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### PHYSICAL MAPPING OF 18S-25S rDNA AND 5S rDNA IN *LUPINUS* VIA FLUORESCENT *IN SITU* HYBRIDIZATION

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**Abstract:** Double-target fluorescent *in situ* hybridization (FISH) was used to determine the genomic distribution of ribosomal RNA genes in five *Lupinus* species: *L. cosentinii* (2n=32), *L. pilosus* (2n=42), *L. angustifolius* (2n=40), *L. luteus* (2n=52) and *L. mutabilis* (2n=48). 18S-25S rDNA and 5S rDNA were used as probes. Some interspecific variation was observed in the number and size of the 18S-25S rDNA loci. All the studied species had one chromosome pair carrying 5S rDNA.

**Key Words:** *Lupinus*, 18S-25S rDNA, 5S rDNA, *In situ* hybridization, Physical Mapping.

#### INTRODUCTION

The *Lupinus* genus (*Fabaceae*) comprises Old and New World species, both wild forms and crops [1]. Lupins are considered to be of polyploid origin; however, their relationships and phylogeny are unclear. Their small-sized and similar chromosomes are difficult to karyotype by conventional cytology [2].

FISH is an efficient method of molecular cytogenetics for identifying the number and physical location of DNA repeated sequences in genomes [3]. FISH for rDNA was often used for the investigation of species difficult to karyotype, providing markers for morphologically indistinct chromosomes [4, 5]. It is a valuable tool in polyploid species analysis and in phylogenetic studies [6-8]. FISH mapping of rDNA was also applied to some *Fabaceae* [9, 10]. Recently, FISH was used to *Lupinus* chromosomes in order to examine the distribution of rDNA sites in *L. hispanicus*, *L. luteus* and their hybrid [11].

In this study, the 18S-25S rDNA and 5S rDNA loci were physically mapped by double-target FISH in five *Lupinus* species: the Old World wild, rough-seeded species *L. cosentinii* and *L. pilosus*, the Old World cultivated, smooth-seeded species *L. angustifolius* cv. Sonet and *L. luteus* cv. Topaz, and one New World

species *L. mutabilis*. The aim was to provide the chromosome landmarks in karyotyping and to get preliminary information on genome organization in the species representing different taxonomic groups of lupins.

## MATERIAL AND METHODS

Seeds of *L. cosentinii* (2n=32), *L. pilosus* (2n=42), *L. angustifolius* cv. Sonet (2n=40), *L. luteus* cv. Topaz (2n=52) and *L. mutabilis* (2n=48) were used. The chromosome preparation and *in situ* hybridization performed generally followed the methods of Schwarzacher *et al.* [3], applied to *Lupinus* [11], with some minor modifications.

Two DNA probes were used. The 18S-25S rDNA from *Arabidopsis thaliana* (in the SK+ plasmid) was labelled with digoxigenin using the Nick Translation System (Roche). The 5S rDNA from *Lupinus luteus* (Acc. No: Z93433) [12] was labelled with biotin using PCR.

DNA denaturation in 70% formamide was performed for 3 min at 76°C. Hybridization was carried out overnight, at 37°C. Probes were detected with 20 µg/ml anti-digoxigenin-FITC (Roche) and 10 µg/ml avidin-Rhodamine (Vector) in 2% BSA. Chromosomes were counterstained with DAPI. The slides were mounted in Vectashield antifade solution (Vector Laboratories). Observations were mainly made at different stages of mitotic metaphase but also during interphase (5 cells per species). The preparations were examined with the OLYMPUS BX 60 System Microscope. The images of DAPI, FITC and rhodamine fluorescence were acquired separately with a black and white CCD camera, interfaced to a PC running the analySIS 3.0 software (Soft Imaging System). Image processing consisted exclusively of signal intensity, contrast, and background adjustments.

## RESULTS AND DISCUSSION

The double-target FISH revealed one pair of strong, large-sized signals of 18S-25S rDNA on a long, subtelomeric SAT chromosome pair in all five *Lupinus* species (Fig. 1 A-E). The signals were extended, covering the major part of the chromosome, including secondary constriction. In *L. pilosus* and *L. angustifolius*, it was clearly visible that it corresponded with almost the whole longer arm. As a rule, 45S rDNA sequences are markers for the nucleolar organizer region (NOR), visible in mitosis as a secondary constriction. Thus, this locus is a cytogenetic marker for the NOR-bearing chromosome pair in *L. cosentinii*, *L. pilosus*, *L. angustifolius*, *L. luteus* and *L. mutabilis*. Its location is not surprising, but the size and intensity of the signal seem to be uncommon.

*L. cosentinii* was an exception, having two more pairs of 18S-25S rDNA loci: one of a medium and another of a small size (Fig. 1 A). All three sites in that species were located on different chromosomes.

Fig. 1. Mitotic metaphase plates of five *Lupinus* species after fluorescent *in situ* hybridization with rDNA probes. The chromosomes were counterstained with DAPI (blue colour), the probe for 18S-25S rDNA was detected by FITC (yellow-green signals) and the probe for 5S rDNA by rhodamine (red signals).  
**A:** *L. cosentinii* (2n=32), **B:** *L. pilosus* (2n=42), **C:** *L. angustifolius* cv. Sonet (2n=40),  
**D:** *L. luteus* cv. Topaz (2n=52), **E:** *L. mutabilis* (2n=48). Bar = 10  $\mu$ m.

In *L. pilosus* and *L. luteus*, the second pair of 18S-25S rDNA loci was sometimes visible as minor signals, more often in interphase nuclei. This concurs with preliminary results for *L. luteus* [11], where two loci of different intensity were observed. Some variation of the rDNA loci number in other species was previously reported [4, 6].

Only two sites (one locus) of 5S rDNA were found in the studied lupins (Fig. 1 A-E). The signals were located on a pair of medium-sized, submedian chromosomes, in different positions. *L. cosentinii* possessed a 5S rDNA locus on the long arm in the distal region, *L. pilosus* on the short arm, at the interstitial position. *L. angustifolius* showed 5S rDNA sites in the pericentromeric region of the short arm, *L. luteus* in the distal region of the short arm. In *L. mutabilis*, the 5S rDNA site was visible as an interstitial signal, presumably on the long chromosome arm.

The detected rDNA sequences, all located on different chromosomes, permitted the unambiguous identification of chromosome pairs bearing these loci. However, they marked only two to four pairs (depending on the species) among numerous chromosomes. The rDNA distribution was similar in *L. pilosus*, *L. angustifolius*, *L. luteus* and *L. mutabilis*, despite the variation in their chromosome number. *L. cosentinii*, the species with the lowest chromosome number ( $2n=32$ ), had the highest number of 18S-25S rDNA loci.

The genus *Lupinus* is supposed to be of polyploid origin, but its ploidy level is unclear and there are several different basic chromosome numbers within the genus [13, 14]. Lupin polyploidy is believed to be ancient. The low loci numbers of both rDNA types found in *Lupinus* seem to contradict the hypothesis of the polyploid origin. In some genera, rDNA loci numbers reflect the process of polyploidization [15]. However, other results indicate that rDNA genes may not be direct markers of polyploid origin. Possibly, some rDNA sites could have been lost during evolution [16]. On the other hand, it can be presumed that some rDNA loci could have been translocated and fused with other rDNA sequences, as suggested for *Brassica* [17]. Such a hypothesis would explain the large size of the rDNA blocks observed in the studied lupins. The rate of chromosomal changes and of their fixation in the genome during the diploidization process of polyploids seems to be high [8]. Thus even numerous, successive chromosomal rearrangements could occur in the long history of lupins, resulting in the creation of contemporary forms – cytogenetically stable, functional diploids.

The *Lupinus* species analyzed in this study belong to three distinct taxonomic groups. The observed distribution of rDNA loci does not reflect the divisions among these groups. However, considering the hypothesis that the genus *Lupinus* developed from the Old World wild, rough-seeded forms [18], it is interesting that two species of that group – *L. cosentinii* and *L. pilosus* – differ markedly in their rDNA loci number.

The obtained results provide a framework for the future comparative mapping of other repetitive DNA sequences and large DNA fragments in *Lupinus*.

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## REFERENCES

1. Gladstones, J.S. (1998) Distribution, origin, taxonomy, history and importance. In: **Lupins as Crop Plants: Biology, Production and Utilization**. (Gladstones, J.S., Atkins, C.A. and Hamblin, J. Eds). CAB International, 1998, 1-37.
2. Naganowska, B. and Ładoń, D. Chromosomes of *Lupinus hispanicus* subsp. *hispanicus* Boiss. et Reut., *L. luteus* L. and their hybrids. **J. Appl. Genet.** 41 (2000) 167-170.
3. Schwarzacher, T. and Leitch, A.R., Heslop-Harrison J.S. DNA:DNA *in situ* hybridization – methods for light microscopy. **Plant Cell Biology: A Practical Approach**. (Harris, N., Oparka, K.J. Eds.) Oxford, Oxford University Press (1994) 1-20.
4. Armstrong, S., Frasz, P., Marshall, D.F. and Jones, G.H. Physical mapping of DNA repetitive sequences to mitotic and meiotic chromosomes of *Brassica oleracea* var. *alboglabra* by fluorescence *in situ* hybridization. **Heredity** 81 (1998) 666-673.
5. Hasterok, R., Jenkins, G., Langdon, T., Jones, R.N. and Maluszynska, J. Ribosomal DNA is an effective marker of *Brassica* chromosomes. **Theor. Appl. Genet.** 103 (2001) 486-490.
6. Hasterok, R. and Maluszynska, J. Nucleolar dominance does not occur in root tip cells of allotetraploid *Brassica* species. **Genome** 43 (2000) 574-579.
7. Sang, Y. and Liang, G.H. Comparative physical mapping of the 18S-5.8S-26S rDNA in three sorghum species. **Genome** 43 (2000) 918-922.
8. Weiss, H. and Maluszynska, J. Chromosomal rearrangement in autotetraploid plants of *Arabidopsis thaliana*. **Hereditas** 133 (2000) 255-261.
9. Ali, H.B.M., Meister, A. and Schubert, I. DNA content, rDNA loci, and DAPI bands reflect the phylogenetic distance between *Lathyrus* species. **Genome** 43 (2000) 1027-1032.
10. Raina, S.N. and Mukai, Y. Detection of a variable number of 18S-5.8S-26S and 5S ribosomal DNA loci by fluorescent *in situ* hybridization in diploid and tetraploid *Arachis* species. **Genome** 42 (1999) 52-59.
11. Naganowska, B., Doležel, J. and Świącicki, W.K. Development of molecular cytogenetics and physical mapping of ribosomal RNA genes in *Lupinus*. **Biologia Plantarum**, in press.
12. Nuc, K.T., Nuc, P.W. and Pawełkiewicz, J. The nucleotide sequence and organization of nuclear 5S rRNA genes in Yellow Lupine. **Bull. Pol. Acad. Sci. Chem.** 41 (1993) 103-106.

13. Atkins, C.A., Smith, P.M.C., Gupta, S., Jones, M.G.K. and Caligari P.D.S. Genetics, Cytology and Biotechnology. In: **Lupins as Crop Plants: Biology, Production and Utilization**. (Gladstones, J.S., Atkins, C.A. and Hamblin, J. Eds.). CAB International, 1998, 67-92.
14. Wolko, B. and Weeden, N.F. Estimation of *Lupinus* genome polyploidy on the basis of isozymic loci number. **Genet. Pol.** 30 (1989) 165-171.
15. Calderini, O., Pupilli, F., Cluster, P.D., Mariani, A. and Arcioni, S. Cytological studies of the nucleolus organizing regions in the *Medicago* complex: *sativa-coerulea-falcata*. **Genome** 39 (1996) 914-920.
16. Thomas, H.M., Harper, J.A., Meredith, M.R., Morgan, W.G. and King, I.P. Physical mapping of ribosomal DNA sites in *Festuca arundinacea* and related species by in situ hybridization. **Genome** 40 (1997) 406-410.
17. Snowdon, R.J., Köhler, W. and Köhler, A. Chromosomal localization and characterization of rDNA loci in the *Brassica* A and C genomes. **Genome** 40 (1997) 582-587.
18. Käss, E. and Wink, M. Molecular phylogeny and phylogeography of *Lupinus* (*Leguminosae*) inferred from nucleotide sequences of the *rbcL* gene and ITS 1+2 regions of rDNA. **Plant Syst. Evol.** 208 (1997) 139-167.