# Triticum dicoccoides for qualitative improvement of durum wheat: Associations of protein loci to grain traits in recombinant inbred lines

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# *Triticum dicoccoides* for qualitative improvement of durum wheat: Associations of protein loci to grain traits in recombinant inbred lines

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**SUMMARY-** The improvement of protein quantity and quality are important goals of durum wheat and other wheat breeding programs. This research is aimed at studying the variability and the associations of the gliadin and glutenin subunit alleles with some grain quality traits by means of a set of recombinant inbred lines (RIL) derived from the cross durum wheat cv. Messapia x *Triticum dicoccoides* acc. MG4343. The results obtained show that: (i) the wild wheat *T. dicoccoides* crossed with durum wheat provides a wide variation for some quantitative traits of grain quality; (ii) there is no correlation between the protein content and the sedimentation value; (iii) HMW and LMW glutenin loci are associated with sedimentation value but they account for only a part of the variation; (iv) the *Gli-A1* and *Gli-B2* loci of *T. dicoccoides* are associated with protein quantity; (v) recombinant inbred lines constitute a good genetic material to study the association between marker loci and quality traits.

**Key words:** Durum wheat, *Triticum dicoccoides*, grain quality, protein content, SDS sedimentation.

***RESUME -*** *"*Triticum dicoccoides *pour l'amélioration de la qualité du blé dur : Associations de loci de protéines avec les caractères du grain chez les lignés recombinantes autogames". L'amélioration de la quantité et de la qualité des protéines sont des objectifs importants des programmes d'amélioration du blé dur ainsi que pour d'autres blés. Cette recherche vise à étudier la variabilité et les associations des allèles des sous-unités de gliadine et gluténine avec certains caractères qualitatifs du blé à travers une série de lignées "inbred" recombinantes (RIL) dérivées du croisement blé dur cv. Messapia x Triticum dicoccoides acc. MG4343. Les résultats obtenus montrent que : (i) le blé sauvage T. dicoccoides croisé avec le blé dur présente une grande variation pour ce qui est de certains caractères quantitatifs de la qualité des graines ; (ii) la teneur en protéines n'est pas corrélée avec la valeur de sédimentation ; (iii) les loci de gluténine HMW et LMW sont associés avec la valeur de sédimentation mais ils n'expliquent qu'une partie de la variation ; (iv) les loci Gli-A1 et Gli-B2 de T. dicoccoides sont associés avec la quantité de protéines ; (v) les lignées "inbred" recombinantes constituent un bon matériel génétique pour l'étude de l'association entre les loci des marqueurs et les caractères qualitatifs.*

***Mots-clés :*** *Blé dur,* Triticum dicoccoides, *qualité du grain, teneur en protéine, Test de Sédimentation SDS.*

## Introduction

In Western Europe and Northern America durum wheat is mostly used for flour and pasta production. Despite the wide variability in quality characteristics within cereals, it is ascertained that the rheological properties of the dough obtained with durum wheat flour are most suitable for the modern procedures for pasta production, compared with those obtained from common wheat or other cultivated species of *Triticum*. This is why, in some countries, where this obligation is not in force, this species is increasingly used as demand rises and higher quality is required.

In the case of durum wheat, quality does not only refer to its nutritional value but chiefly to the technological and organoleptic properties of the finished products. Given that the notion of quality is still subjective and controversial, in any case, in assessing the quality of spaghetti samples, different characteristics should be considered, including cooking and post-cooking resistance, stickiness, consistency, colour, taste, fragrance, etc., whose appraisal is often subjective. Although such properties are differently related to each other, they are indeed the ultimate expression of physical and chemical

processes occurring during kneading; they can reveal differences resulting from the qualitative and quantitative variability of different flour components.

The main commercial, technological and nutritional characteristics considered by breeders and pasta-makers concern kernels (hectolitre weight, 1000 grain weight, seed size, seed shrivelling, yellowberry, pericarp/endosperm ratio, colour), flour (carotenoid content, protein content, gluten quality and quantity, ash content) and pasta (colour, cooking-resistance, stickiness, consistency). All these properties are important and greatly contribute to the value of durum wheat products. Nevertheless, the properties which are most directly related to pasta quality are the ones which determine the technological quality, which is mainly dependent on protein quality and quantity, in particular on the content and quality of water-insoluble protein fraction (i.e. gluten), and carotenoid content, as well as peroxidase which affect pasta colour.

Several research groups (including Della Gatta *et al.*, 1979; Dexter and Matsuo, 1980; Autran and Feillet, 1985) have pointed out a highly positive significant correlation between protein quantity and pasta cooking quality. Under normal cropping conditions, wheats show a low protein content, usually ranging between 10 and 14%. Through the past twenty years, the rise in seed protein content has been mainly obtained by increasing nitrogen fertilization. The genetic improvement of protein content has been particularly hampered, not only by the sharp environmental influence, but also by the fact that a negative correlation was found between the grain yield and seed protein content in segregating populations in all cereals (Cox *et al.*, 1985; Day *et al.*, 1985). Different research groups point out, however, that the seed protein percentage can be increased by genetic manipulation without a simultaneous reduction of productivity (Johnson *et al.*, 1978; Day *et al.*, 1985). An interesting approach to increase wheat protein content consists in using the germplasm of some Triticeae species (Johnson and Waines, 1977; Avivi, 1978; Levy and Feldman, 1989; Blanco *et al.*, 1990; Law *et al.*, 1994).

A high protein content, however, does not always assure the good quality of pasta. It has been by now ascertained that specific protein components are correlated with the technological properties of durum wheat flour. The viscoelasticity of gluten is highly correlated with some gliadin subunits coded by the *Gli-B1* locus (Damidaux *et al.*, 1978). The γ-42 and γ-45 alleles, so called for the relative mobility of the two γ-gliadin subunits in polyacrilamide gels, have been found in every durum wheat collection and always associated with high and low gluten viscoelasticity, respectively. *Gli-B1* locus is strictly linked to the *Glu-B3* locus coding for low molecular weight glutenin subunits (LMW). Pogna *et al.* (1990a) have recently demonstrated that the γ-42 and γ-45 subunits are only genetic markers of grain quality since as a matter of fact the LMW glutenin subunits are functionally responsible for gluten viscoelasticity.

The pasta-making quality of durum wheat flour has been also associated with the high molecular weight (HMW) glutenin subunits (Tomassini *et al.*, 1988; Autran and Galterio, 1989; Boggini and Pogna, 1989; Pogna *et al.*, 1990b). HMW subunits are encoded at the *Glu-1* loci on the long arm of the group 1 chromosomes (1A, 1B and 1D). Each locus consists of two genes encoding a low Mr x-type subunit and a high Mr y-type subunit (Payne *et al.*, 1981). Tetraploid wheat could, theoretically, contain four different subunits. In fact only one or two subunits are present in durum wheat cultivars as a result of the silencing of some genes. The analysis in SDS-PAGE of 65 Italian cultivars bred in the two last decades by 20 different breeders showed a low variability at Glu loci as only 3 alleles (coding for subunits 7+8, 6+8 and 20) were detected (Blanco *et al.*, 1994). A number of surveys on the polymorphism of HMW subunits in wild and cultivated wheats have been reported, notably by Mansur-Vergara *et al.* (1984), Margiotta *et al.* (1987), Levy *et al.* (1988), Branlard *et al.* (1989) in tetraploid wheats, Waines and Payne (1987) and Blanco *et al.* (1994) for A genome diploids. This polymorphism provides a basis for the correlation of specific alleles with differences in grain quality traits.

The objectives of this paper are to study the variability and the associations of the gliadin and glutenin subunit alleles with some grain quality traits by means of a random population of near-homozygous lines termed recombinant inbred lines (RIL). The RIL were derived from two genotypes of durum wheat and *Triticum dicoccoides* contrasting greatly in grain protein content and sedimentation value.

## Materials and methods

The cv. Messapia of durum wheat (low protein content and high sedimentation value) and the accession MG4343 of *Triticum dicoccoides* (high protein content and low sedimentation value) were hybridized and random $F_2$-derived $F_8$ lines were developed by the single-seed-descent method. After the last generation of selfing, each RIL was bulk-harvested to provide seed for replicated field trials and for electrophoretic analysis to classify each RIL for gliadin and glutenin protein subunits.

Total seed protein extraction and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis were carried out with 10% polyacrylamide (Payne *et al.*, 1981). Monomeric prolamins were extracted from crushed single seeds (several seeds for each RIL) with 1.5 M dimethylformamide at a 1:5 w/v ratio. After centrifugation (15 min at 10,000 x g), the clear supernatant was used for electrophoretic separation (Lafiandra and Kasarda, 1985). For this study, the Messapia allele at any protein locus was arbitrarily designated 1 and the MG4343 allele was designated 2. Heterozygotes were designated 1.5. The high-molecular-weight (HMW) glutenin bands were numbered according to the system adopted by Payne and Lawrence (1983) and the asterisk was used to indicate the new subunits detected in the accession of *T. dicoccoides*.

Sixty-five RIL and the two parental lines were sown in a randomized complete block design with four replicates in Bari on December 12, 1992. Plots were 1.2 m long and consisted of two rows, 30 cm apart. 120 kg $ha^{-1}$ of nitrogen were applied during the experiment.

The grain nitrogen content was determined for each entry on ground seeds by the near-infrared technique (NIR) using a Technicon Infralyzer 450 and checked using standard Kjeldahl procedures. The 1000 grain weight was determined on a 15 g sample. The SDS sedimentation value was used as a measure of gluten strength of the flour according to the method proposed by Quick and Donnelly (1980) and modified by Dick and Quick (1983) using 1 g of whole grain meal obtained by grinding the sample in a Udy mill with a 1.00 mm sieve.

The data obtained from each field plot were subjected to analysis of variance to detect genetic variation among RIL and between the two parents. The effects of gliadin and glutenin subunit alleles on grain quality traits was studied by ANOVA and regression analysis.

## Results and discussion

Fig. 1 reports the distribution frequencies of the RIL population and the parental mean values of protein content (%), protein per grain (%), sedimentation value and 1000 grain weight (g). The patterns of variability are those of typical quantitative traits, indicating a large number of segregating genetic factors. No transgressive segregation was observed for all characters, excluding some genotypes showing sedimentation values which exceeded those of the parents.

The phenotypic matrix of correlation between all the parameters measured in the 65 RIL, is presented in Table 1. Protein percentage was negatively correlated to 1000 grain weight, so indicating that high protein content could partly be due to the reduced seed size. However, some lines exhibited both an increase in protein content as well as in 1000 grain weight. A negative correlation between protein content and seed weight was also found by Nevo *et al.* (1986) but not by Avivi (1978) and Levy and Feldman (1989). As expected, protein per grain was positively correlated with 1000 grain weight.

SDS-sedimentation value, grain quality parameter widely known to have a positive relationship to pasta-making quality, showed no relationship with protein content and 1000 grain weight. Thus, it may be possible to select both for high protein content and high sedimentation value.

The cv. Messapia of durum wheat and the accession MG4343 of *T. dicoccoides* differed at all four gliadin (*Gli-1* and *Gli-2*) loci on the short arm of chromosomes 1A, 1B, 6A and 6B (Fig. 2) and at the two HMW glutenin loci (*Glu-1*) on the long arm of chromosomes 1A and 1B (Fig. 3) Messapia had no band at *Glu-A1* and bands 6 + 8 at *Glu-B1*; MG4343 had two new alleles not reported in the catalogue of Payne and Lawrence (1983) and coding bands 1* (*Glu-A1*) and 7* + 22* (*Glu-B1*). For each gliadin

and glutenin locus, the RIL population was divided into two groups: those resembling the cultivated type (Messapia) and those resembling the wild type (MG4343) corresponding to the homozygous genotypes. The observed frequency of alleles at each locus in the whole population of RIL is shown in Table 2. There was generally a good agreement between the observed frequencies of RIL and the expectations from independent random assortment. There was some disagreement for the *Glu-B1* locus because the observed frequencies differed significantly from expected frequencies. The deviation of *Glu-B1* frequency from 0.5 might be an indication of unintentional selection.

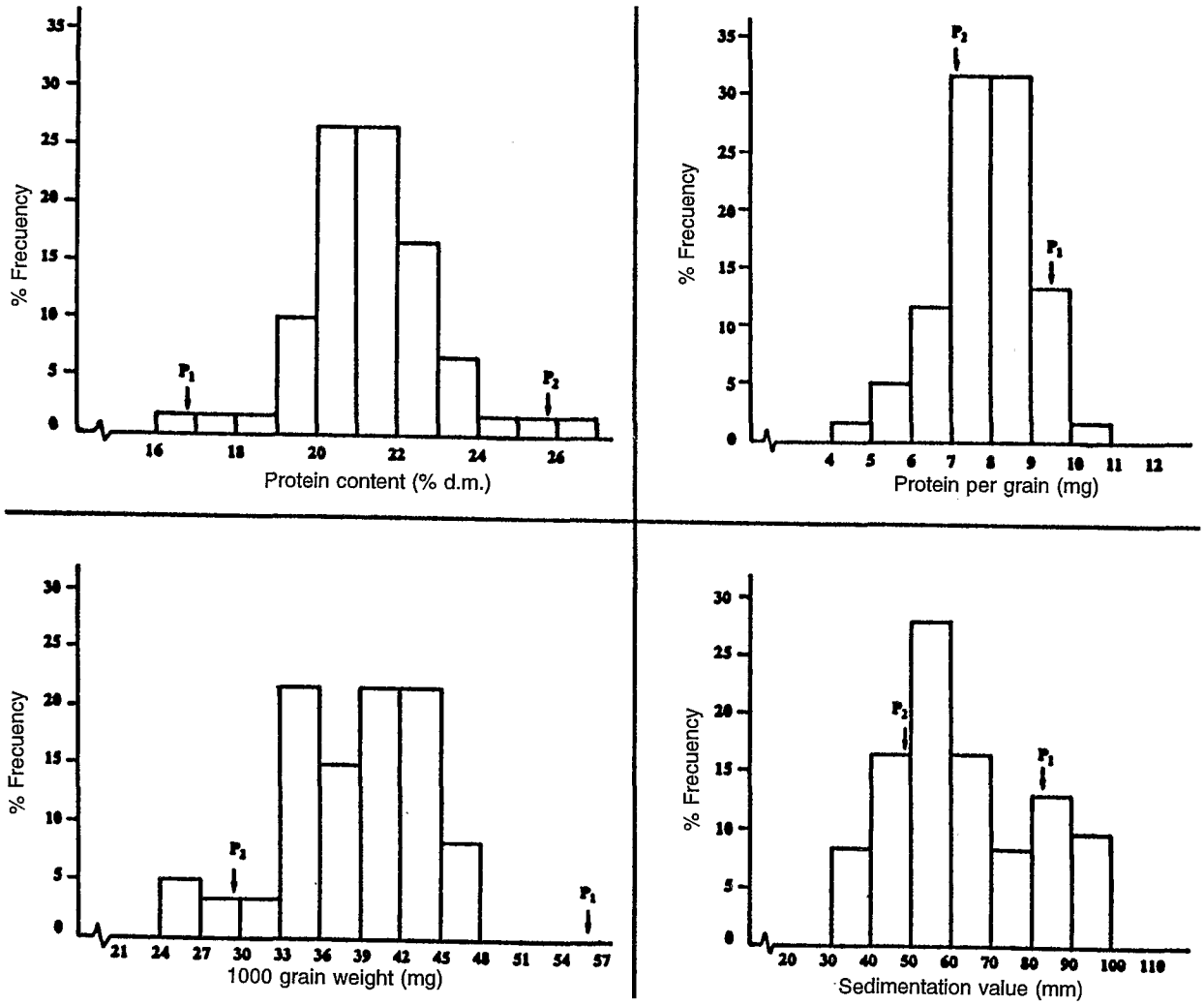


Fig. 1. Frequency distribution of protein content, protein per grain, 1000 grain weight and sedimentation value in a population of recombinant inbred lines derived from a cross between cv. Messapia of durum wheat ($P_1$) and the accession MG4343 of *T. dicoccoides* ($P_2$). Arrows indicate mean values of the two parental lines.

The results of regression analysis are reported on Table 3. $R^2$ values estimate the amount of variation between inbred means, explained by the variation of that protein locus, while the sign of the regression coefficient indicates the distribution of the alleles in the two parental lines: + or - indicate that the allele which increases the character is in the Messapia or in MG4343 parent, respectively. No gliadin locus was associated with sedimentation value, excluding the *Gli-B1* allele of *T. dicoccoides* which significantly decreases that quality parameter. The *Gli-B1* locus is strictly linked to *Glu-B3* locus coding for LMW glutenin subunits on chromosome arm 1BS and it is now considered a marker of quality. Indeed, Pogna *et al.* (1990b) have recently ascertained that *Glu-B3* is functionally responsible for the protein quality. The new allele *Glu-A1* of *T. dicoccoides* coding for the HMW glutenin band 1*

is positively associated with high sedimentation value in comparison with the null allele of cv. Messapia. No effect on sedimentation value was observed between the two *Glu-B1* alleles coding the band 6+8 and 7*+22* in the Messapia and *T. dicoccoides*, respectively. The regression analysis thus pointed out that only *Gli-B1*/*Glu-B3* and *Glu-A1* loci have effects on sedimentation value with $R^2$ values of 22.9% and 14.8% respectively. Therefore, the remaining portion of variation is likely to be contributed by other genes not associated with protein loci. This could explain the variation of sedimentation value among lines having the same protein genotypes.

Table 1. Coefficients of correlation between seed quality traits in 65 recombinant inbred lines derived from crosses between the cv. Messapia of durum wheat and the accession MG4343 of *T. dicoccoides*

| | Protein content | Protein per grain | 1000 grain weight |
|---|---|---|---|
| Sedimentation value | -0.16 | 0.08 | 0.05 |
| Protein content | | 0.15 | -0.34** |
| Protein per grain | | | 0.86** |

** Significant at 1% level

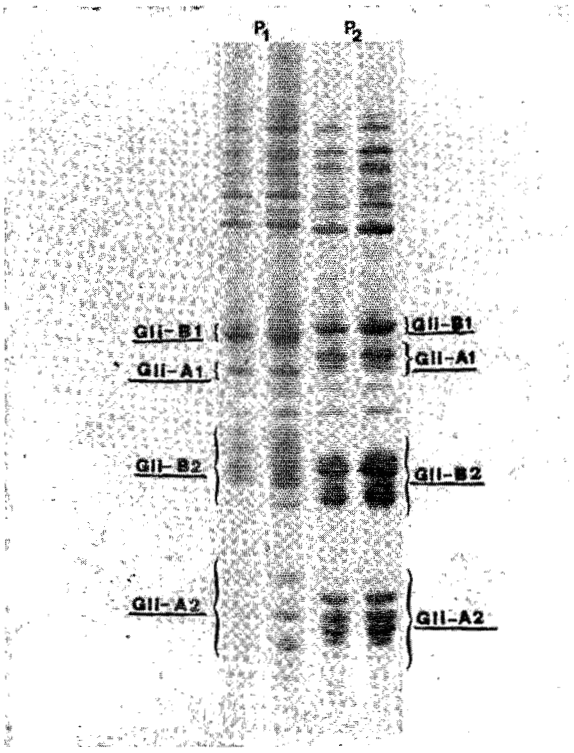


Fig. 2. A-PAGE patterns of gliadins of durum wheat cv. Messapia ($P_1$) and *T. dicoccoides* acc. MG4343 ($P_2$). The main subunits coded by the Gli alleles are shown.

As far as the protein percentage is concerned, *Gli-A1* locus of *T. dicoccoides* was significantly associated with a decrease and *Gli-B2* with an increase of protein content. The last association is really interesting as it was also confirmed by the regression of the same locus with protein per grain, trait which takes account of the grain size. Some studies have attempted to locate genes, or gene complexes, affecting grain protein content on *T. dicoccoides* chromosomes. Levy and Feldman (1989)

crossed four accessions of *T. dicoccoides* with the durum wheat cultivar Inbar and measured protein in the parents, $F_1$ and $F_2$ populations. They suggested that high protein content is associated with black glumes (linked with *Glu-A1* on chromosome arm 1AS), with gliadin (*Gli-B1*) locus on chromosome 1B, with genes for beaked glume (Bkg) and toothed palea (Tp) on group 5 chromosomes, and with the kinky neck gene on group 7. Joppa and Cantrell (1990) measured the protein content in a set of durum wheat -*T. dicoccoides* chromosome substitution lines and found that genes on chromosomes 6B, 2A, 5B, 3A and 6A of *T. dicoccoides* significantly increased the grain protein content of Langdon durum. The present study has confirmed the chromosomes 1A and 6B are of particular interest because of their role in the control of grain protein content.

No association was found between the six protein loci and 1000 grain weight.

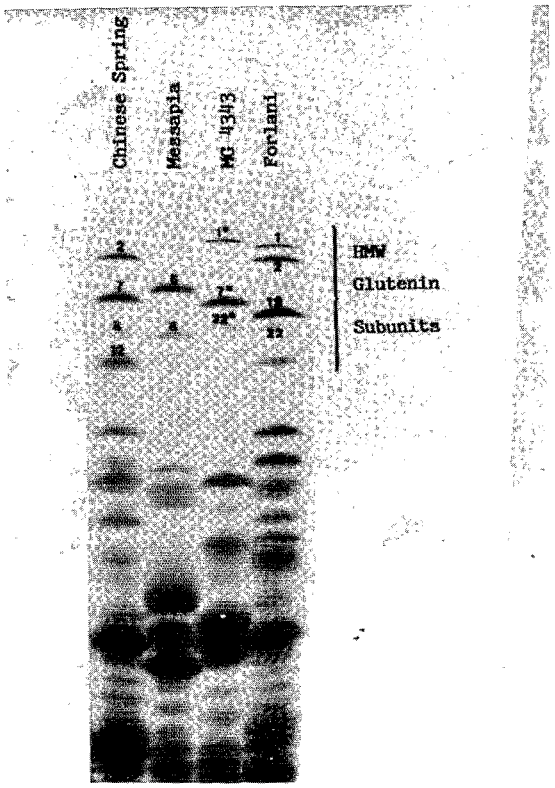


Fig. 3. SDS-PAGE patterns of HMW glutenins of durum wheat cv. Messapia and *T. dicoccoides* acc. MG4343 and of testers cultivars Chinese spring and Forlani in common wheat. The HMW subunits previously described are numbered according to the nomenclature of Payne and Lawrence (1983). Bands 1*, 7* and 22* are subunits not described previously.

Table 2. Frequencies of homozygous genotypes observed among recombinant inbred lines (RIL) from the $F_8$ Messapia ($P_1$) x MG4343 ($P_2$)

| Locus | Observed frequencies | | $X^2$ |
|---|---|---|---|
| | $P_1$ | $P_2$ | (1:1) |
| *Gli-A1* | 31 | 34 | $0.14^{NS}$ |
| *Gli-A2* | 34 | 31 | $0.14^{NS}$ |
| *Gli-B1* | 35 | 28 | $0.78^{NS}$ |
| *Gli-B2* | 33 | 32 | $0.02^{NS}$ |
| *Glu-A1* | 31 | 34 | $0.14^{NS}$ |
| *Glu-B1* | 18 | 47 | 12.94** |

** Significant at 1% level

Table 3. Regression (b) and determination ($R^2$) coefficients computed between protein loci and seed quality traits in 65 recombinant inbred lines derived from crosses between the cv. Messapia of durum wheat ($P^1$) and the accession MG4343 of *T. dicoccoides* ($P_2$)

| Chromosome arm | Locus | Protein subunits | | Sedimentation value (mm) | | Protein percentage (%) | | Protein per grain (mg) | | 1000 seeds weight (g) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $P_1$ | $P_2$ | b | $R^2$ | b | $R^2$ | b | $R^2$ | b | $R^2$ |
| 1AS | *Gli-A1* | γ-ω | gliadins | -1.82 | 0.2 | -0.81* | 6.3 | -0.11 | 0.3 | 0.52 | 0.2 |
| 1BS | *Gli-B1* | γ-45 | γ-42 | -17.00** | 22.9 | -0.33 | 1.1 | 0.26 | 1.3 | 1.63 | 2.2 |
| 6AS | *Gli-A2* | α-β | gliadins | 2.51 | 0.5 | 0.29 | 0.8 | -0.09 | 0.1 | -1.04 | 0.9 |
| 6BS | *Gli-B2* | α-β | gliadins | -2.48 | 0.5 | 1.21** | 14.1 | 0.56* | 6.3 | 0.48 | 0.2 |
| 1AL | *Glu-A1* | null | 1† | 13.41** | 14.8 | -0.63 | 3.8 | -0.27 | 1.5 | -0.19 | 0.2 |
| 1BL | *Glu-B1* | 6+8 | 7†+22† | -6.00 | 2.5 | 0.57 | 2.7 | 0.19 | 0.6 | -0.32 | 0.1 |

† Protein subunits with relative mobility similar to that reported by Payne and Lawrence (1983)
*, ** Significant at 5 and 1% respectively

## Conclusion

Protein content and protein quality are very important grain traits not only for their nutritional value but chiefly for their relationships with the technological properties of durum wheat. The narrow range of variation in genes controlling protein quantity and quality in cultivated wheats is presumably a main constraint for the improvement of these traits in wheats. The genetic resources of wild wheats and Triticeae species in general, show a wide variability in many economically important characteristics, including genes potentially useful for wheat quality improvement.

The present results point to the following important observations:

(i) The wild wheat *T. dicoccoides* crossed with the cultivated durum wheat can provide a wide variation for some quantitative traits of grain quality, notably protein content. Actually, *T. dicoccoides* is being used in breeding programs to increase protein content of cultivated wheats (Gram *et al.*, 1984; Levy and Feldman, 1987; Ciaffi *et al.*, 1991).

(ii) The protein content can be non correlated with protein quality, so that it is possible to select simultaneously for high protein content and high sedimentation value.

(iii) LMW and HMW glutenin loci are associated with sedimentation value but they account for only a part of the variation of that quality parameter. The variation at Glu loci of *T. dicoccoides* can be exploited to identify alleles positively correlated with quality traits, such as the *Glu-A1* allele coding for the 1* glutenin subunits of the present study.

(iv) The *Gli-A1* and *Gli-B2* loci of *T. dicoccoides* are associated with protein quantity. These results confirm previous indications about the localization of protein content loci on chromosome arms 1AS (Levy and Feldman, 1989) and 6B (Joppa and Cantrell, 1990).

(v) Recombinant inbred lines constitute a good genetic material to study the association between protein loci and grain quality traits. Since a genotype is represented by an inbred line, rather than an individual, a more accurate assessment of the genetic component of variance can be made in studying quantitative traits. In addition, when densely saturated maps with genetic and molecular markers are available, the RIL can allow precise localization of QTL on fragment of DNA and then gene isolation.

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