

Relationship Between the Qualitative and Quantitative Compositions of Gluten Protein Types and Technological Properties of Synthetic Hexaploid Wheat Derived from *Triticum durum* and *Aegilops tauschii*

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ABSTRACT

Cereal Chem. 80(3):247–251

The contribution of the diploid wheat species *Aegilops tauschii* (Coss.) Schmal to the technological properties of bread wheat (*Triticum aestivum* L.) was previously studied by the investigation of synthetic hexaploids derived from tetraploid durum wheat (*T. turgidum* L.) and three diploid *Ae. tauschii* lines. The results indicated that bread volume, gluten index, SDS-sedimentation volume, and maximum resistance of gluten were significantly influenced by the *Ae. tauschii* lines. To determine the relationship between technological properties and qualitative and quantitative compositions of gluten proteins, the flours of parental and synthetic lines were extracted using a modified Osborne fractionation. Gliadin and glutenin fractions were then characterized by reversed-phase (RP) HPLC on C₈ silica gel. The HPLC patterns revealed typical differences between synthetic and parental lines. The gliadin patterns of three synthetic lines and the glutenin patterns of two synthetic lines were more similar to that of the diploid *Ae. tauschii* parents involved in the hybrids. In the glutenin pattern of one synthetic line, characteristics from both *Ae. tauschii* and the durum wheat parents were observed. The amount of total gliadin and

gliadin types of the synthetic lines was mostly intermediate between those of the durum and *Ae. tauschii* parents. The amounts of total glutenin and glutenin types (HMW and LMW subunits) of the synthetic lines were generally higher than those of the parental lines, and the ratio of gliadins to glutenins was significantly decreased. High positive correlations were found between the amount of total glutenins, HMW, and LMW subunits and bread volume, maximum resistance and extension area of gluten, and SDS-sedimentation volume. The ratio of gliadins to glutenin subunits had a strong negative influence on these properties. The protein content of the flours and the amount of total gluten proteins were not correlated with any of the technological properties. Results on the relationship between biochemical characteristics and the breadmaking properties indicated that wheat prebreeding would benefit from studies on protein types and quantification in the choice of parents. In addition, the potential of the diploid *Ae. tauschii* for improvement of breadmaking quality should be further exploited.

The diploid wild wheat species *Aegilops tauschii* (Coss.) Schmal. (genome DD) is an ancestor of bread wheat (*Triticum aestivum* L.) (genome AABBDD). Recently, a previous study demonstrated that the contribution of *Ae. tauschii* to breadmaking properties employed three synthetic hexaploid wheats (genome AABBDD) derived from tetraploid *Triticum turgidum* var. *durum* (genome AABB) and three different *Ae. tauschii* parental lines (Hsam et al 2001). The common durum wheat parent had the HMW subunit compositions 7+8. Two *Ae. tauschii* lines had the composition 5+10 and one *Ae. tauschii* line the composition 2+T1+T2. The HMW subunits 2+T1+T2 were described in *Ae. tauschii* (Laguda and Halloran 1988; Williams et al 1993) and in derived synthetic hexaploid wheat lines (Pena et al 1995; Hsam et al 2001). Breadmaking properties of the flours were characterized by microscale extension tests of gluten and microbaking tests. The durum wheat and the *Ae. tauschii* line with HMW subunits 2+T1+T2 developed weak gluten and bread with low loaf volume, whereas the other *Ae. tauschii* lines had relatively strong gluten and high loaf volume. Studies on the synthetic hexaploid lines indicated that HMW subunit alleles of both the durum and *Aegilops* parents were expressed. Bread loaf volumes, gluten maximum resistance and extensibility, dough surface, and other quality characteristics of the synthetic hexaploid wheats were closely related with those of the *Ae. tauschii* wheat involved in each cross (Hsam et al 2001). This clearly demonstrated that the diploid *Ae. tauschii* determined the properties of the synthetic lines much more than the tetraploid durum wheat. The aim of the present work was to further characterize and quantify gluten proteins present in the flour samples by a combined extraction and HPLC procedure and to correlate the technological properties determined previously with the quantities and ratios of the different gluten protein types.

MATERIALS AND METHODS

Wheat Samples

The synthetic wheat amphiploids XX201 (TT89 × TD12), XX227 (TT89 × TD 26), and XX229 (TT89 × TD190) were developed by crossing *T. turgidum* var. *durum* cv. Marocco 182 (accession no. TT89) with *Ae. tauschii* accessions TD12, TD26, and TD190, respectively, using TT89 always as the female parent (Hsam et al 2001). In this article, the accession numbers are abbreviated as TD12 (2N-1), TD26 (2N-2), TD190 (2N-3), TT89 (4N), XX201 (6N-1), XX227 (6N-2), and XX229 (6N-3). Preliminary seed increase of F₃ to F₅ synthetic hexaploid plants and the parental *Ae. tauschii* lines was conducted under glasshouse conditions. All materials were later field-grown at one location in Weihenstephan using a randomized complete block design with two replicates. Mean values between replicates were not significant. The kernels were milled to flours with ash contents of 0.55% using a Quadruplex mill (Brabender). The crude protein contents (N × 5.7) of the flours determined according to the Dumas method are shown in Table I.

Gluten Protein Quantification

Flour samples (100 mg) were stepwise extracted with 0.4M NaCl + 0.067M HKNaPO₄, pH 7.6 (2 × 1.0 mL), with 60% (v/v) aqueous ethanol (3 × 0.5 mL) and with 50% (v/v) 1-propanol + 2M urea + 0.05M Tris-HCl (pH 7.5) + 1% (w/v) dithioerythritol under nitrogen and increased temperature (2 × 1.0 mL) (Wieser et al 1998). Aliquots of the alcoholic extracts were analyzed by reversed phase (RP) HPLC on C₈ silica gel, and quantification was achieved by using a detection wavelength of 210 nm (two determinations) according to the method developed previously (Wieser et al 1998). A linear elution gradient (0 min 24% B, 50 min 56% B) was applied to separate gliadin and glutenin subunits. The integration procedure was automatically handled by software (Beckmann System Gold). The baseline was adjusted to zero when the injector was moved to injection position. Statistical evaluations were performed with program Slide Write Plus (Advance Graphics Software, Carlsbad, CA).

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Technological Properties

The determination of SDS sedimentation volume (SDSS), gluten index (GI), maximum resistance (RE), extensibility (EX) and extension area (EA) of gluten, and bread volume (BV) on a microscale was as described by Hsam et al (2001).

RESULTS AND DISCUSSION

Wheat Properties

For the present work, the flours of *T. durum* (4N), *Ae. tauschii* lines (2N-1, 2N-2, and 2N-3), and the corresponding synthetic hexaploid lines (6N-1, 6N-2, and 6N-3) were taken from a previous study (Hsam et al 2001). The compositions of HMW glutenin subunits were 7+8 (4N), 5+10 (2N-1, 2N-3), 2+T1+T2 (2N-2), 7+8, 5+10 (6N-1, 6N-3) and 7+8, 2+T1+T2 (6N-2). The crude protein content of flours was lowest for 4N (15.5%) and highest for 6N-3 (19.3%) and 6N-2 (19.0%) (Table I). In a previous study, 4N and 2N-2 developed a weak gluten and a bread with low volume, whereas the other *Ae. tauschii* lines had relatively strong doughs and high bread volumes (Hsam et al 2001). The technological properties of the hexaploid lines were closely related with those of the *Ae. tauschii* 2N lines.

HPLC Patterns of Gliadins and Glutenin Subunits

Flours were extracted stepwise with a saline solution (albumins, globulins), 60% ethanol (gliadins) and 50% 1-propanol under

reducing and disaggregating conditions (glutenin subunits) using an extraction procedure on a microscale (Wieser et al 1998). Aliquots of the gliadin and glutenin extracts were then separated by RP-HPLC on C₈ silica gel. The comparison of the gliadin patterns of the *Ae. tauschii* lines demonstrated that 2N-1 differed strongly from 2N-2 (Fig. 1). These genotypes disagreed in the number of ω 5-gliadins and in the number and position of dominating ω 1,2-gliadins. The α -gliadins of 2N-2 were more uniformly distributed over the whole elution range than those of 2N-1. Number and positions of major γ -gliadins were also different. The gliadin pattern of 2N-3 was more similar to that of 2N-1 than to that of 2N-2. Typical for all three *Ae. tauschii* lines was the poor separation of hydrophobic γ -gliadins (elution range 37–46 min). The durum wheat accession 4N was different from the *Ae. tauschii* lines in all elution regions of gliadins; its pattern was similar to those of hexaploid bread wheat investigated previously (Wieser et al 1987; Wieser et al 1994a).

The gliadin pattern of the synthetic line 6N-1 derived from 2N-1 and 4N was more related to that of 2N-1, particularly with respect to ω 5-gliadins and the major α - and γ -gliadins, but in the late elution areas of α - and γ -gliadins, components of 4N could also be recognized very well (Fig. 1). However, the major ω 1,2-gliadin of 6N-1 was present only as a minor component in the parental 2N-1 and 4N lines. The gliadin patterns of the hexaploid lines 6N-2 and 6N-3 were similar to those of the *Ae. tauschii* parental 2N-2 and 2N-3 lines, respectively. Components that were derived from

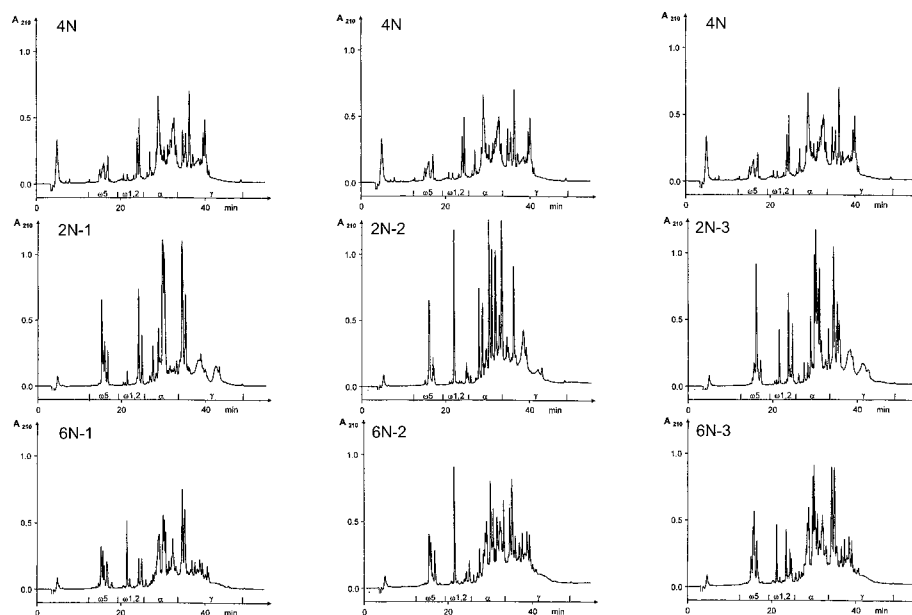


Fig. 1. Reversed-phase HPLC of gliadins on C₈ silica gel.

TABLE I
Crude Protein Content (CP), Quantities, and Ratios of Gluten Protein Types in Flour

No.	Wheat	CP (%)	Gluten Protein (AU)	Gliadins (GLI, AU) ^a					Glutenins (GLU, AU)			Ratio			
				Total	ω 5	ω 1,2	α	γ	Total	ω b	HMW	LMW	GLU/GLU	GLI/HMW	GLI/LMW
1	2N-1	17.6	2,110	1,592	156	133	595	708	518	10	160	348	3.1	10.0	4.6
2	2N-2	18.1	2,149	1,915	117	145	959	694	234	5	56	173	8.2	34.2	11.1
3	2N-3	18.5	2,298	1,871	174	162	732	803	427	6	119	302	4.4	15.7	6.2
4	4N	15.5	1,671	1,394	92	96	637	569	277	6	60	211	5.0	23.2	6.6
5	6N-1	16.4	1,950	1,373	132	105	553	583	577	17	175	385	2.4	7.9	3.6
6	6N-2	19.0	2,211	1,810	138	121	761	790	401	10	109	282	4.5	16.6	6.4
7	6N-3	19.3	2,439	1,867	182	131	798	756	572	16	169	387	3.3	11.1	4.8
CV	(\pm %) ^b	...	1.5	1.9	2.9	2.6	2.6	2.0	2.3	15.3	1.6	3.0

^a Absorbance units (AU) of HPLC corresponding to 1 mg of flour.

^b Average coefficient of variation (two determinations).

durum wheat 4N were only visible in those elution regions with gaps in the *Ae. tauschii* patterns. Hence, the gliadin patterns of the synthetic lines were more similar to those of the *Ae. tauschii* lines involved in each cross. Generally, the gliadin patterns of the hexaploid synthetics themselves were similar to those patterns of common bread wheat (*T. aestivum* L.) (Wieser et al 1994a).

The patterns of glutenin subunits are shown in Fig. 2. Among glutenin types glutenin-bound ω -(ω b)gliadins were negligible. As already observed (Wieser et al 1994b), each HMW subunit was eluted as a major and a minor peak. This can be seen in chromatograms of 2N-1 and 2N-3 (subunits 5+10), 2N-2 (subunits 2+T1+T2), and 4N (7+8). The HMW subunit patterns of the hexaploid synthetic lines reflected very well the combined pattern of the parents. With respect to LMW subunits, 2N-1 and 2N-2 differed distinctly in the patterns of subunits eluted at 35–39 min. 2N-3 was similar to 2N-1, with the exception of a double peak at 45 min. The LMW subunit patterns of the hexaploid synthetic lines 6N-1 and 6N-3 were more similar to those of the *Ae. tauschii* lines, whereas 6N-2 was more compensatory influenced by both durum and the *Ae. tauschii* parents.

Quantities of Gluten Protein Types

The quantification of total gliadins (GLI) and glutenin subunits (GLU) and of the different gluten protein types was performed by

the measurement of HPLC absorbance units at 210 nm, which strongly correlates with protein (Wieser et al 1998). According to previous studies (Bietz and Burnouf 1985; Wieser et al 1994a,b), proteins were eluted according to different surface hydrophobicity in the series ω 5-, ω 1,2-, α -, and γ -type gliadins (gliadin extract) and glutenin-bound ω (ω b)-gliadins, HMW, and LMW subunits (glutenin extract), and could be quantitated without major overlap. The elution borders of the protein types were set to absorbance minima between specific peaks. Table I summarizes the absorbance units (AU) per mg of flour and also includes the ratios of GLI to GLU and glutenin types.

The coefficient of average variation for the total procedure (extraction plus HPLC) was ± 1.5 to $\pm 3.0\%$ (two determinations) with the exception of ω b-gliadins ($\pm 15.3\%$), which occurred in very small amounts. Based on the data in Table I, the crude protein content of the flours was strongly correlated with the amounts of total gluten proteins ($r = 0.95^{***}$) and total gliadins ($r = 0.90^{***}$) but was not correlated with the amount of total glutenin subunits ($r = 0.20^{ns}$). The quantitative data of the parental lines indicated that the *Ae. tauschii* lines 2N-1 and 2N-3 had significantly higher amounts of gluten proteins, total gliadins, and gliadin types (except for α -gliadins of 2N-1), total glutenin subunits, and glutenin types (except for ω b-gliadins of 2N-3) than the durum line 4N. The *Ae. tauschii* line 2N-2 revealed remarkable differences compared with

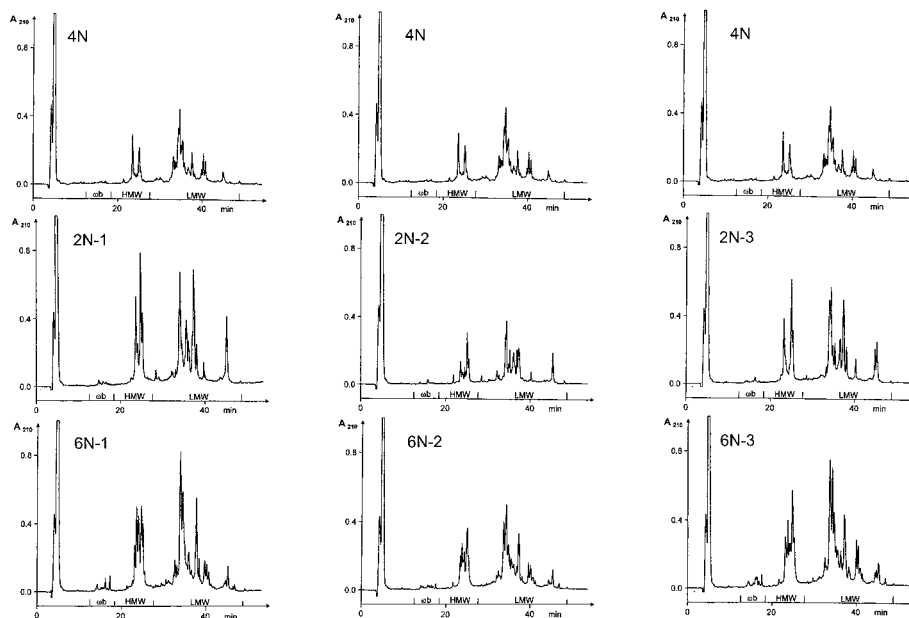


Fig. 2. Reversed-phase HPLC of glutenin subunits on C₈ silica gel.

TABLE II
Correlation Coefficients (r)^a for the Relationship Between Quantities of Gluten Protein Types and Wheat Properties^b

	SDSS	GI	RE	EX	EA	BV
Crude protein	0.31	0.19	-0.21	0.63	-0.04	0.00
Gluten proteins	0.54	0.40	0.03	0.45	0.15	0.27
Gliadins (GLI)	0.09	0.04	-0.47	0.70	-0.35	0.22
ω 5	0.75	0.62	0.41	-0.02	0.47	0.63
ω 1,2	0.37	0.47	-0.11	-0.34	-0.07	0.12
α	-0.23	-0.30	-0.71	0.83	-0.61	0.52
γ	0.23	0.22	-0.24	0.45	-0.11	-0.03
Glutenin subunits (GLU)	0.83	0.66	0.85	-0.38	0.88	0.86
HMW	0.87	0.71	0.87	-0.34	0.91	0.88
LMW	0.82	0.63	0.83	-0.40	0.86	0.86
GLI/GLU	-0.58	-0.47	-0.80	0.64	-0.79	-0.75
GLI/HMW	-0.68	-0.57	-0.81	0.54	-0.82	-0.78
GLI/LMW	-0.54	-0.43	-0.79	0.68	-0.76	-0.72

^a Level of significance: $r = 0.67$ – 0.78 , $P = 0.01$ (**); $r > 0.78$, $P = 0.001$ (***)

^b SDSS, SDS sedimentation volume; GI, gluten index; RE, maximum resistance; EX, extensibility; EA, extension area; BV, bread volume.

2N-1 and 2N-3; the amount of α -gliadins was strongly increased, and that of ω 5-gliadins decreased. The amount of total glutenins and HMW and LMW subunits were much lower and similar to those of the durum wheat parent 4N. Results of the synthetic hexaploid wheat lines revealed that the effects of hybridization on gliadins and glutenin subunits were completely different. In most cases, the amounts of total gliadins and gliadin types were between those of the parental lines (except for ω 5- and γ -gliadins of 6N-2 and for total gliadins and α -gliadins of 6N-1). The amount of glutenin types in the synthetic hexaploids, however, were generally higher compared with the parental lines. A further effect of introgression of gluten protein genes was that the ratios of monomeric proteins (gliadins) to aggregative proteins (total glutenins, HMW, and LMW subunits) were strongly decreased (Table I). All parental and synthetic lines with HMW subunits 5+10 (2N-1, 2N-3, 6N-1, 6N-3) determined by the *Glu-D1* locus were characterized by the highest amounts of HMW subunits.

Correlations Between Protein Quantities and Technological Properties

The correlation coefficients (r) calculated for the relationship of protein quantities and ratios (Table I) to the technological parameters (Hsam et al 2001) are summarized in Table II. Only $r > 0.66^{**}$ ($P < 0.01$) reflected a significant relationship. Neither the crude protein content nor the amount of total gluten proteins correlated with any

of the technological properties ($r < 0.64$). Not even the bread volume (BV) was related to the protein content of the flours ($r = 0.00$), which is usually very important for baking (Pomeranz 1988). In accordance with previous studies (Wieser et al 1994a,b; Wieser and Kieffer 2001), the amount of total gliadins (GLI) and GLI types was not significantly correlated with wheat properties except for GLI and extensibility (EX), for ω 5-gliadins and SDS sedimentation volume (SDSS), and for α -gliadins and maximum resistance (RE) and EX, respectively. RE, extension areas (EA), and BV were strongly correlated with the amount of total glutenins (GLU), HMW, and LMW subunits ($r > 0.82$). The coefficients for HMW subunits were a little bit higher than those for LMW subunits. The regression lines for the relationship of the amount of HMW subunits to RE and BV, respectively, are shown in Fig. 3A and B. A striking outlier for both relationships was the synthetic line 6N-2 with a weak gluten and the lowest BV; the reasons for that are unclear. Additionally, RE, EA, and BV were negatively correlated with the ratios of GLI to GLU and to HMW and LMW subunits ($r = -0.72$ to -0.82) (Fig. 3C and D). The influence of both absolute quantities of glutenin subunits and the ratio of gliadins to glutenins on RE also was observed for 28 bread wheat samples studied previously (Wieser and Kieffer 2001). Furthermore, the amount of GLU, HMW, and LMW subunits were strongly correlated with SDSS ($r = 0.82$ – 0.87) and in a lower degree also with the gluten index ($r = 0.63$ – 0.71). The relations of gluten

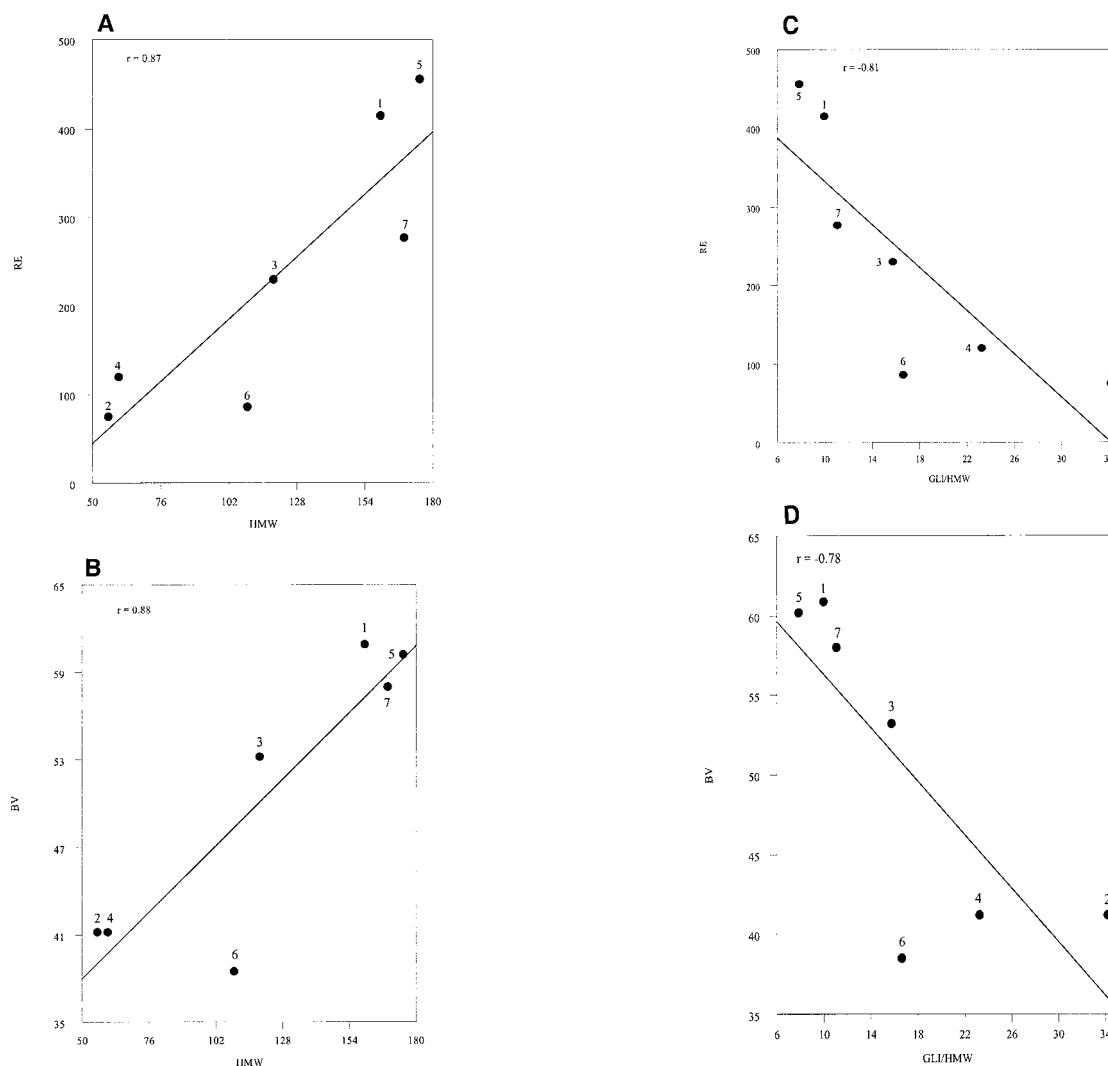


Fig. 3. Correlations between (A) maximum resistance of gluten (RE) and amount of HMW subunits; (B) bread volume (BV) and amount of HMW subunits; (C) maximum resistance of gluten (RE) and ratio GLI to HMW; and (D) bread volume (BV) and ratio GLI to HMW. See Table I for wheat line numbers.

proteins to EX were not uniform; the highest coefficients were observed for α -gliadins ($r = 0.83$), GLI (0.70), and the ratio of GLI to LMW subunits (0.68).

CONCLUSIONS

The HPLC patterns of gliadins and glutenin subunits of the synthetic hexaploid wheat lines were more similar to those of the diploid *Ae. tauschii* lines. Consequently, all gliadin patterns and two of the three glutenin subunits patterns of the synthetic hexaploids corresponded to those of the *Ae. tauschii* parents and not to that of the durum parent. The introgression of gluten protein genes as evidenced quantitatively by the amounts of gliadins and glutenin subunits in the synthetic hexaploids did not always express normal additivity of the parents. This was observed in *Ae. tauschii* parents possessing the HMW subunits 5+10. With regard to the total amount of gliadins and gliadin types, the derived synthetic lines were mostly between the parental lines. However, the amount of total glutenins and glutenin types in synthetic hexaploids were generally higher, and the ratio of gliadins to glutenins was significantly decreased in comparison with the parental lines. Both the amounts of glutenin subunits (positively) and the ratio of gliadins to glutenin subunits (negatively) had a strong influence on the maximum resistance and extension area of gluten and on the bread volume. Results indicated that further studies involving in a wider spectrum of genotypes is needed to fully understand the regulation and expression of gluten protein genes in synthetic hexaploid wheats. New synthetic hexaploid wheats exploiting the wide untapped variation of the wild *Ae. tauschii* species would provide a valuable genetic resource for quality improvement of bread wheat.

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[Received May 14, 2002. Accepted October 21, 2002.]