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Genetic variability of the wild diploid wheat *Triticum urartu* revealed by RFLP and RAPD markers

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Abstract Genetic variability among 49 accessions of *Triticum urartu* was estimated by RFLP and RAPD marker analyses, and the two data sets were compared. One *T. timopheevii* accession and two accessions of *T. durum* and *T. aestivum*, respectively, were included to identify *T. urartu* accessions closely related to these polyploid wheats. Twenty eight RFLP clones and 29 RAPD primers generated 451 and 155 polymorphic bands, respectively. The three accessions from Armenia clustered together and were well separated from all other accessions, which showed less pronounced geographical patterns. Genetic similarity and co-phenetic values calculated with RAPD markers were very similar to those calculated with RFLP markers for the intraspecific comparisons, but not for the interspecific comparisons. The identification of individual *T. urartu* accessions which are more related to polyploid wheats than others was not possible.

Key words *Triticum urartu* · Wheat · A genome · RFLP · RAPD · Genetic variability

Introduction

Triticum urartu Tum. is a wild diploid wheat first described in 1938 as a rare endemic species of Transcaucasia (Jakubizner 1959). Nevertheless, several *T. urartu* accessions were subsequently found in Turkey, Lebanon, Iraq and Iran by Johnson (1975), who demonstrated that this species is sympatric with the wild tetraploid wheats. Initially, morphological, electrophoretical and cytogenetical observations suggested that *T. urartu* is the donor of the B genome of polyploid wheats (Dhaliwal and Johnson 1976; Johnson and Dhaliwal 1978). Dvorák (1976) and Chapman et al. (1976), however, on the basis of the pairing behaviour of the different chromosome sets, showed that *T. urartu* is the donor of the A genome of polyploid wheats like *T. durum* and *T. aestivum*. This was also supported by analysing the polymorphisms of repeated nucleotide sequences (Dvorák et al. 1988, 1993). Further indications in this respect were obtained from RFLP data when examining the relative genetic distance between a few *T. urartu* accessions and different polyploid wheats (Takumi et al. 1993; D'Ovidio et al. 1994).

The *T. urartu*-specific traits are small anthers, a prominent second tooth on the sterile glume, a two-awned spikelet, a pubescent leaf and reddish kernels. These traits make it possible to distinguish this species from *T. boeoticum*, a second diploid A-genome wheat (Kimber and Feldman 1987; Morrison 1993). In *T. urartu* collections additional genetic variability has been observed for several seed characteristics (Waines et al. 1987; Blanco et al. 1994), for storage-protein electrophoretic variants (Waines and Payne 1987), for isozymes (Smith-Huerta et al. 1989; Nishikawa et al. 1992) and for different DNA molecular markers (Vierling and Nguyen 1992; Dhaliwal et al. 1993; Castagna et al. 1994; Le Corre and Bernard 1995). The aim of the present work was: (1) to evaluate, by RFLP (restriction fragment length polymorphism) and RAPD (random amplified polymorphic DNA) marker techniques, the genetic variability present in a wider *T. urartu* collection consisting of 49 accessions sampled from different geographic areas, (2) to identify individual *T. urartu*

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accessions more related to polyploid wheats, and (3) to compare the results obtained using RFLPs with those using RAPDs in order to establish the reliability of the two methods when calculating genetic distances.

Materials and methods

Plant material

Forty nine *T. urartu* (2n = 2x = 14, AA) accessions obtained from four germ plasm collections were analysed (Table 1). One accession of *T. timopheevii* (2n = 4x = 28, AAGG) and two accessions each of durum wheat (*T. durum*, 2n = 4x = 28, AABB, cvs *Creso* and *Primadur*) and of bread wheat (*T. aestivum*, 2n = 6x = 42, AABBDD, cvs *Alpe 1* and *Chinese Spring*) were also included.

Field observation

The 49 *T. urartu* accessions, together with five *T. boeoticum* accessions, were planted in one-row plots 1.5-m long in an experimental farm near S.Angelo Lodigiano (Italy) on October 21, 1993. Morphological traits were observed on five representative main tillers of each accession. The length of the anther and of the awn of the second floret were scored on the central spikelets, 5 days after heading, which occurred between May 22 and June 10, 1994.

DNA isolation and RFLP procedures

DNA was isolated from freeze-dried leaf tissue, which was harvested from about 15 plants per accession (according to a modified CTAB procedure, Murray and Thompson 1980). For Southern blotting, 6–8 μg of DNA were digested with *Bam*HI, *Eco*RI and *Hin*dIII (Boehringer Mannheim, Germany) and by using two units of the enzyme per μg DNA. DNA fragments were separated on 1% agarose gels and alkaline blotted onto Hybond-N^+ nylon membranes (Amersham, Buckingham, UK) for 12–15 h. Probes were labelled with ^{32}P by random priming (Feinberg and Vogelstein 1983). Pre-hybridizations and hybridizations were performed overnight at 65°C in a solution containing 6 × SSC, 0.5% SDS, 5 × Denhart and 100 μg/ml of calf thymus DNA (Sambrook et al. 1989). Membranes were washed at the stringency described by Castagna et al. (1994). X-ray films were exposed at − 80°C for 7–10 days.

RFLP clones

Twenty eight RFLP clones (Table 2) defining DNA polymorphisms on all seven chromosomes of the *Triticeae* genome were chosen. Genomic DNA clones originate from bread wheat (WG, Heun et al. 1991; TAG, Liu and Tsunewaki 1991; PSR, Devos and Gale 1993) as well as from *T. tauschii* (KSU, Gill et al. 1991). A cDNA clone of bread wheat, PSR 128, was supplied by Sharp et al. (1989). In addition, four storage protein-encoding DNA sequences were used for RFLP analysis.

RAPD procedures

PCR reactions were performed in a volume of 25 μl combining 20 ng of genomic DNA, 200 μM each of the dNTPs, 0.2 μM of primer, 1 unit of *Taq* DNA polymerase (Perkin Elmer, Norwalk, USA), 1.5 mM of $MgCl_2$ and 1 × *Taq* DNA polymerase buffer (supplied with the polymerase). Twenty nine random primers (Operon Technologies Inc., Alameda, USA) were tested (Table 3). Amplifications were carried out in a Perkin Elmer GeneAmp PCR System 9600 as follows: 1 cycle of 1 min at 94°C followed by 44 cycles of 1 min at

Table 1 List of the accessions used for RFLP and RAPD analyses

Code	Species	Accession	Origin	Source[a]
1	*T. urartu*	HTRI 6735/83	Armenia	IGK
2	*T. urartu*	MG 26997	Armenia	IDG
3	*T. urartu*	MG 29386	Armenia	IDG
4	*T. urartu*	G3246	Lebanon	KSU
5	*T. urartu*	101005	Lebanon	AFRC
6	*T. urartu*	MG 26994	Lebanon	IDG
7	*T. urartu*	G3221	Iran	KSU
8	*T. urartu*	MG 4256	Iran	IDG
9	*T. urartu*	MG 26992	Iraq	IDG
10	*T. urartu*	MG 29800	Iraq	IDG
11	*T. urartu*	101004	Turkey	AFRC
12	*T. urartu*	MG 27001	Turkey	IDG
13	*T. urartu*	MG 27004	Turkey	IDG
14	*T. urartu*	MG 29404	Turkey	IDG
15	*T. urartu*	MG 29405	Turkey	IDG
16	*T. urartu*	MG 29406	Turkey	IDG
17	*T. urartu*	MG 29408	Turkey	IDG
18	*T. urartu*	MG 29409	Turkey	IDG
19	*T. urartu*	MG 29410	Turkey	IDG
20	*T. urartu*	MG 29414	Turkey	IDG
21	*T. urartu*	MG 29415	Turkey	IDG
22	*T. urartu*	MG 29422	Turkey	IDG
23	*T. urartu*	MG 29423	Turkey	IDG
24	*T. urartu*	MG 29424	Turkey	IDG
25	*T. urartu*	MG 29425	Turkey	IDG
26	*T. urartu*	MG 29426	Turkey	IDG
27	*T. urartu*	MG 29427	Turkey	IDG
28	*T. urartu*	MG 29431	Turkey	IDG
29	*T. urartu*	MG 29434	Turkey	IDG
30	*T. urartu*	MG 29453	Turkey	IDG
31	*T. urartu*	MG 29465	Turkey	IDG
32	*T. urartu*	MG 29468	Turkey	IDG
33	*T. urartu*	MG 29469	Turkey	IDG
34	*T. urartu*	MG 29478	Turkey	IDG
35	*T. urartu*	MG 29495	Turkey	IDG
36	*T. urartu*	MG 29497	Turkey	IDG
37	*T. urartu*	MG 29498	Turkey	IDG
38	*T. urartu*	MG 29499	Turkey	IDG
39	*T. urartu*	MG 29500	Turkey	IDG
40	*T. urartu*	MG 29501	Turkey	IDG
41	*T. urartu*	MG 29502	Turkey	IDG
42	*T. urartu*	MG 29503	Turkey	IDG
43	*T. urartu*	MG 29504	Turkey	IDG
44	*T. urartu*	MG 29505	Turkey	IDG
45	*T. urartu*	MG 29506	Turkey	IDG
46	*T. urartu*	MG 29507	Turkey	IDG
47	*T. urartu*	MG 29508	Turkey	IDG
48	*T. urartu*	MG 29509	Turkey	IDG
49	*T. urartu*	MG 29511	Turkey	IDG
50	*T. timopheevii*	W1899		ISC
51	*T. durum*	Creso		
52	*T. durum*	Primadur		
53	*T. aestivum*	Alpe 1		
54	*T. aestivum*	Chinese Spring		

[a] IGK Institut für Genetik and Kulturpflanzenforshung, Gatersleben, Germany; IDG Istituto del Germoplasma, Bari, Italy; KSU Kansas State University, Manhattan, USA; AFRC Cambridge Laboratory, Norwich, England; ISC Istituto Sperimentale per la Cerealicoltura, Roma, Italy

Table 2 List of the RFLP clones used

Clone	Chromosome location[a]
pTag 1290	1AL, 1BL, 1DL
TAG 368	1B
TAG 365	1B
pTag 1436	1AS, 1BS, 1DS
pLMWTG2	1AS, 1BS, 1DS
WG 996	2H
KSU H9	2D
PSR 666	2AS, 2BS, 2DS
WG 178	3HL
WG 940	3HL
TAG 364	3B
TAG 366	3A
WG 464	4HL
WG 114	4HL
TAG 367	4A
WG 622	4HS
PSR 128	5AL, 5BL, 5DL
WG 541	5H
WG 364	5H
TAG 385	5A, 6A
PSR 915	6AL, 6BL, 6DL
TAG 387	6A
PSR 627	6AS, 6BS, 6DS
pTU1	6AS, 6BS, 6DS
WG 420	7HL
WG 686	7HL
TAG 361	7A, 7B, 7D
WG 719	7HS

[a] According to: Bartels et al. (1986) for the pTAG 1436 clone, which corresponds to *Gli-1*; D'Ovidio et al. (1992) for the pTU1 clone, which corresponds to *Gli-2*; Devos and Gale (1993) for the PSR clones; Gill et al. (1991) for the KSU H9 clone; Heun et al. (1991) for the WG clones; Liu and Tsunewaki (1991) for the TAG clones; Sabelli and Shewry (1991) for the pLMWTG2 clone, which corresponds to *Gli-1/Glu-3*; Thompson et al. (1983) for the pTAG 1290 clone, which corresponds to *Glu-1*

Table 3 List of the random primers used

Primer	Sequence (5′–3′)
OPA-01	CAGGCCCTTC
OPA-02	TGCCGAGCTG
OPA-03	AGTCAGCCAC
OPA-04	AATCGGGCTG
OPA-05	AGGGGTCTTG
OPA-07	GAAACGGGTG
OPA-08	GTGACGTAGG
OPA-09	GGGTAACGCC
OPA-10	GTGATCGCAG
OPA-11	CAATCGCCGT
OPA-12	TCGGCGATAG
OPA-13	CAGCACCCAC
OPA-14	TCTGTGCTGG
OPA-16	AGCCAGCGAA
OPA-18	AGGTGACCGT
OPA-19	CAAACGTCGG
OPA-20	GTTGCGATCC
OPB-03	CATCCCCCTG
OPB-06	TGCTCTGCCC
OPB-17	AGGGAACGAG
OPB-18	CCACAGCAGT
OPJ-09	TGAGCCTCAC
OPJ-10	AAGCCCGAGG
OPJ-15	TGTAGCAGGG
OPM-02	ACAACGCCTC
OPM-07	CCGTGACTCA
OPM-08	TCTGTTCCCC
OPM-09	GTCTTGCGGA
OPM-10	TCTGGCGCAC

94°C, 1 min at 36°C and 1 min at 72°C, followed by a final cycle of 8 min at 72°C. Amplification products were analysed on 1.2% agarose gels run at 100 V for 3 h in TAE buffer (Sambrook et al. 1989) and stained with ethidium bromide. Reproducibility of amplification profiles was tested for each primer on a random sample of five accessions; only clear and stable bands were considered.

Analysis of data

For each genotype, the presence of a band (1) or its absence (0) was entered in a computer file as a binary matrix for all the markers (RFLP and RAPD). Similarities for pairwise accessions were calculated using Jaccard's coefficient. Similarity matrices based on different marker types were compared using the Mantel matrix-correspondence test (Mantel 1967). Since the dimension of the two similarity matrices was $[n*(n-1)]/2$, where n = number of accessions analysed, the Mantel test was performed on 1431 pairs of distance evaluation. Cluster analyses were computed on similarity values using the unweighted pair-group method with arithmetical averages (UPGMA) and the resulting clusters were represented as dendrograms. Estimates of the differences between the two dendrograms, one from RFLP and one from RAPD data, were obtained by computing the co-phenetic values and constructing the relative co-phenetic matrix for each marker type. The co-phenetic matrices were compared using the Mantel matrix-correspondence test. The multivariate analyses were performed employing the NTSYS-pc package, version 1.8 (Rohlf 1993).

Results

All 49 *T. urartu* accessions were grown under field conditions to verify the typical traits of this species (Kimber and Feldman 1987; Morrison 1993). All accessions had a well-developed awn on the second floret of the spikelet and a small second tooth on the outer glumes. Leaf pubescence and short anthers (1.5–3.0 mm long) characterised all accessions and clearly distinguished them from the five *T. boeoticum* accessions grown under the same conditions. These five *boeoticum* accessions had longer hairs on the leaves and longer anthers (about 5.0 mm). Since all 49 *T. urartu* accessions showed the typical morphological traits, they were considered for the molecular-marker analyses.

The RFLP analysis was based on 28 clones distributed throughout the *Triticeae* genome. Three different restriction enzymes were used to give a total of 44 clone/enzyme combinations which generated 451 polymorphic fragments. The number of fragments detected per clone/enzyme combination ranged from 2 (TAG

Table 4 Comparison of genetic similarity estimates based on RFLP and RAPD analyses, calculated using Jaccard's coefficient

Species compared	RFLP			RAPD		
	Mean	Min	Max	Mean	Min	Max
Among *T. urartu*	0.727	0.477	1.000	0.718	0.423	0.982
T. timopheevii vs *T. urartu*	0.159	0.124	0.219	0.263	0.211	0.356
T. durum vs *T. urartu*	0.161	0.135	0.184	0.300	0.218	0.360
T. aestivum vs *T. urartu*	0.135	0.110	0.165	0.304	0.236	0.363

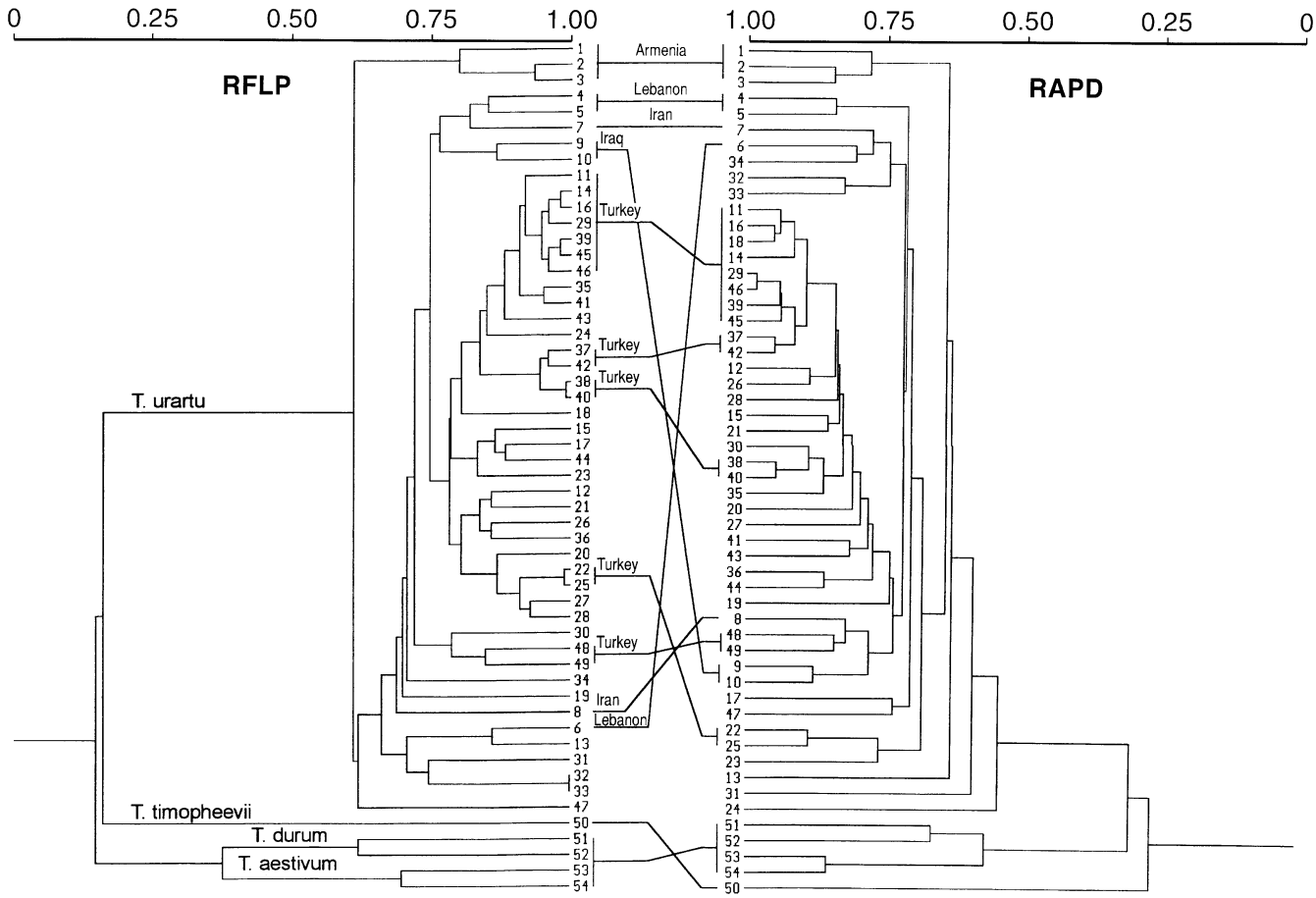

Fig. 1 Comparison of the two dendrograms obtained with RFLPs and RAPDs for the analysed 54 *Triticum* spp. accessions

366/*Bam*HI and WG 464/*Bam*HI) to 27 (WG 420/*Hin*dIII), with an average number of 10.3. When the clone pLMWTG2, corresponding to the *Gli-1*/*Glu-3* genetic locus, was combined with *Eco*RI it revealed the highest number of unique banding patterns and distinguished 35 out of the 54 accessions considered. A total of 161 amplification products was observed using 29 RAPD primers. Primers OPA-02, 05, 13 and OPM-08 amplified 2, 1, 2 and 1 monomorphic bands, respectively. Therefore, 155 polymorphic RAPD bands were obtained. The average number of polymorphic bands per primer was 6.2 with a range from 2 (OPA-03, 18 and OPM-10) to 13 (OPB-03 and OPJ-15). The size of the amplified products ranged from 0.3 to 2.8 kb. RAPD markers were effective in distinguishing all 54 accessions while RFLP patterns failed to distinguish two *T. urartu* accessions (i.e. 32 and 33).

Among the *T. urartu* accessions, the coefficient of similarity ranged from 0.477 to 1.000 (accessions 32 and 33 were identical) for the RFLPs, and from 0.423 to 0.982 for the RAPDs (Table 4). The two methods revealed the same range of similarity values (0.523 and 0.559) and a very similar average genetic distance (0.727 and 0.718) for the *T. urartu* accessions. The average RFLP genetic similarity was 0.159 between *T. timopheevii* and *T. urartu*, 0.161 between *T. durum* and *T. urartu*, and 0.135 between *T. aestivum* and *T. urartu* for the RFLP data, whereas for the RAPD data the

corresponding values were 0.263, 0.300, and 0.304, respectively. Therefore, contrary to what we observed for the intraspecific comparisons, the interspecific RAPD estimates were twice as large as those calculated by RFLPs (Table 4). With both the markers, genetic similarity values for the interspecific comparisons were not very different among the different *T. urartu* accessions considered (see min and max genetic similarity values in Table 4). Moreover, the three polyploid species were so distant to all *T. urartu* accessions, that the identification of a more related accession among the *T. urartu* was not possible.

Cluster analysis (UPGMA) was used to generate two independent dendrograms for the two types of molecular markers (Fig. 1). As expected, in both dendrograms the accessions were grouped according to species. The relative position of the *T. timopheevii* accession, however, was slightly different. Among the *T. urartu* accessions several clusters were recognised. Some of them were maintained in the same position in the two dendrograms. Accessions 1, 2 and 3, collected in Armenia, clustered together and were well separated from the remaining accessions in both dendrograms. Only two accessions from Lebanon (4 and 5), as well as the two originating from Iraq (9 and 10), grouped closely. The Lebanese accessions maintained the same relative position in the two dendrograms. Accession 6, although originating from Lebanon, did not cluster with the others Lebanese accessions 4 and 5. Accessions 7 and 8, both originating from Iran, did not group together. Among the Turkish accessions one large cluster including the accessions 11, 14, 16, 29, 39, 45 and 46 was generated with both DNA markers. The Turkish accessions 18, 37 and 42 clustered within this group based only on RAPD data. In several cases a close relationship between two accessions was observed in both dendrograms, i.e. accession 37 resulted similar to 42, 38 to 40, 22 to 25, 48 to 49.

To compare RFLP with RAPD data, the correlation between the Jaccard's coefficients of similarity obtained with both data sets was calculated (data not shown). A significant but not very high correlation ($r = 0.575$ $P < 0.01$) was obtained for the intraspecific comparisons only. Interspecific correlations (*T. aestivum* versus *T. durum* accessions and diploid versus polyploid species) were not significant. To verify this, we also analysed the correlation between the matrices of co-phenetic values from the two dendrograms shown in Fig. 1. Again, a significant correlation was evident only for the intraspecific comparisons ($r = 0.586$ $P < 0.01$, data not shown), but not for the remaining comparisons.

Discussion

Vierling and Nguyen (1992) analysed six *T. urartu* accessions with RAPDs and found a range of similarity of the Jaccard's coefficient of 0.32–0.68, which is lower than the range reported here for the RAPDs (0.423–0.982). An even lower range (0.63–0.83) was found by D'Ovidio et al. (1994) when studying three genotypes by RFLP analysis. All this can be explained by the larger number of accessions considered here, which was the initial reason for performing this study.

In respect of the geographical origin of the *T. urartu* germ plasm considered here, we found a strong genetic diversity between the three Armenian accessions on one hand and the remaining accessions on the other. In contrast, Vierling and Nguyen (1992) found large genetic distances between one accession from Iraq and their remaining five accessions. Le Corre and Bernard (1995), analysing seven accessions (none from Iraq), found that those from Armenia clustered together with those from Lebanon, whereas those from Turkey grouped together with one from Iran. We found only minor differences among the *T. urartu* accessions from Turkey, Iraq, Iran and Lebanon. Further analyses with even larger collections of *T. urartu* are needed to draw more precise conclusions about the geographical differentiation of *T. urartu*. This should be done by considering also accessions of the other diploid A-genome wheats like *T. monococcum* and *T. boeoticum* (work in progress).

It was not possible with either RAPDs or RFLPs to identify a *T. urartu* accession which was more similar to the polyploid wheats than the others. As discussed by Mori et al. (1995), the presence of more than one genome in the polyploid wheat species generates additional RFLP or RAPD bands/products. Thus, the genetic distance between diploid and polyploid species is over-estimated and differences within species might become less pronounced. An alternative method was applied by Dvorák et al. (1993) to demonstrate that *T. urartu* is the A-genome donor of polyploid wheat species. In this case the variation in repeated nucleotide sequences was assessed among different *Triticum* species and the "repeated sequence correspondence index" was calculated. This index considers only the number and the hybridization intensity of electrophoretic bands observed in the diploid wheats which are also present in the polyploids. The method, however, was not used to identify which of the 14 *T. urartu* accessions considered were more closely related to the polyploid species (Dvorák et al. 1993). Finding individual A-genome wheats closely related to polyploid wheats would be a significant contribution to phylogenetical studies of the *Triticeae*.

In several recent studies, fingerprints based on RFLPs and RAPDs were compared using genotypes from different species. In general, e.g. in *Brassica* by Dos Santos et al. (1994), a satisfactory level of agreement was found between data from the two markers. This indicates that RAPDs alone could be used for this type of analysis, which is very convenient considering

the ease of RAPD experiments. However, Vierling et al. (1994) found that RFLP data are more informative than RAPDs in *Sorghum*. N'Gorang et al. (1994) obtained particular information when using RAPDs or RFLPs in cocoa; in fact, genotypes which had the same geographical origin were grouped together when analysed with RAPDs, whereas some of them clustered apart with RFLPs. The data presented in the present paper, indeed, indicate that RAPDs overestimated the genetic similarity when interspecific comparisons were considered, because RAPDs give twice larger similarity estimates than RFLPs. Similar results were found by Thorman et al. (1994) who compared RFLPs and RAPDs for analysing genetic relationships in different cruciferous species. According to their hypothesis, this "overestimation effect" observed when analysing different species with RAPDs might occur, since bands with similar mobility may represent different DNA sequences. Therefore, non-homologous amplification products may be erroneously considered identical. This fact has also been stressed by Bachmann (1994). Our results indicate that this error is more frequent when distantly related accessions or genotypes are compared. Nevertheless, the high correlation between similarities within a species, here *T. urartu*, justify the use of RAPDs for detecting genetic variation easily. More advanced methods (like AFLP markers, Vos et al. 1995) might offer new alternatives and could make the analysis of larger collections more efficient.

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References

Bachmann K (1994) Molecular markers in plant ecology. New Phytol 126:403–418

Bartels D, Altosaar I, Harberd NP, Barker RF, Thompson RD (1986) Molecular analysis of γ-gliadin gene families at the complex *Gli-1* locus of bread wheat (*T. aestivum* L.). Theor Appl Genet 72:845–853

Blanco A, Zacheo S, Antonacci M, Tribuzio G, Bufano AG (1994) Frumenti diploidi selvatici e miglioramento della qualità del frumento duro. In: Di Fonzo N, Ronga G (eds) La durogranicoltura quale fattore di sviluppo per l'agricoltura meridionale: ricerca di base e analisi di mercato Camera di commercio. Foggia, pp 101–115

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

Chapman V, Miller TE, Riley R (1976) Equivalence of the A genome of bread wheat and that of *T. urartu*. Genet Res 27:69–76

D'Ovidio R, Tanzarella OA, Porceddu E (1992) Isolation of an alpha-type gliadin gene from *Triticum durum* Desf. and genetic polymorphism at the *Gli-2* loci. J Genet Breed 46:41–48

D'Ovidio R, Tanzarella OA, Cenci A, Iacono E, Porceddu E (1994) RFLP analysis in wheat. Isolation and chromosomal assignment of digoxigenin-labelled clones. J Genet Breed 48:73–80

Devos K, Gale M (1993) The genetic maps of wheat and their potential in plant breeding. Outlook Agric 22: 93–99

Dhaliwal HS, Johnson BL (1976) Anther morphology and the origin of the tetraploid wheats. Am J Bot 63:363–368

Dhaliwal HS, Sidhu JS, Minocha JL (1993) Genetic diversity in diploid and hexaploid wheats as revealed by RAPD markers. Crop Improv 20:17–20

Dos Santos JB, Nienhuis J, Skroch P, Tivang J, Slocum MK (1994) Comparison of RAPD and RFLP genetic markers in determining genetic similarity among *Brassica oleracea* L. genotypes. Theor Appl Genet 87:909–915

Dvorák J (1976) The relationship between the genome of *Triticum urartu* and the A and B genomes of *Triticum aestivum*. Can J Genet Cytol 18:371–377

Dvorák J, McGuire PE, Cassidy B (1988) Apparent sources of the A genomes of wheats inferred from polymorphism in abundance and restriction fragment length of repeated nucleotide sequences. Genome 30:680–689

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

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction fragments to a high specific activity. Anal Biochem 132:6–13

Gill KS, Lubbers EL, Gill BS, Raupp WJ, Cox TS (1991) A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). Genome 34:362–374

Heun M, Kennedy AE, Anderson JA et al. (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34:437–447

Jakubizner MM (1959) New wheat species. In: Jenkins BC (ed) Proc 1st Int Wheat Genet Symp. The Public Press Limited, Winnipeg, pp 207–217

Johnson BL (1975) Identification of the apparent B-genome donor of wheat. Can J Genet Cytol 17:21–39

Johnson BL, Dhaliwal HS (1978) *Triticum urartu* and genome evolution in the tetraploid wheats. Am J Bot 65:907–918

Kimber G, Feldman M (1987) Wild wheat: an introduction. Special reports 353, University of Missouri, Columbia, Missouri, USA

Le Corre V, Bernard M (1995) Assessment of the type and degree of restriction fragment length polymorphism (RFLP) in diploid species of the genus *Triticum*. Theor Appl Genet 90: 1063–1067

Liu YG, Tsunewaki K (1991) Restriction fragment length polymorphism (RFLP) analysis in wheat. II. Linkage maps of the RFLP sites in common wheat. Jpn J Genet 66:617–633

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220

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

Morrison LA (1993) *Triticum-Aegilops* systematics: taking an integrative approach. In: Damania AB (ed) Biodiversity and wheat improvement. John Wiley and Sons, Chichester, pp 59–66

Murray M, Thompson WF (1980) Rapid isolation of high-molecular-weight plant DNA. Nucleic Acids Res 8:4321–4325

N'Goran JAK, Laurent V, Risterucci AM, Lanaud C (1994) Comparative genetic diversity studies of *Theobroma cacao* L. using RFLP and RAPD markers. Heredity 73:589–597

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

Rohlf FJ (1993) NTSYS-pc. Numerical taxonomy and multivariate analysis system: version 1.8. Applied Biostatistics, New York

Sabelli PA, Shewry PR (1991) Characterization and organization of gene families at the *Gli-1* loci of bread and durum wheats by restriction fragment analysis. Theor Appl Genet 83 : 209–216

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbour, New York

Sharp PJ, Chao S, Desai S, Gale MD (1989) The isolation, characterization and application in the *Triticeae* of a set of wheat RFLP probes identifying each homoeologous chromosome arm. Theor Appl Genet 78 : 342–348

Smith-Huerta N, Huerta AJ, Barnhart D, Waines JG (1989) Genetic diversity in wild diploid wheats *Triticum monococcum* var. *boeoticum* and *T. urartu* (Poaceae). Theor Appl Genet 78 : 260–264

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

Thompson RD, Bartels D, Harberd NP, Flavell RB (1983) Characterization of the multigene family coding for HMW glutenin subunits in wheat using cDNA clones. Theor Appl Genet 67 : 87–96

Thorman CE, Ferreira ME, Camargo LEA, Tivang JG, Osborn TC (1994) Comparison of RFLP and RAPD markers for estimating genetic relationships within and among cruciferous species. Theor Appl Genet 88 : 973–980

Vierling RA, Nguyen HT (1992) Use of RAPD markers to determine the genetic diversity of diploid wheat genotypes. Theor Appl Genet 84 : 835–838

Vierling RA, Xiang Z, Joshi CP, Gilbert ML, Nguyen HT (1994) Genetic diversity among elite *Sorghum* lines revealed by restriction fragment length polymorphisms and random amplified polymorphic DNAs. Theor Appl Genet 87 : 816–820

Vos P, Hogers R, Bleeker M et al. (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23 : 4407–4414

Waines JG, Payne PI (1987) Electrophoretic analysis of the high-molecular-weight glutenin subunits of *Triticum monococcum*, *T. urartu* and the A genome of bread wheat (*T. aestivum*). Theor Appl Genet 74 : 71–76

Waines JG, Ehdaie B, Barnhart D (1987) Variability in *Triticum* and *Aegilops* species for seed characteristics. Genome 29 : 41–46