Theor Appl Genet (1985) 70:97–101



# Hybridization in the genus *Lens* by means of embryo culture

G. Ladizinsky [1], D. Cohen [1] and F. J. Muehlbauer [2]

[1] Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel
[2] U.S.D.A. – ARS Washington State University, Pullman, Wash, USA

Received August 23, 1984; Accepted November 21, 1984
Communicated by L. Alföldi

**Summary.** The cultivated lentil *L. culinaris* and the wild lentil *L. ervoides* are reproductively isolated from one another due to their hybrid embryo breakdowns. Using embryo culture, vegetatively normal hybrids were obtained. One specific hybrid, heterozygous for a reciprocal translocation, had about 50% gamete viability and produced aborted and viable embryos in a 1 : 1 ratio. In the $F_2$, vegetatively normal and highly fertile plants were selected. With the aid of embryo culture techniques, *L. ervoides* can be included in the wild gene pool of the cultivated lentil.

**Key words:** *Lens culinaris* – *L. ervoides* – Hybridization – Embryo breakdown – Embryo culture

## Introduction

The biological species concept recently has been applied to the genus *Lens* to define the two species *L. culinaris* and *L. nigricans* (Ladizinsky et al. 1984). *L. culinaris* contains the subspecies *culinaris, orientalis* and *odemensis*; the former is the cultivated lentil and the latter two are wild. *L. nigricans* contains two wild subspecies, *nigricans* and *ervoides*. In the taxonomical literature, these subspecies are often considered independent species. Within both biological species, hybrids can be obtained that are completely or partially fertile, depending upon the differences in chromosomal rearrangements between the parental lines. Using conventional crossing procedures, no interspecific hybrids could be obtained between *L. culinaris* and *L. nigricans*. When crosses are made between species, the pods develop for about 7–14 days but later collapse and yield only shrivelled non-viable seeds. Using embryo culture, we obtained *L. culinaris* × *L. ervoides* hybrids (Cohen et al., in press). This paper reports the behavior of these hybrids and their $F_2$ derivatives.

## Materials and methods

The plant material used in this study included the cultivated lentil, *L. culinaris* accessions No. 2 from Israel and No. 7 from Ethiopia, and the wild species *L. ervoides* accessions No. 32 from Israel, and Nos. 90 and 92 from Yugoslavia.

The technique used for embryo culture is reported in Cohen et al. (in press). Fourteen-day-old fertilized ovules were placed on MS medium containing 10% sucrose and supplemented with 0.5 mg/l zeatin. After 7–10 days the embryos were released from the ovular integuments and placed on low (3%) sucrose MS medium which was free of gibberellic acid (GA) and contained 0.3 mg/l zeatin.

To study chromosome associations at meiosis, buds of appropriate size were fixed in 3 : 1 absolute ethanol : acetic acid, stored in 70% ethanol and individual anthers were stained with aceto carmine. Pollen fertility was assessed by staining mature pollen grains with aceto carmine.

## Results

The two *L. culinaris* accessions, Nos. 2 and 7, were fully interfertile. Both accessions had green epicotyls and stems, and glabrous pods. The three *L. ervoides* lines had purple epicotyls and stems, and puberulent pods. Accessions Nos. 90 and 92 were cytogenetically alike and their hybrids were fully fertile. Both differed by one reciprocal translocation from No. 32 (Table 1). Under normal crossing conditions, *L. culinaris* × *L. ervoides* hybrid embryos die 7–14 days after fertilization. To rescue these hybrids, we planted them on artificial medium and kept them under a 12 h photoperiod at 25 °C. Altogether 32 hybrid embryos were planted, from which 12 plantlets were obtained. Roots and

**Table 1.** Chromosomal configuration at MI in *L. culinaris* and *L. ervoides* hybrids

| Cross combination | Chromosome configuration | No. of cells |
|---|---|---|
| *L. culinaris* × *L. culinaris* | | |
| 2× 7 | 7 II | 20 |
| *L. ervoides* × *L. ervoides* | | |
| 90×32 | 5 II+IV | 16 |
| | 7 II | 4 |
| | | 20 |
| *L. culinaris* × *L. ervoides* | | |
| 2×92 | 4 II+VI | 15 |
| | 5 II+IV | 6 |
| | 3 II+III+V | 2 |
| | 3 II+2 IV | 2 |
| | | 25 |
| 2×90 | 4 II+VI | 11 |
| | 5 II+IV | 3 |
| | I+4 II+V | 1 |
| | | 15 |
| 32× 7 | 5 II+IV | 13 |
| | 7 II | 10 |
| | 4 I+5 II | 1 |
| | I+5 II+III | 1 |
| | | 25 |

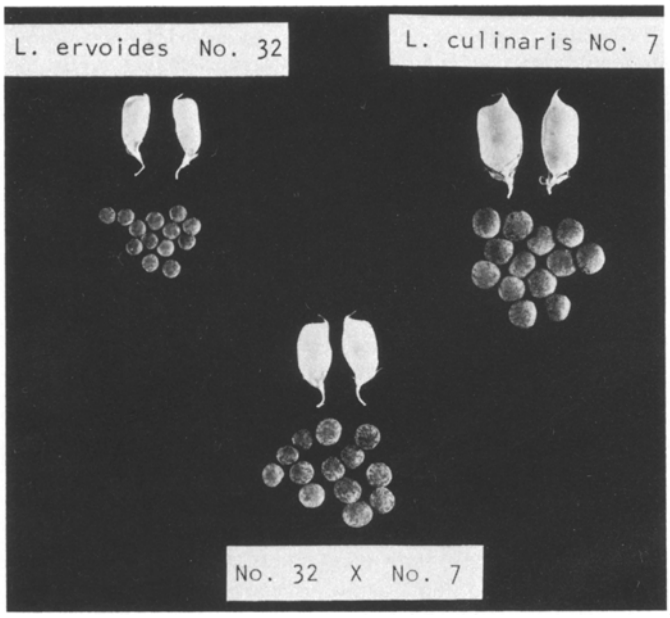


**Fig. 1.** Pods and seeds of *L. culinaris, L. ervoides* and their $F_1$ hybrid (natural size)

leaves developed 25–38 days after planting. Plantlets with roots and 3–4 leaves were transferred to pots containing vermiculite and were watered daily. At this stage we lost all but three of the young plants. The three plants were then transferred to soil and were grown in the greenhouse.

The three $F_1$ hybrids that were similar to the *L. ervoides* parents had purple stems and puberulent pods, and were intermediate to the parents for leaf size, number of leaflets per leaf and pod size (Fig. 1).

Chromosome associations at MI of meiosis in the No. 2×No. 90 and No. 2×No. 92 hybrids indicated that the parental species differed by two chromosomal rearrangements (Table 1 and Fig. 2). Stainable pollen in these two hybrids was 3–5% and no seeds were produced.

A different chromosome pairing pattern was observed in the No. 32×No. 7 hybrid. In this case only a single quadrivalent per cell was formed, indicating that the parental lines differed by a single translocation (Table 1 and Fig. 2). Pollen fertility in the No. 32×No. 7 hybrid was 52% and of the 592 flowers produced on that hybrid, 218 failed to develop pods apparently because of female gamete inviability as a result of the chromosomal rearrangements. The rate of the female gamete inviability could be stimated as $\sqrt{\frac{218}{592}}=0.606$ since in each ovary there were two ovules and when both were inviable no pod development took place. This abortion rate, however, is somewhat higher than that of the pollen.

The 374 pods which developed on the No. 32×No. 7 hybrid, formed two distinct groups. 174 pods ceased to grow between 7–14 days after anthesis (Fig. 3) and yielded shrivelled non viable seeds. Another 200 pods reached maturity and produced 214 seeds. While no attempt was made to distinguish between aborted gametes and embryos in the shrunken and normal pods, it could be estimated that $(1{,}184\times0.606)-436=$ 282 ovules aborted, 214 embryos developed seeds, and the remaining 252 embryos aborted between 7 and 14 days after anthesis. Thus, the ratio between the normal and aborted embryos on the $F_1$ fit a 1 : 1 ratio ($P=0.10$–$0.05$).

Fifty seeds collected from the $F_1$ were planted and all germinated. Five seedlings were albino and died about 10 days after germination, and another five green seedlings died in later stages before maturity. Because the ratio between the green and the albino seedlings approached a 15 : 1 ratio, an additional 117 seedlings were grown to verify that ratio. Of the 117 seedlings, 11 were albino and indicated that, in this hybrid combination, albino seedlings were conditioned by two recessive genes (Table 2).

Segregation was noted also for the epicotyl color, 33 plants had purple epicotyles while 12 had green ones.

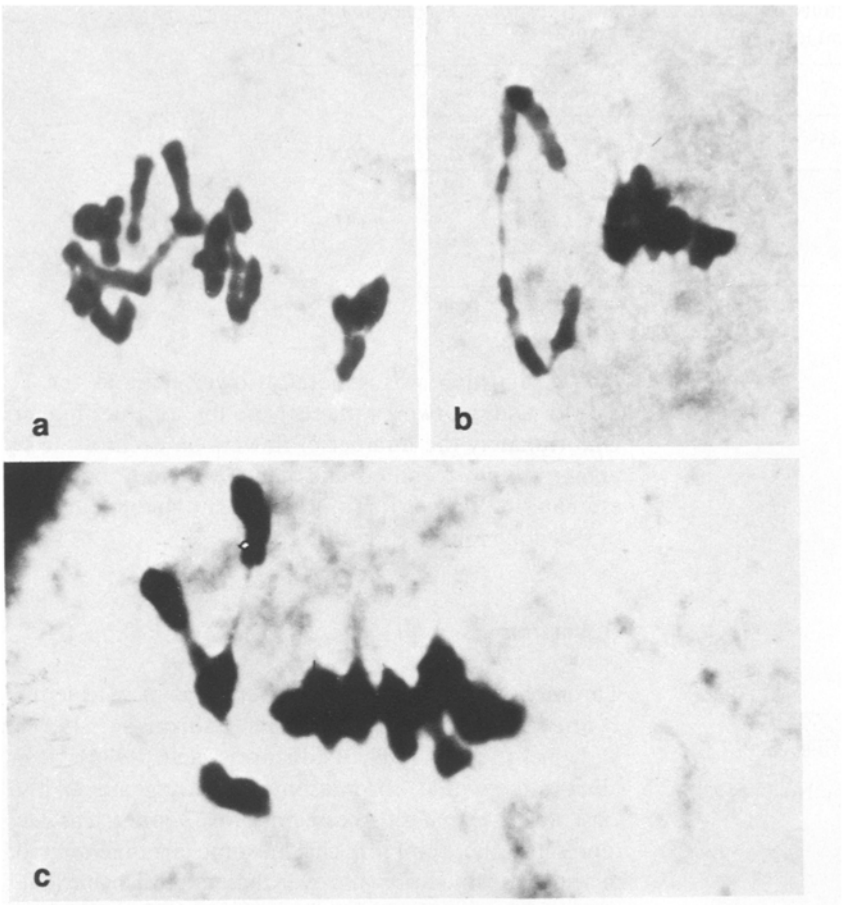


**Fig. 2a–c.** Chromosome associations at MI in *L. culinaris* × *L. ervoides* hybrids. **a** No. 2 × No. 90 (4II + VI); **b** No. 32 × No. 7 (5II + IV); **c** No. 32 × No. 7 (5II + III + I)

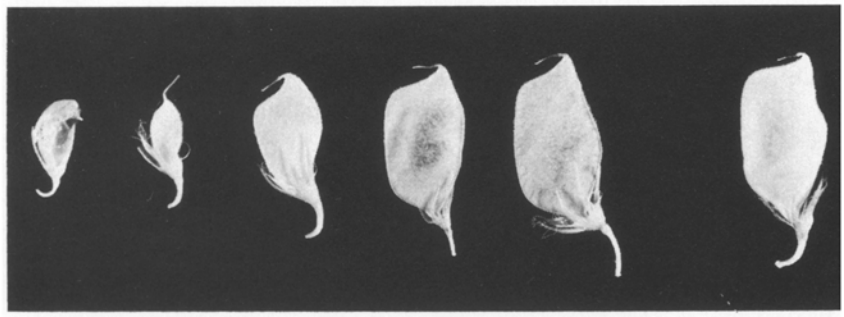

**Fig. 3.** Flower that failed to develop pod (extreme *left*) and 14 day-old seed-bearing pod (extreme *right*) and 14 day-old pods with aborted embryos (in the *middle*) on No. 32 × No. 7 hybrid (×2.5)

**Table 2.** The segregation pattern and P values of 3 characteristics in *L. ervoides* × *L. culinaris* $F_2$ population

| Trait | *P* | | Gene symbol | Parental genotype | |
|---|---|---|---|---|---|
| | 3:1 | 15:1 | | | |
| | | | | *L. culinaris* | *L. ervoides* |
| Albino seedling | | 0.10–0.05 | *Ws* | $Ws_1Ws_1ws_2ws_2$ | $ws_1ws_1Ws_2Ws_2$ |
| Epicotyle coloration | 0.95–0.90 | | *Gs* | *gsgs* | *GsGs* |
| Pod puberulence | | 0.90 | *P, R* | *pprr* | *PPRR* |

**Table 3.** Frequency distribution of pollen fertility among *L. ervoides* × *L. culinaris* $F_2$ plants with 7 bivalents (7 II) and 5 bivalents and quadrivalent (5 II + IV)

| Chromosome association at MI | Pollen fertility % | | | | | | |
|---|---|---|---|---|---|---|---|
| | 15–30 | 31–45 | 46–60 | 61–75 | 75–90 | 90–100 | Total |
| 7 II | 1 | 1 | 2 | 1 | 7 | 1 | 13 |
| 5 II + IV | 3 | 6 | 6 | 3 | 1 | | 19 |
| | | | | | | | 32 |

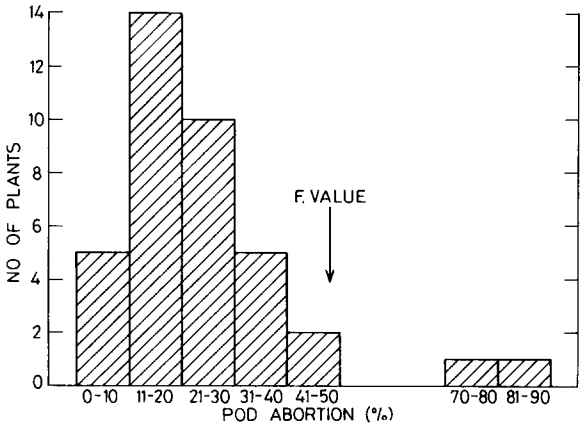


**Fig. 4.** Distribution of pod abortion among the No. 32 × No. 7 $F_2$ family

This ratio indicated that this trait is governed by a single gene (Table 2) as had been found previously in crosses between *L. culinaris* and *L. orientalis* (Ladizinsky 1979 a).

Only two out of 40 $F_2$ plants had glabrous pods similar to those of the *L. culinaris* parent. The rest of the plants had pods with different degrees of pubescence. This segregation pattern indicated that glabrous pod is conditioned by two recessive genes (Table 2).

Chromosome associations at MI of meiosis was examined in 32 $F_2$ plants. In 13 of the $F_2$ plants, 7 bivalents were regularly formed but the other 19 plants had 5 bivalents and a quadrivalent. This fits ($P$= 0.30–0.20) the 1 : 1 ratio expected for segregation of a reciprocal translocation. On the other hand, a wide range of pollen stainability was observed among the $F_2$ plants. While only a single plant had the stainable pollen value of the parental species, the value of 11 plants was lower than that of the $F_1$ hybrid. Furthermore, no consistancy was found between the pattern of chromosome associations at MI and pollen stainability (Table 3).

The rate of pod abortion that resulted from embryo breakdown varied considerably among the $F_2$ plants (Fig. 4). While none were free from pod abortion, the rate of abortion was generally lower than in the $F_1$ hybrid. Only in two $F_2$ plants were these values higher. Unfortunately the number of flowers on each of the $F_2$ plants was not counted and hence we were unable to estimate the proportion of aborted and viable embryos as was done for the $F_1$ hybrids.

## Discussion

Chromosome repatterning was reported in wild lentils *L. orientalis* and *L. nigricans* (Ladizinsky 1979 b, Ladizinsky et al. 1983; Ladizinsky et al. 1984). It is obvious now that chromosomal rearrangements also exist in *L. ervoides*. Accession No. 32 of *L. ervoides* represents the common chromosome arrangement of this species which prevails over the entire distributional range of *L. ervoides*. Accessions 90 and 92, both from Yugoslavia, deviate from this arrangement but they definitely are not representative of that territory since other accessions of *L. ervoides* from Yugoslavia were similar to No. 32 (Ladizinsky et al. 1984).

More interesting, however, is the difference of only one chromosomal interchange between *L. ervoides* No. 32 and *L. culinaris*. It is worth mentioning that among *L. orientalis*, the wild progenitor of the cultivated lentil, there are accessions that differ from the cultivated lentil by up to three chromosomal rearrangements. Thus, from a cytogenetic point of view, *L. ervoides* is closer to *L. culinaris* than some of the *L. orientalis* lines.

It seems, however, that *L. ervoides* and *L. culinaris* differ from each other by additional cryptical rearrangements that cannot be seen at MI of the interspecific hybrids. This is apparently the reason for the low pollen fertility observed in $F_2$ plants with perfect chromosome pairing at meiosis. Such cryptical changes might explain the segregation pattern of the albino seedlings in the $F_2$. The 15 : 1 ratio obtained for the albino seedlings can be interpreted as follows: each of the two species had two loci for chlorophyll production and only one, but not the same one, is active in the two species. Or, and perhaps a more likely explanation, that each species has only one gene for chlorophyll, but

because of a minor translocation it is not located on the same chromosome in *L. culinaris* and *L. ervoides.*

*L. culinaris* and *L. ervoides* are reproductively isolated from one another by a strong postzygotic mechanism. The breakdown of 7–14 day-old interspecific hybrid embryos could be attributed to malfunction of the endosperm or to an undesirable interaction between maternal and hybrid genotypes. The distinction between the two can be made when the anatomical studies of these hybrid embryos is completed. Nevertheless, the genetic mechanism seems peculiar. The complete hybrid embryo breakdown in the interspeficic crosses, regardless of the cross direction, and the 1 : 1 ratio between viable and aborted embryos on the $F_1$ hybrid, suggest the involvement of a single gene and that abortion occurs in the heterozygous state. According to that explanation of inheritance, no embryo breakdown is to be expected on $F_2$ plants because all heterozygous embryos would have already aborted on the $F_1$ hybrid. Furthermore, embryo breakdown on $F_2$ plants indicated that this behavior is of a quantitative nature. The discrepancy between the behavior of the $F_1$ hybrid and the $F_2$ plants apparently cannot be explained by usual genetic mechanisms. An explanation of the behavior of the $F_1$ and the $F_2$ apparently has to be sought by interaction between the maternal cytoplasmic and nuclear genomes, and the hybrid embryo genotype.

Rescue of hybrids by means of embryo culture has been done in many interspecific crosses. These hybrids were usually vegetatively normal but totally sterile indicating that besides embryo breakdown, there are additional mechanisms that assure reproductive isolation. The *L. culinaris* × *L. ervoides* hybrids were vegetatively normal, but, in particular combinations, partially fertile hybrids and reasonably fertile $F_2$ plants were obtained. This indicates that despite the establishment of an effective reproductive barrier between *L. culinaris* and *L. ervoides,* the two genomes have not differentiated from one another to a great extent.

The possibility of obtaining normal and fertile $F_2$ derivatives from crosses between *L. culinaris* and *L. ervoides* via embryo culture, is economically important. With this technique, the wild gene pool of the cultivated lentil can be widened to include *L. ervoides* in addition to *L. orientalis* and *L. odemensis* (Ladizinsky et al. 1984). Thus far we have not succeeded in growing *L. culinaris* × *L. nigricans* hybrids to maturity. Such hybrids might be obtained by improving the embryo culture technique. Nevertheless, the gene pool of *L. nigricans* might already be used for breeding purposes by using *L. ervoides* as a bridge, since *L. nigricans* and *L. ervoides* are cross compatible and their hybrids are partially fertile.

*Acknowledgement.* This research was supported by a grant from the US-Israel Binational Agricultural Research and Development Fund (BARD).

## References

Cohen D, Ladizinsky G, Ziv M, Muehlbauer FJ (in press) Rescue of interspecific *Lens* hybrids by means of embryo culture. Plant Cell Tissue Organ Cult

Ladizinsky G (1979a) The genetics of several morphological traits in the lentil. J Hered 70: 135–137

Ladizinsky G (1979b) The origin of lentil and its wild gene pool. Euphytica 28: 179–187

Ladizinsky G, Braun D, Muehlbauer FJ (1983) Evidence for domestication of *Lens nigricans* (M. Bieb) Godron in S Europe. Bot J Linn Soc 87: 169–176

Ladizinsky G, Braun D, Goshen D, Muehlbauer FJ (1984) The biological species of the genus *Lens.* Bot Gaz 145: 253–261