Theor. Appl. Genet. 55, 145-152 (1979)

Cell Frequencies of Green Somatic-Variations in the *tl* Chlorophyll Mutant of *Nicotiana tabacum* var 'Samsun'

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Summary. The chlorophyll deficient *tl* mutant of *Nicotiana tabacum* var 'Samsun' expresses green, clear and twin, green and clear somatic variations spontaneously on leaves at a low frequency. This character is maintained after both vegetative multiplication and sexual reproduction. However a very important phenotypic variability in the capacity for somatic variation appears in in vitro bud neoformations from leaf fragments of *tl/tl* homozygous plants. This variability is observed in the type of variations and the variation pattern, defined as the frequency and size of the variant areas.

The present work was aimed at determining both the cell frequencies of the events which lead to the somatic variation and the preferential sequence of leaf initial development during which these frequencies are at a maximum. It was limited to plant populations with very different patterns for green variations, some having a high frequency of large variation, others having a high frequency of small variations. They were compared with a population of control plants having a low frequency.

In the case of plants having a high frequency of large green variations, the events leading to somatic variation occurred between the twenty-first and the twelfth cell cycles preceding the end of the initial division phase, the maximum cell frequencies being in the seventeenth and sixteenth cycles. The maximum frequencies appeared extremely high, being on average about 10^{-2}. In plants with a high frequency of small green variations the event occurred between cell cycles nine and one, with mean frequencies of 10^{-3} but without any clearly marked maximum. In the low frequency control plants the event also took place during the last ten cell cycles but with decreasing frequencies from 10^{-4} to 10^{-7}.

The frequency and the starting period of the cell events leading to somatic variation are closely dependent on the state of the cell. This is, on the one hand, strictly linked to the physiology of the plant and, on the other, closely correlated with the stage of differentiation, which may vary according to the genetic back ground of the leaf initial cells.

The results are discussed in relation to comparable observations and the relevant interpretations made on other instability mutants.

Key words: Somatic variation – *Nicotiana tabacum* – Bud neoformation

1 Introduction

The previously described *tl* chlorophyll mutant (Deshayes, 1972) shows spontaneous somatic variations on leaves at a low frequency. The variations appear as single areas of either greener or cleaner leaf tissue, or as areas that contain both greener and cleaner tissue. Plants showing a high frequency of variations have been obtained both in the homozygous state for the gene *tl* and in the heterozygous state *TL/tl*. The high frequency character is controlled by a nuclear genetic factor *H* linked with the gene *tl* and is active in the heterozygous *(H/+)* as well as the homozygous *(H/H)* state (Deshayes, unpublished). This factor *H*, of which the mode of action is still unknown is called the 'unstabling factor'.

Several authors, working with different unstable mutants, observed that the somatic variation pattern, defined by both the frequency and the size of the variations, is sexually transmitted. It is clear, in fact, that in all the cases studied, the cell event which leads to the variation takes place during a relatively short time sequence of the ontogenic development of the organ considered. If this sequence occurs at the beginning of development large variations will be observed, but if, on the contrary, it is situated in the later stages of development, the variations will be small. The timing is genetically determined since it varies according to the genotype, that is according to (1)

040-5752/79/0055/0145/$ 1.60

the allele of the marker gene, the phenotype of which is observed (Brink and Williams, 1973; Sastry, 1976; Fincham and Harrison, 1967; Gavazzi, 1967; MacClintock, 1965), and (2) the allelic form of the genetic system controlling instability: a controlling element (McClintock, 1958, 1965; Peterson, 1965) and a modifier (Harrison and Fincham, 1973; Ashman, 1965; McClintock, 1958).

The present work is aimed at determining the frequency of the events which lead to the somatic variation and the stage in leaf development during which these events preferentially take place. From the results it should be possible to determine the corresponding histological stage.

2 Materials and Methods

The original mutant was characterized by a low frequency of variation; each plant showing a higher number of variations was considered as a plant having a high frequency of variation.

All such plants originated only from in vitro bud neoformations on leaf blade fragments of low frequency plants and were never obtained in sexual progenies of the original mutant. As previously observed (Deshayes, 1976) plants obtained directly by in vitro culture from a low frequency plant did not show any change in their phenotype as compared to the control. However a second successive cycle of neoformations from these plants, resulted in a great diversity of phenotype with regard to their somatic variation ability. High frequency plants with either green (Hf.G) or clear (Hf.C) or twin (Hf.T) variations and high frequency plants with all three types of variations were obtained. In all cases the high frequency character was sexually inherited.

1) In this study families of plants with different patterns for high frequency of green variations were compared. The families came from the progenies of the following:

a) Selfed Hf.G.11, which originated from a *tl/tl* homozygous plant, being homozygous for the gene *tl* but heterozygous for the unstabling factor H and expressing a high frequency of large somatic variations. Plants from the first selfed generation showing a high frequency presented a uniform variation pattern which could be compared with that of the Hf G 11 mother plant. On the other hand, those from the second generation presented a certain heterogeneity and three plants were chosen to represent this variability. No precise observation was made on these plants and the choice depended only on overall differences in the pattern of variation:
– plant 2801 had a pattern of variation similar to that of Hf G 11,
– plant 2862 which although similar to Hf.G.11 had apparently a higher frequency of variations,
– plant 2861 showed larger variations than those observed on Hf.G.11 with a higher frequency.

b) A cross between Hf.C.6, originating from a *tl/tl* homozygous plant and Hf.T.1., originating from a heterozygous plant *(TL/tl)*. One of the plants homozygous for the gene *tl* obtained through this cross and which presented a high frequency of green variations of small size (plant 1344) was chosen for comparison with the three plants 2801-2862 and 2861.

2) Each of these four plants, all having the 'Samsun' '*nn*' genome but different variation patterns, was both selfed and crossed with *tl* pollen lacking the unstabling factor. Because of the sensitivity of 'Samsun' '*nn*' to TMV, all crosses were made with the pollen of a plant homozygous for the gene *tl* into which the gene *N* for hypersensitivity to the virus has been introduced.

In the eight progenies thus obtained (4 × 2), the cell frequency of somatic variations was determined only for plants showing a high frequency pattern. A population of plants homozygous for the gene *tl*, coming from the 6th selfed generation of the original mutant, and therefore with a low frequency of variation, was chosen as a control.

3) In order to obtain satisfactory observations on the green variations, all plants were grown under the same temperature (20° C) and light (16h) conditions to ensure that the chlorophyll deficiency would be highly pronounced (Deshayes, 1972).

4) It has been shown (Deshayes, 1973) that the frequency of variations is not constant, depending on the development stage of the plant, and that the maximum for green variations occurs at level 7 where the reference level 1 corresponds to that of the last prefloral leaf. This leaf level 7 was chosen to determine the variation frequencies per cell.

5) The cell frequency of the appearance of somatic variations at a given stage in leaf development corresponds to the ratio between the number of events which produce a somatic variation at this stage and the number of *tl* cells in the palissade tissue at the same stage; i.e. $f_i = V_i/N_i - N_v$ where:

i = cell cycle which is concerned;
V_i = number of events which occur during cell cycle i;
N_i = total number of cells in the pallisade layer of the leaf initial issues from cell cycle i;
N_v = number of green cells which result of events preceeding cell cycle i.

It has been assumed that:

– cell multiplication is exponential during the entire growth period and that the same applies to both cells with the *tl* phenotype and cells in which that phenotype has changed;
– the variant phenotype is established immediately without requiring a mitosis;
– each variant cell becomes stable and keeps its phenotype throughout leaf growth.

6) For plants having large green variations, cell numbers in adult leaves (N) and cell numbers for each variation, were determined indirectly by measuring the areas of leaves (S_L) and of variant tissue (S_v) on millimeter graph paper; the area of the *tl* zones (S_{tl}) was obtained from the difference. The cell number per leaf unit area was determined microscopically on 4 or 5 plants from each population with at least four replicate countings per *tl* and green leaf fragment. In an area corresponding to 1/196 mm² of leaf surface (measured using a camera lucida) an average of 13.63 cells was found in the *tl* zones (125 countings) and an average of 12.35 cells in the green zones (135 countings). A test of the null hypothesis (Ho) between these two means was highly significant ($t = 3.95$, $\nu = 258$). These represented actually 2671.48 cells for the phenotype *tl* and 2420.60 variant cells per square millimeter leaf area. Under these conditions, the number of cells in the palisade parenchyme of the adult leaf was: $N = 2671.48 \times S_{tl} + 2420.60 \times S_v$. From the value obtained and by knowing the number of cell cycles (m) necessary for the appearence of the variation of maximum size observed on an adult leaf, it is easy to calculate the cell number in the leaf initial at the start of the first events (N_m) and then N_i.

7) The preceding method cannot be applied to plants with small variations. The variations' area was therefore measured using a binocular microscope on discs of 25 mm² each, punched out at random from the leaf blade. Under the condition of observation, 1.2 variant cells corresponded to one square millimetre drawn in the camera lucida. N and N_m were determined as described above and the results were expressed not on the entire leaf or a leaf initial basis, but as the cell number on a leaf disc of 25 mm² and the cell number in this disc at the occurrence of the first variation, respectively.

3 Results

3.1 Leaf Characteristics

Highly significant differences in leaf surface areas ($t = 4{,}72$ $\nu = 131$) occurred between plants obtained by selfing and those obtained with *tl* pollen, the latter having a larger leaf area (Table 1). Since in the latter category the male parent had a part of the 'Xanthi' genome, the differences observed could be attributed to a phenomenon of intervarietal heterosis, the 'Xanthi' variety having smaller leaves than the 'Samsun' variety.

There was very considerable variability in the capacity for somatic variation within each population with regard to both the relative surface area ($\frac{S_v}{S_L}$) and the number of variations. Consequently the variability for the mean surface area of each variation was also important.

Differences between populations obtained by either selfing or crossing were not significant in the case of plants 2801 and 2862. The two progenies originating from plant 2861, however, showed significantly greater variant areas and numbers of variations.

The differences observed in leaf areas made the comparison between populations obtained by selfing and populations obtained by crossing, difficult. In fact, the total variation area took into account neither leaf area nor variation number; the relative variation area did not take the number of variations into account and the mean variation surface area did not take into consideration the leaf surface area. A factor (K) was therefore chosen for comparisons between plants and between populations. Its value was given by multiplying together the relative variant surface area ($\frac{S_v}{S_L}$) and the mean surface area ($\frac{S_v}{n}$). This value had the dimension of a surface and took into consideration the leaf surface area and the number of variations.

Using this criterion, there was clear evidence that (Fig. 1):

1) The variability within a population was considerable.

2) The variability was the same for the population obtained by selfing and for those obtained by crosses in the case of plants number 2801 and 2862. The mean values of K were comparable.

3) The populations originating from plant 2861 were markedly different from those above and the variability observed was more important in the population obtained from the cross with *tl*.

Taking into account this criterion and the variability observed, four groups of plants were defined (Fig. 1). This classification was arbitrary as there was no marked discontinuity in the K value within a population, but it was

Table 1. Leaf characterictics at foliar level 7. The reference leaf 1 is the last prefloral one

Origin		Mean surface area of leaf	Mean surface area of *tl* tissue per leaf	Mean surface area of variant tissue per leaf	Relative surface area of variant tissue $\frac{S_v}{S_L}$	Mean numbers of somatic variations per leaf	Mean surface area of variation per leaf	K[b]
		S_L^a	S_{tl}^a	S_v^a	$\times 10^2$	n	$\frac{S_v}{n}$	$\times 10^2$
2801	Self	9 398.6	8 753.6	645.1	6.9	64.3	10.0	68.8
	$\times tl_x$	11 451.7	11 014.9	436.9	3.8	34.2	12.8	48.8
2862	Self	7 882.1	7 407.0	475.1	6.0	45.4	10.5	63.0
	$\times tl_x$	11 183.2	10 380.4	802.8	7.2	101.4	7.9	56.8
2861	Self	8 396.9	7 451.9	944.9	11.2	99.1	9.5	107.3
	$\times tl_x$	10 291.4	8 479.0	1 822.4	17.6	119.0	15.2	268.2
1344	Self	25.0[c]	24.86	0.14	0.56	53.8	0.003	0.002
	$\times tl_x$	25.0[c]	24.86	0.14	0.56	29.1	0.005	0.003
Control	tl_s	25.0[c]	24.99	0.01	0.04	5.1	0.002	0.00008

tl_s = *tl* mutation with 'Samsun' genetic background tl_x = *tl* mutation with 'Xanthi' background

[a] All surface areas are given in square millimetres

[b] Coefficient K is the product of the relative surface area of variant tissue ($\frac{S_v}{S_L}$) and the mean surface area of variation ($\frac{S_v}{n}$)

[c] Leaf discs

used to account more fully for the variability in the variation frequencies during the cell cycles. Figure 2 represents the differences in the variation patterns according to the K value for the population obtained from plant 2861 after crossing with *tl*. In Table 2, for each population, the mean value of variation numbers per leaf in the case of plants with large variations and per 10 discs in the case of plants with small variations is given.

For the populations coming from plants 2801, 2862 and 2861 the first events appeared between the twenty-first and nineteenth cell cycles preceding the end of the division stage of the leaf initial. The calculated number of cells in the initial N_m confirmed the earliness of variation

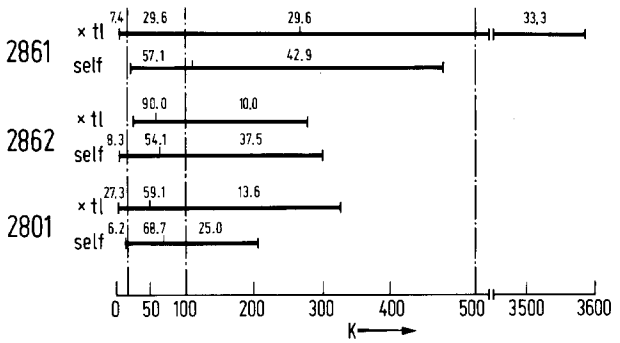

Fig. 1. Variability in the value of the coefficient K within each population. Four groups of plants were defined according to the K value; 0 to 10, 10 to 100, 100 to 500 and above 500. The numbers given for each population correspond to the percentage of plants in each group

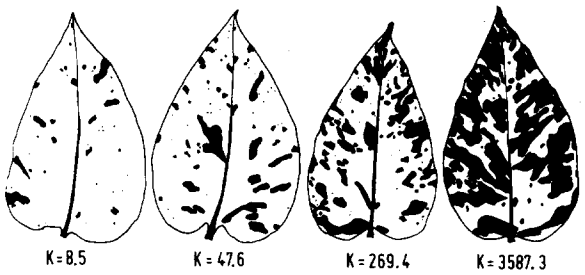

Fig. 2. Variability in the pattern of somatic variation according to the value of the coefficient K within the population originating from a cross between plant 2861 and *tl*

Table 2. Mean numbers of spontaneous somatic variations induced per cell cycle

	2801		2862		2861		1344		Control
m[a]	Self	$\times tl_x$	Self	$\times tl_x$	Self	$\times tl_x$	Self	$\times tl_x$	tl_s
0							16.88	55.33	0.3
1							16.49	76.67	1.0
2							9.47	53.00	1.3
3							4.37	47.33	0.8
4							3.03	28.33	0.3
5							1.72	11.33	0.3
6							1.04	7.33	0.1
7							0.51	7.33	0.3
8							0.17	2.00	0.3
9[c]	25.13	16.27	14.25	28.97	23.50	41.74	0.10	2.00	0.2
10							0.33	–	0.2
11									
12	5.25	2.91	4.04	14.47	6.71	15.81			
13	6.50	2.77	6.04	18.43	21.57	17.93			
14	6.94	2.05	4.54	12.50	14.07	11.33			
15	7.31	3.27	6.29	12.67	15.29	10.96			
16	6.44	2.82	5.58	8.10	9.86	8.07			
17	4.50	1.91	3.29	4.00	5.50	5.89			
18	1.81	1.36	1.00	1.80	2.14	3.52			
19	0.44	0.68	0.33	0.43	0.43	2.07			
20	–	0.14	0.04	0.03	0.07	1.37			
21	–	–	–	0.03	–	0.30			
N_m[b]	47.58	29.07	19.92	14.15	21.17	12.84	65.19	130.38	65.22

[a] m refers to the number of cell cycles which precede the end of the cell division phase in the foliar initium

[b] N_m refers to the number cells in the palisade layer of the foliar initium, at the time of appearance of the first events which lead to somatic variation.
In the case of populations from 1344, N_m refers to the number of cells on leaf discs of 25 sqmm at the moment of the appearance of the first variations

[c] In selfing and backcross populations from plants 2801, 2862 and 2861, all variations which occur after cycle 12 are assigned to cycle 9

appearance as only 15 to 50 cells of the palisade tissue were estimated to be present at the beginning of the first events.

In the case of populations originating from plant 1344 the first events occurred only during the tenth and ninth cell cycles (Table 2). Assuming that leaf areas in these populations were identical to those of others, about 30,000 cells must have been present at the moment of appearance of the first variations.

It should be noted that the origin of the populations, whether from selfing or from crossing, did not affect the time at which the variations appeared. Also, the differences between the *tl* control population and the two populations with small variations had bearing only on the number of events in each cell cycle and not on the number of cycles.

3.2 Cell Frequencies of Somatic Events

The distribution curves for the frequencies of events in the function of the cell cycles show that there was a preferential moment in the development of the leaf initial when somatic variations appeared (Figs. 3 and 4). This was very distinct in the case of plants showing large variations and for plants obtained by selfing; it corresponded to the seventeenth cell cycle preceding the end of the division stage. Although there was a certain variability from one population to another in plants originating from crosses with *tl*, a maximum in the cellular frequencies of somatic events was also observed but it occurred between the 20th and the 16th cell cycles. The pattern of curves representing the cellular frequencies determined for the four groups of plants defined according to their K value, suggests that a positive correlation exists between high cellular frequency and the earliness of the start of events leading to somatic variation.

This 'sensitive period' was less evident in the case of plants showing a late appearance in variations (Fig. 4). The *tl* control plants did not show a maximum in variation frequencies with the cell cycle (Fig. 4).

It 'should be noted (Fig. 4) that in all cases the appearance of the variations was not gradual but occurred suddenly after a certain stage in the development of the leaf initial.

For populations having large somatic variations, the mean values for maximum cellular frequencies reached unexpectedly high values, ranging from 1.19×10^{-2} to 5.29×10^{-2} (Fig. 3). However, whilst the differences between populations for maximum frequencies ranged from 1 to 5, more important values could be attained within the same population. For example, when plant groups were distinguished according to their K value, maximum frequencies of the population obtained by selfing of plant 2862 varied

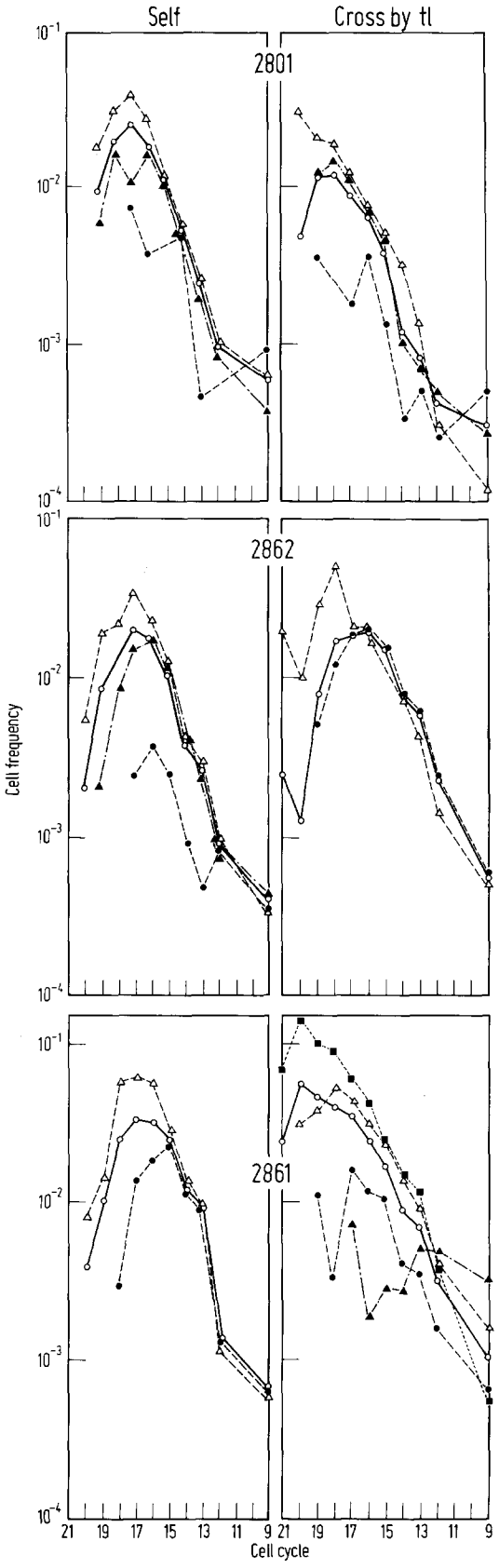

Fig. 3. Cellular frequencies of the somatic events in relation to the cell cycle of populations with large variations. For each population the curve representing the mean frequencies (o–o) is drawn, together with the curves corresponding to the groups of plants defined by K values such as 0 to 10 (▲–▲), 10 to 100 (●–●), 100 to 500 (△–△) and above 500 (■–■)

between 3.8×10^{-3} and 3.5×10^{-2}, that is from 1 to 10; for the population obtained by crossing plant 2861 with *tl*, they were between 5.02×10^{-3} and 1.4×10^{-1}. Within this latter population the actual difference between extreme plants was 1 and 49. However, the variability between populations was generally very low during the 9th cell cycle (Fig. 3).

The cell frequencies were lower in plants showing small variations. They varied between 10^{-4} and 10^{-3}, independent of the cell cycle, and the frequency of the single variant cells ranged from about 2×10^{-5} to 6×10^{-5} (Fig. 4). In the *tl* control population cell frequencies ranged from 3×10^{-4} to 10^{-7}.

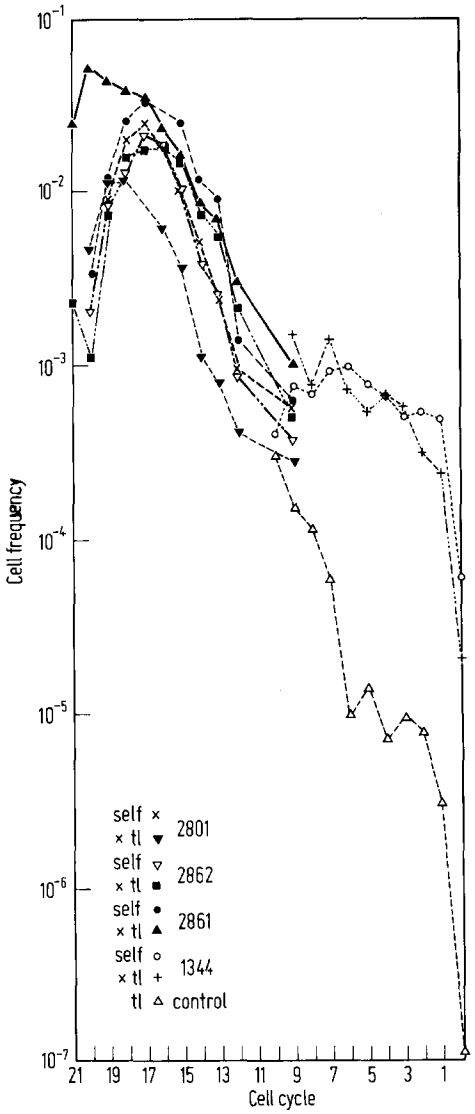

Fig. 4. Mean cellular frequencies of the somatic events in relation to the cell cycle for all populations

4 Discussion and Conclusion

1) The variability observed between plants of the second generation of selfing of the HfVll strain is not due to a simple fluctuation, but to genetic differences. As to the differences observed between plants showing large variations and those with small variations, they are due to different genetic factors (Deshayes, unpublished). It is worth noting that they all originally came from *tl/tl* homozygous plants, with a low variation frequency (see Materials and Methods).

Such a variability in material of common genetic origin or in a population of sister plants has already been observed in maize and *Antirrhinum majus*. Two interpretations have been envisaged for maize; either the genetic modification takes place at the gene locus as in the case of: $a_1^{m\text{-}1}$ (McClintock, 1965), $a_1^{m\text{-}2}$ (McClintock, 1968), R^{st} (Gavazzi, 1967), $R^{\bar{n}j}$ (Brink and Williams, 1973), a^x (Cornu, 1977) – or, it affects the genetic system, *Spm* or *En*, controlling the pattern of variation (McClintock, 1965; Peterson, 1965).

Recently Sastry (1976) proposed a different, but not exclusive, interpretation to explain the variability observed in *Antirrhinum*. Rejecting the possibility of a segregation of modifiers of the variation pattern, he suggested that the drift observed was due to a phenomenon comparable to paramutation.

Since in the present study the phenotype of the chlorophyll deficient tissue was identical in all families and responses to the environment (temperature and light) were the same, there is no reason to think that it is the alleles of the *tl* gene which distinguish plants from each other or which distinguish early occurring strains from late occurring ones. The differences between these strains are therefore located at least at the level of the unstabling factors.

It thus seems possible in the case of the *tl* mutant to speak of 'Mutability of the mutability' as Fincham and Sartry (1974) do. This may occur when the unstabling factor is present in the genome either under an active form (high frequency) or under an inactive or little active form (low frequency). Although, taking into consideration certain of the present observations, the hypothesis of a phenomenon comparable to paramutation cannot be excluded, it hardly seems in agreement with the fact that passage by in vitro bud neoformation induces genetic changes which are at least as important (Deshayes, 1976).

2) There is no significant difference between the plants obtained by selfing and those obtained by crossing with *tl* showing that introduction of the '*Xanthi*' genome does not affect the instability phenomenon and that dosage of the *H* factor has no effect. This situation is apparently in contradiction with the fact that each of two distinct instability factors, associated in one and the same

genome, expresses its own specificity (Deshayes, unpublished). It can, however, be compared with the situation observed by McClintock, 1958 in which an inactive controlling element, *Spm*, associated with an active element, induces the same dose effect as two associated active elements; in the former case the active element keeps this property after meiosis. In addition, the behaviour of the dominant factor *Mod* (Modifier) (McClintock, 1958) which is likely to restore a normal activity of the 'weak' state of *Spm* and which does not present a dose effect is in agreement with the present observations.

3) For a given strain, the development sequence of the leaf initial which corresponds to the maximum frequencies of variations is relatively constant. In plants showing early events, this sequence took place between the 20th and 16th cell cycles which precede the end of the division stage of the leaf initial; it took place between the 9th and 4th cycle for the plants showing late events.

In all cases the peak frequencies appear more or less suddenly as if the genetic system which is responsible is triggered at a particular development stage. It must be stressed that in all plants showing early appearance, none presented a caulinary variant sector, which means that either the variation probability is nil, if not low, in the cells of the lateral zone of the apex, or that the number of plants observed was too small.

4) The number of cells belonging to palisade tissue present in leaf promordium N_m at the beginning of the first events which lead to the variation, correspond to about 15 cells in the case of plants showing early appearance. However, the developmental stage of the leaf initial at this time cannot be determined for two reasons:

a) In cross-sections of the plant apex it can be seen that in the leaf primordia most cells are located in the zone which will give rise to the leaf rib. The two primordial parts of the blade appear laterally but it is impossible to determine in the initial with 15 cells those which will give rise to the palisade parenchyma.

b) It is known (Avery, 1933; Dulieu, 1968) that at an early stage of the initial and primordium most of the cells present are destined to form the leaf apex. Intercalary growth of the basal cells will later give rise to 3/4 of the leaf surface. This is therefore a limiting factor in an eventual determination of a leaf initial stage which would correspond to N_m cells in the palisade layers. In fact no ontogenic effect on the frequency and size of the variation can be observed on adult leaves of plants presenting a high frequency for green variations (Fig. 2). This means that all the events leading to the variation and corresponding to a given cell cycle do not begin at the same moment but at different moments which are always correlated with the same development stage of each primordial part of the leaf blade.

Thus, whether the strain is a late or an early one two aspects of the development must be simultaneously taken into account, one associated with the development stage of the leaf and the other with the development stage of different parts of the leaf blade, that is with the differentiation stage of the cells in the palisade tissue.

5) The variation frequencies per cell and per cycle not only objectively differentiate a 'low frequency' strain from a 'high frequency' strain, but also between two 'high frequency' strains. However the method of calculation used can be criticized on two points:

– It was assumed (see Material and Methods) that a variation could take place in a cell without mitosis. Arguments for and against this choice can be discussed (Hill, 1972), but in any case, the displacement of a cell cycle would induce only slight modifications in the curves representing the variation frequencies according to the cycle. For a given strain, the frequencies corresponding to the occurence of the first events would be halved. Beyond the fifth cycle, the differences would become minimal and even inferior to the error from experimentation.

– Cell divisions in the leaf do not stop in a synchronous way although it was assumed that cell numbers per leaf increase continuously and exponentially. It therefore follows that in the case of strains with late appearing variations, the calculated frequencies of the last cell cycles are slightly underestimated.

Taking these two restrictions into consideration, the cellular variation frequencies per cell and per cycle are still particularly high. Even the control population presents frequencies superior to the usual frequency for spontaneous mutations of about 10^{-4} for the cell cycles 10 to 8. For the plants showing late appearances the mean maximum frequencies obtained are about 10^{-3} (9.7×10^{-4} to 1.5×10^{-3}). As for the populations showing early appearance, the mean frequencies vary according to the population from 1.2×10^{-2} to 5.3×10^{-2} (extreme values varying between 2.3×10^{-3} and 3.4×10^{-1}).

Comparison of spontaneous frequencies obtained on the present material with those observed by other authors on different instability mutants is difficult. In fact, little data on the number of events per cell and per cell cycle are available.

In *Delphinium ajacus*, Demerec (1930) demonstrated that the mutable gene *'rose alpha'* is subject to mutation with a constant frequency from 5×10^{-3} to 8×10^{-4} according to the plant family, during the last 12 cellular generations of sepal development. The mutable gene *'lavenda alpha'*, on the contrary, presents a high mutability rate in the first and late stages of sepal development and a stable, but low, rate during the intermediary phase.

In the case of the r^{38} gene in *Petunia*, Cornu (1977) has shown that maximum frequencies correspond to the 7,6 and 5 cell cycles preceding the end of the division stage of the corolla and ranged from 4×10^{-3} to 5×10^{-3}.

For the R^{st} gene of maize, Gavazzi (1967) observed similar frequencies (4×10^{-3} to 5×10^{-3}) for the events occurring at a stage at which all, half or a quarter of the grain is subject to mutation. Anderson and Eyster (1928) have reported, also in maize, an increase in the mutation rate of the mutable gene p^{vv} during the ontogenic development of the ear. It varied between 1.1×10^{-4} and 6.7×10^{-3} in the case of near self mutations and between 5.9×10^{-4} and 1.4×10^{-2} for mutations of the dark crown type. In *Tradescantia*, the event number per cell calculated by Sparrow and Sparrow (1976) is equal to 2.2×10^{-3} in the case of the mutable clone 0106. These few examples, as well as others for which no cellular frequency has been determined, show that the existence of a preferential moment for the occurrence of the cell events leading to the variation is not unusual. Generally speaking it can be said that the genotype and the environment, mainly the temperature (Harrison and Fincham, 1964; Sand, 1957, 1962; Peterson, 1958; Faberge and Beale, 1942; Rhoades, 1941), also interact in determining the variation pattern. An interpretation on the existence on this sensitive stage, however, cannot be postulated since there is as yet no available data. At the very most a new event in the cell metabolism preceding the genetic event and making it more probable can be imagined.

Acknowledgement

I wish to thank M. Jacky Delbut for excellent technical assistance throughout the work and Miss Douard for typing the manuscript. I am grateful to Drs. Cornu, Dulieu and Dommergues for fruitful discussions of the subject.

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Received March 3, 1979
Communicated by D. von Wettstein

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