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Short communication

Chromosomes of *Lupinus hispanicus* subsp. *hispanicus* Boiss. et Reut., *L. luteus* L. and their hybrids

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Abstract. Cytological observations of mitotic chromosomes were performed for two species and interspecific hybrid lines: *Lupinus hispanicus* subsp. *hispanicus* (line Badajoz 2, cat. no. Wt 96383), *L. luteus* cv. Topaz (cat. no. Wt 98006), *L.* × *hispanicoluteus* (cat. no. Wt 98301 and Wt 98302). It was found that *L. hispanicus*, *L. luteus* and *L.* × *hispanicoluteus* have the same chromosome number 2n = 52. Chromosome morphology is, generally, similar. Centromeres are located mostly in median or submedian region. Chromosome differentiation appears to be very poor, except the first (the longest) pair which is easily discernible in both parental species and in the hybrid.

Key words: cytological analysis, *Lupinus hispanicus*, *L. luteus*, *L.* × *hispanicoluteus*.

Lupinus luteus and *L. hispanicus* form a complex which is one of four distinct cyto-taxonomic groups of the smooth-seeded Old World lupins. It appears to be evolving still. Specific distinction between the two taxa is based on genetic and morphological evidence, although they are closely related and have the same chromosome number 2n = 52 (GLADSTONES 1974, 1998).

From the agronomic point of view *L. luteus* – perhaps the most common lupin in cultivation – has many valuable traits, but some characters of wild *L. hispanicus* could be used for its improvement. Interspecific hybrids of these two species could be interesting as a source of variation to generate new cultivars. However, there are only few reports on interspecific crossings or the production of hybrids between *Lupinus* species. ŚWIĘCICKI et al. (1999) successfully performed experi-

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mental crossing of *L. hispanicus* subsp. *hispanicus* Boiss. et Reut. × *L. luteus* L. They obtained a synthetic, stable hybrid for which the name *Lupinus* × *hispanicoluteus* was proposed.

Cytogenetic studies provide a basis for explaining evolutionary relationships between the members of the genus and contribute to breeding programmes by resolving problems associated with interspecific hybridization. Cytological studies in *Lupinus* species are not as advanced as in many other genera, probably because of difficulties in preparing high quality chromosome spreads, problems with staining and the very small chromosome size. In most cases only chromosome numbers could be determined. For *L. luteus* and *L. hispanicus* chromosomes have been counted, then preliminary mitosis observations and some meiosis studies in *L. luteus* have been conducted (GLADSTONES 1974, KAZIMIERSKI, KAZIMIERSKA 1975, PAZY et al. 1977, ATKINS et al. 1998).

In the present study cytological observations of mitotic chromosomes were performed for two species and two interspecific hybrids: *L. hispanicus* subsp. *hispanicus*, line Badajoz 2 (Wt 96383); *L. luteus* cv. Topaz (Wt 98066); *L.* × *hispanicoluteus* – plant 1 (catalogue number Wt 98301); *L.* × *hispanicoluteus* – plant 2 (catalogue number Wt 98302).

Root tips from seedlings growing in Petri dishes were collected in ice water and refrigerated for 24 h. Then they were fixed in a mixture of absolute ethanol and glacial acetic acid (3 : 1) for 2-3 h at room temperature and for 24 h at 4°C (or refrigerated until used). Next the root tips were hydrolysed in 1 N HCl at 60°C for 5 min, stained in a mixture of haematoxylin and ferric ammonium sulphate (in propionic acid) and squashed.

Metaphase plates were photographed under a Nikon Optiphot-2 microscope on Fuji Superia 100 film. The photographs were scanned and the chromosomes were measured by MultiScan software.

L. hispanicus, *L. luteus* and *L.* × *hispanicoluteus* have the same chromosome number 2n = 52. Chromosome morphology is generally similar (Figures 1a-d). Centromeres are located mostly in median or submedian regions but even with the use of software it was not possible to measure individual chromosome arms because of their small size. No secondary constrictions have been noticed. The absolute length of chromosomes (in μm) and their relative lengths (in % of haploid chromosome set, making comparison of species possible) are given in Table 1.

The chromosome length values measured show that chromosome differentiation in all forms analysed is very poor – except the first (the longest) pair, which is easily discernible in both parental species as well as in hybrids. It is interesting that the size of that pair in *L.* × *hispanicoluteus* is not intermediate between parental ones but closer to *L. hispanicus*. The same was noticed in a study of genome size in these *Lupinus* forms (OBERMAYER et al. 1999). Probably the longest pair – making a considerable part of the whole chromosome set – reflects also DNA content differences.

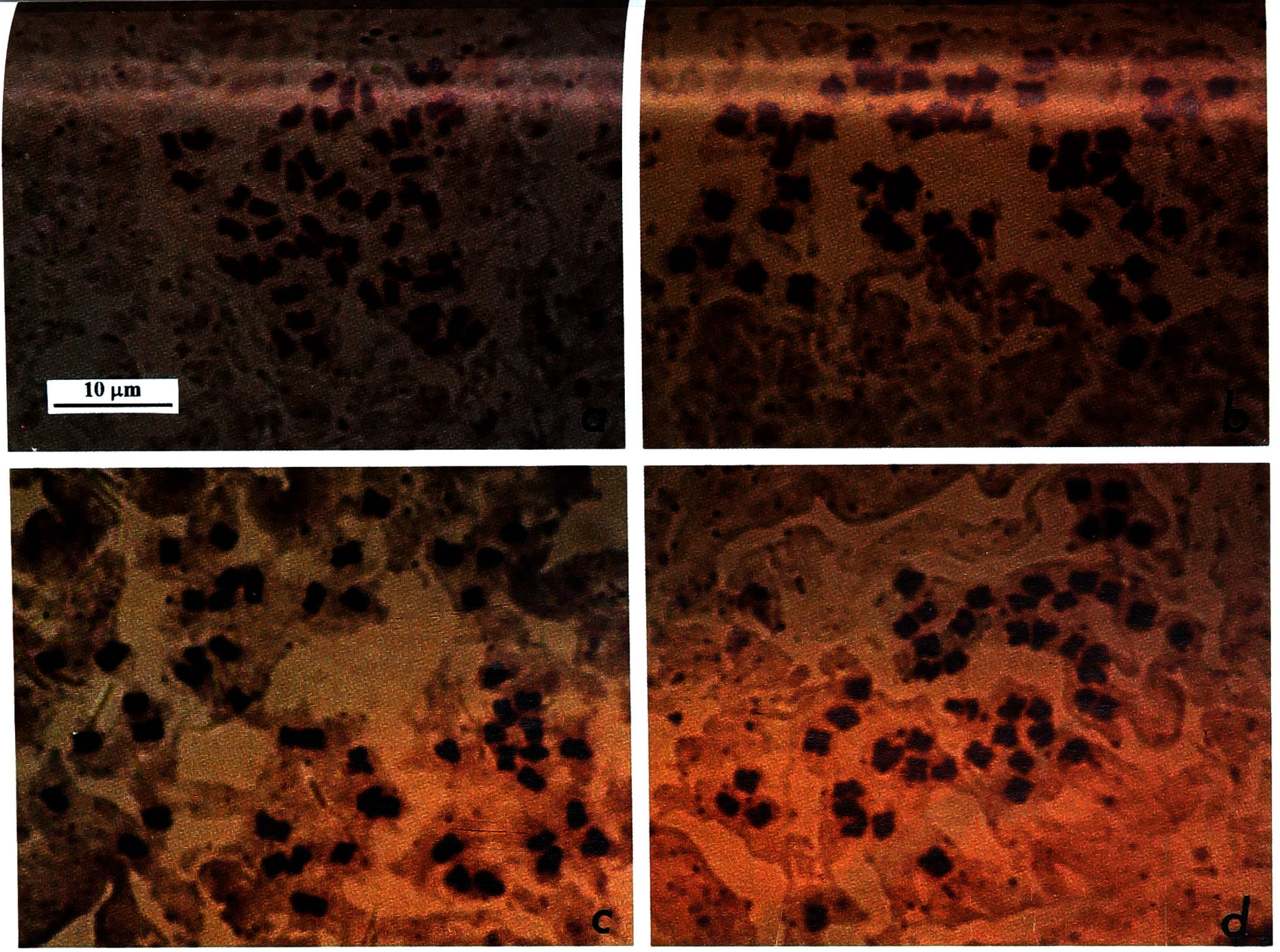

Figure. 1. Metaphase plates: a) *L. hispanicus* (Wt 96383); b) *L. luteus* (Wt 98066); c) *L.* × *hispanicoluteus* plant 1 (Wt 98301); d) *L.* × *hispanicoluteus* plant 2 (Wt 98302)

Table 1. Absolute and relative length of chromosomes of *L. hispanicus*, *L. luteus* and *L.* × *hispanicoluteus*

No. of air	*L. hispanicus* (Wt 96383)		*L. luteus* (Wt 98066)		*L.* × *hispanicoluteus* (Wt 98301)		*L.* × *hispanicoluteus* (Wt 98302)	
	µm	%	µm	%	µm	%	µm	%
1	3.64	6.2	3.92	6.7	3.07	5.7	2.35	5.7
2	2.96	5.1	2.95	5.0	2.75	5.1	1.97	4.7
3	2.78	4.8	2.78	4.7	2.59	4.8	1.95	4.7
4	2.73	4.7	2.63	4.5	2.47	4.6	1.93	4.6
5	2.62	4.5	2.59	4.4	2.43	4.5	1.90	4.6
6	2.61	4.4	2.50	4.3	2.41	4.5	1.89	4.5
7	2.55	4.4	2.46	4.2	2.37	4.4	1.85	4.5
8	2.52	4.3	2.44	4.2	2.31	4.3	1.77	4.2
9	2.46	4.2	2.40	4.1	2.24	4.2	1.70	4.1
10	2.31	4.0	2.37	4.0	2.13	4.0	1.66	4.0
11	2.30	3.9	2.31	3.9	2.10	3.9	1.64	3.9
12	2.28	3.9	2.27	3.9	2.08	3.9	1.59	3.8
13	2.26	3.9	2.24	3.8	2.07	3.9	1.56	3.8
14	2.20	3.7	2.22	3.8	2.04	3.8	1.56	3.8
15	2.17	3.7	2.18	3.7	1.98	3.7	1.55	3.7
16	2.13	3.6	2.12	3.6	1.94	3.6	1.54	3.7
17	2.08	3.6	2.05	3.5	1.88	3.5	1.51	3.6
18	2.04	3.5	2.00	3.4	1.83	3.4	1.44	3.5
19	2.03	3.5	1.98	3.4	1.78	3.3	1.40	3.4
20	1.92	3.3	1.96	3.3	1.76	3.3	1.38	3.3
21	1.89	3.2	1.91	3.3	1.72	3.2	1.36	3.3
22	1.82	3.1	1.89	3.2	1.69	3.2	1.34	3.2
23	1.78	3.0	1.84	3.1	1.65	3.1	1.31	3.1
24	1.63	2.8	1.68	2.9	1.58	2.9	1.24	3.0
25	1.47	2.5	1.57	2.7	1.46	2.7	1.18	2.8
26	1.39	2.4	1.40	2.4	1.33	2.5	1.00	2.4

It is clear that neither identification of all chromosomes nor species differentiation is possible on the basis of chromosome measuring in the analysed *Lupinus* forms. Further analysis of chromosomes in this genus should be performed with the application of molecular cytogenetic techniques like fluorescence in situ hybridization (FISH). Together with molecular mapping they could contribute to a comparison of genomes, understanding the evolution of the genus, and genetic improvement of lupins for agriculture.

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