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Karyotype analysis of *Placea amoena* Phil. (Amaryllidaceae) by double fluorescence *in situ* hybridization

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Abstract — *Placea* Miers ex Lindley is an endemic genus of Chile. There are between 3 and 6 species cited for Chile. *P. amoena* Phil., is a beautiful plant with red tepals and it is growing in the Province of Illapel in Región IV. We identify the physical localization of gene clusters of 5S and 25S rDNA in *Placea amoena* by double fluorescence *in situ* hybridization (FISH). *P. amoena* is 2n = 16, with the chromosome set 2m + 5sm + 1st. The ratio of the longest pair/shortest pair (R) is 2.60 with a karyotype asymmetry index (AsI %) of 63.15. Signals of 5S and 18/25S rRNA genes are seen in 5 of the 8 chromosomal pairs. Chromosome 1 has a 5S rDNA location terminal in the short arm; chromosome 2 contains the same localization, but in a position nearer the centromere; chromosome 3 shows no rDNA localizations; chromosome 4 has two signals, both in the terminal part of the short arm, the 18/25S rDNA at the tip of the arm and 5S rDNA inmediately below it; chromosome 5 has only one 18/25S rDNA signal terminal portion of the short arm; chromosomes 6 and 7 show no localizations; chromosome 8 contains one 18/25S rDNA signal distal in the short arm. The homologues of the long arm of chromosome pair 2 was polymorphic in the length. It would be of interest to localize 5S and 18/25S rRNA in the other species of *Placea* through *in situ* hybridization to help clarify the taxonomic position of each taxa.

Key words: Chile, FISH, 5S and 18S/25S rDNA genes, Karyotype, *Placea amoena*.

INTRODUCTION

Placea Miers ex Lindley is an endemic genus of Chile, belonging to Amaryllidaceae (Kubitzki 1998). The plants are without odor, and they have a tunicated bulb, umbel (flowering in the autumn) a 3-12-flowered, with white, yellow or yellow-orange petals that are striped and veined with red or purple (rarely with purplish dots, or vivid red-purple), and with a 3 or 6-lobed paraperigone (Traub and Moldenke 1949). Its distribution is from Regions IV to VII, including the federal district (Munoz 2000). The number of species of *Placea* cited for Chile vary between 3 and 6 (Traub and Moldenke 1949; Marticorena and Quezada 1985; Kubitzki 1998; Arriagada and Zöllner 1996; Munoz 2000). The only karyotypic information on *Placea* was given by Naranjo (1985), in which he showed 2n = 16 in *Placea arzae*. Another species, *Placea amoena,* grows in the Province of Illapel in Region IV of Chile. It is a beautiful plant with red tepals that conveys upon it high horticultural potential.

Knowledge of the relative physical locations and the number of multicopy rDNA gene loci is very important and useful for the construction of physical maps of chromosomes and for phylogenetic studies (Linares *et al.* 1996; Schrader *et al.* 1997; Galasso *et al.* 1997; Fukui *et al.* 1998; Chen *et al.* 1999; Moscone *et al.* 1999; Heslop-Harrison 2000). Fluorescence *in situ* hybridization (FISH) has been widely used for cytotaxonomical studies within different plant groups (e.g., Guerra *et al.* 1996; Zhang and Sang 1998; Lee *et al.* 1999; Adams *et al.* 2000; Weiss *et al.* 2003). It is also a powerful tool for molecular cytogenetic studies (Fransz *et al.* 1996, 1998; Fukui *et al.* 1998; Jiang and Gill 1994; Kubis *et al.* 1998; Weiss *et al.* 1999; Schrader *et al.* 2000, 2002). This method allows hybridization of known labelled marker sequences or genes preferentially as tandem repeats to homologous chromosomal targets (reviewed by Schwarzacher and Heslop-Harrison 2000). The most common markers are ribosomal genes (5S and 18S/25S rDNA), because they are abundant and highly conserved in all species of higher plants (Schmidt and Heslop-Harrison 1998). In the present paper we identify the physical localization of gene clusters of 5S and 25S rDNA in *Placea amoena* by double FISH.

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MATERIALS AND METHODS

Plant material, chromosome preparation and measurements - Roots (1 to 2 cm long) from seedlings of *Placea amoena* (Chile, Region IV, Illapel, Cuesta El Espino, 2001, KEW-INIA 013) grown on water-moistened filter paper at 24°C were excised and pre-treated with 8-hydroxyquinoline solution (2 mM aqueous solution at 4°C for 24 h), fixed in a freshly prepared mixture of absolute ethanol/glacial acetic acid (3:1) for 24 h and stored in 70% ethanol at –20°C. Before maceration, the root tips were washed 3 times in distilled water for 30 min and then digested with an enzyme mixture of 4% cellulase 'Onozuka R-10' (Serva) and 1% pectolyase Y-23 (Seishin Pharmaceutical) in 75 mM KCl, pH 4.0 for 40 min at 37°C. After a short rinse in distilled water, the root tips were softened in 45% acetic acid for 1 min and squashed. The cover slips were removed after freezing of the slides at –84°C and than air-dried for 1-3 days and stored at –20°C. Chromosomes were measured with the computer-aided program 'MicroMeasure 3.3' (Reeves 2001) and classified according to their arm ratios (long/short; modified after Levan *et al*. 1964) designated by the position of the centromere: 1.0 - 1.7 (metacentric; m), 1.7 - 3.0 (submetacentric; sm), and 3.0 - 7.0 (subtelocentric; st). The karyotype asymmetry index (AsI %) was calculated using the formula described by Arano and Saito (1980). The total genomic lengths of the measured 10 metaphases ranged in diploid chromosome sets between 143 and 238 µm. For a better comparison, each chromosome length was calculated in percent of total genomic length of the corresponding diploid chromosome set. The classified lengths of homologous chromosomes and their respective arms were combined to mean lengths of each of the 8 chromosome types per haploid set and finally over all 10 metaphases including the standard deviation (±) of chromosome arms. Positions of fluorescence signals were calculated in chromosome arms with their relative distance to the centromere between 0 and 100 %.

Preparation of DNA probes and fluorescence in situ hybridization - The 5S rRNA gene-specific probe was amplified and simultaneously labelled with digoxigenin-11-dUTP (Roche Diagnostics) from genomic DNA of *Allium ampeloprasum* L. via PCR using primers that were specific for 5S RNA genes (Gottlob-McHugh *et al*. 1990). The 18/25S rRNA gene-specific plasmid-DNA of the probe VER 17 (Yakura and Tanifuji 1983; kindly provided by Professor S. Tanifuji) was labelled with biotin-16-dUTP using a nick translation mix (Roche Diagnostics) according to the manufacturer's instructions.

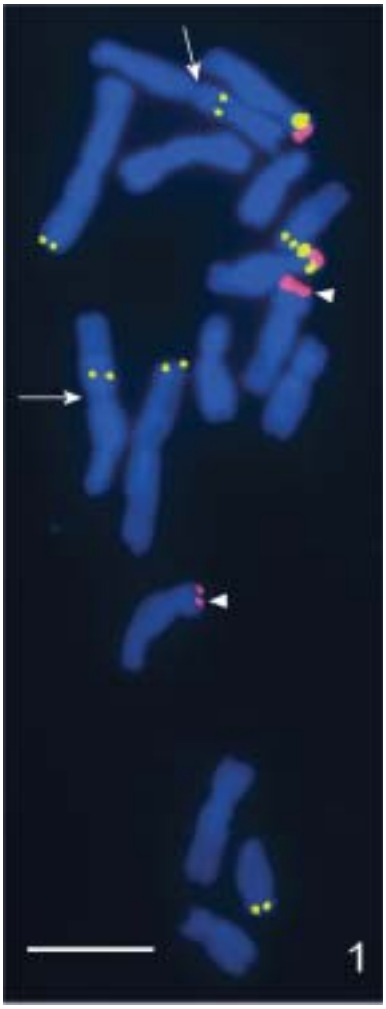
The FISH procedure was performed according to Schrader *et al*. (2000) with little modifications. For double hybridizations biotin was detected with 6 ng/µl of streptavidin-Cy3 (Dianova) and digoxigenin with 6 ng/µl of anti-digoxigenin-FITC (Roche Diagnostics). After washing of the slides three times in 4xSSC with 0.1 % Tween 20 at 42°C the FITC signals were enhanced by 6 ng/µl of anti-sheep-fluorescein (Dianova). Chromosomes were counterstained with 1.0 ng/µl of 4',6-diamidino-2-phenylindole (DAPI; Molecular Probes) for 5 min at 23°C. Photographs were taken using a computer-assisted cooled CCD camera (Visitron Systems). Pseudocoloration and mergence of images were done with the camera software.

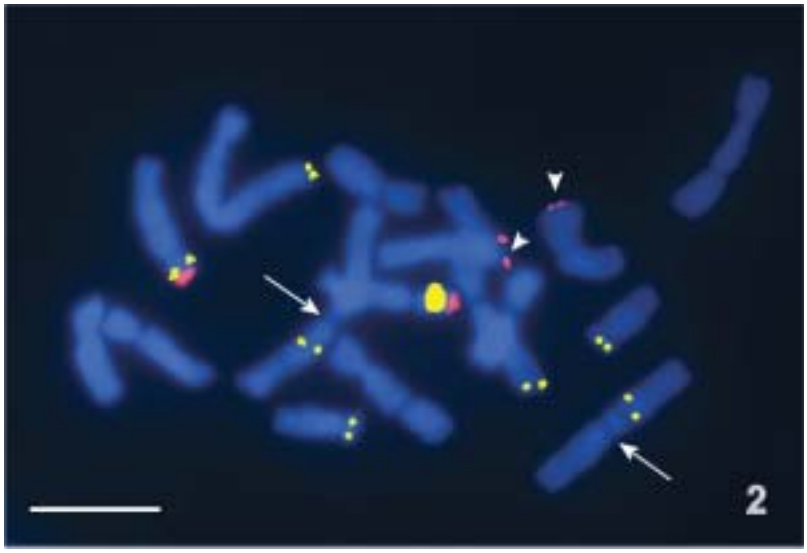
RESULTS AND DISCUSSION

The results of chromosome measurements can be summarized in the table 1 and Fig. 3. *Placea amoena* Phil., is 2n = 16, with the chromosome set 2m + 5sm + 1st, i.e., 2 metacentric pairs, 5 submetacentric pairs, and 1 subtelocentric pair. The ratio of the longest pair/shortest pair (R) is 2.60 with a karyotype asymmetry index (AsI %) of 63.15. Signals of 5S and 18/25S rDNA are seen in 5 of the 8

Table 1 — Average length of chromosomes of *Placea amoena* Phil., calculated in percent of the mean haploid genome length of 10 metaphases (177,77 µm). The position of fluorescence signals * 5S rDNA and ** 18S/25S rDNA are designated by asterisks of calculated values relative to the centromere (%).

Chromosome pair	Long arm (%) ± S.D.	Short arm (%) ± S.D.	Relative length (%)	Total length (µm)	Arm ratio (L/S)	Centromeric index	Chromosome type
1	5.15 ± 0.28	4.69 ± 0.17 *94.9 ± 1.60	9.84	17.49	1.1	0.48	m
2	5.00 ± 0.40	4.19 ± 0.20 *31.5 ± 5.80	9.19	16.34	1.19	0.46	m
3	4.36 ± 0.17	2.30 ± 0.17	6.66	11.84	1.9	0.35	sm
4	4.14 ± 0.26	1.59 ± 0.14 *55.2 ± 9.30 **87.8 ± 2.10	5.73	10.19	2.6	0.28	sm
5	3.84 ± 0.20	1.68 ± 0.10 **89.5 ± 2.90	5.52	9.81	2.29	0.31	sm
6	3.29 ± 0.24	1.61 ± 0.14	4.9	8.71	2.04	0.33	sm
7	2.88 ± 0.14	1.49 ± 0.14	4.37	7.77	1.93	0.34	sm
8	2.92 ± 0.20	0.88 ± 0.00 *63.0 ± 10.0	3.8	6.76	3.32	0.23	st





Figs. 1 and 2 — DAPI-stained metaphase chromosomes (white) of *Placea amoena* Phil., after fluorescence *in situ* hybridization with probes specific for 5S (yellow) and 18S/25S (red) rRNA genes. Arrows indicate a length polymorphism on the long arm of chromosome 2. Arrow heads denote a polymorphism in signal intensity of 128S/25S rDNA on chromosome 5. Scale bar: 10 μm.

chromosomal pairs. Chromosome 1 has a 5S rDNA signal in the terminal portion of the short arm (94.9 ± 1.6 % of the whole arm length away from the centromere); chromosome 2 contains the same localization, but in a position nearer the centromere (31.5 ± 5.8 %); chromosome 3 shows no rDNA localizations; chromosome 4 has two signals, both in the terminal part of the short arm, the 18/25S rDNA at the tip of this arm (87.8 ± 2.1 %) and 5S rRNA inmediately below it (55.2 ± 9.3 %); chromosome 5 has only one 18/25S rDNA signal in the terminal portion of the short arm (89.5 ± 2.9 %); chromosomes 6 and 7 show no localizations; chromosome 8 contains one 18/25S rDNA signal distal of the short arm (63.0 ± 10.0 %). Chromosome 2 has a length polymorphism of the long arm (5.00 ± 0.40 %), that can be seen in Figs. 1 and 2.

Fluorescence *in situ* hybridization using the VER17 probe with 18S/25S rDNA genes produced signals at the ends of the short arms of submetacentric chromosome pairs 4 and 5 (Fig. 3). The intensity of the signals was different (Figs. 1 and 2). Chromosome 4 showed a very strong signal whereas that in chromosome 5 was weaker in one chromosome of the pair (Fig. 2), but nevertheless detectable in all 10 inspected metaphases and also in interphase nuclei (Fig. not shown) of the same preparation. This polymorphism of 18S/25S rDNA loci in homologous chromosomes can reflect differences in the number of repeats originated maybe by unequally crossing over and was observed also in other plant species (Frello and Heslop-Harrison 2000; Shan *et al.* 2003; Weiss *et al.* 2003). Furthermore, also one of the four 5S rDNA loci showed a polymorphism in the signal intensity of the homologues in chromosome 4 (Fig. 1 and 2). A similar variation of 5S rDNA loci was also described in tobacco (Fulnecek *et al.* 2002). In cotton Cronn *et al.* (1996) had shown that in 5S DNA 12% of the nucleotide positions are polymorphic within individual arrays and that the intralocus concerted evolutionary forces are relatively weak. Interestingly, Fulnecek *et al.* (2002) had ob-

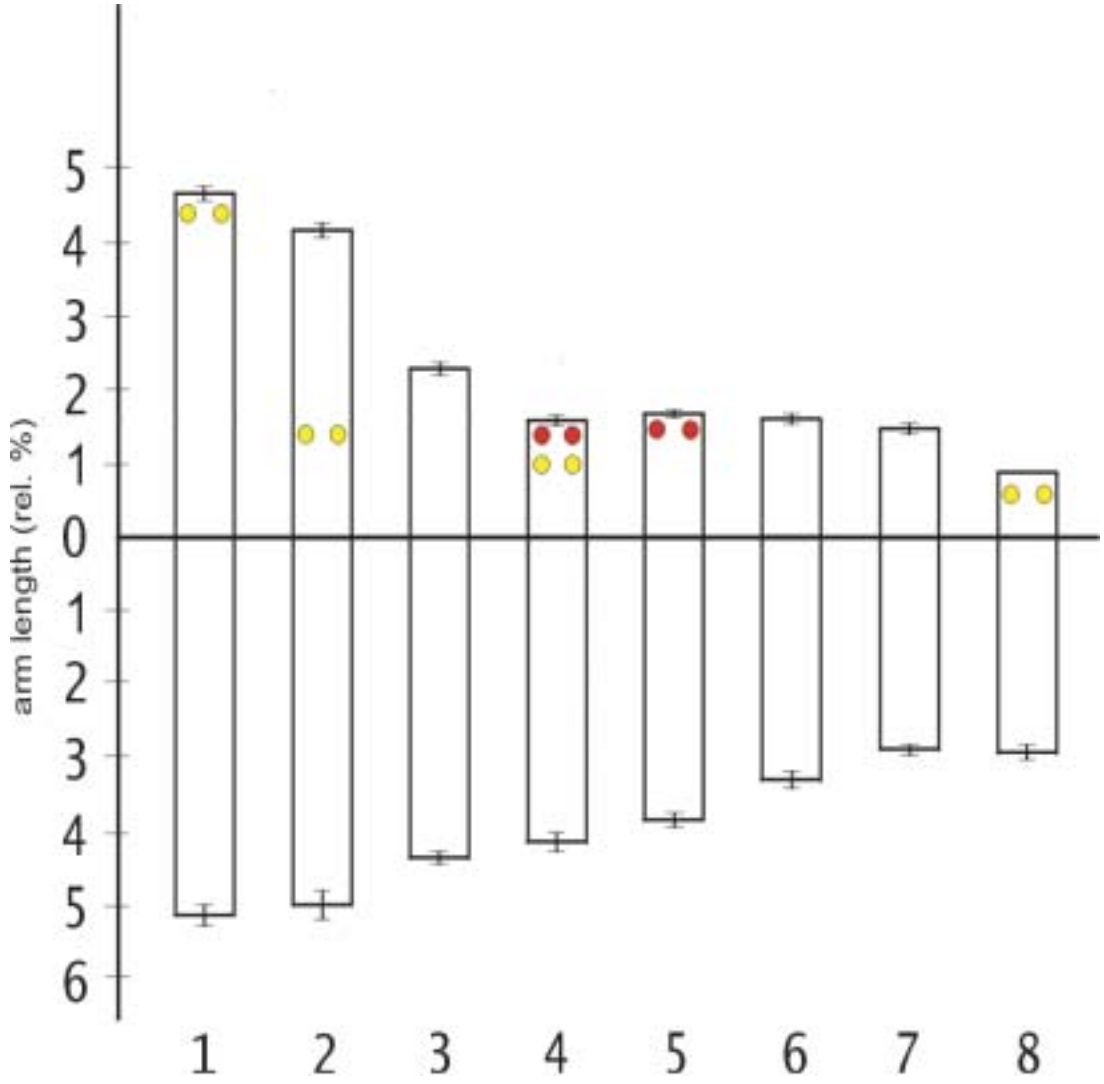


Fig. 3 — Idiogram of the haploid chromosome complement of *Placea amoena* Phil., derived from 10 analysed metaphases. 18S/25S rRNA gene locus, red dots; 5S rRNA gene locus, yellow dots; standard deviations of chromosome arms as bars.

served that 'a decrease in the copy number in one 5S rDNA family in tobacco is often associated with an increase in copy number in the second family suggesting that a mechanism maintaining a relatively fixed number of 5S repeats may be active'.

Altogether, using these two probes in combination, five of the eight pairs of *Placea* chromosomes were identifiable with molecular-cytological markers: one with loci for both gene sequences, chromosome 4 (Fig. 3), one with only a 18/25S rRNA gene locus, chromosomes 5 (Fig. 3), and three with 5S rRNA gene loci in chromosomes 1, 2 and 8. The chromosome 3 was also clear differentiated by the exclusion of chromosome markers and the relations of chromosome length. The remaining chromosomes 6 and 7 were differentiable by their length of chromosome arms after application of the t-Test: long arms with $t = 4.57$ were high significant ($\alpha = 0.1$ %) and the short arms with $t = 1.9$ were not significant. In addition of this statistical analysis and the results of FISH it was possible to discriminate all 8 chromosome pairs in the karyotype of *Placea amoena*.

The length of homologous chromosome 2 reveals a polymorphism in the long arms (Figs. 1 and 2) in five different plants, which is also expressed in the greater standard deviation of this arm (Tab. 1). This phenomenon has been also observed in other plant species such as *Alstroemeria* (BUITENDIJK *et al.* 1998), *Brachycome* (HOUBEN *et al.* 2000), *Scilla* (GREILHUBER and SPETA 1976), and *Triticum*, *Tulpia*, *Secale* and *Allium* (cited by HOUBEN *et al.* 2000). Each of the 5 investigated plants possessed the polymorphism in the heterozygous condition; there is no indication for the homozygous condition. It may be

speculated that the heterozygous condition is of selective advantage and therefore maintained in the population. The nature of this chromatin difference lies presumably in the origin of heterochromatic regions, such as documented in *Brachycome* (HOUBEN *et al*. 2000) and can play a role in genome variation (JONES and REES 1982; NAVAS-CASTILLO *et al*. 1987).

NARANJO (1985) carried out a study of the karyotype of *Placea arzae* Phil., a species with a broader distribution than that of *P. amoena*, occurring in the central Mediterranean Regions V and VI, including the federal district (MUNOZ 2000). There is a discrepancy in the citation of the original locality of the material, however as it is given from Cautín, Villarrica in the Southern Región IX, where the species does not occur naturally. NARANJO (1985) had estimated the karyotype of *P. arzae* as 4m + 6sm + 6 st, with a distinct satellite on chromosome 6. This chromosome is defined as subtelocentric, but in measuring directly from the published idiogram, it would more accurately be called submetacentric (following LEVAN *et al*. 1964). In addition, this species has an ratio (R) of 3, and an index of karyotypic asymmetry (AsI %) of 61.63 (determined also from the idiogram of the haploid complex). These values are very similar to those in *P. amoena*, with R = 2.6 and AsI % = 63.15. The most notable difference between the two species is the presence in *P. arzae* of a satellite on the short arm of chromosome 6. In *P. amoena* no satellite has been observed, even though 10 metaphases have been examined carefully, in which the nucleolar organizing region (NOR) was always clearly seen. Often a satellite dissociates from a chromosome arm during metaphase, but this does not occur in *P. amoena*.

It would be of interest to localize 5S and 18/25S rDNA in *P. arzae* through *in situ* hybridization and compare these data with those for *P. amoena*. It would also be valuable to compare other species of *Placea* in Chile to help clarify the taxonomic position of each taxa. These studies could be extended to include a broader investigation of the family Amaryllidaceae in Chile, which reflect great difficulties taxonomically due to complex morphological variation in genera such as *Hippeastrum, Phycella,* and *Rodophiala* (ARROYO and CUTLER 1984).

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REFERENCES

ADAMS S., LEITCH I., BENNETT M., CHASE M. and LEITCH A., 2000 – *Ribosomal DNA evolution and phylogeny in* Aloe *(Asphodelaceae)*. Amer. J. Bot., 87: 1578-1583.

ARANO H. and SAITO H., 1980 – *Cytological studies in family Umbelliferae 5. Karyotypes of seven species in subtribe Seselinae*. La Kromosomo, 2: 471-480.

ARRIAGADA L. and ZÖLLNER O., 1996 – *The genus* Placea *Miers ex Lindley (Amaryllidaceae) in Chile.* Herbertia, 51: 133-135.

ARROYO S. and CUTLER D., 1984 – *Evolutionary and taxonomic aspects of the internal morphology in Amaryllidaceae from South America and Southern Africa*. Kew Bull., 39: 467-498.

BUITENDIJK J., PETERS A., QUENE R. and RAMANNA M., 1998 – *Genome size variation and C- band variation polymorphism in* Alstroemeria aurea*,* A. ligtu*,* and A. magnifica *(Alstroemeriaceae).* Plant Syst. Evol., 212: 87-106.

CHEN J., STAUB J., ADELBERG J. and JIANG J., 1999 – *Physical mapping of 45S rRNA genes* in Cucumis *species by fluorescence* in situ *hybridization*. Can. J. Bot., 77: 389-393.

CRONN R., ZHAO X., PATERSON A. and WENDEL J., 1996 – *Polymophism and concerted evolution in a tandemly repeated gene family: 5S ribosomal DNA in diploid and alloploid cottons*. J. Mol. Evol., 42: 685-705.

FRANSZ P., STAM M., MONTJIN B., TENHOOPEN R., WIEGANT J., KOOTER J., OUD O. and NANNINGA N., 1996 – *Detection of single-copy genes and chromosome rearrangements in* Petunia hybrida *by fluorescence* in situ *hybridization*. Plant J., 9: 767-774.

FRANSZ P., ARMSTRONG S., ALONSO-BLANCO C., FISCHER T., TORRES-RUIZ R. and JONES G., 1998 – *Cytogenetics for the model species* Arabidopsis thaliana. Plant J., 13: 867-876.

FRELLO S. and HESLOP-HARRISON J., 2000 – Chromosomal variation in Crocus vernus Hill (Iridaceae) investigated by in situ hybridization of rDNA and tandemly repeated sequences. Ann. Bot., 86: 317-322.

FULNECEK J., LIM K., LEITCH A., KOVARIK A. and MATYASEK R., 2002 – *Evolution and structure of 5S rDNA loci in allotetraploid* Nicotiana tabacum *and its putative parental species*. Heredity, 88: 19-25.

FUKUI K., NAKAYAMA S., OHMIDO H., YOSHIAKI H. and YAMABE M., 1998 – *Quantitative karyotyping of three diploid* Brassica *species by imaging methods and localization of 45s rDNA loci on the identified chromosomes*. Theor. Appl. Genet., 96: 325-330.

GALASSO I., BLANCO A., KOTSIOTIS A., PIGNONE D. and HESLOP-HARRISON J., 1997 – *Genomic organization and phylogenetic relationships in the genus* Dasypyrum *analysed by Southern and* in situ *hybridization of total genomic and cloned DNA probes*. Chromosoma, 106: 53-61.

GOTTLOB-MCHUGH S., LEVESQUE M., MACKENZIE K., OLSON M., YAROSH O. and JOHNSON D., 1990 – *Organization of the 5S rRNA genes in the soybean* Glycine max *(L.) Merrill and conservation of the 5S*

rRNA repeat structure in higher plants. Genome, 33: 486-494.

GREILHUBER J. and SPETA F., 1976 – *C-banded karyotypes in the* Scilla hohenackeri *group* S. persica*, and* Poschkina *(Liliaceae).* Plant Syst. Evol., 126: 149-188.

GUERRA M., KENTON A. and BENNETT M., 1996 – *rDNA sites in mitotic and polytene chromosomes of* Vigna unguiculata *(L.) Walp. and* Phaseolus coccineus *L. revealed by* in situ *hybridization*. Ann. Bot., 78: 157-161.

HESLOP-HARRISON J., 2000 – *Comparative genome organization in plants: from sequences and markers to chromatin and chromosomes*. Plant Cell, 12: 617-635.

HOUBEN A., WANNER G., HANSON L., VERLIN D., LEACH C. and TIMMES J., 2000 – *Cloning and characterization of polymorphic heterochromatic segments of* Brachycome dichromosomatica. Chromosoma, 109: 206-213.

JIANG J. and GILL B., 1994 – *Nonisotopic* in situ *hybridization and plant genome mapping: the first 10 years*. Genome, 37: 717-725.

JONES R. and REES H., 1982 – *B-chromosomes*. Academic Press, London, 266 pp.

KUBIS S., SCHMIDT T. and HESLOP-HARRISON J., 1998 – *Repetitive DNA as a major component of plant genomes*. Ann. Bot., 82: 45-55.

KUBITZKI K., (ed.) 1998 – *The Families and Genera of Vascular Plants. III Flowering Plants – Monocotyledons. Lilianae (except Orchidaceae).* Springer, pp. 478.

LEE S., DO G. and SEO B., 1999 – *Chromosomal localization of 5S rRNA gene loci and the implications for relationships within the* Allium *complex*. Chrom. Res., 7: 89-93.

LEVAN A., FREDGA K. and SANDBERG A., 1964 – *Nomenclature for centromeric position on chromosomes*. Hereditas, 52: 201-220.

LINARES C., GONZALEZ J., FERRER E. and FOMINAYA A., 1996 – *The use of double fluorescence* in situ *hybridization to physically map the positions of 5S rDNA genes in relation to the chromosomal location of 18S-5.8S-26S rDNA and a C genome specific DNA sequence in the genus* Avena. Genome, 39: 535-542.

MARTICORENA C. and QUEZADA M., 1985 – *Catálogo de la flora vascular de Chile*. Gayana, Bot., 42: 1-157.

MOSCONE E., KLEIN F., LAMBROU M., FUCHS J. and SCHWEIZER D., 1999 – *Quantitative karyotyping and dual-color FISH mapping of 5S and 18S-25S rDNA probes in the cultivated* Phaseolus *species (Leguminosae).* Genome, 42: 1224-1233.

MUNOZ M., 2000 – *Consideraciones sobre los géneros endémicos de Monocotiledóneas de Chile*. Notic. Mens. Mus. Nac. Hist. Nat., 343: 16-27.

NARANJO C., 1985 – *El cariotipo de* Placea arzae *(Amaryllidaceae).* Bol. Soc. Argent. Bot., 24: 197-199.

NAVAS-CASTILLO J., CABRERO J. and CAMACHO J., 1987 – *Chiasma redistribution in presence of supernumery chromosome segments in grashoppers on the size of the extra segment*. Heredity, 58: 409-412.

REEVES A., 2001 – *MicroMeasure: A new computer program for the collection and analysis of cytogenetic data.* Genome, 44: 239-443.

SCHMIDT T. and HESLOP-HARRISON J., 1998 – *Genomes, genes and junk: the large-scale organization of plant chromosomes.* Trends Plant Sci., 3: 195-199.

SCHRADER O., AHNE R., FUCHS J. and SCHUBERT I., 1997 – *Karyotype analysis of* Helianthus annuus *using Giemsa banding and fluorescence* in situ *hybridization*. Chrom. Res., 5: 451-456.

SCHRADER O., BUDAHN H. and AHNE R., 2000 – *Detection of 5S and 25S rRNA genes in* Sinapis alba, Raphanus sativus *and* Brassica napus *by double fluorescence* in situ *hybridization*. Theor. Appl. Genet., 100: 665-669.

SCHRADER O., AHNE R., ZHAO H., BUDAHN H. and PETERKA H., 2002 – *Hohe Karyotypvariabilität in somatischen Nachkommenschaften von zwei* Allium*-Bastarden.* Vortr. Pflanzenzüchtung, 54: 381-384.

SCHWARZACHER T. and HESLOP-HARRISON P., 2000 – *Practical* in situ *Hybridization*. BIOS Scientific Publishers Ltd, The Bath Press, Bath, Oxford, UK, 203 pp.

SHAN F., YAN G. and PLUMMER J., 2003 – *Cyto-evolution of* Boronie *genomes revealed by fluorescent* in situ *hybridization with rDNA probes*. Genome, 46: 507-513.

TRAUB H. and MOLDENKE H., 1949 – *Amaryllidaceae: tribe Amarylleae*. Stanford, 194 pp.

WEISS H., PASIERBEK P. and MALUSZYNSKA J., 1999 – *An improved non-fluorescent detection system for* in situ *hybridization in plants.* Biotech. Histochem., 75: 49-53.

WEISS H., STUESSY T., SILJAK-YAKOVLEV S., BAEZA C. and PARKER J., 2003 — *Systematic and evolutionary implications of karyotipes of* Hypochaeris *(Asteraceae, Lactuceae) from South America*. Plant Syst. Evol., (241: 171-184).

YAKURA K. and TANIFUJI S., 1983 – *Molecular cloning and restriction analysis of Eco RI- fragments of* Vicia faba *rDNA*. Plant Cell Physiol., 24: 1327-1330.

ZHANG D. and SANG T., 1998 – *Chromosomal structural rearrangement of* Paeonia brownie *and* P. californica *revealed by fluorescence* in situ *hybridization*. Genome, 41: 848-853.

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