total number of tendrils on the plant	60	45
Tendril length in cms.	17.0	17.4
Number of buds/branch	2	3
Total number of buds/plant	14	7
Bud, (LXB) cms.	3.4x0.6	3.8x0.8
Number of flowers/branch	2	1
Total number of flowers/plant	11	4
Calyx, (LXB) cms.	3.1x1.1	3.7x1.3
Corolla length in cms.	2.3	2.7
Ovary, (LXB) cms.	0.6x0.1	0.7x.3
Style length in cms.	0.8	1.4
Stigma length in cms.	0.4	0.6
Filament length in cms.	0.8	1.0
Anther length in cms.	1.0	1.1

Photographs showing the morphological variations due to colchicine treatment.

Fig. 5.1 Reduced growth

Fig. 5.2 Curling of the leaves

Fig. 5.3 Thickness of the leaves increased

FIG. 5.1

FIG. 5.2

FIG. 5.3

Fig. 5.4 Camera lucida drawings of the stomata of diploid and tetraploid P. incarnata.

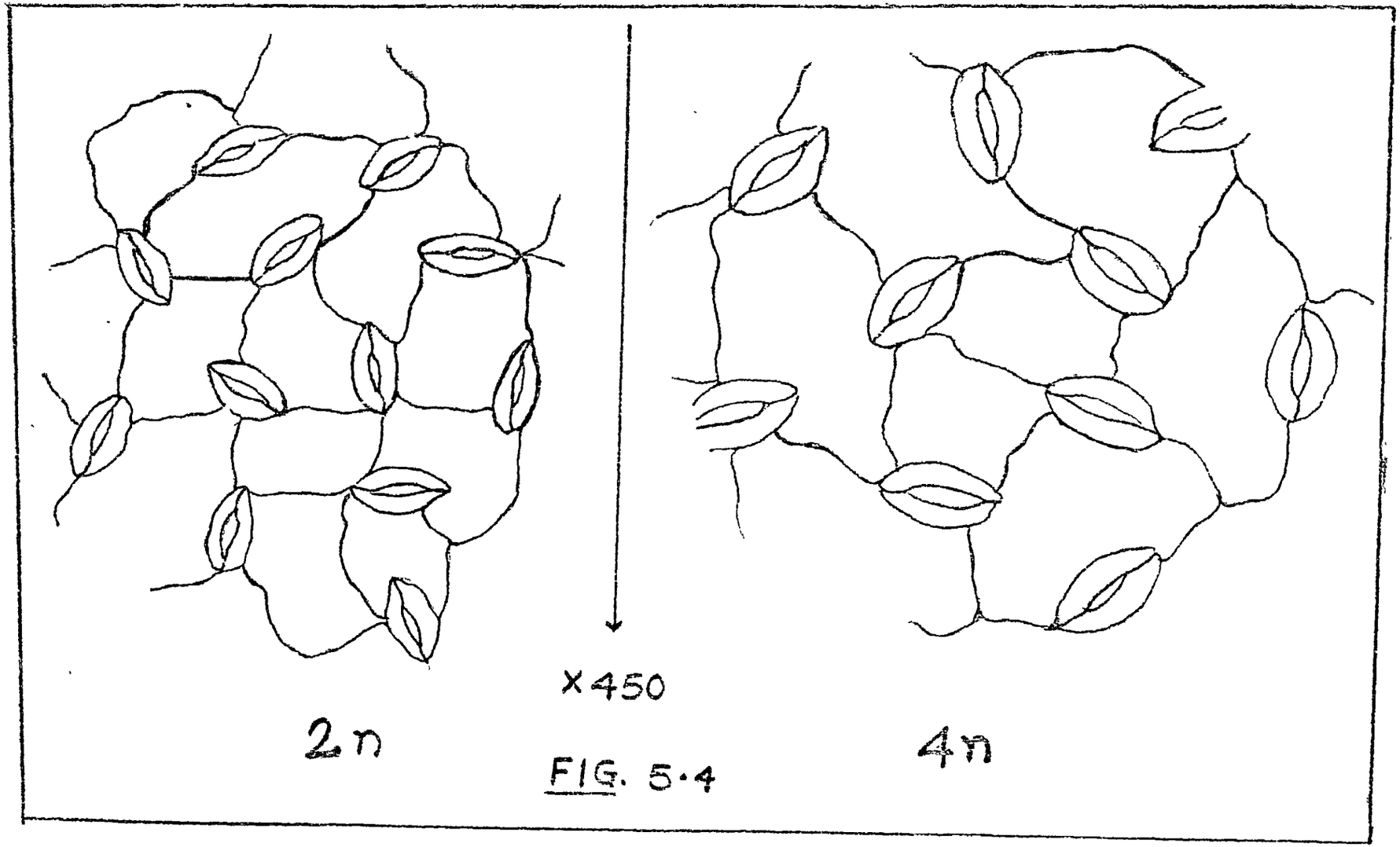

FIG. 5·4

Fig. 5.5-5.6 Morphological anomalies in colchicine (0.2%) treated plant (with coloured contrast).

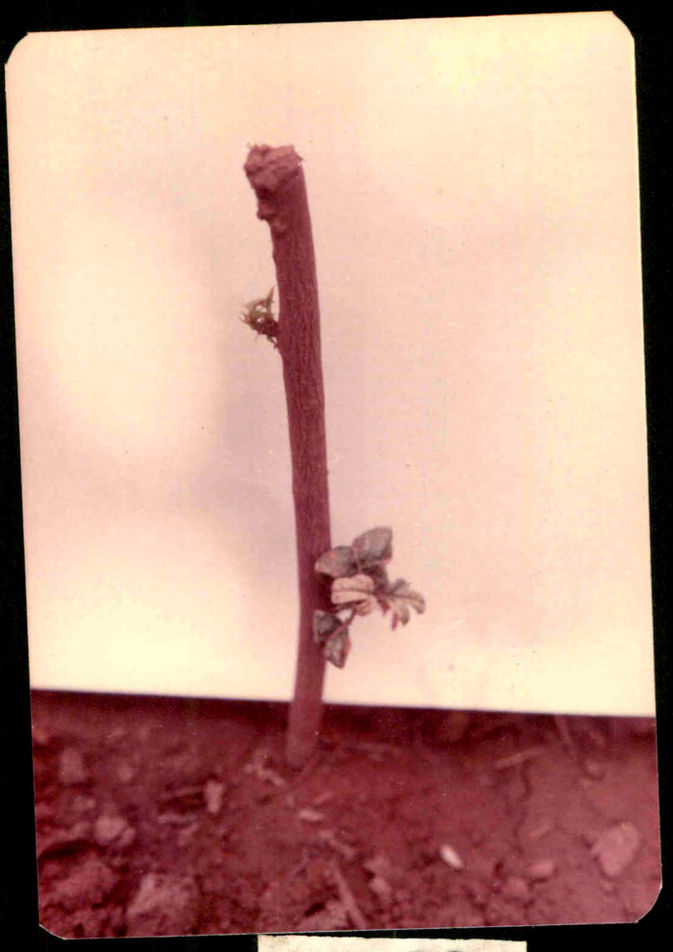

FIG. 5.5

FIG. 5.6

leaves on the tetraploid were reduced as compared to control. But it is found that the length and breadth of leaves in tetraploid (12.2 x 8.41) were higher than diploids (9.42 x 6.8). The tetraploid leaves are thick, large, and dark green. The leaves showed distortions and mosaic pattern with dark green and light green patches (fig. 5.1). The distortions of the leaves consisted of irregular margins, narrowing of the leaves and assymetrical blade development (fig. 5.2). Besides the mosaic effect, sectorial chimeral leaves could be recognized by the contrast in colour, thick texture, strong venation and somewhat greater leaf area (Table 5.1). Identification of such growth ultimately helped in recovering tetraploids. Other useful criteria were the increase in leaf serrations in thick and dark green leaves of the suspected polyploids and short thick petiole. The serrations were also more pointed and deeply cut. Polyploids was found to be due to the presence of more numerous and larger chloroplasts. Number of tendrils on tetraploid were jreduced than the corresponding diploids, but their length was increased.

The number of buds on tetraploid are half the number of buds on the diploid but their size and shape was high. The tetraploid showed delayed emergence of bud. The total number of flowers on the tetraploid are also less than the diploid. Late flowering is a common feature of induced polyploids of different plants (Randolph 1935, Chin, 1946, Tarakowski 1961, Schertz, 1962, Kazimerski and Kazimerska, 1981). In the present study the tetraploids were found to bloom late by at least 3 days. Several explainations have been offered for the late

maturity in tetraploids. The suggestion of Schwanitz (1951) that due to lower permeability accompanied by lower respiration and transpiration, the transport of manufactured food from the site of production to the growing point is upset appears to be a plausible explaination for late flowering in polyploids. Since the flower soon falls it is presumed that there is no parthenogenetic development of embryo. The cause of sterility and lack of fruit setting is found to lie in the premature disintegration of stigma; which prevents the passage of the pollen tube to reach the egg. An interesting point to note here was that the size and shape of the tetraploid organs were increased as compared to diploids (Table 5.1).

The diploid and tetraploid showed 87% and 95% aborted pollens. Induction of tetraploidy breaks the self-incompatibility system (Pandey, 1968). But Pickering (1980) was of the opinion that the self incompatibility remaining as it is or increases due to induction of tetraploids. The present investigation holds good with Pickering's view.

In suspected tetraploid leaves, both the length and breadth of guard cells were greater than those of the diploids, but the number of stomata per unit area decreased considerably in tetraploid leaves (Table 5.1). Morphological change, chromosome count, pollen characters and sterility are the most reliable criteria for the detection of polyploidy than increase in stomatal size (Bali and Tandon, 1957; Francis and Bemis, 1974).

In general induced and spontaneous tetraploids have been found to be characterized by an increase in cell size which may

be reflected in an increase in the size of plant parts, this is so especially for organ with determinate growth (Stebbins, 1950). P. incarnata is not exception to this generalization from present study. Stebbins (1950) believes that sterility is mainly due to some genetically controlled physiological factor of an unknown nature.

EFFECT OF CHEMICAL MUTAGENS DIETHYL SULPHATE AND N-METHYL-N'-NITRO-N-NITROSOGUANIDINE

INTRODUCTION

Historically ornamentals have always been associated with all advanced civilizations of the World (Khoshoo, 1968). Except for the work of a few persons notably Pal (1966) and Pal and Krishnamurthi (1967) exotic as well as indigenous ornamentals have not received the deserved genetic attention in India.

Few studies have been made on the morphological changes due to the chemical mutagen MNNG (Levy and Ashri, 1973). Chemical mutagenesis is assuming increasing importance as a useful tool in mutation breeding. The use of chemically induced mutations in higher plants has been receiving increasing attention (Brock, 1971, Micke, 1975). Mutants are being utilized in genetic and evolutionary studies (Smith, 1971, Singh and Drolsom, 1973, and Conger *et al.* 1976).

MATERIALS AND METHODS

The *P. incarnata* cuttings were treated with various concentrations of DES viz. 0.005 M, 0.01M, and 0.02 M. After 3 months the plants showing visible changes were planted in separate pots.

The cuttings of *P. incarnata* were treated in freshly prepared solution of MNNG (20 mg/l) dissolved in deionized water for 12, 24, 48 and 72 hours. The low concentration of MNNG was

adopted because of its low solubility in water. In longer MNNG treatments, the solution was changed every 24 hours because of its fairly rapid hydrolysis. Its half life at pH 6.15 is 990 min. and at pH 7.85 is 48 min. (Lawley, 1968). The pH of our deionized water was 6.4 and the temperature was considerably lower (18-20°c.). Hence the hydrolysis rate was reduced. After treatments, the cuttings were rinsed in running water and planted in the field. After 3 months the plants showing the visible changes were planted in separate pots. Photographs of the plants showing visible morphological changes were taken.

In the present investigation the characters studied were tabulated in Table 5.2. Stomatal drawings were made at X450 with the help of camera lucida.

RESULTS

Results of effects of DES and MNNG on P. incarnata are presented in Table 5.2.

The observed morphological variations due to DES and MNNG treatments have been shown in figures 5.7 to 5.11 and 5.12 to 5.17 respectively.

DISCUSSION

In case of the height of mutants it was found that the mutants were dwarf with condensed internodes (31-127 cms) as compared to control (167 cms) for both the DES and MNNG treatments. It was found that here the DES treatment is more damaging

Fig. 5.7-5.10 Photographs showing the morphological variations due to various concentrations of DES treatment on *P. incarnata*.

FIG. 5.7

FIG. 5.8

Fig. 5.11 Camera lucida drawings of the stomata of *P. incarnata* treated with various concentrations of DES.

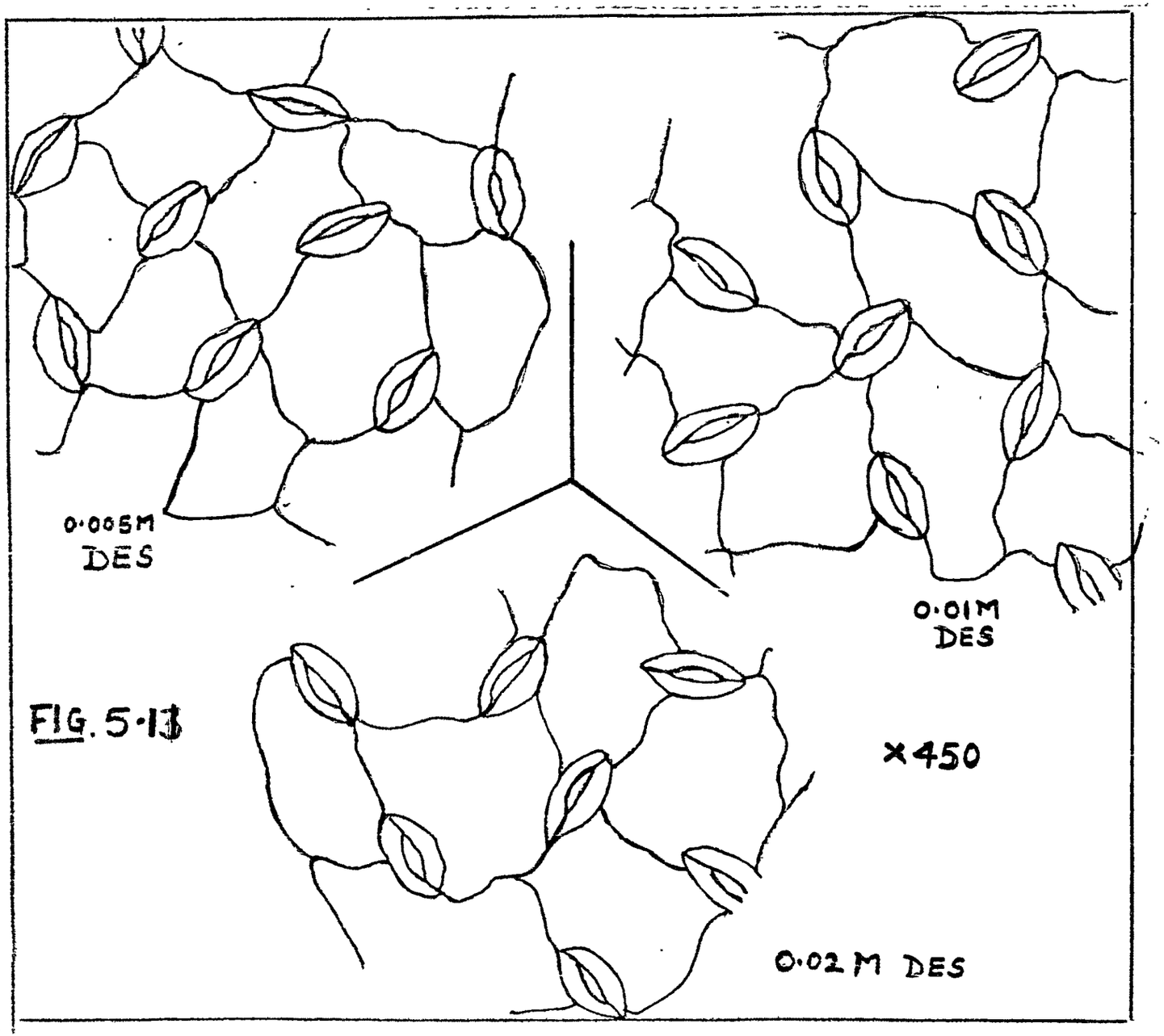

FIG. 5·13

Fig. 5.12-5.16 Photographs showing the morphological variations due to various durations of MNNG treatment on P. incarnata. (C = control, 12 h = 12 h MNNG treatment etc.)

FIG. 5.12 C

FIG. 5.13 12h

FIG. 5.14 24h

FIG. 5.15 48h

48h

FIG. 5.16 72h

Fig. 5.17 Camera lucida drawings of the stomata of P. incarnata treated with various durations of MNNG.

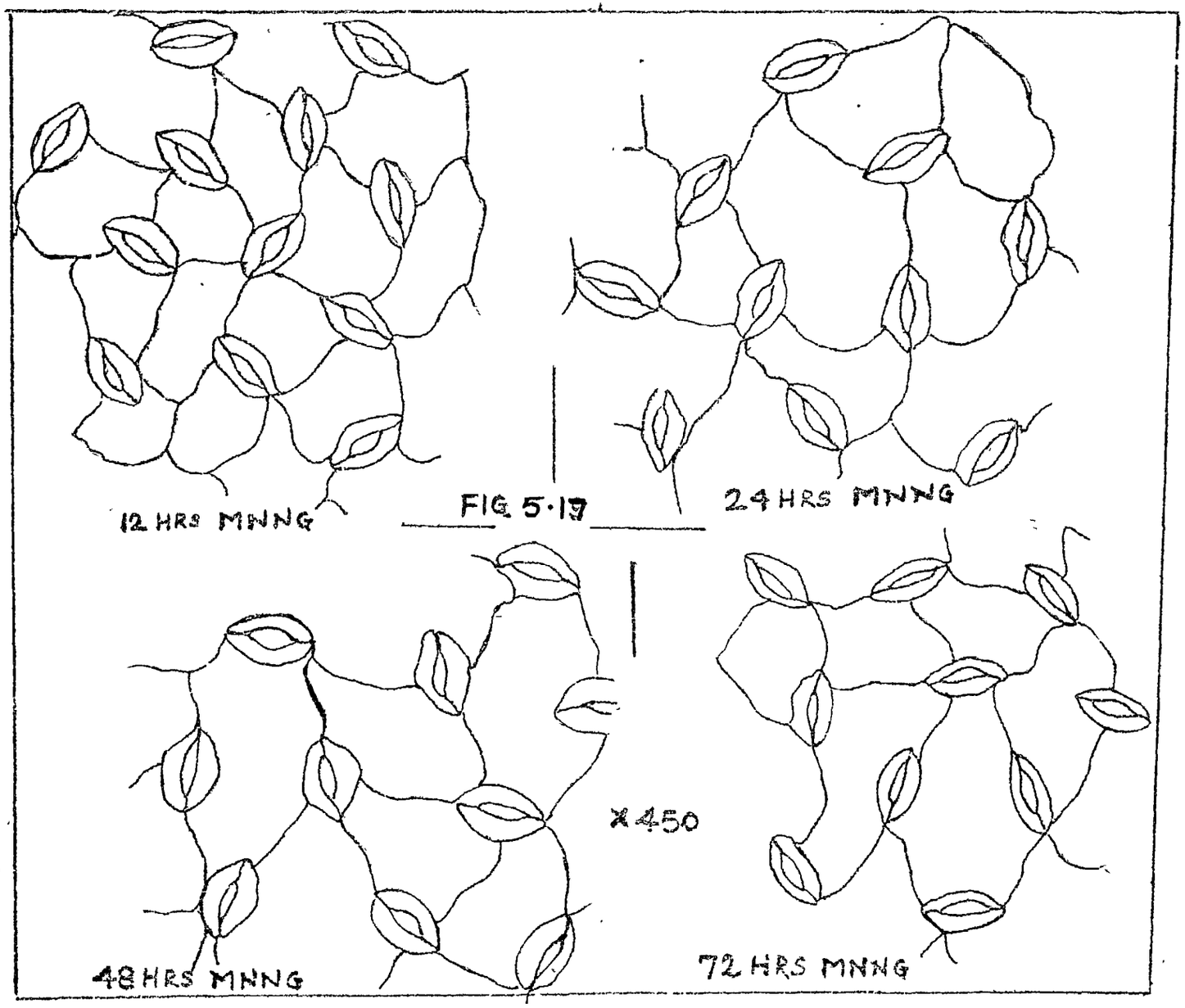

FIG 5·19

than MNNG (Table 5.2). Leaf area is increased for MNNG treatment than DES as compared to control due to considerable amount of cell enlargement. In the present investigation as the duration of MNNG treatment was increased the size of the leaves decreased considerably but for DES treatment the mutant showed variation in respect of size and shape of leaves. The mutant showed changed leaf shape from oblong to trifid. The probable mechanism responsible for the alteration of leaf blade from normal oblong to trifid shape can easily be interpreted on the basis of the arrangement of veins. Although mutant shows the changes in leaf shape they are of minor interest in the study of evolution of leaf shape as these genes were found to be responsible only for increasing the genetically conditioned leaf diversity within the species (Joshnu and Rao, 1972). Leaf character has been considered as the most drastically affected one in mutation research (Singh, 1974).

The number of tendrils on the mutants were increased except for 0.005 M DES and 72 h MNNG treatments than the control. The length of tendril was increased only for 12 h and 48 h MNNG treatments as compared to control. The morphology of the tendril in Passiflora has been contraversial and is variously interpreted (Masters, 1879; Hagerup, 1930, Gangastad 1938, Rendle 1952; Lawrence, 1960).

Number of buds on the mutants were decreased as compared to the control for both DES and MNNG, but flower buds could not be traced for MNNG treatment beyond 48 hours. The length and

breadth of the buds remain somewhat uniform for both the DES and MNNG treatments. In the present study both the DES and MNNG treated mutants showed delayed emergence of bud. The mutants were found to bloom late by 3 days for DES treatment and 2 days for MNNG treatment as compared to control. Rather a continuous flowering was observed for MNNG treatment than DES as compared to control. After the maturation of flowers the abscission takes place early in DES treatment than MNNG. The colour of the flowers were found to be deep blue for MNNG treated mutants as against faint blue for DES treated mutants as compared to control. Several workers working on mutation have reported proportionally higher number of variations in flower colour intensities rather than total colour change (Broertjes and Harten, 1978). Mutagen effectiveness has been explored to uncover the action mechanisms of mutagens' and to establish efficient methods of inducing mutations in plants (Auerbach 1967; Drake 1969; Nilan 1969).

There were not much difference in the size of the floral organs - calyx, corolla, style, stigma, filament, and anther for both the DES and MNNG treated mutants, except the ovary in case of DES treated mutants (0.01M and 0.02 M DES) comparable to control.

In control plant the number of stomata per unit area was 12 which was equal to 12 h MNNG treated mutant. For other mutants it was decreased proportionally as the DES concentration was increased and duration of MNNG treatment was increased (Table 5.2). The size of the stomata was increased for both DES and MNNG treatment. Some mutants (especially 48 h MNNG treated)

exhibited an alternation of the morphology of the whole plant, probably due to pleiotropic action of the mutated gene.