# Relationships among 81 durum genotypes based on RRLPs, gliadins, parentage, and quality traits

**Sorrells M.E., Barbosa J., Nachit M., Ketata H., Autrique E.**

*in*

**Di Fonzo N. (ed.), Kaan F. (ed.), Nachit M. (ed.).**
**Durum wheat quality in the Mediterranean region**

**Zaragoza : CIHEAM**
**Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 22**

**1995**
pages 249-262



# Relationships among 81 durum genotypes based on RFLPs, gliadins, parentage, and quality traits

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**SUMMARY -** Efficient use of durum germplasm is enhanced by understanding the patterns of genetic variation for the traits of interest. RFLP, gliadin components, abd grain quality traits, and coefficients of parentage data were recorded and compared to assess the correspondence between similarity matrices based on these different traits. Individual gliadin and RFLP bands were analyzed for association with quality traits. Quantification of genetic variation between parental material used for hybridization could enhance the level of variation in breeding populations.

**Key words:** RFLP, gliadin, coefficient of parentage, grain quality, correspondence analysis, genetic variation.

***RESUME -*** *"Relations entre 81 génotipes de blé dur basés sur RFLP, gliadines, parenté et caractères de qualité". Une utilisation efficace du germosplasme de blé dur est accrue par la compréhension des mécanismes de variation génétique des caractères d'intérêt. Les RFLP, les composantes gliadine, les caractères de qualité du grain, et les coefficients concernant les données de parenté ont été enregistrés et comparés afin d'évaluer la correspondance entre matrices de similarité basées sur ces différents types de caractères. Les bandes individuelles de gliadine et de RFLP ont été analysées en recherchant leur association avec des caractères de qualité. La quantification de la variation génétique chez le matériel apparenté utilisé pour l'hybridation a permis d'augmenter le niveau de variation dans les populations améliorées.*

***Mots-clés :*** *RFLP, gliadine, coefficient de parenté, qualité du grain, analyse de correspondance, variation génétique.*

## Introduction

Efficient utilization of a germplasm collection is enhanced by knowledge of patterns of genetic variation within the collection for traits of interest. If the geographic distribution of variation for a trait is known, accessions can be sampled from the region where the desired characteristic frequently occurs. Sampling also can be based on patterns of genetic relationship among accessions from different regions. These patterns of genetic relationship may be based on geographic proximity, several qualitative or quantitative morphological traits, isozymes, RFLPs, flavonoids, or other traits.

Genetic variation in gene pools of self-pollinated crops has been analyzed by studying the pedigree relationship between cultivars released over a period of time. Coefficient of parentage estimation of cultivars of oats (Souza and Sorrells, 1988), soybean (Cox *et al.*, 1985a), winter wheat (Cox *et al.*,

1985b), as well as other crops has shown that a restricted number of ancestral genotypes account for a large proportion of the variation present in released cultivars. In barley, the hybridization of different gene pools was traditionally restricted to two-row and six row barley cultivars (Martin *et al.*, 1991).

Durum wheat germplasm collections from the USA and Italy were evaluated for morphological traits (Jain *et al.*, 1975; Porceddu, 1976), flag leaf characteristics (Spagnoletti Zeuli and Qualset, 1990), physiological and morphological traits (Clarke *et al.*, 1991; Yang *et al.*, 1991). Based on a diversity index, greater variation was found for some of the countries that are believed to be the centres of diversity and diversification. Total variation was reported to be significantly different among the characters but not among the countries (Porceddu, 1976). Low genetic diversity in Ethiopia was reported by Yang *et al.* (1991) while another study of 1800 landraces collected in Ethiopia showed great diversity for many traits (Srivastava *et al.*, 1988).

Morphological traits often do not reliably portray genetic relationships because of environmental interactions, epistatic interactions and because the genetic control of the traits is often unknown. Genetic markers such as restriction fragment length polymorphism (RFLP) represent genetic variation at the DNA level, allowing an estimation of the degree of relatedness between individuals without the influence of environmental variation.

Protein content and composition are the most important factors affecting pasta quality. Known relationships exist between gliadin banding patterns separated by polyacrylamide gel electrophoresis (PAGE) and the gluten quality of the endosperm proteins (Kosmolak *et al.*, 1980). In particular, the presence of gliadin band 45 and the absence of 42 has been shown to be associated with strong gluten (Damidaux *et al.*, 1978; Yupsanis and Moustakis, 1988; Pogna *et al.*, 1990). Joppa *et al.* (1983) used Langdon durum aneuploids with chromosomes substituted from the strong gluten variety Edmore to show that chromosome 1B contained the gene(s) responsible for these gliadin bands and the resultant effect on gluten strength and SDS sedimentation. Leisle *et al.* (1981) found a strong association between chaff colour, gliadin bands 42 and 45, and gluten strength with white chaff varieties having strong gluten and band 45 while those with bronze chaff had weak gluten and band 42.

In this study, RFLP, gliadin, quality traits, and pedigree data recorded on 81 durum accessions were compared to assess the correspondence between similarity matrices based on these different types of traits. In addition, individual gliadin and RFLP bands were analyzed for association with quality traits. Quantification of genetic variation between individual genotypes used for hybridization could enhance the level of variation in breeding populations.

## Materials and methods

### Accessions and traits scored

A sample of 81 durum accessions from Algeria, Canada, Cyprus, Ethiopia, Italy, Jordan, Lebanon, Morocco, Syria, Turkey, USA, and improved lines from CIMMYT/ICARDA breeding program were obtained from the ICARDA germplasm collection. RFLP, gliadin, SDS sedimentation, protein content, vitreousness, thousand kernel weight and semolina colour (yellow pigment) data were collected for all of these accessions; however, one accession was not included in the RFLP survey. SDS sedimentation index (SDSI) was calculated by dividing SDS sedimentation volume (ml) by percent protein content.

For RFLP analysis, ten grams of fresh leaf tissue from eight to ten 17 day old plants were frozen in liquid nitrogen, ground and used for DNA extraction. Extraction buffer and procedure used were based on Tai and Tanksley (1990), using one to one volume of chloroform/isoamyl (24:1) instead of potassium acetate and omitting the second DNA precipitation. DNA was cut with the restriction enzyme EcoRI (AAB, Aurora, CO) using the appropriate buffer and conditions specified by the manufacturer. Approximately 20-25 µg of digested DNA were loaded into a 0.9% agarose gel, electrophoresed and transferred to a Hybond N+ membrane (Amersham International plc). Probes were labelled using [$^{32}$P]dCTP by random priming method (Feinberg and Vogelstein, 1983). Filters were washed as described in Anderson *et al.* (1992) and filters were exposed over X-ray film for 5 to 7 days.

Thirty-nine probes from oat and barley cDNA libraries and a wheat genomic library, described in Heun *et al.* (1991), were used. Probes were selected based on their chromosomal location (Anderson *et al.*, 1992). These clones showed hybridization to fragments of the A and B genomes, based on Chinese Spring aneuploids, and were distributed across the 7 chromosome groups.

Gliadins were extracted from seeds, electrophoresed, and assayed as described in the ICARDA technical manual for Crop Quality Evaluation Methods and Guidelines (Williams *et al.*, 1988). A total of 145 distinct electrophoretic bands were present in the sample of 81 accessions, and 127 of these were polymorphic. As with the RFLPs, each distinct band was scored as present or absent in each accession. Allelic relationships were not determined for gliadins or restriction fragments.

SDS sedimentation volume, protein content, thousand kernel weight, percent vitreous kernels, and semolina flour colour, referred to collectively as *quality traits,* were scored for samples from a three-replicate trial at Tel Hadya, Syria under rainfed conditions (230 mm annual rainfall). Genotypic values for these traits were estimated from least squares means, derived from analysis of variance for each trait.

## Coefficient of parentage

The coefficient of parentage (COP) between two individuals is defined as the probability that a random allele at a locus in one individual is identical by descent to a random allele at the same locus in the other individual. COP values (r) were estimated as described by Cox *et al.* (1985b). Assumptions for this estimation were: (i) each parent contributes equally to the genetic composition of the cultivar, (ii) selection is neutral through the generations of selfing, (iii) reselection of a previous cultivar or landrace has assigned relationship of r=0.75 with the parental genotype, and (iv) the relationship of a cultivar with itself r=1. Cultivars without known common parentage were assumed to be unrelated.

Ancestral lines from 37 improved varieties were traced back using several sources of information about genealogies and pedigrees (Zeven and Zeven-Hissink, 1976; Brajcich *et al.*, 1986; Zeven and Reiner, 1991). Additional information was provided by Dr. Elias Elias (North Dakota State University), Dr. Victor Vallega (Istituto Sperimentale per la Cerealicoltora, Rome), and by Dr. Osman Abdalla (CIMMYT, Mexico). The COP values were calculated using a FORTRAN program developed at Kansas State University (Cox and Murphy, 1990). Cultivars were grouped based on COP to ancestral varieties using the unweighted-pair-group-mean with arithmetic (UPGMA) method.

## Data analysis

Autoradiographs were scored based on the presence or absence of bands, generating a matrix of 1 and 0. Informative bands were used to generate a genetic distance matrix using the SIMGEND routine based on Nei's formula (Nei and Li, 1979) from the NTSYS-pc statistical package (Rohlf, 1990). Subroutine SAHN and the UPGMA clustering method were used to determine groups of similar genotypes based on the generated genetic distance matrix. The method used for clustering was UPGMA.

The quality trait data were transformed using the STAND procedure from NTSYS-pc (Rohlf, 1990) to reduce the effect of different scales of measurement. In this transformation, the mean is subtracted from the individual value and the result divided by the standard deviation. The standardized values were used in the SIMINT subroutine of NTSYS-pc (Rohlf, 1990) to compute a matrix of similarities among all pairs of genotypes using the average taxonomic distance. Standard taxonomic distances were used rather than other measures of relationship as they showed greater correlation with measures of similarity based on RFLP and isozyme data (Beer *et al.*, 1993).

## Matrix comparisons

Matrices based on Nei's genetic distance, COP, and quality traits were compared by using the

MXCOMP routine of NTSYS-pc (Rohlf, 1990) that uses the normalized Mantel Z statistic (Mantel, 1967). The statistical considerations for these analyses were discussed by Beer *et al.* (1993).

## Results

### Similarities among accessions

#### *Restriction fragment length polymorphisms*

Thirty-six probes were polymorphic on at least one of the accessions surveyed. A total of 232 fragments were detected resulting in a mean of 5.7 fragments per clone and 4.3 polymorphic bands. From the total number of fragments detected, 165 were polymorphic and were used to estimate the genetic relationships between all possible pairs of genotypes.

Clustering of genotypes based on Nei's genetic distance matrix computed from RFLPs resulted in seven distinctive groups, namely clusters A to G (Fig. 1). Clusters A, B, and C were composed of mostly improved lines but several of the Jordan and Morocco landraces were related to them. In contrast, clusters D, E, F, and G were relatively diverse and the majority were landraces. Cluster D included 12 landraces and one improved line. The landraces of this cluster were collected in Greece (5), Syria (3), Italy (2), Algeria (1), and Cyprus (1). Cluster E consisted of landraces and lines related to Haurani that originated in Jordan (2), Syria (5), Italy (2), and Canada. ND86-10, Akbash, Baladia Hamra, 1293 (Cyprus), and Tripolino, were least related to the rest of the accessions in this study. Unique alleles for the landraces and improved germplasm in this study represented 13% and 2% of the total, respectively.

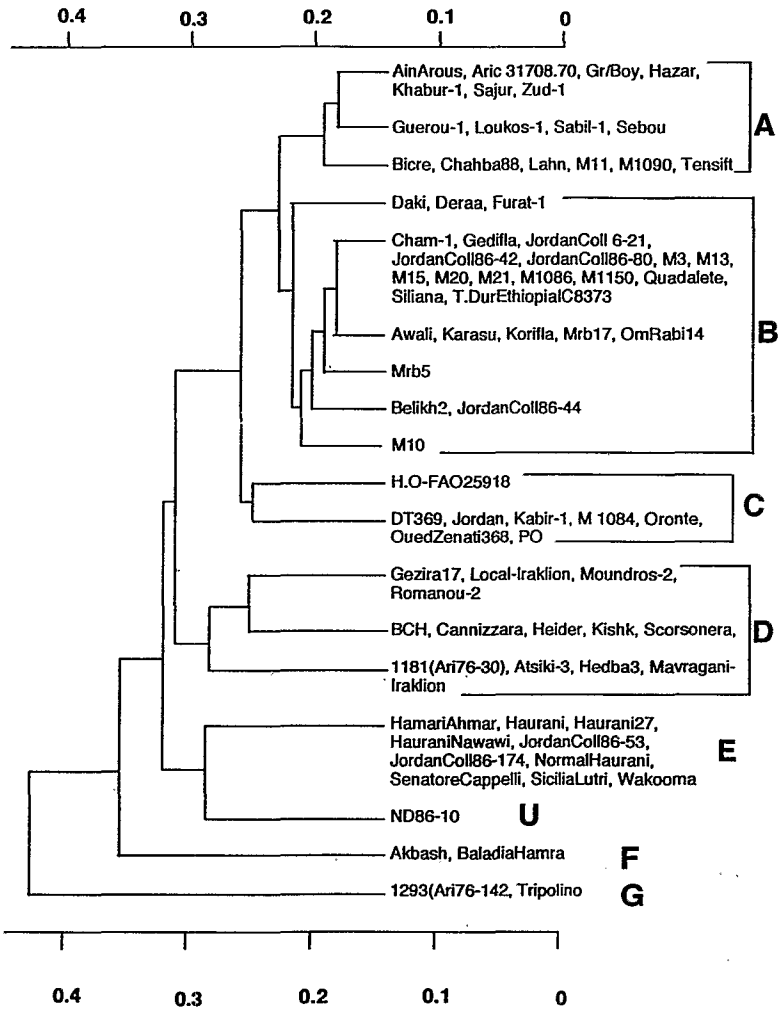


Fig. 1. Relationships based on RFLPs for 81 durum accessions.

In general, similarities among accessions from individual countries was low although landraces from the same region or known to have common origins were closely related as expected.

### *Coefficient of parentage*

Coefficient of parentage was calculated for 37 cultivars with known pedigrees. Fifty ancestral lines were related to the improved lines and included seven different bread wheat parents (primarily Norin10/Brevor as a source of dwarfing genes), seven accessions corresponding to four subspecies of *Triticum turgidum*, twenty four landraces with origin in twelve different countries, and twelve lines with unknown pedigrees.

Fifteen ancestral lines were present in at least 80% of the cultivars. Five of them were present in all pedigrees. These lines included Vernal Emmer, Mindum, Eiti, Norin 10 and Brevor. Overall, the average contribution of these 15 ancestral parents accounted for 72% of the total variation of these cultivars and ranged from 23.5% to 95.7%. The rest of the ancestral varieties contributed to less than 50% of the varieties and the contribution to the improved lines ranged from <0.1% to 50%.

Clustering of genotypes based on the COP value to ancestral lines resulted four distinctive clusters (Table 1). In cluster A, three ancestrals namely S179 (20%) and Dur6 (7%), and Haurani (12%) showed a large contribution. In Cluster B, lines included small contributions from many of the ancestrals but were largely related to Mindum (21%). Improved cultivars in cluster C were largely related to Jennah Khetifa (23%), Mindum (22%), Caravaca (8%) and Barrigon Yaqui (8%). In cluster D, the largest contributors to the pedigrees were Mindum (35%) and Eiti (11%).

### *Gliadin relative mobilities*

A total of 127 gliadin bands with relative mobilities ranging from 20 to 90 were polymorphic for the 81 durums in this study. Clustering based on gliadin bands resulted in 2 large groups and several smaller groups designated A through I (Fig. 2). Group A included 9 landraces, mostly from North Africa and Italy, and 12 improved lines. Cluster E was entirely composed of improved lines while B, C, D, F, G, and H were mostly landraces.

### *Quality traits*

SDSI ranged from 0.9 to 3.6, protein from 8.5 to 14.7, vitreous kernel percentage from 70 to 100, thousand kernel weight from 37.9 to 55.6 and flour colour from 2.0 to 6.5. Standard taxonomic distances based on SDSI, protein content, vitreous kernel count, kernel weight, and flour colour were clustered in groups A through H with two varieties ungrouped (Fig. 3). Cluster A was intermediate for all of the traits and included mostly improved lines along with Haurani and some related landraces (Fig. 4). Cluster G represented those with the best overall quality and included Senatore Cappelli. Cluster H had the poorest quality followed by B. Deraa and Baladia Hamra were not grouped with other genotypes because of unusual combinations of quality traits. Both had low SDSI with above average protein and vitreousness but Deraa had high kernel weight and flour colour while Baladia Hamra was low.

## Linearity of relationship between similarity measures

Correlations between matrices derived from the three different types of traits were low and non-significant. The highest correlation ($r=.06$, $p<0.82$) was between Nei's genetic distance and the average taxonomic distance based on quality traits. Correlation coefficient between COP and Nei's genetic distance was 0.23.

Because of the large number of analyses used for detecting associations, only those F-tests resulting in probabilities of less than 0.01 were considered significant. Four RFLPs were significantly associated with SDSI, 3 with protein content, 4 with vitreousness, none with kernel weight, and 5 with flour colour (Table 2). Those probes hybridize to fragments located on chromosomes 1, 3, 4, 5, and 6 with 5 being the most common.

Table 1. Genotype names, origin and cluster number based on RFLP, gliadin, or quality trait genetic distance, and coefficient of parentage (COP)

| Entry No.† | Genotype name | Origin | Cluster | | | |
|---|---|---|---|---|---|---|
| | | | RFLP | COP | Gliadin | Quality |
| 1 | Hedba 3 | Algeria | D | | U | A |
| 2 | Qued Zenati 368 | Algeria | C | | F | G |
| 7 | 1181 (ARI 76-30) | Cyprus | D | | A | G |
| 8 | 1293 (ARI 76-142) | Cyprus | G | | D | D |
| 10 | T. Dur. Ethiop.IC 8373 | Ethiopia | B | | C | D |
| 12 | Romanou 2 | Greece | D | | G | F |
| 13 | Mavragani-Iraklion | Greece | D | | A | A |
| 14 | Moundrous-2 | Greece | D | | A | H |
| 15 | Atsiki-3 | Greece | D | | A | A |
| 16 | Local Iraklion | Greece | D | | B | B |
| 18 | Tripolino | Italy | G | | C | F |
| 19 | Scorsonera | Italy | D | | B | A |
| 20 | Sicilia Lutri | Italy | E | | A | A |
| 21 | Cannizzara | Italy | D | | A | C |
| 22 | Senatore Cappelli†† | Italy | E | | A | G |
| 23 | Jordan Coll. 86 NO 21 | Jordan | B | | D | C |
| 24 | Jordan Coll. 86 NO 42 | Jordan | B | | D | A |
| 25 | Jordan Coll. 86 NO 44 | Jordan | B | | F | G |
| 26 | Jordan Coll. 86 NO 53 | Jordan | E | | D | E |
| 27 | Jordan Coll. 86 NO 80 | Jordan | B | | D | A |
| 28 | Jordan Coll. 86 NO 174 | Jordan | E | | D | A |
| 29 | M 13 | Morrocco | B | | D | D |
| 30 | M 21 | Morrocco | B | | G | A |
| 31 | M 3 | Morrocco | B | | D | A |
| 32 | M 20 | Morrocco | B | | D | A |
| 33 | M 10 | Morrocco | B | | A | A |
| 34 | M 1084 | Morrocco | C | | C | G |
| 35 | M 1086 | Morrocco | B | | C | E |
| 36 | M 1090 | Morrocco | A | | H | G |
| 37 | M 11 | Morrocco | A | | I | A |
| 38 | M 1150 | Morrocco | B | | H | A |
| 39 | M 15 | Morrocco | B | | C | D |
| 42 | Haurani Nawawi†† | Syria | E | | A | A |
| 43 | Haurani 27 | Syria | E | | D | H |
| 44 | Normal Haurani | Syria | E | | D | F |
| 45 | Haurani | Syria | E | | B | A |
| 46 | Hamari Ahmar | Syria | E | | A | A |
| 47 | BCH | Syria | D | | C | F |
| 48 | Akbash | Syria | F | | A | A |
| 49 | Kishk | Syria | D | | G | G |
| 50 | Baladia Hamra | Syria | F | | A | U |
| 51 | Gezira 17 | Syria | D | | D | D |
| 61 | H.O-FAO 25918 | Turkey | C | | A | C |
| 73 | Lahn | Imp. Var. | A | A | A | C |
| 76 | Mrb 5 | Imp. Var. | B | A | E | D |
| 77 | Mrb 17 | Imp. Var. | B | A | E | A |
| 78 | Tensift 1 | Imp. Var. | A | C | E | A |
| 79 | Guerou 1 | Imp. Var. | A | C | E | C |
| 80 | Gedifla | Imp. Var. | B | C | E | A |
| 81 | Chahba 88 | Imp. Var. | A | A | E | A |
| 82 | Sabil 1 | Imp. Var. | A | D | A | F |

Table 1. (Continued). Genotype names, origin and cluster number based on RFLP, gliadin, or quality trait genetic distance, and coefficient of parentage (COP)

| Entry No.† | Genotype name | Origin | Cluster | | | |
|---|---|---|---|---|---|---|
| | | | RFLP | COP | Gliadin | Quality |
| 83 | Loukos 1 | Imp. Var. | A | C | E | A |
| 84 | Bicre | Imp. Var. | A | C | E | A |
| 85 | Furat 1 | Imp. Var. | B | B | E | G |
| 86 | Nile | Imp. Var. | - | B | C | A |
| 87 | Zud 1 | Imp. Var. | A | B | D | A |
| 88 | Khabur 1 | Imp. Var. | A | B | D | A |
| 89 | Po | Imp. Var. | C | C | A | F |
| 90 | Hazar | Imp. Var. | A | B | A | A |
| 91 | Deraa | Imp. Var. | B | B | U | U |
| 92 | Om Rabi 14 | Imp. Var. | B | A | A | E |
| 93 | Kabir 1 | Imp. Var. | C | C | H | B |
| 94 | Oronte | Imp. Var. | C | A | C | A |
| 95 | Daki = Ceyhan | Imp. Var. | B | B | H | C |
| 96 | Karasu | Imp. Var. | B | B | A | D |
| 97 | Gr/Boy | Imp. Var. | A | B | A | H |
| 98 | Sajur | Imp. Var. | A | B | A | A |
| 99 | Ain Arous | Imp. Var. | A | B | A | A |
| 100 | Sebou | Imp. Var. | A | B | A | A |
| 101 | Quadalete | Imp. Var. | B | - | D | A |
| 102 | Jordan | Imp. Var. | C | C | D | B |
| 103 | Korifla | Imp. Var. | B | B | A | D |
| 104 | Belikh 2 | Imp. Var. | B | B | A | A |
| 105 | Cham 1 | Imp. Var. | B | B | A | D |
| 106 | Siliana | Imp. Var. | B | C | A | D |
| 107 | Awali | Imp. Var. | B | B | E | D |
| 108 | Heider = Marjawi | Imp. Var. | D | B | A | C |
| 110 | Aric31708.70=IC-78 | USA | A | D | D | B |
| 111 | N. DAK 86-10 | USA | E | D | E | C |
| 112 | Wakooma | Canada | E | D | I | A |
| 113 | DT 369 | Canada | C | D | A | G |

†Entries numbers are from the Regional Durum Yield Trial - Low Rainfall (RDYT-LR) 1988 distributed by CIMMYT/ICARDA; entries from 1 to 61 represent landraces

††Ancestral lines based on pedigree information of all improved varieties

Although they were only significant at the 5% level, gliadins with relative mobilities of 42, 43, and 45 are included in the table because of previous reports of association with SDSI. Only 1 gliadin band was associated with protein content and one with flour colour. No other associations were significant at the 1% level.

## Discussion

### Effect of type of trait on relationship estimates

There are a great number of possible combinations of transformations and proximity coefficients

(Sneath and Sokal, 1973) from which to choose. Beer *et al.* (1993) tested several coefficients for a sample of *Avena sterilis* accessions and concluded that the results using different coefficients were similar for both RFLPs and allozymes so the same analyses were used for this study. For morphological traits, they found that standard taxonomic distance using z-transformed data gave the best correlations with RFLPs and allozymes. For this sample of durum accessions, similarity values based on one type of data were not predictive of any similarity values based on another type of trait. Therefore, an RFLP-derived similarity value for any pair of accessions provided little information about the similarity of that pair relative to others when it was based on gliadins, COP, or quality traits.

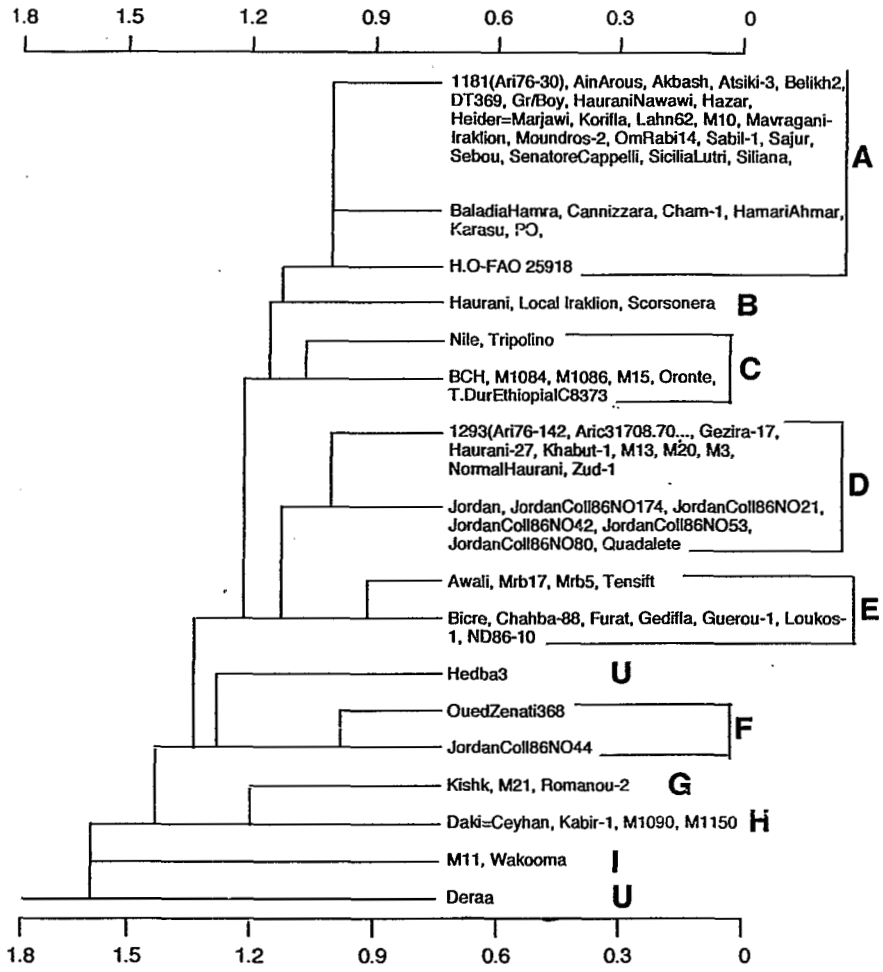


Fig. 2. Relationships based on gliadin banding patterns of 81 durum accessions.

When sampling germplasm for incorporation into a breeding program, if the goal is to maximize genetic diversity, Nei's genetic relationship based on RFLP would likely be the most reliable measure to use. Neither RFLP nor gliadin relationship was sufficiently correlated with the quality relationships to warrant the use of quality traits for measuring genetic diversity. Therefore, selection of germplasm pools for genetic diversity should be based on RFLPs or other traits rather than quality traits. Distance matrices derived from certain morphological or quality traits may be strongly influenced by environmental conditions under which the material is evaluated and single genes can have major effects on those estimates. Also, the effects of quality genes from one variety may be expressed differently in a different background. If the primary goal is improving end-use quality, the breeder may wish to select a pool of germplasm for quality traits first, then, using RFLP-based relationship

coefficients, select the least related set of lines for crossing to elite breeding lines and varieties. Several of the RFLPs observed are strong candidates for mapping and gene tagging loci controlling quality traits.

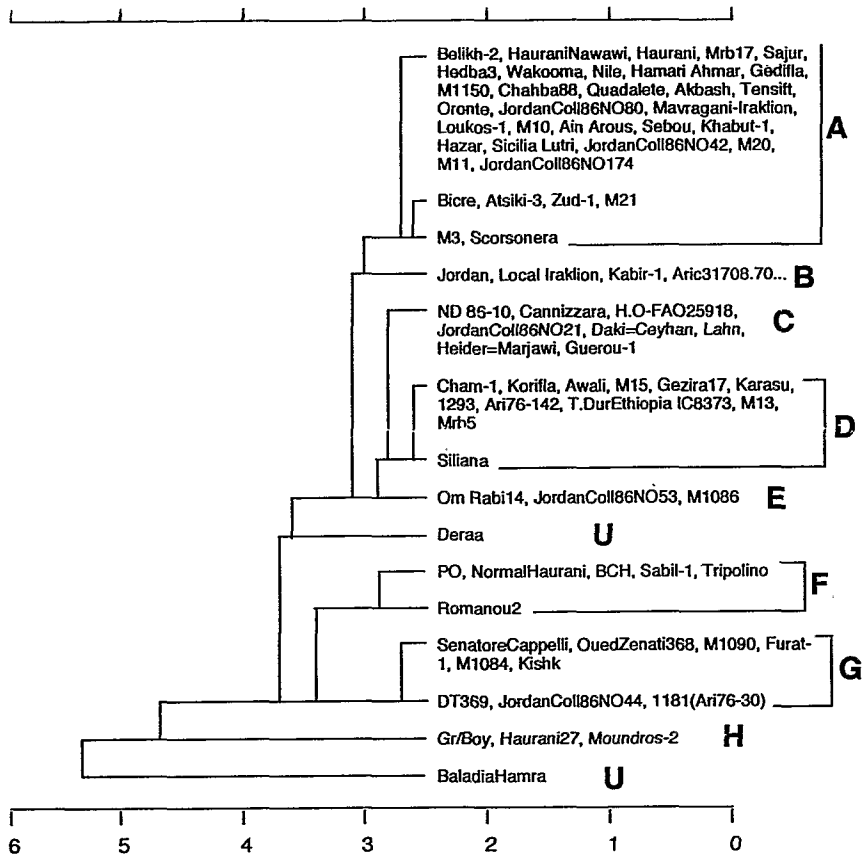


Fig. 3. Relationships based on quality traits for 81 durum accessions.

| | SDSI | Prot | Vit | TKW | Clr |
|---|---|---|---|---|---|
| A | 2.2 | 10.9 | 94 | 48 | 4.1 |
| B | 1.7 | 9.9 | 87 | 50 | 5.3 |
| C | 1.6 | 11.3 | 98 | 43 | 5.4 |
| D | 2.3 | 10.1 | 98 | 44 | 5.6 |
| E | 2.3 | 12.0 | 99 | 40 | 5.2 |
| F | 3.1 | 10.7 | 90 | 48 | 4.3 |
| G | 2.5 | 12.8 | 99 | 51 | 4.0 |
| H | 2.7 | 9.3 | 74 | 47 | 3.2 |
| Deraa | 1.4 | 12.4 | 100 | 53 | 6.0 |
| BalHa | 0.9 | 11.4 | 95 | 43 | 2.0 |
| Mean | 2.2 | 11.0 | 94 | 47 | 4.5 |

Fig. 4. Quality based cluster means for quality traits. Associations between quality traits and gliadin or RFLP bands

Table 2. Mean difference between those accessions possessing and those missing the indicated band for SDSI, protein content, vitreous seed percentage, thousand kernel weight, and kernel colour

| Band | SDSI | Protein | Vitreousness | Kernel wt | Colour |
|---|---|---|---|---|---|
| *RFLP* | | | | | |
| BCD809.24b(3)† | | -0.38*** | | | 0.69** |
| CDO669.31e(4) | -0.27** | | 3.6** | | 0.64** |
| BCD1355.13f(7) | | 0.35*** | | | |
| WG583.5a(5) | -0.27** | | | | |
| BCD1355.13d(7) | | | -0.90** | -4.2** | |
| CDO405.15c(2) | | -0.70** | | | |
| BCD21.37c(5,6) | | 0.80** | | | |
| CDO718.11d(3) | | | 3.8** | | |
| BCD926.8b(5) | | | 6.8*** | | |
| CDO393.27f(1) | | | | | 0.60** |
| BCD1095.36h(2) | | | | | -0.69** |
| BCD21.37e(5,6) | | | | | -0.85*** |
| *Gliadins* | | | | | |
| 42 | -0.23* | | | | |
| 43 | 0.23* | | | | |
| 45 | 0.29* | | | | |
| 78 | | -0.96** | | | |
| 76 | | | | | -0.68** |

*Significant at the 0.05 probability level
**Significant at the 0.01 probability level
***Significant at the 0.001 probability level
†Numbers in parenthesis indicate probable chromosome location

## RFLPs *vs* gliadins or pedigrees for estimating genetic relationships

Based on the results of previous studies, nuclear RFLPs are among the most effective traits for investigating genetic relationships among plants at the intraspecific level (Wang and Tanksley, 1989; Beer *et al.*, 1993). Because it is likely that much of the RFLP is attributable to insertions and deletions (eg Wang and Tanksley, 1989), the probability of two such mutations producing fragment patterns which are indistinguishable (ie convergent evolution) is small. Compared to RFLPs, there is greater likelihood that gliadin electrophoretic patterns that appear identical could have resulted from independent mutations. Any survey that uses seed proteins, isozymes, or DNA probes samples a very small fraction of the total genome; thus, each instance of convergent evolution at a sampled locus leads to an underestimation of mutation at numerous unsampled loci. Also, it is likely that gliadins are subject to selection in breeding programs. This would lead to over-estimation of genetic similarity of lines selected in breeding programs. All of these factors may have contributed to the low correlation between RFLP and gliadin based relationship estimates.

The low correlation between RFLP relationship and COP could result from violation of one of one or more of the assumptions necessary for accurate calculation of COP. Several of the ancestors that were assumed to be unrelated for calculating COP, in fact are known to have a strong relationship based on RFLPs. Using RFLP information one might devise a statistical method for adjusting the COP for ancestors to reflect their known relationship. When we introduced such an adjustment into our

calculations, the correlation did not improve significantly. Selection or drift might also affect the accuracy of the COP values. Finally, while the pedigrees of crosses may be accurate, occasional natural outcrossing in the breeding nursery could result in different RFLP patterns that would not reflect the ancestry.

## Associations between quality traits and gliadin bands or RFLPs

Selectable markers for quality traits could be used in a breeding program to facilitate early generation selection with improved heritability. The associations between a particular band or fragment and a quality trait identified in this study do not demonstrate genetic linkage; however, rather than randomly choosing markers distributed over the entire genome one can test these markers for linkage first in populations segregating for both the marker and the trait. In this study, a loose association was observed between SDSI and the absence of gliadin 42 and the presence of 45 as reported by others (Damidaux *et al.*, 1978; Yupsanis and Moustakis, 1988; Pogna *et al.*, 1990); however, there was much variation unaccounted for by the gliadin patterns observed. Most of the significant RFLPs observed for SDSI were located on chromosome 5, but there were also significant markers located on chromosomes 1 and 6, known locations of gliadin and glutenin genes affecting SDS sedimentation (Jackson *et al.*, 1983; Metovsky *et al.*, 1990; Rogers *et al.*, 1990).

## Implications for germplasm conservation

Neither RFLP nor gliadin distances were highly correlated with distances based on quality traits. Advantages to using RFLPs to estimate relationship include the larger number of loci that can be sampled relative to gliadins and the reduced likelihood of being subject to selection in a breeding program. Because RFLPs provide a better estimate of genetic relationship, the intermating of genotypes with low RFLP-based similarity values would create more genetic variability than would the intermating of dissimilar genotypes based on gliadins. Breeders must weigh this advantage against the considerable expense of generating RFLP data. Other methods such as the random amplified polymorphic DNA (RAPD) method have been developed (Williams *et al.*, 1990) that may be less costly. Preliminary results with these accessions suggest that relationships based on RAPDs and RFLPs are correlated (E. Autrique, pers. comm.). Germplasm managers and breeders need not use one type of data exclusively. One potential strategy is to select genotypes initially on the basis of useful agronomic characteristics or quality traits, and then select from those genotypes, a subset which are mutually dissimilar, on the basis of RFLP or RAPD data. This could maximize opportunities for transgressive segregation because there is a higher probability that unrelated genotypes will contribute unique desirable alleles at different loci.

A future goal of this project is to develop an information base for this germplasm pool consisting of agronomic and quality trait data as well as molecular and biochemical markers, their chromosome location, and their linkage to loci affecting those traits. This information can then be used to identify appropriate gene or marker combinations and relationship parameters that optimize parental selection for hybridization in combined conventional and marker-assisted-selection breeding programs.

**Acknowledgements**

The authors express their sincere thanks to ICARDA, Aleppo, Syria and CIMMYT, Mexico for support for this research and to the coordinators of the seminar on Durum Wheat Quality in the Mediterranean Region for their assistance.

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