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High molecular weight glutenin subunit variation in *Triticum turgidum* var. *dicoccum*

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Summary. Variation in high molecular weight (HMW) glutenin subunit composition among 167 accessions of dicoccum wheat (*Triticum turgidum* L. var. *dicoccum* Schrank) of diverse origins was investigated using one-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). A total of 20 alleles were identified, and 9 of them were found to be different from those previously detected by Payne and Lawrence (1983b) in hexaploid wheat (*Triticum aestivum* L.). The newly discovered alleles enhance the genetic variability available to improve the industrial quality of wheats and some of them may facilitate basic research on the relationship of industrial quality with HMW glutenin subunit number. The novel variants include a *GLU-A1* encoded subunit which has higher molecular mass than any other so far described in tetraploid and hexaploid wheats, and a 'null' *GLU-B1* allele. Dicoccums containing neither *GLU-A1*- nor *GLU-B1*-encoded subunits were also identified. A comparison of the mean number of HMW glutenin subunits contained in various primitive and modern domesticated wheats of different ploidy levels and the identification of wheats containing no HMW glutenin subunits suggest that the occurrence of 'null' *GLU-1* alleles in these species depends on chance rather on an inherent tendency on the part of modern polyploid wheats to suppress the activity of redundant *GLU-1* genes.

Key words: SDS-PAGE – Novel subunits – Null alleles – *GLU-1* loci

Introduction

The high molecular weight (HMW) subunits of glutenin can be readily distinguished from other storage proteins contained in the seed of *Triticum* species by their low mobility during sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Bietz and Wall 1972; see Miflin et al. 1983 for review). Variation in HMW glutenin subunit composition as evidenced by SDS-PAGE provides an additional means for the identification of wheat cultivars (Wagner and Maier 1982) and the study of evolutionary processes (Lawrence and Shepherd 1981; Payne et al. 1981b; Galili and Feldman 1983). Moreover, there is strong evidence which indicates that in common wheat (*Triticum aestivum* L.) the presence of certain HMW glutenin subunits can be related to bread-making quality (Payne et al. 1979; Burnouf and Bouriquet 1980; Moonen et al. 1983). Glutenins probably also have some effect on pasta quality in tetraploid wheat (*Triticum turgidum* var. *durum* Desf.) (Payne et al. 1984b).

In *T. aestivum* (genome AABBDD), the genes coding for the HMW glutenin subunits occur at three complex loci (*GLU-A1*, *GLU-B1* and *GLU-D1*) situated on each of the long arms of chromosomes 1A, 1B, and 1D (Bietz et al. 1975; Lawrence and Shepherd 1981). Generally, each cultivar contains between three and five major subunits, that is two subunits coded by *GLU-D1*, either one or two controlled by *GLU-B1* and either one or none coded by *GLU-A1* (Lawrence and Shepherd 1980; Payne et al. 1981b). However, because of allelic variation at each of these loci, Payne and Lawrence (1983b) were able to describe 11 distinct variants coded by *GLU-B1*, 6 controlled by *GLU-D1* and 3 by *GLU-A1* in a collection comprising about 300 common wheat cultivars of different origins. Further alleles have been recently identified by various research groups (Payne et al. 1984a; Pogna et al. 1985; Vallega 1986; Waines and Payne, unpublished data).

Knowledge of HMW glutenin subunit variants in tetraploid wheat *Triticum turgidum* (AABB) and of their possible relationship with quality parameters in durum is limited, since only Italian (Pogna et al. 1985; Vallega 1986), and a small number of Austrian (Wagner and Maier 1982) and Israeli (Galili and Feldman 1983) tetraploid wheats have been analysed. However, because *T. turgidum* shares with common wheat at least some of the variants associated with improved bread-making quality (this paper, inter alia), the possibility of applying electrophoretical techniques to select tetraploid wheats better suited for bread-making (see Quick and Crawford 1983 for review) appears promising.

The present communication describes HMW glutenin subunit variation in dicoccum wheat (*Triticum turgidum* var. *dicoccum* Schrank), the purported progenitor of both common wheat and durum wheat (McFadden and Sears 1946; Tsunewaki 1966). An extensive screening of this primitive germplasm appears relevant not only because of the possibilities it offers for the detection of novel variants and in the study of the evolution and domestication of commercial wheats.

Materials and methods

Plant materials

In the study, 167 accessions of *Triticum turgidum* var. *dicoccum* were analysed. The collection, comprising wheats from 23 countries of origin, was kindly provided by Dr. J. C. Craddock (USDA Small Grains Collection, Beltsville, MD, USA). Except for those cases where heterozygosity at the *GLU-1* loci was suspected, only one or two grains of each accession were examined by SDS-PAGE. Seed of the HMW glutenin subunit standards (Payne and Lawrence 1983b) was kindly provided by Dr. P. I. Payne. Standards for the more recently identified alleles were, for the most part, not available when this study was carried out. The chromosomal location of the genes coding for the novel subunits found amongst dicoccums was inferred by determining which allelic subunits they replaced.

SDS-polyacrylamide gel electrophoresis

Proteins were extracted from the brush-half of the kernel and fractionated in 10% polyacrylamide gels using the method of Laemmli (1970), as modified by Payne et al. (1981a).

Results and discussion

Novel variants

A total of 20 different *GLU-A1* and *GLU-B1* encoded variants were identified amongst dicoccums. Nine of these variants (three governed by *GLU-A1*, and six by *GLU-B1*) were different from those previously described by Payne and Lawrence (1983b). Throughout the present communication these novel alleles (Figs. 1–3) are designated with progressive Roman numerals, prefixed by the gene symbol of the locus controlling their synthesis (i.e. *GLU-A1-III, GLU-B1-VII,* etc.). The relative frequencies of each of the HMW glutenin variants found amongst dicoccums and examples of the accessions carrying novel alleles are given in Table 1. It should be noted that *GLU-A1-III* and *GLU-B1-VI* correspond to variants already identified in the Italian durum cultivar ‘Lambro’ (Pogna et al. 1985; Vallega 1986).

Novel *GLU-A1* encoded variants were found in dicoccums from Italy, Germany, India and the USSR. Especially noteworthy is *GLU-A1-I* (identified only in P.I. 94683 from the USSR), which codes for a subunit (Fig. 1) having a lower mobility during SDS-PAGE than any of the *GLU-A1*-encoded subunits so far described in tetraploid and hexaploid wheats. This subunit probably has a relatively higher molecular mass and so investigation of its effects on flour and semolina quality will be of particular interest.

GLU-A1-II (Fig. 1) was found only in C.I. 12213 from India. The mobility of the HMW subunit coded by this allele is only marginally higher than that of subunit 1 contained in cv ‘Hope’. *GLU-A1-II,* however, can be distinguished with relative ease when gels are run for a relatively long period of time (i.e. for 20 h or more at 14 mA). *GLU-A1-III* (Fig. 1), which also codes for a single major subunit, was found in 14 dicoccums from Europe and the Middle East. Most dicoccums contain *GLU-B1*-encoded variants which have not been identified in bread wheats (Table 1; Figs. 2 and 3). Among

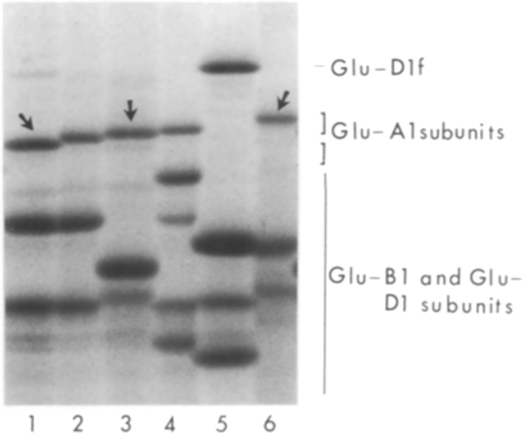

Fig. 1. SDS-PAGE patterns of the HMW glutenin subunits contained in the following wheats: *1: T. dicoccum* P.I. 352359 (*GLU-A1-III*); *2: T. durum* cv ‘Escuro’ (*GLU-A1-IV*); *3: T. dicoccum* C.I. 12213 (*GLU-A1-II*); *4: T. aestivum* cv ‘Hope’ (*GLU-A1a*); *5: T. aestivum* cv ‘Danchi’ (null allele *GLU-A1c*); *6: T. dicoccum* P.I. 94683 (*GLU-A1-I*). The novel *GLU-A1*-encoded subunits identified amongst dicoccums are *arrowed. GLU-A1-IV* (*slot 2*) was detected in two Portuguese wheats (Vallega and Mello-Sampayo, unpublished data). The slowest-moving subunit is coded by a *GLU-D1* allele (*GLU-D1f*) carried by the hexaploid bread wheat cv ‘Danchi’

Table 1. Frequencies of alleles for the loci *Glu-A1* and *Glu-B1* amongst 300 cultivars of bread wheat, *Triticum aestivum*[a], and amongst 167 dicoccum wheat, *T. turgidum* var. *dicoccum*

Locus	Alleles	Subunits	Bread wheat cultivars[a]		Dicoccums	
			Frequency (%)	Variety standard	Frequency (%)	Variety standard
GLU-A1	a	1	28	Hope	56	–
	b	2^x	28	Bezostaya-1	16	–
	c	none	44	Chinese Spring	21	–
	I	(one)	–	–	rare	PI94683
	II	(one)	–	–	rare	CI12213
	III	(one)	–	–	7	PI352359
GLU-B1	a	7	19	Flinor	2	–
	b	7+8	25	Chinese Spring	11	–
	c	7+9	30	Bezostaya-1	–	–
	d	6+8	18	Hope	18	–
	e	20	3	Federation	rare	–
	f	13+16	rare	Lancota	–	–
	g	13+19	rare	Frondoso	rare	–
	h	14+15	rare	Sappo	11	–
	i	17+18	4	Gabo	–	–
	j	21	rare	Zorba	1	–
	k	22	rare	Serbian	2	–
	I	none	–	–	4	PI94640
	II	(two)	–	–	9	PI355505
	III	(two)	–	–	11	PI352354
	IV	(two)	–	–	13	PI94665
	V	(one)	–	–	2	PI94633
	VI	(one)	–	–	13	PI94669

[a] From Payne and Lawrence (1983b)

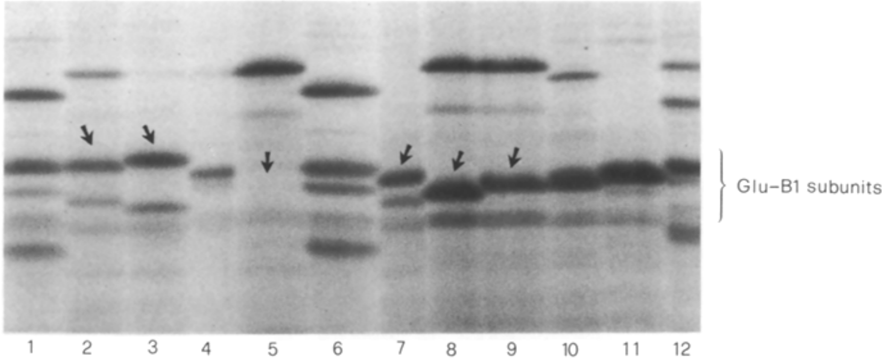

Fig. 2. SDS-PAGE patterns of the HMW glutenin subunits contained in the following wheats: *1: T. aestivum* cv 'Lancota' (*GLU-Blf*); *2: T. dicoccum* P.I. 355505 (*GLU-Bl-II*); *3: T. dicoccum* P.I. 352354 (*GLU-Bl-III*); *4: T. durum* cv 'Trinakria' (*GLU-Ble*); *5: T. dicoccum* P.I. 94640 (*GLU-Bl-I*); *6: T. aestivum* cv 'Sappo' (*GLU-Blh*); *7: T. dicoccum* P.I. 94665 (*GLU-Bl-IV*); *8: T. dicoccum* P.I. 94633 (*GLU-Bl-V*); *9: T. dicoccum* P.I. 94669 (*GLU-Bl-VI*); *10: T. durum* cv 'Lambro' (*GLU-Bl-VI*); *11: T. durum* cv 'Athena' (*GLU-Bl-XI*); *12: T. aestivum* cv 'Federation' (*GLU-Ble*). The novel *GLU-Bl*-encoded variants identified amongst dicoccums are *arrowed*. *GLU-Bl-XI* was recently detected in Italian germplasm (Pogna et al. 1985; Vallega 1986)

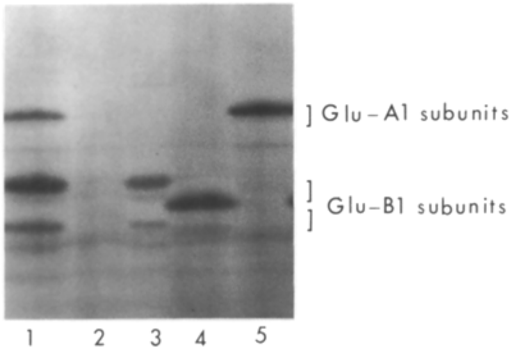

Fig. 3. SDS-PAGE patterns of the HMW glutenin subunits contained in various *T. dicoccum* accessions from the USSR. Note that neither P.I. 94668 (*slot 2*) nor P.I. 94666 (*slot 5*) contain any major *GLU-Bl*-encoded subunits, and that P.I. 94668 (*slot 2*) lacks both the *GLU-A1* and *GLU-Bl* subunits

these novel variants, *GLU-B1-I* (a "null B" allele) is of considerable interest because of its usefulness in the study of HMW glutenin subunit dosage effects on the industrial quality of wheats. Investigations on the relationship between bread making quality and the amount of HMW subunits have already been carried out by Lawrence and Shepherd, who have developed near-isogenic lines of 'Gabo' with null alleles for both *GLU-A1* and *GLU-D1* (cited by Payne et al. 1983 a). Amongst dicoccums, the null *GLU-B1* variant was found in four accessions from the USSR (P.I. 94666, P.I. 94668, P.I. 94675 and P.I. 349045), in two from Iran (P.I. 94635 and P.I. 94640) and in one from Ethiopia (P.I. 94665). Two accessions (P.I. 94668 and P.I. 349045 from the USSR) were found to carry both null A and null B alleles (Fig. 3), which indicates that the HMW glutenin subunits, or at least those detectable by SDS-PAGE, are not critical for the survival of cultivated tetraploid wheats.

Variants *GLU-B1-II* and *GLU-B1-III*, each coding for two major subunits are quite common amongst dicoccums from Europe and Ethiopia, respectively. The slower-moving band coded by *GLU-B1-II*, migrates at a rate intermediate between that of subunit 7 (contained in 'Chinese Spring') and subunit 13 (in 'Lancota'), whereas its faster-moving band has the same mobility as band 18 ('Gabo'). *GLU-B1-III* differs from *GLU-B1b* (bands 7+8 in 'Chinese Spring'), in that its faster-moving band migrates at a slower rate than subunit 8. *GLU-B1-IV*, an allele coding for two subunits, was found in 22 dicoccums of diverse origins. This variant occurs with a relatively high frequency also amongst durums from the Mediterranean area (Vallega, unpublished data). The subunits coded by *GLU-B1-IV* have, respectively, a slower and faster mobility than subunit 15, contained in 'Sappo' (Fig. 2).

GLU-B1-V and *GLU-B1-VI* each code for only one major subunit, but in some gels a less strongly stained band of higher mobility can also be distinguished (Fig. 2). *GLU-B1-VI* was found in 22 dicoccums of diverse origins, whereas *GLU-B1-V* was detected only in a few accessions from Iran and Morocco. The subunits coded by *GLU-B1-V* and *GLU-B1-VI* are faster-moving than the novel subunit found in durum cultivar 'Athena' (Pogna et al. 1985; Vallega 1986).

In a number of European hexaploid cultivars, the 1B chromosome pair has been substituted by a pair of IR chromosomes from rye, and bread wheats with this particular IR (IB) substitution ('Zorba', for instance) carry the *GLU-B1j* allele (subunit '21'), as well as genes conferring resistance to various wheat pathogens (Moonen and Zeven 1984). Two of the dicoccums examined (P.I. 350001 from Yugoslavia and P.I. 355468 from Germany) were found to contain a band with the same mobility as subunit '21'; it will be of interest to study their behaviour with regard to diseases.

Variation of HMW glutenin subunits

The relative mobilities of some of the novel subunits detected amongst dicoccums are only slightly different from those of certain variants found in hexaploid wheats, and their presence might have been therefore overlooked in previous investigations. This is especially true for the *GLU-A1* subunits, which are clustered in a relatively narrow space, and for variants with mobilities resembling those of subunits 7 and 8. Even so, the number of different HMW variants observed amongst tetraploid wheats was strikingly greater than that reported in *T. aestivum* cultivars. In fact, whereas 20 *GLU-A1* and *GLU-B1* alleles were identified amongst dicoccums (Table 1) and 19 were detected in about 240 durums (Vallega, unpublished data), only 14 variants were found in a sample of 300 common wheat cultivars (Payne and Lawrence 1983 b). It should be noted, however, that since many of the hexaploid wheats examined by Payne and Lawrence were obtained by modern breeding methods, their survey probably underestimates the extent of HMW glutenin variation amongst common wheats worldwide. Indeed, more recent studies by Payne et al. (1984 a) and by Waines and Payne (unpublished data) have shown that common wheat landraces contain numerous variants which are not present in modern cultivars.

Dicoccums were found to differ markedly from both common wheats and durums in the frequency of each *GLU-1* allele. For instance, the 'null' *GLU-A1c* allele, observed in only 21% of the dicoccums and mainly amongst those of Ethiopian origin, occurs with a frequency of about 44% in common wheats (Payne and Lawrence 1983 b) and with a still higher frequency amongst durums (Vallega, unpublished data). Moreover, 53% of the dicoccums examined carry *GLU-B1*-encoded variants which so far have not been detected in common wheat cultivars (Table 1), and none of them was found to carry allele *GLU-B1c* (which occurs with a frequency of about 30% amongst common wheats) nor *GLU-B1f* nor *GLU-B1i*.

The *GLU-A1* and *GLU-B1* alleles identified amongst dicoccums could theoretically give rise to 84 different HMW glutenin patterns, but only 30 such combinations were found. Dicoccums displaying the same HMW glutenin patterns, however, almost invariably differed from each other for one or more of their faster-moving proteins, so that the use of SDS-PAGE permitted differentiation of practically all the accessions examined. Detailed lists of the *GLU-1* alleles carried by each of these wheats will be published elsewhere.

Number of HMW glutenin subunits

According to Galili and Feldman (1983), who examined various primitive and modern wheats of different ploidy levels, (a) all dicoccums, but no durums contain *GLU-A1*-encoded subunits and (b) a non-random diploidization process of HMW glutenin subunits affecting mainly the *GLU-A1* locus has occurred in wheats. However, we found *GLU-A1*-encoded subunits and null *GLU-A1* variants occurring with relatively high frequencies both amongst dicoccums and durums. Strains of the more primitive (tetraploid) *T. dicoccoides* and of (diploid) *T. boeoticum* lacking the *GLU-A1* subunits have also been identified (Waines and Payne, unpublished data). Moreover, it should be noted that the mean number of major *GLU-A1* and *GLU-B1* subunits found in dicoccums (2.45 subunits per accession) is similar to that contained in the modern hexaploid cultivars examined by Payne and Lawrence (2.35 subunits), as well as to that contained in durums of certain countries (Vallega and Mello Sampayo, unpublished data). What has been indicated by Galili and Feldman as an example of diploidization might therefore be possibly only the result of random sampling within genetically variable wheat populations on the part of early agriculturalists and modern breeders.

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