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EVALUATION OF WILD SPECIES OF LENTIL FOR AGRO-MORPHOLOGICAL TRAITS

Aditya Pratap*, Jitendra Kumar and Shiv Kumar[1]

Crop Improvement Division,
Indian Institute of Pulses Research, Kanpur-208 024, India

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ABSTRACT

Most of the existing varieties of lentil (*Lens culinaris* ssp. *culinaris*) have been developed mainly through intraspecific hybridization and pureline selection leading to a narrow genetic base in cultivated populations. This makes them vulnerable to a number of biotic and abiotic stresses besides reducing their genetic potential due to lesser hidden variability. Distant hybridization involving wild accessions increases genetic variability and also helps in introgression of desirable genes rendering cultivated species more usable. Keeping this in view, wild accessions of lentil procured from ICARDA, Aleppo, Syria were established and evaluated under local conditions at IIPR, Kanpur. These comprised 88 accessions from *Lens nigricans*, *L. culinaris* ssp. *odemensis*, *L. culinaris* ssp. *orientalis*, *L. culinaris* ssp. *tomentosus*, *L. ervoides*, *L. lamottei* and unknown Lens spp. The results showed significant genetic variation among the wild accessions for all characters except cotyledon colour. PCA analysis of the morphological data resulted in clustering of 88 wild accessions into three groups and distinct position of each genotype was observed within each group. The first three most informative components in PCA analysis individually accounted for 89.35, 4.38 and 2.3% of total variation, respectively and collectively these explained about 95% of the total variability. While more traits and multilocation data need to be considered for getting more reliable results, in general *L. ervoides* was observed to possess useful traits like, plant height, internode length and pods/cluster and therefore could be utilized for genetic improvement of cultivated lentil.

Key words: Distant hybridization, Lentil, *L. ervoides*, PCA, Wild accessions.

INTRODUCTION

Lentil (*Lens culinaris* Medikus ssp. *culinaris*) is an important cool-season food legume and ranks ~~third~~ in production in the world after ~~chickpea and pea~~. In ~~2010~~, global lentil production was about 4.55 million tonnes from an estimated 4.25 million ha area with an average yield of 1070 kg per ha (FAO, 2013). Canada is the largest producer of lentil followed by India Australia and Turkey. During 2011-12, India harvested 0.95 million ton lentils from 1.60 million ha area with an average yield of 594 kg per ha. Evidently, the present productivity of lentil in India is very low in spite of a large number of improved varieties developed for cultivation in different agro-ecological zones of the country. Earlier studies have confirmed that these varieties, mostly developed through intraspecific hybridization and pure line selection, have narrow genetic base (Kumar *et al.* 2004). This makes them vulnerable to several biotic and abiotic stresses besides limiting their realizable yield potential. Introgression of useful genes from wild relatives has been suggested to overcome the problem of narrow genetic base of lentil (Erskine *et al.*, 1998; Rahman *et al.*, 2009). This may help in introgresson of desirable genes or gene combinations into the cultivated backgrounds, thereby rendering them more usable (Pratap *et al.* 2009).

It is well known that wild species are a rich reservoir of useful alien genes, which are no longer available within the cultivated gene pool (Tanksley and McCouch, 1997). Therefore, continuous efforts have been made to collect and conserve wild relatives

*Corresponding author's e-mail: adityapratapgarg@gmail.com
[1]International Centre for Agricultural Research in the Dry Areas, Rabat Office, P.O. Box 6299, Rabat – instituts, Rabat, Morocco

of various food legume crops including lentil in the national and international gene banks . ICARDA global collection of Lens has about 587 wild accessions representing six *Lens* species and subspecies from 26 countries (Kumar *et al.*, 2011). Efforts have also been made to search for genes imparting resistance to biotic and abiotic stresses and other traits among the wild relatives and success of introgression of alien genes from wild relatives has been achieved for few diseases and insect-pests which are controlled by major gene(s) (Ladizinsky et al. 1988, Hajjar and Hodgkin 2007; Fiala et al., 2009; Tullu et al. 2011). Significant advances have recently been made both in the molecular technologies and hybridization procedures that make it possible to transfer alien gene(s) into the cultivated germplasm. However, the use of wild relatives for lentil improvement has remained limited, and that too confined to only a few wild accessions, mainly due to limited access to wild species, difficulties in their establishment, non-synchrony in flowering between cultivated and wild species and various pre- and post-fertilization barriers (Kumar *et al.*, 2011). Further, most of the wild germplasm collection ~~set~~ has largely remained unevaluated for morpho-physiological traits under Indian soil and climatic conditions. Keeping this in view, this study was conducted to establish exotic wild accessions of lentil under controlled conditions at IIPR, Kanpur and evaluate them to identify most promising donors for various yield and yield contributing traits.

MATERIALS AND METHODS

Eighty eight wild accessions of lentil representing six *Lens* species and sub-species were procured from the International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria under standard material transfer agreement during 2008 (Tables 1 and 2). The seeds of these accessions were evaluated in pots at the main research farm of Indian Institute of Pulses Research, Kanpur during 2009-10 and 2010-11. The 12-inch diameter plastic pots were filled with the sterilized mixture of sand, farm yard manure and soil (1: 1: 2). Before sowing, seeds were scarified to overcome the germination problem due to hard seed coat in wild accessions. For seed coat scarification, 10 seeds of each accession were held with the thumb and an excision was made on the reverse side of the seed using a sharp surgical blade. Immediate after the scarification, seeds were incubated on moist filter paper at room temperature for 24 hours in Petri-plates, followed by their direct sowing in the pots. Germination was observed in all the accessions within 6-7 days though only 88 accessions reached the maturity stage. Twelve accessions were lost during the crop development owing to various reasons, the most prominent being very poor initial seedling vigour and consequently drying of plants. Nevertheless, the germination percentage differed within the accessions also and it ranged 40-100% in different wild accessions. Observations on 88 accessions which reached maturity were recorded for 11 morphological traits. Plant height, internode length, rachis length, leaf length, leaf width, pods/cluster and seeds/pod were recorded on ~~minimum~~ four plants per accession while data on presence or absence of tendrils were recorded on three random plants. Data on 100-seed weight and cotyledon colour were taken after the harvest and threshing. All the characters were recorded when these had full expression.

The data of both the years were pooled to work out range and mean. The pooled data were subjected to similarity co-efficient analysis (Jaccard 1908) based on which a dendrogram was constructed using unweighted pair group method with arithmetic average (UPGMA) using NTSYS pc-2.11x (Rolf 1998) software. The data were also subjected to Principal Component Analysis (PCA) using the same software.

RESULTS AND DISCUSSION

Lens gene-pool consists of many wild relatives offering resistance to biotic (Ahmad *et al.*, 1997) and abiotic stresses (Hamdi *et al.*, 1996). Accessions belonging to *L. odemensis* and *L. ervoides* showed drought tolerance (Hamdi and

TABLE 1: Wild accessions of lentil used in the present study.

Name of the species	No. of accessions
Lens nigricans	16
L. culinaris ssp. odemensis	10
L. culinaris ssp. orientalis	22
L. culinaris ssp. tomentosus	06
L. ervoides	31
L. lamottei	02
Lens spp.	01
Total	88

TABLE 2: Wild accessions of lentil evaluated in present study with their country of origin and cotyledon colour.

Name of the genotype	ILWL	Country of origin	Cotyledon colour
L. culinaris ssp. *orientalis*	7	Turkey	Red
Lens spp.	9	Syria	Red
L. culinaris ssp. *tomentosus*	11	Syria	Red
Lens nigricans	13	Italy	Red
L. lamottei	14	France	Red
Lens nigricans	15	France	Red
Lens nigricans	16	France	Red
Lens nigricans	18	France	Red
Lens nigricans	19	Spain	Yellow
L. culinaris ssp. *odemensis*	20	Palestine	Red
L. culinaris ssp. *odemensis*	21	Palestine	Yellow
Lens nigricans	22	Italy	Yellow
Lens nigricans	23	Italy	Red
Lens nigricans	26	Croatia	Red
Lens nigricans	28	BIH	Red
L. lamottei	29	Spain	Red
Lens nigricans	30	Spain	Red
Lens nigricans	31	Spain	Red
Lens nigricans	32	Spain	Red
Lens nigricans	33	Spain	Red
L. culinaris ssp. *odemensis*	35	Turkey	Red
L. culinaris ssp. *odemensis*	36	Turkey	Red
Lens nigricans	37	Turkey	Red
L. culinaris ssp. *odemensis*	39	Turkey	Yellow
L. ervoides	40	Ukraine	Red
L. ervoides	41	Turkey	Red
L. ervoides	42	Italy	Red
L. ervoides	45	Croatia	Red
L. ervoides	48	Croatia	Red
L. ervoides	49	Croatia	Red
L. ervoides	52	Croatia	Red
L. ervoides	55	Palestine	Red
L. ervoides	56	Palestine	Red
L. ervoides	57	Palestine	Red
L. ervoides	58	Turkey	Red
L. ervoides	59	Turkey	Red
L. ervoides	60	Turkey	Red
L. ervoides	62	Turkey	Red
L. ervoides	65	Turkey	Red
L. ervoides	67	Turkey	Red
L. culinaris ssp. *orientalis*	69	Uzbekistan	Red
L. culinaris ssp. *orientalis*	78	Iran	Red
L. culinaris ssp. *orientalis*	82	Iran	Red
L. culinaris ssp. *odemensis*	83	Turkey	Red
L. culinaris ssp. *orientalis*	85	Turkey	Red
L. culinaris ssp. *orientalis*	87	Turkey	Red
L. culinaris ssp. *orientalis*	96	Turkey	Red
L. culinaris ssp. *orientalis*	97	Turkey	Red
L. culinaris ssp. *orientalis*	103	Turkey	Red
L. culinaris ssp. *orientalis*	104	Turkey	Red
L. culinaris ssp. *orientalis*	105	Turkey	Red
L. culinaris ssp. *orientalis*	109	Turkey	Red
L. nigricans	111	Turkey	Red
L. nigricans	112	Turkey	Red
L. culinaris ssp. *orientalis*	122	Syria	Red
L. culinaris ssp. orientalis	124	Syria	Red

L. ervoides	128	Syria	Red
L. ervoides	129	Syria	Red
L. ervoides	130	Syria	Red
L. ervoides	131	Syria	Red
L. ervoides	132	Syria	Red
L. ervoides	133	Syria	Red
L. ervoides	135	Syria	Red
L. ervoides	140	Syria	Red
L. ervoides	142	Syria	Red
L. culinaris ssp. *orientalis*	143	Syria	Red
L. culinaris ssp. *orientalis*	152	Syria	Red
L. ervoides	155	Syria	Red
L. ervoides	159	Syria	Red
L. ervoides	162	Syria	Red
L. culinaris ssp. *odemensis*	164	Syria	Red
L. culinaris ssp. *odemensis*	167	Syria	Yellow
L. culinaris ssp. *odemensis*	173	Syria	Red
L. culinaris ssp. *odemensis*	175	Syria	Yellow
L. culinaris ssp. *orientalis*	176	Syria	Red
L. culinaris ssp. *orientalis*	181	Syria	Red
L. culinaris ssp. *orientalis*	183	Syria	Red
L. ervoides	184	Syria	Red
L. ervoides	186	Syria	Red
L. ervoides	187	Syria	Red
L. culinaris ssp. *orientalis*	189	Turkey	Red
L. culinaris ssp. *orientalis*	192	Syria	Red
L. culinaris ssp. *tomentosus*	194	Syria	Red
L. culinaris ssp. *tomentosus*	195	Syria	Red
L. culinaris ssp. *tomentosus*	196	Syria	Red
L. culinaris ssp. *tomentosus*	198	Syria	Red
L. culinaris ssp. *tomentosus*	199	Syria	Red
L. culinaris ssp. *orientalis*	200	Turkey	Red

Erskine, 1996; Gupta and Sharma, 2006), while cold tolerance and earliness have been observed in *L. culinaris* ssp. *orientalis* (Hamdi *et al.*, 1996). Combined resistance to ascochyta blight and fusarium wilt (ILWL 138) or anthracnose diseases (IG 72653, IG 72646, IG 72651) have also been identified (Bayya *et al.*, 1995, Tullu et al. 2006). Earlier, a few attempts have been made at ICARDA, Aleppo, Syria to evaluate wild *Lens* taxa for agro-morphological traits besides key biotic and abiotic stresses (Erskine and Saxena 1993; Bayya *et al.*, 1995; Hamdi and Erskine, 1996 Ferguson and Robertson 1999; Tullu *et al.* 2006). However, in the Indian context such evaluation has not been done earlier to identify useful donors for the local conditions. To address this and to identify suitable wild accessions which could be used as potential donors, this study was conducted on 88 accessions of lentil representing all the six wild Lens species and sub-species. Though initial seedling vigour was less in wild species as compared to the cultivated check, the wild accessions developed profusely later on and yielded good biomass (Fig. 1). The results showed significant genetic variation among the wild accessions for all characters except cotyledon colour (Tables 2 and 3). A wide range of variability has been reported earlier also by Gupta and Sharma (2006) for yield attributes and biotic and abiotic stresses among 70 accessions of four wild species/ subspecies (*L. culinaris* ssp. *orientalis*, *L. odemensis*, *L. ervoides* and *L. nigricans*). The results of principal component analysis are presented in Figure 2 and mean and range are presented in Table 3. On the basis of 100-seed weight, cultivated lentil germplasm is classified into small (< 2 g), medium (2-2.5 g), large (2.6-3.0 g) and very large (> 3 g) seed size groups (Dixit *et al.* 2011). Following this scale, it was observed that all the wild accessions under evaluation belonged to the small seed size category. The seed size ranged between 0.3 and 1.34 g/100-seeds in different accessions, the highest in *L. culinaris* ssp. *tomentosus* (ILWL 199). Though wild accessions cannot be ideal targets for improving seed size as sufficient variability for seed size exits in the

TABLE 3: Morpho-physiological variation in wild accessions of lentil

Trait	Mean	Range	Minimum	Maximum
Plant height (cm)	24.15	11.33-33.33	*L. culinaris* ssp. ~~*Orientalis*~~ (ILWL143)	*L. ervoides* (ILWL130)
Internode length (cm)	2.37	0.5-4.3	*L. culinaris* ssp. *orientalis* (ILWL 124)	*L. ervoides* (ILWL 140)
Primary branches	3.71	1-7	*L. ervoides* (ILWL142)	*L. culinaris* ssp. *tomentosus* (ILWL195)
Rachis length	1.37	0.5-5.0	*L. culinaris* ssp. *orientalis* (ILWL 87)	*L. nigricans* (ILWL173)
Pods/cluster	1.35	1-3	Most of the accessions (58 accessions)	*L. ervoides* (ILWL56)
Leaf length (cm)	0.74	0.2-1.5	*L. culinaris* ssp. *orientalis* (ILWL124)	*L. ervoides* (ILWL131), *L. culinaris* ssp. *orientalis* (ILWL 200), *L. nigricans* (ILWL32)
Leaf width (cm)	0.26	0.1-0.6	*L. culinaris* ssp. *odemensis* (ILWL 167, ILWL175), *L. culinaris* ssp. *tomentosus* (ILWL196), *L. ervoides* (ILWL 65)	*L. culinaris* ssp. *orientalis* (ILWL78)
Seeds/pod	1.66	1-3	30 accessions	*L. nigricans* (ILWL 28)
100-seed weight	0.74	0.32-1.34	*L. ervoides* (ILWL132, ILWL135)	*L. culinaris* ssp. *tomentosus* (ILWL199)

primary gene-pool itself, involving wild species in lentil hybridization programmes can help to generate the transgressive sergeants for this trait. A very good amount of variability was observed for plant height which ranged between 11.33 and 33.33 cm, the highest being in *L. ervoides* (ILWL 130) and minimum in *L. culinaris* ssp. *orientalis* (ILWL 143).

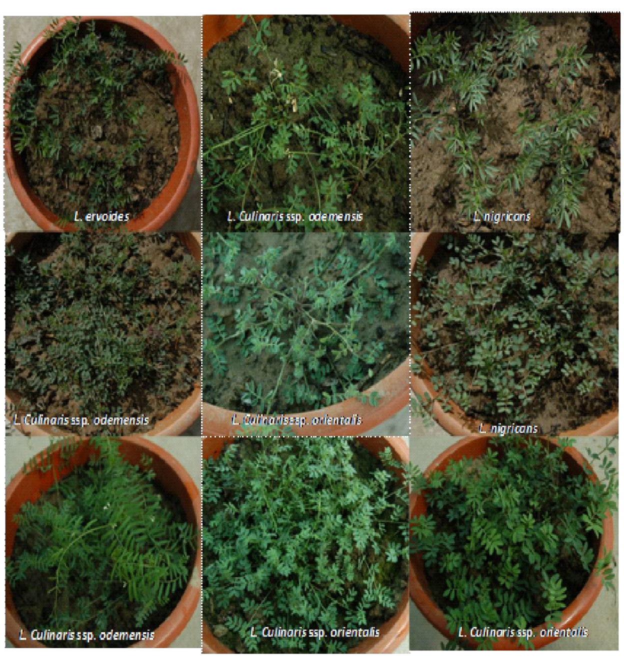

FIG. 1: Morpho-physiological variation in wild accessions of lentil

The internode length ranged between 0.5 and 4.3 cm, the maximum being in *L. ervoides* (ILWL 140) and the minimum in *L. culinaris* ssp. *orientalis* (ILWL 124). It is noticeable that both plant height as well as internode length were maximum in *L. ervoides* and minimum in *L. culinaris* ssp. *orientalis* although their accession numbers were different. This suggests that *L. ervoides* in general has a tendency of taller plants with longer internodes. Singh and Singh (1991) and Pandey *et al.* (1992) indicated that plant height, number of pods/plant and seeds/pod had significant and positive correlations with yield/plant in both, macrosperma and microsperma types.

The primary branches/plant varied between 1-7, the highest number of primary branches being in *L. culinaris* ssp. *tomentosus* (ILWL 195) and the minimum in *L. culinaris* ssp. *orientalis* (ILWL 87). Since the cultivated lentil has 3-4 primary branches/ plant, *L. culinaris* ssp. *tomentosus* can be utilized for increasing this trait in the cultivated lentil. Pandey *et al.*, (1992) and Esmail *et al.* (1994) reported that secondary branches/plant contribute directly to seed yield. Pods/cluster ranged between 1-3. Therefore, increasing branches/plant may be an ideal target for increasing seed yield/plant using wild lentil. While most of the accessions (58) recorded only 1 pod/ cluster, only one accession of *L. ervoides* (ILWL 56) recorded 3 pods/cluster. Though 3 pods/clusters can

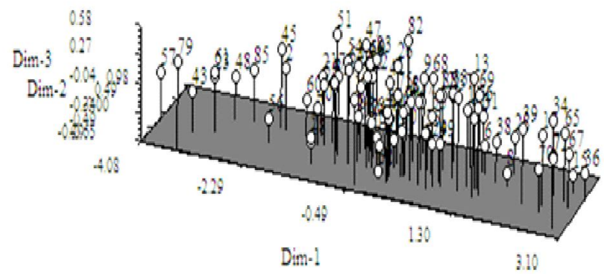

FIG. 2: PCA analysis of the 88 wild accessions of lentil based on phenotypic data

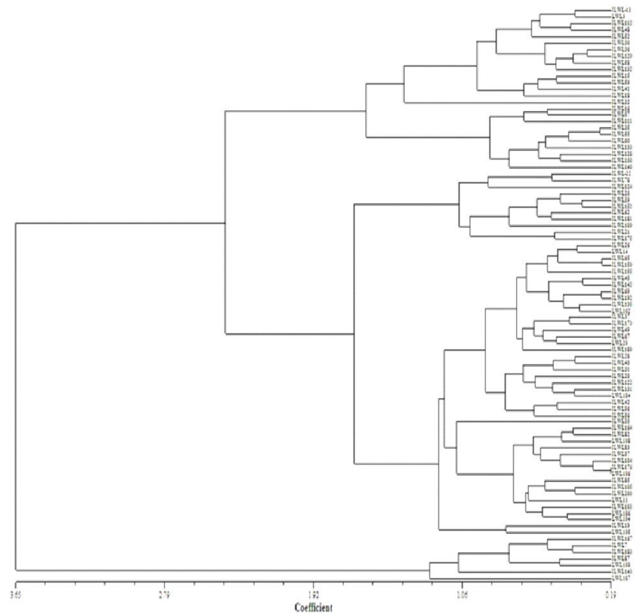

FIG 3: Dendogram based on Jaccard's similarity coefficient using UPGMA method of clustering

be observed in the germplasm of cultivated gene-pool, every cluster of a plant does not have same number of pods. Therefore, this trait can play an important role in increasing seed yield if this trait expresses uniformly within plant. There was considerable variability for leaf length and leaf width also. While leaf length ranged between 0.2-1.5 cm, the average being 0.74 cm, leaf width varied between 0.41-0.6 cm.

Seeds/pod is an important criterion for selection as it directly contributes to seed yield and stability in lentil. While most of the accessions recorded one seed per pod, only one accession of *L. nigricans* (ILWL 28) recorded 3seeds/pod. Among the 88 wild accessions, 34 did not have tendrils while the rest of the entries had medium to large tendrils thereby having a twining habit. Similarly, for cotyledon colour, it was observed that only six accessions had yellow cotyledon colour while the other had ~~pink~~ colour. Noticeably, red cotyledon colour is preferred for consumption in South Asia and therefore, it can be preferred in lentil breeding program.

PCA analysis of the morphological data resulted in clustering of 88 wild accessions into three groups (Table 4). Distinct position of each genotype was observed within each group (Fig. 3). In the first group, there were 10 accessions, 7 being of *L. culinaris* ssp. *orientalis*, while in the third group there were 12 accessions out of which 8 were of *L. ervoides*. Remaining 66 accessions were clustered in the II group. In earlier reports, *L. culinaris* ssp. *orientalis*, *L. culinaris* ssp. *odemensis* and *L. nigricans* ssp. *odemensis* have been grouped in the primary gene-pool while *L. ervoides* and *L. nigricans* fall in secondary and *L. lamottei* and *L. culinaris* ssp. *tomemtosus* in the tertiary gene-pool (Muehlbauer and McPhee 2005). The first three most informative components in PCA analysis individually accounted 89.35, 4.38 and 2.3% of total variation, respectively and collectively these three components explained about 95% of the total

TABLE 4: Grouping of wild lentil accessions on the basis of UPGMA analysis.

Cluster	No. of accessions	Name of ~~accession~~
1	10	*L. nigricans* (ILWL 22), *L. culinaris* ssp. *orientalis* (ILWL 7, 78, 87, 124, 143, 189, 192), *L. ervoides* (ILWL 187) and *L. culinaris* ssp. *tomentosus* (ILWL 199)
II	66	*L. nigricans* (ILWL15, 19, 23, 26, 28, 37, 30, 31, 32, 33, 112) *L. culinaris ssp. odemensis* (ILWL 20, 21, 35, 36, 39, 83, 164, 167, 173, 175), *L ervoides* (ILWL 40, 42, 45, 48, 49, 52, 56, 58, 62, 65, 67, 129, 131, 132, 135, 140, 142, 155, 159, 162, 184, 186), *L. culinaris* ssp. *orientalis* (ILWL 69, 82, 85, 96, 97, 103, 104, 105, 109, 122, 152, 176, 181, 183, 200, *L. culinaris* ssp. *tomentosus* (ILWL 11, 194, 195, 196, 198, *L. lamottei* (ILWL 29, 14) and *Lens* spp. (ILWL 9)
III	12	*L. nigricans* (ILWL13, 16, 18, 111), *L. ervoides* (ILWL 41, 55, 57, 59, 60, 128, 130, 133)

variability. Therefore, more accessions as well as more parameters need to be taken into consideration to represent true genetic variability in wild accessions of lentil. In general *L. ervoides* was observed to possess useful traits like, plant height, internode length and pods/cluster and therefore could be utilized for genetic improvement of cultivated lentil.

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