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

A. Deshayes
Laboratoire de Mutagenèse, Station d'Amélioration des Plantes, I.N.R.A. Dijon (France)

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 background 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)
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 (TT/lh). 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 'Samson' 'mn' genome but different variation patterns, was both selfed and crossed with a pollen lacking the unstabling factor. Because of the sensitivity of 'Samson' 'mn' 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 palisade tissue at the same stage; i.e. \( f = V_i/N_i - N_v \) where:
- \( f \) = cell cycle which is concerned;
- \( V_i \) = number of events which occur during cell cycle i;
- \( N_i \) = total number of cells in the palisade layer of the leaf initial issues from cell cycle i;
- \( N_v \) = number of green cells which result of events preceding 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 mm\(^2\) 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, v = 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 mm\(^2\) 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 mm\(^2\) and the cell number in this disc at the occurrence of the first variation, respectively.