Quantification of genetic relationships among A genomes of wheats

A. Brandolini, P. Vaccino, G. Boggini, H. Özkan, B. Kilian, and F. Salamini

Abstract: The genetic relationships of A genomes of *Triticum urartu* (A^u) and *Triticum monococcum* (A^m) in polyploid wheats are explored and quantified by AFLP fingerprinting. Forty-one accessions of A-genome diploid wheats, 3 of AG-genome wheats, 19 of AB-genome wheats, 15 of ABD-genome wheats, and 1 of the D-genome donor *Ae. tauschii* have been analysed. Based on 7 AFLP primer combinations, 423 bands were identified as potentially A genome specific. The bands were reduced to 239 by eliminating those present in autoradiograms of *Ae. tauschii*, bands interpreted as common to all wheat genomes. Neighbour-joining analysis separates *T. urartu* from *T. monococcum. Triticum urartu* has the closest relationship to polyploid wheats. *Triticum turgidum* subsp. *dicoccum* and *T. turgidum* subsp. *durum* lines are included in tightly linked clusters. The hexaploid spelts occupy positions in the phylogenetic tree intermediate between bread wheats and *T. turgidum*. The AG-genome accessions cluster in a position quite distant from both diploid and other polyploid wheats. The estimates of similarity between A genomes of diploid and polyploid wheats indicate that, compared with A^m, A^u has around 20% higher similarity to the genomes of polyploid wheats. *Triticum timopheevii* AG genome is molecularly equidistant from those of A^u and A^m wheats.

Key words: A genome, *Triticum*, genetic relationships, AFLP.

Résumé : Les relations génétiques entre les espèces à génome A *Triticum urartu* (A^u) et *Triticum monococcum* (A^m) et les blés polyploïdes sont explorées et quantifiées à l'aide de marqueurs AFLP. Quarante et une accessions de blés diploïdes à génome A, trois blés à génome AG, 19 à génome AB, 15 à génome ABD et 1 *Aegilops tauschii* (une espèce donatrice du génome D) ont été analysés. Avec sept combinaisons d'amorces AFLP, 423 amplicons ont été identifiés comme étant potentiellement spécifiques du génome A. Ce nombre a été réduit à 239 en éliminant tous ceux qui étaient présents chez l'*Ae. tauschii*, ces amplicons étant jugés communs à tous les génomes du blé. Des analyses « neighbour-joining » séparent le *T. urartu* du *T. monococcum*. Le *T. urartu* était le plus apparenté avec les blés polyploïdes. Les lignées de *T. turgidum* subsp. *dicoccum* et subsp. *durum* formaient des groupes très proches. Dans cet arbre phylogénétique, les épeautres hexaploïdes occupent des positions intermédiaires entre les blés tendres et le *T. turgidum*. Les accessions à génome AG forment un groupe, mais sont assez distants tant des blés diploïdes que des autres blés polyploïdes. Les mesures de similarité entre les génomes A des blés diploïdes et polyploïdes indiquent que, par rapport au génome A^m, le génome A^u montre environ 20 % plus de similarité avec les génomes des blés polyploïdes. Le *T. timopheevii* à génome AG s'avère équidistant des blés à génome A^u et A^m sur le plan moléculaire.

Mots clés : génome A, *Triticum*, relations génétiques, AFLP.

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Introduction

The genus *Triticum* includes cultivated species cytogenetically associated in 4 groups: einkorn ($2n = 2x = 14$, genome AA), emmer ($2n = 4x = 28$, AABB), *T. timopheevii* ($2n = 4x = 28$, AAGG), and bread wheat ($2n = 6x = 42$, AABBDD) (Sax 1918; Lilienfeld and Kihara 1934; Zohary and Hopf 2000). At the diploid level, 2 species are recognised: (*i*) *Triticum monococcum* L., with a wild form (*T. monococcum* subsp. *boeoticum*), a domesticated form (*T. monococcum* subsp. *monococcum*), and a weedy European form (*T. monococcum* subsp. *aegilopoides*) (Heun et al. 1997), and (*ii*) *Triticum urartu* Tum., existing only as a wild form. At the tetraploid level, 2 different groups are distinguished: (*i*) *Triticum turgidum* L. with 1 wild form (*T. turgidum* subsp. *dicoccoides* (Korn.)Thell.) and several cultivated subspecies; and (*ii*) *Triticum timopheevii* Zhuk., with 1 wild ancestor (*T. timopheevii* subsp. *araraticum* (Jakubz.) Mac

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Key) and 1 cultivated form (*T. timopheevii* subsp. *timopheevii* (Zhuk.) Löve and Löve). At the hexaploid level, 2 cultivated species are described: *Triticum aestivum* L., with several subspecies, and *Triticum zhukovskyi* Menab. et Ericz.

Origin and phylogenetic relationships of wheat polyploid species have been studied using different approaches. Cytogenetic and morphological studies in the past supported the conclusion that *T. monococcum* was the A-genome donor of polyploid wheats (Sax 1922; Kihara 1924; Lilienfeld and Kihara 1934; Zohary et al. 1969). However, after the recognition of *T. urartu* as a new species, cytogenetic (Chapman et al. 1976), immunochemical (Konarev 1983), electrophoretic (Waines and Payne 1987; Ciaffi et al. 1997), and enzymatic data (Nishikawa 1983) indicated that this species is the most likely progenitor of the A genome of polyploid wheats.

Molecular markers have provided new tools in the study of plant evolutionary relationships: restriction fragment length polymorphisms (RFLPs) were used to infer the phylogeny of cultivated wheats, supporting the claim of *T. urartu* as the direct progenitor of polyploid AB and ABD wheats (Dvorak et al. 1988, 1993; Takumi et al. 1993). RFLPs, however, require a large numbers of probe–enzyme combinations to discriminate genotypes and to challenge the genetic relationships of a whole genome. Techniques based on PCR, on the other hand, are faster, cheaper, and less labour intensive. In particular, amplified fragment length polymorphisms (AFLPs) are superior to other markers because of the number of different loci simultaneously analysed in each experiment (Powell et al. 1996; Bohn et al. 1998). The capacity of AFLP to fingerprint the whole genome (Heun et al. 1997; Badr et al. 2000; Martin and Salamini 2000) and the possibility of handling a large number of samples per population considered are also relevant. It is concluded that, when used with appropriate precautions, AFLP fingerprints are also informative for phylogenetic studies (Buntjer et al. 2002).

Quantifying the molecular differences among the A genomes of diploid and polyploid wheats is important: the possibility of creating chimaeric recombinant A^m and A^u chromosomes (unpublished results from our laboratories) is worthwhile only if the 2 genomes differ substantially. We have applied the AFLP procedure to a large sample of *Triticum* species to quantify the level of similarity of A^m and A^u genomes to those of AB, AG, and ABD polyploids.

Materials and methods

Plant material

The 41 A-genome (37 A^m and 4 A^u), 3 AG-genome, 19 AB-genome, 15 ABD-genome, and 1 D-genome wheat listed in Table 1 were provided by several genebanks (see Acknowledgments). The 37 A^m diploid lines were chosen among a group of 338 accessions fingerprinted by Heun et al. (1997) and representative of a wider pool of 1362 accessions morphologically characterized (Empilli 1994). The 4 A^u lines were chosen among a group of more than 100 lines of *T. urartu*, based on maximisation of their genetic distance evaluated by AFLP (data not shown).

DNA isolation and AFLP procedures

DNA from 30 7-day-old seedlings per accession was extracted following a modified cetyltrimethylammonium bromide (CTAB) procedure (Murray and Thompson 1980); AFLP analysis was performed as described by Vos et al. (1995). Briefly, DNAs were digested with *Eco*RI and *Mse*I, and adapter sequences were then ligated to the restricted DNA fragments. PCR was performed in 2 consecutive steps. In the first, the selective pre-amplification, DNAs were amplified with primers complementary to the adapters and having 1 selective nucleotide, either *Eco*RI primer (E) + A or *Mse*I primer (M) + A. In the second step, the preamplification products were used as template for amplification using primer combinations with the following extensions: E-ACC–M-ACC, E-ACG–M-AGC, E-ACG–M-ATT, E-AGC–M-ACT, E-AGT–M-AAC, E-AGT–M-ACA, and E-AGT–M-ACT. For these reactions, only the *Eco*RI primer was labelled with [^{33}P]ATP (Amersham, Little Chalfont, UK). PCR products were separated by electrophoresis in a 4.5% denaturing polyacrylamide gel and visualised by autoradiography.

Data analysis

For each genotype, the presence (1) or absence (0) of amplified fragments was recorded. Two consecutive steps were adopted to select A genome specific bands in polyploids: in the first step, only bands comigrating with diploid *T. monococcum* and *T. urartu* fragments were kept; in the second step, all bands comigrating with D-genome *Ae. tauschii* fragments were eliminated. The reason to consider the diploid *Ae. tauschii* was that its D genome is different from diploid A genomes. This situation allows to spot AFLP bands that, owing to the existing homoeology among A, B, and D genomes, are common to all diploid and polyploidy wheats, but that do not map uniquely to A chromosomes. In the absence of clear indications concerning diploid B- and G-genome donors, the second step was restricted to *A. tauschii*. The correct attribution of AFLP bands to the A genome was tested by fingerprinting few representative diploid wheats along with *Triticum aestivum* 'Chinese Spring' and its nulli-A-tetrasomics. A restricted set of primer combination was used (the nullitetrasomic N2AT2B was missing in this experiment). The results of this control, although partial, supported band assignments carried out in the 2 steps mentioned above.

Similarities between pairwise accessions were estimated by the Dice similarity index (Dice 1945)

$$S_{\mathrm{D}xy} = \frac{n_{xy}}{n_{xy} + \frac{u_{xy}}{2}}$$

where n_{xy} represents the number of bands common to 2 accessions, x and y, and u_{xy} is the number of bands present only in x or only in y. A dendrogram based on the similarity matrix data was generated using neighbour joining (NJ) and the goodness of the tree was tested by bootstrapping (1000 runs). This analysis was performed with the softwares DistAFLP (Mougel et al. 2002) and PHYLIP version 3.6 (Felsenstein 2002).

Consensus genotypes for each species and for each subspecies were obtained by scoring as 0 any gene frequencies

Table 1. Accessions considered in the quantification of A-genome relationships among wheats.

Species		Origin	Passport
A^m			
ID68	*T. monococcum* subsp. *boeoticum*		PRG6150
ID126	*T. monococcum* subsp. *boeoticum*		HTRI6734/89
ID229	*T. monococcum* subsp. *boeoticum*		BGRC36551
ID386	*T. monococcum* subsp. *boeoticum*	Turkey	G1878
ID521	*T. monococcum* subsp. *boeoticum*	Hungary	PI272556
ID604	*T. monococcum* subsp. *boeoticum*	Turkey	PI427470
ID618	*T. monococcum* subsp. *boeoticum*	Turkey	PI427484
ID746	*T. monococcum* subsp. *boeoticum*	Turkey	PI427614
ID758	*T. monococcum* subsp. *boeoticum*	Turkey	PI427627
ID909	*T. monococcum* subsp. *boeoticum*	Iraq	PI427782
ID1075	*T. monococcum* subsp. *boeoticum*	Iraq	PI427949
ID1089	*T. monococcum* subsp. *boeoticum*	Turkey	PI427963
ID1117	*T. monococcum* subsp. *boeoticum*	Lebanon	PI427995
ID1121	*T. monococcum* subsp. *boeoticum*	Lebanon	PI427999
ID1174	*T. monococcum* subsp. *boeoticum*	Turkey	PI538540
ID1182	*T. monococcum* subsp. *boeoticum*	Iraq	PI538548
ID1256	*T. monococcum* subsp. *boeoticum*	Turkey	PI538623
ID1261	*T. monococcum* subsp. *boeoticum*	Turkey	PI538723
ID1303	*T. monococcum* subsp. *boeoticum*	Turkey	PI554540
ID1310	*T. monococcum* subsp. *boeoticum*	Turkey	PI554550
ID1315	*T. monococcum* subsp. *boeoticum*	Turkey	PI 554559
ID1326	*T. monococcum* subsp. *boeoticum*	Turkey	PI554577
SAL1366	*T. monococcum* subsp. *boeoticum*	Armenia	
ID69	*T. monococcum* subsp. *monococcum*	Turkey	G4325
ID127	*T. monococcum* subsp. *monococcum*	Turkey	AT12910/89
ID194	*T. monococcum* subsp. *monococcum*	Austria	BGRC13193
ID279	*T. monococcum* subsp. *monococcum*	Balkans	BGRC42016
ID409	*T. monococcum* subsp. *monococcum*	Turkey	16273
ID494	*T. monococcum* subsp. *monococcum*	Turkey	PI167526
ID495	*T. monococcum* subsp. *monococcum*	Turkey	PI167589
ID500	*T. monococcum* subsp. *monococcum*	Turkey	PI167634
ID576	*T. monococcum* subsp. *monococcum*	Israel	PI393496
ID1157	*T. monococcum* subsp. *monococcum*	Spain	PI518452
ID26	*T. monococcum* subsp. *aegilopoides*	Rumania	PI306532
ID123	*T. monococcum* subsp. *aegilopoides*		ASchgt1/88
ID228	*T. monococcum* subsp. *aegilopoides*	Balkans	BGRC36548
ID520	*T. monococcum* subsp. *aegilopoides*	Hungary	PI272520
A^u			
ID388	*T. urartu*	Lebanon	G3246
ID1122	*T. urartu*	Lebanon	PI428000
ID1264	*T. urartu*	Turkey	PI554479
ID1277	*T. urartu*	Turkey	PI554498
AG (+AAG)			
ID1233	*T. timopheevii* subsp. *araraticum*	Iraq	PI538599
FAR72	*T. timopheevii* subsp. *timopheevii*		W899
FAR77	*T. zhukowskyi*	Georgia	ATRI7262/74
AB hulled			
TD10	*T. turgidum* subsp. *dicoccum*	Italy	
TD15	*T. turgidum* subsp. *dicoccum*	Italy	
TD33	*T. turgidum* subsp. *dicoccum*	Italy	
TD47	*T. turgidum* subsp. *dicoccum*	Italy	
AB naked			
'Aristan'	*T. turgidum* subsp. *durum*	France	
'Aziziah'	*T. turgidum* subsp. *durum*	Italy/Palestine	

Table 1. (*concluded*).

Species		Origin	Passport
'Cappelli'	*T. turgidum* subsp. *durum*	Italy/Tunisia	
'Coll. Jordan'	*T. turgidum* subsp. *durum*	Jordan	
'Muri S 503'	*T. turgidum* subsp. *durum*	Cyprus	
'Razza K'	*T. turgidum* subsp. *durum*	Tunisia	
'Roqueño'	*T. turgidum* subsp. *durum*	Spain	
'Sabil 1'	*T. turgidum* subsp. *durum*	Syria	
'Santa'	*T. turgidum* subsp. *durum*	Greece	
'Taganrog'	*T. turgidum* subsp. *durum*	Ukraina	
'Timilia'	*T. turgidum* subsp. *durum*	Italy	
'Tripolino'	*T. turgidum* subsp. *durum*	Italy	
'Vatan'	*T. turgidum* subsp. *durum*	Tajikistan	
'Villemure'	*T. turgidum* subsp. *durum*	France	
TT3243	*T. turgidum* subsp. *polonicum*		
ABD hulled			
TS24	*T. aestivum* subsp. *spelta*	Italy	
TS58	*T. aestivum* subsp. *spelta*	Italy	
Rouquin	*T. aestivum* subsp. *spelta*	Germany	
Trivento	*T. aestivum* subsp. *spelta*	Italy	
ABD naked			
'Chinese Spring'	*T. aestivum* subsp. *aestivum*	China	
'Akagomugi'	*T. aestivum* subsp. *aestivum*	Japan	
'Daruma 2'	*T. aestivum* subsp. *aestivum*	Japan	
'Gabo'	*T. aestivum* subsp. *aestivum*	Australia	
'Hatif Inversable'	*T. aestivum* subsp. *aestivum*	France	
'Red Egyptian'	*T. aestivum* subsp. *aestivum*	Africa	
'Rieti'	*T. aestivum* subsp. *aestivum*	Italy	
'Squarehead Master'	*T. aestivum* subsp. *aestivum*	England	
'Wilhelmina'	*T. aestivum* subsp. *aestivum*	Holland	
TA3238	*T. aestivum* subsp. *compactum*		
TA3240	*T. aestivum* subsp. *macha*		
D			
ID1608	*Ae. tauschii*	Iran	

up to 0.5 and the remainder as 1; Dice genetic similarities were computed using consensus values and 2 trees, one reporting species and the other reporting subspecies relationships, were built by NJ. Similarly, genetic distances based on the frequency of each band in each group were computed using Nei's distance (1972) with the software AFLP-SURV version 1.0 (Vekemans 2002) and NJ clustering was performed. Bootstrap testings (1000 runs) were performed as outlined.

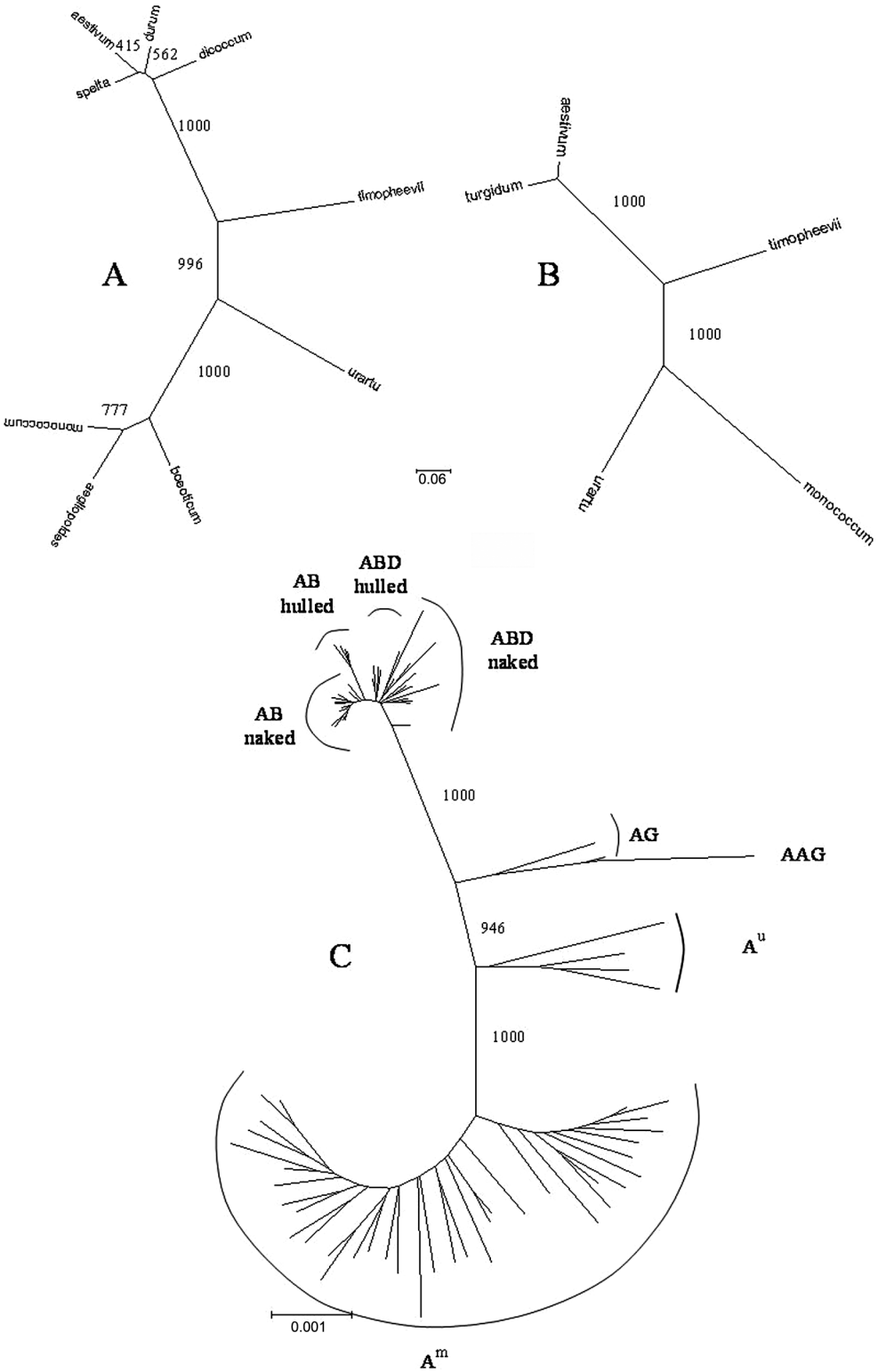
Results

Molecular profiling of the 79 *Triticum* accessions with 7 primer pair combinations yielded 738 polymorphisms (AFLP bands scored as present or absent). During the evaluation of AFLP markers across species, criteria as described by El Rabey et al. (2002) were followed to assign a band to homoeologous loci. Primer combinations differed widely in their ability to amplify polymorphic products: the average number of AFLP polymorphisms per gel was 105, ranging from 55 (E-AGT–M-AAC) to 163 (E-ACG–M-ATT).

Considering as a comparison the diploid A-genome wheats, 423 polymorphic loci were identified as hosting putative A bands. Fragments comigrating with D-genome bands of the *Ae. tauschii* accession were subsequently deleted. This process yielded 239 putative A bands complying with the conditions described, and these were retained for further analysis. A comparison of 'Chinese Spring' and its nulli-A tetrasomics with representative diploids mapped 33 of 63 putative A bands on A chromosomes. This fraction of 52.4% supports the assignment of our AFLP bands to the A genome, given that 'Chinese Spring' was characterized only by 67.6 % of the bands present across hexaploid lines studied.

The wheat accessions grouped following well-known wheat taxonomy (Fig. 1C). All A^m-genome samples (*T. monococcum*) clustered together and were separated from A^u diploids (*T. urartu*). At the polyploids level, the 2 AG- and the AAG-genome accessions clustered in a small group distant from both diploids and from the other polyploids. The AB tetraploids formed 1 group divided into 2 strictly related subgroups: the 4 *T. turgidum* subsp. *dicoccum* formed the first subgroup, whereas all 14 *T. turgidum* subsp. *durum* accessions made up the second; *T. turgidum* subsp. *polonicum* mapped alone. The hexaploid group was represented by 2 subgroups, the first containing all bread wheats

Fig. 1. Neighbour-joining tree of Dice genetic similarities. Bootstrap consensus values from 1000 iterations are indicated. 239 AFLP bands related to genome A were considered (specified in Materials and methods). In A and B, the consensus genotypes were obtained by scoring as 0 gene frequencies up to 0.5 and the remainder as 1; in C, topologies from all accessions studied are reported.



and the second including the spelts, these fitting between 4*x* and 6*x* wheats.

The dendrograms based on consensus genotypes (see Materials and methods) showed a similar picture (Figs. 1A, 1B), with a high level of confidence for most branches; only the relationships between *T. dicoccum* and *T. durum*, as well as between *T. aestivum* and *T. spelta*, showed intermediate values. Similar results were observed using Nei (1972) ge-

Table 2. Dice genetic similarities among genome A accessions and polyploid wheats based on 239 AFLP bands (see Materials and methods).

Genome A line	4X wheats (AB)	6X wheats (ABD)	*T. timopheevii* group (AG + AAG)
Triticum monococcum subsp. *boeoticum*			
ID68	0.365 ± 0.0034	0.363 ± 0.0065	0.510 ± 0.0191**
ID126	0.404 ± 0.0023	0.411 ± 0.0047	0.427 ± 0.0082
ID229	0.394 ± 0.0033	0.410 ± 0.0048	0.473 ± 0.0185
ID386	0.364 ± 0.0029	0.372 ± 0.0045	0.477 ± 0.0182
ID521	0.408 ± 0.0024	0.406 ± 0.0030	0.451 ± 0.0272
ID604	0.386 ± 0.0024	0.374 ± 0.0053	0.458 ± 0.0195
ID618	0.349 ± 0.0029	0.354 ± 0.0045	0.457 ± 0.0196
ID746	0.322 ± 0.0032*	0.330 ± 0.0046*	0.457 ± 0.0123
ID758	0.363 ± 0.0028	0.355 ± 0.0038	0.437 ± 0.0109
ID909	0.382 ± 0.0031	0.384 ± 0.0045	0.483 ± 0.0180
ID1075	0.381 ± 0.0027	0.370 ± 0.0052	0.502 ± 0.0101
ID1089	0.398 ± 0.0038	0.402 ± 0.0054	0.482 ± 0.0221
ID1117	0.345 ± 0.0040	0.362 ± 0.0059	0.454 ± 0.0149
ID1121	0.365 ± 0.0026	0.382 ± 0.0057	0.458 ± 0.0089
ID1174	0.355 ± 0.0026	0.364 ± 0.0057	0.426 ± 0.0023*
ID1182	0.372 ± 0.0035	0.379 ± 0.0050	0.494 ± 0.0171
ID1256	0.422 ± 0.0032	0.445 ± 0.0037**	0.496 ± 0.0074
ID1261	0.376 ± 0.0045	0.387 ± 0.0038	0.486 ± 0.0171
ID1302	0.426 ± 0.0023**	0.436 ± 0.0041	0.491 ± 0.0207
ID1310	0.423 ± 0.0021	0.424 ± 0.0043	0.502 ± 0.0172
ID1315	0.395 ± 0.0030	0.407 ± 0.0047	0.475 ± 0.0188
ID1326	0.408 ± 0.0030	0.422 ± 0.0059	0.477 ± 0.0104
SAL1366	0.386 ± 0.0024	0.384 ± 0.0058	0.478 ± 0.0212
Triticum monococcum subsp. *monococcum*			
ID69	0.330 ± 0.0024	0.339 ± 0.0046	0.439 ± 0.0164
ID127	0.330 ± 0.0024	0.339 ± 0.0046	0.439 ± 0.0164
ID194	0.405 ± 0.0026	0.412 ± 0.0049	0.467 ± 0.0111
ID279	0.350 ± 0.0038	0.353 ± 0.0039	0.419 ± 0.0100*
ID409	0.325 ± 0.0025*	0.335 ± 0.0036*	0.421 ± 0.0176
ID494	0.396 ± 0.0022	0.407 ± 0.0038	0.481 ± 0.0155
ID495	0.385 ± 0.0019	0.383 ± 0.0040	0.483 ± 0.0165**
ID500	0.371 ± 0.0028	0.378 ± 0.0038	0.453 ± 0.0121
ID576	0.393 ± 0.0026	0.403 ± 0.0037	0.475 ± 0.0058
ID1157	0.425 ± 0.0027**	0.431 ± 0.0050**	0.479 ± 0.0139
Triticum monococcum subsp. *aegilopoides*			
ID26	0.348 ± 0.0024	0.359 ± 0.0044	0.429 ± 0.0154
ID123	0.329 ± 0.0029	0.334 ± 0.0042	0.395 ± 0.0195
ID228	0.318 ± 0.0023*	0.328 ± 0.0038*	0.393 ± 0.0112*
ID520	0.387 ± 0.0025**	0.399 ± 0.0040**	0.444 ± 0.0211**
Triticum urartu			
ID388	0.489 ± 0.0024*	0.477 ± 0.0053*	0.473 ± 0.0225*
ID1122	0.507 ± 0.0032**	0.508 ± 0.0049	0.524 ± 0.0306**
ID1264	0.503 ± 0.0039	0.509 ± 0.0046**	0.521 ± 0.0266
ID1277	0.495 ± 0.0026	0.480 ± 0.0066	0.495 ± 0.0203

Note: *, within group lowest similarity; **, within group highest similarity.

netic distances based on AFLP fragment frequencies (data not shown).

The values of Dice genetic similarity for genome A among diploid and polyploid wheats are presented in Table 2. All *T. urartu* samples, compared with any *T. monococcum* accession, showed a closer relationship with the AB and ABD genome wheats. The same clear-cut result was not observed when the *T. timopheevii* group was considered: in this case, ranges of *T. urartu* and *T. monococcum* accessions overlapped partially. These observations are synthesized in

Table 3. Average values of Dice genetic similarities (±SEM) among diploid and polyploid wheats.

		Dice genetic similarity[a]				
		4	15	4	11	3
Species	No. of lines	AB (hulled)	AB (naked)	ABD (hulled)	ABD (naked)	AG + AAG
T. monococcum (wild)	23	0.39±0.003	0.38±0.002	0.39±0.003	0.39±0.002	0.47±0.004
T. monococcum (domesticated)	10	0.38±0.005	0.37±0.003	0.38±0.006	0.38±0.004	0.46±0.006
T. monococcum (feral)	4	0.35±0.005	0.35±0.004	0.35±0.008	0.36±0.005	0.42±0.010
A^m genome	37	0.38±0.003	0.37±0.001	0.38±0.003	0.38±0.002	0.46±0.004
T. urartu. A^u genome	4	0.50±0.004	0.50±0.002	0.50±0.003	0.49±0.005	0.50±0.012

Note: Two hundred thirty-nine AFLP bands related to genome A are considered (specified in Materials and methods).
[a]Similarity varies between 0 and 1 (maximum).

Table 3, reporting the average values of Dice genetic similarities between diploid and polyploid wheats: while the closer relationship between *T. urartu* and AB and ABD wheat accessions is unmistakable, AG wheats show roughly the same similarity with A^m and A^u diploids.

Our estimate of the genetic similarity between genomes A^m and A^u on one side and polyploids AB and ABD on the other indicate that A^u has around 20% higher similarity with A genomes of polyploids. However, *T. timopheevii* (genome AG) appears to be almost molecularly equidistant from A^u and A^m, a finding that may indicate that the G genome is more similar to A genomes than previously thought.

Average values of A-genome genetic similarities recorded for single accessions of wheats revealed differences, from 3.6% to 25.8% (values with asterisks in Table 2). This finding highlights the necessity of using a congruous number of accessions when similar types of analyses are carried out.

Discussion

The quest for the donor of genome A to the polyploid wheats was settled in the last 20 years based on different approaches (Chapman et al. 1976; Konarev 1983; Waines and Payne 1987; Nishikawa 1983), including those based on molecular markers (Dvorak et al. 1988, 1993; Takumi et al. 1993). The available results indicate that *T. urartu* is the genome A progenitor of polyploid wheats, a case of paternal contribution (Provan et al. 2004).

On the other hand, several studies indicate a high level of similarity between the chromosomes of *T. monococcum* and *T. aestivum* (Dubcovsky et al. 1995; Luo et al. 2000), suggesting that A^u and A^m genomes, notwithstanding their high sterility when crossed, have not diverged very significantly since their separation from a common progenitor. A quantification of their difference, however, has not yet been provided.

AFLPs are a reliable and powerful method for the detection of DNA polymorphisms because they are highly reproducible, show a high multiplex ratio (Powell et al. 1996), and allow genome-wide scanning of genetic variation (Heun et al. 1997; Badr et al. 2000). This is not the case of sequence-based studies, which are limited to restricted genome regions having mutation rates that may vary widely.

AFLPs have been included among "discontinuous" DNA markers (Martin and Salamini 2000): they describe variations of a DNA sequence scattered along the genome, whereas, upon comparison of gene sequences, the nucleotides considered are contiguous. The capacity of AFLP to portray nucleotide variation is well recognised (Innan et al. 1999; Mougel et al. 2002), and they provide a measure of π, the genomic nucleotide diversity index of Nei and Li (1979). In this paper, AFLP markers were exploited to assess the differences existing among A genomes of diploid A^m and A^u wheats and their polyploid relatives. In this context, a drawback of AFLPs is the existence of fragment length homoplasy, i.e., the comigration of non-homologous fragments. In barley, it has been demonstrated that different genotypes have comigrating AFLP bands derived from orthologous DNA sequences. In the same *Hordeum* genus, more caution has to be used in interspecific comparisons, but the same criterium can be adopted (Badr et al. 2000). In *Triticum* species, the A, B, D, and G genomes are derived from a common ancestor and, being molecularly similar, they may share the same AFLP bands. This situation raises questions on genome assignment of specific AFLPs.

To elucidate and quantify the relationship between the genomes of diploid and polyploid wheats, only A bands are useful, given that the B genome is common to tetraploid and hexaploid species and that the D genome is present only in hexaploid wheats. *Triticum urartu* and *T. monococcum* have, by definition, only AFLP A bands. The problem arises when polyploid wheats must be fingerprinted and the search is restricted to AFLP A bands. In such a case, our approach to identify specific A bands involved 2 steps: (*i*) in polyploid wheats, only bands comigrating with those of diploid A wheats were considered; (*ii*) bands comigrating with those of the *Ae. tauschii* were discarded as a way to minimise identical-by-descent fragment homoplasies. This procedure reduced the original 423 polymorphic bands, observed in *T. monococcum* and *T. urartu*, as well as in the polyploids, to 239 putative A bands. As a final control, the correct classification of the putative A bands of 'Chinese Spring' was assessed by comparison with its nulli-A-tetrasomics. The results showed that 53% of the 'Chinese Spring' bands were related to the A genome, a satisfactory value considering that only 67% of putative AFLP A bands in our set of hexaploid lines were present in 'Chinese Spring'.

Our results demonstrate that the A^u genome of *T. urartu* is more similar by around 20% to the A genome of AB and ABD wheats, compared with the A^m genome of *T. monococcum*. This difference is much smaller when the comparison with the AG genome of *T. timopheevii* is considered. As

evident from Fig. 1C, the estimate is not affected by the pooling of the hexaploid *T. zhukovskyi* (A^uA^mG genome) with the tetraploid AG wheats. The comparable similarity existing between genome A of the 2 diploids and the same genome from the *T. timopheevii* group might be due to the mode of origin of this last species. Our finding supports the hypothesis that the G genome is more similar, as previously thought, to A genomes.

The AFLP-based phylogenetic tree obtained from the putative A-bands is consistent with the cyto-taxonomical data on species relationships in the genus *Triticum*. *Triticum urartu* and *T. monococcum* subsp. *boeoticum* are sympatric diploid wheats, with a geographical distribution partially overlapping that of wild tetraploids. Accessions of the 2 species were first discriminated by seed protein electrophoretic pattern and based on few morphological characters (Johnson and Daliwal 1976). Since its recognition, *T. urartu* has been suggested as the donor of the A genome to the polyploid wheats. Our results support the obvious conclusion that *T. urartu* and *T. monococcum* are indeed different species. That the A genome of polyploid wheats derives from *T. urartu,* and that only a limited part of the broad variation existing in the diploid genepool is present in the polyploid wheats, is an accessory, confirmatory result of our study.

The 2 AG and 1 AAG genome accessions group far from all of the other species. *Triticum timopheevii* and *T. zhukovskyi* wheats are still cultivated in a limited area of the Caucasus. They are probably the result of a limited number of hybridisations generating small gene pools. Unpublished results of our laboratory show, as previously suggested (Zohary and Hopf 2000), that AG domesticated wheats derive from *T. araraticum*, but fail to prove where geographically this event has taken place. This leaves some uncertainties on the putative donor of the G genome, because of the missing correlation between the geographical distribution of putative wild donors and a sample of domesticated accessions. Measures of genomic similarity provided in this paper open the discussion on a testable hypothesis: is *T. araraticum* derived from interspecific crosses between *T. monococcum* subsp. *boeoticum* and *T. urartu*?

Triticum aestivum subsp. *spelta*, considered the transition form of naked hexaploid wheats (Mc Fadden and Sears 1946), was later reported to have a more recent appearance in fossil records. In our phylogenetic trees, this wheat has a topology intermediate between hexaploid and tetraploid wheats. Molecular fingerprinting of spelts from Italy and Germany strengthens the hypothesis of Liu and Tsunewaki (1991) and Dvorak and Luo (2001), which propose the introgression of non-free-threshing emmer genes into *T. aestivum* subsp. *aestivum* as the most likely origin of European spelts.

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