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EFFECT OF TREATMENTS OF COLCHICINE, DIETHYL SULPHATE AND TRIETHYLAMINE IN TOMATO (*LYCOPERSICON ESCULENTUM* MILL.)

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ABSTRACT

The present investigation was undertaken in one variety of Tomato, *Best of All*, to compare the cytogenetic effects of treatments of seeds of tomato with colchicine, diethyl sulphate and triethylamine and also to compare the morphological and physiological effects of these chemicals.

Lowest number of seeds germinated from diethyl sulphate treatment. Plants originating from colchicine, diethyl sulphate and triethylamine treatments showed many variant types. Interesting among these were those showing spotting on leaves originating from diethyl sulphate treatment, dwarf ones originating from colchicine and diethyl sulphate treatments and those with abnormal leaves originating from treatment with all the three chemicals.

Mitotic irregularities included clumped chromosomes and fragments at metaphase and bridges and fragments at anaphase while in meiosis clumped chromosomes, univalents, trivalents and quadrivalents were noted at metaphase I. Anaphase I irregularities included cells with fragments, bridges with or without fragments, lagging chromosomes and unequal separation of chromosomes. In metaphase II cells with aneuploid chromosome numbers and those with fragments were noticed while in anaphase II cells were found with fragments and unequal separation of chromosomes.

The importance of detecting interesting mutants in the M_2 generation and the importance of finding translocations and inversions have been pointed out.

INTRODUCTION

In this preliminary investigation an attempt has been made to compare the cytogenetic effects of treatments of seeds of tomato with colchicine, diethyl sulphate and triethylamine and also to compare the morphological and physiological effects of these chemicals.

As regards the previous work with these chemicals it may be pointed out that colchicine is well known as a *polyplodizing* agent (*Eigsti & Dustin*, 1957) and the mutagenic action of diethyl sulphate was for the first time observed by Heiner *et al.*, (1960) in barley, where they also observed that it induced a remarkably high frequency of mutation and a negligible frequency of gross chromosomal aberrations and the pattern of mutation types were also different from diethyl sulphate treatment in comparison with gamma rays. Later, the favourable reports on the mutagenic efficiency of diethyl sulphate was confirmed by the works of Konzak *et al.*, (1961) in barley and Ashri and Goldin (1965) in peanuts. So far as triethylamine is concerned, Lüers and Röhrbom (1963) observed that tri-functional ethylamines were more mutagenic than bi-functional ones and these gave a higher frequency of mutations than the monofunctional ethylamines.

MATERIALS AND METHODS

Seeds of one variety of tomato, *Best of All*, obtained from Sutton and Sons, Calcutta, were utilized for the present investigation.

Dry seeds were given 24 hours treatment with (i) distilled water, (ii) 0.2% solution of colchicine, (iii) saturated solution of diethyl sulphate and (iv) 0.5% solution of triethylamine. After the treatments were over, colchicine, diethyl sulphate and triethylamine treated seeds were thoroughly washed in distilled water and were sown in pots as also the control ones. Seedlings were transplanted in the field a month after sowing.

Because of high percentage of mortality at the seedling stage and the lack of adequate field facilities, only a limited number of plants were available for field studies.

In order to study the mitotic chromosomes, colchicine, diethyl sulphate and triethylamine treated seeds as well as those from distilled water treated ones were allowed to germinate on blotting papers in petridishes. Suitable roottips were pretreated with saturated solution of paradichlorobenzene for 2 hours. After this treatment they were fixed in acetic acid: absolute alcohol (1 : 3) for about 24 hours and then preserved in 70% alcohol until use. Roottips were treated with 9 : 1 acid-dye mixture (9 c.c. of 2% aceto-orcein : 1 c.c. N/HCl) and were squashed and stained in 2% aceto-orcein.

For meiotic studies suitable flower buds were fixed in acetic acid : absolute alcohol (1 : 3) for about 24 hours and were then preserved in 70% alcohol until use. Pollen mother cells were stained in 2% aceto-carmine for studying the chromosomes.

Pollen grains were stained in 1% aceto-carmine to score sterility.

OBSERVATIONS

It can be observed from Table I that lowest number of seeds germinated from diethyl sulphate treatment. Number of branches per plant did not show much difference among the treatments but those originating from distilled water treatment were earliest to flower while those originating from diethyl sulphate treatment took the longest time to flower where fruiting also took the longest time. Pollen sterility was, however, highest in those originating from triethylamine treatment. Number of fruit plants was highest in those originating from distilled water treatment while the number of seeds per fruit was highest in those originating from triethylamine treatment and lowest in those originating from diethyl sulphate treatment.

Plants originating from colchicine, diethyl sulphate and triethylamine treatments showed many variant types. Interesting among these were those showing spotting on leaves originating from diethyl sulphate treatment, dwarf plants originating from colchicine and diethyl sulphate treatments and other variant types listed in Table II.

Roottip mitosis showed cells with fragments, clumped chromosomes, those with increased chromosome numbers (from colchicine treatment), at metaphase, and fragments, bridges and irregular grouping of chromosomes at anaphase originating from treatment with the chemicals.

In meiosis, metaphase I irregularities included cells with fragments, univalents, trivalents, quadrivalents and those with clumped chromosomes while in anaphase I lagging chromosomes, cells with fragments, bridges with or without fragments an unequal separation of chromosomes were noticed. In metaphase II, cells with aneuploid chromosome number and those with fragments were seen. Other cells were found with clumped chromosomes. In anaphase II cells were found with fragments and unequal separation of chromosomes.

DISCUSSION

One interesting thing observed was the presence of yellow spots on leaves in plants originating from diethyl sulphate treatment. This has been reported earlier by Blixt (1965) in his work with ethyl methane sulphonate in peas. As regards the finding of minute and dwarf plants and those with retarded growth and others with bushy habit, it can be said that Gunckel and Sparrow (1954) commented that these resulted from induced physiological and biochemical disturbances. Evans and Sparrow (1961) hypothesized that radiation induced growth inhibition was due to genetic loss resulting from chromosomal aberrations.

Fasciation of floral organs has also been observed in tomato by Bose and Banerjee (1968a) after treatment with X-rays and colchicine and by Saha (1968) after treatment with physical and chemical mutagens. White (1948) while reviewing on fasciation commented that it has been taken to be due to increase in the number of growing points and their subsequent fusion by some while others have taken it to be due to flattening or enlargement of one growing point. The lack of finding any polyploid plant may be due to the fact that the treatment with colchicine was for 24 hours only, since Chowdhury (1955) observed that the best result for the induction of polyploidy in tomato by seed treatment was pre-soaking the seeds in water for 24 hours and then immersing in colchicine (0.2%) for 6 to 8 days.

Mitotic aberrations observed from roottip cells, like fragments and anaphase bridges detected from the treated series, were of the same type as observed by Yagyu and Morris (1957) after X-ray and neutron irradiated tomato seeds and recently by Bose and Banerjee (1968a) after treatment of tomato seeds with X-rays and colchicine. The bridges were attributed by Yagyu and Morris (*l.c.*) to be resulting from the union of centric segments of broken chromosomes which appeared usually in pairs. They also found single bridges and added that these bridges and fragments resulted from original breakage and reunion. Gaul (1964) observed that bridge formation at mitotic anaphase resulted from the fusion of two centromeric bearing fragments.

Typical meiotic aberrations like univalents, trivalents and quadrivalents at metaphase I, lagging chromosomes and unequal separation at anaphase I and bridges with or without fragments, also at this stage, was observed. These types of aberrations were noted earlier by Lesley and Lesley (1956) and more recently by Bose and Banerjee (1968a; b) and Saha (1968). Lesley and Lesley (*l.c.*) noted translocations to be of more frequent occurrence than inversions while the fragments were of rare occurrence. Earlier, Gottschalk (1951) and Barton (1954) noted many translocations originating after X-ray treatment of tomato pollen and pollen mother cells. The quadrivalents must have resulted from reciprocal

TABLE I

Plant growth, flowering and fruiting in Tomato (L. esculentum Mill.*) following treatment with colchicine, diethyl sulphate and triethylamine*

Observations		Treatments			
		DW*	Colchicine**	DES***	TEM****
No. of seeds per treatment		75	75	75	75
No. of seeds germinated		65	59	56	58
% of germination		86.66	78.00	74.00	77.33
No. of seedlings transplanted		12	15	18	20
No. of plants surviving till maturity		12	13	14	18
No. of branches per plant	a	6.36±0.60	6.20±0.87	5.19±0.51	6.63±1.90
	b	(2—10)	(0—7)	(1—9)	(1—11)
No. of flowers per plant	a	17.73±0.92	18.88±1.20	18.01±1.56	21.68±1.10
	b	(6—29)	(0—27)	(3—31)	(1—42)
% of pollen sterility		12.65	15.29	17.93	21.24
First date of flowering (in days)	a	119.98±9.18	133.56±6.29	148.32±2.57	145.05±5.89
	b	(108—138)	(130—149)	(141—160)	(139—154)
1st date of fruiting (in days)	a	139.04±7.29	149.21±7.12	161.13±2.90	154.11±3.77
	b	(127—149)	(141—162)	(150—173)	(142—165)
No. of fruits per plant	a	15.64±5.30	12.72±10.41	11.07±3.95	12.32±5.82
	b	(5—24)	(0—20)	(3—18)	(0—19)
No. of seeds per fruit	a	110.23±13.16	106.41±14.62	82.20±8.55	113.94±15.01
	b	(41—167)	(47—160)	(29—94)	(32—168)

DW* = Distilled water; Colchicine** = (Colchicine 0.2% soln.);
DES*** = (Diethyl sulphate, saturated soln.); TEM**** = (Triethylamine, 0.5% soln.);
a = Mean±S.E.; b = Range.

TABLE II

Frequency and types of variants observed following treatment with colchicine, diethyl sulphate and triethylamine

Treatments	No. of plants observed	No. of variants	Frequency	Description of the variants
DW	12	—	—	— — —
Colchicine (0.2% soln.)	13	6	2	Dwarf plant with rolled leaves and prominent pinkish vein, sterile.
			2	Unbranched, leaves with virus like mottling, decreased number of floral organs.
			1	Bushy plant with rough, small, deep green leaves with fasciated floral organs and flattened gynoecium.
Diethyl sulphate (Saturated soln.)	14	5	3	Slender stemmed and poorly branched plant. Some of the leaves showing yellow spots.
			2	Dwarf plant with increased number of floral organs.
Triethylamine (0.5% soln.)	18	8	3	Plants with minute terminal branching. Leaves rough, completely rolled inwards with few flowers.
			3	Plants with slender stems with deeply lobed leaves and big-sized flower buds.
			2	Large flattened gynoecium with increased number of floral organs.

translocation. The importance of finding translocations in cytogenetic analysis and breeding work has been stressed by Hagberg (1959) and Hagberg and Akerberg (1961) who also emphasized their importance with special reference to cytogenetic analysis of mutants and directed mutation breeding.

Darlington and Upcott (1940) and Rees (1952) explained the formation of bridges without fragments to be resulting from failure of division of end genes brought about by nucleic acid upset. Rees (*l.c.*) took fragmentation to be resulting from severing by cell wall formation of loop. Bridges with fragments, however, were due to the presence of heterozygous inversions (*Darlington*, 1937; *Swanson*, 1957). Walters (1950) while expressing the same opinion pointed out that, besides inversions, bridges and fragments could also result from breakage and reunion of chromosomes. The absence of fragments in most of the cells where bridges were found may also be due to smallness of the fragments and their disappearance in earlier divisions or their origin from chromatic portions. This opinion was expressed by Bora *et al.*, (1961) in their studies on X-ray and neutron induced meiotic abnormalities in *Arachis hypogoea* and *Plantago ovata*. Bose (1968) also expressed the same opinion in his studies on *Setcreasea brevifolia*. The second division bridges could be resulting from breakage and reunion as also from inversion crossing over.

The above study has shown the origin of variant types after treatment with the chemicals and has also revealed the presence of those with translocations and inversions in the M_1 generations. It would be desirable to continue this study in the M_2 generation for the detection and isolation of desirable mutants, like those showing male sterility, the importance of which has been pointed out by Rick and Robinson (*l.c.*) and Verkerk (1959). Cytogenetic studies, especially in those having translocations, could be of immense practical importance for the isolation of induced translocated types (*Elliott*, 1958; *Hagberg*, 1959; *Hagberg and Akerberg*, 1961 and *Bose and Banerjee*, 1968a).

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