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Allelic variation at the *Gli-A1^m*, *Gli-A2^m* and *Glu-A1^m* loci and breadmaking quality in diploid wheat *Triticum monococcum*

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Summary

Fifty-six accessions of *Triticum monococcum* and one accession each of *T. beoticum* and *T. sinskajae* were analysed for their storage protein compositions and breadmaking quality as determined by the SDS-sedimentation test. In total 30 different alleles at the *Glu-A1^m* locus coding for high-molecular-weight glutenin subunits (HMW-GS), 25 alleles at the *Gli-A1^m* locus coding for ω- and γ-gliadins and 45 alleles at the *Gli-A2^m* locus controlling the synthesis of α/β-gliadins were detected. Most accessions contained one x-type and one y-type HMW-GS and two genotypes were null for both types of subunits. Two polypeptides within the mobility range of HMW-GS in SDS-PAGE were shown to be ω-type gliadins encoded by genes on the short arm of chromosome 1A. *T. sinskajae* and several 'monococcum' accessions were shown to share the same alleles at *Gli-A1^m*, *Gli-A2^m* and *Glu-A1^m*, confirming *sinskajae* as a subspecies of *T. monococcum*. The SDS-sedimentation volumes of most accessions were very low (11–35 ml), a few accessions showing mean sedimentation volumes as high as 90–93 ml. Through the comparison between biotypes occurring in some accessions of 'monococcum', good bread-making quality was found to be associated with the presence of alleles *y*, *c* and *i* at the *Gli-A1^m locus*. All accessions were resistant to leaf rust and rich in protein ($\geqslant$ 16·5%), and most of them showed resistance to powdery mildew.

1. Introduction

Elasticity and extensibility of doughs are strongly affected by allelic variation at the genes coding for high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS) in both durum and bread wheats (Payne *et al.* 1980; Payne, 1987; Gupta & Shepherd, 1988; Pogna *et al.* 1990). The genes coding for HMW-GS are located on the long arms of group 1 chromosomes at loci designated as *Glu-1*; each locus contains two genes, one encoding an x-type subunit, the other a y-type subunit according to their mobilities in SDS-PAGE (Payne *et al.* 1981). The genes coding for LMW-GS occur on the short arms of group 1 chromosomes at the *Glu-3* loci (Singh & Shepherd, 1988) which are tightly linked to the *Gli-1* loci encoding for ω-, γ- and a few β-gliadins (Payne, 1987). Furthermore, α- and most β-gliadins are encoded by genes tightly clustered at a single locus on each of the group 6 chromosomes (Payne, 1987).

Little is known about genetic variability and inheritance of the storage proteins, glutenin and gliadins, in A-genome diploid species *Triticum monococcum* (Waines & Payne, 1987; Metakovsky & Baboev, 1992*a*; Blanco *et al.* 1994). Moreover, to our knowledge, there is no report of studies on the relationship between storage protein composition and breadmaking quality in this primitive species. *T. monococcum* has been considered as a rich source of high protein content and disease resistance for cultivated polyploid wheats (Lawrence *et al.* 1958; The, 1975; Fedak, 1985) but very few attempts have been carried out to develop commercial cultivars of 'monococcum' (Borojevic, 1956; Waines, 1983). In fact, this species shows high protein, lysine and carotene contents, resistance to diseases and salinity tolerance as compared to durum or bread wheat (Lawrence *et al.* 1958; Sharma *et al.* 1981; The, 1975; Waines *et al.* 1987; Pasquini *et al.* 1989; Gorham *et al.*

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Table 1. *Plant height (PH, cm), heading date (HD, days from May 1st), resistance to powdery mildew (M) and leaf rust (LR), and allele composition at the Gli-A1^m, Gli-A2^m and Glu-A1^m loci in diploid wheat accessions*

Code	Species	Source and accession code	PH	HD	M	LR	Allele at *Glu-A1^m*	*Gli-A1^m*	*Gli-A2^m*
1376	*T. beoticum*	AFRC 103005 CL	—	—	—	—	x	s	ah
2	*T. monococcum*	UA CI 13691	60	17	—	—	a	a	l
4	*T. monococcum*	UA PI 94740	70	24	—	—	a	b, o	m
42	*T. monococcum*	UA PGR 10403	65	34	MR	R	b	e	g
365	*T. monococcum*	ISC Line 1006	75	20	Sg	R	ab	y, b	ae
359	*T. monococcum*	INTA ARG 22796	95	30	—	—	o	d	a
1327	*T. monococcum*	IDG 4242	120	19	Sg	MR	n, q, r	b, l	y, z
1331	*T. monococcum*	IDG MG 4278	105	28	R	R	s, a	b, f	z, ab
1358	*T. monococcum*	AFRC 104002 CL	—	—	S	—	y	f	ae
1359	*T. monococcum*	AFRC 104003 CL	75	34	—	—	w	q	af
1360	*T. monococcum*	AFRC 104004 CL	110	21	—	R	w	r	ag
1378	*T. monococcum*	MPI Einkorn	115	20	Sg	R	y	b	ai
1379	*T. monococcum*	MPI Winterform	115	24	R	R	aa, z	b, x	ak, aj
1408	*T. monococcum*	BGRC 27605	85	21	Sg	R	a	a	an
111	*T. m.* spp *atriaristatum*	IGK ATRI 2124-741	58	39	—	—	g	m	q
113	*T. m.* spp *atriaristatum*	IGK ATRI 2399-74	70	39	R	R	h	l	i
101	*T. m.* spp *flavescens*	IGK ATRI 612-74	—	—	—	—	a	b	j
117	*T. m.* spp *flavescens*	IGK ATRI 4309-74	80	28	MS	—	l	b	s
114	*T. m.* spp *halbohornemanii*	IGK ATRI 3409-79	90	26	Sg	MR	i	w	i
1401	*T. m.* spp *halbohornemanii*	BGRC 13190	115	26	MR	R	o	h, w, g	at, d
105	*T. m.* spp *hornemanii*	IGK ATRI 895-74	50	52	—	—	c	b	j
106	*T. m.* spp *hornemanii*	IGK ATRI 896-74	58	38	—	—	d	w	o, j
110	*T. m.* spp *hornemanii*	IGK ATRI 2001-74	70	40	MR	—	c	x	k
116	*T. m.* spp *hornemanii*	IGK ATRI 4304-74	—	—	—	—	j, c	m	h
1402	*T. m.* spp *hornemanii*	BGRC 13191	80	38	MS	MR	o	b	au
1403	*T. m.* spp *hornemanii*	BGRC 13192	85	35	R	R	a	g	d
1404	*T. m.* spp *hornemanii*	BGRC 13193	85	35	R	R	ae	b	d
1405	*T. m.* spp *hornemanii*	BGRC 13194	85	35	R	R	ae	h	e
1406	*T. m.* spp *hornemanii*	BGRC 13195	90	32	R	R	ae	h, i	e
1407	*T. m.* spp *hornemanii*	BGRC 13196	90	35	R	R	ae	i	f
1409	*T. m.* spp *hornemanii*	BGRC 42018	95	35	R	R	a	i	f
119	*T. m.* spp *laetissimum*	IGK ATRI 4321-75	100	25	S	—	n	b	t
1348	*T. m.* spp *laetissimum*	RAC 486	105	23	Sg	R	t	e	ae
1349	*T. m.* spp *laetissimum*	RAC 1496	100	20	—	—	u	t	ad
112	*T. m.* spp *macedonicum*	IGK ATRI 2126-74	—	—	—	—	h	a	k
118	*T. m.* spp *macedonicum*	IGK ATRI 4329-74	90	27	—	—	m	a	g
357	*T. m.* spp *macedonicum*	INTA ARG 22553	—	—	—	—	o	d	a
360	*T. m.* spp *macedonicum*	INTA ARG 22928	—	—	—	—	p	j	x
121	*T. m.* spp *monococcum*	IGK ATRI 11360-80	90	30	Sg	R	o	a	u
358	*T. m.* spp *nigricultum*	INTA ARG 22554	90	31	—	—	o	c	w
361	*T. m.* spp *nigricultum*	INTA ARG 22929	95	31	R	R	o	y	x
1351	*T. m.* spp *nigricultum*	RAC 1498	110	29	R	R	t	c	w
1400	*T. m.* spp *nigricultum*	BGRC 13189	105	30	R	R	ae	b, c	as, av
1352	*T. m.* spp *pseudomacedonicum*	RAC 2372	100	26	R	R	v	j	q
1395	*T. m.* spp *pseudoflavescens*	BGRC 13177	110	24	MR	R	ac	j	c
1497	*T. m.* spp *sofianum*	RAC 1497	105	21	MR	R	t	y	x
1399	*T. m.* spp *sofianum*	BGRC 13187	105	28	MR	R	ad	y	aq
103	*T. m.* spp *vulgare*	IGK ATRI 617-74	—	—	—	—	a	k	b
104	*T. m.* spp *vulgare*	IGK ATRI 618-74	95	33	MR	—	a	k	b
108	*T. m.* spp *vulgare*	IGK ATRI 1985-74	60	35	—	—	e, b	d, n	v, a
109	*T. m.* spp *vulgare*	IGK ATRI 1990-74	—	—	—	R	f	c	h
115	*T. m.* spp *vulgare*	IGK ATRI 3637-74	65	41	MR	—	a	p	r
124	*T. m.* spp *vulgare*	IGK A SCHGT 2-88	77	30	—	—	o	d	a
1394	*T. m.* spp *vulgare*	BGRC 3522	105	26	R	R	a	d	ao
1396	*T. m.* spp *vulgare*	BGRC 13178	115	27	MS	R	ac	j	c
1397	*T. m.* spp *vulgare*	BGRC 13182	90	30	R	—	ad	u	ap
1398	*T. m.* spp *vulgare*	BGRC 13183	95	28	MR	R	ae	v	aq
127	*T. sinskajae*	IGK ATRI 12910-89	—	—	—	—	o	n	v

MPI = Max-Planck Institut, Germany; IDG = Istituto del Germoplasma, Italy; INTA = Instituto Nacional de Technologia Agropecuaria, Argentina; AFRC = Cambridge Laboratory, UK; IGK = Institut fur Genetic und Kulturpfanzenforschung, Germany; RAC = Recherches Agronomique de Changins, Switzerland; BGRC = Institut Pflanzenbau Braunschweig,

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1991; D'Egidio *et al.* 1993) but is inferior to cultivated wheats for yield potential and breadmaking quality (Guzy *et al.* 1989; Vallega, 1992; D'Egidio *et al.* 1993).

The main objective of the present work was to determine allelic variation at loci coding for storage proteins in 56 accessions of 'monococcum' and one accession each of *T. beoticum* and *T. sinskajae*. The association between the presence of certain alleles and breadmaking quality, and resistance to powdery mildew and leaf rust have been studied as well.

2. Materials and Methods

(i) *Plant material*

Seeds of 56 accessions of *Triticum monococcum* and one accession each of *T. beoticum* and *T. sinskajae* were grown in single rows 3 m long in Rome. After recording plant height, days to head and morphological uniformity in each accession, spikes from each plant were collected by hand picking and threshed manually. Protein content of grains was determined by the Kjeldhal method (N × 5·7) and expressed on a dry weight basis.

(ii) *Electrophoretic analysis*

Gliadins were extracted from single seeds for 2 h at 50 °C with 80 μl of aqueous 70% ethanol. After centrifugation for 5 min at 20000 ***g***, the supernatant was divided into 20 μl aliquots. One aliquot was mixed with 20 μl of a solution containing 60% (w/v) of glycerol and 0·05% (w/v) of pyronin G and separated at pH 3·1 by A-PAGE as described previously (Pogna *et al.* 1990). Unreduced gliadins of some genotypes were also fractionated by SDS-PAGE according to Dachkevitch *et al.* (1993). One gliadin aliquot was mixed with 40 μl of a solution containing 16·5% glycerol, 0·1 M-Tris-HCl, pH 6·8, 3·5% (w/v) SDS and 0·016% (w/v) pyronin Y, and fractionated by SDS-PAGE using a 15% separating gel, pH 8·4. Two-dimensional A-PAGE × SDS-PAGE of gliadins from some relevant accessions was carried out following the method of Payne *et al.* (1984) with minor modifications. After A-PAGE in the first dimension, the gel was cut into thin strips parallel to the direction of electrophoresis and each strip was incubated at 37 °C for 45 min in an equilibration solution containing 2·4% (w/v) SDS, 0·07 M-Tris-HCl, pH 6·8, and 5% (v/v) 2-mercaptoethanol, mounted on top of a new gel and fractionated by SDS-PAGE in the second dimension as described above.

To reveal total storage proteins, the residual crushed seed-ethanol mixture was mixed with 250 μl of water, 25 μl of 2-mercaptoethanol and 125 μl of a stock solution containing 0·2 M-Tris-HCl, pH 6·8, 7% (w/v) SDS, 30% (v/v) glycerol. After incubation for 1·5 h at room temperature and for 30 min at 80 °C, proteins were fractionated by SDS-PAGE using a 15% separating gel, pH 8·4.

(iii) *SDS-sedimentation test*

Hulled grains were milled at 2 g/s grinding rate using a Udy Cyclone Mill (Tecator AB, Sweden) fitted with a 0·5 mm sieve. The whole meal samples were then used for the SDS-sedimentation test as previously described (Pogna *et al.* 1990). Whole meals from cv. Salmone (high-quality bread wheat), cv. S. Pastore (poor quality bread wheat) and cv. Latino (poor quality durum wheat) were used as control.

(iv) *Resistance to powdery mildew and leaf rust*

Resistance to leaf rust and powdery mildew was determined in a greenhouse under standardized controlled conditions. The seedlings were infected at the first leaf stage with two biotypes (V4 and A4) of *Erysiphe graminis* spp *tritici* and one biotype (12513) of *Puccinia recondita* spp *tritici*. This latter biotype is virulent to resistant genes *Lr 3ka, Lr 14b, Lr 15, Lr 17, Lr 30* and avirulent to *Lr 1, Lr 2a, Lr 2b, Lr 9, Lr 19* and *Lr 24*. Biotype V4 is virulent to *Pm 4a* and avirulent to *Pm 8* and *Pm 13* whereas biotype A4 is avirulent to all the three genes. Recording of reactions to the pathogen was done about 10 d later using a 0–4 scale (0 = no visible disease, 4 = large pustules with abundant mycelia and conidia, no chlorosis or necrosis). On the basis of this scale, the accessions were classified into three groups i.e. resistant or moderately resistant (infection types from 0 to 1, and from 1+ to 3−, respectively), susceptible (infection types from 3 to 4) and segregant (resistant and susceptible plants).

3. Results

(i) *High-molecular-weight glutenin subunit (HMW-GS) compositions*

Amongst the 58 diploid wheat accessions analysed (Table 1), 30 different HMW-GS patterns were obtained by SDS-PAGE fractionation of total endosperm protein. In particular, most accessions contained one x-type subunit with relative mobility slightly slower than that of subunit 5 in the reference bread wheat cultivar Centauro, plus one y-type subunit slightly faster than subunit 7 (Fig. 1*a*). However, seven accessions showed only the x-type subunit

Germany; UA = University of Alberta, Canada; ISC = Istituto Sperimentale Cerealicoltura, Italy. S = Susceptible; R = Resistant; MR = Moderately resistant; MS = Moderately susceptible; Sg = Segregant.

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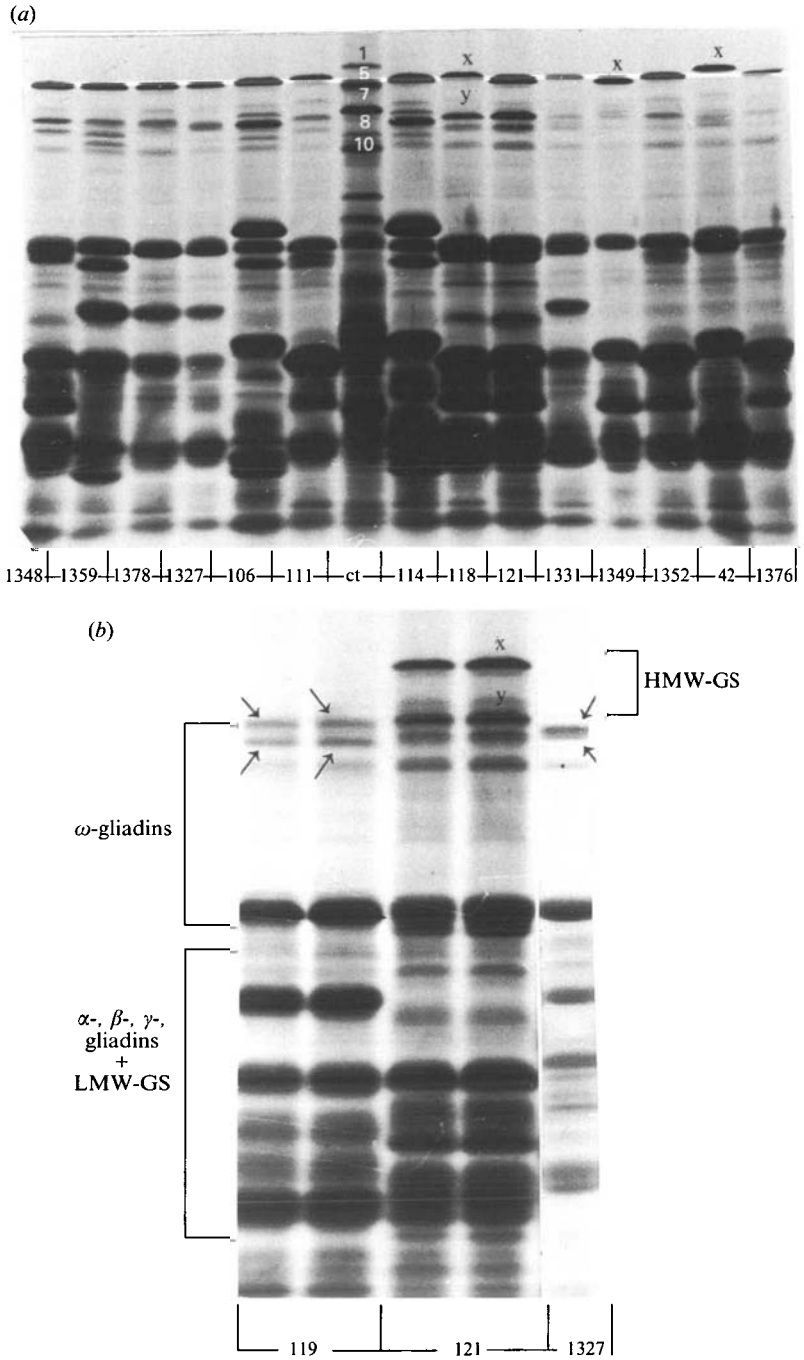

Fig. 1. SDS-PAGE fractionation of total protein from single seeds of 15 accessions of *T. monococcum*. HMW glutenin subunits in the bread wheat cv. Centauro (ct) are numbered. Arrows indicate HMW ω-gliadins. Biotypes 2 and 3 in accession 1327 are shown in (*a*) and (*b*) respectively. x, y = x- and y-type HMW glutenin subunits.

(Fig. 1 *a*, accessions 42 and 1349 for example), whereas accessions 119 and 1327 (biotype 3) were quite unique in lacking both subunits (Fig. 1 *b*). Each of the 30 different HMW subunits or pairs of subunits were assigned to a specific allele designated by a lower case letter following the locus symbol *Glu-A1*^{m}. Besides the HMW-GS, there were 4–5 bands which fell within the mobility range of HMW-GS. This aspect will be considered later.

Each of 56 accessions showed the same HMW-GS pattern in the three seeds analysed electrophoretically. However, when 26 accessions were screened using eight or more seeds each, three of them were found to contain two or more genotypes having different HMW-GS. In one group of heterogeneous accessions the genotypes in each accession possessed the same gliadin pattern and are therefore related genetically (Fig. 2, accession 116). On the contrary, a second group of heterogeneous accessions consisted of admixtures of genotypes having contrasting gliadin compositions and plant morphology (Fig. 2, accession 1379).

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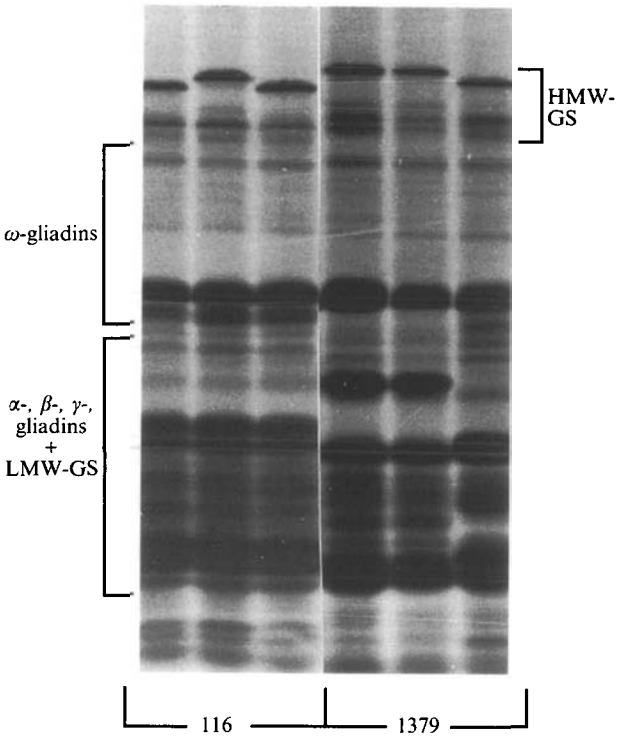

Fig. 2. SDS-PAGE of total proteins from three single seeds each of accessions 116 and 1379 of 'monococcum'.

Furthermore, different accessions showed the same HMW-GS pattern (Table 1). For example, the HMW-GS assumed to be encoded by allele *o* at the *Glu-A1*^{m} locus (Fig. 1, accession 121) occurred in nine accessions including *T. sinskajae*, whereas alleles *a* and *ae* were found in 11 and six accessions respectively.

(ii) *Gliadin compositions*

When fractionated by A-PAGE, gliadins from the diploid wheats were shown to consist of 13–26 bands having a wide variability in mobility and intensity. The same group (or block) of ω- and γ-gliadins in the upper region of the patterns occurred in two or more accessions and was therefore assumed to be encoded by tightly linked genes at the complex *Gli-A1*^{m} locus on chromosome 1A. This assumption was confirmed by F_2 segregation data from four crosses involving eight 'monococcum' genotypes (manuscript in preparation), and is in accordance with earlier results by Metakovsky & Baboev (1992*a*). Each block of gliadins consisted of 2–9 components; in particular, some accessions showed 4–6 strong ω-gliadins (Fig. 3*a*), whereas a few accessions had no ω-gliadins (Fig. 3*a*, accessions 358 and 1351).

In total 25 different blocks (*Gli-A1*^{m} alleles) were identified, alleles *a*, *b*, *c*, *d* and *y* occurring in 60% of the accessions analysed (Table 1). Similarly, 45 different blocks of α/β-gliadins were assigned to the *Gli-A2*^{m} locus on chromosome 6A (Table 1). These blocks consisted of 6–8 bands in the lower region of the A-PAGE patterns (Fig. 3*a*). Ten accessions contained two or three genotypes which differed from each other in the alleles at *Gli-A1*^{m} (Fig. 3*b*, accession 365, and accessions 4 and 1406), at *Gli-A2*^{m} (accession 106) or at both loci (Fig. 3*b*, accession 1400, and accessions 108, 1327, 1331, 1379 and 1401). Since accessions 108, 1327, 1331 and 1379 were also heterogeneous at the *Glu-A1*^{m} locus coding for HMW-GS, they are likely to be off-types resulting from errors during the maintenance of the wheat collection. On the contrary, the one-locus heterogeneous accessions consisted of two or more genotypes (or biotypes) related genetically.

The gliadin proteins were also fractionated by two-dimensional electrophoresis A-PAGE × SDS-PAGE (Fig. 4). In total, 18–23 major components and up to ten ω-gliadins were observed in the different accessions. In addition to the major proteins, there were two minor polypeptides with molecular weights similar to those of the y-type HMW-GS (Fig. 4, arrows). These polypeptides also occurred in the two-dimensional fractionations of unreduced gliadins in which the reducing agent 2-mercaptoethanol was not added to the solution used to equilibrate the A-PAGE strips before electrophoresis in the second dimension (data not shown). Moreover, they appeared as two bands in the one-dimensional SDS-PAGE fractionations of the alcohol-soluble prolamins in the absence of a reducing agent (Fig. 5). The polypeptides were assumed to be HMW ω-type gliadins. This conclusion is supported by the observation that each variant of this band pair occurred in accessions having a peculiar *Gli-A1*^{m} allele.

Finally, there were three or more characteristic streaks in the two-dimensional map of gliadins (Fig. 4, arrowheads) and these are likely to be low-molecular-weight glutenin subunits (LMW-GS) because they showed molecular weights and an electrophoretic behaviour typical of LMW-GS in polyploid wheats. As observed for LMW-GS in bread wheat (Dachkevitch *et al.* 1993), these proteins appeared as curved streaks in the upper part of the two-dimensional A-PAGE × SDS-PAGE maps of unreduced gliadins, suggesting their polymeric nature (data not shown).

(iii) *Quality characteristics*

Variation in the quantity and type of storage protein in both bread and durum wheat strongly affect the viscoelastic properties of dough (Payne, 1987; Pogna *et al.* 1990). Therefore, the storage protein profiles of the 58 accessions were correlated with their bread-making quality as measured by the SDS-sedimentation test. In particular, the biotypes isolated in each of six heterogeneous accessions were compared to each other for their sedimentation volumes and protein content. The mean protein content of the diploid

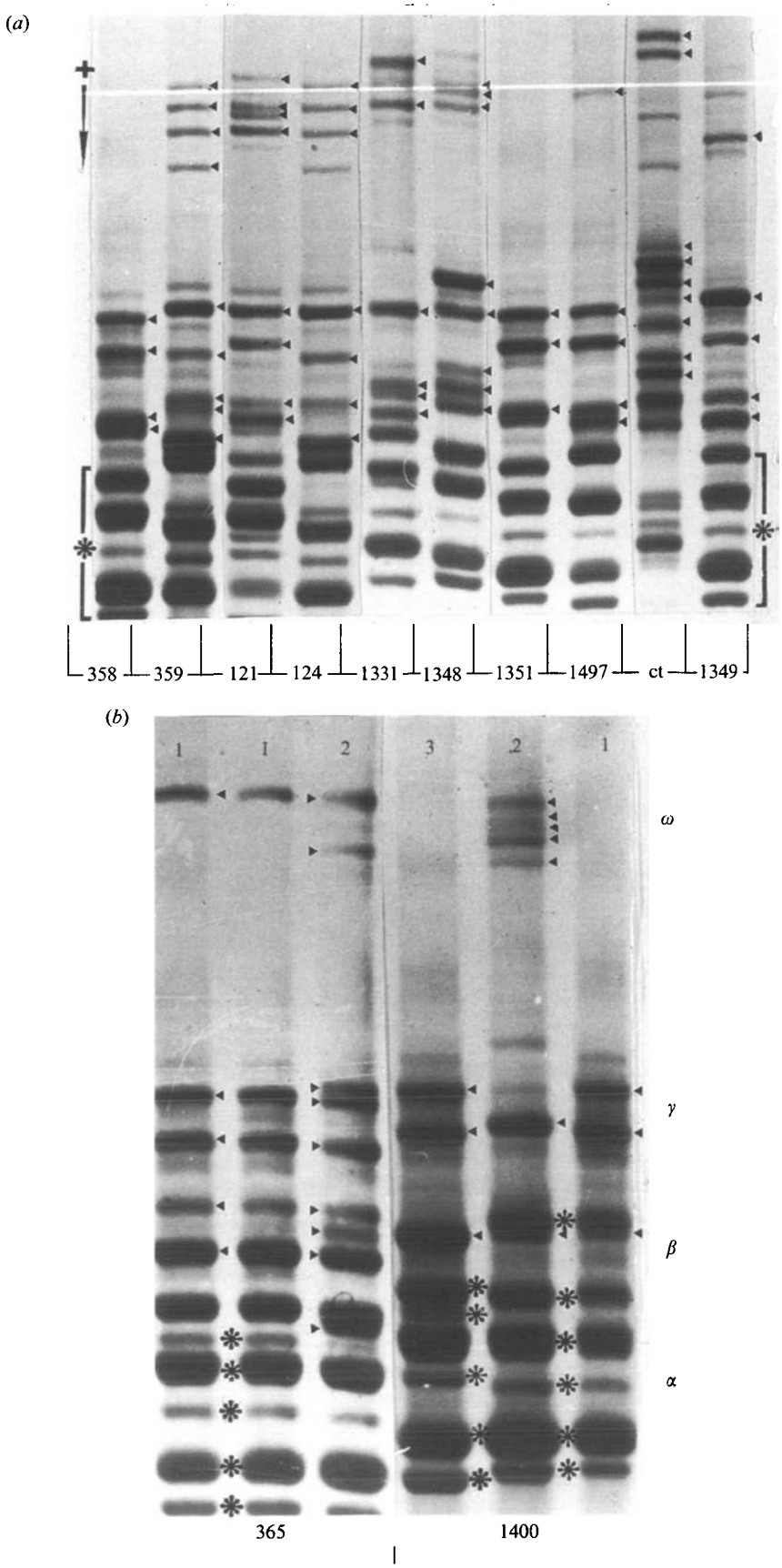

Fig. 3. A-PAGE of gliadins from (*a*) one single seed each of 9 accessions and (*b*) three single seeds of accessions 365 and 1400 of *T. monococcum*. Arrowheads and asterisks indicate gliadin blocks encoded by the *Gli-A1*^{m} and *Gli-A2*^{m} locus, respectively. 1, 2, 3 = biotypes 1, 2 and 3, respectively. ct = bread wheat cv. Centauro.

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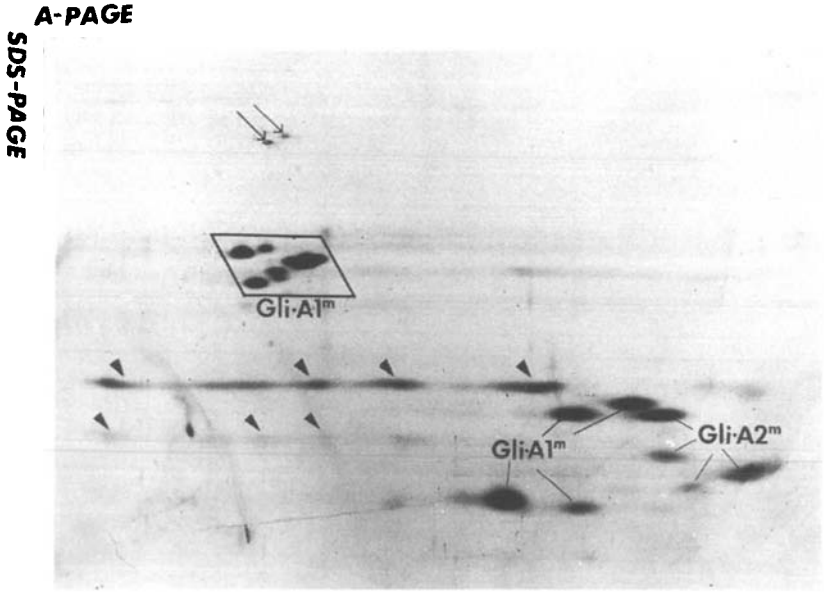

Fig. 4. Two-dimensional A-PAGE × SDS-PAGE of gliadins from accession 115 of 'monococcum'. Arrows indicate HMW ω-gliadins, arrowheads show major LMW glutenin subunits. The gliadin blocks encoded by genes at *Gli-A1*^{m} and *Gli-A2*^{m} are indicated.

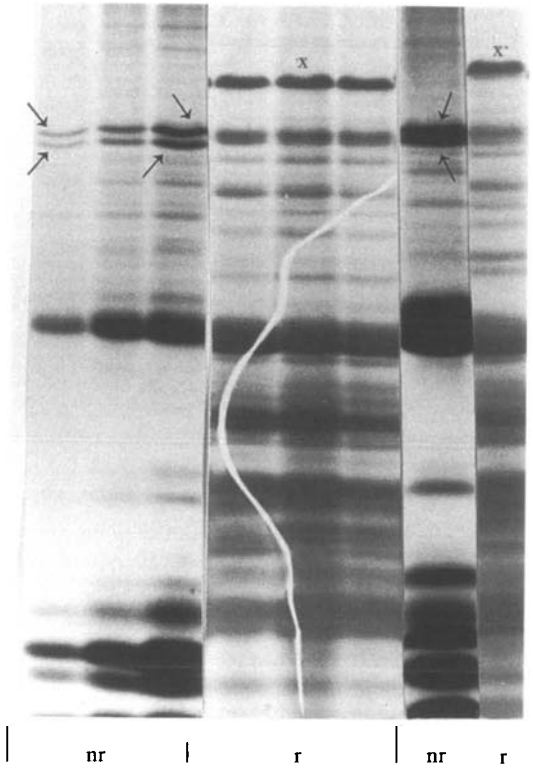

Fig. 5. SDS-PAGE fractionation of unreduced (nr) and reduced (r) alcohol-soluble proteins from (lanes 1–6) accession 4 and (lanes 7 and 8) accession 1331 (biotype 1) of *T. monococcum*. Arrows indicate HMW ω-gliadins. x = x-type HMW-GS.

wheat accessions was 18·5%, the range of variation being 16·5 to 22·9% (Table 2). The SDS-sedimentation volumes ranged between 11 and 100 ml as compared to 28–89 ml in the control wheat cultivars Salmone, S. Pastore and Latino. The biotypes in each of the heterogeneous accessions 1327, 1331, 365, 1400, 1401 and 1406 showed contrasting sedimentation volumes (Table 2). In particular biotype 2 of accession 1406 had an average sedimentation volume (62 ml) significantly high as compared to that of biotype 1 (46 ml). The protein differences between these biotypes arise from allelic variation at the *Gli-A1*^{m} locus, both biotypes sharing alleles *e* and *ae* at *Gli-A2*^{m} and *Glu-A1*^{m} respectively (Fig. 6*a* and *b*, lanes 10 and 11). Similarly, biotype 1 in accession 1401 had a mean sedimentation volume of 55 ml as compared to 21·8 ml in its counterpart biotype 2 (Table 2). These biotypes contain the same allele at the *Glu-A1*^{m} locus but possess contrasting alleles at *Gli-A1*^{m} and *Gli-A2*^{m} (Fig. 6*a* and *b*, lanes 8 and 9). Biotype 1 in accession 365 (Fig. 3*b*, lanes 1 and 2), which contains allele *y* at *Gli-A1*^{m} (Table 2), gave a very high sedimentation volume compared to biotype 2 (Fig. 3*b*, lane 3), which possesses allele *b*. These biotypes share the same alleles at *Gli-A2*^{m} and *Glu-A1*^{m}. Another interesting finding was that allele *c* at *Gli-A1*^{m} in the high-quality biotypes 1 and 3 of accession 1400 (Fig. 6*b*, lanes 6 and 7, and Table 2) also occurs in accessions 109, 358 and 1351 (Fig. 3*a*), which showed mean sedimentation volumes of 75, 86 and 90 ml, respectively. This allele does not code for any ω-gliadins. Finally, the genotypes in each of the accessions 1327 and 1331 possess contrasting alleles at all the three storage protein loci (Fig. 6*a*, *b*, lanes 1–5) and, therefore, are considered as off-types. However, it is worth noting that the *Glu-A1*^{m}-null biotype 3 in accession 1327 (Figs 1*b* and 6*a*, lane 3) gave an SDS-sedimentation volume not significantly lower than that of biotype 2 in which both x- and y-types HMW-GS are expressed (Fig. 6*a*, lane 2 and Table 2).

As noted above, most accessions were found to have one or more common alleles at the storage

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Table 2. *Mean values for protein content and SDS-sedimentation volume in 58 accessions of diploid wheat and SDS-sedimentation volume of some relevant genotypes*

Accession No.	Biotype	Allele at			No. of lines analysed	SDS-sedimentation volume (ml)		Protein content (% d.wt.)	
		Glu-A1^m	*Gli-A1^m*	*Gli-A2^m*		Mean	Range	Mean	Range
1327	1	*q*	*b*	*z*	3	31·5	30–33	18·5	18·0–19·1
	2	*r*	*l*	*y*	1	15·0	—	18·9	—
	3	*n*	*l*	*y*	2	14·5	14–15	20·4	20·3–20·5
1331	1	*a*	*b*	*z*	2	22·5	16–29	21·7	21·8–21·6
	2	*s*	*f*	*ab*	8	68·4	61–87	21·1	20·5–21·9
365	1	*ab*	*y*	*ae*	2	90·0	85–94	18·8	18·6–18·9
	2	*ab*	*b*	*ae*	2	55·7	45–65	18·9	20·2–19·7
1401	1	*o*	*g*	*d*	8	55·0	52–59	19·8	19·5–20·0
	2	*o*	*h*	*at*	2	21·8	19–29	18·9	18·5–19·4
1406	1	*ae*	*h*	*e*	1	46·0	—	19·3	—
	2	*ae*	*i*	*e*	3	62·0	55–72	19·4	19·0–19·7
1400	1	*ae*	*c*	*as*	6	93·6	89–100	18·1	18·0–18·6
	3	*ae*	*c*	*av*	1	92·0	—	20·4	—
1403	—	*a*	*g*	*d*	10	81·2	70–95	22·0	21·1–22·5
All accessions (58)	—	—	—	—	101	25·5	11–100	18·5	16·0–22·9
Control cvs									
Salmone (bread wheat)	—	—	—	—	—	87·5	86–89	14·5	—
S. Pastore (bread wheat)	—	—	—	—	—	31·0	28–34	13·5	—
Latino (durum wheat)	—	—	—	—	—	34·0	33–35	14·0	—

protein loci *Gli-A1^m*, *Gli-A2^m* and *Glu-A1^m* (Table 1), suggesting different degrees of similarity amongst the accessions. In particular, *T. sinskajae* and accession 108 of 'monococcum' both showed alleles *n* and *v* at *Gli-A1^m* and *Gli-A2^m* respectively, suggesting a close genetic relationship between these two species. Moreover, *T. sinskajae* and several accessions belonging to spp *vulgare*, *nigricultum*, *macedonicum*, *hornemanii* and *albohornemanii* share allele *o* at the *Glu-A1^m* locus (Table 1). On the contrary, *T. beoticum* was shown to have unique alleles at the three storage protein loci.

(iv) *Agronomical characteristics and resistance to leaf rust and powdery mildew*

There was a wide range of values for plant height and heading time amongst the 58 accessions analysed (Table 1). Some accessions (2, 105, 111 and 106) were as short as 50–60 cm whereas a few accessions (2, 1327, 1378 and 1394) reached anthesis 31–35 days earlier than the latest accession 105. All accessions proved to be resistant to leaf rust whereas 35 out of 45 accessions showed resistance to both biotypes V4 and A4 of powdery mildew. Furthermore, seven accessions were segregating from high resistance to moderate susceptibility to powdery mildew, some of these being heterogeneous also for storage protein composition.

4. Discussion

The banding patterns of HMW-GS of the endosperm of 'monococcum' are quite distinctive with respect to those of both durum and bread wheats. In particular, in most present-day accessions both x- and y-type subunits of HMW-GS encoded at *Glu-A1* are expressed, whereas in cultivated wheats y-type subunits are absent, the only exception being two Swedish bread wheat lines selected from interspecific crosses involving wild wheat species (Margiotta *et al.* 1995). Our observations are in general agreement with the earlier report by Waines & Payne (1987). However, seven and two accessions in our collection were shown to lack y-type or both types of subunits respectively, these unusual genotypes being absent in the 132 accessions analysed by the authors mentioned above. In the mobility range of HMW-GS there was a series of less prominent bands slightly faster than y-type subunits. According to Waines & Payne (1987) these are the product of proteolytic activity against y-type subunits. However, two of these bands are shown here to be HMW ω-gliadins encoded by genes on the short arm of chromosome 1A. Our conclusion is further supported by the finding of one *Gli-A1*-encoded polypeptide in the HMW-GS region in *Triticum beoticum* (Metakovsky & Baboev, 1992*b*).

The A-PAGE and SDS-PAGE fractionations of gliadins from the present collection of 'monococcum' showed two blocks of proteins, one in the ω/γ-region, the other in the α/β-zone of the gel, which occurred in two or more accessions. Taking into account the work of Metakovsky & Baboev (1992*a*), these blocks are assumed to be encoded by the *Gli-A1^m* locus on chromosome 1A, and by the *Gli-A2^m* locus on chromosome 6A respectively. In total at least 30 different alleles at *Gli-A1^m* and 45 alleles at *Gli-A2^m* have been detected in the 58 accessions analysed here.

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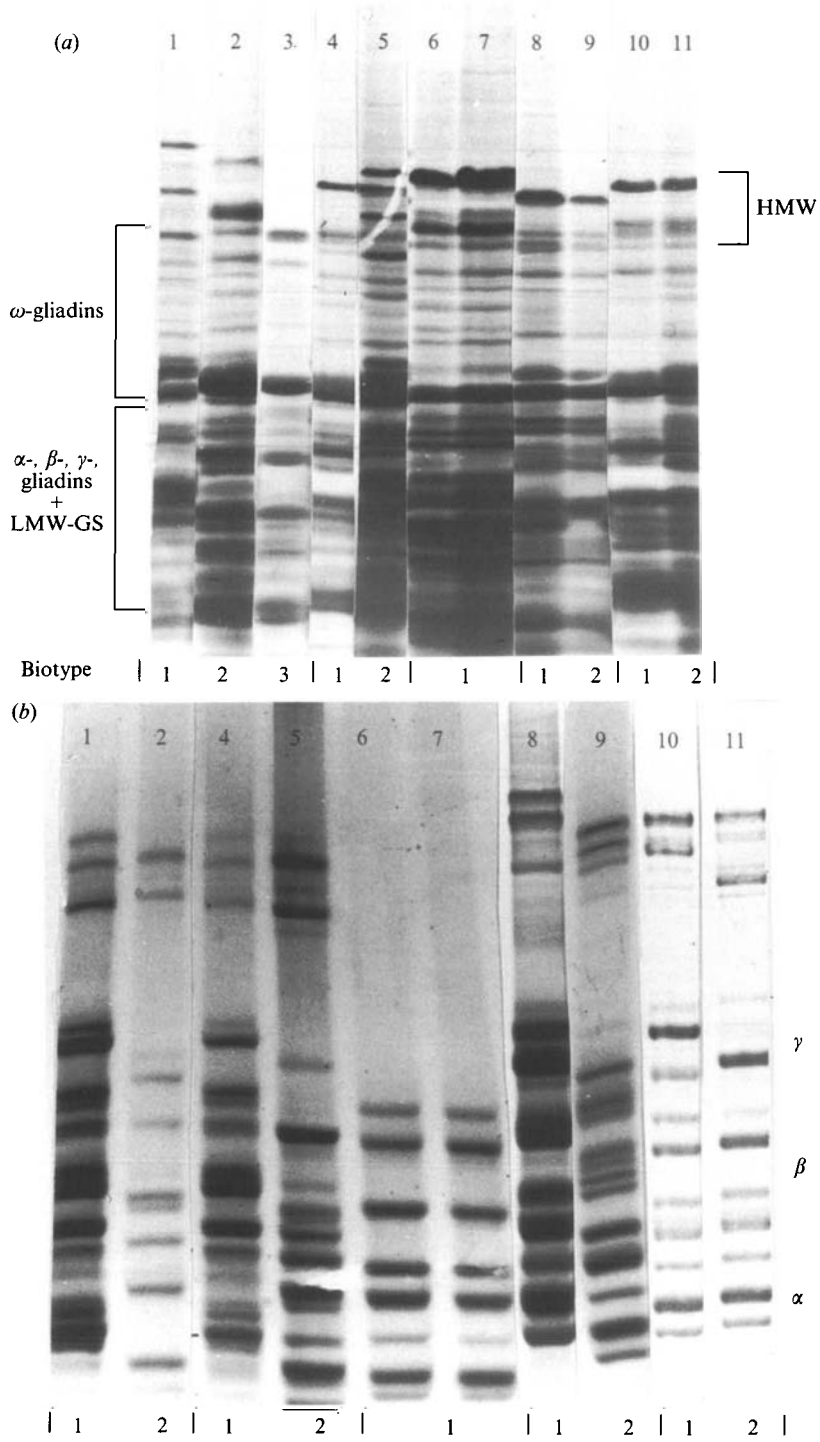

Fig. 6. (*a*) SDS-PAGE of total protein and (*b*) A-PAGE of gliadins from biotypes of (lanes 1–3) accession 1327, (lanes 4 and 5) accessions 1331, (lanes 6 and 7) accession 1400, (lanes 8 and 9) accession 1401 and (lanes 10 and 11) accession 1406 of *T. monococcum*.

These figures are comparable with those (50 and 70 alleles, respectively) observed in 109 accessions of 'monococcum' by Metakovsky & Baboev (1992*a*). Two findings from the present work are worth noting. First, most accessions were shown to contain up to ten strong ω-gliadins encoded by genes at *Gli-A1*^{m} as compared with 0–3 bands in the cultivated wheats (Metakovsky, 1991). This is in agreement with the observation that more storage protein genes are expressed in 'monococcum' than in polyploid wheats (Galili *et al.* 1988). Secondly, in most accessions the major γ-gliadin band encoded by the *Gli-A1*^{m} locus has a molecular weight inferior to those of α/β gliadins (Fig. 4), whereas in bread and durum wheats the opposite situation occurs. This indicates that two-dimensional A-PAGE × SDS-PAGE fractionations may provide relevant information when gliadin patterns are used to study phylogenesis or systematics

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in wheat. In this context it is worth noting that the gliadin and glutenin subunit patterns of *T. sinskajae* were indistinguishable from those of several accessions of 'monococcum', confirming the recent nomenclature which considers this wheat as a different subspecies of *T. monococcum* (Sharma & Waines, 1981; Castagna *et al.* 1994).

HMW-GS and LMW-GS have been found to have a pronounced effect on dough viscoelastic properties in polyploid wheats (Payne, 1987; Gupta & Shepherd, 1988; Pogna *et al.* 1990). On the other hand, *T. monococcum* has been considered as a valuable source of genes for increased protein content (Johnson & Waines, 1977; Fedak, 1985) or genes encoding y-type HMW-GS (Waines & Payne, 1987) for the improvement of breadmaking quality of polyploid wheats. To our knowledge, this is the first report on the relationship between breadmaking quality and allelic variation at genes coding for storage protein in this diploid species. Comparison of biotypes naturally occurring in a few accessions suggests the presence of alleles *y* and *i* at the *Gli-A1*^{m} locus to be associated with superior breadmaking quality. Furthermore, all accessions or biotypes containing allele *c* at *Gli-A1*^{m} but different alleles at *Glu-A1*^{m} or *Gli-A2*^{m}, showed high sedimentation volumes, suggesting a positive effect of this allele on gluten quality. These conclusions are supported by the recent findings that a strong correlation exists between sedimentation volume and breadmaking quality, as measured by Alveograph W and bread volume, in 25 'monococcum' at Istituto Sperimentale Cerealicoltura, S. Angelo Lodigiano (B. Borghi, personal communication, manuscript in preparation).

Positive effects on dough strength due to allelic variation at *Gli-A1* have been observed by several authors in bread wheat (Payne *et al.* 1987; Gupta *et al.* 1991; Gupta *et al.* 1994), and have been ascribed to allelic variation at the closely linked *Glu-A3* locus coding for LMW-GS. The four streaks observed in the two-dimensional A-PAGE × SDS-PAGE map of gliadins clearly indicate that at least four families of LMW-GS with different molecular weights occurs in *T. monococcum*. A particular extraction procedure has been recently developed to obtain gliadin-free glutenin subunits before fractionation by one- or two-dimensional electrophoresis (Singh *et al.* 1991). This method is currently being used to separate LMW-GS in diploid wheat.

Breadmaking quality has also been associated to quantitative increase in HMW-GS because the bread wheat cultivars which contain one *Glu-A1*-encoded subunit proved to be of superior quality when compared with *Glu-A1*-null varieties (Payne *et al.* 1979; Halford *et al.* 1992). No clear correlation between the number of HMW-GS and the SDS-sedimentation volumes was found in the accession analysed here: for example, several genotypes with two expressed *Glu-A1*^{m} subunits showed sedimentation volumes (11–15 ml) similar to that obtained in biotype 3 of accession 1327 which lacks HMW-GS.

A few accessions gave sedimentation volumes as high as 90–93 ml. In addition to confirming the finding of Blanco *et al.* (1994) that some accessions of 'monococcum' show good grain characteristics, the results in the present study offer an opportunity to investigate the biochemical bases of bread-making quality and the gluten structure at the diploid level, and could help the breeders developing commercial cultivars of 'monococcum' with good grain quality. The high protein content of the accessions analysed, along with their resistance to leaf rust and powdery mildew are consistent with earlier reports (Lawrence *et al.* 1958; The, 1975; Sharma *et al.* 1981; Pasquini *et al.* 1989; D'Egidio *et al.* 1993), and greatly increase the economical prospects of *T. monococcum*.

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