Pak. J. Bot., 33(2): 181-186, 2001.

STUDIES ON INHERITANCE OF SOME ISOZYMES IN LENTIL (*LENS CULINARIS*)

M. EHTISHAM-UL-HAQ, M. MUNIR, BASHIR A. MALIK AND NASIR M. MINHAS

Department of Plant Breeding and Genetics, University of Arid Agriculture, Rawalpindi, Pakistan.

Abstract

Inheritance of three isozymes i.e., Leucine aminopeptidase-1, Phosphoglucoisomerase-1, and Phosphoglucomutase-2 in lentil was investigated. The plants were grown in greenhouse and the isozyme analysis was done by starch gel electrophoresis. χ^2 procedure was applied to test the goodness-of-fit for the observed and hypothesized ratios. All the three isozymes showed a monogenic inheritance pattern with co-dominant alleles for fast and slow allozymes. Heterozygous plants can be recognized at early stages and selfed plants may be discarded immediately. Their use in lentil improvement has been discussed.

Introduction

Lentil is an important pulse crop. It possesses an ability to tolerate extreme arid conditions and is therefore successfully grown as major winter (Rabi) crop in barani tracts of both Pakistan and India. As it performs well even on relatively low moisture and poor fertility conditions, it can be grown on such areas which are normally considered as unsuitable for other cash crops.

The objective of this study was to investigate the inheritance of isozymes of the enzyme leucine amino peptidase (LAP), phosphoglucoisomerase (PGI) and phosphoglucomutase (PGM). Isozymes are the different forms of the same enzyme which catalyse the same biochemical reaction but are coded by different loci. One isozyme may have further variants called allozymes and they are under the control of same locus but different alleles. Both isozymes and allozymes differ in their electrophoretic mobility due to variations in their molecular weights and charge ratio. Isozymes can be used as genetic markers and have numerous applications in plant breeding. They are also used to resolve the phylogenetic relationships among different taxa (Lazaro & Aguinagalde, 1998). The most important use of isozymes can be their linkage relationship to economically important quantitative traits (Vaillaincourt, 1989). Use of certain morphological traits and isozymes as genetic markers in lentil has been increasing in the recent past (Vaillancourt & Slinkard, 1992).

The starch gel electrophoresis apparatus for isozyme analysis has only recently been set up in the evaluation laboratory of Plant Genetic Resources Institute (PGRI), at National Agricultural Research Center, Islamabad. These facilities were used to analyze the local varieties and advanced lines of lentil. Skibinski *et al.*, (1984) reported variation at a polymorphic locus of enzyme aspartate amino transferase in a sample of 298 accessions of lentil germplasm from ICARDA. Two alleles Aat^F and Aat^S were reported with global frequencies of 0.51 and 0.49, respectively. Weeden *et al.*, (1984) showed that resistance to *Fusarium* wilt was controlled by a locus closely linked to Est-s, coding a

seed esterase. They also reported that resistance to bean yellow mosaic virus can be predicted with 98% accuracy by allozyme PGM-p linkage. Zamir & Ladizinsky (1984) examined the genetics of 8 enzymes in lentil and concluded that the allozymes at each locus behaved in a co-dominant manner and segregated in the expected Mendelian fashion. Linkage tests between these loci and epicotyl colour revealed 2 linkage groups that involved 5 loci; the rest were independent of each other. Vaillancourt (1989) studied the mode of inheritance and linkage of morphological markers and isozymes in lentil at the University of Saskatchewan Canada. All 17 isozymes exhibited codominant segregation for a single gene. Such studies in lentil have not been reported in Pakistan.

Materials and Methods

The research material comprised of 20 lentil genotypes and 18 hybrids between these genotypes. These hybrids and the varieties/lines were obtained from the Pulses Programme and gene bank of Plant Genetic Resources Institute (PGRI), National Agricultural Research Centre (NARC), Islamabad.

The following four categories of lentil genotypes were used:

a. Four commercial varieties viz., Masoor-85, Masoor-93, Mansehra-89, and Dashat of lentil (*Lens culinaris*) released in Pakistan.
b. Seven exotic varieties / lines of lentil (*Lens culinaris*) viz., 66546, Red Chief, Laird, ILL-7200, ILL-87104, LS-92-24 and LS-92-25.
c. Five advanced lines of lentil (*Lens culinaris*) viz., 91516, 91517, NL-96, NL-731 and M-932.
d. Four accessions of wild lentil (*Lens orientalis*) viz., LO-1, LO-5,LO-7 and LO-78.

Isozyme analyses was carried out for leucine amino peptidase (LAP), phosphoglucoisomerase (PGI) and phosphoglucomutase (PGM) using the starch gel electrophoresis (Tinnemore, 1990). Leaves from the young seedlings were collected and crushed in extraction-buffer. Paper wicks were soaked in crude extract of leaves and were loaded on starch gel. The gel was run for 3-4 hours at 45 mA. After this gel was removed and thin slices of the gel were cut and put into different containers having stains for different isozymes. After overnight staining, the gel slices were read for the presence of different isozymes and allozymes.

First Year: During 1996-97 Rabi season, the putative hybrids (Table 1) and their parents were sown in pots in greenhouse of PGRI. The putative hybrids were screened for the true hybrids and the selfed plants through isozyme and allozyme analysis.

Second Year: The F_2 population of the cross No.14 from Table 1 (Mansehra-89 X LO-78) was raised in pots in greenhouse of PGRI, during 1997-98 Rabi season and the segregating ratios for homozygous and heterozygous allozymes were counted. The ratios calculated were tested for expected ratios by using the Chi-square (χ^2) test for goodness-of-fit (Steel & Torrie, 1980). The probability for each χ^2 value was checked from MS Excel version 5.

Table 1. The hybrids used for isozyme and allozyme analyses.

S. No.	Female parent		Male parent
1.	91516	X	66546
2.	Dashat	X	LO-5
3.	Dashat	X	R. Chief
4.	ILL-7200	X	Masoor-85
5.	ILL-87104	X	Mansehra-89
6.	LAIRD	X	LO-5
7.	LS-92-24	X	66546
8.	LS-92-24	X	91517
9.	LS-92-24	X	DASHAT
10.	LS-92-24	X	LO-7
11.	LS-92-25	X	91517
12.	LS-92-25-	X	NL-731
13.	Mansehra-89	X	LO-1
14.	Mansehra-89	X	LO-78
15.	Mansehra-89	X	Masoor-85
16.	Mansehra-89	X	NL-731
17.	Mansehra-89	X	NL-96
18.	M-932	X	Mansehra-89

Results and Discussion

First Year:

A. A number of accessions which were parents of the hybrid material were analysed. Two zones of activity were found on the gel for each of the enzyme in all accessions studied. The slow moving zone was designated as 1 (e.g., LAP-1) and fast moving as 2 (e.g., LAP-2). Then the allozymes variation for each isozyme was studied. The results of allozyme variation are given in Table 2 which shows that the maximum variation was present at LAP-1 locus. There were two allozymes i.e., a slow moving allozyme (S) and a fast moving allozyme (F). The accessions studied are quite diverse at this locus. On the other hand, LAP-2 zone was not very clear and it was not possible to score it for S or F allozymes. Similarly at PGI-1 zone, two allozymes F and S were recognized but no variation was found at PGI-2 locus and F or S symbol could not be designated to the isozyme present. At PGM-1 locus, considerable variation was found and two allozymes F and S were identified. At PGM-2 locus all the accessions showed the same allozyme designated as F, while only accession LO-78 (*Len orientalis*) showed a slow moving allozyme called S.

B. In the second part of the 1st year study, 18 putative F_1 plants were grown in pots in the green house. These plants were analysed for allozyme variation for the three isozymes i. e., LAP-1, PGI-1 and PGM-2.

The results of this analysis presented in Table 3 shows that when both the parents differed for the allozymes, the hybrid contained both the allozymes, F and S, and was designated as FS. This indicates that alleles for the allozymes are co-dominant and both are present in F_1 plants.

Table 2. Allozyme variation present in the isozymes in the acccssions of lcntil.

S.No.	Accessions	LAP-1	LAP-2	PGI-1	PGI-2	PGM-1	PGM-2
1.	66547	S	N.S.*	F	N.V.**	F	F
2.	91516	S	N.S.	F	N.V.	F	F
3.	66546	S	N.S.	F	N.V.	F	F
4.	Dashat	S	N.S.	F	N.V	S	F
5.	LO-5	F	N.S.	F	N.V.	S	F
6.	R. Chief	F	N.S.	F	N.V.	F	F
7.	ILL-7200	F	N.S.	F	N.V.	S	F
8.	M-85	S	N.S.	F	N.V.	S	F
9.	ILL-87104	S	N.S.	F	N.V	F	F
10.	M-89	F	N.S.	F	N.V.	S	F
11.	Laird	S	N.S.	F	N.V.	S	F
12.	LS-92-24	F	N.S.	F	N.V.	S	F
13.	LO-7	S	N.S.	F	N.V.	S	F
14.	91517	S	N.S.	F	N.V	F	F
15.	LS-92-25	F	N.S.	F	N.V.	F	F
16.	NL-731	S	N.S.	F	N.V.	S	F
17.	LO-1	S	N.S.	F	N.V.	F	F
18.	LO-78	S	N.S.	F	N.V.	F	F
19.	M-932	S	N.S.	F	N.V	F	F
20.	LO-6	S	N.S.	F	N.V.	F	F

*N.S. = No variation, **N.S. = Not scorable, S = Slow, F = Fast

Table 3. Allozyme variation in putative F_1 plants in lentil genotypes.

S.No.	Female	Male	LAP-1	PGI-1	PGM-2	Hybrid	Selfed
1.	91516	66546	S*	F	FS	+	-
2.	Dashat	LO-5	FS	F	FS	+	-
3.	Dashat	R. Chief	S	F	S	-	+
4.	ILL-7200	M-85	F	F	S	-	+
5.	ILL-87104	M-89	FS	F	S	+	-
6.	Laird	LO-5	FS	F	FS	+	-
7.	LS-92-24	66546	FS	F	S	+	-
8.	LS-92-24	91517	FS	F	FS	+	-
9.	LS-92-24	Dashat	FS	F	S	+	-
10.	LS-92-24	LO-7	FS	F	FS	+	-
11.	LS-92-25	91517	FS	F	FS	+	-
12.	LS-92-25-	LN-731	FS	FS	FS	+	-
13.	M-89	LO-1	FS	F	S	+	-
14.	M-89	LO-78	FS	FS	FS	+	-
15.	M-89	M-85	FS	F	FS	+	-
16.	M-89	NL-731	FS	F	S	+	-
17.	M-89	NL-96	FS	F	S	+	-
18.	M-932	M-89	S	F	F	-	+

*S = slow, F = fast, FS = both fast & slow, + present, and - absent.

Table 4. Allozyme variation in F_2 population for three isozymes in lentil genotypes

S.No.		Parents	LAP-1	PGI-1	PGM-2
1.	Female	Mansehra-89	FF	FF	FF
2.	Male	LO-78	SS	SS	SS
3.	Hybrid	F_1	FS	FS	FS

Table 5. Chi-square values of F_2 phenotypes for 1:2:1 segregation ratio for fast, heterozygous and slow isozyme alleles.

S. No.	Isozyme	Fast	Heterozygous	Slow	df	X^2	Prob.
1.	LAP-2	14	44	16	2	2.75	0.25
2.	PGI-1	15	36	22	2	1.35	0.50
3.	PGM-2	15	41	17	2	1.21	0.54

Those plants which were not in fact hybrid and got selfed at the time of crossing could easily be distinguished by allozyme analysis since they contained only one allozyme band which was present in the female parent. Using this technique, the grown putative F_1 plants were screened and the plants which selfed were recognized (Table 3).

Second Year: In the second year, the allozyme segregation was analysed in one F_2 population i.e., Mansehra-89 X LO-78. The genotypes of parents and F_1 are shown in Table 4.

Both parents i.e., Mansehra-89 and LO-78 had different alleles at all the three loci, and the F_1 hybrid exhibited heterozygous genotype in which both the alleles showed their presence in a co-dominant manner.

The results of χ^2 test of F_2 population are shown in Table 5. In F_2 population three types of plants were found on the basis of presence of F allozyme, S allozyme or both, F and S allozymes, at all the three loci. Those having only F allozyme have genotype FF; those having S allozyme have SS genotype and heterozygous plants have FS genotype. The segregation pattern shows that the F_2 plants are segregating in 1 FF: 2 FS : 1 SS ratio. The probability for this ratio at the three loci is 0.25 (fair fit), 0.50 (good fit), and 0.54 (good fit) respectively. So no significant difference was found between the actual ratios and the expected ratios.

The segregation ratios of 1:2:1 have been reported by Muehlbauer *et al.*, (1989) for various isozymes in lentil:B-D-galactosidase-1; alcohol dehydrogenase-1; fructose kinase; leucineaminopeptidase-1;leucineaminopeptidase-2; and shikimic dehydrogenase. Zamir & Ladizinsky (1984) also reported the similar mode of inheritance for 8 enzyme systems in lentil. These included: glutamic-oxaloacetic transaminase, malic enzyme, phosphoglucomutase, alcohol dehydrogenase, 6-phospho-glutamate dehydrogenase, shikimic dehydrogenase and isocitrate dehydrogenase. According to them the allozymes at each of the studied locus behaved in a co-dominant manner and segregated in the expected Mendelian fashion. These findings are in complete agreement with Gaur & Slinkard (1990).They studied the inheritance of 13 isozymes in chickpea lines and reported a co-dominant inheritance which fit well in 1:2:1 ratio. These results are also supported by the findings of Vaillancourt & Slinkard (1992) in lentil . They reported monogenic inheritance of 11 isozymes. Similarly Isshiki-s *et al.*, (1997) found polymorphism for glucose-6-phosphate isomerase in amphidiploids of *Brassica* spp.

The allozymic polymorphism present in different lentil varieties/ accessions can assist in a number of breeding problems. Plant variety protection agencies often accept isozyme "fingerprints" as supporting evidence that new cultivars are unique. Allozyme diversity can also be used to characterize germplasm accessions and to estimate any genetic change over time in future. Isozymes also have an important use as genetic markers especially for disease resistance. Two isozyme loci have proven useful markers for disease resistance genes in pea. Resistance to *Fusarium* wilt (Race 1) is controlled by a locus closely linked to Est-s, coding a seed esterase. Allozymes of PGM-p can be used to predict the inheritance of alleles at Mo, the locus which controls resistance to bean yellow mosaic virus, with 98% accuracy (Weeden *et al.*, 1984).

The equipment and materials needed for the screening of the isozyme banding pattern (Zymogram) of plants is relatively inexpensive and it is possible to screen a large number of plants rapidly. Furthermore, the process is non-destructive to plants and the plants can be retained for other purposes. Isozyme markers are also used in genetic mapping and their linkage with any monogenic or quantitative trait can improve the breeding efficiency. The lentil crop in Pakistan often faces the problems of rust and *Ascochyta* blight diseases. In a population segregating for a number of isozymes loci along with resistance to diseases can be used to find any possible linkage between disease resistance and any isozyme; and this could eliminate the use of pathological laboratory work and inoculation to screen the resistant lines.

References

Baranski, R. 2000. Application of rapid glucose-6-phosphate isomerase assay in white cabbage breeding. *Acta Physiologiae Plantarum*, 22: 45-51.

Gaur, P. M. and A. E. Slinkard. 1990. Genetic control and linkage relations of additional isozyme markers in chickpea. *Theor. Appl. Genet.*, 80: 648-656.

Isshiki, S., M.M. Hossain and K. Fujieda.1997. Segregation of isozymes in selfed seedlings of synthetic amphidiploids between *Brassica oleracea* and *B. campestris*. *Bulletin of the Faculty of Agriculture, Saga University, Japan*, 82:17-22.

Lazaro, A. and I. Aguinagalde. 1998. Genetic diversity in *Brassica oleracea* L. (Cruciferae) and wild relatives (2n=18) using isozymes. *Annals of Botany*, 82: 821-828.

Muehlbauer, F.J., N.F. Weeden and D.L. Hoffman. 1989. Inheritance and linkage relationships of morphological and isozyme loci in lentil (*Lens* Miller). *J. Hered.*, 80: 298-303.

Skibinski, D.O.F., D. Rasool and W. Erskine. 1984. Aspartate aminotransferase allozyme variation in a germplasm collection of the domesticated lentil (*Lens culinaris*). *Theor. Appl. Genet.*, 68: 411-448.

Steel, R.G.D. and J.S. Torrie. 1980. *Principles and Procedures of Statistics*. Mc Graw Hill Co. Inc., New York.

Tinnemore, D. 1990. *Laboratory Procedures for Starch Gel Electrophoresis used in Pea, Lentil and Chickpea.* Grain Legume genetics and physiology research unit. Pullman, Washington, USA.

Vaillancourt, R. 1989. *Inheritance and linkage of morphological markers and isozymes in lentil.* Ph.D. thesis. University of Saskatchewan, Canada..

Vaillancourt, R. and A.E. Slinkard. 1992. Inheritance of new genetic markers in lentil (*Lens* Miller). *Euphytica*, 64: 227-236.

Weeden, N.F., R.W. Robinson and F. Ignart. 1984. Linkage between isozyme locus and one of the genes controlling resistance to watermelon mosaic virus 2 in *Cucurbita ecuadorensis*. *Cucurbit Genet. Coop.*, 7: 86-87.

Zamir, D. and G. Ladizinsky. 1984. Geneties of allozymes variants and linkage groups in lentil. *Euphytica*, 33: 329-336.

(Received for publication 24 February 2000)