DNA Polymorphism in Some Samples of European Emmer

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Abstract: Phylogenetic relationships among twenty two tetraploid wheats of *Triticum dicoccon* coming from various European countries and from Near East region were estimated by means of RAPD polymorphism. Based on neighbour-joining clustering method we find out, that all tested accessions were clustered with taxonomic classification in line. Samples from Yugoslavia, Turkey, India, Ethiopia and an American variety Vernal created one cluster and all belong to the supraconvariety *asiaticum.* Our analyse proved that Slovak relic *dicoccon* wheats are related to the Yugoslavian emmer and belong to the convar. *serbicum.* All the rest European accessions together with accessions from Morocco and Israel were included in the second very clearly separated cluster of genotypes which can be classified in supraconvar. *dicoccon*. Iranian accession is phylogenetic intermediate stage between these two supraconvarieties.

Keywords: wheat+ emmer; DNA polymorphism; taxonomy; phylogeny

Emmer wheat (*Triticum dicoccon* Schrank) is among the most ancient cereal crops of the Old World's agriculture. It was probably the first domesticated crop at all, the specific site of its domestication within the Fertile Crescent has been discovered recently using AFLP analysis (Özkan *et al*. 2002). Emmer taxonomy is not explicit. There is a tendency to classify the whole tetraploid wheat variability under the common name *Triticum turgidum* L. This approach does not allow a clear taxonomic differentiation between hulled and naked wheat taxa (Szabó & Hammer 1996). The intraspecific classification of emmer as a separate species by Dorofeev *et al*. (1979) is rather complex. There are two basic principles of classification: geographical and botanical. Four subspecies (supraconvar.) representing geographical distribution of *T. dicoccon* material are being used: subsp. *dicoccon* (European material), subsp. *marocanum* (material from Morocco), subsp. *asiaticum* (material from Asia) and subsp. *abyssinicum* (Ethiopian material). A category of convariety indicates groups of botanical varieties.

In the present study molecular marker techniques, namely RAPD (Random Amplified Polymorphic DNA) was used in an attempt to determine genetic diversity levels and phylogenetic relationships of a set of *T. dicoccon* accessions originated from several countries of their traditional cultivation within Europe, Asia and Africa.

MATERIAL AND METHODS

Plant material used for the molecular analyses was obtained from the Gene Banks in Gatersleben and Wageningen (Table 1).

DNA isolation. High molecular weight genomic DNAs were isolated from 0.5–1 g leaf material of

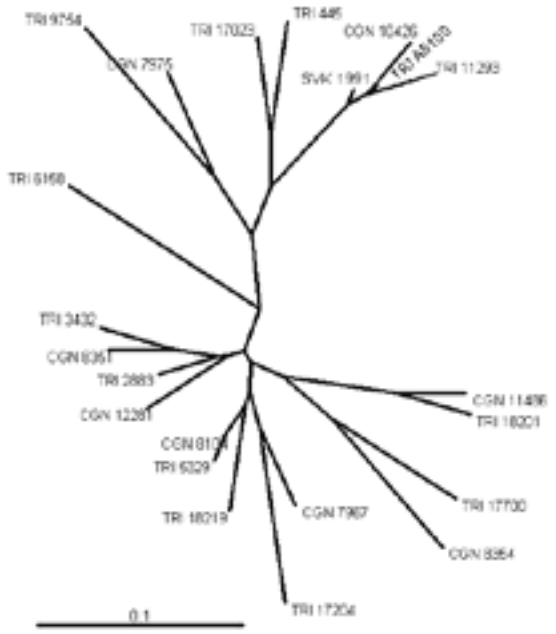

Figure 1. Radial tree of the analysed emmer accessions

10 day-old wheat seedlings following the procedure described by Doyle and Doyle (1990). DNAs were treated with RNAse A at 37°C, and RNA-free DNA was prepared using the phenol-isoamylalcohol-chloroform extraction method. Quality of genomic DNAs was examined by agarose gel electrophoresis. The 5 primers listed in Table 2 that had been shown to be highly polymorphic among wild *T. dicoccoides* populations (Fahima *et al*. 1999) were selected from the sets of RAPD primer kits, namely A and F (Operon Biotechnologies, Kőln, Germany). Each amplification reaction (25 μl) contained 10 ng genomic DNA, 1 × PCR buffer (Qiagen GmbH, Hilden, Germany), 25 mmol/l $MgCl_2$, 100 g 10-nucleotid primer (Operon Biotechnologies, Cologne), 0.4 mmol/l dNTP Mix (Fermentas, Vilnius, Lithuania) and 1.0 U Hot Star Taq polymerase (Qiagen GmbH, Hilden, Germany). DNA amplification was performed using a Whatman Biometra GmbH Thermal Cycler programmed for 45 cycles of 1 min at 94°C (denaturation), 2 min at 37°C (primer annealing), and 2 min at 72°C of primer polymerisation.

Neighbour-joining clustering method was used for Jaccard's coefficient calculation.

RESULTS AND DISCUSSION

Relations among the analysed accessions of emmer obtained on the base of DNA polymorphisms are given in the radial tree (Figure 1) and in the cladogram (Figure 2).

All the accessions differed in agreement with the taxonomical classification in line. We can distinguish two main groups of accessions on the radial tree. One branch of this tree is created by the accessions from Turkey, former Yugoslavia, Slovakia, India, Iran and Ethiopia. The American variety Vernal (TRI 445) is very related to the Turkish genotype TRI 17023 and probably its origin is from this country. All these mentioned emmer belong to the *asiaticum* supraconvariety. The accessions from the Western Europe create the most part of the second big branch of the radial tree. Together with the European emmer were the accessions from Kuwait, Morocco, Ukraine and Israel placed into the same group. All these mentioned samples of emmer can be classified into the *dicoccon* supraconvariety. The Iranian accession TRI 6158 is phylogenetically intermediate between the *asiaticum* and *dicoccon* supraconvarieties.

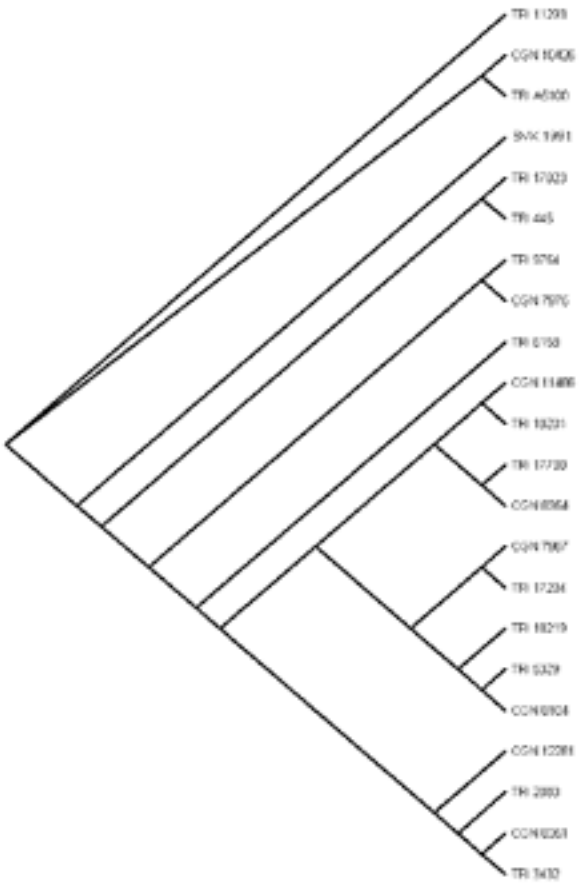

Figure 2. Cladogram of the analysed emmer accessions

Table 1. Origin of *Triticum dicoccon* accessions

Accession number at the Gene Bank	Name of cultivar	Acronym country of origin	Donor institution
SVK-1991	Branc emmer	SVK	UK Bratislava[1]
TRI 11293	–	SVK	IPK Gatersleben[2]
TRI 2883	Kahler Emmer	DEU	IPK Gatersleben
TRI 18201	Kihapti	FRA	IPK Gatersleben
TRI 34 32	Abessinischer Emmmer	KUW	IPK Gatersleben
TRI 9754	NP 202	IND	IPK Gatersleben
TRI 17 700	Roncal	ESP	IPK Gatersleben
TRI 17 023	–	TUR	IPK Gatersleben
TRI 17 204	–	ITA	IPK Gatersleben
TRI 18 219	–	UKR	IPK Gatersleben
TRI 53 29	May Emmer	CHE	IPK Gatersleben
TRI 445	Vernal	USA	IPK Gatersleben
TRI 6158	–	IRN	IPK Gatersleben
TRI A5100	–	YUG	IPK Gatersleben
CGN 7975	Abessinischer Emmer	ETH	CGR Wageningen[3]
CGN 8351	Brauner Sommer	DEU	CGR Wageningen
CGN 7967	Roter Emmer	DEU	CGR Wageningen
CGN 11488	Weisser Begrannter	DEU	CGR Wageningen
CGN 8104	–	ISR	CGR Wageningen
CGN 8354	–	ISR	CGR Wageningen
CGN10426	–	YUG	CGR Wageningen
CGN 12281	–	MAR	CGR Wageningen

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[3]CGR = Centre for Genetic Resources Wageningen, The Netherlands

Table 2. Ten-nucleotid primers used for RAPD

Primer	3'→5'sequence	Molecular weight	GC content (%)
OPA-03	AGTCAGCCAG	2997	60
OPA-12	TCGGCGATAG	3068	60
OPF-15	CCAGTACTCC	2948	60
OPA-19	CAAACGTCGG	3037	60
OPA-20	GTTGCGATCC	3019	56

The evolution relations among the emmer accessions can be visible from the cladogram (Figure 2). If we suppose the emmer evolution started in Turkey (Özkan *et al*. 2002), we can state that it aimed in two directions in this case. The one lead up to the development the Yugoslavian and Slovak genotypes and the second, the opposite, to the development of the Ethiopian, Indian, Israeli and finally to the west European *dicoccon* varieties.

Comparing our results with the Dorofeev classification (Dorofeev *et al*. 1979) it could be stated there is similarity in two main geographic groups: *asiaticum* and *dicoccon*. Geographical supraconvarieties *marocanum* and *abyssinicum* are probably a component part of the big *dicoccon* supraconvariety. It should be proved by more samples from Iran if the Iranian emmer is the independent supraconvariety.

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References

Dorofeev F., Filatenko A.A., Migushova E.F., Udashin R.A., Iacubziner R.R. (1979): Wheat. Flora of Cultivated Plants. Leningrad, Kolos, Russia.

Doyle J.J., Doyle J.L. (1990): Isolation of plant DNA from fresh tissue. Focus, **12**: 13–15.

Fahima T., Sun G.L., Beharev A., Krugman T., Beiles A., Nevo E. (1999): RAPD polymorphism of wild emmer wheat populations, *Triticum dicoccoides*, in Israel. Theoretical and Applied Genetics, **104**: 17–29.

Özkan H., Brandolini A., Schäfer-Pregl R., Salamini F. (2002): AFLP analysis of collection of tetraploid wheats indicates the origin of emmer and hard wheat domestication in southeast Turkey. Molecular Biology and Evolution, **19**: 1797–1801.

Szabo A., Hammer K. (1996): Notes on the taxonomy of farro: *Triticum monococcum*, *T. dicoccon* and *T. spelta*. In: Padulosi S. *et al*. (eds): Hulled Wheats. Proc. Int. Workshop, Castelvecchio Pascoli, 2–40.

GeneThresher® Methylation Filtering Technology – A Path to Rapid Gene and Marker Discovery in Plants

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Abstract: The genomes of many plants are known to be composed of a large fraction of repetitive DNA, while a small portion is dedicated to genes. The bulk of the repetitive DNA constitutes transposable elements and is heavily methylated. GeneThresher® methylation filtering technology takes advantage of these differential methylation patterns by filtering genomic shotgun libraries to exclude methylated sequences. The result is a gene-enriched library. Random shotgun sequencing of plant gene space, enabled by GeneThresher technology, is a rapid and cost-effective strategy for comprehensive gene discovery in crops. We have applied GeneThresher to plants that span the major branches of the plant kingdom including species representing monocots, dicots, gymnosperms, and non-vascular plants with a last common ancestor estimated at 500 million years ago. Gene enrichment was achieved in all plants tested suggesting that GeneThresher will be effective across the whole plant kingdom. GeneThresher subclone libraries appear to be a random unbiased representation of the gene set and represent the 5', internal, and 3' portions of genes with equal frequency. Exons, introns, promoters, non-coding RNAs, and simple sequence repeats are preferentially represented while representation of interspersed repeats is minimized in the GeneThresher libraries. Using GeneThresher methylation filtering technology, we have tagged more than three quarters of the *Sorghum* gene set after only 0.3 × coverage of the *Sorghum bicolor* genome. DNA sequence obtained from these libraries provides a robust view of the functional parts of the genome and enables the design of DNA microarrays that can rapidly catalog complete gene sets of large plant genomes. In addition, GeneThresher data can help in developing research tools that achieve more rapid and precise plant improvements.