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RAPD polymorphism of wild emmer wheat populations, *Triticum dicoccoides*, in Israel

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Abstract Genetic diversity in random amplified polymorphic DNAs (RAPDs) was studied in 110 genotypes of the tetraploid wild progenitor of wheat, *Triticum dicoccoides*, from 11 populations sampled in Israel and Turkey. Our results show high level of diversity of RAPD markers in wild wheat populations in Israel. The ten primers used in this study amplified 59 scorable RAPD loci of which 48 (81.4%) were polymorphic and 11 monomorphic. RAPD analysis was found to be highly effective in distinguishing genotypes of *T. dicoccoides* originating from diverse ecogeographical sites in Israel and Turkey, with 95.5% of the 100 genotypes correctly classified into sites of origin by discriminant analysis based on RAPD genotyping. However, inter-population genetic distances showed no association with geographic distance between the population sites of origin, negating a simple isolation by distance model. Spatial autocorrelation of RAPD frequencies suggests that migration is not influential. Our present RAPD results are non-random and in agreement with the previously obtained allozyme patterns, although the genetic diversity values obtained with RAPDs are much higher than the allozyme values. Significant correlates of RAPD markers with various climatic and soil factors suggest that, as in the case of allozymes, natural selection causes adaptive RAPD ecogeographical differentiation. The results obtained suggest that RAPD markers are useful for the estimation of genetic diversity in wild material of *T. dicoccoides* and the identification of suitable parents for the development of mapping populations for the tagging of agronomically important traits derived from *T. dicoccoides*.

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Introduction

Wild emmer wheat, *Triticum dicoccoides* is the tetraploid progenitor of cultivated hexaploid bread wheat. It has been the focus of many genetic, ecological, physiological and cytogenetic studies. The rich genetic diversity of wild emmer wheat for multiple disease resistances, agronomic traits of economic significance and environmental adaptations, has been reviewed previously (Nevo 1983, 1989, 1995). Populations of wild wheat are geographically structured and are predictable in terms of ecological and allozyme markers. Of special relevance to the present paper are the previous studies scoring the allelic variation at about 42 protein coding loci in 37 populations from Israel and Turkey (Nevo and Beiles 1989). From the distribution and frequencies of these defined alleles, it was concluded that natural selection was responsible for some of the difference between populations in localities with different climates and soil types. The present study explores the variation in RAPD band patterns and its relationship to the allozymic diversity in these populations.

Random amplified polymorphic DNA (RAPD) markers represent amplification products from a polymerase chain reaction (PCR) utilizing arbitrary primers and genomic DNA (Williams et al. 1990). Most variation among individuals for RAPDs probably arises from base-pair substitutions or insertions/deletions that modify (or eliminate) the primer site, or from insertions in the genomic sequence that separate the primer sites to a distance that will not permit amplification (Williams et al. 1990). RAPD markers have been demonstrated to be useful for the studies of taxonomic identities, systematic relationships, population genetic structure, species hybridizations, and parentage identifications (for reviews see McClelland and Welsh 1994; Penner 1996).

RAPDs were also proved to be useful as genetic markers in the case of self-pollinating species with a relatively low level of intraspecific polymorphism, such as hexaploid wheat, *Triticum aestivum* (e.g. Devos and Gale 1992; Joshi and Nguyen 1993 b) and cultivated barley, *Hordeum vulgare* (e.g. Barua et al. 1993; Chalmers et al. 1993; Tinker et al. 1993). RAPDs were also used for population genetic studies of wild barley, *Hordeum spontaneum* (e.g. Dawson et al. 1993; Baum et al. 1997; Owuor et al. 1997; Nevo et al. 1998). The application of RAPDs in wheat at first encountered problems of poor levels of reproducibility and low polymorphism, which were attributed to the large genome size and the high proportion of repetitive DNA characteristic of this species (Devos and Gale 1992). Several approaches were proposed to circumvent these problems, including the combination of RAPDs with denaturing gradient gel-electrophoresis (He et al. 1992) and pre-digestion of the DNA template with frequently cutting restriction enzymes (Koebner 1995). However, other studies suggested that careful optimization and strict control of PCR conditions are essential for the application of the RAPD technique in wheat (Joshi and Nguyen 1993 a, b; Penner 1996). Since then numerous studies have employed RAPD markers for various applications in wheat, including the estimation of genetic diversity among diploid (Vierling and Nguyen 1992), tetraploid (Joshi and Nguyen 1993 a) and hexaploid (He et al. 1992; Joshi and Nguyen 1993 b) wheats, tagging disease resistance genes (e.g. Schachermayr et al. 1994; Fahima et al. 1995; Talbert et al. 1996; Sun et al. 1997), pest resistance genes (e.g. Dweikat et al. 1994), and other important genes, such as the gene governing cadmium uptake (Penner et al. 1995).

In a previous study we demonstrated the efficiency of RAPD analysis for the identification of markers linked to the yellow rust resistance gene, *Yr15*, using the near-isogenic lines approach (Sun et al. 1997). In the present study, we have applied RAPD markers for the differentiation and estimation of genetic diversity among different populations of *T. dicoccoides* originating primarily from northern Israel, and earlier studied for allozyme diversity (Nevo et al. 1982; Nevo and Beiles 1989).

Materials and methods

Ecological background of wild emmer wheat

Wild emmer wheat, *T. dicoccoides* (genomic constitution AABB), is the tetraploid, predominantly self-pollinated, wild progenitor from which modern tetraploid and hexaploid cultivated wheats were derived (Zohary 1970). Wild emmer is distributed over the Near East Fertile Crescent, in Israel, Jordan, Lebanon, Syria, east Turkey, north Iraq and west Iran (Harlan and Zohary 1966). The center of distribution and diversity of *T. dicoccoides* is found in the catchment area of the upper Jordan Valley in Israel and its vicinity (Nevo and Beiles 1989). Wild emmer ranges over a wide altitudinal amplitude. Robust, early maturing phenotypes grow in the winter-warm slopes facing the sea of Galilee, as low as 100 m below sea level. More slender and late flowering types occur in higher and cooler elevations, reaching 1500 m on Mount Hermon (Zohary 1970). The populations of wild emmer are dense and lush in the catchment area of the upper Jordan Valley (Golan Heights, upper eastern Galilee mountains, etc.). However, towards their marginal and peripheral areas, both in Israel and Turkey, they become semi-isolated or isolated, and smaller in size. This distributional pattern has a dramatic effect on their population genetic structure and differentiation (Nevo and Beiles, 1989).

Plant material and isolation of DNA

Individual plants of *T. dicoccoides* were collected at random, at least 1-m apart, from populations differing in major ecological properties. These collection sites and populations have been described in detail elsewhere (Nevo et al. 1982; Nevo and Beiles 1989). The genotypes used for the present study are conserved in the cereal gene bank of the Institute of Evolution, University of Haifa. Altogether, 110 genotypes from 11 populations were analyzed with RAPD markers, ten populations from Israel and one from Turkey. The locations of the tested populations are listed in Table 1. Figure 1 shows the geographic distribution of the ten Israeli populations. The map location of the Diyarbakir population in Turkey can be found in Fig. 1 of Nevo and Beiles (1989). The Israeli climate data is from the Atlas of Israel (1970) and publications of the Meteorological service of Israel.

Five to ten seeds of each genotype were germinated in the dark for 5–6 days at room temperature. The dark-grown shoots were frozen in liquid nitrogen and ground with a small amount of glass powder in a mortar, and the DNA was isolated as described by Junghans and Metzlaff (1990).

RAPD analysis

A series of optimization experiments using 11 DNA samples (one genotype from each population) was conducted in which concentrations of template DNA, primers, dNTPs, and *Taq* polymerase were varied to determine which conditions gave the strongest and most reproducible patterns. Reproducibility was gauged by comparing duplicate reactions, between which the only source of variation should have been the pipetting error in adding the master mix and template DNA. These duplicate reactions were usually adjacent to one another in the thermocycler, and products were run side by side on the same gel. Using the thermal cycling regime of Williams et al. (1990), low reproducibility was obtained for at least half of the primers tested. Dilution of *Taq* polymerase with storage buffer (Perkin-Elmer), before adding into the reaction mix, significantly increased the intensity of products and the reproducibility of band patterns. The optimized reaction conditions for all RAPD patterns scored in this study are as follows: PCR reaction mixtures of 20 μl contained 20 ng of template DNA, 0.5 U of Thermostable DNA Polymerase and incubation buffer (IV) from Advanced Biotechnologies (Leatherhead, Surrey, UK), 2 mM $MgCl_2$, 0.1 mM of each: dCTP, dGTP, dTTP and dATP, and 12 ng of a single 10-mer primer obtained from Operon Technologies or the University of British Columbia (Table 2). Amplifications were performed in a DNA Thermo-cycler (Perkin Elmer) programmed for 45 consecutive cycles each consisting of 1 min at 94°C, 2 min at 37°C and 2 min at 72°C. Following amplification, the samples were subjected to electrophoresis in 1.4% agarose gels, stained with 0.5 μg/ml of ethidium bromide and viewed under ultra-violet light.

Data scoring and statistical analysis

The gels were scored for the presence or absence of only those major bands which showed a reproducible pattern among genotypes. Following Lynch and Milligan (1994) we regarded each band as a locus with two alternative alleles: present (1) or absent (0). We also based our analysis on the assumptions, proposed by Lynch and Milligan (1994), that alleles from different loci do not co-migrate, and that each locus can be treated as a two-allele system. The main concern of Lynch and Milligan (1994) was the estimation of indices in RAPD analysis, when heterozygotes are not distinguished. That problem does not exist in *T. dicoccoides* because it is a self-pollinating species with a quite limited rate of outcrossing (estimated t approximately 0.005; Golenberg 1987). Hence, we have assumed

Table 1 Geographical and climatical data for 11 populations of *T. dicoccoides* in Israel and Turkey. Symbols of variables: geographical: Ln = longitude (decimals), Lt = latitude (decimal), Al = Altitude (m); temperature: Tm = mean annual temperature, Ta = mean August temperature, Tj = mean January temperature, Td = seasonal temperature difference, Tdd = day-night temperature difference, Trd = mean number of tropical days; water availability: Rn = mean annual rainfall (mm), Rd = mean number of rainy days, Huan = mean annual humidity, Hu14 = mean humidity at 14:00, Dw = mean number of dew nights in summer, Ev = mean annual evaporation, Rv = mean interannual variability of rainfall, Rr = mean relative variability of rainfall; edaphic: So = soil type, 1 = terra rossa, 2 = rendzina, 5 = basalt

No.[a]	Population	Ln	Lt	Al	Tm	Ta	Tj	Td	Tdd	Rn	Rd	Hu14	Huan	Dw	Trd	Ev	So	Rv	Rr
1	Mt. Hermon	35.73	33.30	1300	11	21	3	18	6	1400	66	48	60	60	0	150	1	30	20
8	Gamla	35.74	32.88	200	19	26	9	17	12	470	50	43	58	58	60	155	5	39	26
9	Rosh Pinna	35.52	32.95	700	18	25	9	16	10	697	50	48	58	50	35	150	1	35	22
11	Tabigha	35.53	32.90	0	24	32	15	17	10	436	45	45	57	58	120	160	5	39	25
16	Mt. Gilboa	35.42	32.50	150	21	28	12	16	12	400	44	43	58	40	160	165	1	34	24
17	Mt. Gerizim	35.28	32.20	800	17	23	8	15	9	700	47	45	60	42	0	155	1	38	25
18	Gitit	35.40	32.10	300	21	29	13	16	12	360	39	39	55	25	100	170	1	38	24
19	Kokhav Hashahar	35.34	31.95	600	20	28	12	16	12	400	40	45	59	30	25	165	1	38	22
30	Bat-Shelomo	35.02	32.60	75	20	26	13	13	10	650	55	58	68	77	30	150	2	24	20
33	Givat Koach	34.92	32.03	75	20	26	12	14	12	540	46	50	64	65	105	160	1	32	26
36	W. Diyarbakir, Turkey	39.63	37.89	850	13	27	2	25	–	546	65	–	46	–	–	–	5	–	–

[a] Population numbers are according to Nevo and Beiles (1989)

Fig. 1 Geographic distribution of the ten tested populations of wild emmer wheat in Israel. For names of the numbered populations see list in Table 1. The numbers of populations are according to Nevo and Beiles (1989)

100% homozygosity and analyzed the data accordingly. The identification of 59 scorable bands led to the construction of a 110 genotypes × 59 loci data matrix which was analyzed for diversity within and between populations. Polymorphism at the 5% level (*P*), gene diversity (*He*) and genetic distance coefficients (*D*) (Nei 1978), were calculated by the BIOSYS-1 program (Swofford and Selander 1989). The computation of the Spearman rank correlation between RAPD gene diversity (*He*) values obtained in this study and allozyme *He* values obtained previously (Nevo and Beiles 1989) was carried out by using SAS procedure PROC CORR (SAS Institute 1996). Various discriminant analysis procedures (SAS Institute 1996) were carried out as follows: (1) SAS procedure PROC STEPDISC was used for choosing the loci which are the best differentiating factors between the 11 populations; (2) SAS procedure PROC NEIGHBOR, which is suitable for classification when the classes have a radically non-normal distribution, was used for correct classification of plants into their respective populations, based on the loci chosen by PROC STEPDISC; and (3) SAS procedure PROC CANDISC was used to generate a graphical display of the first three parametric canonical discriminant functions. Stepwise multiple regression (MR), with ecogeographical variables as the independent variables, was conducted to find out the best predictors of *P*, *He* and representative allele frequencies at each of the polymorphic loci (SAS Institute 1996). Spatial autocorrelation analysis was conducted by the SAAP program, release 2.3 (Sokal and Wartenberg 1983).

Results

Initial screening of more than 100 primers resulted in the identification of ten primers which yielded

Table 2 List of the primers used in the RAPD analysis, their sequence, number of bands and size range

Primer[a]	Sequence (5′ → 3′)	Total no. of bands	No. of scorable bands	Size range of scorable bands (bp)
1. OPA-12	TCGGCGATAG	14	5	430–840
2. OPA-19	CAAACGTCGG	14	6	500–920
3. OPA-20	GTTGCGATCC	14	6	600–1600
4. OPF-15	CCAGTACTCC	15	6	460–930
5. OPT-17	CCAACGTCGT	16	6	400–1170
6. UBC-486	CCAGCATCAG	14	6	600–1100
7. UBC-494	TGATGCTGTC	12	5	490–930
8. UBC-537	CGAAAGGACT	17	6	530–880
9. UBC-551	GGAAGTCCAC	11	5	500–1130
10. UBC-561	CATAACGACC	16	6	650–1690

[a] Primers were obtained from Operon Technologies (OPA-12, 19, 20, OPF-15 and OPT-17), or from the University of British Columbia (UBC-486, 494, 537, 551 and 561). In the text "OP" and "UBC' were omitted

informative, polymorphic products resolvable by gel electrophoresis (Table 2). Usually, products below 300 bp or above 3 kb gave faint and non-reproducible bands, hence most of the scored products are in the range of 0.3–2 kb. The RAPD primers amplified between 5 and 15 bands per genotype; however only 5–6 of them were scorable, with a molecular size range of 400–1700 bp (Table 2). Using ten RAPD primers, a total of 59 bands were scored. Figure 2 shows an example of such a typical RAPD pattern using the F-15 primer. Only the six bands at 460, 550, 600, 740, 830 and 930 bp were considered; there are more diffuse bands visible in some genotypes, but they were not considered because of their ambiguous nature.

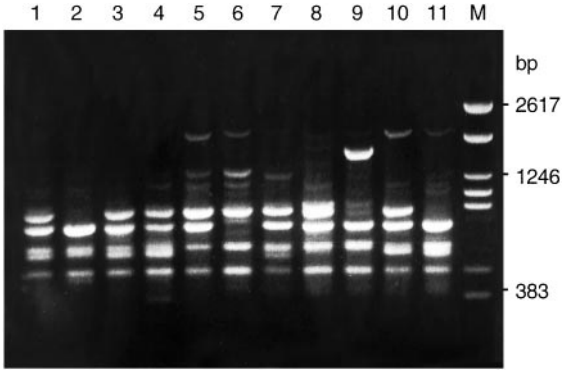

Fig. 2 Amplification products generated from genomic DNA of 11 genotypes representing 11 populations of *T. dicoccoides* with RAPD primer F15. Each lane shows the RAPD profile of one representative genotype from one wild wheat population. *Lane 1* Gitit 18-35; *lane 2* Givat-Koach 17-31; *lane 3* Kokhav Hashahar K-36; *lane 4* Gerizim M-35; *lane 5* Mt. Hermon G-49; *lane 6* Tabigha H-54; *lane 7* Rosh Pinna C-6; *lane 8* Gamla 36-35; *lane 9* Bat-Shelomo I-30; *lane 10* Diyarbakir, Turkey T10-67; *lane 11* Gilboa L-61; *lane M* molecular-weight marker, pBR322 digested with *Alw*441/*Mval*

Pattern of variation of RAPD loci

The ten selected primers were used to examine the level of polymorphism detectable in the ten populations of *T. dicoccoides* sampled in Israel and the one from Turkey. All ten primers detected polymorphism within the 110 *T. dicoccoides* genotypes tested. Out of the 59 RAPD loci scored, 48 (81.4%) were polymorphic among the 110 wild wheat geonotypes originating from ten different geographic locations in Israel and one location in Turkey, while 11 (18.6%) were monomorphic in all of the 11 tested populations. An example of the polymorphism detectable with primer F-15 is given in Fig. 2. The allele frequencies for the 48 polymorphic loci appear in the Appendix.

RAPD genetic diversity

A summary of the genetic data for each of the 11 populations of *T. dicoccoides* is given in Table 3. Mean levels of the proportion of polymorphic loci, *P* (5%), and the gene diversity, *He* (Nei 1978), of the 11 populations of wild emmer wheat were 0.524 and 0.188, respectively. The range of variation in *He* between the wild wheat populations was large, 0.138–0.213. The highest value of *He* (0.213) was obtained in the high-altitude and arid-cold steppic population of Mt. Hermon which represents one of the most stressful xeric-cold habitats in Israel. High *He* values (0.212) were also obtained for the Rosh-Pinna and Tabigha populations. In contrast, the populations from Mt. Gerizim in Israel and Diyarbakir in Turkey were characterized by low levels of diversity, with *He* values of 0.138 and 0.149, respectively.

RAPD versus allozyme gene-diversity profiles

The genetic-diversity profiles obtained in this study with RAPD markers were compared to those obtained

Table 3 Summary of genetic variation, based on 59 RAPD loci in 11 populations of *T. dicoccoides* in Israel and Turkey

No.[a]	Population[b]	RAPD proportion of polymorphic loci (*P*)[c]	Allozyme[d] proportion of polymorphic loci (*P*)	RAPD gene diversity *He*[e] (SE)	Allozyme[d] gene diversity *He*
1	Mt. Hermon	0.593	0.214	0.213 (0.027)	0.060
8	Gamla	0.627	0.308	0.212 (0.025)	0.102
9	Rosh-Pinna	0.593	0.238	0.212 (0.026)	0.093
11	Tabigha	0.525	0.167	0.203 (0.028)	0.054
16	Mt. Gilboa	0.542	0.143	0.192 (0.026)	0.044
17	Mt. Gerizim	0.407	0.167	0.138 (0.024)	0.059
18	Gitit	0.509	0.293	0.189 (0.027)	0.116
19	Kokhav Hashahar	0.509	0.190	0.187 (0.027)	0.058
30	Bat-Shelomo	0.525	0.214	0.201 (0.028)	0.085
33	Givat Koach	0.492	0.100	0.178 (0.026)	0.026
36	W. Diyarbakir, 22 km	0.441	0.167	0.149 (0.025)	0.055
Average		0.524	0.200	0.188	0.068
Range		0.407–0.627	0.100–0.308	0.138–0.213	0.026–0.116

[a] Population numbers are according to Nevo and Beiles (1989)
[b] The RAPD study included a sample size of ten accessions per population, while the allozyme study included 25–48 (average of 38) accessions per population
[c] *P* = Proportion of polymorphic loci (5%)
[d] Allozyme data is according to Nevo and Beiles (1989)
[e] *He* = Gene diversity, equivalent to the expected heterozygosity under panmixia (Nei 1978)

previously with allozyme markers (Nevo et al. 1982; Nevo and Beiles 1989) (Table 3). All of the populations studied here showed a higher proportion of polymorphic loci (*P*) and higher gene diversity (*He*) values for RAPD loci as compared to allozyme loci. The average RAPD values for *P* and *He* were 0.524 (range 0.407–0.627) and 0.188 (range 0.138–0.213), as compared to allozyme values of 0.200 (range 0.100–0.308) and 0.068 (range 0.026–0.116), respectively. These results show that RAPD loci are more polymorphic than allozyme loci among the wild wheat populations from Israel and Turkey.

The correlation between RAPD gene diversity (*He*) values and allozyme *He* values was also tested. The results, presented in Fig. 3, show a positive ($r = 0.414$) Spearman rank correlation between RAPD and allozyme *He* values. It is interesting to note that all of the populations that show high allozyme *He* values also showed high RAPD *He* values. However, there were many cases in which low allozyme *He* values were associated with high RAPD values. There were no opposite cases in which high allozyme *He* values were associated with low RAPD *He* values. It is noteworthy that the previous allozyme analysis (Nevo and Beiles 1989) included 423 *T. dicoccoides* individuals (on average 38 genotypes per population), of the same 11 populations tested here, while the RAPD analysis included only 110 genotypes (ten genotypes per population). We would expect a higher correlation level between RAPDs and allozymes, if the analysis had been based on identical plant material with the same sample size.

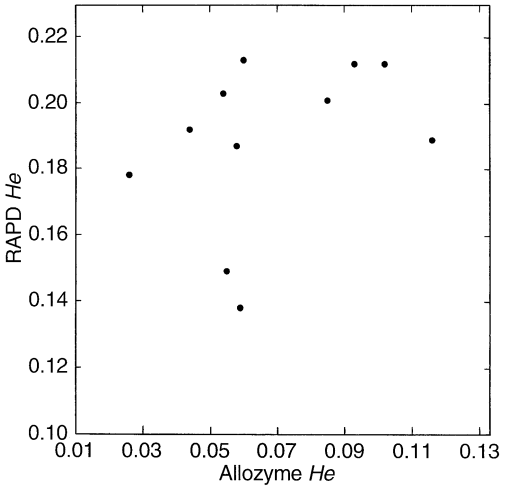

Fig. 3 Correlation between RAPD and allozyme gene diversity profiles. The Spearman rank correlation was tested between the *He* values produced by RAPD and allozyme profiles

Genetic distance

Coefficients of genetic distance, *D*, were calculated for paired comparisons of the 11 populations, based on the normalized identity of all loci between each of the populations (Nei 1978). The results are given in Table 4. The mean value of *D* was 0.089, range 0.017–0.165. The highest genetic distance (0.165) was obtained between the population of Mt. Gerizim and the population of Tabigha, while the most similar populations were at

Table 4 Coefficients of genetic distance (*D*; Nei 1978) among 11 populations of *T. dicoccoides* in Israel and Turkey. *D*: mean = 0.089, range = 0.017–0.165

Population	1	8	9	11	16	17	18	19	30	33
1. Mt. Hermon										
8. Gamla	0.075									
9. Rosh-Pinna	0.053	0.053								
11. Tabigha	0.108	0.072	0.088							
16. Mt. Gilboa	0.117	0.129	0.101	0.155						
17. Mt. Gerizim	0.075	0.123	0.106	0.165	0.111					
18. Gitit	0.057	0.069	0.090	0.110	0.101	0.089				
19. Kokhav Hashahar	0.055	0.073	0.055	0.074	0.067	0.062	0.071			
30. Bat-Shelomo	0.037	0.064	0.050	0.070	0.069	0.063	0.071	0.017		
33. Givat Koach	0.133	0.127	0.065	0.131	0.117	0.133	0.120	0.076	0.078	
36. Diyarbakir	0.069	0.133	0.106	0.131	0.089	0.074	0.100	0.083	0.065	0.130

Bat-Shelomo and Kokhav-Hashahar with a genetic distance of 0.017. The estimates of *D* were geographically independent. They displayed large *D*s, i.e. sharp genetic differentiation over very short geographic distances, against low *D*s between geographically distant populations. For example, the genetic distance obtained between the Mt. Gilboa population and the Tabigha population, located only about 50 km apart, was 0.155, whereas the genetic distance between the Bat-Shelomo population in Israel and the Diyarbakir population from Turkey, separated by 750 km, was only 0.065. Many other such examples occur and are given in Table 4.

Discriminant analysis

The discriminant analysis succeeded in differentiating significantly, on the basis of allele presence or absence, between almost all of the 11 populations. The summary of results is presented in Table 5 and Fig. 4. The STEPDISC procedure chose the following 15 loci as the best differentiating factors: A12-460, F15-740, F15-830, A20-600, F15-600, A19-850, A20-1200, 494-490, 551-790, 551-500, F15-550, 486-600, A20-1600, 561-1690, and A19-530 (Table 5). The correct classification of individual genotypes into their respective population according to their geographic distribution was 95.5%. A graphical illustration of the first, second and third canonical discriminant functions, which represent 36.9%, 16% and 12% of the sum of the eigenvalues of the ten discriminant functions, respectively, is displayed in Fig. 4, based on the presence/absence of the 48 polymorphic RAPD loci. These results indicate that RAPD markers can be used to differentiate between *T. dicoccoides* genotypes and to classify them according to their respective populations, based on multilocus analysis. Figure 4 shows that the genotypes of most of the populations are clustered together. The separation of Tabigha and Gamla from the other eight Israeli populations by the first three canonical discriminant functions (Fig. 4) is of special interest due to the ecological factor shared by these two populations. The two populations are located on basalt soil, while all other eight Israeli populations are located on limestone rocks. However, it should be noted that while the Turkish population from Diyarbakir is also located on basalt soil, it was not clustered together with the Isareli basalt populations, Tabigha and Gamla, although it is well separated from the other Israeli populations. Several other Israeli populations also showed clear separation from each other, such as Hermon, Givat Koach, Gitit and Gerizim. Most of these are isolated marginal populations that can also be distinguished by their unique ecogeographical conditions.

Spatial autocorrelation

Spatial autocorrelation analysis is a statistical analysis approach for quantifying spatial relations among a set of univariate data observations. It gives a measure of the level of correlation of the observed values (in our case allele frequencies) at each locality with values of the same variables at other geographic sections. The method has been extended in biology to include the computation of correlograms for spatial autocorrelation. These show the autocorrelation coefficients (and their significance levels) as a function of the distance between the pairs of localities being considered, and summarize the patterns of geographic variation exhibited by the response surface of any given variable (Sokal and Oden 1978 a, b). We calculated Moran's I autocorrelation coefficient of allele frequencies across the entire geographic range given in our study including all 11 populations. We partitioned the space into four distance classes, so that each class contained equal numbers of locality pairs, except for the 4th class which contained the ten pairs of the Turkish population with

Table 5 Summary table of 15 chosen loci obtained by stepwise discriminant analysis, based on the allele frequencies of 48 polymorphic loci among 11 populations of *T. dicoccoides* in Israel and Turkey

Locus	Number in	Partial R^2	*F* Statistic	Prob > *F*	Wilk's lambda
1. A12-460	1	0.7105	24.300	0.0001	0.2895
2. F15-740	2	0.5636	12.658	0.0001	0.1263
3. F15-830	3	0.5405	11.410	0.0001	0.0580
4. A20-600	4	0.4913	9.273	0.0001	0.0295
5. F15-600	5	0.4408	7.488	0.0001	0.0165
6. A19-850	6	0.4086	6.496	0.0001	0.0098
7. A20-1200	7	0.3460	4.921	0.0001	0.0064
8. 494-490	8	0.3267	4.463	0.0001	0.0043
9. 551-790	9	0.3153	4.191	0.0001	0.0029
10. 551-500	10	0.3405	4.647	0.0001	0.0019
11. F15-550	11	0.3292	4.367	0.0001	0.0013
12. 486-600	12	0.3080	3.917	0.0002	0.0009
13. A20-1600	13	0.2835	3.442	0.0008	0.0006
14. 561-1690	14	0.2886	3.488	0.0007	0.0005
15. A19-530	15	0.2828	3.351	0.0010	0.0003

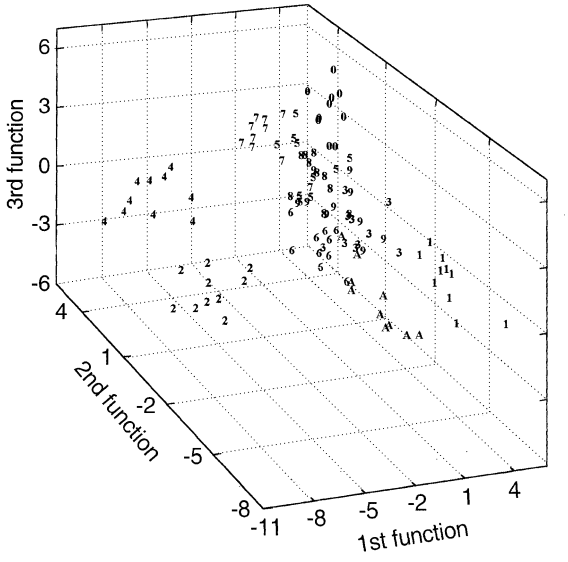

Fig. 4 Plot of canonical discriminant functions 1, 2 and 3 based on the 48 polymorphic RAPD loci. Symbols: *1* = Mt. Hermon; *2* = Gamla; *3* = Rosh Pinna; *4* = Tabigha; *5* = Mt. Gilboa; *6* = Mt. Gerizim; *7* = Gitit; *8* = Kokhav Hashahar; *9* = Bat-Shelomo; *0* = Givat-Koach; *A* = Diyarbakir. The proportion of the eigenvalues of functions 1, 2 and 3, from the sum of the eigenvalues of the ten discriminant functions, are 36.9%, 16% and 12%, respectively

all of the Israeli populations. The following are the main results (Table 6):

1) *Average coefficients.* All of the average autocorrelation coefficients over all tested alleles in all distance groups were very low with only a small deviation from the expected value. Note that in each column in Table 6, positive and negative values of low, medium, and sometimes high and significant estimates, sum up into low negative averages. This indicates that there is no similar pattern across loci in any of the distance classes.
2) *Low-order shorter distance* (*0–48 km*) *autocorrelations.* Positive, negative, and no autocorrelations were intermixed across loci:

2.1) positive autocorrelations (>0.1) were displayed by 14 RAPD loci; however, only three of them are significant ($P<0.05$; F15-550, A19-850 and A19-500),
2.2) negative autocorrelations (>-0.1) were displayed by 22 RAPD loci; although none of them is significant, their abundance suggests the existence of an opposite pattern to the expected highest positive autocorrelations in low distances.
3) *Scattered positive autocorrelations.* Generally, positive correlations were more frequent between low- and medium-order distance groups, whereas negative autocorrelations were more frequent in high-order distance groups.

Multiple regression analysis of environmental variables and RAPD polymorphisms

A test of the best predictors of *P*, *He* and representative allele frequencies at the 48 polymorphic loci in the ten Israeli populations was conducted by stepwise multiple regression (MR) analysis, employing these characters as dependent variables and geographic, climatic and edaphic factors as independent variables. The following environmental variables were included in the analysis: (1) *geographical*: longitude (Ln), latitude (Lt), altitude (Al); (2) *climate* means: (i) *temperature*; annual (Tm), January (Tj), August (Ta), seasonal temperature difference (Td), daily temperature difference (Tdd), number of tropical days (Trd), evaporation (Ev), (ii) *moisture*; annual rainfall (Rn), number of rainy days (Rd), number of dewy nights in summer (Dw), annual humidity (Huan), humidity at 14 : 00 (Hu14), inter-annual rainfall variation (Rv), coefficient of variation in rainfall (Rr); and (3) three *edaphic* dummy variables, one per each of the soil types: basalt (Ba), rendzina (Ren), and terra rossa (Tr). The test involved the ten Israeli populations (the Turkish population was not included in this test

Table 6 Spatial autocorrelation analysis of allele (present) frequencies at 48 loci of 11 *T. dicoccoides* populations in Israel and Turkey. Moran's I coefficients for each allele in four distance classes are given. The expected I is − 0.111 for classes 1–3 and − 0.100 for class 4

Allele	Distance classes (km)			
	0–48	48–88	88–200	200–700
N:	15	15	15	10
486-1100	− 0.25	− 0.01	− 0.07	− 0.49**
486-810	− 0.30	0.01	− 0.04	− 0.01
486-750	0.22	− 0.19	− 0.36	− 0.01
486-690	− 0.31	− 0.11	0.08	− 0.09
486-600	− 0.14	− 0.21	0.01	− 0.07
551-880	0.11	− 0.21	− 0.24	− 0.07
551-790	− 0.11	− 0.11	− 0.11	− 0.01
551-500	0.28	− 0.27	− 0.34	− 0.04
494-750	− 0.29	− 0.18	0.13	− 0.19
494-580	− 0.35	− 0.02	0.03	− 0.24
494-530	− 0.04	− 0.22	− 0.08	− 0.07
494-490	− 0.21	0.04	− 0.16	− 0.01
F15-930	− 0.27	− 0.11	0.04	− 0.07
F15-830	− 0.21	0.09	− 0.22	− 0.02
F15-740	− 0.11	− 0.11	− 0.11	− 0.01
F15-600	− 0.08	− 0.06	− 0.20	− 0.02
F15-550	0.35*	− 0.15	− 0.53*	− 0.04
F15-460	− 0.33	0.08	− 0.08	− 0.02
537-800	− 0.27	− 0.07	0.00	− 0.08
537-700	0.03	− 0.36	− 0.01	− 0.23
537-560	0.00	− 0.28	− 0.05	− 0.23
561-1690	− 0.02	0.13	− 0.44	0.00
561-1420	− 0.09	− 0.24	0.00	− 0.02
561-1020	0.03	− 0.42	0.06	− 0.06
561-810	0.17	− 0.25	− 0.25	− 0.02
561-730	− 0.17	− 0.17	0.00	0.00
561-650	0.13	− 0.56*	0.10	− 0.02
A20-1600	− 0.29	− 0.07	0.03	− 0.01
A20-1400	− 0.25	− 0.17	0.08	− 0.02
A20-1200	− 0.26	0.10	− 0.17	− 0.03
A20-1000	0.15	− 0.12	− 0.37	− 0.02
A20-600	− 0.26	− 0.08	0.00	− 0.56***
A19-920	− 0.11	− 0.04	− 0.19	− 0.01
A19-850	0.35*	− 0.60**	− 0.08	− 0.07
A19-780	− 0.06	− 0.03	− 0.24	− 0.07
A19-700	0.06	− 0.23	− 0.16	− 0.08
A19-530	0.11	− 0.22	− 0.22	− 0.02
A19-500	0.33*	− 0.08	− 0.58*	0.00
T17-1170	− 0.33	0.21	− 0.22	0.00
T17-800	− 0.22	− 0.03	− 0.08	− 0.17
T17-650	0.12	− 0.34	− 0.12	− 0.13
T17-470	− 0.04	0.04	− 0.33	− 0.01
T17-400	0.22	− 0.10	− 0.46	− 0.22
A12-720	− 0.32	− 0.09	0.08	− 0.02
A12-690	0.12	0.17	− 0.62**	− 0.08
A12-490	0.14	− 0.57*	0.09	− 0.28
A12-460	− 0.08	− 0.32	0.07	− 0.19
A12-430	0.08	− 0.25	− 0.17	− 0.02
Average	− 0.058	− 0.141	− 0.135	− 0.086
SE	0.030	0.026	0.027	0.017

*,**,***, significant ($p < 0.05$, $p < 0.01$ and $p < 0.001$) autocorrelation

due to missing ecological data). The results are given in Table 7.

Latitude, temperature and water-availability factors, singly or in combination, explained a significant proportion of the variation in polymorphism of RAPDs. The best two variable-predictors of *P* and *He*, explaining significantly 0.7–0.8 of their variance, were Lt and Tdd (latitude and day-night temperature difference). A three–variable combination involving both geographic (latitude) and climate (day-night temperature difference and mean annual temperature), LtTddTm, accounted significantly ($p = 0.001$) for 0.94 of the variance in *P*, while the combination LtTddBr (latitude, day-night temperature difference and mean relative variability of rainfall) accounted significantly ($p = 0.01$) for 0.88 of the variance in *He*.

RAPD loci can be classified into several categories in terms of their prime ecological predictors: (1) water factors (Rn, Hu, Ev, Rd): 537-800, 537-560, 561-1690, A19-500, T17-650; (2) soil type (Ba, Tr, Ren): 551-790, 551-500, 494-530, F15-740, 561-650, A19-850, A19-530; (3) Temperature (Ta, Td, Tm, Trd): 486-750, 486-600, 486-1100, 537-700, A12-490, A20-600, T17-800, T17-470, T17-400; (4) water + temperature (Hu, Rr, Trd, Tm): 486-600, A12-720, 537-700; (5) soil + temperature (Ba, Ren, Tr, Tj, Tdd, Trd, Ev): T17-800, 486-1100, 494-530, F15-550, A20-1000; (6) soil + water (Ba, Ren, Rd, Dw); T17-650, F15-830.

Discussion

RAPD analysis of the wheat genome

The main objective of the present study was to assess RAPD diversity among emmer wheat populations originating mainly from Israel and to test for relationships with ecological parameters and any correlation with allozyme diversity. RAPD analysis revealed extensive polymorphism between the different genotypes of wild emmer wheat in Israel and Turkey, with 81% of the 59 scorable bands showing polymorphism within this wild wheat germplasm. RAPD markers were shown previously to be useful for wheat genome analysis. However, strict PCR conditions are required in order to avoid problems of poor reproducibility believed to be associated with the large genome size and the high proportion of repetitive DNA characteristic of the wheat genome (e.g. Devos and Gale 1992; Joshi and Nguyen 1993 a, b; Koebner 1995). It is well documented, in wheat and in other plant species, that not all of the amplified RAPD bands are scorable and useful as markers. In the present study RAPD primers amplified between 5 and 15 bands per genotype; however, only 5–6 of them were scorable. This problem may be, in part, due to the relatively low resolving power of the agarose gels commonly used for RAPD analysis (Penner 1996). He et al. (1992) observed a higher level of scorable and reproducible polymorphisms in wheat when PCR samples were subjected to denaturing gradient-gel electrophoresis, as compared to agarose gels.

Table 7 Coefficient of multiple regressions (R^2) of genetic indices and allele frequencies, as the dependent variables, and environmental variables in ten populations of *T. dicoccoides* in Israel, as independent variables. The following is a list of 18 loci for which no variable met the 0.15 significance level for entry into the model: 486-810, 486-690, 551-880, 494-750, 494-490, F15-930, F15-600, F15-460, 561-1420, 561-810, 561-730, A20-1400, A20-1200, A19-920, A19-780, A19-700, T17-1170, A12-460

Genetic indices	Stepwise model					
	Ecogeographical variables			Ecological variables		
P	Lt 0.526*	Lt Tdd 0.781**	Lt Tdd Tm 0.936***	Td 0.277^{ns}		
He	Lt 0.485*	Lt Tdd 0.698*	Lt Tdd Rr 0.876**			
Alleles						
486-1100	Trd 0.752**	Trd Tr 0.848**		Trd 0.752**	Trd Tr 0.848**	
486-750	Ln 0.491*	Ln Trd 0.745**	Ln Trd Td 0.835**	Td 0.374$^{@}$	Td Trd 0.561$^{@}$	
486-600	Trd 0.608**	Trd Rr 0.799**	Trd Rr Al 0.887**	Trd 0.608**	Trd Rr 0.799**	Trd Rr Hu14 0.872**
551-790	Ba 0.444*			Ba 0.444*		
551-500	Ba 0.929***			Ba 0.929***		
494-580	Ba 0.279^{ns}			Ba 0.279^{ns}		
494-530	Ba 0.898***	Ba Ev 0.945***	Ba Ev Rr 0.971***	Ba 0.898***	Ba Ev 0.945***	Ba Ev Rr 0.971***
F15-830	Ln 0.359$^{@}$	Ln Ren 0.602*	Ln Ren Dw 0.824*			
F15-740	Ba 0.444*			Ba 0.444*		
F15-550	Lt 0.499*	Lt Tr 0.638*		Tr 0.375$^{@}$	Tr Ti 0.785**	
537-800	Hu14 0.355$^{@}$	Hu14 Rv 0.566$^{@}$		Hu14 0.355$^{@}$	Hu14 Rv 0.566$^{@}$	
537-700	Trd 0.400$^{@}$	Trd Br 0.722*	Trd Rr Ba 0.840**	Trd 0.400$^{@}$	Trd Rr 0.722*	Trd Rr Ba 0.840**
537-560	Rn 0.489*	Rn Lt 0.666*		Rn 0.489*		
561-1690	Ev 0.507*	Ev Dw 0.747**	Ev Dw Tr 0.863**	Ev 0.507*	Ev Dw 0.747**	Ev Dw Tr 0.863**
561-1020	Rv 0.296^{ns}			Rv 0.296^{ns}		
561-650	Ren 0.464*			Ren 0.464*		
A20-1600	Al 0.342$^{@}$			Tj 0.202^{ns}		
A20-1000	Ln 0.407*	Ln Huan 0.589*		Tj 0.323$^{@}$	Tj Ba 0.690*	Tj Ba Tdd 0.872**
A20-600	Trd 0.334$^{@}$	Trd Rr 0.533$^{@}$		Trd 0.334$^{@}$	Trd Rr 0.533$^{@}$	
A19-850	Ba 0.603**			Ba 0.603**		
A19-530	Ba 0.878***			Ba 0.878***		
A19-500	Rd 0.585**			Rd 0.585**		
T17-800	Trd 0.398$^{@}$	Trd Tr 0.611*		Trd 0.398$^{@}$	Trd Tr 0.611*	
T17-650	Rd 0.898**	Rd Ba 0.934***		Rd 0.898**	Rd Ba 0.934***	
T17-470	Tm 0.853***	Tm Trd 0.952***	Tm Trd Rr 0.975***	Tm 0.853***	Tm Trd 0.952***	Tm Trd Rr 0.975***
T17-400	Ln 0.525*	Ln Trd 0.673*		Td 0.413*	Td Trd 0.621*	Td Trd Ta 0.785*
A12-720	Al 0.356$^{@}$			Trd 0.313$^{@}$	Trd Hu14 0.562$^{@}$	

Table 7 Continued

Genetic indices	Stepwise model	
	Ecogeographical variables	Ecological variables
A12-690	Lt	Tdd
	0473*	0.291^{ns}
A12-490	Ln	Td
	$0.313^{@}$	$0.309^{@}$
A12-430	Lt	
	0.284^{ns}	

Level of significance: *** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$
$^{@} = p < 0.10$; $^{ns} = p > 0.10$
Symbols of variables are explained in the text and in Table 1

RAPD markers were employed previously to assess polymorphism among wild and cultivated tetraploid wheats (Joshi and Nguyen 1993 a). A higher level of polymorphism was observed among different accessions of wild emmer wheat from Israel, Turkey and Jordan than in cultivated durum wheat. The study of Joshi and Nguyen (1993 a) included only 17 genotypes of *T. dicoccoides* from six locations in Israel and three from unknown locations in Turkey and Jordan, which represents only a small fraction of the genetic variation harbored within this species. The present study included 110 genotypes collected from 11 different sites in Israel and Turkey and cover a wide range of ecogeographical conditions across the distribution range of the species in these countries.

Genetic structure of wild emmer wheat populations

Wild emmer wheat grows in lush and extensive stands in the catchment area of the Upper Jordan Valley, in the eastern upper Galilee Mts. and the Golan Heights in Israel. However, elsewhere in the Fertile Crescent, populations of wild emmer are semi-isolated or isolated, and largely display a patchy structure. At least in Israel, but possibly also elsewhere across the range of wild emmer in the Fertile Crescent, populations are subdivided into demes or clumps of varying sizes, including large, medium, and small patches (Nevo et al. 1982; Nevo and Beiles 1989). RAPD analysis was found to be highly effective in distinguishing genotypes and populations of *T. dicoccoides* originating from diverse ecogeographical sites in Israel and Turkey. In the present study 95.5% of the 110 genotypes were correctly classified into sites of origin by discriminant analysis on the basis of RAPD genotyping. The clear separation of isolated marginal populations (e.g. Hermon, Givat Koach, Gitit and Gerizim) from the other populations by discriminant analysis, as illustrated in Fig. 4, demonstrates the influence of unique ecogeographical conditions on the genetic structure of these populations. The soil type at each location also plays an important role in genetic differentiation, as can be seen by the separation of the populations located on basalt soil (e.g. Tabigha, Gamla and Diyarbakir) from those located on terra rosa or a rendzina type of soil.

Correlation with environmental variables

Wild emmer wheat is distributed over the Fertile Crescent including Israel, Syria, Jordan, Turkey and Iran. These natural populations are highly polymorphic for morphological characters, as well as for various economically important traits, such as disease resistances, grain quality, photosynthetic yield, salt tolerance, herbicide resistance, etc. (for a review see Nevo 1983, 1989, 1995). Although major collection areas such as Mt. Hermon, Rosh Pinna, Gamla, Bat-Shelomo and Tabigha are at a relatively similar longitude and latitude, they differ significantly in altitude. These locations, for example, are at 1300, 700, 200, 75 and 0 m, respectively, above the sea level (Table 1). Along with these features there are a number of other environmental factors that are different for these locations, such as climatic conditions, water availability and soil type (Nevo et al. 1982; Nevo and Beiles 1989). Our results demonstrate high levels of diversity of RAPD markers in these wild wheat populations. These polymorphic RAPD markers showed significant correlates with climatic factors and may be useful in a predictive sense for sampling strategies. Latitude, temperature and water availability factors, singly or in combination, explained a significant proportion of the variation in the polymorphism of RAPDs. The reason why latitude was found to be associated with RAPD variation could be explained by the sharp gradient of climatic conditions from north to south in Israel, with increasing temperatures and decreasing water availability towards the semi-arid zones in southern Israel. When a multiple regression analysis of environmental variables and RAPD polymorphism was run separately for ecogeographical and ecological variables, there were few cases in which climatic factors replaced latitude (e.g. F15-550 and A12-690; Table 7). It is noteworthy that the list of climatic factors included in our analysis is not

representive of all the possible components involved in the determination of the real climate. Thus, we conclude that latitude involves climatic components.

Spatial autocorrelation

The autocorrelation analysis predicts that migration will cause high positive correlations (similarity) in the low-order distance groups (i.e. between neighbors), starting from the first one. Migration is expected to result in a similarity between loci and alleles. This prediction is not realized in our results. In contrast, RAPD loci of wild emmer differ drastically in their autocorrelation pattern between loci. In addition, positive autocorrelations emerge at intermediate distance groups, thus negating migration as an important evolutionary factor. The analysis also predicts that genetic drift will not create any autocorrelative pattern. Our data is structured, and so negates randomness. Despite the between-loci variation, a general tendency of more positive correlations on the left-hand side of the table, and more negative correlations on the right-hand side, are evident.

Environmental selection is also partly autocorrelated and affects loci differentially. This is supported by our data for the following three reasons: (1) the variation among loci; (2) positive correlation in different distance groups, and not necessarily the first ones; and (3) the predominance of negative correlations in the larger distance groups is expected due to the decreasing ecological similarity which often occurs with increasing distance.

Genetic distance versus geographical distance

The absence of a significant relationship between geographic separation and genetic distance, *D*, indicates a sharp local differentiation, rather than a gradual change, in allele frequencies across the range of *T. dicoccoides* in Israel, as was previously found for allozymes (Nevo et al. 1982, 1991, Nevo and Beiles 1989). Population divergence does not always follow the isolation by distance model of Wright (1943). Quite often it is easier to find a greater genetic difference between close populations than among populations which are far apart. This was clearly demonstrated by local short transects of different soil types at Tabigha (Nevo et al. 1988 a) and the micro-difference of sun-shade differentiation at Yehudiyya (Nevo et al. 1988 b). Likewise, a detailed allozymic analysis between Kazrin and Yehudiyya in the Golan heights showed drastic multilocus differentiation over a short transect of 8 km (Golenberg and Nevo 1987). This confirms the island population genetic model of wild emmer wheat (Nevo and Beiles 1989). Thus, the genetic structure of wild emmer wheat populations in Israel is mosaic. This patchy genetic distribution appears to reflect the underlying ecological heterogeneity at both and micro- and macro-scales (Golenberg and Nevo 1987; Nevo et al. 1988 a, b, 1991; Nevo and Beiles 1989). The high polymorphism and genetic variation found within and between populations could be explained by spatio-temporal selection. Moreover, the micro-environmental variation, coupled with the limited migration of *T. dicoccoides*, can explain the within-population variation.

Genetic diversity detected at allozyme versus RAPD loci

RAPD markers have proved to be useful for population genetic analysis in plants. Some studies have directly compared the patterns of variation detected at allozyme versus RAPD loci. Overall patterns were generally in good agreement, although the levels of genetic variation and the fine-scale genetic structure differed sometimes between the two kinds of markers (Puterka et al. 1993; Isabel et al. 1995; Peakall et al. 1995; Lanner-Herrera et al. 1996). In the present study we have compared the genetic diversity at previously studied allozyme loci (Nevo and Beiles 1989) with the RAPD loci examined here in wild emmer wheat populations. Our results show complete agreement between RAPD and allozyme genetic-diversity profiles. However, RAPDs yielded much higher values of diversity than allozymes. Allozymes have provided useful insights into the genetic structure of wild emmer wheat (Nevo and Beiles 1989), although the number of scorable loci is limited and variation is detected only at coding loci. On the other hand, the RAPD technique (Williams et al. 1990) provides a powerful tool for obtaining a large number of anonymous loci of both coding and non-coding sequences, although it is commonly considered that most RAPD loci consist of non-coding sequences (Williams et al. 1990). The results, showing a higher diversity of RAPD loci, may be partially explained by the conserved nature of the coding sequences, primarily of housekeeping genes, sampled by allozymes versus the non-coding sequences sampled by RAPDs. The parallelism between RAPD and allozyme patterns indicates that similar primarily deterministic (selection) evolutionary forces are involved in shaping the genomic structure of both RAPD and allozyme loci, i.e. across coding and non-coding genomic regions.

The adaptive nature of RAPD polymorphisms in wild emmer

The evidence presented in this study supports the hypothesis that RAPD polymorphisms are at least partly adaptive and are determined by natural diversifying selection. The evidence includes the association of

RAPD loci with both climatic and soil factors. Genetic differentiation also appears to match, to a large extent, the heterogeneous ecological background. Genetic differentiation is not, however, correlated with geographic distance, but rather with local ecological conditions of soil and climate. Spatial autocorrelation analysis reveals different significant spatial patterns for different RAPD loci, ruling out migration as a differentiating factor. Likewise, the general order of negative and positive correlations rules out random drift as a major agent of genetic differentiation in wild emmer. Our overall results indicate that genetic diversity among localities displays weak and non-significant correlations with geography and stronger ones with ecology. These results match those found earlier using allozyme markers (Nevo et al. 1982; Nevo and Beiles 1989).

The wild gene pool of emmer wheat

The DNA analysis by PCR used in the present work corroborates the evidence of genetic diversity and divergence found in natural populations of *T. dicoccoides* across Israel (Nevo et al. 1982; Nevo and Beiles 1989). These showed great variability both between and within populations, emphasizing the importance of this wild wheat relative in future breeding programs and confirming the proposal that it may be an important source for wheat improvement (Nevo 1983, 1989, 1995). This idea was first suggested by Ahronson (1913), and was later elaborated by many authors. Multidisciplinary studies of wild wheat in Israel, conducted at the Institute of Evolution (reviewed in Nevo 1983, 1989 and 1995) and elsewhere (e.g. Feldman 1979; Grama et al. 1983; Joppa and Cantrell 1990), indicate that wild wheat harbors rich genetic sources for wheat improvement, not only regionally but also locally. Thus, sampling strategies must be designed accordingly, considering both macro- and micro-ecological divergence.

The results obtained suggest that RAPD markers are valuable for the estimation of genetic diversity in wild material of *T. dicoccoides*. These results will be useful in future sampling strategies and mapping studies for the identification of suitable parents to tag agronomically important traits in wheat derived from *T. dicoccoides*. This approach has proven to be useful for selecting suitable parents for the mapping of stripe rust resistance genes derived from *T. dicoccoides* based on a microsatellite analysis of highly resistant wild wheat lines (Fahima et al. 1998). Further work is now in progress to test the adaptive evolutionary significance of RAPDs and microsatellites in natural populations of wild emmer wheat, both at the macro- and micro-geographic scales. Our current results, displaying significant correlations between RAPDs, allozyme and ecological diversities, suggest that the former is subject to natural selection and may serve not only as genetic markers but also as adaptive multilocus genomic probes.

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Appendix

Allele (present) frequencies at 48 polymorphic loci[a] in 11 populations of *T. dicoccoides* from Israel and Turkey. Population numbers refer to those listed in Table 1

Locus	Population										
	1	8	9	11	16	17	18	19	30	33	36
486-1100	0.0	0.1	0.1	0.1	0.5	0.0	0.2	0.0	0.0	0.2	0.6
486-810	0.7	0.2	0.8	0.3	0.5	0.3	0.1	0.6	0.4	1.0	0.4
486-750	0.6	0.7	0.6	0.6	0.7	0.2	0.7	0.1	0.3	0.2	0.4
486-690	0.1	0.2	0.5	0.2	0.3	0.0	0.3	0.1	0.0	0.8	0.0
486-600	0.3	0.2	0.2	0.4	0.9	0.0	0.4	0.2	0.2	0.2	0.1
551-880	0.8	0.6	0.2	0.6	0.7	0.9	1.0	1.0	0.9	0.5	0.5
551-790	1.0	1.0	1.0	0.6	1.0	1.0	1.0	1.0	1.0	1.0	1.0
551-500	0.0	0.5	0.1	0.7	0.1	0.0	0.0	0.0	0.1	0.0	0.0
494-750	0.6	0.6	0.5	0.0	0.9	0.7	0.5	0.7	0.5	0.1	0.1
494-580	0.4	0.8	0.4	0.7	0.2	0.8	0.5	0.6	0.6	0.6	0.9
494-530	0.1	0.7	0.0	0.8	0.1	0.1	0.3	0.2	0.1	0.0	0.0
494-490	0.5	0.8	1.0	1.0	1.0	1.0	0.3	0.8	1.0	1.0	0.9
F15-930	0.2	0.9	0.6	0.0	0.3	0.0	0.4	0.1	0.5	0.6	0.1
F15-830	1.0	1.0	1.0	1.0	0.8	1.0	1.0	1.0	1.0	0.3	1.0
F15-740	1.0	1.0	1.0	0.4	1.0	1.0	1.0	1.0	1.0	1.0	1.0
F15-600	0.9	1.0	0.4	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0

Appendix (Continued)

Locus	Population										
	1	8	9	11	16	17	18	19	30	33	36
F15-550	0.3	0.3	0.8	0.5	0.8	1.0	1.0	0.6	0.5	1.0	0.5
F15-460	1.0	0.9	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0
537-800	0.5	0.1	0.6	0.9	0.2	0.3	0.0	0.8	0.7	0.7	0.2
537-700	0.4	0.6	0.5	0.0	0.1	0.7	0.4	0.4	0.2	0.5	0.0
537-560	0.9	0.4	0.8	0.2	0.2	0.8	0.5	0.4	0.5	0.8	1.0
561-1690	0.2	0.4	0.2	0.4	0.6	0.1	0.8	0.5	0.3	1.0	0.4
561-1420	0.7	0.7	0.9	0.9	0.8	0.1	0.8	0.8	0.8	0.5	0.8
561-1020	0.8	0.9	0.9	0.9	0.4	0.8	0.9	0.8	0.6	0.7	0.9
561-810	1.0	1.0	0.9	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0
561-730	0.1	0.1	0.1	0.0	0.1	0.2	0.1	0.0	0.1	0.2	0.1
561-650	0.3	0.1	0.3	0.4	0.5	0.5	0.2	0.4	0.7	0.3	0.3
A20-1600	0.9	0.9	0.8	0.8	1.0	1.0	0.6	0.8	0.5	0.2	0.8
A20-1400	1.0	0.9	1.0	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0
A20-1200	0.2	0.5	0.7	0.5	1.0	1.0	0.1	0.8	0.5	0.9	0.8
A20-1000	0.9	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
A20-600	0.2	0.0	0.1	0.4	0.7	0.0	0.1	0.4	0.1	0.1	1.0
A19-920	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0
A19-850	0.1	0.8	0.6	0.7	0.0	0.0	0.2	0.0	0.0	0.4	0.0
A19-780	0.9	0.9	0.8	1.0	0.9	1.0	1.0	1.0	1.0	0.9	1.0
A19-700	0.0	0.2	0.1	0.0	1.0	0.5	0.1	0.4	0.5	0.3	0.6
A19-530	1.0	0.8	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0
A19-500	0.7	0.8	0.9	0.9	1.0	1.0	0.9	1.0	0.9	0.9	0.9
T17-1170	0.7	0.9	0.8	0.6	0.9	0.9	0.7	0.9	0.8	0.9	0.8
T17-800	0.1	0.2	0.3	0.1	0.6	0.2	0.4	0.1	0.1	0.2	0.0
T17-650	0.9	0.2	0.3	0.1	0.3	0.3	0.0	0.0	0.5	0.1	0.6
T17-470	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
T17-400	0.5	0.5	0.5	0.5	0.9	0.7	0.6	0.6	0.7	0.9	0.9
A12-720	0.6	0.8	0.8	0.7	0.8	0.3	0.7	0.6	0.8	1.0	0.8
A12-690	1.0	1.0	0.9	1.0	0.5	0.8	0.8	0.7	0.9	0.3	1.0
A12-490	0.7	0.9	0.8	0.8	0.4	0.2	0.8	0.8	0.5	0.5	0.2
A12-460	0.4	1.0	1.0	1.0	0.7	0.0	0.1	1.0	0.9	1.0	0.1
A12-430	1.0	1.0	1.0	1.0	1.0	0.9	1.0	0.9	1.0	1.0	1.0

[a] List of the 11 monomorphic loci: 486-510, 551-1130, 537-880, 537-650, T17-950, A12-840, A20-800, 537-530, 494-930, 551-560, 486-930

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