

Cytotaxonomic characterization of cultivars and accessions of *Lens culinaris* Medik. (Fabaceae) from Chile and Canada

Caracterización citotaxonómica de cultivares y procedencias de *Lens culinaris* Medik. (Fabaceae) de Chile y Canadá

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ABSTRACT

The lentil (*Lens culinaris* Medik.) belongs to the legume family (Fabaceae), and it possesses benefits at nutritional, sustainable, and crop rotation system levels. Its occurrence and consumption in Chile originated from imports from Canada, but molecular tools now group Canadian lentils apart from those of the Mediterranean agro-ecological zones in Chile. Isoenzyme studies have shown a narrow genetic distance between different strains of Chilean germplasm. This present work characterizes Chilean cultivars and regional accessions and compares them with those in Canada using the cytotoxic tools of chromosome formula, chromosomal asymmetry index, total chromosome length (TLC), and a detailed architectural examination of each chromosome. We found chromosome stability among all lentils as $4m+3sm$. Results showed a low TLC but higher inter-chromosomal asymmetry in the accessions from Hualqui (Biobío Region) ($CV_{CL} = 31.25$), a unique secondary constriction from Yumbel (Ñuble Region), and a higher TLC in Collanco (Biobío Region). Even though there was no evident karyotypic separation between Chilean and Canadian lentils, there was greater heterogeneity in TLC among cultivars in Chile than among those from Canada. It is unknown what physiological differences these cytological variants might possess. Because *L. culinaris* in Chile is of agronomic importance, studies of conservation are recommended to safeguard cytotoxic races as a national genetic resource.

Keywords: cytotoxicity, karyotypic asymmetry, lentils, secondary constrictions.

RESUMEN

La lenteja (*Lens culinaris* Medik.) pertenece a la familia de las leguminosas (Fabaceae), y posee beneficios a nivel nutricional, sustentable y de sistema de cultivos de rotación. Su ocurrencia y consumo en Chile se origina de las importaciones desde Canadá, pero las herramientas moleculares agrupan a las lentejas canadienses aparte de aquellas que se cultivan en zonas agroclimáticas de tipo mediterráneas como las de Chile. Estudios isoenzimáticos han demostrado una reducida distancia genética entre diferente material de germoplasma de la lenteja chilena. El presente trabajo caracteriza cultivares chilenos y procedencias regionales y los compara con aquellos de Canadá usando herramientas citotaxonómicas como fórmula cromosómica, índice de asimetría intercromosomal, longitud total de los cromosomas (TLC), y un detallado examen de la arquitectura de cada cromosoma. Encontramos estabilidad cromosómica entre todas las lentejas como $4m+3sm$. Los resultados mostraron un bajo TLC, pero una mayor asimetría intercromosomal

en las procedencias de Hualqui (Región del Biobío) ($CV_{CL} = 31,25$), una única constricción secundaria de Yumbel (Región de Ñuble), y un mayor TLC en Collanco (Región del Biobío). Aún cuando no hubo evidente separación cariotípica entre las lentejas Chilenas y Canadienses, hubo mayor variación en el TLC de los cultivares de Chile que de los de Canadá. Se desconoce qué diferencias fisiológicas podrían poseer estas variantes citológicas. Debido a que *L. culinaris* es de importancia agronómica en Chile, se recomiendan estudios de conservación para resguardar las razas citotaxonómicas como un recurso genético nacional.

Palabras clave: asimetría cariotípica, citotaxonomía, constricciones secundarias, lentejas.

INTRODUCTION

The understanding and conservation of plant diversity provides benefits for humans, such as in prospecting for natural chemical compounds helpful in medicine (Tharanathan & Mahadevamma 2003; Al-Islam *et al.* 2012) or in use of phytodiversity for improvement of cultivated plants to meet challenges of future nutritional demands (Govindaraj *et al.* 2015). In this context it is especially important to evaluate genetic diversity in crops of agronomic importance.

Many cultivated crops belong to the legume family (Fabaceae), which consists of about 800 genera and 20000 species (Lewis *et al.* 2005). According to Stagnari *et al.* (2017), the benefits offered by legumes can be classified at three levels: good nutrition (Tharanathan & Mahadevamma 2003; Al-Islam *et al.* 2012), high productivity (Liting *et al.* 2019), and a stable system of cultivation (Pacajes *et al.* 2002; Zander *et al.* 2016), all of which provide high significance for many zones of the world. Among the legumes, there exists a subgroup called the dry legumes (pulses), which are harvested for their dry seeds.

An important dry leguminous crop world-wide is the lentil (*Lens culinaris* Medik.). This annual, herbaceous, and autogamous species originated in the near East, and it was one of the crops from which agriculture developed in the Old World (Zohary 1999). In 2018, the lentil was the fifth most productive dry leguminous crop in the world with six million tons harvested (FAOSTAT 2020). In this same year, the largest producers were Canada, India, USA and Turkey, in that order, with Canada producing two million tons and 13953 hg ha^{-1} (FAOSTAT 2020).

The lentil was introduced to Chile in the 17th century with the arrival of the Spanish, and it became an important domesticated crop within the country. In 1979 Chile produced up to 31690 tons, but 2018/2019 production dropped significantly to only 1400 tons and 7642 hg ha^{-1} (FAOSTAT 2020). Lentils are now being grown by small farmers only in Maule, Ñuble, La Araucanía, and Biobío regions, the latter region showing the highest yields (ODEPA 2019). Due to low

domestic production, Chile now imports a large portion (97%; 14726 t) of its crop from Canada (ODEPA 2019).

Diversity within cultivated *L. culinaris* is classified principally on the basis of the diameter of its seeds, these being the microspermas (2-6 mm) and macrospermas (6-9 mm) (Barulina 1930), with the latter plants also being taller and having heavier seeds (Sharma *et al.* 1995). The macrospermas also flower and fruit over a longer period of days.

Recent studies with molecular markers on germplasm of *L. culinaris* throughout the world have revealed three agro-ecological zones, the northern temperate region, the Mediterranean, and southern Asia (Khazaei *et al.* 2016). Studies on genetic diversity of *L. culinaris* in Chile are few, and hence there is no clear picture of the status of this crop within the country. The Instituto de Investigaciones Agropecuarias (INIA) has made an effort to generate cultivars that possess seeds of high caliber, e.g., Calpun-INIA and Super Araucana-INIA (Tay *et al.* 2001; Peñaloza *et al.* 2007) and resistant to "la roya" (a devastating leaf rust, *Uromyces fabae* (Pers.) de Bary). Rodríguez *et al.* (1999) completed an isozyme study that revealed low genetic distance among samples of Chilean germplasm, with 87.2% of Chilean lentils being incorporated in the same phenetic grouping. These results suggest that low genetic diversity exists within the naturalized Chilean lentils.

Another approach to recognize patterns of genetic variation within diverse crop plants is use of karyological data. These methods permit evaluation of differences in the number, form, and size of chromosomes, band polymorphisms, and architectural alterations in the presence of secondary restrictions or nuclear organizing regions (NORs; Guerra 2008). General cytotaxonomic studies on *L. culinaris* have revealed a chromosome number of $2n = 14$ (Bhattacharjee 1953; Sinha & Acharia 1972; Balyan *et al.* 2002; Jha *et al.* 2015). The morphology of the chromosomes varies from metacentric, submetacentric, to subtelocentric or acrocentric (Sinha & Acharia 1972; Manara & Manara 1979; Ladizinsky & Muehlbauer 1993; Rehman & Altaf 1994; Balyan *et al.* 2002; Gaffarzadeh-Namazi *et al.* 2007; Jha *et al.* 2015). Size of the individual chromosomes varies from 3.0 to 12.5 μm ,

depending upon which cultivar is examined, whereas the total length of the chromosomes (TLC) ranges from 28 to 121 μm (Sinha & Acharia 1972; Naithani & Sarbhoy 1973; Manara & Manara 1979; Rehman & Altaf 1994; Gaffardazeh-Namazi *et al.* 2007; Jha *et al.* 2015). Secondary constrictions range from 0 to 3 (Battacharjee 1953; Sinha & Acharia 1972; Naithani & Sarbhoy 1973; Kumar *et al.* 2001; Galasso *et al.* 2001; Balyan *et al.* 2002; Gaffarzadeh-Namazi *et al.* 2007; Jha *et al.* 2015).

In the present study, we contribute to a better understanding of Chilean lentils by characterizing cytotaxonomically selected Chilean cultivars and accessions and comparing these with accessions from Canada. Specific data involve chromosome formula, indexes of chromosomal asymmetry, total length of chromosomes, and a detailed examination of the architecture of each of the chromosomes.

MATERIALS AND METHODS

PLANT MATERIAL

Five accessions and two cultivars of *Lens culinaris* Medik. were utilized from Chile, plus three cultivars from Canada (Líder, Martini, Campo Lindo) obtained from Chilean supermarkets. For the Chilean accessions, these were obtained from small farmers in distinct localities of the Ñuble (Yumbel, Ninhue) and Biobío (Collanco, Hualqui, Los Ángeles) Regions. Finally, the Chilean cultivars, Calpun-INIA and Super Araucana-INIA, were obtained from the Instituto de Investigaciones Agropecuarias (INIA).

PREPARATION OF PLANT MATERIAL

Seeds of *L. culinaris* were thoroughly hydrated in Petri dishes at room temperature (18-22 °C), germinating after 4 d. This was followed by an antimetabolic treatment of 8-hydroxyquinoline in the darkness at 4 °C for 24 h. Later, the germinated seeds were fixed in ethanol/acetic acid (ratio 3:1) and kept in the darkness at 4 °C. The root tips were subsequently excised from the seeds and washed with distilled water, followed by maceration with HCl 0.5 N at 45 °C for 17 min. Immediately the root tips were washed in distilled water and placed on a microscope slide to which was added a drop of distilled water to titrate the tissue. The root tips were then squashed in a drop of 1% aceto-orcein for observation under the microscope.

Seven metaphase plates were observed for each cultivar and accession. Data obtained were: long arm/short arm ratio of the chromosomes following the modified method of Levan *et al.* (1964); and indices of the interchromosomal (coefficient of variation of chromosome length; CV_{CL}) and intrachromosomal asymmetry (mean centromeric asymmetry; M_{CA}) of the karyotypes (Peruzzi & Eroglu 2013). For measuring

dimensions of the chromosomes, the software Micromasure (Reeves 2001) was used. Statistical analyses were done with the software Past 3.26b (Hammer *et al.* 2001).

RESULTS AND DISCUSSION

UNIFORMITY OF CHROMOSOMAL FORMULA

As can be observed in the metaphase plates (Fig. 1), all samples of *L. culinaris* analyzed in this study showed $2n = 14$ chromosomes, as has been reported previously (Bhattacharjee 1953; Sinha & Acharia 1972; Balyan *et al.* 2002; Jha *et al.* 2015). The chromosomal formula was mostly $4m+3sm$ (Fig. 2) with no differences being detected between Chilean and Canadian cultivars and accessions, probably due to the frequent introduction of Canadian lentils to Chile. There were two exceptions: Collanco and Campo Lindo, which had $3m+4sm$. Chromosome 1 in these two samples can be defined as submetacentric (following Levan *et al.* 1964), although the standard deviation might permit one alternatively to consider it as metacentric. The important point is that these samples were effectively distinct from the others.

In studies in other regions of the world, uniformity of the chromosome formula of $4m+3sm$ has also been documented, e.g., in cultivars from Iran (Gaffarzadeh-Namazi *et al.* 2007) and also from India, but here with a slight difference of $4m+2sm+1st$ (Jha *et al.* 2015).

VARIATION IN NUMBER OF SECONDARY CONSTRICTIONS

Among the cultivars and accessions from Canada and Chile, there is variation in number of secondary constrictions, 4 to 5 from those in Canada and 4 to 6 from those in Chile (Fig. 3). In Chile this variation is due principally to the six secondary constrictions from Yumbel, which has a distinctive secondary subtelomeric constriction on chromosome 2 (Fig. 3). This constriction is related to the ribosomal gene 5S, which is positioned in a subtelomeric zone of chromosome 2 (Balyan *et al.* 2002) or 1 (Galasso *et al.* 2001). Three nuclear organizing regions (NORs) have been reported previously (Galasso *et al.* 2001; Balyan *et al.* 2002), one of 5S rDNA in a subtelomeric zone on chromosome 1 or 2, which correlates with the secondary subtelomeric constriction observed in Yumbel, and another for the 18S-5.8S-25S rDNA in an easily observable centromeric zone on chromosome 3 or 4 (Fig. 2). In addition, we also observed a secondary subcentromeric constriction on chromosome 6 that is related to the position of 5S rDNA on the same chromosome (also reported by Balyan *et al.* 2002 and Galasso *et al.* 2001). Another secondary constriction that has not been reported previously is a centromeric type found on chromosome 1 (Fig. 1). Given these variations in the

presence of secondary constrictions, it would be important in the future to obtain more information, especially from fluorescence in situ hybridization (FISH).

TOTAL LENGTH OF CHROMOSOMES (TLC)

A broader variation in total length of chromosomes (TLC) exists among Chilean cultivars and accessions than among those from Canada, with 150.86-234.28 μm in the former, and 176.11-190.92 μm in the latter (Fig. 4). Hualqui possesses TLC of 150.86 μm , the lowest among samples examined, and significantly differentiated from the highest TLCs in Yumbel and Collanco ($p = 0.04$ and $p = 5.5 \times 10^{-5}$, respectively) (Table 2). Collanco, with the largest TLC of 234.28 μm , is differentiated significantly from Ninhue, Los Ángeles, and Hualqui ($p = 3.1 \times 10^{-4}$, $p = 6.0 \times 10^{-3}$ and $p = 5.5 \times 10^{-5}$, respectively) (Table 2). Other studies have reported TLC for cultivars of *L. culinaris* in different countries that range from 28 to 121 μm (Sinha &

Acharia 1972; Naithani & Sarbhoy 1973; Manara & Manara 1979; Rehman & Altaf 1994; Gaffarzadeh-Namazi *et al.* 2007; Jha *et al.* 2015), revealing that this character is quite variable. The results obtained in the present study indicate TLC even higher than those reported previously.

INTERCHROMOSOMAL ASYMMETRY

Differences in the asymmetric karyotypes among Canadian and Chilean cultivars and accessions reveal clusters that are somewhat distinct from each other between the two regions (Fig. 5). Separating these data into cultivars and accessions, however, shows that only Hualqui (Fig. 6) is separated from all other samples. With exclusion of these data, the cultivars and accessions from Chile and Canada are completely overlapping. The interchromosomal asymmetry (CV_{cl}) of the Hualqui accession was an average 31.25, significantly differentiated from all the rest of the cultivars or accessions (Table 1).

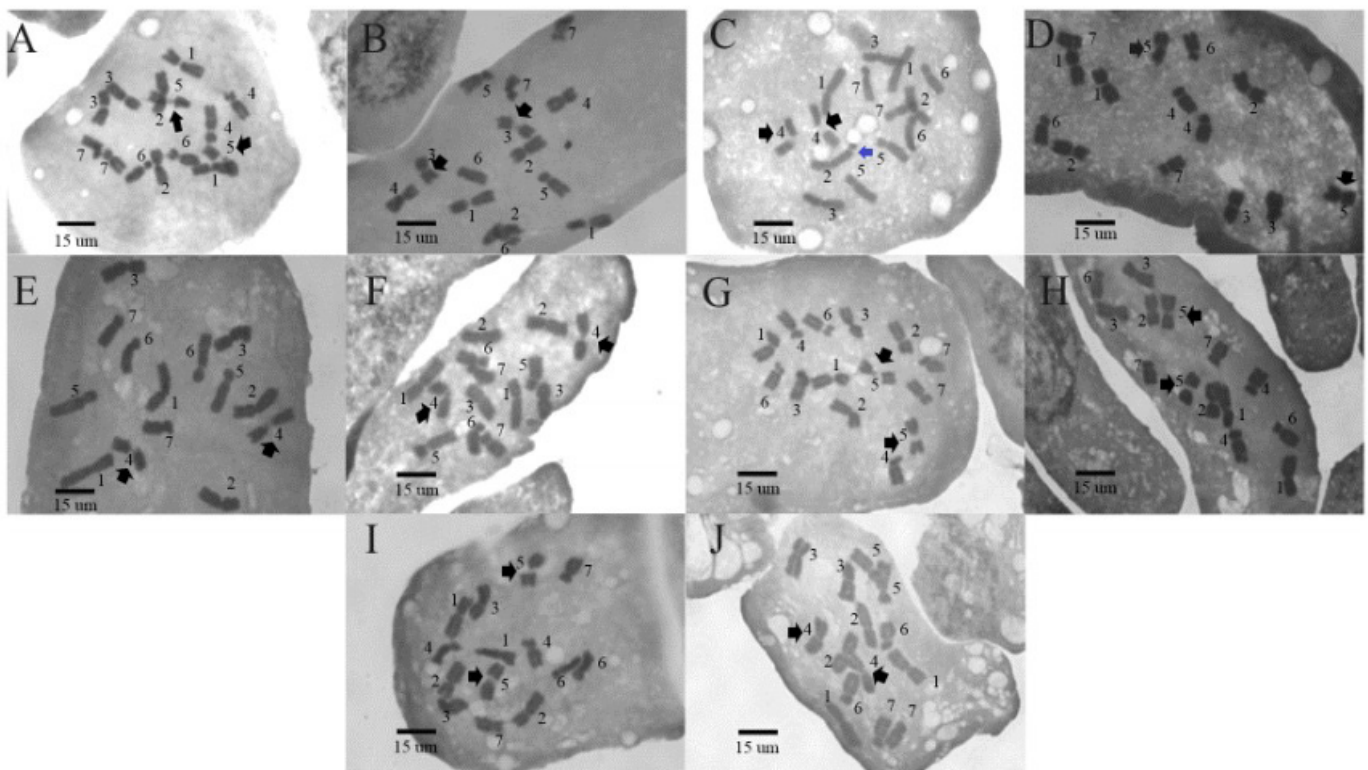


FIGURE 1. Metaphase plates from each cultivar and accession of *Lens culinaris*, with chromosomes numbered: Calpún-INIA (A), Super Araucana-INIA (B), Yumbel (C), Ninhue (D), Collanco (E), Hualqui (F), Los Ángeles (G), Campo Lindo (H), Líder (I), Martini (J). Black arrows indicate the most representative secondary constrictions. The blue arrow indicates the single subtelomeric secondary constriction. / Placas metafásicas de cada cultivar y procedencia de *Lens culinaris*, con cromosomas numerados: Calpún-INIA (A), Super Araucana-INIA (B), Yumbel (C), Ninhue (D), Collanco (E), Hualqui (F), Los Ángeles (G), Campo Lindo (H), Líder (I), Martini (J). Las flechas negras indican las constricciones secundarias más significativas. La flecha azul indica la única constricción secundaria subteloamérica.

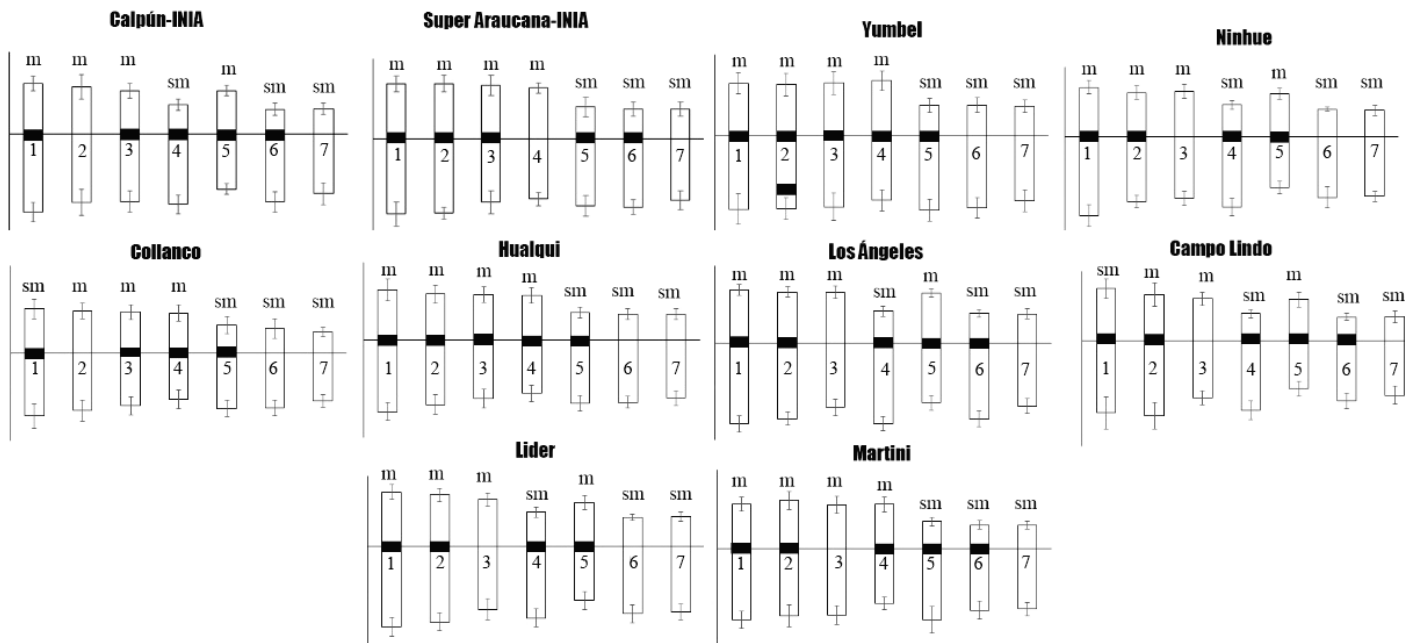


FIGURE 2. Idiograms of the cultivars and accessions of *Lens culinaris* evaluated in this study. The chromosomes are ordered according to their length; whiskers indicate standard deviations. The black areas represent secondary constrictions observed in metaphase plates. / Ideogramas de los cultivares y procedencias de *Lens culinaris* evaluados en este estudio. Los cromosomas están ordenados de acuerdo a su longitud, cada uno lleva su desviación estándar. Las áreas negras representan las constricciones secundarias observadas en las placas metafásicas.

TABLE 1. Tukey test for comparisons of the coefficient of variation of chromosome length (CV_{CL}) index of the Hualqui accession with all other cultivars and accessions. / Test de Tukey para la comparación del índice de coeficiente de variación de la longitud del cromosoma (CV_{CL}) de la procedencia de Hualqui con respecto a los demás cultivares y procedencias.

Comparisons with Hualqui	CV_{cl} -p value
Calpún-INIA	1.80×10^{-11}
Super Araucana-INIA	1.81×10^{-11}
Ninhue	1.80×10^{-11}
Collanco	1.81×10^{-11}
Yumbel	1.79×10^{-11}
Los Ángeles	1.79×10^{-11}
Campo Lindo	1.79×10^{-11}
Líder	1.80×10^{-11}
Martini	1.79×10^{-11}

TABLE 2. Significant differences in Dunn test comparisons for total chromosome length (TLC). All p-values are adjusted by Bonferroni correction. / Diferencias significativas en comparaciones del test de Dunn para la longitud total del cromosoma (TLC). Todos los valores p están ajustados a la corrección de Bonferroni.

Comparisons	TLC -p value
Hualqui - Yumbel	0.04307
Hualqui - Collanco	0.00006
Collanco - Los Ángeles	0.00602
Collanco - Ninhue	0.00031

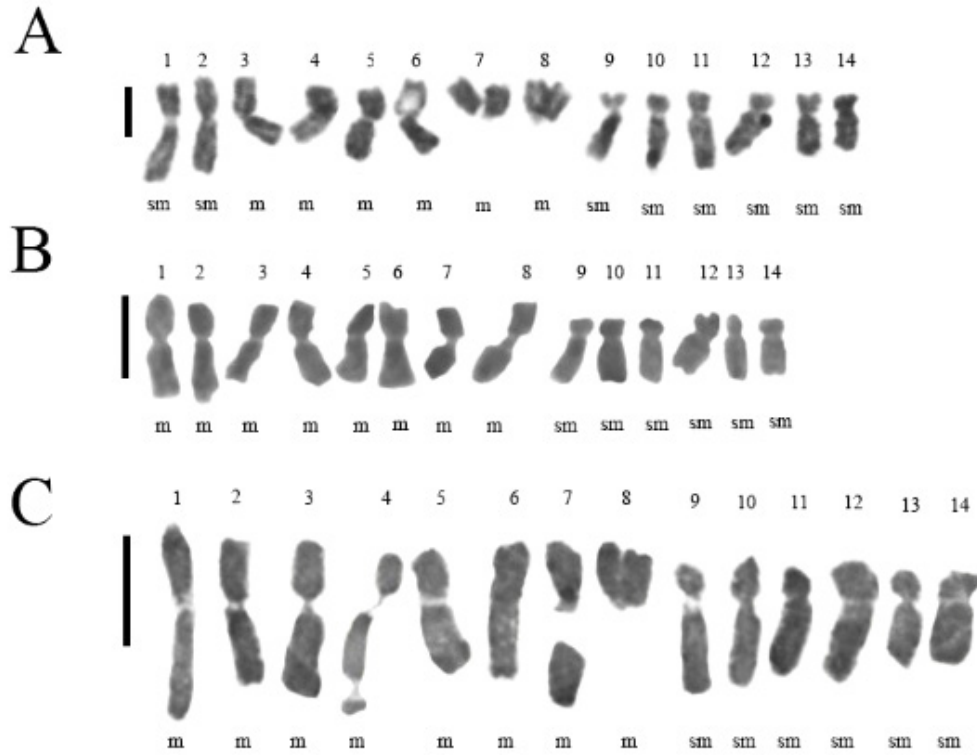


FIGURE 3. Karyotypes from the accessions of Collanco (A), Hualqui (B), and Yumbel (C). Scales = 10 μ m. / Cariotipos de las procedencias de Collanco (A), Hualqui (B), y Yumbel (C). Escala = 10 μ m.

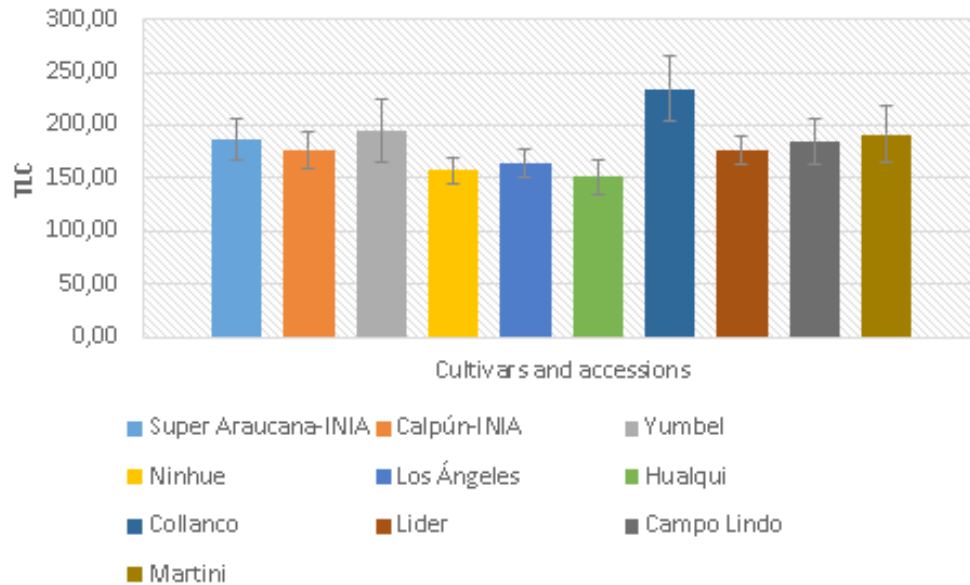


FIGURE 4. Total chromosome length (TLC) for each cultivar and accession evaluated in this study. / Longitud total del cromosoma (TLC) para cada cultivar y procedencia evaluada en este estudio.

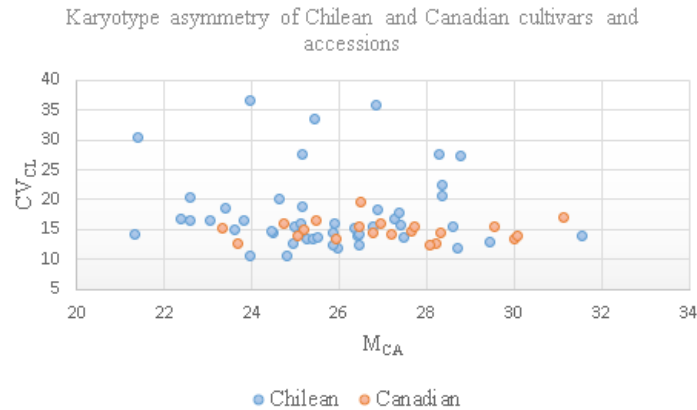


FIGURE 5. Karyotypic asymmetry of the coefficient of variation of chromosome length (CV_{CL}) and mean centromeric asymmetry (M_{CA}) for the Chilean and Canadian cultivars and accessions of *Lens culinaris*. / Asimetría cariotípica del coeficiente de variación de la longitud del cromosoma (CV_{CL}) y asimetría centromérica media (M_{CA}) para los cultivares y procedencias de *Lens culinaris* chilenos y canadienses.

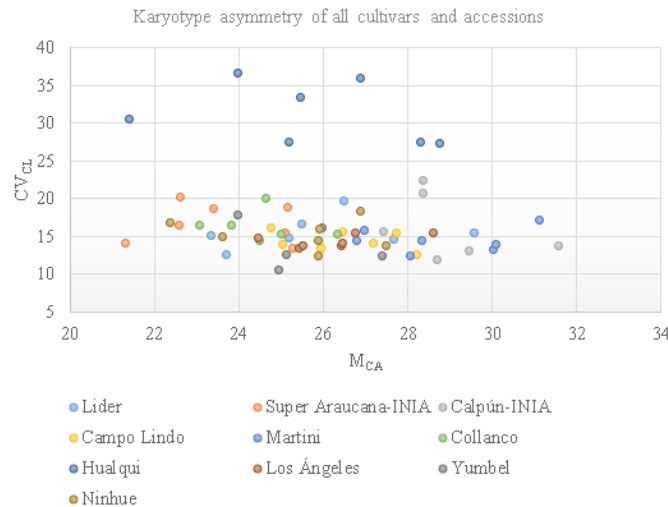


FIGURE 6. Asymmetric karyotype of the indices coefficient of variation of chromosome length (CV_{CL}) and mean centromeric asymmetry (M_{CA}) for each cultivar and accession of *Lens culinaris* evaluated in this study. The blue ellipse includes values from plants of Hualqui. / Asimetría cariotípica de los índices coeficiente de variación de la longitud del cromosoma (CV_{CL}) y asimetría centromérica media (M_{CA}) para cada procedencia y cultivar de *Lens culinaris* evaluado en este estudio. La elipse azul incluye valores de Hualqui.

Differences in intrachromosomal asymmetry (M_{CA}) also exist between the Chilean 'Calpún-INIA' and 'Super Araucana-INIA' (Fig. 6; Pino-Palma & Baeza-Perry 2019). One possible explanation for the interchromosomal asymmetric karyotype in Hualqui might be chromosomal rearrangements, possibly deletions, given that the lowest TLC occurs in this accession (Fig. 4). Vu *et al.* (2015) indicated that repair of a rupture of the DNA double helix can generate insertions or deletions, which can determine the size of the genome as consequence of the net value between insertions and deletions. Polymorphisms in some chromosomal bands have

been found in cultivars of *L. culinaris* from Iran (Gaffarzadeh-Namazi *et al.* 2007), which also might reflect processes such as chromosomal rearrangements, such as have been documented in cultivars of wheat (Schneider *et al.* 2003). In studies of other Chilean species, such as *Alstroemeria ligtu* L., differences in asymmetrical karyotypes have been suggested to correlate with environmental factors, such as type of soil, habitat, and distribution (Baeza *et al.* 2016). In addition, in two subspecies of *Alstroemeria presliana* Herb., karyotypic asymmetry appears to correlate with phenotypic variations in intensity of color of tepals and anthers (Baeza *et al.* 2015).

CONCLUSIONS

In this karyological study of *Lens culinaris*, we have shown the broadest variation of total length of chromosomes (TLC) to occur within Chile. We found some Chilean accessions with distinct cytotaxonomic characteristics, such as Hualqui with marked interchromosomal asymmetry. Yumbel possesses a secondary subtelomeric constriction on chromosome 2, and Collanco has the highest TLC. It is not known that the physiological implications might be from these variations, but further study and understanding of *L. culinaris* in Chile is certainly warranted. With further sampling, it might be possible to detect consistent cytotaxonomic signatures for each of these regions, distinct from those in Canada, which would permit identification of origin for any marketable product. The agronomic importance of this species in Chile recommends protection of these cytotaxonomic variants as a national genetic resource.

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