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Use of RAPD markers to determine the genetic diversity of diploid, wheat genotypes *

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Summary. The genetic diversity of two diploid wheat species, *Triticum monococcum* and *Triticum urartu* (2n = 2x = 14), was assessed using random primers and the polymerase chain reaction (PCR). Electrophoretic analysis of the amplification products revealed a higher incidence of polymorphism in *T. urartu* than *T. monococcum*. Pair-wise comparisons of unique and shared polymorphic amplification products, were used to generate Jaccard's similarity coefficients. These were employed to construct phenograms using an unweighted pair-group method with arithmetical averages (UPGMA). The UPGMA analysis indicated a higher similarity among *T. monococcum* than *T. urartu*. Analysis of RAPD data appears to be helpful in determining the genetic relationships among genotypes.

Key words: RAPD – Genetic diversity – Phenograms — *Triticum monococcum* – *Triticum urartu*

Introduction

Williams et al. (1990) described an assay based on the amplification of random DNA segments with short primers of arbitrary nucleotide sequence. They have named these DNA markers RAPD for Random Amplified Polymorphic DNA. These short primers were used to reproducibly amplify segments of genomic DNA from a wide variety of species (Williams et al. 1990; Caetano-Anilles et al. 1991; Martin et al. 1991). Their results show that single primers can be used to amplify genomic DNA and that polymorphisms can be detected between the amplification products of different individuals.

Polymorphism between individuals can arise through: (1) nucleotide changes that prevent amplification by introducing a mismatch at one priming site; (2) deletion of a priming site; (3) insertions that render priming sites too distant to support amplification; and (4) insertions or deletions that change the size of the amplified product (Williams et al. 1990). This type of polymorphism makes RAPD markers well suited for studies of genetic diversity, genetic relationships, genetic mapping, plant breeding, DNA fingerprinting, and population genetics.

RFLP mapping in *Triticum aestivum* (2n = 6x = 42) has been hampered by low levels of intervarietal polymorphism, complexities arising from polyploidy, and the large genome size (Gale et al. 1990). Because of these complications, we chose to study *Triticum monococcum* and *Triticum urartu* (A genome, 2n = 2x = 14). The objectives of this study were to determine the extent of inter- and intra-specific genetic diversity using RAPD markers and thus establish the relationships of genotypes within each species.

Materials and methods

Plant material

Seven genotypes of *T. monococcum* (2n = 2x = 14, A genome, M1, M2, M3, M4, M6, M7, M9, M10) and six genotypes of *T. urartu* [2n = 2x = 14, A genome, U23 (Lebanon), U24 (Lebanon), U26 (Turkey), U27 (Iran), U28 (Armenia), U29 (Iraq)] were kindly provided by Dr. B. S. Gill (Kansas State University). Seeds were planted in the greenhouse and all plants were selfed for seed increase.

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DNA isolation and PCR amplification

DNA was isolated from fresh leaves according to Sambrook et al. (1989). Amplification reactions contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 2×10^{-5} M tetramethyl ammonium chloride (TMAC) (Wood et al. 1985), 200 µM each of dATP, dCTP, dGTP and dTTP, 60 ng primer (random 10-mers, Kits A, F and P, Operon Technologies, Alameda, Calif.), 100 ng genomic DNA and 0.5 units *Taq* DNA polymerase (Perkin Elmer Cetus). DNA amplification was performed in a Perkin Elmer Cetus DNA Thermal Cycler programmed for 43 cycles of 1 min at 95 °C, 1 min at 37 °C, 1.5 min at 72 °C, using the fastest available temperature transition. Amplification products were analyzed by electrophoresis in 1.4% agarose gels and visualized by ethidium bromide staining.

Data analysis

For each individual amplification, the most cathodal product was designated a and subsequent products were designated b, c, d, and so on. Data were scored on the presence or absence of amplification products. If a product was present in a genotype it was designated 1, if no shared product was present in other genotypes they were designated 2. This type of scoring was done for each amplification product across all genotypes within a species.

Pair-wise comparisons of genotypes, based on both unique and shared polymorphic products, were used to generate similarity coefficients (Jaccard 1908). These were used to construct phenograms using the unweighted pair-group method with arithmetical averages (UPGMA) employing the Numerical Taxonomy and Multivariate Analysis System, version 1.21 (Applied Biostatistics, Inc.).

Results and discussion

The PCR has proved to be a powerful tool for the identification of polymorphism in cereals. Using wheat, barley, rye and wheat-barley addition lines, Weining and Langridge (1991) detected polymorphism using conserved, semi-random and random primers. With different combinations of primers, they were able to detect both inter-and intra-specific diversity.

In our study, products of 0.3 to 4.0 kilobase pairs were amplified by the 60 random primers tested (Fig. 1). In *T. monococcum*, 41 amplified products showed polymorphism and 62 products were shared among all genotypes. In *T. urartu* 73 products showed polymorphism and 53 products were shared among all genotypes. Even though we screened two fewer genotypes in *T. urartu*, there were 32 more polymorphic amplification products in *T. urartu* than in *T. monococcum*. In addition to the overall greater number of polymorphic products, genotypes of *T. urartu* had almost double the number of unique products compared to *T. monococcum* genotypes. The *T. urartu* genotypes also had a greater number of unique products than those of *T. monococcum*. In *T. urartu* there was a large range in the number of unique products from none in U28 to 12 in U29. Genotypes U23, U25 and U26 had one unique product and U27 had

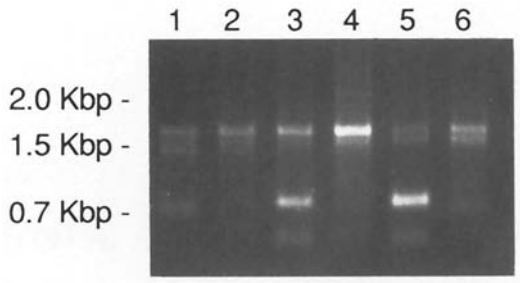

Fig. 1. Ethidium bromide-stained agarose gel of amplification products. (*1*) U23 (*2*) U25 (*3*) U26 (*4*) U27 (5) U28 (*6*) U29

three. In addition to the unique products, there were 55 polymorphic products that were shared among the various genotypes.

T. monococcum had a smaller range in the number of unique products. Genotypes M1, M2, M4, and M6 had no unique products, while M1 had one and M7, M9 and M10 had three. There were only 31 polymorphic products that were shared among the *T. monococcum* genotypes. Two amplifications, all in *T. urartu*, had reproducibly different amounts of product as visualized by ethidium bromide staining which may indicate that the genotypes differ in the number of copies of that particular product (Fig. 1). None of the 60 random primers had amplification product profiles that were identical for both *T. monococcum* and *T. urartu*, but numerous amplifications produced profiles that contained shared products between the two species. Since these taxa are closely related, the observation that amplification with certain primers produced shared products in both species was expected. That the product profiles between the two species were not identical is also reasonable since the interspecific hybrids are highly infertile. It is not surprising that there was a higher level of polymorphism in *T. urartu* than in *T. monococcum*, since *T. urartu* is a wild species and *T. monococcum* is a primitive cultivar.

The UPGMA analysis of Jaccard's similarity coefficients indicated a higher similarity among the *T. monococcum* genotypes than those of *T. urartu*. The coefficient of Jaccard is a similarity coefficient that omits consideration of negative matches. Jaccard's coefficient is: a/(n-d) where a = the positive matches (two genotypes scored 1 for a product); n = sample size; and d = negative matches (two genotypes scored 2 for a product). Since there are several ways in which a genotype may lose a product, it may be argued that basing similarity on the mutual absence of a character is improper. Because of this, phenograms were produced by clustering matrices based on Jaccard's coefficient. The similarity of the *T. monococcum* genotypes was 0.41 to 0.81. If the most divergent genotype (M10) is excluded, this range shrinks to 0.67–0.81 (Fig. 2). The similarity of the *T. urartu* genotypes was 0.32–0.68 (Fig. 3).

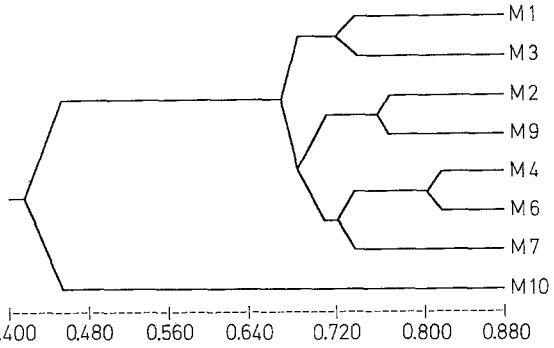

Fig. 2. Phenogram of *T. monococcum* genotypes. Scale on bottom is Jaccard's coefficient of similarity

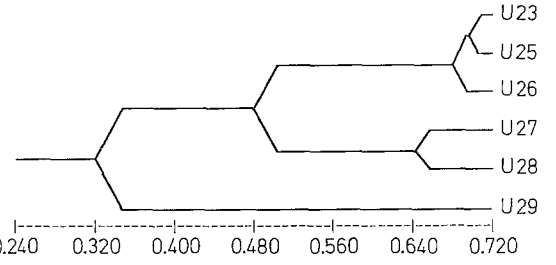

Fig. 3. Phenogram of *T. urartu* genotypes. Scale on bottom is Jaccard's coefficient of similarity

The phenogram clustering of *T. urartu* genotypes supports the expected grouping based on what we know of the origin of these genotypes. Genotypes U23 and U25 are the most similar (0.71) and both were collected in Lebanon. The first clustered group, U23, U25 and U26, were all collected from cooler climates in Lebanon and Turkey. The second cluster, including U27 and U28, were collected in mountainous areas of Iran and Armenia. The most divergent genotype, U29, was collected in the presumed center of wheat origin, between the Tigris and Euphrates rivers. Interestingly, U29 had the greatest number of unique products with 12; the next closest genotype (U27) had only three unique products. This supports the theory that the center of distribution or origin is the center of variation (Vavilov 1951). Phylogenetic analysis using parsimony (PAUP version 2.4.1, 1985; data not shown) based on shared/derived character analysis produced a similar topology. These results support the validity of the UPGMA analysis based on the similarity of the genotypes.

The exact origin of *T. monococcum* genotypes is not known, neither is the effect of domestication. When acquired thermal-tolerance data were matched with the clustering data, there appears to be a correlation of heat tolerance with clustering. The two most heat-tolerant genotypes, M1 and M3, cluster together and the two least heat-tolerant genotypes, M2 and M9, cluster together (Vierling and Nguyen 1992).

Though RAPD is a powerful tool, there are limitations associated with the procedure. We believe that an annealing temperature of 37°C and the addition of trimethyl-ammonium chloride (TMAC) to the amplification reactions gives a high stringency of primer to genomic DNA annealing. Under these conditions, both qualitative and quantitative polymorphisms are reproducible. One limitation of this analysis is that the complete sequence of the amplification products is not known. A primer might produce identically sized products in two genotypes, but possible divergence within the internal sequences can not be detected. We believe we have overcome this problem by using numerous primers. A second limitation is that very small differences in product size may be difficult or impossible to detect. Again, this can be overcome with numerous primers, using a higher concentration of agarose, analysing products in question in adjacent lanes, or using acrylamide gels.

The data indicate that RAPD analysis is a powerful tool for determining the extent of genetic diversity among diploid wheat genotypes. Overall, *T. urartu* genotypes were less similar than those of *T. monococcum*. Due to the apparent correlation of the known origin of *T. urartu* genotypes and the acquired thermal tolerance data in *T. monococcum*, the RAPD analysis is of value in helping determine genetic relationships among genotypes. This is especially useful for genotypes that have undergone similar selection pressures and will be of particular importance when dealing with germplasm collections. Knowing the relationships and phenotypes of individuals or genotypes would also be extremely useful for determining the best choice of parents for a mapping population or when introgressing traits into breeding populations. In addition, this technique would make an excellent tool for monitoring and determining the genetic diversity present in germplasm collections. As shown by the *T. monococcum* UPGMA analysis, RAPD products are also useful for clustering genotypes whose origin is unknown.

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