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INVESTIGATIONS OF THE INHERITANCE OF FLOWER VARIEGATION IN *MIRABILIS JALAPA* L. 3. SOMATIC CHROMOSOME NUMBER, 4. DISTRIBUTION OF THE PIGMENTS AND 5. CHROMATOGRAPHIC STUDIES

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SUMMARY

The somatic chromosome number was found to be 58 for plants with uniformly coloured flowers as well as for plants with variegated flowers. It is concluded that flower variegation is not caused by variation of somatic chromosome number, if any.

Yellow pigments occur in all cells of the flower, while violet pigmentation is restricted to the epiderm. Violet pigment is also limited to the epiderm of the hypocotyl and the lower epiderm of the cotyledon. In the epiderm of the flower in ordinary and glandular hairs and in the epiderm of the hypocotyl R can only manifest itself in the presence of Y, whereas in the lower epiderm of the cotyledon R can do so independent of Y.

Yellow pigments are composed of several betaxanthins, whereas the violet pigment probably consists of one betacyanin. Y conditions the production of a precursor(s) of betaxanthins while R converts part of this (these) precursor(s) into betacyanin. In the lower epiderm of the cotyledon the production of betaxanthins is hampered. Their precursors are present and can be changed into betacyanin.

PART 3. SOMATIC CHROMOSOME NUMBER

INTRODUCTION

In the course of the investigations of the inheritance of flower variegation (ENGELS et al., 1975; SPITTERS et al., 1975) the somatic chromosome number of plants with uniformly coloured and variegated flowers has been determined. There was a possibility that the variegation is caused by variation of somatic number.

MATERIAL AND METHODS

Seeds harvested on plants 1 (yellow/red variegated), 4 (white) and 5 (red), as described by ENGELS et al. (1975), were germinated. Chromosomes were counted in the cells of root-tips of seedlings which had been kept for 8 hours at about 4 °C. Prior to counting, the root-tips were put for 2 h in 2% colchicine, then rinsed with water, fixed in ethanol-chloroform-ice acetic acid (6:3:1), again rinsed with water and macerated in 1N HCL for 9 min at 58–60 °C. After staying in the Feulgen staining fluid for at least 1 h, followed by 10 min in a pectinol solution the material was post-treated with carmin acetic acid or orcein and squashed. The slides were observed under a phase contrast microscope.

RESULTS

The somatic chromosome number of the uniformly coloured plants (29 cells counted) and of the variegated plants (20 cells counted) proved to be $2n = 58$. The chromosomes are very small, three pairs being longer than the others (Fig. 1). Occasionally satellites have been observed for the biggest pair.

Our counting ($2n = 58$) agrees with the numbers given in the literature: SHOWALTER

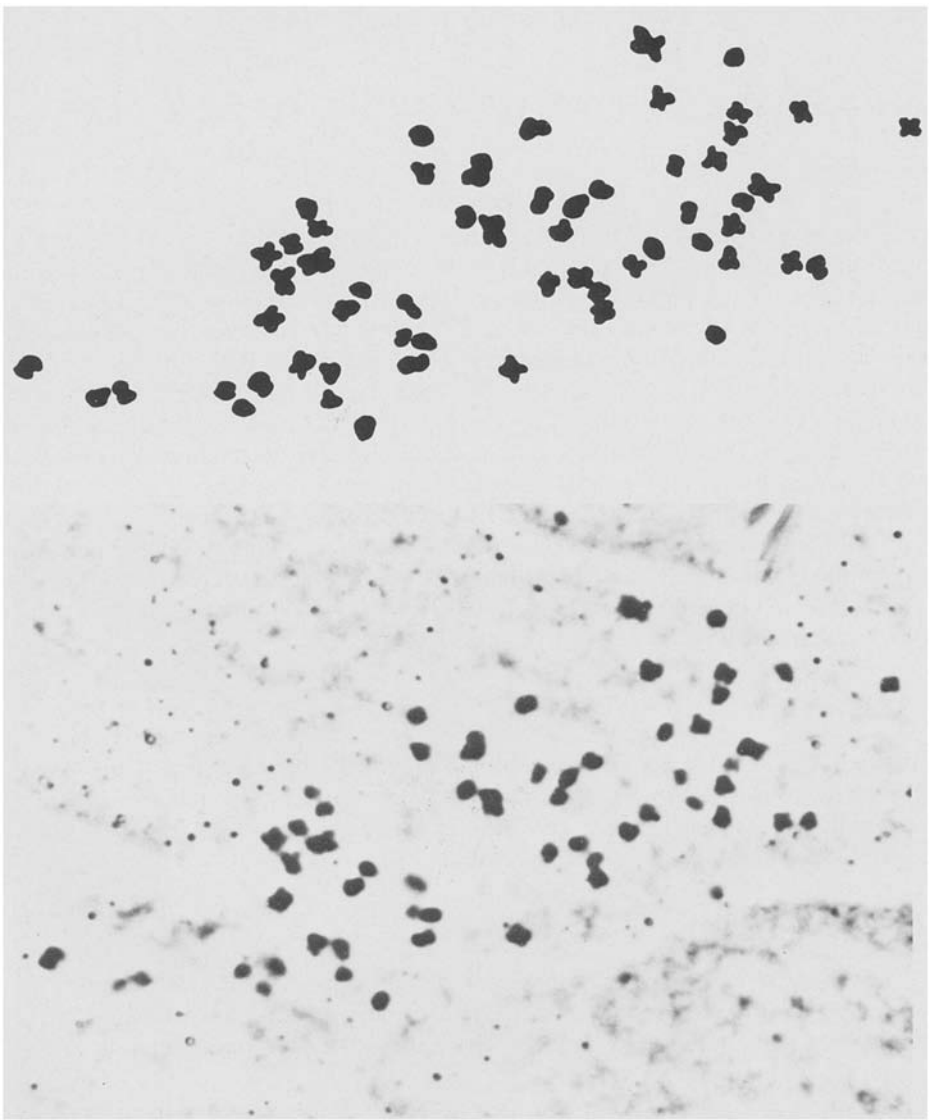

Fig. 1. Mitotic metaphase in *Mirabilis jalapa*, showing $2n = 58$. The drawing is prepared from several different photographs. Enlargement ca. × 2200.

(1935), 58; PRAKKEN (1944), 58; STROUN et al. (1960), 58; KRUSZEWSKA (1961), 58; NAKAJIMA (1958) found $2n = 54$. An early figure published by TISCHLER (1928) and based on work done before 1909 is c. 32.

Like STROUN et al. (1960) no variants of number have been observed in the rootlets of the plants with fully coloured flower or with variegated flowers. Although it is possible that variants of number occur in the flower (calyx), from the above data and the data collected in our investigation (ENGELS et al., 1975; SPITTERS et al., 1975; VOSSELMAN et al., 1975; this article) it can be concluded that flower variegation cannot be caused by variation of somatic chromosome number, if any.

PART 4. DISTRIBUTION OF THE PIGMENTS

INTRODUCTION

During the investigations of the inheritance of pigment production in uniformly coloured and variegated flowering plants of *Mirabilis jalapa* L. a study was made of the distribution of the pigments in the various parts of the perianth. SHOWALTER (1934) already pointed out that the colour of various parts of a uniformly coloured flower may differ in intensity. For instance the colour of the limbs of the perianth differs slightly from that of the perianth. This could be caused by different pigments, but is more likely brought about by a tapering-off of the inner layers of the limbs towards the edge. These inner layers could contain a different pigment or pigment mixture than the epiderm. The boundary between a coloured group of cells and the differently coloured adjacent tissue could be distinct or not. In the last case, a vagueness of boundary could be caused by diffusion of the pigments into neighbouring cells. Ordinary hairs could be of great help because of their lineary arranged cells. Hairs of *M. jalapa* have been described by INAMDAR (1968/69) but he does not mention the presence of pigments.

MATERIAL

In our work hairs and strips of the petal surface on the perianth and strips of the cotyledons were made. Flowers of yellow, red, white, white/red and white/yellow flowering plants were used. These were studied microscopically. See part 1 (ENGELS et al., 1975) for a description of the parental plants.

RESULTS AND DISCUSSION

The perianth of *M. jalapa* exists of an upper and a lower epiderm with parenchyma between them. The epiderms contain plastids. Ordinary hairs occur on both of them, while many glandular hairs are present where the epiderm covers the ends of the main ribs. These hairs differ in shape.

In yellow and pale yellow flowers the cells of the epiderm and parenchyma contain yellow pigments only. The epiderm cells are, on the average, a paler yellow than the parenchyma cells. The ribs are an intense yellow. In reddish coloured flowers the

violet pigments are mainly restricted to the epiderm, while the parenchymatous cells remain yellow. In rose red flowers the violet pigmentation is much more intensive than in orange flowers. In the rose red flowers a few of the neighbouring parenchymatous cells are also violet. Nearer to the limbs, the parenchymatous tissue becomes thinner, which results in a slightly different colour of the limbs of reddish flowers. In variegated flowers there is no distinct boundary between differently coloured groups of cells.

From the centre of differently coloured parts of the adjacent tissues the cells are decreasingly less intense in colour.

So the colour of a flower is determined by the presence of yellow pigments in the cells of the parenchyma and possibly the epiderm and by the presence of violet pigments in the cells of the epiderm.

Violet pigments very likely diffuse to neighbouring cells, from cells producing these pigments, hence making the boundary between violet pigmented tissue and neighbouring tissue diffuse.

In white flowers no pigments have been observed.

Cells of ordinary hairs of flowers with a violet epiderm are either violet or limpid. Sometimes violet cells are seen near to the tip of the hair, while limpid cells occur between these cells and the violet cells of the foot of the hair. Very rarely a pale yellow pigmentation has been observed in these hairs. Hairs from yellow and pale yellow flowers are mostly limpid or, rarely, pale yellow. In contradiction to ordinary hairs glandular hairs of uniformly coloured flowers show a large variation of colours. The cells of the foot of the glandular hair in general have the colour of the adjacent epidermous cells. Towards the tip of the hair the colouring becomes less intensive especially for the red flowers. The following sequences have been observed in glandular hairs (from foot to tip): 1) rose red to orange to yellow, 2) rose red to rhodamine purple to rose red to rhodamine purple, 3) orange to rhodamine purple to orange, and 4) rose red to yellow to pale rhodamine purple to rose red.

It is interesting that production of the violet pigments is restricted to the epiderm, because this has also been observed for the epiderm of the hypocotyl and the lower epiderm of the cotyledon (VOSSELMAN et al., 1975). This means that R (ENGELS et al., 1975) can only manifest itself in the epiderm of these plant parts. But in the flower, the glandular and ordinary hairs and the epiderm of the hypocotyl Y is necessary for the production of violet pigments. For the cells of the lower epiderm of the cotyledon it appears that R may express itself irrespective of the presence of Y.

PART 5. CHROMATOGRAPHIC STUDIES

INTRODUCTION

Investigation of the genetics of the flower colour was accompanied by chromatographic studies as it was hoped that these would indicate the pigments and their concentration, since together they determine the colour.

The genus *Mirabilis* belongs to the Nyctaginaceae which are classified under the Caryophylales. These are characterized by betaxanthins and betacyanins. Dosage

effects play a role because the alleles Y and R are incompletely dominant over alleles y and r respectively (ENGELS et al., 1975).

REZNICK (1955) investigated red, yellow and variegated flowers of *M. jalapa*. In his work he observed that red flowers give one red spot, which has another Rf value than the red spot of the red hypocotyl of *Beta vulgaris*. Its Rf value is equal to that of one of the red spots of the flowers of *Bougainvillea spectabilis*. This species also belongs to the Nyctaginaceae.

PIATELLI et al. (1964, 1965) investigated red and yellow flowers respectively. They found in red flowers the aglycones betanin and isobetanin. In yellow flowers they observed nine betaxanthins of which eight could be identified. Furthermore, they observed two phenols: tyramine and dopamine.

The colour inheritance of the hypocotyl[1] colour of *Beta vulgaris* is identical to that of the flower colour of *M. jalapa*. The hypocotyl of red table beet (genotype YYRR) possesses three betacyanins and two betaxanthins (NILSSON, 1970). URBAN (1955) found betaxanthins in yellow orange fodder beets. In red varieties he also observed betacyanins. The difference in intensity of red was caused by different concentrations of the pigments. Not by an absence or presence of certain pigments. No whitish beetroots have been investigated. Thus betacyanins are only observed in the presence of betaxanthins. This may point to a chemical reaction conditioned by Y to produce betaxanthins, or its precursor(s) while R induces a partial production of betaxanthins or its precursor(s) into betacyanins. Other minor genes must play a role with the production of different betaxanthins and betacyanins. The same processes probably occur in *M. jalapa*.

MATERIAL

Flowers of white, yellow, pale yellow, magenta rose, orange, scarlet red, rose red and rhodamine purple flowering F_2-plants of various crosses were used. These plants grew in the greenhouse. The flowers were crushed and pigments extracted with BAW (12:3:5). Whatman III paper was used. With a UV lamp (type TL 900, wave length 350 nm) the chromatograms were studied. For spectrophotometric studies spots were cut out and the pigment eluted. A Double Beam Spectrophotometer Hitachi Perkin-Elmer 124 was used. This apparatus was connected to a Hitachi Perkin-Elmer 165 Recorder for the preparation of 'total' spectrograms.

RESULTS

The results of the work have been presented in Table 1. In white flowers no betaxanthins and betacyanins are found. The difference between the yellow and red flowers is the presence of a magenta spot (spot 1) in the chromatograms of the latter. This must be a betacyanin or more than one in case they have the same Rf-value. The only chromatogram deviating from red flowers, though in a small degree, is that of rhoda-

[1] The red table beet and the upper $\frac{1}{2}$–$\frac{3}{4}$ part of the fodder beet is a thickened hypocotyl, while the sugar beet is mainly a thickened root.

Table 1. Chromatographic results.

Colour of spot		Intensity	Rf	Max.	Max.	Flower colour[4]			
daylight	UV light 350 nm	in UV light[2]	(BAW 12:3:5)	H_2O (nm)	H_2O + HCl (nm)	white	yellow	rose red, orange, magenta rose[3]	rhod. purple
1. magenta	magenta	5	0.017	536	536	−	−	+	+
2. yellowish	blue green	2	0.07	456	456	−	+	+	±
3. yellowish[1]	yellow green	3	0.09	480	477	−	+	+	+
4. yellowish	yellow green	1	0.14	475	475	−	+	+	−
5. yellowish	blue	4	0.20	476	458	−	+	+	+
6. yellowish	green	4	0.22	473	455	−	+	+	±
7. yellowish	green blue	2	0.28	471	471	−	+	+	+
8. −	pale blue	5	0.51	−	−	+	+	+	+
9. −	blue	5	0.61	−	−	+	+	+	+
10. pale yellow	red	1	0.94	−	−	−	+	+	+

[1] This spot is composed of two pigments, which could often not be distinctly seen.
[2] 5 intensive – 1 vague.
[3] The colours have been grouped. The difference is the proportion of the component pigments between the yellow pigments at one side and the red pigments at the other side.
[4] + = present, ± = vague to very vague, − = absent.

mine purple flowers. In the latter, pigment 4 (spot 4) seems to be absent, while pigments 2 and 5 are only present in small quantities.

The genotype of rhodamine purple plants is YyRR. In the presence of two R-alleles one Y-allele only apparently results in a limited production of some betaxanthins, causing the difference in colour of the flower. For other genotypes more refined investigations to determine the amount of pigments are needed for a study of the relation between genotype and types of pigments and their concentrations. However, the dosage effects of Y and R (see Table 1 in ENGELS et al., 1975), which result in different concentrations of the pigments, determine together with environmental influences the colour of the flower. This allows only a rough grouping of the plants into genotype within reddish and within yellowish classes. Therefore, it is concluded that our method of chromatographic studies is not better than 'observation by eye' in determining the genotype of a certain plant.

It is difficult to compare the results obtained by REZNIK (1955) and PIATELLI et al. (1965) with our results. Maybe that differences in growing conditions, laboratory procedures and genetic background have played a role. However, it is very likely that the two fluorescent spots of the white and coloured flowers (Table 1) are the same two phenols identified by PIATELLI et al. (1965) as tyramine and dopamine.

It is also probable that, as with *Beta vulgaris*, Y conditions the production of betaxanthins, or its precursor(s) and that R effects that of betacyanins, by converting a precursor of betaxanthin into a betacyanin.

In the lower epiderm of the cotyledon these precursors must be present because, although the production of betaxanthins is blocked, betacyanin can be produced when R is present.

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