SINET: Ethiop. J. Sci., 27(2):153–160, 2004
 ISSN: 0379–2897

ISOZYMES, PROTEIN AND ODAP VARIABILITY OF GRASSPEA (*LATHYRUS SATIVUS* L.) IN ETHIOPIA

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ABSTRACT: Variability of grass pea landrace populations for isozymes, Oxalyl Diamino Propanoic acid (ODAP) and protein contents were investigated following standard procedures to detect genetic variation. The result indicated that phenotypic polymorphism was observed for the three enzymes, the highest being for esterase. The degree of differentiation (FST) of the individual loci ranged from 0.031 (for ACP-3) to 0.784 (for AAT-2). The highest polymorphism was detected in populations collected from Gondar region indicating the need for priority germplasm collection and site selection for *in situ* conservation in this region. The ODAP and protein analyses also revealed presence of variability among the populations. Fortunately, four entries with low ODAP contents ranging from 0.14% to 0.18% were identified in populations collected from different regions indicating the variation of this trait within population and between regions. These lines with low ODAP content are obviously considered important for further breeding program.

Key words/phrases: Ethiopia, grass pea , isozyme, *Lathyrus sativus*, *ODAP*

INTRODUCTION

Grass pea (*Lathyrus sativus* L.) is produced as a major crop in Bangladesh, China, India, Nepal, Pakistan and Ethiopia (Haque and Manan, 1989; Hanbury *et al*., 1999). It is the third important pulse crop after fababean and chickpea in Ethiopia with 142,170 ha of production area and 1,047,440 q of production (CSA, 1998). It is grown in the off-season (planted in September or October and harvested in January /February) under residual moisture on vertisols in altitudes ranging from 1600-2700 metres above sea level (m.a.s.l.) in the different administrative regions of the country. The ability of grass pea to tolerate both drought and flooding conditions, its low cost of production, its high protein content, and its capacity to ameliorate soil fertility all make it an important subsistence and insurance crop for poor farmer (Wuletaw Tadesse *et al*., 1997).

Excessive consumption of grass pea seeds in humans result in an irreversible disorder of the lower limbs (neurolathyrism), due to the toxin-Oxalyl Diamino Propanoic acid (ODAP) present in the seeds (Haque and Manan, 1989; Roy *et al*., 1989). Identification of varieties with low ODAP content is the primary objective in grass pea improvement for which a wide genetic base is highly required. Germplasm collection and characterisation is one of the strategies to increase the genetic base for the above objective. Diversity studies are highly important to guide such germplasm collection and selecting sites for *in situ* conservation (Abebe Demissie and Bjørnstad, 1997).

Isozymes are practical and useful biochemical markers as well as good estimators of genetic variability in plant populations (Hamrick and Godt, 1997). The most commonly used measures of intrapopulational variation are the percent of polymorphic loci, the number of alleles per locus, the effective number of alleles per locus, and the mean proportion of loci hetrozygous per individual. This last parameter is the expected mean hetrozygosity, assuming Hardy-Weinberg equilibrium. Studies aiming at obtaining insight into the total variation of isozymes are thus of paramount important to determine genetic variability of populations and to asses diversity, which, of course, is importance in germplasm collection and conservation.

Campbell and Tiwari (1997) had reported the presence of considerable variation for seed ODAP concentration in *Lathyrus sativus* genotypes collected from different countries. Analysis for ODAP and protein contents is therefore important to detect the genetic variation in grass pea landrace populations, for which previous studies are lacking in Ethiopia. Hence this initiative has been taken to determine variability of isozymes, ODAP and protein contents in landrace populations of Ethiopian grass pea germplasm.

MATERIALS AND METHODS

Isozyme analysis

Ten landrace populations of grass pea (Table 1) selected based on diversity index, region of origin, and ODAP content (the detail is indicated in Wuletaw Tadesse, 1999) were used for this study. Twenty-five seedlings per accession were studied. Three and seven days old leaf samples were compared for extraction and better resolution. The three days old leaf gave better resolution and hence extraction was made from them. 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 gel electrophoresis was carried out using 12% starch gel.

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 15.1 g 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 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 in 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 for this study. The three enzymes analysed were: esterase **(EST)**, aspartate amino transferase **(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 buffer system, the gel was cut into 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**. In buffer system 2, the second slice was used for **ACP**.

The following staining recipes were used following protocols developed by Wendel and Weeden (1990) and Chamberlin (1998).

F**or 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 mg beta napthyl acid phosphate, 50 mg fast blac kk salt, and 0.5 ml 10% $MgCl_2$ were used.

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 were used. **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% 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 BIOSIS-1 software (Swofford and Selander, 1989) which is formulated following the method of Nei (1978). A tentative genetic interpretations of the banding patterns was made based on the reported structure of each enzyme in different plant species (Wendel and Weeden, 1990) and particularly in related genera such as *Pisum, Lens* and *Vicia*, for which the information is already available. Correlation of isozme diversity index (He) with morphologica diversity index was carried out for each of the ten populations using Mstat c software.

ODAP and protein contents analyses

Fifty grass pea landrace populations collected from different administrative regions of Ethiopia (Fig. 1) were planted and characterized for morphological traits at Adet Research Centre, Ethiopia during the 1998/99 crop season (the detail is indicated in Wuletaw Tadesse, 1999). Three genotypes from each population, total 150 genotypes, were selected based on the morphological evaluation and were harvested separately. ODAP content for these genotypes was determined following the method developed by Rao *et al*. (1964) at the Department of Chemistry, Addis Ababa University. Similarly, protein content for the 50 grass pea populations was determined using the method developed by Association of Analytical Chemists (AOAC) (1990) at the Institute of Biodiversity Conservation and Research, Ethiopia.

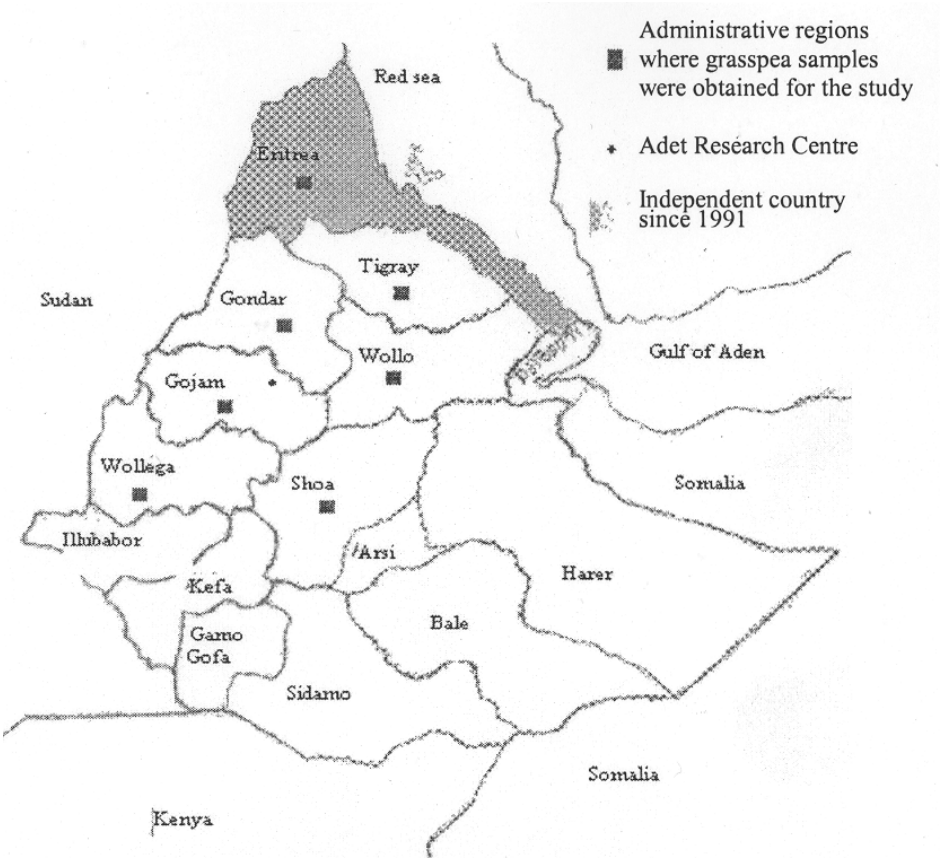

Fig. 1. Map of Ethiopia indicating the former administrative regions.

RESULTS AND DISCUSSION

Isozymes variability

The genetic variability at seven loci in all populations is indicated in Table 1. The mean number of alleles per locus ranged from 1.6 to 2.1 with a mean of 1.84. The lowest being for population # 219950 while the highest being for population # 236705.

The percentage of polymorphic loci ranged from 57.1 to 85.7 with a mean of 74.3 (Table 1). The lowest range was for population # 219950 and the highest range was for population #s 236705 and 46024. In this case, a locus is said polymorphic if more than one allele was detected. As per this criterion, polymorphism was detected in all populations. The highest polymorphism was detected from populations collected in Gondar region. The highest polymorphism at morphological level was also recorded from populations of this region (Wuletaw Tadesse, 1999).

Table 1. Genetic variability at 7 loci in ten grass pea populations.

Population	Origin	Altitude (m)	Mean no. of alleles/locus	% loci polymorphic	He*
A. 208449	Gojam	2300	1.7	71.4	0.189
B . 207499	Gondar	2600	2.0	71.4	0.214
C. 46035	Wollo	2375	1.9	71.4	0.081
D. 219945	Tigray	1870	1.9	85.7	0.302
E. 46024	Shoa	2460	1.9	85.7	0.297
F. 219950	Tigray	2230	1.6	57.1	0.196
G. 226001	Wollo	2400	1.9	71.4	0.170
H. 46027	Shoa	2420	1.7	71.4	0.196
I. 236711	Gojam	1840	1.7	71.4	0.170
J. 236705	Gondar	1800	2.1	85.7	0.241
Mean			1.84	74.3	0.205

*He= expected hetrozygosity at 5%

Mean hertrozygosity for the populations ranged from 0.081 in population # 46035 to 0.302 in population # 219945, with a mean of 0.205 (Table 1). As indicated in Table 2, marked differences in the extent of differentiation (FST) were shown between many loci. The populations were differentiated among themselves 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).

Table 2. Summary of F-statistics at all loci.

Locus	F_{ST}	Number of allels
AAT-1	0.630	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	2.57

The degree of differentiation (F_{ST}) of the individual loci ranged from 0.031 for ACP-3 to 0.784 for AAT-2. The mean F_{ST} value, 0.346, is medium as compared to the average F_{ST} value for inbreeding species (0.510) reported by Hamrick and Godt (1990). High F_{ST} value reflects adaptation to strong environmental dissimilarities or high level of genetic drift maintained by restricted gene flow between populations (Abebe Dermissie and Bjørnstand, 1997). The medium mean FST value in grass pea could be attributed to the similarity of its growing conditions since grass pea is a drought tolerant crop grown mostly in the off-season under residual moisture, in addition to other factors .

The unbiased minimum distance between populations ranged from 0.001 to 0.341 (Table 3). The highest distance (0.341) was between population # 219950 from Tigray region and population # 46035 from Wollo region. These two populations were from different regions with much physical distances and absence of common marketing and social interactions among the rural farmers of the different regions, indicating that isolation by geographic region and physical distance is one of the important factors responsible for the observed genetic distance disparity.

Oxalyl di-amino propanoic acid (ODAP) variation

ODAP variability of the 50 grass pea landrace populations and 150 entries are indicated in Figs 2 and 3, respectively.

The variation of ODAP between entries ranged from 0.149 to 0.916% (Table 4). Fortunately, out of the 150 entries (three from each population), 4 entries were in the safe range (below 0.2%). The first entry with very low ODAP content (0.14%) was 46027-r2-3, which is a selection from population # 46027 collected from Shoa Region.

Table 3. Matrix of genetic distance coefficients.

Population	A	B	C	D	E	F	G	H	I	J
A	*****									
B	0.067	*****								
C	0.117	0.174	*****							
D	0.068	0.031	0.164	*****						
E	0.109	0 .039	0.183	0.001	*****					
F	0.212	0.152	0.341	0.151	0.155	*****				
G	0.262	0.231	0.213	0.240	0.245	0.097	*****			
H	0.058	0.044	0.140	0.008	0.021	0.132	0.228	*****		
I	0.078	0.040	0.202	0.030	0.037	0.204	0.278	0.067	*****	
J	0.179	0 .180	0.120	0.180	0.214	0.285	0.166	0.168	0.255	*****

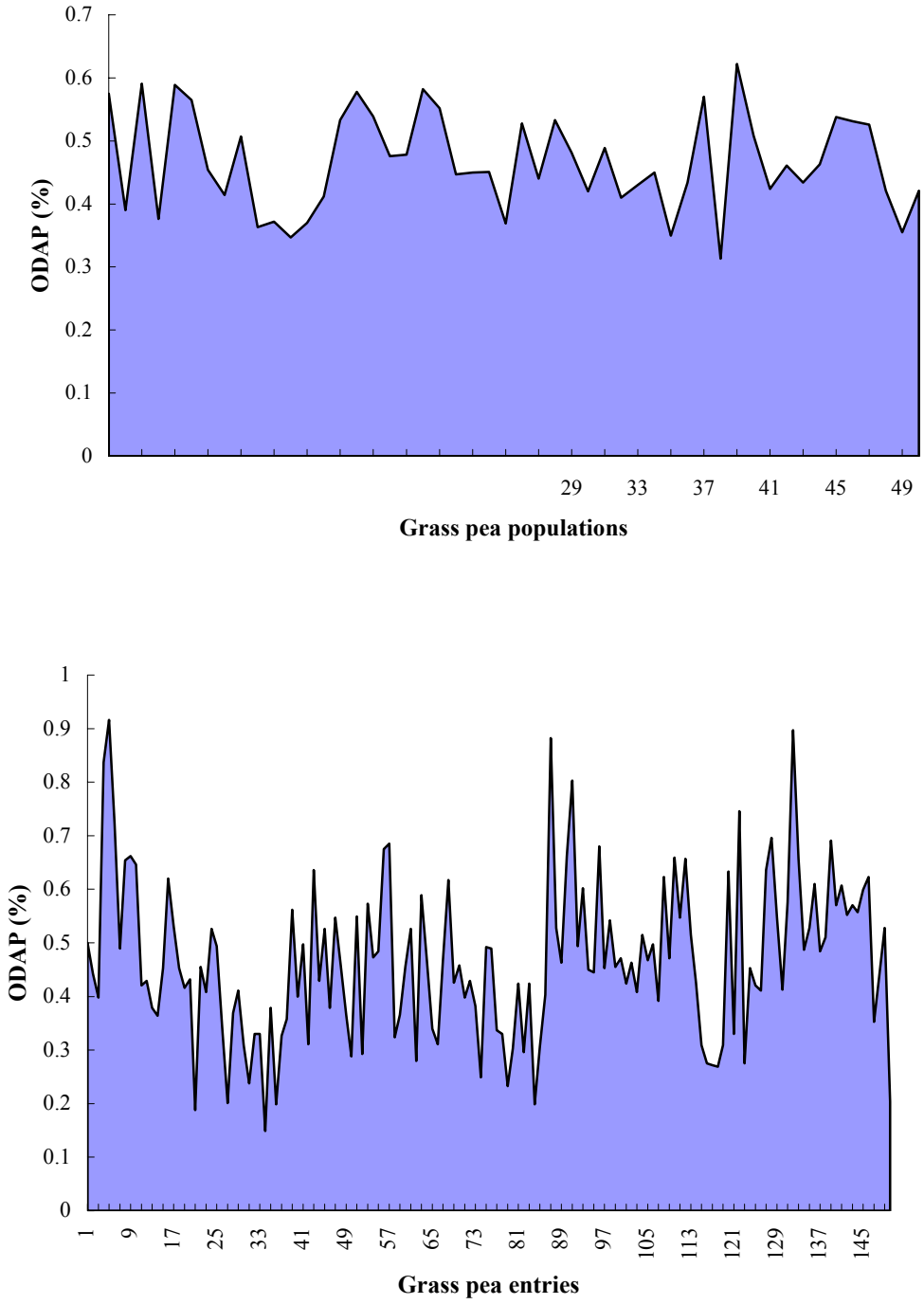

Fig. 3. Variability of ODAP in 150 grass pea entries.

Table 4. Variability in ODAP and Protein contents of 50 grass pea populations at Adet, Ethiopia, 1999.

Variables	Mean± SE	Minimum	Maximum
ODAP in 50 Populations (%)	0.470±0.01	0.31	0.62
ODAP in 150 entries (%)	0.469±12.10	0.149	0.916
Protein contents (%)	28.7±0.25	23.93	31.94

Three other entries, 46106-r2-3, 46033-r2-2 and 207497-r1-5, were selections from populations of Wollo, Gonder and Tigray Regions, respectively. This result indicates the presence of variability for ODAP among regions. The presence of genetic variation in landrace populations of grass pea was also reported by Hanbury *et al.* (1999).

Further collection and characterization of germplasms mainly from these areas would be highly important in order to identify genotypes with low ODAP content and thereby to combat the menace of lathyrism.

Kaul *et al.* (1986) had indicated that environment does not strongly mask the effects of genetic factors influencing ODAP content in seeds. On the other hand, Wuletaw Tadesse (1998) indicated that varieties have different ODAP content at different locations. Lambein and Kuo (1997) also indicated that the ODAP content of genotypes is negatively associated with zinc content of the soil. The reports on the effect of environment on ODAP content are not yet in the same line. Accordingly, these genotypes should be tested for ODAP stability across the potential grass pea growing areas before intending to release them as an interim measure to tackle lathyrism. Maximum care, however, needs to be taken during experimentation to reduce out-crossing between the low ODAP genotypes and the local cultivar since about 12% out-crossing is expected in grass pea (Kaul, 1990).

Variability in protein content

The variability in protein content among the 50 grass pea landrace populations is indicated in Fig 4. The highest protein percentage (31.94%) was recorded for population # 219946, which is collected from Tigray Region at 2080 m.a.s.l. and the lowest protein content (23.93%) was recorded for population # 46030, collected from Gojam region at 2300 m.a.s.l. (Table 4).

Correlation between morphological and isozyme diversity indices

The association between morphological diversity estimates (Shanon Weaver diversity index) and genetic diversity estimates from isozyme data (expected hetrozygosity estimate, H) at population level was negative and non significant (r =-0.25). Yunus *et al.* (1991) also observed the absence of correlation of isozyme data with morphological data in grass pea. Similar result was obtained by many authors for other crops (Price *et al.*, 1984; Abebe Demisse and Bjørnstad, 1997; Seifu Tsegaye, 1997). 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.

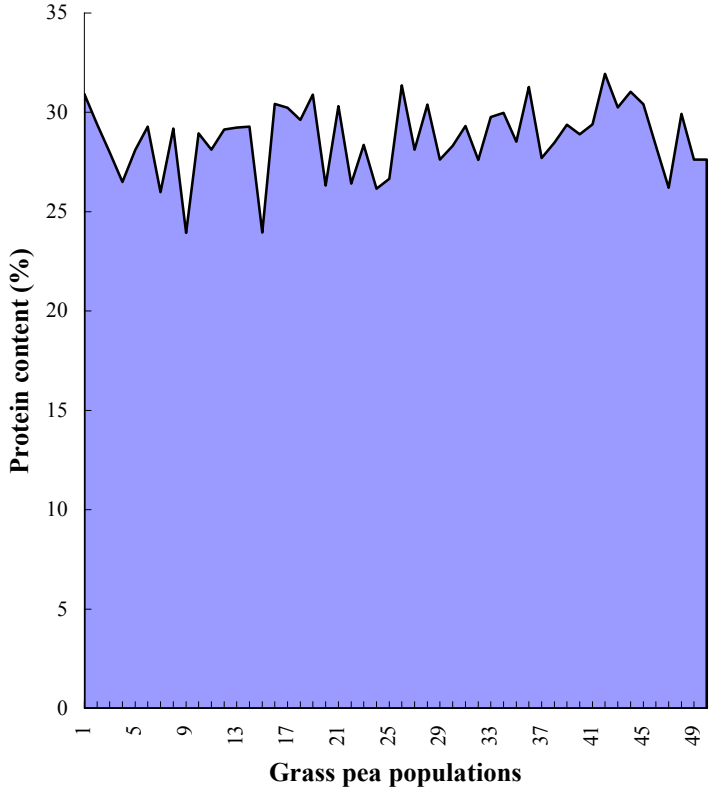

Fig. 4. Variability of protein content in 50 grass pea populations.

SUMMARY AND CONCLUSION

In this study the variability of landrace populations of grass pea for isozymes, protein and ODAP contents were determined. In the isozyme study, a mean FST value of 0.346 is obtained. This value is medium as compared to the average FST value for inbreeding species (0.510) reported by Hamrick and Godt (1990).

The highest allelic polymorphism was detected from populations collected in Gondar Region indicating the presence of much genetic diversity of grass pea in this region. Thus, it is advisable to give priority for this region during germplasm collection for *ex situ* conservation. Furthermore, sites for *in situ* conservation needs to be selected in the region so as to tackle the increasing rate of genetic erosion in grass pea due to the expansion of high yielding varieties of cereals, especially wheat and maize through the Extension Program. The absence of correlation between morphological and isozyme diversity indices indicates that there is no single best marker in diversity study. Therefore, further study is called for using molecular markers such as Restriction Length Polymorphism (RFLP) and Randomyl Amplified DANN (RAPD).

The variation of ODAP between entries ranged from 0.14 to 0.91% while the variability in protein content among populations ranges from 23.93 to 31.94%. The ODAP analyses from single plants enabled the identification of entries within the safe range of ODAP content (below 0.2%), indicating the importance of landraces in screening for low ODAP content and thereby to tackle the menace of lathyrism. These lines with low ODAP will be used in content breeding program so as to exploit the immense potential of this hardy pulse and thereby utilize it widely for sustainable agricultural development, especially in the drought prone areas of the country. However, stability study is important before releasing these lines since ODAP is highly influenced by environmental factors (Dixit *et al*., 1997; Wuletaw Tadesse, 1998).

ACKNOWLEDGEMENTS

This study is financially supported by the Institute of Biodiversity Conservation and Research, Ethiopia. The first author wishes to express his gratitude to the Amhara National Regional State for covering personal cost during the study.

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