CHROMOSOMAL CHARACTERIZATION OF THREE NATIVE AND ONE CULTIVATED SPECIES OF Lathyrus L. IN SOUTHERN BRAZIL

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ABSTRACT

Mitotic metaphase chromosomes and interphase nuclei of nine populations of three South American species of Lathyrus (L. pubescens, L. nervosus and L. crassipes) and six populations of the cultivated species L. odoratus were analyzed. All populations had 2n = 2x = 14 chromosomes. There were significant differences among populations within each species and among species in the number of metacentric, submetacentric and subtelocentric chromosomes, the number and location of secondary constrictions, chromosome length (longest and shortest), total haploid complement, arm ratio, and centromeric index. L. odoratus showed the highest tendency towards karyotype symmetry whereas the three South American species showed a moderate tendency towards asymmetry, with L. pubescens being the most asymmetrical. Silver staining was used to identify the nucleolar organizer regions (NORs) and the number of nucleoli per interphase nucleus in each species. In L. pubescens and L. nervosus, the NORs were located on the secondary constriction of the long arm of pair 7, in L. crassipes, the NOR was proximal being located in the pair of metacentric chromosomes, and in L. odoratus there were four terminal NORs on the short arms of pairs 4 and 5. The four species had a maximum of four nucleoli per interphase nucleus, indicating the presence of four regions with active ribosomal genes in each case.

INTRODUCTION

The genus Lathyrus L. of the family Leguminosae consists of annual and perennial species, most of which are self-pollinating (Rees and Narayan, 1977; Narayan and McIntyre, 1989). According to Allkin et al. (1983), the genus comprises approximately 190 species and varieties, with centers of diversification in the Old and New Worlds. These are located in temperate zones. All species have 2n = 2x = 14 chromosomes, with the basic number n = x = 7. Although there is no variation in chromosome number, there are variations in chromosome size, in centromere location, and in the number, size and location of secondary constrictions (Sharma and Datta, 1959; Roy and Singh, 1967; Federov, 1969; Fouzard and Tandon, 1975; Broich, 1989; Battistin and Fernández, 1994), and in DNA content, involving euchromatin and heterochromatin, as well as repetitive and non-repetitive DNA sequences (Lavania and Sharma, 1980; Narayan and Durrant, 1983; Kuryan and Narayan, 1987; Murray et al., 1992). Another important variation observed in these species is in the number and location of nucleolar organizer regions (NORs) (Nazeer et al., 1982; Murray et al., 1992; Battistin and Fernández, 1993).

The objective of the present study was to compare the chromosomal characteristics within and between four species of Lathyrus.

MATERIAL AND METHODS

Fifteen populations of four species of Lathyrus L. were collected (Table I). Four mitotic metaphases were selected at random from plants of each population. Metaphases were obtained by the method of Battistin and Fernández (1994). The number of chromosomes, karyotype formula, mean chromosome length (µm), total length of the haploid complement, mean long/short arm ratio, and centromeric index were determined. These were compared among populations within each species by analysis of variance and by the t-test, and between species by the Tukey test (Steel and Torrie, 1960). The karyogram was mounted by arranging the chromosomes in pairs in decreasing order of size according to the method of Singh (1993). Chromosome nomenclature based on centromere location followed that proposed by Levan et al. (1964), i.e., metacentric (m), submetacentric (sm) and subtelocentric (st).

Eight metaphases and 300 interphase nuclei selected at random from four plants of each species were analyzed for NOR bands. The NORs and nucleoli were identified by AgNO₃ staining as described by Howell and Black (1980) for L. odoratus, and Herickoff et al. (1992) for the remaining species.

RESULTS AND DISCUSSION

The 15 populations of the four Lathyrus species studied were diploid, with 2n = 2x = 14 chromosomes (Table II). All species of Lathyrus from Europe, North America, Australia, New Zealand and South America, including polyploid and aneuploid individuals, have the same basic number x = 7 (Khawaja, 1988; Broich, 1989; Murray et al., 1992; Battistin and Fernández, 1994; Schifino-
A conserved chromosome number is a common phenomenon in the species of this genus. Populations 1 and 2 (Table II) of *L. pubescens* had similar karyotypes, with only submetacentric and subtelocentric chromosomes. However, they diverged in the position of secondary constrictions (Figure 1). In the karyotype of population 1, the secondary constriction was proximal on the short arm of pair 1, followed by a macrosatellite, whereas in population 2, the constriction was on the long arm of pair 7, followed by a macrosatellite. Populations 20 and 21 of *L. pubescens*, all populations of *L. nervosus* and population 13 of *L. crassipes* also showed similarities in their karyotypes, with metacentric, submetacentric and subtelocentric chromosomes, which differed only in number.

Table I - Details of the species, collection sites and number of plants studied in 15 populations of four species of *Lathyrus*.

| Species                      | Voucher No.* | Pop. No. | Origin                        | Number of plants |
|------------------------------|--------------|----------|-------------------------------|-----------------|
| *Lathyrus pubescens* Hook et Arn. | SMDB 3493    | 1        | Caçapava do Sul/RS - Brazil  | 5               |
|                              |              | 2        | Bage/RS - Brazil             | 4               |
|                              |              | 20       | São Gabriel/RS - Brazil      | 8               |
|                              |              | 21       | Lavras do Sul/RS - Brazil    | 7               |
| *Lathyrus nervosus* Lam.     | SMDB 3825    | 13       | Caçapava do Sul/RS - Brazil  | 4               |
|                              |              | 14       | Bage/RS - Brazil             | 6               |
|                              |              | 15       | Torres/RS - Brazil           | 4               |
| *Lathyrus crassipes* Hook et Arn. | SMDB 3813    | 11       | Caçapava do Sul/RS - Brazil  | 8               |
|                              |              | 13       | Santa Maria/RS - Brazil      | 10              |
| *Lathyrus odoratus* L.       | SMDB 4117    | 1        | ISLA SA, Lote 1293, Porto    | 5               |
|                              |              | 2        | ISLA SA, Lote 1635, Porto    | 5               |
|                              |              | 3        | Royal Fleur, Lote 76, France | 5               |
|                              |              | 4        | Royal Fleur, Lote 74, France | 5               |
|                              |              | 5        | Royal Fleur, Lote 73, France | 5               |
|                              |              | 6        | Feltrin, Lote 9014, Farroupilha/RS - Brazil | 5 |

* Specimens deposited in the Santa Maria Department of Biology (SMDB), Federal University of Santa Maria, Rio Grande do Sul, Brazil.

Table II - Chromosome number (2n), karyotype formula, total length of the longest chromosome (TLLC), total length of the shortest chromosome (TLSC), total haploid complement (THC), ratio long arm/short arm (LA/SA) and centromeric index (CI), in 10 populations (Pop.) of four *Lathyrus* species.

| Species/pop. | 2n | Karyotype | TLLC (µm) | TLSC (µm) | THC (µm) | LA/SA | CI |
|--------------|----|-----------|-----------|-----------|-----------|-------|----|
| *L. pubescens* |    |           |           |           |           |       |    |
| Pop. 1       | 14 | 10m+4st   | 12.45 ± 0.41 - 8.30 ± 1.15 | 34.95 ± 0.99 | 2.53 ± 0.26 | 29.53 ± 1.27 |
| Pop. 2       | 14 | 10m+4st   | 11.83 ± 1.26 - 8.33 ± 0.49 | 35.63 ± 3.20 | 2.48 ± 0.10 | 29.50 ± 0.91 |
| Pop. 20      | 14 | 2m+8sm+4st| 13.60 ± 0.89 - 9.23 ± 1.64 | 38.93 ± 1.42 | 2.53 ± 0.05 | 29.18 ± 0.29 |
| Pop. 21      | 14 | 2m+8sm+4st| 11.93 ± 1.15 - 7.93 ± 0.69 | 34.50 ± 1.75 | 2.58 ± 0.10 | 28.75 ± 0.45 |
| *L. nervosus* |    |           |           |           |           |       |    |
| Pop. 13      | 14 | 2m+10sm+2st| 12.48 ± 2.82 - 8.28 ± 0.90 | 38.50 ± 4.28 | 2.23 ± 0.05 | 31.65 ± 0.71 |
| Pop. 14      | 14 | 2m+10sm+2st| 10.10 ± 1.66 - 7.28 ± 0.81 | 31.55 ± 2.86 | 2.15 ± 0.13 | 32.38 ± 1.42 |
| Pop. 15      | 14 | 2m+10sm+2st| 10.85 ± 2.16 - 7.80 ± 0.96 | 32.85 ± 5.97 | 2.13 ± 0.10 | 32.85 ± 0.72 |
| *L. crassipes* |   |           |           |           |           |       |    |
| Pop. 11      | 14 | 6m+8sm    | 8.38 ± 1.41 - 6.45 ± 1.39 | 26.10 ± 4.88 | 2.25 ± 0.06 | 31.85 ± 0.86 |
| Pop. 13      | 14 | 4m+8sm+2st| 9.50 ± 1.19 - 7.10 ± 0.92 | 29.03 ± 3.14 | 2.03 ± 0.05 | 34.03 ± 0.66 |
| *L. odoratus* |    |           |           |           |           |       |    |
| Pop. 1       | 14 | 4m+10sm   | 9.28 ± 1.07 - 6.25 ± 0.25 | 27.58 ± 2.69 | 1.80 ± 0.14 | 37.10 ± 2.07 |
| Pop. 2       | 14 | 2m+12sm   | 8.80 ± 1.79 - 5.85 ± 0.44 | 25.70 ± 3.63 | 2.00 ± 0.12 | 34.53 ± 1.96 |
| Pop. 3       | 14 | 6m+8sm    | 12.00 ± 0.91 - 7.90 ± 0.70 | 34.08 ± 3.18 | 2.07 ± 0.26 | 34.05 ± 2.29 |
| Pop. 4       | 14 | 4m+10sm   | 11.00 ± 2.73 - 7.20 ± 1.19 | 32.03 ± 6.38 | 1.90 ± 0.12 | 34.58 ± 1.23 |
| Pop. 5       | 14 | 2m+12sm   | 9.83 ± 1.29 - 6.38 ± 1.02 | 27.15 ± 3.64 | 2.15 ± 0.13 | 33.40 ± 1.13 |
| Pop. 6       | 14 | 2m+12sm   | 11.55 ± 2.63 - 8.84 ± 2.16 | 36.13 ± 9.60 | 2.10 ± 0.14 | 33.93 ± 1.32 |

Lowercase letters indicate significant differences (P < 0.05, t-test). The results are shown as the mean ± SD when appropriate.
Study of chromosomes in *Lathyrus* L.

*Lathyrus pubescens*

A
B
C
D

*Lathyrus nervosus*

E
F
G

*Lathyrus crassipes*

H
I

Continued on next page
This finding suggests a more restricted degree of homology among these populations compared to the others. The similarity between the karyotypes of *L. pubescens* and *L. nervosus* was reported by Battistin and Fernández (1994). Another characteristic shared by the above populations was the location of secondary constrictions on the long arm of pair 7, followed by a macrosatellite. An exception was population 20 of *L. pubescens* which, in addition to the secondary constriction on pair 7, had a proximal secondary constriction on the short arm of pair 1, similar to population 1.

Population 11 of *L. crassipes* (Table II and Figure 1H) and all the populations of *L. odoratus* (Table II and Figure 1J-O) had metacentric and submetacentric chromosomes which differed in number. Two distal secondary constrictions were identified on the short arms of pair 4 in population 6 of *L. odoratus*. No secondary constrictions were seen in the remaining populations.

Although the material originated from different locations, the karyotypes of the *L. odoratus* populations maintained a stable composition of chromosome types (metacentric and submetacentric), in contrast to popula-
tions of the native species, L. pubescens, L. nervosus and L. crassipes. This difference may indicate that during evolution L. odoratus reached greater stability, with a higher symmetry present in its chromosomes than in the three South American species.

Another morphological trait that showed significant variation among populations within and among species was chromosome length (Tables II and III). The mean lengths of the largest chromosomes did not differ significantly among species (Table III); the highest value was found in L. pubescens (12.45 µm) and the lowest in L. crassipes (8.94 µm). There were only slight differences in the mean length of the smallest chromosome among the populations of L. odoratus. L. pubescens had the greatest mean length (8.44 µm), which was significantly different from that of L. crassipes (6.78 µm) and L. odoratus. For the total haploid length (µm), which reflects the size of the karyotype, there were significant differences among populations within each species, except for L. crassipes. The largest mean was that of L. pubescens (36 µm), which differed significantly from the smallest mean, found in L. crassipes (27.56 µm), and also from L. odoratus. The mean for L. nervosus differed significantly from that for L. crassipes. Variation in chromosome size is often the result of the amplification or deletion of a chromatin segment during species diversification. According to Rees and Narayan (1977), the within- and between-species variation in chromosome size in Lathyrus indicates marked differences in the amount of DNA affecting complement size; a high percentage of this DNA is moderately repetitive. Murray et al. (1992) indicated that at the molecular level the changes in the amount of DNA occurred over a very short time during formation of the species.

Comparative analyses of the arm ratios allowed us to characterize each karyotype. The populations of L. pubescens and L. nervosus showed no important variations, whereas the populations of L. crassipes and L. odoratus diverged in their means. The arm ratios varied (Table III), the highest value being found in L. pubescens and the lowest in L. odoratus.

The centromeric index (CI) was stable among the populations of L. pubescens and L. nervosus, whereas it differed significantly among populations of L. crassipes and L. odoratus. L. odoratus had the highest means for all populations when compared with the means for populations of the three South American species (Tables II and III). A similar relationship was observed when the means were compared among species (Table III). In the South American species, L. pubescens had the lowest means compared to L. nervosus and L. crassipes (Tables II and III), showing clearly that two opposite trends have occurred in karyotype symmetry within the genus.

The arm ratios and CI data revealed opposite trends in karyotype symmetry among the Lathyrus species studied. L. odoratus, with the highest mean CI and the lowest arm ratio, showed the greatest tendency towards karyotype symmetry, while L. pubescens, L. nervosus and L. crassipes tended towards karyotype asymmetry. L. pubescens was the most asymmetrical of these species and had the lowest CI and the highest arm ratio. Levan et al. (1964) described these karyotypes as having a moderately tendency towards asymmetry.

Based on the chromosomal traits studied here, the exotic species L. odoratus was the most symmetrical, a tendency towards more primitive species in this genus (Stebbins, 1971). On the other hand, karyotype asymmetry in the three South American species suggests that evolutionarily they are more recent species. Of these three species, L. crassipes is considered to be the most primitive and L. pubescens the most recent. Rees and Narayan (1977) and Murray et al. (1992) indicated that L. pubescens is outstanding because of its greater mean karyotype length, indicative of a greater chromatin gain in its chromosome complement over a shorter period than in the other three species.

NOR bands

Silver nitrate staining of mitotic metaphases (Figure 2) allowed the identification of NORs in the chromosomes of the four species examined. Population 20 of L. pubescens and population 13 of L. nervosus were studied. Both showed NORs on the secondary constrictions of the long arm of pair 7. The NOR of L. pubescens stained deeply (Figure 2a), whereas that of L. nervosus stained weakly (Figure 2c). In population 13 of L. crassipes, the NOR was located in the proximal region of metacentric pair 3 (Figure 2e). In population 6 of L. odoratus, four deeply stained terminal NORs were seen on the short arms of pairs 4 and 5 (Figure 2g). Nazeer et al. (1982) and Murray et al. (1992) also identified two chromosome pairs with terminal NORs in L. odoratus. These investigators also found three NORs in the secondary constrictions of L. sativus and a pair of chromosomes with AgNO₃-positive secondary constrictions in L. blepharicarpus, L. cassius and L. hirsutus. Not all secondary constrictions are NOR sites.

### Table III - Chromosome characteristics in the four species of Lathyrus studied.

| Species       | Chromosome length (µm) | TLLC | TLSC | THC   | LA/SA | CI    |
|---------------|------------------------|------|------|-------|-------|-------|
| L. pubescens  | 12.45µs                | 8.44µs |       | 36.00µs | 2.53µs | 29.24µs |
| L. nervosus   | 11.14µs                | 7.78µs | 34.23µs | 2.17µs | 32.29µs |
| L. crassipes  | 8.94µs                 | 6.78µs | 27.56µs | 2.14µs | 32.94µs |
| L. odoratus   | 10.41µs                | 7.08µs | 30.44µs | 2.00µs | 34.74µs |

Lowercase letters indicate significant differences at the 5% level (Tukey test). TLLC = Total length of the longest chromosome, TLSC = total length of the shortest chromosome, THC = total haploid complement, LA/AS = ratio long arm/short arm, CI = centromeric index.
but all NORs are located in secondary constrictions. Each species has a characteristic number and size, which indicated the quantity of active ribosomal genes. Silver nitrate staining also showed the maximum number of nucleoli in interphase cells. Each species had a maximum of four nucleoli per cell, indicating the existence of four regions with active ribosomal genes. The fact that only one NOR pair was observed in metaphases from *L. pubescens* (Figure 2a,b), *L. nervosus* (Figure 2c,d) and *L. crassipes* (Figure 2e,f) may reflect the presence of small NORs represented by two smaller nucleoli in each cell, which may have impeded the detection of NORs in metaphase chromosomes. In *L. odoratus*, the four interphase nucleoli reflected the four NOR bands in the metaphases (Figure 2g,h).
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RESUMO

Cromossomos em metáfases mitóticas e núcleos interfásicos em 9 populações de 3 espécies sul-americanas de Lathyrus (L. pubescens, L. nervosus e L. crassipes) e 6 populações da espécie cultivada L. odoratus foram analisados. Todas as populações apresentaram 2n = 2x = 14 cromossomos. As diferenças significativas observadas entre as populações dentro de cada espécie e entre as espécies foram: número de cromossomos metacêntricos, submetacêntricos e subteloceêntricos; número e localização das constritações secundárias; comprimento dos cromossomos (maior e menor); complemento total haplóide; razão braço longo/braço curto e índice centromérico. L. odoratus é a espécie com maior tendência simétrica em seu cariótipo, enquanto que nas outras espécies sul-americanas os cariótipos têm tendência moderada para assimetria, sendo L. pubescens o mais assimétrico. Com nitrato de prata foi possível identificar as NORs e o número máximo de quatro núcleos em cada núcleo interfásico, nas espécies L. odoratus, L. nervosus e L. crassipes. As quatro espécies apresentam uma constrição secundária do braço longo do par 7, em oridade de nucléolos por núcleo interfásico em cada espécie.

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