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Null alleles for gliadin blocks in bread and durum wheat cultivars

D. Lafiandra [1], G. Colaprico [2], D. D. Kasarda [3] and E. Porceddu [1]

[1] Institute of Agricultural Biology, University of Tuscia, Via S. Camillo de Lellis, I-01100 Viterbo, Italy
[2] Germplasm Institute, C.N.R., Bari, Italy
[3] Western Regional Research Center, USDA, ARS, Albany, CA 94710, USA

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Summary. Wheat gliadin proteins are coded by clusters of genes (complex loci) located on the short arms of chromosomes of homoeologous groups 1 and 6 in bread (6×) and durum (4×) wheats. The proteins expressed by the various complex loci have been designated gliadin blocks. In a survey of accessions from the Germplasm Institute (C.N.R., Bari, Italy) collection, several different accessions have been found that lack particular blocks of proteins (null alleles). In some bread wheat accessions, seeds do not express gliadins that are coded by chromosomes 1D and 6A in normal cultivars. Similarly, some durum wheat accessions lack ω-gliadin components coded for by genes on chromosomes 1A and 1B. The missing proteins do not result from the absence of whole chromosomes, but may be the consequence of partial deletion of these genes at a complex locus or result from their silencing.

Key words: Gliadins – Deletion – Gene silencing

Introduction

In wheat, the utilization of gel electrophoretic techniques along with the availability of aneuploids, has resulted in major advances in the understanding of genetic aspects of the storage protein components of the gliadin and glutenin fractions.

Gliadins are coded by genes located on the short arms of homoeologous chromosomes of groups 1 and 6 of the A, B and D genomes of bread wheats (Wrigley and Sheperd 1973; Payne et al. 1982; Lafiandra et al. 1984) and those on chromosomes of groups 1 and 6 of the A and B genomes of durum wheats (du Cros et al. 1983; Lafiandra et al. 1983).

Genetic analysis has indicated that gliadin genes are clustered in complex loci that include several genes, but which act like single genes in crossing experiments (Mecham et al. 1978). The proteins expressed by these gene clusters have been termed "blocks" by Sozinov and Poperelya (1980). Multiple allelic forms of these blocks have been described (Sozinov and Poperelya 1982; Metakovsky et al. 1984).

The information obtained about the nature and dispersion of complex loci has provided new and powerful tools for comparing physical and genetic chromosomal maps and for identification of translocations, substitutions, deletions and other forms of chromosomal rearrangements. Such genetic studies also have the potential to define silent genes or the silencing of complex genetic loci, perhaps as a consequence of the disturbance of regulatory nucleotide sequences that affect DNA transcription.

Investigations of protein block variation in wheat landraces have demonstrated the occurrence of seeds with unusual alleles and also the absence of specific components in these landraces (Damania et al. 1983; Damania 1983). In this paper, we provide additional evidence for spontaneous mutations that have resulted in a loss of functional expression of specific complex loci or parts of them in accessions of both bread and durum wheats.

Materials and methods

The material used in this study was obtained from the bread and durum wheat world collection preserved at the Germplasm Institute, National Research Council of Italy, Bari. Most of the bread wheats analysed were from the materials collected by missions to Nepal in 1979 sponsored by the International Board for Plant Genetic Resources (Erskine et al. 1979), with accession numbers MG 27079, MG 27080, MG 27081, MG 27115, MG 27155 and MG 27163; these accessions might best be considered landraces or landrace cultivars rather than improved cultivars. The bread wheat cultivar 'Raeder' from the United States, with accession number MG 24690, was also analysed.

Durum wheats were represented by accessions MG 18197 and MG 41078 both collected in Algeria. One- and two-

dimensional (two-pH) polyacrylamide gel electrophoresis were carried out on gliadins extracted from single seeds according to the procedures described by Lafiandra and Kasarda (1985).

Results

Bread wheats

One-dimensional polyacrylamide gel electrophoretic patterns (lactate, pH 3.1) of gliadins extracted from single seeds of three bread wheat landrace accessions are shown in Fig. 1. For convenience, we shall simply refer to the accessions as cultivars, even though they do not generally correspond to improved cultivars. Samples in adjoining pairs of lanes were obtained from seeds of the same cultivars. Patterns in lanes 1, 3 and 5 represent typical gliadin patterns of normal hexaploid wheats; components are spread over the entire range of mobility from the α- to the ω-region. Patterns in lanes 2, 4 and 6, which were from seeds found in the same cultivars, indicate deficiencies in slow-moving ω-gliadin components and some components with mobilities close to the borderline between the β- and γ-regions.

The result of two-dimensional separation of the same gliadin extracts from two of these cultivars (corresponding to lane 3 and 4 of Fig. 1) is shown in Fig. 2; the gliadin pattern of the apparently normal hexaploid wheat is on the left and the equivalent pattern, but which is null for 1D-coded ω-gliadins, on the right. In addition, the deficiency of a component near the γ/β borderline and the absence of a major γ-component that was obscured by another component in the one-dimensional separation are indicated by arrows pointing to the equivalent components in the normal sample.

Two-dimensional separation of gliadin components from the bread wheat cultivar 'Chinese Spring' is shown in Fig. 3, along with chromosomal assignments for the corresponding genes based on previously reported results (Lafiandra et al. 1984), but with improved electrophoretic patterns obtained by the method of Lafiandra and Kasarda (1985). The improved electrophoretic patterns resulted in resolution of a minor component in the β-region that had not been resolved previously, and the gene for the new component was assigned to chromosome 6A by techniques previously described (Lafiandra et al. 1984). Comparison of the patterns in Figs. 2 and 3 suggests that the components missing from the deficient pattern of Fig. 2 are coded by genes of chromosome 1D. We have noted similar absences of 1D-coded components in other cultivars from Nepal, such as accessions MG 27115, MG 27155 and MG 27163, in common wheats from other sources, and in improved (commercial) cultivars. Likely examples of similar absences may be noted in

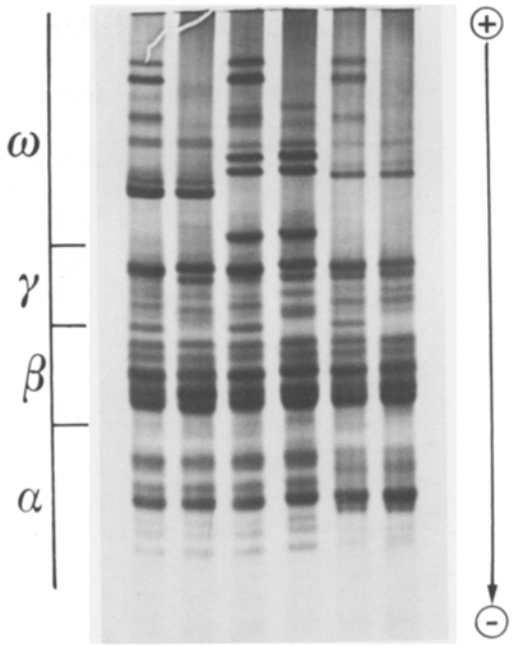

Fig. 1. One-dimensional electrophoretic separation at pH 3.1 of gliadins from normal lines and lines lacking slow ω-components; from *left:* MG 27081 (*lanes 1, 2*), MG 27079 (*lanes 3, 4*), MG 27080 (*lanes 5, 6*)

the gliadin biotype patterns of the US cultivars 'Newana' and 'Wanser', as shown in Figs. 12 and 14 of Mecham et al. (1985), where ω- and γ-gliadin components likely to be coded by genes of chromosome 1D are missing from the pattern of 'Wanser' biotype III. However, ω- and γ-gliadin components likely to be coded by genes of chromosome 1B are missing from the pattern of 'Newana' biotype III.

We also observed gliadin component absences for blocks of genes other than those residing in chromosome 1D. In Fig. 4, we compare the gliadin patterns obtained from a normal seed and one from the same cultivar (Raeder) that yields a pattern missing for several components in the α-region and a minor component in the β-region. Comparison of these patterns with that of 'Chinese Spring' (Fig. 3) indicates that the missing components almost certainly correspond to an allelic block of genes located on chromosome 6A. The chromosome number was found to be normal for this line and for those previously mentioned.

Durum wheats

The presence of seeds with missing gliadin components is not confined to bread wheat. We found a similar pattern among durum wheat accessions as well. Again, it seems useful to describe the different patterns found among durum wheat accessions (we shall refer to the

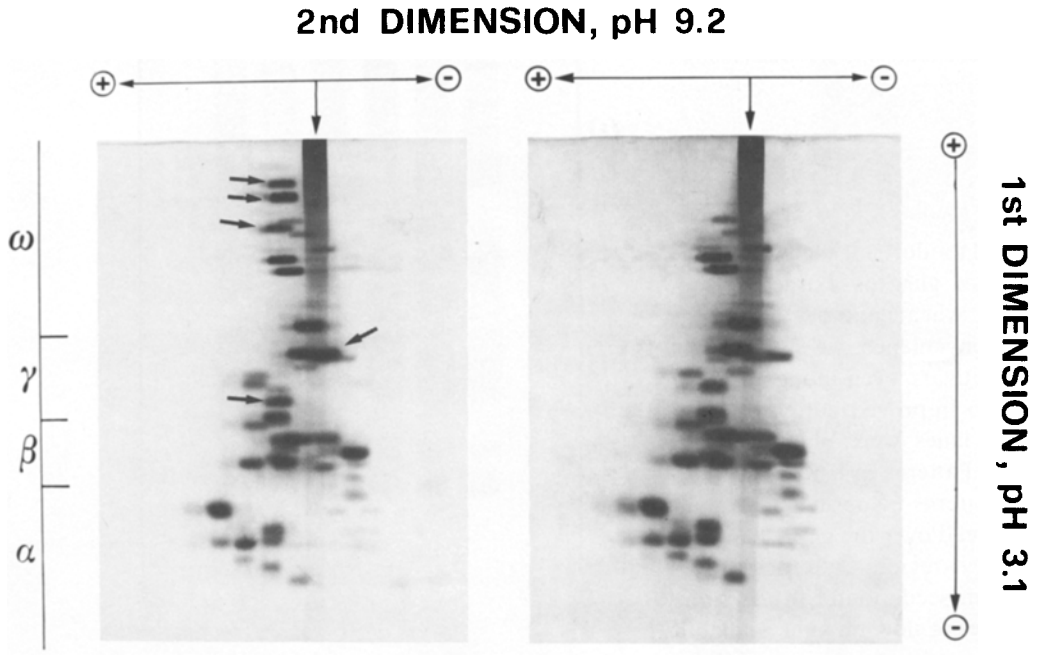

Fig. 2. Two-dimensional (two-pH) electrophoretic pattern of gliadins from a normal line and a line without slow ω-components (corresponding respectively to the sample in *lanes 3* and *4* in Fig. 1)

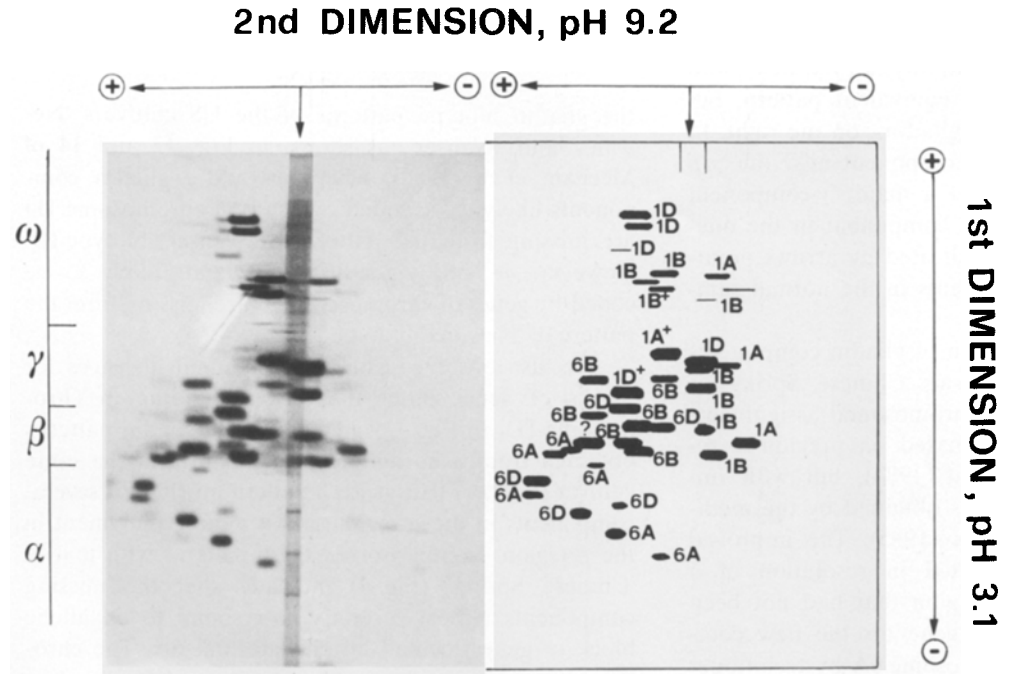

Fig. 3. Two-dimensional electrophoretic pattern of gliadin components and their chromosomal assignment in the bread wheat cultivar 'Chinese Spring'

accessions as cultivars) in relation to the likely chromosomal assignments of the genes coding for the various gliadin components of these tetraploids. Such assignments have been made (du Cros et al. 1983; Lafiandra et al. 1983) for at least one durum wheat cultivar, 'Langdon', by using chromosome substitution lines of this cultivar prepared by Joppa et al. (1973). The results of Lafiandra et al. (1983), who used the two-pH, two-dimensional approach, are shown in Fig. 5, which includes the 'Langdon' gliadin pattern and a diagram

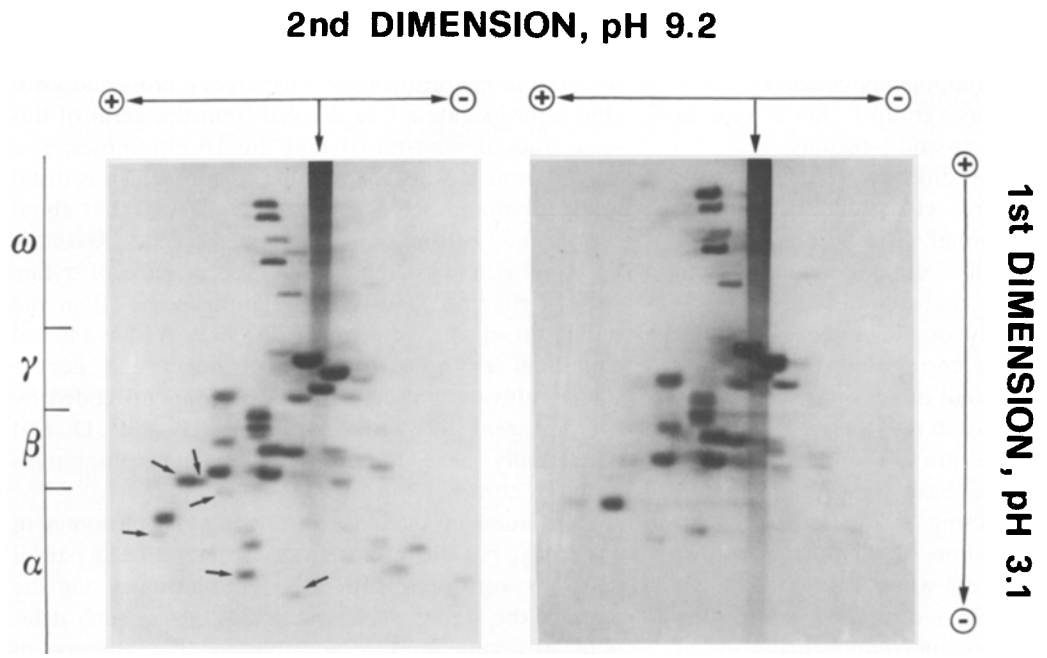

Fig. 4. Two-dimensional electrophoretic pattern of gliadins from a normal seed (*left*) and from the same hexaploid cultivar (MG 24690), without components controlled by genes on the chromosome 6A

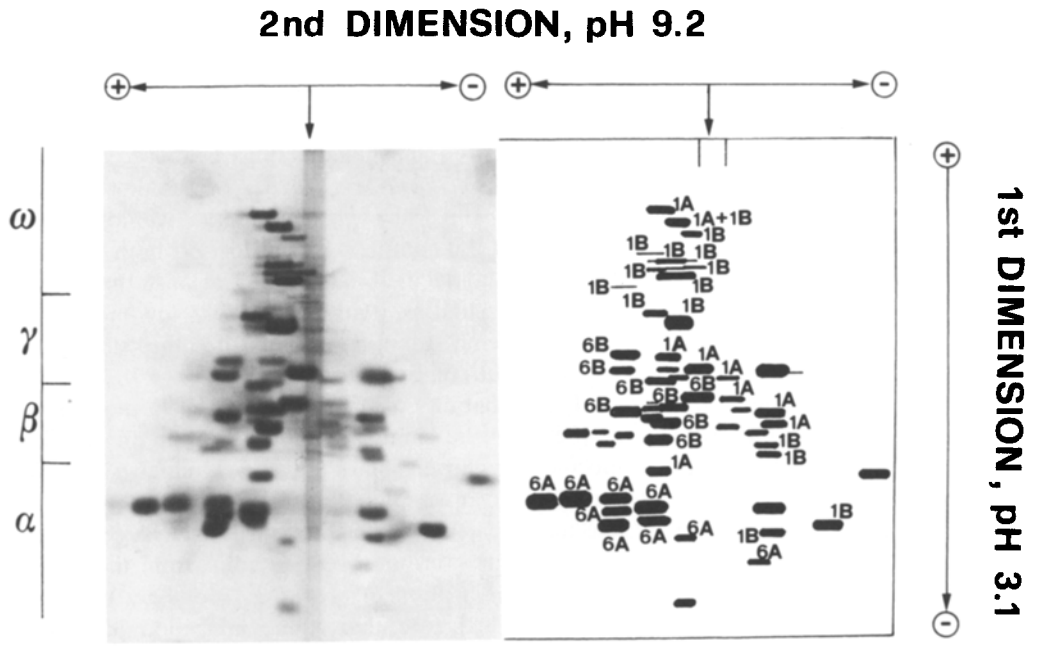

Fig. 5. Two-dimensional electrophoretic pattern of gliadin components and their chromosomal assignment in the durum wheat cultivar 'Langdon'

indicating the chromosomal assignments of the genes coding for the various gliadin components. Components coded by genes on both chromosomes 1A and 1B are present in the ω-region, 6A components are present in the α-region, while 6B-controlled are present in the β- and γ-regions. Several components with a positive charge at pH 9.2 are present in durum wheats. Insofar as assignments could be made, these components were coded by genes on chromosomes 1A and 1B even though some of them have electrophoretic mobilities in

the first-dimension correspoding to α- and β-gliadins, which are usually coded by chromosomes of homoeologous group 6. The major components coded by chromosomes of homoeologous group 1 in hexaploid wheats appear mainly in the ω- and γ-regions.

Except for a few minor differences, these results agree with those of du Cros et al. (1983) that were obtained by a two-dimensional procedure combining isoelectric focusing in the first dimension and lactate electrophoresis (pH 3.1) in the second. These minor differences consisted mainly of the absence of some components that probably correspond to the more basic proteins in our second-dimension separation; these components may be lost in the isoelectric focusing first dimension through a failure of the pH gradient to extend sufficiently into the basic range, a common problem with isoelectric focusing.

Two-dimensional separation of gliadins from different seeds of the same durum wheat cultivar is shown in Fig. 6. When the patterns are compared, that on the right is deficient in several protein components (arrowed in Fig. 6), which are present in the apparently normal pattern on the left. Comparison of these with the pattern of 'Langdon' in Fig. 5 indicates that the genes coding for the missing components should be located on chromosome 1A. Some minor components coded for by genes on chromosome 1A of 'Langdon' do not appear in the normal pattern of the durum cultivar, but this may be ascribed to a difference in the allelic blocks of the two cultivars.

A very special case is represented by the patterns compared in Fig. 7 which indicate that one of the seeds shows simultaneous absence of all major ω-gliadin components controlled by genes on both chromosomes 1A and 1B. By comparison with the pattern of Fig. 5, the slower components should be coded by the genes on chromosome 1A and the faster components by those on chromosome 1B. Curiously, components in the α-, β-, and γ-regions of the electrophoretic pattern deficient in the ω-gliadins appear essentially normal; yet some of these components should also be coded by the same complex loci on chromosomes 1A and 1B that code for the ω-gliadins.

Discussion

The presence of material without slow ω components was first reported by Wrigley and Shepherd (1974) and then by Autran (1975) for the bread wheat cultivars 'India' and 'Darius' respectively.

Variant forms with gliadin component deficiencies were then reported by Damania et al. (1983) among seeds of bread wheat landraces from Nepal. Payne et al. (1984b), using two-dimensional techniques, showed that the entire block of 1D components was missing in the French cultivar 'Darius'. Payne et al. (1984a) also found a seed lacking all the proteins coded by the *Gli-B1* locus in the offspring of a triparental cross and were able to propagate a line derived from the germ of this seed. They demonstrated that the 1B chromosome of this line was lacking the satellite region, which is distal to the ribosomal RNA genes; this indicated that about $^2/_3$ of the chromosome arm remained. Recently, Pogna et al. (1985) reported the occurrence of a line lacking gliadins controlled by chromosome 1B in the Italian bread wheat cultivar 'S. Pastore'. We have found additional examples of seeds of bread and durum wheat cultivars that lack gliadin components coded by complex gene loci on chromosomes 1B and 1D, and additionally have found seeds missing components coded by chromosome 6A.

We found no evidence of missing chromosomes in our study, but did not attempt to determine if partial deletions were present in these chromosomes. On the basis of the studies of Payne et al. (1984a) such deletions are sometimes responsible for the absence of specific groups of proteins, particularly those corresponding to an entire allelic block (although it should be possible for a deletion to remove only part of a gene cluster corresponding to an allelic block).

Our results shown in Fig. 7 are puzzling. The ω-gliadins presumed to be coded normally by the short arm loci of both chromosomes 1A and 1B are missing, while β-, γ-, and ω-gliadins are unchanged. The genes for some of the β- and γ-gliadins must belong to the same allelic blocks (*Gli-A1* and *Gli-B1* loci) as those for the ω-gliadins. Thus, the present results would require deletions in the complex gliadin loci on both chromosomes 1A and 1B that caused loss of only the subloci for ω-type gliadins (and presumably low-molecular-weight glutenin subunits), leaving the other subloci for γ-type gliadins on both chromosomes.

The probability of such a double deletion occurring at precisely the same homologous point on two homoeologous chromosomes must be extremely small. We cannot offer any explanation for our result, but speculate that there may be a difference in DNA sequences controlling transcription of ω-gliadins from those controlling transcription of γ-gliadins coded by the complex *Gli-1* loci, and that stable inhibition of such a controlling sequence has occurred for the ω-gliadin genes. Deletion of or damage to a promoter sequence on one chromosome would not affect transcription of the other; either 1A or 1B ω-gliadins would be synthesized unless an inhibitor had been produced that could bind to equivalent sites on the homoeologous chromosomes and prevent transcription of all ω-gliadin genes. This is obviously a highly speculative suggestion, but we put it forward to focus attention on the difficulty in explaining our observation.

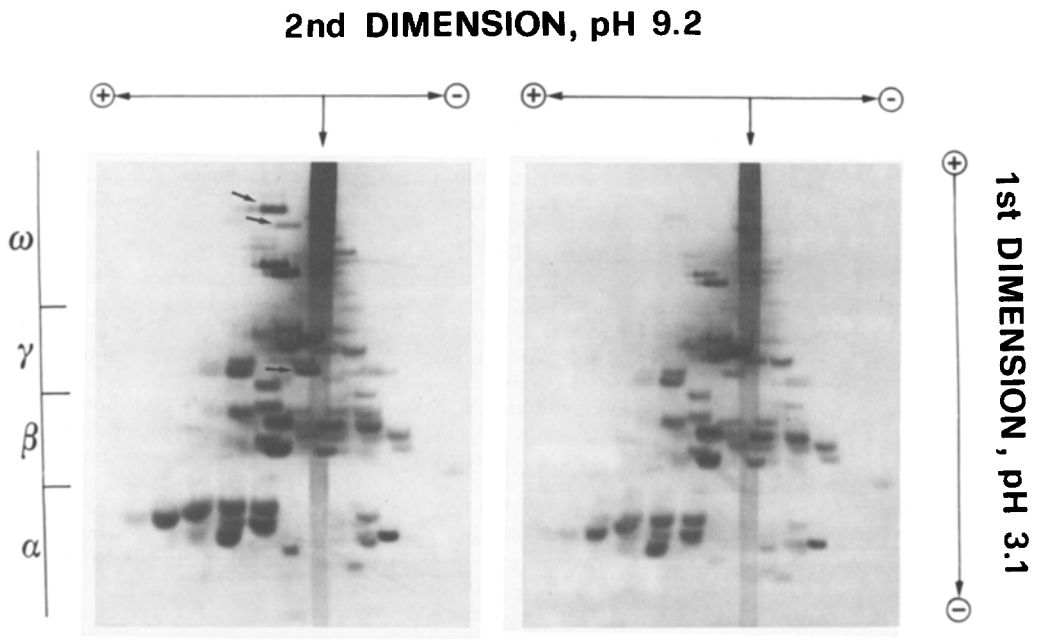

Fig. 6. Two-dimensional electrophoretic pattern of gliadin components from a normal durum wheat seed (*left*) and one from the same cultivar (MG 41078) without 1A controlled gliadins (*right*)

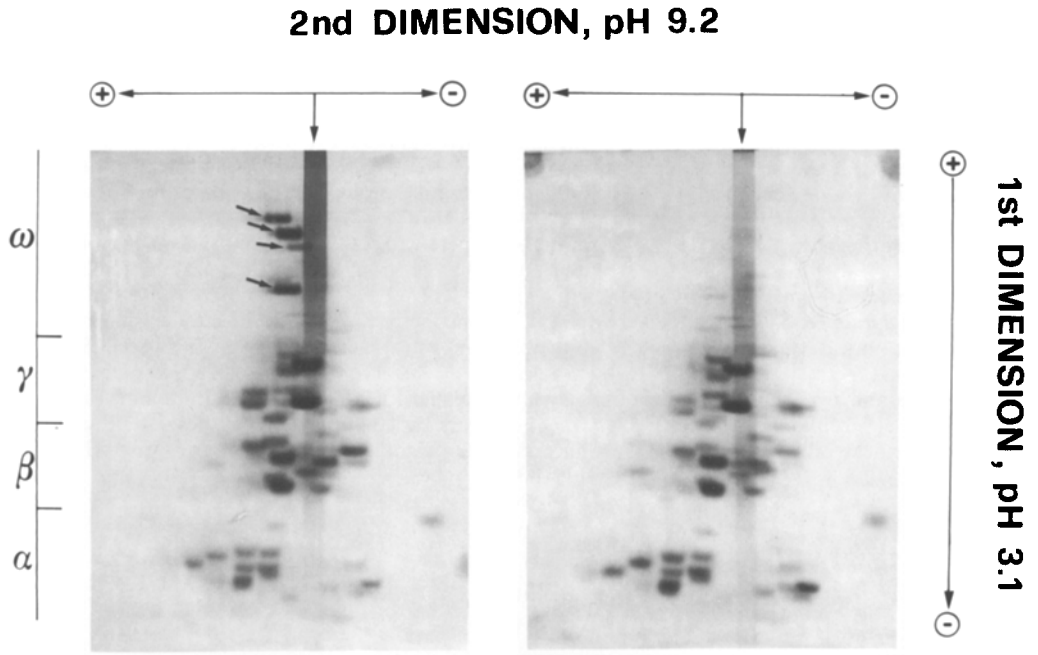

Fig. 7. Two-dimensional electrophoretic pattern of gliadin components from a normal durum wheat seed (*left*) and a seed without the major ω-components (MG 18197)

Study of various mutations that have effected deletions (or additions) of protein components in seeds found in landraces, unimproved cultivars and improved commercial cultivars should be useful in elucidating gene organization and control at the complex loci coding for wheat storage proteins. These mutations are easily recognized by protein electrophoresis of single seeds. A combination of cytogenetic and molecular biological methods involving recombinant DNA (Payne et al. 1984a; Forde et al. 1985) will be useful in

determining the origin of the changes in protein patterns. These changes could affect the processing characteristics of flour and semolina derived from null lines; understanding the changes may provide new insights into the molecular basis for end-use quality in bread and durum wheat.

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