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Effects of Nitrogen mustard on somatic cells (root tips) of *Phaseolus radiatus* L.*

by

MASIHULLAH KHAN, A. SHIVRAJ and B. V. RAMANA RAO**

Synopsis: The effects of Nitrogen mustard (HN_2) on somatic cells (root tips) of *Phaseolus radiatus* L. are reported in this paper. The most prominent effect of nitrogen mustard was the induction of stickiness leading to the formation of bridges at anaphase. Occurrence of persisting bridges, probably due to specific action of the chemical on a chromosome, was recorded.

Introduction: Since the discovery of the mutagenic action of mustard gas and allied compounds by Auerbach and Robson in 1943, on Drosophila, attempts were made by a number of workers on various crop plants like Onion, Maize, Wheat, Barley and Rice to isolate beneficial mutants. Many chemicals are now being added to the list of chemical mutagens. Of all these chemicals nitrogen mustard and its derivatives were found to be the most effective. They are of importance because of their action on specific regions of the chromosome. Present study was undertaken to determine the exact concentration of the chemical capable of inducing mutations in green gram.

Material and Method: A bold seeded variety of green gram of the Maharashtra State, China Mung 781, was selected and nitrogen mustard i.e. dichloroethyl-methyl-amine hydrochloride of Boots Pure Drug Co. (bis-beta-chloroethyl amine Hcl) was used in the experiment.

Seeds were disinfected with 0·1% mercuric chloride solution before treatment for 2 minutes. They were treated when the primary roots were 0·7 cm. long. Fresh solutions of Nitrogen mustard were prepared for every treatment. Four concentrations were used viz., 0·00005%, 0·0001%, 0·0005%, 0·001% and the duration was 5, 10 and 15 minutes.

The following technique was adopted for the preparation of the slides.

1. Root tips were fixed after 4, 8 and 24 hours of treatment in 1:3 acetic alcohol for 24 hours and washed in 70% alcohol and then in water.
2. Hardening of the cells was done by treating the root tips with 10% formallin for 6 hours.
3. Root tips were directly transferred to 4% NaOH for 12 hours to macerate the cells.
4. They were washed well in water several times and were immersed in 10% acetic acid to eliminate all remaining traces of alkali.
5. Finally they were stored in 70% alcohol for future use.

Slides were prepared using aceto-carmine stain and they were examined for various aberrations before they were made permanent. Camera lucida drawings were made under oil immersion using 100 × objective and 6 × eye piece.

** Department of Botany, College of Agriculture, Osmania University, Hyderabad.

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Results and Discussion: 1. *Morphological effects:* Stunted root growth and earlier differentiation of vascular tissue was observed in all the treatments. This may be due to the action of nitrogen mustard on prosthetic groups of important enzyme systems as considered by Goodman and Gillman (1956). In wheat Bhaduri *et al.* (1958) reported the premature differentiation of vascular bundles following nitrogen mustard treatment.

2. *Cytological effects:* There was an increase in the size of the nucleus and the nucleolus with the increasing doses. Size of the nucleus varied from 43·27 μ to 51·75 μ while that of nucleolus from 17·99 μ to 23·98 μ. The size of the nucleus and nucleolus in control was 40·50 μ and 16·87 μ respectively.

(a) *Laggards:* Laggards were found in all the treatments (Table 1, Fig. 1). Since no laggards were observed in the control it suggests that the chemical might have affected the centromeres thereby detaching the chromosome from the spindle fibres.

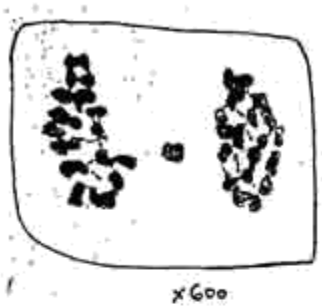
(b) *Stickiness and bridges:* Marked stickiness in chromosomes was found in all the treatments. At metaphase chromosomes looked melted and got attached to each other (Fig. 2). Bridges resulted due to non-separation of such chromosomes (Fig. 3). Butler *et al.* (1948) revealed depolymerisation of DNA, *in vitro* with nitrogen mustard causing stickiness. Severe stickiness in case of wheat also was reported by Bhaduri *et al.* (1958) following nitrogen mustard treatment resulting in the formation of bridges.

In addition to this, six cells showed persisting bridges in 0·0001 of the chemical even upto next metaphase of the daughter cells (Fig. 4). This may be due to specific action of the chemical on the chromosome as suggested by (Auerbach 1946, Bhaduri 1958). However, the occurrence of such persisting bridges is very interesting.

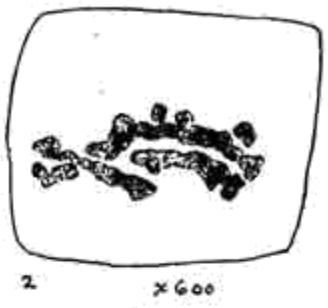
TABLE 1

Cytological effects of Nitrogen mustard.

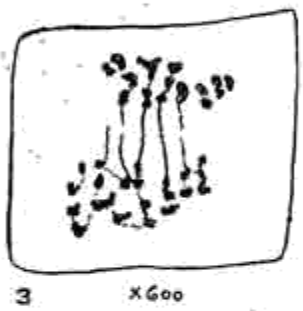
S. No.	Treatment		Total No. of cells found at anaphase	No. of cells showing laggards	No. of cells showing stickiness	No. of cells showing bridges	Percentage of cells showing abnormalities
	Dose	Duration					
1.	Control		171	0	0	0	
2.	·00005	5 mts.	120	3	9	15	22·5
3.	·00005	10 ,,	209	4	1	11	7·6
4.	·00005	15 ,,	224	1	2	11	6·2
5.	0001	5 ,,	204	4	1	41	22·5
6.	·0001	10 ,,	191	1	3	34	19·8
7.	·0001	15 ,,	353	2	4	67	20·6
8.	·0005	5 ,,	208	1	...	20	10·0
9.	·0005	10 ,,	119	1	2	28	26·0
10.	·0005	15 ,,	183	3	2	22	14·9
11.	·001	5 ,,	214	2	2	22	12·1
12.	·001	10 ,,	89	2	...	15	19·1
13.	·001	15 ,,	111	2	1	9	9·9



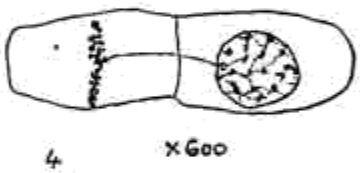
Cell showing a laggard chromosome.



Cell showing severe stickiness of chromosomes at metaphase.



Anaphase with continuous and broken bridges.



Persisting bridge even after cell division.

(c) *Dosage and duration of treatment:* During the present study it was found that the green gram is very sensitive to HN_2. The effective dose of the chemical varies from species to species. In case of maize even low concentrations (0·0005% to 0·001%) gave positive results (Bhaduri *et al.* 1958). Novick *et al* (1949) used HN_2 of 0·001% and 0·0005% in case of onion. It was found that 0·0005% was not effective whereas 0·001% for 30 minutes produced marked effects. Bhaduri *et al* (1958) used 0·00001% to 0·01% for wheat and the duration was 15 minutes with lower concentration to 7 minutes with higher concentrations. They found the lower concentrations with shorter duration of treatment were sufficient for inducing mutations in wheat and they are of the opinion that the nascent volatile amine produced as a result of the hydrolysis of the salt in solution is at once effective irrespective of the concentration.

In the present study it appears that the low concentrations are more effective in inducing mutations in green gram, because of the occurrence of more number of chromosomal abnormalities in 0·0005% for 10 minutes duration.

Summary: The effects of HN_2 on somatic cells (root tips) of *Phaseolus radiatus* L. were studied. The concentrations used were 0·00005%, 0·0001%, 0·0005% and 0·001% and the duration was 5, 10 and 15 minutes for each dose.

The effective concentration of the chemical in inducing chromosomal abnormalities was found to be 0·0005% with 10 minutes duration. The most prominent effect of nitrogen mustard was the induction of stickiness leading to the formation of bridges at anaphase. Occurrence of persisting bridges, probably due to specific action of the chemical on a chromosome, was recorded.

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