ISOZYME AND MORPHOLOGICAL POLYMORPHISM IN GRASSPEA

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Abstract

Genetic diversity for 20 isozymes of 13 enzyme systems and 8 morphological traits was determined in 348 accessions of grasspea (Lathyrus sativus L.) from 10 geographic regions. Accessions from the Near East were the most diverse, based on their high values for diversity index, number of allele per locus, observed heterozygosity and proportion of polymorphic loci; suggesting that grasspea originated in the Near East. Accessions from the Near East and North Africa had the lowest genetic distance, suggesting a common origin. Accessions from South Asia and Sudan-Ethiopia were genetically close even though these regions are widely separated geographically. Accessions from Southern Peninsular Europe and North America were genetically close, suggesting that most grasspea introductions into North America came from Southern Peninsular Europe. The large genetic distance between accessions from South America and those from other regions probably reflects a diverse origin of the few (2) accessions from that region.

Introduction

Grasspea belongs to the genus Lathyrus which includes over 160 species and 45 subspecies (Allkin et al. 1986). Several species are used as animal fodder, including *L. hirsuta* and *L. palustris,* and some are known for their ornamental use, especially *L. odorutus, the* sweet pea. Only one species *L. sutivus, the* grasspea, khesari or chickling pea, is used as a food crop as well as a fodder crop. Two important features of this crop have made it popular in subsistence agriculture; 1) tolerance to water-logged conditions and 2) tolerance to drought. Grasspea is a minor pulse crop grown principally in the Indian subcontinent, North Africa and other parts of the Mediterranean basin.

The study of genetic polymorphism in a species is of little direct consequence to plant breeders. However, it is useful for several reasons, e.g., identifying cultivars, analyzing the pattern of gene flow, determining phylogenetic relationships among closely related species, identifying duplication of accessions, and selecting areas for additional plant exploration. Codominant expression and absence of epistasis and pleiotropism make isozyme analysis an efficient tool for population genetic studies. The present experiment was conducted with the following objectives: 1) determine the extent of morphological and isozyme polymorphism in the available grasspea germplasm, and 2) determine the relationship between the geographic origin of the accessions and patterns of morphological and isozyme polymorphism.

Materials and methods

A sample of 348 *Lathyrus sutivus* accessions from the USDA, Regional Plant Introduction Station, Pullman, WA; Zentral Institut fur Genetic and Kulturpflanzen Forschungen der DDR, Gaterslaben; and Agriculture Canada, Morden, MB was assayed for morphological and isozyme polymorphism. Genetic variation was assessed by analyzing eight morphological traits and 20 isozyme loci of 13 enzyme systems (Table 1 and Table 2). Due to the presence of intra-accession variability five individuals were assayed from each accession. Isozymes were assayed by starch gel electrophoresis on crude protein extracts of cotyledon tissue from freshly germinated seeds. The

accessions were assigned to 10 regions based on their geographic origin (Table 3). Allelic frequency was calculated by region for each isozyme using Proc. Freq (SAS Institute 1985). The genetic diversity index of a population per locus (Marshall and Allard 1970) and Nei's (1973) genetic distance were calculated from the allelic frequencies. Clustering of isozymes among populations was depicted by a dendrogram constructed on genetic distance. Clustering of morphological traits was depicted by a scatter diagram obtained through a principal component analysis.

Table 1. Isozymes for different enzyme systems used in starch gel electrophoresis studies of grasspea.

	Enzyme commission (E.C.) no.	Designation	Polymorphic isozyme
Aconitase	4.2.1.3	AC0	AC0-1,-2
Alcohol dehydrogenase	1.1.1.1	ADH	ADH-4
Asparate aminotransferase	2.6.1.1	AAT	AAT-1,-2,-3
Endopeptidase	3.4.-.-	ENP	ENP
Esterase	3.1.1.-	EST	EST-1,-2
Glutamate dehydrogenase	1.4.1.2	GDH	GDH
Isocitrate dehydrogenase	1.1.1.42	IDH	IDH¶
Leucine aminopeptidase	3.4.11.1	LAP	LAP-1,-2
Malic enzyme	1.1.1.40	ME	ME-3
6-phosphogluconate dehydrogenase	1.1.1.44	PGD	PGD-1,-2
Phosphoglucomutase	5.4.2.2	PGM	PGM
Shikimate dehydrogenase	1.1.1.25	SKDH	SKDH
Triosephosphate isomerase	5.3.1.1	TPI	TPI-1,-2

¶Monomorphic isozyme.

Results

Five alleles were found for the isozymes ACO-1, EST-l, and EST-2. Four alleles were observed for ME-3, PGD-1, PGD-2, TPI-1 and TPI-2. Three alleles were found for AAT-2, GDH, and SKDH. Two alleles were found for ACO-2, ADH, AAT-1, AAT-3, ENP, LAP-l, LAP-2 and PGM. The isozyme IDH showed only one allele and thus was monomorphic.

Table 2. Morphological traits and their variant forms.

Flower colour	Maturity	Seed shape	Coat colour	Seed struc.	Seed coat luster	Coat pattern	Seed¶ weight
blue	early	angular	black	wrinkle	dull	no	
bicolor	late	globular	tan	smooth	shiny	dottet	
white		brown					
		grey					
		green					
		white					

¶ Quantitative trait

The estimates of the number of alleles per locus (A) by region ranged from 1.3 to 2.4 and averaged 2.02 (Table 3). Percent polymorphic loci (P) ranged from 30 to 80% and averaged 64%. The estimates of average diversity index (H) across regions ranged from 0.107 to 0.273 and averaged 0.225. Based on the average diversity calculated for individual regions, the two most diverse regions were Near East (H=0.273) and North Africa (H=0.261). The highest genetic diversity in the Near East was consistent with the other measures of diversity: number of alleles per locus (A), and proportion of polymorphic loci (P) and observed heterozygosity (H_o).

Table 3. **Summary** of diversity data for isozyme traits in grasspea by region. The estimates of average number of alleles per locus (A), genetic diversity (H), observed heterozygosity (H_o), and proportion of polymorphic loci (P) are based on 20 isozyme loci.

Region	N[1]	A	H	H_o	P
South Asia	390	2.30	0.243 ± 0.046	0.0196	0.75
Near East	390	2.40	0.273 ± 0.049	0.0282	0.80
USSR	95	2.05	0.247 ± 0.052	0.0117	0.75
Southern Peninsular Europe	74	2.05	0.215 ± 0.052	0.0127	0.65
Northwest Central Europe	25	1.80	0.240 ± 0.069	0.0140	0.60
Eastern Europe	170	2.20	0.248 ± 0.051	0.0124	0.75
North Africa	85	2.05	0.261 ± 0.057	0.0147	0.60
Sudan-Ethiopia	450	2.25	0.197 ± 0.045	0.0146	0.70
South America	10	1.30	0.107 ± 0.037	0.0000	0.30
North America	50	1.75	0.223 ± 0.053	0.0190	0.50
Mean		2.02	0.225	0.0147	0.64

[1]Number of individuals

Overall similarity for isozyme polymorphism among regions was summarized by the genetic distance (D) which is the transformed version of the Nei's (1972) genetic identity coefficient (I) (Table 4). The genetic distance ranged from 0.011 for the Near East-North Africa pair to 0.229 for the USSR-South America pair. The genetic distance between Near East and other regions was low which is reflected by the lowest value of mean genetic distance of this region. Nei's genetic distance was used to determine the relationship between geographic origin and genetic variation for isozyme traits via a cluster analysis and results are presented as a dendrogram (Pig 1). The major branch of the dendrogram separates the South American accessions from the rest of the accessions (average genetic distance = 0.148; Table 4). The first major subbranch separates the Eastern European accessions from the remaining accessions, suggesting that, after South America, Eastern European accessions are distantly related to the rest of the accessions as indicated by its large average genetic distance (0.112). Accessions from the Near East and North Africa formed the first cluster due to their low genetic distance (0.011). Accessions from Southern Peninsular Europe and North America formed a cluster (genetic distance=0.022). Accessions from South Asia and Sudan-Ethiopia formed a separate, closely related cluster.

Table 4. Estimates of gene identity (I) and genetic distance (D) for isozyme traits in grasspea in ten regions. Values above and below the diagonal are the estimates of gene identity (I) and genetic distance (D), respectively.

	South Asia	Near East	USSR	South Penin. Europe	N.W. Centr. Europe	East. Europe	North Africa	Sudan-Ethio	South America	North America
	1	2	3	4	5	6	7	8	9	10
1	_____	0.965	0.913	0.955	0.918	0.881	0.946	0.956	0.921	0.956
2	0.036	_____	0.970	0.977	0.953	0.923	0.989	0.936	0.972	0.972
3	0.090	0.031	_____	0.926	0.929	0.888	0.965	0.889	0.795	0.925
4	0.046	0.024	0.077	_____	0.961	0.923	0.961	0.935	0.882	0.978
5	0.086	0.048	0.074	0.040	_____	0.900	0.948	0.914	0.852	0.966
6	0.127	0.080	0.119	0.080	0.106	_____	0.917	0.879	0.815	0.924
7	0.056	0.011	0.036	0.039	0.053	0.086	_____	0.904	0.836	0.966
8	0.045	0.066	0.118	0.068	0.090	0.129	0.101	_____	0.835	0.933
9	0.082	0.029	0.229	0.126	0.160	0.204	0.180	0.181	_____	0.869
10	0.045	0.028	0.078	0.022	0.035	0.080	0.034	0.070	0.141	_____
Mean genetic distance										
	0.068	0.039	0.095	0.058	0.077	0.112	0.066	0.096	0.148	0.059

Accession means of one quantitative trait and seven categorical traits were subjected to PCA. The first and second principal components (PRIN1 and PRIN2) accounted for 55.5 and 18.3% of the total variability, respectively. Thus, the first two principal components accounted for 73.8% of the total variation, indicating that the relationships among these accessions could be visualized through two dimensional plotting of the first two principal components. When scores of the accessions for the PRIN1 were plotted against the scores for PRIN2 the accessions from region 1 (South Asia) and 8 (Sudan-Ethiopia) formed a discrete cluster except for a few outliers (Fig 2). The distribution of the accessions from the 10 geographic regions was nonrandom, leading to the recognition of more or less distinct clusters of accessions corresponding to a particular geographic region.

Figure 1. Dendrogram for regions based on Nei's genetic distance for isozyme traits in grasspea.

Figure 2. Scatter diagram for principal component analysis of morphological traits in grasspea. The description of each region is given in Table 4.

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Discussion

In the present study all three estimates of genetic diversity, as well as observed heterozygosity, were highest for accessions from the Near East region. These results suggest that, like other old world pulses (lentil, pea, chickpea), the grasspea also originated and underwent diversification in the Near East. Kislev (1989) suggested that domestication of grasspea began in the Balkan peninsula as a result of expansion of Near East agriculture into this region, which is consistent with maximum genetic diversity for grasspea accessions from the Near East. The cluster analysis

showed that Near East and North Africa formed the first cluster due to their low genetic distance. Similarities in physical features and in the culture of the inhabitants (Freeman and Morris 1965) might lead to the spread of grasspea germplasm from the Near East to North Africa. On the other hand the presence in the same cluster of geographically distant region such as South Asia and Sudan-Ethiopia may be because particular forms and landraces are more frequent within the germplasm from these region than from other regions.

It can be concluded that 1) significant variability exists for isozyme and morphological traits in grasspea; 2) accessions from the Near East showed the highest level of genetic diversity and this area should be explored further by grasspea breeders as a source of valuable genetic material; 3) accessions from South Asia and Sudan-Ethiopia were genetically close, even though these regions are widely separated geographically, 4) accessions from Southern Peninsular Europe and North America were genetically close, suggesting that most grasspea introductions into North America came from Southern Peninsular Europe, and 5) the large genetic distance between the accessions from South America and those from other regions probably reflects a diverse origin of the few (2) accessions from that region.

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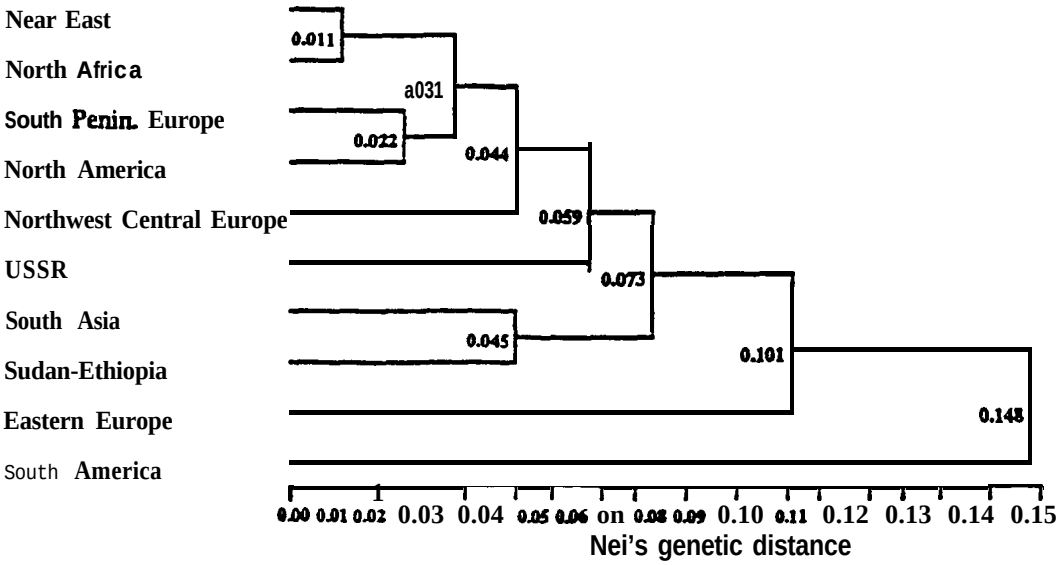

Figure 1. Dendrogram for regions based on Nei's genetic distance for isozyme traits in grasspea.

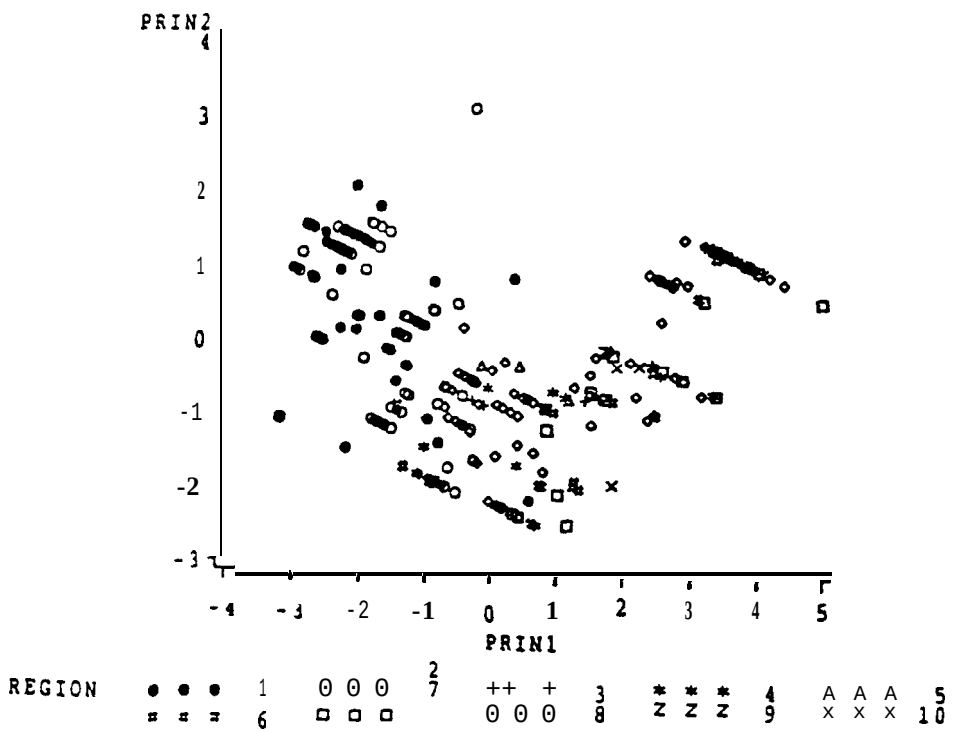

Figure 2. Scatter diagram for principal component analysis of morphological traits in grasspea. The description of each region is given in Table 4.