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Phenotypic polymorphism of six enzymes in the grasspea (*Lathyrus sativus* L.)

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Summary

The phenotype variation in six enzymes, 6-phosphogluconate dehydrogenase (6-PGD), malate dehydrogenase (MDH), peroxidase (PRX), isocitrate dehydrogenase (IDH), galactose dehydrogenase (GD) and glutamate oxaloacetate transaminase (GOT), was investigated using horizontal starch gel electrophoresis in 52 accessions of the grasspea, *Lathyrus sativus*. Phenotypic polymorphism was observed for all six enzymes. High phenotypic polymorphism (Pj) was observed for PRX and 6-PGD, while there was little polymorphism for GOT, with only two accessions showing variation. There was no correlation between phenotypic polymorphism and region of origin, or groupings of accessions made on the basis of flower colour. Tentative genetic interpretations of banding patterns are given for five of the enzyme systems. The level of apparent heterozygosity was higher than expected in this predominantly autogamous species. The level of variation in the grasspea is discussed in terms of its potential for exploitation through plant breeding.

Introduction

Allozyme markers are often used to investigate systematic problems or to measure levels of variation within and among populations (Hamrick & Godt, 1990). Furthermore an understanding of the way genetic variation is partitioned among populations is of primary importance for the conservation of genetic diversity of plant species. Few studies on the grasspea, *Lathyrus sativus* L., a minor pulse crop grown principally in the Indian subcontinent and also in North Africa and other parts of the Mediterranean basin, have been carried out to evaluate its variation patterns. On the basis of morphological characters, Jackson & Yunus (1984) showed that the grasspea is differentiated into several distinct forms, primarily on the basis of flower colour, seed size and size of leaves.

Genetic analysis of isozymes has been carried out in genera of the tribe Vicieae. The garden pea (*Pisum sativum*) has become an important model for isozyme studies (Przybylska et al., 1982; Weeden, 1985; Parzysz et al., 1986; Weeden & Marx, 1987; Weeden, 1988). Other studies have been carried out with *Lens* (Zamir & Ladizinsky, 1984; Pinkas et al., 1985; Hoffman et al., 1986; Tadmor et al., 1987) and *Vicia* (Yamamoto & Plitmann, 1980; Mora et al., 1983; Suso & Moreno, 1986; Mancini et al., 1989). Only one study of isozyme diversity in *Lathyrus* has been published by Yamamoto et al. (1986).

The gene pools of *L. sativus* (sensu Harlan & De

Wet, 1971) were described by Yunus & Jackson (1991). It is clear that the primary gene pool of *L. sativus* is extensive, including both primitive landrace forms as well as more advanced varieties, even though formal plant breeding appears to have had little impact on the development of this cultigen. The wild progenitor of *L. sativus* has not yet been identified with any certainty. The secondary gene pool is rather limited, comprising two species, *L. amphicarpos* and *L. cicera*. All other *Lathyrus* species can be placed in the tertiary gene pool. Future improvement of the grasspea will consequently be directed at exploitation of the primary gene pool. Given the variation in morphological characters in this cultigen, a study of isozyme variation may be considered as a useful adjunct to more conventional studies of plant morphology.

In this paper we report the study of isozyme diversity in 52 accessions of *L. sativus* representing its wide geographical range. The analysis of isozyme phenotypic polymorphism is a useful initial step for identifying genetic variation in plant species, even when the genetic control of banding patterns has not been determined. Since the genetic basis of many isozymes has been reported in many plant species, including those in several closely-related genera of the tribe Vicieae, a tentative genetic interpretation of isozyme patterns in the grasspea can be made by extrapolation.

Materials and methods

Isozyme analysis was carried out on 52 accessions of *L. sativus* (Table 1), the majority of which were studied for morphological variation by Jackson & Yunus (1984). The accessions were obtained from botanic gardens and research institutes, and represent the geographic range of this cultigen, from Portugal to Afghanistan and India, as well as in-

Table 1. The geographic origin and flower colour of the *L. sativus* accessions studied for isozyme diversity

Acc. no.	Flower colour	Provenance	Acc. No.	Flower colour	Provenance
404	Mixed	Spain	479	Mixed	USSR (Georgian SSR)
406	Mixed	Spain	480	Blue	USSR (Tajik SSR)
428	Mixed	Turkey	481	White	USSR (Ukrainian SSR)
429	Blue	Turkey	484	White	USSR (Krasnodar Territory)
432	White	Portugal	485	White	USSR (Kuibyshev region)
433	Mixed	Hungary	488	Mixed	Bulgaria
434	Mixed	Hungary	489	White	Czechoslovakia
435	White	USSR (Tambor region)	491	Blue	India
438	Blue	India	492	Mixed	Czechoslovakia
439	Blue	India	494	Blue	USSR (Azerbaijan SSR)
441	Blue	India	495	White	USSR (Orel region)
442	White	USSR (Voronezh region)	498	White	USSR (Kuibyshev region)
444	White	USSR (Georgian SSR)	499	Blue	Australia
448	Blue	USSR (Tajik SSR)	502	Mixed	USSR (Krasnodar territory)
452	Blue	USSR (Azerbaijan SSR)	504	White	Australia
454	Blue	Turkey	506	Blue	Afghanistan
455	Blue	Afghanistan	507	White	Chile
459	White	USSR (Mordovian SSR)	556	White	India
466	Mixed	USSR (Azerbaijan SSR)	558	Blue	India
467	White	USSR (Orenburg region)	565	Blue	Tunisia
468	Blue	France	567	White	Crete
472	Mixed	USSR (Moldavian SSR)	580	Blue	Iran
473	Mixed	Bulgaria	585	Blue	Iran
474	White	USSR (Tatar SSR)	586	Blue	Israel
475	White	USSR (Tambor region)	587	Mixed	Crete
476	Blue	Turkey	588	White	Tunisia

cluding three accessions from Australia and Chile. They also encompassed the range of flower colours, namely white, blue, and blue and white, described by Jackson & Yunus (1984).

Ten seedlings per accession were studied. Leaf samples were taken from 3–4 week old seedlings grown in a glasshouse between March and August 1989. Only the youngest leaves were used. Crude extracts were prepared by macerating leaves in 50 μl of extraction buffer (0.1 M Tris-HCl pH 7.2 with 13 mM (0.1% v/v) 2-mercaptoethanol). The perspex extraction trays were kept on crushed ice during maceration to prevent denaturation of the enzymes. Extracts were absorbed on to wicks made from Whatman No. 3MM chromatography paper. Horizontal electrophoresis was carried out in 12% starch gels. Two buffer systems were used: Buffer System A 0.005 M L-histidine monohydrochloride pH 7.0 (gel buffer), and 0.2 M trisodium citrate pH 7.0 (electrode buffer) (Nickerson-Zwaanesse, B.V., unpublished); Buffer System B 0.009 M Tris and 0.003 M citric acid pH 7.0 (gel buffer), and 0.135 M Tris and 0.043 M citric acid pH 7.0 (electrode buffer) (Shaw & Prasad, 1970). Thirty samples were run on each gel, plus two wicks dyed with bromophenol blue to act as a marker control. Reference extracts from the *L. sativus* accession 476 were run on each gel for comparison.

Electrophoresis was carried out at 4° C with a constant current of 30 mA until the tracker dye moved about 1 cm from the origin, after which the wicks were removed and the current reduced to 25 mA. Each electrophoretic separation took approximately 6 hours for both the buffer systems used.

Six enzymes were selected for detailed analysis after a preliminary survey of 13 enzymes, since they gave consistent results with this species. The six enzymes assayed were 6-phosphogluconate dehydrogenase (6-PGD), malate dehydrogenase (MDH-NAD) and peroxidase (PRX) with Buffer System A, and isocitrate dehydrogenase (IDH-NADP), glutamate oxaloacetate transaminase (GOT) and galactose dehydrogenase (GD) with Buffer System B.

Each gel was cut into four slices. The top slice was discarded since most enzymes did not stain well in this slice. For Buffer System A, the second slice was used for 6-PGD, and the third for MDH. The second slice of the cathodal strip was used for PRX. For Buffer System B, IDH was stained in the second slice, GD in the third and GOT in the fourth. Stain recipes were obtained from Shaw & Prasad (1970) for PRX and modified for 6-PGD (0.5 M Tris-HCl pH 7.1 5 ml, distilled water 45 ml, 6-phosphogluconic acid Na_3 10 mg, NADP 5 mg, NBT 10 mg, PMS 2 mg), from Tanksley & Orton (1983) for GD and GOT, and from Wendel & Weeden (1990) for MDH. For IDH, the following recipe from Nickerson-Zwaanesse, B.V. (unpublished) was used: 0.1 M-Tris-HCl pH 7.5 50 ml, 1 M $MgCl_2$ 0.5 ml, DL-isocitric acid 50 mg, NADP 8 mg, NBT 10 mg, PMS 2 mg. Variation in banding patterns was determined by the migration from the origin towards the anode, or cathode for PRX, and the width of the bands.

An assessment of isozyme phenotypic polymorphism was made using the overall banding patterns. Phenotypic polymorphism (Pj) and weighted phenotypic polymorphism (P) were calculated according to Kahler et al. (1980). A tentative genetic interpretation of the banding patterns was made, based on the reported structure of each enzyme in different plant species (Weeden & Wendel, 1990), and particularly in related genera such as *Pisum, Lens* and *Vicia,* where the information was available. *L. sativus* is a diploid species (2n = 14).

Results

All observed phenotypes for the six enzymes studied are shown diagrammatically in Fig. 1, and the number of accessions and frequencies in the total number of plants in which they were observed are given in Table 2.

6-phosphogluconate dehydrogenase (6-PGD, EC 1.1.1.44). Sixteen phenotypes were observed for this enzyme. Phenotype 1 with two bands was the most common, and observed in all accessions except 565 from Tunisia. In three accessions, 556 and 558 from India and 580 from Iran, all 10 plants had phenotype 1. Phenotypes 2, 3, 5 and 6 were observ-

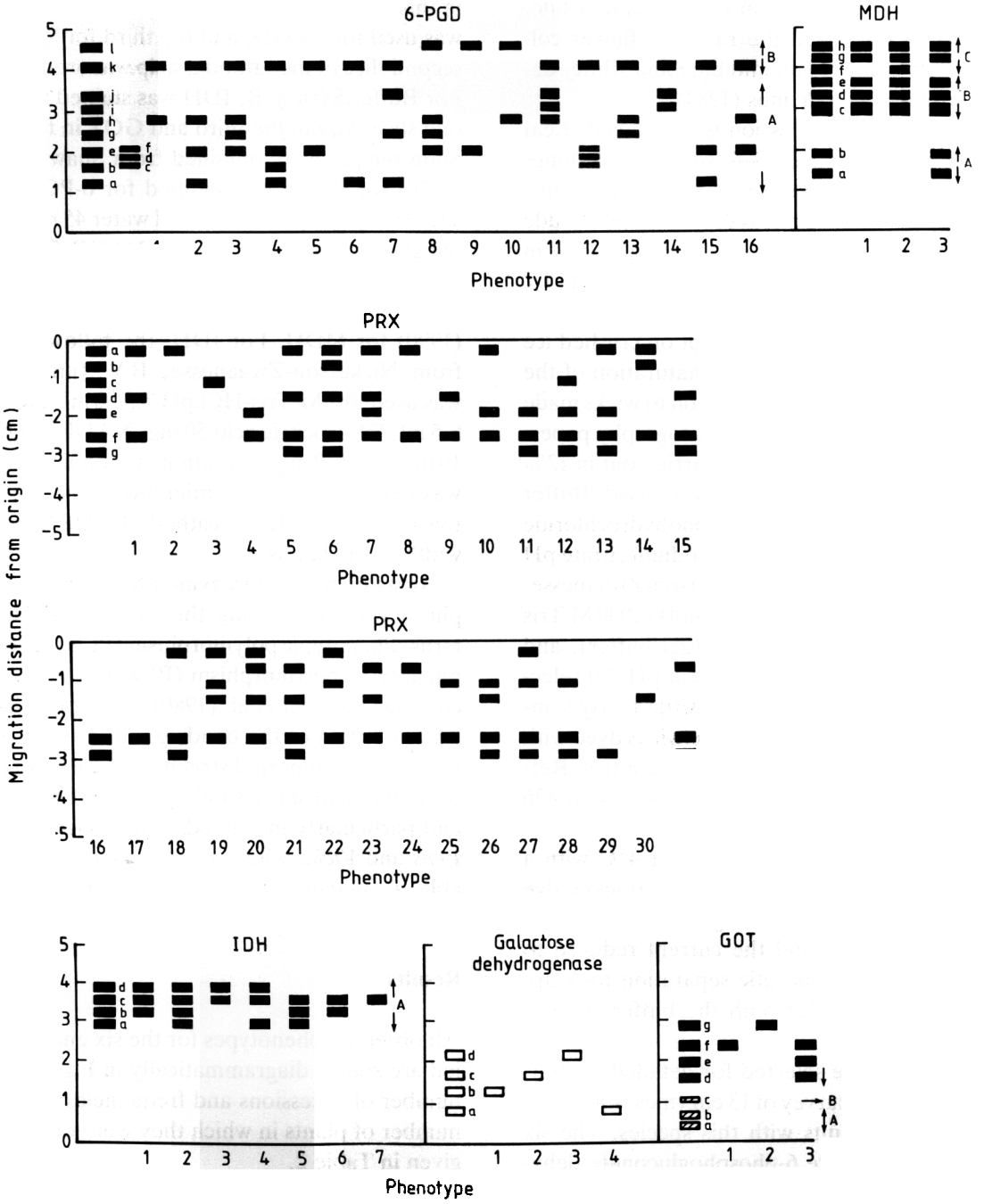

Fig. 1. Phenotypes of six isozymes from 52 accessions of the grasspea, *Lathyrus sativus*.

ed in more than 30% of the accessions, and phenotype 15 in 13% of the accessions. The remaining 10 phenotypes were less common in less than 6% of the accessions. Of these phenotypes, 7, 11, 13 and 14 were found in accessions only from the Soviet Union. Phenotypes 4 and 16 were seen in three and two accessions respectively from widely separated geographic regions.

The isozymes of 6-PGD have been reported as dimers in castor bean (Simcox & Dennis, 1978) and in spinach (Schnarrenberger et al., 1973). The two distinct zones of activity in *L. sativus* can be interpreted as two loci, A and B. At locus B a single band representing one of two alleles was present, and one of these was seen in nearly 98% of all plants. At locus A the patterns can be interpreted as several alleles and both single and multiple bands were present. Almost 58% of plants were homozygous at locus A. However, almost 40% of plants showed three bands in this zone, and can be interpreted as heterozygous for this locus. In four phenotypes (13–16) only two bands were seen in this zone. There are several explanations for this phenomenon. Such a pattern may represent homozygosity with gene duplication (Gottlieb, 1982), or heterozygosity with a null allele (Hvid & Nielson, 1977). Another possibility is co-migration of bands, but this and other explanations can be verified only by formal genetic analysis.

Malate dehydrogenase (NAD) (MDH, EC 1.1.1.37). There were only three phenotypes, and phenotype 1 with five bands was represented in all accessions, and almost 98% of plants studied. Phenotype 2 was found only in eight accessions, and in no more than two plants per accession. Phenotype 3 was rare and

Table 2. The frequency of isozyme phenotypes for 6 enzymes in 52 accessions and individuals of *L. sativus* (n = 520)

Phenotype number	GOT		MDH		GD		IDH		6-PGD		PRX	
	Accs.	% popn.	Accs.	% popn.	Accs.	% popn.	Accs.	% popn.	Accs.	% popn.	Accs.	% popn.
	52	99.4	52	97.9	45	74.0	52	80.0	51	57.7	45	30.2
2	2	<1.0	10	1.9	18	17.5	11	7.1	29	11.0	3	<1.0
3	1	<1.0	1	<1.0	3	1.2	25	11.3	17	6.2	3	<1.0
4					9	7.3	3	<1.0	3	1.1	1	1.2
5							1	<1.0	20	8.5	23	7.3
6							2	<1.0	23	8.0	6	1.2
7							1	<1.0	1	<1.0	2	<1.0
8									1	<1.0	22	8.1
9									1	<1.0	30	13.8
10									1	<1.0	4	<1.0
11									2	<1.0	2	<1.0
12									1	<1.0	1	<1.0
13									3	<1.0	2	<1.0
14									1	<1.0	4	<1.0
15									7	2.7	9	2.5
16									2	<1.0	8	1.9
17											12	4.4
18											8	1.7
19											4	<1.0
20											12	3.5
21											7	1.5
22											11	3.7
23											17	6.2
24											11	2.1
25											8	2.1
26											1	<1.0
27											7	1.5
28											1	<1.0
29											2	<1.0
30											1	<1.0
31											2	<1.0

only observed in one plant from accession 504 from Australia. There were three zones of enzymic activity. The zone nearest to the origin generally had diffuse bands, but in one plant only two clear bands were observed (phenotype 3). The other two zones were close together but were usually separated as shown in phenotype 1, where the zone nearest to the anode consisted of two bands, and the other had three bands. Occasionally, a single band was detected between these two zones (phenotype 2).

In MDH (NAD) both three and four dimeric loci have been reported in maize (Goodman et al., 1980). In *L. sativus* the banding patterns can be interpreted as three polymorphic loci. Phenotype 1 was the most common with two bands at locus C and three bands at locus B. The latter locus certainly shows heterozygosity. Another explanation of the multiple bands could be gene duplication, since this phenomenon has been reported for MDH (Gottlieb, 1982).

Cathodal peroxidase (PRX, EC 1.11.1.7). Peroxidase exhibits both anodal and cathodal bands, but only the cathodal region was analysed because anodal bands were diffuse and difficult to interpret. In *L. sativus*, PRX banding patterns were complex with a total of seven bands, but the number of bands in a phenotype ranged from one to five. The complex banding patterns were represented by 31 phenotypes. The maximum number of phenotypes in a single accession was eight, and the minimum was two. Phenotypes 1, 5, 8, 9 and 23 were common, observed in more than 30% of the accessions. Fifteen phenotypes were present in fewer than 10% of the plants studied, and 14 were found in fewer than 1% of the plants.

Weeden (1985) and Weeden & Marx (1987) reported a similar complexity in the dimeric peroxidase system in *P. sativum* which has four isozyme loci. Due to the complexity of the banding patterns in *L. sativus* it was not possible with any confidence to make a genetic interpretation.

Isocitrate dehydrogenase (NADP) (IDH, EC 1.1.1.42). The banding pattern of IDH (NADP) was clearly seen in one well-defined zone but with a faint and diffuse stained region closer to the origin. A maximum of four bands was observed and seven phenotypes scored. Phenotype 1 was the most common and seen in 80% of all plants; 19 accessions (37%) had only phenotype 1. Phenotypes 2 and 3 were less common, represented in 11 and 25 accessions respectively, and in 7% and 11% of all plants. The rare phenotype 7 was seen in accession 473 from Bulgaria.

A single isozyme locus and dimeric structure for IDH were reported in *Pisum sativum* (Weeden, 1985; Ni et al., 1987). Using these reports as models, the patterns seen in *L. sativus* appear to represent a single locus, but it is difficult to suggest what the diffusely stained region might represent, since in *Beta,* for instance, it sometimes shows three bands or diffuse staining (Adel Sabir, personal communication). The multiple bands seen in six of the phenotypes indicate heterozygosity, yet the presence of two and four bands in phenotypes 3 and 6, and 2, respectively, are difficult to explain. Only one plant from a total of 520 studied (0.2%) had phenotype 7 with a single band, which can be interpreted as a homozygous locus.

Galactose dehydrogenase (GD, EC 1.1.1.48). The staining method for this enzyme exhibits achromatic bands. Only one zone of activity was observed, and the bands can be interpreted as four alleles at the same homozygous locus. Phenotype 1 was the most common, in 74% of plants studied, and observed in all but five accessions (455, 481, 485, 499 and 567). Almost 50% of the accessions had only phenotype 1. There were 18 accessions with phenotype 2, but only three accessions (455, 506 and 585) with phenotype 3. Phenotype 4 occurred in nine accessions, and in all 10 plants from the Australian accession 499. This is one of the least frequently studied enzymes in plant species. Given the single band patterns observed, this enzyme could be either a monomer or a dimer in *L. sativus.*

Glutamata oxaloacetate transaminase (GOT, EC 2.6.1.1). Most of the plants analysed showed one darkly stained zone with another two zones near to the origin. Since these showed little enzymic activity they could not be analysed consistently. Three phenotypes were observed and the single band of

Table 3. Phenotypic polymorphism (Pj) and weighted polymorphism (P) for 6 enzymes

Accession	6-PGD	MDH	PRX	IDH	GD	GOT	Average Pj	Average P
404	0.32	0.00	0.82	0.42	0.00	0.00	0.26	0.09
406	0.58	0.00	0.76	0.00	0.00	0.00	0.22	0.05
428	0.54	0.18	0.32	0.00	0.00	0.00	0.17	0.09
429	0.46	0.18	0.80	0.32	0.50	0.34	0.43	0.35
432	0.18	0.00	0.46	0.34	0.00	0.00	0.16	0.06
433	0.46	0.00	0.60	0.34	0.18	0.00	0.26	0.12
434	0.56	0.00	0.76	0.00	0.00	0.00	0.22	0.05
435	0.50	0.00	0.18	0.32	0.48	0.00	0.25	0.18
438	0.76	0.00	0.76	0.00	0.00	0.00	0.25	0.06
439	0.54	0.00	0.68	0.48	0.48	0.00	0.36	0.21
441	0.62	0.00	0.76	0.48	0.42	0.00	0.33	0.19
442	0.58	0.00	0.86	0.48	0.00	0.00	0.32	0.13
444	0.62	0.00	0.82	0.00	0.00	0.00	0.24	0.06
448	0.18	0.00	0.60	0.42	0.00	0.00	0.20	0.08
452	0.74	0.00	0.76	0.00	0.32	0.00	0.30	0.13
454	0.66	0.00	0.86	0.18	0.00	0.00	0.28	0.08
455	0.32	0.00	0.84	0.54	0.32	0.00	0.34	0.18
459	0.42	0.00	0.80	0.18	0.34	0.00	0.29	0.14
466	0.32	0.00	0.70	0.00	0.00	0.00	0.17	0.03
467	0.46	0.00	0.80	0.18	0.00	0.00	0.24	0.07
468	0.46	0.00	0.70	0.00	0.00	0.00	0.19	0.04
472	0.64	0.18	0.82	0.00	0.00	0.00	0.27	0.11
473	0.48	0.00	0.82	0.58	0.48	0.00	0.39	0.22
474	0.48	0.00	0.72	0.58	0.00	0.00	0.30	0.12
475	0.72	0.00	0.68	0.00	0.00	0.00	0.23	0.06
476	0.34	0.18	0.54	0.18	0.18	0.00	0.24	0.15
479	0.62	0.18	0.78	0.56	0.18	0.00	0.39	0.22
480	0.58	0.00	0.46	0.18	0.00	0.00	0.20	0.07
481	0.66	0.00	0.82	0.42	0.00	0.00	0.32	0.11
484	0.46	0.00	0.58	0.42	0.48	0.00	0.32	0.20
485	0.70	0.00	0.80	0.32	0.00	0.00	0.30	0.10
488	0.46	0.18	0.76	0.18	0.50	0.00	0.65	0.23
489	0.56	0.32	0.54	0.18	0.42	0.00	0.34	0.25
491	0.46	0.00	0.48	0.32	0.42	0.00	0.33	0.18
492	0.74	0.00	0.78	0.00	0.54	0.00	0.34	0.18
494	0.48	0.00	0.82	0.54	0.00	0.00	0.31	0.12
495	0.66	0.00	0.68	0.00	0.00	0.00	0.22	0.05
498	0.66	0.00	0.58	0.56	0.42	0.00	0.37	0.21
499	0.32	0.00	0.68	0.18	0.00	0.00	0.20	0.06
502	0.34	0.00	0.86	0.00	0.00	0.00	0.20	0.04
504	0.58	0.18	0.72	0.00	0.00	0.00	0.25	0.10
506	0.54	0.00	0.58	0.18	0.56	0.18	0.34	0.24
507	0.78	0.00	0.46	0.18	0.00	0.00	0.24	0.08
556	0.00	0.00	0.54	0.18	0.00	0.00	0.12	0.04
558	0.00	0.00	0.48	0.00	0.00	0.00	0.08	0.01
565	0.76	0.00	0.66	0.00	0.42	0.00	0.31	0.15
567	0.70	0.32	0.72	0.42	0.00	0.00	0.36	0.20
580	0.00	0.00	0.56	0.00	0.00	0.00	0.09	0.02
585	0.66	0.00	0.84	0.46	0.62	0.00	0.43	0.25
586	0.74	0.00	0.45	0.48	0.00	0.00	0.28	0.11
587	0.58	0.00	0.78	0.00	0.48	0.00	0.31	0.16
588	0.58	0.00	0.86	0.00	0.00	0.00	0.24	0.06
Mean	0.51	0.04	0.69	0.23	0.17	0.01		

Table 4. Phenotypic polymorphism (Pj) for 6 enzymes in 52 accessions of *L. sativus* grouped according to geographical origin

Region	No. accs	6-PGD	MDH	PRX	IDH	GD	GOT	Average Pj	Average P
W. & S.W. Europe	4	0.39	0.00	0.69	0.19	0.00	0.00	0.21	0.06
C. & S. Europe	8	0.57	0.10	0.72	0.21	0.33	0.00	0.32	0.18
USSR	20	0.54	0.12	0.71	0.26	0.11	0.00	0.27	0.09
W. Asia	9	0.47	0.06	0.64	0.26	0.40	0.06	0.30	0.18
Tunisia	2	0.67	0.00	0.76	0.00	0.21	0.00	0.27	0.10
India	6	0.35	0.00	0.67	0.24	0.22	0.00	0.25	0.12
Chile		0.78	0.00	0.46	0.18	0.00	0.00	0.24	0.18
Australia	2	0.45	0.09	0.70	0.09	0.00	0.00	0.22	0.08

phenotype 1 was seen in over 99% of plants. This was the least variable enzyme studied, with only two accessions (429 and 506) showing variation. Accession 429 from Turkey had all three phenotypes, whereas 506 had the single-banded phenotypes 1 and 2.

In *Pisum sativum*, four loci are present (Weeden, 1985), but in *Vicia faba* (Mancini et al., 1989) and *Lens culinaris* (Zamir & Ladizinsky, 1984) only three GOT loci have been observed. In spinach and pea, the enzyme is a dimer (Huang et al., 1986; Weeden & Marx, 1987). In *L. sativus,* three putative loci were detected, and at locus C where analysis was possible, there were apparently three alleles. Only one plant was heterozygous, and all other plants were homozygous.

Phenotypic polymorphism. Peroxidase showed the highest average polymorphism (Pj) with a value of 0.69, and all accessions were polymorphic for this enzyme (Table 3). The second highest value was shown by 6-PGD, with a value of 0.51, and 94% of accessions were polymorphic. IDH and galactose dehydrogenase were less polymorphic with values of 0.23 and 0.17 respectively, and 63% and 40% of accessions were polymorphic for these enzymes. For MDH only 17% of accessions were polymorphic, with an average value of 0.04, and for GOT the value was even lower at 0.01, represented by only 4% of the accessions. Among the accessions, the weighted average polymorphism (P) ranged from 0.01 (accession 558 from India which was polymorphic only for PRX) to 0.35 (accession 429 from Turkey which was the only one polymorphic for all six enzyme systems). Only one other accession, 580 from Iran was polymorphic for a single enzyme, PRX, with an average Pj of 0.09, and P of 0.02. Grouping accessions according to geographical areas of origin (Table 4) or according to flower colour forms (Table 5) as indicated by Jackson & Yunus (1984), showed no significant differences between average polymorphism or the weighted average.

Discussion

The grasspea, *L. sativus,* is a morphologically variable species, based on an analysis of floral and vegetative characters (Jackson & Yunus, 1984). The isozyme data presented here also show considerable polymorphism although there is no apparent

Table 5. Phenotypic polymorphism (Pj) for 6 enzymes in 52 accessions of *L. sativus* grouped according to flower colour

Flower colour	No. accessions	6-PGD	MDH	PRX	IDH	GD	GOT	Average Pj	Average P
White	19	0.54	0.04	0.66	0.25	0.11	0.00	0.27	0.12
Blue	20	0.47	0.02	0.68	0.25	0.21	0.03	0.28	0.13
Mixed blue and white	13	0.51	0.06	0.74	0.16	0.18	0.00	0.27	0.13

correlation with morphology. Furthermore the isozyme variation cannot be interpreted on a geographical basis.

Although we did not carry out formal genetic analyses of these isozyme banding patterns, analogy with similar systems in closely related genera such as *Pisum, Lens* or *Vicia* and other plant species had enabled us to speculate on the extent of genetic variation at particular isozyme loci. Even though our interpretations must be regarded as tentative until further analyses are undertaken by study of progenies, it is clear that the banding patterns, which we have observed, do represent allelic variation at several loci.

The important feature to emerge from this study is that *L. sativus,* an autogamous species as we have noted during our own experimental work, is variable with regard to four of the enzymes studied, and several of the hypothesized loci appear to be heterozygous. The frequency of putative heterozygous loci is greater than we would have expected given our knowledge of the breeding system of *L. sativus,* and by analogy with the breeding systems of other grain legumes in which there is a high frequency of autogamy. Differences in terms of alleles or allelic frequencies might be expected on a geographic basis, on a large or small scale, since in other species correlations between isozyme phenotypes and environment have been reported in *Avena barbata* (Kahler et al., 1980) and *Triticum dicoccoides* in Israel (Nevo et al., 1982). Furthermore, allozyme variation in *T. dicoccoides* is correlated with spikelet variation, even on a mosaic pattern between collecting sites.

A high level of phenotypic polymorphism typifies some *L. sativus* accessions. Given the fact that the secondary gene pool of this species is rather limited, and utilization of the tertiary gene pool is difficult because hybridization is difficult, it is apparent to us that exploitation of the primary gene pool of *L. sativus* is the most appropriate strategy to employ for the improvement of this cultigen. If the level of apparent heterozygosity in *L. sativus* which we have detected is a reflection of wider heterozygosity in this species, then crosses between different forms are likely to lead to the release of a considerable amount of hidden variation. It might then be possible to make selections for forms with lower concentrations of the neurotoxin, β-N-oxalyl-L-α,β-diaminopropionic acid or ODAP, which causes neuro-lathyrism, or indeed to select non-toxic forms which might allow wider utilization of this minor pulse. Given its agricultural importance in India and in several other countries, and given that with increasing stresses being placed on the agricultural environment world wide now and in the future (Jackson et al., 1990), the grasspea clearly has the potential to increase in importance as a grain legume. What is needed is a concerted breeding effort to exploit the variation which we have demonstrated exists in this underexploited crop genetic resource.

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