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THE ORIGIN OF LENTIL AND ITS WILD GENEPOOL

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SUMMARY

Following hybridization experiments and cytogenetic analysis of interspecific hybrids three chromosome interchanges were found between the cultivated lentil *L. culinaris* and *L. nigricans*, and only one between the cultivated species and *L. orientalis*. This indicates that the latter species is more likely to be wild progenitor of lentil. The partial fertility of the interspecific hybrids indicate further that both *L. nigricans* and *L. orientalis* should be included in the wild genepool of lentil, and their variation can be exploited by relatively simple hybridization techniques. The wild lentils *L. orientalis* and *L. nigricans* are morphologically very similar but reproductively strongly isolated from one another by the albino seedling of their hybrids. It has been suggested that the populations of *L. orientalis* that gave rise to the cultivated lentil still possess a similar chromosome arrangement as in *L. culinaris* and are also capable of forming normal hybrids with *L. nigricans*. According to these considerations it is unlikely that lentil originated from populations at the south western corner of the distribution area of *L. orientalis*.

INTRODUCTION

The genus *Lens* MILLER. comprises five annual species of which only *L. culinaris* MEDIK. is cultivated. Of the wild species *L. montbretii* (FISCH & MEY.) DAVIS & PLIT., *L. ervoides* (BRIGN.) GRANDE, *L. nigricans* (BIEB.) GODR., and *L. orientalis* (BOISS.) M. POPOV, only the latter two bear morphological similarity to the cultivated lentil. These three species also have the same chromosome number ($2n = 14$). Already BARULINA (1930) in her monograph on the genus *Lens*, suggested that the cultivated lentil originated from *L. orientalis*. This species has a vast distribution in central and southwest Asia as shown in Fig. 1 which is based on BARULINA (1930), supplemented by other literature sources and by data collected on field trips throughout Israel and Turkey. Recently, detailed morphological comparisons between the cultivated lentil and *L. nigricans* and *L. orientalis* have supported the idea that *L. culinaris* originated from *L. orientalis* (WILLIAMS et al., 1974). These authors, however, have not eliminated the possibility that some of the variation of the cultivated species was introduced via introgression from *L. nigricans*. HEGI in Flora von Mittel Europa stressed further the morphological similarity between *L. culinaris* and *L. nigricans* and treated the latter as sub-species of the cultivated lentil. Thus, the different grouping proposed for these three taxa might indicate considerable interrelationships between them. WILLIAMS et al. (1974) demonstrated that *L. culinaris*, *L. orientalis* and *L. nigricans* differ from one another by

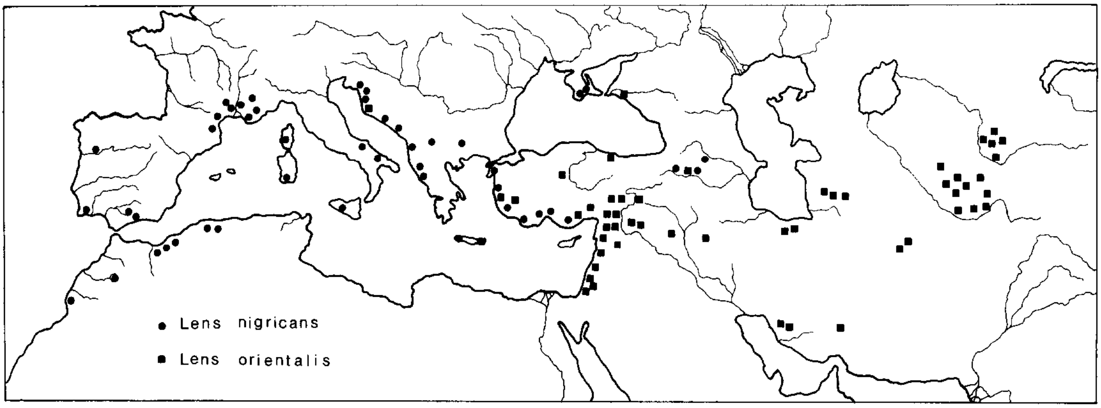

Fig. 1. The distribution of the wild lentils *L. nigricans* and *L. orientalis*.

characters of quantitative nature but their ranges of variation overlap considerably. According to the various keys to the genus *Lens* (BARULINA, 1930; BALL, 1968; DAVIS & PLITMANN, 1970; WILLIAMS et al., 1974) stipule shape is the only diagnostic character by which *L. nigricans* can be separated from *L. culinaris* and *L. orientalis*. While in the latter two taxa the stipules are oblong or elliptic lanceolate, entire, those of *L. nigricans* are semi hastate, entire or dentate. Our examination of stipule shape in forty-five cultivars from the Mediterranean countries (unpublished) showed that this trait is conspicuously plastic and variable. Differences were found between stipules on the same plant and between plants of different cultivars. Some of them even had well defined semi hastate, dentate stipules. DAVIS & PLITMANN (1970) mention that *L. nigricans* is occasionally cultivated in Turkey. It is possible that they refer to *L. culinaris* with semi hastate stipules. A thorough survey of stipule shape in *L. orientalis* has not been made but it is interesting to note that while *L. nigricans* is confined to Southern Europe and West Turkey (Fig. 1) some populations have been reported from an area typical of *L. orientalis*. It is, thus, tempting to believe that these are in fact *L. orientalis* populations with rather semi hastate stipules.

The place of domestication of lentil is also not clear. Of the vast distribution area of *L. orientalis*, that between Hindu Kush and the Himalaya was selected by BARULINA (1930) as the area where the more primitive, small seeded, lentil evolved. Attempts to identify the place of domestication based on the occurrence of carbonized lentil seeds in archeological sites are also not without complications, since there is no way to distinguish between wild and cultivated forms found in these remains. Thus, RENFREW (1969, 1973) by accepting *L. nigricans* as the wild progenitor of lentil infers southern Europe as the place where lentil evolved. ZOHARY (1972) on the other hand, favoring Barulina's idea that lentil originated from *L. orientalis*, interpreted the occurrence of carbonized lentil seeds in neolithic settlements in the Middle East as a sign that lentil was domesticated throughout the Fertile Crescent.

The purpose of the present paper is to bring new kind of evidence regarding the origin of lentil and its wild relatives. This information results from breeding experiments and cytogenetic analysis of hybrids between the cultivated species and the wild *L. orientalis* and *L. nigricans*.

Table 1. Cultivated and wild lentils used in crosses.

Species	Origin
L. culinaris	
No 2	Israel
No 7	Ethiopia
No 13	Chile
L. orientalis	
No 22	approx. 15 km s.w. of Jerusalem, Israel
No 23	1 km w. of Jerusalem, Israel
No 24	Mnt. Gilboa, Israel
No 26	Yeftach, Upper Galilee, Israel
L. nigricans	approx. 40 km w. of Edremit, Turkey

MATERIALS AND METHODS

The various accessions of *Lens* species used in crosses are shown in Table 1. Crosses were made in the greenhouse. Buds approaching anthesis were emasculated and pollinated with appropriate pollen. For karyotype studies, root tips were placed in cold water (0–4°C) for 20–24 h., fixed in 3;1 absolute Ethanol acetic acid and stored in 70% Ethanol. Chromosomes were stained by Feulgen method after hydrolization in 1 N HCl for 10 min at 60°C. For meiotic studies buds were fixed in 3 : 1, stored in 70% Ethanol and individual anthers were stained with aceto-carmine. Pollen fertility was determined by staining mature anthers with aceto-carmine. At least 500 pollen grains were scored for each parental line and hybrid pollen grains were considered normal if they had a rounded shape and darkly stained cytoplasm. Seed set was calculated by the number of flowers which developed pods and by the number of seeds in these pods.

RESULTS

The *L. culinaris* lines employed in this study represented extremes in several characteristics. Line No 13 was tall with very few branches, semi hastate stipules and large seeds. Lines No 2 and No 7 had a bushy growth habit, elliptic stipules and small seeds. They all had 14 chromosomes and their karyotype comprised two pairs of large submetacentric chromosomes, two pairs of metacentrics, one of which possessed a secondary constriction and large satellite, and three acrocentric chromosomes (Fig. 2). At meiosis 7 bivalents were formed and the fertility was high.

The *L. orientalis* lines represented the entire distribution range of this species in Israel. They varied in their growth habit and seed color but all had blue flowers. The karyotype of *L. orientalis* was very similar to that of *L. culinaris*. Meiosis and seed set were normal in these *L. orientalis* lines.

The single *L. nigricans* line used in this study was collected in Turkey. It had pronounced prostrate growth habit and semi hastate, dentate stipules. The karyotype of this line was similar to that of *L. culinaris* by having two metacentric pairs one with a secondary constriction and large satellite, and three acrocentric pairs of chromosomes.

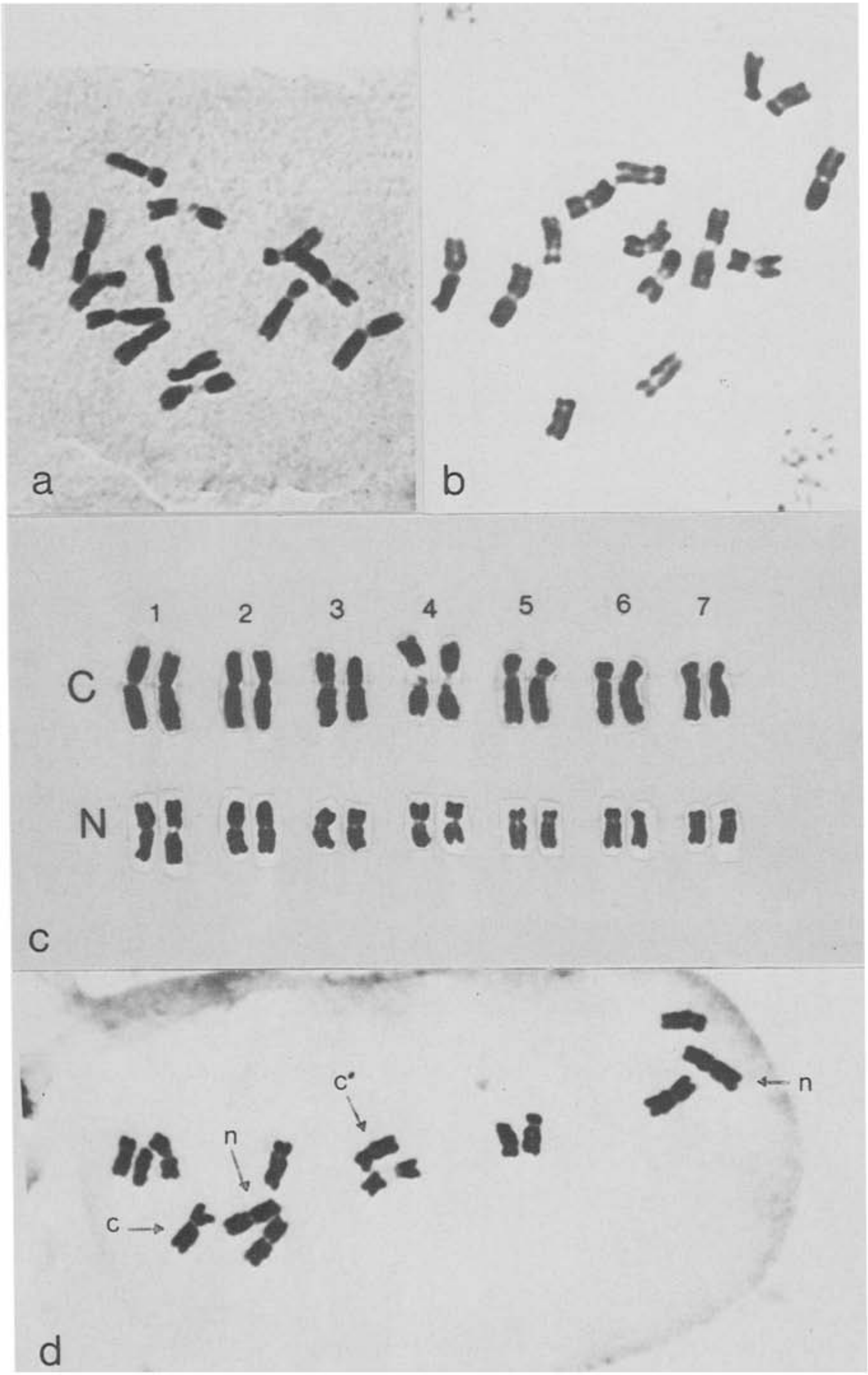

Fig. 2. Mitotic chromosomes of lentil species. a) *L. culinaris*; b) *L. nigricans*; c) the karyotypes of *L. culinaris* (C) and *L. nigricans* (N), note the secondary constriction and the large satellite in chromosome No 4; d) mitotic chromosomes in *L. culinaris* × *L. nigricans* hybrid, (c) chromosomes derived from *L. culinaris*, (n) derived from *L. nigricans*.

But instead of two pairs of submetacentric chromosomes, as in *L. culinaris*, there were two pairs of metacentric chromosomes in *L. nigricans* (Fig. 2). At meiosis 7 bivalents were regularly formed and fertility was high.

To facilitate crossing between *L. culinaris* and the two wild species the cultivated type was used as the female parent. Eighty-five crosses between *L. culinaris* and *L. orientalis* yielded 32 hybrid seeds. Eight hybrid seeds were obtained from crossing two cultivated lines with *L. nigricans*. In crosses between the two wild species *L. orientalis* served as the female parent and five seeds resulted from 30 crosses. All the hybrids between the cultivated and the wild species developed normally and were analyzed cytogenetically.

L. culinaris (No 2) × *L. culinaris (No 13)*. The branching pattern and stipule shape of the hybrids from this combination were intermediate between the parental lines. At metaphase I 7 bivalents were regularly formed and fertility was normal. No breakdown was noticed among the F_2 populations from these hybrids. Segregation for several traits occurred in the F_2 but the details will be published elsewhere.

L. culinaris × *L. orientalis*. Nine different hybrids between three *L. culinaris* and *L. orientalis* lines were grown. The growth habit of all the hybrids was semi erect, and

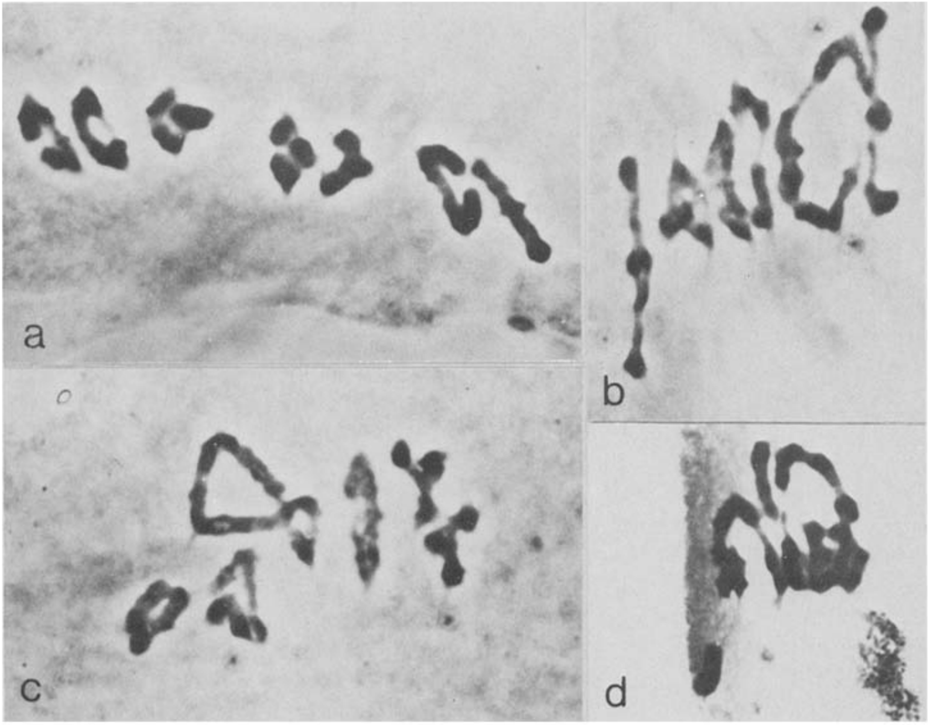

Fig. 3. Meiotic chromosomes in lentil hybrids. a) 7 II in intervarietal hybrid in *L. culinaris;* b) 5 II + IV in *L. culinaris* × *L. orientalis*, note that only two of the four centromerers of the quadrivalent are active; c) 5 II + IV in the same hybrid as in b, note the alternate orientation of the heteremorphic quadrivalent; d) 2 II + IV + VI in *L. culinaris* × *L. nigricans*.

Table 2. Chromosome association and fertility of parental lines and hybrids in *Lens*.

Parental lines and hybrids	Chromosome association in metaphase I							Mean xta per cell	% pollen fertility	% flowers to pods	Number of seeds per pod
	Number of cells	I	Rod II	Ring II	III	IV	V–VII				
L. culinaris											
No 2	30		1.32	5.68				12.69	92	89	1.50
No 7	30		1.11	5.88				13.10	94	90	1.64
No 13	30		1.13	5.68				12.93	88	85	1.04
L. orientalis											
No 22	30		1.24	5.76				12.80	96	87	1.62
No 24	30		1.18	5.82				12.84	93	90	1.70
No 26	30		1.26	5.74				13.02	98	92	1.66
L. nigricans	30		1.82	5.18				12.55	94	94	1.75
L. culinaris (2) × *L. culinaris* (13)	30		1.56	5.42				12.60	91	86	1.25
L. culinaris × *L. orientalis*											
No 2 × No 22	16		1.75	1.35		0.75		12.12	70	78	1.50
No 2 × No 24	16		1.75	4.50		0.35		12.25	37	68	1.24
No 2 × No 26	30	0.06	1.73	4.36		0.43		12.03	51	71	1.12
No 13 × No 22	30	0.06	1.20	4.46		0.63		12.50	82	64	1.08
No 13 × No 24	30		1.60	4.33		0.53		12.16	48	38	1.04
No 7 × No 23	30		1.53	3.93		0.76		12.0	41	83	1.21
L. culinaris × *L. nigricans*											
No 7 × N	60	2.06	2.30	1.01	0.78	0.60	0.08	8.20	18	22	1.06
No 2 × N	26	1.08	2.50	0.92	0.38	0.92	0.73	9.07	20	18	1.03

intermediate between the parental species. The flower color of these F_1 hybrids was blue, but paler than that of *L. orientalis*. Six of the hybrids were examined cytologically. Chromosome association at metaphaseı in all the hybrids was characterized by 5 bivalents and a quadrivalent in many cells (Table 2). The chromosomes involved in this aberration differed markedly in size and formed conspicuously heteromorphic quadrivalent (Fig. 3). Most of these quadrivalents showed adjacent orientation either in a ring or chain configuration. While the chromosome aberration had some effect on pollen fertility the seed set was relatively high (Table 2).

The F_2 populations of *L. culinaris* × *L. orientalis* developed normally and segregated for characters such as growth habir, flower color, pod dehiscence and seed coat color.

L. culinaris × *L. nigricans*. The hybrids between two lines of lentil and *L. nigricans* developed normally. They had markedly decumbent growth which was less pronounced than in *L. nigricans*. Most of the stipules of these plants had a semi hastate shape and occasionally they were dentate. Flower color was blue and the tendrils were well developed, much more so than in the parental species.

Chromosome association at metaphase ı of this hybrid combination was very irregular. Mean chiasmata number per cell was reduced in comparison with the parental lines which resulted from a relatively large number of univalents and overwhelming proportion of rod bivalents (Table 2). Heteromorphic bivalents were observed occasionally and multivalent associations were quite common, particularly trivalents and quadrivalents. But in several cell chains of 5, 6, or even 7 chromosomes were observed (Fig. 3, Table 2). In the various cells of *L. culinaris* × *L. nigricans* hybrids the chromosomes associated in 24 different ways of which the 2ı + 3ıı + 2ııı type was the most common (9%). Furthermore, cells in which a quadrivalent and pentavalent, or trivalent and two quadrivalents were observed indicated that the chromosomes of the two parental species differed by three interchanges. Despite the irregularity of chromosome association at meiosis the fertility of *L. culinaris* × *L. nigricans* hybrids was relatively high and many seeds were produced (Table 2).

L. orientalis × *L. nigricans*. Crosses in this combination were attempted with a single accession (No 23) of *L. orientalis*. The four seedlings which developed from the hybrid seeds were albino and died about 10 days after germination. One of these seedlings was successfully grafted to a normal plant. It produced 3 small leaves and remained alive for more than 4 months but no flowers were formed.

DISCUSSION

The three *L. culinaris* lines employed in the present study were morphologically distinct from one another and represented a considerable part of the vast geographical area where lentil is currently growing. The similar karyotypes of these lines and the normal meiosis of their hybrids indicate that they share a similar linear arrangement of their chromosomes. It is pertinent to note that the karyotype of 14 *L. culinaris* lines studied by SHARMA & MUKHOPADAY (1963) was essentially similar to that found in this study. On the other hand, WILLIAMS et al. (1974) proposed a different karyotype for *L. culinaris*. It will be interesting to verify these differences by breeding experiments.

The performance of hybrids between large and small seeded lentils supports the contention of WILLIAMS et al. (1974) that lentil should not be subgrouped according to the seed size. These two seed types are easily hybridized with one another, their F_1 hybrids are normal and in the F_2 segregation occurs in many traits including seed size.

The *L. orientalis* lines used in this study represented only a small fraction of the distribution range of this species, perhaps its most south western corner. Cytogenetically these accessions were apparently uniform and they differed from the *L. culinaris* lines by a single chromosome interchange. This rearrangement most probably involved segments of similar size and hence no difference in the karyotypes of these two taxa was noticed.

The single *L. nigricans* line employed by us could possibly be considered as a representative of the eastern fringe of the distribution area of this species. The karyotype of this line is very similar to that reported by WILLIAMS et al. (1974) for *L. nigricans*. Cytogenetically *L. nigricans* differs from *L. culinaris* by three chromosome rearrangements. However, it can be concluded from the fertility of the hybrids that these aberrations do not necessarily prevent gene exchange between the two species.

Most interesting, however, is the behavior of the hybrids between *L. orientalis* and *L. nigricans*. As already mentioned these two wild species are very much alike morphologically, but they apparently differ in their ecological requirements. While *L. orientalis* is adapted mainly to steppes and relatively dry conditions, *L. nigricans* is more a Mediterranean element (Fig. 1). The adaptation of these two sibling species to different growing conditions was apparently coupled with physiological changes, affecting the process of chloroplast formation or chlorophyll synthesis in their hybrids and thus forming a strong, practically absolute reproductive barrier. From the pattern of chromosome association at meiosis in the hybrids of *L. culinaris* with these two wild lentils it can be concluded further that *L. orientalis* and *L. nigricans* differ from one another also by at least two, possibly four, chromosome rearrangements.

Our present knowledge of the physiological dissimilarity and the cytogenetic difference between *L. orientalis* and *L. nigricans* comes from a single cross combination. Obviously further study is needed in order to reach a more concrete conclusion regarding the genetic relationships between them. It is important however to note that each of the parent lines of the *L. orientalis* × *L. nigricans* hybrids examined by us represents a border region of the species distribution range. That area is also a zone where the distributions of the two species overlap. It would be interesting to examine the extent to which the reproductive barriers have been built up in populations of *L. orientalis* and *L. nigricans* from other areas in their distribution range.

With the information obtained in this study we are now in a much better position to assess the origin of the cultivated lentil. The normal meiosis and fertility of the *L. culinaris* intraspecific hybrids indicate that this cultigen possibly has a monophyletic origin and that chromosome repatterning played a minor role, if any, in the evolution of this crop. It can be concluded further that *L. culinaris* is much closer to *L. orientalis* than to *L. nigricans*. However, since *L. orientalis* lines used in this study differed from the cultivated species by a reciprocal translocation, and were unable to form normal hybrids with *L. nigricans* it is quite unlikely that they were involved in the origin of the cultivated type. Thus, it is apparent that lentil was not domesticated at the southwestern part of the Fertile Crescent in the Middle-East.

Although it is more probably that lentil originated from *L. orientalis* the role of *L. nigricans* in the evolution of lentil should not be overlooked. Introgression between these two taxa evidently can take place but its actual magnitude is necessarily low due to rare outcrossing in these plants (WILSON & LAW, 1972) and their low coincidence in nature. Nevertheless, it is possible that the *L. culinaris* reported with semi hastate stipules is a consequence of gene flow from *L. nigricans*.

Exploitation of the wild genepool for breeding purposes is a common practice in an increasing number of cultivated plants. In many cases the wild relatives still possess important variation that no longer exists in their cultivated counterparts. An outstanding example is the remarkable number of disease resistant genes that are floating in the wild genepool and their rarity among the cultivated lines. The information now available on the wild genepool of lentil indicates that for breeding purposes *L. orientalis* and *L. nigricans* can be exploited almost equally and their variation can easily be utilized. At the moment, however, the potential variation of these wild species is practically unknown, mainly because of their poor representation in world collections. Considering the close genetic affinity between *L. culinaris* and both *L. orientalis* and *L. nigricans* systematic collection and evaluation of these wild species is fully justifiable. This material could be an important source for lentil breeding in the future.

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