Variation in the ribosomal RNA units of the genera *Lens* and *Cicer*

P.B. Patil¹, P.L. Vrinten², G.J. Scoles¹ & A.E. Slinkard¹

¹ Department of Crop Science and Plant Ecology, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan, Canada S7N SA8; ² Department of Crop Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

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Summary

Investigation of the organization of the tandemly repeated 5S and 18-25S ribosomal RNA (rRNA) genes was carried out on members of the genera *Lens* and *Cicer* using restriction endonuclease digestion and Southern hybridization. The 5S rRNA unit (gene + spacer) in *Lens* (0.38 to 0.50 kb) and *Cicer* (0.40 to 0.44 kb) is the largest among the legumes. In *L. nigricans* and *L. culinaris* ssp. *odemensis* a second repeat size was detected. Restriction maps for the 18-25S units of *Lens* and *Cicer* species were developed. The 18-25S rRNA unit varied from 8.3 to 9.8 kb in *Lens* and 10.5 to 11.4 kb in *Cicer*. The only detectable variability in the *Lens* and *Cicer* 18-25S rRNA unit was in the length of the intergenic spacer (IGS) region, except for a HindIII site in the IGS region of *L. nigricans* ssp. *nigricans* and in all *Cicer* species.

Similarities in the size of both 5S and 18-25S rRNA units were noted between *L. culinaris* ssp. *culinaris* and *L. culinaris* ssp. *orientalis*, which supports previous evidence that *L. culinaris* ssp. *orientalis* is the progenitor of the cultivated lentil. Differences in the arrangement of both 5S and 18-25S rRNA units were noted between *L. nigricans* ssp. *nigricans* and *L. nigricans* ssp. *ervoides*, indicating either divergent evolution of these subspecies or alternatively incorrect taxonomy.

On the basis of the size of the 5S rRNA unit, *C. bijugum*, *C. chorassanicum* and *C. echinospermum* formed a group with a slightly smaller unit than the other *Cicer* species. Similarities in the length of both 5S and 18-25S rRNA units of *C. arietinum* and *C. reticulatum* possibly confirm *C. reticulatum* as the progenitor of *C. arietinum*. *Cicer cuneatum* has a smaller 18-25S rRNA unit than the other *Cicer* species as a result of a smaller intergenic spacer.

Introduction

Lentil (*Lens culinaris* ssp. *culinaris* Medik.) and chickpea (*Cicer arietinum* L.) are important food legume crops of the Old World. Both are now being grown in the new world, particularly in Idaho and Washington in the U.S.A. and in Western Canada (Slinkard, personal commun.). In these areas, breeding programs are underway to develop adapted cultivars. As in any breeding program, the wild relatives of these crops may serve as a secondary gene pool for desirable agronomic traits, but this requires knowledge of the taxonomic relationships of the cultivated and wild species. The taxonomic relationships of members of these two genera have been studied only in the last 20 years and are still not fully understood.

Lentil is an ancient crop that was domesticated in the Fertile Crescent area of S.W. Asia approximately 9000 years ago (Zohary & Hopf, 1988). Based on studies of crossability and fertility of hybrids, Ladizinsky et al. (1984) suggested that the genus *Lens* consists of only two species, *L. culinaris* and *L. nigricans*, with three subspecies in *L. culinaris* (ssp. *culinaris* (the cultivated lentil), ssp. *orientalis* and ssp. *odemensis*) and two subspecies in *L. nigricans* (ssp. *nigricans* and ssp. *ervoides*). Several other traits have also been investigated in the genus *Lens* in order to better understand the taxonomy of the group including seed protein pro-
files (Ladizinsky, 1979), isozymes (Hoffman et al., 1986), morphology (Hoffman et al., 1988), nuclear DNA RFLP (Havey & Muehlbauer, 1989) and chloroplast DNA RFLP (Muench et al., 1991). These studies confirm the taxonomy proposed by Ladizinsky et al. (1984), although some contradictions remain, particularly with regards to the taxonomic status of L. n. ssp. ervoides (Hoffman et al., 1986; Muench et al., 1991). Ladizinsky et al. (1984) found two accessions of L. n. ssp. nigricans which differed from other L. n. ssp. nigricans accessions by four interchanges and a pericentric inversion and, hence, were reproductively isolated, although each produced partially fertile hybrids with L. n. ssp. ervoides. These have been referred to as having a differentiated cytotype (Ladizinsky et al., 1984). Isozyme studies by Hoffman et al. (1986) confirmed the differentiation of these two accessions from other accessions of L. n. ssp. nigricans.

The genus *Cicer* is best known by the cultivated chickpea (*C. arietinum* L.), which is an important food legume in many Old World countries. In addition the genus *Cicer* includes eight other annual species (van der Maesen, 1987). Interspecific relationships among these annual species have been studied using crossability (Ladizinsky & Adler, 1975, 1976a, b) and seed protein profiles (Ahmad & Slinkard, 1992). On the basis of crossability, seven of the nine annual *Cicer* species were classified into three groups: Group I included *C. arietinum, C. reticulatum* and *C. echinospermum*, group II included *C. bijugum, C. judaicum* and *C. pinnatifidum*, and group III contained only *C. cuneatum*. Of the two annual species which were not included in these crossability studies, one of them (*C. chorasanicum*) was placed in group II and the other (*C. yamashitae*) was placed in a fourth group, based on seed protein profile studies (Ahmad & Slinkard, 1992). Based on crossability studies, Ladizinsky & Adler (1976b) proposed that *C. reticulatum* was the progenitor of the cultivated chickpea. Ahmad & Slinkard (1992), using seed protein profiles, also proposed that *C. reticulatum* was the progenitor of the cultivated chickpea.

Variation in the length of 5S and 18-25S ribosomal RNA units has been used as a taxonomic tool in the Triticeae (Dvorak & Appels, 1982; Scoles et al., 1988), maize (Zimmer et al., 1988), soybean (Doyle & Beachy, 1985) and many other species. This study investigated the organization of the 5S and 18-25S ribosomal genes in members of the genera *Cicer* and *Lens* in order to further our understanding of the relationships among the various species within *Lens* and *Cicer*.

**Materials and methods**

To provide plant material for DNA extraction, plants of both genera were grown in a greenhouse at a temperature of 20–25°C with supplemental light provided by a mixture of fluorescent and incandescent lights to extend daylength to 16 h. A number of accessions of each of the five *Lens* subspecies recognized by Ladizinsky et al. (1984) (including the two accessions of *Lens nigricans* ssp. nigricans with the differentiated cytotype) were grown in hydroponic tanks supplied with a modified Hoagland solution. Twelve cultivated chickpea lines (seven desi and five kabuli type) and two accessions of each of the eight wild *Cicer* species (except for *C. cuneatum*, where only one accession was available) were grown in a 2:1:1 soil, peat-moss,