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INDUCED MUTATIONS IN CUCUMBER (*CUCUMIS SATIVUS* L.) II. MUTANT OF GIGANTISM[1]

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Summary. In this paper a mutant induced by a 0.06% concentration of ethyleneimine in cucumbers is described. It is characterized by a general gigantism, particularly of leaves, flowers and seeds, which frequently attain two-fold larger size than the initial forms. The mutant may be identified already at the first leaf stage. Anatomical studies showed that changes observed in the mutant are mainly a result of changes in the size of cells. The cell size is significantly differentiated and in some cases, as for instance in the palisade and spongy parenchyma of leaves it is even smaller than that of the initial form. As a result of that leaf blades of the mutant are thinner. Disharmonies in the growth of testae and embryos cause that despite considerably larger testae, the embryos with cotyledons are of the similar size as those of the original form.

Cytoembryological studies showed that the mutant has a diploid chromosome number, a little lower pollen fertility and considerably reduced seed fertility. That last character is likely to be a result of irregularities in the development of the mutant embryos.

A genetic analysis showed that the described changes are determined by a single recessive gene, designated as *gig*.

As shown by preliminary studies (Kubicki — in press), ethyleno-imine appeared to be a very successful chemical mutagen in cucumbers. Several dozens mutations have been isolated and genetically established until now. Some of them, like for instance mutations of growth and sex, particularly interesting from the viewpoint of their usefulness in breeding, have been already represented in the literature (Kubicki 1974, 1975, 1977, 1978, 1979, 1980).

The present paper is the first of the series devoted to macromutations obtained by the first author.

MATERIAL AND METHODS

An object of the studies was an induced mutant obtained by treating seeds of the inbred line of Borszczagowski variety by 0.06% concentration of ethyleno-imine. A detailed description of the method was presented in part I of the publication (Kubicki — in press).

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The mutant was subjected to investigations of its morphological, anatomical and cytological traits.

All the observed traits of the mutant *gig* were compared to corresponding traits of the initial form (*B*). Statistical measurements were verified by the Student's *t*-test. The last part of the paper presents the way of inheritance of the described mutation.

RESULTS AND DISCUSSION

1. MORPHOLOGICAL TRAITS

The mutant is distinguishable already at the first leaf stage (Table 1). That leaf in the mutant is considerably larger, has a wrinkled surface with the margins turned downwards like an umbrella with uneven margination of the leaf blade and without lobating, typical of the original form. That leaf is attached to a significantly longer petiole and because of its large surface area and weight, it hangs down or rests on the ground. The described changes intensify in the mutant at the phase of four and six leaves (Table 1). This chiefly concerns leaves, leaf petioles and internodes, which are remarkably larger in the mutant in the original form. Fig. 1 shows differences in the leaf size, shape and surface area. A strong leaf wrinkle

Table 1. Selected morphological traits of the mutant *gig* and initial form (measurements in cm)

Traits at different developmental stages	Mutant *gig*	Initial form
A. First leaf stage:		
1. Hypocotyle length	2.98	3.60
2. Cotyledon length	3.30	4.08
3. The first internode length	1.80	2.30
4. The first leaf breadth	11.20	8.70
5. Petiole length of the 1st leaf	4.40	1.20
6. Plant height	9.60	10.60
B. Stage of four leaves:		
1. Hypocotyle length	5.00	8.60
2. The length of 1st internode	3.53	2.52
3. The first leaf breadth	21.38	15.36
4. Petiole length of 1st leaf	7.23	5.56
5. Plant height	15.60	15.50
C. Stage of six leaves:		
1. Length of 4th internode	10.02	5.60
2. Breadth of 5th leaf	30.89	19.10
3. Petiole length of 5th leaf	11.80	8.56
4. Plant height	48.17	38.33
D. Full flowering:		
1. Diameter of flower corolla	7.80	3.40
2. Ovary at the stage of:		
a) green bud — length	1.12	1.48
breadth	0.54	0.60
b) full flowering — length	1.52	2.15
breadth	0.60	0.90
c) 10 days after flowering — length	3.50	9.20
breadth	4.15	3.70
d) 25 days after flowering — length	14.70	15.10
breadth	9.20	7.60

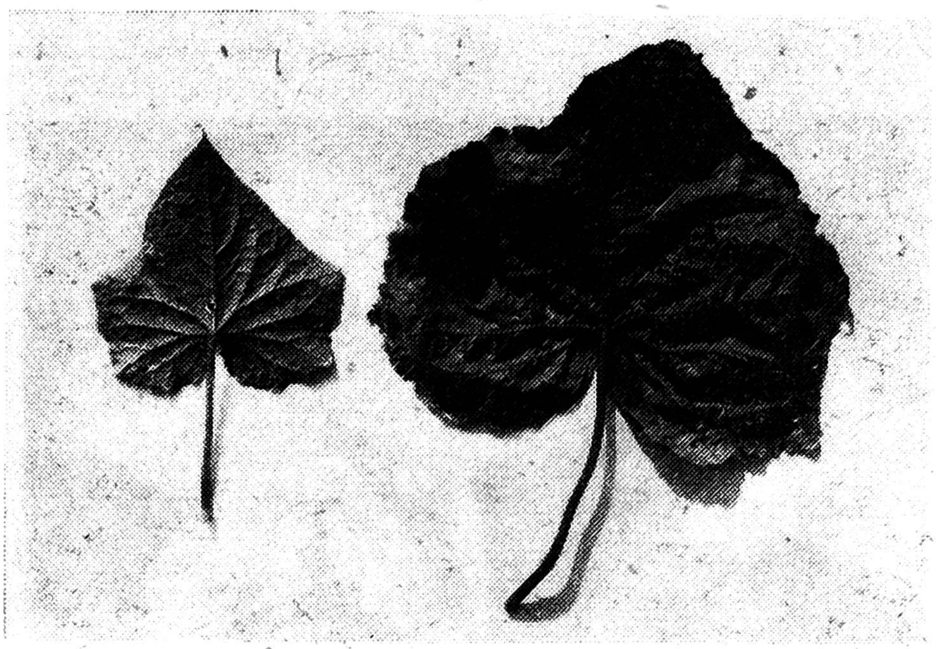

Fig. 1. A leaf of the initial form (left) and mutant *gig* (right)

Fig. 2. Habitus of the mutant *gig*

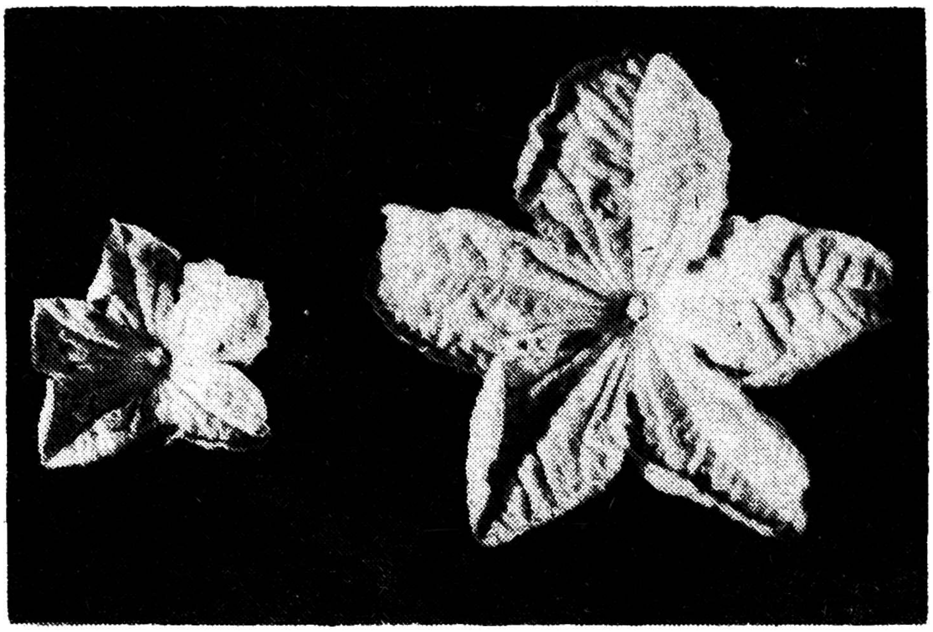

Fig. 3. A flower of the initial form (left) and mutant *gig* (right)

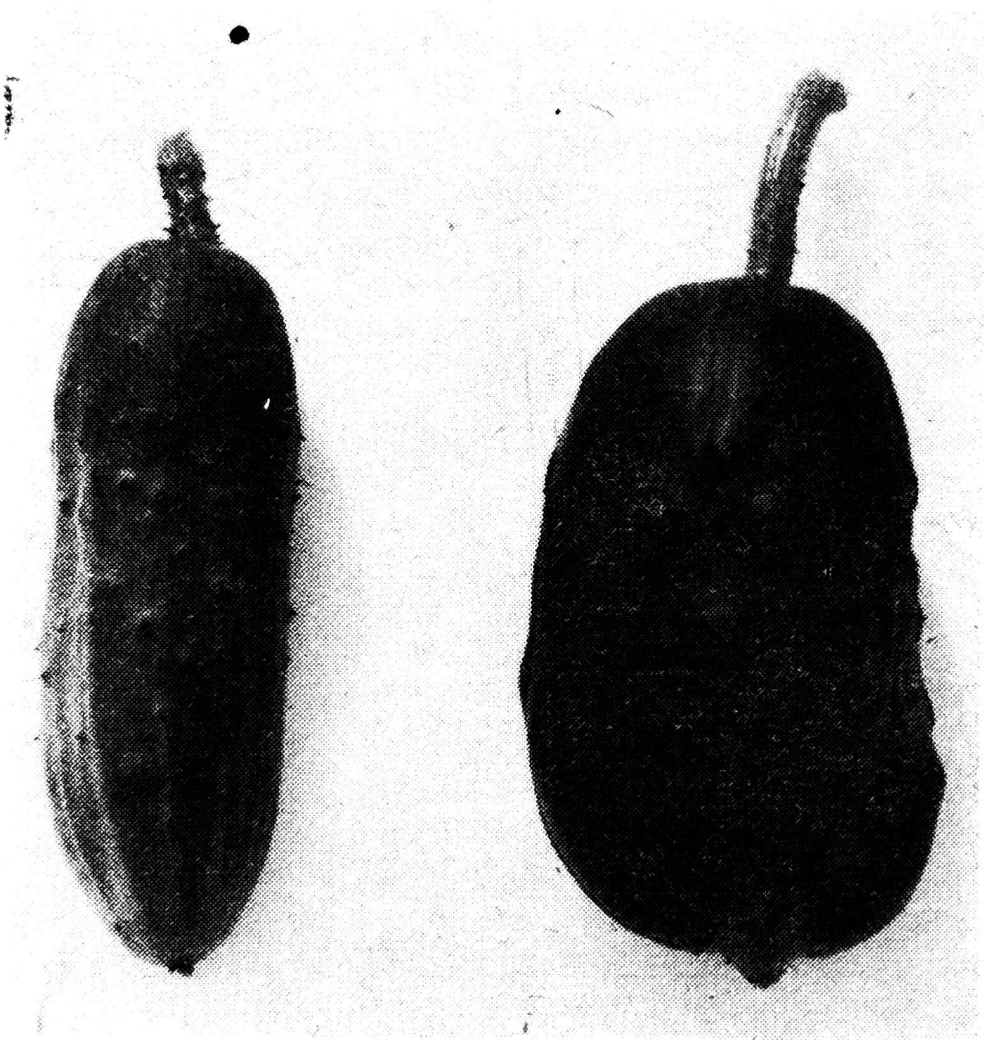

Fig. 4. A fruit of the initial form (left) and mutant *gig* (right)

in the mutant is a result of uneven growth rate of nervuration and the remaining part of the leaf blade. Fig. 2 presents the general view of the mutant with visible, characteristic of it traits, such as large, wrinkle, umbrella-like leaves turned downwards and drooping on long petioles.

Similar differences concern the petal size of the corolla male and female flowers (Fig. 3, Table 1). The size of the corolla petals in the mutant is twofold larger. These differences, however, do not concern the ovary size of the female flowers, which as

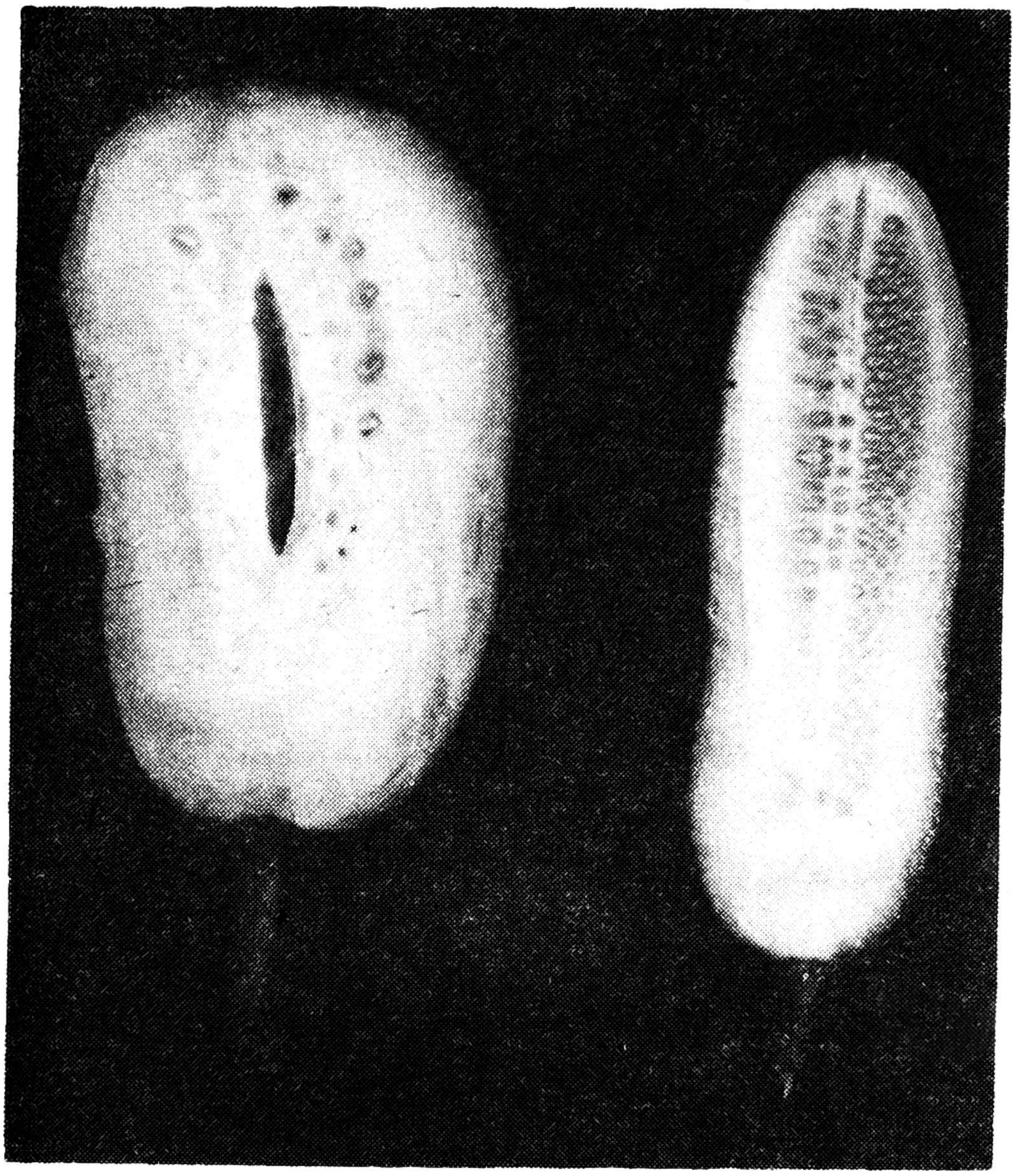

Fig. 5. A fruit section of the initial form (left) and mutant *gig* (right)

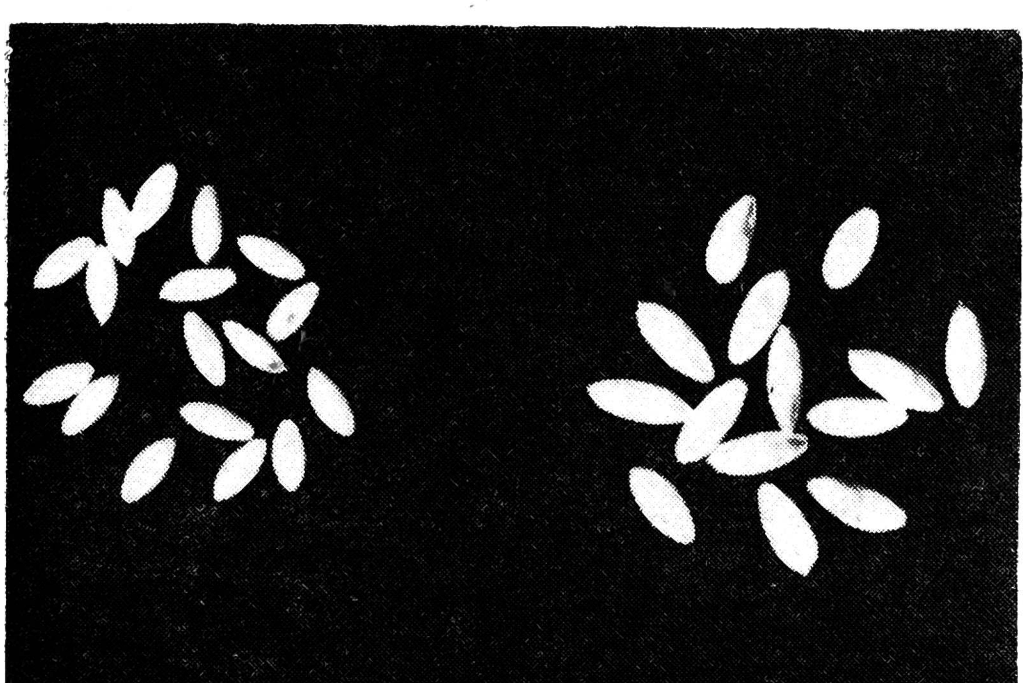

Fig. 6. Seeds of the initial form (left) and mutant *gig* (right)

Table 2. Seed size of mutant *gig* and initial form (in mm)

Form	Length	Width	Thickness	Length without testae
Initial	9.34	3.64	1.32	8.91
Mutant *gig*	12.29	4.83	1.25	8.83
NURα=0.05	0.38	0.24	0.15	0.35

follows from Table 1 are even smaller in the mutant. It is also seen to some extent in Figs. 4 and 5, which present unripe fruits. The mutant fruits are shorter but markedly thicker, more bulgy and less shapely, resembling slightly tetraploid forms. A cross-section of fruits (Fig. 5) shows regularly arranged seeds filling the original form, whereas the mutant has considerably less seeds and the entire cross-section differs by a significantly thicker texture and seed cavity, in which some empty cavities may also occur. The mutants seeds are on the average 2,32 mm longer and 1.8 mm wider (Fig. 6). No differences in the seed thickness or embryo size were observed (Table 2). It follows from that external differences in the seed size concerns the testae, but not cotyledons. Large testae in the mutant present as if large "packing", which is not well filled, makes the impression that the seeds are underdeveloped.

2. ANATOMICAL TRAITS

Since the general symptoms of the mutant gigantism concerning chiefly the leaves, flowers and seeds should result from an increased size or number of cells, the corresponding anatomical studies were carried out.

Tables 3 and 4 contain measurements of anatomical traits of the leaves and leaf petioles. From these data it follows that epidermal cells in the mutant are larger

Table 3. The size of the anatomical leaf traits of the initial form and mutant *gig* (in microns)

Form	Thickness of			Size of vascular bundle in the main nervure	Leaf thickness		Length of	
	epidermis	palisade karenchyma	spongy karenchyma		in the main nervure	1000 μ apart from the main nervure	racheidal cells	epidermal cells
Initial	26.0	78.2	188.5	336.0	956.0	354.0	26.1	52.2
Mutant *gig*	21.5	53.2	169.1	406.0	956.0	290.0	33.6	73.9
NURα=0.05	3.2	10.5	12.9	54.0	—	26.0	1.2	2.5

Table 4. The size of anatomical traits of the leaf petiole of the initial form and mutant *gig* (in microns)

Form	Thickness of		Size of vascular bundle	Number of vascular bundles	Length of assimilation karenchyma cells
	epidermis	axial roll			
Initial	4,5	217,0	85,1	14.0	237.0
Mutant *gig*	18,6	241,0	129,6	21.2	399.0
NURα=0.05	9,1	38,3	22,2	5.6	134.0

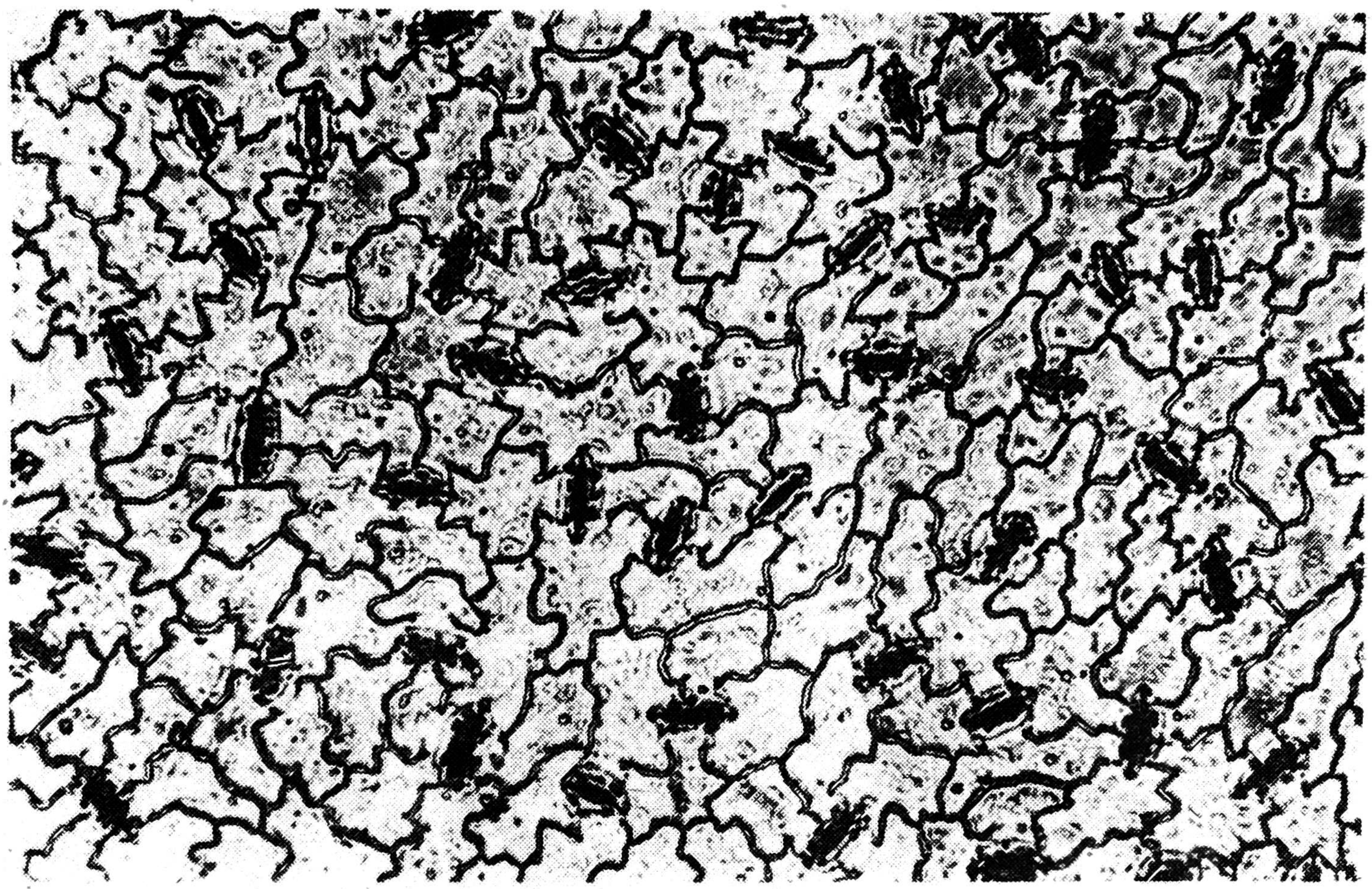

Fig. 7. Epidermal cells of the initial form (×650)

Fig. 8. Epidermal cells of the mutant *gig* (×650)

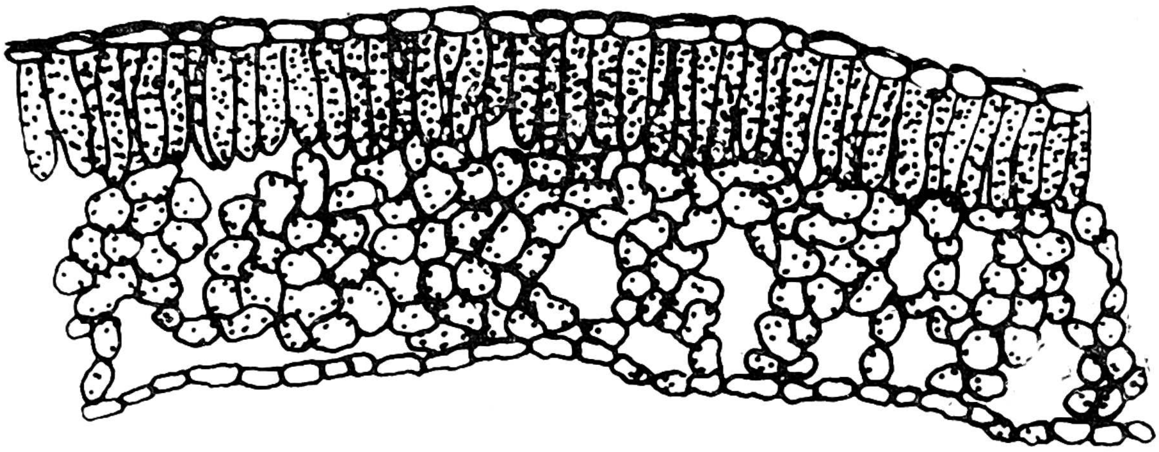

Fig. 9. A longitudinal leaf section of the initial form (×850)

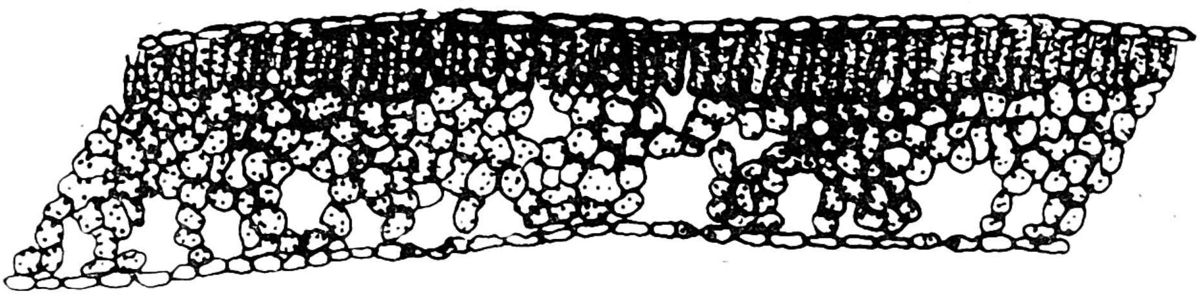

Fig. 10. A cross-section of the leaf of the mutant *gig* (×850)

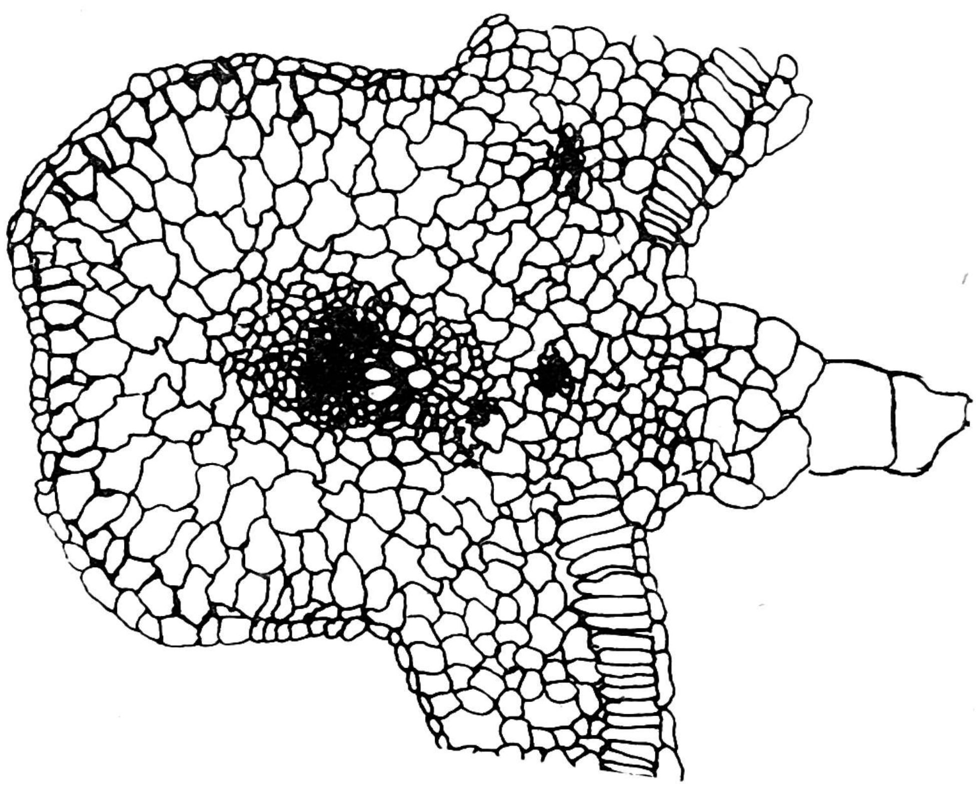

Fig. 11. A cross-section of the leaf main nervure of the initial form (×223)

mainly due to their considerably larger length. The length of tracheidal cells in the mutant is also significantly larger. This is supported by the attached pictures (Fig. 7, 8).

Since the mutant leaves look thinner, their cross-section was also studied. It appeared that the layer of the palisade and spongy karenchyma and epidermis

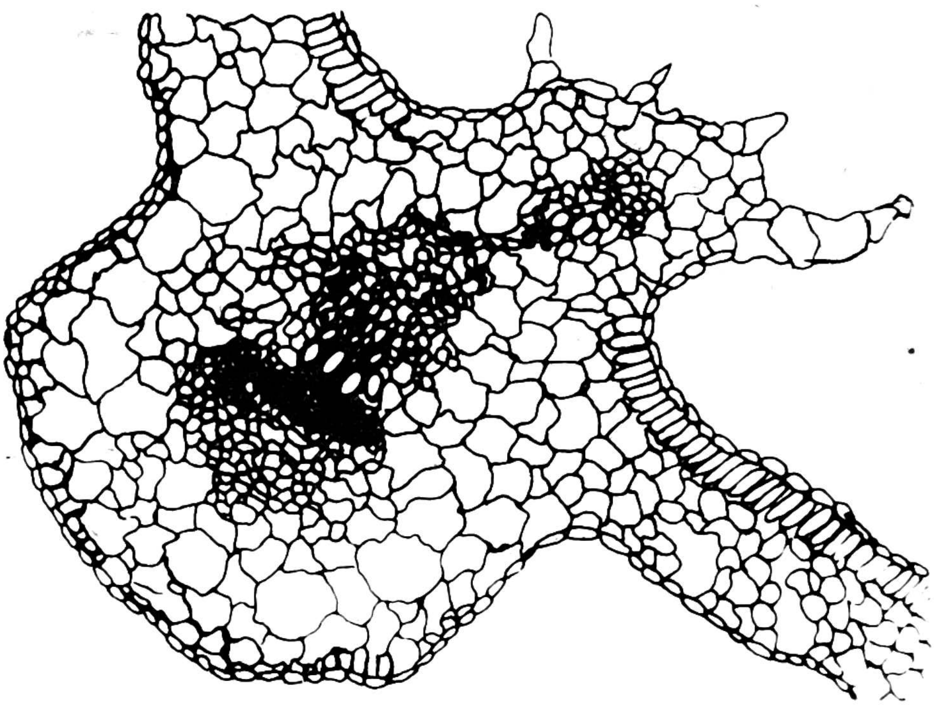

Fig. 12. A cross-section of the main nervure of the mutant *gig* (×223)

in the mutant is significantly thinner, as a result of which the thickness of the entire leaf is smaller (Table 3 and Figs. 9, 10). Somewhat different is the pattern of a longitudinal section along the main nervure of the leaf blade (Figs. 11 and 12). From this it follows that the size of the vascular bundles in the main nervure is larger in the mutant as a result of which the leaf thickness at the place of the main nervure is similar (Table 3). Such traits of the bundles as the length, the cell number at the base and the number of segments do not differ significantly. In view of the fact that the mutant leaf petioles are considerably larger and thicker (Fig. 1) they were analysed anatomically. Results are presented in Table 4 and in Figs. 13, 14. They indicate that the mutant leaf petioles have a considerably thicker epidermis

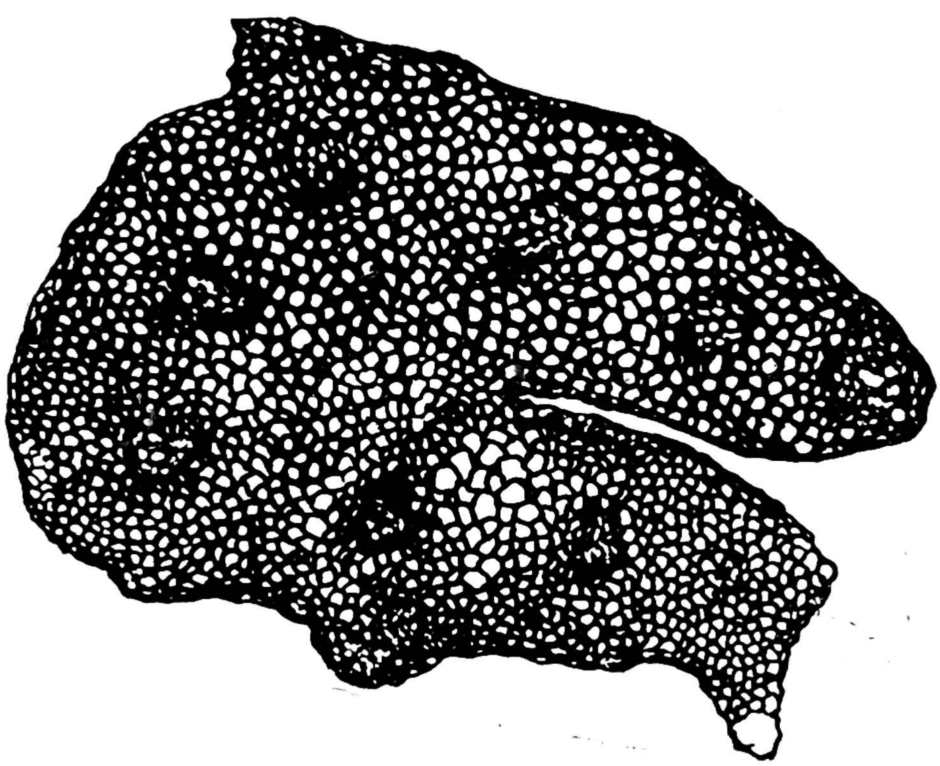

Fig. 13. A cross-section of the leaf petiole of the initial form (×25)

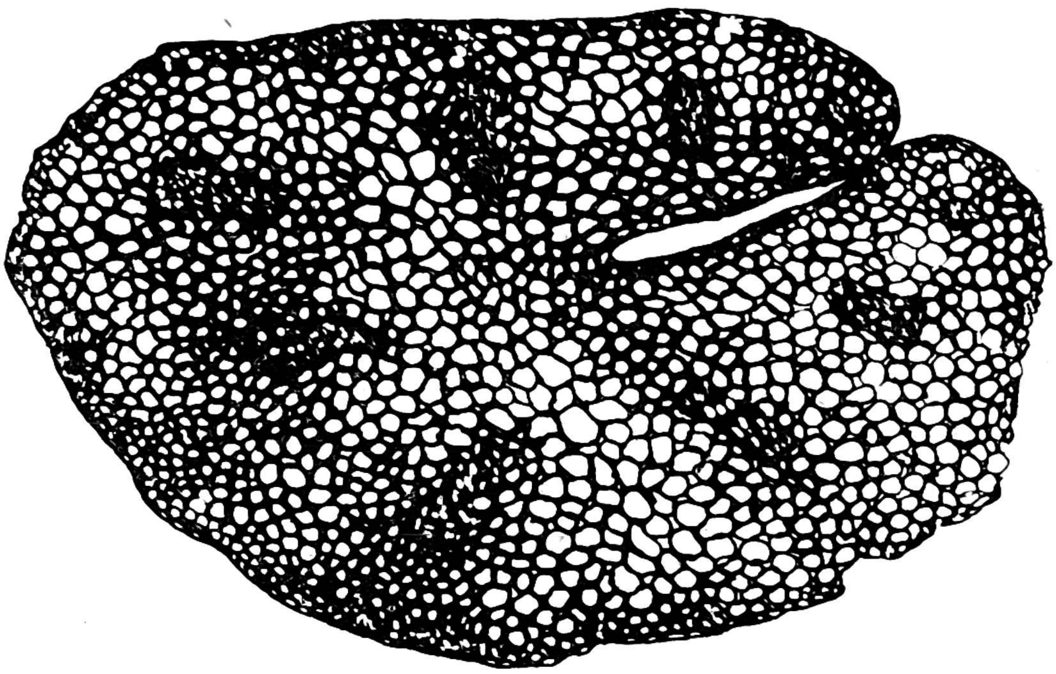

Fig. 14. A cross-section of the leaf petiole of the mutant *gig* (×25)

which is larger and is more rich in the number of vascular bundles. In addition to that, cells of the assimilation parenchyma on a longitudinal section of the petiole are markedly longer in the mutant.

3. CYTOEMBRYOLOGICAL TRAITS

In view of the fact that the mutant was characterized by a lower seed fertility and displayed certain changes characteristic of polyploids, it was subjected to cytoembryological studies. They showed that the course of embryogenesis in the mutant is analogical to that of the original form, except a pronounced delay in the development of the embryo and ovules. May be it is the cause of a lower seed fertility of the mutant. Studies of pollen viability showed no pronounced difference either in the size of pollen grains or in pollen viability (in the mutant the percentage of viable pollen was 88.2, that in the original form — 97.7 and in the heterozygote — 98.2). Though such a high per cent of viable pollen in the mutant did not indicate its polyploid origin, its chromosome number was examined. It appeared that it is a normal diploid, showing no changes either in the number or morphology of chromosomes.

It should, therefore, be suggested that the observed numerous changes in the morphological, anatomical and cytological traits occurred due to a pointwise mutation.

A corresponding genetic analysis was carried out to make sure, what is the character of these changes.

4. GENETIC ANALYSIS OF THE MUTANT *GIG*

Since the described mutant may be reproduced in the established hoxozygous form, it was crossed to the original inbred line and the character of the F_1, F_2 and backcross was examined. As follows from Table 5, the mutant traits described in the

Table 5. Genetic analysis of hybrids obtained after crossing the mutant (*gig*) with the initial form

	Plant number					
	obtained		expected		Chi^2	P
	normal	mutated	normal	mutated		
P_1 (initial form)	44	—	44	—	—	—
P_2 mutant (*gig*)	—	31	—	31	—	—
F_1	47	—	47	—	—	—
F_2	381	111	369	123	1.55	>0.20
$F_1 \times P_2$	76	65	70.5	70.5	0.86	>0.30

present paper were determined by a single recessive gene designated by the symbol *gig* in view of its phenotypic symptoms of gigantism. A certain defficiency of mutated plants in segregating F_2 and Bc_1 generations would indicate that this gene in a homozygous state decreases plant viability, which may be a result of a pleiotropic effect of its action.

It is an interesting fact that the *gig* gene does not manifest itself to the same degree in all the parts of a plant. Though its basic phenotypic effects result from the increase of the cell size, it was found that in some cases these cells can be smaller.

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MUTACJE INDUKOWANE U OGÓRKA (*CUCUMIS SATIVUS* L.) II. MUTANT GIGANTYZMU

Streszczenie

Opisano mutanta otrzymanego w wyniku indukowania etylenoiminą (stężenie — 0.06%). Charakteryzuje się on ogólnym gigantyzmem, szczególnie liści, kwiatów i nasion, które często osiągają dwukrotnie większe rozmiary w porównaniu z formą wyjściową. Mutant może być

zidentyfikowany już w stadium pierwszego liścia. Badania anatomiczne wykazały, że obserwowane u tej formy zmiany są wynikiem głównie zmian wielkości komórek. Wielkość ich jest znacznie zróżnicowana, a w niektórych przypadkach jak np. w miękiszu palisadowym i gąbczastym liści, komórki są nawet mniejsze niż u formy wyjściowej. Rezultatem tego jest np. mniejsza grubość blaszki liściowej mutanta. Występujące we wzroście okrywy nasiennej i zarodków dysharmonie, powodują, że mimo znacznie większej okrywy nasiennej wielkość zarodków z liścieniami jest podobna jak u formy wyjściowej.

Badania cytoembriologiczne wykazały, że mutanta charakteryzuje diploidalna liczba chromosomów, nieco obniżona płodność ziarn pyłku i znacznie obniżona płodność nasion. To ostatnie jest prawdopodobnie wynikiem zaburzeń rozwoju zarodków zmutowanej formy.

W wyniku analizy genetycznej stwierdzono, że opisane w pracy zmiany uwarunkowane są jednym recesywnym genem, który oznaczono symbolem *gig*.

ИНДУЦИРОВАННЫЕ МУТАЦИИ У ОГУРЦОВ (*CUCUMIS SATIVUS* L.) II. МУТАНТ ГИГАНТИЗМА

Резюме

В настоящей работе описан мутант, индуцированный 0,06% этилено-имином. Характеризуется он общим гигантизмом, особенно листьев, цветов и семян, которые часто достигают двукратно больших размеров по сравнению с первоначальной формой. Мутант можно идентифицировать уже в стадии первого листа. Анатомические исследования показали, что наблюдаемые у мутанта изменения являются главным образом результатом увеличения клеток. Однако, величина их довольно различна, а в некоторых случаях, как например в палисадной и губчатой мякоти листьев, они могут быть меньше, чем у первоначальной формы. В результате этого листовые пластинки мутанта тоньше. Дисгармония, выступающая в росте семенной оболочки и зародышей, причиняется к тому, что несмотря на значительно большие семенные оболочки, зародыши с семядолями имеют подобную величину, как у первоначальной формы.

Цитоэмбрилогические исследования показали, что мутант характеризуется диплоидальным числом хромосом, несколько пониженной плодородностью зерен пыльцы и значительно обниженной плодородностью семян, что вероятно является результатом нарушений в развитии зародышей.

В результате генетического анализа установлено, что описанные выше изменения обусловлены одним рецессивным геном, который обозначено символом *gig*.