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Allozyme and morphological variability, outcrossing rate and core collection formation in lentil germplasm

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Summary. A survey of qualitative genetic variation at 3 morphological trait loci, 17 isozyme loci and a putative isozyme locus (amylase) was made for 105 lentil (*Lens culinaris* Medikus) germplasm accessions from Chile, Greece and Turkey. New alleles were found for *Lap-1, Me-2, Pgm-c, Pgm-p* and *6-Pgd-c*. The average proportion of polymorphic loci per population was 0.19, with a range of 0 to 0.42 over populations. Germplasm from Chile was equally variable to that from Greece and Turkey on the basis of individual loci and in a multilocus sense, despite its post-Columbus introduction to the New World. Evidence was found from associations between allelic states at different loci of a complex multilocus structure of lentil populations. A single multilocus genotype represented 10.2% of all plants sampled. The rate of outcrossing varied from 2.2% and 2.9% in Turkish and Greek landraces to 6.6% among Chilean populations. Using the survey data, a random sampling strategy for core collection formation was compared with two stratified sampling methods. The advantage of stratified sampling over random sampling was only significant at $P=0.28$.

Key words: *Lens culinaris* – Isozymes – Genetic diversity – Core collection – Cross-pollination

Introduction

To maintain and exploit crop germplasm resources efficiently, an understanding of the variation, its assortment between and within accessions and the population structure of the collection is required. This information dictates the practical details of most aspects of genetic resources activity. Part of this activity currently receiving attention is the use of core collections to streamline the evaluation of germplasm (Frankel and Brown 1984). International germplasm collections often contain so many accessions that they are formidable to potential users. A representative sample of the collection may be easier to handle than the entire collection, for example, by a national programme or as the starting point of extensive evaluation planned for some key trait. Such a representative sample of the collection is a core collection which aims, with minimum redundancy, to include the genetic diversity in a species in a condensed (and hence manageable) assembly of accessions.

Several sampling methods to select entries for the core collection have been suggested, ranging from random sampling to stratified sampling based on known groups with sample size either constant, logarithmic or proportional to the group size (Brown 1989). But a comparison between sampling methods can only be made with information from a survey of genetic variability in germplasm. In lentil, as in most other crops, the necessary detailed information is currently lacking.

Regional groups in lentil (*Lens culinaris* Medikus) germplasm have, however, already been identified following stepwise discriminant analysis of a world collection (Erskine et al. 1989). Close resemblance was found between germplasm from climatically similar, neighbouring countries, indicating that adaptation to the ecological environment has been a major evolutionary force in cultivated lentil. These groups can form the basis of stratification of sampling for a core collection.

Although estimates of variation between and within lentil accessions have been made for quantitative traits (Erskine and Choudhary 1986), information on discretely inherited characters is required to compare different

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Table 1. Plant Inventory numbers (PI) and collection site of accessions from Chile, Greece and Turkey

Chile		Greece		Turkey		Turkey	
Plant Inventory no. (PI)	Collection site	Plant Inventory no. (PI)	Collection site	Plant Inventory no. (PI)	Collection site	Plant Inventory no. (PI)	Collection site
299144	La Ligua, Aconcagua	209858	Athens	169517	Bornova, Izmir	175758	Kayseri
299148	Mulchen, Bio-Bio	297757	Arahova	169518	Elmali, Antalya	175759	Bozaliyan, Yozgat
299149	Matanzas, Santiago	297758	Larissa	169519	Selimiye, Antalya	176602	Partek, Tunceli
299150	Zapallar, Aconcagua	297761	Egleuvi	169523	Koycegiz, Mugla	176604	Yildizili, Sivas
299151	Longotoma, Aconcagua	297762	Heani	169527	Antalya	176607	Konya
299153	San Carlos, Nuble	297763	Lassion, Rhodopis	169531	Kirklareli	176609	Aksehir, Konya
299156	Navidad, Santiago	297764	Paleohorion, Karditsa	169534	Edirne	176610	Ayfon, Ayfon
299310	Quinteros, Valparaiso	297765	Korynos, Kastorias	169542	Cannakale	176986	Jommece, Kastamonu
299311	Curepto, Talca	297766	Lahania, Redos	169552	Bandirma	176987	Konya
299312	Licancheu, Santiago	297767	Kavakli, Redopis	169556	Gokce Viran	176988	Silivri, Istanbul
299313	Parral, Linares	297768	Furenou, Redopis	172938	Cinar, Diyarbakir	176993	Kagizman, Kars
299314	Pupuya, Talco	297770	Pella, Giannitsa	172944	Pazarcik, Maras	176994	Sarkoy, Tekirdag
299345	Palquibudis, Curico	297771	Dara	172947	Darende, Malatya	176995	Yalova, Istanbul
299346	Lora, Curico	297773	St Irene, Kefallinia	172949	Malatya	177430	Kure, Ankara
299347	Tuman, Santiago	297774	Oreskia, Serres	172953	Elazig	177432	Merzifan, Amasya
299348	Los Molles, Valparaiso	297775	Pedikovrisi, Arkadia	173718	Acimanuk, Mus	177433	Alaca, Corum
299349	Santiago	297776	Ahladini, Ilias	174238	Muradiye, Van	177437	Hinanis, Hakari
299351	Malleco	297777	Dafni, Serres	174246	Suruc, Urfa	177438	Yuksekora, Hakari
299352	Chimbarongo, Colchagua	297778	Ptolemais	174249	Gaziantep	177439	Cukurca, Hakari
299353	Renaico, Malleco	297779	Oreccastron, Ionnion	174251	Safalar, Elazig	178928	Mardin
299354	Machali, O'Higgins	297780	Peregeniani, Ionnion	175746	Coru, Tekirdag	178931	Diyarbakir
299355	Malloa, O'Higgins	297789	Pyrgi, Kefallinias	175748	Arac, Kastamonu	178934	Kars
299357	Rosario, O'Higgins	297790	Molle, Kefallinias	175751	Ayvacik, Canakkale	178939	Isparta
299358	Natijilla, O'Higgins			175753	Salihli, Manisa		
299360	Laja, Bio-Bio			175754	Erzincan		
299361	Cauquenes, Maule			175755	Ismail, Corum		
299362	Tijeral, Malleco			175757	Kirsehir		
299363	Yumbel, Concepcion						
299364	Santa Fe, Bio-Bio						
299365	Caimanes, Coquimbo						
299373	Illapel, Coquimbo						
299374	Portezuelo, Nuble						

sampling strategies for core collection formation. One such survey was made on a single locus, namely aspartate aminotransferase (Skibinski et al. 1984).

This study aimed to survey the variation for a much wider range of qualitative characters in a lentil collection from three countries, Chile, Greece and Turkey, so that the data generated may be used to estimate outcrossing rates and test various sampling strategies for core collection formation.

Materials and methods

A total of 105 accessions of lentil were included in the survey of genetic variability, with 32 accessions from Chile, 23 from Greece and 50 from Turkey. These countries were selected to represent two different regional groups, with germplasm from Greece and Turkey previously identified as coming from the same regional group and Chilean germplasm selected as representative of a group with a less ancient history of local adaptation (Erskine et al. 1989). Seed was supplied by the United States Department of Agriculture Western Regional Plant Introduction Station (WRPIS), Pullman, Washington. Turkish accessions were collected by Dr. J. Harlan in 1948/49; Greek accessions by Dr. D. A. Panos in 1953 and by the Institute of Fodder Crops, Larissa, in 1963; and Chilean accessions were collected in 1964. Plant Inventory numbers (PI) and origins of the accessions are given in Table 1.

Ten plants per accession were assayed for three morphological characters, each controlled by a single locus (Table 2), and for nine enzyme systems.

Samples were prepared for electrophoresis using a TRIS-HCl grinding buffer (Soltis et al. 1983). Samples were then absorbed onto 5 mm × 12 mm Whatman No. 9 filter paper wicks, and 22 samples (including checks) were placed onto each starch gel. Samples were run both on a pH 8.1 TRIS-citrate/lithium borate gel system and on a pH 6.5 histidine gel system as described by Selander et al. (1971). Slices from the TRIS-citrate/lithium borate gels were assayed for aspartate aminotransferase (AAT), alcohol dehydrogenase (ADH), leucine aminopeptidase (LAP) and malic enzyme (ME), and left overnight to observe amylase (AMY) activity. Slices from the histidine gels were assayed for diaphorase (DIAP), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucomutase (PGM) and shikimic dehydrogenase (SKDH) activity.

Extracts from fresh leaf tissue were analysed for all enzyme systems except ADH, for which extracts were made from immature pods.

The assays for ADH, 6PGD and LAP were slightly different or identical to those described in Shaw and Prasad (1970). Assays for AAT, PGM and SKDH have been described by Weeden and Gottlieb (1980) and the assays for ME and DIAP have been described by Weeden (1984).

Table 2. Mean allelic relative frequencies and proportion of polymorphic populations of different alleles for each country and the significance probability (P) from chi-square tests of independence for fixed ratio over countries. Percentage heterozygotes are also indicated for each country. Values in parentheses are standard deviations over accessions

Locus symbol and name	Allele	Allelic proportion				Proportion polymorphic population			
		Chile	Greece	Turkey	*P*	Chile	Greece	Turkey	*P*
gs	Gs	0.887	0.670	0.962	0.000	0.47	0.83	0.12	0.001
Epicotyl colour	gs	0.113	0.330	0.038		(0.50)	(0.38)	(0.33)	
yc	Yc	0.114	0.168	0.374	0.000	0.19	0.22	0.32	0.368
Cotyledon colour	yc	0.886	0.832	0.626		(0.39)	(0.41)	(0.47)	
scp	scp	0.886	0.822	0.793	0.000	0.75	0.61	0.46	0.410
Seed coat	scp^d	0.104	0.141	0.177		(1.73)	(0.49)	(0.50)	
pattern	scp^s	0.003	0.0	0.016					
	scp^m	0.006	0.038	0.014					
adh	a	0.122	0.978	0.168	0.003				
Alcohol dehydrogenase	b	0.878	0.022	0.832					
Amy	+	1.0	1.0	0.994	0.372	0	0	0.04	*
Amylase	−	0.0	0.0	0.006		(0)	(0)	(0.20)	
Aat-mb	a	0.750	1.0	1.0	0.000	0.38	0	0	0.000
Aspartate amino transferase-1	b	0.250	0.0	0.0		(0.5)	(0)	(0)	
Aat-p	a	0.920	0.762	0.375	0.000	0.38	0.44	0.56	0.317
Aspartate amino transferase-2	b	0.080	0.238	0.625		(0.48)	(0.50)	(0.50)	
Aat-m	a	0.736	0.329	0.227	0.011	0.67	0.26	0.30	0.017
Aspartate amino transferase-3	b	0.264	0.671	0.773		(0.48)	(0.44)	(0.46)	
Aat-c	a	1.0	1.0	0.996	0.366	0	0	0.02	*
Aspartate amino transferase-4	b	0.0	0.0	0.004		(0)	(0)	(0.14)	
Dia-2	a	0.0	0.0	0.010	0.064	0	0	0.04	*
Diaphorase-2	b	1.0	1.0	0.990		(0)	(0)	(0.196)	
Lap-1	a	0.681	0.845	0.708	0.000	0.47	0.39	0.48	0.778
Leucine amino	b	0.286	0.155	0.271		(0.50)	(0.49)	(0.50)	
peptidase-1	c	0.033	0.0	0.020					
Me-1	a	0.089	0.195	0.149	0.000	0.16	0.22	0.38	0.067
Malic enzyme-1	b	0.911	0.805	0.851		(0.36)	(0.41)	(0.49)	
Me-2	a	0.850	0.996	0.940	0.000	0.16	0.04	0.12	0.427
Malic enzyme-2	b	0.038	0.0	0.028		(0.36)	(0.20)	(0.33)	
	c	0.112	0.004	0.032					
Pgm-c	a	0.003	0.0	0.002	0.616	0.03	0	0.04	*
Phosphoglucomutase-1	b	0.997	1.0	0.996		(0.17)	(0)	(0.20)	
	c	0.0	0.0	0.002					
Pgm-p	a	0.029	0.057	0.0	0.000	0.44	0.44	0.20	0.0351
Phosphoglucomutase-2	b	0.377	0.392	0.427		(0.50)	(0.50)	(0.40)	
	c	0.594	0.551	0.573					
6 Pgd-p	a	0.793	0.946	0.466	0.000	0.28	0.09	0.36	0.052
6 Phosphogluconate dehydrogenase-1	b	0.207	0.054	0.534		(0.45)	(0.28)	(0.48)	
6 Pdg-c	a	0.006	0.0	0.0	0.102	0.03	0	0	*
6 Phosphogluconate dehydrogenase-2	b	0.994	1.0	1.0		(0.17)	(0)	(0)	
% Heterozygotes		2.01	2.61	5.94	0.011				

* Some expected frequencies <1

The alleles detected and their loci for the observed morphological traits and enzyme systems are given in Table 2. The nomenclature of *a, b, c* was used to identify alleles, with *a* as the fastest allele on the gel and the others named in descending order (Weeden et al. 1988). Genetic variability was described by allelic frequencies, and the proportion of polymorphic loci per population was determined as suggested by Gottlieb (1981) for each country and for each population. Tests of independence were made for each locus to detect differences among countries in allelic frequency by comparing the number of plants of different alleles from each country. Tests of independence were also made to detect variations among countries in the proportion of polymorphic loci per population by comparing each country's number of monomorphic and polymorphic populations.

Chi-square tests of independence were made of the allele distribution at the nine most polymorphic loci (*gs, scp, yc, Aat-p, Aat-m, Lap-1, Me-1, Pgm-p* and *6Pgd-p*) to ascertain if the genotypic state at one locus depends on the genotypic state at another locus. A multilocus diversity index was used to summarize multilocus genotypic diversity within and among populations as $H_j = 1\text{-}\hat{E}\ g^2_i$ in which g_i is the frequency of the ith 9-locus genotype in the jth population (Garcia et al. 1989). The value of H_j is zero when only one 9-locus genotype is present in a population, and values of H_j increase as the number of genotypes

increases, approaching unity when there are equally frequent genotypes in the population.

Stepwise discriminant analysis on the basis of country of origin was undertaken using the proportions of the most frequent allele at each locus as variables (Erskine et al. 1989). Characters for inclusion were selected stepwise to minimize Wilk's lambda between groups. Canonical variate analysis was then used to represent the multivariate data on orthogonal axes such that the maximum discrimination is obtained between groups, when tested against variation within groups (Seal 1964).

Three sampling methods were compared for core collection formation using all data except that of *Adh-2* for which only 353 plants were sampled. (1) Random: ten random samples of 10% of all accessions were selected and the number of alleles assessed. (2) Stratified sampling with constant allocation per geographic group: using a constant proportion of accessions per geographic group, ten samples were selected at random with five accessions each from Chile and Turkey/Greece. (3) Stratified sampling with the allocation per group proportional to group size: ten samples were selected at random with each geographic region represented proportionally by the number of accessions (Chile: 3 accessions, and Turkey/Greece: 7 accessions).

Results

Allelic frequencies and diversity of the germplasm

The survey of variation covered a total of 1048 plants for three loci of morphological traits, 17 known isozyme loci (Zamir and Ladizinsky 1984; Muehlbauer et al. 1989; Vaillancourt 1989) and one putative isozyme locus – amylase (*Amy*). Table 2 gives the allelic frequencies at each locus for the three countries and the probability (*P*) of significant differences between countries in allelic frequency.

Morphological loci. Green epicotyl (*gs*) was rare in Turkey, but common in Greece. Consequently, 83% of Greek populations were polymorphic, compared with 12% of polymorphic Turkish populations.

The incidence of red cotyledon (*Yc*) was highest in Turkish populations, but differences among countries in the proportion of polymorphic populations were not significant.

The locus seed coat pattern (*scp*) had four alleles, the highest number of alleles per locus among the loci investigated. Marbled seeds were common among Greek germplasm, and there were no spotted seed. In contrast, a small amount of material from Chile and Turkey had marbled and spotted seed.

Isozyme loci. Four loci were monomorphic, namely *Dia-1, Dia-3, Dia-4* and *Skdh*. The other 14 isozyme loci were polymorphic.

Adh-2: Only 353 plants were surveyed for the *Adh-2* locus. Consequently, data for this locus are presented overall and on a country basis, but not for individual populations. Striking differences were found among countries in allelic frequencies with the allele *Adh-2 a* very frequent in Greece but less frequent in Chile and Turkey.

Amy: Three plants from Turkey had no observable amylase activity. They bred true and were probably homozygous for a nul allele at a putative *Amy* locus.

Aat-mb: There was no diversity for this locus in the germplasm from Greece and Turkey, which was fixed for the fast allele (*a*), whereas the Chilean material contained both alleles (*a* and *b*) with the *b* allele at the relatively high frequency of 0.25.

Aat-p: This locus was frequently polymorphic for two alleles (*a* and *b*) in all three countries. The allelic frequencies varied over countries with the frequency of the fast allele (*a*) 0.92 in Chile, compared to only 0.375 in Turkey.

Aat-m: The fast allele (*a*) was most common in Greece and Turkey, but in Chile the slow form (*b*) was prevalent.

Aat-c: Chilean and Greek germplasm was monomorphic for the fast allele (*a*) at this locus. But the rare slow allele (*b*) was found in a single population among genetic resources from Turkey.

Dia-2: The locus *Dia-2* was variable only in Turkish lentils, where a rare fast (*a*) allele was recorded.

Lap-1: Lap-1 was a highly polymorphic locus with three alleles. Alleles *a* and *b* were found at high frequencies, and a new, slow allele (*c*) was identified at a low frequency. Heterozygotes of *Lap-1 c* with both *Lap-1 a* and *Lap-1 b* were found, and segregation was observed in their progeny, demonstrating Mendelian inheritance of the new allele.

Me-1: Two alleles were evident for this locus, both of which were widespread, but the slow allele (*b*) was always more common than the fast allele (*a*), which reached its highest frequency in Greek germplasm.

Me-2: There were three alleles for the locus *Me-2,* with the fastest allele (*a*) predominant throughout. In Greece, all populations except one were monomorphic for this allele. A new very slow allele (*c*) was found from Chile and Turkey. Being relatively uncommon, the new allele was not found in hybrid combination in heterozygotes; consequently its pattern of inheritance is not yet known.

Pgm-c: Allele *b* was the most frequent among the three alleles found. The fast allele *a* occurred at a low frequency in germplasm from Chile and Turkey, and a new slow allele *c* was found in two Turkish populations. Because the new locus did not appear in hybrid combination, its inheritance is unknown.

Table 3. Mean number of alleles per locus and mean percentage of polymorphic loci over loci and populations and the mean number of multilocus genotypes per population and mean multilocus diversity index (H_j) over populations in the three countries. Standard errors are in parentheses

	Chile	Greece	Turkey
Mean number alleles/locus	1.20 (0.13)	1.19 (0.09)	1.18 (0.11)
Mean % of polymorphic loci	21.4 (17.7)	17.7 (7.4)	17.5 (10.1)
Mean no. multilocus genotypes/population	3.7 (1.98)	4.5 (1.88)	3.8 (1.91)
Mean multilocus diversity value/population (H_j)	0.530 (0.267)	0.664 (0.140)	0.532 (0.247)

Pgm-p: This was a frequently polymorphic locus with three alleles. The fast allele *Pgm-p a* was rarest, followed in ascending order by *b* and *c*. There were highly significant differences among countries in allelic frequencies. Heterozygotes for the new locus (*Pgm-p a*) were not seen, because it was relatively infrequent; consequently its pattern of inheritance is not yet known.

6Pgd-p: Both alleles at this locus showed frequencies greater than 0.4 in Turkish germplasm which was, consequently, highly polymorphic. In contrast, material from Greece was less variable, and the fast allele was predominant.

6Pgd-c: Most populations were fixed for the slow allele *b* at this locus, but in a single Chilean population two plants were found with a fast allele *a* for *6Pgd-c*.

Diversity among and within countries and populations

The average proportion of polymorphic loci per population was 0.19, and the range over populations was from 0 to 0.42. The average number of alleles per locus was 1.19. Overall there were no significant differences among countries in variability either in the proportion of polymorphic populations or the average number of alleles per locus over loci and populations (Table 3). However, countries differed for individual loci in the proportion of polymorphic populations. For example, Chilean material had a higher proportion of polymorphic populations than Greece and Turkey for *Aat-m* and *Aat-mb*, Greek germplasm had the most polymorphic populations for *gs* and fewest for *6Pgd-p*, and Turkish material had the fewest polymorphic populations for *Pgm-p*.

Discriminant analysis revealed overall that 80% of the populations were correctly classified to their country of origin and that Turkish germplasm was the most distinctive with 86% of the Turkish populations correctly classified. Greek material was the least distinct with only 65% of the populations correctly classified (Table 4). Loci, in descending order of discrimination between material of different origins, were *Aat-p, gs, Aat-m, 6Pgd-p, scp, Me-2, 6Pgd-c, Pgm-p, Me-1* and *Aat-c*. The individual populations and country centroids are plotted on the first two canonical variates in Fig. 1.

Independence tests of the genotypic state at one locus with the gentoypic state at other loci were made among

Table 4. Results of classification as predicted group membership from discriminant analysis of lentil populations from Chile, Greece and Turkey

Origin	No. accessions	Predicted group membership (%)		
		Chile	Greece	Turkey
Chile	32	81.3	18.8	0
Greece	23	26.1	65.2	8.7
Turkey	50	8.0	6.0	86.0

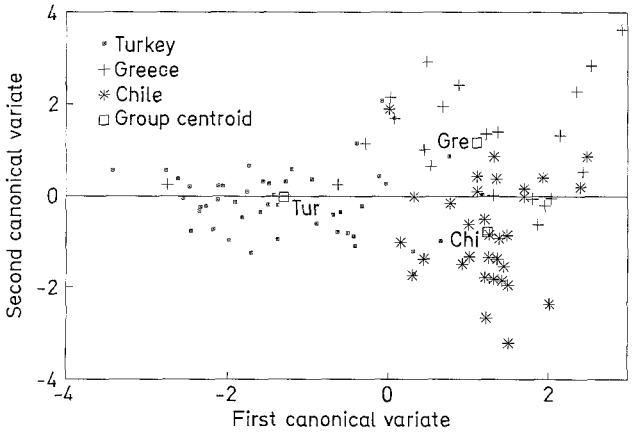

Fig. 1. Scatter diagram of the first two canonical variable mean values for 105 populations of lentil from three countries of origin, together with the group centroids for the countries identified by abbreviations

the nine most polymorphic loci (Table 5). Of the 36 tests, 21 combinations were significant at $P<0.05$. Having established the non-random distribution of alleles at different loci, the number of different multilocus genotypes per population was examined. Populations of ten plants contained between one and nine different multilocus genotypes, with an average of four. The value of the multilocus diversity index (H_j) varied from 0 in some populations, those with only a single multilocus genotype, to greater than 0.9, with an overall mean of $H_j=0.56$ over populations. A total of 10.2% of all sampled plants were of a single multilocus genotype. Differences among countries in the average number of multilocus genotypes per population and the multilocus diversity index (H_j) were non-significant (Table 3). The distribution of populations

Table 5. Probabilities from chi-square tests of independence of distribution of alleles at nine polymorphic loci within germplasm surveyed

	Epicotyl colour	Cotyledon colour	Seed coat pattern	Aspartate amino-transferase		Leucine amino-peptidase	Malic enzyme-1	Phospho-glucomutase-2
	(*gs*)	(*yc*)	(*scp*)	(*Aat-p*)	(*Aat-m*)	(*Lap-1*)	(*Me-1*)	(*Pgm-p*)
yc	0.089							
scp	1.000	0.000						
Aat-p	0.089	0.000	0.008					
Aat-m	1.000	0.000	0.187	0.000				
Lap-1	0.0117	0.071	1.000	0.000	0.002			
Me-1	0.129	0.014	0.029	0.074	0.294	0.021		
Pgm-p	0.000	0.000	1.000	0.002	1.000	1.000	0.705	
6Pgd-p	0.000	0.046	0.011	0.000	0.000	0.000	0.002	0.259

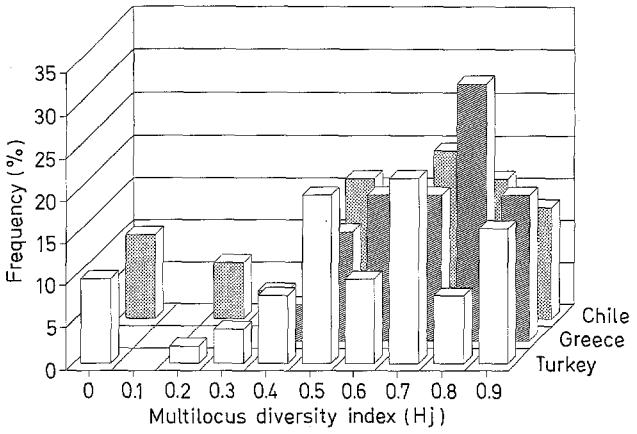

Fig. 2. Distribution of multilocus diversity indices of accessions from Chile, Greece and Turkey expressed as a frequency (%)

by country for the multilocus diversity index is shown in Fig. 2.

Heterozygosity

Co-dominance at isozyme loci allows the recognition of heterozygotes. The proportion of non-discernible outcrosses was approximately 0.1 from the multilocus estimator of Shaw et al. (1981). A total of 35 plants out of 1048 showed heterozygosity at one or more loci. Their progeny segregated, representing an overall outcrossing rate of 3.7%. Significant differences among countries in the rate of outcrossing varied from 2.2 and 2.9% in Turkish and Greek germplasm up to 6.6% in genetic resources originating from Chile.

Sampling for core collections

Ten samples of ten random accessions captured a mean total number of 27.7 alleles with a standard deviation of 4.3 from a total of 38 possible alleles. Using stratified sampling with a constant number (5) of accessions per geographic group, the mean total of 29.1 (SD = 1.8) alleles was captured. A stratified sampling procedure using a proportional number of accessions per geographic group (3 accessions from Chile and 7 accessions from Greece/Turkey) yielded a mean total of 29.6 alleles (SD = 1.4). Although differences between sampling methods were only significant at $P = 0.28$, stratified sampling appeared marginally superior to random sampling; this is in accordance with theoretical expectations (Brown 1989).

Discussion

The level of outcrossing is central to the breeding system and population structure of a species. An earlier measurement of outcrossing rate in lentil from Washington, USA, showed a level of less than 0.08% based on the marker locus cotyledon colour (*yc*) (Wilson and Law 1972), and a second estimate gave 0.9% outcrossing from diverse germplasm grown in Syria, based on the aspartate amino-transferase plastid locus (*Aat-p*) (Skibinski et al. 1984). Although single-locus estimates of outcrossing tend to underestimate the true rate of outcrossing by failing to account for non-discernible outcrossing between similar genotypes, this does not explain the observed high outcrossing rate, based on nine loci, among Chilean germplasm of 6.6%. This is an order of magnitude above previous estimates. All of the accessions studied have been maintained in the WRPIS germplasm collection for over 20 years. Sections of the collection are regrown annually at Pullman, Washington, and the difference among countries in outcrossing rates probably reflects year to year variation (R. Hannan, personal communication). The lentil flower is normally cleistogamous, and an insect vector is required to effect cross-pollination. A search for the causal vector insect is now warranted, particularly since problems in maintaining varietal purity during seed multiplication were recently encountered in Washington.

In the only previous extensive survey of allozyme diversity in lentil germplasm, 59% of the lentil accessions were polymorphic for the aspartate aminotransferase-2 locus (*Aat-p*). A comparison with surveys of wild and cultivated barley germplasm indicates that lentil was more variable than other self-pollinated species (Skib-

inski et al. 1984). The locus *Aat-p* was shown in this survey to be among the most polymorphic in lentil. The earlier, high estimate polymorphism within populations was biased, being based on a single very variable locus. The revised estimate of 19% of loci polymorphic is typical of other self-pollinated species (Skibinski et al. 1984).

The history of lentil cultivation in Chile is short in comparison to its ancient cultivation in Greece and Turkey and begun only after the arrival of Columbus in the New World. However, the variation in germplasm from Chile was equal to that within the Greek and Turkish material measured as an average over loci and on a multilocus basis.

An important feature of the variability uncovered by the germplasm survey is that the genotypic state at a given locus often depends on the genotypic state at other loci. Of the 36 tests of independence of the allelic distribution at pairs of loci, a total of 21 showed significant departures from random association of alleles at pairs of loci (Table 4). Linkage tests among many of these loci have been made (see summary of Muehlbauer et al. 1989), and the only linkage between these loci is between epicotyl colour (*gs*) and aspartate aminotransferase (*Aat-p*), explaining only one of the many associations found in this study. These results indicate a complex multilocus organization of lentil populations, with one multilocus genotype found in 10.2% of all plants. Studies on other predominantly self-pollinating species, both wild and cultivated, increasingly indicate that adaptive changes occur not only through changes in allelic frequency, but also through the reorganization of allelic ingredients into new multilocus allelic combinations adapted to specific habitats (Allard 1988; Garcia et al. 1989). These associations can develop between unlinked or loosely-linked loci in inbreeders since they are not broken up each generation by recombination. In contrast, outbreeders have a less highly structured multilocus organization.

Lentil is among the first crops domesticated (Hansen and Renfrew 1978). Its adaptation to the farming systems of dry areas is the result of centuries of natural selection. The components of this close adapation are, or should be, instructive to plant breeders trying to introduce/develop improved cultivars. There is considerable evidence from cereals and grain legumes that genetically heterogeneous populations produce more stable yields than genetically homogeneous populations (summarized in Simmonds 1979). The extent of variation within lentil landraces is clear from this and previous studies (Erskine and Choudhary 1986; Skibinski et al. 1984), but the relationships between diversity, multilocus organization and adaptation in lentil clearly merit further study.

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