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Genetic polymorphisms of α- and β-amylase isozymes in wild emmer wheat, *Triticum dicoccoides*, in Israel

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Summary. α- and β-amylase isozyme diversity was studied electrophoretically by thin-layer polyacrylamide gel isoelectrofocusing in the tetraploid wild emmer wheat, *Triticum dicoccoides*, the progenitor of all cultivated wheats. We analyzed 225 plants from 23 populations encompassing the ecological spectrum of *T. dicoccoides* in Israel. The results were as follows: (a) Band and multilocus genotype polymorphisms abound and vary within and between the four amylase components: malt, green (α-amylases), and dry and germinating seeds (β-amylases). (b) The number of bands of malt, green, and dry and germinating seeds were 20, 6, 11 and 13, respectively, generating 40, 6, 51, and 51 patterns or multilocus genotypes (MGP), respectively. The MGPs vary drastically within and between populations, from monomorphic in some populations with a single pattern to highly polymorphic ones. (c) Mean H_e values for malt, green, and germinating and dry seeds are 0.053, 0.055, 0.088, and 0.077, respectively; mean number of bands per individual was 11.8, 4.4, 7.6, and 4.0, respectively. (d) The percentages of 50 bands and 148 multilocus genotype patterns (MGP) (in parenthesis) were classified into widespread, sporadic, and localized: 84.4 (10.8), 8.9 (12.2), 6.7 (77.0), respectively. Notably, 89.2% of the patterns were not widespread, but sporadic and localized. (e) The mean value of genetic distances among populations (Nei's D) for the four amylase groups is $D = 0.136$, 0.175, 0.288 and 0.307, respectively, not displaying geographical correlates. (f) Most of the α- and β-amylase diversity is between populations ($G_{st} = 68–75\%$). (g) Significant environmental correlates occur between either bands or patterns and climatic diversity (water and primarily temperature factors). (h) Significant associations of multilocus amylase bands occur across Israel. Likewise, significant gametic phase disequilibria, D, occur within populations and are positively correlated with climatic variables, primarily that of temperature. (i) Discriminant analyses correctly classified (95–100%) the 23 wild emmer populations into their ecogeographical region and soil type. (j) Autocorrelation analysis showed that there is no correlation between bands and geographic distance and excluded migration as a major factor of amylase differentiation.

These results suggest that diversifying climatic and edaphic natural selection rather than stochastisity or migration is the major evolutionary force driving amylase differentiation at both the single and multilocus levels. Furthermore, wild emmer harbors high levels of α- and β-amylase diversity both as single bands and as multilocus adaptive genetic patterns. These are exploitable both as genetic markers for quantitative loci (QTLs) and as adaptive genetic resources to improve wheat germination and growth through classical breeding and/or biotechnology.

Key words: Amylases – *Triticum dicoccoides* – Allozyme polymorphisms – Natural selection – Wheat improvement

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Introduction

Starch is the chief storage fuel in most plants. Seed germination and early seedling growth in cereals are dependent upon the enzymatic hydrolysis of the starchy endosperm into metabolizable sugars. Among other germinating enzymes, α-amylase is the one that is primarily responsible for the endoglycolytic cleavage

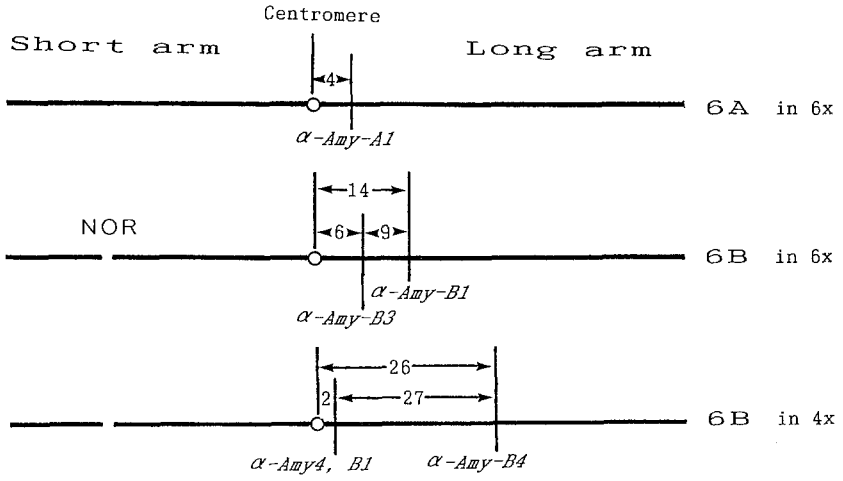

Fig. 1. Linkage maps of chromosomes 6A and 6B of wheat (K. Nishikawa et al. in preparation)

of amylase and amylopectin (reviewed in Akazawa and Hara-Nishimura 1985).

The genetics of the α- and β-amylases is well characterized in wheat (Nishikawa and Noburhara 1971; Nishikawa et al. 1975, 1979, 1980; Hart 1983; Gale and Ainsworth 1984; Ainsworth et al. 1985; Baulcombe et al. 1987; Hutlly et al. 1988). α-amylase isozymes are polymorphic, represent good genetic markers and, are duplicated in polyploid wheat (Nishikawa and Noburhara 1971; Nishikawa et al. 1979, 1980; Hart 1983). In wheat, α-amylase production is controlled by three triplicate sets of genes involving 27 isozymes (Gale et al. 1983). Amylases have also been described in some related species (Knox et al. 1987; Khursheed and Rogers 1988; Ainsworth et al. 1987). The linkage map of α-amylase on chromosomes 6A and 6B appears in Fig. 1 (from K. Nishikawa et al., in preparation).

β-amylase has been assayed in mature grains and shown to be controlled by two series of homoeologous genes in hexaploid wheat: the *β-Amy-1* series with loci on chromosome arms 4Aα (β-Amy-A1) and 4DL (β-Amy-D1) (Joudrier and Cauderon 1976; Joudrier 1980; Ainsworth et al. 1983) and the *β-Amy-2* series with loci on 5AL (β-Amy-A2) and 5BL (β-Amy-B2) (Ainsworth et al. 1983; Dabrowska 1983). Furthermore, using RFLP markers β-amylases have also been mapped to the short arms of group 2 chromosomes (Sharp et al. 1988). The updated mapping position of α- and β-amylases appear in Milne and McIntosh (1989), Hart and Gale (1989), and Nishikawa (1991).

Our previous studies of allozyme polymorphisms in *T. dicoccoides* in the Near East Fertile Crescent have been reviewed and re-analyzed in Nevo and Beiles (1989). The genetics of α-amylase has been valuable in wheat evolutionary studies, but little knowledge is available on the population genetics and ecology of amylases in wild wheat (e.g., Nishikawa et al. 1988b). In the present article we demonstrate that the complex multigene families of α- and β-amylases of *T. dicoccoides* are ecogeographically structured and significantly explicable through ecological factors at both the single and multilocus levels. These properties enable their utilization in wheat improvement.

Materials and Methods

Wild emmer wheat, *T. dicoccoides* (*T. turgidum* var 'dicoccoides' in Kimber and Feldman 1987; genomic constitution AABB) is the tetraploid, predominantly self-pollinated, wild progenitor from which modern tetraploid and hexaploid wheats were derived (Zohary 1970; Feldman 1976; Kimber and Feldman 1987).

Sampling

In this study of wild emmer, *T. dicoccoides*, 23 populations from the entire ecological spectrum of wild emmer in Israel were investigated. Altogether, we analyzed 225 plants that had been collected between 1979 and 1987. Seeds were stored and multiplied at the Institute of Evolution, University of Haifa, for further studies. For the locations and ecogeographical background of all of the tested populations see Fig. 1 and Table 1 in Nevo and Beiles (1989).

Electrophoretic procedures

Sample solutions of α-amylase were extracted with 1 ml of 0.05 *M* TRIS-HCl buffer (pH 7.0) from the endosperm of a single seedling 4 days after germination and incubated at 70 °C for 15 min to inactivate the β-amylase. A sample solution of β-amylase from 2 dry seeds and one 3-day-old seedling was extracted with 0.05 *M* TRIS-HCl containing DTT and EDTA (pH 7.0) and dialyzed in 0.02 *M* acetic buffer (pH 3.6) for 1 night in order to remove α-amylase activity. Electrophoresis was carried out by thin-layer (0.5 mm) polyacrylamide gel isoelectrofocusing (pH range 4.0–8.0 by Pharmalyte) instead of the disc isoelectrofocusing used in previous reports (Nishikawa and Nobuhara 1971; Nishikawa et al. 1975). The revised method will be described in detail elsewhere.

Statistical analyses

We employed uni- and multivariate statistics using SPSS-x (1986), and used both nonparametric (Spearman rank correlations) and parametric (multiple regression and principle component procedures) analyses. Correlations, discriminant analyses, as well as autocorrelation (Sokal and Oden 1987a, b) and G_{st} analysis (Nei 1973) were used. We italicized Nei's *D* (genetic distance) and used the unitalicized D for gametic phase disequilibria in order to distinguish between both D's.

For environmental correlations we also used principle components involving an overall of 15 climatic factors as well as three climatic subsets, one comprising 8 water variables, the second 7 temperature variables, and the third 3 geographical variables. The symbols for the levels of significance are: @ = $P < 0.10$, * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$.

Results

Space limitation prevents us from presenting most of our tables (11 tables and 2 figs.); these can be obtained upon request from the senior author.

Variation pattern

The α-amylase isozyme bands as well as the updating of the banding patterns of the β-amylases in *T. dicoccoides* appear in Fig. 2a, b.

Our data set is displayed at two different levels for both the α- and β-amylases. The first level involves band frequencies, which presumably represent putative alleles of many clustered loci. The second level is expressed as an individual "band pattern", representing clusters of genes, referred to here as multilocus genotype pattern (MGP). Variation among individuals in the MGP patterns of both α- and β-amylases is shown in Fig. 3a, b. The typing has been done separately for α-amylase active in malt, α-amylase active in green parts, β-amylase active in germinating seeds, and β-amylase active in dry seeds. The details on the MGPs and their frequencies can be obtained from the senior author.

α-Amylase

The frequency of all of the bands of α-amylase are shown in Table 1. The degree of polymorphism varies between malt and green as well as among bands and populations. In malt extracts we found 23 bands; however, we used only 19 bands, representing 19 putative loci, in the analyses and excluded 3 artefacts (20 = 22; 6 = 7; 1 = 1′) and band 20a, which is allelic to band 11. We found only 6 bands in the green parts (Fig. 2a). Three bands (3, 4, and 6) in the malt section and 2 bands (5 and 8) in the green section were fixed in all of the plants and populations. Two bands in malt (5 and 23) appear only in the Tabigha population.

The 19 malt bands generated 40 different MGPs (Table 2). One MGP (no 19) appeared in 10 popula-

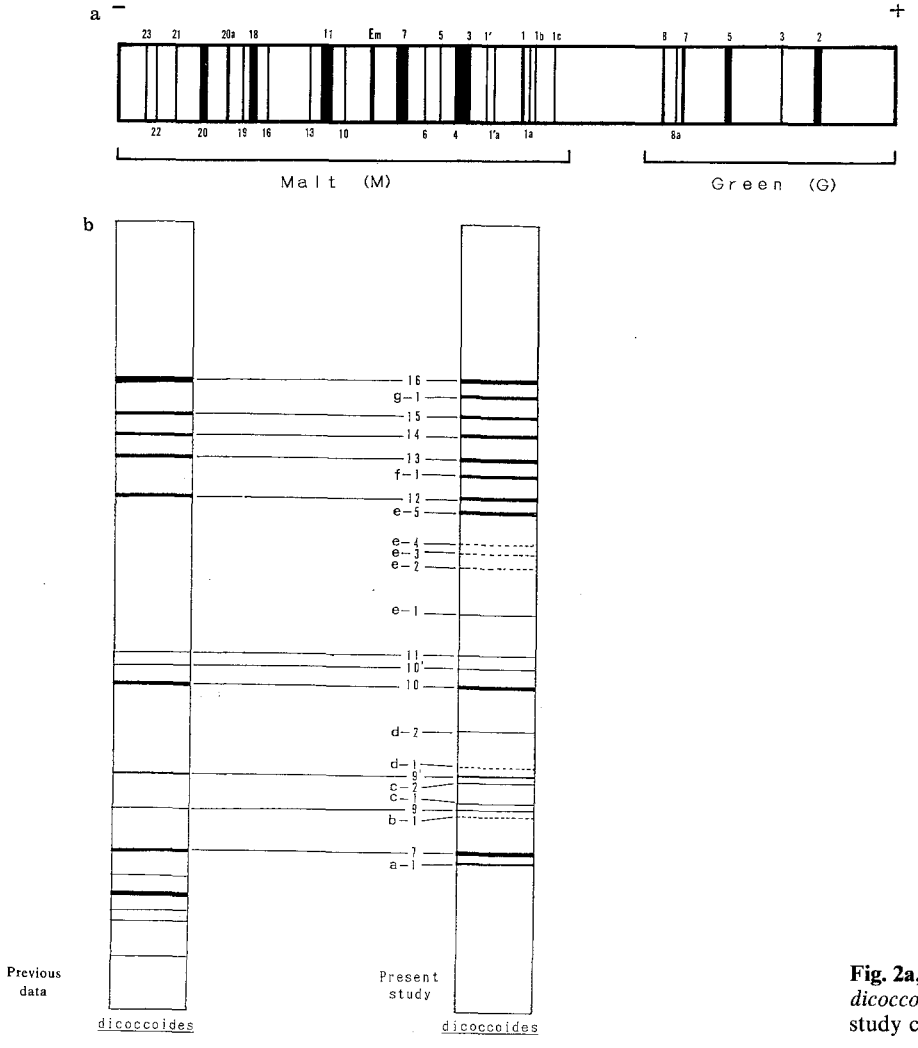

Fig. 2a, b. Amylase bands in *Triticum dicoccoides* **a** α-amylase, **b** β-amylase in this study compared with previous data

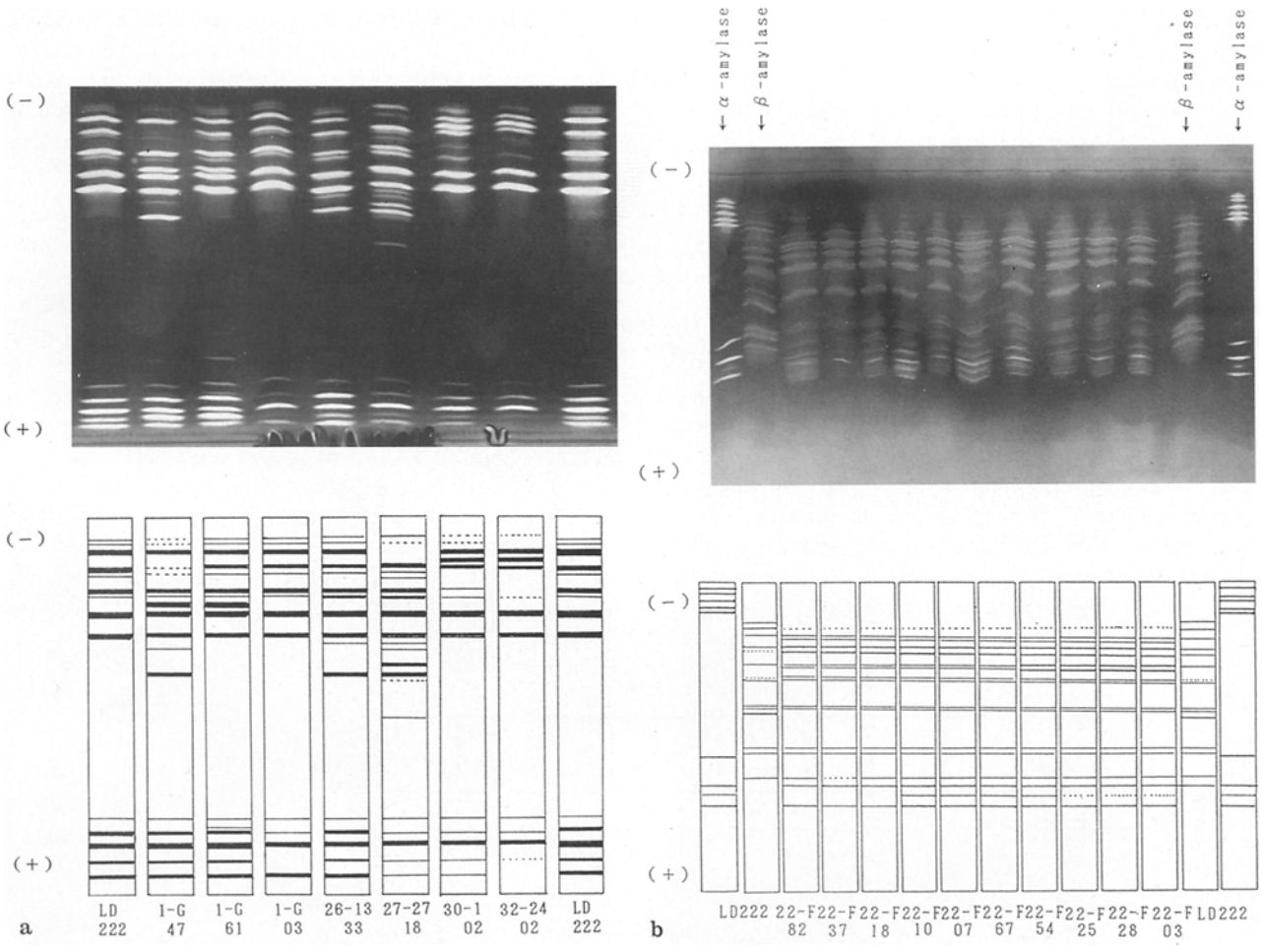

Fig. 3a, b. Amylase phenotypes of wild emmer wheat, *Triticum dicoccoides*. **a** α-amylase, **b** β-amylase

tions; 1 (no 21) in 7 populations; 1 (no 10) in 5 populations; 2 (nos 9 and 16) in 4 populations; and 2 (nos 15 and 25) in 3 populations each. These 7 MGPs were used in the correlation studies. Each of the other 33 MGPs were restricted to a single or a pair of populations. In 4 populations a fixation of 1 MGP occurred, whereas the Gitit population reached the maximal diversity level: 10 different MGPs in 10 plants.

The 4 polymorphic bands of the 6 bands found in the green parts generated 6 different MGPs. Two of them (3 and 6) appear only in two populations; 1 in 3 populations; whereas the remaining 3 MGPs appear in many localities and were used in the correlation analysis. Nine populations were fixed for a single MGP (Table obtainable upon request).

β-Amylase

The frequency of all of the bands of β-amylase appear in Table 3 and Fig. 2b. The degree of polymorphism varies between bands and populations. In germinating seeds we found 11 bands and in dry seeds, 13 bands. Only 1 band, 10a, is confined to a single population (Yehudiyya). Two bands (9a, 10b) appear in 2 populations. Generally, there is less amylase activity in dry seeds than in germinating seeds. Some populations appear highly polymorphic in both germinating and dry seeds (i.e., Mt. Gerizim and Qazrin, though less polymorphic in dry seeds). In contrast, some populations displayed high monomorphism in both germinating and dry seeds (Rosh Pinna and Bet Meir).

The 11 bands of germinating seeds generated 51 MGPs (Table obtainable upon request). Only 1 MGP (no 4) is wide-spread, appearing in 6 populations. Two MGPs (6 and 29) appear in 3 populations, whereas 42 MGPs were confined to single populations. Nine populations were fixed to a single MGP (i.e., Rosh Pinna, Gitit, etc.). The 13 bands found in dry seeds also generated 51 MGPs (Table obtainable upon request). One MGP (no 51) appears in 5 populations; another (no 22) in 4 populations, whereas 40 MGPs were confined to single populations. Three populations were fixed to a single MGP (Rosh Pinna, Kokhav Hashahar and Bet-Meir).

Table 1. Geographical distribution of 26 bands revealed by electrophoretical analysis of α-amylase in 23 populations of *Triticum dicoccoides* in Israel

Number of population	*n*	Bands[a]																											Mean number of bands
		Malt																					Green parts							
		23	21	20	20a	19	18	16	13	11	10	Em	6	5	4	3	1'a	1	1a	1b	1c	8	8a	7	5	3	2		
1 Mt. Hermon	10	0	1.0	1.0	0	0	1.0	1.0	0	1.0	1.0	0.70	1.0	0	1.0	1.0	0	0.30	0	0	0	1.0	0.30	1.0	1.0	1.0	1.0		16.5
5 Qazrin	10	0	1.0	1.0	0.10	0	1.0	0.90	0	1.0	1.0	0	1.0	0	1.0	1.0	0	1.0	0	0	0	1.0	0	1.0	1.0	0.70	1.0		17.7
7 Yehudiyya	10	0	1.0	1.0	0	0	1.0	1.0	0	1.0	1.0	0.20	1.0	0	1.0	1.0	0	0.90	0	0	0	1.0	0	1.0	1.0	0.50	1.0		17.5
8 Gamla	9	0	1.0	1.0	0	0.11	1.0	1.0	0	1.0	1.0	0.11	1.0	0	1.0	1.0	0.11	1.0	0.11	0.11	0.11	1.0	0.11	1.0	1.0	1.0	1.0		18.8
9 Rosh-Pinna	9	0	1.0	1.0	0.67	0	1.0	0.33	0	0.33	1.0	0	1.0	0	1.0	1.0	0	0.11	0	0	0	1.0	0	1.0	1.0	0.22	1.0		14.8
11 Tabigha	10	0.70	1.0	1.0	0	0	0.70	1.0	0.90	1.0	1.0	0	1.0	0.70	1.0	1.0	0.10	1.0	0	0	0	1.0	0	1.0	1.0	0.40	1.0		19.2
16 Mt. Gilboa	9	0	1.0	1.0	0.22	0	1.0	0.78	0.44	0.78	1.0	0	1.0	0	1.0	1.0	0	0.89	0	0	0	1.0	0	1.0	1.0	1.0	1.0		18.0
17 Mt. Gerizim	10	0	1.0	1.0	0	0	1.0	0.30	0.20	1.0	1.0	0	1.0	0	1.0	1.0	0	0	0	0	0	1.0	0	1.0	1.0	1.0	1.0		15.5
18 Gitit	10	0	0.70	0.90	0	0.10	0.50	0.70	0.50	1.0	1.0	0.30	1.0	0	1.0	1.0	0	0.20	0	0	0	1.0	0	1.0	1.0	1.0	1.0		16.0
19 Kokhav Hashahar	10	0	1.0	0.90	0.30	0	1.0	0.70	0	0.70	1.0	1.0	1.0	0	1.0	1.0	0	1.0	0	0	0	1.0	0	0.70	1.0	0.70	1.0		16.9
20 Taiyiba	10	0	1.0	1.0	0.50	0	0.90	0.60	0	0.60	0.80	0	1.0	0	1.0	1.0	0	0.10	0	0	0	1.0	0	0.20	1.0	0.20	1.0		13.8
21 Sanhedriyya	10	0	1.0	1.0	0.70	0	1.0	0.20	0	0.30	1.0	0	1.0	0	1.0	1.0	0	0.90	0	0	0	1.0	0	1.0	1.0	1.0	1.0		17.0
22 Bet-Meir	10	0	1.0	1.0	0.70	0	1.0	0	0	1.0	1.0	1.0	1.0	0	1.0	1.0	0	1.0	0	0	0	1.0	0	0.20	1.0	0.20	1.0		16.1
23 J'aba	10	0	0.70	1.0	0.20	0	1.0	0.80	0	0.80	1.0	0.10	1.0	0	1.0	1.0	0	0.30	0.10	0.10	0.10	1.0	0	0.40	1.0	0.40	1.0		14.5
24 Amirim	9	0	1.0	1.0	0.11	0	1.0	0.89	0.44	0.89	1.0	0	1.0	0	1.0	1.0	0	0.89	0	0	0	1.0	0	1.0	1.0	0.44	0.89		17.3
25 Nahef	10	0	0	1.0	0	0	1.0	0	0	1.0	1.0	0.10	1.0	0	1.0	1.0	0	0.90	0	0	0	1.0	0	0.90	1.0	0.90	1.0		14.1
26 Ahihud (Achihood)	10	0	1.0	1.0	0	0	1.0	1.0	0	1.0	1.0	0	1.0	0	1.0	1.0	0	1.0	0	0	0	1.0	1.0	1.0	1.0	1.0	1.0		19.0
27 Nesher	10	0	1.0	0	0	0	1.0	0	0	1.0	1.0	0	1.0	0	1.0	1.0	0	1.0	1.0	1.0	1.0	1.0	0	0	1.0	0	0		14.0
28 Beit-Oren	10	0	1.0	1.0	0	0.20	1.0	0	0	1.0	1.0	0	1.0	0	1.0	1.0	0	1.0	0	0	0	1.0	0	0.10	1.0	0.10	0.10		13.5
29 Daliyya	10	0	1.0	1.0	0	0	1.0	1.0	1.0	1.0	1.0	0	1.0	0	1.0	1.0	0	0	0	0	0	1.0	0	1.0	1.0	0	1.0		16.0
30 Bat-Shelomo	10	0	1.0	1.0	0.40	0	1.0	0.10	0	0.70	1.0	0	1.0	0	1.0	1.0	0	0.70	0	0	0	1.0	0	1.0	1.0	1.0	1.0		16.4
32 Yabad	10	0	1.0	1.0	0.70	0	1.0	0	0	0.30	1.0	0	1.0	0	1.0	1.0	0	0	0	0	0	1.0	0	1.0	1.0	1.0	0		13.0
33 Givat-Koach	9	0	1.0	1.0	0	0	1.0	0	1.0	1.0	1.0	0	1.0	0	1.0	1.0	0	0	0	0	0	1.0	0	1.0	1.0	0.44	1.0		15.4
Total	225																												
Number of polymorphic populations:		1	2	2	11	3	3	11	5	9	1	6	0	1	0	0	2	12	2	2	2	0	2	6	0	12	2		
Mean frequency		0.03	0.93	0.95	0.20	0.02	0.96	0.53	0.19	0.84	0.99	0.15	1.0	0.03	1.0	1.0	0.01	0.61	0.95	0.95	0.95	1.0	0.06	0.80	1.0	0.62	0.87		
Mean "H_e"		0.02	0.04	0.02	0.18	0.03	0.05	0.16	0.09	0.15	0.01	0.07	0	0.02	0	0	0.02	0.13	0.02	0.02	0.02	0	0.03	0.08	0	0.20	0.02		

[a] The following bands are duplicates: 1 = 1'; 6 = 7 (in Malt); 20 = 22

Table 2. Geographical distribution of 40 multilocus genotype patterns (MGP) of 19 bands revealed by electrophoretical analysis of α-amylase in malt from 23 populations of *Triticum dicoccoides* in Israel

Number of population	n	MGP of α-amylase in malt																																						"H_e"
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	
1 Mt. Hermon	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	5	–	1	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.66
5 Qazrin	10	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.18
7 Yehudiyya	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	7	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.46
8 Gamla	9	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1	–	1	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.52
9 Rosh-Pinna	9	–	–	–	–	–	–	–	–	–	6	–	–	–	–	–	–	–	–	1	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.49
11 Tabigha	10	1	5	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	0.64
16 Mt. Gilboa	9	–	–	–	–	1	–	–	–	1	–	–	–	–	3	–	–	–	–	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.67
17 Mt. Gerizim	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	1	–	–	–	–	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.46
18 Gitit	10	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1	–	–	–	–	–	–	–	–	1	–	–	1	–	1	1	–	1	1	1	–	1	–	–	–	–	0.91
19 Kokhav Hashahar	10	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	0.46
20 Taiyiba	10	–	–	–	1	–	–	–	–	1	3	–	–	–	–	–	–	–	–	–	–	4	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	0.72
21 Sanhedriyya	10	–	–	–	–	–	–	–	–	7	–	–	–	–	–	–	–	–	–	1	–	1	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.48
22 Bet-Meir	10	–	–	–	–	–	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.42
23 J'aba	10	–	–	–	–	–	–	–	1	1	–	–	–	–	–	–	–	–	–	–	1	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	0.72
24 Amirim	9	–	–	–	–	–	–	–	–	–	1	–	–	–	4	–	–	–	–	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.59
25 Nahef	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	9	0.18
26 Ahihud (=Achihood)	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.0
27 Nesher	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10	–	–	–	0.0
28 Beit-Oren	10	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.32
29 Daliyya	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.0
30 Bat-Shelomo	10	–	–	–	–	–	–	1	–	–	3	–	–	–	–	–	–	–	–	1	–	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.64
32 Yabad	10	–	–	–	–	–	–	–	–	–	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.42
33 Givat-Koach	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.0
Total	225	1	5	1	1	1	7	2	3	10	20	1	1	2	7	13	12	5	1	44	1	15	9	3	1	14	10	1.	3	1	1	1	1	1	1	1	1	10	3	1	9	

Table 3. Geographical distribution of 24 bands revealed by electrophoretical analysis of β-amylase in 23 pupulations of *Triticum dicoccoides* in Israel

Number of population	n	Bands																							Mean number of bands
		in germinating seeds											in dry seeds												
		16	16a	15	14	13	13a	12	12a	12b	12c	12d	12e	11	10′	10	10a	10b	9′	9′a	9′b	9	9a	7	7a	
1 Mt. Hermon	10	1.0	0.40	1.0	0.40	0.60	0.40	1.0	1.0	0.40	0.60	0	0	1.0	0.70	0.80	0	0	1.0	0	0	1.0	0	0	0	11.3
5 Qazrin	10	0.40	0.70	0.80	0.70	0.80	0.10	1.0	0.70	1.0	0.50	0	0	0.80	0.50	0	0	0	0.70	0	0	1.0	0	0.80	0.80	11.2
7 Yehudiyya	10	0.20	0	1.0	0.60	1.0	0.60	1.0	0.60	1.0	0	0	0.10	1.0	0	0.10	1.0	0	1.0	0	0	1.0	0	1.0	0	11.2
8 Gamla	9	0.89	1.0	0.89	1.0	0.89	1.0	0.78	0	0.56	0.56	0	0	1.0	0	0.67	0	0	0.89	0	0	1.0	0	0.89	0.89	12.9
9 Rosh-Pinna	9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0	1.0	1.0	0	0	1.0	1.0	1.0	0	0	1.0	0	0	1.0	0	1.0	0	15.0
11 Tabigha	10	1.0	1.0	1.0	1.0	1.0	0.10	1.0	1.0	1.0	1.0	0	1.0	1.0	0	0	0	0	0.90	0.90	0.90	0.90	0.40	0.90	0.50	16.5
16 Mt. Gilboa	9	0.56	0.89	0.67	1.0	0.56	0	1.0	1.0	0	0	0	0	0	0	0.56	0	0	1.0	0	0	0.78	0	1.0	0	9.2
17 Mt. Gerizim	10	0.30	0.70	0.90	0.80	0.90	0.70	0.90	0	1.0	0.50	0	0	0.70	0	0	0	0.30	0.50	0.10	0.20	0.50	0.20	0.50	0.30	10.0
18 Gitit	10	1.0	1.0	1.0	0	1.0	0	0	0	1.0	1.0	0	0	1.0	1.0	1.0	0	0	1.0	0	0	0.50	0	0.70	0.70	11.9
19 Kokhav Hashahar	10	1.0	1.0	1.0	1.0	1.0	1.0	0.60	0	1.0	1.0	0.10	0	0	0	0	0	0	0	0	0	0	0	0	0	8.7
20 Taiyiḇa	10	1.0	0.90	1.0	0.80	1.0	0.90	0.80	0	1.0	1.0	0	0.80	1.0	0.20	0	0	0	0	0	0	0	0	0	0	10.4
21 Sanhedriyya	10	1.0	1.0	1.0	1.0	1.0	0	1.0	1.0	1.0	1.0	1.0	1.0	0.80	0.70	0	0	0	0	0	0	0	0	0	0	12.5
22 Bet-Meir	10	1.0	0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0	0	1.0	1.0	0	0	0	1.0	0	0	1.0	0	1.0	0	14.0
23 J'aba	10	0	0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0	0	1.0	1.0	0.40	0	0	0	0	0	0	0	0	0	10.4
24 Amirim	9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0	1.0	1.0	0	0	0.78	0	0	0	0	0	0	0	0	0	0	0	9.8
25 Nahef	10	1.0	0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0	1.0	0.90	0.90	0.50	0	0	1.0	0	0	1.0	0	1.0	0	15.3
26 Ahihud (Achihood)	10	1.0	1.0	1.0	0	1.0	0	1.0	0	1.0	1.0	0	0	1.0	0.50	0	0	1.0	1.0	1.0	1.0	0	0	0	0	12.5
27 Nesher	10	1.0	0.50	1.0	0.10	1.0	0	0.40	0	0.70	0.70	0	0	0	0	0.50	0	0	0.70	0	0	0.50	0	0.60	0.60	8.3
28 Beit-Oren	10	1.0	0	1.0	1.0	1.0	0	1.0	0	0.90	0.40	0	0	1.0	1.0	0.50	0	0	1.0	0	0	0	0	1.0	0.50	11.3
29 Daliyya	10	1.0	0	1.0	1.0	0	1.0	1.0	0	0.80	0.40	0	0	1.0	1.0	0.50	0	0	1.0	0	0	1.0	0	1.0	0	11.7
30 Bat-Shelomo	10	0.80	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.90	0.80	0	0	1.0	0.80	0.10	0	0	0.70	0	0	0.90	0	0.10	0	13.1
32 Yabad	10	0.50	1.0	1.0	1.0	0.90	1.0	0.50	0	0.70	0.80	0.30	0	0.30	0.80	0	0	0	0.60	0	0	0.60	0	0	0	9.5
33 Givat-Koach	9	1.0	0	1.0	0	1.0	0	1.0	0	1.0	1.0	0	0.78	0.56	1.0	1.0	0	0	0	0	0	0	0	0	0	9.3
Total	225																									
Number of polymorphic populations:		7	6	4	6	6	6	6	2	7	9	2	3	7	8	10	0	1	7	2	2	7	2	7	7	
Mean frequency		0.81	0.61	0.97	0.76	0.90	0.56	0.87	0.40	0.87	0.75	0.06	0.20	0.78	0.53	0.33	0.04	0.06	0.65	0.09	0.09	0.55	0.03	0.50	0.19	
Mean "H_e"		0.12	0.10	0.05	0.10	0.08	0.08	0.10	0.04	0.11	0.17	0.03	0.04	0.11	0.13	0.18	0	0.02	0.11	0.02	0.02	0.12	0.03	0.10	0.12	

Table 4. Geographical distribution of genetic indices of the bands and MGPs revealed by electrophoretical analysis of α- and β-amylases in 23 populations of *Triticum dicoccoides* in Israel

Number of population	*n*	α-Amylase										β-Amylase									
		Malt					Green parts					Germinating seeds					Dry seeds				
		MGPs no.	H_e	Number of bands	H_e	P	MGPs no.	H_e	Number of bands	H_e	P	MGPs no.	H_e	Number of bands	H_e	P	MGPs no.	H_e	Number of bands	H_e	P
1 Mt. Hermon	10	4	0.660	11.2	0.050	0.13	2	0.420	5.3	0.070	0.17	3	0.640	6.8	0.262	0.55	3	0.620	4.5	0.057	0.15
5 Qazrin	10	2	0.180	13.0	0.016	0.09	2	0.420	4.7	0.070	0.17	7	0.820	6.7	0.278	0.73	6	0.760	4.6	0.145	0.38
7 Yehudiyya	10	3	0.460	13.0	0.030	0.13	2	0.500	4.5	0.083	0.17	3	0.640	6.0	0.160	0.36	2	0.180	5.2	0.028	0.15
8 Gamla	9	4	0.519	13.7	0.052	0.26	2	0.198	5.1	0.033	0.17	4	0.617	7.6	0.175	0.55	4	0.519	5.3	0.080	0.31
9 Rosh-Pinna	9	3	0.494	10.6	0.075	0.22	2	0.346	4.2	0.058	0.17	1	0.0	9.0	0.0	0.0	1	0.0	6.0	0.0	0.0
11 Tabigha	10	4	0.640	14.8	0.089	0.26	2	0.480	4.4	0.080	0.17	2	0.180	9.1	0.016	0.09	4	0.660	7.4	0.145	0.54
16 Mt. Gilboa	9	4	0.667	13.0	0.084	0.26	1	0.0	5.0	0.0	0.0	4	0.617	5.7	0.148	0.36	3	0.593	3.3	0.065	0.15
17 Mt. Gerizim	10	3	0.460	10.5	0.032	0.09	1	0.0	5.0	0.0	0.0	5	0.680	6.7	0.238	0.73	6	0.800	3.3	0.275	0.69
18 Gitit	10	10	0.900	11.0	0.150	0.43	1	0.0	5.0	0.0	0.0	1	0.0	6.0	0.0	0.0	3	0.620	5.9	0.103	0.23
19 Kokhav Hashahar	10	4	0.480	12.5	0.070	0.22	2	0.420	4.4	0.140	0.33	3	0.580	8.7	0.060	0.18	1	0.0	0.0	0.0	0.0
20 Taiyiba	10	6	0.780	10.4	0.117	0.39	2	0.320	3.4	0.107	0.33	4	0.480	8.4	0.091	0.36	3	0.560	2.0	0.049	0.15
21 Sanhedriyya	10	4	0.480	12.0	0.066	0.22	1	0.0	5.0	0.0	0.0	1	0.0	10.0	0.0	0.0	3	0.460	2.5	0.057	0.15
22 Bet-Meir	10	2	0.420	12.7	0.018	0.04	2	0.320	3.4	0.107	0.33	1	0.0	9.0	0.0	0.0	1	0.0	5.0	0.0	0.0
23 J'aba	10	6	0.800	10.7	0.131	0.43	2	0.480	3.8	0.160	0.33	1	0.0	8.0	0.0	0.0	2	0.480	2.4	0.037	0.08
24 Amirim	9	4	0.667	13.0	0.071	0.26	3	0.568	4.3	0.115	0.33	1	0.0	9.0	0.0	0.0	2	0.346	0.8	0.027	0.08
25 Nahef	10	3	0.540	9.3	0.034	0.13	2	0.180	4.8	0.060	0.33	1	0.0	9.0	0.0	0.0	3	0.580	6.3	0.066	0.23
26 Ahihud (Achihood)	10	1	0.0	13.0	0.0	0.0	1	0.0	6.0	0.0	0.0	1	0.0	7.0	0.0	0.0	2	0.500	5.5	0.039	0.08
27 Nesher	10	1	0.0	12.0	0.0	0.0	1	0.0	2.0	0.0	0.0	6	0.760	5.4	0.182	0.45	5	0.720	2.9	0.183	0.38
28 Beit-Oren	10	2	0.320	11.2	0.014	0.04	2	0.180	2.3	0.090	0.50	3	0.580	6.3	0.060	0.18	2	0.500	5.0	0.077	0.15
29 Daliyya	10	1	0.0	12.0	0.0	0.0	1	0.0	4.0	0.0	0.0	3	0.640	6.2	0.073	0.18	2	0.500	5.5	0.039	0.08
30 Bat-Shelomo	10	5	0.760	11.4	0.087	0.22	1	0.0	5.0	0.0	0.0	4	0.480	9.5	0.075	0.27	5	0.680	3.6	0.099	0.38
32 Yabad	10	2	0.420	9.0	0.037	0.09	1	0.0	4.0	0.0	0.0	8	0.860	7.7	0.213	0.55	4	0.740	2.3	0.131	0.31
33 Givat-Koach	9	1	0.0	11.0	0.0	0.0	2	0.494	4.4	0.082	0.17	1	0.0	6.0	0.0	0.0	4	0.667	3.3	0.065	0.15
Mean		3.4	0.463	11.8	0.053	0.17	1.7	0.232	4.4	0.055	0.15	3.0	0.373	7.6	0.088	0.24	3.1	0.499	4.0	0.077	0.21
Total	225																				

[a] P = proportion of polymorphic bands (or loci)

Genetic summary

A summary of the genetic data for each of the 23 populations of *T. dicoccoides* is given in Table 4. The following results were indicated: Mean H_e values of MGP for α- (malt and green parts) and β- (germinating and dry seeds) amylases were: 0.463, 0.232, 0.373 and 0.499, respectively. The equivalent H_e for the bands (assuming that each band represents a locus complemented by a null allele unseen on the gel) were: 0.053, 0.055, 0.088 and 0.077, respectively. The mean number of bands per individual per population was 11.8, 4.4, 7.6, 4.0, respectively. Notably, the highest number of amylase bands was in malt, then in germinating seeds, both representing areas of high amylase metabolism.

Geographic patterns of band and multilocus genotype (MGP) distribution

The major pattern of band and MGP distribution was regional and local, and not clinal. A regional distribution of bands (appearance in two or more regions) characterizes all forms of α- and β-amylases. In contrast, a local distribution (appearance in only one region and mostly only in 1 population) characterizes the MGPs.

To assess the various kinds of bands and multilocus distributions, we followed the classification proposed by Marshall and Brown (1975). For this classification we defined eight regions: (1) Eastern Galilee, (2) Golan Heights, (3) Mt. Hermon, (4) Western and Central Galilee (5) Mt. Carmel, (6) Coastal Plain, (7) Samaria, (8) Judea. Each of the 50 bands and 148 MGPs found in the 23 populations of *T. dicoccoides* in Israel were classified into one of the following classes. *Common* bands or MGPs occurred in at least 1 sample with a frequency ≥ 10%: (a) *widespread*, common occurrence in more than two regions: 38 (84.4%) bands and 16 (10.8%) MGPs; (b) *sporadic*, common occurrence in two regions: 4 (8.9%) bands and 18 (12.2%) MGPs; (c) *localized*, common occurrence in only one region: 3 (6.7%) bands and 114 (77.0%) MGPs (Table obtainable upon request). *Rare* bands or MGPs were not observed in this study due to the small local sample size ($n = 10$ or 9). The percentage of the variants was computed by ignoring the fixed bands. Note that 89.2% of the MGPs were not widespread, but rather localized or sporadic. An opposite trend was found in the band proportions. These figures suggest that populations of wild emmer wheat in Israel differ considerably in their MGP content.

Genetic distance

Coefficients of genetic distance, *D*, were calculated for pair comparisons of all 23 populations based on the normalized identity of all bands (putative loci) among all population pairs (Nei 1972). The mean value of *D* for each of the four amylase groups is: α-amylase, malt, $D = 0.136$ (range 0.01–0.479); green parts, $D = 0.175$ (range 0.0–1.099); β-amylase, germinating seeds, $D = 0.288$ (range 0.0–0.788); dry seeds, $D = 0.307$ (range 0.010–0.857). The estimates of *D* were not generally geographically dependent. They displayed large *D*'s i.e., sharp genetic differentiation over very short geographic distances (i.e., Nesher and Beit-Oren, 10 km apart, in malt bands, $D = 0.249$), against low *D*'s between geographically distant populations (Qazrin and Ahihud, 70 km apart, in malt bands, $D = 0.001$).

Genetic differentiation within and among populations

The gene diversity of a subdivided population (H_t) can be analyzed into its components (Nei 1973). With the exception of 1 pair of bands that are known to be allelic (20a and 11 in malt), we considered each band to be representive of a locus of the amylase multigene family. For the present analysis we arbitrarily defined a null allele when the band was absent. A table summarizing the average estimates of the diversity (H_s) and the proportion expressed between populations (G_{st}) for each amylase group in malt, green, and germinating and dry seeds is obtainable upon request. In general, β-amylase, in both germinating and dry seeds, presents up to double the diversity of the α-amylase. H_t was 0.30 in β-amylase as compared to 0.15 in α-amylase. Overall, most of the amylase diversity was between populations. The G_{st} values were very high across the four amylase groups ($G_{st} = 68$–75%).

Environmental correlations (single and multiclimatic factors, PC) with α- and β-amylase polymorphisms

Single climatic variables

Detailed Spearman rank correlation matrices of bands and MGPs of α- and β-amylases, first with individual climatic factors and second with multiclimatic factors expressed as principal components (PC) of climate, are obtainable upon request.

α-Amylase

In malt. The most striking result of this matrix in α-amylase of malting seeds was the correlation between band no 18 and annual rainfall and annual humidity ($r_s = 0.528^{**}, 0.548^{**}$, respectively) and between band 16 with seasonal temperature difference ($r_s = 0.610^{**}$). Bands 18, 16, and 13 were correlated with different climatic factors. Likewise, MGP no 21 was correlated with altitude and the number of hot and dry days ($r_s = 0.615^{*}, 0.552^{*}$, respectively). MGP no 9 was correlated with the second PC factor of water variables ($r_s = -0.512^{*}$).

In green parts. As in the malt, bands and MGPs were significantly correlated with climatic factors.

β-Amylase

In germinating seeds. The most striking result of this matrix in β-amylase of germinating seeds was the cor-

relation between band 15 and the second climatic factor combining water availability and temperature variables, which explained 28.5% of the variance ($r_s = -0.428$*). Likewise, band 12b was correlated with the number of rainy days and relative rainfall variation (RV) ($r_s = -0.485$* and 0.512*, respectively). MGP no 4 was correlated with the number of hot and dry days ($r_s = 0.463$*) and MGP no 29 with daily temperature difference ($r_s = 0.479$*).

In dry seeds. The frequency of band 9 was significantly correlated with the third factor of climate, which explained 7.2% of the variance, and the second factor of the subsection of water variables (16% of the variance) ($r_s = 0.483$* and 0.416*, respectively). Band 10′ was significantly correlated with humidity, seasonal temperature difference, and longitude ($r_s = 0.417$*, -0.458*, and -0.512*, respectively). Band 9 was significantly correlated with latitude ($r_s = 0.527$**). Significant correlations were also found between MGPs and climatic variables. For example, MGPs nos 14 and 22 were significantly correlated ($r_s = 0.582$** and 0.560**) with plants growing on rendzina (grey calcareous soil). Likewise, MGP no 22 was correlated with the third factor of climate ($r_s = 0.423$*).

Multiple regression analysis

Several tests of multiple regression (MR) were conducted to find out the best predictors of the dependent genetic diversity, H_e, of bands and MGPs and the independent climatic variables (Table obtainable upon request).

α-Amylase
Significant coefficients of MR (R^2) were found explaining the variance of malt MGP frequencies. For example, the variance in the frequency of MGP no 21 was explained by a 3-variable combination (altitude, number of rainy days, and January temperature) ($R^2 = 0.420$*).

β-Amylase
Significant explanation of the variance also occurred between multilocus of β-amylase patterns and climate. The following significant R^2 have been found: in germinating seeds, the variance of band 16a was explained by a 3-variable combination (dew, growing on rendzina, and evaporation) ($R^2 = 0.528$**). The variance in MGP no 29 was explained by a 3-variable combination, relative rainfall, annual humidity, and growing on terra rossa ($R^2 = 0.469$**). In dry seeds the variance of band 9a was explained by a 3-variable combination (growing on basalt, annual temperature, and daily temperature difference) ($R^2 = 0.512$**). The variance in MGP no 33 was explained by a 3-variable combination (relative rainfall, no of tropical days, and growing on rendzina) ($R^2 = 0.433$*).

Genetic association of amylase bands across Israel

Examples of amylase band associations across Israel in malt, green, and dry and germinating seeds, expressed as deviation from the expected pairwise band combinations, are obtainable upon request. Notably, a considerable amount of band association occurs across the geographic range studied above than that which might be expected by chance.

α-Amylase
Malt. Among the 12 polymorphic bands suitable for the analysis, 25 out of 66 (37.9%) possible combinations were significant ($P < 0.05$).
Green parts. Among the four polymorphic bands (2, 3, 7, 8a) 4 out of the 6 (66.7%) possible combinations were significant ($P < 0.01$).
Malt-green. Among the 48 possible combinations of polymorphic bands 19 (39.6%) were significant ($P < 0.05$).

β-Amylase
Dry seeds. Among all of the 13 polymorphic bands 48 out of 78 (61.5%) possible combinations were significant ($P < 0.05$).
Germinating seeds. Among all of the 11 polymorphic bands, 27 out of 55 (49.1%) combinations were significant ($P < 0.05$).
Dry-germinating seeds. Among the 143 possible combinations of polymorphic bands 58 (40.6%) were significant ($P < 0.05$).

α, β-Amylase
Malt-dry seeds. Among the 156 possible combinations 48 (30.8%) were significant ($P < 0.05$).
Malt-germinating seeds. Among the 132 possible combinations of polymorphic bands 41 (31.1%) were significant ($P < 0.05$).
Green-dry seeds. Among the 52 possible combinations 16 (30.8%) were significant ($P < 0.05$).
Green-germinating seeds. Among the 44 possible combinations 11 (25%) were significant ($P < 0.05$).

Genetic differentiation at the two-band (= locus?) level: gametic phase disequilibria (D)

We calculated gametic phase disequilibria within populations, D, and correlated climatic variables and principle components (PC) with the percentage of significant gametic phase disequilibria (D) from the total number of D's within a population (Table 5). The above correlation was made after exclusion of D's based on band frequencies less than 0.12 or more than 0.88. The percentage of D is significantly correlated with seasonal temperature difference and with the second factor of the PC combining all temperature variables ($r_s = 0.482$* and 0.479*, respectively). D is also approaching significant correlation with longitude,

Table 5. Gametic phase disequilibria of amylases within populations of *Triticum dicoccoides* in Israel. Only D's based on frequencies between 0.2 and 0.8 are counted. Significance by χ^2 or Fisher exact tests

Number of population	*n*	Number of polymorphic bands		Gametic phase disequilibria (D)				
				Total number	Number significant			
		All	0.2–0.8		<0.05	<0.01	All	%
1 Mt. Hermon	10	11	11	55	4	7	11	20.0
5 Qazrin	10	15	13	78	5	3	8	10.3
7 Yehudiyya	10	9	6	16	0	3	3	20.0
8 Gamla	9	17	4	6	2	1	3	50.0
9 Rosh-Pinna	9	4	3	3	1	0	1	33.3
11 Tabigha	10	14	6	15	3	4	7	46.7
16 Mt. Gilboa	9	10	8	28	4	3	7	15.6
17 Mt. Gerizim	10	19	15	105	1	6	7	6.7
18 Gitit	10	11	9	36	0	1	1	2.8
19 Kokhav Hashahar	10	7	5	10	0	4	4	40.0
20 Taiyiba	10	12	9	36	2	1	3	8.3
21 Sanhedriyya	10	5	4	6	0	0	0	0.0
22 Bet-Meir	10	3	3	3	1	0	1	33.3
23 J'aba	10	11	7	21	1	1	2	9.5
24 Amirim	9	7	3	3	0	0	0	0.0
25 Nahef	10	7	1	0	–	–	–	–
26 Ahihud (=Achihood)	10	1	1	0	–	–	–	–
27 Nesher	10	10	9	36	12	3	15	41.7
28 Beit-Oren	10	8	6	15	0	1	1	6.7
29 Daliyya	10	3	3	3	0	0	0	0.0
30 Bat-Shelomo	10	11	6	15	0	1	1	6.7
32 Yabad	10	11	10	45	3	2	5	11.1
33 Givat-Koach	9	3	3	3	0	0	0	0.0
Total	225							

relative humidity, and the first PC factor of 3 geographical variables ($r_s = 0.422^{@}$, $0.387^{@}$ and $0.397^{@}$, respectively). We conclude that the percentage of significant D is ecogeographically structured and affected by climatic heterogeneity.

Discriminant analysis

We conducted several stepwise discriminant analyses (SPSS-x 1986) maximizing the overall multivariate F ratio between categories in each test. The analyses were based on band and MGP frequency or on genetic indices and involved a maximum of 20 polymorphic variables. The results for band frequencies are given in Fig. 4, and the data plus the figure of soil type are obtainable upon request. For all 23 populations analyzed, the analyses succeeded to differentiate significantly, on the basis of band and MGP frequencies of amylases between: (1) populations of wild emmer wheat growing in different ecogeographical regions in Israel (central, northern, western, and southeastern marginal regions); and (2) populations growing on different soil types: basalt, terra rossa, and rendzina. The correct classification of wild emmer populations

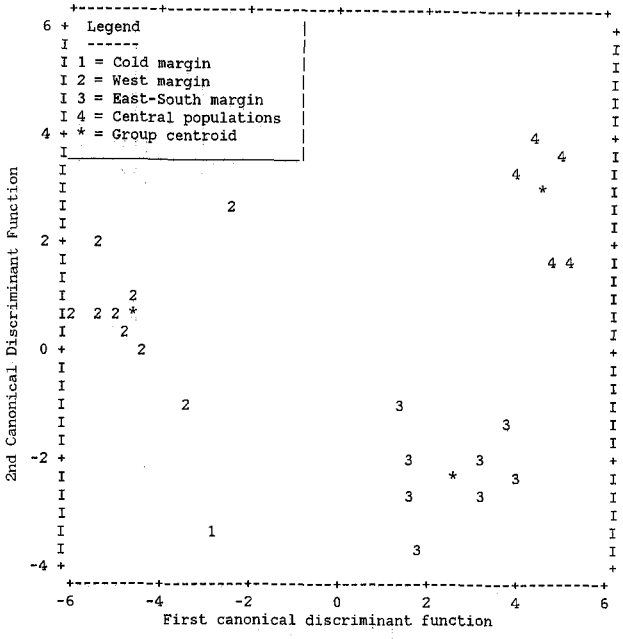

Fig. 4. Discriminant analysis of 23 populations of *Triticum dicoccoides* in Israel into four ecogeographical regions.

into their respective ecogeographical regions or soil types was 95–100%.

Autocorrelation

Spatial autocorrelation analysis gives a measure of the level of correlations of the observed values (band frequencies) at each locality with values of the same variables at other geographic sections. (Sokal and Oden 1978a, b).

We calculated Moran's *I* autocorrelation coefficients of bands across the entire geographic range including all 23 populations. We partitioned the space into six distance classes so that each class contained equal numbers of locality pairs. The results are obtainable upon request. The following are the main results. For α-amylase we analyzed 15 polymorphic bands. The average *I* appears to be independent of the geographical distance. The geographic pattern varies between single bands. There was no continuous monotonic decrease with distance. The fourth distance class (66–91 km) includes 3 positive and 2 negative significant correlations, thus displaying inconsistency and opposite trends. For β-amylase we analyzed 21 polymorphic bands. Again, the average *I* appears to be independent of geographic distance, and no band showed a continuous monotonic change in *I*. Only if we ignore the first distance group (0–28 km) does 1 band (dry seed 9′) display a monotonic change in *I* from the second to the sixth distance group (0.41** to −0.39**). There was not a single significant *I* in the first distance group in the entire matrix (of α- and β-amylases). Thus, migration can not explain these results.

Discussion

The evidence presented here for the multigene families of α- and β-amylases in 23 Israeli populations of wild emmer wheat, *T. dicoccoides*, parallels basically, in population genetics terms, that of single gene and multilocus diversity encoded by 42 allozyme gene loci (Nevo and Beiles 1989).

Theoretical evolutionary considerations

The amylase multigene families display an "Archipelago" pattern of population genetic structure parallel to that of allozymes in wild emmer wheat and wild barley (Nevo and Beiles 1989). The evidence of α- and β-amylases in natural populations of *T. dicoccoides* illustrates the evolutionary driving forces causing amylase differentiation, and its potential adaptedness. Out of the potential conceivable forces, mutation, genetic drift, migration, and natural selection, the latter appears to be the predominant differentiating and orienting force for the following reasons.

The amylases' pattern is nonrandom, structured, and correlated with ecology across the Israeli range of *T. dicoccoides*, negating genetic drift as an explanatory model. The predominance of ecogeographically structured polymorphisms (displaying significant climatic and soil correlates) excludes randomness and strongly suggests the operation of ecologically diversifying natural selection. Furthermore, the significant correlation of α- and β-amylases with climatic factors, primarily temperature variables, suggests that many of the selection processes in different temperature regimes operate on multilocus genotype patterns, (MGP), in addition to those directly affecting single bands. Finally, the autocorrelation analysis excludes migration as a major differentiating forces; again relegating selection to an important evolutionary role in amylase differentiation. We conclude that as in the single loci allozyme case and as concluded from both micro- and macrogeographic differentiation (Nevo and Beiles 1989), the amylase multigene families are basically governed by climatic and edaphic diversifying natural selection. This conclusion bears directly on the utilization of this amylase variation.

Wild emmer and wheat improvement

The wild gene pool of emmer wheat can contribute to wheat improvement by providing (1) direct elite genetic resources, and (2) genetic markers for chromosomally identifying QTLs of agronomic interest. We have reviewed (1) earlier (Nevo 1983, 1988; Nevo and Beiles 1989) and suggested allozyme markers for identifying agronomic traits of interest (Nevo 1987). Israel is particularly rich in the genetic resources of wild cereals due to its extraordinary physical and biotic diversity (Nevo 1983, 1986, 1988; Carver and Nevo 1990; Nevo et al. 1991, 1992; Snape et al. 1991). This is particularly true for wild emmer wheat, whose center of origin is northern Israel.

The Numerous genes and rich polymorphisms of both α- and β-amylases of wild emmer described previously (Nishikawa et al. 1988a, b) and in the present study may provide unique genetic resources of amylase genes of agricultural importance for improving wheat germination and growth both in optimal as well as in adverse and stressful environments. The chromosomal location of most of the α- and β-amylase genes is known (Hart and Gale 1989; Milne and McIntosh 1989; Nishikawa 1991). Some have been cloned (Lazarus et al. 1985; Huang et al. 1990), and others from wild emmer are clonable. Most sequence motifs are expected to be common to most of the actively transcribed genes in plants. However, specific sequences are expected in the rich amylase allelic

diversity described here in wild emmer as well as in other genes regulating gibberellic acid (GA_3), which in turn regulates the amylases. The unique expression and effects on germination and growth of the wild emmer genes can be tested biotechnologically in *T. aestivum*. It is expected that amylase genes and alleles, and their regulator GA_3 genes, may be unique in some wild emmer populations, particularly in steppic ones. These steppic populations are adapted to rapid germination and quick growth in order to shorten the annual cycle and escape the early hot and dry summer (Nevo and Beiles 1989).

In addition to their direct utilization as described above, α- and β-amylase isozymes can provide useful markers for chromosomal locations of QTLs (Ainsworth et al. 1987; Nishikawa et al. 1988a, b; Nishikawa 1991). Biochemical loci are superior over morphological loci that affect the gross phenotype since they and their alleles are easily identifiable. The wheat α-amylase genes are useful markers because the products of six individual loci of *α-Amy-1* and three of *α-Amy-2* can be resolved simultaneously, whereas the β-amylase genes are less valuable (Ainsworth et al. 1987). We are currently using amylase probes in our laboratory to trace herbicide resistant gene(s) (Snape et al. 1991) on chromosome 6B of wild emmer wheat.

The transformation and expression of foreign genes is now also progressing speedily (Schell 1987). Clearly, both theoretically and practically, the challenges of exploring and utilizing wisely the genome of wild wheat for future utilization in wheat improvement are wide open and very promising, also in the case of the amylase multigene families.

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