ACTA SOCIETATIS
BOTANICORUM POLONIAE
vol. XXXVI — nr 2
1967

Mutations in *Petunia*

II. Plastid mutations

S. MUSZYŃSKI

In previous report (Muszyński 1964) we described an induced chlorophyll deficiency in diploid *Petunia axillaris*. After X-ray irradiation of petunia seed one plant of the variegata type was found. It was a sectorial chimaera. There were no chloroplasts present in the white sectors of the leaves. Microscopic examination showed that L I and L II tissue layers were normal green, deeper lying cells being deprived of chloroplasts. This was confirmed later by the fact that only normal green plants were observed in the selfed progeny.

Another series of our experiments included neutron irradiation of the seed of tetraploid *Petunia hybrida superbissima purpurea*. The treatment was performed at the Brookhaven National Laboratory at thermal neutron flux of $2.9-3.4\times10^9$ n/cm^2/sec. and irradiation time ranging from 30—90 min. Among 3000 plants grown in 1963 some showed chlorophyll deficiency of the *maculata* type. They had small white or yellow spots on the leaves.

The same type of chlorophyll deficiency occurred in the progeny of selfed NI *maculata* plants, but other types of chlorophyll deficient plants, such as *albina xantha* and various *viridis* were also observed. The seeds from one of the N2 *maculata* plants were sown in 1966. We observed again a wide range of phenotypic expression of the chlorophyll deficiency. The seedlings had white, yellow, mosaic or normal green cotyledons. All white seedlings and a part of the yellow ones died at a very early stage of development, but the other part developed mosaic plants. The *maculata* N3 plants were examined more throughly to find whether the occurrence of variegated tissue was due to a plastid mutation. Very young leaves of the plants were uniformly yellow, becoming variegated after reaching full development.

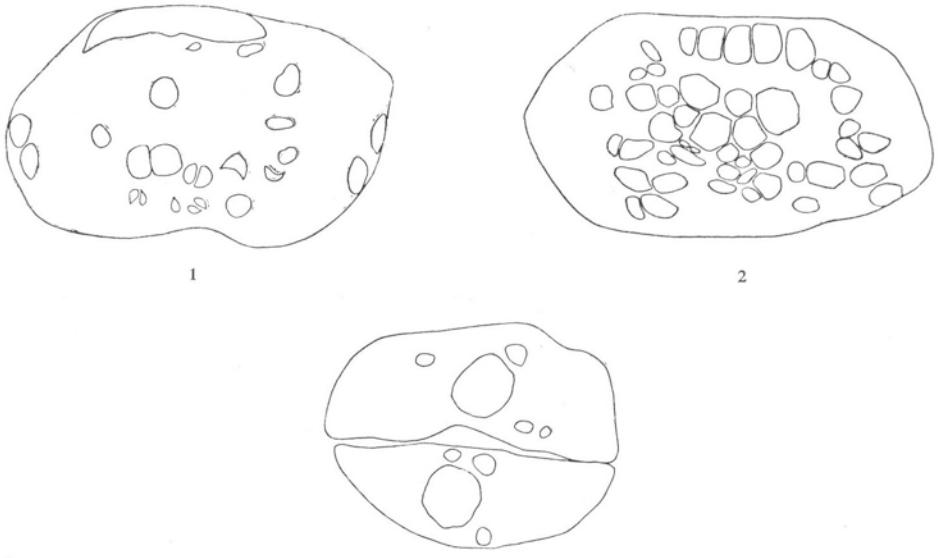
Microscopic examination was performed on free-hand slices of leaves. The slices were prepared from leaves of 0.5—2.0—3.0—4.0 cm thus representing all stages of leaf growth. It was shown that in the young leaves the yellow colour of the leaf blade was due to the absence of normal green chloroplasts. Only colourless and very pale greenish-yellow plastids were observed. The chloroplasts differed widely in size and had mostly abnormal shapes (Tab. 1, figs. 1, 2, 3). It is very interesting to note that the cells of leaf parenchyma were also changed. The change was most apparent in the palisade layer where large inter-cellular areas were observed. In the young leaves numerous cells with mixed chloroplasts were present

Table 1

Chloroplast types found in muteted *Petunia* plants

Colour of the plastids	green	pale-green	colourless
Shape of the plastids	lenticular	regularly round, normal size lenticular, smaller than normal chloroplasts comma-like, very small angular, various size lenticular, very big	round of various size,

(fig. 1). In the elder leaves the situation was almost the same, the cells with mixed chloroplasts being not so numerous. The grown up leaves showed green spots of irregular size and shape, non uniformly distributed over the leaf blade. In the cells forming the green patches on the leaves two kind of normal green chloroplasts of



Figs. 1—3. Chloroplast types found in muteted *Petunia* plants. 1 — Cell with mixed chloroplasts; 2 — cell with pale green, angular chloroplasts; 3 — guard cells with angular chloroplasts

different shape were present. One type was of normal round shape whereas the other type had a very characteristic comma-like shape. The chloroplasts of the latter type were smaller in size. In the cells of white tissue only abnormal chloroplasts were found. Cells with mixed chloroplasts were very rare. In some cases also cells with a very small number of normal green chloroplasts were observed. The cells

with abnormal chloroplasts had abnormal shape and reduced size. On the other hand the size of the cells with normal chloroplasts was often markedly increased (fig. 4).

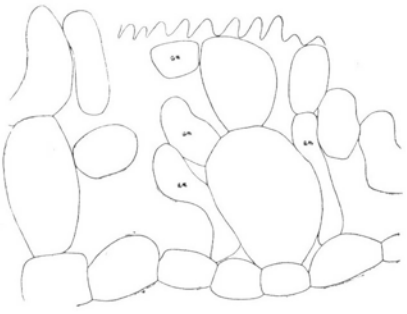


Fig. 4. Fragment of the palisade layer of the grown up leaf with cells containing normal green plastids (without designation) and abnormal ones (designated *an*).

CONCLUSIONS

The presence of cells with mixed chloroplasts provides evidence of plastidome mutation. Such cells are numerous in young leaves and rare in the elder ones because of the segregation of chloroplasts during cell division combined with the reduced propagation rate of abnormal chloroplasts. The presence of normal green chloroplasts may be due to the delayed chlorophyll formation in mutated plastids or rather to back-mutations of defective chloroplasts as postulated by Michaelis. Further research on the subject is planned.

Department of Genetics
Agricultural University
Warsaw, Poland

REFERENCES

Bartels F., 1957, Ungewöhnlich grosse Chloroplasten bei Epilobium-Bastarden, Protoplasma 48: 398—402.

Gustafsson A., 1940, The mutation system of the chlorophyll apparatus, Lunds Univ. Arsskr. N. F. Avd. 2: 1—40.

Michaelis P., 1965, Uber Kerninduzierte Plasma- und Plastiden-Abänderungen, Z. Naturforschung 20 b: 264—267.

Muszyński S., 1964, A case of sectorial chimaera obtained after irradiation of Petunia seed, Acta Agrobot. 16: 171—173.

Mutacje u petunii

II. Mutacje plastomu

STRESZCZENIE

Wyodrębniono u *Petunia hybrida superbissima purpurea* chloroplasty o zmienionym kształcie: soczewkowate, przecinkowate, kanciaste oraz olbrzymie, i o zmienionej barwie: jasnozielone oraz bezbarwne. Obecność komórek z mieszanymi chloroplastami świadczy o mutacji plastydów. Komórki takie są częściej spotykane w liściach młodszych, a rzadziej w liściach starszych. Spowodowane jest to segregacją plastydów podczas podziałów komórkowych. Chloroplasty zmutowane dzielą się wolniej od normalnych. Wystąpienie plam zielonych na liściach jednolicie żółtych może być spowodowane faktem wolniejszej syntezy chlorofilu w zmutowanych chloroplastach lub też mutacjami powrotnymi chloroplastów.