EVALUATION OF WILD SPECIES OF LENTIL FOR AGRO-MORPHOLOGICAL TRAITS

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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 introgression 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 (Tankesley and McCouch, 1997). Therefore, continuous efforts have been made to collect and conserve wild relatives...
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. omdemensis and L. ervoides showed drought tolerance (Hamdi and

| Name of the species                  | No. of accessions |
|--------------------------------------|-------------------|
| Lens nigricans                       | 16                |
| L. culinaris ssp. omdemensis         | 10                |
| L. culinaris ssp. orientalis         | 22                |
| L. culinaris ssp. tomentosus         | 06                |
| L. ervoides                          | 31                |
| L. lamottei                          | 02                |
| Lens spp.                            | 01                |
| Total                                | 88                |
| Name of the genotype | ILWL | Country of origin | Cotyledon colour |
|----------------------|------|------------------|-----------------|
| L. culinaris ssp. orientalis | 7    | Turkey           | Red             |
| L. nigricans         | 9    | Syria            | Red             |
| L. culinaris ssp. tomentosus | 11   | Syria            | Red             |
| L. lamottei          | 14   | Italy            | Red             |
| L. nigricans         | 15   | France           | Red             |
| L. nigricans         | 16   | France           | Red             |
| L. nigricans         | 18   | France           | Red             |
| L. nigricans         | 19   | Spain            | Yellow          |
| L. culinaris ssp. odemensis | 20   | Palestine        | Red             |
| L. culinaris ssp. odemensis | 21   | Palestine        | Yellow          |
| L. nigricans         | 22   | Italy            | Yellow          |
| L. nigricans         | 23   | Italy            | Red             |
| L. nigricans         | 26   | Croatia          | Red             |
| L. nigricans         | 28   | BiH              | Red             |
| L. lamottei          | 29   | Spain            | Red             |
| L. nigricans         | 30   | Spain            | Red             |
| L. nigricans         | 31   | Spain            | Red             |
| L. nigricans         | 32   | Spain            | Red             |
| L. nigricans         | 33   | Spain            | Red             |
| L. culinaris ssp. odemensis | 35   | Turkey           | Red             |
| L. culinaris ssp. odemensis | 36   | Turkey           | Red             |
| L. 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             |
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 agromorphological 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
primary gene-pool itself, involving wild species in lentil hybridization programmes can help to generate the transgressive sergentes 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).

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. 1: Morpho-physiological variation in wild accessions of lentil](image_url)
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 3 seeds/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. tomentosus 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 |
|---------|-------------------|-------------------|
| I       | 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* (ILWL 15, 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* (ILWL 13, 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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