Theoretical and Applied Genetics 41, 18–20 (1971)

The Effect of Gibberellic Acid on Chromosomal Aberrations in *EMS* and *MMS* Treated *Pisum sativum* Linn.

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Summary. The present experiments revealed a reduction in seedling height and damage at chromosomal level in *Pisum* treated with *EMS* and *MMS*. Further it was observed that GA_3 post-mutagen treatment could reduce this damage considerably by reducing the number of aberrations as well as chromosomal translocations. The GA_3 treatment also increased seedling height as well as pollen fertility.

It is known that duplications as well as translocations do provide an important source of genetic variability which may yield plants of useful types and which could be used further for breeding of desirable plants.

A large number of reciprocal translocations has been induced by various mutagens. Reciprocal translocations are by far the most common structural rearrangement found and are easily transferred to later generations. They are not only of theoretical interest but are also of practical application. Hagberg (1964) and Konzak *et al.* (1964) have indicated the possibilities of usefully employing reciprocal translocations in breeding programmes. Lamm and Miravalle (1959) have described a tester set of translocations in *Pisum*.

The present experiments were undertaken to induce duplications and translocations in *Pisum* using *EMS* and *MMS* as well as to see if gibberellic acid (GA_3) treatment had any effect in modifying production of these duplications and translocations.

Material and Methods

Pea seeds of the variety 'Bounville' were presoaked in water for two hours and then treated with one dose each of *EMS* (0.25%) and *MMS* (0.025%) for ten hours. The solutions of mutagens were prepared in phosphate buffer adjusted to pH 7.0. At the end of the 10-hour period, the seeds were washed in distilled water for 10 minutes and resoaked in a 1000 ppm solution of gibberellic acid (GA_3) for 8 hours. At the end of the treatment the seeds were again washed with distilled water. Four replications, each of 25 treated seeds were sown in wooden flats for recording seedling height. Another set of 100 seeds was sown in the field in 4 plots. The seedling height was recorded after 10 days. For mitotic studies, 25 treated seeds were placed in a petri dish lined with wet filter paper and the root tips were collected and fixed from the germinating seeds. The slides for mitotic studies were prepared by hydrolysing the root tips with a mixture of 9 parts of 2% aceto orcein and 1 part of *N HCL* and then staining with 1% aceto orcein.

For meiotic studies, the floral buds were fixed in Carnoy's fixative (6 parts ethyl alcohol, 3 parts chloroform and 1 part acetic acid) and squashed in 1% aceto carmine stain.

Results and Discussion

The data presented in Table 1 show that there was a reduction in seedling height in *EMS*- and *MMS*-treated plants. However, this damage was neutralised when the seeds were given post-mutagen treatment of 1000 ppm GA_3. The effect of GA_3 was much more evident in *EMS*-treated material than in the *MMS*. In *EMS*-treated material the increase in height was quite significant and even surpassed the control by about 25 per cent. The increase in height in *MMS* treatment was more or less equal to that of the control.

Gaur and Notani (1960) using maize, Haber and Luippold (1960) with wheat and Mathur (1961) with potato have reported the modification of physiological damage by mutagens through post-treatment with gibberellic acid. Kumar (1967) has reported that GA_3 post-mutagen treatment could reduce the cytological damage in gamma-irradiated barley.

Table 1. *Seedling injury and mitotic studies*

Mutagenic treatment	*Seedling height (cms.)	Mitotic studies×			
		bridges	rods	dots	% aberrations per cell
Control	5.16	Nil	Nil	Nil	0.00
EMS 0.25%	3.40	49	14	27	0.45
EMS 0.25% + GA 1000 ppm	6.80	35	17	7	0.30
MMS 0.025%	3.00	48	25	33	0.53
MMS 0.025% + *GA*	5.00	46	4	32	0.41

* Seedling height as recorded after 10 days
× No. of cells scored — 200

Table 2. *Meiotic studies and pollen fertility*

Treatment	No. of cells analysed	Metaphase anomalies like rings, chains, dots fragments, etc.	Anaphase and telophase anomalies like bridges, laggards, dots, rods, fragments, etc.	Total -aberrant cells	% aberration per cell	% age fertile pollen grain
EMS 0.25%	260	46	25	71	0.27	67
EMS 0.25% + *GA* 1000 ppm	232	19	27	46	0.20	73
MMS 0.25%	278	42	41	83	0.30	62
MMS 0.025% + *GA*	196	22	17	39	0.20	74

Table 3. *Metaphase I and II meiotic anomalies*

Treatment	No. of cells analysed	Rings				Chains				Fragments, dots, rods, etc.	Total No. of cells showing anomalies	% aberration per cell
		Rings of 3 chromosomes	Rings of 4 chromosomes	Rings of 6 chromosomes	Total No. of rings	Chain of 2 chromosomes	Chain of 3 chromosomes	Chain of 4 chromosomes	Total no. of chains.			
EMS 0.25%	260	5	9	3	17	11	1	3	15	14	46	0.18
EMS 0.25% + *GA* 1000 ppm	232	1	1	—	2	1	2	—	3	14	19	0.08
MMS 0.025%	278	10	7	—	17	7	3	1	11	14	42	0.15
MMS 0.025% + *GA* 1000 ppm	196	1	1	1	3	2	—	1	3	16	22	0.11

Table 4. *Anaphase I and II meiotic anomalies*

Treatment	No. of cells analysed	Bridges	Laggards, rods, dots, etc.	Total	%age aberrations per cell
EMS 0.25%	260	23	2	25	0.09
EMS 0.25% + *GA* 1000 ppm	232	19	8	27	0.12
MMS 0.025%	278	35	6	41	0.15
MMS 0.025% + *GA*	196	14	3	17	0.09

The mitotic studies (Table 1) show that GA_3 can effectively modify the cytological damage caused by chemical mutagens in the same way as it does with physical mutagens (Kumar, 1967). However, the response to GA_3 treatment was variable. A marked reduction in the number of bridges and dots was observed in *EMS* + GA_3-treated material. The number of rods was reduced after treatment with *MMS* + GA_3 but not after treatment with *EMS* + GA_3.

In the meiotic studies (Tables 2, 3 and 4), GA_3 had more effect on the metaphase than on the anaphase in *EMS*-treated material. The rings and chains were reduced whereas there was no effect on dots and rods. These results corroborate the evidence obtained in the mitotic studies.

In *MMS*-treated material, GA_3 treatment had a marked effect both at the metaphase and anaphase stages, the aberrations per cell decreasing considerably at both stages.

From these results it is evident that GA_3 is effective in reducing chromosomal damage, but at the moment it is difficult to indicate how this comes about. Varner (1964) has shown that gibberellic acid controls the synthesis of α amylase in barley endosperm, and that it induces the synthesis of RNA which in turn helps to repair the cytological damage caused by physical and chemical mutagens. Gibberellic acid probably has something to do with the changed structural arrangement of the DNA molecules (after treatment with *EMS* and *MMS*). Alternatively, increased protein synthesis stimulated by GA_3 may be helping in the repair mechanism as suggested by Varner (1964).

From the results presented here, it appears that post-mutagen treatment with gibberellic acid could be used with advantage in reducing chromosomal aberrations. Gibberellic acid should be used only if one is interested in reducing the chromosomal aber-

rations with a view to obtaining an increased number of mutations; it should not be used if one is interested in obtaining duplications and translocations.

The duplications and translocations obtained are undergoing further observations to determine which chromosomes are involved.

Acknowledgements

The authors are grateful to Dr. H. N. Mehrotra, Professor and Head of the Department and to Dr. B. K. Srivastava, Director, for the interest taken and facilities provided. The senior author is grateful to the Department of Atomic Energy, Government of India, for the award of a Fellowship as well as the funds for carrying out this project.

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Received November 25, 1969

Communicated by H. Stubbe

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