

Prediction of pedigree relationships in durum wheat varieties based on agronomic, morphological and molecular traits

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Abstract

For successful crop improvement, genetic diversity among crossed varieties is fundamental. Generally, varieties exhibiting great genetic distances are less related to each other and their original genetic materials may not have common pedigree. Genetic distances could be estimated by the use of morphological and agronomic traits or various molecular markers. The purpose of this study was to estimate the genetic distances among 10 durum wheat (*Triticum turgidum* L.) varieties and see whether they could be utilized as prediction criteria for genetic relationships as they are expressed by their pedigree. Genetic distances were estimated based on data from molecular analyses using RAPD and SSR markers, morphological characteristics according to Community Plant Variety Office (CPVO), and various agronomic and quality characteristics (AQC). Clustering of the varieties was based on genetic distances and pairwise comparisons were made among and within the four methods used (i.e. RAPD, SSR, CPVO, AQC) along with the pedigree data. It was concluded that CPVO and AQC data predicted more effectively the genetic relationship, as it is revealed by the pedigrees, as compared to molecular methods based on RAPD and SSR data. Yet, the molecular markers as well as the data from other methods were unreliable for an accurate prediction of the genetic diversity as it is expressed by pedigrees.

Key words: Agronomic traits, Durum wheat, Morphological traits, Pedigree prediction, RAPD, SSR.

Abbreviations: AQC - agronomic and quality characteristics; CPVO - Community plant variety office; RAPD - random amplified polymorphic DNA sequences; SSR - simple sequence repeats.

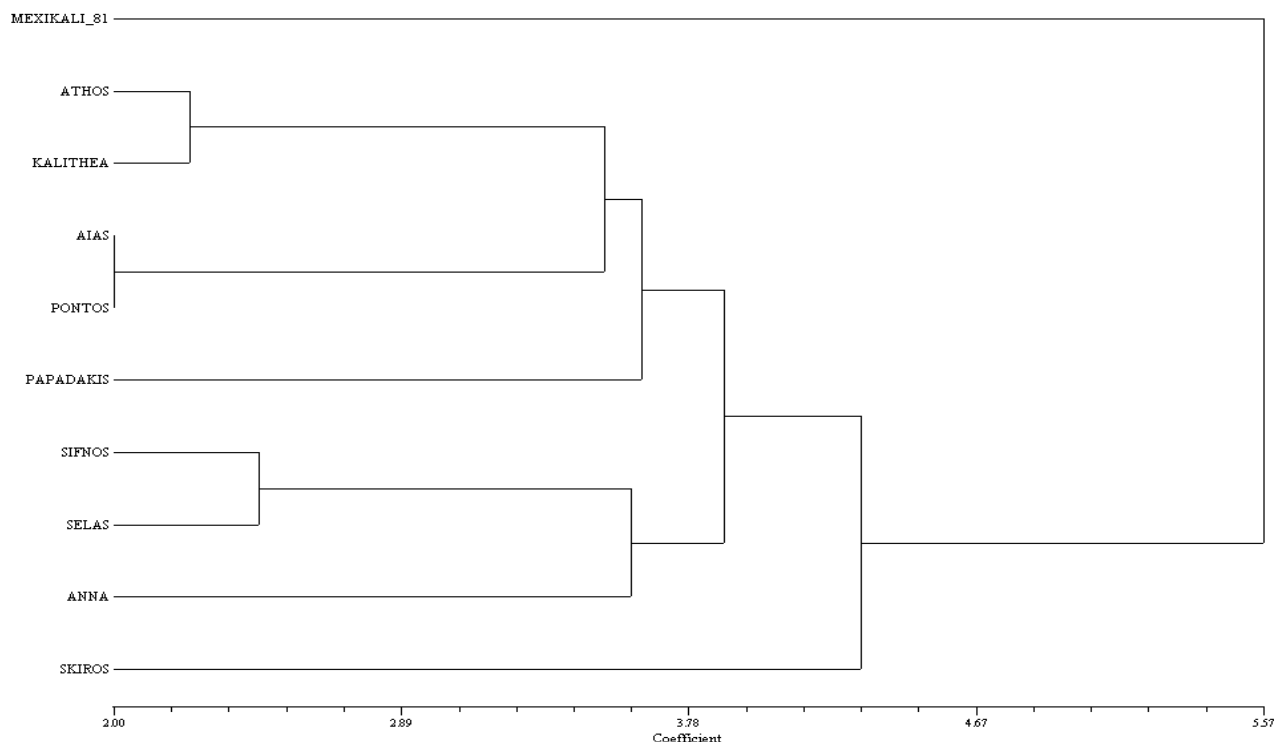
Introduction

One of the important decisions of a plant breeder is the choice of parents to produce genetic variability. Crosses between genetically divergent parents are expected to have a larger genetic variance among progenies than crosses between closely related parents (Messmer et al., 1993). Conventionally, a commercial cultivar adapted to the region in question and selected for its proven performance in this area, is frequently the choice for one parent. The other parent may be chosen because it has a different pedigree and it complements a recognized weakness of the first. This decision however is more difficult when the breeders have to choose parents from elite genetic material of unknown pedigree. Since genetic diversity and genetic relatedness among elite breeding material is essential for successful breeding programs (Fehr, 1987; Mukhtar et al., 2002), the estimation of genetic distances could be the answer to the problem. Criteria for the estimation of genetic distances other than pedigree records (Cox et al., 1985) are morphological traits (Maric et al., 2004) and molecular markers (Bohn et al., 1999). Morphological characters however are limited in number, they are not reliable and their relationship to other data may be influenced by environmental conditions, epistatic interactions and the largely unknown genetic control of traits (Smith and Smith, 1989). In contrast, genetic markers proved to be more effective than morphological

traits for classification purposes (Mitrick et al., 1997). In addition, RAPD and SSR molecular markers have been used to study genetic relationships between durum wheat cultivars and evaluation of plant genetic resources in wheat genotypes (Vierling and Nguyen, 1992; Sun et al., 1998; Dograr et al., 2000; Soleimani et al., 2002; Mukhtar et al., 2002). Finally, DNA fingerprinting is used to characterize genetic material, to detect genetic variability and for genome mapping (Holton, 2001). Furthermore, for the plant breeder, knowledge of genetic diversity and relationships among breeding materials is more essential for selecting parental combinations. When pedigree data are not available, the simple sequence repeat markers (SSR) are preferable for genetic diversity assessment (Hamza et al., 2004; Cao et al., 2000) due to their advantages compared with other types of molecular markers (Holton, 2001). Theoretically, the SSR assays are more robust than RFLPs and RAPDs and more transferable than AFLPs where the polymorphisms are often difficult to transfer to more sequence specific PCR applications (Maccaferri et al., 2003). Microsatellite markers have also shown high level of polymorphism even in closely related varieties (Plaschke et al., 1995) and uniform distribution in the wheat genome (Roder et al., 1998). These advantages would be useful for the study of pedigrees or related genotypes. The objective of this study was to determine the correspondence between the

Table 1. Varieties used in pairwise comparisons and their pedigree.

Group	Code	Varieties	Pedigree
G1	C1	Papadakis	(Athos × Mexicali 81) × Mexicali 81
	C3	Pontos	Selection from Mexicali 81
	C4	Anna	Mexicali 81 × Santa
	C7	Sifnos	Limnos × Mexicali 81
	C5	Mexicali 81	Selection from Mexicali 75
	C8	Selas	Selection from Stork (which is similar to Mexicali 75)
G2	C2	Aias	Selection from Yavaros
G3	C6	Athos	Selection from Appulo (originating from Capeiti 8)
	C9	Kallithea	Selection from Capeiti 8
G4	C10	Skiros	Selection from WAHA”S”-PL “S”-RUFF/GTA”S”-ROL (ICARDA)

**Fig 1.** Dendrogram of 10 durum wheat cultivars based on RAPD molecular markers (Euclidian UPGMA $r = 0.94$)

pedigree based genetic relationship among 10 durum wheat varieties and the genetic distances estimated by other methods, i.e. RAPD, SSR, morphological traits (CPVO), and various important agronomic traits (including yield) and quality characteristics (AQC). This could define which of the method is the most appropriate to trace more effectively the genetic relationship among the varieties and therefore to select the most promising genetic material suitable to pairwise crossing for breeding programs.

Results

The genetic distances estimated by the methods utilized: (i) molecular analysis by RAPDs and SSRs, (ii) morphological traits by CPVO and (iii) agronomical characteristics, exhibited discrepancies from the known pedigrees. Based on RAPD and SSR data, the cv. Mexicali 81 exhibited the greatest genetic distance among the varieties used in this experiment (Table 2 and 3). This variety however, exhibited high genetic distance even from related varieties (Table 1) such as Papadakis (5.83 and 3.32), Pontos (5.29 and 3.16) and Anna (5.66 and 3.46) for RAPD and SSR data,

respectively, and from Selas (5.83) based on RAPD analysis (Tables 1, 2, 3). On the other hand, varieties such as Pontos and Aias, and Selas and Aias, which are not related to each other (belong to different pedigree groups) (Table 1), exhibited the lowest genetic distances (Table 3) according to SSR data. However, the data from RAPD analysis (Fig. 1) estimated successfully the close relationship between Athos and Kallithea, and Sifnos and Selas varieties (Table 1). In addition, clustering of the related varieties derived from Mexicali 81 (Pontos, Papadakis, Sifnos, Selas, Anna), was successful. In contrast, Mexicali 81 was clustered together with Athos and Kallithea, the ones that have no relation to each other neither to Mexicali 81 according to the pedigree data. The data from the SSR analysis (Fig. 2) predicted accurately the pedigree relation between Sifnos and Papadakis, and Anna and Pontos (Table 1). In contrast, Mexicali 81 was clustered together with Athos and Skiros, although the pedigree data were not confirmed this relation. According to CPVO data, Mexicali 81 exhibited the greatest euclidean distances in most of the variety pairs, but this variety exhibited even greater genetic distances (15.5, 15.0, 15.5) from the related varieties Papadakis, Pontos, and Anna,

Table 2. Genetic distances between ten durum wheat varieties based on RAPD data.

		C1	C2	C3	C4	C5	C6	C7	C8	C9
Papadakis	C1									
Aias	C2	3.87								
Pontos	C3	3.61	2.00							
Anna	C4	4.24	3.87	4.12						
Mexicali 81	C5	5.83	5.57	5.29	5.66					
Athos	C6	3.46	3.16	3.32	3.46	4.80				
Sifnos	C7	4.00	3.61	3.61	3.74	5.39	3.16			
Selas	C8	4.24	3.87	4.12	3.46	5.83	3.74	2.45		
Kallithea	C9	3.61	3.74	3.87	4.12	5.66	2.24	3.87	4.36	
Skiros	C10	4.47	4.00	4.12	4.69	6.08	3.87	4.47	4.90	4.00

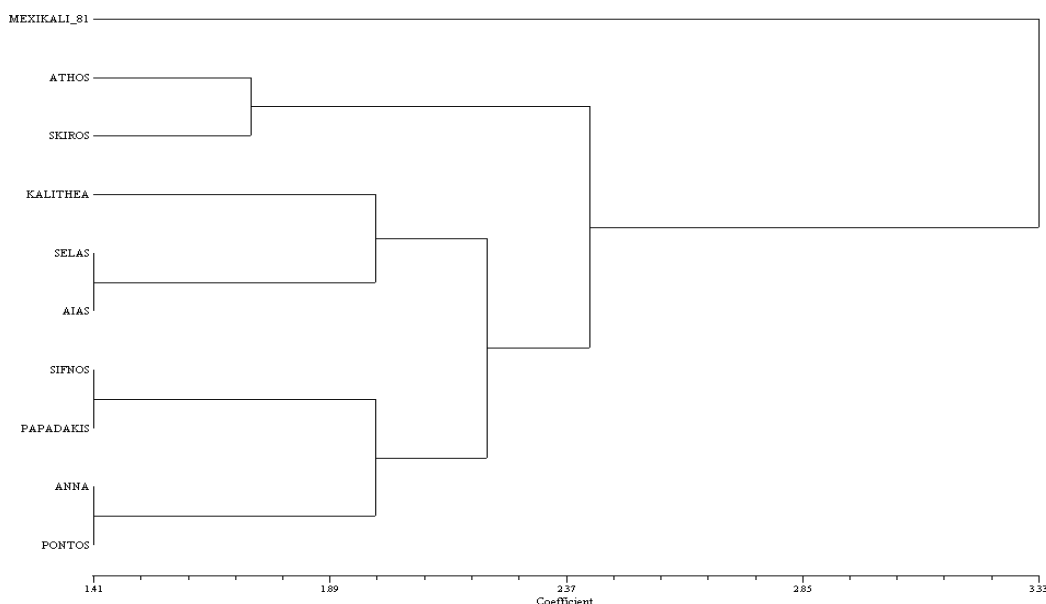


Fig 2. Dendrogram of 10 durum wheat cultivars based on SSR molecular markers (Euclidian UPGMA $r = 0.86$)

respectively (Tables 1 and 4). These data are in an agreement with the ones originated from molecular analysis. Additionally, varieties Pontos and Aias, and Anna and Aias, showed very low genetic distance (6.9 and 6.2), as it was expected by the pedigree data.

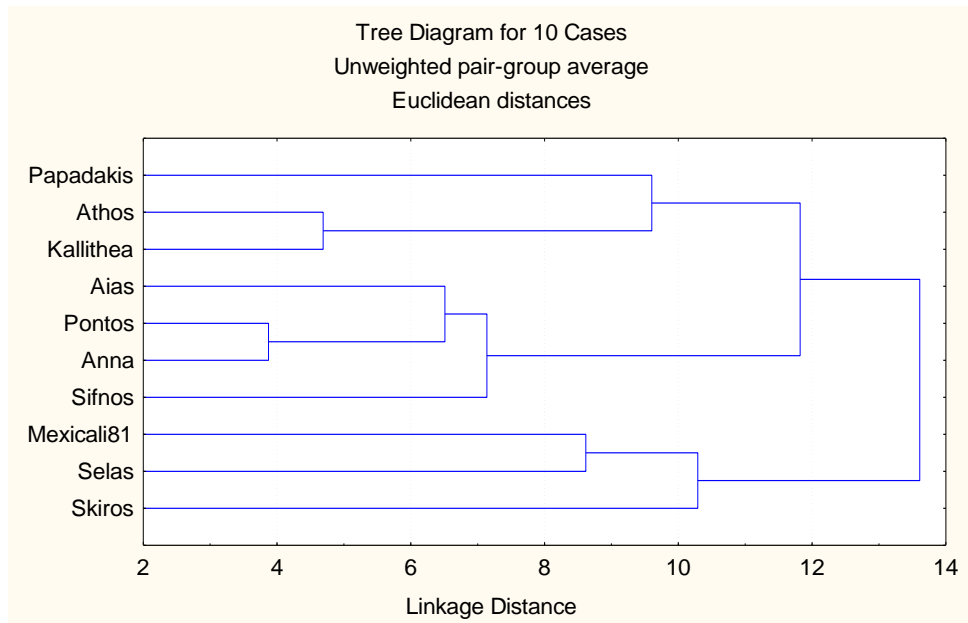
Discussion

Based on the above data, it was concluded that CPVO trait method was more effective than RAPD and SSR methods in identifying relations based on the pedigrees (Fig. 3), as CPVO results identified the relatedness of 4 pairs varieties correctly compared to pedigree results (Papadakis – Athos, Athos – Kallithea, Pontos – Anna, and Mexicali 81 – Selas) (Table 1). Additionally, Mexicali 81 and other related cultivars clustered together. Finally, the closely related varieties according the pedigree (Papadakis, Athos, and Kallithea), were also clustered together. For agronomic and quality data, Mexicali 81 exhibited the greatest euclidean distances in most of the pairs but exhibited even greater genetic distances from the related varieties Papadakis,

Pontos, Anna, Sifnos and Selas (Table 1). Additionally, the genetic distances between the varieties Pontos and Aias, and Selas and Aias were the lowest ones (Table 5). Generally, the agronomic method (Fig. 4) produced more satisfactory results as compared to the ones obtained from RAPD and SSR methods since it identified 4 pairs of varieties closely related to each other (Anna – Papadakis, Papadakis – Selas, Pontos – Sifnos, Athos – Kallithea). Furthermore, varieties derived from Mexicali 81 were clustered together (Anna, Papadakis, Selas, Pontos, Sifnos). However, the common parent Mexicali 81 was not included in this group. Instead it was close to Athos and Kallithea. These data indicate that the highest values of the genetic distance were observed in variety Mexicali 81 with other, either related or unrelated, varieties. Furthermore, no relationship was observed between the genetic distances estimated with the methods used and the one based on the pedigree. Similarly, Smith and Smith (1989) reported that morphological data did not provide a measure of relatedness, but reflected genetic relatedness estimated either by pedigree or by genetic data. Finally, Maric et al. (2004) in a study of genetic diversity among Croatian wheat cultivars by RAPD markers, morphological traits and pedigree records,

Table 3. Genetic distances between ten durum wheat varieties based on SSR data.

		C1	C2	C3	C4	C5	C6	C7	C8	C9
Papadakis	C1									
Aias	C2	2.00								
Pontos	C3	2.24	1.73							
Anna	C4	1.73	2.24	1.41						
Mexicali 81	C5	3.32	3.00	3.16	3.46					
Athos	C6	2.83	2.00	1.73	2.24	3.46				
Sifnos	C7	1.41	2.00	2.24	1.73	3.32	2.83			
Selas	C8	2.00	1.41	1.73	2.24	3.00	2.45	2.00		
Kallithea	C9	2.65	1.73	2.45	2.83	3.46	2.00	2.65	2.24	
Skiros	C10	3.00	2.24	2.00	2.45	3.74	1.73	3.00	2.65	2.45

**Fig 3.** Dendrogram of 10 durum wheat cultivars based on CPVO Euclidean distances

also concluded that no significant correlations were observed among the methods used. It could be concluded that the data obtained from agronomic traits (CPVO and AQC data) produced more reliable results as compared to the ones obtained from molecular data for prediction of pedigree relationships of the ten durum wheat varieties. Therefore, the molecular markers used (RAPD and SSR methods), are not capable alone to estimate the genetic distances and therefore unable for the prediction of the genetic diversity as it expressed by pedigrees. This suggests that pedigree information in wheat will continue to be useful to inexpensively identify diverse parents for a breeding program.

Materials and methods

Plant material

Nine durum wheat varieties Anna, Athos, Aias, Kallithea, Mexicali 81, Papadakis, Pontos, Selas, Sifnos, derived from breeding programs of the Cereal Institute in Thessaloniki (Kotzamanidis et al., 2008) and the variety Skiros were

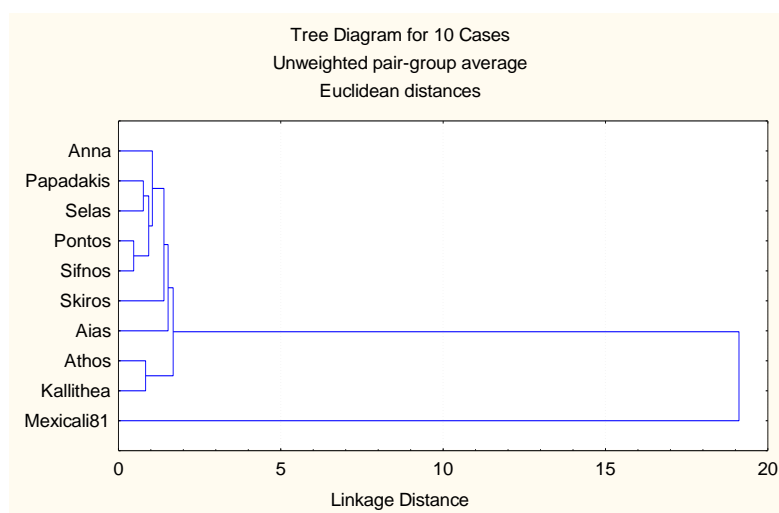
studied. These varieties are grouped in four groups (G_1 , G_2 , G_3 , G_4) according to the information obtained from their pedigrees (Table 1). The pedigree of the varieties used is given in Table 1. Chosen varieties were standardized for two years (2001-02 and 2002-03) for morphological characteristics to exclude off-types. Genetic distances of the ten varieties were calculated using: (a) DNA analysis from RAPD and SSR data (NTSYS program) (Rolf, 1998), following a protocol according to Kotzamanidis et al. (2008), (b) Morphological characteristics according to CPVO and various agronomic and quality characteristics.

Field experiments

Field experiments were conducted in two different areas (experimental farm of Cereal Institute, Thessaloniki and the Experimental Station of NAGREF, Agios Mamas) to measure various agronomic and quality characteristics of the ten varieties for two growing seasons (2003-04 and 2004-05). Randomized Complete Block designs (RCBD) with ten treatments (varieties) and four replications were used and each plot consisted of 7 rows, 4 m long and 0.25 m apart.

Table 4. Euclidean distances between ten durum wheat varieties based on CPVO data.

		C1	C2	C3	C4	C5	C6	C7	C8	C9
Papadakis	C1									
Aias	C2	11.7								
Pontos	C3	10.6	6.9							
Anna	C4	10.0	6.2	3.9						
Mexicali 81	C5	15.5	14.5	15.0	15.5					
Athos	C6	10.3	13.3	13.0	12.8	17.1				
Sifnos	C7	9.2	8.0	6.2	7.2	13.6	12.2			
Selas	C8	14.8	13.0	12.6	12.7	8.6	16.5	11.0		
Kallithea	C9	8.9	12.8	12.6	12.1	16.2	4.7	11.6	15.7	
Skiros	C10	11.7	11.4	10.9	11.2	11.5	13.9	9.4	9.1	13.8

**Fig 4.** Dendrogram of 10 durum wheat cultivars based on Agronomic Euclidean distances**Table 5.** Euclidean distances between ten durum wheat varieties based on agronomic and quality data.

		C1	C2	C3	C4	C5	C6	C7	C8	C9
Papadakis	C1									
Aias	C2	1.3								
Pontos	C3	0.8	1.4							
Anna	C4	1.2	1.9	1.2						
Mexicali 81	C5	18.7	18.6	18.8	19.5					
Athos	C6	1.7	2.0	1.7	1.8	19.2				
Sifnos	C7	0.8	1.5	0.5	1.0	18.8	1.5			
Selas	C8	0.8	1.6	1.1	0.8	19.2	1.9	1.0		
Kallithea	C9	1.4	2.1	1.6	1.4	19.4	0.8	1.4	1.5	
Skiros	C10	1.3	1.4	1.5	1.4	19.7	1.9	1.5	1.3	1.7

Within each plot, two rows randomly selected of the middle five rows were used for measurements (outside rows served as borders). Measurements were conducted in specified parts of each row, 50 cm long for both sides of the row and for all replications.

Agronomic and morphological characteristics measurements

Agronomic and quality characteristics measured were: total number of tillers per plant, number of fertile tillers per plant, number of kernels on the spike, kernel weight per spike. Especially for the last two measurements, 5 spikes were used separately within the specified areas of the two middle rows in the plots and means were calculated. In each plot, seven more characteristics were measured for all replications:

heading date, plant height, yield, 1000-kernel weight, total protein, vitreosity (referring to the translucent or glassy appearance of endosperm) and black points on the grain. For the ten durum wheat varieties, 26 morphological characteristics were described according to the wheat Protocol for distinctness, uniformity and stability tests of the Community Plant Variety Office of the European Union (CPVO, 2002). Twenty plants (randomly selected) from each variety were used for each characteristic (five plants from each replication).

Statistical analysis

ANOVA and calculation of genetic distances were based on Snedecor and Cochran (1980) and SPSS manual (1998), and were performed on SPSS and Statistica software packages. In

addition, genetic distances were based on the Euclidean model of distances with unweighted pair-group average according to previous work on similar data (Beer et al., 1993; Rolf, 1998; Kotzamanidis et al., 2006). Data were standardized to become comparable and fit in Euclidean model (SPSS, 1998).

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