STUDIES ON THE SPONTANEOUS PLASTID MUTATION IN *DAHLIA VARIABILIS* DESF.

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Abstract. *Three plants of* Dahlia variabilis *were observed to have variegated leaves. On studying these spontaneously arising plastid mutations the stomatal cells (Mixed Stomata) were found to have both green and colourless plastids. Comparative studies of the number of plastids and the stomatal size in the mixed and the normal green stomata were also conducted.*

Introduction

Correns (1909) was the first to discover green-white plants of *Mirabilis jalopa*. After him till recently several workers including Caspari (1936), Ephrussi (1953), Jinks (1964), Wilkie (1964), Nanney (1957), Sager and Ramanis (1963) have added to the extrachromosomal inheritance especially the mode of inheritance of plastids in the green plants.

Plastids are the selfduplicating particles like mytochondria and microsomes which are extranuclear in nature. The transmission of these organelles in the next generatation is independent of the nuclear duplication. Rhoades (1947), however, discovered a gene which is responsible for the transmission of plastid abnormality in the *Maize* plants. He has proved that in the homozygous condition this gene can change the plastids to a colourless state. He named this factor as Iojap which is located on chromosome VII.

Cytoplasmic inheritance as it is termed in such cases forms an entirely different system of transmission and gives unlike results in the reciprocal crosses, and cannot be explained by the Mendel's laws.

The studies of plastids in the variegated plants offer a great problem due to the indistinguishability of green i.e. normal plastids from the albino in the meristematic regions (Von Wettstein 1957, 1961; Murakami 1962; Burk, Stewart and Derman 1964). The young plastids in their colourless phase are identical to the chondriosomes but here the differentiation can be done by the help of microchemical or by staining as described by different authors (W. Schwarz, 1931; Guilliermond, 1931 b; Metzner, 1952 b, 140).

The problem of behaviour and segregation of green plastids from the colourless in a variegated plant is twofold. The plastids in the young region are colourless so indistinguishable from one another. When the colour develops in the mature leaves and plastids can be distinguished from one another the segregation has already completed. In still maturer leaves the colourless plastids start degenerating and nothing distinguishable can be seen. The senior author tried different types of variegated leaves and was unable to find the mixed cells i.e., the cells with albino and green plastids. The problem of differentiation of albino plastids from the green ones at a very young stage in *Nicotiana* has been

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studied by Susalla and Mahlberg (1977). They have reached the conclusion that the young plastids in the cells of green and albino apices cannot be distinguished as to internal organization even with the help of electron microscope.

Michaelis (1951, 1954 and 1967) working with *Epilobium* observed 172 variegated plants and found that 50 had mixed cells, 30 were without mixed cells, i.e., cells having either colourless or green plastids, and in the rest he found it difficult to differentiate the mutant plastids from the normal ones due to little difference between them.

Materials and methods The fresh leaves were taken from the plants growing at Pakistan Forest Institute, Peshawar. To study the position of plastids in the stomata of the lower epidermis and for the comparative study of the size of the stomata the peeled skin was placed on a clean slide in a drop of water covered with a coverslip. The slide with the material was placed in a desiccator connected with a water suction pump to remove the air bubbles. The material was observed under a microscope (Olympus) with magnifications eyepiece 15X and objective oil 100X. The diagrams were drawn with the help of a camera lucida (Atago) and the areas were measured by using a planimeter (Allbrit).

Observations. *Dahlia variabilis* is grown in the beds and the gardens of Pakistan Forest Institute, Peshawar. Two plants out of 458 were with the variegated leaves. The leaves were having different sizes of colourless sectors (Fig 1 and Fig 2). In one plant these patches were the result of leaf deformation since the size of the cell or the cell division itself was unable to keep pace with the green portions of the leaves during development, whereas the other plant did not show such deformation and had only white patches on the normal green leaves. A count was also made in the same plant growing in the beds near Forest Rest House, Changa Manga. It was noticed that one plant out of 150 had the same type of variegation.

Table 1

The size of the stomata with normal plastids.

Mid Class Value	40	50	60	70	80	90	100
Frequency	3	9	28	39	15	5	1

Mean : 66 $cm^2 \pm 0.84805183$ (S.E.)

Table 2

The size of the stomata with the mixed plastids.

Mid Class Value	40	50	60	70	80	90	100
Frequency	20	34	29	16	1	—	—

Mean : 55 $cm^2 \pm 1.0200019$ (S.E.)

Fig 1. Plant showing variegated leaves.

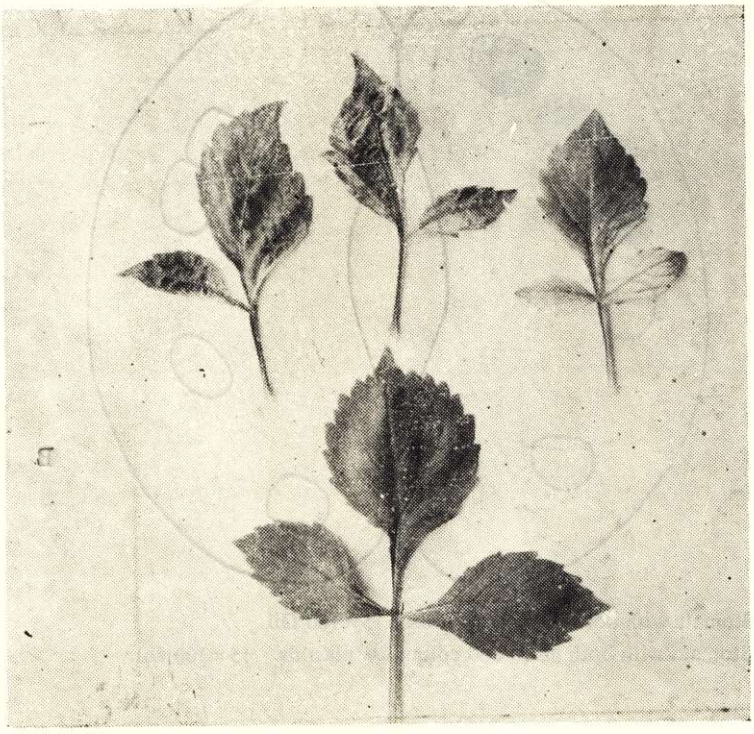

Fig 2. Variegated leaves and a normal leaf.

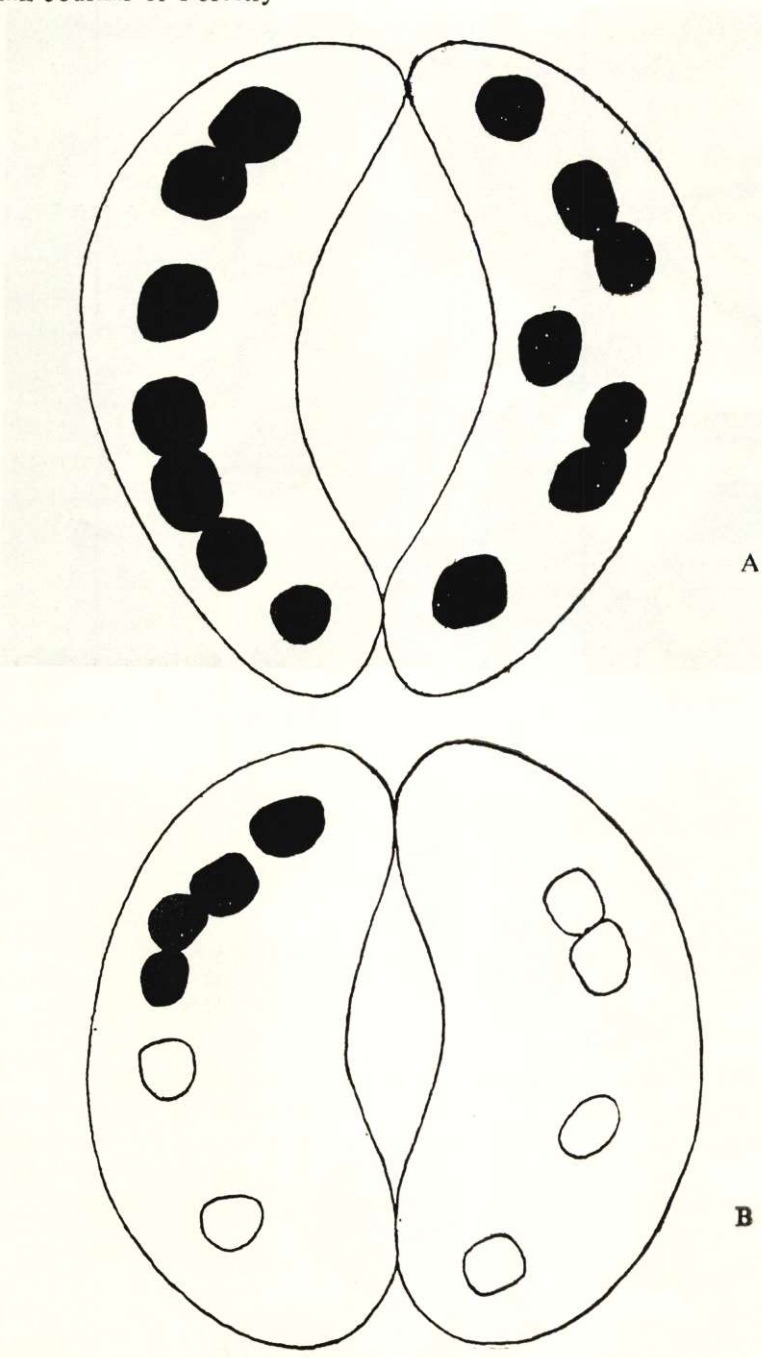

Fig. 3. a. Stomata with normal green plastids. 15 × 100 Oil.
b. Stomata with both green and colourless plastids. 15 × 100 Oil.

Table 3

Number of plastids per stomata of the normal green leaves without any mixed cell.

Mid Class Value	0	3	6	9	12	15	81	21	24
Frequency	1	2	3	4	11	29	35	14	1

Mean : 16 ± 0.4697471

Table 4

Number of normal plastids per stomata in a mixed cell i.e., one with normal green plastids along with the colourless ones.

Mid Class Value	3	6	9	12	15	18
Frequency	1	3	7	8	67	14

Mean : 14 ± 0.2862

Table 5

Number of colourless plastids per stomata in a mixed cell.

Mid Class Value	1	2	3	4	5	6
Frequency	35	30	25	8	2	1

Mean : 2 ± 0.11281521

All the three types mentioned above showed the colourless and the green plastids lying together in the same stomatal cells. In the young leaves the number of mixed cells i.e., the stomatal cells having normal green plastids along with the colourless ones (Fig 3) is greater than that in the older leaves due to two reasons. First, the sectors established by the separation of the two types of plastids during cell division are completed. Second, in the mature leaves the colourless plastids start diffusing even in the complete colourless stomata. The colourless plastids are difficult to identify due to the diffusion and the degeneration.

The sizes of the stomata with the normal plastids and that with the mixed type are 66 $cm^2 \pm 0.848$ and 55 $cm^2 \pm 1.02$ respectively (Table 1 and 2). The average number of plastids per stomata was also counted (Table 3, 4 and 5) The average number of plastids in the green leaves' stomata was 16 ± 0.47 whereas the average number of green plastids

in the mixed stomatal cells was 14 ± 0.286 and the average number of colourless plastids in the mixed type of cells was 2 ± 0.113. The average total number of plastids in a mixed cell is:

$$14 \pm 0.286$$
$$2 \pm 0.116$$
$$16 \pm 0.399$$

Discussion. The two plants studied belong to the group for which the explanation is rather easier. Three types of variegation have been described.

(1) The mixed type in which both the mutated and the normal plastids lie within the same cell The origin of this type is from a zygot which has the normal and the colourless plastids while by the further divisions the cells got both the types of plastids from the mother cell, thus resulting in the variegation and the cells with the mixed plastids can be observed. It is purely a plastid mutation.

(2) The type of variegation which is produced due to the effect of certain genes and chromosomes where no mixed plastids can be observed.

(3) The type in which the cytoplasm inhibits or allows a plastid to develop into a normal green plastid. This factor of the cytoplasm is responsible for the changes in plastids and is its secondary effect. In this case we are not expecting the normal and the mutated plastids within the same cell. It is a working hypothesis without much proof.

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