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Seed storage proteins of wild wheat progenitors and their relationships with technological properties

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A large collection of wild wheat progenitors, consisting of diploid (*Triticum boeoticum* Boiss. and *Triticum urartu* Tum.) and tetraploid wheats (*Triticum dicoccoides* Korn.) was evaluated for certain grain quality parameters such as protein content and the SDS-Sedimentation test. The variation in protein content was larger in *T. dicoccoides*, ranging from 16 to 27 %, compared to diploid wheat (20–28 %). Some accessions appeared to be very promising for gluten properties, as measured by the SDS-test, when compared with some durum wheat cultivars. To determine the relationships between particular protein components and gluten properties, diploid, tetraploid wheats, and synthetic amphiploids (AABB × DD) were analysed by different electrophoretic procedures. Attention was focused on the study of the allelic variation at loci that in cultivated wheats play the major role in determining gluten quality (*Glu-1*, *Gli-1* and *Glu-3*). The range of allelic variation at the loci examined is remarkable, and genetic variants unique to wild wheats and positively related to gluten quality are reported.

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Wild species of *Triticum* are considered a rich source of variation for both morphological and physiological characters. Particular attention must be given to storage proteins, glutenins and gliadins, which have proved to be important for technological properties both in durum and bread wheats. Glutenins include both high (HMW) and low-molecular weight (LMW) subunits; HMW subunits are controlled by genes located on the long arm of chromosomes 1A, 1B, and 1D at loci designated as *Glu-1*. Molecular analyses have shown that each *Glu-1* locus contains two genes (HARBERD et al. 1986), one encoding a high MW x-type subunit, the other a low MW y-type subunit.

Gliadins are all encoded by genes located on the short arm of the homoeologous groups 1 and 6 chromosomes, at loci designated respectively as *Gli-1* and *Gli-2*. The *Gli-1* loci correspond to a family of tightly associated genes which code for three structurally distinct protein types, indicated as omega, gamma gliadins, and LMW-glutenin subunits. Loci encoding this last group of proteins were designated *Glu-3* (SINGH and SHEPHERD 1988). Each of these loci displays allelic variation which is partly responsible for qualitative differences between wheat varieties.

Gluten viscoelasticity, an important factor in pasta cooking quality, is strongly related to some gliadin components and to LMW-glutenin subunits, coded by genes at complex loci *Gli-B1* and *Glu-B3*, respectively. Only two alleles, named 42 and 45, according to their relative mobility in polyacrylamide gels at pH 3.1, have been detected at these loci in the durum wheat world collection; they are related, respectively to poor and good gluten viscoelasticity. In bread wheat a recent study on the variation of the protein components, controlled by genes at the linked *Gli-D1* and *Glu-D3* loci, showed that low variation is present at both loci, with two main types identified according to the electrophoretic patterns of gliadins and LMW-glutenin subunits (MASCI et al. 1991); they resemble analogous patterns found in cultivars 'Cheyenne' and 'Chinese Spring'. Limited variation is also present for HMW-glutenin subunits encoded by *Glu-1* loci, which are responsible for baking differences of bread wheats.

Evaluation of wild genetic resources for technologically useful characteristics, in terms of types and amount of storage proteins is only in its initial stages. The present paper reports on the evaluation of some grain quality parameters, such as protein content and SDS-Sedimentation, in a representa-

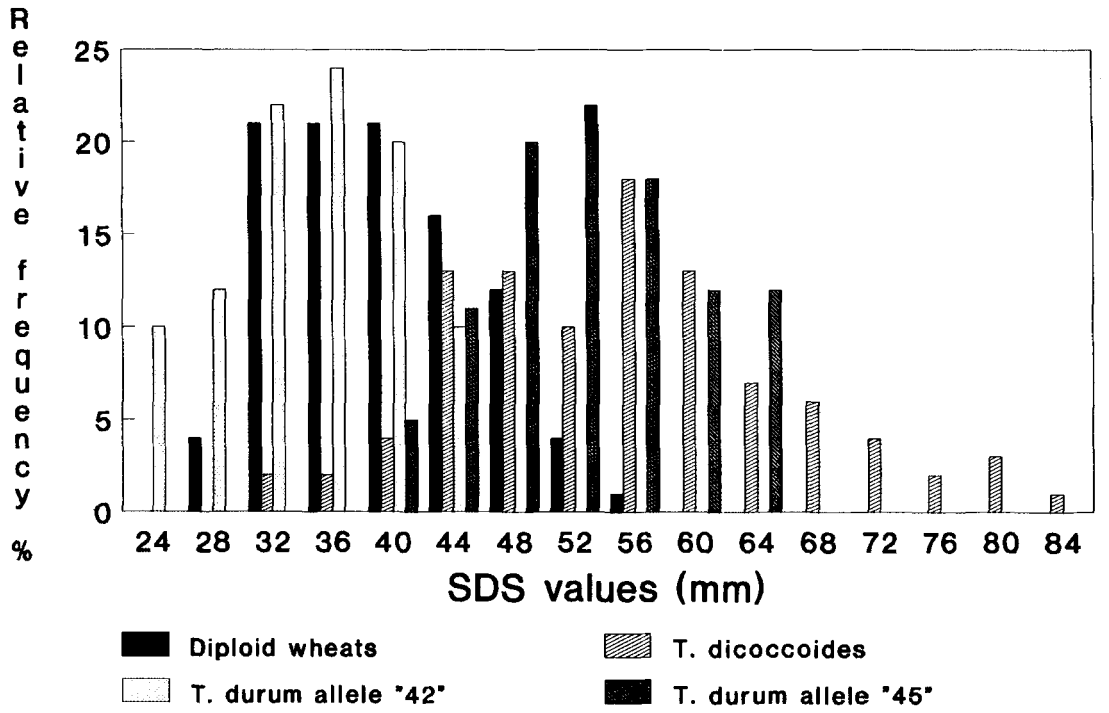

Fig. 1. Frequency distribution of SDS-Sedimentation values in diploid and tetraploid wild wheat accessions and in *T. durum* varieties separated on the basis of their allelic variation at the *Gli-B1* locus.

tive sample of the wild progenitors. The relationships between particular protein components and potentially positive gluten properties of wild wheats have also been considered.

Materials and methods

A total of 545 wild relatives of wheat were utilised in this study. It included 315 accessions of *Triticum dicoccoides* Korn. from Jordan and Turkey; 138 accessions of *Triticum urartu* Tum. from Lebanon, Turkey and Iran; and 92 assessions of *Triticum boeoticum* Boiss. from Lebanon, Turkey, Iran and Iraq. Synthetic amphiploids (AABB × DD), developed by L. R. Joppa, by crossing the durum wheat cultivar 'Langdon' with different accessions of *Triticum tauschii* were also included in this analysis.

Gliadin and HMW-glutenin subunits, were analysed by two different electrophoretic procedures, A-PAGE and SDS-PAGE, according to the methods of KHAN et al. (1985) and PAYNE et al. (1981). Gluten strength was estimated by SDS-Sedimentation (DICK and QUICK 1983). Protein content was determined by the Kjeldal method (N × 5.7).

Results

Grain quality parameters of wild diploid and tetraploid wheats

Large and significant variation of protein content was detected among the wild progenitors analyzed, ranging from 20 to 28 % in diploid wheats and from 16 to 27 % in *T. dicoccoides*, confirming the finding that wild wheat relatives have a wider range of variation for grain protein content than cultivated ones, and that an even larger variation is present in *T. dicoccoides*.

Gluten strengths, as measured by the SDS-test, of the wild diploid and tetraploid wheats are reported in Fig. 1. The SDS-values of these wild wheats were compared with those obtained for 130 durum wheat cultivars separated on the basis of their allelic variation at the *Gli-B1* locus. The range of

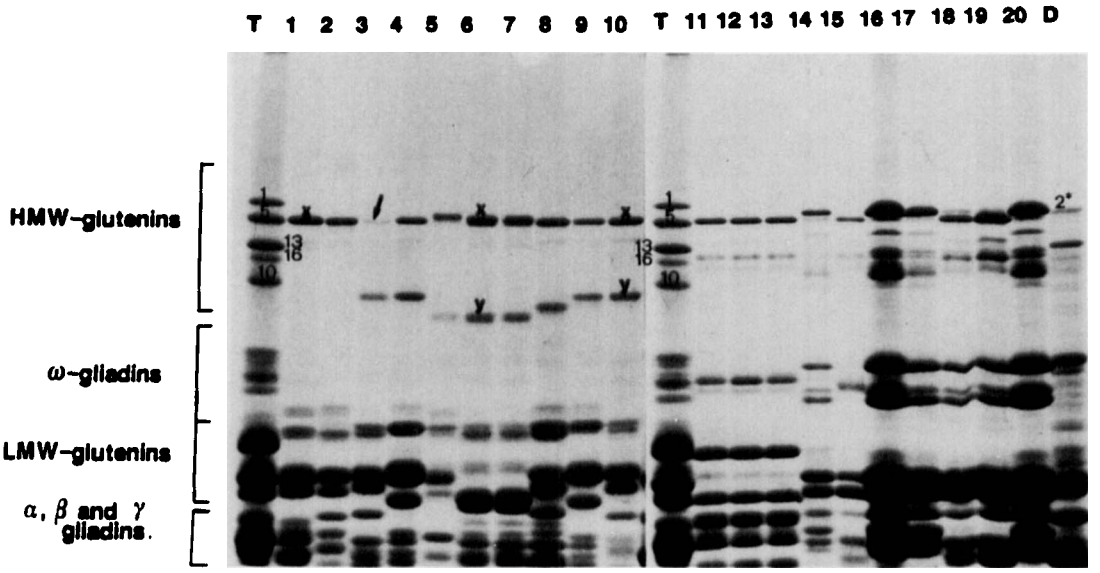

Fig. 2. SDS-PAGE of total proteins from 10 accessions of *T. urartu* (lanes 1–10) and 10 accessions of *T. boeoticum* (lanes 11–20). The HMW-glutenin subunits of the bread and durum wheat cultivars, 'Torim' (T) and 'Duramba' (D), have been numbered according to PAYNE and LAWRENCE (1983).

variation of *T. dicoccoides* (32–84 mm) was larger than those of *T. durum* (24–64 mm) and diploid wheats (28–56 mm). Approximately 10 % of *T. dicoccoides* accessions had SDS-Sedimentation values significantly higher than the best durum wheat, in this respect.

Generally, the diploid wheats had the same range of variation as *T. durum* cultivars possessing the allele 42 (24–44 mm); only 15 % of these wheats (particularly *T. urartu*) had SDS-Sedimentation values higher than 44 mm.

Variation of storage proteins and their relationships with potential gluten properties of wild progenitors

Diploid wheats

The SDS-PAGE migration patterns of total proteins from representative samples of *T. urartu* and *T. boeoticum* accessions, are reported in Fig. 2, together with total proteins from the bread and durum wheat cultivars 'Torim' and 'Duramba'. HMW-glutenin subunits of many *T. boeoticum* accessions (lanes 16–20) had a major subunit of low mobility (x-type) and a series of less prominent subunit bands of faster mobility with one dominating (y-type). In only three of the accessions from Turkey (lanes 11–13) were two well distinct subunits observed; in this case the mobility of the lowest y subunit is intermediate between those of the 1Bx and 1By subunits of cultivated wheats. The mobility of the x subunit varies in different accessions, but their range of mobility is similar to that of 1Ax and 1Dx subunits of cultivar 'Torim'. The HMW-glutenin subunit patterns of *T. urartu* (lanes 1–10) were quite distinctive from *T. boeoticum* in containing one major 1Ax and 1Ay subunit in agreement with previous findings (WAINES and PAYNE 1987). In 15 % of *T. urartu* accessions, the 1Ay subunit was not expressed (lanes 1–2), whereas only four accessions from Lebanon showed a faint x subunit (lane 3). The range of the mobility of the *T. urartu* 1Ax subunits is similar to that observed in *T. boeoticum*. Conversely, the y subunits have a higher electrophoretic mobility than the main 1Ay subunits observed in *T. boeoticum*. Altogether twelve alleles for *Glu-A1* locus, seven in *T. urartu* and five in *T. boeoticum*, were found in the 230 accessions of the diploid wild wheats. A high level of polymorphism was also present in diploid wild wheats for the *Gli-A1* encoded proteins. In particular, four different allelic variants for *T. boeoticum* and nine for *T. urartu* were observed (data not shown).

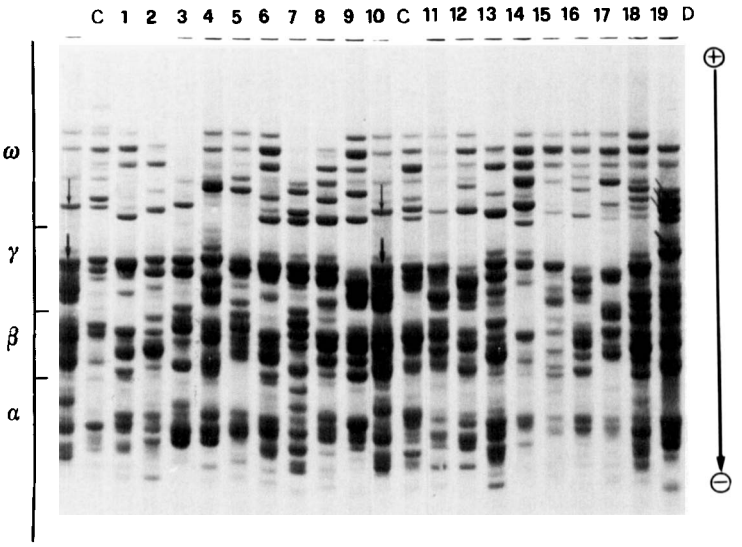

Fig. 3. One-dimensional electrophoretic separation of gliadins from 19 *T. dicoccoides* accessions representative of the different allelic variants observed at the *Gli-B1* locus. Vertical arrows indicate the omega-gliadin 35 and the gamma 45 in cultivar 'Creso' (C). The omega-gliadins 33-35-38 and the gamma-component 42 are also indicated by diagonal arrows in cultivar 'Duramba' (D).

Results of analysis of variance of data for gluten strength, as measured by SDS-test, carried out using allelic variants at *Gli-A1* and *Glu-A1* as the source of variation, indicated that variation in gluten properties could be accounted for by allelic variation in *Glu-A1*, whereas variation for *Gli-A1* encoded proteins did not affect gluten quality in these wild wheats. In particular, mean sedimentation values of the *T. urartu* accessions possessing the *Glu-A1* alleles reported in lanes 4 and 8 in Fig. 2 were significantly higher than those of accessions showing alternative allelic variants. Also the HMW-glutenin subunits of the *T. boeoticum* accessions reported in lanes 16, 17 and 20 were associated with larger sedimentation values than those showing the alternative alleles.

Triticum dicoccoides

Electrophoretic migration patterns of gliadins from some accessions of *T. dicoccoides*, representative of the different allelic variants detected at the *Gli-B1* locus are shown in Fig. 3, together with gliadins from the durum wheat cultivars 'Creso' and 'Duramba', included for comparison. Appreciable variation occurs, but only that which is due to the fast moving omega and slow moving gamma regions, where gliadin components controlled by 1B chromosome are usually located, is considered here. Some accessions were found to possess the main *Gli-B1* coded gamma gliadins with same electrophoretic mobilities (lanes 3 and 4 or lanes 8 and 9 in Fig. 3), but they contained different gliadin components in the fast moving omega region and unlike LMW-glutenin subunits (lanes 3 and 4 or lanes 8 and 9 in Fig. 4). Conversely, other accessions that possessed the same omega and gamma gliadins coded at *Gli-B1* locus (lanes 2 and 7 in Fig. 3) were not uniform in LMW-glutenin subunits (lanes 2 and 7 in Fig. 4). Altogether 19 alleles for *Gli-B1/Glu-B3* loci were found in the 315 accessions of *T. dicoccoides*.

The SDS-PAGE migration patterns of total proteins indicated that each accession of *T. dicoccoides* possesses from one to four major components in the region of the HMW-glutenin subunits (Fig. 4 and 5). In accordance with previous nomenclature (PAYNE et al. 1981), subunits coded by the same chromosome have been split into two groups, x and y, according to their mobilities in SDS-PAGE. A total of 40 different SDS-PAGE migration patterns were observed resulting from the combination of 16 HMW-glutenin subunit patterns controlled by the A genome and 18 subunit

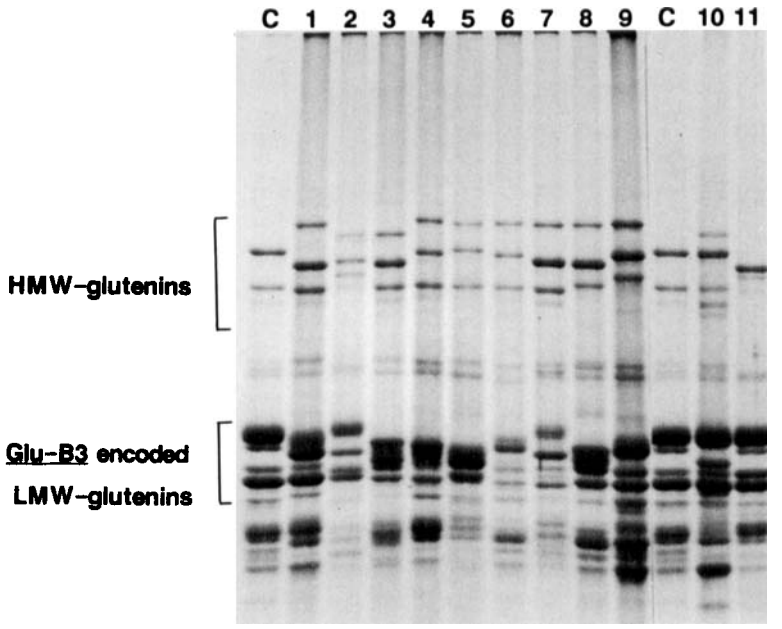

Fig. 4. SDS-PAGE of total proteins from some *T. dicoccoides* accessions representative of the different *Glu-B3* alleles. 1 – 11 = *T. dicoccoides* accessions; C = cultivar 'Creso'.

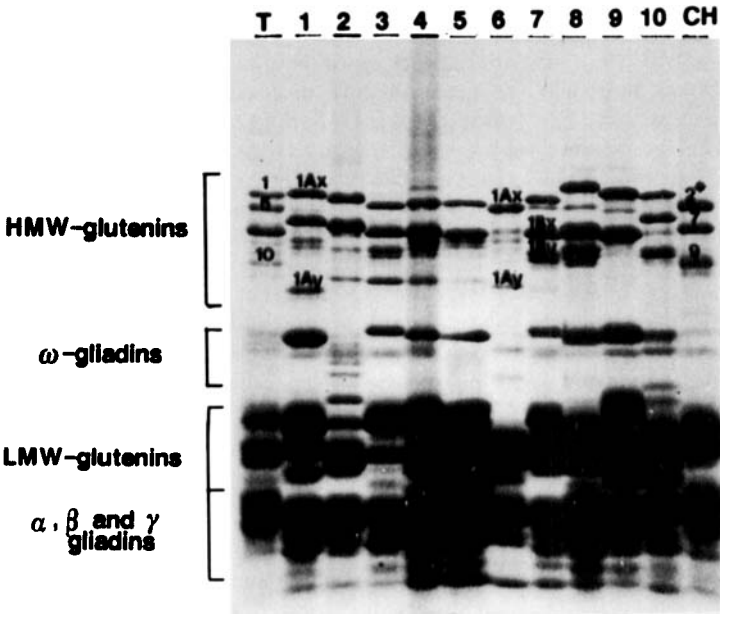

Fig. 5. SDS-PAGE of total proteins of various accessions of *T. dicoccoides* showing 10 different allelic variants at the *Glu-A1* and *Glu-B1* loci. The HMW-glutenin subunits of the bread wheat cultivars, 'Torim' and 'Cheyenne', have been numbered according to PAYNE and LAWRENCE (1983).

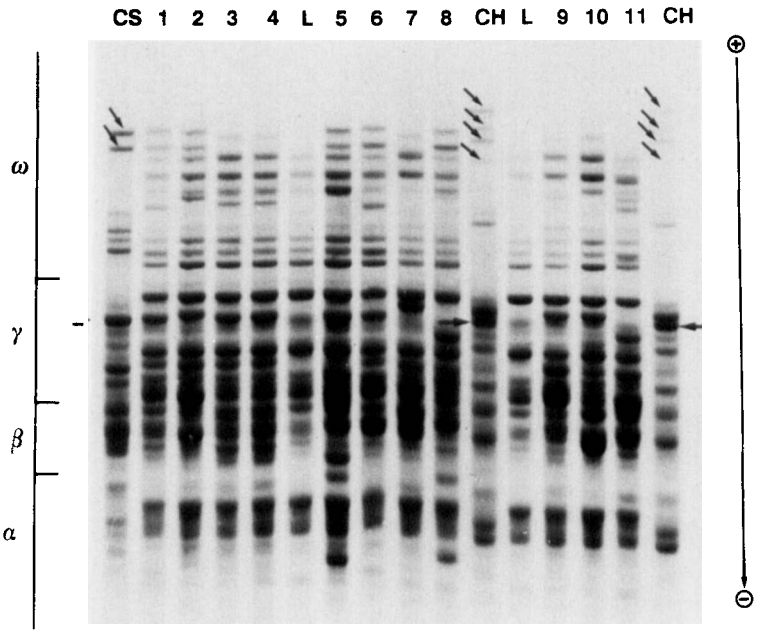

Fig. 6. One-dimensional electrophoretic separation of gliadins from 11 synthetic hexaploids and durum wheat cultivar 'Langdon' (L). Arrows indicate the gliadin components encoded by the *Gli-D1* locus in bread wheat cultivars 'Chinese Spring' (CS) and 'Cheyenne' (CH).

patterns from the B genome. Usually, two major HMW-glutenin subunits produced by *Glu-B1*, as in bread and durum wheats, were present, although occasionally only one was detected (lane 9 in Fig. 5). The normal situation for *Glu-A1* is the presence of one subunit, whereas only 10 % of the accessions have the null alleles. Only 6 (lanes 1–6 in Fig. 5) out of the 16 different allelic variants detected at *Glu-A1* locus showed both 1Ax and 1Ay subunits. An interesting novel *Glu-A1* subunit, with a low mobility on SDS-PAGE (lane 8 in Fig. 5), was detected in some accessions from Jordan.

Variation at the *Gli-B1/Glu-B3* loci was mostly responsible for differences in gluten quality. The SDS-test values for *T. dicoccoides* accessions, grouped according to the allelic variation detected at the *Gli-B1/Glu-B3* loci indicated that some allelic variants (2, 7, 14 and 19) appear to be very promising for the improvement of gluten viscoelastic properties of durum wheats.

Synthetic amphiploids (AABB × DD)

Electrophoretic separation of gliadin proteins and HMW-glutenin subunits from the 11 synthetic hexaploids and the durum wheat cultivar 'Langdon' are reported in Fig. 6 and 7, respectively. Gliadins and total proteins from the bread wheat cultivars 'Chinese Spring' and 'Cheyenne' were included in the gels for comparisons. Gliadin component controlled by 1D in 'Cheyenne' and 'Chinese Spring', as deduced by chromosomal assignment data (LAFIANDRA et al. 1984) are indicated in Fig. 6. The comparison of the gliadin components in the slow moving omega regions between the different synthetic hexaploid and the two cultivars used as control, revealed that extensive variation occurs at *Gli-D1* locus in the *T. tauschii* accessions used to produce the 11 synthetic hexaploids, and that no accessions have the *Gli-D1* gliadin components of 'Chinese Spring' and 'Cheyenne'. Nine allelic variants were detected among the synthetic hexaploids (Table 1 and Fig. 6).

Similarly, no accessions were found to possess the two main allelic variants (subunits 2 + 12 and subunits 5 + 10) detected at *Glu-D1* in hexaploid cultivated wheats (Fig. 7). Seven allelic variants at the *Glu-D1* locus were observed in the 11 amphiploids (Table 1 and Fig. 7). Analysis of variance, calculated on SDS-Sedimentation values of

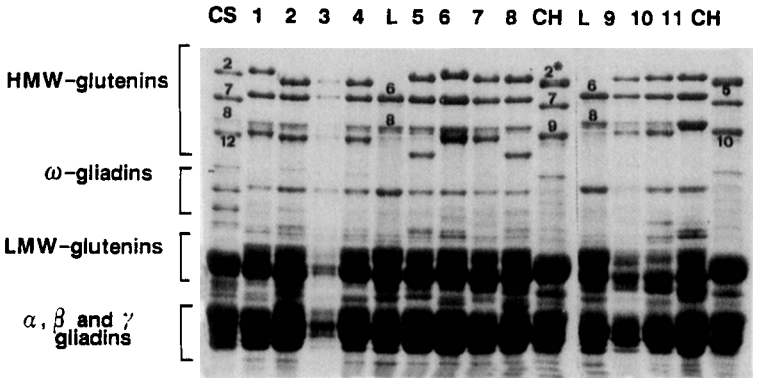

Fig. 7. SDS-PAGE of total proteins from 11 synthetic hexaploids and durum wheat cultivar 'Langdon' (L). The *Glu-1* HMW-glutenin subunits from bread wheats 'Chinese Spring' (CS) and 'Cheyenne' (CH) are indicated.

Table 1. Allelic variation at the *Glu-D1* and *Gli-D1* loci and mean SDS-Sedimentation values of synthetic hexaploid wheats

Amphidiploids	*Glu-D1*	*Gli-D1*	SDS-test*
1) J83 AE 34	1	1	72.67 c
2) J83 AE 386	2	2	89.67 d
3) J83 AE 396	2	3	92.00 d
4) J83 AE 419	2	3	89.67 d
5) J84 AE 42	3	4	57.67 a
6) J84 AE 42	4	5	75.00 c
7) J83 AE 434	5	6	63.00 b
8) J88 AE 342	3	7	71.33 c
9) J88 AE 338	6	8	91.00 d
10) J83 AE 421	6	8	93.33 d
11) J83 AE 355	7	9	60.00 ab

* Means with the same letters are not different at 1 % level

synthetic hexaploids, allowed the 11 amphiploids to be classified into three groups (Table 1). The first includes the five synthetic hexaploids that have higher SDS-test mean values, which range from 94 to 90 mm, the second contains three amphiploids with intermediate mean SDS-values (ranging from 75 to 72 mm), whereas the third includes those possessing lower SDS-test mean values (63–57 mm).

The better baking quality, as in group 1, could be ascribed to particular 1D protein components not detected in the other amphiploids. The synthetic hexaploids 9 and 10 possess the same allelic variants at the *Glu-D1* and *Gli-D1* loci (Fig. 6 and Fig. 7) which are different from those detected for amphiploids 2, 3 and 4. The synthetic hexaploid 2 has the same HMW-glutenin subunits as 3 and 4, but differs for the *Gli-D1* gliadin components. The second and third groups include two synthetic hexaploids, 8 and 5, which possess the same HMW-glutenin subunits (Fig. 7). The significant difference between these two amphiploids in baking quality, as measured by SDS-test, could be ascribed to the different *Gli-D1* gliadin components (Fig. 6).

Conclusion

The variation in the amount and type of grain proteins is the main factor responsible for the differences in bread, pasta making quality, and nutritional properties of flours. An approach to improve pasta and bread-making attributes relies on the possibility of using the tremendous variation for storage proteins existing in wild relatives of *Triticum* species. At present it appears impossible to predict which wild species will yield the most valuable contributions to wheat quality breeding. Thus it seems plausible to focus attention mainly on the progenitors, because these will recombine with wheat without the need for special methodology.

The first results on the transfer of genes from wild relatives for the improvement of technological quality in cultivated wheats are very promising. The potentiality of using *T. dicoccoides* not only as a donor of genes for higher protein content but possibly for improving gluten quality in durum wheats was recently reported by CIAFFI et al.

(1991). Some progenies, derived from crosses between durum wheat cultivar 'Creso' and an accession of *T. dicoccoides*, appeared to be very promising for gluten properties. The contribution of *T. dicoccoides* genes to these lines was primarily due to its high protein content and its allelic combination, with both x and y HMW-glutenin subunits present at the *Glu-A1* locus. A novel approach to improving technological quality of cultivated wheats would be to increase the dosage of genes actively expressing HMW-glutenin subunits. One way of achieving this would be to replace existing genes at the *Glu-A1* locus by equivalent genes from different accessions of *T. urartu* or *T. dicoccoides* which direct the synthesis of both x and y HMW-subunits.

References

CIAFFI, M., BENEDETTELLI, S., GIORGI, B., PORCEDDU, E. and LAFIANDRA, D. 1991. Seed storage proteins of *Triticum turgidum* ssp. *dicoccoides* and their effect on technological quality in durum wheat. — *Plant Breed.* (in press)

DICK, J. and QUICK, J. S. 1983. A modified screening test for rapid estimation of gluten strength in early-generation durum wheat breeding lines. — *Cereal Chem. 60*: 315–318

HARBERD, N. P., BARTELS, D. and THOMPSON, R. D. 1986. DNA restriction-fragment variation in the gene family encoding high molecular weight (HMW) glutenin subunits of wheat. — *Biochem. Genet. 24*: 579–595

KHAN, K., HAMADA, A. S. and PATEK, J. 1985. Polyacrylamide gel electrophoresis for wheat identification: effect of variable on gel properties. — *Cereal Chem. 62*: 310–313

LAFIANDRA, D., KASARDA, D. D. and MORRIS, R. 1984. Chromosomal assignment of genes coding for the wheat gliadin protein components of the cultivar Cheyenne and Chinese Spring by two-dimensional electrophoresis. — *Theor. Appl. Genet. 68*: 531–539

MASCI, S., PORCEDDU, E. and LAFIANDRA, D. 1991. Two-dimensional electrophoresis of 1D encoded B and D glutenin subunits in common wheats with similar omega gliadins. — *Biochem. Genet. 29*: 403–413

PAYNE, P. I. and LAWRENCE, G. J. 1983. Catalogue of alleles for the complex gene loci, *Glu-A1*, *Glu-B1*, and *Glu-D1* which code for high-molecular-weight subunits of glutenin in hexaploid wheat. — *Cereal Res. Commun. 11*: 29–35

PAYNE, P. I., HOLT, L. M. and LAW, C. N. 1981. Structural and genetical studies on the high-molecular weight subunits of wheat glutenin. I. Allelic variation in subunits among varieties of wheat (*Triticum aestivum*). — *Theor. Appl. Genet. 60*: 229–236

SINGH, N. K. and SHEPHERD, K. W. 1988. Linkage mapping of genes controlling endosperm storage proteins in wheat. I. Genes on the short arms of group I chromosomes. — *Theor. Appl. Genet. 75*: 634–642

WAINES, J. G. and PAYNE, P. I. 1987. Electrophoretic analysis of the high-molecular weight glutenin subunits of *T. monococcum*, *T. urartu* and the A genome of bread wheat (*T. aestivum*). — *Theor. Appl. Genet. 74*: 71–76