ISSN 1479-2621

Plant Genetic Resources **4**(3); 159–171
DOI: 10.1079/PGR2006115

High genetic diversity in a world-wide collection of *Lathyrus sativus* L. revealed by isozymatic analysis

José Francisco Gutiérrez-Marcos*, Francisca Vaquero, Luis Enrique Sáenz de Miera and Francisco Javier Vences

Area de Genetica, Facultad de Biologia, Universidad de Leon, Campus Vegazana, Leon 24071, Spain

Received 16 September 2005; Accepted 6 February 2006

Abstract

Grasspea (*Lathyrus sativus* L.) is an annual, herbaceous, drought-resistant legume and staple crop in Asian and African countries. Little is known about the nature and the amount of genetic diversity present in existing grasspea seed collections, yet this information is pivotal for future breeding programmes, such as those striving to reduce high neurotoxin levels present in seeds. Here we report on the level of genetic diversity within a world-wide collection of *L. sativus*, determined by isozymatic analysis. Although grasspea is generally considered a predominantly self-pollinating species, we found that the population genetic structure of these accessions showed a considerable outcrossing rate of 36%. The identification of a mixed mating system in *L. sativus* has significant implications for collecting and multiplying genetic resources for conservation and for future breeding purposes. In addition, we determined the genetic closeness of grasspea accessions from different geographical regions around the world. While we noticed an allelic richness in this species that was conserved across the regions, we did not find any evidence of high genetic identity between accessions, even when originating from the same geographical location. Instead, we found that greater genetic variability existed at the intra-regional level than at the inter-regional level.

Keywords: Genetic resources; genetic variability; grasspea; isozymes; *Lathyrus sativus*; mating system

Introduction

Grasspea or *Lathyrus sativus* L. (also referred to as vetchling, khesari or chickling vetch) is an annual, herbaceous legume of the family *Fabaceae*. *L. sativus* has been traditionally considered a cultivated form of its genetically closest wild species, *L. cicera* (Hopf, 1986). The principal centre for the domestication of *L. sativus* is thought to have been the Balkan Peninsula, due to the Near East agricultural expansion into the Mediterranean basin and from there throughout the world (Kislev, 1989). Currently, grasspea is widely grown as a winter season crop in several South-East Asian countries (India, Bangladesh, Pakistan and Nepal) and East African countries (Egypt, Somalia, Sudan and Ethiopia), where it is cultivated for livestock and human consumption (Zohary and Hopf, 1988). Grasspea is an attractive crop in these countries because of its tolerance to both drought and excessive rainfall (McCutchan, 2003). This is mainly due to its very robust and penetrating root system, which allows cultivation on a variety of soil types, ranging from very poor soils to heavy clays. The robust growth of grasspea, together with its ability to fix atmospheric nitrogen, makes this species highly suitable for

* Corresponding author: Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK.
E-mail: jose.gutierrez@plant-sciences.oxford.ac.uk

cultivation under very adverse climatic and agronomical conditions (Campbell, 1997).

One of the major disadvantages of the cultivation of this species is that its excessive consumption causes lathyrism, a neurodegenerative disorder attributed to the presence of a glutamate analogue neurotoxin in the seeds (Getahun *et al.*, 2003). The presence of neurotoxin in grasspea seeds could be reduced or eliminated through breeding programmes. A vital component of all breeding programmes is the awareness of the genetic diversity and of genetic resources for any given species. Marker-mediated characterization of germplasms can reveal variations in accessions. This not only helps germplasm managers, but also provides useful information that can be used to enhance the efficiency of a breeding programme (Farooq and Azam, 2002).

The utilization of different genetic markers in population genetic studies has been essential to undertake germplasm collection for *ex situ* conservation and to identify geographic areas with high genetic diversity for *in situ* conservation (Bekele, 1983; Abebe and Bjørnstad, 1996; Seifu, 1997). Unfortunately, the absence of a definitive correlation between different types of markers in previous works (Yunus *et al.*, 1991; Tadesse and Bekele, 2001, 2003) suggests that there is no one suitable marker for genetic diversity studies. Isozymatic analysis by gel electrophoresis has been demonstrated to be a valuable tool as a genetic marker to estimate genetic diversity, and to analyse evolutionary patterns in natural populations. This is mainly because of their variability, codominant expression and absence of epistatic interactions (Brown and Weir, 1983). Furthermore, associations between isozymatic genotypes and environmental factors have been detected (Allard *et al.*, 1993; Pérez de la Vega *et al.*, 1994), as well as those between agronomic traits and isozymatic patterns (Singh, 2001; Singh *et al.*, 1991).

Grasspea has been traditionally considered a predominantly self-pollinating species (Campbell, 1997). As a consequence, most studies on genetic diversity in this species using a range of morphological (De la Rosa and Martín, 2001), isozymatic (Przybylska *et al.*, 1998; Chowdhury and Slinkard, 2000) or molecular markers (Croft *et al.*, 1999), have only been conducted on very small sample sizes. It is however worth noting that cross-pollination by insects has also been reported in grasspea (Rahman *et al.*, 1995). Therefore the genetic diversity estimates for this species may be somewhat underestimated.

We therefore undertook a necessary study to understand better the nature and the extent of genetic diversity present in existing grasspea seed collections. Using isozymatic markers, we report here on the high levels of genetic diversity identified in a world-wide collection of *Lathyrus sativus*. From our findings, it can be deduced that: (i) the seed collections we have examined show a high genetic variability randomly distributed over all geographical regions; (ii) this high level might be related to the substantial levels of cross-pollination detected; and (iii) from this estimated genetic variability, there is no evidence that very high genetic identity exists between accessions.

Material and methods

Plant material

A total of 2987 individuals belonging to 110 different world-wide samples of grasspea *L. sativus* L. ($2n = 2x = 14$) were analysed. Accessions were kindly supplied by several germplasm banks and botanical gardens (listed in Table 1). For each accession, crude extracts from cataphyls of 20-day-old germinated seeds (average number of 27 seeds per accession) were analysed for nine isozymatic systems (14 loci), as described previously (Gutiérrez *et al.*, 2001).

Electrophoretical procedures

Horizontal starch gel electrophoresis was performed for nine different isozymatic systems: aspartate amino transferase (AAT, EC 2.6.1.1), phosphoglucose isomerase (PGI, EC 5.3.1.9), phosphoglucose mutase (PGM, EC 2.7.5.1), peroxidase (PRX, EC 1.11.1.7), leucin aminopeptidase (LAP, EC 3.4.11.1), malate dehydrogenase (MDH, EC 1.1.1.37), esterase (EST, EC 3.1.1.–), alkaline phosphatase (ALP, EC 3.1.3.2) and 6-phosphogluconic dehydrogenase (PGD, EC 1.1.1.44). Electrophoretic and staining procedures were carried out following the methods previously described for these enzymes (Gutiérrez and Vences, 1992; Gutiérrez *et al.*, 1994, 2001). Genotypes of the isozymes were determined genetically by segregation analysis (Gutiérrez and Vences, 1992; Gutiérrez *et al.*, 2001).

Statistical procedures

From the scored genotypes of the 2987 individuals analysed, the Garret software program (L. E. Sáenz de Miera, unpublished) was used to calculate the following genetic diversity parameters: the observed number of alleles for each locus (A); the average number of alleles per locus (A/L) for each accession; the effective number of alleles per locus ($ne = 1/\Sigma x_i^2$, where x_i^2 is the the frequency of the ith allele in each

https://doi.org/10.1079/PGR2006115 Published online by Cambridge University Press

Table 1. Geographical origin of the 110 grasspea accessions, and different variability indices at both accession and regional levels

Accession no.	Name	Origin[a]	A/L	ne	H_I	H_S	%P	F_{IS}
Africa								
PI283570	Africa-Al	Algeria (8)	1.9	1.3	0.112	0.178	57.14	0.371
PI283546	Africa-Eg	Egypt (8)	1.7	1.3	0.093	0.162	57.14	0.426
PI19381	Africa-Et1	Ethiopia (8)	2.1	1.3	0.074	0.172	71.43	0.570
PI193814	Africa-Et2	Ethiopia (8)	1.9	1.4	0.093	0.170	64.29	0.453
PI193815	Africa-Et3	Ethiopia (8)	1.9	1.5	0.119	0.276	71.43	0.569
PI193816	Africa-Et4	Ethiopia (8)	1.9	1.3	0.061	0.164	71.43	0.628
PI195998	Africa-Et5	Ethiopia (8)	2.4	1.5	0.123	0.259	92.86	0.525
PI195999	Africa-Et6	Ethiopia (8)	2.0	1.5	0.168	0.243	71.43	0.309
PI283569	Africa-Li	IIberianPya (8)	1.9	1.4	0.118	0.203	50.00	0.419
MG100291	Africa-Mor1	Morocco (5)	1.9	1.5	0.114	0.244	71.43	0.533
PI283560	Africa-Mor2	Morocco (8)	2.0	1.4	0.074	0.206	71.43	0.641
PI283564	Africa-Sud	Sudan (8)	2.1	1.4	0.148	0.228	71.43	0.351
LSLAT45473	Africa-Tun	Tunisia (3)	1.9	1.4	0.143	0.212	64.29	0.325
S2 S	Africa-Tun	Tunisia (1)	1.9	1.4	0.059	0.230	71.43	0.743
S84 92	Africa-Tun	Tunisia (1)	1.3	1.1	0.011	0.061	21.43	0.820
Average			1.9	1.4	0.104	0.201	65.24	0.512
Pool			3.8	1.8	0.104	0.378[b]	100.00	
Anatolian P.								
S34 85	AnatolianP-Az	Azerbaijan (1)	1.7	1.3	0.114	0.188	50.00	0.394
S32 85	AnatolianP-Geo	Georgia (1)	1.6	1.4	0.105	0.207	42.86	0.493
S97 83	AnatolianP-Tur1	Turkey (1)	1.5	1.3	0.091	0.145	35.71	0.372
TR12621	AnatolianP-Tur2	Turkey (7)	1.9	1.5	0.121	0.258	64.29	0.531
TR15373	AnatolianP-Tur3	Turkey (7)	1.6	1.4	0.091	0.213	57.14	0.573
TR39180	AnatolianP-Tur4	Turkey (7)	1.9	1.4	0.079	0.213	71.43	0.629
TR47649	AnatolianP-Tur5	Turkey (7)	2.4	1.5	0.152	0.258	78.57	0.411
Average			1.8	1.4	0.106	0.207	57.10	0.481
Pool			3.4	1.9	0.106	0.374[b]	92.86	
Asia–Russia								
LSLAT46578	Asia-Russia01	Russia (3)	1.8	1.4	0.083	0.214	57.14	0.612
PI283551	Asia-Russia02	Russia (8)	2.4	1.6	0.155	0.312	85.71	0.503
PI283552	Asia-Russia03	Russia (8)	1.6	1.4	0.071	0.213	42.86	0.667
PI422522	Asia-Russia04	Russia (8)	1.4	1.1	0.051	0.075	28.57	0.320
PI422531	Asia-Russia05	Russia (8)	1.9	1.5	0.097	0.240	57.14	0.596
S28 87	Asia-Russia06	Russia (1)	1.4	1.3	0.127	0.148	35.71	0.142
S33 85	Asia-Russia07	Russia (1)	1.7	1.5	0.121	0.219	50.00	0.447
S35 85	Asia-Russia08	Russia (1)	1.5	1.2	0.050	0.133	42.86	0.624
S36 85	Asia-Russia09	Russia (1)	1.5	1.3	0.133	0.179	42.86	0.257
S37 85	Asia-Russia10	Russia (1)	1.4	1.3	0.121	0.187	42.86	0.353
S39 85	Asia-Russia11	Russia (1)	1.3	1.2	0.048	0.119	28.57	0.597
Average			1.6	1.3	0.098	0.181	46.80	0.469
Pool			3.2	1.7	0.098	0.375[b]	100.00	
South-East Asia								
PI212312	SEAsia-Af1	Afghanistan (8)	1.8	1.5	0.106	0.254	57.14	0.583
PI219923	SEAsia-Af2	Afghanistan (8)	1.7	1.5	0.087	0.233	50.00	0.627
PI317439	SEAsia-Af3	Afghanistan (8)	1.6	1.3	0.087	0.190	57.14	0.542
PI317441	SEAsia-Af4	Afghanistan (8)	1.5	1.2	0.071	0.150	42.86	0.527
LSLAT44079	SEAsia-Ind1	India (3)	1.6	1.4	0.075	0.210	57.14	0.643
PI163293	SEAsia-Ind2	India (8)	1.9	1.3	0.089	0.160	57.14	0.444
PI391430	SEAsia-Ind3	India (8)	1.3	1.1	0.019	0.043	21.43	0.558
PI391431	SEAsia-Ind4	India (8)	1.7	1.4	0.076	0.193	42.86	0.606
PI442801	SEAsia-Ind5	India (8)	1.6	1.3	0.067	0.164	50.00	0.591
PI227450	SEAsia-Ir1	Iran (8)	2.4	1.6	0.097	0.262	64.29	0.630
PI251413	SEAsia-Ir2	Iran (8)	1.4	1.2	0.071	0.114	35.71	0.377
PI218082	SEAsia-Pak	Pakistan (8)	1.4	1.1	0.009	0.043	28.57	0.791
Average			1.7	1.3	0.071	0.165	47.00	0.568
Pool			3.2	1.7	0.071	0.318[b]	85.71	

https://doi.org/10.1079/PGR2006115 Published online by Cambridge University Press

Table 1. *Continued*

Accession no.	Name	Origin[a]	A/L	ne	H_I	H_S	%P	F_{IS}
Cyprus								
MG112390	Cyprus01	Cyprus (5)	2.4	1.5	0.111	0.244	85.71	0.545
MG112398	Cyprus02	Cyprus (5)	2.3	1.5	0.140	0.250	85.71	0.440
MG112403	Cyprus03	Cyprus (5)	2.1	1.4	0.092	0.242	71.43	0.620
MG112406	Cyprus04	Cyprus (5)	3.0	1.6	0.144	0.327	100.00	0.560
MG112408	Cyprus05	Cyprus (5)	1.9	1.3	0.132	0.208	64.29	0.365
MG112410	Cyprus06	Cyprus (5)	2.0	1.5	0.138	0.265	78.57	0.479
MG112411	Cyprus07	Cyprus (5)	2.1	1.5	0.157	0.273	71.43	0.425
MG112412	Cyprus08	Cyprus (5)	2.2	1.4	0.146	0.226	78.57	0.354
MG112414	Cyprus09	Cyprus (5)	2.4	1.6	0.208	0.297	92.86	0.300
MG112416	Cyprus10	Cyprus (5)	2.3	1.6	0.137	0.262	78.57	0.477
MG112417	Cyprus11	Cyprus (5)	2.7	1.6	0.184	0.276	92.86	0.333
MG112418	Cyprus12	Cyprus (5)	2.4	1.6	0.141	0.296	85.71	0.524
MG112419	Cyprus13	Cyprus (5)	2.1	1.4	0.124	0.205	78.57	0.395
Average			2.3	1.5	0.142	0.255	81.80	0.441
Pool			3.9	1.6	0.142	0.308[b]	100.00	
Northern Europe								
S2 87	NEurope-Bel	Belgium (1)	1.1	1.1	0.105	0.072	14.29	−0.458
PI283588	NEurope-Czech1	Czech Rep. (8)	2.0	1.3	0.083	0.189	57.14	0.561
LSLAT48777	NEurope-Czech2	Czech Rep. (3)	1.9	1.4	0.092	0.211	57.14	0.564
S72 83	NEurope-Czech3	Czech Rep. (1)	1.4	1.2	0.029	0.138	35.71	0.790
S88 83	NEurope-Czech4	Czech Rep. (1)	1.6	1.4	0.071	0.177	42.86	0.599
MG100292	NEurope-Fr	France (5)	1.9	1.5	0.167	0.246	57.14	0.321
S101 83	NEurope-Fr1	France (1)	1.8	1.4	0.114	0.207	50.00	0.449
S3 85	NEurope-Fr2	France (1)	1.5	1.3	0.153	0.146	35.71	−0.048
DRF 02	NEurope-Ger1	Germany (2)	2.0	1.6	0.168	0.280	71.43	0.400
LSLAT44473	NEurope-Ger2	Germany (3)	2.0	1.4	0.101	0.195	57.14	0.482
PI209789	NEurope-Ger3	Germany (8)	1.6	1.4	0.124	0.183	50.00	0.322
PI283595	NEurope-Pol1	Poland (8)	1.9	1.4	0.103	0.200	57.14	0.485
PL 114661	NEurope-Pol2	Poland (8)	2.3	1.7	0.176	0.330	71.43	0.467
PL114613	NEurope-Pol3	Poland (8)	2.1	1.5	0.131	0.249	64.29	0.474
PL114668	NEurope-Pol4	Poland (8)	1.7	1.3	0.086	0.159	50.00	0.459
Average			1.8	1.4	0.116	0.195	51.40	0.416
Pool			3.9	1.9	0.116	0.388[b]	100.00	
Iberian Peninsula								
LSLAT45675	IberianP-Por	Portugal (3)	1.9	1.5	0.158	0.252	57.14	0.373
BG13223	IberianP-Sp01	Spain (6)	1.6	1.3	0.067	0.173	42.86	0.613
BG13261	IberianP-Sp02	Spain (6)	2.3	1.6	0.145	0.292	71.43	0.503
BG1487	IberianP-Sp03	Spain (6)	2.2	1.5	0.117	0.233	71.43	0.498
BG2259	IberianP-Sp04	Spain (6)	2.2	1.4	0.158	0.242	78.57	0.347
BG3712	IberianP-Sp05	Spain (6)	1.9	1.4	0.104	0.230	64.29	0.548
BG4211	IberianP-Sp06	Spain (6)	2.2	1.5	0.129	0.265	71.43	0.513
BG4212	IberianP-Sp07	Spain (6)	2.3	1.5	0.161	0.253	85.71	0.364
BG4216	IberianP-Sp08	Spain (6)	1.9	1.4	0.113	0.244	71.43	0.537
BG4217	IberianP-Sp09	Spain (6)	2.3	1.6	0.104	0.322	78.57	0.677
BG4218	IberianP-Sp10	Spain (6)	1.9	1.4	0.140	0.217	57.14	0.355
Average			2.1	1.5	0.126	0.248	68.18	0.484
Pool			3.3	1.6	0.126	0.319[b]	100.00	
Italian Peninsula								
MG100955	ItalianP01	Italy (5)	1.9	1.5	0.159	0.228	64.29	0.303
MG110263	ItalianP02	Italy (5)	2.1	1.5	0.093	0.288	71.43	0.677
MG110434	ItalianP03	Italy (5)	1.6	1.4	0.121	0.184	50.00	0.342
MG110435	ItalianP04	Italy (5)	1.7	1.4	0.143	0.196	57.14	0.270
MG110492	ItalianP05	Italy (5)	1.9	1.5	0.097	0.225	71.43	0.569
MG110955	ItalianP06	Italy (5)	1.6	1.4	0.113	0.191	35.71	0.408
MG110956	ItalianP07	Italy (5)	1.8	1.4	0.115	0.196	57.14	0.413
MG110957	ItalianP08	Italy (5)	2.3	1.4	0.183	0.215	78.57	0.149
MG111983	ItalianP09	Italy (5)	1.6	1.4	0.124	0.206	50.00	0.398
MG111985	ItalianP10	Italy (5)	2.4	1.4	0.090	0.241	71.43	0.627
MG112251	ItalianP11	Italy (5)	1.9	1.5	0.162	0.279	64.29	0.419

https://doi.org/10.1079/PGR2006115 Published online by Cambridge University Press

Table 1. *Continued*

Accession no.	Name	Origin[a]	A/L	ne	H_I	H_S	%P	F_{IS}
MG113089	ItalianP12	Italy (5)	1.9	1.6	0.187	0.292	78.57	0.360
MG113090	ItalianP13	Italy (5)	2.1	1.4	0.095	0.236	71.43	0.597
Average			1.9	1.4	0.125	0.225	63.20	0.438
Pool			3.1	1.6	0.125	0.306[b]	92.86	
Ukraine								
S38 85	Ukraine-Ukr1	Ukraine (1)	1.6	1.4	0.095	0.210	50.00	0.548
S41 85	Ukraine-Ukr2	Ukraine (1)	1.3	1.2	0.050	0.107	21.43	0.533
LSLAT45277	Ukraine-Bul1	Bulgaria (3)	1.6	1.2	0.079	0.132	35.71	0.402
LSLAT41982	Ukraine-Bul2	Bulgaria (3)	2.1	1.5	0.104	0.242	71.43	0.570
HU030191	Ukraine-Hun1	Hungary (4)	1.9	1.3	0.092	0.164	64.29	0.439
HU040191	Ukraine-Hun2	Hungary (4)	2.0	1.3	0.127	0.198	71.43	0.359
HU050191	Ukraine-Hun3	Hungary (4)	1.6	1.3	0.071	0.154	35.71	0.539
HU060191	Ukraine-Hun4	Hungary (4)	1.7	1.3	0.067	0.170	50.00	0.606
LS118229	Ukraine-Hun5	Hungary (3)	1.9	1.4	0.104	0.217	57.14	0.521
LSLAT46179	Ukraine-Hun6	Hungary (3)	1.9	1.4	0.106	0.228	71.43	0.535
PI255368	Ukraine-Ser	Serbia (8)	1.5	1.3	0.093	0.163	50.00	0.429
LSLAT43580	Ukraine-Rus1	Ukraine (3)	2.1	1.5	0.109	0.268	78.57	0.593
S42 85	Ukraine-Rus2	Ukraine (1)	1.6	1.2	0.043	0.120	42.86	0.642
Average			1.8	1.3	0.088	0.175	53.90	0.511
Pool			3.4	1.7	0.088	0.334[b]	92.86	
Mean per accession (mean of averages)			1.9	1.4	0.108	0.205	59.40	0.480
Mean per region (mean of pools)			3.5	1.7	0.108	0.344	96.03	
Pool of all accessions			5.2	1.8	0.110	0.380	100.00	

A/L, number of alleles per locus; ne, effective number of alleles; H_I, observed heterozygosity; H_S, expected heterozygosity; P, percentage of polymorphic loci at 0.99 level (Nei, 1975); F_{IS}, genetic differentiation.

[a] 1, Universite de Pau et Pais L'adour, Pau, France; 2, Institut für Nutzpflanzenforschung, Berlin, Germany; 3, Zentralinstitut für Genetik und Kulturpflazenforschung, Gatersleben, Germany; 4, Hungarian Plant Genetic Resources, Tapioszele, Hungary; 5, National Research Council Germplasm Institute, Bari, Italy; 6, Banco de Germoplasma INIA, Alcala de Henares, Spain; 7, Plant Genetic Resources Institute, Izmir, Turkey; 8, Western Regional Plant Introduction Station, USDA-ARS, Pullman, WA, USA.

[b] H_S pool value = $H_{T(R)}$ value in Table 2.

locus); and the percentage of polymorphic loci at 0.99 level (Nei, 1975).

In addition, Nei's (1973) genetic diversity statistics (H_T, H_S, D_{ST}, G_{ST}) were calculated. H_S (expected intra-population gene diversity) represents the average expected number of heterozygous individuals under Hardy–Weinberg equilibrium in each subpopulation. H_T (expected gene diversity for the entire population) refers to the expected number of heterozygous individuals under Hardy–Weinberg equilibrium when all individuals are taken as belonging to a single population (non-subdivided population). Finally, D_{ST} (expected inter-population gene diversity) was calculated from $D_{ST} = H_T - H_S$. The relative magnitude of the gene differentiation among subpopulations was subsequently measured as $G_{ST} = D_{ST}/H_T$.

Also, deviations of genotype frequencies in a subdivided population were measured by Wright's (1951) *F*-statistics (F_{IS}, F_{ST} and F_{IT}) or fixation indices, which estimate the deviation between the number of heterozygotes observed and the expected under Hardy–Weinberg equilibrium at three different levels. For a given locus, if the observed heterozygosity proportion of heterozygous individuals is H_I, then F_{IS} is defined as $F_{IS} = (H_S - H_I)/H_S$, and measures the degree of genetic differentiation within subpopulations; F_{ST} is calculated as $F_{ST} = (H_T - H_S)/H_T$, which measures the genetic differentiation between subpopulations; F_{IT}, which represents the genetic differentiation within a population can be calculated as $F_{IT} = (H_T - H_I)/H_T$. The outcrossing rate t ($t = (1 - F_{IS})/(1 + F_{IS})$) (Wright, 1951) and the number of migrants Nm ($Nm = 0.25(1 - F_{ST})/F_{ST}$) (Slatkin, 1987) were also calculated.

Nei's genetic identity (I) and distance ($D = -\ln I$) indices (Nei, 1973) between accessions pairs were calculated from their allelic frequencies. A genetic distance matrix was then used to elaborate a dendrogram by the unweighted pair-group method of arithmetical means (UPGMA) algorithm (Sneath and Sokal, 1973) using the MEGA version 3.0 software program (Kumar *et al.*, 2004).

Finally, the TFPGA 1.3 sofware program (Miller, 1997) was used to determine Nei's genetic identity (I) and distance (D) at the regional level. A loci bootstrap procedure was then carried out using 1000 replicates to confirm the topology of the dendrogram.

https://doi.org/10.1079/PGR2006115 Published online by Cambridge University Press

Results

Genetic diversity in Lathyrus sativus

To facilitate analysis of the data generated in this study, the 110 accessions were categorized into nine groups based on their geographical location. These regions comprised Africa, Anatolian Peninsula (Anatolian P.), Asia–Russia, South-East Asia (SE Asia), Cyprus, Northern Europe, Iberian Peninsula (Iberian P.), Italian Peninsula (Italian P.) and Ukraine (Table 1).

We found an elevated number of polymorphisms for all isozymes analysed in all accessions, revealing a significant number of alleles present in each accession and each region (Table 1). The average number of alleles per locus (A/L) ranged from 1.6 (Asia–Russia) to 2.3 (Cyprus), and the effective number of alleles (n*e*) was greater than 1.0 for most loci. We also found the percentage of polymorphic loci (P) to be 100 in five of the regions, while SE Asia possessed the lowest value of less than 90 (Table 1).

Seven isozymatic alleles were specific to three geographical regions (i.e. three in African accessions, three in Cypriot accessions and one in Northern European accessions), whereas the other six regions characteristically showed common alleles, some of which were more abundant (see Tables A1 and A2 in the Appendix).

We then calculated the observed heterozygosity (H_I) and the expected heterozygosity (H_S and H_T) values (Nei, 1973) for each accession, region and for all the regions pooled in a single group (Tables 1 and 2). From these data we were able to calculate the inter-population variability for each region ($D_{ST(R)}$), for the nine regions ($D_{S(R)T}$) and for the pooled 110 accessions (D_{ST}), respectively. To dissect the genetic structure of the subdivided populations, we then calculated the genetic differentiation ($G_{ST} \approx F_{ST}$) and the Wright's *F*-statistics (F_{IS} and F_{IT} fixation index) at the same hierarchical levels (Table 2). For all calculations, the expected heterozygosity was higher than the observed heterozygosity. Similar results were obtained for each of the individual accessions, although only two accessions belonging to Northern Europe showed a H_I value higher than H_S (see Table 1). With the exception of Asia–Russia, the intra-population (H_S) component of the variability was always higher than the inter-population component ($D_{ST(R)}$) at the regional level ($D_{ST(R)}/H_S$) (Table 2). This result was also observed when the 110 accessions were either not grouped (D_{ST}), or when the accessions belonging to a particular region were counted as a single population ($D_{S(R)T}$, pool-regions). The average genetic differentiation ($G_{ST(R)}$) between accessions for each region (i.e. intra-region average gene differentiation) was 39.8%; slightly lower than the 46.1% that was calculated for samples when they were not grouped (intra-population level, G_{ST}). These data indicate that the intra-population variability contributes around 53.9% of the total variability found within the 110 accessions studied. Further, the calculated $G_{S(R)T}$ for pooled regions was 0.094, indicating that the genetic variability within regions accounts for 90.6% of the total estimated variability.

Because the unusual occurrence of out-crossing has been reported in *L. sativus* (Rahman *et al.*, 1995), we decided to also calculate the rate of outcrossing for all

Table 2. Genetic diversity (Nei, 1975) and genetic variability (Wright, 1951) values for each geographical region

	n	$\overline{H_I}$	$\overline{H_S}$	$H_{T(R)}$	$D_{ST(R)}$	$D_{ST(R)}/\overline{H_S}$	$G_{ST(R)}$	$\overline{F_{IS}}$	$F_{IT(R)}$	*t*
Africa	15	0.104	0.201	0.378	0.181	0.919	0.479	0.512	0.735	0.399
Anatolian P.	7	0.106	0.207	0.374	0.167	0.807	0.447	0.481	0.711	0.350
Asia–Russia	11	0.098	0.181	0.375	0.194	1.068	0.516	0.469	0.739	0.361
SE Asia	12	0.071	0.165	0.318	0.153	0.928	0.481	0.568	0.777	0.276
Cyprus	13	0.142	0.255	0.308	0.053	0.209	0.173	0.441	0.540	0.388
Northern Europe	15	0.116	0.195	0.388	0.194	0.995	0.499	0.416	0.708	0.412
Iberian P.	13	0.126	0.248	0.319	0.076	0.312	0.238	0.484	0.606	0.352
Italian P.	13	0.125	0.225	0.306	0.081	0.358	0.264	0.438	0.586	0.391
Ukraine	13	0.088	0.175	0.334	0.155	0.865	0.464	0.511	0.733	0.324
Average		0.108	0.205	0.344	0.140	0.723	0.398	0.480	0.683	0.361
		$\overline{H_{I(R)}}$	$\overline{H_{S(R)}}$	H_T	$D_{S(R)T}$	$D_{ST(R)T}/\overline{H_{S(R)}}$	$G_{S(R)T}$	$\overline{F_{I(R)S(R)}}$	$F_{I(R)T}$	
Pool-regions	9	0.108	0.344	0.380	0.036	0.104	0.094	0.685	0.717	
		$\overline{H_I}$ total	$\overline{H_S}$ total	H_T	D_{ST}	$D_{ST}/\overline{H_S}$	G_{ST}	$\overline{F_{IS}}$ total	F_{IT}	
Total (samples)	110	0.110	0.205	0.380	0.176	0.856	0.461	0.472	0.714	

n, number of subpopulations in each hierarchical level; $\overline{H_I}$, average observed heterozygosity; $\overline{H_S}$, average expected heterozygosity (intra-population diversity); H_T, expected heterozygosity; D_{ST}, inter-population diversity; $G_{ST} \approx F_{ST} = D_{ST}/H_T$, differentiation among subpopulations; $\overline{F_{IS}}$, genetic differentiation within subpopulations; F_{IT}, total genetic differentiation. $_{(R)}$, parameters calculated at the regional level.

https://doi.org/10.1079/PGR2006115 Published online by Cambridge University Press

110 accessions based on the inbreeding coefficient (F_{IS}). We found that the outcrossing rate (*t*-value) ranged from 27.6% (SE Asia) to 41.2% (Northern Europe) (Table 2).

Genetic similarity between Lathyrus sativus accessions

Taking into account the 14 polymorphic loci analysed, we calculated the Nei's genetic identity (*I*) for each pair of accessions and arranged the data into a Nei's genetic identity matrix (110 × 110 matrix, data not shown). The average genetic identity between accessions was 0.778 (SD = 0.099) with a maximum of 0.991 and a minimum of 0.417. We then carried out a cluster analysis using the UPGMA algorithm to generate a dendrogram that displayed the genetic distances (*D*) between accessions. We found that, with the exception of the Euro-Mediterranean accessions, most accessions were not arranged according to geographical origin (Fig. 1).

To determine the genetic identity between regions we calculated two different genetic identity matrices (Table 3). The first was made by considering all accessions of each region as a single population. From this parameter, the average genetic identity between regions was 0.934 (SD = 0.034), with values ranging from 0.974 (Asia–Russia versus Anatolian P.) to 0.841 (Italian P. versus Anatolian P.). A second matrix was made by calculating the mean genetic identity between regions. This was based on the average genetic identity value calculated for every possible pairing of any accession from

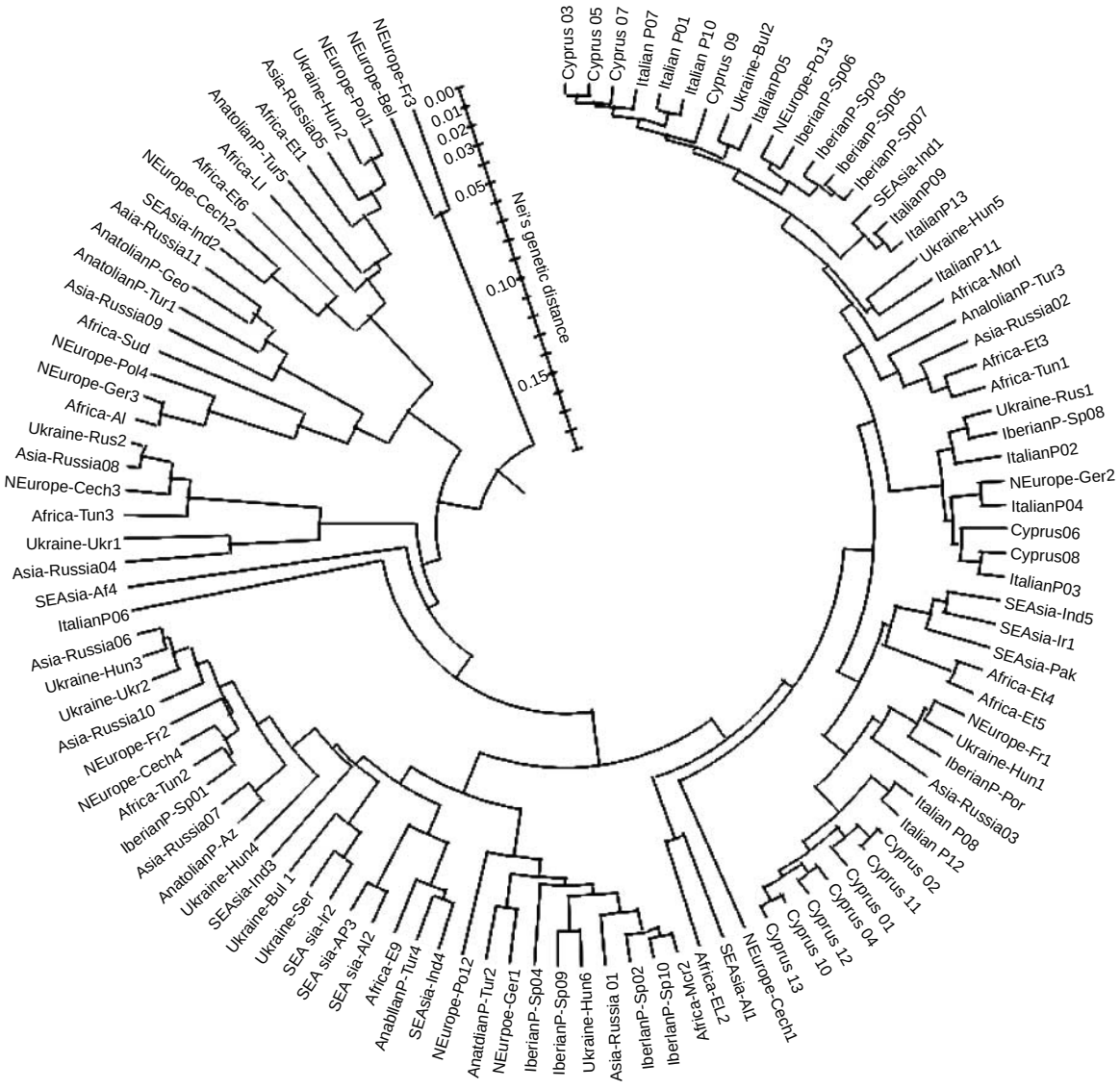

Fig. 1. Nei's genetic distances between 110 grasspea accessions.

https://doi.org/10.1079/PGR2006115 Published online by Cambridge University Press

Table 3. Nei's genetic identities between regions

	Africa	Anatolian P.	Asia–Russia	SE Asia	Cyprus	Northern Europe	Iberian P.	Italian P.	Ukraine
Africa	**0.758**	0.745	0.742	0.771	0.753	0.741	0.799	0.767	0.774
Anatolian P.	0.941	**0.759**	0.764	0.752	0.755	0.746	0.754	0.715	0.763
Asia–Russia	0.953	0.974	**0.751**	0.749	0.745	0.748	0.771	0.719	0.775
SE Asia	0.964	0.927	0.940	**0.800**	0.831	0.744	0.817	0.801	0.791
Cyprus	0.913	0.878	0.885	0.9559	**0.921**	0.762	0.864	0.891	0.805
Northern Europe	0.955	0.960	0.973	0.935	0.894	**0.744**	0.788	0.751	0.770
Iberian P.	0.954	0.877	0.920	0.9486	0.937	0.944	**0.888**	0.870	0.830
Italian P.	0.921	0.841	0.867	0.932	0.966	0.899	0.970	**0.889**	0.787
Ukraine	0.966	0.941	0.970	0.965	0.923	0.968	0.963	0.914	**0.804**

Above diagonal bold values: calculated mean of genetic identities between accessions from two regions. Below diagonal bold values: calculated genetic identities between regions when all accessions from a region are considered as belonging to a single population. Diagonal bold values: calculated mean of genetic identities between accessions from a region.

each region. In this matrix the mean genetic identity between regions was 0.776 (SD = 0.040) with values ranging from 0.891 (Cyprus versus Italian P.) to 0.715 (Italian P. versus Anatolian P.). From this matrix we also calculated the mean intra-region genetic identity (0.813, SD = 0.069). The highest value was found in Cyprus accessions (0.921), Italian P. (0.889) and Iberian P. (0.888).

We then generated a dendrogram for Nei's genetic distance between regions by considering all accessions as a single population (Fig. 2). The consistency of the dendrogram topology was calculated by a loci bootstrap procedure (see Material and Methods). This analysis placed accessions from Euro-Mediterranean regions (Iberian P., Italian P. and Cyprus) in a cluster separate to a second group of accessions from Euro-Asiatic and African regions (Fig. 2).

Discussion

In this paper we report the isozymatic genetic structure for a large collection of *L. sativus* accessions, from which we are able to reach several conclusions about the genetic variability and mating system of this species.

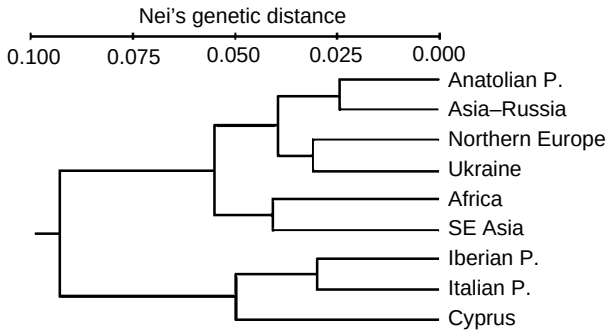

Fig. 2. Nei's genetic distances between regions. Note that three of the eight nodes have bootstrap values higher than 50%.

We found (see Table 1) that the mean number of alleles per locus observed (A/L = 3.5) in each region was higher than previously described for a collection of 348 accessions (five individuals per accession) also derived from different geographical regions (Chowdhury and Slinkard, 2000). The mean A/L per accession was 1.9, also slightly higher than those estimated from a collection of 10 accessions from Ethiopia (Tadesse and Bekele, 2001) and from the analysis of 10 *Lathyrus* species (Ben Brahim *et al.*, 2002). Although some of the geographical regions chosen for this study are very extensive, the mean A/L per accession was also higher in the smaller geographical regions (Cyprus, A/L = 2.3; Iberian P., A/L = 2.1; Italian P., A/L = 1.9) compared to those in previously studied collections (Tadesse and Bekele, 2001; Ben Brahim *et al.*, 2002). From our study, it is noticeable that not only is there a conserved allelic richness across all these regions, but also that this richness is present even in agronomically selected accessions.

Further, we discovered that the mean number of effective alleles per accession (n*e*) was about five-tenths lower than the observed value (n*e* = 1.4, A/L = 1.9). The pool n*e* value was slightly higher than the average n*e* value in each region, but in all cases n*e* values were in the range described for other plant species (Berg and Hamrick, 1997). Two factors might explain why n*e* values were, in most cases, much lower than A/L. First, most isozymatic loci had several alleles but only one or two were common in all accessions. Second, there might be some accession-specific alleles present at very low frequency in some accessions. These specific alleles could also explain why the difference between the observed (A/L) and the effective number (n*e*) was always higher at the accession level than at the regional level.

The percentage of polymorphic loci (%P) was high for all nine regions, with a mean per region of 96%.

https://doi.org/10.1079/PGR2006115 Published online by Cambridge University Press

These values are substantially higher than those previously reported for this species when studying 20 isozymatic loci (Chowdhury and Slinkard, 2000). In this study, the average values of polymorphic loci for each region ranged between 46.9% for Asia–Russia and 81.8% for Cyprus, but the total polymorphism in each region (pool) was equal or close to 100%. Consequently, each accession was polymorphic for different loci. The decrease in the %P value from pool to average for each region coincided with a decrease in n*e*. This indicates that some of the alleles present at very low frequencies in accessions belonging to one region were also present in other accessions of this region.

In our study, the expected Hardy–Weinberg heterozygosity was always higher than the observed frequency. From the F_{IT} Wright's parameter calculated for each region $F_{IT(R)}$ and between regions $F_{I(R)T}$, we discovered a deficit of heterozygotes. This could be explained by the spatial variance in allele frequencies (Wahlund effect) (Hedrick, 1983). Because in our study the accessions were grouped in nine categories according to their geographical location, we would expect a Walhund effect. Given that in all cases the F_{ST} ($\approx G_{ST(R)}$) was greater than 0.173 (and with an average value of 0.398), preferential local mating must have an important effect on the genetic structure of these accessions. In addition, because grasspea has been traditionally considered a self-pollinating species, we would also expect this factor to contribute to a deficit of heterozygotes in our study. Surprisingly, our calculated F_{IS} values indicated a high value of cross-pollination (where *t* values ranged from 0.276 to 0.412), five times greater than cross-pollination values previously reported for this species (Rahman *et al.*, 1995). Although the floral structure of grasspea is suggestive of a totally autogamous breeding system (Rahman *et al.*, 1995), out-crossing in natural populations has been described (Yunus *et al.*, 1991; Rahman *et al.*, 1995; Chowdhury and Slinkard, 1997). Similarly, other legumes have been found not to be absolute inbreeders, and should therefore be regarded as mixed mating species, in which self-pollination and outcrossing both occur at significant rates. Mixed mating legumes have been reported in recent years, for instance for *Centrosema* spp. (Penteado *et al.*, 1996), *Medicago truncata* (Vitale *et al.*, 1998) or *Phaseolus vulgaris* L. (Ibarra-Perez *et al.*, 1997). The mixed mating system in *L. sativus* is a major factor to be taken into consideration when collecting and multiplying seed stocks for future conservation of genetic resources in this species. Also, careful attention must now be taken to prevent contamination by out-crossing when isolating *L. sativus* lines for the production of pure breeds. Clearly, the existence of a mixed mating system in *L. sativus* adds further complexity to our understanding of the level and extent of genetic diversity in this species. Interestingly, most of the genetic diversity in grasspea was found within each geographic region tested ($H_S/H_{T(R)}$). These results are in agreement with those previously reported when using isozymatic markers (Chowdhury and Slinkard, 2000). The highest genetic diversity levels ($H_{T(R)}$) were found in Northern Europe, Africa, Asia–Russia and Anatolia. The lowest genetic variability values were found in Mediterranean accessions (Iberian P., Italian P. and Cyprus). Furthermore, these Mediterranean accessions also showed the lowest $G_{ST(R)}$ values, indicating that they are most similar. This behaviour is especially marked in Cyprus. Some of the homogeneity for genetic variability found across the 13 accessions from Cyprus could be explained by the rate at which genes are exchanged between accessions due to pollen or seed dispersal. This phenomenon can be evaluated by the number of migrants (*Nm*), which for Cyprus had a value five times higher than any of the other regions (data not shown). Although it has been shown that *Nm* values have great limitations in some population studies, comparisons between large groups of accessions are likely to be more informative (Whitlock and McCauley, 1999).

Our calculations for Nei's genetic identity values between accessions revealed that they do not share a high degree of identity (average value = 0.778, SD = 0.099). These values are similar to those reported by Tadesse and Bekele (2001) for a collection of 10 grasspea accessions. Surprisingly, these genetic identity values were about five-tenths lower than those described for other species (Crawford, 1983), including wild populations of legumes (Papa and Gepts, 2003).

The UPGMA dendrogram (Fig. 1) showed that especially most of the accessions belonging to Cyprus were grouped in three main clusters. Furthermore, these accessions displayed very low distance between them. This framework was not clearly observed for the rest of the accessions, which appeared not to be distributed in relation to their geographical origin.

When we calculated the average genetic identity between accessions from different regions, it was in most cases lower than the average genetic identity between accessions from within a region (Table 3). On the other hand, when all accessions from a region were considered as a single population, the average genetic identity values were always higher (Table 3), suggesting that most accessions from a region shared distinct isozymatic alleles and with similar allelic frequencies. When we plotted a dendrogram for these genetic distances (Fig. 2), we found two clearly distinct groups; the first included Euro-Mediterranean regions (Cyprus, Iberian P. and Italian P.). The second group included regions from two main geographical areas, the Africa–SE Asia regions and the central Euro-Asiatic regions

https://doi.org/10.1079/PGR2006115 Published online by Cambridge University Press

(Anatolian P., Asia–Russia, Northern Europe and Ukraine). The latter were grouped in two small clusters (Anatolian P. and Asia–Russia; Northern Europe and Ukraine), according to geographical proximity.

In conclusion, our study has revealed that considerable genetic diversity exists in grasspea seed collections throughout the world. Because genetic diversity is an essential prerequisite for crop improvement, our findings indicate that the primary gene pool of *L. sativus* is a valuable resource suited to the genetic improvement of this crop species. Increased exploration, characterization and utilization of grasspea genetic resources are long awaited and required to develop this neglected crop to its full potential for the benefit of mankind.

Acknowledgements

We would like to acknowledge Dr John Pannell and Dr Liliana M. Costa (University of Oxford) for critical suggestions for the manuscript.

References

Abebe D and Bjørnstad A (1996) Phenotypic diversity of Ethiopian barleys in relation to geographical regions, altitudinal range, and agro-ecological zones: as an aid to germplasm collection and conservation strategy. *Hereditas* 124: 17–29.

Allard RW, Garcia P, Saenz-de-Miera LE and Perez de la Vega M (1993) Evolution of multilocus genetic structure in *Avena hirtula* and *Avena barbata*. *Genetics* 135: 1125–1139.

Bekele E (1983) Some measure of gene diversity analysis on landrace populations of Ethiopian barley. *Hereditas* 98: 127–143.

Ben Brahim N, Salho A, Chtorou N, Combes D and Marrakcho M (2002) Isozymic polymorphism and phylogeny of 10 *Lathyrus* species. *Genetic Research and Crop Evolution* 49: 427–436.

Berg EE and Hamrick JL (1997) Quantification of genetic diversity at allozyme loci. *Canadian Journal of Forest Research* 27: 415–424.

Brown AHD and Weir BS (1983) Measuring genetic variability in plant populations. In: Tanksley SD and Orton TJ (eds) *Isozymes in Plant Breeding and Genetics, Part A*. Amsterdam: Elsevier, pp. 219–239.

Campbell CG (1997) *Grass Pea Lathyrus sativus L. Promoting the Conservation and Use of Underutilized and Neglected Crops*. Gatersleben and Rome: Institute of Plant Genetics and Crop Plant Research and International Plant Genetic Resources Institute.

Chowdhury MA and Slinkard AE (1997) Natural outcrossing in grasspea. *Journal of Heredity* 88: 154–156.

Chowdhury MA and Slinkard AE (2000) Genetic diversity in grasspea (*Lathyrus sativus* L.). *Genetic Research and Crop Evolution* 47: 163–169.

Crawford DJ (1983) Phylogenetic and systematic inferences from electrophoretic studies. In: Tanksley SD and Orton TJ (eds) *Isozymes in Plant Genetics and Breeding, Part A*. Amsterdam: Elsevier, pp. 257–287.

Croft AM, Pang ECK and Taylor PWJ (1999) Molecular analysis of *Lathyrus sativus* L. (grasspea) and related *Lathyrus* species. *Euphytica* 107: 167–176.

De la Rosa L and Martín I (2001) Morphological characterisation of Spanish genetic resources of *Lathyrus sativus* L. *Lathyrus Lathyrism Newsletter* 2: 31–34.

Farooq S and Azam F (2002) Molecular markers in plant breeding II. Some pre-requisites for use. *Pakistan Journal of Biological Sciences* 5: 1141–1147.

Getahun H, Lambein F, Vanhoorne M and Van der Stuyft P (2003) Food-aid cereals to reduce neurolathyrism related to grass-pea preparations during famine. *Lancet* 362: 1808–1810.

Gutiérrez JF and Vences FJ (1992) Genetic control of isoenzymatic loci in grass pea *Lathyrus sativus* L. Paper presented at the Second International Food Legume Research Conference (IFLRC-II), Cairo.

Gutiérrez JF, Vaquero F and Vences FJ (1994) Allopolyploid vs. autopolyploid origins in the genus *Lathyrus* (Leguminosae). *Heredity* 73: 29–40.

Gutiérrez JF, Vaquero F and Vences FJ (2001) Genetic mapping of isozyme loci in *Lathyrus sativus* L. *Lathyrus Lathyrism Newsletter* 2: 74–78.

Hedrick PW (1983) *Genetics of Populations*. Boston: Jones and Bartlett.

Hopf M (1986) Archaeological evidence of the spread and use of some members of the Leguminosae family. In: Barigozzi C (ed.) *The Origin and Domestication of Cultivated Plants*. Oxford: Elsevier, pp. 35–60.

Ibarra-Perez FJ, Ehdaie B and Waines JG (1997) Estimation of outcrossing rate in common bean. *Crop Science* 37: 60–65.

Kislev ME (1989) Origins of the cultivation of *Lathyrus sativus* and *Lathyrus cicera* (Fabaceae). *Economic Botany* 43: 262–270.

Kumar S, Tamura K and Nei M (2004) MEGA3: integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Briefings in Bioinformatics* 5: 150–163.

McCutchan JS (2003) A brief history of grasspea and its use in crop improvement. *Lathyrus Lathyrism Newsletter* 3: 18–23.

Miller MP (1997) Tools for population genetic analysis (TFPGA) 1.3: a Windows program for the analysis of allozyme and molecular population genetic data. Computer software available from http://www.marksgeneticsoftware.net.

Nei M (1973) Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Science USA* 70: 3321–3323.

Nei M (1975) *Molecular Population Genetics and Evolution*. Amsterdam: North-Holland.

Papa R and Gepts P (2003) Asymmetry of gene flow and differential geographical structure of molecular diversity in wild and domesticated common bean (*Phaseolus vulgaris* L.) from Mesoamerica. *Theoretical and Applied Genetics* 106: 239–250.

Penteado MI, Saénz de Miera LE and Pérez de la Vega M (1996) Genetic resources of *Centrosema* spp.: genetic changes associated to the handling of an active collection. *Genetic Research and Crop Evolution* 43: 85–90.

Pérez de la Vega M, Sáenz de Miera LE and Allard RW (1994) Ecogeographical distribution and differential associations in Spanish *Avena sativa* L. *Theoretical and Applied Genetics* 88: 56–64.

Przybylska J, Zimniak-Przybylska Z and Krajewski P (1998) Diversity of seed albumins in the grasspea (*Lathyrus sativus* L.): an electrophoretic study. *Genetic Research and Crop Evolution* 45: 423–431.

Rahman MM, Kumar J, Rahman MA and Afzal MA (1995) Natural outcrossing in *Lathyrus sativus* L. *Indian Journal of Genetics* 55: 204–207.

Seifu T (1997) *Genetic Diversity and Structure of Tetraploid Wheat Landraces of the Central Highlands of Ethiopia*. Svalov, Sweden: Swedish University of Agricultural Sciences.

Singh SP (2001) Broadening the genetic base of common bean cultivars. *Crop Science* 41: 1659–1675.

Singh SP, Gepts P and Debouck DG (1991) Races of common bean (*Phaseolus vulgaris*, Fabaceae). *Economic Botany* 45: 379–396.

Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science* 236: 787–792.

Sneath PHA and Sokal R (1973) *Numerical Taxonomy*. San Francisco: Freeman.

Tadesse W and Bekele E (2001) Isozymes variability of grasspea (*Lathyrus sativus* L.) in Ethiopia. *Lathyrus Lathyrism Newsletter* 2: 43–46.

Tadesse W and Bekele E (2003) Phenotypic diversity of Ethiopian grass pea (*Lathyrus sativus* L.) in relation to geographical regions and altitudinal range. *Genetic Research and Crop Evolution* 50: 497–505.

Vitale M, Pupilli F, Labombarda P and Arcioni S (1998) RAPD analysis reveals a low rate of outcrossing in burr medic (*Medicago polymorpha* L.). *Genetic Research and Crop Evolution* 45: 337–342.

Whitlock MC and McCauley DE (1999) Indirect measures of gene flow and migration FST1/(4Nm + 1). *Heredity* 82: 117–125.

Wright S (1951) The genetical structure of populations. *Annals of Eugenics* 93: 323–354.

Yunus AG, Jackson MT and Catty JP (1991) Phenotypic polymorphism of six enzymes in the grasspea (*Lathyrus sativus* L.). *Euphytica* 55: 33–42.

Zohary D and Hopf M (1988) *Domestication of Plants in the Old World*. San Francisco: Freeman.

https://doi.org/10.1079/PGR2006115 Published online by Cambridge University Press

Appendix

Table A1. Summary of the allelic distribution in grasspea accessions across regions

		Locus														
		ACP-1	*ACP-2*	*EST-1*	*EST-2*	*GOT-1*	*GOT-2*	*GOT-3*	*LAP-1*	*MDH-1*	*PGD-1*	*PGD-2*	*PGM-1*	*PGM-2*	*PRX-1*	ΣA
Africa	A	4	2	2	5	4	4	4	3	2	4	4	3	4	7	52
	ne	1.2	1.7	1.5	3.1	1.1	2.0	2.0	2.2	1.0	1.6	1.5	1.1	2.3	2.4	
Anatolian P.	A	3	2	3	5	3	3	4	3	3	3	3	1	3	7	46
	ne	1.3	1.2	1.5	3.3	1.0	1.6	2.5	1.7	1.0	1.9	2.5	1.0	2.0	4.5	
Asia–Russia	A	2	2	2	4	3	4	4	3	3	2	5	2	3	6	45
	ne	1.2	1.5	1.5	2.2	1.3	2.0	2.1	1.5	1.0	1.6	2.4	1.3	1.9	2.9	
SE Asia	A	4	2	2	5	2	5	3	3	1	1	3	3	5	6	45
	ne	1.5	1.6	1.2	3.1	1.0	2.1	1.2	2.3	1.0	1.0	1.0	1.2	2.6	3.4	
Cyprus	A	4	3	4	4	3	3	3	5	6	2	5	3	4	4	53
	ne	1.8	1.7	1.2	2.5	1.1	1.7	1.1	1.4	1.1	1.1	2.4	1.0	1.6	2.9	
Northern Europe	A	3	2	3	4	3	4	5	3	4	2	7	3	5	6	54
	ne	1.5	1.9	1.3	1.9	1.1	2.5	2.7	1.6	1.0	1.4	3.2	1.0	1.9	3.2	
Iberian P.	A	4	2	2	4	2	3	3	3	2	2	4	3	4	7	45
	ne	1.4	1.6	1.6	1.8	1.0	2.2	1.0	1.9	1.0	1.0	2.2	1.1	2.1	2.4	
Italian P.	A	3	3	2	4	1	3	3	4	4	3	5	2	3	4	44
	ne	1.4	1.6	1.3	2.6	1.0	2.3	1.1	1.2	1.0	1.3	2.2	1.0	1.9	2.8	
Ukraine	A	4	2	2	5	2	4	3	3	3	1	6	3	3	6	47
	ne	1.9	1.7	1.3	2.1	1.4	2.5	1.2	1.9	1.0	1.0	2.2	1.1	1.9	3.0	
Mean per region	A	3.4	2.2	2.4	4.4	2.6	3.7	3.6	3.6	3.1	2.2	4.7	2.6	3.9	5.9	48.2
	ne	1.5	1.6	1.4	2.5	1.1	2.1	1.7	1.7	1.0	1.3	2.2	1.1	2.0	3.1	
Pooled accessions	A	4	3	4	5	4	6	6	5	6	4	7	3	5	7	69
	ne	1.4	1.9	1.4	2.6	1.1	2.3	1.5	2.2	1.0	1.2	2.1	1.1	2.2	3.2	

A, observed number of alleles; ne, effective number of alleles in each region (pooled data). Pooled accessions: all accessions were pooled in a single population.

Table A2. Isozymatic allelic presence (+) and absence (−) in grasspea accessions grouped in nine geographical regions

Locus/allele	*Acp-1*				*Acp-2*			*Est-1*				*Est-2*					*Got-1*				*Got-2*						*Got-3*					
	1	2	3	4	1	2	3	1	2	3	4	1	2	3	4	5	1	2	3	4	1	2	3	5	6	7	1	2	3	4	5	6
Africa	+	+	+	+	−	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	−	+	+	−
Anatolian P.	+	+	+	−	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	+	+	+	−	−	−	+	+	+	+	−	−
Asia–Russia	+	+	−	−	−	+	+	+	+	−	−	+	+	−	+	+	+	+	−	+	+	+	+	−	+	−	+	+	+	+	−	−
SE Asia	+	+	+	+	−	+	+	+	+	−	−	+	+	+	+	+	+	+	−	−	+	+	+	−	+	+	+	+	−	+	−	−
Cyprus	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	−	−	−	+	+	+	−	−	−
Northern Europe	+	+	+	−	−	+	+	+	+	+	−	+	+	−	+	+	+	+	+	−	+	+	+	−	+	−	+	+	+	+	−	+
Iberian P.	+	+	+	+	−	+	+	+	+	−	−	+	+	−	+	+	+	+	−	−	+	+	+	−	−	−	+	+	+	−	−	−
Italian P.	+	+	−	+	+	+	+	+	+	−	−	+	+	−	+	+	+	−	−	−	+	+	+	−	−	−	+	+	+	−	−	−
Ukraine	+	+	+	+	−	+	+	+	+	−	−	+	+	+	+	+	+	+	−	−	+	+	+	−	−	+	−	+	+	+	−	−

Locus/allele	*Lap-1*					*Mdh-1*						*Pgd-1*				*Pgd-2*							*Pgm-1*			*Pgm-2*					*Prx-1*						
	1	2	3	4	5	1	2	3	5	6	7	1	2	3	5	1	2	3	4	5	6	7	1	2	3	1	2	3	4	5	1	2	3	4	5	6	7
Africa	+	+	+	−	−	+	+	−	−	−	−	+	+	+	+	+	+	+	−	−	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+
Anatolian P.	+	+	+	−	−	+	+	+	−	−	−	+	+	+	−	+	+	+	−	−	−	−	−	+	−	+	+	−	+	−	+	+	+	+	+	+	+
Asia–Russia	+	+	+	−	−	+	+	+	−	−	−	+	+	−	−	+	+	+	+	−	+	−	+	+	−	+	+	+	−	−	+	+	+	+	+	+	−
SE Asia	+	+	+	−	−	+	−	−	−	−	−	−	+	−	−	+	+	+	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
Cyprus	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	−	−	+	+	+	+	+	+	+	+	−	+	+	+	+	−	−	+	−
Northern Europe	+	+	+	−	−	+	+	+	−	+	−	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Iberian P.	+	+	+	−	−	+	+	−	−	−	−	+	+	−	−	+	+	+	−	−	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
Italian P.	+	+	+	+	−	+	+	+	+	−	−	+	+	+	−	+	+	+	−	−	+	+	+	+	−	+	+	+	−	−	+	+	+	−	−	−	−
Ukraine	+	+	+	−	−	+	+	+	−	−	−	−	+	−	−	+	+	+	+	+	+	−	+	+	+	+	+	−	+	−	+	+	+	+	+	+	−