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Genetic variations in accessions of *Lathyrus sativus* L.

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Eighteen grass pea (*Lathyrus sativus* L.) accessions (donated from USDA germplasm) collected from different geographical regions were evaluated for variations of seed weight, and seed protein content. Environmental factors may not be most appropriate for explaining variations in seed weight. The presence of small-seeded accessions in Eastern Africa possibly indicate this sub-region as a new center of origin of *L. sativus.* There were no correlations between protein content and seed weight indicating genetic independence. Multivariate analysis (cluster and factor analysis) based on protein analysis data showed a high genetic variability among the accessions of different geographical regions and a low variability among the accessions of the same region.

Key words: *Lathyrus sativus*, accessions, seed weight, protein, genetic variability.

Introduction

A major goal of genetic resource conservation is to conserve as wide a representation as possible of the array of extant genetic variations of target taxa (FERGUSON et al. 1998). This is irrespective of the relative frequency of any gene or linked gene complex in germplasm. Satisfying this objective is dependent in part on the efficiency of selection of species and location for the sampling of the genetic diversity. Most species display a complex of genetic variations along their range of distribution (MCCALL et al. 2004, MILLER and SCHAAL 2006). For landraces, this is a function of species characteristics, such as breeding system, migration and dispersal mechanisms, which determine the movement of genes among populations (ERSKINE 1997, HERLIHY and ECKERT 2004); biotic pressure, for example, competition, predation and local anthropogenic influence and biotic selection intensities determined by location (FERGUSON et al. 1998).

Genetic conservation strategies are initially concerned with understanding of the genetic variation within species and then by the geographical distribution of genetic variation. Such a study will increase sampling efficiency for meeting genetic resource management (FRANKEL et al. 1995, FERGUSON et al.1998).

Several qualitative and quantitative biochemical and molecular markers loci encoding storage proteins, isozymes or restriction fragment length polymorphism are currently available for measuring variation between closely related germplasm sources (ZORO BI 1999). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of seed

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storage proteins has proven a simple and effective method for distinguishing among cultivars of the largely cross-fertilized pasture grasses and legumes despite their high innate genetic variability (FERGUSON and GRAB 1986, GARDINAR and FORDE 1988, SAMMOUR 1988, CLARK et al.1989). Similar techniques have been used very extensively for cultivar identification in breeding crops (COOKE 1995) but to a lesser extent for the differentiation of cultivars of outbreeding species (GILLILAND 1989, SAMMOUR 1999). Seed protein electrophoresis has also become a useful tool in evolutionary studies to determine species relationships (SMARTT 1990, PRZYBYLSKA et al. 1999). The seed protein profiles reflect genetic affinities within a taxon and even between different biological entities (MAHMOUD et al. 2006, VAUGHAN and DENFORD 1968).

A few groups have studied genotype specificity, intra-specific variation, and genetic diversity in relation to geographical origin among accession of *L.sativus* by means of seed storage proteins; globulins,albumins, total seed proteins (PRZYBYLSKA et al. 1999, DELLA GATTA et al. 2002). They showed that the SDS-PAGE of albumins and globulins of different grass peas, even of the same geographic origin, have variations in number, width and intensity of bands, concluding that geographical origin does not influence specific seed protein content and its polymorphism.

In this study, we have used 100-seeds weight, protein content of the seed meal and SDS-PAGE of the total seed proteins to detect intra-specific variation in *L. sativus* and its relation to geographical origin.

Materials and methods

The present study covered 18 accessions of *L. sativus* (Tab. 1), obtained from the germplasm collection of the USDA, ARS, WRPIS Washington State University, Regional Plant Introduction Station, 59 Johnson Hall, P.O. 646402 Pullman, Washington, United States, 99164-6402.

Total proteins were extracted separately from 0.02 g air-dried seed meals of 18 *L. sativus* accessions in 1000 μL extraction buffer (0.0625 M Tris HCl pH 6.8, 2% SDS, 10% sucrose and 0.002% bromophenol blue) without and with 5% b-mercaptoethanol, for 24 hours at –4°C. After that time, the extracts were centrifuged for 10 minutes at 1000 g. Concentration of proteins in each sample was determined by using the Bradford method (BRADFORD 1976) and the final concentration was adjusted to 2 mg protein/ml of sample buffer. From each accession 30 μL of the extracted protein was boiled in a water bath for 5–10 min and loaded on sodium dodecyl sulfate-polyacrylamide gel containing 12.5% polyacrylamide (LAMMELI 1970) for electrophoresis. The gels were stained with Coomassie blue and visualized in white fluorescent light. Bovine serum albumin (67 KDa), ova albumin (43 KDa), β-lactoglobulin (36.5 KDa) and myoglobulin (18 KDa) were used as marker proteins.

Total seed proteins were quantitatively estimated by the method of BRADFORD (1976).

Chromosome numbers of the studied accessions were determined in root tips pretreated with 0.05% colchicines solution and fixed in 3:1 alcohol: acetic acid. Preparations were made using the Feulgen squash method and were made permanent by mounting in Canada balsam.

The band identification was based on electrophoretic mobility and by numerous side-by-side comparisons of protein extracts. The estimation of genetic diversity within and among the samples was based on 32 reproducibly scored bands identified in the zones of highest variation of protein profile (ranging from 110 to 15 KDa). The genetic diversity among the populations was evaluated by the Jaccard similarity index, cluster analysis and factor analysis. The analysis was performed using the frequencies of scored bands calculated for the populations. A dendrogram was constructed through the complete linkage-joining rule, using the software package »SYSTAT for Windows«, Version 7.0 copyright (C) 1997, SPSS INC.

Results

The total protein contents in seeds of *L. sativus* range from 28.7 to 41 with a mean value of 34.2 % (Tab. 1). The lowest protein content was found in an accession from Ethiopia and the highest in an accession from Italy. Most of the accessions examined (95%) had protein contents higher than 300 mg g^{-1}. We found significant variations in European accessions, where five accessions showed protein variations of 316–408 mg g^{-1}. The Asian accessions showed the narrowest range of protein content variations.

The measured buffer-extracted proteins in the examined accessions showed the pronounced effect of the supernumerary chromosome on the quantity of the total protein in the accession from Italy, while it had no effect on the protein quantities of some other accessions (Tab. 1).

For a better characterization of the *Lathyrus* germplasm investigated, relationships among protein content and 100-seeds weight were considered (Fig. 1). The distribution of the points indicates clearly the absence of correlation between protein content and 100--seeds weight. Nevertheless, it may be noticed that the protein content tends to be less variable for lower values of 100-seeds weight. In other words, a small range of 100-seeds weight variation corresponds to a narrow range of protein content variation.

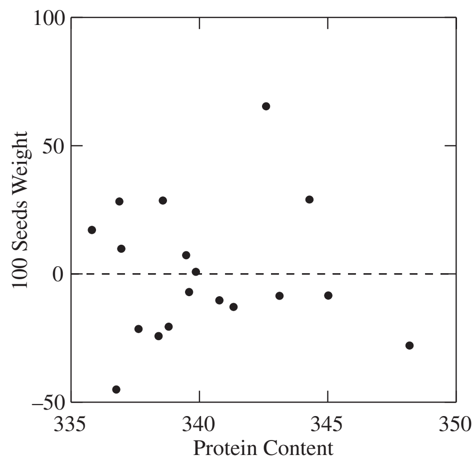

Fig. 1. Regression line showing the relationships among protein content and 100-seeds weight of 18 of *Lathyrus sativus* accessions.

Tab. 1. Origin, sub-region, accession number, total protein content (TPC), chromosome number and B chromosome in accessions of *L.sativus* L.

No.	Species .	Origin	Sub-region	Accession No.	Mean of TPC ± SD	Wt of 100 seeds in grams	Chromo-some No.	B Chromo-some
1	L.sativus	Egypt	Northern Africa	PI 283546	340.680 ± 09.348	14.19	14	0
2	*L.sativus*	Libya	Northern Africa	PI 283569	373.320 ± 06.120	23.41	14	0
3	*L.sativus*	Sudan	Sudano-Sahelian	PI 283564	314.160 ± 07.067	11.16	14	0
4	*L.sativus*	Ethiopia	Eastern Africa	PI 358601	291.720 ± 07.067	07.73	14	0
5	*L.sativus*	Tunisia	Northern Africa	PI 283597	320.280 ± 15.402	31.54	14	1
6	*L.sativus*	USSR	Eastern Europe	PI 210342	316.187 ± 14.122	09.54	14	1
7	*L.sativus*	Iran	Near East (Middle East)	PI 422533	332.520 ± 15.402	13.64	14	0
8	*L.sativus*	Afghanistan	Central Asia	PI 317438	318.240 ± 06.120	11.98	14	1
9	*L.sativus*	Bangladesh	Southern and Eastern Asia (Indian Subcontinent)	W6 12869	346.800 ± 09.348	08.14	14	1
10	*L.sativus*	Turkey	Near East (Middle East)	PI 577136	367.200 ± 12.240	11.51	14	0
11	*L.sativus*	Spain	Mediterranean Europe	PI 283563	336.600 ± 00.000	24.94	14	0
12	*L.sativus*	Germany	Western Europe	PI 209789	330.480 ± 06.120	16.10	14	0
13	*L.sativus*	Italy	Mediterranean Europe	PI 422540	408.000 ± 09.348	19.89	14	1
14	*L.sativus*	Serbia	Central Europe	PI370600	346.800 ± 09.348	13.40	14	0
15	*L.sativus*	Canada	Northern America	PI 283558	328.440 ± 09.348	17.24	14	1
16	*L.sativus*	Hungary	Central Europe	PI 422543	334.560 ± 17.667	20.99	14	0
17	*L.sativus*	India	Southern and Eastern Asia (Indian Subcontinent)	PI 391431	365.160 ± 12.740	07.98	14	0
18	*L.sativus*	Pakistan	Southern and Eastern Asia (Indian Subcontinent)	PI 426886	352.920 ± 03.533	05.75	14	1

The scatter plot for the 100-seeds weight (Fig. 2) showed that small seeds were most frequent in Asia (India and Pakistan) and Africa (Ethiopia and Sudan). The approximate ranges in protein content and seed weight of the *Lathyrus sativus* accessions analyzed are between 29–40% and 7.7–31.5 g. As regards the frequency distributions for both traits in relation to the geographical origin of the accessions, it is interesting to note that accessions with high protein content were detected in Italy, Libya and Turkey, and the largest seed sizes in Tunisia, Libya and Spain (Tab. 1). Such a seeds could be utilized in breeding programmes after further evaluation and characterization.

When proteins extracted from a composite sample of each accession of a mixed-breeding-systems (breeding/outbreeding) legume such as *L. sativus* (Fig. 3) were separated by

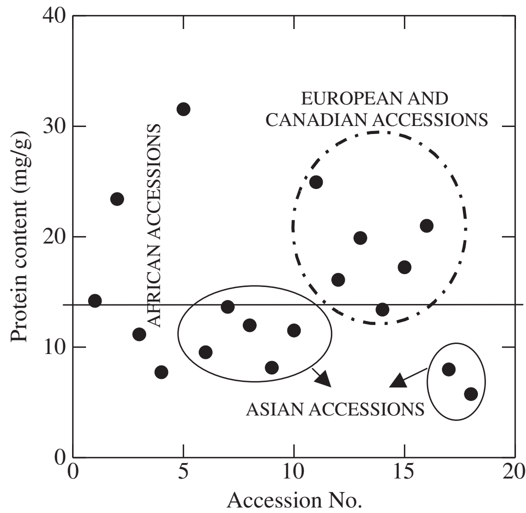

Fig. 2. Scatter plot of protein contents of 18 *L. sativus* accessions.

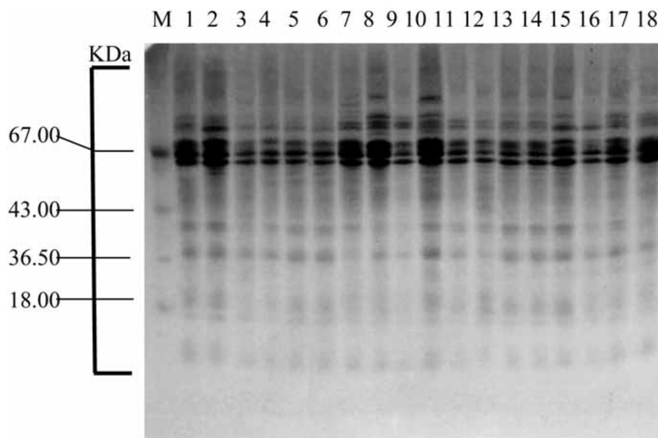

Fig.3. Electrophoregrames produced by SDS-PAGE analysis of seed proteins of 18 accessions of *L. sativus*, numbered as in Table 1.

SDS-PAGE under non-reducing and reducing conditions, the patterns of bands obtained were different for each accession. Theses differences were most marked amongst the proteins of higher molecular weight near the top of the gel.

The genetic distances, Jaccard's coefficient of similarity, among *Lathyrus sativus* accessions were based on protein data (Tab. 2). Jaccard's similarity coefficients ranged from 0.950 (between an accession from Tunisia and accessions from the USSR, Iran and Afghanistan) to 0.077 (between the accession from Egypt and accessions from the USSR, Iran and Afghanistan), with an average of 0.428.

The dendrogram produced using Euclidean distance matrix on average linkage (Fig. 4) shows two major groups of germplasm; one group consisted of closely related germplasm from Africa, except the accession from Tunisia, and the second group comprised germplasm from other countries. The second group was divided into two subgroups; subgroup 1 included most of the Asian accessions and subgroup 2 contained the European accessions. These subgroups, however, were partly region-specific.

An arbitrary genetic distance of 0.22 was used to divide countries into six groups on the basis of protein data. All accesssions from Africa with the exception of that from Tunisia were clustered in cluster1, with Jaccard coefficient ranging from 0.815 to 0.643. The Turkish accession was isolated from the other germplasm in cluster 2. The Indian and Pakistani accessions, collected from the center of diversity of *L. sativus,* formed cluster 4. The majority of Europian germplasm was clustered in cluster 5.

The matrix of eigenvectors and values of the principal components (PCs) resulting from the interactions of protein data (Tab. 3) shows that the protein data influencing 72.427% of the variability accumulated up to the first three components.

The magnitude and direction of a factor loading within a PC determines the importance and relationship between variables within a component. Principal Component 1 accounted

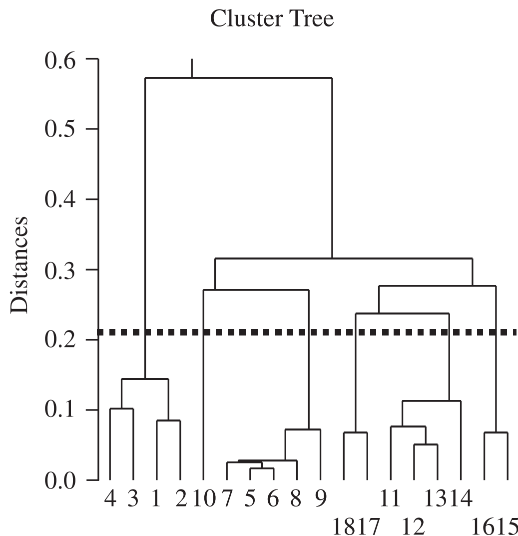

Fig. 4. Dendrogram showing the genetic relationships among of 18 accessions of *L. sativus* based on genetic distance of SDS-PAGE. Horizontal axis indicates genetic distance at 0.22%.

Tab. 2. Jaccard binary similarity coefficients

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1.000																	
2	0.815	1.000																
3	0.778	0.710	1.000															
4	0.643	0.700	0.786	1.000														
5	0.079	0.095	0.125	0.075	1.000													
6	0.077	0.093	0.122	0.073	0.950	1.000												
7	0.077	0.093	0.122	0.073	0.950	0.905	1.000											
8	0.077	0.093	0.122	0.073	0.950	0.905	0.905	1.000										
9	0.108	0.122	0.154	0.103	0.810	0.773	0.773	0.857	1.000									
10	0.167	0.175	0.150	0.100	0.444	0.429	0.429	0.429	0.393	1.000								
11	0.143	0.154	0.128	0.105	0.423	0.407	0.462	0.462	0.480	0.267	1.000							
12	0.143	0.154	0.158	0.135	0.370	0.357	0.407	0.407	0.480	0.226	0.800	1.000						
13	0.121	0.105	0.108	0.083	0.308	0.296	0.346	0.346	0.360	0.207	0.737	0.833	1.000					
14	0.194	0.200	0.237	0.154	0.429	0.414	0.464	0.464	0.538	0.323	0.625	0.773	0.714	1.000				
15	0.146	0.156	0.214	0.167	0.294	0.286	0.324	0.324	0.294	0.364	0.387	0.433	0.379	0.438	1.000			
16	0.184	0.190	0.256	0.205	0.313	0.303	0.344	0.344	0.355	0.265	0.464	0.519	0.462	0.517	0.846	1.000		
17	0.135	0.146	0.179	0.128	0.393	0.379	0.429	0.379	0.345	0.290	0.520	0.462	0.400	0.464	0.452	0.433	1.000	
18	0.143	0.154	0.222	0.167	0.370	0.357	0.357	0.357	0.370	0.310	0.500	0.440	0.375	0.444	0.433	0.414	0.810	1.000

Tab. 3. Origin, matrix of eigenvectors and values of the principal components for protein data of *L. sativus* L. accessions

No.	Species	Origin	Principal components		
			C1	C2	C3
1	L.sativus	Egypt	–0.470	0.559	0.547
2	*L.sativus*	Libya	–0.527	0.502	0.529
3	*L.sativus*	Sudan	–0.429	0.573	0.564
4	*L.sativus*	Ethiopia	–0.533	0.499	0.465
5	*L.sativus*	Tunisia	0.820	–0.294	0.456
6	*L.sativus*	USSR	0.793	–0.336	0.427
7	*L.sativus*	Iran	0.838	–0.257	0.386
8	*L.sativus*	Afghanistan	0.840	–0.268	0.404
9	*L.sativus*	Bangladesh	0.796	–0.114	0.426
10	*L.sativus*	Turkey	0.416	–0.192	0.160
11	*L.sativus*	Spain	0.730	0.426	–0.081
12	*L.sativus*	Germany	0.701	0.531	–0.135
13	*L.sativus*	Italy	0.650	0.492	–0.233
14	*L.sativus*	Serbia	0.695	0.484	0.023
15	*L.sativus*	Canada	0.471	0.337	–0.429
16	*L.sativus*	Hungary	0.509	0.493	–0.282
17	*L.sativus*	India	0.601	0.321	–0.144
18	*L.sativus*	Pakistan	0.564	0.384	–0.060
Variance Explained by Components			07.570	3.077	02.390
Percent of Total Variance Explained			42.058	17.093	13.276
Accumulated Eigenvectors			42.058	59.0 150	72.326

for 42.058% of the variation in protein data and was negative for Egypt, Libya, Sudan and Ethiopia and positive for the other accessions (Tab. 3). Principal Component 2 accounted for 17.093% of the variation and was negative for Tunisia, the USSR, Iran, Afghanistan, Bangladesh, Turkey and positive for the rest of the accessions. Principal Component 3 accounted for 13.276% of the variation and was negative for Spain, Germany, Italy, Canada, Hungary, India, and Pakistan and positive for the others.

Discussion

Evaluation of crop germplasm is essential to ensure its efficient and effective use. In the present investigation, high genetic variation was observed for SDS-PAGE, total protein of the seed meal and 100-seed weight. This indicated that improvement through simple selection for these traits is possible. However, broadening the genetic base from diverse sources is recommended to include most of the genetic determinants of these traits (LAGHETTI et al. 1998, GHAFOOR *et al*. 2001).

Traditionally, the variation in seed size is a trade-off with seed number . However, it is useful to consider whether a plant can vary its position in this trade-off in response to environmental conditions or if seed size is solely a genetic trait . The constant weight of the seed of *Ceratonia siliqua* supports the suggestion that seed weight is a genetic trait (REES 1997). On the other hand, the change in the seed size of *Hakea sericea* (DELLA GATTA et al. 2002, VENABLE 1992) and *L. sativus* accessions in the present study in response to changing environmental conditions suggested that the parent plant could control its seed number, seed size fitness ratio in response to environmental conditions. The variation of the seed size among different population of the species was attributed to the development process or the life cycle of the plant (WESTOBY et al. 1997). In other words, WESTOBY et al. (1997) believe that the seed size trait is embedded in the life cycle of the plant. However this variation, which is a development process, may itself enhance fitness. In much the same way that genetic variation in a population makes the population more able to adapt to change, variation in seed size in an individual plant may make that plant more able to adapt to a changing environment.

The origin of *Lathyrus sativus* is unknown; however, its presumed center of origin is Southwest and Central Asia (SMARTT 1990), Mediterranean and central Asia (DUKE 1981) or Central Asia and Abyssinia (VAVILOV 1951). Due to the smallest seed size, we suggest that the center of origin of *Lathyrus sativus* is Southern and Eastern Asia (Indian Subcontinent) and Eastern Africa. CHOWDHURY and SLINKARD (2000) suggested that the Near East and North Africa regions included the most variability for isozyme systems, which can indicate the grass pea area of origin. It was noticed that trends in diversity of *L. sativus* are similar to those found in other pulses, such as lentils and broad beans, in that smaller--seeded forms were found in southern and southwest Asia, whereas around the Mediterranean region almost all were highly cultivated forms with large white seeds and flowers (VAVILOV 1951, JACKSON and YUNUS 1984). This notation is in agreement with the diversity found in this study, where the smaller seeds were of the accessions from Pakistan, India and the larger seeds of those from Libya and Spain. In addition, small seeds were also recorded in Eastern Africa (Ethiopia).

The protein content found in this study is different from the values reported by GRANATI et al. (2003) and DELLA GATTA et al. (2002). The difference may be attributed to methodological or accession differences. In terms of methodology, GRANATI et al. (2003) and DELLA GATTA et al. (2002) used the most common protocol (Kjeldahl method) to determine the amount of total seed proteins. This protocol does not allow discrimination among the different sources of nitrogen in digested tissue, and the protein content is estimated using a conversion factor (VARGAS et al. 2000). The significant variation in protein content among the different accessions may be due to environmental factors such as geographical area, season of collecting, elevation, and annual temperature, precipitation and soil fertility. More studies are needed to determine the effects of the environment on the amount of the total seed proteins in seeds of *L. sativus*. Our data also reveal that there is more variation among accessions from a given sub-region, e.g. South African sub-region. This finding is important because it indicates that major environmental factors such as those discussed above might not be the most appropriate for explaining the levels of variation found in this study. The same finding was reported by VARGAS et al. (2000) in his study on phaseolin seed proteins in *Phaseolus lunatus L.* Genetic differences among accessions, which will be

discussed below, may explain some of the differences in protein content reported in this investigation.

The association between protein content and seed weight was not statistically significant. The absence of correlation supports the conclusion that both traits are under independent genetic control, as already evidenced by DIXIT et al. (1995) and GRANATI et al. (2003). Further research is needed to determine exactly the genetically control of this correlation. Thus accessions high in both protein content and/or 100-seeds weight may be useful parents in breeding improved genotypes. The smaller seeded accessions appear to be most promising as far as some combinations with a high but not the highest protein content are concerned. These facts call for more large collections to be analyzed and for more extensive research work to ascertain the seed storage protein content and its relationships with other seed traits.

In this investigation, each accession showed an identical electrophoretic pattern. These individual electrophoregrams of the accessions can be used as passport data for their genetic identity and can be used a good tool for testing core collection concepts and organizing genetic diversity in *L. sativus*. The differences between accessions were most marked amongest the proteins of higher molecular weight near the top of the gel and three subunits of molecular weights of 40 KDa (subunit of beta-Lathyrin), 24 KDa (major albumin) and 20 KDa (lectin) (ROSA and FERREIRA 2000). The three main types of unglycosylated subunits (alpha-lathyrin) with molecular weights between 50 and 66 kDa showed no variation. Each of alpha-lathyrin subunits produce, upon reduction, a heavy and a light polypeptide chain, by analogy with 11S in studied accessions (data not shown).

Cluster analyses based on SDS-PAGE analysis of protein peptides were more reliable, where most of the accessions of each group were collected from the same geographical region. Only a few accessions were scattered and separated from the main group. Of these, accession 5 was collected from Tunisia and grouped with the Asian accessions, 6 from USSR and grouped with Asian collection, 15 from Canada and collected with European accessions. This showed a sort of correlation between SDS-PAGE data and geographic origin.

Our SDS-PAGE results revealed that the total amount of variability accounted for the principal components was 72.727%; this percentage indicated that the accessions show a good association, due, probably, to parallel evolution and extensive breeding in the different geographical regions. The variability within the investigated accessions agrees with previous morpho-agronomic (POLIGNANO et al. 2005, TAVOLETTI et al. 2005, KUMAR and DUBEY 2003, TADESSE and BEKELE 2003), biochemical (CHOWDHURY and SLINKARD 2000; TADESSE and BEKELE 2001, 2004) and molecular studies (CROFT et al. 1999, CHTOUROU-GHORBEL et al. 2002, POLIGNANO et al. 2003, BELAID et al. 2006). However, the degree of this variability is different with the different tools used; whereas, RAPDs detected a relatively low genetic variation, isozymes and SDS-PAGE of storage proteins observed a high genetic diversity. The variability is also different among the sub-regions of the same geographical region, as we found in this study.

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