GENETICAL ANALYSIS OF LOW-MOLECULAR-WEIGHT GLUTENIN SUBUNITS OF WHEAT BY TWO-DIMENSIONAL ELECTROPHORESIS

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INTRODUCTION

In the endosperm of bread wheat (T. aestivum L.) glutenins represent about 30% of total proteins. They play a major role in producing the unique viscoelastic properties of wheat flour dough and in determining variations in the breadmaking properties of different cultivars (Payne et al. 1984a).

Glutenins are polymeric proteins, in which several subunits are cross-linked by disulfide bonds, so that their molecular weight ranges from about 80,000 to several million (Kasarda 1989). Glutenin subunits can be fractionated on the basis of their mobility in sodium dodecyl sulphate polyacrylamide electrophoresis (SDS-PAGE), under reducing conditions to break down the disulfide bonds. They have been classified in: high molecular weight glutenin subunits (HMW-GS, 80-120 kDa) and low molecular weight glutenin subunits (LMW-GS, 30-50 kDa) (Payne and Corfield 1979). LMW-GS are coded by three Glu-3 loci located on the short arm of the chromosomes of the group 1 (Glu-A3, Glu-B3 and Glu-D3) (Payne et al. 1984b). Several studies, based on different electrophoretic approaches, have been focused on the description of allelic variation occurring at the Glu-3 loci.

LMW-GS alleles have proved to be difficult to distinghish because, under reducing conditions, they overlap to some of the classical gliadins in one-dimensional SDS-PAGE.

Several techniques have been developped to remove gliadins from LMW-GS electrophopretic patterns. One of them consists in performing a two-step electrophoresis: glutenins are trapped in the first centimeter of a non-reducing gel and then fractionated, after reduction, in a SDS-PAGE gel (Gupta and Shepherd 1987, 1990; Khelifi and Branlard 1991). Another possibility is to use a particular procedure for extraction: gliadins are washed away from the flour with alcohol or DMSO, then the glutenins are subjected to reduction and alkylation before being fractionated in one-dimensional SDS-PAGE (Singh et al. 1991; Gupta and McRitchie 1991) or A-PAGE (Morel, submitted).

Two-dimensional electrophoresis techniques have also been applied to LMW-GS separation (Jackson et al. 1983; Masci et al. 1991).

In this work we present a new two-dimensional A-PAGE/SDS-PAGE technique to fractionate LMW-GS. Several F_7 lines, deriving from the cross between the spring bread wheat cultivars Neepawa and Costantino were analysed and a description of the main components of each LMW-GS allele was achieved.

MATERIALS AND METHODS

Plant material

Single F_7 plants derived from the cross between the spring bread wheat cultivars Neepawa and Costantino were multiplied in the fields at the Experimental Institute for Cereal Research, S.Angelo Lodigiano, Italy.

Gliadin extraction

Gliadin were extracted from ten crushed grains of each plant with 70% (v/v) ethanol for 2 hours at 50°C. They were fractionated by SDS-PAGE as previously described (Dachkevitch et al. 1993).

LMW-GS extraction

LMW-GS were extracted as described by Singh et al. (1991), with the following modifications. After the reduction, glutenins were alkylated overnight at 20°C. An aliquot (150 µl) was precipitated with cold acetone and the dried pellet was resuspended with 50 µl of a solution containing 6M urea, 30% (w/v) glycerol and 25 mM acetic acid. Seven µl were then loaded on the acid gel for the first dimension.

Two-dimensional electrophoresis (A-PAGE x SDS-PAGE)

Acid-PAGE gels and electrode buffers were prepared as described by Clements (1988). Polyacylamide gels (T=12%, C=3.1%) contained 2M urea, 0.1% ascorbic acid, 0.014% ferrous sulfate $7H_2O$ and 0.75% glacial acetic acid, pH 3.1. For each gel, 40 ml of this solution were deareated under vacuum and then 55 µl of 0.6% (v/v) H_2O_2 were added for polymerization. The gels (160 x 180 x 1.5 mm) were run for 4.5 hours at 500 V at 18°C. After the run the gels were cut into single tracks and incubated for 30 min in 62.5 mM Tris-HCl pH 6.8 containing 2% SDS and 40% (w/v) glycerol. The tracks were then loaded on a SDS-PAGE gel for the second dimension.

For SDS-PAGE, running gels were prepared at 15% acrylamide concentration, pH 8.4 (T=15%, C=0.5%); stacking gels were T=5.6%, C=10%. The gels (160 x 180 x 1.5 mm) were run at 40 mA/gel at 18°C and stopped 30 min after the tracking dye had reached the bottom of the gel. They were stained in 12.5% (w/v) trichloroacetic acid with 0.14% (w/v) of Coomassie Brilliant Blue R250, and destained with distilled water.

RESULTS

Before electrophoretic analysis of LMW-GS alleles, gliadin patterns were determined by SDS-PAGE in each F_7 line. In fact, gliadin patterns of the two parental cultivars, Neepawa and Costantino, had been largely described in a previous work (Dachkevitch et al. 1993). The Gli-1 locus, coding for gliadins, and the Glu-3 locus, coding for LMW-GS, are tightly linked on the short arm of the chromosomes of the group 1 (Payne et al. 1984b). The Glu-B3 locus was found to be 1.8-2.0 cM from Gli-B1 (Singh and Shepherd 1988; Pogna et al. 1990); no recombination was found between the two loci on the chromosomes 1A and 1D (Singh and Shepherd 1988). Therefore, LMW-GS composition could be derived on the basis of gliadin patterns. Gliadin composition of F_7 lines is shown in Table 1.

Fig. 1 shows two-dimensional maps of reduced and alkylated glutenins from Neepawa (A) and Costantino (B). On the left side of the figure (top) HMW-GS are indicated.

LMW-GS patterns appear to be composed by a high number of spots with different staining intensities. Some gliadins are also present in the samples (brackets), in spite of the treatment with alcohol before the extraction of glutenins.

The components coded at each Glu-3 locus (Glu-A3, Glu-B3 and Glu-D3) were identified by the comparison between the 2-D maps of the two parental cultivars and those of the F_7 lines carrying different alleles at the Gli-1 loci (Figs. 1 and 2).

For example, by the comparison between lines R6 and R8 (Fig. 2, A and B), which have the same alleles for chromosomes 1A and 1D, but different alleles for chromosome 1B, subunits coded at Glu-B3 could be identified (*arrows*).

Lines R6 and R12 (Fig. 2, A and C) differ only for chromosome 1D alleles: the subunits coded at the Glu-D3 locus are indicated by *arrowheads*.

Finally, comparing Neepawa (Fig. 1A) and line R19 (Fig. 2D), which carries the Neepawa alleles for chromosomes 1B and 1D and the Costantino allele for chromosome 1A, subunits coded at Glu-A3 were recognized (*open triangles*).

F_7 lines resulted to be very useful for the description of LMW-GS alleles in Neepawa and Costantino. For example, the unusual position of Glu-A3 polypeptide in Neepawa, respect to the position in SDS-PAGE of the Glu-A3 allele of other cultivars (unpublished results), made its identification uneasy by one-dimensional electrophoresis, so that Neepawa was thought to carry a null allele at this locus.

It was not possible, however, to identify with this material the genes coding for all the spots resolved in the 2-D map. Some proteins are present in the two parental cultivars and in all the lines we analysed (*white stars*); some others are present or absent without any relationship to the genetic background (*solid triangles*). Further analyses should be performed to attribute these spot to a locus or a chromosome.

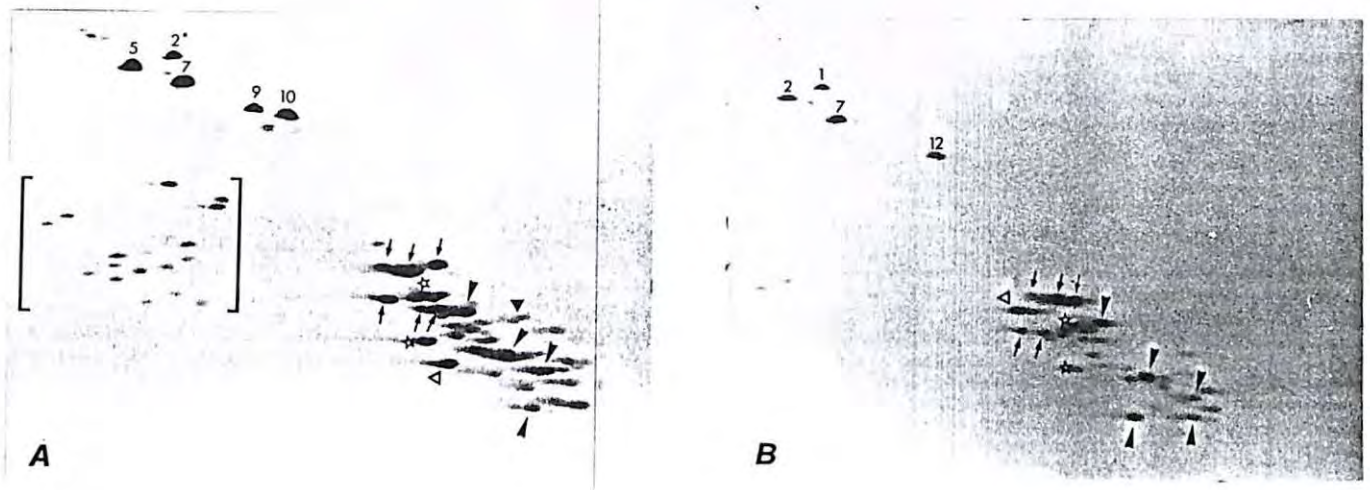

Fig. 1 : Two-dimensional fractionation of LMW-GS from Neepawa (A) and Costantino (B).
On the left side (top) HMW-GS are numbered. *Open triangles* show the subunits coded by Glu-A3; *arrows* show the subunits coded at Glu-B3; *arrowheads* show the subunits coded at Glu-D3.

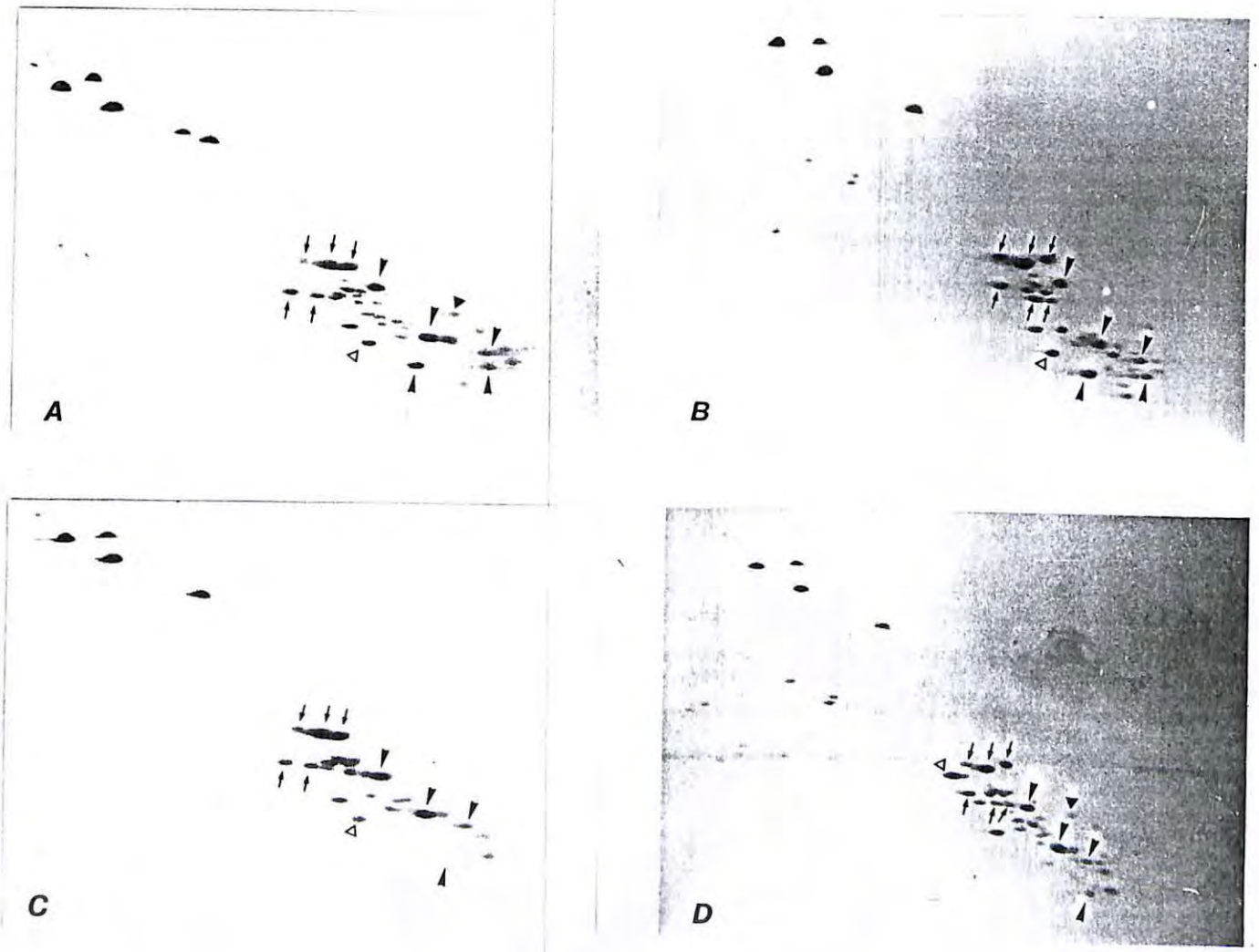

Fig. 2 : Two-dimensional fractionation of LMW-GS from lines R6 (A), R8 (B), R12 (C) and R19 (D).
Open triangles shows the subunits coded at Glu-A3; *arrows* show the subunits coded at Glu-B3; *arrowheads* shows the subunits coded at Glu-D3.

TABLE 1

Composition of ω-gliadins alleles coded at the Gli-1 loci in F_7 lines.

Lines	Gli-A1	Gli-B1	Gli-D1
R6	N	C	C
R8	N	N	C
R12	N	C	N
R19	C	N	N

N = Neepawa; C= Costantino.

DISCUSSION

F_7 recombinant lines derived from the cross Neepawa x Costantino represented an interesting material to describe LMW-GS alleles in the two parental cultivars, characterized by contrasting technological quality and coding for different alleles at the Gli-1 loci.

Due to the tight linkage between Gli-1 and Glu-3 loci on the short arm of chromosomes 1A, 1B and 1D in wheat, gliadins can be considered as reliable markers for LMW-GS alleles identification. For this reason, our analysis was based on F_7 lines gliadin composition, as revealed by one-dimensional, unreduced SDS-PAGE (Dachkevitch et al. 1993).

Glutenins from recombinant lines were first analysed by one-dimensional electrophoresis: A-PAGE according to Morel (Cereal Chem., submitted) and 15%, pH 8.4 SDS-PAGE. By both techniques a large number of polypeptides was resolved. Anyway, overlapping of subunits with similar electrophoretic mobility did not allow an exhaustive description of the alleles.

A-PAGE and SDS-PAGE were then combined in an original, two-dimensional system. This 2-D technique gave a good and easily reproducible resolution of LMW-GS alleles. Through the comparison of the 2-D maps of parents and recombinant lines, the main subunits coded at each Glu-3 locus could be identified. In particular, it was possible to recognize Glu-A3-encoded subunit in Neepawa, that was thought to carry a null allele.

Several 2-D systems have been employed to fractionate LMW-GS alleles: IEF or NEPHGE in the first dimension, followed by SDS-PAGE; Acid-PAGE in the first dimension, followed by Basic-PAGE or SDS-PAGE (Jackson et al. 1983; Masci et al. 1991). The system we propose in this work is based on original protocols, that were found to give an improved resolution when applied in one-dimensional electrophoresis. It can be therefore considered as a possible alternative to other electrophoretic methods.

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ANALYSE GÉNÉTIQUE DES SOUS UNITÉS GLUTÉNINES DE FAIBLE POIDS MOLÉCULAIRE DU BLÉ PAR ÉLECTROPHORÈSE BIDIMENSIONNELLE

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Les sous-unités gluténines de faible poids moléculaire (LMW-GS) représentent environ 30 % des protéines du gluten de blé (*Triticum aestivum* L.). Sous le contrôle génétique de trois différents loci (*Glu-3a, Glu-3B, Glu-3D*) situés sur les chromosomes homéologues du groupe 1, elles sont à la base de l'aptitude à la transformation boulangère du blé. Suivant les variétés, le nombre de LMW-GS varie de 25 à 30 sous-unités, de poids moléculaire compris entre 30 kDa et 50 kDa. La détermination de leur variation allélique fait l'objet de nombreuses études et repose sur l'analyse des diagrammes électrophorétiques. Du fait des fortes homologies de taille entre les LMW-GS, la séparation obtenue par SDS-PAGE ne permet de distinguer qu'une dizaine d'électromorphes. Au contraire, par IEF le nombre d'électromorphes dépasse 50 pour ces mêmes LMW-GS, le degré d'amidation des glutamines, acide aminé présent à hauteur de 34 à 42 % chez les LMW-GS, variant selon un mécanisme mal connu.

Nous avons développé une technique d'électrophorèse bidimensionnelle originale : PAGE-acide des gluténines réduites et alkylées pour la première dimension et SDS-PAGE à 15% d'acrylamide, pH 8.4 pour la deuxième dimension. Cette technique a été appliquée à l'étude des lignées F7 dérivées du croisement entre Costantino, cultivar italien et Neepawa, d'origine canadienne, porteurs d'allèles différents aux trois loci *Glu-3* et aux qualités technologiques très contrastées. Les gels 2-D qui assurent une bonne résolution des LMW-GS nous ont permis d'effectuer une description complète des sous-unités contrôlées par les différents allèles.