B- and C-type low molecular weight glutenin subunits in tetraploid wheat germplasm

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ABSTRACT

Electrophoretic analyses of B and C-type low molecular weight glutenin subunits (LMW-GSs) in several tetraploid wheat accessions and in durum wheat lines, carrying a 1BL.1RS translocation and null at *Gli-2* locus, have allowed the identification of new variants at the *Gli-1*/*Glu-3* loci.

INTRODUCTION

The B and C-type low molecular weight glutenin subunits (LMW-GSs) are structural components of the glutenin polymer affecting dough viscoelastic properties. They are encoded by genes at *Glu-3*, *Gli-1* and *Gli-2* loci present on the short arms of the homoeologous group 1 and 6 chromosomes in tetraploid and hexaploid wheats[1]. The B-type, considered typical LMW-GS, have been also subdivided into LMW-s, LMW-m, LMW-i, according to their first amino acid residue of the mature proteins. In durum wheat allelic variation at the linked *Gli-B1/Glu-B3* loci has also been associated with pasta-making quality. A consistent relationship between the presence of a gliadin protein, termed γ-45 and gluten strength and the presence of a different gliadin protein, γ-42 and gluten weakness[2] has been demonstrated. This was later shown to be due to genetic linkage of genes coding for these allelic gliadin components and those encoding for two groups of low-molecular-weight glutenin subunits, designated LMW-2 and LMW-1, the former group of subunits being the functionally active polypeptides determining gluten strength[3].

The C group of LMW-GS is quantitatively present in a lower amount, with respect to the B group, and has been studied to a limited extent[4]. N-terminal amino-acid sequencing has shown that they are made up of α/β and γ–gliadin-like components. This led to the hypothesis that they are encoded by genes present at the *Glu-3 locus* and possibly at the *Gli-1* and *Gli-2* loci.

It has been suggested that the presence of gliadin-like subunits in glutenin preparations is very likely due to mutations that affect the number and/or the distribution of cysteine residues[5]. It is likely that mutated genes have acquired one extra or less cysteine residue, so that the encoded polypeptides frequently have odd numbers of cysteine residues. An odd number of cysteines makes such subunits act as chain terminators of growing glutenin polymer chains, which would presumably have a negative effect on flour quality[6].

In order to increase knowledge about the role played by LMW-GS on quality characteristics of durum wheat and detect novel variation, B and C-type subunits have been extracted from different varieties and lines of tetraploid wheats and analysed by one- and two-dimensional electrophoresis and chromatographic techniques (RP-HPLC).

MATERIALS AND METHODS

Plant material. A collection of 237 accessions of tetraploid wheats *T. turgidum* ssp. *turanicum,* ssp. *polonicum*, ssp. *carthlicum*, aneuploid lines of the cv. Langdon, a durum wheat line carrying a 1BL.1RS translocation and a durum line null at *Gli-A2* locus, have been analysed along with different cultivars and lines of durum and bread wheats used as references.

Electrophoretical analyses. Total proteins have been extracted from crushed endosperm halves following fractionated precipitation with hydro-alcoholic solvent and gliadin analysed by A-PAGE (pH 3.1). In order to obtain glutenin fractions enriched in B or C subunits, we used different precipitation steps involving increasing concentrations of propan-1-ol, based mainly on the modified methods described by Verbruggen et al. [7] and Masci et al.[4]. Total proteins and the above fractions enriched of B+C, B- and C-type LMW-GSs have been separated by SDS-PAGE (1DE) and subsequently by IEFxSDS-PAGE (2DE) following the modified procedure reported by Ikeda et al.[8].

Chromatographical analyses. Fractions containing LMW-GSs have been prepared for reversed-phase high performance liquid chromatography (RP-HPLC). Analyses have been performed essentially according to the procedure of Marchylo et al. [9].

RESULTS AND DISCUSSION

Electrophoretic separation of different groups of LMW-GS present in the durum wheat cultivar Svevo is reported in Fig.1. Though some overlap is evident between the B- and C- fractions, the results can be used in further studies.

A similar separation of the B and C-type LMW-GSs present in the durum wheat cultivar Langdon and derived D-genome substitution lines, have permitted complete chromosomal assignment of components of the two group of subunits after their electrophoretic and chromatographic separations indicating association of the majority of C-type subunits to the *Gli-1* and *Gli-2*

loci. The procedure adopted to obtain glutenin fractions enriched in B- or C-type subunits, has been applied to identify novel LMW-GS alleles in the *T. turgidum* ssp. *turanicum, polonicum*, *carthlicum* collection showing, however, the difficulties in resolving the electrophoretic and chromatographic patterns even if carried out by different precipitation steps.

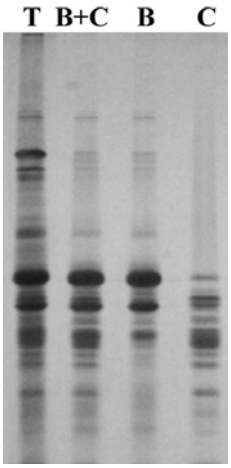

Figure 1. SDS-PAGE separation of total proteins (T), B+C, B- and C-type LMW-GSs present in the durum wheat cv. Svevo.

Additional information on the C-type subunits were deduced by the 2DE of durum wheat cultivar Svevo and a derived near isogenic line in which a deletion on the short arm of the chromosome 6A is responsible of the absence of the entire *Gli-A2* associated gliadin components. In this case, electrophoretic separation showed that absence of 6A encoded gliadin components was associated to the absence of some C-type subunits compared to Svevo (Fig. 2).

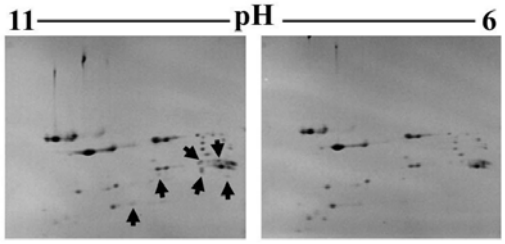

Figure 2. Comparison of enriched B- and C-type LMW-GS fractions from cv. Svevo (a) and Svevo null at *Gli-2* locus, separated by 2D gel electrophoresis.

RP-HPLC of the glutenin fractions extracted from different tetraploid wheats indicated the existence of variation in the B-type subunits, as reported in *T. polonicum* by other authors[10], with most of the glutenin alleles similar to that of cultivated wheat. Analysis of C-type subunits showed extensive allelic variation compared to the one present in durum wheat cultivars (Fig. 3 and 4). Out of 237 accessions of domesticated tetraploid wheats analysed, 26 of *T. turanicum*, 19 of *T. carthlicum* and 20 of *T. polonicum* LMW-GS alleles have been detected. The new alleles provide the basis for further studies, at the molecular level, on these glutenin fractions to assess their structural organization in the gluten complex.

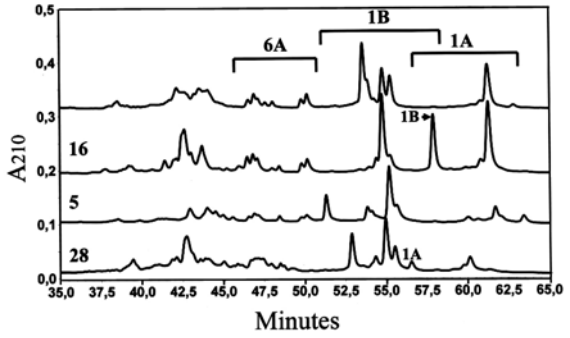

Figure 3. RP-HPLC separations of C-type LMW-GSs from durum wheat cv. Svevo and accessions of *T. turanicum* (16), *T. polonicum* (5), *T. carthlicum* (28).

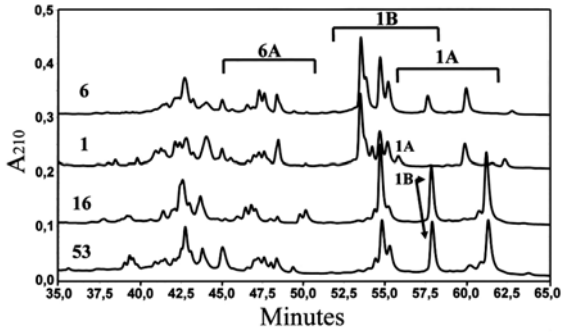

Figure 4. RP-HPLC separations of C-type LMW-GSs from accessions 6, 1, 16, 53 of *T. turanicum*.

Comparison of electrophoretic and chromatographic analyses of B- and C-type LMW-GSs from tetraploid wheat *T. turgidum* ssp. *durum* and ssp. *carthlicum*, *turanicum* and *polonicum* have indicated the existence of a large variation in this material providing additional information about the association to *Glu-3, Gli-1* and *Gli-2* loci. The role of structural and quantitative variation associated to different allelic subunits will be assessed in order to shed light on this complex protein mixture strongly affecting wheat technological properties.

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ACKNOWLEDGEMENT

This work is supported by AGRO-GEN public/private laboratory for Plant Genomics (Lab.6 n. DM 18092). We also thank Dr. E. De Angelis and M. Lestingi for technical assistance.