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AN INVESTIGATION INTO THE ANATOMY OF THE SHOOT APEX OF PETUNIA HYBRIDA IN CONNECTION WITH THE RESULTS OF TRANSFORMATION EXPERIMENTS

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SUMMARY

From observations of flower colour chimeras of *Petunia* the conclusion can be drawn that dominant alleles of corolla colour genes can only express themselves in those cells in which these alleles are present. Anatomical studies of shoot apices of eight-day-old seedlings show that at this stage of development of the plant three independent cell layers are already present in the shoot apex, viz., two tunica layers and a corpus. In his interpretation of the results of his transformation experiments with *Petunia*, Hess did not take the existing differentiation of the shoot apex in his seedling material into account.

1. CHIMERAS RESULTING FROM THE INCIDENCE OF MUTATIONS IN SOMATIC CELLS

In the course of studies of the flower colour genetics of *Petunia* an appreciable number of flowers were observed in which mutations in the floral apex had produced a larger or smaller sector with a deviating pigmentation of the corolla. These always sharply delimited sectors indicate that the phenotypic expression of dominant alleles of corolla colour genes remains restricted to those cell groups in which the alleles in question are present.

In other cases plants are found in which the deviating flower colour is not confined to a part of the corolla but occurs in the whole flowers, again within a sharply delimitated sector of the individual plant. In a specimen with deep magenta flowers which was heterozygous for a pigment intensity factor, for instance, a sector was observed in which the branches bore flowers of an aberrant type.

These aberrant flowers were pale magenta except in the midpetaline areas where a fairly broad zone remained deep magenta. By taking cuttings of the aberrant branches plants were obtained which produced flowers of the deviating type exclusively. Selfing of these plants yielded a progeny segregating in flower colour into 75% deep magenta and 25% pale magenta. Flowers with the aberrant corolla type were not produced, however; nor were they observed in subsequent generations of this progeny. This permits the conclusion that in the aberrant flowers (and the "completely" aberrant plants) the mutation responsible for the colour of the corolla is located in the epidermis alone, whereas the subdermal layer of the shoot apex from which the gametic cells are derived did

not undergo such an alteration of the genotype. This is clearly indicative of a periclinal chimera. The independence of the dermal cell layer in respect of the subdermal one was maintained for the several years during which this particular chimerical form was propagated vegetatively.

In those incidental cases in which a sharply delimited sector of a whole plant exhibited an aberrant corolla pigmentation this sector never exceeded one third of the plant body. The obvious deduction is that this must be attributed to the presence of at least three initials of the outer tunica layer (the dermatogen) of the shoot apex. In other instances somatic mutations were observed which had an influence on the chlorophyll synthesis resulting in changes in the colour of the leaves. Since the chlorophyll containing mesophyll is most probably of subdermal derivation (compare SATINA & BLAKESLEE 1941), these leaf pigment mutations must have occurred in the subdermal layer of the shoot apex. As the extent of this aberrant leaf colour also never exceeded one third of the whole individual, there is, accordingly, good reason to assume the presence of at least three initials of the subdermatogenic cell layer of the shoot apex.

2. THE STRUCTURE OF THE SHOOT APEX

It follows from the studies of flower and inflorescence initiation in *Petunia* by CORNU & BUGNON (1971) that in this taxon the shoot apex consists of three manifestly independent cell layers. In view of the fact that HESS (1969a) used eight-day-old seedlings of *Petunia*, we tried to ascertain if this differentiation of the shoot apex is already completed at this early developmental stage. Shoot tips were fixed in CRAF I and III, dehydrated in a tertiary butanol series, embedded in Paraplast and sectioned. The 5 μm sections were stained with Safranin-Astra Blue. *Fig. 1* shows a longitudinal (median) section of a shoot apex of such a seedling. The absence of periclinal division walls in the cell layers 1 and 2 indicates that a differentiation into two tunica layers and a corpus has already taken place. This agrees with the restriction of somatic mutations, apparently having occurred in the dermatogen, to the epidermis, and with the absence of such aberrant features in the sexually produced progeny.

3. TRANSFORMATION EXPERIMENTS WITH PETUNIA

Hess published the first results of his transformation experiments in 1969. Eight-day-old seedlings of a white-flowering mutant (34d10) were brought in contact for 48 hours with DNA extracted from a red-flowered cultivar, or with DNA from the same white-flowering mutant. The red-flowered cultivar, indicated as the "cyanidin type" on account of the nature of the corolla pigment, was reported by the author to be homozygous for two genetical factors each of which is capable of inducing anthocyanin synthesis in the corolla. The white-flowering strain was a homozygous recessive for both factors. Of the 201 plants treated with the heterologous DNA which reached the flowering stage, 53 (i.e., 27%) produced reddish to red flowers. The reddish colouration was mostly

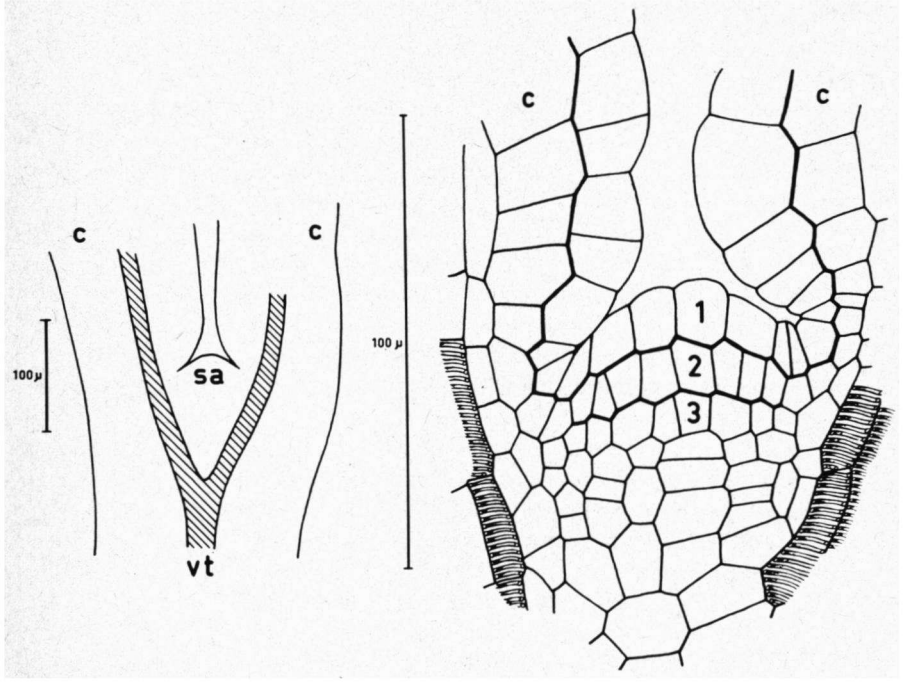

Fig. 1. Longitudinal section of a shoot apex of an eight-day-old seedling of *Petunia*. In the more detailed figure at the right the two already independent tunica layers (1 and 2) and corpus (3) are indicated.
v.t.: vascular tissue, s.a.: shoot apex, c: cotyledons.

weak, but occasionally it was fairly intense. Of the 63 plants that came to flower after the treatment with homologous DNA, six (i.e., 9%) exhibited a faint red colouration. Hess is of the opinion that in the latter case the pigment synthesis must be ascribed to the effect of environmental factors, since plants of the 34d10 strain occasionally produce progeny with faintly pigmented corollas. He points out that the group of plants treated with the heterologous DNA not only exhibited a much higher percentage of pigment-producing individuals, but also much clearer differences in the degree of pigmentation, a fairly deep corolla pigmentation only having been observed in the group treated with heterologous DNA. Of the 53 plants of the first group showing corolla pigmentation a number selected for the deepest colour were subsequently studied genetically by means of selfings. The number of individuals analysed in this way was not stated, but from more recent papers by the same worker (HESS 1969b, 1970) it can be deduced that presumably not more than two plants were studied in this way. The F_1 produced after selfing and the F_2 subsequently obtained bore pigmented flowers exclusively. Hess believes that these results can be explained by assuming that, as a result of the treatment with heterologous DNA, the seedlings of the white-flowered mutant 34d10 had

become homozygous for at least one of the two pigment-inducing factors of the red-flowering plants, in other words, that the change must be attributed to a transformation initiated by the DNA of the "cyanidin type".

In a second publication on the subject (HESS 1969b) it was stated that a duplication of the experiment yielded similar results. In a discussion of his results the author compares the frequencies of transformation with those found in transformation experiments with bacteria and arrives at the following conclusion: "*Die Transformationshäufigkeit lag bei rund 15%, also durchaus im Rahmen dessen, was man bei Bakterien hätte erwarten dürfen*". Although Hess immediately continues with the remark that *Petunia* is a pluricellular organism and not a one-celled one, it must be concluded that he visualises the transformation in a higher plant *(Petunia)* as the incorporation of exogenous DNA in the chromosomes in very much the same way as the incorporation of donor-DNA in recipient bacterial cells takes place.

From a more recent publication by the same worker (HESS 1972) it appears that a continuation of the transformation experiments with Petunia on a larger scale did not yield the same results. In his first set of experiments he had obtained four so-called "transplantation homozygotes", from about 800 treated seedlings, but in later experiments over 12,000 white-flowered seedlings of the strain 34d10 yielded only one "transplantation homozygote" after treatment with DNA extracted from the red-flowered "cyanidin type" cultivar. In addition he could establish that three plants exhibiting corolla pigmentation were heterozygous for one of the factors inducing anthocyanin production. One of these three individuals was regarded as a chimera, because not all flowers of this plant contained a red pigment. The author remarks that the incidence of chimeric specimens in the experiments may be expected. Hess could not explain the discrepancy between the results of his first series of experiments (HESS 1969a, 1969b) and those of his later ones (HESS 1972). The first experiments having mostly been carried out in Cologne-Vogelsang and the later ones in Hohenheim, Hess does not preclude the possible effect of the different environmental conditions in the two experimental sites.

4. DISCUSSION

If, in the experiments reported by Hess, exogenous DNA indeed became incorporated in *Petunia* chromosomes in a fashion comparable with the incorporation of donor-DNA in bacterial cells, one might expect that, owing to the architecture of the shoot apex and the characteristics of floral pigmentation chimeras of *Petunia*, anthocyanin pigmentation would occur in sharply delimited sectors of the individual plants. In his reports relating to this first experiments, HESS (1969a, 1969b) did not mention the incidence of chimerical forms at all. As it is inconceivable that such a striking phenomenon as sectorial pigmentation would have been left unmentioned, one must suppose that all plants recorded as red-flowered in the experiments exhibited a uniform pigmentation of all flowers in all parts of the plant.

Only in more recent experiments carried out at Hohenheim and not readily comparable with the series begun at Cologne-Vogelsang did HESS (1972) note specimens in which pigmentation did not occur in all flowers. However, no incidence of a clear-cut sectorial distribution of pigmentation being mentioned, the occurrence of chimeras is by no means certain. In some cases the presence or absence of pigmentation is attributable to the effect of the environment. During genetical studies of floral pigmentation in *Petunia* carried out in Amsterdam, plants which produced such small quantities of floral pigments that the corolla seemed to be practically white, were repeatedly noticed. Changes in the environmental factors may induce such plants to produce a number of much more conspicuously pigmented flowers. In such cases there is never any sectorial distribution of the pigments formed, either.

As also pointed out by Hess, this hypothesis for explaining the appearance of pigmented individuals in his experiments implies that heterozygosity for the "transplanted" (dominant) factor will be the rule. That all four specimens with anthocyanin pigmentation, when studied genetically, proved to be homozygous for at least one anthocyanin factor, is attributed by Hess to his choice of plants with the most deeply coloured flowers, so that a selection of homozygosity had taken place. However, he completely disregards the fact that, in view of the autonomy of the tunica layers in the shoot apex, the production of anthocyanins in the epidermis does not necessarily mean that the gametic cells derived from the inner (subdermal) tunica layer also contain factors regulating anthocyanin synthesis.

In his first transformation experiments out of 800 seedlings treated with the heterologous DNA Hess obtained four plants which were homozygously red-flowered (i.e., 0.5%). Since the more deeply pigmented corollas of these plants are according to this author caused by homozygosity of colour-producing factors, and the alleles of factors inducing anthocyanin formation only become expressed in the cell in which they themselves occur, all coloured epidermal cells must have contained at least two dominant alleles for anthocyanin formation. As pointed out before, there are good reasons to assume that there are at least three dermatogen initials in the shoot apex, and this implies that in all three cells an anthocyanin-inducing allele must have become incorporated twice. In view of the homogeneously pigmented progeny the same double transformation must be postulated for the initials of the subdermal cell layer of the shoot apex. If one assumes that the incorporation of genetic material in the chromosomes is basically comparable with the transformation process in bacteria, there is no a priori reason to suppose that the incorporation of one gene in a particular chromosome increases the chances of the incorporation of that same gene in another chromosome of the same, or of an adjoining, cell. If one starts from the high transformation frequency of 15% mentioned by Hess, the chances of a simultaneous homozygous modification of all initials of the dermal and subdermal tunica layers in the shoot apex are nevertheless extremely small. Even in the improbable situation of a single initial of each of the two tunica layers, the percentage of 0.5 is much higher than one might expect starting from the above-

mentioned transformation frequency. It becomes quite clear from the above-mentioned considerations that Hess did not take the existing differentiation within the shoot apex into account when he attempted to interpret his experimental results. A satisfactory explanation of the experimental results reported by Hess is not possible on the basis of the available data. One might consider the possibility of a frequent replication of certain DNA fragments penetrated into a cell followed by a dispersal of the fragments to the adjoining cells by means of a process resembling a virus infection. The high frequency of incorporation of exogenous DNA into the chromosomes might in this instance be ascribed to a strong multiplication within the cells, so that a large number of replicates of the DNA fragments in question become available for incorporation. Whatever the case may be, the appreciable percentages of plants with floral pigmentation after treatment with heterologous DNA recorded by Hess in his first series of transformation experiments suggest a process comparable to a virus infection rather than to bacterial transformation.

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