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Mutations in *Petunia*

III. Morphological changes observed in M_1 and M_2 generations after irradiation of seeds

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I. Effect of gamma rays on morphology of M_1 plants

The seeds of a diploid wild species, *Petunia axillaris*, were treated with gamma rays. The treatment was performed in Radiobiological Laboratory of Plant Breeding Institute at Radzików, by the use of ^{60}Co source.

Ten doses of gamma rays ranging from 1 kr to 10 kr were applied. The irradiated seeds were sown in a greenhouse. Two-week old seedlings were planted in the field.

The observed effects of gamma radiation concerned the following features of M_1 plants:

— germination of irradiated seeds (in per cent);
— height of two-week old seedlings;
— number of leaves of the seedlings;
— height of mature plants;
— number of generative organs formed on the plants during the growing season;
— chlorophyll changes.

Gamma irradiation of petunia seeds caused a marked decrease in germination of the seeds. The dose-effect curve had a typically sigmoidal shape (fig. 1). The threshold value of the dose was reached somewhat above 4 kr and the LD_{50} amounted to 6 kr. The LD_{100} was not achieved in the experiment as there were some plants surviving the highest dose applied.

Gamma rays had also a marked influence on the growth of the seedlings. It is interesting to note that a statistically proved stimulation of the growth of the seedlings was observed (fig. 2). The stimulation occurred, however, in the seedling stage only. Two-weeks old seedlings reached the height of 5,4 cm at 1 kr, of 6,0 cm at 2 kr, of 6,9 cm at 3 kr and again of 5,4 cm at 4 kr. It is evident that the doses 2 kr and 3 kr had a growth stimulating effect.

The number of leaves of two-week old seedlings was slightly but significantly increased after irradiation of the seeds. The mean number of six leaves per seedling was observed after doses in the range from 1 to 5 kr while at the higher doses seven leaves per seedling were present. The increase in the number of leaves is probably related to the decrease in the height of the seedlings.

The height of mature M_1 plants as measured by the height of their main stems, was not influenced by gamma irradiation of the seeds. The growth stimulating

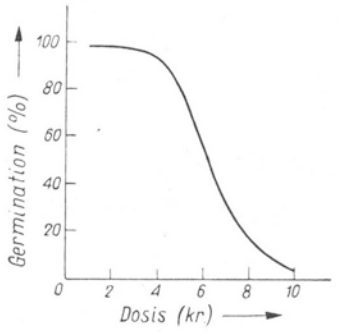


Fig. 1

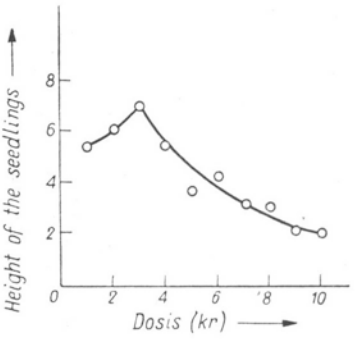


Fig. 2

Fig. 1. Dose — effect curve for germination of gamma irradiated seeds of *Petunia axillaris*
Fig. 2. The effect of gamma seed irradiation on the subsequent growth of the seedlins as measured by the height of the two-week-old seedlins

effect observed in the seedlings did not occur in mature plants. The highest dose applied caused significant growth depression: 46,3 cm in comparison with 51,8 cm at 1 kr.

There was a marked decrease in the number of generative organs formed on the M_1 plants during the growing season (Tabl. 1). The decrease was particulary evident in the number of capsules which reached maturity.

Only one chlorophyll mutation was found among the 650 M_1 plants. One plant which survived the highest dose showed a chlorophyll-less sector. This was a mericlinal chimera with L II tissue layer deprived of chloroplasts. Some branches of the plant formed periclinal chimeras.

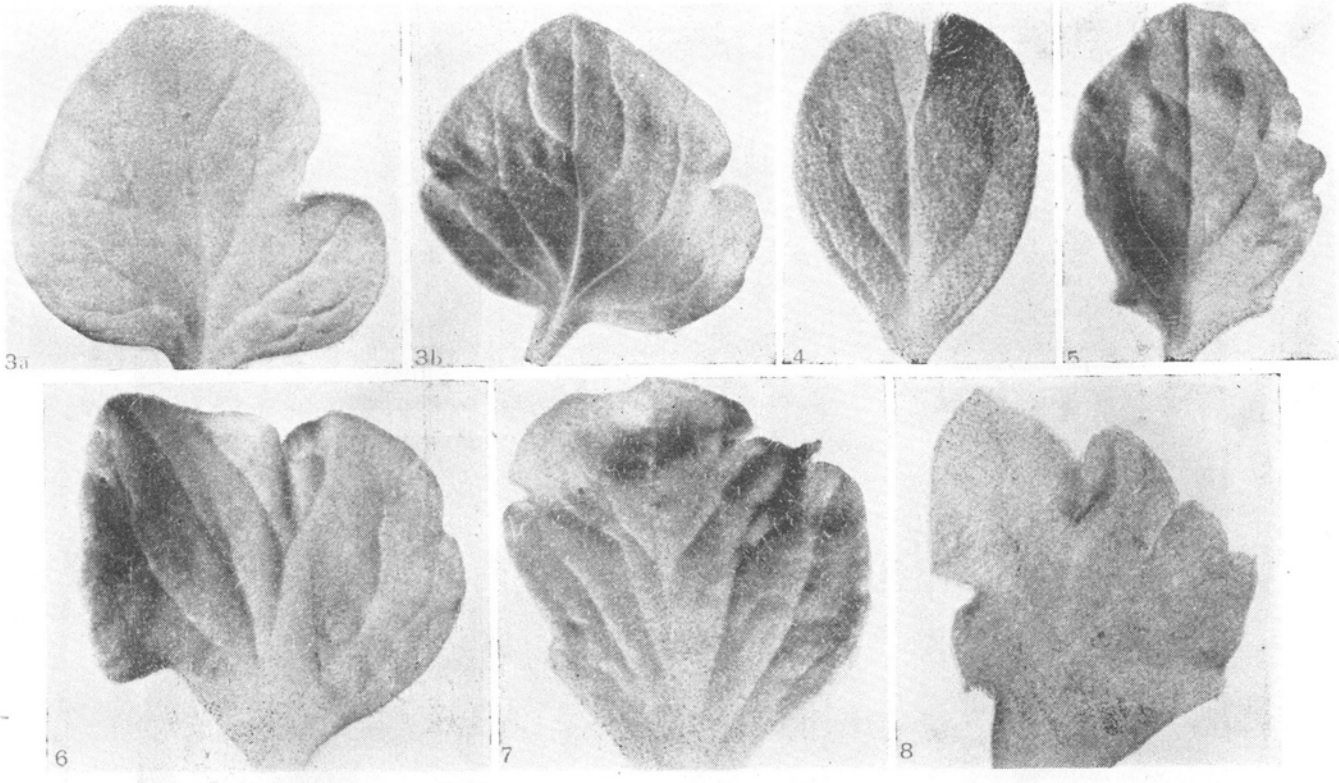
Table 1

Number of generative organs, found on M_1 plants at the end of the growing season, after gamma irradiation of the seeds

Dose (kr)	All generative organs	Mature capsules only	Unmatured capsules only	Flowers only	Flower buds only
1	32.2	18.8	10.0	1.2	2.2
2	30.0	17.2	9.5	0.7	2.1
3	32.3	16.5	10.7	1.9	3.2
4	31.2	17.9	9.8	1.1	2.4
5	23.5	10.0	8.8	2.2	2.6
6	11.8	2.8	5.9	1.6	1.5
7	17.8	6.3	7.3	1.3	2.7
8	17.0	1.9	9.5	3.0	2.9
9	23.0	3.0	12.0	4.3	3.0
10	21.3	3.3	14.0	1.7	3.0

(Note: The results after higher doses are incnoclusive because of the small number of plants observed).

Plate I



Phot. W. Guzewski

Fig. 3 *a*, *b* — Slight asymmetry of leaf blades after thermal neutron treatment of the seeds of tetraploid petunia; fig. 4 — splitting of the top of the leaf; fig. 5 — pronounced deformations of the leaf blade; fig. 6 — pronounced asymmetrical development of the leaf blade; figs 7, 8 — pronounced deformations of the leaves

Plate II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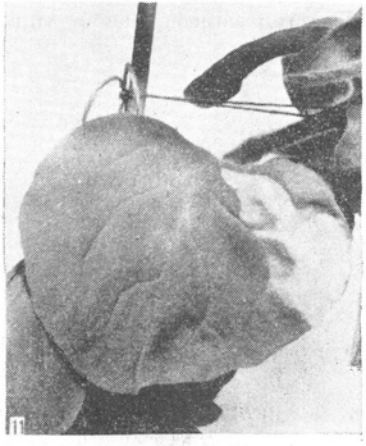
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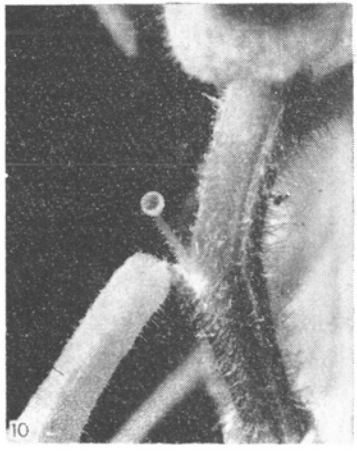
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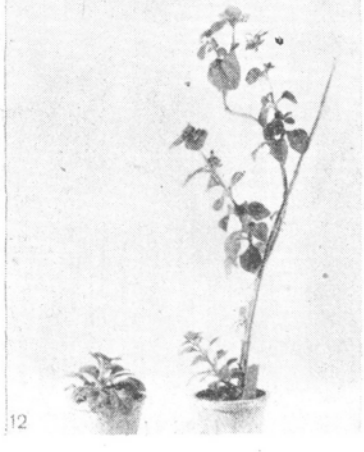
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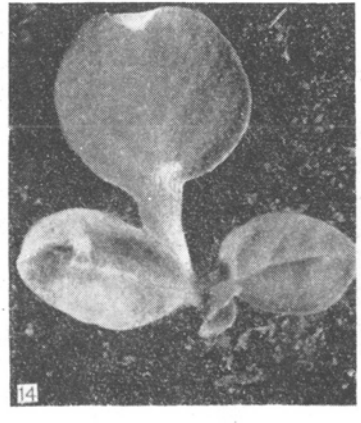
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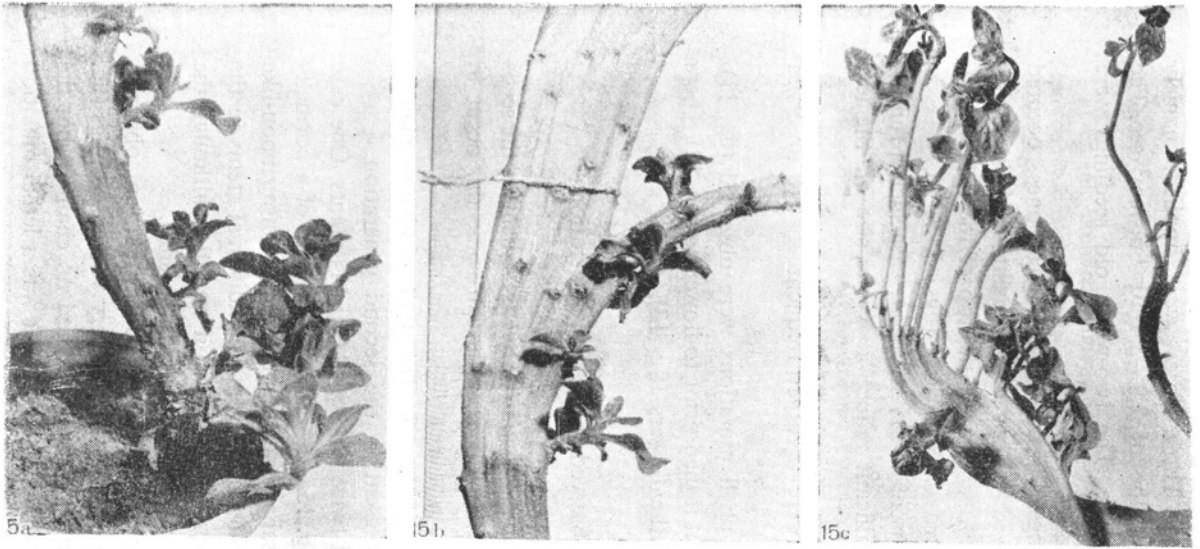

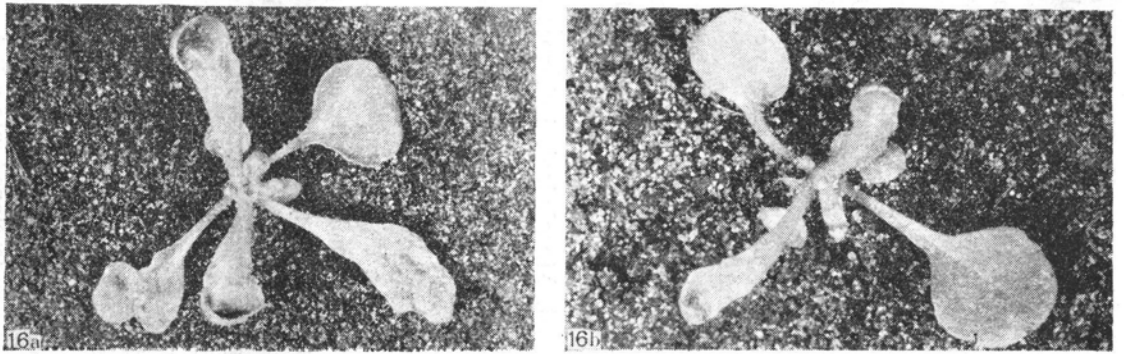

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Fig. 9 — Funnel-like leaf; fig. 10 — funnel-like leaf with a significant reduction of the size of leaf; fig. 11 — A chlorophyll-less sector of the leaf due to a mutation in one cell only; fig. 12 — a darf mutant (left) in comparison with control plant (right); fig. 13 — a flower with split corolla (right); fig. 14 — sedling with a double leaf; fig. 15. Fasciation of the main stem: a — the basal part of the plant, b — middle region of the plant, c — the upper part of the plant; fig. 16. A lethal mutation causing deformation of the leaves of the seedlings into "spoon-like" structures

II. Effect of neutrons on the morphology of M_1 plants

Seeds of tetraploid garden petunia, *Petunia hybrida superbissima purpurea*, were irradiated with thermal neutrons. The treatment was performed at the Brookhaven National Laboratory (USA). Three doses were applied, namely irradiation during 30—60—90 minutes at the of $2.9—3.4 \cdot 10^{-9}$ $n_{th}/cm^2 \cdot sec$. The irradiated seeds were sown in a greenhouse. The two-week old seedlings were planted in the field.

Several types of morphological changes, mostly teratological ones, occured among the M_1 plants. Leaf blades showed various developmental abnormalities, ranging from slight asymmetry (fig. 3 *a*, *b*), splitting of the top of the leaf (fig. 4), marked asymmetry (fig. 6) to pronounced deformations (figs. 5, 7, 8).

In two cases funnel-like leaves (fig. 9) were observed. In one case such a funnel-like structure was associated with a significant reduction of the size of the leaf (fig. 10).

The bifurcations of the main stems were a rather frequent change observed among M_1 plants.

In some cases very interesting chlorophyll mutations were observed (fig. 11). In one case a dwarf plant was obtained but it died prior to flowering (fig. 12). There was one plant with splitted corollas of the flowers (fig. 13).

III. Morphological changes observed in the M_2 generation

There were no changes observed in *Petunia axillaris* after treatment of the seeds with gamma rays as well as with thermal neutrons, although some did occur in *Petunia hybrida superbissima purpurea*.

In one case a double leaf was observed (fig. 14). This type of change may be due to chromosomal aberrations still present in the second generation.

Fasciation of the main stem occurred twice among 12,000 M_2 plants. One case of fasciation was especially interesting (fig. 15a, b, c): the main stem of a normally developing plant became thicker in its basal part, changing its shape into ribbon-like structure in the middle region and splitting into seventeen individual stems in the upper part. It is supposed that the fasciation was due to a mutation although the plant died prior to flowering and genetical analysis was impossible.

The second morphological change, presumably due to a mutation, observed in the M_2 generation, was a deformation of the leaves of very young seedlings (fig. 16a, b). Only the cotyledons and the very first leaves showed no changes, but the following leaves developed into a "spoon-like" structures. It was a lethal mutation because the seedlings died in a very early stage.

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Mutacje u petunii. III. Zmiany morfologiczne, obserwowane w pokoleniach M_1 i M_2 po napromieniowaniu nasion

Streszczenie

I. Wpływ promieni gamma na morfologię roślin M_1

Działanie promieni gamma na nasiona dzikiego gatunku diploidalnego, *Petunia axillaris*, spowodowało istotny spadek siły kiełkowania nasion. Siewki, wyrosłe z nasion, które otrzymały dawkę 2 kr i 3 kr, wykazywały stymulację wzrostu, jednakże efekt ten był przejściowy i nie wystąpił u roślin dojrzałych. Wyższe dawki spowodowały znaczne zmniejszenie się ilości dojrzałych w ciągu okresu wegetacyjnego torebek nasiennych.

II. Wpływ neutronów na morfologię roślin M_1

Zaobserwowano szereg zmian morfologicznych o charakterze teratologicznym u roślin, wyrosłych z nasion, napromienionych neutronami termicznymi. Opisano zmiany kształtu liści, wśród których na szczególną uwagę zasługuje liść lejkowaty przy jednoczesnej redukcji wielkości blaszki liściowej.

III. Zmiany, obserwowane w pokoleniu M_2

Nie zaobserwowano zmian morfologicznych u *Petunia axillaris*, natomiast u *Petunia hybrida superbissima purpurea* zanotowano wystąpienie fascjacji pędów oraz mutacji letalnej, powodującej deformację i zahamowanie rozwoju liści u siewek.