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Problems of interpreting results obtained in studies of somaclonal variation in gliadin proteins in wheat

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Summary. It is quite likely that somacloning increases the frequency of mutations: this has, in fact, been claimed for gliadin proteins. However, in such instances it is important to distinguish between true mutations and presumed changes in regenerants due to cross pollination or admixture. We present herein arguments that some so-claimed somaclonal variants in gliadin-coding genes are unlikely to be due to mutations since they would have had to involve directed changes at several loci simultaneously.

Key words: Somaclonal variation – Blocks of gliadin

Regeneration of adult plants from wheat-embryo culture is now a routine procedure in many laboratories around the world. Regenerated wheat plants may differ from the initial (parent) variety or line in a number of characters, including agronomically important ones (Larkin et al. 1984), as has been shown in various plant species (Larkin and Scowcroft 1981; Skirvin 1978). In reality, these differences or somaclonal variations can be a new and powerful source of genetic variation for biotechnology and plant breeding (Larkin and Scowcroft 1981).

To use somaclonal variation effectively, it is necessary to understand the mechanisms of its occurrence. A primary task is to describe this phenomenon correctly and impartially. First of all it is important to discriminate between variations induced by conditions of in vitro culture and those differences which arise as the result of genetic heterogeneity of the initial material, either from cross-pollination or from admixture (Appleyard et al. 1979). For this purpose the parental genotype(s) must be clearly defined. An excellent set of genotype markers in wheat is provided by the gliadin proteins. The genetics of gliadins have been well studied (see Payne et al. 1984 for review). Six gliadin-coding loci are located on the short arms of chromosomes 1A, 1B, 1D, 6A, 6B, and 6D respectively. Each of these complex loci control the synthesis of a group (or block) of electrophoretically resolved components of gliadin (Sozinov and Poperelya 1980).

A block is inherited as a codominant Mendelian character (Mecham et al. 1978; Sozinov and Poperelya 1980). Extensive multiple allelism is found for each locus. Allelic variants of blocks differ in number, electrophoretic mobility, and in the staining intensities of the components. Catalogues of the allelic variants of these blocks have been formulated (Sozinov and Poperelya 1980; Metakovsky et al. 1984) and are at present being further developed. A standard electrophoretic procedure has been used to identify variants of blocks in a sample (Bushuk and Zillman 1978): gliadin-coding genes, scattered over 12 chromosomes in hexaploid wheat, can serve as independent markers for a considerable part of the wheat genome.

It is possible to identify 25–30 components of gliadin in a one-dimensional electrophoretic spectrum of gliadin from bread wheat, and up to 50 spots (components) by two-dimensional fractionation. It has been shown that gliadins are simple proteins and that the synthesis of each of them is controlled by one gene or several identical genes (Payne et al. 1984). Hence, we can assume that at least 50 different gliadin-coding genes control the synthesis of gliadin components. About the same figure has been obtained by means of recent molecular-biological methods (Kreis et al. 1985).

The majority of mutation events in gliadin-coding genes (including point mutations, deletions, rearrangements, etc.) can probably be traced by gliadin electrophoresis. One or two changes in the gliadin spectrum – for example, appearance, disappearance, or alteration of mobility of one or two components – indicate that 2–4% (1–2 out of 50) of all gliadin-coding genes are damaged. There is no reason to believe that gliadin-coding genes have any special tendency towards somaclonal variation compared to other genes, so this percentage of damage can be expected to be characteristic of the whole wheat genome. On the other hand, it is statistically improbable that all mutations would accidentially fall on gliadin-coding genes. We might expect that a plant having a high percentage of damaged genes would hardly be viable. Hence, there is good reason to expect only single (if any) changes in the electrophoretic pattern of regenerants.

If mutations in gliadin-coding genes are caused by in vitro growth conditions, new unknown and, of course, uncatalogued blocks would appear. If a known block of gliadins other than the parental one appears in the regenerant, one has to suggest that directed alterations (mutations) have occurred simultaneously in several gliadin genes. Such an event is improbable.

One can imagine the existence of several tightly linked gliadin-coding clusters controlling different blocks in the same locus, so that only one of these clusters functions and others are silent. In this case variation may be due to a switching off of the working cluster and a switching on of a silent one. Taking into account the present knowledge of gliadin genetics, this cumbersome explanation also seems unlikely. A much more plausible explanation is cross-pollination during propagation or genetic heterogeneity in the initial material. A genetic approach to the analysis of the electrophoretic spectra (particularly determination of allelic variants in gliadin blocks) allows this heterogeneity to be traced, thus establishing the exact cause of variation.

Differences in gliadin composition between parental forms and the regenerants have been found by several workers who interpreted them as being due to somaclonal variation in gliadin-coding genes.

Larkin et al. (1984) analysed 290 SC_2 grains from 31 families of 'Yaqui 50E' regenerants. They found an average of 3.0 changes per gliadin spectrum and numerous variations in other characters. Our analysis of two gel photographs presented by these authors indicates that in family 2–29, genetic segregation of allelic variants of parental and "new" blocks occurred (at least for chromosomes 1B and 6A) and that there is a substitution in the gliadin-coding locus on chromosome 1D. Family 1A–35 clearly has a substitution of blocks controlled by chromosomes 1A, 1D and 6A. Using a recent catalogue of gliadin blocks (Metakovsky et al. 1984), we determined that parental GLD 1B2 and "new" GLD 1B4 (or 1B1), which are allelic blocks controlled by chromosomes 1B, segregated in family 2–29. Parental GLD 1D5 block, controlled by chromosome 1D in family 2–29, was substituted by GLD 1D9 and in family 1A–35 by GLD 1D1. We suggest that these substitutions of blocks are due to segregation after inadvertent cross-pollination of parent or SC_1 plants. Since the technique of gliadin separation used by the authors differs from ours (Bushuk and Zillman 1978), it is difficult to determine with certainty which other blocks are present in their spectra.

Nevertheless, the evident presence of substitutions and segregations of known blocks in regenerants does not permit the results illustrated in Larkin et al. (1984) to be interpreted as somaclonal variation of gliadin-coding genes.

Maddock et al. (1985) studied 590 regenerants. Variation was found in 5 lines and in one grain from another line. The photographs presented by the authors are of high quality, so it can be clearly seen that the variant grain in the variety 'Highbury' (in RH 48) has a substitution in the gliadin-coding locus on chromosome 1B: probably the GLD 1B8 block has been substituted for by GLD 1B2 (see catalogue in Metakovsky et al. 1984). It is of interest that the GLD 1B8 block is usually linked with the red colour of the spike, while GLD 1B2 is associated with white spike colour (Koval et al. 1986). The RB 20 line of variety 'Bersee' has many changes in the gliadin spectrum and seems to carry substitutions for gliadin-coding loci on chromosomes 1B, 1D and 6D. To define the blocks in RB 20, a more detailed analysis is needed (including an analysis of the hybrids obtained using the RB 20 line).

In a footnote to Maddock et al. (1985), Dr. P. I. Payne is quoted as saying that regenerant RT 43 is a contaminating seed of 'Chinese Spring'. Our assessment of the RT 43 spectrum is the same. Maddock et al. also reported spectrum changes (in RT 13 and RT 16) involving additional minor bands and considerable streaking of gliadin zones. They attributed these changes to the presence of a heritable character affecting total gliadin synthesis. We usually explain this type of spectrum by technical errors in preparing samples for electrophoresis. However, a heritable factor such as a protease may be involved: this would not, however, involve mutations of gliadin-coding genes.

The RT 26 regenerant seems to have a true mutant spectrum. To be sure of this, a more careful analysis of the parental form is needed. Studying spectra of single grains of different varieties, we found similar changes with a frequency of about 10^{-3}. It is possible to find differences in gliadin spectra even between grains of an inbred line. One such example was given by Cooper et al. (1986). One out of 16 grains of the 'ND 7532' line analysed in this work differed from the others to the same extent as RT 26 of Maddock et al. (1985) differs from its parent variety 'Timmo'.

Cooper et al. (1986) found 4 families with altered gliadin spectra among 32 families of regenerants with changed agronomic characters. Judging by the photograph presented by the authors, the initial line, 'ND 7532' differs from the control variety 'Marquis' only in allelic variants of two gliadin-coding loci. Apparently, the line 'ND 7532' has GLD 1A3 block which includes components with RMU 26 and RMU 48 (RMU in accordance with Cooper et al. (1986)), whereas 'Marquis' has GLD 1A1 (the main component with RMU 50, see catalogue in Metakovsky et al. (1984)). 'Marquis' and 'ND 7532' are very likely also different in allelic states for the gliadin-coding locus on chromosome 6D. Blocks controlled by this chromosome are different in electrophoretic mobility of components having RMU of about 57–58 and 76–77. The difference between allelic variants of blocks would have been more distinct if a standard electrophoretic procedure (Bushuk and Zillman 1978) had been used.

Judging by the photograph presented by Cooper et al. (1986), in regenerants 1083–3 and 1083–7 either block GLD 1A3 has been substituted by GLD 1A1 or this pair of allelic blocks segregate. In addition, in 1082–9 genetic segregation of the alleles of the gliadin-coding locus on chromosome 6D is observed. We therefore suggest that the appearance of the gliadin band with RMU 50 is a result of accidental cross-pollination by 'Marquis' or a related variety rather than a consequence of mutation in the gliadin-coding gene caused by in vitro conditions.

Thus we believe that the absence of a thorough genetic approach to the analysis of electrophoretic spectra has caused incorrect interpretations of some results obtained in the reports reviewed. We maintain that simple scoring of changes in gliadin spectrum is not enough to prove that somaclonal variation in gliadin genes has occurred. Some of these workers claimed, as evidence of somaclonal variation, that the "new" pattern differed from those of any wheats in their greenhouses. We do not accept this as sufficient evidence since our experience (and that of others such as Appleyard et al. (1979)) shows that varieties can carry well-hidden off-types. It should be taken into account that an increase in cross-pollination frequency can be caused by extraordinary environmental factors (in vitro cultivation being undoubtedly one of them).

Estimation of the effect of in vitro cultivation on variability of gliadin-coding genes requires statistic comparisons of the frequency of mutational changes (i.e. the appearance of indeed new, previously unknown combinations of gliadin components) in regenerants and in parental material involving very large numbers of grains.

Can somaclonal variations be induced in gliadin genes? What is the frequency and mechanism of its occurrence? We believe answers to these questions will be given by future studies.

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