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Breeding of the Wild Genepool of Lentil

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Abstract

The wild species of the genus Lens were collected in their reported areas of distribution to provide living material for the evaluation of the genus and to provide material for use in lentil crop improvement programs. During the trips to southern Europe, Turkey, and Israel, seeds of 112 collections were assembled and included 48, 28, 34, and 2 accessions of L. orientalis, L. nigricans, L. ervoides, and L. montbretii, respectively,. Based on crossability and fertility of hybrids between these accessions, the genus was grouped in two biological species, L. culinaris and L. nigricans. Members of the different species are cross incompatible. Within species, considerable chromosome repatterning occurs, but as a rule, F₁ hybrids are easily obtained and are fertile or partially fertile. Three subspecies were defined in L. culinaris: ssp. culinaris, the cultivated lentils, ssp. orientalis, the wild lentils with lanceolate stipules, and ssp. odemensis, characterized by horizontal semi-hastate stipules. Previously, ssp. odemensis was considered as L. nigricans. Lens nigricans is composed of two subspecies: ssp. nigricans with semi-hastate stipules oriented parallel to the stem and ssp. ervoides, semi-hastate or lanceolate stipules and smaller leaves, calyx teeth, pods, and seeds. Lens montbretii was shown not to be a Lens species, but more correctly classified within Vicia. Viable hybrids were obtained between L. culinaris ssp. culinaris and L. nigricans ssp. ervoides through embryo culture. Analysis of the F₁ hybrid indicated that the reproduction barrier was controlled by a single mendelian factor, thus all the various members of the genus Lens can be considered as belonging to one genepool.

Evaluation of the material for traits of economic importance indicated little variation for protein content and amino acid profiles. There was no resistance to pea seedborne mosaic virus; however, there were accessions of ssp. nigricans that had tolerance to cold temperatures and which likely carry genes for winterhardiness.

Introduction

Lentil crops are grown mainly in West Asia, North Africa, South Europe, and are of major importance in the Pacific Northwest of the United States where it is grown in rotation with cereals. Various accessions of cultivated lentil are maintained by ICRISAT in India, ICARDA in Aleppo, Syria, and by USDA at Pullman, Washington. The USDA at Pullman, Washington currently maintains about 1400 accessions. Though this is a relatively large number, representing lentils from 31 countries, the variability is insufficient in such traits as disease resistance, winter hardiness, photoperiod response, food quality, and other characteristics to provide the needed germplasm for significant cultivar improvement.

Lentil is a member of the genus Lens which, until this study, was presumed to include four wild species some of which have wide distribution and ecological adaptation. However, the information accumulated so far on the wild gene pool is insufficient to draw general conclusions about possible exploitation for practical breeding purposes. Particularly, adequate collections of the wild species were lacking.

Exploitations of the wild gene pool in breeding programs is a common practice in an increasing number of crop plants (potato, wheat, oats, barley, and more recently, chickpea). If information on the wild gene pool in lentil were available, it could be used to facilitate the improvement of the cultivated lentil in the U.S. and worldwide.

Even though wild Lens species have been widely reported, those reports are based mainly on nonliving Herbarium specimens and until this research no material was available for breeding purposes. Therefore, collections of living material had to be made in order to study the genus and to provide lentil breeders with germplasm to broaden the genetic base and improve cultivated forms. Improvement of cultivated forms with the wild gene pool will depend on variability present for such traits that might include disease resistance, dinitrogen fixation, winter hardiness, stress tolerance, photoperiod response, growth habit, and food quality. The feasibility of using the wild species in breeding programs will depend on cross-fertility and chromosome affinities of the wild species with the cultivated forms.

Prior to this study, the genus Lens was presumed to contain, besides the cultigen, four wild species: L. orientalis, L. nigricans, L. ervoides and L. montbretii, all annuals. The taxonomic positions of the latter species was quite dubious and it was apparently an intermediate form between Lens and Vicia. Morphologically, the wild species, L. orientalis and L. nigricans, are very similar to the cultigen. The similarity is well reflected in various taxonomical treatments of the genus Lens. Williams et al. (1974) treated L. orientalis as a sub species of L. culinaris and considered L. nigricans as a different species while, according to Heggi in flora of central Europe, L. nigricans deserved no more than subspecific rank of the cultigen. L. orientalis was proposed as the wild progenitor of L. culinaris by Barulina (1933), but only lately were the cytogenetic relationships between the cultigen and both L. orientalis and L. nigricans established (Ladizinsky). Early work by Ladizinsky showed that L. orientalis differed from the cultigen by a

single chromosome rearrangement, while L. nigricans differed by three rearrangements. Surprisingly, the hybrids between L. nigricans and L. orientalis were albino and died shortly after germination.

The objectives of the project were therefore to assemble living material of the wild relatives of lentil, determine cross-fertility and chromosome affinities of the wild species with cultivated forms, and assess the variability within the wild genepool and its potential for breeding purposes.

Methods and Procedures

To assemble living material of the wild Lens species, collection trips were made in 1980, 1981, and 1982 to reported distribution areas of the genus. The 1980 collection trip was made in southern Europe and included the areas in Greece, Crete, Yugoslavia, Italy, France, and Spain. The 1981 collection trip was made to Turkey. In addition, several collection trips were made in Israel.

To determine cross-fertility and chromosome affinities of the wild species with cultivated forms inter- and intra-specific hybrids were obtained by emasculating buds approaching anthesis and immediately pollinating the stigmas with ripe pollen from the male parent. For study of chromosome pairing in meiosis, immature anthers were fixed in a 3:1 solution of absolute ethyl alcohol and glacial acetic acid and later stained with aceto-carmin. Pollen fertility (% stainable pollen) was determined by staining mature pollen grains with aceto-carmin.

Attempts were also made to overcome incompatibility barriers between the cultigen and L. nigricans ssp. ervoides by means of tissue culture. L. culinaris ssp. culinaris No. 7 from Ethiopia and L. nigricans ssp. ervoides No. 32 from Israel were used in this study. Fourteen-day-old fertilized ovules were removed aseptically and placed on culture medium that contained the following ingredients per liter: Murashige and Skoog salt mixture made by Gibco, sucrose 100 g, Myro inositol 100 mg, Thiamine HCl 1 ml, nicotinic acid 1 ml, pyridoxine HCl 1 ml. The solution was brought to pH 5.7 and solidified by 8 g. Agar. After sterilization, IAA 0.2 mg, Gibberellic acid 0.5 mg and ziatine 0.5 mg were added. The transplanted fertilized ovules were kept in the dark at 23°C for 3 days, and then placed in 12 hr daylight. After two weeks, the ovules were transferred to a low sugar medium that differed from the previous medium by having 30 g sucrose per liter, having no gibberellic acid and IAA 0.5 mg and ziatine 0.2 mg per liter. After another week, the embryos were released from the integuments to improve seedling growth.

To assess the variability within the wild genepool and its potential for breeding purposes, 42 accessions of L. orientalis, 24 accessions of L. nigricans, 24 accessions of L. ervoides, and 60 lines of L. culinaris were evaluated for 24 characters under field conditions at Spillman Agronomy Farm, Pullman, WA, USA, during the summer of 1982. The L. culinaris lines chosen for this study included small and large-seeded cultivars and experimental lines, plus Plant Introductions from the same areas of distribution as the wild lentils. Seeds of the wild accessions were scarified by soaking in concentrated sulfuric acid for 15 min followed by

a tap water rinse. Seeds of cultivated lentils were not scarified. All seeds were dusted lightly with Captan to control seed rot and damping off. Since indigenous strains of Rhizobium leguminosorum are present in Palouse area soils, inoculum was not applied.

Twenty seeds of each entry were hand planted into single rows 1 m long, spaced 0.6 m apart, and replicated twice. Entries were planted in numerical order within specie groups in the first replication and were randomized within specie groups in the second replication. Several light irrigations were applied until the seedlings became established. Number of days from planting to first flower was recorded for each plant. For evaluating the remaining 23 characters, five plants were randomly chosen from each row. In a few rows, where emergence was poor, less than five plants per row were evaluated. Where possible, end-of-row plants were not selected.

Vegetative characteristics were recorded within a few days of first flower in the following manner: plant habit was scored 1 = completely prostrate to 5 = nearly erect; leaf pubescence of upper leaf surface was scored as 1 = slight, 2 = medium, 3 = heavy; number of leaflets per leaf was scored from the node of first flower to last fully expanded leaf as 1 = 2 to 4 leaflets to 6 = 12 to 14 leaflets; rachis length was measured to the nearest mm at the node of first flower; leaflet length of the basal leaflet of the fully expanded leaf at the node of first flower was measured to the nearest mm; leaflet width of the same leaflet was measured to the nearest 0.5 mm; anthocyanin in the leaflets was scored as 1 = anthocyanin and 2 = no anthocyanin; branching was scored as 1 = unbranched or 2 = branched; stipule shape was scored at the node of first flower and above as 1 = lanceolate, 2 = semihastate, or 3 = mixed. Stipule margin was scored as 1 = entire, 2 = dentate, 3 = mixed.

Floral characters were evaluated in the first open flower or next open flower above and recorded in the following manner: corolla color of the standard petal was scored as 1 = white, 2 = slightly colored, or 3 = deeply colored. Corolla length was measured from the tube to the tip of the standard along the posterior and recorded to the nearest 0.5 mm. Sepal length of the same flower was measured from the tube to the end of the sepal on the posterior of flower to the nearest 0.5 mm. The corolla tube of the same flower was measured to the nearest 0.5 mm. Number of flowers per peduncle were scored from the node of first flower and above as 1 = only 1, 2 = 1 to 2, or 3 = more than 2.

The following characters were evaluated as the first pod matured: peduncle length was measured at the node of first flower to the nearest mm. The arista or awn terminating the same peduncle was measured to the nearest 0.5 mm. Pod length and width were measured to the nearest 0.5 mm. Pod pubescence was scored as 1 = none (glabrous), 2 = light, short or 3 = heavy, long; number of seeds per pod were scored as 1 = 1 only, 2 = 1 to 2, or 3 = more than 2.

As the plants matured, pod dehiscence was scored as 1 = non-dehiscent, 2 = shattering, 3 = strongly dehiscent. Cotyledon color was scored as 1 = orange or 2 = yellow. Insect netting was placed under the plants of the wild accessions to collect seeds for further study.

Data were initially subjected to the Variance Component Estimation Procedure of the Statistical Analysis System to determine which characters had little variation among species means relative to environmental variation (Goodman and Paternioni, 1969). Such characters were eliminated from further analysis. The remaining data were subjected to Principal Component Analysis of the Statistical Analysis System. Correlation matrix was utilized and replication means were pooled.

Protein content and amino acid profiles were determined for the wild accessions using micro-Kjeldahl analyses and the amino acid analyzer available at Bioanalytical Research Services of Washington State University.

The collection was also evaluated for resistance to pea seedborne mosaic virus, response to vernalization, and for winter hardiness.

Description of Research Results

A. Collection:

As a result of the collections trips, over 112 accessions of wild Lens species were obtained and made available for study. The number of accessions collected in the different countries of the Mediterranean basin are shown in Table 1.

Table 1. Number of wild lentil accessions collected in the Mediterranean basin.

Species	Greece	France	Israel	Italy	Spain	Turkey	Yugoslavia
<u>L. orientalis</u>	8		8			32	
<u>L. nigricans</u>	1	5	2	5	6	4	5
<u>L. ervoides</u>			5	2		14	13
<u>L. montbretii</u>						2	

B. The pattern of distribution and the ecology of the four wild species are as follows:

L. orientalis was not found in Europe but was common in Turkey. L. orientalis was found in elevations of 1000 - 1800 m in the Mediterranean part of Turkey, but in rather restricted populations. A similar pattern of distribution was also found on the Anatolian plateau. In southeast Turkey, L. orientalis is much more abundant and is found in elevations of 600 -1300 m. In Israel, L. orientalis is restricted to hilly areas of the country. The populations there are local and fairly small. Where L. orientalis was found, it was associated with small annuals, particularly wild medics and clovers, and on soils of calcareous origin that were shallow, stoney or gravelly.

L. nigricans. In southern European herbaria, there is a rich collection of the species. Most of that material was collected toward the end of the 19th century or at the beginning of the 20th century. During the field trip to S. Europe, it became apparent that the distribution of this species is very localized and restricted. The populations found were extremely small. Some were composed of only a few plants and occupying only a few square meters. L. nigricans was always found in herbaceous habitats among annual legumes, mainly wild medics and clovers and on shallow gravelly soil. No clear soil preference could be observed. It was found on calcareous, metamorphic, basaltic and igneous bed rocks.

In Greece, Yugoslavia, central and northern Italy, France, and central Spain, the association between L. nigricans and habitats formed by man was obvious. L. nigricans was found on uncultivated terraces, abandoned plantations, as a weed in vineyards, near ruins, and near caves that were inhabited in ancient times. Only in Mostaz, Yugoslavia, Bari area in Italy, and Almeria area in Spain was L. nigricans found in natural habitats and those populations were quite large.

L. ervoides: The distribution of L. ervoides partially overlapped the distribution of L. orientalis and L. nigricans. We found this species in Turkey, Israel, Italy, and Yugoslavia. In two locations in Yugoslavia mixed populations of these two species were observed. In one location in Turkey a mixed stand of L. ervoides and L. orientalis were detected on shallow, gravelly soil among dwarf shrubs. Occasionally they were found under pine trees where very few annuals survive. Similar to L. orientalis and L. nigricans, no bedrock preference was observed for L. ervoides.

L. montbretii differed from the other Lens species by being sturdy and hairy, up to 10 leaflet pairs per leaf, large cylindrical pods with 2-3 globular seeds. We have examined the chromosomes of L. montbretii and found the number to be $2n = 12$; other Lens species have $2n = 14$. Furthermore, the karyotype of L. montbretii is considerably assymetrical and contains one metacentric chromosome, 4 acrocentric chromosomes, and a small metacentric chromosome that has a small sattelite.

The remarkable differences between L. montbretii and the other Lens species suggest that L. montbretii should not be included in the genus Lens, but more correctly classified within Vicia as V. montbretii (for additional information see publication #2).

Small disjunct populations of the wild lentil L. nigricans were found in secondary and man made habitats throughout southern Europe. Much larger populations of this species were found in primary habitats in S. E. Spain, S. Italy and along the Adriatic coast of Yugoslavia. The majority of the L. nigricans populations were cytogenetically alike. One population differed from those by one reciprocal translocation and another two by four chromosomal rearrangements. The L. nigricans accessions from S. Europe were invariably cross incompatible with L. nigricans of the Middle East and with the cultivated lentil, L. culinaris. The latter two were cross compatible and their hybrids partially fertile. The existence of small L. nigricans populations in man made habitats was interpreted as if they originated from types that escaped cultivation. Being cross incompatible with L. culinaris, these L. nigricans populations apparently were derived from a domesticated form of L. nigricans (for more information, see Ladizinsky et al., publication #4 attached).

C. Crossability and chromosome affinities within the genus:

According to crossability relations and fertility of hybrids, the various members of the genus Lens were grouped in two biographical species, L. culinaris and L. nigricans. Members of different species are cross incompatible because hybrid embryos abort two weeks after pollination. Hybrid seeds which are rarely produced, are albino, and die shortly after germination. If a mature plant is obtained, it is totally sterile as a result of irregular chromosome associations at meiosis. Within species, considerable chromosome repatterning occurs, but as a rule, F_1 hybrids are easily obtained and are fertile or partially fertile. Three subspecies were defined in L. culinaris; ssp. culinaris, which encompasses the cultivated lentils; ssp. orientalis, which represents the wild lentils with lanceolate stipules (cytogenetically some accessions of this subspecies are closely related to ssp. culinaris); and ssp. odemensis, characterized by semi-hastate stipules that form horizontal positions on the stem. In conventional taxonomic treatments of the genus ssp. odemensis is considered as L. nigricans. Lens nigricans is composed of two subspecies: ssp. nigricans with stipules considerably semi-hastate and dentate at their base and pointed upward in a parallel position to the stem; and ssp. ervoides with semi-hastate or lanceolate stipules is distinguished by its smaller leaves, calyx teeth, pods, and seeds. The two subspecies rarely form mixed stands in nature, but their hybrids are partially fertile (for more information, see Ladizinsky et al., publication #3 attached).

Attempts to overcome incompatibility between the cultigen and L. nigricans ssp. ervoides were quite successful. The fertilized ovules of the parental lines No. 7 and No. 32 started forming roots and shoots about 4 weeks after transplanting. Eventually, about 90% of the ovules formed plantlets. Plantlets with sufficiently developed shoot and roots were planted in pots containing a 1:1 soil vermiculite mixture and were covered with plastic bags to avoid dehydration. Most of the plantlets developed into mature plants that were as normal as those grown from seeds.

The development of the No. 32 x No. 7 F_1 embryos was slower compared to the parents and if not released from the integuments, they died. Shoots and roots of the F_1 hybrids were formed about 8 weeks after the transplanting.

One of the F_1 hybrids developed to a mature plant and was examined. Morphologically it was intermediate of the parents, particularly in the leaf shape and size of the flowers, the pods, and the seeds. It resembled the cultivated parent by the stipule shape and awned peduncle, and the wild parent by the hairy pod. Pollen fertility of the F_1 hybrid was 45% and at meiosis a quadrivalent was observed in 56% of the M1 cells. Of the 592 flowers produced on the hybrid, 194 have not developed into pods, another 194 developed pods that collapsed about 8-20 days after anthesis, and 204 flowers developed seed bearing pods.

The F_1 hybrid was crossed to the ervoides No. 32 as the male parent. Of the 18 successful crosses, 8 collapsed about 8-16 days from the crossing and 10 produced seed bearing pods.

The obtaining of a hybrid between the cultivated lentil, L. culinaris ssp. culinaris and L. nigricans ssp. ervoides, indicates that the two crossability groups of the genus Lens can be bridged by culturing their F_1 hybrids in an appropriate medium.

The behavior of the F_1 hybrid examined by us sheds new light on the evolution of the genus Lens and the genetics of the reproductive barrier between the two crossability groups. It is now obvious that cytogenetically L. culinaris ssp. L. culinaris is closer to L. nigricans ssp. L. ervoides than to the members of its own crossability group ssp. odemensis and some accessions of ssp. orientalis. More than that, it seems that the reproductive isolation between the two groups, which is due to the endosperm breakdown of the F_1 hybrid, is governed by a single mendelian factor. This can be inferred from the pattern of the pod setting on the F_1 hybrid and the results of the backcross to the ervoides parent. Assuming that one gene controls the embryo breakdown (eb) in the L. culinaris ssp. culinaris x L. nigricans ssp. ervoides hybrid and the breakdown is manifested only in the heterozygous condition in the endosperm, i.e. EbEbeb or ebebEb, the F_1 hybrid will always collapse regardless of the cross direction. The F_1 hybrid obtained via embryo culture was heterozygous, EbEb, and upon selfing 4 kinds of endosperms could be expected, EbEbEb, EbEbeb, ebebEb and ebebeb, of which only the homozygous are functional. Consequently, a 1:1 ratio between aborted and seed bearing pods is expected. The results obtained are in good agreement with this assumption ($\chi^2 = 0.24$, $P = 0.6-0.7$).

The practical outcome of this study is that, for breeding purposes, the various members of the genus Lens can be considered as one genepool.

From the variance component analysis (Table 2) specie means were significantly different for all characters except number of seeds per pod. Number of days to first bloom was the only character that was significantly different across replications. This may in part be due to the delayed emergence in parts of the first replication. No significant species x rep interaction was noted. Five characters (pod pubescence, presence of anthocyanin, number of flowers over peduncle, cotyledon color, and number of seeds per pod were found to have a low ratio of $\sigma^2 \text{ specie} / (\sigma^2 \text{ error} + \sigma^2 \text{ rep} + \sigma^2 \text{ specie} \times \text{rep})$. This indicated that the variation in these characters was largely due to environmental and/or experimental variation (see Goodman and Paternoni, 1969) rather than actual specie differences. These five characters were excluded from further analysis.

Plot means of the remaining 19 characters were averaged over replications and subjected to principal component analysis. A correlation matrix was used in calculating the principal components rather than a variance-covariance matrix since different scales of measurement were used in evaluating the characters (some qualitative, others quantitative) (Goodman 1973). The variance of each principal component or principal axis is called its eigenvalue, which are given in Table 3. The first three principal components calculated accounted for 80.8% of the total variance. Therefore, the separation of points can be portrayed by two dimensional plots involving the first three principal axis.

The plot of the first principal component versus the second principal component separated the points into the four species groups. The points represent the accession or lines of each species as described in the materials and methods section and were given a letter designation corresponding to the species in which each were classified. The greatest degree of separation occurred along the first principal axis. The L. culinaris spp. culinaris lines occupied a band on the right side of the plot with the macrosperma types making up the extreme end and microsperma types occupying the bank nearest the wild species. L. nigricans spp. ervoides accessions were grouped to the left of the other species along the first principal furthest axis from spp. culinaris. L. culinaris spp. orientalis and L. nigricans spp. nigricans accessions occupied nearly the same position along the first principal axis but were separated from one another along the second principal axis. Some degree of overlap appears to be present between spp. orientalis and spp. nigricans. Of the wild species, spp. orientalis was the closest to the cultivated types.

The plot of the first principal axis versus the second principal axis spread the points more in the second dimension, thus separating spp. orientalis from spp. nigricans. As before, spp. orientalis occupies a position close to the left edge of the spp. culinaris scatter belt while spp. ervoides is the furthest removed. This plot tends to place spp. ervoides closer to spp. nigricans. In the plot of the second principal axis versus the third principal axis L. orientalis and L. culinaris show the greatest degree of overlap, while most L. ervoides and L. nigricans remained in discreet groups.

The eigenvectors (Table 4) represent how much a given character contributes to a given principal component. Separation along the first principal axis is due to a combination of mostly quantitative characters and a few qualitative characters. Some of the more prominent ones include rachis length, sepal length, pod dehiscence, leaflet width, pod width, corolla color, corolla tube length, corolla length, and pod length. It is the linear combination of these correlated characters that tend to separate the culinaris, orientalis-nigricans, and ervoides groups. The second and third principal components separated spp. orientalis from spp. nigricans. The variables contributing to these components tended to have a higher proportion of qualitative characters which included stipule shape, stipule margin, and growth habit. Stipule shape and stipule margin are highly discriminatory and have been used in taxonomic schemes to differentiate L. nigricans from L. orientalis and L. culinaris. Lens nigricans spp. nigricans tends to be more prostrate in growth habit as compared to L. culinaris spp. orientalis. Some quantitative characters contribute to the second and third principal components. These include arista length, peduncle length, leaflet length, and days to first bloom. Strongly aristate peduncles are characteristic of L. nigricans spp. nigricans and would tend to differentiate between the two species. Lens culinaris spp. orientalis tends to have shorter peduncles (Table 5) than L. nigricans spp. nigricans or L. nigricans spp. ervoides.

When a principal components analysis was conducted on measured characters only (excluding days to bloom), the L. orientalis and L. nigricans groups merged into one group, clearly demonstrating that these taxa are closely related, being separated by characters of a qualitative nature. L. culinaris spp. culinaris and L. nigricans spp. ervoides tended to remain distinct.

To determine relative winterhardiness of the wild species 22, 19, 15, and 21 accessions of subspecies orientalis, nigricans, ervoides, and culinaris, respectively, were planted in the fall of 1981. Germination of wild lentil species was poor when compared to germination when planted the following spring. This was attributed to possible post harvest dormancy in wild lentils. However, fall stand counts of nigricans were significantly greater than the orientalis and ervoides. None of the ervoides accessions germinated in the fall and two nigricans collections (LN23 and LN25) tolerated the winter temperatures better than the rest of the wild material. One cultivated lentil line (WH80) tolerated the cold temperatures and later fungal attack by Pythium much better than the two nigricans accessions. Lens culinaris spp. orientalis contained both winter and summer growth habit types (prostrate and erect). Lens nigricans spp. ervoides primarily had a spring type growth habit (erect). The growth habit of nigricans accessions was mainly winter type (prostrate). The relative post harvest dormancy of wild lentil species and their growth habits seemed to be related to their geographical distribution and the habitats where they were collected.

Representative subspecies accessions of nigricans, ervoides and culinaris were vernalized for 35 days at 1 - 20C and planted in the field. Vernalization caused earlier flowering and a tendency toward upright growth habit. Collections from southerly locations and low elevations responded similarly to vernalization. As a group, accessions from more northerly locations appeared to have a greater response to vernalization. The significance of vernalization requirement as a survival mechanism in nature needs further study.

Unfortunately, resistance to pea seedborne mosaic virus and other viruses was not found in the wild species collection. Amino acid profiles for the wild species (Table 6) were quite variable; however, there was little apparent variation for the sulphur containing amino acids (methionine and cystine). These amino acids are the most limiting in lentils and in grain legumes in general. It appears that the wild species have limited value for improving protein quality in the cultivated types.

Evaluation of research achievement with respect to the original proposal.

With respect to the original plan, the project was successful in collecting and assembling living material of the wild relatives of the cultivated lentil. This material was then used extensively, as planned, to determine cross fertility and chromosome affinities of the wild species with the cultivated forms. Based on these studies, it is now clear that ssp orientalis and spp. odemensis accessions can be used directly for breeding purposes; whereas, ssp. nigricans and spp. ervoides were shown to be incompatible with the cultigen. However, the incompatibility of spp. ervoides with the cultigen can be overcome through embryo culture and it is now clear that all subspecies of the genus Lens can be utilized in breeding programs.

Evaluations of the material for traits of economic importance indicated a surprising similarity between the subspecies for amino acid profiles and protein content and it appears that improvement of lentil

protein content and quality may not be possible through the use of the wild species. Evaluations for virus resistance have indicated that the material is lacking in resistance to pea seedborne mosaic and that the wild species do not contain a new source of resistance to the virus. Agronomic evaluations have shown a degree of environmental stress tolerance (winter hardiness) within spp. nigricans.

The research has led to a revised classification of the genus Lens into groups which are cross compatible within species, but cross incompatible between species (see publication #3). Also, new insights into domestication of lentils were obtained and it now appears likely that Lens species other than L. culinaris ssp. culinaris were at one time domesticated.

Description and results of cooperation

Cooperation in the collection phases of the project made it possible to obtain seeds of over 120 accessions of the wild Lens species. These accessions are now available for breeding purposes and have also been made available to other lentil breeders worldwide. The collection is being maintained by our lentil breeding program at Pullman, by the USDA Plant Introduction Station, and by the International Centre for Agricultural Research in the Dry Areas.

Cooperation with the Israeli counterpart made it possible to group the material into compatible and incompatible subspecies such that successful hybridization and utilization of the wild species in breeding programs is now possible and predictable. The thorough understanding of the genus Lens that we now have would not have been possible without the successful completion of this project.

Theses

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Inventions

None

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Table 2. Variance component analysis of specie means for each character.

Character	Character type	$\hat{\sigma}^2$ (Sp)	$\hat{\sigma}^2$ (Rep)	$\hat{\sigma}^2$ (Sp $\times$ Rep)	$\hat{\sigma}^2$ (Error)	$\frac{1}{2+3+4}$	$\frac{1}{1+2+3+4}$
POD DHS	Qual	1.238**	0.000	0.001	0.049	25.27	0.962
STIP SHP	Qual	0.700**	0.000	0.001	0.051	13.72	0.932
RACH LEN	Quan	245.157**	0.042	-0.881	31.083	7.88	0.887
SEPL LEN	Quan	3.178**	0.002	-0.012	0.469	6.75	0.871
CORO COL	Qual	0.985**	0.000	-0.003	0.151	6.52	0.867
TUBE LEN	Quan	0.170**	0.000	-0.001	0.027	6.30	0.863
GR HABIT	Qual	1.706**	0.000	-0.005	0.285	5.99	0.857
CORO LEN	Quan	0.849**	0.000	-0.003	0.156	5.44	0.845
LLT LEAF	Qual	1.850**	0.000	-0.009	0.357	5.18	0.838
STIP MAR	Qual	0.700**	0.000	-0.001	0.037	4.32	0.812
POD WID	Quan	2.071**	0.004	-0.017	0.631	3.26	0.765
LLT LEN	Quan	15.741**	-0.008	-0.082	4.848	3.25	0.765
ARIS LEN	Quan	5.012**	-0.002	-0.026	1.551	3.23	0.764
LLT WID	Quan	1.993**	-0.001	-0.013	0.706	2.82	0.738
POD LEN	Quan	4.741**	0.007	-0.037	2.073	2.28	0.695
PED LEN	Quan	94.008**	-0.055	-0.785	41.550	2.26	0.693
TENDRIL	Qual	0.133**	0.000	-0.003	0.151	1.30	0.566
LEAF PUB	Qual	0.255**	0.000	-0.006	0.211	1.21	0.547
D BLOOM	Quan	38.028**	1.144*	-0.185	36.427	1.01	0.503
POD PUB	Qual	0.105**	0.000	-0.003	0.112	0.94	0.484
ANTHO	Qual	0.039**	0.000	-0.001	0.075	0.57	0.342
FLWR PED	Qual	0.075**	0.001	-0.005	0.197	0.38	0.276
COT COL	Qual	0.048**	0.000	-0.004	0.137	0.35	0.259
SEED POD	Qual	0.0003	0.000	-0.0003	0.0125	0.02	0.023

Table 3. Variances and cumulative percentages of variance of the principal components.

Character	Eigenvalues	Cumulative percent of Eigen value
1	12.103	63.7
2	1.774	73.0
3	1.469	80.8
4	0.710	84.5
5	0.602	87.7
6	0.476	90.2
7	0.420	92.4
8	0.266	93.8
9	0.202	94.9
10	0.169	95.7
11	0.168	96.6
12	0.133	97.3
13	0.115	97.9
14	0.113	98.5
15	0.086	99.0
16	0.070	99.3
17	0.066	99.7
18	0.037	99.9
19	0.021	100.0

Table 4. Eigenvectors indicating the contribution of each character to a given principal component.

Variable	Eigenvectors		
	λ_1	λ_2	λ_3
PPD DHS	-0.265	0.124	-0.005
STIP SHP	-0.190	-0.173	0.481
RACH LEN	0.272	-0.041	0.140
SEPL LEN	0.266	0.062	0.039
CORO COL	0.257	0.090	-0.057
TUBE LEN	0.256	0.207	-0.068
GR HABIT	0.235	-0.278	-0.199
CORO LEN	0.248	0.215	-0.130
LLT LEAF	0.253	0.094	-0.104
STIP MAR	-0.084	0.549	0.383
POD WID	0.258	0.047	0.040
LLT LEN	0.218	-0.286	0.353
ARIS LEN	0.205	0.417	0.025
LLT WID	0.260	0.035	0.131
POD LEN	0.244	0.012	0.048
PED LEN	0.222	-0.096	0.400
TENDRIL	0.212	-0.165	0.216
LEAF PUB	0.166	0.310	-0.202
D BLOOM	-0.158	0.272	0.363

Table 5. Species Means

Variable	L.O.	Ln	Le	Lc	Overall
POD DHS	2.82 $\pm$	3.00	3.00	1.02	
STIP SHP	1.00	2.00	2.83	1.00	
RACH LEN	15.10	16.15	10.05	40.53	
SEPL LEN	3.85	3.96	2.60	6.44	
CORO COL	2.82	2.78	2.94	1.15	
TUBE LEN	1.56	1.62	1.05	2.03	
GR HABIT	3.34	2.03	2.97	4.96	
CORO LEN	4.89	4.73	3.42	5.66	
LLT LEAF	3.70	3.30	2.08	5.22	
STIP MAR	1.00	1.93	1.08	1.00	
POD WID	5.57	5.68	4.54	7.64	
LLT LEN	10.00	10.20	13.32	17.37	
ARIS (AWN) LEN	3.47	5.28	0.41	5.65	
LLT WID	3.32	3.47	2.35	5.40	
POD LEN	10.81	11.01	9.63	14.13	
PED LEN	23.85	28.72	28.67	42.68	
TENDRIL	1.03	1.00	1.00	1.64	
LEAF PUB	1.81	1.89	1.00	2.24	
D BLOOM	62.88	71.39	66.70	56.85	
POD PUB	1.15	1.00	1.77	1.02	
ANTHO	2.00	1.67	1.61	2.00	
FLWR PED	1.85	1.74	1.77	2.26	
COT COL	1.03	1.39	1.00	1.38	
SEEP POD	1.98	2.00	1.96	2.00	

Table 6. Amino acid profiles of three cultivars of *L. culinaris* and various wild species accessions (g amino acid per 100 g dry seed flour).

Amino acid	<i>L. culinaris</i> l/		<i>L. culinaris</i> ssp. <i>orientalis</i> (38)		<i>L. culinaris</i> ssp. <i>odemensis</i> (4)		<i>L. nigricans</i> ssp. <i>nigricans</i> (16)		<i>L. nigricans</i> ssp. <i>ervoides</i> (22)		<i>Vicia montbretii</i> (1)	
	$\bar{x}$	Range	$\bar{x}$	Range	$\bar{x}$	Range	$\bar{x}$	Range	$\bar{x}$	Range	$\bar{x}$	Range
ASX	2.64	2.57-2.77	2.88	2.26-4.07	2.47	2.14-2.78	2.75	2.10-3.27	2.87	2.39-3.84	2.32	
THR	0.97	0.93-1.01	0.99	0.84-1.32	0.93	0.81-0.99	0.97	0.58-1.40	0.94	0.82-1.24	0.82	
SER	1.46	1.38-1.51	1.32	0.85-1.58	1.30	1.14-1.37	1.45	1.08-1.75	1.38	1.10-1.73	1.23	
GLX	3.98	3.80-4.16	4.12	3.24-5.40	3.42	2.90-3.68	3.95	2.95-4.87	3.91	3.00-5.09	3.26	
PRO	0.89	0.78-0.97	1.00	0.76-1.50	0.86	0.73-0.93	0.96	0.73-1.23	0.95	0.77-1.18	0.80	
GLY	1.01	0.98-1.03	1.11	0.54-1.42	0.98	0.82-1.08	1.05	0.83-1.46	1.03	0.80-1.40	0.87	
ALA	1.10	1.05-1.15	1.10	0.73-1.52	0.99	0.85-1.09	1.07	0.84-1.47	1.02	0.90-1.24	0.89	
CYS	0.42	0.40-0.43	0.36	0.25-0.45	0.33	0.27-0.42	0.31	0.23-0.41	0.39	0.29-0.56	0.30	
VAL	1.05	1.02-1.09	1.26	0.94-1.59	1.06	0.84-1.15	1.19	0.87-1.53	1.17	0.90-1.54	0.95	
NET	0.28	0.25-0.31	0.28	0.21-0.38	0.27	0.21-0.33	0.26	0.20-0.31	0.28	0.20-0.40	0.26	
ILE	0.96	0.91-1.03	1.09	0.74-1.47	0.96	0.81-1.06	1.09	0.92-1.34	1.06	0.50-1.40	0.94	
LEU	1.79	1.75-1.84	1.89	1.43-2.34	1.68	1.42-1.80	1.92	1.52-2.36	1.85	1.37-2.37	1.64	
TYR	0.77	0.72-0.83	0.76	0.50-0.90	0.70	0.62-0.75	0.77	0.59-1.07	0.78	0.60-1.22	0.64	
PHE	1.27	1.19-1.31	1.26	0.99-1.65	1.11	0.93-1.21	1.28	0.98-1.56	1.24	0.92-1.54	1.08	
KH3	0.75	0.62-0.84	0.92	0.59-1.27	0.92	0.73-1.25	0.88	0.52-1.40	0.78	0.54-1.18	1.22	
LYS	1.70	1.67-1.73	1.87	1.35-2.15	1.59	1.32-1.77	1.75	1.42-2.16	1.72	1.19-2.40	0.82	
HIS	0.60	0.57-0.62	0.66	0.50-0.85	0.59	0.48-0.68	0.62	0.49-0.75	0.67	0.51-1.11	0.53	
ARG	1.82	1.61-1.98	1.94	1.41-2.82	1.73	1.48-1.90	2.01	1.37-2.59	2.23	1.30-3.91	1.85	
Total	23.4	22.8-24.3	24.7	19.7-31.2	21.9	18.5-23.8	24.3	18.7-29.5	24.3	19.4-30.6	20.4	

l/ Number of cultivars or accessions analyzed.