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Assessment of the type and degree of restriction fragment length polymorphism (RFLP) in diploid species of the genus Triticum

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Abstract The A genome of the Triticeae is carried by three diploid species and subspecies of the genus *Triticum*: *T. monococcum* ssp. *monococcum, T. monococcum* ssp. *boeoticum*, and T. *urartu*, the A-genome donor of bread wheat. These species carry many genes of agronomic interest, including disease resistances, and may also be used for the genetic mapping of the A genome. The aim of this study was to evaluate the variability present in a sample of 25 accessions representative of this group using RFLP markers. Twenty probes, consisting of genomic DNA or cDNA from wheat, were used in combination with four restriction enzymes. A high level of polymorphism was found, especially at the interspecific level. Selecting the most informative enzymes appeared to be of great importance in order to obtain a stable structure for the diversity observed with only 20 probes. The results are largely consistent with taxonomy and data relating to geographical origins. The probes were also tested on 14 wheat cutivars. A good correlation coefficient was found for their informative values on wheat cultivars and diploid lines. Whether the group of species studied here would be useful for genetic mapping remains to be determined. Nevertheless, RFLP markers will be useful to follow genes that can possibly be introgressed from these species into cultivated wheat.

Key words *T. monococcum* ssp. *monococcum* · *T. monococcum* ssp. *boeoticum* · *T. urartu* · RFLP · Diversity

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Introduction

The wheat genus *Triticum* L. is a complex taxonomic group including diploid, tetraploid and hexaploid species, among which is bread wheat, *Triticum aestivum* L. Em. Thell., the most important cereal crop in the world. As genetic variability among wheat varieties is rather low (Branlard and Chevalet 1984), breeders have increasingly turned to the wild or primitive cultivated *Triticum* species, or else to related genera, in order to introgress new genes. Surprisingly, the two diploid species of the genus *Triticum, T. monococcum* L. (2n=2x=14, AA) and *T. urartu* (2n=2x=14, AA), have rarely been used for breeding programmes, although they possess the A genome in common with tetraploid (2n=4x=28, AABB) and hexaploid (2n=6x=42, AABBDD) cultivated wheats.

T. monococcum L. includes two subspecies. *T. monococcum* ssp. *boeoticum* is a wild form whereas the second subspecies, *T. monococcum* ssp. *monococcum*, is a domesticated form (Bell 1987) grown in Europe and the near East both as a forage grass and for human nutrition (Percival 1921). *T. urartu* has been taxonomically separated from *T. monococcum* ssp. *boeoticum* only relatively recently, after hybrid sterility was established (Johnson and Dhaliwal 1976). Cytogenetic (Dvorak 1976) and biochemical (Nishikawa 1984) data, and a more recent study based on repeated nucleotide sequence polymorphism (Dvorak et al. 1992), established that *T.urartu* was the A-genome donor of all polyploid wheats, while the A genome of *T. monococcum* ssp. *boeoticum* is present only in *T. zhukovskyi* (2n=6x=42, AAAAGG).

T. monococcum ssp. *boeoticum* is widely distributed throughout the eastern Mediterranean, from the Balkans to west Iran and from the Caucasus to south Israel, often as a weed in cultivated cereal fields. The much-more restricted area of *T. urartu* is within that of *T. monococcum* ssp. *boeoticum* (from Turkey to Iran and from Azerbaidjan to Lebanon).

All these species carry many genes of agronomic interest, so that some recent authors have proposed the devel-

opment of modern cultivars of *T. monococcum* (Waines 1983; Vallega 1992). The most attractive of these traits are a good adaptability to poor and dry soils (Waines 1983) and the presence of numerous resistances to diseases (especially rusts and powdery mildew), and to insects (Gill et al. 1983; Perrino and Porceddu 1990).

Moreover, the greater simplicity of diploidy, allied to a presumed greater genetic variability than that observed in polyploid wheats, might be used for an efficient genetic mapping of the A genome: such a project has already been successful for the D genome in the diploid species *T. tauschii* (Gill et al. 1991). The first necessary step is the evaluation of the amount and structure of the genetic variability. Current knowledge concerning *T. monococcum* and *T. urartu* was provided by isozyme markers (Smith-Huerta et al. 1989) and, more recently, by random amplified DNA (RAPD) markers (Vierling and Nguyen 1992).

The aim of the present work was to evaluate the genetic variability available in a representative collection of the three species and subspecies of the group, maintained at the INRA Plant Breeding Station of Rennes (France), using RFLP techniques.

Materials and methods

Plant material

Twenty-five accessions representative of the three taxa *T. monococcum* ssp. *monococcum*, *T.monococcum* ssp. *boeoticum* and *T. urartu* were used (Table 1). Each accession consisted of one line. Fourteen genotypes of bread wheat, *T. aestivum* L. Em. Thell., were also included: Courtot, Cappelle, Magnif 27, Prinqual, Rex, Apollo, Pernel, Thesée, Eureka, Renan, Bezostaja, RE607, RE714 and RE813.

Table 1 Plant material used in this study

T. monococcum ssp. monococcum	
M1	Czechoslovakia
M2	France
M3	Asia Minor
M4	Azerbaïdjan
M5	Albania
M6	Turkey
M7	?
M8	Spain
M9	Greece
M10	Afghanistan
M11	Yugoslavia
T. monococcum ssp. boeoticum	
B1	Asia Minor
B2	Asia Minor
B3	?
B4	Iran
B5	Iran
B6	Iran
B7	Armenia
T. urartu	
U1	Iran
U2	Armenia
U3	Armenia
U4	Turkey
U5	Turkey
U6	Lebanon

Detection of RFLPs

Total DNA was extracted from young leaves following a rapid procedure adapted from Dellaporta et al. (1983). Four restriction enzymes were used: *Eco*RI, *Eco*RV, *Hin*dIII and *Dra*I. DNA fragments were separated by electrophoresis on 0.8% agarose gels, then blotted onto hybond N+ membranes (Amersham).

Twenty probes, consisting of genomic DNA (seven probes) or cDNA (13 probes) from bread wheat, were used; all were obtained from the John Innes Centre , Norwich, England. They were chosen for their distribution over the genome, one or two probes being located on each chromosome arm, except for 5S. Since certain probe/enzyme combinations gave illegible autoradiographs, each of the 20 probes used were combined either with one, two, three or four enzymes. In total, 57 probe/enzyme combinations were employed.

Probes were labelled non-radioactively with Digoxigenin-11-dUTP (Boehringer Mannhein) using a PCR (polymerase chain reaction)-amplification procedure. Probe-DNA hybridizations were performed in sealed plastic bags at 65°C; membranes were then washed and developed at 37°C. All procedures were as described in Lu et al. (1994).

Data handling and analysis

Each fragment detected was treated as a single character. Its presence or absence was scored assuming that fragments migrating identically between two individuals revealed homologies in genomic DNA sequences. Nei's genetic similarities (Nei 1987) between all genotypes were calculated using the Splus (Statistical Sciences Inc., 1992) program. Dendrograms were constructed according to the unweighted pair-group method (UPGMA) using the Hclust procedure in Splus. Standard errors for the nodes were determined following a jackknife procedure (Mosteller and Tukey 1978) by dropping each probe in turn and then re-drawing the corresponding tree.

Values of the polymorphic index were calculated for each probe-enzyme combination. This index was first described by Botstein et al. (1980) as the "polymorphism information content" (PIC), and then often re-used under different names (Graner et al. 1990; Lubbers et al. 1991; Anderson et al. 1993).We used the formula:

$$PICk=n/(n-1)\ (1-\Sigma_j\ p_{jk}^2)$$

where n is the population size and pjk the frequency of the jth pattern revealed by the probe k (combined with one given enzyme).

The value obtained for one probe represents the probability that this probe might detect different patterns between two randomly chosen members of the population under investigation. Mean polymorphic indices were also calculated for each enzyme from the data of every probe used in combination with the enzyme.

Mean genic diversities were estimated within each taxonomic group following Nei (1987).

Results

Each probe/enzyme combination detected between one and three fragments per line, with a mean of 1.16. All 20 probes revealed different patterns among the 25 accessions with at least one enzyme. Thus, 84.2% (48/57) of the probe/enzyme combinations were polymorphic.

The average probe polymorphic index was 0.44 with values ranging from 0 to 0.84 (Table 2). *Dra*I proved to be the most informative enzyme, while *Eco*RI and *Hin*dIII showed the lowest index values.

Table 2 Compilation of probe and enzyme polymorphic index values

	*Eco*RI	*Eco*RV	*Hin*dIII	*Dra*I
PSR161	0.42	0.42	0.42	0.42
PSR596	–	0.00	–	0.42
PSR158	0.00	0.42	–	–
PSR162	–	0.42	0.74	0.42
PSR107	–	0.42	0.42	0.42
PSR135	0.42	–	0.42	0.69
PSR388	–	–	0.00	0.50
PSR1196	0.78	0.73	0.48	0.84
PSR125	0.58	0.44	–	0.66
PSR156	0.42	0.48	0.42	0.68
PSR163	0.42	0.42	0.42	0.44
PSR1051	–	0.72	0.00	0.62
PSR144	–	0.38	0.38	–
PSR360	0.42	0.74	0.00	0.42
PSR370	–	0.62	–	–
PSR141	–	–	–	0.70
PSR463	0.00	0.00	0.72	0.00
PSR103	–	–	0.64	0.72
PSR129	–	0.42	–	0.53
PSR165	0.42	0.42	0.42	0.42

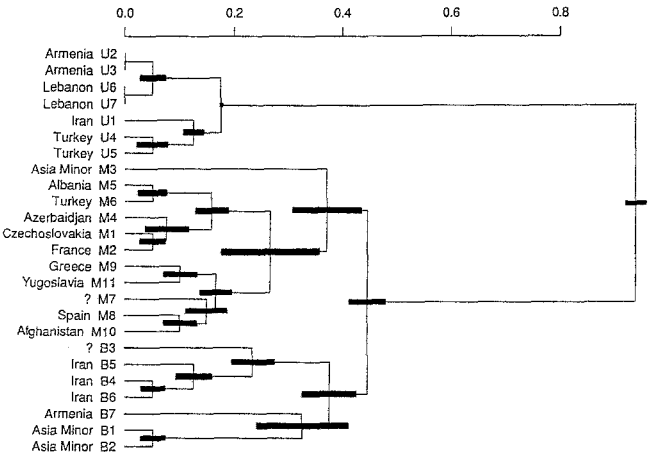

Fig. 1 Dendrogram based on Nei's genetic similarity. *Black bars* represent one 95% confidence interval for the node

Each probe/enzyme combination revealed a mean of 2.84 different patterns among the 25 accessions, but two different classes of clones could be distinguished. Five probes detected variability only at the interspecific level (only two different patterns were revealed, one specific to *T. monococcum*, the other specific to *T. urartu*). Three among those five probes were located on chromosome 1 while the two others were on chromosomes 2 and 7. The remaining 15 probes detected both inter- and intra- specific variability, the mean number of different patterns among the 25 accessions being 3.65 (from two to six). Six probes were noteworthy in that they revealed patterns unique to one particular accession.

Relationship between species, subspecies, and accessions

Selecting the most informative enzymes appeared to be of great importance in order to obtain a stable structure for the diversity observed with a limited number of probes. We found that a set of 20 probes, each combined with one randomly taken enzyme among those used, was not more informative than a sample of only ten probes combined with their most polymorphic enzyme (data not shown). However, when enzymes were thus selected according to their polymorphic index, 20 probes efficiently separated all 20 accessions.

The dendrogram based on Nei's genetic similarity clusters the two species *T. urartu* and *T. monococcum* separately, the latter being clearly divided into two branches corresponding to its two subspecies *monococcum* and *boeoticum* (Fig. 1).

The relationships observed between accessions in *T. uratu* and *T. monococcum* ssp. *boeoticum* are mainly consistent with their geographical origins, since accessions collected in the same country cluster together. Such a correlation was not established for the *T. monococcum* ssp. *monococcum* lines, due to their wide geographical origins.

Mean genic diversity within each taxonomic group

Mean genic diversities were calculated as described by Nei (1987) considering the same 20 loci as above. The values obtained are uniformly low for all species and subspecies, ranging between 0.150 and 0.260. The highest value was for *T. monococcum* ssp. *boeoticum*, the lowest for *T. urartu*. Genetic diversity in the cultivated subspecies *T. monococcum* (0.234) was close to that observed in its wild progenitor *T. boeoticum*.

Genetic similarity between the A genomes of *T. urartu* and *T. monococcum* and the A genome of the wheat cultivar Courtot

Thirteen probes were used to evaluate the proportion of fragments shared by the bread wheat cv Courtot with one or several diploid accessions. Common fragments were revealed for 85% (11/13) of the probe/enzyme combinations. Among the 14 fragments in common, three were common to Courtot and all diploid lines, two were present in lines of *T. monococcum* only, and nine were present in lines of *T. urartu* only. A higher similarity was thus observed between Courtot and *T. urartu* than between Courtot and *T. monococcum*.

Comparative informativeness of clones on diploid lines and on wheat varieties

The total DNAs of 14 bread-wheat cultivars were digested with *Dra*I and hybridised with 15 probes previously used

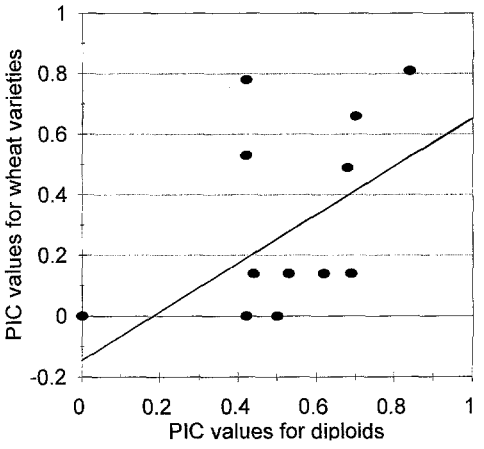

Fig. 2 Regression of the PIC values of the probes tested on 14 wheat cultivars against the PIC values obtained on 25 lines of diploid wheat

also in combination with *Dra*I. Polymorphic indices were calculated as above.

The average index was lower than with diploids, with a value of 0.25. A positive correlation of 0.507 was found between the polymorphic indices calculated on diploid lines and their values obtained on wheat varieties. Some clones having a good value on diploid lines were not very informative on wheat varieties and, conversely, others were very informative on wheat varieties but not on diploid lines (Fig. 2). The results obtained could neither be explained by the nature (gDNA or cDNA) of the clones, nor by the kind of polymorphism (intra- or inter-specific) they detected among the diploid accessions.

Discussion

The taxonomic classification into three species and subspecies among the diploid *Triticum* was strongly confirmed. Whereas species identification is classically based on difficult comparisons of spike morphology, five probes among the 20 tested here are usable for a rapid (though more costly) identification of seed lots, at least among our collection. With 20 probes a stable structure among accessions was obtained since the dendrogram was not strongly modified by deleting each probe in turn, the locations of the main nodes for which confidence intervals overlap being positively correlated when jackknife was applied (data not shown). Moreover, some probes revealed patterns identifying individual accessions. An apparent similarity between genetic distances calculated from RFLP data and geographical proximity was found for most wild accessions, although this result certainly needs to be confirmed on a greater number of accessions.

All these results support the use of RFLP markers for the identification and the management of germ-plasm collections constituted of diploid, autogamous species.

The values of genic diversity that were estimated did not take into account any intrapopulation variability, since each accession was represented by one line. However, previous studies based on isoenzymes (Smith-Huerta et al. 1989) have described very low amounts of genetic diversity within populations of *T. monococum* ssp. *boeoticum* or *T. urartu*. Values obtained in the present study may then be considered as estimators of the actual values; they are consistent with previous results based on isoenzymes or RAPDs, in that low values were found for all species and subspecies, the greatest diversity being observed for *T. monococcum* ssp. *boeoticum* which was already considered as the ancestor (Asins and Carbonel 1986 a).

The bread wheat cv Courtot is a French semi-dwarf cultivar which, like cv Chinese Spring, possesses the primitive genomic structure of bread wheat. Fragments common to Courtot and to all diploid lines probably correspond to conserved sequences from the ancestral A genome, while the higher similarity observed with *T. urartu* confirms that this species, which diverged more recently, is the A-genome donor of bread wheat.

In addition to the analysis of taxonomy and genetic diversity, the second aim of this study was to discuss whether this set of diploid lines might be used to generate a segregating population for an efficient genetic mapping of the A genome. A high level of RFLP was found between *T. urartu* and *T. monococcum*, but interspecific crosses are unusable because of F_1 hybrid sterility, the mechanism of which still remains unexplained (Shang et al. 1988). Nevertheless, the amount of RFLP among pairs associating one genotype of *T. monococcum* ssp. *monococcum* with one of *T. monococcum* ssp. *boeoticum* is still high enough for genetic mapping.

However, the correlation between the values of polymorphism information content (PIC) for the probes on the diploid lines and on the set of wheat cultivars was low. The B genome of hexaploid bread wheat is known to be more variable than either the A or D genomes (Sharp et al. 1989). The probability of having different patterns between two randomly taken wheat varieties may thus not be correlated with any initial variability in the A genome. As a consequence, the clones mapped in *T. monococcum* will not all be informative in breeding populations of polyploid wheats. However, the map will be useful for tagging genes of agronomic interest that might be introgressed from a A-genome diploid wheat into bread or durum wheat.

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