Isozymes variability of grasspea (*Lathyrus sativus* L.) in Ethiopia.

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Introduction

Grasspea (*Lathyrus sativus* L.) is the third important pulse crop after faba bean and chickpea in Ethiopia (CSA, 1998). It is grown in the off season (planted in September/October and harvested in January/February) on residual moisture in vertisols at altitudes ranging from 1600-2700 m.a.s.l., across the different administrative regions of the country. The ability of grasspea to tolerate both drought and flooding conditions, the low cost required for its production, its high protein content and capacity to ameliorate soil fertility make it an important subsistence and insurance crop of the poor family [(2)].

Excessive consumption of grasspea seeds by humans can result in an irreversible disorder of the lower limbs, known as "neurolathyrism", caused by the toxin oxalyl-diaminopropanoic acid (ODAP) present in the seeds [(6,9)]. Identification of varieties with low ODAP content is the primary objective in grasspea improvement for which a wider genetic base of germplasm is essential. Germplasm collection and characterisation is one of the strategies to increase the genetic base to meet the above objective. Diversity studies are highly important to guide such germplasm collection and selecting sites for *in situ* conservation [(1)].

Isozymes are practical and useful genetic and biochemical markers as well as good estimators of genetic variability in plant populations [(5)]. The most commonly used measures of intra-population variation are the percent of polymorphic loci, the effective number of alleles per locus and the mean proportion of loci heterozygous per individual. The last parameter is the expected mean heterozygosity assuming Hardy-Weinberg equilibrium. Studies to evaluate the total variation of isozymes are thus of paramount importance in order to determine genetic variability of populations and to assess diversity, which of course is important in germplasm collection and conservation. This study was carried out to determine the isozyme variability of in Ethiopian grasspea populations.

Materials and Methods

Isozyme analysis was done in ten accessions of grasspea selected from five clusters based on morphological diversity, i.e. two populations from each cluster were used. The selection of the two populations from each cluster was based on diversity index, origin and ODAP content. Twenty-five seedlings per accession were studied. Three and seven days old leaf samples were compared for extraction and better resolution. The three day old leaf gave better resolution and hence these were used for extraction. Crude extracts were prepared by macerating leaves in two drops of extraction buffer (0.05M sodium phosphate, pH 7.0, plus 0.2M 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 3MM chromatography paper. Horizontal electrophoresis was carried out in 12% starch gels.

Two buffer systems were used:

1. *Lithium borate buffer pH 8.3:* the gel buffer for this system contains 5.4 g tris base and 1.28 g anhydrous citric acid. The electrode buffer contains 1.2 g lithium hydroxide and 11.9 g boric acid (pH 8.3).
2. *Histidine tris citrate buffer pH 7.5:* the gel buffer for this system contains 8.3 g histidine-HCl and 0.03 g EDTA. The electrode buffer for this system contains 15.1g tris-base and 7.3 g citric acid. Twenty five samples were run on each gel plus two wicks dyed with bromophenol blue to act as a marker control. Electrophoresis was carried out at 4°C with a constant current of 70 milli ampere (250 volts) for lithium borate gels and 50 milli ampere (200 volts) for tris-citrate gels. Gels were run approximately 8 cm within 4-5 hours.

Three enzyme systems were selected for detailed analysis after a preliminary survey of five enzymes (ACP:EC 3.1.3.2, AAT:EC 2.6.1.1, EST:EC 3.1.1, PRX:EC 1.11.1.7, and LAP:EC 3.4.11.1) since they gave consistent results with this species. The three enzymes analysed were esterase (EST), aspartate aminotransferase (AAT) and acid phosphatase (ACP). Buffer system 1 was used for EST and AAT, while

buffer system 2 was used for ACP. In the first gel system, the gel was cut in to three slices. The top slice was discarded since most enzymes did not stain well on it. For buffer system 1, the second slice was used for EST and the third slice for AAT. For buffer system 2, the second slice was used for ACP.

The following staining recipes were used following protocols developed in the literature [2, 11].

For ACP: 50 ml 0.4 M sodium acetate buffer pH 5.0 which was used to pre-soak the gel for 20 minutes at 4°C, 50 milligram beta napthyl acid phosphate, 50 milligram fast blac kk salt, and 0.5 ml 10%$MgCl_2$.
For AAT: 50 ml 0.1 M tris-HCl (pH 8.5), 18 mg alpha ketoglutaric acid, 65 mg DL-aspartic acid, 250 mg PVP, 50 mg disodium EDTA, 710 mg Na_2HPO_4 and 200 mg fast blue BB salt.
For EST: 20 ml distilled water, 20 ml 0.2 M NaH_2PO_4, 10 ml 0.2 M Na_2HPO_4, 2 ml 1% α-naphtyl acetate and 125 mg fast blue BB salt and 1 ml acetate were used.

Variation in banding patterns was determined by the migration from the origin towards the anode. Isozyme zones were designated to define the general area on the zymogram within which the bands migrated. The zones were numbered from the fastest to the slowest migration from the point of insertion of the wicks in the gel. Scoring was made for those bands which were clearly visible. An assessment of isozyme phenotypic polymorphism was made using the overall banding patterns. Phenotypic polymorphism, genetic distance, degree of differentiation (FST) and hetrozygosity were determined using Biosys software [7]. A tentative genetic interpretation of the banding patterns was made based on the reported structure of each enzyme in different plant species [11] and particularly in related genera such as *Pisum, Lens* and *Vicia*, where the information was available.

Results and Discussion

Population variability. The genetic variability at seven loci in all populations is presented in Table 1. The mean number of alleles per locus ranged from 1.6 to 2.1, the lowest in population 219950 and the highest in population 236705. The polymorphic loci ranged from 57.1 to 85.7%, the lowest in population 219950 and the highest in populations 236705 and 46024. A locus is polymorphic if more than one allele is detected. As per this criteria, polymorphism was detected in all populations. The highest polymorphism was detected in populations collected from the Gondar region. This is in line with morphological data (not reported here). Mean heterozygosity for the populations ranged from 0.081 in population 46035 to 0.313 in population 226001.

Table 1. Genetic variability at 7 loci in all populations.

Population	Origin	Altitude (m)	Mean no. of alleles	% loci per locus	He* polymorphic
208449	Gojam	2300	1.7	71.4	0.189
207499	Gondar	2600	2.0	71.4	0.214
46035	Wollo	2375	1.9	71.4	0.081
219945	Tigray	1870	1.9	85.7	0.302
46024	Shoa	2460	1.9	85.7	0.297
219950	Tigray	2230	1.6	57.1	0.196
226001	Wollo	2400	1.9	71.4	0.170
46027	Shoa	2420	1.7	71.4	0.196
236711	Gojam	1840	1.7	71.4	0.170
236705	Gondar	1800	2.1	85.7	0.241

*He is expected heterozygosity

Marked differences in the extent of differentiation (FST) were shown between many loci (Table 2). The populations were differentiated markedly for AAT-1, AAT-2, and EST-2. The level of differentiation was low for ACP-3 (0.031) and for ACP-1 (0.118). The degree of differentiation (FST) of the individual loci ranged from 0.031 for ACP-3 to 0.784 for AAT-2. The mean FST value (0.346) is medium as compared to the average FST value for inbreeding species (0.510) [4]. Grasspea is both drought and flooding tolerant. In Gojam and Gondar it is grown in flooded vertisols, while in Wollo and Tigray it is grown in moisture stress conditions. Accordingly, the high FST value reflects adaptation to strong environmental dissimilarities or high level of genetic drift maintained by restricted gene flow between populations. Similar observations have been noted in barley [1].

The distance between populations ranged from 0.001 to 0.341 (Table 3). The highest distance (0.341) was between population 219950 from Tigray and population 46035 from Wollo. These two populations were from different regions separated by some distance, indicating that geographic isolation is one of the important factors responsible for the observed genetic distance disparity.

Correlation between morphological and isozyme diversity indices. The association between morphological diversity estimates (Shannon Weaver diversity index) and genetic diversity estimates from isozyme data (expected hetrozygosity estimate, H) at population level was negative and not significant (r = -0.25). Yunus *et. al*. 1991 also observed the absence of correlation of isozymes with morphological data in grasspea. The absence of correlation between markers indicates that there is no one best marker that can be used for diversity study. Hence, it is important to study diversity by using both morphological and molecular markers. Similar results have been reported by many authors in other crops [1,8,10].

Table 2. Summary of FST at all loci

Locus	FST	No. of alleles
AAT-1	0.6302	2
AAT-2	0.784	2
EST-1	0.188	2
EST-2	0.353	4
ACP-1	0.118	2
ACP-2	0.157	3
ACP-3	0.031	3
Mean	0.346	

Table 3. Matrix of genetic distance coefficients. Below diagonal: unbiased minimum distance [7].

	Population									
Population	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	-									
2	0.067	-								
3	0.117	0.174	-							
4	0.068	0.031	0.164	-						
5	0.109	0 .039	0.183	0.001	-					
6	0.212	0.152	0.341	0.151	0.155	-				
7	0.262	0.231	0.213	0.24	0.245	0.097	-			
8	0.058	0.044	0.140	0.008	0.021	0.132	0.228	-		
9	0.078	0.040	0.202	0.030	0.037	0.204	0.278	0.067	-	
10	0.179	0 .180	0.120	0.180	0.214	0.285	0.166	0.168	0.255	-

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