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

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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-1*F and *Aat-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-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-
lecion 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 in-breeding 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 phenoxethanol 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 height (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 un lodged 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 = 0–0% 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 occurred on 18th September. Fifty kg of P2O5 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/m² and 4 m² 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-1 F) and the other slow migrating (Aat-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-1 F /Aat-1 F (1,495 plants), Aat-1 F /Aat-1 S (18 plants) and Aat-1 S /Aat-1 S (1,450 plants). This gives overall allele frequencies for Aat-1 F and Aat-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-1 F. The categories 0 to 10 represent accessions containing from zero to ten Aat-1 F/Aat-1 F plants. Accessions with heterozygotes or less than 10 plants were assigned to the