

## INFLUENCE OF GENETIC DIVERSITY ON CAUSE AND EFFECT RELATIONSHIPS IN *LENS CULINARIS* GERMPLASM UNDER RAIN-FED ECO-AGRICULTURAL SYSTEM

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### Abstract

Due to emerging demands of organic foods, lentil, one of the most primitive legumes was investigated for genetic diversity including cause and effect relationships among various clusters under eco-agricultural system. The 73 lentil genotypes were investigated for qualitative and quantitative traits to identify the potential lines under rain-fed conditions for organic farming using no chemical fertilizers for crop production. Variation existed for all the qualitative traits including orange cotyledon colour in 27 genotypes which is a preferred trait by Asian consumers including Pakistan. Five clusters revealed that average intra-clusters distances were more or less similar, whereas inter-cluster distance indicated higher level of genetic diversity. First three PCs contributed more than  $\frac{3}{4}$  of the variability and the results were in coordination with clustering pattern amongst 73 genotypes. The populations contributing the first PC were late in maturity possessed higher number of branches, pods, better biomass and grain yield. The PC<sub>2</sub> was more contributed by seeds pod<sup>1</sup> and seed diameter, whereas pod length and harvest index contributed 13% variability. The cause and effect relationships indicated differential response for selection of lentil genotypes suitable for eco-agricultural system within each cluster.

### Introduction

Lentil (*Lens culinaris* Medik) is a self pollinated crop of the legume family ( $2n = 14$ ) that contains 25% protein, 2% minerals, 59% other carbohydrates, and 343 calories per 100 g, whereas the fiber and fat contents are 0.7% each (Piecyk *et al.*, 2012, Hamayun *et al.*, 2011), hence known as poor man's meat. It is grown during winter season in Pakistan on an area of 31 thousand hectares with an annual production of 15 thousand tones with an average seed yield of 466 kg ha<sup>-1</sup> (Anon., 2009-10). Lentil production in Pakistan has been reduced due to non-availability of improved cultivars associated with biotic and abiotic stresses, although Erskine *et al.*, (2011) has mentioned that lentil as a potential crop for future food security in developing countries. The ultimate aim in most of the plant breeding programs is the enhancement of crop productivity per unit area that has not been addressed, especially in lentil (Sylvester-Bradley *et al.*, 2012; Khan *et al.*, 2013).

Seed yield is the ultimate expression of different yield contributing traits in lentil that is determined through cause and effect relationship in a biological web of the plant traits (Li *et al.*, 2011). Inability to visualize the small differences in quantitative traits among single plants have led to frequent attempts to find associated traits that are more amenable to visual selection. The correlation coefficient gives a measure of the relationship between traits and provides the degree to which various characters of a crop are associated with productivity (Turi *et al.*, 2012) Selection based on yield components is advantageous if different yield related traits have been well documented (Kiær *et al.*, 2012; Chigeza *et al.*, 2012; Rehman *et al.*, 2012, Sultan *et al.*, 2012). Eco-agriculture is the most primitive farming system that was interrupted during the last few centuries

with incorporation of modern farming system coupled with inorganic fertilizer application and chemical sprays (Yixing *et al.*, 2011). The main reason for this intervention was world's growing population, restrictions on trade of agricultural commodities and unjustified human division on the basis of socio-economic status of various countries (Townsend & Porder, 2012). With the emerging knowledge on hazards to human health by inorganic fertilizers and pesticides, man has started rethinking for eco-friendly agricultural production (Sarkar *et al.*, 2012; Shinwari *et al.*, 2012). Lentil is believed to be among the oldest crops that man domesticated (Badr & El-Shazly, 2011) and has quick response to primitive agricultural system as well as to climate change (El-Beltagy & Madkour, 2012), hence was used to have insight to investigate the potential of lentil germplasm for eco-agricultural response. According to Tullu *et al.*, (2011), huge lentil genetic resources have been assembled throughout world that include cultivated as well as wild *Lens* and lot of work has been accomplished but the research reported in this manuscript has been conducted for the first time, especially in Pakistan.

To initiate any strategic crop improvement program, the pre-requisite information includes the magnitude of genetic diversity, nature of population structure, complexity of interrelated traits and their cause-effect equation, contributing towards the economic yield, (Akbar *et al.*, 2011) *i.e.*, grain yield in case of lentil (Hamblin *et al.*, 2011). Keeping in view the need of the present era, the lentil germplasm was evaluated under rain-fed conditions without any inorganic application to assess the genetic diversity and interrelationships among genetic parameters that will enable us to shift breeding methodologies to meet the requirements of the present lentil consumers.

## Materials and Methods

The 73 genotypes of lentil including three checks (Mansehra 89, Masoor 2000, Shiraz) were planted in a triplicate randomized complete block design (RCBD) at National Agriculture Research Center (NARC), Islamabad, Pakistan (30° 42' N and 37° 08' E, 540 masl) during October 22, 2010 and harvested in April 2011. The experiment was laid out keeping two rows of 5 m length with rows and plants distance of 30 and 10 cm, respectively. The experiment was planted under rain-fed conditions and no chemicals (fertilizers, pesticides, fungicides and herbicides) were applied to ensure eco-agricultural scenario to select the future lentil genetic resources for the development of eco-friendly agricultural system. The area where the experiment was conducted did not receive any chemical fertilizer during last five years that is a pre-requisite for selecting germplasm suitable for organic farming (van Bueren *et al.*, 2012). At maturity ten competitive plants were sampled from each genotype within each replication and data were recorded on individual plant basis. The data were recorded for plant height, branches plant<sup>-1</sup>, pods plant<sup>-1</sup>, biological yield plant<sup>-1</sup>, pod length, seed pod<sup>-1</sup>, seed diameter, seed thickness and grain yield plant<sup>-1</sup>, whereas harvest index was expressed in percent of grain yield over the biological yield. Other traits, *viz.*, 100-seed weight in grams, pod color, color of testa, pattern of testa, color of pattern on testa and cotyledon color were recorded on line basis.

The qualitative traits were grouped in discrete classes, and the measurable traits were analyzed for inter and intra-accessions variation and then the averaged values were analyzed for multivariate techniques using cluster and principal component analyses with the help of computer software, STATISTICA 7.0 and SPSS 13.0 following the

methods by (Sneath & Sokal, 1973). The data were standardized prior to cluster analysis to avoid variance due to different measurements for agronomic traits. Other genetic parameters including genotypic and phenotypic variances, heritability (broad sense), genotypic, phenotypic and environmental correlation coefficients (Steel *et al.*, 1997), and path analysis were carried out according to Singh & Chaudhary (1985) using a computer software in Basic using the methodology for path analysis as described by Dewey & Lu (1959). Inter and intra-cluster genetic diversity was plotted in scattered diagram on the basis of five clusters constructed for agronomic traits with the help of computer software SPSS 13.0.

## Results

The frequency distribution for plant descriptors with discrete classes and likely to be employed for varietal identification is presented in Fig. 1. A considerable variation was observed for all the traits and 27 genotypes produced orange cotyledon. The analysis of variance including broad sense heritability and genetic advance (at 5% level) are presented in the Table 1. The genetic parameters indicated significant inter-genotypic differences for all the traits, whereas intra-genotypic differences were insignificant for all the traits, except seeds pod<sup>-1</sup>, seed diameter, biomass and grain yield plant<sup>-1</sup> that revealed the prevalence of homogeneous mixture in a valuable number that can be isolated, especially for seeds pod<sup>-1</sup> and seed diameter which are quite stable traits in lentil. The ratio between additive variance and total variance indicated varying degrees of heritability estimates that indicated the scope of simple selection for improving biomass in lentil which is also important indicator for grain yield in lentil.

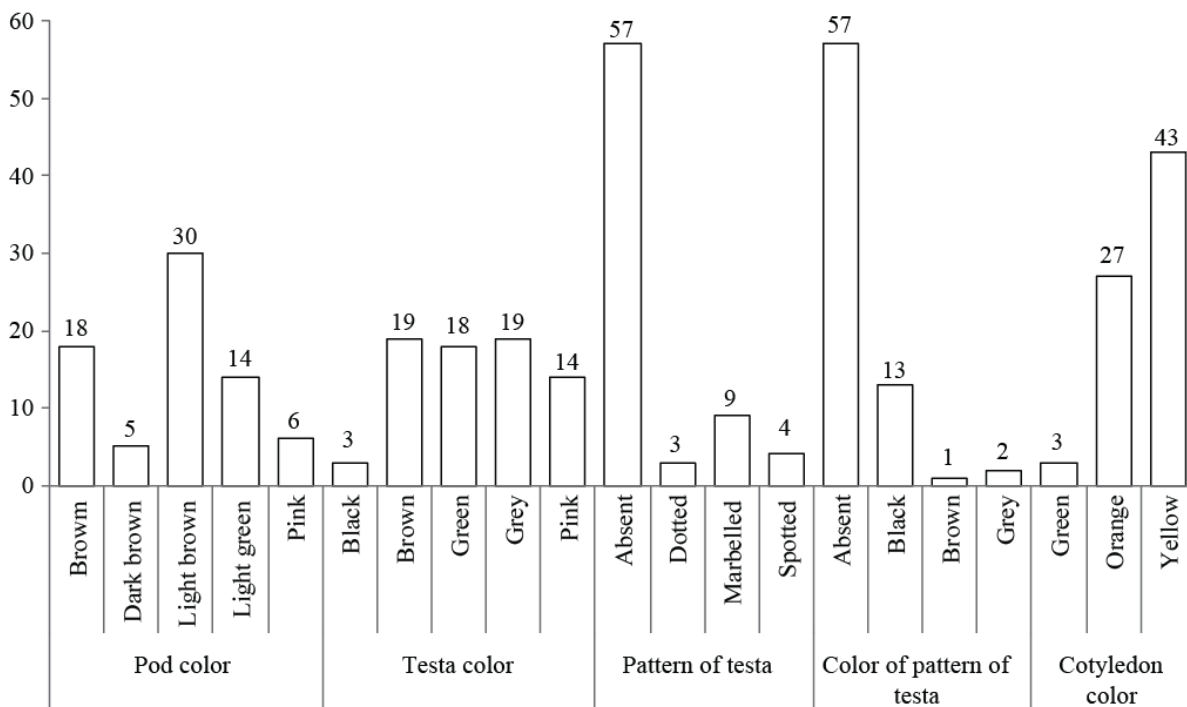


Fig. 1. Frequency distribution for qualitative traits in *Lens culinaris* germplasm.

**Table 1. Analyses of variance and estimation of genetic parameters in *Lens culinaris* germplasm.**

	PH	BR	PODS	PL	SPP	SD	ST	HI	BM	GY
Mean squares (inter-genotypes)	329.11**	49.72**	12823.72**	91.09**	19.42**	1.25**	4.59**	251.70**	5.01**	0.32**
Mean squares (intra-genotypes)	23.81	15.63	1345.00	17.42	1.80*	1.38*	0.48	64.10	0.31*	0.08*
Mean squares (error)	19.80	11.45	1264.82	7.90	0.92	0.14	0.59	95.11	0.13	0.08
CD <sub>1</sub>	3.90	2.96	31.17	2.46	0.84	0.32	0.67	8.54	0.31	0.16
CD <sub>2</sub>	5.12	3.89	40.97	3.23	1.11	0.43	0.89	11.23	0.42	0.22
$\sigma_g^2$	30.93	3.82	1155.89	8.31	1.85	0.11	0.40	15.65	0.48	0.02
$\sigma_p^2$	50.73	15.27	2420.71	16.22	2.77	0.25	0.99	110.77	0.62	0.06
Heritability (broad sense)	0.61	0.25	0.47	0.51	0.66	0.44	0.40	0.14	0.78	0.43
Genetic advance at 5% gain	8.95	2.01	15.30	4.25	2.28	0.45	0.82	3.05	1.27	0.23

\*and \*\* is significant at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively. PH- plant height (cm), BR- branches plant<sup>-1</sup>, PODS- pods plant<sup>-1</sup>, PL- pod length (mm), SPP- seeds pod<sup>-1</sup>, SD- seed diameter (mm), ST- seed thickness (mm), HI- harvest index (%), BM- biomass plant<sup>-1</sup> (g) and GY- grain yield plant<sup>-1</sup> (g).  $\sigma_g^2$  and  $\sigma_p^2$  are genotypic variance and phenotypic variance, respectively

The genotypic, phenotypic and environmental correlations are presented in Table 2 and partitioning of genetic correlation in cause and effect relationships is presented in Table 3. Pods plant<sup>-1</sup> were positively associated with plant height, branches, pod length and seed thickness at both genotypic and phenotypic levels, whereas environmental correlations were negligible for most of the cases indicating the broader relevance of results for practical utilization in lentil improvement. Biological yield has positive association with grain yield indicating the impact of total herbage mass for transport of grain filling that is further enhanced through seeds pod<sup>-1</sup>. In general, genotypic correlations were higher than corresponding phenotypic ones for all the traits that demonstrated the negligible environmental influence upon the traits expression. Although the relationship seems to be a true indicator for improving grain yield through exploitation of present results, hence knowledge of correlation alone is often misleading as the correlation observed may not be always true, especially in biological network of associated responses due to various interactions. Two characters may show correlation just because they are correlated through a common third one. In such cases, it becomes necessary to study a method which takes into account the causal relationship between the variables in addition to the degree of such relationship. Path coefficient analysis measures the direct influence of one variable upon the other and permits the separation of correlation coefficients into components of direct and indirect effects that is often of practical utilization in plant improvement. Partitioning of genotypic correlation in cause and effect relationship provides more precise information on contribution of various characters in a biological phenomenon and thus forms the basis for selection to improve the yield. Table 3 presents the direct and indirect effects on grain yield contributed by various traits in the 73 genotypes of lentil. Although pod length and seed thickness exhibited negligible correlation with grain yield but the direct effects were maximum by both of these traits that was mainly through indirect effects contributed reciprocally

towards grain yield. It could be concluded that the lentil genotypes having longer pods with better seed thickness could be the base material, especially for breeding cultivars for organic farming.

Based on UPGMA, five clusters were observed at one forth dissimilarity for 10 agronomic traits amongst 73 genotypes (Fig. 2). The cluster I consisted of 12 genotypes, cluster II of 19, cluster III of 21 genotypes, cluster IV of 14 and the cluster V consisted of 7 genotypes. The average genetic distance within clusters was quite similar ranging from 3.7 to 3.9 (for ten samples genotype), whereas inter-cluster genetic distance ranged from 3.8 to 5.5 (Table 4). The genotypes grouped in the cluster I exhibited the maximum breeding value coupled with higher magnitude of inter-genotypic genetic distance. Hence better genotypes are expected from this cluster those will represent maximum genetic diversity and a mini core collection can be established keeping 10% genotypes from each cluster with average distance corresponding to inter and intra-genotypic distances coinciding the average distance for individual clusters (Table 4). Results for principal components analyses were in coordination with the clustering pattern on UPGMA (Fig. 3), and the traits with better average values for the first cluster exhibited higher portion of variance for the first factor with Eigen value of 4.5 (Table 5). The character expressions were low for plant height (0.38) and pod length (0.39), whereas other contributed closer to linearity. First three PCs contributed more than three fourth of the variability amongst 73 genotypes. The populations contributing the first PC were late in maturity and possessed higher number of branches and pods and obviously better biomass and grain yield. The PC<sub>2</sub> contributing 21% of the total variability was more contributed by seeds pod<sup>-1</sup> and seed diameter, whereas pod length and harvest index were attributed towards the third PC that contributed 13% variability.

Direct and indirect effects of different seed yield components indicated that pod length and seed thickness exhibited positive direct effect in determining grain yield. Other important yield contributing traits did not

contributed directly, rather indirect effects were contributed mainly through pod length and seed thickness. The information on correlation and path analysis generated on huge germplasm are often misleading to draw conclusion, hence the germplasm was grouped in to five clusters based on UPGMA using Ward's method. The basic genetic parameters including correlation and path analysis were conducted for individual clusters that provided useful information to draw concrete conclusion on selection criterion within each cluster (Table 6). The accessions of the cluster I and cluster III exhibited significant differences among all the accessions for all the traits, whereas others clusters were insignificant for pod length, biomass and grain yield (cluster II), branches, pods and grain yield (cluster IV), whereas inter-cluster differences were insignificant for branches, pods, seed thickness, harvest

index and grain yield for the cluster V. It was observed that biomass could be the main selection criterion for improving lentil for eco-agriculture in Pakistan due to highest genetic correlation as well as high direct effects for the all the clusters except cluster II, where harvest index was in linearity for correlation and the direct effects. The Fig. 4 presents the positions of five clusters on the basis of within and between clusters genetic diversity and it was observed that the cluster I exhibited the maximum inter-cluster diversity with an above average for both types of genetic diversity indexes. Other clusters maintained above average intra-cluster diversity but low to medium or inter-cluster diversity. The accessions falling in the upper left and in the lower right are likely to produce better recombinants for development of lentil cultivars for future harvest through eco-agricultural system.

**Table 2. Genotypic ( $r_g$ ), phenotypic ( $r_p$ ) and environmental ( $r_e$ ) correlation among ten measureable traits in *Lens culinaris*.**

Variable		PH	BR	PODS	PL	SPP	SD	ST	HI	BM
BR	$r_g$	0.24*								
	$r_p$	0.14								
	$r_e$	0.09								
PODS	$r_g$	0.36**	0.68**							
	$r_p$	0.26*	0.53**							
	$r_e$	0.13	0.47**							
PL	$r_g$	0.58**	0.72**	0.90**						
	$r_p$	0.32**	0.30**	0.47**						
	$r_e$	-0.01	0.08	0.05						
SPP	$r_g$	0.08	-0.26*	-0.38**	-0.12					
	$r_p$	0.08	-0.12	-0.21	-0.05					
	$r_e$	0.07	-0.02	0.01	0.05					
SD	$r_g$	0.03	0.17	0.28*	0.17	-0.34**				
	$r_p$	-0.01	0.04	0.12	0.04	-0.19				
	$r_e$	-0.04	-0.01	-0.02	-0.06	-0.01				
ST	$r_g$	0.47**	0.52**	0.89**	0.95**	-0.15	0.30**			
	$r_p$	0.21	0.04	0.39**	0.56**	-0.06	0.10			
	$r_e$	-0.03	-0.03	0.01	0.25*	0.02	-0.05			
HI	$r_g$	-0.31**	-0.53**	-0.10	-0.25*	-0.06	0.23*	0.04		
	$r_p$	-0.09	-0.16	-0.04	-0.34**	-0.04	0.05	0.45**		
	$r_e$	-0.01	-0.08	-0.03	-0.43**	-0.05	-0.01	0.61**		
BM	$r_g$	0.06	-0.29*	-0.41**	-0.08	0.62**	-0.64**	-0.08	-0.02	
	$r_p$	0.07	-0.11	-0.23*	-0.04	0.46**	-0.36**	-0.02	-0.01	
	$r_e$	0.11	0.03	-0.02	0.03	0.04	0.03	0.05	0.01	
GY	$r_g$	0.20	-0.09	-0.18	0.14	0.34**	-0.25*	-0.01	-0.38**	0.28*
	$r_p$	0.14	-0.05	-0.07	0.04	0.17	-0.21	-0.01	-0.09	0.23*
	$r_e$	0.07	-0.03	0.02	-0.05	-0.02	-0.02	-0.01	0.01	0.19

\* and \*\* is significant at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively. PH= Plant height (cm), BR= Branches plant<sup>-1</sup>, PODS= Pods plant<sup>-1</sup>, PL= Pod length (mm), SPP= Seeds pod<sup>-1</sup>, SD= Seed diameter (mm), ST= Seed thickness (mm), HI= Harvest index (%), BM= Biomass plant<sup>-1</sup> (g) and GY= Grain yield plant<sup>-1</sup> (g)

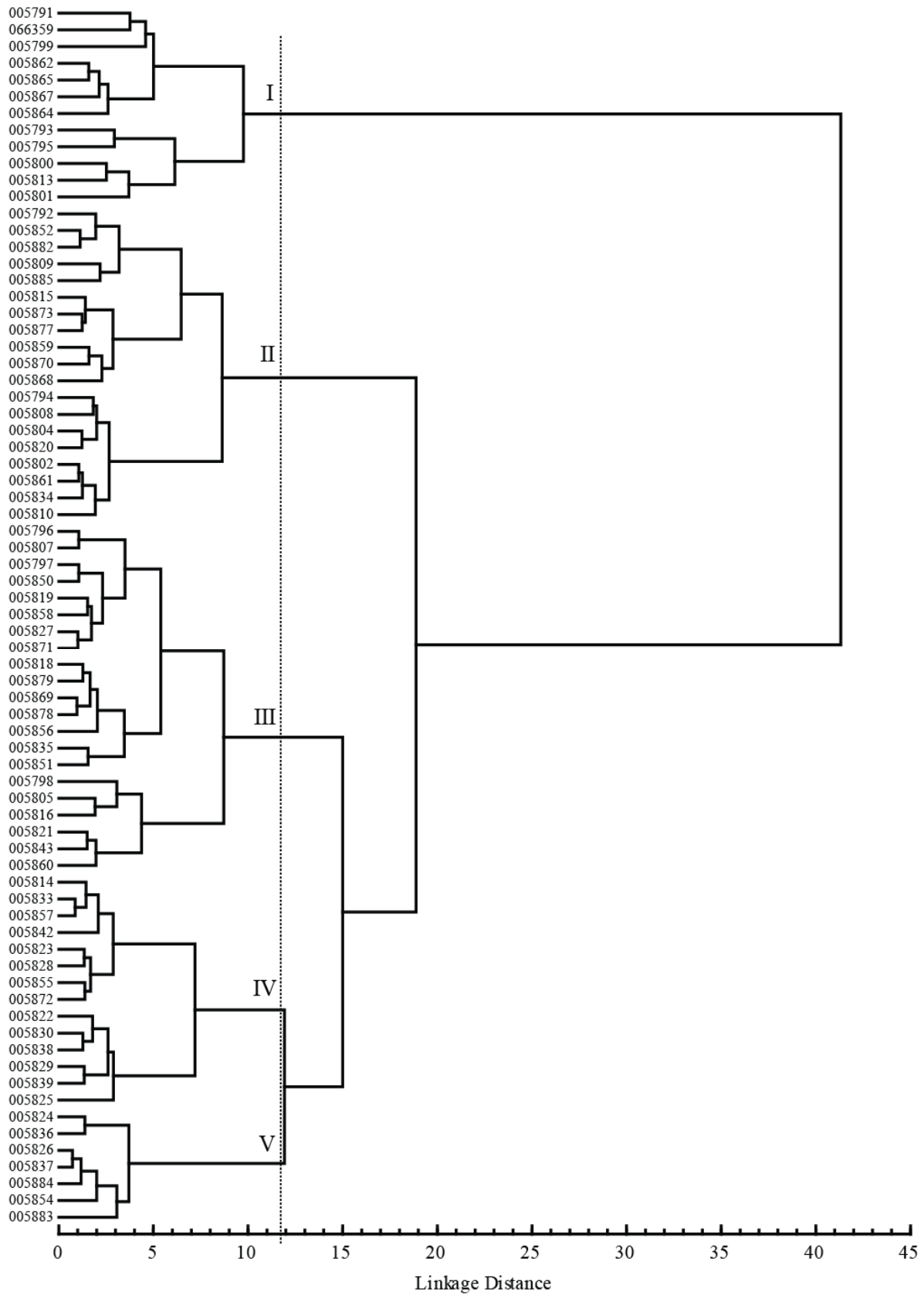


Fig. 2. Clustering pattern in *Lens culinaris* based on agronomic traits. The roman characters represent the clusters at one fourth dissimilarity index.

**Table 3. Direct and indirect effects of independent variables toward grain yield in *Lens culinaris*.**

	PH	BR	PODS	PL	SPP	SD	ST	HI	BM	$r_G$ with GY
PH	<b>-1.64</b>	-0.23	-4.38	2.82	-0.02	-0.12	3.75	0.34	-0.31	0.20
BR	-0.39	<b>-0.95</b>	-8.26	3.54	0.09	-0.57	4.19	0.59	1.67	-0.09
PODS	-0.59	-0.65	<b>-12.05</b>	4.42	0.13	-0.96	7.12	0.11	2.29	-0.18
PL	-0.94	-0.69	-10.91	<b>4.88</b>	0.04	-0.57	7.58	0.28	0.48	0.14
SPP	-0.13	0.25	4.68	-0.60	<b>-0.33</b>	1.15	-1.22	0.07	-3.51	0.34
SD	-0.05	-0.16	-3.47	0.84	0.11	<b>-3.34</b>	2.44	-0.25	3.64	-0.25
ST	-0.77	-0.50	-10.80	4.65	0.05	-1.02	<b>7.94</b>	-0.04	0.48	-0.01
HI	0.51	0.50	1.25	-1.23	0.02	-0.78	0.33	<b>-1.10</b>	0.10	-0.38
BM	-0.09	0.28	4.88	-0.41	-0.20	2.15	-0.68	0.02	<b>-5.66</b>	0.28

PH= Plant height (cm), BR= Branches plant<sup>-1</sup>, PODS= Pods plant<sup>-1</sup>, PL= Pod length (mm), SPP= Seeds pod<sup>-1</sup>, SD= Seed diameter (mm), ST= Seed thickness (mm), HI= Harvest index (%), BM= Biomass plant<sup>-1</sup> (g) and GY= Grain yield plant<sup>-1</sup> (g).  $r_G$  with GY is genetic correlations of corresponding trait with grain yield

**Table 4. The average agronomic performance of five clusters in *Lens culinaris*. The figures in parenthesis are the frequency of genotypes in clusters.**

	Cluster 1 (12)	Cluster 2 (19)	Cluster 3 (21)	Cluster 4 (14)	Cluster 5 (7)
Plant height (cm)	42.6 ± 5.9	37.2 ± 4.8	35.0 ± 4.0	32.3 ± 3.8	38.7 ± 6.9
Branches plant <sup>-1</sup>	11.0 ± 3.3	7.1 ± 1.0	7.6 ± 1.1	6.2 ± 0.8	6.5 ± 0.9
Pods plant <sup>-1</sup>	140.5 ± 37.6	92.2 ± 18.9	64.1 ± 15.9	58.5 ± 9.3	51.9 ± 6.6
Biologic yield plant <sup>-1</sup> (g)	12.6 ± 2.6	7.4 ± 1.1	5.7 ± 1.5	4.8 ± 1.1	5.9 ± 1.0
Pod length (mm)	9.8 ± 1.5	10.2 ± 1.0	9.8 ± 1.3	10.7 ± 0.9	12.7 ± 0.6
Seed pod <sup>-1</sup>	1.7 ± 0.4	1.7 ± 0.3	1.4 ± 0.2	1.5 ± 0.4	1.0 ± 0.1
Seed diameter (mm)	1.7 ± 0.4	1.7 ± 0.3	1.4 ± 0.2	1.5 ± 0.4	1.0 ± 0.1
Seed thickness (mm)	2.8 ± 0.7	2.0 ± 0.3	1.2 ± 0.4	1.4 ± 0.3	1.4 ± 0.2
Grain yield plant <sup>-1</sup> (g)	2.8 ± 0.7	2.0 ± 0.3	1.2 ± 0.4	1.4 ± 0.3	1.4 ± 0.2
Harvest index (%)	23.6 ± 2.9	29.7 ± 3.8	22.7 ± 4.4	31.3 ± 3.1	26.0 ± 2.4
Intra-cluster $D_g$	3.8	3.8	3.9	3.9	3.7
Inter-cluster $D_g$	5.5	3.8	3.9	3.9	4.4

$D_g$ -Genetic diversity

**Table 5. Principal component analysis for *Lens culinaris* germplasm based on ten agronomic traits.**

Traits	Communality	PC <sub>1</sub>	PC <sub>2</sub>	PC <sub>3</sub>
		Eigen vector		
		4.5	2.1	1.3
		44.8	21.0	13.0
		44.8	65.8	78.7
Plant height (cm)	0.38	0.50	-0.36	0.05
Branches plant <sup>-1</sup>	0.72	0.65	-0.22	-0.50
Pods plant <sup>-1</sup>	0.82	0.90	-0.08	-0.06
Biologic yield plant <sup>-1</sup> (g)	0.94	0.92	-0.31	0.02
Pod length (mm)	0.39	-0.30	-0.41	0.36
Seed pod <sup>-1</sup>	0.89	0.42	0.82	-0.18
Seed diameter (mm)	0.88	0.40	0.83	-0.18
Seed thickness (mm)	0.97	0.92	-0.06	0.36
Grain yield plant <sup>-1</sup> (g)	0.97	0.92	-0.05	0.36
Harvest index (%)	0.84	-0.07	0.51	0.76

**Table 6. Cluster-wise analyses of variance and estimation of genetic parameters in *Lens culinaris* germplasm.**

		PH	BR	PODS	PL	SPP	SD	ST	HI	BM	GY
Cluster 1	Intra-cluster	*	ns	ns	ns	*	ns	ns	ns	ns	ns
	Inter-cluster	**	*	**	**	**	*	*	**	**	ns
	Heritability	0.48	0.11	0.24	0.22	0.72	0.41	0.12	0.57	0.16	0.02
	$r_g$	-0.12	-0.43	0.57	0.07	-0.42	0.14	0.08	0.04	0.98	-
	Direct effect	-0.19	-0.13	1.68	1.38	0.22	0.12	0.05	-0.26	0.13	-
Cluster 2	Intra-cluster	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Inter-cluster	**	**	*	ns	**	*	*	**	ns	ns
	Heritability	0.55	0.11	0.09	0.02	0.52	0.31	0.10	0.41	0.01	0.04
	$r_g$	0.08	0.49	0.92	-0.35	-0.16	-0.12	-0.09	0.98	-0.31	-
	Direct effect	-1.07	-0.73	-1.55	0.24	-0.78	-0.11	0.14	2.53	1.16	-
Cluster 3	Intra-cluster	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Inter-cluster	**	**	**	**	**	*	*	*	**	*
	Heritability	0.43	0.14	0.26	0.27	0.67	0.25	0.08	0.09	0.18	0.07
	$r_g$	0.51	0.31	0.76	-0.21	-0.33	-0.21	0.15	0.40	0.96	-
	Direct effect	0.64	-0.90	1.42	1.02	1.07	0.32	0.11	0.20	0.49	-
Cluster 4	Intra-cluster	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Inter-cluster	**	ns	ns	**	**	*	ns	**	**	ns
	Heritability	0.46	0.06	0.07	0.31	0.38	0.21	0.15	0.41	0.21	0.05
	$r_g$	0.22	0.99	0.98	0.06	0.29	-0.51	0.06	-0.70	0.96	-
	Direct effect	-0.02	-0.01	0.03	-0.22	0.15	0.12	-0.19	0.36	0.96	-
Cluster 5	Intra-cluster	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Inter-cluster	**	ns	ns	*	*	*	ns	ns	*	ns
	Heritability	0.74	0.08	0.05	0.16	0.11	0.18	0.06	0.11	0.11	0.03
	$r_g$	-0.33	-0.95	0.13	-0.82	0.97	-0.38	0.12	-0.52	0.99	-
	Direct effect	-0.48	0.77	-0.49	-0.79	0.87	0.19	-0.34	-0.91	0.37	-

\*, \*\* and ns is significant at  $p \leq 0.05$ ,  $p \leq 0.01$  and insignificant, respectively. PH= Plant height (cm), BR= Branches plant<sup>-1</sup>, PODS= Pods plant<sup>-1</sup>, PL= Pod length (mm), SPP= Seeds pod<sup>-1</sup>, SD= Seed diameter (mm), ST= Seed thickness (mm), HI= Harvest index (%), BM= Biomass plant<sup>-1</sup> (g) and GY= Grain yield plant<sup>-1</sup> (g)

## Discussion

Variability among qualitative traits explained almost all the classes described anywhere else that indicated the worth of the lentil germplasm assembled in the genebank (Toklu *et al.*, 2009). Among the variation observed, the orange cotyledon can be selected from the material and can be utilized for varietal identification (Carovic-Stanko *et al.*, 2011) and these were the first to be used as markers in plant breeding (Stuessy, 1990; Liu *et al.*, 2012). Genetic parameters with insignificant intra-genotypic variance indicated the scope of simple selection, especially for the traits with higher heritability and genetic advance (Jatoi *et al.*, 2011; Tyagi & Khan, 2011). Contrarily, the seeds pod<sup>-1</sup>, seed diameter, biomass and grain yield plant<sup>-1</sup> revealed the prevalence of homogeneous mixture that was suggested to isolate, especially for seeds pod<sup>-1</sup> and seed diameter which are highly heritable traits in lentil. Higher levels of genotypic correlations coupled with higher direct effects for the traits (pod length and seed thickness) highlighted the impact of these traits for enhancing lentil productivity under eco-agricultural system, especially where environmental influence is negligible. Vlachostergios *et al.*, (2011) examined lentil varieties for organic farming

systems developed through conventional breeding and similarly we expect to have base material for eco-agricultural systems of lentil that has a quick response to climate change.

The clusters and their performance indicated the selection of better genotypes for organic farming as well as establishment of a mini core (Wei *et al.*, 2011), but with addition investigation of molecular information for this material (Bonman *et al.*, 2011; Carpio *et al.*, 2011). Linearity of numerical techniques increased the scope of selection, especially from individual clusters with track from direct effects in relation with genotypic correlations. Combined information of correlation and path analyses on high number of genotypes, *i.e.*, 73 in this study often mislead to draw conclusion, hence in this case correlation and path analysis were conducted on individual cluster in addition to combined data set that provided concrete conclusion on selection criterion within each cluster. On the basis of present results, the lentil germplasm preserved in the genebank at NARC possessed alleles for adaptation under eco-agricultural system, and the genotypes which are more or less homogeneous as revealed by intra-genotypic variance are expected from lentil germplasm for development of lentil cultivars suitable for organic farming that is today's demand for future human healthcare.

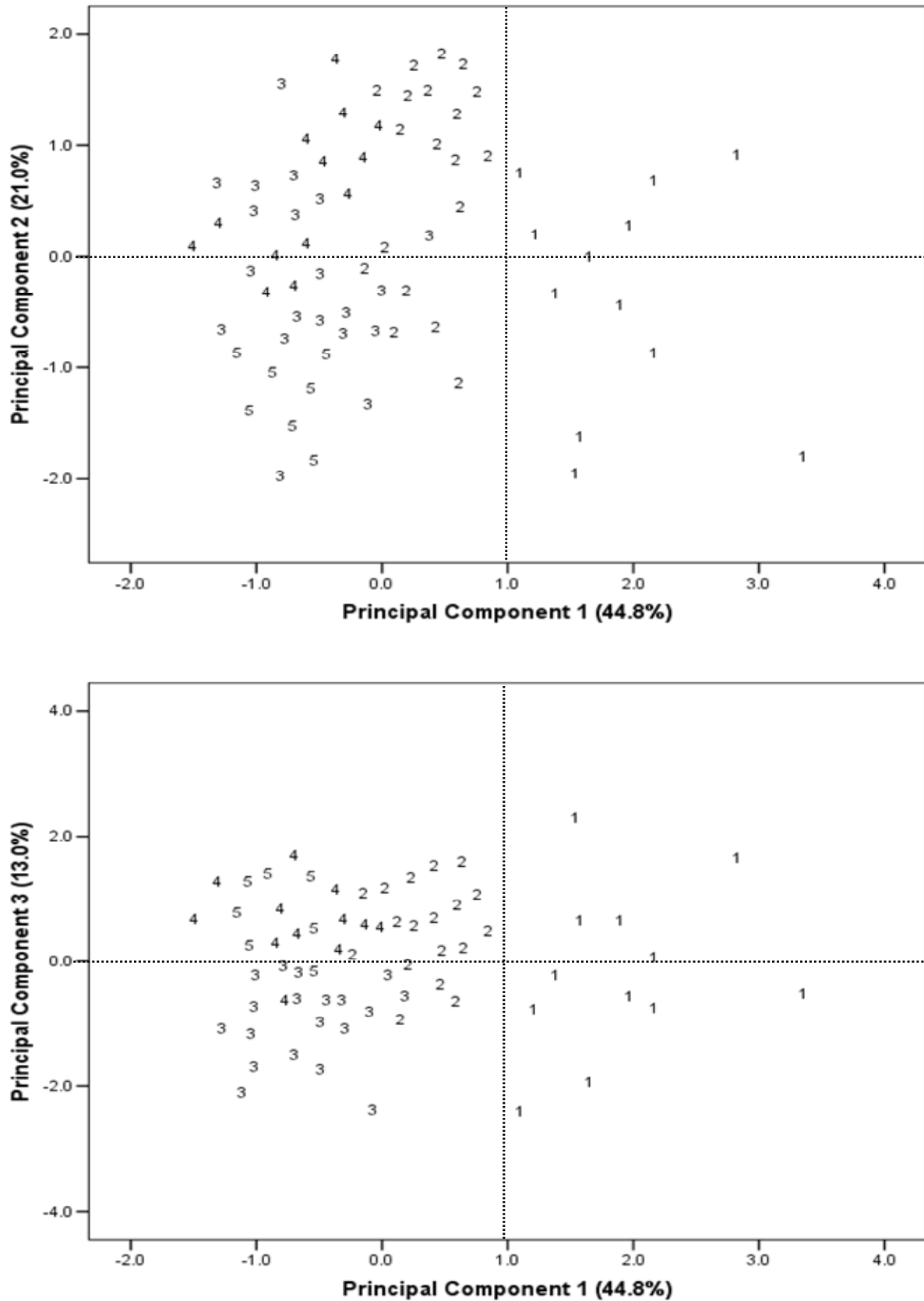


Fig. 3. Scattered diagram for PC<sub>1</sub>-PC<sub>2</sub> (above) and PC<sub>1</sub>-PC<sub>3</sub> (below) in *Lens culinaris* based on agronomic traits. The numeric characters represent the clusters based on UPGMA.



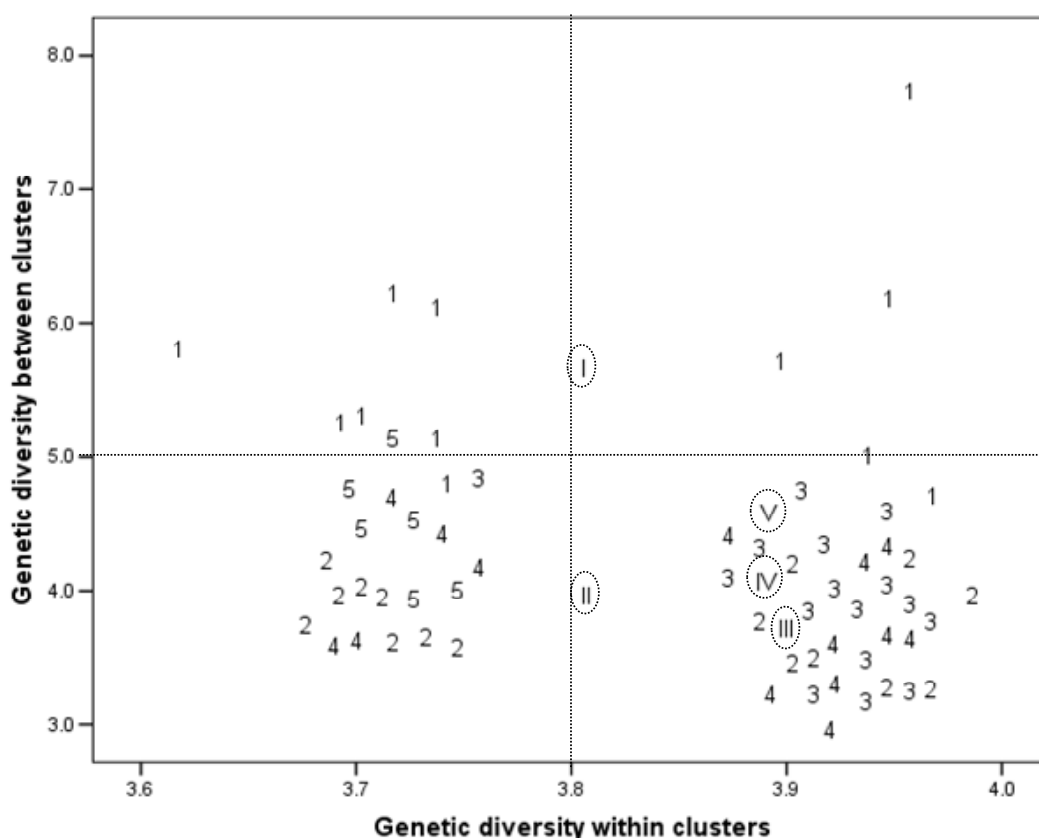


Fig. 4. Scattered pattern of five clusters based on agronomic traits in *Lens culinaris*. The numeric characters represent the clusters based on UPGMA, whereas the encircled roman characters represent the average genetic distance of within and between clusters.

Erskine *et al.*, (2011) conducted a study to re-examine world germplasm of lentil at two sites in Pakistan (Islamabad and Faisalabad) and addressed lentil movement and explained their genetic bottlenecks. They concluded that lentil germplasm of Afghanistan perhaps harbored recessive alleles for time to flower from introgression with *Lens culinaris* ssp. *Orientalis*. Most of the reports in crop species are candidate gene-based, genomic studies and new types of populations with better selection impact have been developed including classical mapping for rare in germplasm collections, such as disease resistance genes or alleles introgressed from exotic germplasm (Rafalski, 2010). The advancement in genomic sequences of most of the crops along with the ability to perform high-throughput re-sequencing has enhanced our understanding of the history of plant domestication and to accelerate crop improvement (Morrel *et al.*, 2012), but conventional breeding techniques are still valid and without this, the modern molecular methodologies are no more than a cosmetic sciences that has got popularity due to huge investment in this field (Mumtaz *et al.*, 2011). Path coefficient analysis on the total germplasm did not reveal concrete understanding, whereas on subsets of five clusters proved more practical inference for lentil improvement through various traits within specific cluster using cause and effect relationship. Although the future of crop improvement is expected on plant genomes, new mapping strategies and evolutionary analysis to direct and optimize the discovery and use of genetic variation, anyhow the use

of variation could only be possible through the breeders' skill (Fridman & Zamir, 2012), and simply if we rule out the selection, the whole pyramid of modern biotech tools may collapse without increasing agricultural productivity. According to Reynolds *et al.*, (2012) improvement in co-ordination of agricultural research is vital for food security. Although advances in biotechnology has promised research, but gaps exist that may reduce the impact on crop productivity, hence coordinated globalized approach to agricultural research through the implementation of Global Crop Improvement Networks (GCIN) is vital for ensuring food security. Lentil being a potential pulse crop of Asian countries should at least be coordinated for development through any Asian Network, especially for organic farming under changing climate scenario for which the present findings could be the baseline information.

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