GENETIC DIVERSITY AND POPULATION STRUCTURE OF PIGEON PEA

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ABSTRACT

Eight varieties of pigeon pea (*Cajanus cajan* L.) including landraces, released varieties and promising lines of Nepal were compared for the amplitude of polymorphism at the loci of four enzyme systems: alcohol dehydrogenase (ADH), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH) and peroxidase (POX). Polymorphism was observed among different populations and a total of 25 alleles and 9 loci occurring with different frequencies were detected. Cluster analysis of isozyme data with 4 enzyme systems had helped to place pigeon pea varieties into two distinct groups. Landraces (*Chanki* and *Pajawa* populations) were genetically different from improved varieties (Bageshwori and Rampur Rahar 1). ICPL-84072 and *Chanki* possessed highest number of alleles per locus (2.6). ADH-1 was highly diverse (Ht=91) with highest mean gene diversity within populations (Hs=0.82). IDH-1 showed high coefficient of gene differentiation with highest gene diversity among populations (Gst=0.54; Dst=0.29). On an average, genetic variation within populations (0.47) was higher than among population variation (0.17). Landraces and promising lines were most diverse with high values of diversity parameters. *Chanki*, a commonly grown landrace exhibited high gene diversity.

Key words: *Cajanus cajan*, genetic diversity, polymorphism, isoenzymes

INTRODUCTION

Pigeon pea (*Cajanus cajan* L.) is a multipurpose legume crop grown as a sole crop or intercrop in Terai farming systems. It is used diversely as dal (soup of seed), feed (leaves for livestock), firewood, or materials for making huts and baskets. It is well adapted even in marginal lands.

High diversity of pigeon pea has been reported by Bajracharya *et al.* (1999), Sherchand *et al.* (1999), Rana *et al.* (2000). Diversity of pigeon pea varied with respect to environments (Joshi *et al.*, 2004a). *Chanki* and *Pajawa* are common landraces and *Chanki* is grown widely in large areas by many households. Begeshwori and Rampur Rahar-1, the two improved varieties were released by Nepal Agricultural Research Council for Central and Western Nepal (NARC, 2000). In addition to many landraces, five wild species are found in Nepal (Neupane, 1999). Diversity is being maintained by farmers to meet their diverse needs in diverse environments. Maesen (1990) has grouped all pigeon pea cultivars under primary gene pool. The tertiary gene pool of pigeon pea consisting of wild species is not crossable with primary gene pool.

An understanding of genetic diversity of landraces and varieties is essential for *in situ* and on-farm conservation programs. Most studies on diversity of pigeon pea are based on morphological and phenological characteristics rather than actual population genetic structure of crop varieties. Population structures along with genetic relationship and diversity are useful in developing strategy for conservation and utilization of crop genetic resources.

Isozymes have been used in many crops for study of genetic diversity and population structure e.g. barley (Bajracharya *et al.*, 2003a), flax (Mansby *et al.*, 2000), grass pea (Chowdhury and Slinkand, 2000), chickpea (Kazan *et al.*, 1993), and broad bean (Torres *et al.*, 1995) etc. They are also used to verify farmers' naming that was used to describe landraces (Bajracharya *et al.*, 2003b). Isozymes being co-dominant marker system, it is possible to assign a genotype as an individual from its electrophoretic profile (Brown, 1979; Wendel and Weeden, 1989). This approach is relatively simple and allows data to be collected quickly from large sample sizes and low cost compared to other molecular techniques.

Pigeon pea is a self-pollinated and biennial crop. Because of its different breeding systems, one can relate population structure and breeding system with maintenance of diversity on-farm. Farmers grow pigeon pea in small areas. If there is diversity in small area it could help in policy formulation for on-farm management of agricultural biodiversity. Pigeon pea is one of the mandated crops for *in situ* conservation of agro biodiversity in Nepal. The aim of the present

study was to use isozymes to assess genetic diversity, population structure and genetic relationship among eight pigeon pea populations. The specific objectives were measured the extent and distribution of genetic diversity, to relate genetic diversity and farmer division making, to measure intra/inter varietal diversity, to study genetic diversity between landraces and modern varieties and to determine consistency between farmer names and genetic distinction.

MATERIALS AND METHODS

Plant materials

Eight populations of pigeon pea comprising of two landraces from Bara (Central Tarai), two released varieties for Central and Western Tarai regions of Nepal and four promising lines introduced from India were included in the study. Each variety/line was considered as a population and 10 samples from each population were collected from 10 farmers for landraces, 10 production fields for improved varieties and 10 different research trials for promising lines. Samples of improved varieties were obtained from Regional Agriculture Research Station (RARS), Nepalgunj.

Isozyme analysis

Equal proportions of plumules of 3-5 seedlings of each sample raised over moistened filter paper in controlled condition at 30oC for 7 days in dark were used for enzyme extraction in L-Ascorbic acid buffer (0.1 M Ascorbic acid & glycerol, and pH 7.4) following the methods of GEVES (GEVES, 1993). The extracted leaf samples were electrophoresed in tris buffer (0.400 M Tris, pH 8.0 with 105 M citric acid) using 12% starch gel (potato starch S-4501, Sigma company, USA) for overnight at 4oC and at constant 4 watt power supply. After electrophoresis, the gels were sliced horizontally and stained for enzymes: alcohol dehydrogenase (ADH, EC 1.1.1.1), isocitrate dehydrogenase (IDH, EC 1.1.1.42), malate dehydrogenase (MDH, EC 1.1.1.37) and peroxidase (POX, EC 1.11.1.7) dipping the slices in respective enzyme trays and enzymes for staining were prepared following the manual developed by Biotechnology Unit, 2000 (BU, 2058). Isozyme banding patterns were scored based on zone of activity

Table 1: Frequency of allele for 4 isozymes in 8 pigeon pea populations

Locus	Allele	Populations							
		Bageshwori	*Chanki*	ICPL-84072	ICP-7035	*Pajawa*	Pusa-9	Pusa-14	Rampur Rahar-1
Adh-1	a	0.00	0.23	0.23	0.23	0.29	0.00	0.00	0.00
	b	0.00	0.31	0.38	0.23	0.36	0.00	0.00	0.00
	n	0.00	0.46	0.38	0.54	0.36	0.00	0.00	0.00
Adh-2	a	0.00	0.64	0.43	0.54	0.60	0.00	0.00	0.00
	b	0.90	0.29	0.29	0.23	0.33	1.00	0.90	1.00
	n	0.10	0.07	0.29	0.23	0.07	0.00	0.10	0.00
Idh-1	a	0.20	1.00	0.83	0.67	1.00	0.20	0.00	0.10
	b	0.70	0.00	0.17	0.33	0.00	0.70	1.00	0.80
	n	0.10	0.00	0.00	0.00	0.00	0.10	0.00	0.10
Mdh-1	a	0.00	0.00	0.30	0.80	0.50	0.00	0.00	0.00
	n	0.00	0.00	0.70	0.20	0.50	0.00	0.00	0.00
Mdh-2	a	1.00	0.23	0.47	0.56	0.53	0.63	0.50	1.00
	b	0.00	0.23	0.47	0.44	0.41	0.38	0.50	0.00
	n	0.00	0.54	0.07	0.00	0.06	0.00	0.00	0.00
Pox-1	a	0.20	0.20	0.10	0.30	0.10	0.00	0.30	0.50
	b	0.60	0.40	0.20	0.10	0.40	0.90	0.40	0.00
	n	0.20	0.40	0.70	0.60	0.50	0.10	0.30	0.50
Pox-2	a	0.00	0.00	0.10	0.10	0.00	0.00	0.00	0.60
	b	0.60	0.70	0.50	0.30	0.50	1.00	1.00	0.20
	n	0.40	0.30	0.40	0.60	0.50	0.00	0.00	0.20
Pox-3	a	0.30	0.70	0.40	0.80	0.90	1.00	0.90	0.00
	n	0.70	0.30	0.60	0.20	0.10	0.00	0.10	0.00
Pox-4	a	0.30	0.00	0.10	0.20	0.00	1.00	0.90	0.60
	b	0.30	0.70	0.40	0.60	0.70	0.00	0.00	0.00
	n	0.40	0.30	0.50	0.20	0.30	0.00	0.10	0.40

and number of bands developed for respective enzyme. Each enzyme activity was presumed as locus and number of bands was recorded for each zone of activity/locus and individual band seen for the active zone was scored as presence (1) and absence (0) for further numerical analysis of the genetic similarity and distance (Wendel and Weeden, 1989). Most anodal active zone for each enzyme system is coded as locus 1 and based on the migration of band in gel, the most anodal band of each locus is coded alphabetically as "a", "b" so on. A locus with two or more alleles is considered as polymorphic (Chang and Kang, 1996; Nei, 1975).

Data analysis

A total of 16 bands were used as isozyme characters. Based on combination of the bands and their banding pattern, zymograms were developed. Genetic variability in each population was assessed for mean number of alleles per locus (A), mean number of alleles per polymorphic locus (Ap), proportion of polymorphic loci (P) and mean gene diversity or variation within (Hs) and among (Dst) populations and coefficient of gene differentiation (Gst) according to the unbiased method of Nei (1973) using Genestat software (Lewis, 1992). Further, genetic variations among populations were measured in all possible pair-wise comparisons using Nei's genetic distance (1973) and cluster analysis (UPGMA) using the computer softwre, NTSYS PC (Version 2.7) (Rohlf, 1998).

RESULTS

A total of 8 populations and 10 samples from each population were assayed for 4 enzymes and 9 zones of activity as loci. A total of 16 alleles were detected. One to four loci were resolved for different enzymes and all loci were polymorphic over the populations. Maximum of 4 loci were observed for POX. However, two improved varieties, Bageshwori and Rampur Rahar-1 showed no zone of activity for ADH and MDH but all loci were found in these two landraces. Two populations, ICP-7035 and ICPL-84072 had more homogenously distributed alleles than other populations. Frequency of alleles ranged from 0.06 to 1 (Table 1). All loci except Idh-1 were polymorphic in two landraces, *Chanki* and *Pajawa*. Pusa-9 had least number of polymorphic loci. Promising lines, ICPL-84072 and ICP-7035 had all polymorphic loci. Except Idh-1 in *Chanki* and Adh-2 and Mdh-2 in *Pajawa* all other loci were polymorphic. Some of the samples across populations, that were observed with no activity were assumed to have null

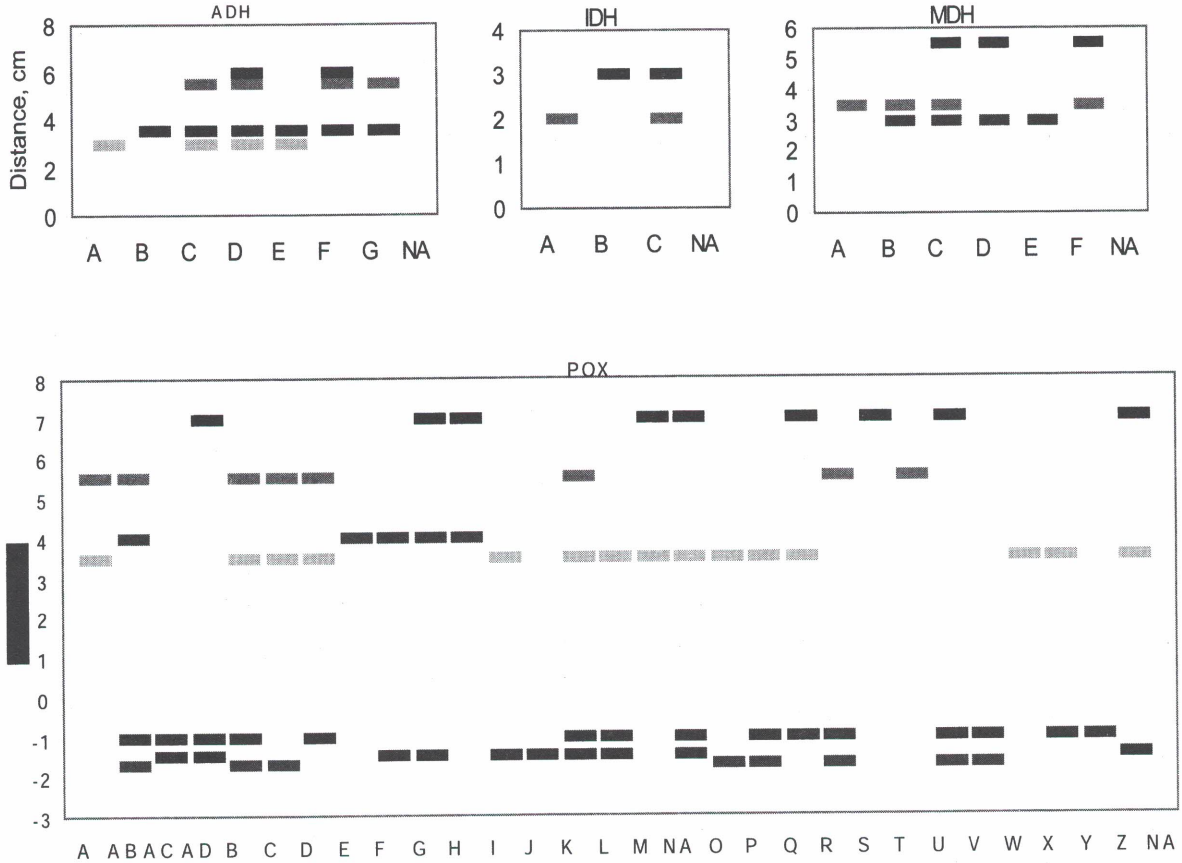

Fig 1: Zymograms of 4 isozymes namely ADH(alcohol dehydrogenase), IDH(isocitrate dehydrogenase), MDH(malate dehydrogenase) & POX(Peroxidase) in eight pigeon pea populations. Letters A to Z represent different alleles & NA represents null allele.

Table 2: Frequency of zymograms in 8 pigeon pea populations. Abbreviation A to Z represents different alleles and NA refer null allele.

Population	Isozymes			
	ADH	IDH	MDH	POX
Bageshwori	A (0.8)	A (0.7)	A (1)	A (0.2)
	NA (0.2)	B (0.2)		B (0.2)
		NA (0.1)		C (0.1)
				D(0.1)
				E (0.1)
				F (0.1)
				G (0.2)
Chanki	B (0.5)	B (1)	B (0.3)	B (0.2)
	C (0.1)		NA (0.7)	O (0.1)
	D (0.3)			P (0.3)
	NA (0.1)			Q (0.1)
				R (0.1)
				S (0.1)
				T (0.1)
ICPL-84072	B (0.1)	B (0.8)	A (0.2)	B (0.1)
	C (0.1)	C (0.2)	B (0.3)	E (0.1)
	D (0.3)		C (0.2)	O (0.1)
	G (0.1)		D (0.1)	P (0.1)
	NA (0.4)		E (0.1)	V (0.1)
			NA (0.1)	W (0.1)
				Y (0.1)
				Z (0.1)
				NA (0.2)
ICP-7035	B (0.4)	B (0.5)	A (0.1)	P (0.4)
	D (0.3)	C (0.5)	B (0.1)	S (0.1)
	NA (0.4)		C (0.7)	V (0.1)
			F (0.1)	AB (0.1)
				AC (0.1)
				AD (0.1)
				NA (0.1)
Pajawa	B (0.3)	B (1)	A (0.2)	B (0.2)
	C (0.1)		B (0.2)	O (0.1)
	D (0.3)		C (0.5)	R (0.1)
	E (0.1)		NA (0.1)	U (0.1)
	F (0.1)			V (0.3)
	NA (0.1)			W (0.1)
				X (0.1)
Pusa 14	A (0.9)	A (1)	B (1)	K (0.4)
	NA (0.1)			L (0.3)
				M (0.1)
				N (0.2)
Pusa 9	A (1)	A (0.7)	A (0.4)	K (0.9)
		B (0.2)	B (0.6)	L (0.1)
		NA (0.1)		
Rampur Rahar-1	A (1)	A (0.8)	A (1)	F (0.1)
		B (0.1)		G (0.1)
		NA (0.1)		H (0.4)
				I (0.2)
				J (0.2)

Table 3: Descriptive statistics of alleles and loci in eight pigeon pea populations based on 9 loci studied in 10 samples per population

Population	Alleles per locus (A)	Alleles per polymorphic locus (Ap)	Proportion of polymorphic loci (P)	Mean gene diversity (H)
Bageshwari	1.778	2.500	0.667	0.529
Chanki	2.111	2.571	0.778	0.516
ICPL-84072	2.667	2.667	1.000	0.518
ICP-7035	2.556	2.556	1.000	0.491
Pajawa	2.333	2.500	0.889	0.435
Pusa-9	1.222	2.333	0.333	0.345
Pusa-14	1.444	2.200	0.556	0.411
Rampur Rahar-1	1.333	2.500	0.444	0.542
Mean	1.931	2.478	0.708	0.473
SE	0.200	0.052	0.089	0.025

Number of alleles unique to these populations was zero.

allele effect. Therefore a total of 25 alleles including the null alleles of respective enzymes were used in analysis for genetic similarity and distances among populations.

Isozyme variation

Zymograms based on allelic combination of the polymorphic loci were sketched and shown (Figure 1). Minimum of 3 (IDH) to 29 different patterns zymograms and maximum of 7 different bands (POX) were observed. None of the bands were observed common over the populations of pigeon pea under study.

In ADH, two zones of enzyme activity were detected and 7 zymograms and 4 different alleles were observed in 8 populations of pigeon pea (Fig. 1). Zymogram A was common in Bageshwori and Pusa 9. *Pajawa* was most diverse for this enzyme with maximum of 6 zymograms (Table 2). IDH detected a single zone of activity with two alleles and 3 zymograms were observed (Figure 1). Zymogram A was again common in Bageshwori and Pusa 9, whereas B was in *Chanki* and ICPL-84072. Others occurred in low frequencies (Table 2).

MDH resolved two zones of activities and 6 zymograms with 3 different alleles (Figure 1). The most common zymograms were A (100%) in Bageswori and C (70%) in ICP-7035. ICPL-84072 was most diverse for this enzyme with 6 zymograms (Table 2). Zymograms A and B were common in Bageshwori and Rampur

Table 4: Gene diversity in 4 isozymes of eight pigeon pea populations

Locus	Total gene diversity (Ht)	Within population gene diversity (Hs)	Percentage mean gene diversity within population (Hs/ Ht, %)	Among populations gene diversity (Dst)	Coefficient of gene differentiation (Gst)
Adh-1	0.912	0.820	89.85	0.093	0.102
Adh-2	0.912	0.330	62.06	0.202	0.380
Idh-1	0.535	0.248	46.40	0.287	0.536
Mdh-1	0.929	0.78	83.93	0.149	0.161
Mdh-2	0.524	0.395	75.53	0.128	0.245
Pox-1	0.644	0.515	79.96	0.129	0.200
Pox-2	0.540	0.385	71.30	0.155	0.287
Pox-3	0.547	0.375	68.57	0.172	0.314
Pox-4	0.660	0.413	62.47	0.248	0.375
Mean	0.647	0.473	71.32	0.174	0.268
SE	0.054	0.066	*	*	*

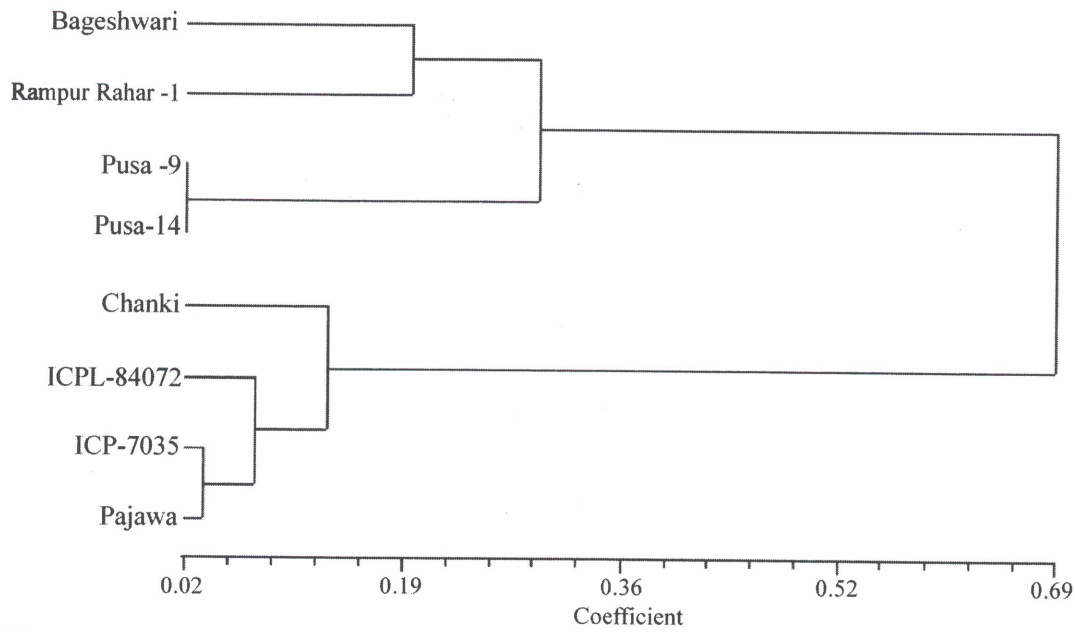

Fig 2: Dendrogram of eight pigeon pea populations based on Nei's genetic distance

Rahar -1 and C in ICP-7035 with 70% frequency. POX showed 4 zones of activities and was observed with highest variables of zymograms. Twenty-nine zymograms and 7 different alleles were detected. Zymogram K was common in Pusa 9 with 70% frequency. This enzyme exhibited high variation within and among populations, each with 2 to 8 zymograms.

Genetic diversity and structure

The number of alleles per locus ranged from 1.2 (Pusa 9) to 2.7 (ICPL-84072) with mean value of 1.9 across populations (Table 3). The percentage of polymorphic loci (P) ranged from 33% (Pusa-9) to 100% (ICPL-84072 and ICP-7035) with an average of 71%. The average diversity index (H) among populations ranged from 0.345 (Pusa-9) to 0.542 (Rampur Rahar-1) with an average of 0.473. Four populations with the greatest diversity were Bageshwori, *Chanki*, ICPL-84072, ICP-7035 and Rampur Rahar-1. The highest genetic diversity was observed in ICPL-84072 as indicated by the higher values for A (2.667), P (100%) and H (0.518).

The summary of gene diversity at the locus level is shown in Table 4 which reveals the consequence of diverse genetic structures within and among pigeon pea populations under study. The average total genetic diversity (Ht) over eight populations was 0.65 and Mdh1 showed the highest (0.93) diversity. Adh-1, Mdh-1 and Pox-4 showed marked total diversity with values higher than the mean value (Table 4). The mean intra and inter populations diversities over eight populations were 0.47 (Hs) and 0.17 (Dst) with range of 0.25- 0.82 and 0.09 – 0.29 respectively. Likewise the mean coefficient of gene differentiation (Gst) was 0.27. It is obvious that the diversity in pigeon pea has resulted due to the within population variation rather than the variation among the populations. Twenty seven % of the total genetic diversity (Ht=0.65) accounted for by the inter-population variation and 73% was due to within-population variation. It indicated that each population of pigeon pea under study was composed of divergent individuals with different genetic structures.

Clustering and relatedness

Cluster analysis using isozyme data resulted dendrogram (Figure 2) which illustrates the genetic relationships and divergence among the pigeon pea populations of different origins. Clustering of eight populations using UPGMA and Nei's genetic distance resulted in two distinct groups. Two promising lines Pusa 9 and Pusa 14 were identical and clustered together with improved varieties Bageshwori and Rampur Rahar-1. On the other hand, two landraces (*Chanki* and *Pajawa*) and two promising lines (ICPL-84072 and ICP-7035) were grouped together into a cluster. The cluster reflected the sharing of frequencies of alleles and magnitude of genetic relatedness among these diverse populations of pigeon pea. Two landraces, *Chanki* & *Pajawa* were genetically different from each other, although they were clustered into the same group.

Isozyme profiles

Isozyme profiles are useful for varietal identification. Bands along with their mobility for loci of eight populations are given in Figure 3. Bands that travelled faster like *Chanki*, and *Pajawa* and ICPL-84072 had higher number of bands. Each cultivar had its own distinct isozyme profile. Many researchers had developed isozyme profiles of crop

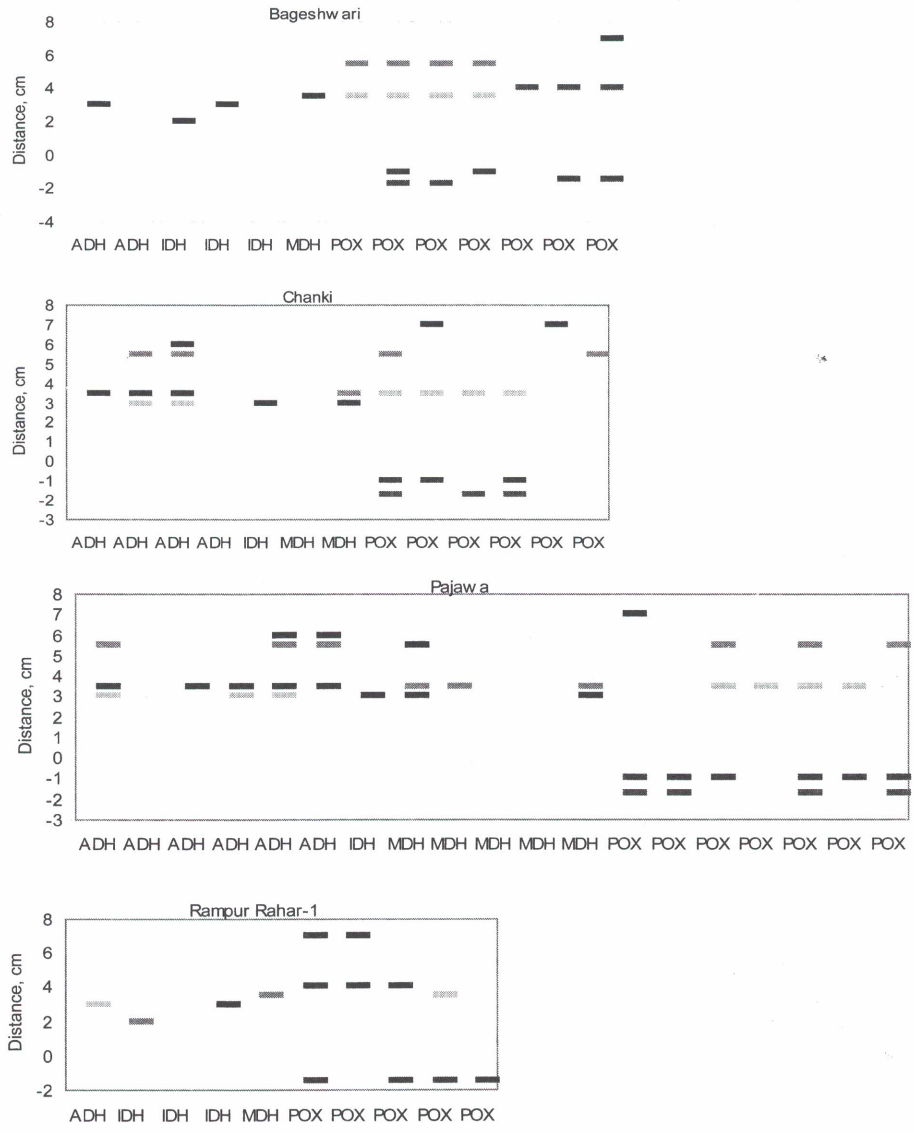

Fig 3: Isozyme profiles of four pigeon pea populations for isozymes

species (Romero *et al.*, 1993; GEVES, 1993; Joshi and Bimb, 2004;). Distinct esterase pattern in pigeon pea was reported (Mallikarjuna and Moss, 1995). This profile could be used to identify the variety.

DISCUSSION

Knowledge about genetic structure and relationships among the populations provides information on population divergence, which is important for conservation and exploitation of genetic resource for crop improvement. The values measured for diversity parameters based on allelic information exhibited the differences in pigeon pea populations and helped to quantify the extent of genetic variation in each of the population for studied enzymes. This preliminary study was successful to establish allozyme variability in pigeon pea of Nepal for the first time. Genetic diversity in pigeon pea demonstrated high variation within population and therefore each population was composed of different individuals, which was a desirable variability for selection and improvement in a breeding programme. Higher null allele value was due to sick samples or lack of genetic variation. In some populations, zone of activities was not resolved which resulted in zero frequency of allele. These cases showed probably why Ht was so high for Adh-1 and Mdh-1. Further analysis of F2 progeny of crosses would be necessary to verify locus and banding patterns.

One hundred local germplasms of pigeon pea have been collected and characterized in Nepal. Bageshwori, a variety developed from the local selection is highly resistant to sterility mosaic disease (Neupane, 1999). Agromorphological variation within *Chanki* and *Pajawa* was reported by Bajracharya *et al.* (1999). Sherchand *et al.* (1998) reported 12 different farmer named landraces in Bara and Rana *et al.* (2000) reported 5 landraces in Kachorwa. Farmers were

consistent in naming pigeon pea in Kachorwa (Bajracharya *et al.*, 1999). Two farmers named landraces *Chanki* and *Pajawa* are genetically different. Farmer's descriptions are based on plant type, seed color, size, raceme type and taste. In Kachorwa 27.2% households grow pigeon pea and *Chanki* was the dominant. Pigeon pea growers have one landrace per household (Rana *et al.*, 2000). Pigeon pea was one of the crops grown under least external inputs and its pulses fetches highest price in the market among the pulses. However, only limited households from resource poor grow the crop (Rana *et al.*, 2000). In Kachorwa, it is prestigious crop and grown either in Khet land or in marginal bari land as a sole or mix. Because of its importance at household levels, crop improvement works should be initiated utilizing existence diversity.

Two promising lines of pigeon pea introduced from India namely ICPL-84072 & ICP-7035 were closely related. The major landraces were separated from improved varieties by genetic distance in the dendogram. Similarly Pusa series were separated from ICP series. These series were promising lines being evaluated in Nepalgunj. Even though Bageshwori was of Nepalese origin and Rampur Rahar-1 was of Indian origin these varieties were grouped in the same cluster.

Two landraces of pigeon pea namely *Chanki* and *Pajawa* exhibited higher diversity than Bageshwori and Rampur Rahar-1. It indicates that landraces are genetically more diverse than improved varieties. No private alleles were detected in any of the populations. Genetic statistics were computed based on bulked samples. In the present study all farmers' plots under each variety were considered as a population and each farmer's plot was treated as a sample. Therefore, bulked samples were used to make more representative of concerned plots. For an in depth isozyme analysis, large sample size with individual sample treatment as a single field should be considered as a population, and be further studied.

Pigeon pea is an often self-pollinated and biennial crop. So, the individual plants from which seeds are collected may be heterozygous. Detailed study including progeny analysis is necessary to verify the banding patterns and to know the nature of enzyme (either monomeric or dimeric) in pigeon pea.

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