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MUTAGENESIS BY RADIOWAVES IN *ANTIRRHINUM MAJUS* L.

CORNELIA HARTE
Institut für Entwicklungsphysiologie, Universität zu Köln, Köln (W. Germany)
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SUMMARY

Pollen of *Antirrhinum majus* was irradiated with radiowaves (γ = 1.5 m; field strength 1.5 V/m) in 3 series for 4, 12 and 43 3/4 h, then crossed on styles of emasculated untreated flowers. After selfing of the M_1 generation an increase in embryonic lethality and in mutations for characters of the seedlings and young plants was observed. These observations confirmed the mutagenic action of the treatment that was described earlier in *Vicia faba* and *Oenothera hookeri*.

INTRODUCTION

The mutagenic action of short radiowaves was observed first by ZINECKER-BAUER as inducing chromosome mutations in roots of *Vicia faba*[9]. She found a high frequency of chromosomal abnormalities after exposure of the roots to the radiation of a small transmitter. In other experiments involving exposure of pollen, pollen mother cells and whole plants of *Oenothera hookeri* to radiation of the same transmitter for several hours or to the radiation of a radiostation for a whole vegetative period, the induction of mutations with the following effects were clearly demonstrated: lethals, reduced viability during embryogenesis, morphological changes and chromosomal mutations[2–7]. Parallel to these experiments the pollen of *Antirrhinum majus* L. Sippe 50, a well-known genetical material, was irradiated in the same way to test the mutagenic effect in the progenies.

DESCRIPTION OF THE RADIATION TREATMENT

Flowers of *Antirrhinum majus* Sippe 50 were placed in a petri dish shortly after, or just before, anthesis and exposed to radiowaves in a closed room. The source of radiation was the same transmitter with dipole aerial that had been used for the experiment with *Vicia faba*[1] and the treatment of pollen and inflorescences of *Oenothera hookeri* (l.c.) (γ = 1.5 m; distance between the flowers and the aerial 0.95 m; field strength 1,5 V/m). The duration of the treatment in experiment I was 4 h; in experiment II, 12 h; in experiment III, 43.75 h; given on 3 consecutive days with 12.75, 12 and 19 h exposure. The treatments for experiments I and II were given on the same day. All experiments were repeated once (series a and b). The irradiated pol-

len was crossed onto styles of emasculated flowers of plants in the greenhouse. For the controls (experiment K) flowers were handled in the same way as for the experimental treatments, so that the only difference between experimental pollen and the controls was the exposure to radiowaves.

INVESTIGATION OF THE M_1 GENERATION

Table 1 shows the data for the M_1 generation. The seeds were sown in petri dishes on moist filter paper and the seedlings transplanted into seed-beds. Because of severe infections of the seedlings in all groups, the influence of the pollen treatment on lethality in the M_1 generation could not be determined. The development of the surviving plants was normal. All flowering plants of the M_1 generation and the controls were selfed. The failure of propagation of some plants is not correlated with the type of treatment of the pollen from which they originated.

TABLE I

INVESTIGATION OF THE M_1 AND M_2 GENERATIONS

Experiment No.	*Treatment (h)*	*Number of capsules*	*Number of seeds per capsule*	*% germinated seeds*	*Plants selfed*	*% of plants with good seed set*	*Test for lethal M_2 embryos in samples of 100 seeds*	
							Number of families tested	*% empty seeds*
Ia	4	2	88.5	90.3	92	65.2	60	4.4
Ib	4	5	64.4	92.2	180	61.6	111	5.0
IIIa	12	5	56.2	88.2	137	67.1	93	15.5
IIb	12	3	90.3	90.7	134	52.9	71	10.0
IIa	43.75	4	121.0	87.6	293	65.1	188	19.9
IIIb	43.75	4	127.7	92.9	278	63.6	177	13.7
Ka	Control	2	74.5	90.6	29	68.9	20	4.9
Kb		3	109.6	95.4	208	52.8	109	5.4

INVESTIGATION OF THE M_2 GENERATION

Testing for lethal mutations

From each single plant progeny with enough seeds a sample of 100 seeds was sown in a petri dish on moist filter paper and the percentage of germinating, non-germinating and empty seeds was counted. In addition, from progenies with a low seed set a sample of 50 seeds was tested in the same way. The results of this group did not differ significantly from those with the 100-seed samples; they will not be considered further. In the progenies of the controls the mean of empty seeds without an embryo was about 5%. The variance between the samples was according to the expectation of random samples taken from one basic lot. The progenies of plants from pollen exposed to the higher levels of irradiation gave a higher percentage of empty seeds (Table 1, Fig. 1). The reason for the difference in the means can be found by considering the whole distribution. The frequency of progenies with a high percentage of empty seeds shows a correlation with the duration of the pollen treatment.

In experiment I the distribution of the percentage of empty seeds per single plant progeny is, with one exception (60% empty seeds), the same as in the progenies

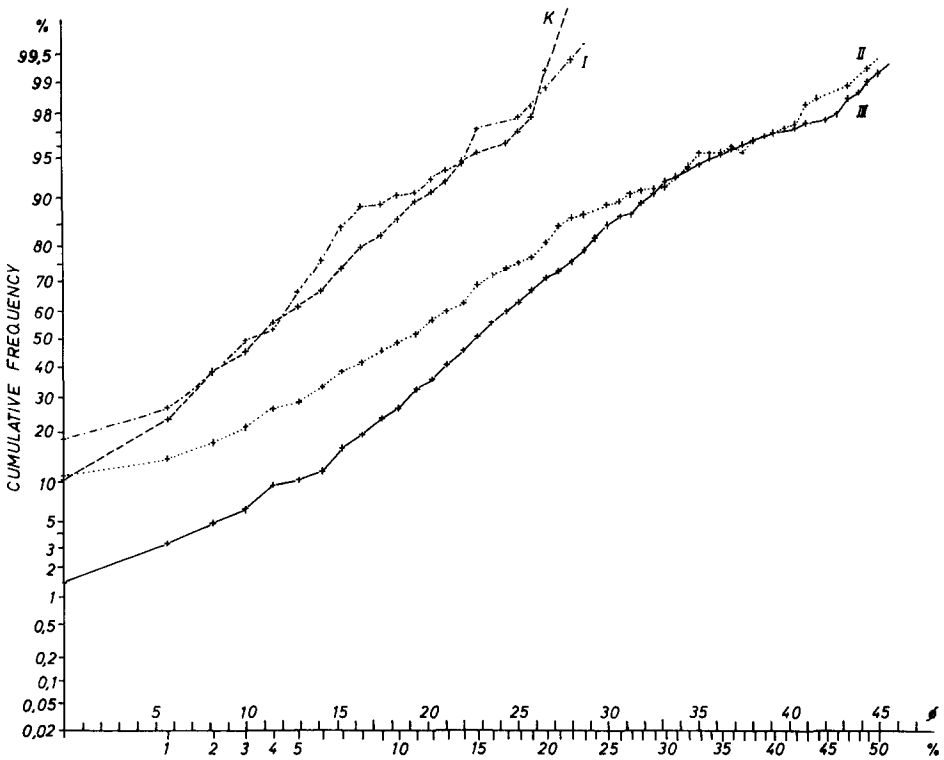

Fig. 1 Cumulative frequency of M_2 progenies from selved M_1-plants, classified according to the percentage of empty seeds (degenerating embryos). Abscissa: the lower scale gives the percentage of empty seeds in a sample of 100 seeds per progeny, the upper scale the corresponding value of phi after angular transformation of the percentage. Ordinate: probability scale of the cumulative relative frequency of progenies with the given percentage of empty seeds, K, controls; I–III, selfed M_1-plants originated from pollenirradiation; I, 4 h; II, 12 h; III, 43.3/4 h.

of control plants. In experiments II and III, progenies with a higher percentage of empty seeds were found with a frequency that cannot be interpreted as a statistical deviation from a general mean. If the distribution of the percentage of empty seeds in samples of 100 seeds is plotted in a graph with a probability scale as ordinate and an angula rtransformation of the percentages on the abscissa, the resulting graph is not a straight line as expected for a homogeneous population. (For description of the method see HARTE[5].) This leads to the interpretation that the samples were taken from a non-homogeneous population that can be divided into several sub-sets. One group of progenies corresponds with the samples of progenies from the controls. Other plants show an increase in embryonic lethality in their progenies.

This observation is interpreted as the consequence of an accumulation of genes with lethal or vitality reducing affect during embryogenesis of the homozygotes, which will segregate after selfing of heterozygotes. The distribution of progenies with normal and with increased embryonic lethality overlap, so it is impossible to decide for each single plant progeny whether the mother plant contained lethal genes. By the method of separating the curves into different sub-populations it can be estimated that, in experiment I, 99% of the M_1 plants, in experiment II 50% and in experiment III

approximately 20% of the M_1 plants belonged to the original population. The rest, 1% in experiment I, 50% in experiment II and 80% in experiment III, must have been heterozygous for genes with lethal effect. These could have been point or chromosome mutations, but they must have been induced by the pollen treatment.

Testing for mutations with effects in morphological characters

From all progenies with enough seeds, a second sample of seeds was sown in soil in flower-pots for the purpose of observing characteristics of the seedlings and young plantlets, to detect mutations with morphological effects during these early developmental stages. Abnormalities were only counted as "mutations" when several seedlings of the same progeny showed the same character. This procedure is the same as was followed in earlier experiments on mutagenesis in Antirrhinum by other authors (STUBBE[8],). The following abnormalities, which met the above criteria, were observed: differences in colour and form of the cotyledons when compared with the normal phenotype (yellow or light green cotyledons; green cotyledons with yellow basis; green cotyledons and yellow leaves; pointed cotyledons; round cotyledons), malformed leaves, narrow leaves, degeneration of the primary vegetative point and growth of regeneration shoots from the hypocotyl. Anomalies of the growth of the stem and the development of leaves and flowers after the seedling stages were not considered.

In experiments I, II and III, 45 progenies with segregating mutations were observed in a total of 843, that is 5.2%. Some of the progenies in this generation could not be investigated thoroughly because of severe infections. The low rate of germination in many progenies, as shown in results of the germination test, gave many progenies in which the number of seedlings was too low for observable segregation to be expected, especially in series II and III. For these reasons the given mutation rate can be considered as underestimated. For the same reasons the results of the three experiments cannot be used to relate mutation rate with dose of irradiation.

In the controls a segregation for an abnormal character of the seedlings was found in several progenies. One of the plants used as a female parent in the control crosses must have been heterozygous for a spontaneous mutation. The number of remaining progenies is not sufficient for the determination of the mutation rate in the pollen used for the control crosses.

As a comparison the spontaneous mutation rate of *Antirrhinum majus*, given as $< 1\%$ by several authors (STUBBE[8],), has to be used. The relative frequency of at least 5.3% segregating progenies is undoubtedly an increase compared with the spontaneous mutation rate.

DISCUSSION

In prior experiments with *Oenothera hookeri* the mutagenic effect of exposure to short radiowaves was clearly demonstrated. In experiments with *Antirrhinum majus* described here, the mutagenic effect of this treatment is confirmed by the increase in embryonic lethality after selfing the M_1 plants and the increase in relative frequency of segregating progenies with seedling anomalies. These are the experimental facts. A sufficient explanation of the physical and radiobiological basis for this effect of the treatment cannot be given.

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