

## GENETIC DIVERGENCE IN LENTIL GERMPLASM FOR BOTANICAL DESCRIPTORS IN RELATION WITH GEOGRAPHIC ORIGIN

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### Abstract

Three hundred and seventeen accessions of lentil collected from all over the country were characterized for stem colour, pedicle colour, growth habit, tendrils, hairiness, leaf pubescence, leaflet size, pod pigmentation, pod indehiscence, presence/absence of beak, seed coat colour, seed coat pattern, seed coat pattern colour and cotyledon colour. Out of these accessions, 76 were heterogeneous that needs to evaluate carefully to isolate pure lines. Inter and intra-accession variation indicated the presence of local wealth for lentil that is yet needed to collect, therefore further exploration missions are suggested to collect germplasm from remote areas of NWFP, NA and Baluchistan with maximum emphasis to interiors rather than closer to motorable roads. High variability was observed for growth habit, leaf pubescence, leaflet size, seed coat colour, seed coat pattern and seed coat pattern colour that could be expanded and exploited for developing breeding material and to use for Marker Assisted Selection. Though clusters analysis grouped together accessions with greater genetic similarity, the cluster did not necessarily include all the accessions from same origin. Low level of association between genetic diversity and geographic distribution is expected to be due to less representation of accessions from particular area that is needed to be studied uniformly.

### Introduction

Genetic variation between and within populations of crop species is a major interest of plant breeders and geneticists (Hayward & Breese, 1993). Moore & Collins (1983) considered that monitoring of germplasm is necessary during maintenance for predicting potential genetic gain in breeding programme. Barulina (1930) first recorded detailed morphological descriptions of lentil landraces and species from Asia. World lentil germplasm displayed a wide range of diversity on the basis of morphological traits (Erskine & Witcombe, 1984), hence local germplasm has never been characterized in detail (Anon., 1996). The traditional approach of characterization and evaluation involves cultivation of accession subsamples and their morphological and agronomic description; a procedure facilitated by the use of internationally recognized descriptor lists (Erskine & Williams, 1980). Morphological characterization is the first step in the classification and description of any crop germplasm (Smith & Smith, 1989; Singh & Tripathi, 1985).

Clusters on the basis of plant descriptors have been described by Singh, (1988), Caradus *et al.*, (1989), Peeters & Martinelli, (1989) and Clements & Cowling, (1994). Although multivariate analysis on quantitative traits provides a good evaluation of landraces by identifying those that should be further evaluated at the genetic level, but according to most researchers it should not be disassociated from botanical descriptors (Muehlbauer & Slinkard, 1981; Erskine & Witcombe, 1984; Baylon & Singh, 1986; Rouamba *et al.*, 1996; Kurlovich, 1998; Peyghambari, 2003).

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Numerical taxonomic techniques have been successfully used to classify and measure the pattern of phenotypic diversity in the relationship of germplasm collections in a variety of crops by many scientists as in lentil (Ahmad *et al.*, 1997, Tahir *et al.*, 1994) blackgram (Ghafoor *et al.*, 2001), mungbean (Singh, 1988), pea (Amurrio *et al.*, 1995), soybean (Perry & Matntosh, 1991) and alfalfa (Smith *et al.*, 1995; Warburton & Smith, 1993). Lentils are considered to be one of the oldest food crops of mankind (Zohary, 1972; Ladizinsky, 1979; Hawtin *et al.*, 1980; Cubero, 1981; Anon., 2003). In Pakistan, lentil is annually grown as a winter-season (rabi) as second important pulse over an area of 44800 ha with 26200 tones production next to chickpea under rainfed areas. Nutritionist rank lentil as an excellent source of diet which is high in protein, a major source of complex carbohydrates, high in fibers, rich in vitamins A and B, potassium and iron, low in sodium and fat that regulate growth and development (Anon., 2003). The researcher can use genetic similarity information to make decision regarding the choice for selecting superior genotypes for improvement or to be utilized as parents for the development of future cultivars through hybridization. The present study was conducted to assess extent of genetic diversity in lentil germplasm collected from geographical regions of Pakistan on the basis of qualitative traits.

### Materials and Methods

Three hundred and seventeen accessions of lentil collected from all over the country were characterized for 14 traits. Out of these 241 were homogeneous, whereas others were heterogeneous for one or the other trait (Sultana, 2003). Homogeneous accessions were analyzed and are presented in the present paper. Of these, 166 accessions were collected from Punjab, 27 from Baluchistan, 38 from Sindh, 4 from NWFP, 2 from Northern Areas (NA) and 4 from unknown region. The experiment was planted at Plant Genetic Resources Programme, National Agricultural Research Center, Islamabad (33.40°N and 73.07°E). The germplasm was collected during the last two decades that represents a wide agro-ecological variation from dry mountainous region to irrigated plains and sandy arid regions of Pakistan (Bhatti *et al.*, 1997). The experiment was planted in augmented design during winter including Mansehra 89 as checks in this study after every 10 test entries. One row of 4 meter length for each accession was planted with 30 and 5 cm inter and intra-row spacing, respectively. Recommended cultural practices were followed throughout the crop season.

Ten plants were sampled at random to study inter and intra-accession variation for each accession. Data were recorded for qualitative traits like stem colour, pedicle colour, growth habit, tendrils, hairiness, leaf pubescence, leaflet size, pod pigmentation, pod indehiscence, presence/absence of beak, seed coat colour, seed coat pattern, seed coat pattern colour and cotyledon colour following IBPGR Descriptors (Anon., 1985). The data were grouped according to provincial distribution, agro-ecological zones and altitude for comprehensive pattern of geographical distribution. The crop agro-ecological regions were followed by Agro-Ecological Regions of Pakistan (Anon., 1980).

### Results and Discussion

Out of total germplasm, 76 accessions were heterogeneous in nature for one or the other trait that indicated the presence of primitive land races in the form of heterogeneous mixture that is needed to evaluate carefully to isolate pure lines from this material due to diverse collecting sites (Bhatti *et al.*, 1997). Differentiation according to geographical regions of origin is useful in substantiating the postulated regions of diversity or gene centres

(Perry & McIntosh, (1991). The rare alleles, each only occurring in one or two apparently random populations can be considered to be mutants, migration or the results of other coincidental events (Van Hintum & Elings, 1991; Ghafoor *et al.*, 2002). Heterogeneous nature is also attributed to genetic material that exchanged frequently among growers and less research on this crop, followed by some degree of contamination by mixture or out crossing with other landraces (Pecetti *et al.*, 1996).

Variation between and within populations of crop species is useful for analysing and monitoring germplasm during the maintenance phase and predicting potential genetic gain in a breeding programme (Hayward & Breese, 1993; Moore & Collins, 1983). For qualitative characters, a considerable level of variability was observed for growth habit, leaf pubescence, leaflet size, seed coat colour, seed coat pattern and seed coat pattern colour that could be exploited for developing future breeding material in lentil breeding programme (Table 1). Two classes were observed for stem colour, hairiness, tendril, pod pigmentation (anthocianin), pod indehiscence, beak on the pod and cotyledon colour, whereas for other traits more than one class were observed, especially for seed traits that represents the world classification as reported by Erskine & Witcombe (1984). Muehlbauer & Slinkard (1981) reviewed the genetics of *Lens* and listed 12 genes which account for morphological and seed variation in lentil. It was observed critically that seed traits in lentil are difficult to record that need standardization.

**Table 1. Method for measuring and classification of plant descriptors in 241 accessions of *Lens culinaris***

<b>Plant characters</b>	<b>When and where measured</b>	<b>Classification</b>	<b>M 89</b>
Stem colour	Observed at Seedling stage when plants were 2-3" high	Purple (233), green (8)	Purple
Pedicle colour	At 50% flowering	Purple (4), green (237)	Purple
Tendrils	At the time of pod formation when plants were full grown	Present (108), absent (133)	Present
Leaf pubescence	Before maturity when plants were full grown	Absent (50), slight (134), dense (57)	Slight
Leaflet size	Observed on fully expanded leaves on the lower flowering nodes	Small (118), medium (85), large (38)	Medium
Pod pigmentation	Before maturity when pods were filled but not turned brown	Present (6), absent (235)	Absent
Pod indehiscence	At the time of maturity observed carefully to scored this trait up to one week after maturity	Present (3), absent (238)	Absent
Beak	At physiological maturity of pods	Present (118), absent (123)	Absent
Seed coat colour	After harvesting but less than three months old	Olive green (4), grey (35), brown (64), black (12), pink (126)	Olive green
Seed coat pattern	After harvesting but less than three months old	Absent(8),dotted(139), spotted (11), marbled (36), complex (47)	Absent
Seed coat pattern colour	After harvesting but less than three months old	Absent (8), grey (208), brown (11), black (14)	Absent
Cotyledon colour	After harvesting but less than three months old	Yellow (5), red (236)	Yellow

The concept of using morphological marker in plant breeding is therefore not new and most of the researchers considered characterization first step in description and classification of crop germplasm (Singh & Tripathi 1985; Smith & Smith 1989; Shanmugam & Shreerangaswamy 1982; Smith *et al.*, 1991). Qualitative characters are important for plant description (Kurlovich, 1998) and are mainly affected by the consumer's preference, socio-economic scenario and natural selection. Nakayama *et al.*, (1998) reported that foxtail millet landraces with low amylose allele were distributed only in Southeast Asia mainly because of preference followed by selection. Ghafoor *et al.*, (2001) considered that these traits reflect some biotic/abiotic stresses association. Similarly in Pakistan lentils with orange cotyledon are preferred over yellow although later has higher yield potential. It is suggested to use orange cotyledon donor parent to develop desirable high yielding lentils. Tschermak-Seysenegy (1928) described the first qualitative gene of red cotyledon colour in lentil dominant to yellow cotyledon colour.

According to Ladizinsky (1979) pod indehiscence was probably one of the first characters selected by man in the early days of agriculture. In a tiny plant like wild lentil is also equipped with an effective seed dispersal mechanism. Cultivation is almost unthinkable before an indehiscent pod mutant. As in the cereals of the Middle East the transition from wild to domesticated lentil was a single-step event due to one mutation. As it is a single gene character, and in a crop like lentil, once the pod indehiscent accession is selected, it could be maintained forever. Erskine & Witcombe (1984) classified the ground colour of the seed coat (seed coat colour) of lentil into five classes: green, pink, brown, gray or black. They also classified seed coat patterns and seed coat patterns colour into absent, dotted, spotted, marbled or complex and absent, olive green, gray, brown or black, respectively. Similar findings were observed for seed traits in the present material that indicated the importance of local wealth that is yet to be exploited.

Eight clusters were observed on the basis of average linkage and Euclidean dissimilarity coefficient matrix explored the inter-cluster distance among the accessions (Table 2). Cluster I, represented 18.3% of total material consisted of 44 accessions, cluster II represented 6.6% (16 accessions), cluster III 18.7% (45 accessions), cluster IV 17.4% (42 accessions), cluster V 7.1% (17 accessions), cluster VI 5.8% (14 accessions), cluster VII 17.0% (41 accessions), and cluster VIII of 22 accessions. Dissimilarity coefficient ranged from minimum value of 0.53 (Cluster I vs Cluster III) to maximum value of 1.03 (Cluster II vs Cluster VI). Cluster II consisting 16 accessions was obviously different from all other clusters with a genetic distance ranging from 0.83 (cluster III) to 1.03 (cluster VI). Cluster VIII vs V and cluster VII vs VI exhibited higher genetic distances that could be exploited for lentil breeding. Similar types of research was conducted by Malik *et al.*, (1984); Bakhsh *et al.*, (1991) and Singh & Singh (1993), who reported high genetic variance in cultivated lentil. Therefore, these clusters might be consisted of diverse accessions on the basis of qualitative traits. The selected accessions from various clusters are suggested to be used in crop improvement in future (Singh *et al.*, 1997). Inter-cluster distance is a good indicator to select diverse parental lines. It is suggested that superior pure-lines from diverse cluster may be chosen for hybridization due to better performance of hybrids (Ghafoor *et al.*, 2000; Iqbal *et al.*, 2003). Table 3 presents the genetic status of each cluster indicating that among all the traits growth habit, tendrils, beak, seed coat pattern and seed coat pattern colour gave the maximum diversity across the clusters. More than 50% of clusters were with polymorphism for stem colour, pedicle colour, leaf pubescence, leaflet size, pod pigmentation and seed coat, whereas in case of pod indehiscence, hairiness and cotyledon colour low level of variation was observed. Clusters IV, VII and I exhibited high degree of variance, respectively that indicated the usefulness of cluster analysis for botanical descriptors.

**Table 2. Inter cluster distance based on average linkage for botanical descriptors in lentil germplasm.**

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7
Cluster 2	0.959						
Cluster 3	0.530	0.839					
Cluster 4	0.715	0.964	0.672				
Cluster 5	0.717	0.989	0.786	0.615			
Cluster 6	0.619	1.032	0.568	0.646	0.539		
Cluster 7	0.634	0.916	0.608	0.699	0.592	0.817	
Cluster 8	0.672	0.897	0.783	0.534	0.877	0.986	0.680

**Table 3. Inter and intra-cluster heterogeneous status of lentil germplasm classified in eight clusters for average linkage.**

Qualitative traits	C-1	C- 2	C-3	C- 4	C-5	C-6	C-7	C-8
Stem colour	+-	+	+-	+-	+	+	+-	+
Pedicle colour	+-	-	-	+-	-	+-	+-	-
Growth habit	+-	+-	+-	+-	+-	+-	+-	+-
Tendril	+-	+-	+-	+-	+-	+-	+-	+-
Hairiness	+	+	+	+	+	+	+-	+
Leaf Pubescence	+-	+-	+-	+-	+	+	+-	+-
Leaflet size	+-	+-	+	+-	+-	+-	+-	+-
Pod pigmentation	-	-	-	+-	+-	-	+-	+-
Pod indehiscence	-	-	+-	+-	-	-	-	-
Beak	+-	+-	+-	+-	+-	+-	+-	+-
Seed coat colour	+-	+-	+	+-	+-	+	+-	+-
Seed coat pattern	+-	+-	+-	+-	+-	+-	+-	+-
Seed coat pattern colour	+-	+-	+-	+-	+-	+-	+-	+-
Cotyledon colour	+-	+	+-	+-	+	+	+	+

C- Cluster, + Presence, - Absence, +/- Polymorphism

Germplasm categorized on the basis of distribution in provinces revealed that more than 70% of the germplasm in cluster I, III, IV, V, VI and VII was from Punjab that represents the cultivation of this crop in various provinces, respectively (Tahir *et al.*, 1994). Figure 1 gives a picture for distribution of eight clusters in three geographic parameters (provinces, agro-ecological zones and altitude). The material collected from Punjab and Sindh represented almost all the clusters, whereas germplasm did not classify equally in other provinces. Cluster II comprised accessions mostly collected from Baluchistan. Similarly, germplasm categorized on the basis of agro-ecological zones revealed that zones 6 and 7 represented only 2 clusters followed by material from zone 1 and 9 where three clusters were observed. More than 50% germplasm of each cluster was distributed in zone 4 and zone 5 except for cluster II. Cluster II showed 75.0% representation from zone 9 (Baluchistan). About 85% of germplasm in clusters I, III, IV, V, VI and VII was collected within the range of 0-500 masl, whereas 56.3% accessions of cluster II were from 1001-1500 masl. In comparison with the aforementioned cluster, cluster II was observed with maximum variation on the basis of altitude for qualitative characters.

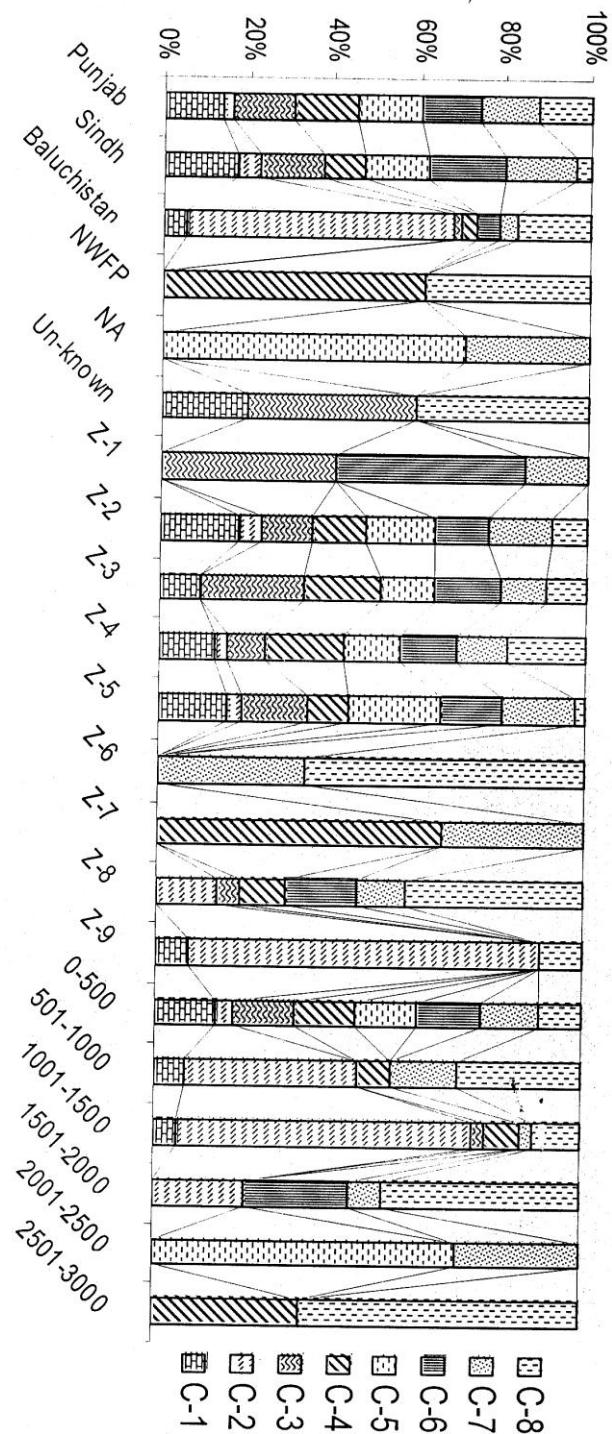


Fig. 1. Inter-relationship of genetic diversity based on botanical descriptors and three geographic parameters (Provinces, Agro-ecological Zones and Altitude) in lentil germplasm.

Though clusters analysis grouped together accessions with greater genetic similarity, the cluster did not necessarily include all the accessions from same origin. Gupta *et al.*, (1991), Dias *et al.*, (1993) Amurrio *et al.*, (1995) and Rabbani *et al.*, (1998) also reported lack of association between morphological traits and origin.

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