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AN INVESTIGATION INTO SPONTANEOUS REVERSIONS IN A DWARF MUTANT OF PETUNIA HYBRIDA IN CONNECTION WITH THE INTERPRETATION OF THE RESULTS OF TRANSFORMATION EXPERIMENTS

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SUMMARY

A spontaneous mutant of *Petunia* is described, which is characterised by the small size of its shoots, leaves and flowers. Apart from showing these size differences, the mutant has more rounded, suborbicular leaves with a wrinkled surface.

In his transformation experiments with *Petunia* Hess obtained plants which according to his description agree in all details with this "dwarf" mutant, including the frequent occurrence of shoots with characters corresponding with those of the original plant form. He assumed that this particular type of plant had arisen as the result of a transplantation of a gene for leaf shape.

However, our studies of the dwarf mutant revealed that the phenomena described by Hess can be explained as well by the incidence of somatic mutations and reversions in one of the two tunica layers of the shoot apex, rather than by the transplantation of gene material.

The study of revertants of the dwarf mutant yielded data pointing to a predominant influence of the dermal layer on the morphogenesis of the whole plant.

1. A DWARF MUTANT OF PETUNIA HYBRIDA

In 1962, among a progeny obtained by selfing, plants were observed whose constituting parts were all so much smaller than those of the parent plant that they may be regarded as dwarfs. These plants, not previously recorded, did not only differ from the parent plant in size but also in several other respects. The relatively broader, and consequently more rounded, suborbicular leaves had an undulating margin and a wrinkled surface. The internodes were so short that the stems were almost hidden by the foliage. The flowers were not only smaller, but also differed in shape because the limb of the corolla was much more deeply incised *(fig. 2c)*.

Selfings, mutual crosses of dwarfs, F1 and F2 of dwarf × normal and the backcross F1 × dwarf indicated that the dimunitive phenotype is induced by mutation of only one gene. Individuals heterozygous for this mutation exhibit the normal habit and flower shape. A striking peculiarity of such mutants is the fairly high rate of back mutations in somatic cells which, if they occur in meristematic tissue, reveal themselves quite clearly by changed morphological features. If such a reversion occurs in a leaf primordium or in a floral apex, a larger or smaller area of the leaf or the flower exhibits the morphological characteristics of the normal form depending on the relative degree of ontogenetic

Fig. 1. Dwarf mutant with a side-shoot bearing leaves of normal size and shape, originated by a spontaneous reversion in the second tunica layer.

differentiation of the meristematic tissues and on the location of the mutated cell in the primordial organ. A reversion in a cell of the apical meristem produces in the mature shoot a sharply delimited sector bearing leaves of normal size and shape. The extent of this sector is dependent on the location of the reverted cell in the vegetation point. Leaves and flowers developing on the borderline of such a sector will partly retain their dwarf features. These reverted sectors are of different sizes but never extend over more than about one-third of the total circumference of the stem. If within the limits of such a sector an axillary bud develops into a side-branch, the latter exhibits the normal size and shape of stem and leaves *(fig. 1)*. Such reversions occur both in young and in older plants. For this reason the percentage of dwarf specimens with reverted side-shoots increases as the plants are under observation longer. During a period of 5 months in a lot of 1480 dwarfs 21 individuals (i.e., 1.4%) were found which had developed normal side-branches. In another lot of 1707 dwarf mutants, in a similar period 37 of such reversions (i.e. 2.2%) were noted.

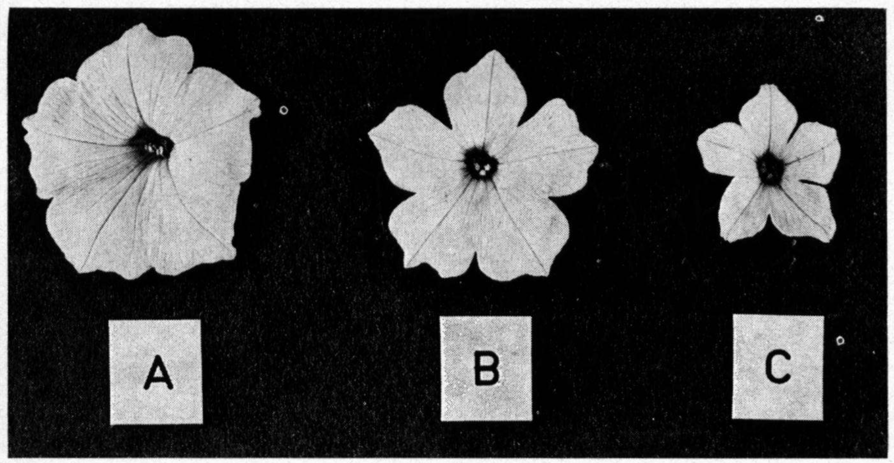

Fig. 2. a. flower of normal type, developed on a dwarf mutant as the result of a spontaneous reversion in the dermal layer (= first tunica layer of the shoot apex); c: flower of the dwarf mutant; b: flower of an "intermediate" type, originated on a dwarf mutant after a spontaneous reversion in the subdermal layer (= second tunica layer) of the shoot apex.

The reverted side branches are of two types, viz. one, to be referred to as type A, with normal flower *(fig. 2a)* and one, type B with flowers of a size and morphology intermediate between those of normal plants and dwarfs *(fig. 2b)*.

It is noteworthy that the form with the intermediate flower type is by far the most common. Out of 58 revertants only 3 belonged to type A.

2. THE STRUCTURE OF THE SHOOT APEX

For an interpretation of sudden somatic changes in higher plants it is always necessary to involve the structure of the shoot apex in the deductions.

An anatomical study of eight-day-old seedling of *Petunia hybrida* had already revealed that in this phase of development of the plant three mutually independent cell layers can be distinguished in the apical growing zone (Bianchi & Walet-Foederer 1974). The examination of shoot apices of flowering specimens showed that this situation is maintained in adult plants. *Fig. 3* shows a camera lucida drawing of a median longitudinal section through such an apex after fixation in Craf IV and embedding in paraplast (section 7 μm, stained in safranin with Astra Blue counter-staining).

In the outer two cell layers of the shoot apex periclinal division walls were never observed. This points to the presence of three mutually independent groups of initials, two for the two tunica layers and one for the corpus. These observations agree with the figures of Cornu & Bugnon (1971) in their study of the formation of the inflorescence in *Petunia hybrida*.

Since the tissues of the flowers are presumably almost completely and those

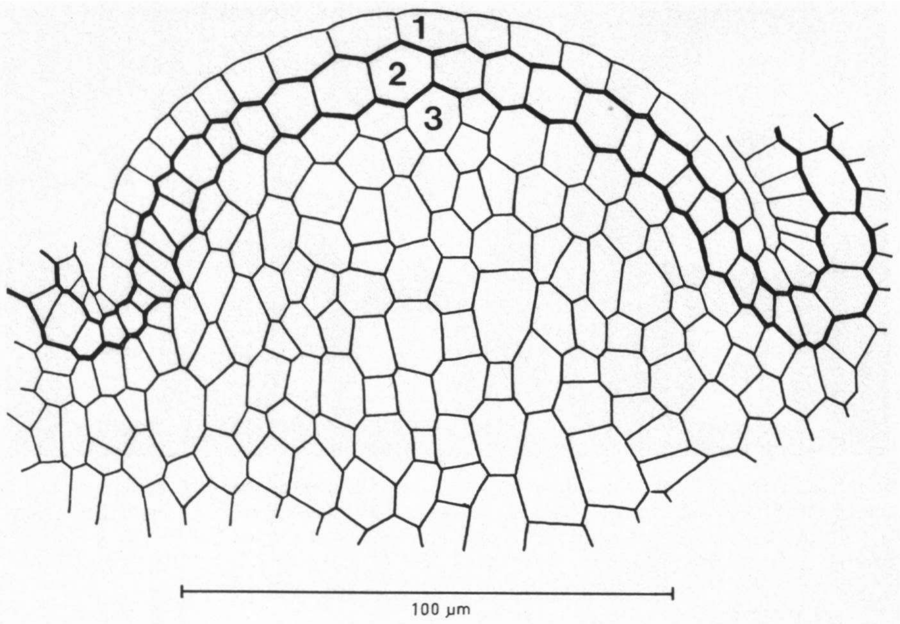

Fig. 3. Longitudinal section of the shoot apex of a flowering specimen of *Petunia*, showing the two independent tunica layers (1 and 2) without periclinal division walls and the corpus (3).

of the leaves for their greater part, derived from the two tunica layers alone (compare SATINA & BLAKESLEE 1941), one may accept that only back mutations occurring in these layers will have a manifest influence upon the shape and the size of the flowers and the leaves.

3. GENETICAL EXPERIMENTS WITH REVERTANTS

By propagating cuttings taken from reverted shoots plants were obtained which show a normal aspect as far as the shape and size of their stems and leaves are concerned. Selfings were made of 3 plants of type A and 16 of type B produced in this way. In addition 13 of these were back-crossed with the dwarf mutant, by using the diminutive flowers both of the individual on which the reverted shoot had originated and of other dwarf specimens. In the majority of these crosses the revertant was used as the mother plant, but in a few cases also the reciprocal crosses were carried out.

3.1. Crosses with the type A revertants

In *table 1* the results are shown of the selfings of three revertants with normal flowers.

It appears that the offspring of all selfings contains only dwarfs. Backcrosses of the revertants PU 3253–1 and PW 5066–1 with dwarf yielded 150 and 261

Table 1. Self-pollinations of type A revertants.

	Parents			Offspring		
Fam.	♀ type A		♂ type A	tot.	norm.	dwarf
PW 5007	PU 3277-1	×	PU 3277-1	91	0	91
PW 5074	PU 3253-1	×	PU 3253-1	174	0	174
PW 5132	PW 5066-1	×	PW 5066-1	63	0	63

descendants, respectively, which were all dwarfs again. The results of these crosses and back-crosses indicate that although these revertants had reassumed the normal morphology and dimensions, they remained homozygous for the factor for dwarf in the subepidermal layer of the shoot apex from which the gametic cells are derived.

If one starts from the fact that the tissues of the flowers and the leaves are to a very large extent formed out of the outer two layers of the apical meristem, the obvious corollary is that the type A revertants arose by a somatic back mutation in the outer tunica layer. These revertants clearly have the character of a periclinal chimera, the genetic constitution of the dermal layer apparently playing a predominant role in the morphogenesis of the whole shoot.

3.2. Crosses with the type B revertants

Table 2 shows the results of selfings of 16 different type B revertants. Generally speaking, these selfings yielded offsprings exhibiting phenotypic segregations

Table 2. Self-pollinations of type B revertants.

	Parents			Offspring			
Fam.	♀ type B		♂ type B	tot.	norm.	dwarf	% dw.
PW 5051	PU 3205A-178	×	PU 3205A-178	89	75	14	15.7
PW 5054	PU 3235A- 1	×	PU 3235A- 1	138	105	33	23.9
PW 5056	PU 3235B- 1	×	PU 3235B- 1	34	21	13	38.2
PW 5057	PU 3236 - 2	×	PU 3236 - 2	84	64	20	23.8
PW 5059	PU 3243 - 1	×	PU 3243 - 1	120	85	35	29.2
PW 5063	PU 3243 - 4	×	PU 3243 - 4	156	138	18	11.5
PW 5068	PU 3245A- 1	×	PU 3245A- 1	293	223	70	23.9
PW 5070	PU 3250 - 1	×	PU 3250 - 1	55	42	13	23.6
PW 5073	PU 3251A- 1	×	PU 3251A- 1	102	75	27	26.5
PW 5076	PU 3254A- 1	×	PU 3254A- 1	264	208	56	21.2
PW 5079	PU 8208 - 13	×	PU 8208 - 13	156	116	40	25.6
PW 5081	PU 8209 - 15	×	PU 8209 - 15	282	208	74	26.2
PW 5084	PU 8222 - 62	×	PU 8222 - 62	210	167	43	20.5
PW 5086	PU 8229 - 4	×	PU 8229 - 4	222	168	54	24.3
PW 5088	PU 8229 - 25	×	PU 8229 - 25	216	168	48	22.2
PW 5095	PU 8233 - 44	×	PU 8233 - 44	93	68	25	26.9
Total				2514	1931	583	23.2

not significantly differing from a ratio of 3 normal : 1 dwarf. Only in the families PW 5051 and PW 5063 a χ^2 test for a 3 : 1 ratio indicated a significant under-representation of the dwarf phenotype ($P = 0.04$ and < 0.001, respectively).

The results of these selfings point to a heterozygosity for the factor for dwarf habit of at least that layer of the shoot apex from which the gametic cells are derived. It follows that the type B revertants originated by the incidence of a back mutation in the second tunica layer. The under-representation of dwarfs recorded in a few cases is most probably attributable to a decreased vitality of the mutants owing to a poor development of the root system.

The results of crosses between 13 type B revertants and dwarfs are summarised in *table 3*. All these crosses yielded a 1 normal : 1 dwarf segregation. A χ^2 test for a 1:1 segregation applied to each offspring and to the total of segregated offsprings yielded P values which were > 0.10 without exception.

Table 3. Crosses of type B revertants with the dwarf mutant.

Fam.	Parents			Offspring			
	♀ type B		♂ dwarf	tot.	norm.	dwarf	% dw.
PW 5053	PU 3205A-178	×	PU 3205A-178	107	53	54	50.5
PW 5058	PU 3236 - 2	×	PU 5152 - 4	115	55	60	52.2
PW 5060	PU 3243 - 1	×	PU 5007 - 2	213	110	103	48.4
PW 5065	PU 3243 - 4	×	PU 5007 - 1	80	44	36	45.0
PW 5066	PU 3245A- 1	×	PU 5152 - 4	126	67	59	46.8
PW 5067	PU 3245A- 1	×	PU 3245A- 1	118	68	50	42.4
PW 5071	PU 3250 - 1	×	PU 5152 - 2	27	13	14	51.9
PW 5072	PU 3250 - 1	×	PU 5007 - 2	107	51	56	52.3
PW 5006	PU 3254A- 1	×	PU 3254A- 1	78	38	40	51.3
PW 5078	PU 3254A- 1	×	PU 5007 - 1	39	16	23	60.0
PW 5080	PU 8208 - 13	×	PU 8208 - 13	174	84	90	51.7
PW 5083	PU 8209 - 15	×	PU 5007 - 2	144	82	62	43.1
PW 5087	PU 8229 - 4	×	PU 8229 - 4	150	80	70	46.7
PW 5089	PU 8229 - 25	×	PU 8229 - 25	97	46	51	52.6
PW 5091	PU 8229 - 25	×	PU 5007 - 2	120	59	61	50.8
PW 5092	PU 8233 - 19	×	PU 8233 - 19	192	92	100	52.1
PW 5094	PU 8233 - 19	×	PU 5007 - 2	289	133	156	54.0
	♀ dwarf		♂ type B				
PW 5077	PU 3254A- 1	×	PU 3254A- 1	69	36	33	47.8
PW 5082	PU 8209 - 15	×	PU 8209 - 15	165	79	86	52.1
PW 5085	PU 8222 - 62	×	PU 8222 - 62	69	39	30	43.5
PW 5090	PU 8229 - 25	×	PU 8229 - 25	99	55	44	44.4
PW 5093	PU 8233 - 19	×	PU 8233 - 19	86	38	48	55.8
Total				2664	1338	1326	49.8

These results completely confirm the conclusion drawn from the results obtained from selfings that the type B revertants originated by a back mutation in the second tunica layer so that they became heterozygous for the factor causing the dwarf habit form.

Since the anatomical study of the architecture of the shoot apex had revealed the presence of three independent cell strata and the frequency of back mutation in somatic cells in the dwarf mutants appeared to be high, one may expect such mutations to occur in all three independent cell systems of the apical meristem. Starting from the assumption that the type A and type B revertants arose by mutations in the outer and in the subdermal tunica layer respectively, conceivably also revertants resulting from similar mutations in the corpus may be found. Since the tissues of leaves and flowers are largely derived from the two tunica layers, reversions taking place in the corpus have hardly any bearing on the morphology of these organs. They may have some influence on the structure of the stem, however, because the cauline tissues are to a considerable extent formed by cells derived from the corpus of the apical meristem.

In a number of cases on dwarf mutants shoots originated with manifestly longer internodes than is usual in the diminutive phenotype. The leaves of such aberrant shoots, although smaller and darker than normal leaves, differ from those of the dwarf type in their slightly larger size and smoother surface. The flowers of such shoots correspond almost entirely with those of the dwarfs, but in some specimens the filaments are somewhat lengthened so that the anthers are exerted from the corolla-tube. Seed from selfings of such flowers produced a typical dwarf offspring exclusively. The morphological characteristics of the atypical shoots and the results of the selfings, agree with the above-mentioned suppositions.

Experiments are in progress in which attempts are made to rear individuals from tissue cultures of corpus cells of reverted shoots with longer internodes. If the conclusion that this aberrant shoot type is the result of a back mutation in the corpus is right, such individuals will have a normal appearance and they will be heterozygous for the factor causing the dwarf growth habit.

4. HESS' TRANSFORMATION EXPERIMENTS WITH PETUNIA

In his transformation experiments HESS (1970a, b, 1972, 1973) used a red-flowered cultivar which was, on the ground of the chemical nature of its floral pigment, indicated as the "cyanidin type" and a white-flowered mutant 34d10. Apart from a difference in the colour of the corolla, these two phenotypes also exhibited a difference in their leaf morphology. The cyanidin type had more rounded leaves with a wrinkled surface, whereas 34d10 bore the normal, more oblong and flat leaves. Crossing experiments with the two types had shown that "round" is dominant over "oblong" and that this difference in leaf shape is determined by one single gene.

After specimens of young seedlings of 34d10 had been treated with DNA extracted from the cyanidin type, they all developed into plants with the oblong leaf-shape characteristic of 34d10. However, one of the plants yielded a progeny after selfing which contained about 25% specimens with the rounded leaves characteristic of the cyanidin type. These plants which appeared to be homozygous for their leaf shape were referred to by Hess as L1 plants. From the results of crosses between L1 individuals and 34d10 plants, the conclusion could be drawn that the suborbicular leaf type of L1, unlike the original situation, became recessive in respect of the normal oblong type of leaf. Moreover 4% to 8% of the L1 individuals exhibited the peculiarity of producing, in a larger or smaller portion of the plant body, shoots bearing leaves of the oblong 34d10 leaf type. Such reverted shoots also bore flowers of the normal shape. Hess is of the opinion that the more rounded leaf-shape of the L1 plants must be ascribed to the transplantation of a gene for leaf shape, i.e. to a genetical transformation. From the occurrence of L1 plants bearing side-shoots with the 34d10 phenotype he drew the conclusion that the original gene material of the DNA receptor was still present. Flowers of the aberrant shoots of such L1 plants were selfed. The 1476 descendants obtained from these selfings were all agian of the L1 type. Hess concludes that the transplanted gene material responsible for the suborbicular leaf shape also remained present in the whole plant. He is of the opinion that all observed phenomena point to a situation comparable to the one described by Fox & Yoon (1970) in their transformation experiments with *Drosophila* and from which they deduced their exosome model. The transplanted gene is supposed to have become associated with the original gene locus, but not incorporated into the linear chromosome structure. The most important consequence of this model is the presence of two sources of genetic information on the same, single locus, providing the possibility of alternative transcription which may result in phenotypic mosaics.

5. DISCUSSION

A comparison of Hess' L1 plants with the dwarf mutants described in the present paper shows that there is a remarkably close agreement in their characteristics. The leaves of L1 plants are not only suborbicular with a wrinkled surface, but judging from Hess's (1970a, 1972) figures also markedly smaller than those of the 34d10 mutant from which the L1 plants originated. These figures also show that the internodes of L1 individuals are so short that they are completely hidden behind the foliage and that the flowers are considerably smaller and have a more deeply incised corolla limb. The aberrant form is also recessive in this case. Moreover, both Hess' L1 plants and our dwarf mutants exhibit the peculiar feature of producing offshoots with internodes of normal length, larger and flatter leaves and normally shaped flowers. Finally, in either case progenies of seed from flowers borne on sidebranches of normal appearance can be obtained which consist exclusively of diminutive plants.

Since in the case of the dwarf mutant no previous DNA treatment had taken place, this form cannot be attributed to a gene transplantation. Therefore, the possibility that the L1 plants are not the result of transformation, but originated by somatic mutation must by no means be excluded. The remarkable correspondence in morphological characters between L1 plants and dwarf mutants and the high incidence of reversions in somatic cells in the two types of plants may even indicate that both forms are the result of a mutation on the same locus.

That Hess did not find a single mutant with the leaf morphology of the cyanidin type in 10,000 individuals of 20 generations of the line 34d10 produced in the course of 9 years is irrelevant in this connection. The mutant allele being recessive, not the number of controls is of importance but the number of progenies obtained by selfings. This latter number was not reported, but the 10,000 controls in question could not very well have belonged to more than at most a few hundred progenies. For the checking of such a rare happening as the occurrence of a dwarf mutation such a number is much to small.

The claim that a duplication of the transformation experiment yielded a similar result as the first might be accepted as an important argument in favour of the supposition that the L1 plants arose as the result of gene transplantation rather than of mutation, but the concise description of the duplicated experiment (HESS 1973) does not at all prove that the aberrant individuals recorded after the application of heterologous DNA were indeed identical with the L1 plants of the first experiment. In the second experiment in which 20 descendants could be reared from a 34d10 ovary treated with DNA extracted from cyanidin type plants, two individuals of the offspring of 20 exhibited the leaf shape of the cyanidin type. However, selfings did not yield progenies consisting exclusively of plants with small suborbicular leaves, as one might have expected from true L1 plants. In how far the two plants under discussion agreed with the L1 type in leaf size, internode length and flower shape cannot be ascertained from the description of the experimental results. There is no mention of the occurrence of specimens with side branches reverted to the 34d10 type either. That this second experiment was a successful attempt to duplicate the first in which L1 plants appeared remains to be seen.

The striking resemblance between Hess' L1 type of plant and the dwarf mutants amply characterised in the present publication renders it likely that in both cases a mutant originated with a high frequency of back mutation. For an explanation of the features of Hess' L1 plants, in our opinion, the assumption of a transplantation of gene material with an exosome-like behaviour is not at all obligatory.

The genetical analysis of dwarf mutants has made it quite clear that an interpretation of sudden changes in somatic cells of higher plants is only permissible if the architecture of the shoot apex is taken into account. The study of revertants of the dwarf mutant yielded data pointing to a predominant influence of the two tunica layers on the morphogenesis of the whole plant; the contribution of the dermal layer being in some respects greater than that of the second tunica layer.

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