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GENOME IDENTIFICATION OF THE *TRITICUM CRASSUM* COMPLEX (POACEAE) WITH THE RESTRICTION PATTERNS OF REPEATED NUCLEOTIDE SEQUENCES[1]

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Species of the *Triticum crassum* complex, tetraploid and hexaploid *T. crassum*, hexaploid *T. juvenale*, and hexaploid *T. syriacum*, share a similar morphology. Variation in the restriction profiles of nuclear repeated nucleotide sequences is employed in identification of genomes of these species. The data show that hexaploid *T. crassum* originated from hybridization of the tetraploid cytotype of *T. crassum* with *T. tauschii*. *Triticum juvenale* and *T. syriacum* originated from hybridization of tetraploid *T. crassum* with *T. umbellulatum* and *T. searsii*, respectively. Tetraploid *T. crassum* appears to be an ancient allotetraploid that originated from hybridization of primitive *T. tauschii* with an ancient species in the evolutionary lineage leading to the section *Sitopsis* of the genus *Triticum*.

The *Triticum crassum* complex includes the tetraploid ($2n = 4x = 28$) and hexaploid ($2n = 6x = 42$) cytotypes of *T. crassum* (Boiss.) Aitch. & Hemsl., *T. juvenale* Thell. ($2n = 6x = 42$), and *T. syriacum* Bowden ($2n = 6x = 42$). All species showed the same restriction patterns of chloroplast (cp) DNAs (Ogihara and Tsunewaki, 1988), which suggests that tetraploid *T. crassum* was the likely female parent of the hexaploid species. Each hexaploid species had, however, a different male parent. *Triticum tauschii* (Coss.) Schmalh. is the most likely male parent of hexaploid *T. crassum* (Kihara, 1949, 1957, 1963; Kihara, Yamashita, and Tanaka, 1959; Shigenobu and Sakamoto, 1977; Jaaska, 1981; Nakai, 1982; Kimber and Zhao, 1983; Zhao and Kimber, 1984; Zhang and Dvorak, 1992); *T. umbellulatum* (Zhuk.) Bowden is the most likely male parent of *T. juvenale* (Kihara, 1957, 1963; Chennaveeraiah, 1960; McGinnis and Melnyk, 1962; Kimber and Yen, 1989); and *T. searsii* is the most likely male parent of *T. syriacum* (Zhang and Dvorak, 1992).

The origin of tetraploid *T. crassum* is uncertain. The chloroplast genome of *T. crassum* differs from the chloroplast genomes of all other *Triticum* species by at least six mutations (Ogihara and Tsunewaki, 1988). Cluster analysis of nucleotide sequences of the chloroplast gene encoding the large subunit of ribulose 1,5-bisphosphate carboxylase (*rbc*L) in *T. crassum*, *T. tauschii*, *T. caudatum*, *T. urartu*, and *T. aestivum* indicates that the chloroplast genome of *T. crassum* is more similar to that of *T. tauschii* than to the chloroplast genomes of the other species (Terachi, Ogihara, and Tsunewaki, 1988; Tsunewaki, 1989). *Triticum tauschii* was also suggested to be a donor of one of the nuclear genomes of tetraploid *T. crassum* (Kihara, 1949; Chennaveeraiah, 1960; Jaaska, 1981), but chromosome pairing in interspecific hybrids revealed substantial differentiation of these genomes (Lilienfeld, 1951; Kimber and Zhao, 1983). The origin of the second genome of tetraploid *T. crassum* is even more obscure. Kihara (1949) first designated this genome with a new symbol (J) to indicate its lack of homology to other *Triticum* genomes. Later, on morphological grounds, he changed this symbol to M^{cr} to indicate relationship of this genome to that of *T. comosum* (Kihara, 1954, 1963; Kihara, Yamashita, and Tanaka, 1959). Although numerous studies have failed to verify the presence of the M genome in tetraploid *T. crassum* (Chennaveeraiah, 1960; Jaaska, 1981; Masci et al., 1992; Zhang and Dvorak, 1992) the genome formula DM is still used for tetraploid *T. crassum* as evidenced by all recent reviews of the genus (Löve, 1984; Kimber and Feldman, 1987; Waines and Barnhart, 1992).

The objective of this paper was to analyze the genome origin and evolution of *T. crassum* complex with particular emphasis on tetraploid *T. crassum* by employing variation in the restriction patterns of repeated nucleotide sequences. This technique was very useful for understanding the genome origin of other polyploid species of *Triticum* (Dvorak, McGuire, and Cassidy, 1988; Dvorak and Zhang, 1990, 1992; Talbert et al., 1991; Zhang and Dvorak, 1991, 1992; Zhang, Dvorak, and Waines, 1992; Dvorak et al., 1993). The utility of this phylogenetic tool has been further advanced by demonstration that it facilitates discrimination between extinction of one of the progenitors of a polyploid and divergence due to ancient origin of a polyploid (Dubcovsky and Dvorak, 1994).

MATERIALS AND METHODS

Plants—Table 1 lists taxa, accession numbers, and origin of plants used in this study. Seeds of the accessions used in this study were deposited in the germplasm repository of the Department of Agronomy and Range Science, University of California Davis, and are available on request. *Triticum longissimum* (Schweinf. et Muschl.) Bowden was not included because no restriction fragments hybridizing with repeated nucleotide sequences distinguished it from the closely related *T. sharonense* (*Aegilops sharonensis* Eig.) (Zhang and Dvorak, 1992; Zhang, Dvorak, and Waines, 1992).

DNA hybridization—Nuclear DNAs isolated from leaves of single plants (Dvorak, McGuire, and Cassidy, 1988) were digested with six restriction endonucleases (*Alu*I, *Dde*I, *Dra*I, *Hae*III, *Mbo*I, and *Tag*I) and electro-

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TABLE 1. Taxa, accession numbers, origin, and sources of the plant materials used.

Species	Accession	Origin	Source[a]
Triticum urartu Thum.	G3135	Lebanon	J. G. Waines
T. monococcum L.	G2528	Iran	J. G. Waines
T. speltoides (Tausch) Gren.	TS02	Israel	M. Feldman
T. sharonense[b] (*Aegilops sharonensis* Eig.)	TH01	Israel	M. Feldman
T. bicorne Forssk.	TB10	Israel	M. Feldman
T. searsii[b] (*Aegilops searsii* Feldman et Kislev)	TE27	Israel	M. Feldman
T. muticum (Boiss.) Hackel	TK136-736	Turkey	R. J. Metzger
	TK136-40	Turkey	R. J. Metzger
	TK136-737	Turkey	R. J. Metzger
	A1	Unknown	R. Johnson
T. caudatum (L.) Godron et Gren.	Rub 74	Unknown	E. R. Sears
	PI551120	Greece	H. E. Bockelman
	PI254863	Iraq	H. E. Bockelman
	PI542197	Turkey	H. E. Bockelman
	PI263554	Turkey	H. E. Bockelman
T. comosum (Sibth. et Smith) Richter	G659	Unknown	J. G. Waines
	G601	Unknown	J. G. Waines
	G5037	Greece	J. G. Waines
	G5034	Turkey	J. G. Waines
	G1515	Greece	J. G. Waines
	G3566	Unknown	J. G. Waines
T. uniaristatum (Vis.) Richter	G3586	Turkey	J. G. Waines
	G1439	Greece	J. G. Waines
	G3585	Turkey	J. G. Waines
	G1297	Turkey	J. G. Waines
	G1296	Greece	J. G. Waines
T. umbellulatum (Zhuk.) Bowden	G1164	Turkey	J. G. Waines
	G1210	Turkey	J. G. Waines
	G3772	Unknown	J. G. Waines
T. tauschii (Coss.) Schmalh.	KU2075	Iran	Kyoto Univ.
	KU2025	Afghanistan	Kyoto Univ.
	KU2377	Iran	Kyoto Univ.
	KU2001	Pakistan	Kyoto Univ.
T. crassum (Boiss.) Aitch. & Hemsl. (4*x*)	G604	Iran	J. G. Waines
(4*x*)	G719	Iraq	J. G. Waines
(4*x*)	G398	Iran	J. G. Waines
(6*x*)	PI317393	Afghanistan	H. E. Bockelman
(6*x*)	PI392330	USSR	H. E. Bockelman
T. juvenale Thell.	PI276993	Unknown	H. E. Bockelman
	PI330485	Unknown	H. E. Bockelman
	PI542193	Turkey	H. E. Bockelman
	PI266815	Unknown	H. E. Bockelman
	PI289577	Unknown	H. E. Bockelman
T. syriacum Bowden	85TR02-4	Unknown	G. Kimber
	85TR01-33	Unknown	G. Kimber

[a] M. Feldman, Weizmann Institute Science, Rehovot; R. J. Metzger, University of Oregon, Corvallis, OR; E. R. Sears, University of Missouri, Columbia, MO; H. E. Bockelman, National Small Grain Collection, USDA-ARS, Aberdeen, ID; R. Johnson, Cambridge Laboratory, Norwich UK; J. G. Waines, University of California, Riverside, CA; G. Kimber, University of Missouri, Columbia, MO.

[b] There are no valid names in *Triticum* for taxa known as *Aegilops sharonensis* Eig. or *A. searsii* Feldman et Kislev. For the sake of consistency we will call these taxa *T. sharonense* and *T. searsii* throughout the paper.

phoretically fractionated in 1.7% agarose gels. The DNAs were transferred to Hybond N+ nylon membranes (Amersham, IL) by capillary transfer. Prehybridization and hybridization were performed as described by Dubcovsky and Dvorak (1994). Probes were prepared from inserts of 38 pUC18 plasmids harboring fragments of repeated sequences isolated from libraries of nuclear DNA of *T. tauschii* (ten clones designated pTtUCD), *T. comosum* (seven clones designated pTcUCD), *T. longissimum* (nine clones designated pTlUCD), *T. speltoides* (Tausch.) Gren. (three clones designated pTsUCD), *T. kostchyi* (Boiss.) Bowden (eight clones designated pTkUCD), and *T. urartu* Thum. (one clone designated pTuUCD) (Zhang and Dvorak, 1992; Zhang, Dvorak, and Waines, 1992; Dvorak et al., 1993; P. Resta and J. Dvorak, unpublished data). Seventy out of the 73 probe-enzyme combinations were previously used by Dubcovsky and Dvorak (1994) to elucidate the genomic origin of *T. cylindricum* (Host) Ces., Pass. & Gib., *T. triunciale* (L.) Raspail, and *T. ventricosum* (Tausch) Ces., Pass. & Gib. Twenty-one of these probe-enzyme combinations also were used in a study of the genome origin of hexaploid *T. crassum* and *T. syriacum* (Zhang and Dvorak, 1992). Inserted DNA fragments were excised with *Eco*RI-*Hind*III or amplified using the Polymerase Chain Reaction. Membranes were washed in 2 × Na citrate buffer (SSC) and 0.5% Na dodecylsulphate (SDS) for 30 min, 1 × SSC and 0.5% SDS for 30 min, and 0.2 × SSC and 0.5% SDS for 30 min at

65 C. All accessions listed in Table 1 were hybridized with each probe. Generally, two enzyme-probe combinations were used per probe.

Data analysis—Southern blots including 12 diploid species of *Triticum* and *T. crassum* (4x and 6x), *T. juvenale,* and *T. syriacum* were hybridized with cloned repeated nucleotide sequences. The autoradiograms of the restriction profiles obtained with different clones were compared with each other to eliminate duplicates. Only marker bands for each diploid species were employed in the analysis (Dvorak and Zhang, 1990; Zhang and Dvorak, 1991; Zhang, Dvorak, and Waines, 1992; Dubcovsky and Dvorak, 1994). A marker band is defined as a restriction fragment that is observed in the restriction profiles of all analyzed accessions of a diploid species but not in those of other diploid species under the same hybridization conditions. For each probe, all the diploid and polyploid DNAs were hybridized and washed in a single hybridization bottle to assure similar hybridization conditions. Additionally, DNAs of each relevant diploid species were included in all blots as controls.

Marker bands also were identified for groups of diploid species (Zhang, Dvorak, and Waines, 1992; Dubcovsky and Dvorak, 1994) that branched from each internal node of a phylogenetic tree of *Triticum* based on variation in restriction fragments of repeated nucleotide sequences (Dvorak and Zhang, 1992; Fig. 5). It is assumed that DNA variants shared by a group of extant diploid species that branched from an internal node but are absent in all other species were present in the ancestral species of such a group and can be considered marker bands for this ancestral species. Thus, if a diploid ancestor of a polyploid is extinct, marker bands for the internal node from which it branched off, or which it represented, will be present in the polyploid.

The proportion of marker bands for a diploid or group of diploids shared with a polyploid is called repeated nucleotide sequence correspondence (RSC). The RSC varies from 1.00, if all marker bands of a diploid or a group of diploids are encountered in a polyploid, to 0.00, if none is. The relationship between RSC and the presence of a genome in a polyploid is not absolute because of the possibility of evolutionary reversals and parallelisms in the amplification or loss of sequence variants. Hence, RSCs were compared statistically with each other and with the extremes, 1.00 and 0.00, using 2×2 contingency tables and Fisher's exact test (SAS Institute, Inc., 1986).

RESULTS

The 73 enzyme-probe combinations investigated produced 827 bands in the autoradiograms. The extremely low intraspecific variation in repeated sequence restriction patterns observed in the few accessions per species analyzed here and in Dubcovsky and Dvorak (1994) was similar to that observed in previous studies involving larger numbers of accessions (Dvorak and Zhang, 1992). Two hundred and thirty-one bands were discarded as noninformative because they were in the hybridization profiles of all diploid species. Of the remaining 596 bands, 135 were marker bands for diploid species and 96 were marker bands for groups of species (Tables 2, 3). Two phylogenetic analyses were performed: one using all marker bands, and the other only one marker band per probe. The rationale for the latter was to eliminate the possibility of scoring the same evolutionary event more than once. This second analysis included 93 marker bands for diploid species and 71 marker bands for groups of species (Table 3, numbers in parentheses). However, if a probe showed two marker bands in a diploid species but only one in a polyploid, both marker bands were considered since they must represent independent evolutionary events. No statistically significant ($P > 0.05$) differences were found between RSCs obtained by these two analyses.

Tetraploid Triticum crassum—No marker band of any diploid species except for *T. tauschii* (Fig. 1) and *T. speltoides* was found in tetraploid *T. crassum.* The RSC of 0.65 between *T. tauschii* and tetraploid *T. crassum* was significantly different from zero and from one (Fisher exact test, $P < 0.01$), but that of *T. speltoides* (RSC = 0.07) was not significantly different from zero ($P = 0.24$) (Table 3). Search for marker bands for groups of diploid species representing internal nodes of the phylogenetic tree of *Triticum* (Fig. 5) indicated the highest correspondence (RSC = 0.80) between *T. crassum* and the group including all species of *Triticum* without *T. tauschii.* This RSC was significantly different from 0 ($P < 0.01$) but not from 1 ($P = 0.11$). The only other RSC significantly different from zero was that for group of species, *T. speltoides, T. sharonense, T. bicorne,* and *T. searsii* included in *Triticum* section *Sitopsis* (RSC = 0.50, $P < 0.05$; Table 3; Fig. 2). This RSC was also significantly different from 1.

Profiles of all accessions of tetraploid *T. crassum* showed five restriction fragments not found in any diploid species of *Triticum* (Fig. 3); these are henceforth designated *T. crassum* marker bands.

Hexaploid Triticum crassum—All restriction fragments in tetraploid *T. crassum,* including the five *T. crassum* marker bands, were also in the hexaploid cytotype (Fig. 3). However, the hexaploid differed significantly ($P < 0.05$) from the tetraploid in the RSC with *T. tauschii* (Table 3). All marker bands detected in *T. tauschii* were in the hexaploid cytotype (Fig. 4). Those that were also in tetraploid *T. crassum* had double intensity in hexaploid *T. crassum* (Fig. 1).

All restriction fragments found in hexaploid *T. crassum* were either in tetraploid *T. crassum* or in *T. tauschii.* No new band was found in hexaploid *T. crassum.*

Triticum juvenale—All restriction fragments in tetraploid *T. crassum,* except for one of the five *T. crassum* marker bands, were in *T. juvenale* (Fig. 3). *Triticum juvenale* showed six of the seven marker bands of *T. umbellulatum* (Fig. 3). There was a complete correspondence between *T. juvenale* and all groups of species that included *T. umbellulatum* (Table 3). All *T. juvenale* bands were shared with either tetraploid *T. crassum* or *T. umbellulatum,* and there were no new restriction fragments.

Triticum syriacum—All restriction fragments present in tetraploid *T. crassum,* including the five *T. crassum* marker bands, were in *T. syriacum* (Fig. 3). *Triticum syriacum* showed nearly perfect correspondence with *T. searsii* and the group *T. searsii-T. sharonense-T. bicorne*

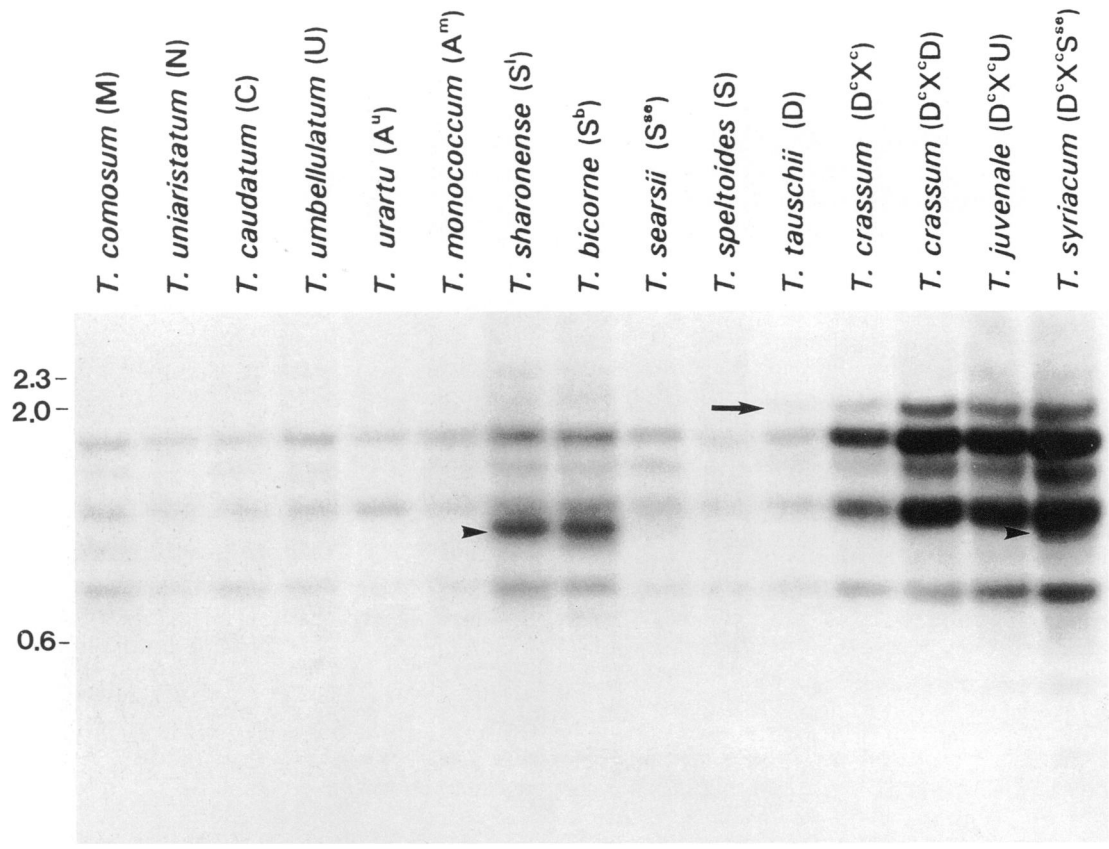

Fig. 1. *Dde*I restriction profiles of nuclear DNAs hybridized with pTlUCD10. Four micrograms of DNA per lane were loaded for the diploid species, 8 μg for the tetraploid, and 12 μg for the hexaploid species. The large arrow indicates a marker band for *T. tauschii* present in all species of the *T. crassum* complex. Note the increased intensity of the band in hexaploid *T. crassum*. The small arrow indicates a marker band for the group of species including *T. sharonense, T. bicorne,* and *T. searsii* present in *T. syriacum*. Molecular size markers in kb from *Hind*III bacteriophage λ are shown on left.

(Table 3). *Triticum syriacum* showed significantly better correspondence ($P < 0.01$) with the species of *T.* section *Sitopsis* than *T. crassum* (Table 3). Marker bands for *T.* section *Sitopsis* that were also present in tetraploid *T. crassum* had double intensity in *T. syriacum* (Fig. 2). Finally, all restriction fragments seen in the hybridization profiles of *T. syriacum* were in those of tetraploid *T. crassum* or *T. searsii*.

DISCUSSION

The restriction fragments of tetraploid *T. crassum* (including the marker bands for *T. crassum*) were all found in the hybridization profiles of the hexaploid species. These findings clearly showed that tetraploid *T. crassum* was one of the parents of the hexaploid taxa. The same unique cpDNA type found in all four species (Ogihara and Tsunewaki, 1988) also indicates this relationship. Additional supporting evidence is provided by isozyme variation (Jaaska, 1981), chromosome pairing in interspecific hybrids (Kihara, 1949, 1963; Kihara, Yamashita, and Tanaka, 1959), and karyotype analysis (Chennaveeraiah, 1960).

The perfect repeated sequence correspondence of *T. tauschii* with hexaploid *T. crassum* but not with *T. juvenale* and *T. syriacum* showed that the third genome pair in hexaploid *T. crassum* is an unmodified D genome. An unmodified D genome in hexaploid *T. crassum* was suggested also by Kihara, Yamashita, and Tanaka (1959) and Zhang and Dvorak (1992). Hexaploid *T. crassum* differs from the tetraploid cytotype in ploidy level and in the presence of one unmodified D genome. These characteristics suggest that the *T. crassum* cytotypes are different biological species. However, the diagnostic morphological characters for the different taxa described within *T. crassum* (Eig, 1928; Zhukovsky, 1928) are not diagnostic characters for the cytotypes. Clearly, a detailed morphotaxonomic study is needed to reconcile the taxonomy of tetraploid and hexaploid *T. crassum* with the biological species concept. At this point, we prefer to consider tetraploid and hexaploid *T. crassum* as cytotypes.

All four markers bands for *T. searsii* were in *T. syriacum*. Although this number of marker bands is small, it adds three new marker bands to the 15 *T. searsii* marker bands investigated previously (Zhang and Dvorak, 1992). Both the present and previous (Zhang and Dvorak, 1992) studies showed that the third pair of genomes in *T. syriacum* was contributed by *T. searsii*.

The third pair of genomes of *T. juvenale* was contributed by *T. umbellulatum*. However, an RSC of 0.86 suggested that the U genome in *T. juvenale* has diverged from that of *T. umbellulatum*. Divergence of a similar magnitude also may have occurred between *T. juvenale* and tetraploid

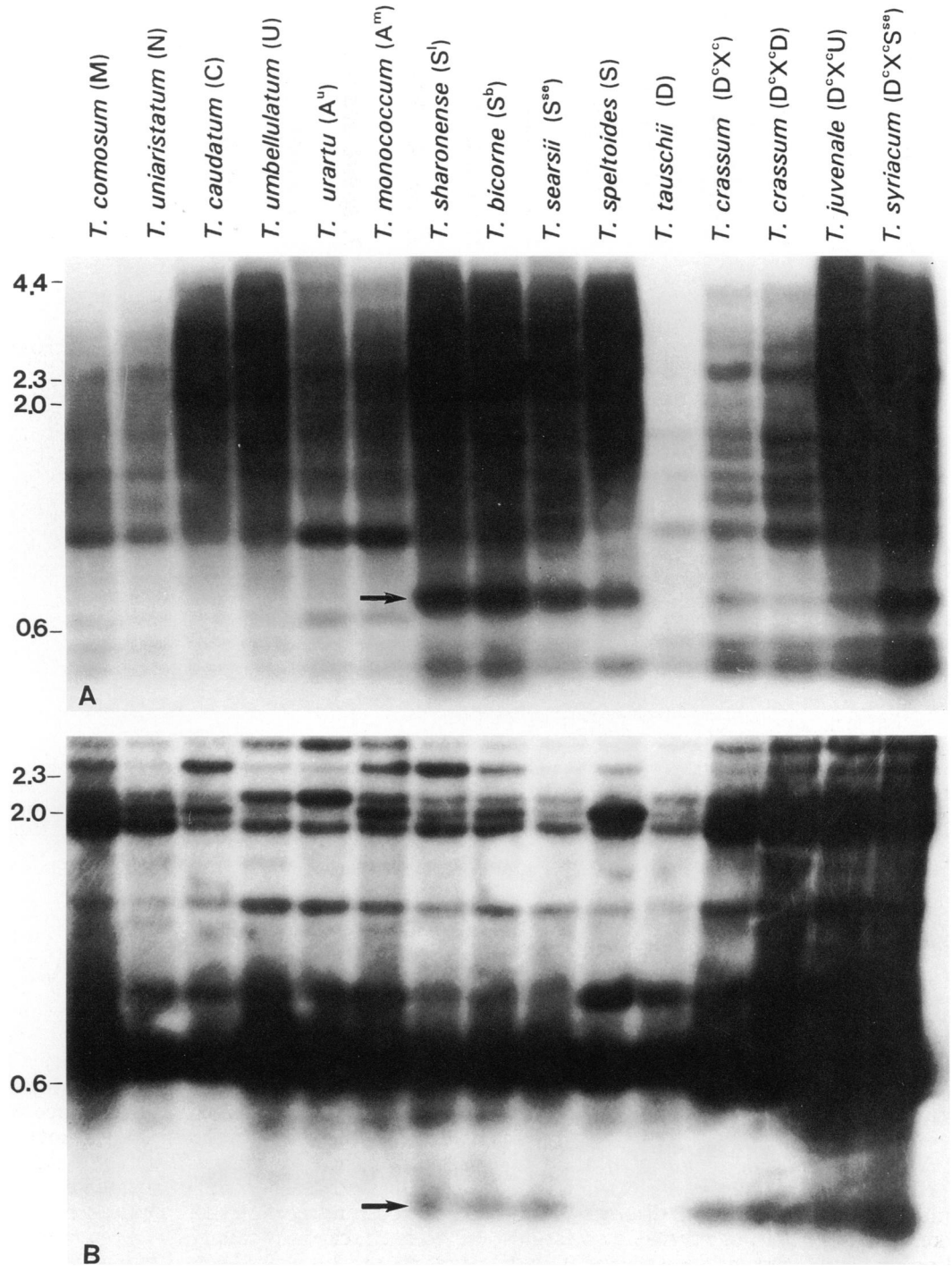

Fig. 2. A. *Dde*I restriction profiles of nuclear DNAs hybridized with pTsUCD4. B. *Hae*III restriction profiles of nuclear DNAs hybridized with pTkUCD8. Four micrograms of DNA per lane were loaded for the diploid species, 8 μg for the tetraploid, and 12 μg for the hexaploid species. The arrows indicate marker bands for the species from *Triticum* section *Sitopsis* (faint in *T. speltoides* in B). Note their presence in all species of the *T. crassum* complex. Note also the increased intensity of the marker bands in *T. syriacum*. Molecular size markers in kb from *Hind*III bacteriophage λ are shown on left.

T. crassum because one of the five marker bands for tetraploid *T. crassum* was absent in *T. juvenale* but not in the other two hexaploid taxa. Differentiation of the U genome of *T. umbellulatum* and the U genome of *T. juvenale* was suggested also by Kimber and Yen (1989) from their investigation of chromosome pairing in interspecific hybrids. It is possible that the origin of *T. juvenale* predates the origin of hexaploid *T. crassum* and *T. syr-*

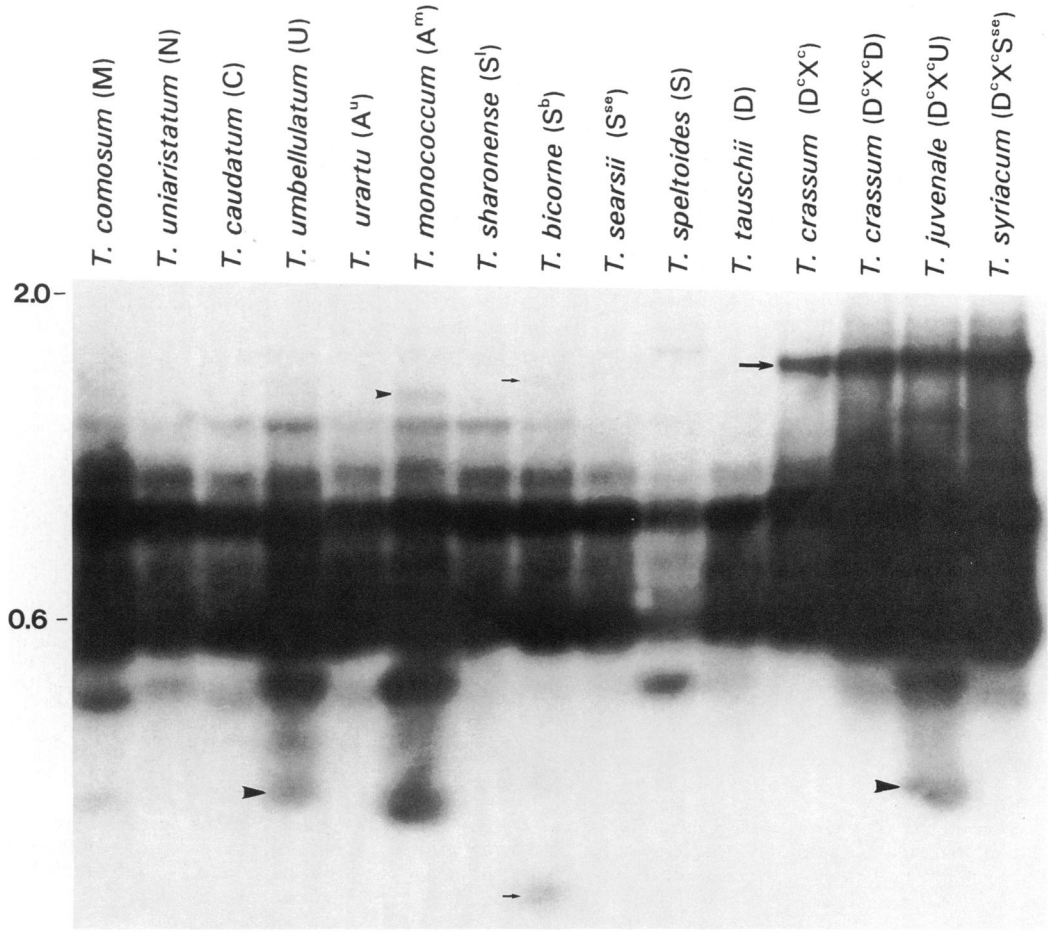

Fig. 3. *Tag*I restriction profiles of nuclear DNAs hybridized with pTkUCD4. Four micrograms of DNA per lane were loaded for the diploid species, 8 μg for the tetraploid, and 12 μg for the hexaploid species. The large arrow indicates a marker band for tetraploid *T. crassum*. The band is present in all species of the *T. crassum* complex and is absent in all diploid species of *Triticum* (arrow). These DNA fragments have lower molecular weight than the fragments constituting the top faint bands in *T. umbellulatum, T. urartu, T. monococcum,* and *T. speltoides*. Note also that the marker band for *T. umbellulatum* is present in *T. juvenale* (large arrowhead), and the marker bands for *T. bicorne* (small arrows) and *T. monococcum* (small arrowhead) are absent from all polyploid species of the complex. Molecular size markers in kb from *Hind*III bacteriophage λ are shown on left.

iacum, allowing some divergence to occur between the genomes of *T. juvenale* and its tetraploid and diploid parents.

Since both genomes of tetraploid *T. crassum* occur in the hexaploid species of the complex, the elucidation of the genome origin of tetraploid *T. crassum* is essential for the understanding of the evolution of the *T. crassum* complex. Previous cytogenetic studies found the D genome of *T. tauschii* in tetraploid *T. crassum* (Kihara, 1949; Lilienfeld, 1951; Kimber and Zhao, 1983). This was confirmed here. *Triticum tauschii* was the only diploid species of *Triticum* showing an RSC with *T. crassum* significantly different from zero. However, both molecular and cytogenetic studies agree that a substantial modification of the D genome in tetraploid *T. crassum* has occurred; the RSC between *T. tauschii* and *T. crassum* was only 0.65. Because intraspecific variation of repeated nucleotide sequences within *T. tauschii* is low (Dvorak and Zhang, 1992; Dubcovsky and Dvorak, 1994), it is unlikely that this poor correspondence reflects existence of *T. tauschii* populations not included in this study with repeated sequence profiles of *T. crassum*.

The second genome of tetraploid *T. crassum* is probably a modified S genome (Figs. 2, 5). An RSC of 0.50 between *Triticum* section *Sitopsis* and *T. crassum* was significantly different from zero ($P < 0.05$). Because RSCs of all species in the section, except for *T. speltoides,* were zero, it appears that the source of the second pair of *T. crassum* genomes was the ancestor of *Triticum* section *Sitopsis* or an extinct species that branched off from this internode. *Triticum speltoides* has the widest distribution of *Triticum* section *Sitopsis* and is the only outbreeder (a primitive character) in the section. It is, therefore, likely that the ancestor of *Triticum* section *Sitopsis* resembled modern *T. speltoides.*

There are several other lines of evidence that are consistent with a genome related to the S genome in *T. crassum.* Masci et al. (1992) reported a common 2.0 kb restriction fragment in *T. searsii, T. longissimum,* and all species of the *T. crassum* complex when hybridized with an alpha/beta-gliadin sequence. Although chromosome

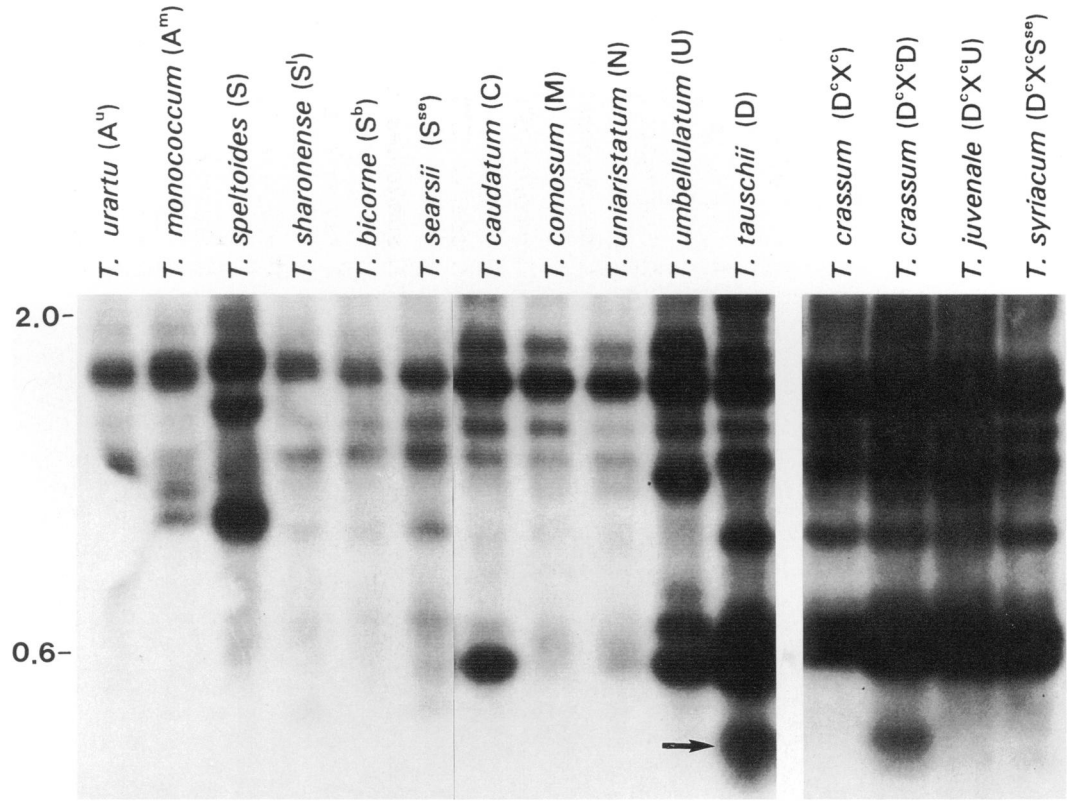

Fig. 4. *Alu*I restriction profiles of nuclear DNAs hybridized with pTtUCD10. Four micrograms of DNA per lane were loaded for the diploid species, 8 μg for the tetraploid, and 12 μg for the hexaploid species. The arrow indicates a marker band for *T. tauschii* in hexaploid *T. crassum*. Note the absence of the band from all other species of the *T. crassum* complex. Molecular size markers in kb from *Hind*III bacteriophage λ are shown on left.

TABLE 2. Probe-enzyme combinations that resulted in detection of marker bands.

Species or group of species[a]	Enzyme-probe combinations
A^u	c^b2($T1^c$), 113(H1), u19(T1)
A^m	c1(A1), c2(H1,T2), c6(H1), k4(T1), 11(M1), t1(T1), u19(T2)
S	c2(T1), c4(D1), c6(H2), k1(D1), k3(T1), k9(H1), 11(Dr2), 12(T1,M1), 13(T1), 14(D2,M1), 16(H1), 114(H2), s2(M1), s6(D1), t1(T1), t6(T1), t7(D2), t8(H1,A1), u19(H1)
S^{sh} or S^{sh} + S^l	c2(T1), c7(T1), k3(T1), t8(A1)
S^b	c1(A1), c7(H1), k4(T2), 11(A1), 13(A1), 14(M2), t8(H1)
S^{se}	c1(A1), c4(Dr1), c6(H1), k2(A1)
T	c3(T2), c5(H1), c6(H1), k3(H1), k9(H1), t4(H3)
C	c4(D1), c5(A1,H2), k4(H1), k9(Dr2), 13(A2), 14(M1), 16(A2,H2), t1(T1), t8(H2), t10(T1), u19(T1)
M	c2(H1), c4(Dr1), k4(A1), 11(M3), 13(A1), t10(T1)
N	c2(H1), k3(H3,T1), 11(Dr3,M1), 12(T2,M1), 17(H1), t3(H1), u19(H1)
U	k3(T1), k4(A1,T1), 12(T1), 110(H1), 114(H1), t10(A1)
D	c4(Dr1), k2(D2,T1,A2), k4(H1), k6(A1), k10(T1), 14(D2), 16(H3), 110(D1), s2(A1), t1(Dr1), t7(Dr1), t10(A2)
A^u + A^m	k2(A1), k3(H1,T2), k4(A1,T1), 17(H1), s4(D1), t5(T1), t7(Dr1), u19(T5,H2)
S^{sh} + S^b + S^{se}	c1(T1), c3(T1), 13(T1), 16(H1), 17(H1), 110(D2), k9(H1), s4(D1), t10(T1)
S + S^{sh} + S^b + S^{se}	c6(A1), k1(D1), k2(A1,T1), k8(H1), 12(T1), 13(A1), 17(H1), s4(D1), t2(H1)
C + U	c3(T2,H1), c6(A1), k2(D1), k3(T1), k9(H2), 13(T1), 14(D3), 16(H1), 17(H1), 110(H1), t2(H2,T1), t8(A2)
M + N	c2(T1), c3(T1), c5(A1), c6(A1), c7(T1), k10(M2), 16(A3,H1), 17(H1), s4(D1), t2(H1,T1), t3(H1), t6(T1), t8(A1)
C + M + N + U	c2(H1), c7(H1), k2(A1), t10(T2)
All sp. − D	c3(T1), c6(H1), k2(A1,T1), k3(H1,T1), k4(T1), k5(A1), k6(A1), k8(T1), k10(T1), 13(T1), 17(H1), t5(A1), t6(A1), t9(T1)

[a] *Triticum urartu* (A^u), *T. monococcum* (A^m), *T. speltoides* (S), *T. sharonense* (S^{sh}), *T. longissimum* (S^l), *T. bicorne* (S^b), *T. searsii* (S^{se}), *T. muticum* (T), *T. caudatum* (C), *T. comosum* (M), *T. uniaristatum* (N), *T. umbellulatum* (U), *T. tauschii* (D).

[b] Sources of clones were *T. comosum* (c), *T. kotschyi* (k), *T. longissimum* (l), *T. speltoides* (s), *T. tauschii* (t), and *T. urartu* (u).

[c] Indicates restriction endonuclease and number of marker bands detected. A = *Alu*I, D = *Dde*I, Dr = *Dra*I, H = *Hae*III, M = *Mbo*I, T = *Taq*I).

TABLE 3. Repeated nucleotide sequence correspondences (RSCs) of *T. crassum* (4*x* and 6*x*), *T. juvenale,* and *T. syriacum* with each diploid species and group of species of *Triticum* representing internal nodes in the phylogenetic tree (Dvorak and Zhang, 1992). Numbers of marker bands and RSCs obtained when only a single marker band per probe was used are in parentheses.

Species or group of species[a]	No. of marker bands	RSC			
		Triticum crassum (4*x*)	*Triticum crassum* (6*x*)	*Triticum juvenale*	*Triticum syriacum*
A^u	3 (3)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
A^m	10 (7)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S	27 (19)	0.07[b] (0.10)[b]	0.08[b] (0.10)[b]	0.07[b] (0.10)[b]	0.08[b] (0.10)[b]
S^{sh}	4 (4)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S^b	9 (7)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S^{se}	4 (4)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	1.00 (1.00)
T	9 (6)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
C	19 (11)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
M	8 (6)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
N	15 (7)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
U	7 (6)	0.00 (0.00)	0.00 (0.00)	0.86[c] (0.83)[c]	0.00 (0.00)
D	20 (13)	0.65[bc] (0.62)[bc]	1.00 (1.00)	0.65[bc] (0.62)[bc]	0.69[bc] (0.64)[bc]
$A^u + A^m$	17 (8)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
$S^{sh} + S^b + S^{se}$	10 (9)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.90[c] (0.88)[c]
$S + S^{sh} + S^b + S^{se}$	10 (10)	0.50[bc] (0.50)[bc]	0.50[bc] (0.50)[bc]	0.50[bc] (0.50)[bc]	1.00 (1.00)
C + U	20 (12)	0.00 (0.00)	0.00 (0.00)	1.00 (1.00)	0.00 (0.00)
M + N	18 (13)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
C + M + N + U	5 (5)	0.20[b] (0.20)[b]	0.20[b] (0.20)[b]	1.00 (1.00)	0.20[b] (0.20)[b]
All − D	16 (14)	0.80[c] (0.77)[c]	0.90[c] (0.89)[c]	1.00 (1.00)	1.00 (1.00)
All *Triticum*	231 (33)	0.99[c] (0.95)[c]	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)

[a] *Triticum urartu* (A^u), *T. monococcum* (A^m), *T. speltoides* (S), *T. sharonense* (S^s), *T. bicorne* (S^b), *T. searsii* (S^{se}), *T. muticum* (T), *T. caudatum* (C), *T. comosum* (M), *T. uniaristatum* (N), *T. umbellulatum* (U), *T. tauschii* (D).

[b] Significantly different from 1 ($P < 0.05$).

[c] Significantly different from 0 ($P < 0.05$).

pairing in critical hybrids between tetraploid *T. crassum* and most diploid species of *Triticum* has not been investigated, available data for hexaploid *T. crassum* and *T. juvenale* are consistent with a modified S genome in the complex. However, since hybrids between polyploid and diploid species are expected to show some variation due to intraspecific polymorphism of the genes affecting homologous and homoeologous chromosome pairing (Dvorak, 1972; Kimber and Athwal, 1972) these data should be considered with caution. Hybrids involving hexaploid *T. crassum* and different diploid *Triticum* species (Melnyk and McGinnis, 1962) show the highest mean arm pairing frequency (c, in the sense of Driscoll, Bielig, and Darvey, 1979) for *T. tauschii* (0.512) and *T. speltoides* (0.507, 0.471). These values are followed by *T. muticum* (0.454), *T. sharonense* (0.415), *T. caudatum* (0.350), and *T. umbellulatum* (0.321) (c values were calculated from data published by Melnyk and McGinnis assuming two arms pairing in trivalents, three in quadrivalents, and four in higher multivalents). Hybrids involving *T. juvenale* (McGinnis and Melnyk, 1962) show a similar pattern; the order being *T. umbellulatum* (0.583), *T. speltoides* (0.396, 0.449), *T. tauschii* (0.386), *T. sharonense* (0.320), *T. muticum* (0.279), *T. caudatum* (0.263), *T. longissimum* (0.253), and *T. uniaristatum* (0.195). High pairing of the *T. umbellulatum* chromosomes indicates a U genome in *T. juvenale.* Chromosome pairing in hybrids involving *T. crassum* or *T. juvenale* with tetraploid *Triticum* species also parallels this trend. Hybrids with tetraploid *T. peregrinum* Hackel (syn. *Aegilops variabilis* Eig) that has a pair of *T. longissimum* or *T. sharonense* (S^1) genomes (Zhang, Dvorak, and Waines, 1992) showed higher mean arm chiasma frequency than hybrids involving tetraploid species without an S genome, such as *T. ovatum* (L.) Raspail, *T. columnare* (the transfer of *Aegilops columnaris* Zhuk. to *Triticum* by Morris and Sears [1967] was incomplete), and *T. triunciale* (L.) Raspail (McGinnis, 1956; McGinnis and Melnyk, 1962; Melnyk and McGinnis, 1962; Kimber, Pignone, and Sallee, 1983). Hybrids involving *T. speltoides* exhibited higher chiasma numbers per cell than other species of *T.* section *Sitopsis.* This is paralleled by the presence of a small number of marker bands of *T. speltoides* in all species of the complex. Previous study also detected some *T. speltoides* marker bands in hexaploid *T. crassum* (Zhang and Dvorak, 1992). *Triticum speltoides* is the only species of *T.* section *Sitopsis*

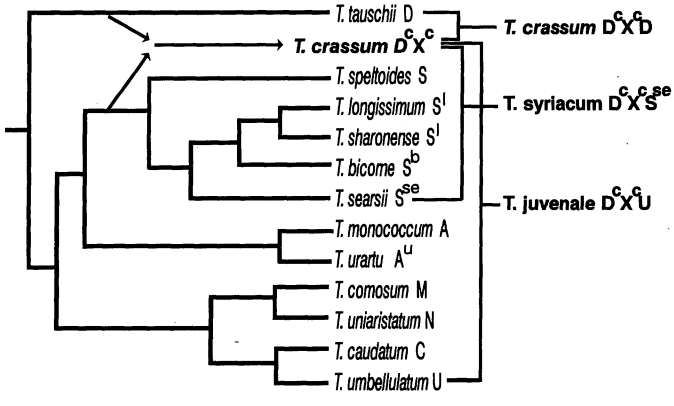

Fig. 5. Phylogenetic tree of the diploid species of *Triticum* based on variation in the restriction patterns of repeated nucleotide sequences (Dvorak and Zhang, 1992) and postulated origin of tetraploid and hexaploid *T. crassum, T. juvenale,* and *T. syriacum.*

whose present geographic distribution is in contact with that of *T. tauschii* and overlaps with that of *T. crassum* (Kihara, 1963). Hence tetraploid *T. crassum* could have originated as suggested above.

Weak repeated sequence correspondence between diploid *Triticum* species and tetraploid *T. crassum* potentially could be caused by the absence of the exact diploid ancestors from the panel of diploid species we investigated. Simulation of this situation showed that the absence of one of the ancestors usually resulted in a low RSC for one of the genomes and perfect correspondence for the other (Dubcovsky and Dvorak, 1994), but this is not the case here. An ancient origin of a polyploid followed by evolutionary divergence is expected to produce low RSCs in both genomes, which is the case for tetraploid *T. crassum*. However, the unlikely possibility of recent origin and absence of both diploid ancestors from the population of diploid species analyzed would have the same result. It must be kept in mind that this situation, unlikely as it is, cannot ever be ruled out.

Introgressive hybridization is another potential cause for poor fit between the genomes of polyploid and diploid relatives. Three lines of evidence suggested that pivotal-differential hybridization (Zohary and Feldman, 1962) is irrelevant in this case. In the first place, both genomes of *T. crassum* are modified. Pivotal-differential hypothesis is expected to result in modification of only a single genome pair of a tetraploid. Occasional recombination between the introgressed genome and the pivotal genome would not alter the restriction profiles. In the second place, the RSC between tetraploid *T. crassum* and *Triticum* section *Sitopsis* as a group is significantly higher than the RSC between tetraploid *T. crassum* and any of the individual species of *Triticum* section *Sitopsis*. However, introgressive hybridization is expected to result in similar RSCs for the diploid species that was hybridized and the internodes of the phylogenetic branch leading to this diploid. In the third place, introgression does not occur in the uniparentally inherited cpDNA. The apparent modification of the cpDNA relative to the rest of diploid and polyploid species in *Triticum* thus provides a potent argument against any form of introgressive hybridization as a cause of the poor fit between the genomes of diploid *Triticum* species and those of *T. crassum*. On the contrary, differentiation of *T. crassum* cpDNA parallels differentiation of the nuclear genomes of tetraploid *T. crassum* from the genomes of *T. tauschii* and its progenitor of *Triticum* section *Sitopsis*. Thus, these inferences are compatible with the possibility that *T. crassum* is an ancient tetraploid whose genomes have diverged from those of its diploid ancestors. A previous investigation of the evolution of hexaploid *T. crassum* and *T. syriacum* following the same methodology tentatively concluded that the hexaploid species arose from hybridization of a *T. crassum*-like tetraploid that originated from an ancient hybridization event involving a primitive *T. tauschii* and a species in the evolutionary lineage of *Triticum* after the divergence of *T. tauschii* (Zhang and Dvorak, 1992). The genome of the latter species was designated X^c. Present results largely agree with these conclusions. The advantage of working with the tetraploid *T. crassum* rather than the hexaploid *T. crassum* and *T. syriacum*, and the availability of more marker bands for the branch preceding the divergence of *Triticum* section *Sitopsis*, allowed us to suggest that the X^c genome was probably contributed by a species that existed later, after the divergence of the branch leading to *Triticum* section *Sitopsis*. Nevertheless, we prefer to retain the designation $D^cD^cX^cX^c$ for the *T. crassum* genomes until the affiliation of the second genome with *Triticum* section *Sitopsis* is confirmed by an independent line of evidence.

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