STUDIES ON YELLOW-INCONSTANT, A MUTATING CHARACTER OF *PHARBITIS NIL*.

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CONTENTS.

INTRODUCTION.

A NUMBER of mutating characters have been described in *Pharbitis Nil,* the Japanese morning glory (Imai, 1927 *c*), and of yellow-inconstant, in which green tissues frequently occur in yellow leaves, the writer has already published a preliminary account (Imai, 1927 *a*), while more recently Miyazawa (1929) has also written on this subject. During the past three years I have collected further data now presented in this paper.

THE IDENTIFICATION OF YELLOW-INCONSTANT.

The "Matsushima" variegation, or yellow-inconstant, character was identified by the occurrence of green areas on the yellow leaves. The green tissues, the presence of which is due to a reversional mutation of yellow, cannot, however, be observed on all individuals carrying the yellow-inconstant gene. Owing to this fact, the identification of yellow-inconstant was at times impossible, and it was often erroneously recorded as yellow. In my own strain of yellow-inconstant about 80 per cent. of seedlings were "false yellow," whereas in Miyazawa's mutant strain of yellow-inconstant, the figure was about 24 per cent., the difference being mainly due to the different mutability of the two strains. Under such circumstances, it is difficult to analyse data in which segregation of yellow-inconstant occurs together with that of normal yellow. In 1927, when making observations on yellow-inconstant, I called attention to the presence of fine green spots on the yellow leaves, even on plants apparently yellow. Sometimes the green spots cannot be detected on particular leaves of a yellow-inconstant plant, but further observation on the other leaves will reveal its phenotype. In doubtful cases a hand lens or binocular microscope of low power may help in the detection of the spots. If the plants are making a luxuriant growth the identification is easy. The discovery of the phenomenon by which the character can be identified, *i.e.* by the occurrence of fine green spots on the yellow-inconstant leaves, is of much help in its investigation. The fine green spots are due to mutations occurring at a late stage in the cell generations during the ontogeny of leaves. The mutation is so frequent during somatogenesis that every individual of yellow-inconstant can be distinguished by the fine green spots.

THE YELLOW LOCUS.

Yellow-inconstant is recessive to normal green (Imai, 1927 *a*; Miyazawa, 1929), and gives yellow-inconstant in F_1 when it is crossed with yellow (Miyazawa, 1929). In the latter case segregation for yellow-inconstant and yellow occurs in F_2, with some green mutants. The occurrence of "false yellow" among the yellow-inconstant segregates, however, obscures the genetic relation of yellow-inconstant to yellow, as well as to the green normal. As stated above, we can now identify the yellow-inconstant segregates very accurately by the presence of fine green spots on leaves, and the new data bring out the following points:

(1) Yellow-inconstant appears as recessive, almost exactly in the proportion expected, in F_2 of the cross between yellow-inconstant and normal, as indicated by the data in Table I.

TABLE I.

F_2 *from the cross of yellow-inconstant by normal.*

Cross	+	y^i	y	Total
422 × 435	133	40	0	173
190 × 421	302	99	0	401
Total	435	139	0	574

(2) The relation between yellow-inconstant and yellow also is very simple, yellow-inconstant being dominant to yellow. The majority of F_1 plants obtained by crossing yellow-inconstant with yellow showed the former character. Actually I obtained 53 yellow-inconstant F_1 from reciprocal matings, with two green mutants. The results of selfing the yellow-inconstant hybrids are shown in Table II.

TABLE II.

F_2 *from the cross of yellow-inconstant by yellow.*

Cross	+	y^i	y	Total
421 × 430	1	123	45	169
420 × 34	7	241	77	325
Total	8	364	122	494

The occurrence of a few green plants in this table is due to the mutable property of yellow-inconstant, and they may be here counted as yellow-inconstant, because of their origin from yellow-inconstant. The data show a simple Mendelian segregation, with yellow a recessive to yellow-inconstant.

The F_2 of the green plants obtained among the regular yellow-inconstant F_1 showed segregation for normal and yellow, but not for yellow-inconstant, as indicated in Table III.

TABLE III.

F_2 *of green* F_1 *from the cross of yellow-inconstant by yellow.*

Cross	+	y^i	y	Total
421 × 430	156	0	55	211
420 × 34	88	0	28	116
Total	244	0	83	327

The regular F_1 between yellow-inconstant (y^i) and yellow (y) should be y^i/y in its genotype, but the green F_1 is expected to be $+/y$, for the presence of + is due to the transformation of y^i.

As repeatedly proved, yellow is allelomorphic to normal and, as mentioned above, yellow-inconstant also is allelomorphic to normal and to yellow; therefore the three genes, normal, yellow-inconstant and yellow, constitute a triple series of allelomorphs situated at the yellow locus. This locus is in the yellow chromosome (Imai, 1929). Linkage studies also favour the interpretation in terms of multiple allelomorphs (Imai, in press).

Miyazawa's yellow-inconstant appeared as a mutation in his experimental plants, so that his strain is not related to my original strain. From the behaviour of the two strains, especially the numerical proportions of greens and "false yellows," I am inclined to regard them as genetically different.

MUTATING STAGES.

My own yellow-inconstant strain gives 11·5 per cent. of yellow seedlings with green tissues in cotyledons, the others (setting green mutants aside for the present) being apparently yellow; the actual number observed was 317 of the former and 2430 of the latter. Of the seedlings with green-yellow-mosaic cotyledons, about 51 per cent. (123 among 243) grew up into "false yellows," whereas only about 2 per cent. (5 in 237) of seedlings with yellow cotyledons changed into mosaic, others remaining as "false yellow." The green areas on these mosaic plants can, however, generally be traced back to an embryonic origin. To fix the distribution of green parts on the yellow-inconstant plants, a spiral diagram of the phyllotaxy is convenient (Imai, 1927 *a*). As stated elsewhere (Imai, 1927 *b*), right- and left-handed phyllotaxy occur at random, and consequently both dextrorsal and sinistrorsal diagrams are required. As pointed out by Miyazawa (1929), discontinuity sometimes occurs in the distribution of the mutated cells over the plant body, especially at the junction of the hypocotyl and its upper stem. Furthermore, confusion of cell arrangement in the formation of organs may frequently occur, changing the successive distribution of the mutated cells. The fact that almost all remarkable mosaic plants in the writer's yellow-inconstant strain can be traced back to an embryonic origin for their mutated cells, together with low frequency in the occurrence of massive green tissues of an isolated distribution in the foliage, reveals to us a marked difference of mutability in the different stages of ontogeny. Sometimes small, more rarely large green areas of an isolated distribution occur on the otherwise yellow leaves of the yellow-inconstant; they may have an origin in the cell generations of the post-embryonic somatogenesis, occurring at so early a stage that the propagation of the mutated cells admits of the formation of massive green parts. As already stated, the yellow-inconstant leaves have fine green spots, which probably originate at a late stage during ontogeny. Taking these facts together, we have roughly three periods for the mutating frequency; *i.e.* the mutability is high during embryonic development, low in the post-embryonic somatogenesis, and again high at a late stage of the cell generations in the formation of leaves. The mutating frequency of Miyazawa's yellow-inconstant strain, however, is much higher than in my original strain, and mutations in the general somatogenesis seem to occur more frequently.

TYPES OF BUD VARIATIONS.

The mosaic yellow-inconstant plants sometimes put forth bud variations of green, or chimerical branches. The bud variations may be green, which is solid green without any yellow cells; periclinal (in a narrow sense) with yellow "skin" and green "core," and the reversal, which also has periclinal (in a broad sense) tissues of green "skin" and yellow "core," as well as sectorial chimeras. Some mosaic plants exhibit manifold bud variations, producing sectorial and periclinal chimeras, green and reversal, and so on.

In the higher plants the tissues of the general plant body are considered to originate from three layers, a problem fully discussed by Chittenden (1927). The original first layer develops into epidermis. Anatomical studies of some periclinal plants show the extent of propagation of the original second layer; that is, for instance, in the stem this layer propagates into outer cortex. The remaining inner region, including the inner cortex and central cylinder, is developed from the original third layer. Therefore, the developmental extension of the three original layers does not entirely coincide with the respective three constituents, dermatogen, periblem and plerome, of the meristem. Noack's theory (1922) is not convincing enough to account for the facts presented by various chimerical plants. Some complicated chimeras were reported and questioned by Chittenden, but the complexity may be induced by recurrent somatic mutations (Imai, 1928).

In the case of yellow-inconstant, it is difficult to determine the colour of the plastids in the guard cells of the epidermis of the leaves, and consequently examination must be confined to the sub-epidermal and more internal regions. The plastid colour of normal green mesophyll is so green that we can readily distinguish it from the yellow under the microscope. The mesophyll of the leaves of *Pharbitis Nil* is composed of one thick layer of palisade, with at times two small cells connected end to end, and several layers of spongy parenchyma arranged irregularly with much intercellular space. The periclinal leaf is composed of yellow sub-epidermal and green inner regions, the former of which includes the palisade and a sub-epidermal region of spongy layer. In the reversal, on the other hand, the palisade and the sub-epidermal region of spongy tissue are green, enclosing a yellow "core." Outwardly, the presence of the yellow "core" in reversal leaves is frequently inconspicuous, and they may be mistakenly classed as green, but careful inspection will reveal its identity. For the periclinal and reversal the tissues of the calyx may give better preparations of the bicoloured arrangement. Miyazawa (1929) seems to doubt the occurrence of the periclinal chimera described by the writer (Imai, 1927 *a*), but he has himself figured a yellow leaf with greenish (light-coloured in his diagram) centre (Miyazawa's Fig. 4, *L*), which is evidently a periclinal composed of yellow "skin" and green "core." The production of a green part on the periclinal leaf is regarded as due to the protrusion of the inner green tissue through the yellow "skin," a phenomenon frequently observed in the writer's yellow-inconstant as well as in chimerical plants generally.

The tissue development of the embryo is different from the otherwise general somatogenesis. In the early developmental stage of the embryonic tissues, the differentiation of the three original layers has not yet taken place; therefore the production of solid green branches may occur, by chance, when the cells have occupied the "Anlage" of buds. In the later somatogenesis, however, this occurrence must be rare, because of the differentiation of the three original layers. Let us attempt to consider more definitely the mechanism of the so-called bud variations, which occurred after the differentiation of the three layers of different origin. Suppose a mutation occurs in an epidermal cell and the mutated cell becomes propagated in the epidermal layer covering the growing point of a bud; in such a case, we shall have a bud variation with the mutant epidermis covering the prototypic inner constituents. If this occurs in the second layer, a bud variation with the mutant sub-epidermal region, covering the prototypic "core" and being covered by the unaltered epidermis, will occur; and if in the third layer, a bud variation with the mutant "core" covered by the prototypic "skin" may be produced. These three bud variations are of primary types, but they may sometimes be transformed into the other different types during tissue development. It is well known that periclinal or reversal plants at times give branches with homogeneous tissues; and also that they rarely do so with inverse arrangement of heterogeneous tissues, from periclinal to reversal or *vice versa*. This does not denote the occurrence of a new mutation, but is due to confusion in the distribution of cells or tissues during development. Because of this confusion, a bud with a growing point composed of the second and third original layers casting off the first, or composed of the first layer excluding the second and third, or others, may occur. Under such conditions, secondary types of bud variations may be produced. When the given bud variations are indifferent to the embryonic origination, the possibility of those with homogeneous tissues must be rare, since this production may be induced by three, rather infrequently occurring factors: firstly, the mutation; secondly, confusion in the arrangement of tissues; and thirdly, the resultant situation of the cell layers on the growing points of buds, taking place successively one after the other. From this point of view the so-called bud variations observed in various plants must be critically examined. East (1917) drew attention to the possibility of a connection between periclinal chimeras and bud variations, and suggested a periclinal explanation for the nectarine. Asseyeva (1927) found the bud variations of the potato to be chimeras, and she isolated the prototypic forms from the mutant potato tubers by the removal of the "eyes." When the mutation can be traced back to an embryonic origin, however, bud variations homogeneous for the mutant tissues may be produced. Actually, green bud variations of this sort were observed at times in yellow-inconstant.

THE OFFSPRING OF CHIMERICAL PLANTS.

An examination of the offspring of bud variations related to yellow-inconstant has already been made (Imai, 1927 *a*), proving their correspondence with the genetical constitution of the sub-epidermal region of the mother branches. The later data confirm this. The offspring of the prototypic and varied branches of mosaic plants are shown in Table IV.

TABLE IV.

Offspring of chimerical yellow-inconstant plants.

Plant	Type of branch	Green	Green variegated	False yellow	Total
3–1	False yellow	3	4	65	72
	Sectorial	50	5	52	107
	Periclinal	1	3	26	30
3–15	Reversal	88	4	21	113
	Green	31	1	9	41
5–1	Periclinal	1	5	90	96
5–3	False yellow	0	0	15	15
	Reversal	35	2	11	48
	Green	27	0	8	35
6–1	False yellow	1	5	56	62
	Sectorial	40	4	77	121
	Green	44	1	10	55
6–6	False yellow	0	0	2	2
	Green	89	3	26	118
8–1	Periclinal	0	2	27	29
10–3	False yellow	1	8	155	164
	Periclinal	1	2	34	37
9	Chimerical plants	299	45	298	642

The results correspond fairly with the genotypes of the sub-epidermal tissues, from which the following generation originated through fertilisation.

GREEN MUTANTS.

In the writer's strain, when only the offspring of the so-called false yellow, on which no massive green areas were discernible by close examination, are taken into account, the production of the green, green variegated and false yellow seedlings is 1·9 per cent., 11·3 per cent. and 86·8 per cent. (52, 317 and 2430 in number) respectively. When it is considered that some of the greens thus appearing might have been produced by gametic mutation, in addition to those which have had a chance to develop into green cotyledonous seedlings by the overcoming of, or the monopolistic distribution of, the green cells mutated in the early stage of the embryonic ontogeny, the production of the green seedlings is not as high as we expect it to be, compared to that of the mosaic. Hence the green mutants thus making their appearance should not be regarded as necessarily originating by mutation at gametogenesis. Furthermore, the occurrence of fine green spots on the otherwise yellow leaves may give the same corresponding distribution of the genetically green cells in the germinal tissues. If this is the case, a few green mutants may be produced in the following generation, with a somatic origin, but occurring in the mother sporophyte. Hence the green mutants cannot here be classified in respect of their origin. In Miyazawa's yellow-inconstant strain, the proportion of green, green variegated and false yellow plants is 14·0 per cent., 61·6 per cent. and 24·4 per cent. respectively. The proportion borne by the green to the green variegated is similar to that in the writer's strain, suggesting a type of mutation in the two cases similar but differing in frequency.

TABLE V.

Offspring of green mutants in the yellow-inconstant strain.

Plant	Green	Green variegated	False yellow	Total
2	32	3	8	43
3	19	1	5	25
48	96	5	29	130
72	92	2	20	114
13 (1)	18	0	4	22
(8)	48	3	14	65
18 (3)	30	1	8	39
(5)	14	1	4	19
Total	349	16	92	457

The green mutants appearing in yellow-inconstant have been proved to be heterozygous for yellow-inconstant. Fresh data favouring this are given in Table V.

The proportion of green segregates is 76·4 per cent., which surplus, however, is not remarkable. In Miyazawa's strain it amounts to 83·5 per cent., owing to the high mutability of his yellow-inconstant strain.

SUMMARY.

1. The seedlings or plants carrying the gene "yellow-inconstant" sometimes are characterised by green variegated areas on the otherwise yellow foliage. The presence of green tissues is due to reversional mutations occurring in somatogenesis. The majority of the offspring are apparently yellow, without any distinct areas of green. Nevertheless close examination of the leaves of yellow-inconstant, and even of false yellow, reveals fine green spots, which are due to vegetative mutations occurring at a late stage of cell division in the ontogeny of the leaves.

2. Yellow-inconstant is dominant to yellow, and recessive to normal green. The three genes, normal, yellow-inconstant and yellow, constitute triple allelomorphs.

3. The frequency of mutations of yellow-inconstant to normal green on the yellow-inconstant sporophyte is not uniform throughout its growth, but can be roughly divided into three periods; for the mutability is high in the embryonic development, low in the post-embryonic somatogenesis, and again high at a late stage of cell division in the ontogeny of the leaves.

4. Yellow-inconstant sometimes puts forth bud variations of solid green, as well as sectorial and periclinal chimeras. In almost all of the remarkable mosaic plants of yellow-inconstant in the writer's strain mutated green cells can be traced back to an embryonic origin.

5. The production of solid green bud variations may not be rare, when mutation occurs during embryonic development. In the later somatogenesis, however, this occurrence must be rare, because of the differentiation of the three original layers. The bud variations induced by such an origination may be composed of heterogeneous tissues, with prototypic parts enclosed or being enclosed by the mutant parts, but not of homogeneous mutant tissues.

6. The genetic aspect of the offspring of various types of bud variations in the yellow-inconstant such as green, periclinal, reversal and sectorial, corresponds fairly to the respective genotypes of the subepidermal tissues of the mother branches.

7. The green mutants appearing in the yellow-inconstant are heterozygous for yellow-inconstant, giving nearly simple Mendelian segregation in their offspring. The deficit of the recessive yellow-inconstant segregates

is not much in the writer's strain, whereas it is remarkable in Miyazawa's strain, owing to its high mutability.

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