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Restriction Fragment Length Polymorphism (RFLP) for protein disulfide isomerase (PDI) gene sequences in *Triticum* and *Aegilops* species

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Abstract RFLP variation revealed by protein disulfide isomerase (PDI) coding gene sequences was assessed in 170 accessions belonging to 23 species of *Triticum* and *Aegilops*. PDI restriction fragments were highly conserved within each species and confirmed that plant PDI is encoded either by single-copy sequences or by small gene families. The wheat PDI probe hybridized to single *Eco*RI or *Hin*dIII fragments in different diploid species and to one or two fragments per genome in polyploids. Four *Aegilops* species in the Sitopsis section showed complex patterns and high levels of intraspecific variation, whereas *Ae. searsii* possessed single monomorphic fragments. *T. urartu* and *Ae. squarrosa* showed fragments with the same mobility as those in the A and D genomes of *Triticum* polyploid species, respectively, whereas differences were observed between the hybridization patterns of *T. monococcum* and *T. boeoticum* and that of the A genome. The single fragment detected in *Ae. squarrosa* was also conserved in most accessions of polyploid *Aegilops* species carrying the D genome. The five species of the Sitopsis section showed variation for the PDI hybridization fragments and differed from those of the B and G genomes of emmer and timopheevi groups of wheat, although one of the *Ae. speltoides Eco*RI fragments was similar to those located on the 4B and 4G chromosomes. The similarity between the *Eco*RI fragment located on the 1B chromosome of common and emmer wheats and one with a lower hybridization intensity in *Ae. longissima*, *Ae. bicornis* and *Ae. sharonensis* support the hypothesis of a polyphyletic origin of the B genome.

Key words Protein disulfide isomerase · *Triticum*, *Aegilops* · Phylogeny · RFLPs

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Introduction

Protein disulfide isomerase (PDI) (EC 5.3.4.1) is an enzyme located in the cell endoplasmic reticulum (ER) of plants (Shimoni et al. 1995a; Coughlan et al. 1996) and vertebrates (Freedman et al. 1994), loosely associated to the luminal surface, where it catalyzes the formation of disulphide bonds that stabilize the tertiary and quaternary structures of many extracellular proteins (Freedman et al. 1994). PDI cDNA sequences have been cloned from plant species such as alfalfa (Shorrosh and Dixon 1991), barley (Chen and Hayes 1994), wheat (Shimoni et al. 1995b), maize (Li and Larkins 1996) and castor bean (Coughlan et al. 1996). They all contain a C-terminal KDEL signal sequence for ER retention, a putative N-glycosylation site and two thioredoxin-like catalytic sites, which are also present in the vertebrate and yeast PDIs (Freedman et al. 1994). Alfalfa, maize and castor bean PDIs are encoded by single copy sequences, whereas two independent loci were detected in barley. The maize PDI gene is located in the short arm of chromosome 4, between the *zpr10* and *adh2* loci (Li and Larkins 1996), within a conserved region potentially orthologous to wheat (Ahn et al. 1993).

Restriction fragment length polymorphism (RFLP) analyses of nuclear DNA, using single-copy (Takuni et al. 1993; Ogihara et al. 1994; Mori et al. 1995; Sasanuma et al. 1996) or repeated sequences (Dvorak and Zhang 1990; Dvorak et al. 1993) as probes, and of organellar DNA (Ogihara and Tsunewaky 1988; Miyashita et al. 1994), have been successfully used for establishing interspecific relationships in wheats. Intraspecific variation has also been investigated using molecular markers in diploid (Takuni et al. 1993; Castagna et al. 1994), tetraploid (Miyashita et al. 1994; Mori et al. 1995) and hexaploid wheats (Siedler et al. 1994) and in the *Aegilops* species of the Sitopsis section (Miyashita et al. 1994; Sasanuma et al. 1996).

Four fragments that hybridized with a PDI probe containing most of the sequence coding for the mature protein were recognized in the DNA of common wheat cv.

Chinese Spring (CS), digested either with *Eco*RI, *Bam*HI or *Hin*dIII (Ciaffi et al. 1999). Southern analysis of CS aneuploid lines indicated that each fragment corresponds to a different gene sequence located in chromosome arms 4BS, 4DS, 4AL or 1BS, respectively. The restriction fragments assigned to homoeologous group 4 were highly conserved in both hexaploid and tetraploid cultivated wheats, whereas the 1B fragment was highly variable (Ciaffi et al. 1999). These findings suggested the means of carrying out further analyses, aimed at assessing RFLP variation for PDI gene sequences in *Triticum* and *Aegilops*. The results of this investigation are reported in the present paper.

Table 1 Accessions of *Triticum* and *Aegilops* species used in this study

Species	Genome	Form	Number of genotypes
Diploids			
T. urartu Tum AA	AA	Wild	15
T. boeoticum Boiss	AA	Wild	10
T. monococcum L.	AA	Cultivated	7
Ae. squarrosa L.	DD	Wild	15
Ae. speltoides Tausch.	SS	Wild	10
Ae. searsii Feld. and Kis	S^sS^s	Wild	9
Ae. longissima Bowden	S^lS^l	Wild	15
Ae. bicornis Forsk.	S^bS^b	Wild	7
Ae. sharonensis Eig	$S^{sh}S^{sh}$	Wild	7
Tetraploids			
T. durum Desf	AABB	Cultivated	4
T. dicoccum Schulb.	AABB	Cultivated	2
T. polonicum L.	AABB	Cultivated	2
T. dicoccoides Korn.	AABB	Wild	20
T. timopheevi Zhuk.	AAGG	Cultivated	8
T. araraticum Jakubz.	AAGG	Wild	15
Ae. cylindrica Host.	CCDD	Wild	5
Ae. ventricosa Tausch.	DDUnUn	Wild	6
Ae. crassa Boiss.	DDMM	Wild	3
Hexaploids			
T. aestivum L.	AABBDD	Cultivated	4
T. zhukovsky Men. and Er.	AAAAGG	Cultivated	2
Ae. crassa Boiss.	DDDDMM	Wild	1
Ae. vavilovii Chen.	DDMMSS	Wild	2
Ae. juvenalis Eig.	DDMMUU	Wild	1

Materials and methods

A set of 170 accessions belonging to different *Triticum* and *Aegilops* species, as specified in Table 1, were used in the analyses. Experimental methods, including DNA extraction, electrophoresis in agarose gels, blotting onto Hybond N+ (Amersham) membranes, hybridization and digoxigenin labelling by the polymerase chain reaction (PCR), were the same as described in Ciaffi et al. (1999). Southern analyses were carried out on DNA digested with *Eco*RI and *Hin*dIII and hybridized with a digoxigenin-labelled cDNA clone of wheat PDI (GeneBank Ac. N. U11496)(Shimoni et al. 1995b).

Results

Aegilops species of the Sitopsis section (S genome)

The 5 *Aegilops* species in the Sitopsis section (*Ae. speltoides*, *Ae. searsii*, *Ae. longissima*, *Ae. bicornis* and *Ae. sharonensis*) generally showed more complex hybridization patterns and higher intraspecific variation for PDI RFLP than the other diploid species of *Triticum* and *Aegilops* examined (Fig. 1). Polymorphism was observed among the 5 Sitopsis species and between these and the B genome of cultivated wheats. *Ae. speltoides*, in particular, showed a marked polymorphism, with accessions showing from three (lane 1, Fig. 1a) to six restriction fragments (lane 9, Fig. 1a). Seven *Ae. speltoides* accessions out of ten possessed a strong hybridization fragment, similar to that located on the 4BS chromosome arm of CS and cultivated wheats (lanes 1–5 and lanes 8–9 in Fig. 1a). Although unexpected, fragments similar to those located in the 4-A (lanes 5, 9, 10) and 4D (lanes 6–10) chromosomes of CS were also present. In addition, seven accessions (lane 3 and lanes 5–10 in Fig. 1a) showed a well-conserved fragment, smaller than that located in 1B chromosome. Conversely, *Ae. searsii* showed a single highly conserved restriction fragment detected by the PDI probe (lanes 11–19, Fig. 1a). Accessions of *Ae. longissima*, *Ae. bicornis* and *Ae. sharonensis* showed four faint conserved restriction fragments, one of them similar to that located

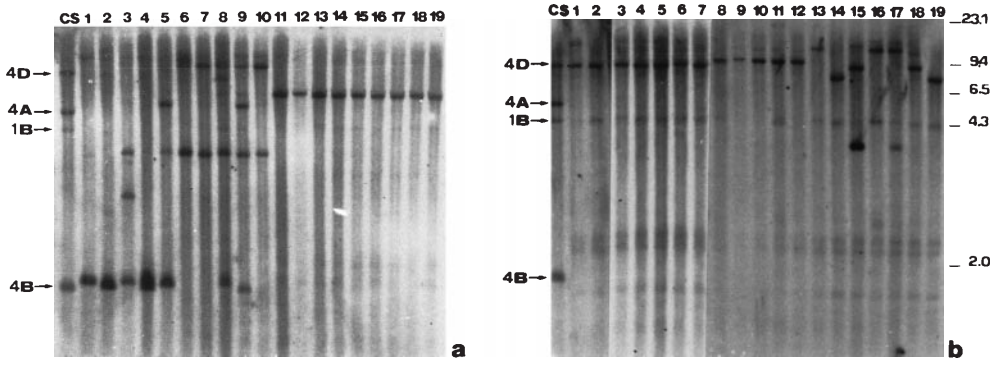

Fig. 1a, b Southern analysis of genomic DNA digested with *Eco*RI from Chinese Spring (CS) and from different accessions of the *Aegilops* species belonging to the Sitopsis section. **a** *lanes 1–10 Ae. speltoides, 11–19 Ae. searsii*. **b** *lanes 1–7 Ae. longissima, 8–12 Ae. bicornis, 13–19 Ae. sharonensis*. The deduced chromosomal location of each fragment is indicated for cv. CS on the *left side* of each picture. The molecular-weight marker (kilobases) is reported on the *right side* of the **Fig. 1b**

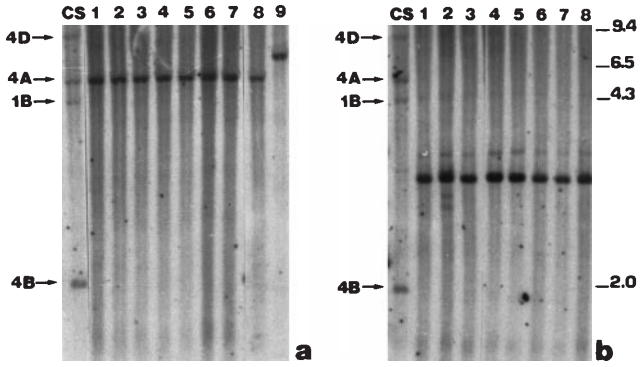

Fig. 2a, b Southern analysis of genomic DNA digested with *Eco*RI from CS and several accessions of *T. urartu* (**a**), *T. boeoticum* (**b**, *lanes 1–3*) and *T. mococcum* (**b**, *lanes 4–8*). The deduced chromosomal location of each fragment is indicated for cv. CS on the *left side* of each picture. The molecular-weight marker (kilobases) is reported on the *right side* of **Fig. 2b**

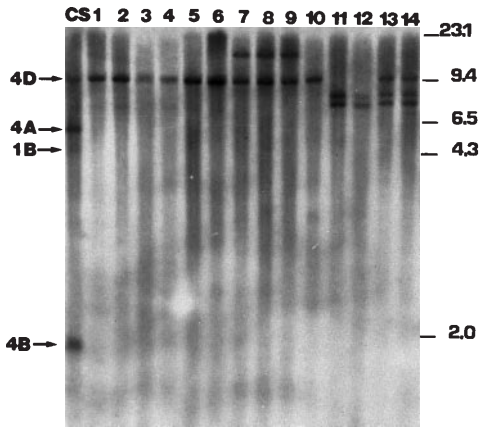

Fig. 3 Southern analysis of genomic DNA digested with *Eco*RI from CS and from different accessions of the *Aegilops* species belonging to the D genome cluster. *Lanes 1–6 Ae. squarrosa, 7–9 Ae. ventricosa, 10 Ae. cylindrica 11 Ae. vavilovii, 12 Ae. juvenalis, 13 Ae crassa* (4x), *14 Ae. crassa* (6x). The deduced chromosomal location of each fragment is indicated for cv. CS on the *left side* of the pictur. The molecular-weight marker (kilobases) is reported on the *right side*

in the 1B chromosome of CS (lanes 1–19, Fig. 1b). *Ae. longissima* exhibited an additional strong hybridization fragment similar to that located in the 4D of CS (lanes 1–7, Fig. 1b), whereas the corresponding fragment of the *Ae. bicornis* accessions was slightly larger (lanes 8–12, Fig. 1b). *Ae. sharonensis* accessions showed additional, strongly hybridizing, highly polymorphic fragments (lanes 13–19, Fig. 1b).

Wild and cultivated einkorn wheats (A genome)

Southern analyses of *Eco*RI-digested DNA from *T. urartu*, *T. boeoticum* and *T. monococcum* accessions and from CS indicated that the wheat PDI probe hybridized to a single fragment in all of the *T. urartu* accessions analyzed (Fig. 2a). This fragment had the same mobility as that in the 4A chromosome of cultivated wheats in 14 out of the 15 *T. urartu* accessions (lanes 1–8, Fig. 2a) but was larger in 1 accession from Syria (lane 9, Fig. 2a). *T. boeoticum* and *T. monococcum* showed two conserved fragments (Fig. 2b), one of them with a strong hybridization signal. Both, however, had a very different mobility in *T. urartu.* Only one *T. boeoticum* accession (from Iraq) showed two additional faint fragments (lane 2, Fig. 2b).

Aegilops species with the D genome

All of the analyzed accessions of *Ae. squarrosa* showed a single restriction fragment (lanes 1–6 in Fig. 3) having the same mobility as the one located in the 4D chromosome of CS and cultivated wheats (Ciaffi et al. 1999). The same fragment was also observed in most accessions of polyploid *Aegilops* species carrying the D genome: *Ae ventricosa* (lanes 7–9, Fig. 3), *Ae. cylindrica* (lane 10) and *Ae. crassa* (lanes 13, 14). It was absent only in *Ae. vavilovii* and *Ae. juvenalis* (lanes 11, 12). The wheat PDI clone also hybridized to a larger additional *Ae. ventricosa* fragment (lanes 7–9, Fig. 3), which most likely is derived from *Ae. uniaristata* (genome UnUn), the other putative diploid progenitor of this species. Both tetraploid (lane 13) and hexaploid (lane 14) accessions of *Ae. crassa* (genome DDMM and DDDDMM, respectively) showed two additional fragments, both faster than the one located in the D genome. The same fragments were also present in hexaploids *Ae. vavilovii* (DDMMSS, lane 11) and *Ae. juvenalis* (DDMMUU, lane 12), both of which, however, lacked the D-genome fragment. The two additional fragments might derive from the M genome of *Ae. comosa*, one of the putative progenitors, with *Ae. squarrosa*, of the tetraploid and hexaploid species *Ae. crassa*, *Ae. vavilovii* and *Ae. juvenalis*.

Wild emmer *T. dicoccoides* (AABB)

The RFLP variation for PDI gene sequences assessed in *T. dicoccoides* accessions collected across the entire natural distribution area of this species revealed the presence of four different restriction patterns (Fig. 4). The hybridization fragment assigned to the 4BS chromosome arm of CS and cultivated wheats was highly conserved, whereas fragments located in the 1B and 4A chromosomes showed some variation. Three out of the four patterns (e.g. lanes 1–3 in Fig. 4) were characterized by variation only for the 1B fragment and were identical to those detected in cultivated emmer wheats (Ciaffi et al. 1999). The 1B fragment was absent (e.g., lanes 3–9) in 70% of the *T. dicoccoides* accessions, whereas only 2 out of the 20 accessions showed the commonest pattern in cultivated tetraploids (Ciaffi et al. 1999) (lane 2). The remaining 4 accessions were characterized by the presence of a smaller 1B fragment (lanes 1 and 10, Fig. 4). Only 2

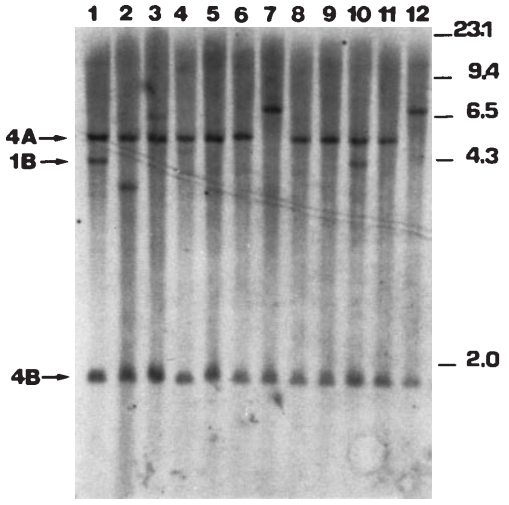

Fig. 4 Southern analysis of genomic DNA digested with *Eco*RI from 12 accessions of the wild tetraploid wheat *T. dicoccoides*. The deduced chromosomal location of each fragment is indicated in one accession of *T. dicoccoides* on the *left side* of the picture. The molecular-weight marker (kilobases) is reported on the *right side*

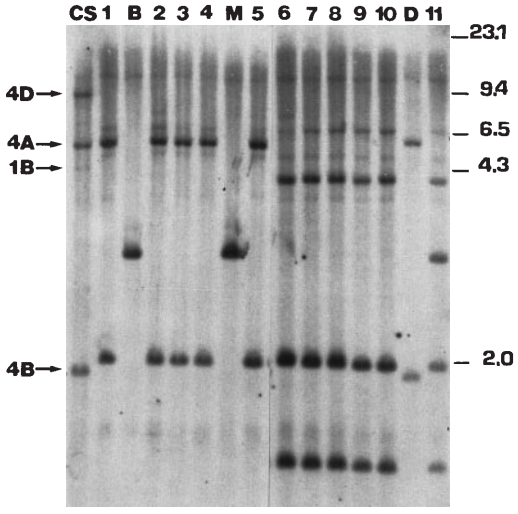

Fig. 5 Southern analysis of genomic DNA digested with *Eco*RI from different accessions of *T. araraticum* (*lanes 1–5*), *T. timopheevi* (*6–10*) and *T. zhukovsky* (*11*) compared to that of *T. boeoticum* (B), *T. monococcum* (M) and *T. dicoccoides* (D). The deduced chromosomal location of each band is indicated for cv. CS on the *left side* of the picture. The molecular-weight marker (kilobases) is reported on the *right side*

accessions from Israel possessed a 4A fragment (lanes 7 and 12) larger than that usually present in cultivated wheats (Ciaffi et al. 1999). It is interesting to note that this fragment showed a mobility identical to that detected in the previously mentioned Syrian accession of *T. urartu* (lane 9 in Fig. 2a).

Tetraploids (AAGG) and hexaploids (AAAAGG) with the G genome

When hybridized with the wheat PDI probe, *Eco*RI-digested DNA from wild *T. araraticum* accessions displayed a pattern with two strong hybridization fragments (lanes 1–5 in Fig. 5). The mobility of one of them (estimated size of 5.3 kb) was similar to the one located in the A genome of common and emmer wheats (lanes CS and D in Fig. 5); the other fragment (about 2.0 kb) was slightly slower than that located in the 4BS chromosome arm of cultivated polyploids and always present in wild emmer *T. dicoccoides* (Fig. 4, and lane D in Fig. 5). This 2.0-kb fragment could correspond to a PDI-related sequence located in the 4G homoeologous chromosome. None of the *T. araraticum* accessions had the fragment located in the 1B chromosome of common and emmer wheats, but they did show some additional weakly hybridizing larger fragments. Cultivated *T. timopheevi* accessions (lanes 6–10 in Fig. 5) showed a highly conserved pattern, characterized by the presence of seven hybridization fragments, four of them (10.4, 6.5, 5.0 and 2.0 kb size) similar to those observed in *T. araraticum*. The hybridization pattern of the hexaploid *T. zhukovskyi* (lane 11) was very similar to that of *T. timopheevi*, except for the presence of an additional strong hybridization fragment having the same mobility as that found in

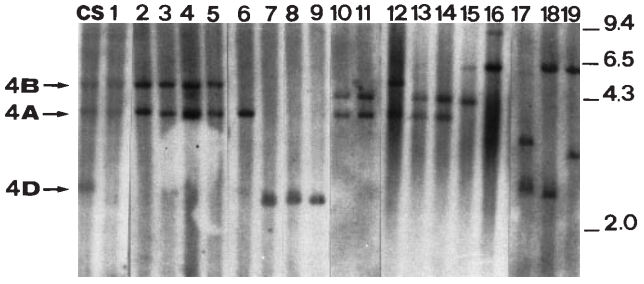

Fig. 6 Southern analysis of genomic DNA digested with *Hin*dIII from CS and some representative lines of tetraploid and diploid species of the *Aegilops* and *Triticum*. *Lanes 1–2 T. durum, 3 T. dicoccum, 4 T. polonicum, 5 T. dicoccoides, 6 T. urartu, 7 T. boeoticum, 8–9 T. monococcum, 10–11 T. araraticum, 12 T. dicoccoides, 13–14 T. timopheevi, 15 Ae. speltoides, 16 Ae. longissima, 17 Ae. searsii, 18 Ae. bicornis, 19 Ae. sharonensis*. The deduced chromosomal location of each band is indicated for cv. CS on the *left side* of the picture. The molecular-weight marker (kilobases) is reported on the *right side*

T. boeoticum and *T. monococcum* (lanes B and M in Fig. 5).

In order to further assess the relationships among accessions belonging to the emmer and timopheevi tetraploid groups, and between these and wild diploid species, we also digested DNA from some representative genotypes of *Triticum* and *Aegilops* with *Hin*dIII and hybridized it with the same PDI probe (Fig. 6). The pattern of the species belonging to the emmer group (lanes 1–5 and 12 in Fig. 6) consisted of two fragments of about 4.5 and 3.7 kb size, respectively, corresponding to those located in chromosomes 4B and 4A, respectively (Ciaffi et al. 1999). *T. urartu* (lane 6 in Fig. 6) showed a hybridization fragment having the same mobility as that located

in the A genome of common and emmer wheats, whereas the single hybridization fragment detected in *T. boeoticum* (lane 7) and *T. monococcum* (lanes 8 and 9) was different, confirming the results obtained from *Eco*RI analysis (Fig. 2). The two *Hin*dIII fragments of *T. araraticum* (lanes 10, 11 in Fig. 6) were similar to those from *T. timopheevi* (lanes 13, 14); furthermore they had the 4A chromosome fragment in common with the emmer group. Differences observed in the *Eco*RI digestion patterns between *T. timopheevi* and *T. araraticum* seemed to be due to the presence in *T. timopheevi* of an additional *Eco*RI restriction site within the 5.3-kb fragment common to all species of the emmer group and to *T. araraticum*. The size of the two hybridization fragments in *Eco*RI digests of *T. timopheevi* (4.0 and 1.3 kb) is consistent with this hypothesis. The other *Hin*dIII restriction fragment detected in *T. araraticum* and *T. timopheevi*, likely deriving from the G genome, was slower than that located in the 4BS chromosome arm of CS. All 5 *Aegilops* species of the Sitopsis section (lanes 15–19 in Fig. 6) showed different patterns. Their hybridization fragments also differed from those located in the B and G genomes, although one fragment characteristic of some accessions of *Ae. speltoides* (lane 15 in Fig. 6) appeared to be more similar to the fragment deriving from the G genome (lanes 10, 11, 13 and 14) than to that located in the 4B chromosome of cultivated wheats (lanes CS, 1–5 and 12). Furthermore, some accessions of *Ae. longissima*, *Ae. bicornis* and *Ae. sharonensis* (lanes 16, 18 and 19 in Fig. 6) had a 6.3-kb *Hin*dIII fragment in common.

Discussion

RFLP for PDI coding sequences, assessed in different *Triticum* and *Aegilops* species, indicated that PDI fragments were highly conserved within each species and confirmed that plant PDI is encoded either by single-copy sequences or by small gene families, as already reported for common and emmer cultivated wheats (Ciaffi et al. 1999). In fact, the wheat PDI probe hybridized to a single fragment in a number of the diploid species analyzed, from *T. urartu* to *Ae. squarrosa*, and to one or two fragments per genome in the polyploids. The *Aegilops* species of the Sitopsis section showed a more complex pattern and a much higher level of intraspecific variation, with the exception of *Ae. searsii*, which possessed a single, well-conserved, PDI fragment.

A comparison of the PDI RFLP patterns of the Sitopsis species clearly identified two distinct groups in the section: one formed by *Ae. speltoides* alone and the second comprising *Ae. longissima*, *Ae. bicornis*, *Ae. sharonensis* and *Ae. searsii*. This distinction agrees with the original classification of Eig (1929), reviewed by Feldman (1978), who divided the Sitopsis section into the subsections: *Truncata*, formed by *Ae. speltoides* alone, and *Emarginata*, comprising *Ae. longissima*, *Ae. searsii*, *Ae. sharonensis* and *Ae. bicornis*. This classification is also consistent with the characteristics of the 5S and 18–26S rRNA loci (Dvorak et al. 1989; Badaeva et al. 1996) and with the RFLPs of nuclear (Sasanuma et al. 1996) and chloroplast DNAs (Ogihara and Tsunewaki 1988). *Ae. searsii* showed a PDI hybridization pattern very different from that of other *Emarginata* species providing additional support to the distinction between *Ae. longissima* and *Ae. searsii* proposed by Feldman (1978).

These results also agree with those of Sasanuma et al. (1996) who, using random clones, found that the four species of the *Emarginata* subsection had a level of intraspecific variation similar to that detected in the *Triticum* species. The present results indicate that, whereas the PDI hybridization patterns of *Ae. searsii*, *Ae. longissima* and *Ae. bicornis* were highly conserved, different patterns were identified in *Ae. sharonensis*. Polymorphism in *Ae. speltoides* was even higher, in agreement with previous reports based on nuclear (Sasanuma et al. 1996) and chloroplast (Miyashita et al. 1994) DNA polymorphism analyses. While the high intraspecific variation of *Ae. speltoides* can be ascribed to its outbreeding reproductive system, *Ae. sharonensis*, like other species of the Sitopsis section and *Triticum* genus, is essentially an inbreeder. One explanation could be the remote origin and larger distribution area of the species, whereas *Ae longissima*, *Ae searsii* and *Ae. bicornis* may be more recent and adapted to some peculiar narrow environment. Further investigations are necessary to clarify these points.

Our results also show that the single hybridization fragment detected in *T. urartu* after either *Eco*RI or *Hin*dIII digestions had the same mobility as that of a fragment present in *T. dicoccoides* and cultivated polyploids, and also located in the 4A chromosome of CS, whereas all the fragments of *T. boeoticum* had a different mobility, independently of the restriction enzyme used (Figs. 2 and 6). Even a rare fragment detected in a Syrian accession of *T. urartu* (lane 9, Fig. 2a) was present in 2 *T. dicoccoides* accessions from Israel (lanes 7 and 12, Fig. 4). These findings are in line with the hypothesis that *T. urartu*, rather than *T. boeoticum*, donated the A genome to *T. dicoccoides* and, through it, to cultivated emmer and common wheats; this conclusion in agreement with studies based on RFLP analysis using single-copy (Takuni et al. 1993) or repeated (Dvorak et al. 1993) DNA sequences. The similarity in the PDI hybridization patterns of *T. monococcum* and *T. boeoticum* also confirm that the former is the domesticated form of *T. boeoticum*.

Similarly, our results indicate that the A genomes of the emmer and timopheevi wheat groups are both closer to the A genome of *T. urartu* than to that of *T. boeoticum*, as found by Dvorak et al. (1993) on the basis of the variation in repeated nucleotide sequences, but they are in disagreement with previous studies on the immunological properties of seed storage proteins (Konarev 1983), which led to the conclusion that the A genome of common and emmer wheats could have been contributed by *T. urartu*, whereas the A genome of the timopheevi group

would derive from *T. boeoticum*. The present results also indicate that *T. monococcum* could be an ancestor of *T. zhukovskyi*, a species which was isolated from mixed crops of *T. monococcum* and *T. timopheevi* and considered to be an amphiploid between the 2 species (Upadahya and Swaminathan 1963). The *Eco*RI hybridization pattern of *T. zhukovskyi* is in fact identical to that of *T. timopheevi* except for the presence of an additional strong hybridization fragment having the same mobility as the one detected in *T. boeoticum* and *T. monococcum*.

As for the B and G genomes, the present results provide evidence that only *Ae. speltoides* in the Sitopsis section showed a PDI *Eco*RI restriction fragment similar to those located in the 4B and 4G chromosomes of emmer and timopheevi wheats, respectively. Southern analysis of *Hin*dIII-digested DNA showed that a fragment present in several *Ae. speltoides* accessions has a mobility more similar to that of one in the G genome of *T. araraticum* and *T. timopheevi* than to the fragment located in the 4B chromosome of common and emmer wheats. In addition, none of the *T. araraticum*, *T. timopheevi* and *Ae. speltoides* accessions analyzed contained the 1B fragment present in common and emmer wheats. Two hypotheses can equally explain the origin of the PDI gene sequences in the 1BS chromosome arm of polyploids: (1) a translocation from an *Aegilops* different from *Ae. speltoides* or (2) duplication and translocation events occurring at polyploid level. The similarity observed between the 1B chromosome *Eco*RI fragment of common and emmer wheats and the fragment with a lower hybridization intensity in *Ae. longissima*, *Ae. bicornis* and *Ae. sharonensis* strongly supports the first hypothesis.

The RFLP for PDI gene sequences in the B and G genomes of wild and cultivated wheats seem to lead to the following conclusions. The RFLP pattern of PDI gene sequences in the S genome of *Ae. speltoides* is closer to that in the G genome of the timopheevi group than to that found in the B genome of common and emmer wheats. This confirms the results obtained from RFLP analysis of chloroplast (Ogihara and Tsunewaki 1988; Miyashita et al. 1994) and nuclear (Dvorak and Zhang 1990) DNAs and genomic *in situ* hybridization (Jiang and Gill 1994).

The PDI gene sequences in the B genome may derive from at least two different *Aegilops* species of the Sitopsis section. This would imply a polyphyletic origin of the B genome, as first shown by Zohary and Feldman (1962) and later supported by karyotype (Giorgi and Bozzini 1969) and enzyme (Nishikawa et al. 1992) analyses and satellite distribution patterns (Dennis et al. 1980). Whereas our present results give information limited to PDI gene sequence RFLPs, a greater number of loci, distributed all over the genome, and a wider array of accessions must be analyzed before some final conclusion can be drawn on the origin of the B genome.

In addition to the differences shown by the PDI hybridization fragments in B and G genomes, present results indicate that *T. dicoccoides* had a much higher level of intraspecific variation than *T. araraticum*, as also found by Mori et al. (1995), suggesting a much earlier origin of *T. dicoccoides*. This conclusion is also supported by cytological studies (Jiang and Gill 1994) and by RFLP and PCR-RFLP analyses of chloroplast and mitochondrial DNAs (Miyashita et al. 1994; Wang et al. 1997).

The wheat PDI probe hybridized to a single *Eco*RI fragment in all of the accessions of *Ae. squarrosa* analyzed, irrespective of the subspecies, *eusquarrosa* or *strangulata*. The hybridization fragment was identical to that located in the D genome of cultivated polyploids belonging to the aestivum group (Ciaffi et al. 1999); it was also well conserved in most of the wild polyploid species carrying the D genome, with the exception of the hexaploids *Ae. vavilovii* and *Ae. juvenalis*. These findings support the hypothesis that the D-genome cluster does not show a classic pivotal-differential pattern of evolution because the D genome also presents increasing levels of differentiation (Kimber 1987). Our results also agree with the classification of Kimber and Zhao (1983), who divided the D-genome cluster into three groups, based on homology with *Ae. squarrosa*: (1) *Ae. ventricosa* and *Ae. cylindrica*, with essentially unmodified D genomes; (2) tetraploid and hexaploid forms of *Ae. crassa*, with slightly modified D genomes; and (3) *Ae. vavilovii* and *Ae. juvenalis*, which show greatly modified D genomes.

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References

Ahn S, Anderson JA, Sorrels ME, Tanksley SD (1993) Homoeologous relationships of rice, wheat and maize chromosomes. Mol Gen Genet 241: 483–490

Badaeva ED, Friebe B, Gill BS (1996) Genome differentiation in Aegilops. 1 Distribution of highly repetitive DNA sequences on chromosomes of diploid species. Genome 39:293–306

Castagna R, Maga G, Perenzin M, Heun M, Salamini F (1994) RFLP-based genetic relationships of Einkorn wheats. Theor Appl Genet 88: 818–823

Chen F, Hayes PM (1994) Nucleotide sequence and developmental expression of duplicated genes encoding protein disulfide isomerase in barley (*Hordeum vulgare* L.). Plant Physiol 106: 1705–1706

Ciaffi M, Dominici L, Tanzarella OA, Porceddu E (1999) Chromosomal assignment of gene sequences coding for protein disulphide isomerase (PDI) in wheat. Theor Appl Genet 98: 405–410

Coughlan SJ, Hastings C, Winfrey RJ Jr (1996) Molecular characterisation of plant endoplasmic reticulum. Identification of protein disulfide-isomerase as the major reticuloplasmin. Eur J Biochem 235: 215–224

Dennis ES, Gerlach WL, Peacock WJ (1980) Identical polypyrimidine-polypurine satellite DNA in wheat and barley. Heredity 44: 349–366

Dvorak J, Zhang HB (1990) Variation in repeat nucleotide sequences sheds light on the phylogeny of the wheat B and G genomes. Proc Natl Acad Sci USA 87: 9640–9644

Dvorak J, Zhang HB, Kota RS, Lassner M (1989) Organization and evolution of the 5 S ribosomal RNA gene family in wheat and related species. Genome 30: 680–689

Dvorak J, Di Terlizzi P, Zhang H-B, Resta P (1993) The evolution of polyploid wheats: identification of the A genome donor species. Genome 36: 21–31

Eig A (1929) Monographisch-kritische Ubersicht der Gattung Aegilops. Beih. Report. Spec Nov Regni Veg 55: 1–228

Feldman M (1978) New evidence on the origin of the B genome of wheat. In: Ramanujam S (ed) Proc 5th Int Wheat Genet Symp. Kapour Art Press,. New Delhi, pp 120–132

Freedman RB, Hirst TR, Tuite MF (1994) Protein disulphide isomerase: building bridges in protein folding. Trends Biochem Sci 19: 331–336

Giorgi B, Bozzini A (1969) Karyotype analysis in *Triticum*: III analysis of the presumed diploid progenitors of polyploid wheats. Caryologia 22: 279–287

Jiang J, Gill BS (1994) Different species-specific chromosome translocations in *Triticum timopheevi* and *T. turgidum* support the diphyletic origin of polyploid wheats. Chromosome Res 2: 59–64

Kimber G (1987) Evolutionary patterns in the wheat group. In: Lasztity R, Bekes F (eds) Proc 3rd Int Workshop Gluten Proteins. Budapest, Hungary, pp 47–51

Kimber G, Zhao YH (1983) The D genome of the Triticeae. Can J Genet Cytol 25: 581–589

Konarev VG (1983) The nature and origin of wheat genomes on the data of grain protein immunochemestry and electrophoresis. In: Sakamoto S (ed) 6th Proc Int Wheat Genet Symp. Maruzen, Kyoto, pp 65–75

Li CP, Larkins BA (1996) Expression of protein disulfide isomerase is elevated in the endosperm of the maize floury-2 mutant. Plant Mol Biol 30: 873–882

Miyashita NT, Mori N, Tsunewaki K (1994) Molecular variation in chloroplast DNA regions in ancestral species of wheat. Genetics 137: 883–889

Mori N, Liu YG, Tsunewaky K (1995) Wheat phylogeny determined by RFLP analysis of nuclear DNA. 2. Wild tetraploid wheats. Theor Appl Genet 90: 129–134

Nishikawa K, Furuta Y, Yamada T, Kudo S (1992) Genetic studies of alpha-amylase isozymes in wheat. VII. Variation in diploid ancestral species and phylogeny of tetraploid wheat. Jpn J Genet 67: 1–15

Ogihara Y, Tsunewaky K (1988) Diversity and evolution of chloroplast DNA in *Triticum* and *Aegilops* as revealed by restriction fragment analysis. Theor Appl Genet 76: 321–332

Ogihara Y, Shimizu H, Hasegawa K, Tsujimoto H, Sasakuma T (1994) Chromosome assignment of four photosynthesis-related genes and their variability in wheat species. Theor Appl Genet 88: 383–394

Sasanuma T, Miyashita NT, Tsunewaki K (1996) Wheat phylogeny determined by RFLP analysis of nuclear DNA. Intra- and interspecific variations of five *Aegilops* Sitopsis species. Theor Appl Genet 92: 928–934

Shimoni Y, Zhu X, Levanoy H, Segal G, Galili G (1995a) Purification, characterization, and intracellular localization of glycosylated protein disulfide isomerase from wheat grains. Plant Physiol 108: 327–335

Shimoni Y, Segal G, Zhu X, Galili G (1995b) Nucleotide sequence of a wheat cDNA encoding protein disulfide isomerase. Plant Physiol 107: 281

Shorrosh BS, Dixon RA (1991) Molecular cloning of a putative plant endomembrane protein resembling vertebrate protein disulfide-isomerase and a phosphatidylinositol-specific phospholinase. Proc Natl Acad Sci USA 88: 10941–10945

Siedler H, Messmer MM, Schachermayr GM, Winzeler H, Winzeler M, Keller B (1994) Genetic diversity in European wheat and spelt breeding material based on RFLP data. Theor Appl Genet 88: 994–1003

Takuni S, Nasuda S, Liu Y-G, Tsunewaki K (1993) Wheat phylogeny determined by RFLP analysis of nuclear DNA. 1. Einkorn wheat. Jpn J Genet 68: 73–79

Upadahya MD, Swaminathan MS (1963) Genome analysis in *Triticum zhukovskyi*, a new hexaploid wheat. Chromosoma 14: 589–600

Wang GZ, Miyashita NT, Tsunewaki K (1997) Plasmon analyses of *Triticum* (wheat) and *Aegilops*: PCR-single-strand conformational polymorphism (PCR-SSCP) analyses of organellar DNAs. Proc Natl Acad Sci USA 94: 14570–14577

Zohary D, Feldman M (1962) Hybridization between amphidiploids and the evolution of polyploids in the wheat (*Aegilops-Triticum*) group. Evolution 16: 44—61