Theor Appl Genet (1997) 94:982–990 

M. J. De Jeu · J. Lasschuit · A. G. J. Kuipers
S. A. Kamstra · R. G. F. Visser

# Characterization and localization of repetitive DNA sequences in the ornamental *Alstroemeria aurea* Graham

Received: 24 November 1996 / Accepted: 20 December 1996

**Abstract** Three repetitive DNA sequences were isolated from a genomic DNA library of the ornamental *Alstroemeria aurea* Graham. Two repeats, A001-I and A001-II, were quite homologous and highly *A. aurea*-specific. A001-I was a 217-bp sequence with several telomeric TTTAGGG repeats at the 5′ end and a unique sequence of 98 bp at the other end. The third repeat, A001-IV, was a 840-bp sequence which contained two sub-sequences of 56 and 74 bp respectively, previously found in chloroplast (cp) DNA of tobacco and spinach and to a lesser extent in the cpDNA of maize and rice. Repeat A001-IV was not species-specific and its hybridization signal was weaker than the other repeats. Fluorescence in situ hybridization (FISH) revealed the *A. aurea*-specific repeats to be located in the heterochromatic regions of all *A. aurea* chromosomes. The differences in FISH pattern make them useful tools for karyotype analysis. The non-species-specific sequence A001-IV gave a dispersed signal over all the *Alstroemeria* chromosomes in an interspecific hybrid. The potential use of these repetitive DNA sequences for the study of phylogenetic relationships within the genus *Alstroemeria* is discussed.

**Key words** *Alstroemeria aurea* · Repetitive DNA · FISH-fluorescence in situ hybridization · Heterochromatin · Phylogeny

## Introduction

All higher-plant genomes have repetitive DNA sequences, comprising in some species, e.g. cereals, more than 90% of their total DNA content (Flavell 1980; Anamthawat-Jonsson and Heslop-Harrison 1993). Highly repetitive DNA sequences may be organized as tandemly repeated elements in the genome such a satellite DNA (Singer 1982; Broun and Tanksley 1993), as dispersed repeats within the genome (Moore et al. 1991), or as clusters of interspersed repeats (Zhao and Kochert 1993). In situ hybridization has demonstrated that satellite DNA sequences are often associated with heterochromatic regions (Maluszynska and Heslop-Harrison 1991; Anamthawat-Jonsson and Heslop-Harrison 1993; Cheung et al. 1994; Irifune et al. 1995).

Besides variation in the amount of repetitive DNA in plant genomes, its constitution also varies between species. Species-specific repetitive sequences have been isolated from several crops, for example, potato (Visser et al. 1988), *Brassica napus* (Gupta et al. 1992), *Thinopyrum elongatum* (Bournival et al. 1994), *Hordeum vulgare* (Svitashev et al. 1994) and rice (Aswidinnoor et al. 1991; Mawal 1995). These repetitive sequences turned out to be very useful as molecular genetic tools for plant breeding.

*Alstroemeria aurea* ($2n = 2x = 16$) is a monocotyledonous ornamental, taxonomically classified in the Alstroemeriaceae, order *Liliales*. The family Alstroemeriaceae not only includes *Alstroemeria* but three other genera: *Bomarea* Mirbel, the monotype *Leontochir ovallei* Phil. and *Schickendantzia* Pax (Dahlgren and Clifford 1982). *Alstroemeria* species have a very large genome with nuclear DNA contents (2C-values) varying from 36.5 to 78.9 pg (Bharathan et al. 1994; Buitendijk et al. 1997). The genus contains over 60 species growing in South-America, mainly in Chile and Brazil (Bayer 1987; Ravenna 1988; Aker and Healy 1990). In *A. aurea* seven of the eight chromosomes have large constitutive heterochromatic regions as revealed by the Giemsa C-banding technique (Buitendijk and Ramanna 1996). Thus, it is expected that, together with the large size of the genome, the *A. aurea* genome contains a mass of highly repetitive DNA.

Communicated by F. Salamini

M. J. De Jeu (✉) · J. Lasschuit · A. G. J. Kuipers · S. A. Kamstra · R. G. F. Visser
The Graduate School of Experimental Plant Sciences, Department of Plant Breeding, Wageningen Agricultural University, P.O. Box 386, NL-6700 AJ Wageningen, The Netherlands

In order to test if species-specific repetitive sequences, used as probes in fluorescence in situ hybridization, might be useful molecular tools for genome and chromosome identification in introgression studies in *Alstroemeria* the present work was carried out. Repetitive DNA sequences of the Chilean species *A. aurea* were isolated, characterized and localized. The species-specificity was studied in dot blots and Southern blots with 21 Chilean and Brazilian *Alstroemeria* species and representatives of nine other genera.

## Materials and methods

### Plant material

Different accessions of *A. aurea* were used in this study. In Table 1 the accession numbers and the origin of all 21 diploid ($2n = 2x = 16$) *Alstroemeria* species used for testing species-specificity are listed. The Chilean species were described by Bayer (1987), the Brazilian species by Uphof (1952). To test the genus-specificity, genomic DNA of *Lilium* and *Tulipa* was received from CPRO-DLO, Wageningen, NL, and of *Bomarea* and *Leontochir* from the Jodrell Laboratory, Kew, UK. For FISH root tips of *A. aurea* A003 and of the hybrid *A. aurea* A001 × *A. inodora* accession P002 was employed.

### Genomic DNA isolation and cloning

Genomic DNA of the *Alstroemeria* species, as well as of *Hordeum*, *Manihot*, *Solanum*, *Lycopersicon* and *Petunia*, was isolated from 2 g of young leaves from greenhouse-grown plants according to Dellaporta et al. (1983). Prior to DNA isolation, the material was frozen in liquid nitrogen and stored at $-80^{\circ}$. DNA of *A. aurea* accession A001 was digested with *Sau*3A and ligated into *Bam*HI-digested pUC19, which was used to transform competent cells of *E. coli* DH5α. Plasmid-containing colonies were selected on LB plates (Sambrook et al. 1989) with 0.008% X-gal, 1 mM of IPTG and 100 µg/l of ampicillin, grown at 37°C.

### Blotting and hybridization

Colony hybridization was performed according to Grunstein and Hogness (1975). Southern blots were made on Hybond-$N^+$ membrane (Amersham) according to the manufacturer's recommendations. Slot blots were produced as described by Kuipers et al. (1996).

Hybridization probes were made from either *Sau*3A-digested genomic *A. aurea* DNA or *A. aurea* repeats isolated from pUC19 by *Eco*RI-*Hin*dIII digestion, agarose-gel electrophoresis and purification with QIAEX (Qiagen). $^{32}$P dCTP was incorporated through random primed DNA labelling by using the Megaprime DNA labelling system (Amersham). Hybridizations were performed as recommended by Amersham. After autoradiography, membranes were de-probed by incubating in 0.4 N NaOH at 45 °C for 30 min and in 0.1 × SSC, 0.1% SDS, 0.2 M Tris pH 7.5 at 45 °C for 30 min.

### Sequencing

Sequence analysis was performed on 1 µg of plasmid DNA with standard forward or reverse M13 sequencing primers using the ABIPRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer) and an automated sequencer (Applied Biosystems). All sequences were analysed for homology to known sequences using the BLAST programs (Altschul et al. 1990) at the WWW site of the NCBI (http://www.ncb-i.nlm.nih.gov/Recipon/bs/seq.html). In all cases BLASTN was used in combination with the database 'nr'. PCgene (IntelliGenetics) was used for all other sequence analyses.

### Fluorescence in situ hybridization

In situ hybridization on mitotic metaphase chromosomes was performed according to Kuipers et al. (1996). Repetitive sequences were

**Table 1** Names, accession numbers and origin of 21 *Alstroemeria* species

| Species | Accession | Origin |
|---|---|---|
| *A. angustifolia* ssp. *angustifolia* Herbert | AN5S | Chile |
| *A. aurea* Graham | A001, A002, A003, A004, A005, A006, A011, A016, A017 | Chile |
| *A. diluta* Bayer | AD4K | Chile |
| *A. exserens* Meyen | AO8Z, AO5S | Chile |
| *A. garaventae* Bayer | AH6Z | Chile |
| *A. hookeri* ssp. *cumminghiana* Herbert | AQ2S | Chile |
| *A. hookeri* ssp. *hookeri* Loddiges | AP2S, 332-11 | Chile |
| *A. modesta* Philippi | AK3W | Chile |
| *A. ligtu* ssp. *incarnata* Bayer | AJ4S | Chile |
| *A. ligtu* L. ssp. *ligtu* | AL5S, F051-1, J091-1, J091-4 | Chile |
| *A. ligtu* ssp. *simsii* Sprengel | AM1S, L111, K101-1 | Chile |
| *A. mutabilis* Kunze | AY1K | Chile |
| *A. pallida* Graham | AG2W | Chile |
| *A. paupercula* Philippi | AE2W | Chile |
| *A. pelegrina* L. | AR, C024-4, C025, C031 | Chile |
| *A. philippii* Baker | AS3Z | Chile |
| *A. pulchra* Sims ssp. *pulchra* | AB8S, AB9Z | Chile |
| *A. magnifica* ssp. *magnifica* Herbert | Q001-5 | Chile |
| *A. brasiliensis* Sprengel | A6-1, R001 | Brazil |
| *A. inodora* Herbert | P002, P003, P004, P006 | Brazil |
| *A. psittacina* Lehm | D032-1, D9206-2 | Brazil |

labelled with digoxigenine-11-dUTP using a nick translation mix (Boehringer Mannheim) or with biotin-16-dUTP (Boehringer Mannheim) by PCR-labelling. The PCR mix contained 200 ng of the plasmid containing the repetitive sequence, 50 pmol of the M13 forward and the M13 reverse primer, 1 unit Ampli*Taq* (Perkin Elmer), labelling mix (200 µM of each dNTP; 1:20 biotin-16-dUTP:dT-TP), 1 × Perkin Elmer PCR buffer. The PCR-profile was 1 × 7 min 95°C, 1 min 60°C, 2 min 72°C, then 29 × times 45 s 95°C, 1 min 60°C, 2 min 72°C, followed by a 10-min final extension at 72°C.

Before detection the slides were washed for 5 min in buffer 1 (0.1 M Tris HCl, 0.15 M NaCl, pH 7.5). Detection of digoxigenine-11-dUTP was performed with 20 µg/ml of anti-dig-FITC (Boehringer Mannheim) and 20 µg/ml of Rabbit-anti-sheep-FITC (Vector Laboratories). Biotin-16-dUTP was detected with 4 µg/ml of streptavidin-CY3 (Jackson Immuno Research Laboratories), 10 µg/ml of biotionylated-anti-streptavidin (Vector Laboratories) and 4 µg/ml of streptavidin-CY3. For each detection or amplification step, slides were incubated for 5 min with 100 µl of blockbuffer [1% Blocking reagent (Boehringer Mannheim) in buffer 1], followed by detection with the appropriate antibody in 100 µl of blockbuffer and 3 washes of 5 min each in buffer 1 at 37 °C. Chromosome preparations were counterstained with 1 µg/ml of DAPI (4,6-diamidine-2-phenylindole) and mounted in 5 µl of Vectashield (Vector Laboratories). Slides were examined with a Zeiss Axiophot fluorescence microscope with appropriate filters for DAPI, FITC and CY3. Photographs were taken on 400 ASA colour negative film. All double exposures were made with a Chromatec triple band filter with single excitation filters (Dan Pinkel System, Zeiss) for each fluorochrome.

## Results

### Isolation and characterization of repetitive sequences

After transformation of *E. coli* DH5α with *Sau*3A-digested DNA from *A. aurea* A001 and ligation into pUC19, 180 colonies were selected for the experiments. Following two cycles of colony hybridization with labelled total genomic *A. aurea* DNA, four colonies designated as A001-I, A001-II, A001-III and A001-IV were found to give strong hybridization signals in both cycles, indicating the possible presence of repetitive sequences.

### Sequence analysis (PCgene)

In Figs. 1 and 2 the sequences of the isolated repeats are represented. A001-I and A001-III (both 217 bp) were completely identical. Hence, the results of all other analyses are only described for A001-I. A001-II was 90.53% identical to A001-I; two subfragments present in A001-I were lacking from A001-II, whereas some bases differed (Fig. 1). Both sequences were slightly A-T rich (A001-I: 54.4%; A001-II: 59.2%). A001-IV was a differently organized, slightly A-T rich (56.2%), sequence of 840 bp (Fig. 2).

A001-I possessed an internal *Sau*3A-site, which was absent from A001-II, although regions flanking this site were well conserved in all two sequences. A001-I contained several unique restriction sites, including an internal *Hae*III-site which was absent from A001-II. Several telomeric direct repeats were found in A001-I and A001-II. These are shown in Fig. 1 as shaded blocks. In addition, eight inverted repeats (of 6 bp or more) were found in A001-I, of which two were palindromic and one was a triple inverted repeat. A001-II contained only one inverted repeat of 6 bp. In A001-IV several direct repeats of 7–9 bp were found. Fifteen

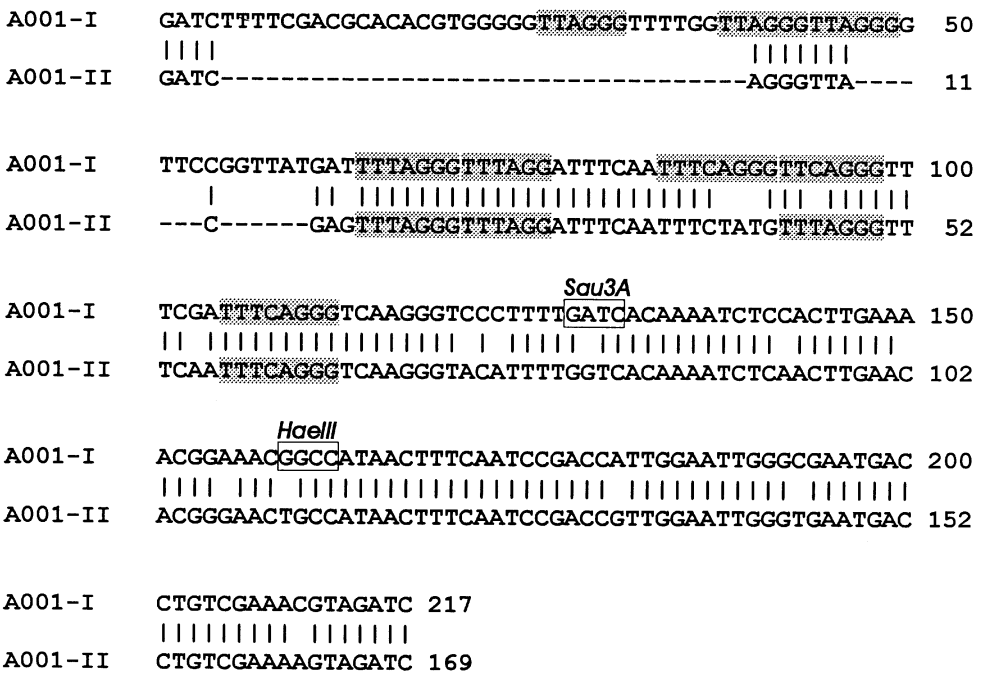


**Fig. 1** Sequences of the isolated repeats A001-I and A001-II. Homology between the two sequences is shown by the connection of identical bp. The internal *Sau*3A and *Hae*III restriction sites are marked. The telomeric repeats are marked as *shaded blocks*. The EMBL accession number for A001-I is Y10977, and for A001-II is Y10978

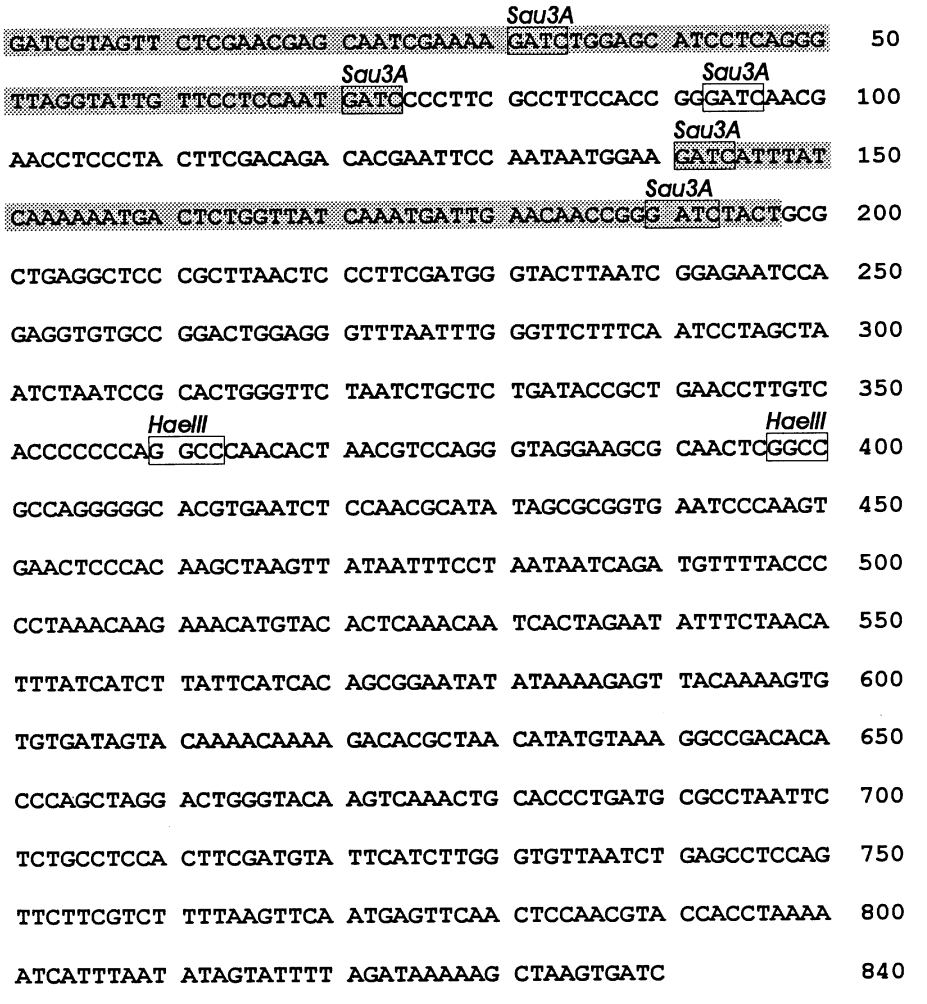


**Fig. 2** Sequence of A001-IV. The internal *Sau*3A and *Hae*III restriction sites are marked. The *shaded blocks* represent the two sub-sequences of 74 and 56 bp respectively, which show homology with chloroplast DNA. The EMBL accession number is Y10979

inverted repeats were present with a minimum repeat length of 7 bp, of which one was palindromic (8 bp). Five internal *Sau*3A sites and three *Hae*III sites were found among several other restriction sites (Fig. 2).

## Sequence analysis (BLASTN)

A001-I and A001-II showed homology with telomeric and subtelomeric repeats from varying species origin. No complete homologies with known sequences were found. A001-IV contained subfragments of 74 and 56 bp (see Fig. 2, shaded blocks) which were 85–91% homologous with sequences of the chloroplast genome of tobacco, spinach and, to a lesser extent, rice and maize. No complete homologies with known sequences were found.

## Specificity analysis

The three repeats were tested on a Southern blot with *Sau*3A-digested DNA of different *A. aurea* accessions, all of which gave identical hybridization patterns. For A001-I and A001-II a tandemly repeated hybridization was present (Fig. 3 A).

The three repeats were tested for species-specificity on a Southern blot with *Sau*3A-digested DNA of various Chilean *Alstroemeria* species. The repeats A001-I and A001-II gave strong hybridization with the DNA of *A. aurea* A001 and *A. mutabilis*. There was a faint hybridization signal with the DNA of *A. angustifolia* spp. *angustifolia*, *A. exserens*, *A. hookeri* spp. *hookeri* and *A. cumminghiana* (Fig. 3 B). Probe A001-IV gave hybridization with the DNA of all species, although the reactions with *A. pulchra* spp. *pulchra*, *A. exserens* and *A. hookeri* spp. *hookeri* accession (AP2S) were faint (Fig. 3 C).

In addition, slot-blot analysis performed on slot blots with genomic DNA from different *Alstroemeria* species, hybridized with the probes A001-I and A001-II, with an exposure time of 1 day, and A001-IV, with an exposure time of 10 days, revealed the following results (Table 2):

(1) A001-I gave very strong hybridization signals with all three *A. aurea* accessions and a clear signal with the

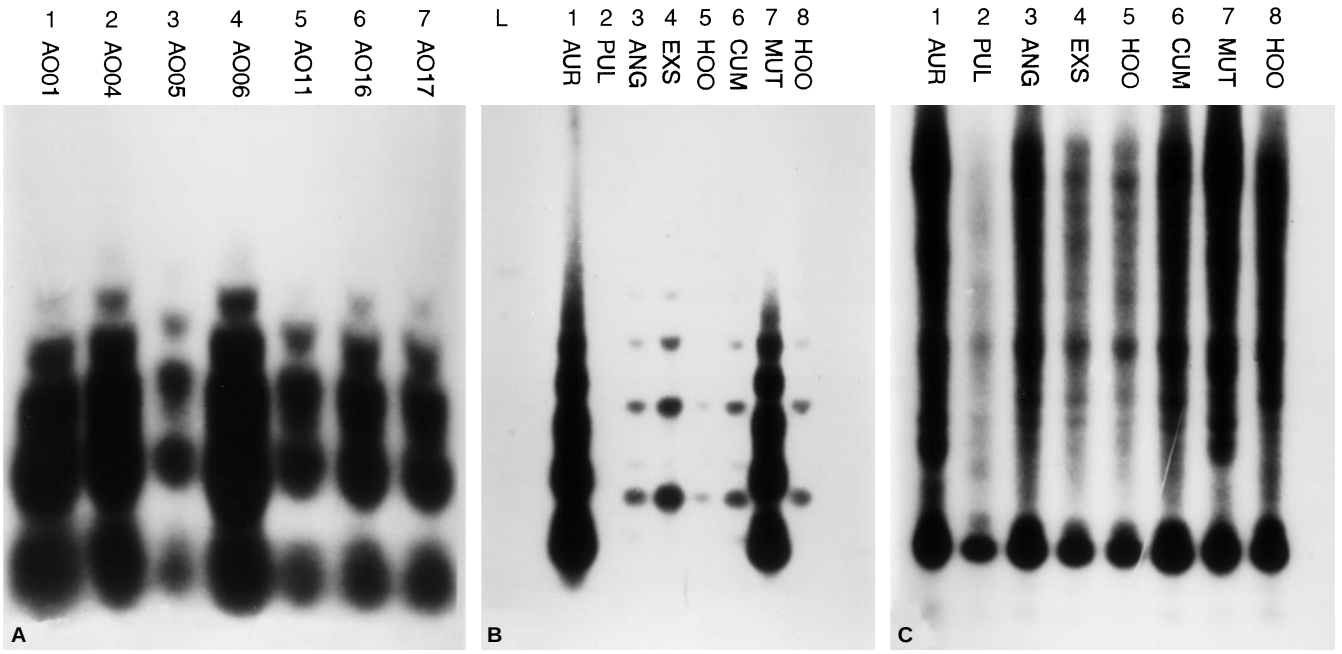


**Fig. 3A, B, C** Southern-blot analyses for a specificity test. **A** Southern blot analysis of different *Sau*3A-digested *A. aurea* accessions with probe A001-I. All *A. aurea* accessions give the same hybridization pattern. *Lane 1* = A001, *lane 2* = A004, *lane 3* = A005, *lane 4* = A006, *lane 5* = A011, *lane 6* = A016, *lane 7* = A017. **B** Southern-blot analysis of total *Sau*3A-restricted genomic DNA of *Alstroemeria* species with probe A001-I. Note a clear hybridization signal with *A. aurea* and *A. mutabilis* and a faint signal with *A. angustifolia* spp. *angustifolia*, *A. exserens*, *A. hookeri* spp. *cumminghiana* and *A. hookeri* spp. *hookeri*. *L* = kb ladder, *lane 1* = *A. aurea*, *lane 2* = *A. pulchra* spp. *pulchra*, *lane 3* = *A. angustifolia* spp. *angustifolia*, *lane 4* = *A. exserens*, *lane 5* = *A. hookeri* spp. *hookeri* (AP2S), *lane 6* = *A. hookeri* spp. *cumminghiana*, *lane 7* = *A. mutabilis*, *lane 8* = *A. hookeri* spp. *hookeri* (332-11). **C** Same blot as in **B** probed with A001-IV. This probe hybridizes with the DNA of all species although there is some difference in intensity of the signal

following species: *A. ligtu simsii* (K101-1), *A. pallida* (AG) *A. garaventae* (AH), *A. hookeri* spp. *hookeri* (AP), *A. hookeri* spp. *cumminghiana* (AQ), *A. angustifolia* spp. *angustifolia* (AN), *A. exserens* (AO).
(2) A001-IV gave a signal which was less specific. All species hybridized a little after 10 days of exposure time. Hybridization was strongest with *A. aurea*, and somewhat less strong with *A. garaventae* (AH), *A. hookeri* spp. *hookeri* (AP), *A. hookeri* spp. *cumminghiana* (AQ), *A. angustifolia* spp. *angustifolia* (AN), *A. exserens* (AO), *A. psittacina* (D), *Bomarea* and *Leontochir* (Table 2).

The three repeats were tested for genus-specificity on a Southern blot with *Sau*-digested DNA of different genera. The repeats A001-I and A001-II did not hybridize with digested genomic DNA of *Lilium* (Liliaceae), *Tulipa* (Liliaceae), *Bomarea* (Alstroemeriaceae), *Hordeum* (Gramineae), *Manihot* (Euphorbiaceae), *Solanum* (Solanaceae) and *Lycopersicon* (Solanaceae) under conditions that yielded a strong signal with *A. aurea* DNA (Fig. 4). Hybridization with repeat A001-IV gave a weak hybridization signal with *A. aurea* and no signal with other genera (data not shown).

## Genomic organization

Southern blots with total genomic DNA of six *Alstroemeria* species, digested with *Sau*3A, *Hae*III or *Mse*I, were hybridized with the repeats A001-I, A001-II and A001-IV. Identical hybridization patterns as tandemly repeated arrays were obtained with the A001-I and A001-II repeats as probes only in the lanes of *A. aurea* digested by all three enzymes. No hybridization with the total genomic DNA of the other species was found. Hybridization with A001-IV resulted in a faint smear with all *Alstroemeria* species digested with *Sau*3A and *Mse*I. In the *Hae*III digestion the lanes with *A. ligtu* spp. *ligtu*, *A. pelegrina*, *A. sierrae* and *A. psittacina* showed two clear bands, one of approximately 800 and one of 1600 bp (data not shown).

## Chromosomal localization

Figure 5 depicts the localization of the repeats on mitotic metaphase chromosomes. A001-I and A001-II were localized on the interstitial and telomeric bands of seven of the eight *A. aurea* chromosomes (Fig. 5 A). The hybridization pattern of both repeats coincided with

**Table 2** Slot-blot analysis of species-specificity of repeats A001-I and A001-IV

| Species | Probe A001-I[a] | Probe A001-IV[b] |
|---|---|---|
| *A. angustifolia* ssp. *angustifolia* | + | + |
| *A aurea* (A001, A002, A003) | +++ | ++ |
| *A. diluta* | 0 | − |
| *A. exserens* | + | + |
| *A. garaventae* | + | + |
| *A. hookeri* ssp. *cumminghiana* | + | + |
| *A. hookeri* ssp. *hookeri* | + | + |
| *A. modesta* | 0 | − |
| *A. ligtu* ssp. *incarnata* | 0 | − |
| *A. ligtu* ssp. *ligtu* (AL5S, F051-1, J091-1, J091-4) | 0 | − |
| *A. ligtu* ssp. *simsii* (AM1S, L111) | 0 | − |
| (K101-1) | + | − |
| *A. pallida* | + | − |
| *A. paupercula* | 0 | − |
| *A. pelegrina* | 0 | − |
| *A. philippii* | 0 | − |
| *A. magnifica* | 0 | − |
| *A. inodora* | 0 | − |
| *A. psittacina* | 0 | + |
| *Bomarea* | 0 | + |
| *Leontochir* | 0 | + |
| potato | 0 | 0 |
| petunia | 0 | 0 |

0, no hybridization
−, faint hybridization
+, hybridization
++, clear hybridization
+++, strong hybridization
[a] Exposure time 1 day
[b] Exposure time 10 days

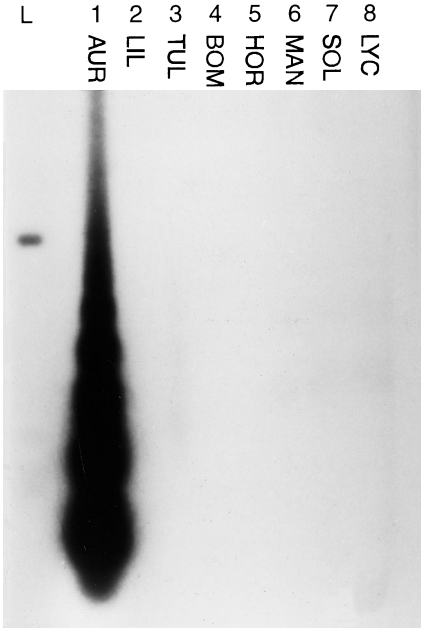


**Fig. 4** Southern-blot analysis of total genomic *Sau*3A-restricted DNA of different genera tested with probe A001-I. Only *A. aurea* gives a strong hybridization signal. *L* = kb ladder, *lane 1* = *A. aurea*, *lane 2* = *Lilium*, *lane 3* = *Tulipa*, *lane 4* = *Bomarea*, *lane 5* = *Hordeum*, *lane 6* = *Manihot*, *lane 7* = *Solanum*, *lane 8* = *Lycopersicon*

known Giemsa C-bands according to Buitendijk and Ramanna (1996), so that individual chromosomes could be recognized (numbers 1–8). Repeat A001-IV was dispersed on all chromosomes in and between the C-bands. In Fig. 5 B the chromosomes of the hybrid *A. aurea* × *A. inodora* showed a dispersed hybridization pattern of A001-IV without any difference between the two genomes.

## Discussion

In this study, sequences were isolated with the constitution of repetitive DNA, having several internal and inverted repeats. The presence or absence of an internal *Hae*III-site in A001-I can be linked with the occurrence of a ladder pattern in the Southern-blot hybridization. The internal *Sau*3A-site was absent from A001-II although regions flanking this site were well conserved. The internal *Sau*3A-site is most likely due to incomplete *Sau*3A digestion, since A001-I was picked up twice. We could speak of a family of tandemly arranged A-T rich repeats of 169–217 bp long. The third repeat had also a constitution of repetitive DNA with several internal and inverted repeats and a high number of internal restriction sites, which may be due to the

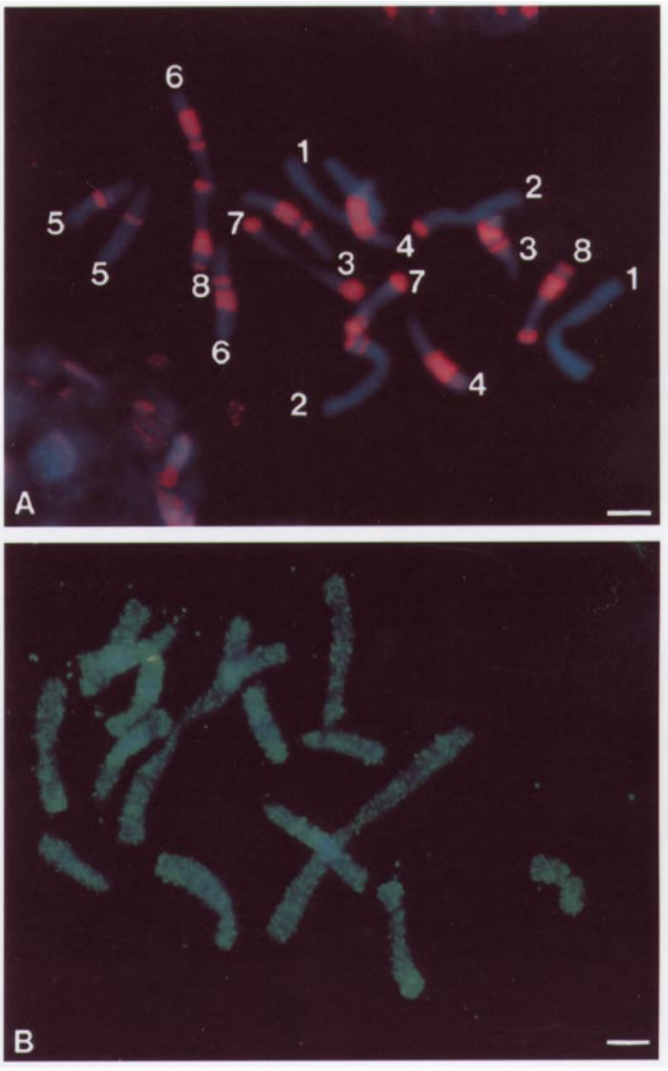


**Fig. 5A, B** Fluorescence in situ hybridization of mitotic metaphase chromosomes. **A** FISH of *A. aurea* accession A003 using A001-I as a probe (CY3 fluorescence, DAPI counterstain). The hybridization pattern coincides with the Giemsa C-bands resulting in the characterization of the chromosomes according to Buitendijk and Ramanna (1996) with the numbers 1–8. Bar = 10 μm. **B** FISH of the hybrid *A. aurea* × *A. inodora* with the probe A001-IV (FITC fluorescence, DAPI counterstain). This probe gives a dispersed signal on all chromosomes without any discrimination between *A. aurea* and *A. inodora* chromosomes. Bar = 10 μm

length of 840 bp. This could also be caused by incomplete digestion.

The family of tandemly arranged repeats contained several copies of the telomere-like repeats as were also isolated by Richards and Ausubel (1988) in *Arabidopsis thaliana*. These telomeric repeats are highly conserved in most plants ranging from the unicellular green alga *Chlorella vulgaris* (Higashiyama et al. 1995) to wheat (Cheung et al. 1994) and *Lycopersicon esculentum* (Ganal et al. 1991), which implies that these motifs cannot be responsible for the species-specificity. Presumably the other part of the sequence accounts for the *A. aurea*-specificity that was found for the repeats A001-I and A001-II which were similarly organized in all tested *A. aurea* accessions. Of the other species that were examined, only one (*A. mutabilis*) hybridized strongly with the *A. aurea*-specific probes. This species seems to be closely related to *A. aurea* according to Bayer (1987). The slot-blot analyses revealed that *Alstroemeria* species with large chromosomal heterochromatic regions, like *A. ligtu* spp. *simsii* (K101-1), *A. angustifolia* spp. *angustifolia*, *A. exserens*, *A. garaventae*, *A. hookeri* spp. *cumminghiana* and *A. hookeri* spp. *hookeri* (Buitendijk et al. 1996; Ramanna personal communication), gave a faint hybridization with the *A. aurea*-specific probes. This may indicate that the *A. aurea*-specific sequences have similarities with sequences located in the heterochromatic regions of other species as well. However, *A. ligtu* spp. *ligtu*, the *A. ligtu* spp. *incarnata* and *A. ligtu* spp. *simsii* (AM1S and L111) gave no hybridization, although they too possess heterochromatic bands. In this respect, Kuipers et al. (1997) found a clear distinction between the heterochromatic bands (and the chromosomes) of *A. aurea* and *A. ligtu* spp. *ligtu* in genomic in situ hybridization of the interspecific hybrid. This finding is further confirmed by our conclusion that the heterochromatic bands of certain *A. ligtu* species do not contain the repeats A001-I and A001-II which are highly repetitive in *A. aurea*. An exception is *A. ligtu* spp. *simsii* K101-1 which needs to be studied in more detail.

The two *A. aurea*-specific repeats were tandemly arranged and the hybridization pattern was similar to the GISH-banding pattern shown by Kuipers et al. (1997) and the Giemsa C-banding pattern (Buitendijk and Ramanna 1996). Apparently a family of A001-I-II-like repeats constitutes a major part of the highly repetitive, species-specific, fraction in the genomic DNA of *A. aurea*. In several other studies the repetitive sequences have been found to be located near to, or associated with, heterochromatic regions. Mukai et al. (1992) localized highly repetitive rye DNA sequences in dark C-banded regions of wheat chromosomes, although not all C-bands showed hybridization sites. In *Allium*, Irifune et al. (1995) found the repetitive DNA sequences associated with the major C-banding terminal regions of all chromosomes. Cheung et al. (1994) isolated TTTAGGG repeats in wheat which were located in interstitial regions. So localization of repetitive repeats on the chromosomes varies and seems frequently to be associated with C-banded heterochromatin. Also, in the present study, it was found that the *A. aurea*-specific probes were closely related to the heterochromatic (C-banded) regions. This is in agreement with the A-T richness of the isolated sequences.

The non-species-specific probe A001-IV gave a dispersed signal all over the *Alstroemeria* chromosomes. In the slot-blot analysis it hybridized also with *Leontochir* and *Bomarea*, although in the Southern blot no hybridization with *Bomarea* was found. An explanation

for this difference could be the 10-day exposure time for the dot-blot analyses as compared with the 1-day exposure time in the Southern blot. The 840-bp-long repeat contained small parts of sequences homologous with chloroplast DNA. Since it was located on all metaphase chromosomes in the FISH, we can conclude that this sequence was part of the nuclear DNA. Because of the variation in hybridization signal in the dot blots, this repeat might be useful in phylogenetic studies, especially in the field, of the relatedness between genera within the family Alstroemeriaceae.

Repetitive DNA sequences, and especially species-specific repeats, have proved to be useful tools in studies on the relatedness of species and genera in both dicots, for example *Arabidopsis* (Kamm et al. 1995), *Brassica* (Gupta et al. 1992) and *Cucumis* (Zentgraf et al. 1992), and monocots, for example in cereals (Aswidinnoor et al. 1991; Tsujimoto and Gill 1991; Vershinin et al. 1994; Cabrera et al. 1995) and *Allium* (Irifune et al. 1995). Our study generated repetitive DNA sequences in a monocotyledonous ornamental which are potentially very important for phylogenetic studies in the genus *Alstroemeria*. The *A. aurea*-specific probes are indispensable for determining the relatedness between species with large heterochromatic bands. These probes are also useful for analysing interspecific hybrids in early stages of development (De Jeu et al. 1995). They can also be employed for introgression studies, the determination of recombinant chromosomes and the characterization of addition lines. Furthermore, with the use of the isolated repetitive probes more information can be generated concerning the chromosome structure of *Alstroemeria*.

**Acknowledgements** Sequencing was done at the Department of Molecular Biology, Wageningen Agricultural University. Fabrice Chevalier was involved in this study as an ERASMUS-exchange student. The authors are grateful to Drs. Mark Chase, Han Sandbrink, and Jaap Van Tuyl for providing the DNA of several genera. We also thank Dr. Evert Jacobsen and Dr. Hans de Jong for critical reading of the manuscript and for helpful comments.

## References

Aker S, Healy W (1990) The phytogeography of the genus *Alstroemeria*. Herbertia 46:76–87

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Anamthawat-Jonsson K, Heslop-Harrison JS (1993) Isolation and characterization of genome-specific DNA sequences in Triticeae species. Mol Gen Genet 240:151–158

Aswidinnoor H, Nelson RJ, Dallas JF, McIntyre CL, Leung H, Gustafson JP (1991) Cloning and characterization of repetitive DNA sequences from genomes of *Oryza minuta* and *Oryza australiensis*. Genome 34:790–798

Bayer E (1987) Die Gattung *Alstroemeria* in Chile. Mitt Bot Staatssamml München 24:1–362

Bharathan G, Lambert G, Galbraith DW (1994) Nuclear DNA content of monocotyledons and related taxa. Am J Bot 81:381–386

Bournival B, Obanni M, Abad A, Ohm H, Mackenzie S (1994) Isolation of a new species-specific repetitive sequence from *Thinopyrum elongatum* and its use in the study of alien translocations. Genome 37:97–104

Broun P, Tanksley SD (1993) Characterization of tomato DNA clones with sequence similarity to human minisatellites 33.6 and 33.15. Plant Mol Biol 23:231–242

Buitendijk JH, Ramanna MS (1996) Giemsa C-banded karyotypes of eight species of *Alstroemeria* L. and some of their hybrids. Ann Bot 78:449–457

Buitendijk JH, Boon EJ, Ramanna MS (1997) Nuclear DNA content in twelve species of *Alstroemeria* L. and some of their hybrids. Ann Bot (in press)

Cabrera A, Friebe B, Jiang J, Gill BS (1995) Characterization of *Hordeum chilense* chromosomes by C-banding and in situ hybridization using highly repeated DNA probes. Genome 38:435–442

Cheung WY, Money TA, Abbo S, Devos KM, Gale MD, Moore G (1994) A family of related sequences associated with $(TTTAGGG)_n$ repeats are located in the interstitial regions of wheat chromosomes. Mol Gen Genet 245:349–354

Dahlgren RMT, Clifford HT (1982) Monocotyledons. A comparative study. Academic Press, London New York

De Jeu MJ, Lasschuit J, Chevalier F, Visser RGF (1995) Hybrid detection in *Alstroemeria* by use of species-specific repetitive probes. Acta Hortic, Ornam Plant Improv 420:62–64

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: version II. Plant Mol Biol Rep 1:19–21

Flavell RB (1980) The molecular characterization and organization of plant chromosomal DNA sequences. Annu Rev Plant Physiol 31:569–596

Ganal MW, Lapitan NLV, Tanksley SD (1991) Macrostructure of the tomato telomeres. Plant Cell 3:87–94

Grunstein M, Hogness S (1975) Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. Proc Natl Acad Sci USA 72:3961–3965

Gupta V, LakshmiSita G, Shaila MS, Jagannathan V, Lakshmikumaran MS (1992) Characterization of species-specific repeated DNA sequences from *B. nigra*. Theor Appl Genet 84:397–402

Higashiyama T, Maki S, Yamada T (1995) Molecular organization of *Clorella vulgaris* chromosome I: presence of telomeric repeats that are conserved in higher plants. Mol Gen Genet 246:29–36

Irifune K, Hirai K, Zheng J, Tanaka R, Morikawa H (1995) Nucleotide sequence of a highly repeated DNA sequence and its chromosomal localization in *Allium fistulosum*. Theor Appl Genet 90:312–316

Kamm A, Galasso I, Schmidt T, Heslop-Harrison JS (1995) Analysis of a repetitive DNA family from *Arabidopsis arenosa* and relationships between *Arabidopsis* species. Plant Mol Biol 27:853–862

Kuipers AGJ, van Os DPM, de Jong JH, Ramanna MS (1997) Molecular cytogenetics of *Alstroemeria*: identification of parental genomes in interspecific hybrids and characterization of repetitive DNA families in constitutive heterochromatin. Chromosome Res 5:31–39

Maluszynska J, Heslop-Harrison JS (1991) Localization of tandemly repeated DNA sequences in *Arabidopsis thaliana*. Plant J 1:159–166

Mawal Y, Lagu M, Moon E, Chang S, Chung M-C, Wu H-K, Gupta V, Ranjekar P, Wu R (1995) *Bam*HI and *Hin*dIII repetitive DNA families in the rice genome. Genome 38:191–200

Moore G, Cheung W, Schwarzacher T, Flavell R (1991) BIS 1, a major component of the cereal genome and a tool for studying genomic organization. Genomics 10:469–476

Mukai Y, Friebe B, Gill BS (1992) Comparison of C-banding patterns and in situ hybridization sites using highly repetitive and total genomic rye DNA probes of 'Imperial' rye chromosomes added to 'Chinese Spring' wheat. Jpn J Genet 67:71–83

Ravenna P (1988) New or noteworthy species of *Alstroemeria*. Phytologia 64:281–288

Richards EJ, Ausubel FM (1988) Isolation of a higher eukaryotic telomere from *Arabidopsis thaliana*. Cell 53:127–136

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Singer MF (1982) Highly repeated sequences in mammalian genomes. Int Rev Cytol 76:67–112

Svitashev S, Bryngelsson T, Vershinin A, Pedersen C, Säll T, von Bothmer R (1994) Phylogenetic analysis of the genus *Hordeum* using repetitive DNA sequences. Theor Appl Genet 89:801–810

Tsujimoto H, Gill BS (1991) Repetitive DNA sequences from polyploid *Elymus trachycaulus* and the diploid progenitor species: detection and genomic affinity of *Elymus* chromatin added to wheat. Genome 34:782–789

Uphof JCT (1952) A review of the genus *Alstroemeria*. Plant Life 8:37–53

Vershinin A, Svitashev S, Gummesson P-O, Salomon B, von Bothmer R, Bryngelsson T (1994) Characterization of a family of tandemly repeated DNA sequences in Triticeae. Theor Appl Genet 89:217–225

Visser RGF, Hoekstra R, Van der Leij FR, Pijnacker LP, Witholt B, Feenstra WJ (1988) In situ hybridization to somatic metaphase chromosomes of potato. Theor Appl Genet 76:420–424

Zentgraf U, King K, Hemleben V (1992) Repetitive sequences are valuable as molecular markers in studies of phylogenetic relationships within the genus *Cucumis*. Acta Bot Neerl 41:397–406

Zhao X, Kochert G (1993) Clusters of interspersed repeated DNA sequences in the rice genome (*Oryza*). Genome 36:944–953