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Aspartate aminotransferase allozyme variation in a germplasm collection of the domesticated lentil (*Lens culinaris*)

D. O. F. Skibinski [1], Dlear Rasool [1] and W. Erskine [2]

[1] Department of Genetics, University College of Swansea, Singleton Park, Swansea SA2 8PP, Wales, UK
[2] ICARDA, P.O. Box 5466, Aleppo, Syria

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Summary. Variation at a polymorphic Aspartate aminotransferase locus was assayed in a sample of 298 accessions from the ICARDA germplasm collection of the domesticated lentil (*Lens culinaris*). Two alleles $Aat\text{-}1^F$ and $Aat\text{-}1^S$ were detected with global frequencies of 0.51 and 0.49, respectively. Fifty-nine percent of accessions were polymorphic for both alleles. The frequency of outcrossing was estimated from the observed heterozygosity to be about 1%. This is higher than direct estimates of outcrossing and implicates selection in favour of heterozygous gene combinations. Significant variation in allele frequency and in the occurrence of polymorphic accessions was observed between countries or geographic areas. Significant associations were observed between the allozymes and agronomic characters. In particular high frequency of $Aat\text{-}1^F$ appeared to be associated with late flowering and maturity and low yield.

Key words: Lentil – Allozyme – Geographic variation – Outcrossing – Yield – *Lens culinaris*

Introduction

Electrophoretic studies of allozyme variation have provided considerable information on the genetic structure of plant populations. It has proved possible to test various hypotheses concerning levels of genetic heterozygosity in populations of inbreeding and outbreeding plants (Brown 1979) and to obtain estimates of natural outcrossing rates (e.g. Brown et al. 1978; Ellstrand et al. 1978; Jain 1979; Shaw et al. 1981). Observations of association with environmental factors and differences in viability (Clegg and Allard 1972, 1973; Clegg et al. 1978; Nevo et al. 1981) and in germination rate (Brown et al. 1976) between genotypes have provided evidence for the adaptive nature of allozyme variation, as have the observations in inbreeding plants of levels of heterozygosity in excess of those expected from known rates of outcrossing (Allard et al. 1972; Brown et al. 1978; Brown 1979) and of marked linkage disequilibrium between loci (Clegg and Allard 1972; Brown et al. 1977; Brown 1979). Associations between allozyme variants and morphological characters, including characters of agronomic importance, have also been reported (Marshall and Allard 1970; Hamrick and Allard 1975; Jensen et al. 1979) and a number of studies have investigated the changes of allozyme frequencies in experimental populations (Allard et al. 1972; Stuber et al. 1980). Stuber et al. (1982) have suggested that manipulation of allozyme allele frequencies could lead to improvement in yield and ear number in maize. Allozymes may also be useful in cultivar and varietal identification (Tanksley and Jones 1981; Arus et al. 1982; Cardy and Kannenberg 1982) and in verifying artificial crosses.

Although considerable data is available on micro- and macrogeographic variation in natural populations, fewer studies have been made of accessions from germplasm collections of domesticated plants. Kahler and Allard (1981) have assayed variation at esterase loci in over 1,500 accessions of domestic and wild barley and found substantial differences within and between accessions. Stuber and Goodman (1983) assayed variation at two phosphoglucomutase loci in nearly 1,000 collections of maize from Latin America, the USA and Canada. A number of different alleles were observed although a single allele predominated at both loci in most collections.

Other more limited studies have been made in a variety of crops (e.g. Rick and Fobes 1975; Rick et al. 1974; Quiros 1979; Jain et al. 1980).

The domesticated lentil (*Lens culinaris*) occupies about 3% of the total world area sown to pulses though is more important in some countries and particularly in Asia (Nygaard and Hawtin 1981). Most of the cultivated area is sown to land races though in a few countries these are being replaced by improved cultivars (Sohl and Erskine 1981).

This paper concerns a study of allozyme variation for the enzyme Aspartate aminotransferase (AAT) in a collection of lentil accessions from the germplasm col-

lection held at the International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria. AAT was chosen for study as it had been previously identified as polymorphic (Skibinski and Savage 1981). The extent of geographical variation in allozyme frequencies and the association of allozyme variants with agronomic characters are considered. In addition inbreeding statistics are used to obtain an estimate of percentage outcrossing from the allozyme data.

Materials and methods

Two hundred and ninety-eight lentil accessions obtained from the ICARDA collection were used in the study. They were chosen to cover a wide geographic range and 32 countries are represented. The gene pool of the lentil contains a high frequency of primitive varieties or land races (Solh and Erskine 1981), and the majority of accessions chosen for the study were of this type. A sample of seeds selected at random with regard to colour and size were germinated in compost for each accession. When the seedlings were 2 weeks old a few young leaves from each plant were removed for electrophoresis. The leaves were prepared by homogenising in three drops of 2% aqueous phenoxyethanol solution and then centrifuging to obtain a clear supernatant. The supernatant was absorbed on filter paper pieces which were applied to a horizontal starch gel containing 26.4 g starch in 230 ml of gel buffer. A discontinuous buffer system (Ashton and Braydon 1961) was used. Electrophoresis was carried out for 4 h using a current of 50 mA with cloth wicks about 10 cm apart. The gels were stained for AAT after the method of Shaw and Prasad (1970). Ten plants were assayed electrophoretically for the majority of accessions, although a few were assayed for 9,8 or 7 plants.

The accessions were assayed for a range of agronomic characters at ICARDA and covariation between AAT allozyme variation and variation in these characters has been assessed. The following characters were measured:

1. Days to flowering (DFL) – Time in days from the first day of rainfall after sowing to when 50% of the plants in a plot flowered.
2. Time to maturity (MAT) – Time in days from the first day of rainfall after sowing to when 90% of the pods in a plot were golden brown.
3. Plant hight (HT) – A visual estimate in cm of the average height of plants from the ground to the tip of the foliage.
4. Height of lowest pod (PD) – The visual average in cm of the height above ground of the lowest pod in unlodged plants at maturity.
5. Number of seeds per pod (SDPD) – Average number of seeds per pod in 30 randomly chosen pods.
6. 100 seed weight (SDWT) – Average weight in gm of two samples of 100 randomly chosen seeds, measured in grams.
7. Seed yield (YLD) – Yield of seed from the central, guarded area of a plot after sun-drying converted to kg/ha.
8. Biological yield (BYLD) – Yield of dried, mature plants after pulling from the central, guarded area of a plot converted to kg/ha.
9. Harvest index (HI) – Proportion of biological yield represented by seed yield, calculated as a ratio.
10. Susceptibility to cold – Accessions planted before winter in 1979 were scored on a 1–3 scale for damage after a 47 day snow cover had melted. 1 = 100–60% survival, 2 = 60–30% survival, 3 = 30–0% survival.
11. Ground colour of testa – Colours green, pink, brown, mixed green and pink were scored. Grey and black seeds also occurred but they were not analysed as separate classes. Accessions scored as polymorphic had two or more colours at frequencies above 10%.
12. Presence of pattern on testa – A variety of spotted and marbled patterns occurred. Accessions were scored as pattern absent, polymorphic, or monomorphic (using same 10% criteria as in 11).
13. Testa pattern colour – For accessions with testa patterns, colours black, olive, grey and brown and polymorphic or monomorphic (using 10% criteria) were scored.
14. Cotyledon colour – Colours orange/red, and yellow and polymorphic (using 10% criteria) were scored.

Characters 1 to 9 were scored in two growing seasons 1978 to 1979 and 1979 to 1980. In the 1978 to 1979 season the planting date was 14th November and date of first rainfall 30th November. In the 1979 to 1980 season planting occurred between the 10th to 25th November and the first rainfall occured on 18th September. Fifty kg of P_2O_5 was applied per hectare. In each growing season the accessions were planted in separate plots of six five-metre rows per plot with 25 cm between rows. Plant density was 200 plants/m^2 and 4 m^2 of plants were harvested.

Results

Aspartate aminotransferase variation

Two loci *Aat-1* and *Aat-2* were observed in *Lens culinaris*. In the total of 2,963 plants assayed no allozyme variation was detected at the slower migrating *Aat-2* locus while at the *Aat-1* locus two alleles, one fast migrating ($Aat\text{-}1^F$) and the other slow migrating ($Aat\text{-}1^S$) were detected. Heterozygotes at the *Aat-1* locus showed the three banded pattern characteristic of a dimeric enzyme locus. Seeds collected from such heterozygous plants segregated all three genotypes confirming mendelian inheritance. No other AAT allozyme mobility variants were observed. However, one plant had no observable activity at the *Aat-1* locus. It's subsequent growth was poor but a few seeds were set. On germination these also showed no activity. The plant was thus probably homozygous for a null allele. Obvious null heterozygotes were not observed.

Allele and genotype frequencies and estimate of outcrossing rate

Of the total of 2,963 plants assayed electrophoretically the genotypic proportions were $Aat\text{-}1^F/Aat\text{-}1^F$ (1,495 plants), $Aat\text{-}1^F/Aat\text{-}1^S$ (18 plants) and $Aat\text{-}1^S/Aat\text{-}1^S$ (1,450 plants). This gives overall allele frequencies for $Aat\text{-}1^F$ and $Aat\text{-}1^S$ of 0.51 and 0.49, respectively. Column 2 of Table 1 gives the distribution of accessions having different values of the frequency of $Aat\text{-}1^F$. The categories 0 to 10 represent accessions containing from zero to ten $Aat\text{-}1^F/Aat\text{-}1^F$ plants. Accessions with heterozygotes or less than 10 plants were assigned to the

Table 1. Means values of agronomic characters for gene frequence categories in 1978–1979 and 1979–1980 season. The number of accessions for each category are given in column two. Numbers in parentheses give the number of accessions on which the means are based. The column heading abbreviations are explained in Materials and methods section. *N* is number of accessions

Category	*N*	DFL	MAT	HT	PD	SDPD	SDWT	YLD	BYLD	HI
Season 1978–79										
0	24	130 (24)	167 (24)	30 (24)	17.08 (24)	1.47 (23)	3.49 (23)	1281 (23)	4418 (24)	0.285 (23)
1	16	131 (16)	169 (16)	31 (16)	18.06 (16)	1.41 (16)	3.61 (16)	1358 (15)	4719 (16)	0.291 (15)
2	6	124 (6)	164 (6)	30 (6)	17.50 (6)	1.51 (6)	3.73 (6)	1456 (5)	4097 (6)	0.354 (5)
3	14	128 (14)	166 (14)	30 (14)	17.21 (14)	1.36 (14)	3.81 (14)	1548 (14)	4908 (14)	0.302 (14)
4	5	123 (5)	164 (5)	29 (5)	15.00 (5)	1.41 (5)	3.69 (5)	1518 (5)	4191 (5)	0.358 (5)
5	8	130 (8)	171 (8)	30 (8)	17.38 (8)	1.40 (8)	4.33 (8)	1433 (6)	4282 (8)	0.292 (6)
6	12	131 (12)	169 (12)	32 (12)	18.67 (12)	1.25 (12)	4.17 (12)	1107 (10)	4121 (12)	0.247 (10)
7	6	133 (6)	174 (6)	30 (6)	18.00 (6)	1.50 (4)	4.62 (6)	1315 (4)	4273 (6)	0.278 (4)
8	8	128 (8)	167 (8)	31 (8)	17.75 (8)	1.39 (8)	4.41 (8)	1806 (7)	4774 (7)	0.292 (6)
9	20	131 (20)	168 (20)	30 (20)	16.90 (20)	1.38 (20)	3.94 (20)	1213 (20)	4418 (20)	0.273 (18)
10	33[a]	134 (32)	171 (32)	31 (32)	18.18 (32)	1.41 (28)	3.46 (32)	908 (28)	3729 (31)	0.240 (28)
Season 1979–80										
0	41	144 (41)	176 (2)	30 (2)	17.00 (2)	1.43 (40)	3.83 (41)	1704 (41)	5798 (41)	0.296 (41)
1	15	143 (15)	179 (1)	29 (1)	15.00 (1)	1.40 (15)	3.94 (15)	1706 (15)	6174 (15)	0.283 (15)
2	5	145 (5)	–	–	–	1.37 (5)	3.53 (5)	2080 (5)	7273 (5)	0.280 (5)
3	7	138 (7)	–	–	–	1.40 (7)	3.81 (7)	1908 (7)	4963 (7)	0.337 (7)
4	6	136 (6)	179 (1)	25 (1)	13.00 (1)	1.56 (6)	3.66 (6)	2178 (6)	6362 (6)	0.347 (6)
5	7	141 (7)	179 (1)	29 (1)	13.00 (1)	1.68 (7)	3.25 (7)	1946 (7)	6187 (7)	0.310 (7)
6	1	145 (1)	–	–	–	1.07 (1)	5.04 (1)	1555 (1)	6240 (1)	0.250 (1)
7	8	143 (8)	–	–	–	1.33 (8)	4.15 (8)	1579 (8)	5718 (8)	0.299 (8)
8	9	142 (9)	179 (1)	33 (1)	17.00 (1)	1.42 (9)	3.70 (9)	1664 (9)	5518 (9)	0.322 (9)
9	15	143 (15)	–	–	–	1.44 (15)	3.72 (15)	1724 (15)	5917 (15)	0.305 (15)
10	32	144 (32)	167 (2)	33 (2)	20.00 (2)	1.42 (32)	3.48 (32)	1539 (32)	5564 (32)	0.283 (32)

[a] Includes one accession which was not scored for any for the agronomic characters in the table

most appropriate category determined by the frequency of *Aat-1*^{F}. One hundred and seventy-six out of 298 or 59% of the accessions are polymorphic for both alleles; no doubt this estimate would have been higher had more plants been assayed per accession. As expected for a predominantly self-pollinating species there is a large deficit of heterozygotes compared with panmictic expectation. This is quantified here using inbreeding statistics (Wright 1951; Jain and Workman 1967). The deficit of heterozygotes within a subdivision of a population is given by:

$$F_{IS} = (F_{IT} - F_{ST})/(1 - F_{ST}) \quad (1)$$

where F_{IT} measures the overall deficit in the pooled accessions and F_{ST}, called the standardised gene frequency variance, measures the gene frequency differentiation among subdivisions. F_{IT} is equal to $1 - H_o/H_e$ where H_o and H_e are respectively observed and expected heterozygosity under panmixia and F_{ST} is equal to $\sigma_p^2/2\bar{p}(1-\bar{p})$ where σ_p^2 is the variance among, and $\bar{p}$ the average gene frequency over, subdivisions. For the genotype frequency data given above $F_{IT} = 1 - 0.0061/0.4999 = 0.988$. The quantity $F_{IT}\sqrt{N}$, where N is sample size is normally distributed with zero mean, and variance equal to one under the null hypothesis that $F_{IT} = 0$ (Brown 1970), that is assuming Hardy-Weinberg equilibrium. Here $F_{IT}\sqrt{N} = 53.77$ with $P \ll 0.001$ and the null hypothesis may thus be firmly rejected. σ_p^2 may be estimated as the variance in the frequency of *Aat-1*^{F} among accessions and equals 0.157; F_{ST} therefore equals $0.157/2$ $(0.51 \times 0.49) = 0.314$. Substituting into equation 1 above gives $F_{IS} = 0.982$. The standard error of F_{IS} computed from an equation given by Rasmussen (1964) is 0.003. The rate of outcrossing can be estimated by the equation (Falconer 1981):

$$t = (1 - F_{IS})/(1 + F_{IS})$$

when $F_{IS} = 0.982$, $t = 0.009$. Thus the rate of outcrossing within accessions is estimated to be 0.9%.

Geographic variation

Table 2 gives information on the geographic distribution of the *Aat-1* variants for each country represented in the sample of accessions. The countries are arranged according to geographic area and average or total values are given for each area. The total number of accessions at 275 is lower than in Table 1. In Table 2 only

Table 2. Geographic variation at the *Aat-1* locus in 275 lentil accessions

Location	No. of accessions	*Aat-1*^{F} frequency	Fixed *Aat-1*^{S}	Polymorphic	Fixed *Aat-1*^{F}
Austria	1	0.90	0	1	0
France	1	0.95	0	1	0
W. Germany	2	0.95	0	1	1
Belgium	1	0.00	1	0	0
Hungary	7	0.69	1	3	3
Yugoslavia	2	0.65	0	2	0
Czechoslovakia	1	0.50	0	1	0
Central and N. Europe	15	0.66	2	9	4
Spain	3	0.77	0	3	0
Italy	6	0.38	2	3	1
S. Europe	9	0.58	2	6	1
Egypt	2	0.50	1	0	1
Ethiopia	9	0.26	4	4	1
Morocco	2	0.73	0	2	0
Tunisia	1	0.00	1	0	0
N. and N.E. Africa	14	0.37	6	6	2
Cyprus	2	0.75	0	1	1
Turkey	49	0.49	10	30	9
Iraq	11	0.22	6	4	1
Palestine	2	0.85	0	2	0
Greece	17	0.73	2	9	6
Jordan	16	0.45	1	15	0
Lebanon	11	0.52	1	7	3
Syria	41	0.58	3	32	6
S. W. Asia	149	0.57	23	100	26
USSR	10	0.62	2	4	4
Iran	22	0.11	16	4	2
Afghanistan	8	0.43	2	5	1
India	4	0.58	1	2	1
Pakistan	11	0.83	0	10	1
Central and S. Asia	45	0.49	19	21	5
Mexico	16	0.25	6	9	1
Guatemala	1	0.10	0	1	0
Costa Rica	1	0.00	1	0	0
Central America	18	0.12	7	10	1
Chili	13	0.89	0	4	9
Argentina	1	1.00	0	0	1
Peru	1	1.00	0	0	1
S. America	15	0.96	0	4	11

land race material is included: the excluded accessions are mass selections made on the basis of seed colour from land race material.

A number of tests have been applied to the data. First, discarding 15 countries where only one or two accessions were assayed, a 3 (number of 'fast', 'slow' or polymorphic accessions) $\times 17$ (countries) test of independence gives $G_{32}=94.851$ ($P<0.001$). This was partitioned first into a comparison of number of fast versus number of slow accessions $G_{15}=43.510$ ($P<0.001$) and second in a comparison of number of polymorphic versus number of fast plus slow ($G_{16}=51.341$ $P<0.001$). The first test can be seen as providing evidence of significance gentic differences between countries. The second test provides evidence of significant variation in the frequency of polymorphic accessions between countries. The same tests were applied to the totals for the geographic areas (including

the USSR as an area). For the 3×8 (geographic areas) table, $G_{14}=49.118$ ($P<0.001$). The comparison of fast versus slow accessions gives $G_7=32.568$ ($P<0.001$) and comparisons of number of polymorphic versus fast plus slow gives $G_7=16.550$ ($P<0.05$). Also, the country frequency of *Aat-1*^{F} was compared among geographic areas by one way analysis of variance with variation between countries within geographic areas providing the error variance (USSR was here excluded). The test gave a significant result ($F_{6,24}=3.176$, $P<0.05$). Thus there appear to be significant genetic differences between geographic areas over and above that attributable to differences between countries.

It is not easy, however, to interpret the differences between countries and areas in terms of geographic trends. From a low value in central and S. Asia there is some indication that the frequency of *Aat-1*^{F} increases towards the West and N. West through into Europe, although the frequency is low in North and N.E. Africa. In America there is a rather clearer contrast between Central America, where the frequency of *Aat-1*^{F} is low, and S. America, where the frequency is high. There are also some quite striking differences between countries in the frequency of polymorphic accessions, for example between Iran and Syria, the latter having a much higher frequency than the former.

Correlations with agronomic characters

The mean values for agronomic characters 1 to 9 for each of the eleven gene frequency categories are shown separately for 1979 and 1980 in Table 1. For each character the variation between the categories has been assessed with a one way analysis of variance.

The significance levels are shown in the first two rows in Table 3. It appears that for the 1978–79 season there are significant differences between categories for the characters days to flowering and maturity, seed yield and harvest index. None of the other characters show significant differences between frequency categories. The second two rows of Table 3 give significance levels for an analysis of variance in which the accessions have been classified into three categories, monomorphic fast, polymorphic and monomorphic slow, rather than eleven. A similar pattern of significant results again emerges for 1978–79, yet here biological yield is also significant.

Days to flowering shows a tendency to increase and seed yield to decrease with increasing frequencing of *Aat-1*^{F}. Superimposed on this there is, however, some indication of bimodality with intermediate frequencies of the alleles being associated with higher yield and earlier flowering. A large part of these significant effects may, however, be attributed to category 10, those accessions having the highest frequency of *Aat-1*^{F}. For 1978–79 this category has a low seed and biological yield and harvest index and is late flowering. Similar though non-significant results are obtained for the 1980 season. Seed weight also has lower averages for category 10 in both seasons though the statistical analyses on this character are non-significant.

Correlations between the frequency of *Aat-1*^{F} and the agronomic characters have also been computed over accessions. The results are consistent with those of the analysis of variance. Rather small though significant correlations were obtained with days to flowering (0.17, $P<0.05$), seed yield (−0.22, $P<0.01$), biological yield (−0.18, $P<0.05$) and harvest index (−0.20, $P<0.05$). Correlations with the other characters were smaller and non-significant. A factor analysis using the method of principal components was made on the matrix of correlation coefficients among the agronomic character 1 to 9. The flowering, maturity and yield characters could be made to load heavily on a single factor. However, scores for this factor were found to correlate no more strongly with the frequency of *Aat-1*^{F} than seed yield alone. It was also found that the partial correlation between the frequency of *Aat-1*^{F} and yield, controlling for days to flowering and seed weight both separately and together, was in each case −0.17 ($P<0.01$). Thus a substantial part of the correlation with yield occurs independently of these other characters. The correlation of *Aat-1*^{F} frequency and yield is, however, not independent of geography. It was shown in the previous section through contingency tests that accessions fixed for *Aat-1*^{F} were not distributed at random with respect to country or

Table 3. Significance levels for analysis of agronomic characters 1–9 for 1978–79 and 1979–80 seasons. Levels are given for analysis of all eleven gene frequency categories and for analysis for classification into three (two monomorphic and one polymorphic) categories

		DFL	MAT	HT	PD	SDPD	SDWT	YLD	BYLD	HI
All categories	1978–79	**	***	–	–	–	–	**	–	*
	1979–80	–	–	–	–	–	–	–	–	–
Three categories	1978–79	**	**	–	–	–	–	**	*	**
	1979–80	–	**	–	–	–	–	–	–	–

*** $P<0.001$; ** $P<0.01$; * $P<0.05$; – not significant

Table 4. Percentage of accessions falling into three gene frequency categories for various seed characters and cold tolerance. *N* is number of accessions

	Fixed *Aat-1*^{S}	Polymorphic	Fixed *Aat-*^{F}	*N*
Testa ground colour				
Green	11	33	56	9
Pink	16	59	25	49
Green + pink	25	62	13	132
Brown	20	57	23	90
Polymorphic	24	62	14	142
Monomorphic	18	56	26	154
Pattern on testa				
Absent	23	58	20	160
Polymorphic	19	64	17	90
Monomorphic	19	54	27	48
Testa pattern colour				
Black	14	70	16	57
Olive	23	64	14	22
Grey	12	69	19	26
Brown	29	36	36	14
Polymorphic	32	37	32	19
Monomorphic	17	65	18	119
Cotyledon colour				
Orange/red	22	60	17	98
Yellow	21	58	22	166
Orange/red + yellow	18	62	21	34
Cold tolerance				
1. 100–60% survival	29	55	16	55
2. 60–30% survival	14	64	22	125
3. 30– 0% survival	24	56	20	117

geographic area. It can be seen from Table 2, however, that the accessions fixed for *Aat-1*^{F} are distributed over a majority of countries. Thus their later flowering and maturity and lower yield cannot be linked to their originating from one or a few countries.

Table 4 gives the percentage of accessions in each of the three gene frequency categories, fixed for *Aat-1*^{S}, polymorphic, and fixed for *Aat-1*^{F} for the seed characters scored at ICARDA and for cold tolerance. Taking account of the numbers of accessions scored there are no substantial differences between the different classes for each character. Contingency tests were used to analyse the association between gene frequency categories and the classes of each character. The tests applied are shown in Table 5. Significant results were obtained in comparing polymorphic and monomorphic accessions for testa ground colour and in comparing accessions monomorphic for green or pink with those polymorphic for green and pink. Other tests give non-significant results.

Discussion

The percentages of natural outcrossing in the lentil has been determined in a series of experiments using cotyledon colour as a marker (Wilson and Law 1972). Estimates of between 0.01% and 0.08% were obtained for crosses between plants of different lines. These values are substantially lower than the value of 0.9% obtained here from the analysis of inbreeding statistics. This kind of discrepancy is a very common feature of studies of in-

Table 5. Contingency tests for association of gene frequency categories with seed characters and with cold tolerance classes

Comparison	df	G	*P*
Testa ground colour			
Green, pink, brown	4	4.169	not significant
Green or pink, green + pink	2	7.675	<0.05
Polymorphic, monomorphic	2	6.914	<0.05
Pattern on testa			
Absent, present	2	0.625	not significant
Polymorphic, monomorphic	2	2.161	not significant
Testa pattern colour			
Black, olive, grey, brown	6	6.952	not significant
Polymorphic, monomorphic	2	5.226	not significant
Cotyledon colour			
Orange/red, yellow	2	0.761	not significant
Orange/red or yellow, orange/red + yellow	2	0.244	not significant
Cold tolerance			
1, 2, 3	4	6.287	not significant

breeding plants (Brown 1979), and the most probably explanation is that selection in favour of heterozygotes is increasing their frequency above that predicted from the theory of inbreeding of neutral genes. The selection might be operating directly on the allozyme locus or on linked heterozygous loci or heterozygous chromosome segments.

Selective effects of this sort have been proposed in a number of studies (Marshall and Allard 1970; Brown et al. 1974; Rick et al. 1977). Explanation of the heterozygote excess in the lentil awaits comparative studies on fitness components of the different allozyme genotypes. Barulina (1930) mapped the geographic distribution of botanical varieties or grex of *Lens culinaris* (see also Cubero 1981). The grex represent associations of characters found in the crops distribution. Lower $Aat\text{-}1^F$ frequencies may tie in to a certain extent with the occurrence of the eastern grex *pilosae* and *asiaticae* with higher frequency being characteristic of the Europe botanical varieties. The interpretation of the pattern of geographic variation in terms of large scale clines should, however, be regarded with caution in view of the substantial differences in allele frequency between accessions within countries and between countries within geographic areas. The pattern of variation probably reflects both the evolutionary history of the species (Cubero 1981) and adaptation of land races to local environmental factors.

In South America the post-Columban introduction of lentils was first to Chile and from there to Argentina and Peru. In central America a much earlier phenology is found and material from Chile is ill-adapted. The large difference in $Aat\text{-}1^F$ frequency between Central and South America is consistent with there being a separate introduction to Central America. The source of this introduction cannot be deduced from the gene frequency data available at present.

Brown (1979) uses the ratio $F_{ST}/(1-F_{ST})$, which is the amount of genetic variation between subdivisions of a population divided by the amount of genetic variation within subdivisions, as a comparative measure of genetic differentiation. In this study the ratio has a value of 0.314/(1–0.314) or 46%. Brown (1979) gives values of the ratio of $F_{ST}/(1-F_{ST})$ for a number of outbreeding and inbreeding plants. The averages are 20% for the former and 106% for the latter, reflecting greater geographic differentiation in inbreeding plants. The value of 47% obtained here is rather low but within the range (from 5% to 550%) of values listed for inbreeders. The maximum value of $F_{ST}/(1-F_{ST})$ that could have been obtained in this study is about 100%, had all accessions been fixed for one or other allele. The lower value therefore reflects the large number of polymorphic accessions. Of interest in this respect is the comparison with the results of Kahler and Allard (1981) who reported on allozyme variation in 1506 barley accessions. Scoring four or more plants per accession an average of 6% polymorphic accessions per locus were found. In this study 176/298 or 59% polymorphic accessions were found. A correction can be made based on the binomial theorem to give the expected number of accessions that should have been detected as polymorphic had four plants rather than ten been assayed. This works out at around 30%. The high value for the lentil is possibly a reflection of the high proportion of land race material. It is interesting, however, that 15% of polymorphic accessions were obtained for wild barley (*Hordeum spontaneum*) which is still much lower than 30% obtained here. Unfortunately it is difficult to make judgements about the role of selection in maintaining diversity within accessions without knowledge of the extent and patterns of gene flow between the populations which the accessions represent.

When large scale geographic surveys are made of allozyme variation, the expectation, or hope, is that the variation will become correlated with taxonomic groupings previously defined on the basis of morphological or other characters. In this way the allozyme variation can serve a useful purpose in confirming or refining taxonomic boundaries whether or not, differences have arisen by drift or selection. It is interesting, therefore, that the $Aat\text{-}1^F$ polymorphism is largely uncorrelated with seed colours and patterns, and with seed size, characters important in defining the grex varieties of Barulina. Seed size in particular has also been used to define subspecific categories microsperma and macrosperma (see Cubero 1981). With this in mind the evidence of significant correlations with reproductive characters flowering, maturity and yield, the widespread nature of the *Aat-1* polymorphism, the apparent excess of heterozygotes over neutral inbreeding expectations all point to the polymorphism being affected and perhaps maintained by selection of some kind. The apparent yield advantage of accessions which are polymorphic or where $Aat\text{-}1^S$ predominates is quite substantial and may prove to be of some practical benefit to lentil breeders. It is, however, essential to examine the yield characters of individual plants of the different genotypes in similar genetic backgrounds before any firm conclusions can be reached.

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