Variation of HMW glutenin and γ-gliadin subunits in selected accessions of *Triticum dicoccon* (Schrank) and *T.spelta* (L.).

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Abstract
Ten accessions of emmer (*T.dicoccon* Schrank) and ten of spelt (*T.spelta* L.) have been analysed, using electrophoresis, for their HMW glutenin subunits and γ-gliadin fraction. Emmer has been discovered to be more polymorphic than spelt. The highest variation has been associated to HMW glutenins. New alleles, provisionally assigned to *Glu-A1* and *Glu-B1* loci have been detected in some accessions of emmer. The whole meals of tested accessions were also analysed for protein content and SDS-sedimentation test. Relationships between the SDS-test value, the quality index and the electrophoretic pattern are also discussed.

Keywords: bread-making quality, diversity, electrophoresis, germplasm, hulled wheat.

Introduction
Over a long period of time the hulled wheat species: *Triticum monococcum* (L.), *T.dicoccon* (Schrank) and *T.spelta* (L.) have been almost entirely displaced by wheat which gives higher yield and it is easier to thresh. During the last decades interest in these ancient wheats is increasing, this is due to their adaptability to poor soils, to the low input techniques used for their growth and to attractive nutritional attributes. In addition, the hulled wheat species variability could constitute a useful gene reservoir for breeding programs of both bread and durum wheat. Crossbreed between *T.aestivum* or *T.durum* cultivars and hulled wheats have been already described (Codianni *et al* 1995; Radic *et al* 1997).
The interest towards these ancient wheats is stimulating the evaluation of their utilisation to prepare several derivatives (i.e. bread, biscuits, pasta, etc.). The study of the main seed storage proteins (glutenins and gliadins) is consequently essential because they are related to the quality of end-products (Mac Ritches *et al* 1990). Depending on the quantity and composition of each protein fraction, dough with different characteristics may be obtained. Presently, the genetic diversity of gliadin and glutenin (HMW and LMW subunits) within hulled wheat species germplasm is still underinvestigated. The available data are rare and sometimes controversial (Rodriquez-Quijano *et al* 1990; Galterio *et al* 1994; Liu and Shepherd 1996).
This paper presents the results relative to an electrophoretic screening of emmer and spelt accessions selected in the past years for their attractive agronomic (Perrino *et al* 1993) and/or biochemical performances (Piergiovanni *et al* 1996) from the germplasm collection (about 600 accessions) of hulled wheats held at Germplasm Institute-CNR of Bari (Italy).

Materials and methods
None of the tested accessions (10 of *T.dicoccon* (Schrank) and 10 of *T.spelta* (L.)) was heterogeneous both for gliadin and glutenin fraction. This is due to the purification carried out

during the previous studies. The original material, when heterogeneous, did not contained more than four genotypes, that were separated and singly tested (Perrino *et al* 1993).
Gliadins was extracted from single seed with an N,N-dimethylformammide solution (1.5 M) (1:5 w:v) and fractionated by electrophoresis on polyacrylamide gel (7%) in potassium lactate (Lafiandra et al 1984). The cultivars: Creso, Flavio, Latino and Manital were used as references for the subunits γ-45, 40, 42 and 43.5, respectively.
Glutenins was extracted, from single seed, using a TRIS-HCl buffer (pH 6.8) containing pyronin Y (1%) and β-mercaptoethanol (5%) (1:10 w: v) and analysed on 10% polyacrylamide gel (Payne *et al* 1981). The cultivars: Cheyenne, Chinese Spring, Creso, Manital, Melchior, Newton, Solitario and Trinakria were used as references for the subunit identification (Table 1).
The protein content was measured by NIR technique; SDS-test was carried out according to Dick and Quick (1983) with a 2% SDS solution. Only whole meals were tested. The quality index (QI) was calculated as described by Halverson and Zeleny (1988). Data were statistically analysed by SNK (Student-Neuman-Keuls) test.

Results and discussion

It is known that highly significant correlation exist between gluten viscoelasticity and two chromosomes 1B encoded the gliadin components γ-42 and γ-45 (Damidaux *et al* 1978). In addition a link has been demonstrated between the *Gli-B1* locus coding for γ-42 and γ-45 gliadins and the *Glu-3* locus coding for LMW glutenin subunits, primarily responsible for gluten viscoelastic properties of durum wheat (Pogna *et al* 1990). The analysis of gliadins by acid PAGE of emmer and spelt accessions was limited to the γ-gliadin fraction. A very low variation was observed only for spelt (Table 2). The gliadin γ-45, which is related to good gluten quality, was common to all emmer accessions as well as to seven spelt accessions. The other spelt accessions showed different subunits: γ-43.5 has been found in the profile of two accessions (MG 15433/1 and MG 27201/1), while a subunit, the calculated mobility of which, is 44.2, was detected in the profile of MG 27182/4.
These results do not agree with by Galterio *et al* (1994) that, analysing three Italian populations of emmer observed an appreciable variation of gliadin fraction. They identified several new alleles attributable to the *Gli-A1*, *Gli-B1* and *Gli-A2* loci and did not observed the γ-45 or γ-42 gliadin subunits in any of tested seeds.
19 HMW glutenin subunits of different mobility were identified; each accession synthesises from two to five subunits (Fig. 1). The *Glu-B1* locus showed much more allelic variation than the other loci. At the *Glu-A1* locus the subunit 1, which is related to the good quality of bread wheat, was the unique observed in *T.spelta*, it was the most frequent among the emmer accessions (Table 2). The null form, quite common among the Italian varieties of *T.durum* (its frequency is about 86%) (Boggini and Pogna 1990), was observed in the profile of two accessions. New subunits with electrophoretic mobility slightly faster or slower than that of known bands, named 1^+ and 2^-, were detected in the profile of the emmer accessions MG4378/1 and MG5380/1. Among the subunits encoded by the *Glu-B1* locus the 6+8 was the most frequent pair, it was found in six spelt accessions (Table 2). It is interesting to observe the presence of the subunit pair 13+16 in three spelt accessions this allele, rare or with low frequency in wheat (Payne and Lawrence 1983), is associated with good bread-making quality. A low allelic variation at the *Glu-B1* locus in spelt has been recently described by

Table 1. H

Cultivar na
T. aestivum
Cheyenne
Chinese sp
Manital
Melchior
Newton
T. durum
Creso
Solitario
Trinakria

Table 2. E

MG code
T. dicocco
4378/1
5282/2
5285/1
5333/1
5380/1
5400/5
5507
27201/4
30782
30835/1
T. spelta
4451/1
5285/3
5320/2
15347/1
15398/1
15433/1
15451/1
15577/1
27182/4
27201/1

eous, did not contained
no *et al* 1993).
mmide solution (1.5 M)
%) in potassium lactate
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fer (pH 6.8) containing
nd analysed on 10%
Chinese Spring, Creso,
ferences for the subunit

carried out according to
were tested. The quality
ny (1988). Data were

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is interesting to observe
allele, rare or with low
vith good bread-making
en recently described by

Table 1. HMW glutenin pattern of reference cultivars.

Cultivar name	*Glu-A1*	*Glu-B1*	*Glu-D1*
T. aestivum			
Cheyenne	2*	7 + 9	5 + 10
Chinese spring	N	7 + 8	2 + 12
Manital	2*	17 + 18	2 + 12
Melchior	N	7 + 9	3 + 12
Newton	1	7 + 8	4 + 12
T. durum			
Creso	N	6 + 8	
Solitario	N	13 + 16	
Trinakria	N	20	

Table 2. Electrophoretic patterns (gliadin and HMW glutenin fraction).

MG code	γ-gliadin	*Glu-A1*	*Glu-B1*	*Glu-D1*
T. dicoccon				
4378/1	45	1 + 1⁻	7 + 8	
5282/2	45	N	13⁻ + 16	
5285/1	45	2*	17 + 18	
5333/1	45	1	8⁻ + 9	
5380/1	45	2⁻	17 + 18	
5400/5	45	1	7⁻ + 8⁻	
5507	45	N	7 + 8⁻	
27201/4	45	1	6 + 8	
30782	45	1	6 + 8	
30835/1	45	1	7⁻ + 8⁻	
T. spelta				
4451/1	45	1	7 + 8	2 + 12
5285/3	45	1	6 + 8	2 + 12
5320/2	45	1	6 + 8	5 + 10
15347/1	45	1	6 + 8	2 + 12
15398/1	45	1	13 + 16	2 + 12
15433/1	43.5	1	13 + 16	2 + 12
15451/1	45	1	6 + 8	2 + 12
15577/1	45	1	6 + 8	2 + 12
27182/4	44.2	1	13 + 16	2 + 12
27201/1	43.5	1	6 +8	5 + 10

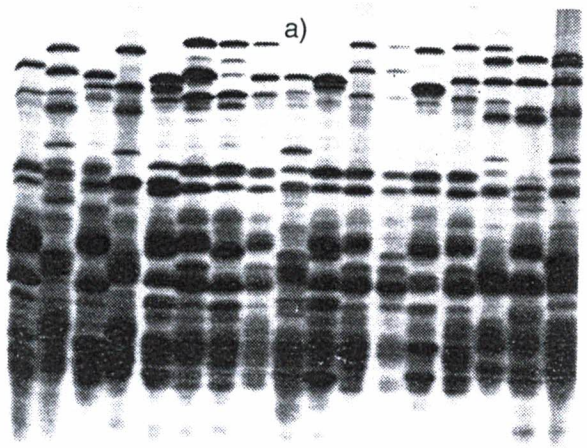

Figure 1a. HMW electrophoresis of *T.dicoccon* accessions from left to right: Creso; Chinese spring; Solitario; Manital; MG 5285/1; MG 4378/1; MG 5333/1; MG5400/5; MG5507; MG 5282/2; MG 27201/4; MG 30782; MG 5380/1; MG 30835/1; Newton; Melchior; Cheyenne.

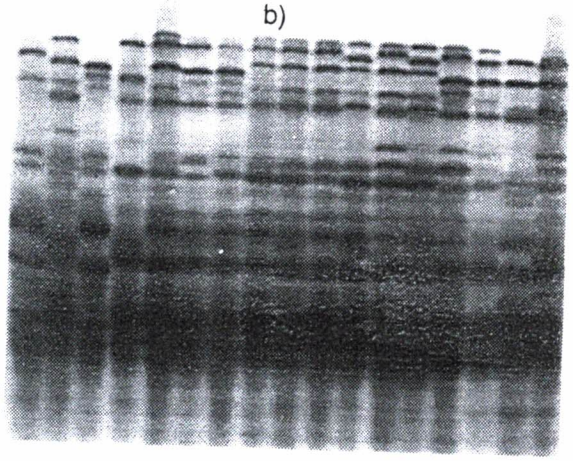

Figure 1b. HMW electrophoresis of *T.spelta* accessions from left to right: Creso; Chinese spring; Solitario; Manital; MG 4451/1; MG 15398/1; MG 15433/1; MG 15451/1; MG 15577/1; MG 5285/3; MG 27201/1; MG 15347/1; MG 5320/2; MG 27182/4; Newton; Melchior; Cheyenne.

right: Creso; Chinese
400/5; MG5507; MG
Melchior; Cheyenne.

ight: Creso; Chinese
MG 15451/1; MG
G 27182/4; Newton;

Table 3. Protein content, SDS-test and Quality index of tested lines.

MG Code	Protein (% dm)	SDS-test (mm)	QI
T. dicoccon			
4378/1	14.9 be	49 e	3.3 eh
5282/2	14.1 de	37 eg	2.6 fi
5285/1	15.3 be	34 eg	2.2 hi
5333/1	15.6 be	30 fg	1.9 i
5380/1	15.5 be	45 ef	2.9 ei
5400/5	14.4 ce	50 e	3.5 dg
5507	13.3 e	24 g	1.8 i
27201/4	13.4 e	33 eg	2.4 gi
30782	15.6 be	70 d	4.5 cd
30835/1	15.5 be	48 ef	3.1 eh
mean	14.8	42	2.8
T. spelta			
4451/1	17.1 ad	94 ac	5.5 ab
5285/3	19.6 a	78 cd	4.0 ce
5320/2	17.5 ad	103 ab	5.9 a
15347/1	19.1 a	71 d	3.7 df
15398/1	18.1 ab	112 a	6.2 a
15433/1	17.7 ac	104 ab	5.9 a
15451/1	19.2 a	85 bd	4.5 cd
15577/1	16.5 ae	79 cd	4.8 bc
27182/4	16.0 be	99 ab	6.2 a
27201/1	17.5 ad	101 ab	5.8 a
mean	17.8	93	5.2

Values with the same letter are not significantly different.

Rodriquez *et al* (1990), who analysing 118 Spanish hexaploid wheat landraces of spelt observed only three patterns. The pair 13+16 resulted prevalent (87% of the accessions); no samples shown the 6+8 pair, which is the most frequent pair in the present screening. A wide variation at the *Glu-B1* locus characterised the emmer accessions (Fig 1a). Three new subunits, named 7^{+}, 8^{-} and 13^{-}, with electrophoretic mobility close to known bands, were identified in five accessions; two of which showed the new pair $7^{+} + 8^{-}$ (Table 2). These results agree with Galterio *et al* (8) that observed some subunits attributable to novel alleles at the *Glu-B1* locus in three Italian populations of emmer. The presence of the subunit pairs 7+8 (MG 4378) and 17+18 (MG 5380/1) is of interest since they are uncommon in durum wheat commercial cultivars. Finally, only two alleles were observed at the *Glu-D1* locus in hexaploid accessions. According to Rodriquez *et al* (1990) subunit pair 2+12 resulted the most frequent (Table 2).

The tested accessions were also analysed for protein content and SDS-test. The quality index was calculated to a more correct comparison of the results. Higher protein contents characterised *T.spelta* (mean value: 17.8 vs. 14.8%); three accessions showed values superior to 19% (Table 3). As expected, spelt show also the highest SDS test values, three accessions exceed the threshold of 100 mm. It is interesting to underline that for this species the highest QI value (6.2) is associated with the presence of the subunit pair 13+16 in the HMW glutenin fraction. The accession MG 15433/1, also posses this pair, but show a slightly inferior QI (5.9). This could be attributable to the differences in both γ-gliadin and not considered protein fractions. Very different the trend relative to emmer, which QI values, are distributed on a broad range (from 1.8 to 4.5) (Table 3). The high heterogeneity observed in the electrophoretic profiles of these accessions do not allow to establish reliable relationships between the SDS test and/or QI and the presence of specific protein subunits. The accessions showing the null form at *Glu-A1* locus had very low QI value, according to the literature (Halverson and Zeleny 1988)

Conclusions

Though a low number of accessions has been screened in this study, some interesting conclusions can be drawn. Emmer appeared to have a higher polymorphism than spelt relatively to HMW glutenin fraction. Moreover Liu and Sheperd (1996) have described a large amount of variation for this species also for LMW glutenin fraction. Consequently, *T.dicoccon* can be considered a richer source of genome diversity for breeders. Comparing the extent of subunit variation patterns relative to each chromosome (1A, 1B and 1D), the highest polymorphic is the chromosome 1B for both hulled wheat species. This is consistent with previous studies on other polyploid wheat, which attribute to the genome B a high polymorphism. Finally, much more information is required to better understand the relationships between the gliadin and HMW glutenin variation and the technological performances of these ancient wheats.

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