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Electrophoretic analysis of the high-molecular-weight glutenin subunits of *Triticum monococcum, T. urartu,* and the A genome of bread wheat (*T. aestivum*)

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Summary. The high molecular weight (HMW) subunit composition of glutenin was analysed by sodium dodecyl sulphate, polyacrylamide gel electrophoresis (SDS-PAGE) in the A genome of 497 diploid wheats and in 851 landraces of bread wheat. The material comprised 209 accessions of wild *Triticum monococcum* ssp. *boeoticum* from Greece, Turkey, Lebanon, Armenia, Iraq, and Iran; 132 accessions of the primitive domesticate *T. monococcum* ssp. *monococcum* from many different germplasm collections; one accession of free-threshing *T. monococcum* ssp. *sinskajae;* 155 accessions of wild *T. urartu* from Lebanon, Turkey, Armenia, Iraq, and Iran; and landraces of *T. aestivum,* mainly from the Mediterranean area and countries bordering on the Himalayan Mountains. Four novel HMW glutenin subunits were discovered in the landraces of bread wheat, and the alleles that control them were designated *Glu-A1d* through *Glu-A1g,* respectively. The HMW subunits of *T. monococcum* ssp. *boeoticum* have a major, "x" subunit of slow mobility and several, less prominent, "y" subunits of greater mobility, all of which fall within the mobility range of HMW subunits reported for bread wheat. In *T. monococcum* ssp. *monococcum* the range of the banding patterns for HMW subunits was similar to that of ssp. *boeoticum.* However, two accessions, while containing "y" subunits were null for "x" subunits. The single accession of *Triticum monococcum* ssp. *sinskajae* had a banding pattern similar to that of most ssp. *boeoticum* and ssp. *monococcum* accessions. The HMW subunit banding patterns of *T. urartu* accessions were distinct from those of *T. monococcum.* All of them contained one major "x" and most contained one major "y" subunit. In the other accessions a "y" subunit was not expressed. The active genes for "y" subunits, if transferred to bread wheat, may be useful in improving bread-making quality.

Key words: Glutenin – *Triticum* – Breadmaking – SDS-PAGE

Introduction

Allelic variation of high molecular weight (HMW) subunits of glutenin in 185 cultivars of bread wheat (*Triticum aestivum*) has been described by Payne et al. (1981).

About 20 different, major subunits were distinguished by sodium dodecyl sulphate, polyacrylamide gel electrophoresis (SDS-PAGE), although each cultivar usually contained between three and five major subunits. All the HMW subunits are controlled by genes on the homoeologous group 1 chromosomes at the *Glu-1* loci; two subunits are translated from chromosome 1D (one 1Dx subunit and one 1Dy subunit), one or two subunits from chromosome 1B (one 1Bx subunit or 1Bx subunit with 1By subunit), and either one subunit of the 1Ax type from chromosome 1A or none at all (Payne et al. 1981).

The extent of allelic variation for chromosome 1A-encoded HMW subunits is much less than that for the other two chromosomes, for only three alleles have so far been distinguished (Payne and Lawrence 1983). Allele *a* codes for subunit 1, allele *b* for subunit 2*, whereas the "null" allele *c,* although represented by a gene (Thompson et al. 1983), is not apparently translated into a protein that can be distinguished by gel electrophoresis. However, variation in storage protein patterns have been shown to be more extensive in bread-wheat landraces of ancient agriculture (Payne et al. 1984) and especially in diploid species related to wheat (Law and Payne 1983).

In this paper, variation in the HMW glutenin subunits coded by the A genome of hexaploid wheat and

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of A-genome diploid species of the genus *Triticum* are described.

Materials and methods

Nomenclature and species description

The following nomenclature has been adopted in the text:

Triticum monococcum L. subspecies *boeoticum*. A wild diploid wheat with a brittle rachis and tough glumes.

T. monococcum L. subspecies *monococcum*. A cultivated diploid wheat with a less brittle rachis but tough glumes.

T. monococcum L. subspecies *sinskajae*. A free-threshing cultivated diploid wheat with a less brittle rachis.

T. urartu Tum. A distinctive diploid species, wild and reproductively isolated from *T. monococcum*.

For brevity, the three subspecies of *T. monococcum* are referred to in the text as 'boeoticum', 'monococcum', and 'sinskajae'. In other classifications (e.g. Dorofeev and Korovina 1979) the three subspecies of *T. monococcum* above are given species status.

A total of 497 diploid wheats from the University of California, Riverside, germplasm collection were analysed. It included 209 accessions of 'boeoticum' from Lebanon, Turkey, Iraq, Iran and Armenia; 132 accessions of the domesticate 'monococcum' from many different germplasm collections; one accession of 'sinskajae' from the germplasm collection of the Vavilov Institute, Leningrad; and 155 accessions of 'urartu' from Lebanon, Turkey, Iraq and Iran (Johnson 1975).

SDS-PAGE

Total protein was usually extracted from segments of three grains and fractionated by SDS-PAGE using 10% gels as described previously (Payne et al. 1980, 1981).

Results

Analysis of HMW glutenin subunits coded by the A genome of bread wheat varieties and landraces

The banding pattern of HMW glutenin subunits from 'Chinese Spring' and 'Sicco', two varieties of bread wheat used as controls in this study, are shown in diagrammatic form in Fig. 1. 'Sicco' contains five HMW subunits, but only one of them, subunit 1, is coded by genes on chromosome 1A; subunits 7+9 are coded by chromosome 1B, and 5+10 by chromosome 1D (Payne et al. 1981). 'Chinese Spring' only contains four HMW subunits: subunits 7+8 coded by chromosome 1B and 2+12 by chromosome 1D. Like many other varieties, 'Chinese Spring' lacks a HMW subunit coded by the A genome. The only other chromosome 1A subunit found commonly in bread wheat varieties is subunit 2*, which is allelic to subunit 1 (Payne et al. 1981) and has a similar mobility in SDS-PAGE to subunit 2 of 'Chinese Spring' (Fig. 1).

In accordance with previous nomenclature (Payne et al. 1981), subunits coded by the same chromosome

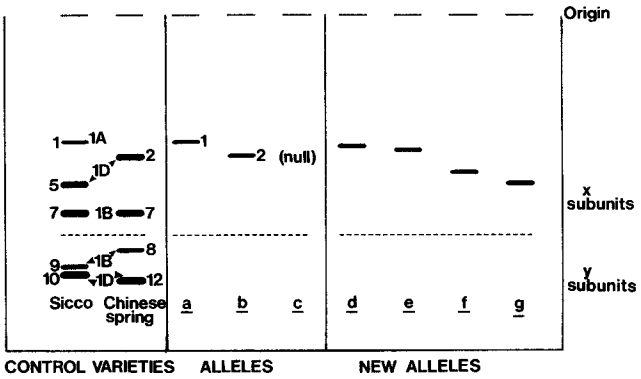

Fig. 1. Diagrammatic SDS-PAGE of HMW glutenin subunits controlled by chromosome 1A from different varieties and landraces of bread wheat. On the left-hand side, the subunits of the control varieties, 'Sicco' and 'Chinese Spring', are displayed. The latters *a*, *b*, and *c* refer to different allelic forms at *Glu-A1* as described by Payne and Lawrence (1983). The four new alleles discovered in landraces have been given consecutive letters

have been split into two groups, x and y, according to their mobilities in SDS-PAGE (Fig. 1). Thus, of the HMW glutenin subunits of 'Sicco', 1, 5, 7 are 1Ax, 1Dx and 1Bx subunits, respectively, and 9 and 10, 1By and 1Dy subunits.

During the screening of landraces of primitive agriculture from countries in the Mediterranean area of Europe and Africa, Eastern Europe, and the countries bordering the Himalayan Mountains, other HMW subunits of glutenin, provisionally assigned to chromosome 1A, were found in addition to subunits 1 and 2*. Their relative mobilities are shown in Fig. 1. The evidence that they are chromosome 1A-encoded is (1) they are expressed at the normal dosage for 1A subunits (thin bands which are less intense than subunit bands of chromosome 1D) and (2) the landraces containing them have their full complement of positively identified 1B and 1D subunits. In the case of two of the novel subunits, designated alleles *Glu-A1e* and *Glu-A1g* in Fig. 1, it has been proved that they are chromosome 1A-encoded. The HMW subunit coded by allele *Glu-A1g* has a mobility which is intermediate between subunits 2 and 5 of chromosome 1D (Fig. 2). The genotype containing it (landrace 1,600), collected by the Bangor and Lyallpur Universities Expedition to Northern Pakistan in 1974 (Witcombe 1975), was crossed to wheat variety 'Sicco' and the F_1 backcrossed as the female with 'Sicco'. A selection of progeny were then analysed by SDS-PAGE (Fig. 2). For each individual, the novel subunit segregated as a strict alternative to subunit 1 and only two combinations were detected: 2 doses of the novel subunit with 1 dose of subunit 1, and 3 doses of subunit 1 in the absence of the novel subunit. In addition to showing that the novel subunit

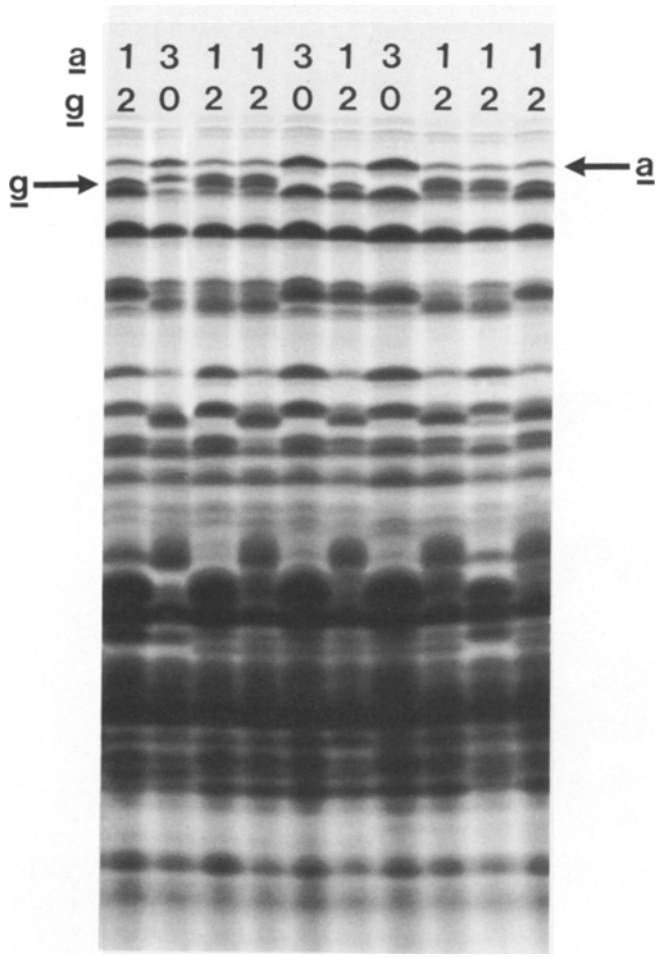

Fig. 2. SDS-PAGE of progeny from the ('Sicco' × landrace 1,600) × 'Sicco'. The two chromosome 1A variants are allele *a* (subunit 1) from 'Sicco' and allele *g* from landrace 1,600 from North Pakistan. All progeny either have two doses of *g* and one dose of *a* or zero doses of *g* and 3 doses of *a*, as expected for allelic proteins

gene (*Glu-A1g*) must belong to chromosome 1A, the results suggest that it is also allelic to the gene coding for subunit 1.

HMW glutenin subunits of accessions of T. monococcum

In total, 209 accessions of 'boeoticum' were analysed by SDS-PAGE and separations of 20 of them are shown in Fig. 3. The proteins which fractionate with similar mobilities to the HMW glutenin subunits of bread wheat control varieties 'Sicco' (Fig. 3, slot 1) and 'Chinese Spring' (Fig. 3, slot 22) occur as a discreet group. In a separate experiment, a protein sample from 'boeoticum' was fractionated by SDS-PAGE without prior reduction with 2-mercaptoethanol. The HMW subunits together with others of lower molecular weight were not detected by electrophoresis, presumably because they were present as large disulphide linked aggregates that could not penetrate the pores of the polyacrylamide gel. These properties are typical of the glutenin proteins of bread wheat, and it was assumed from this evidence that the HMW subunits in 'boeoticum' and other diploids to be described are the equivalent of HMW glutenin subunits.

The HMW subunits of all the 'boeoticum' accessions analysed, including those in Fig. 3, have a major subunit of low mobility (an "x" subunit) and a series of less prominent subunit bands of faster mobility with one dominating ("y" subunits). The mobility of the "x" subunit varies in different accessions, but their range of mobilities are similar to those of 1Ax and 1Dx glutenin subunits of bread wheat. The "x" subunits usually have mobilities intermediate between those of subunit 2 and 5 of bread wheat (Fig. 3) although in about 12% of accessions the mobility was slower, between those of subunits 1 and 2. In three accessions from Greek Thrace and Kiziltepe, Turkey, the "x" subunit had the same mobility as subunit 1 and, at the other extreme, the "x" subunit had a slightly greater mobility than that of subunit 5 in two accessions from Etzurum and Ankara, Turkey.

The cluster of "y" subunit bands in 'boeoticum' are reminiscent of the 1By and 1Bz subunits of bread wheat, which are often similarly subdivided into several components of similar mobilities (Holt et al. 1981). The range of electrophoretic mobilities of "y" subunits amongst the accessions is significantly less than the range of the "x" subunits.

A corresponding analysis by SDS-PAGE of 13 accessions of 'monococcum' is shown in Fig. 4. In all, 132 accessions were analysed. The banding patterns of the HMW subunits were similar to those of 'boeoticum'. The range of mobilities of the "x" subunits was slightly narrower, however, and the average mobilities of all the "x" variants was slightly greater. In two accessions of 'monococcum', G1560 (Pi 191146) and G1559 (PI 190940) – both from Spain – no HMW "x" subunits were detected, though the "y" subunits were still present (Fig. 4, slots 2 and 3). In two accessions represented by G1561 (PI 191381) – originally from Ethiopia, via Spain – the cluster of "y" subunits had strikingly faster mobilities than normal (Fig. 4, slot 1).

The single accession of 'sinskajae' gave an SDS-PAGE band pattern for HMW subunits that was indistinguishable from those of several accessions from the other two subspecies of *T. monococcum.*

HMW glutenin subunits of accessions of T. urartu

Three quarters of the 155 accessions examined were quite distinctive from *T. monococcum* in containing one

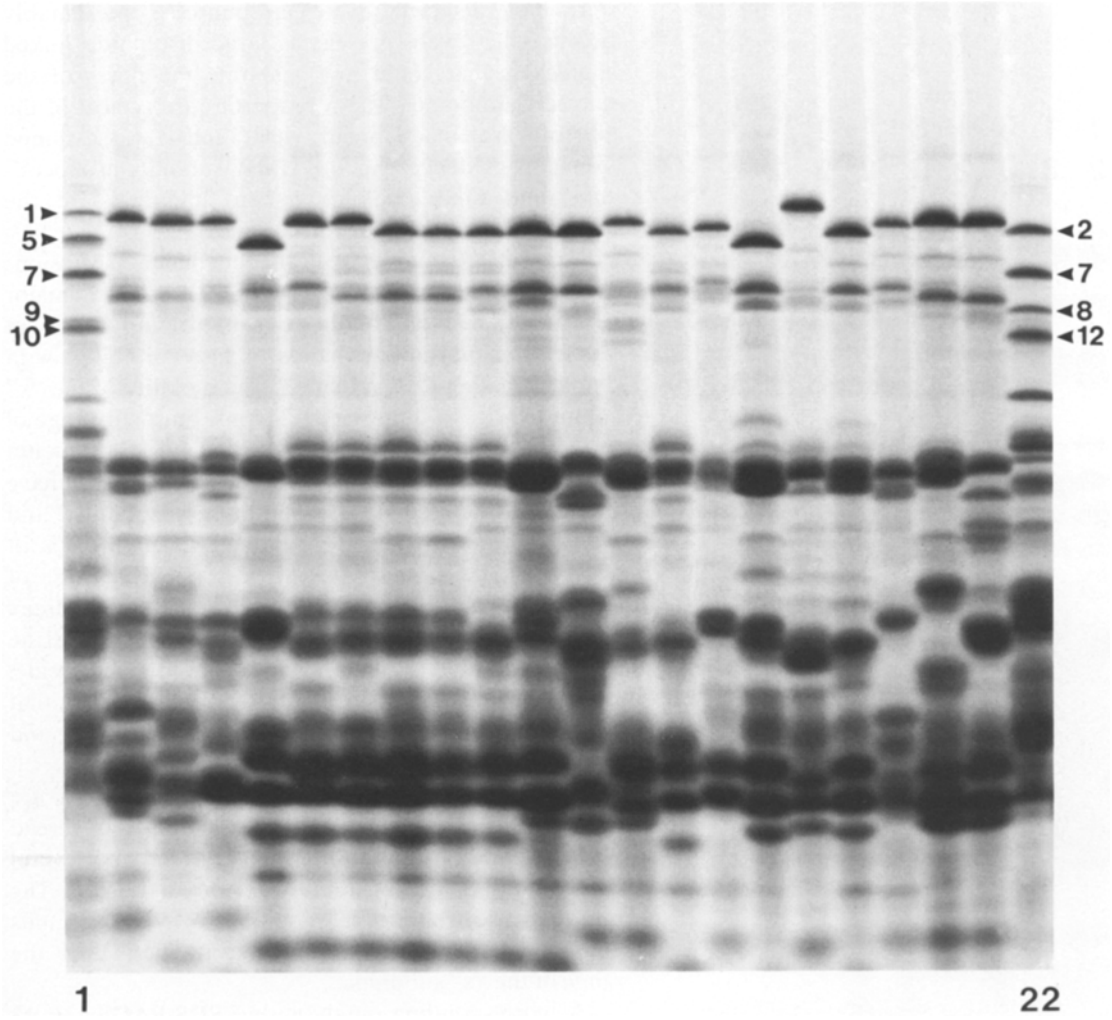

Fig. 3. SDS-PAGE of 20 accessions of *T. monococcum* ssp. *boeoticum*: slot 1, 'Sicco' bread wheat control; slots 2–21, G1215, G1195, G1194, G1174, G1173, G1172, G1170, G1169, G1076, G1074, G1065, G1016, G982, G668, G643, G642, G641, G640, G639, G559, respectively; slot 22, 'Chinese Spring' control. The subunits of the two bread wheats have been numbered according to Payne and Lawrence (1983)

major 1Ax subunit and only one major 1Ay subunit (Fig. 4). In the remaining accessions, the 1Ay subunit was not expressed (Fig. 4, slots 25 and 29). No accessions showed the multitude of minor 1Ay subunits so characteristic of *T. monococcum*. The 1Ax subunit had a mean mobility slightly slower than subunit 2 of Chinese Spring and a mobility range similar to 1Ax subunits of *T. monococcum*. The 1Ay subunits generally had a mobility slightly greater than subunit 12 of 'Chinese Spring'. There were only minor differences in the mobilities of 1Ay subunits between accessions except for the presence of one subunit in 30 accessions with a much faster mobility (Fig. 4, slot 21, arrow). Of 78 accessions from Lebanon, 75 have the 1Ay subunit slightly faster than subunit 12 of 'Chinese Spring', whereas in 3, it is not expressed. In Turkish, Armenian, Iraqi, and Iranian accessions the 1Ay null allele is far more prevalent.

Discussion

The electrophoretic banding patterns of HMW subunit proteins of the endosperm of 'monococcum', a domesticated einkorn with a tough rachis; 'sinskajae', a free-threshing einkorn; and 'boeoticum', a wild diploid wheat, are all similar to each other. These findings are therefore consistent with recent nomenclatural and biosystematic treatments, based mainly on intercrossing, which puts these three wheats as different subspecies of the same species, *Triticum monococcum* (Sharma and Waines 1981; Waines 1983). The electrophoretic

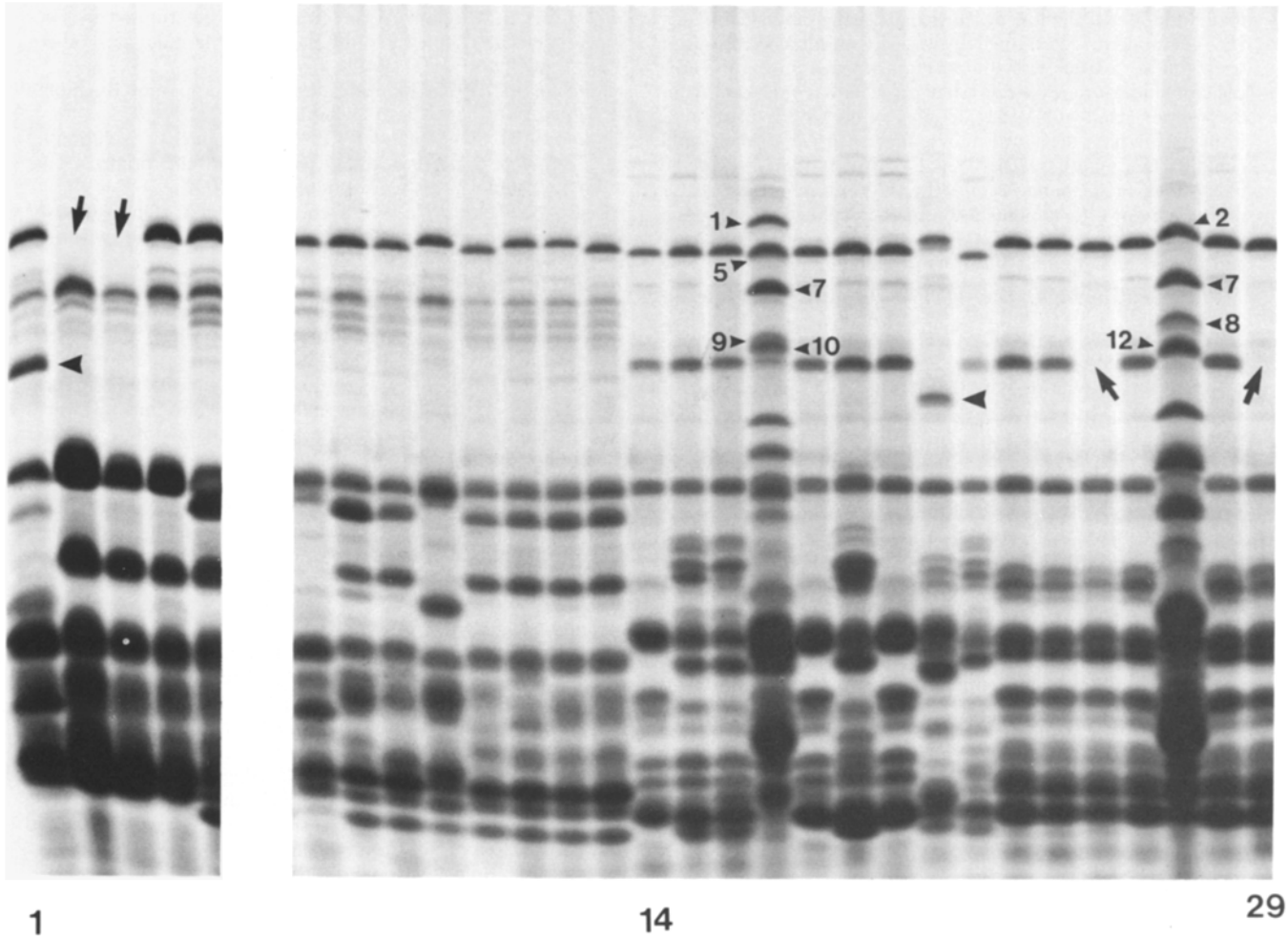

Fig. 4. SDS-PAGE of 13 accessions of *T. monococcum* ssp. *monococcum* and 14 accessions of *T. urartu:* slots 1–13, 'monococcum' G1561, G1560, G1559, G1556, G1554, G2944, G2927, G2926, G2924, G2923, G2922, G2920, and G2919; slots 14–16, 'urartu' G3252, G3250, and G3249; slot 17, 'Sicco' bread wheat control; slots 18–26, 'urartu' G3247, G3246, G3243, G3206, G3204, G3202, G3200, and G3199; slot 27, 'Chinese Spring'; slots 28–29, 'urartu' G3198 and G3197. The large arrows with tails indicate deleted HMW glutenin subunits and the large arrows without tails the presence of 1 Ay subunits with unusually great electrophoretic mobilities

banding patterns of equivalent proteins from 'urartu' are, by contrast, quite distinctive, and this reflects the reproductive isolation of this wild wheat from *T. monococcum.* Hybrids can only be obtained between the two if *T. monococcum* is the female parent, and the F_1 is normally self-fertile (Johnson and Dhaliwal 1976; Sharma and Waines 1981). 'Urartu' is therefore considered to be a separate species, *T. urartu.* It is generally accepted that both *T. urartu* and *T. monococcum* contain the AA genome and are thus closely related to the A-genome donor of bread wheat (*T. aestivum,* AABBDD), and this is the reason in this study for the comparison of the HMW glutenin subunits of the latter with those of the two diploid species.

The genetics and the molecular biology of the HMW glutenin subunits of wheat are being studied intensively and some interesting results are beginning to emerge. The bread wheat variety 'Chinese Spring', whilst not producing HMW subunits coded by chromosome 1A, nevertheless contains a locus, *Glu-A1* of two genes, one containing the coding sequence of a 1Ax subunit and the other, a 1Ay subunit (Forde et al. 1985; Harberd et al. 1986). Similarly, *Glu-B1* and *Glu-D1* of 'Chinese Spring' each contain two genes, one gene each for the "x" and "y" subunits but both of these are expressed in the developing endosperm. Whilst 1Ax subunits are produced in some varieties of bread wheat, 1Ay subunits are never detected. Presumably the 1Ay genes became null in the diploid wild ancestors of the A genome prior to the formation of bread wheat. As shown in this study, most present-day diploid *T. monococcum* wheats produce both "x" and "y" subunits, although many accessions of *T. urartu* do not do so, especially those from Turkey.

The heterogeneity of the 1Ay subunit band in *T. monococcum* is similar to that observed for the 1By and 1Bz subunits of the B genome of bread wheat (Holt et al. 1981). In

that study, it was demonstrated that the position of the major, 1By component, determined by two-dimensional electrophoresis, was correlated with the position of the minor, 1Bz components, indicating that the latter proteins were derived from the former (Holt et al. 1981). This has now been confirmed from the finding, discussed above, that each *Glu-1* locus only contains two genes (Harberd et al. 1986). It therefore seems likely that in *T. monococcum*, the 1Ay proteins seen as a multiplicity of minor bands are also the products of the same gene and arose by proteolytic activity, either in vitro during endosperm development or during protein extraction.

In bread wheat, the HMW subunits of glutenin confer the strength and elasticity of doughs that are needed to make bread. Experiments have shown that null alleles in wheat, which decrease the percentage of HMW subunits to total endosperm protein, have deleterious effects on bread-making properties (Lawrence and Shepherd, unpublished data quoted by Payne et al. 1984; Payne and Holt, unpublished data). A novel approach to improving quality in bread wheat, therefore, would be to increase the dosage of genes actively expressing HMW glutenin subunits as discussed by Law and Payne (1983). One way of achieving this would be to replace existing genes at the *Glu-A1* locus by equivalent genes from one of the accessions of *T. urartu* which direct the synthesis of both x and y HMW subunits.

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