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High molecular weight glutenin subunit variation in wild and cultivated einkorn wheats (*Triticum* spp., *Poaceae*)

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Abstract: Variation in high molecular weight (HMW) glutenin subunit composition among wild and cultivated einkorn wheats (2n = 2x = 14, AA) was investigated using one- (SDS-PAGE and urea/SDS-PAGE) and two-dimensional (IEF × SDS-PAGE) electrophoretic analyses. The material comprised 150 accessions of *Triticum urartu*, 160 accessions of *T. boeoticum*, 24 accessions of *T. boeoticum* subsp. *thaoudar* and 74 accessions of primitive domesticated *T. monococcum* from many different germplasm collections. The biochemical characteristics of HMW-glutenin subunits of *T. boeoticum* and *T. monococcum* were highly similar to one another but distinctly different from those of *T. urartu*. All the species analysed were characterised by large intraspecific variation and only three HMW-glutenin subunit patterns were identical between *T. boeoticum* and *T. monococcum*. Consistent with the distinct nature of *T. urartu*, all its HMW-glutenin patterns were different from those found in *T. boeoticum* and *T. monococcum*. The differences detected between these species might reflect their reproductive isolation and are consistent with recent nomenclatural and biosystematic treatments that recognise *T. urartu* as separate species from *T. boeoticum* and *T. monococcum*. The presence of three distinct glutenin components in some accessions of the species studied seems to be evidence for the existence of at least three active genes controlling the synthesis of the HMW-glutenin subunits in the A genome of wild and primitive domesticated diploid wheats. Results indicate also that HMW-glutenin subunits could represent useful markers for the evaluation of genetic variability present in different wild diploid wheat collections and subsequently for their conservation and future utilisation.

Wild species of *Triticum* L. and *Aegilops* L. provide a useful source of new genetic variation for wheat improvement. There are numerous examples of successful transfers of genes carrying resistance to various pathogens, environmental stresses or nutritionally and technologically useful characteristics from wild diploid relatives into the genome of polyploid wheats (see Gale & Miller 1987, Appels & Lagudah 1990, for reviews). The further evaluation of genetic variability needs to include a detailed study of the genetic structure and differentiation of wild populations

throughout the species distribution in order to identify populations with rich allelic genepools.

The increasing use of wild relatives in wheat breeding has also led to the need to understand the genome structure and differentiation of these species and its evolutionary relationships with cultivated wheats in more detail.

The genus *Triticum* comprises wild and cultivated diploid (einkorn), tetraploid (emmer) and hexaploid (dinkel) species. The origins and the phylogenetic relationships between these species have been studied using various genetic, morphological and geobotanical methods (see KIMBER & SEARS 1987, KERBY & KUSPIRA 1987 for reviews). Einkorn wheat comprises three species: *T. boeoticum* BOISS., *T. urartu* TUM. and *T. monococcum* L. (MILLER 1987), the first two of which are wild and the last the cultivated form. These species share a variant of the wheat A genome, present in domesticated tetraploid (AABB or AAGG) and hexaploid (AABBDD or AAAAGG) wheats (KIMBER & SEARS 1987, KERBY & KUSPIRA 1987, KIMBER & FELDMAN 1987).

Triticum boeoticum is widely distributed throughout the eastern Mediterranean countries of Armenia, Azerbaijan, Bulgaria, Crimea, Greece, Hungary, Iran, Iraq, Lebanon, Syria and Turkey (JOHNSON & DHALIWAL 1976). Two morphological types of *T. boeoticum* are recognised: subsp. *aegilopoides* with a single fertile floret and usually a single awn to each spikelet and subsp. *thaoudar* with two fertile florets and usually two awns per spikelet. They are completely inter fertile (JOHNSON & DHALIWAL 1976) but the geographic distribution of the subsp. *aegilopoides* is much greater than that of the subsp. *thaoudar*.

Domesticated diploid wheat *T. monococcum* is still cultivated in marginal farmlands of south-eastern Europe and Turkey (KIMBER & FELDMAN 1987, VALLEGA 1992). It resembles the wild diploids, but the ears are generally less fragile, broader and more dense with shorter awns (MILLER 1987). Recent nomenclatural and biosystematic treatments, based mainly on inter crossing, put *T. boeoticum* and the cultivated form of diploid wheats as different subspecies of the same species: *T. monococcum* (SHARMA & WAINES 1981, WAINES 1983). Since *T. monococcum* has only a single fertile floret to each spikelet, its progenitor was almost certainly the single-seeded. *T. boeoticum* subsp. *aegilopoides*. The cultivated diploid is unlikely to have been the A genome donor because the archaeological data, although not comprehensive, does not show any evidence of its existence before the wild tetraploids of emmer wheat.

Triticum urartu was identified in 1937 but its existence remained fairly obscure until JOHNSON (1975) began investigating this species as possible donor of the B genome to the polyploid wheats. Its distribution is restricted mainly to the fertile crescent region and is distributed in Armenia, Azerbaijan, Iran, Iraq, Lebanon, Syria and Turkey (JOHNSON 1975, MILLER 1987). JOHNSON (1975) argued that *T. urartu* could be a source of the B genome, but it was shown that *T. urartu* chromosomes pair with the A genome chromosomes of the hexaploid wheat *T. aestivum*. Thus *T. urartu* must have an A genome like *T. monococcum* (DVORAK 1976); its discovery opened the origin of the A genomes in polyploid wheats to question (DVORAK & al. 1988).

Biochemical, genetical and molecular aspects of high molecular weight (HMW) glutenin subunits have received a good deal of attention in recent years due to their importance in determining the flour processing properties of cultivated wheats. Genes controlling HMW-glutenin subunits are located on the long arm of

homoeologous group 1 chromosomes at loci designated *Glu-1* (PAYNE & al. 1981). Molecular analyses have shown that each *Glu-1* locus contains two genes, one encoding a larger x-type subunit, the other a smaller y-type subunit (HARBERD & al. 1986). Promising sources of genes coding for novel HMW-glutenin subunits are represented by diploid relatives of wheat *T. boeoticum*, *T. monococcum* and *T. urartu* (WAINES & PAYNE 1987).

The aim of this work is to analyse and to compare the variability of HMW-glutenin subunits in several accessions of diploid *Triticum* species by using different one- and two-dimensional electrophoretic procedures.

Materials and methods

Plant material. A total of 438 diploid wheats from different germplasm collections was analysed. The plant material was obtained from the Germplasm Institute of Bari (Italy), from the International Center for Agricultural Research in Dry Areas (ICARDA, Syria) and from the Plant Germplasm Institute of Kyoto (Japan). It included 160 accessions of *T. boeoticum* from Armenia, Iraq, Iran, Turkey and Lebanon; 24 accessions of *T. boeoticum* subsp. *thaoudar* from Turkey, Iran and Iraq; 150 accessions of *T. urartu* from Lebanon, Syria, Turkey, Iran and Iraq and 74 accessions of *T. monococcum* from Italy, Greece, Turkey and Russia.

The nomenclature supplied for seeds of the 160 accessions of *T. boeoticum* from different sources of germplasm collections did not indicate the subspecies. However, these can be considered belonging to the subsp. *aegilopoides* because all contained a single seed to each spikelet.

The bread and durum wheat cvs Torim, Lambro, Duramba and Creso were also included in the electrophoretic analyses for comparison.

Electrophoretic analyses. Crushed seeds were treated with 50% propanol. The residue remaining after propanol extraction was resuspended in 1 ml of Tris HCl 0.125 M pH 6.8 buffer containing 1% sodium dodecyl sulphate (SDS) and continually stirred for 20 min, to further remove monomeric and small oligomeric proteins. Residual proteins were extracted and analysed on SDS-PAGE gels (T = 10%, C = 2.67) according to PAYNE & al. (1981). Urea SDS/PAGE was carried out as reported by LAFIANDRA & al. 1993a). Total proteins from some accessions of diploid wheats were also analysed by two-dimensional electrophoretic techniques, combining isoelectric focusing (IEF) in the first dimension with SDS-PAGE in the second essentially as described by HOLT & al. (1981).

Results

Variation of HMW-glutenin subunits in *Triticum urartu*. SDS-PAGE migration patterns of HMW-glutenin subunits from representative samples of *T. urartu* accessions are shown in Fig. 1a, together with those from bread and durum wheat cultivars Torim, Lambro and Duramba that possess the three major allelic variants detected in the durum and bread world collection at the *Glu-A1* locus. The pattern of HMW-glutenin subunits from most of the *T. urartu* accessions is formed by two distinct electrophoretic moving zones, exhibiting one major x subunit (slower mobility) and one major y subunit (faster mobility) in agreement with previous findings (WAINES & PAYNE 1987). In all the cases, subunits of the y group appeared as a closely migrating doublet, that always co-migrated and were formed by a wide band (the slowest) and a faint band (the fastest). Generally the slow-moving zone was

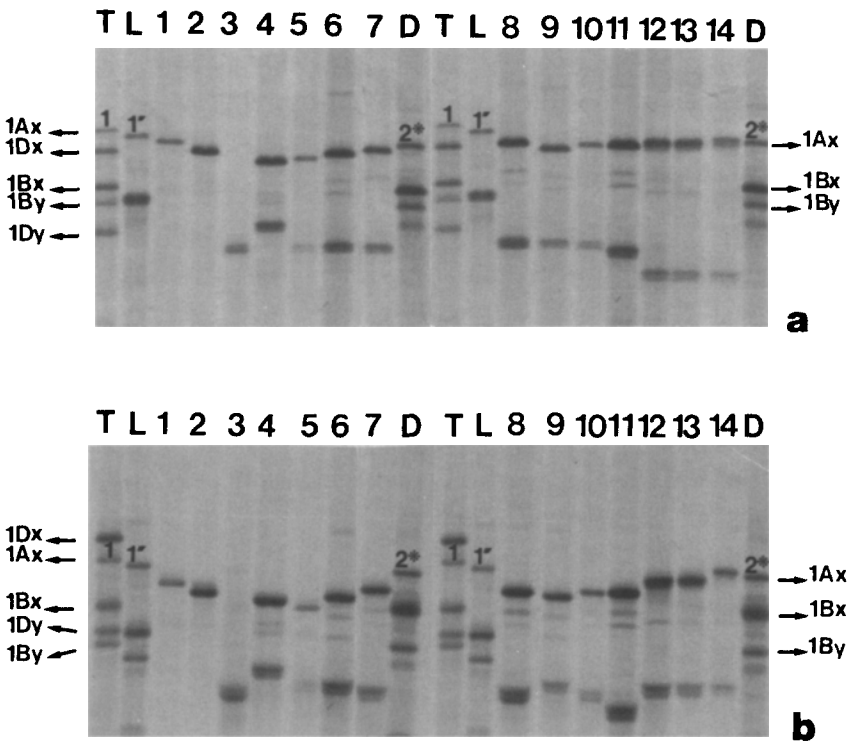

Fig. 1. *a* SDS-PAGE *b* urea/SDS-PAGE electrophoretic separations of HMW-glutenin subunits from *Triticum urartu* accessions 1–14. Numbers indicate the *Glu-A1* alleles in bread wheat cv. Torim (T) and in durum cvs Lambro (L) and Duramba (D). The designation of the subunit chromosomal groups is also given in cvs Torim (T) and Duramba (D)

represented by only one wide band, although in some Turkish accessions two closely migrating bands were observed (lanes 12, 13 and 14 in Fig. 1a). The y subunit was not expressed in 18% of the *T. urartu* accessions (lanes 1 and 2 in Fig. 1a), whereas contrary to the findings of WAINES & PAYNE (1987) and GALILI & al. (1988), a moderate degree of gene inactivity (3%) was also found for the x subunit (lane 3 in Fig. 1a). The range of variation for electrophoretic mobilities of the x subunits amongst the *T. urartu* accessions was significantly lower than that of the y subunits, being similar to that of the 1Ax and 1Dx of wheat cultivars used as control. Most of the x subunits showed electrophoretic mobilities greater than those of the three major allelic variants detected in the durum and bread wheat world collection (subunits 1, 1' and 2*) and equal to or narrower than the subunit 1Dx 5 present in cv. Torim. Only some Turkish accessions possessed x subunits having mobilities intermediate to those of subunits 1' and 2* (lane 14 in Fig. 1a). In all the *T. urartu* accessions analysed the y subunits had electrophoretic mobilities faster than the subunit 1Dy 10 of cv. Torim, except for the presence of one subunit in some Lebanese accessions with a slower mobility (lane 4 in Fig. 1a).

Urea/SDS-PAGE was carried out on all the diploid species in order to provide some information about the biochemical differences between the HMW-glutenin subunits of these species and to detect additional variability for these proteins. In the presence of urea the migration patterns of *T. urartu* x subunits showed a similar behaviour to those of the 1Ax subunits of the cultivars used as control (Fig. 1b). All the x subunits controlled by A genomes in both cultivated and wild wheats had a faster electrophoretic mobility than the 1Dx subunit of cultivar Torim. However, the relative electrophoretic mobility of the *T. urartu* x subunits did not change in comparison with the SDS-PAGE. Generally the *T. urartu* x subunits showed electrophoretic mobilities greater than those of the 1Ax subunits from the cultivated wheats except for the presence in some Turkish accessions of one subunit with slower mobility than the subunit 2* of cv. Duramba (lane 14 in Fig. 1b). Conversely, comparison of SDS-PAGE HMW-glutenin subunit patterns of *T. urartu* with those in Urea/SDS-PAGE indicated that, in the latter, the y subunits migrated much faster than the 1Dy and 1By subunits of wheat controls.

Furthermore urea/SDS-PAGE enabled the detection of a much greater heterogeneity, in particular for the y subunits, which appeared more sensitive to the addition of urea to the SDS-containing gels than the x subunits. Subunits with identical apparent M_rs by SDS-PAGE (e.g. lines 6 and 7, or 8, 9 and 10 in Fig. 1a) were revealed to be different by urea/SDS-PAGE showing a different electrophoretic mobility (same lines in Fig. 1b). Other y subunits changed the relative mobilities in the presence of urea. For example some Iraqi accessions showed a y subunit with higher apparent M_rS in SDS-PAGE (lane 11 in Fig. 1a) than those of some accessions from Turkey (lanes 12, 13 and 14 in Fig. 1a) but in the gels containing urea the relative electrophoretic mobilities of these subunits were reversed (compare lane 11 with lanes 12, 13 and 14 in Fig. 1b).

A total of 22 different HMW-glutenin patterns were detected in the 150 accessions of *T. urartu* studied, resulting from the combination of 15 x and 11 different y subunits. Out of the 22 allelic variants detected 7, 4 and 3 occurred only in the Turkish, Syrian and Lebanese materials, respectively, and 2 were present only in the Iranian and Iraqi accessions, respectively, whereas the remaining 4 had common origins between Lebanese and Syrian materials (2), between Iranian and Turkish materials (1) and between Iraqi and Iranian materials (1).

Some alleles were unique to specific populations collected in a particular geographic area (e.g. lane 2 from Turkey, lane 3 from Syria and lane 4 from Lebanon in Fig. 1a, b). Other alleles were found only in two populations from close locations in Lebanon and Turkey, respectively (lanes 5 and 8 in Fig. 1a, b). Other were widespread in different locations for each country (Fig. 1a, b; e.g. lanes 13 and 14 in Turkey, lane 6 in Syria). Finally, out of the 4 alleles of common origins, two were widespread across different locations of Syria and Lebanon (lanes 1 and 9 in Fig. 1a, b), whereas the other two were sporadic, being found only in two and five accessions from Iran and Turkey (lane 7) and in one and two accessions from Iraq and Iran (lane 10), respectively.

Two-dimensional separation (IEFxSDS-PAGE) of total proteins from *T. urartu* accessions with different HMW-glutenin patterns are shown in Fig. 2. In all the *T. urartu* accessions analysed two spots were present on the two-dimensional maps that could be assigned to the y subunit. The major component, that comprises some

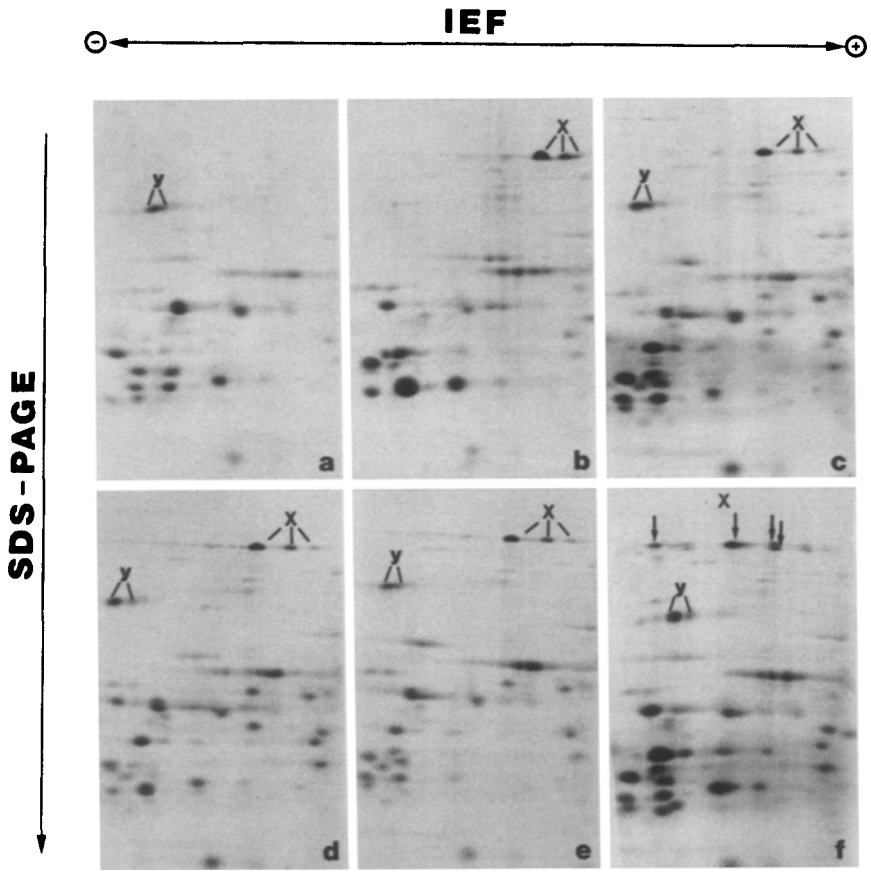

Fig. 2. Two-dimensional fractionation (IEFxSDS-PAGE) of total proteins from *Triticum urartu* accessions with different HMW-glutenin patterns. The components belonging to the x- and y-type subunit groups are indicated. Arrows in *f* indicate the major spots of the three distinct groups detected for the x subunit in the two-dimensional analyses of some Turkish accessions

70–80% of the total, was slightly more basic that the minor one. Generally the x subunits were resolved into three components with similar M_rs and in which the minor spots had a slightly more acid isoelectric point (pI) than the major one (Fig. 2b–e). In these cases the major component of the x subunit group was acidic in contrast to the near neutral and/or relatively basic isoelectric points of the major component of the y subunit. The range of variation for relative pI of the main bands of the x subunit (4.5–6.3) was larger than that of the major components of the y subunit (6.5–7.3). However, in some Turkish accessions, that on SDS-PAGE and urea/SDS-PAGE showed two closely migrating bands for the slow-moving zone of HMW-glutenin pattern (lanes 12, 13 and 14 in Fig. 1a, b), the x subunits were fractionated into several components with very different pI (Fig. 2f). In these accessions it was possible to distinguish three distinct groups for x subunits. The first included six components, that appeared as a closely migrating doublet and in which the major bands had relative isoelectric point similar to those detected in most *T.*

urartu accessions (pI = 5–5.5). The second contained a component with a slightly more basic pI, whereas the third included three components that were much more basic (pI = 7.0–7.5) than those of the other two groups and of the main y subunit.

Variation of HMW-glutenin subunits in *T. boeoticum*. The HMW-glutenin subunit patterns of most of *T. boeoticum* accessions analysed were quite different from those present in *T. urartu*, exhibiting a major subunit of low mobility (x subunit) and a series of less prominent subunit bands of faster mobility with one dominant (y-subunit) (Fig. 3a). However, in 10% of *T. boeoticum* accessions only one well-distinguished subunit of lower mobility (x subunit) was observed (lanes 1, 2 and 11 in Fig. 3a), probably because the gene for the y subunit was inactive. None of these accessions, in fact, showed the multitude of minor bands that are characteristic of the cluster of y subunits. It is worth mentioning also that only in five Turkish accessions, other than the cluster of y subunit bands, an additional component with greater mobility was present in the faster moving zone (lane 3 in Fig. 3a). The range of the mobilities of the *T. boeoticum* x subunits was similar to that observed in *T. urartu*, although the average mobilities of the all the x variants were slightly slower. About 40% of accessions possessed x subunits having mobility intermediate to those of the subunits 1 and 2* of cvs Torim and Duramba, whereas only in three Iraqi accessions the x subunit had electrophoretic mobility faster than the 1Dx subunit of cv. Torim (lane 6 in Fig. 3a). The range of variation for electrophoretic mobilities of the dominating y subunits in *T. boeoticum* was significantly lower than that detected in *T. urartu*. The main y subunits usually had mobilities intermediate between those of

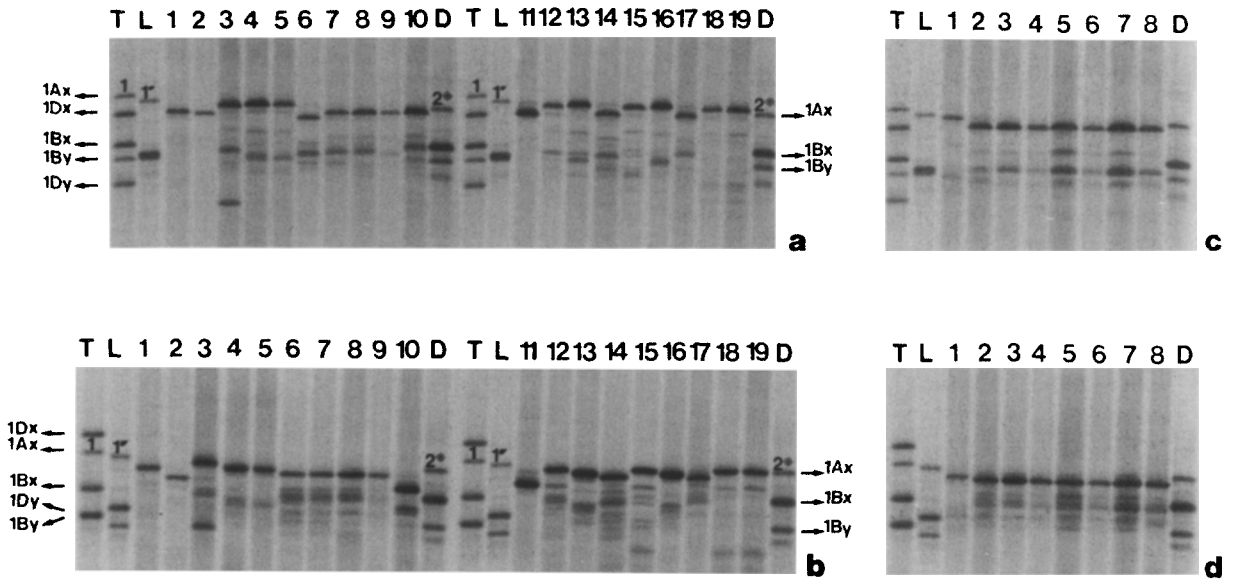

Fig. 3. *a, c* SDS-PAGE and *b, d* urea/SDS-PAGE electrophoretic separations of HMW-glutenin subunits from 19 *Triticum boeoticum* and eight *T. thaoudar* accessions, respectively. Numbers indicate the *Glu-A1* alleles in bread wheat cv. Torim (T) and in durum cvs Lambro (L) and Duramba (D). The designation of the subunit chromosomal groups is also given in cvs Torim (T) and Duramba (D)

the 1Bx and 1By from cultivated wheats with the exception of the presence in some Turkish and Lebanese accessions of the main components with mobilities greater than the 1By subunit (lane 15 in Fig. 3a) and equal to or narrower than the 1Dy subunit from the cv. Torim (lanes 18 and 19 in Fig. 3a), respectively.

In urea/SDS-PAGE all the x subunits migrated faster than the 1Dx subunit of cv. Torim but did not change their relative electrophoretic mobility with respect to the 1Ax subunits of cultivars used as controls (Fig. 3b). On the contrary, the migration patterns of y subunits showed a different behaviour from that observed in *T. urartu*. In presence of urea the cluster of *T. boeoticum* y subunits migrated much slower than that of *T. urartu*, with the major components in most of the accessions close to the x subunits and having an electrophoretic mobility similar to that of 1Bx subunits of control wheats (Fig. 3b). Only in some Turkish and Lebanese accessions the relative electrophoretic mobility of the main y subunit did not change in comparison with SDS-PAGE (lanes 15, 18 and 19 in Fig. 3a, b).

SDS-PAGE and Urea-SDS-PAGE analyses of residual proteins from *T. boeoticum* revealed the presence of a wider polymorphism for HMW-glutenin subunits than in *T. urartu*. A total of 36 different patterns was detected in the 160 accessions of *T. boeoticum* studied, deriving from the combination of 14 x and 18 main y different subunits. The highest variation was found in the material from Turkey and Iraq, with 12 and ten different HMW-glutenin subunit patterns, respectively, whereas seven and five allelic variants were present in Iranian and Lebanese accessions. Only two patterns occurred in the Armenian material, although the number of accessions analysed (eight) was relatively low in comparison with those from the other countries. In *T. boeoticum* more than in *T. urartu*, accessions collected from the same geographic area had similar electrophoretic pattern for HMW-glutenin subunits, but these patterns often differed substantially from those of accessions collected from more distant geographical areas. For example, as shown in Fig. 3a, b, most of the patterns detected in Iraqi (like those reported in lanes 12, 13 and 14), Turkish (lanes 1, 2, 3, 4), Iranian (lanes 9 and 10) and Lebanese (lanes 11 and 16) materials were unique to specific populations or accessions collected in a particular location. Some other allelic variants were present in populations from close locations in Iraq (lane 6), Turkey (lane 5) and Iran (lane 17), respectively. Occasionally, however, we found some HMW-glutenin patterns that were widespread across geographically distant sites in Turkey (e.g. lanes 7 and 15) and in Lebanon (lane 18), whereas no allelic variants had common origin between the different countries.

A corresponding analysis by SDS-PAGE and urea/SDS-PAGE of eight accessions of *T. thaoudar* is shown in Fig. 3c, d, respectively. In all the 24 accessions of *T. thaoudar* analysed, the HMW-glutenin patterns were similar to those of *T. boeoticum* (subsp. *aegilopoides*). Out of the 24 accessions analysed, 22 (lanes 2–8) showed an identical pattern formed by two different electrophoretic moving zones. The slow-moving zone was represented by only one band whose migration was slightly faster than the subunit 2* of cv. Duramba. The fast-moving zone was composed of different bands with the major one in SDS-PAGE located between 1Bx and 1By subunits of cultivated wheats. In presence of urea the cluster of y subunits showed the same behaviour as that from *T. boeoticum* (Fig. 3d). This allelic variant was common in different accessions from Turkey, Iran and Iraq and coincided with one observed in

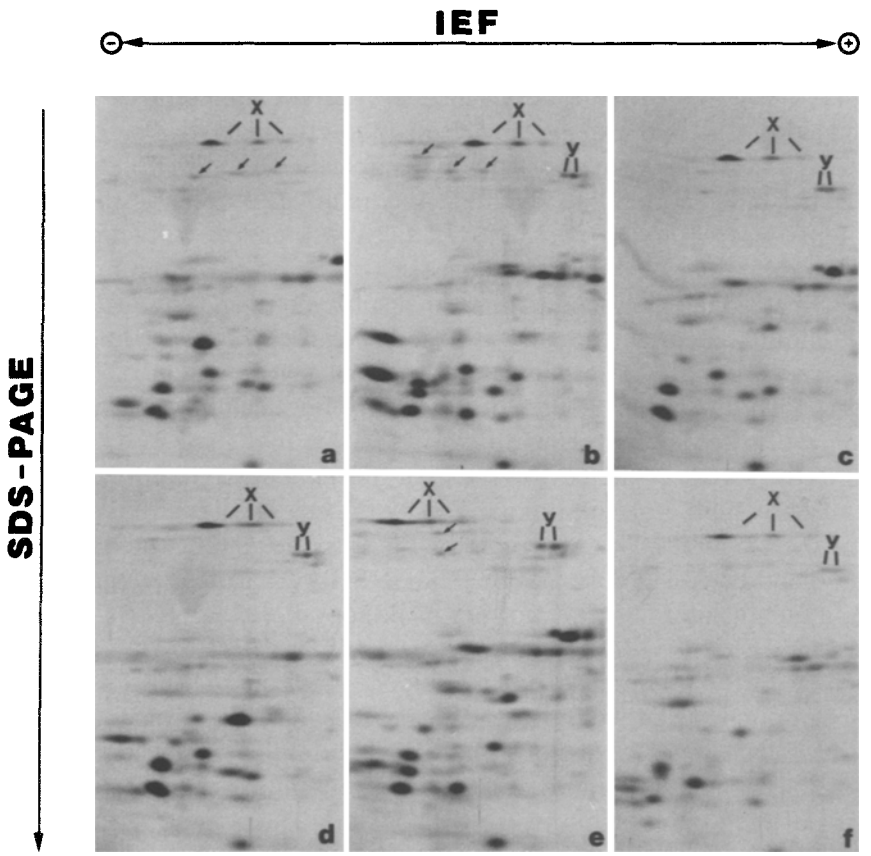

Fig. 4. Two-dimensional fractionation (IEFxSDS-PAGE) of total proteins from *T. boeoticum* accessions with different HMW-glutenin patterns. The components belonging to the x- and y-type subunit groups are indicated. Arrows in the region of HMW-glutenin subunits of some accessions indicated some minor components that differed from both x- and y-subunit groups for their pI and M_rs

T. boeoticum accessions collected in different locations from Turkey (lane 7 in Fig. 3a, b). The other HMW-glutenin pattern detected in *T. thaoudar* (lane 1 in Fig. 3c, d) was found in two accessions from close locations in Turkey and appeared similar to one present in different *T. boeoticum* accessions from the same country (lane 5 in Fig. 3a, b).

As observed also in *T. urartu*, the HMW-glutenin subunits of *T. boeoticum* detected by one dimensional SDS-PAGE split into several components during IEFxSDS-PAGE: three for the x subunit, with the major component having a more basic pI than the minor ones, and two for the y subunit, in which the main band was slightly more acidic than the minor one (Fig. 4). Multiple minor bands with different pI and M_rs have also been observed in the two-dimensional map of the HMW-glutenin subunits from *T. boeoticum*. Most of these faint minor bands were clearly related to the main y subunit, whereas some others differed from both x and y subunit groups for their pI and M_rs. It is interesting to note also that some minor

components, not detected in one dimensional SDS-PAGE, were present in the HMW-glutenin subunit region of the accessions where the gene for the y subunit was inactive (Fig. 4a). This was probably explained by the two different extraction procedures used in one and two-dimensional electrophoretic analyses. Before SDS-PAGE and urea/SDS-PAGE, the residue from the sample used for gliadin extraction was resuspended in a buffer containing SDS to further remove monomeric and small oligomeric proteins, whereas in IEFxSDS-PAGE all the proteins were fractionated, including also the monomeric ones. This suggested that some of the minor bands detected in two-dimensional analyses did not form disulphide-linked aggregates and therefore were eliminated after the SDS wash. We have observed that some components, extracted with 70% ethanol, were present in the region of HMW-glutenin subunits when the reducing agent (dithiothreitol) was eliminated from the protein sample (results not shown). Therefore it is possible that some of the minor bands detected in the region of the HMW-glutenin subunits were gliadin polypeptides having unusually high molecular mass. The hypothesis was confirmed by the finding of a gene in *T. boeoticum* that was closely linked to the gliadin-coding locus, producing a polypeptide with a molecular mass in SDS-PAGE similar to that of an average HMW-glutenin subunit (METAKOVSKY & BABOEV 1992).

Comparison of IEFxSDS-PAGE of total proteins from *T. urartu* and *T. boeoticum* revealed further differences on the biochemical characteristics of the HMW-glutenin subunits of the two diploid wild species. Contrary to the case of *T. urartu*, in all the *T. boeoticum* accessions analysed, the major component of the x group was more basic than the major one of the two y-subunits (Fig. 4). The relative isoelectric points of the main x subunits were more variable (ranging from 6.0 to 7.5) than those of the major component of y subunits (4.5–5.0). The same behaviour for the relative isoelectric point was observed in *T. thaoudar* (results not shown).

Variation of HMW-glutenin subunits in *T. monococcum*. Electrophoretic analyses carried out on the 74 accessions of *T. monococcum* indicated that the HMW-glutenin subunit patterns were much more similar to those of *T. boeoticum* than to those of *T. urartu*. The HMW-subunits of most *T. monococcum* accessions presented a major subunit of low mobility (x-type), whose migration in SDS-PAGE and urea/SDS-PAGE was usually slightly faster than the subunit 2* of cv. Duramba, and a series of different bands of faster mobility with one dominant (y-type), which are characteristics of *T. boeoticum* (Fig. 5a, b). The dominating y subunits usually had SDS-PAGE mobilities intermediate between those of 1Bx and 1By subunits of cultivated wheats, whereas in urea/SDS-PAGE their migrations were equal to or narrower than that of 1Bx subunits of cvs Torim and Duramba. Only one accession from Turkey showed a distinctive banding pattern of HMW-glutenin, containing in addition to the cluster of y subunit bands a component with greater mobility in the faster moving zone (lane 4 in Fig. 5a, b).

Two-dimensional analyses (IEFxSDS-PAGE) of total proteins from different accessions of *T. monococcum* indicated also that the main x and y-subunits could be distinguished from those of *T. urartu* on the basis of significant differences of their relative isoelectric points. Similarly to that observed in *T. boeoticum*, the dominating y subunit was more acidic (pI ranging from 4.3 to 4.8) than the major x subunit (pI ranging from 5.8–7.0) (Fig. 6).

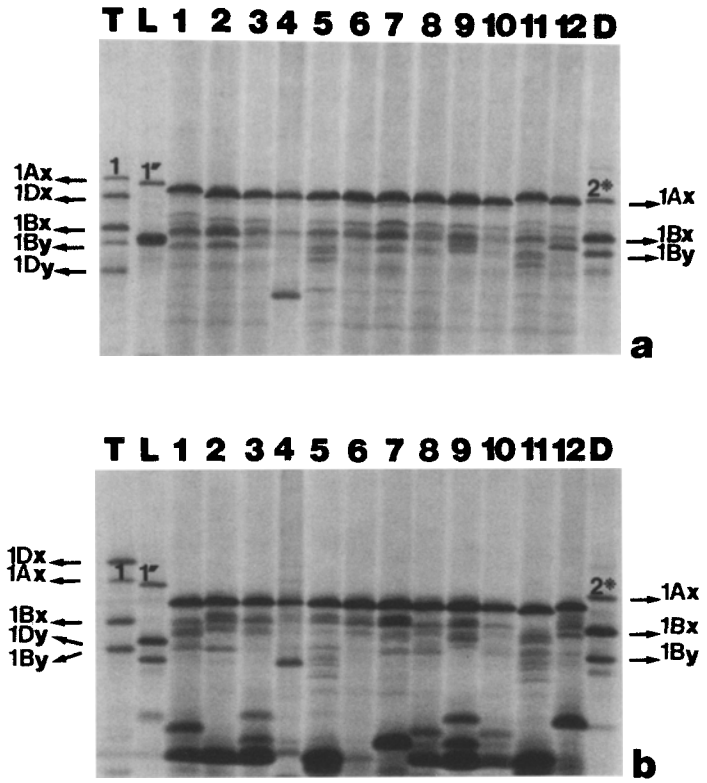

Fig. 5. *a* SDS-PAGE *b* urea/SDS-PAGE electrophoretic separations of HMW-glutenin subunits from 12 *Triticum monococcum* accessions. Numbers indicate the *Glu-A1* alleles in bread wheat cv Torim (T) and in durum cvs Lambro (L) and Duramba (D). The designation of the subunit chromosomal groups is also given in cvs Torim (T) and Duramba (D)

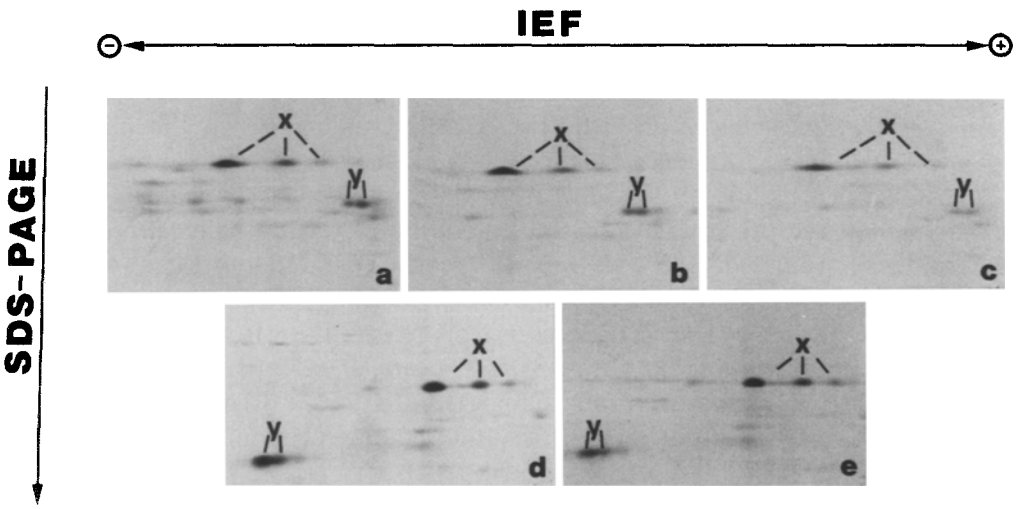

Fig. 6. Two-dimensional fractionation (IEFxSDS-PAGE) of HMW-glutenin subunits from *Triticum monococcum* (*a*, *b*, *c*) and *T. urartu* (*d*, *e*) accessions. The components belonging to the x- and y-type subunit groups are indicated

A relatively lower level of polymorphism was found for *Glu-1* encoded proteins in *T. monococcum* than in *T. urartu* and *T. boeoticum*. A total of 14 HMW-glutenin patterns was identified amongst the 74 accessions analysed with the most common occurring in 27 different genotypes from Turkey, Russia and Greece (lanes 8 and 10 in Fig. 5a, b). The highest variability was observed in the material from Turkey and Greece, with 5 and 4 different HMW-glutenin patterns, respectively, whereas 3 and 2 allelic variants were present in Russian and Italian accessions.

The close relationships between *T. boeoticum* and *T. monococcum* was confirmed by the finding that out of the 36 and 14 HMW-glutenin subunit patterns detected in wild and domesticated diploid wheats, respectively, three were identical, whereas no allelic variants in common were found among these wheats and *T. urartu*. For example, the same HMW-glutenin subunit pattern found in 22 *T. thaoudar* accessions from Turkey, Iran and Iraq (lanes 2–8 in Fig. 3c, d) and in 18 *T. boeticum* accessions collected in different locations from Turkey (lane 7 in Fig. 3a, b) coincided with one observed in eight *T. monococcum* accessions from Turkey (lane 1 in Fig. 5a, b).

Discussion

The present results confirm the phylogenetic relationships among the three diploid *Triticum* species determined by isozyme analyses (SMITH-HUERTA & al. 1989, JAASKA 1993), electrophoretic studies of the non-prolamin fraction of seed storage proteins (JOHNSON 1975, DVORAK 1976, KONAREV 1979), restriction fragment length polymorphism (RFLP) analysis using single copy or repetitive DNA sequences (DVORAK & al. 1988, GALILI & al. 1991, TAKUNI & al. 1993) and are consistent with recent nomenclatural and biosystematic treatments that recognise *T. urartu* as separate species from *T. boeoticum* and *T. monococcum* (WAINES & BARNHART 1992, MORRISON 1993).

The patterns and biochemical characteristics of HMW-glutenin subunits of *T. boeticum*, with both the subspp. *aegilopoides* and *thaoudar*, and *T. monococcum* were highly similar to one another but distinctly different from those of *T. urartu*. The differences in isoelectric points detected for HMW-glutenin subunits in *T. urartu*, *T. boeoticum* and *T. monococcum* will strictly reflect differences in the nucleotide sequence of their encoding genes. On the other hand the different behaviour of y-type HMW-glutenin subunits of *T. urartu* from that observed in *T. boeticum* and *T. monococcum* in the presence of urea indicated additional biochemical differences between these proteins in the three diploid species. Such differences are probably responsible for the decrease in electrophoretic mobility of the y-subunits of *T. boeoticum* and *T. monococcum* in the presence of urea, resulting from altered shape of the molecule or differential binding of SDS. The similarity in the one (SDS-PAGE and urea/SDS-PAGE) and two-dimensional (IEFxSDS-PAGE) migration patterns of HMW-glutenin subunits of *T. urartu* and those of the A genome in *T. dicoccoides* with both x- and y-subunits (LAFIANDRA & al. 1993b, CIAFFI & al. 1993a, and unpubl. results) supports the hypothesis that *T. urartu*, rather than *T. boeoticum*, is the donor of the A genome to the cultivated polyploid wheats.

In addition to phylogenetic studies, HMW-glutenin subunits could represent useful markers for the evaluation of genetic variability present in different wild

diploid wheat collections and subsequently for their conservation and future utilisation. The genetic diversity in enzyme loci of the diploid wild species *T. boeoticum* and *T. urartu* was low compared to the very large variation for HMW-glutenin subunits detected in these species (SMITH-HUERTA & al. 1989, JAASKA 1993). These protein markers could be used in a complementary way to enzyme markers for population-genetical studies (FELSENBURG & al. 1991, CIAFFI & al. 1993b, LAFIANDRA & al. 1993b). The high polymorphism and gene diversity of HMW glutenin loci and the tendency of some alleles to occur locally and sporadically could lead to a more detailed marking of genotypes in relation to the environment than is possible with enzyme markers. On the other hand, the large number of enyzme loci and their distribution throughout the genome facilitate the coverage of a larger part of the genome.

The HMW-glutenin subunits of all diploid species analysed fractionated into several components during IEFxSDS-PAGE (Figs. 2, 4, 6). HOLT & al. (1981) also found that the x and y HMW-glutenin subunits of cultivated wheats detected by one-dimensional SDS-PAGE split into several components during isoelectric focusing. It has been suggested by the same authors that these multiple bands are either products of the same gene or, alternatively, products of closely linked and duplicated genes. The finding that each *Glu-1* locus in cultivated wheats only contains two genes (HARBERD & al. 1986) clearly supports the first hypothesis. Our results also indicates that the multiple bands detected for both x and y subunits in most of diploid wheat accessions are controlled by a single gene. In all the patterns, the three (slower electrophoretic moving zone) or the two (faster moving zone) subunits were all present or absent (Figs. 2, 4, 6); inactivation that is a common phenomenon in HMW glutenin subunits of cultivated polyploid wheats and also occurs in diploid wheats never affected only one of these subunit groups. These subunit groups also showed a strong correlation in M_rs and relative isoelectric point. If they were encoded by different genes, their allelic variation should not have been related. As pointed out by LEVY & al. (1988) there are several molecular mechanisms to produce two or more polypeptides from a single gene; one of them could imply proteolytic activity either during endosperm development or protein extraction. This last mechanism could be responsible for faster moving double bands observed in one-dimensional analyses (SDS-PAGE and urea/SDS-PAGE) in all the *T. urartu* accessions. This seems to be the case for the minor bands showing similar pI in IEFxSDS-PAGE detected in the y and x subunit groups of most diploid wheats analysed but not for those observed for the x subunit group of some *T. urartu* accessions from Turkey, where in the zone of the x subunits several components are present with very different isoelectric points (Fig. 2f). In addition, some *T. boeoticum* and *T. monococcum* accessions showed two well separated components in the slower migrating pattern that could be not classified as minor bands (lanes 3 in Fig. 3 and lanes 4 in Fig. 5). The appearance of more than two main components could be also explained by the existence of heterozygous plants for HMV-glutenin loci. Consequently, plants of the different accessions showing more than two main bands were self-pollinated and the progeny of each plant was analysed by SDS-PAGE. In all cases the electrophoretic pattern was the same as that observed in parentals. This indicated that in some diploid wheats there must be at least a third gene for HMW-glutenins, although further genetic and molecular analyses should be made in order to confirm this hypothesis.

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