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ORIGINAL PAPER

The origin of the C-genome and cytoplasm of *Avena* polyploids

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Abstract The contribution of C-genome diploid species to the evolution of polyploid oats was studied using C-genome ITS-specific primers. SCAR analysis among *Avena* accessions confirmed the presence of C-genome ITS1-5.8S-ITS2 sequences in the genome of AACC and AACCDD polyploids. In situ hybridization and screening of more than a thousand rRNA clones in *Avena* polyploid species containing the C-genome revealed substantial C-genome rRNA sequence elimination. C-genome clones sequenced and Maximum Likelihood Parsimony analysis revealed close proximity to *Avena ventricosa* ITS1-5.8S-ITS2 sequences, providing strong evidence of the latter's active role in the evolution of tetraploid and hexaploid oats. In addition, cloning and sequencing of the chloroplastic trnL intron among the most representative *Avena* species verified the maternal origin of A-genome for the AACC interspecific hybrid formation, which was the genetic bridge for the establishment of cultivated hexaploid oats.

Introduction

Genus *Avena* L. (Poaceae) belongs to the tribe Aveneae and contains diploid, tetraploid and hexaploid species, with the basic chromosome number seven ($x = 7$). All diploid species contain either the A-genome or the C-genome, which are also present in the AABB/AACC tetraploid and AACCDD hexaploid species. No diploid species have been found containing either the B-genome or the D-genome (Rajhathy and Thomas 1974). In addition, minor genic differences have been reported between the A-genome, B-genome and D-genome (Oinuma 1952; Leggett and Markhand 1995; Katsiotis et al. 1997; Linares et al. 1998; Irigoyen et al. 2001), justifying the proposed genomic designation of A' for the B-genome and A" for the D-genome (Leggett and Markhand 1995; Katsiotis et al. 2000; Loskutov 2008). High chromosome affinity between the abovementioned genomes complicates the unravelling of the phylogeny and the identification of ancestral species for *Avena* polyploids. Molecular markers such as RFLPs, RAPDs, AFLPs and SSRs (O'Donoughue et al. 1995; Alicchio et al. 1995; Ronald et al. 1997; Kianian et al. 1999; Jin et al. 1999; Nocelli et al. 1999; Li et al. 2000; Drossou et al. 2004) have adequately described species and genome relationships, but there is little information concerning phylogenetic studies. Recently, Nikoloudakis et al. (2008) were able to reveal the association of *A. longiglumis* Dur. and *A. strigosa* Scheb. to AACC and AACCDD polyploids, by using the Internal Transcribed Spacers (ITS) and the Inter-Genic Spacer (IGS) nucleotide polymorphism. However, identification of the C-genome species contributing to the speciation of the cultivating oat was not possible.

It is noteworthy that during all of the abovementioned studies (molecular markers and nucleotide sequences), it has been observed that all *Avena* allopolyploids cluster with the A-genome diploids, leading to the conclusion that the C-genome has undergone major genomic alterations. In addition, dominance of the A-genome over the C-genome rRNA sequences and sequence elimination of the latter (Jellen et al. 1994; Fominaya et al. 1995; Yang et al. 1999; Shelukhina et al. 2007) complicates phylogenies. As a result, C-genome ancestry in polyploids remains uncertain. Furthermore, there is no available information based on

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cytoplasmic sequences permitting us to unravel the evolution of the AACC and the AACCDD polyploid species.

The objective of the present study was to reveal insights into the origin of the C-genome in polyploid oats by means of detecting and studying rRNA clones from polyploids. In addition, sequence results of the chloroplastic trnL intron among 26 *Avena* spp. accessions were used to verify the maternal origin of the interspecific hybrid formation and the oat speciation.

Materials and methods

Plant material and DNA isolation

Twenty-nine *Avena* taxa along with *Arrhenatherum elatius* L. were included in the present study. Their ploidy level, genomic designation and accession number are listed in Table 1. Total genomic DNA was extracted from 2-week-old leaves using standard CTAB protocol. The purity and quantity of genomic DNA was determined spectrophotometrically and confirmed using 0.8% agarose gel electrophoresis.

SCARs and trnL PCR amplification

Based on previous knowledge from sequences of ITS1-5.8S-ITS2 arrays in *Avena* spp. (Nikoloudakis et al. 2008), specific primers were designed for C-genome species and were used for Sequence-Characterised Amplified Regions (SCARs; Fig. 1). The 50-μL reaction contained 40 ng of total DNA, 1 mM $MgCl_2$, 200 μM dNTPs, 15 pmole of primer CITS1F (5′-ACA CTG TGC CTA GCT CGG TGA TG-3′), 5 pmole of primer CITS2R (5′-TGC CGC ATC CTG TTC CTC GAT GG-3′) and 1 U of *Pfx* polymerase (Invitrogen, Carlsbad, CA). The cycling profile consisted of an initial denaturation step for 4 min, followed by 40 cycles of 1 min at 94°C, 30 s at 68°C and 40 s at 72°C, with a final elongation step of 8 min at 72°C, before cooling to 10°C.

For the chloroplastic trnL intron amplification, the 25-μL reaction contained 40 ng of total DNA, 1.5 mM $MgCl_2$, 100 μM dNTPs, 7.5 pmole of primer trnLC (5′-CGA AAT CGG TAG ACG CTA CG-3′), 7.5 pmole of primer trnLD (5′-GGG GAT AGA GGG ACT TGA AC-3′) and 1 U *Taq* polymerase (Promega, Madison, WI). The cycling profile consisted of an initial denaturation step for 4 min, followed by 35 cycles of 30 s at 94°C, 30 s at 59°C and 45 s at 72°C, with a final elongation step of 8 min at 72°C, before cooling to 10°C. PCR products were cloned and sequenced.

Analysis and sequencing of ITS1-5.8S-ITS2 C-genome clones

Primers for the amplification of the whole ITS1-5.8S-ITS2 fragment from rRNA loci of all genomes were 5′-ACG AAT TCA TGG TCC GGT GAA GTG TTC G-3′ (18S) and 5′-TAG AAT TCC CCG GTT CGC TCG CCG TTA C-3′ (26S), while conditions were as previously described by Nikoloudakis et al. (2008). Cloning of PCR products was carried out using the user-friendly cloning kit (NEB, Beverly, MA) according to manufacturer's instructions. Clones carrying inserts were identified using blue/white colony selection. Two-hundred individual ITS1-5.8S-ITS2 clones of each AACC/AACCDD polyploid species (*A. insularis* Ladiz. accession A, *A. maroccana* Gdgr. accession 2190, *A. sativa* L. cv. 'Image', *A. fatua* L. accession N127, *A. sterilis* L. accession 4704) and 70 clones of *A. longiglumis* accession 9089, used as negative control, were screened in SCAR reactions using the C-genome-specific primers (CITS1F and CITS2R). Each bacterial colony was picked with a sterilized toothpick and diluted in 20 μL of ddH_2O. One microliter was used as template in a 25 μL bulked reaction (in groups of ten), using 1.5 mM $MgCl_2$, 100 μM dNTPs, 7.5 pmole of each primer and 1 U *Taq* polymerase (Promega). Each bulk producing a strong amplification signal was further analysed in separate PCR reactions using individual clones as template. Positive clones were sequenced using the M13 universal primers.

Data analysis

The obtained sequences were checked for homology using the NCBI BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). Nucleotide multiple alignments were performed with ClustalW algorithm (Thompson et al. 1994) using the default parameters. For phylogenetic analysis, the Maximum Likelihood (ML) method as implemented in the phyML software (Guindon and Gascuel 2003) was used. An approximate Likelihood-Ratio Test (aLRT) was performed using the SH-like parameter. Trees were depicted with the TreeView software (Page 1996).

In situ hybridization

Cytological procedures have been described by Katsiotis et al. (1996). Probe pTa71 was used to identify the 18S-5.8S-26S rRNA sites in hexaploid *A. sativa* cv. 'Sun II' (AACCDD) chromosomes.

Results

Development of C-genome-specific SCAR markers

One of the objectives in the present study was to reveal whether C-genome rRNA sequences are present in AACC and/or AACCDD allopolyploids and if so, to what extent. Initial experiments involved an ITS1-5.8S-ITS2

Table 1 Species, ploidy, genome constitution and NCBI accession numbers of taxa used for the ITS1-5.8S-ITS2, SCAR and the trnL intron analyses

Species	Ploidy level	Accession number	Genome constitution	NCBI accession number (trnL-intron)	Clone (ITS1-5.8S-ITS2)	NCBI accession number (ITS1-5.8S-ITS2)
EG 88744	2*n* = 8*x* = 56		$C_pC_pAACCDD$	–		–
A. canariensis	2*n* = 2*x* = 14	Cc 7041	A_cA_c	EU276591		–
A. damascena	2*n* = 2*x* = 14	Cc 7261	A_dA_d	EU276595		–
A. longiglumis	2*n* = 2*x* = 14	CIav 9071	A_lA_l	EU276597		–
A. prostrata	2*n* = 2*x* = 14	Cc 7060	A_pA_p	EU276593		–
A. atlantica	2*n* = 2*x* = 14	Cc 7429	A_sA_s (?)	EU276596		–
A. brevis	2*n* = 2*x* = 14	CIav 9005	A_sA_s	EU276612		–
A. hirtula	2*n* = 2*x* = 14	Cc 7046	A_sA_s	EU276610		–
A. lusitanica	2*n* = 2*x* = 14	CAV 6317	A_sA_s	EU276609		–
A. strigosa	2*n* = 2*x* = 14	JIC 2080	A_sA_s	EU276589		–
A. clauda	2*n* = 2*x* = 14	JIC 2201	C_pC_p	–		–
A. eriantha	2*n* = 2*x* = 14	Cc 7056	C_pC_p	EU276592		–
A. ventricosa	2*n* = 2*x* = 14	Cc 7064	C_vC_v	EU276611		–
A. barbata	2*n* = 4*x* = 28	JIC 2048	AABB	EU276607		–
A. vaviloviana	2*n* = 4*x* = 28	PI 412767	AABB	EU276608		–
A. insularis	2*n* = 4*x* = 28	A	AACC	EU276598	A-73	EU252120
				–	A-118	EU252121
				–	A-137	EU252122
A. insularis	2*n* = 4*x* = 28	B	AACC	EU276613		–
A. insularis	2*n* = 4*x* = 28	C	AACC	EU276614		–
A. insularis	2*n* = 4*x* = 28	D	AACC	EU276615		–
A. insularis	2*n* = 4*x* = 28	E	AACC	EU276600		–
A. maroccana	2*n* = 4*x* = 28	JIC 2190	AACC	EU276590	2190-34	EU252116
				–	2190-79	EU252117
				–	2190-138	EU252118
A. maroccana	2*n* = 4*x* = 28	Cc 7070	AACC	EU276604		–
A. murphyi	2*n* = 4*x* = 28	Cc 7120	AACC	EU276594		–
A. macrostachya	2*n* = 4*x* = 28	Cc 7068	$C_mC_mC_mC_m$	EU276601		–
A. fatua	2*n* = 6*x* = 42	Cc 7668	AACCDD	EU276605		–
A. fatua	2*n* = 6*x* = 42	N127	AACCDD	–	N127-39	EU252123
				–	N127-76	EU252124
				–	N127-115	EU252125
A. macrocarpa	2*n* = 6*x* = 42	19437	AACCDD	EU276602		–
A. sativa	2*n* = 6*x* = 42	cv 'Image'	AACCDD	EU276606	Image-173	EU252115
A. sterilis	2*n* = 6*x* = 42	Cc 4704	AACCDD	EU276603	4704-148	EU252119
Arrhenatherum elatius	2*n* = 2*x* = 14	JIC 2518	?	EU276599		–

Single-Stranded Conformation Polymorphism (SSCP) analysis that was performed using 55 *Avena* spp. accessions. However, identification of C-genome-specific products in AACC and AACCDD polyploids was not possible (data not shown). Therefore, a more sensitive/analytical approach such as SCAR markers was used to detect putative C-genome rRNA sequences.

Alignment of 71 ITS1-5.8S-ITS2 *Avena* spp. clones was performed as reported by Nikoloudakis et al. (2008) and a pair of C-genome specific primers was designed (the forward primer located on ITS1 and the reverse primer on ITS2). Attention was paid to minimise homology to the A-genome ITS sequences (Fig. 1). To test their resolution, 11 genotypes were analysed in a SCAR reaction. A single band of the expected size (approximately 280 bp) was obtained by using template DNA from diploids (C_pC_p and C_vC_v), a tetraploid ($C_mC_mC_mC_m$), a synthetic octoploid (EG 88744) (*A. eriantha* Dur. X *A. sativa* - $C_pC_pAACCDD$) and

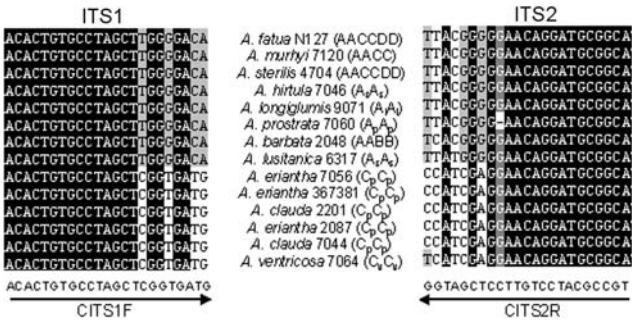

Fig. 1 Part of the ITS nucleotide polymorphism observed among 14 *Avena* spp. accessions. C-genome-specific primers are indicated by *arrows*. Genome types in *parentheses*

polyploids (AACC and AACCDD). No amplification signal was detected for species having either the AA or the AABB genomes (Fig. 2).

Once able to amplify C-genome rRNA sequences in allopolyploids, it was examined whether those sequences remained unaffected or were significantly altered by losing copy numbers. Therefore, individual clones from AACC/AACCDD polyploids were analysed as described in 'Materials and methods', to estimate a rough C-genome/A-genome rRNA sequence ratio. One to eight positive clones per taxon (with an average of three clones/200 colonies) were detected and identified as C-genome clones (*A. insularis* A, three clones; *A. maroccana* 2190, three clones; *A. sativa* cv. 'Image', one clone; *A. fatua* N127, eight clones; *A. sterilis* 4704, one clone).

Analysis of the ITS1-5.8S-ITS2 C-genome locus in *Avena* polyploids

Eleven clones (one to three from each species) were sequenced to use them in phylogenetic studies. Their length ranged from 590 to 594 bp and an alignment was performed with other C-genome *Avena* spp. sequences available in the NCBI nucleotide database. The whole sequence was used for the phylogenetic study. Molecular data were analysed with the ML method and sequences were clustered according to their genomic constitution (Fig. 3). Polyploid derived C-genome clones were clustered in the same group with *A. ventricosa* Bal. Ex coss. and *A. macrostachya* Bal. ex Coss. clones, separated from all C_p-genome sequences. Significant branch support values (0.97) reflect the divergence between the C_p-genome and C_v-genome and provide strong evidence that the C-genome in polyploids has a C_v origin. In addition, the perennial tetraploid *A. macrostachya* ($C_mC_mC_mC_m$) was clustered within the C_v group, making plausible the role of *A. ventricosa* to the establishment of *Avena* spp. polyploids. The topology and divergence between the above genome constitutions was also supported by NJ clustering and high bootstrap values (data not shown).

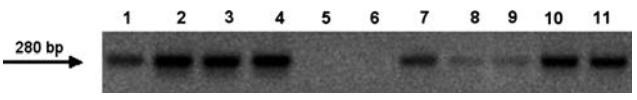

Fig. 2 C-genome rRNA query among species with different genome constitution using SCAR markers. *Lanes* are as follows: (*1*) EG 88744 (C_pC_pAACCDD); (*2*) *A. clauda* JIC 2201 (C_pC_p); (*3*) *A. macrostachya* Cc 7068 ($C_mC_mC_mC_m$); (*4*) *A. ventricosa* Cc 7064 (C_vC_v); (*5*) *A. longiglumis* CIav 9071 (A_lA_l); (*6*) *A. vaviloviana* PI 412767 (AABB); (*7*) *A. insularis* A (AACC); (*8*) *A. maroccana* JIC 2190 (AACC); (*9*) *A. fatua* N127 (AACCDD); (*10*) *A. sterilis* Cc 4704 (AACCDD); (*11*) *A. sativa* cv. 'Image' (AACCDD)

Analysis of the trnL intron in *Avena*

Twenty-six clones from the most representative *Avena* spp. belonging to all known genome types along with *Arrhenatherum elatius* were sequenced and aligned. Clones ranged from 396 to 410 bp. The trnL intron was generally conserved; however, major differences were revealed between the A-genome and C-genome. The majority of diploids and polyploids containing the A-genome had a 5 bp deletion, while in the case of *A. damascena* Rajhathy et Baum it was extended to 14 bp (Fig. 4). Significant branch support values (0.86) clearly separate C-genome taxa from A-genome and polyploid species. Differentiation of diploid and polyploid A-genome-containing species was not possible in general. However, high affinity was observed among *A. longiglumis* (A_lA_l) and *A. atlantica* (A_sA_s?), *A. prostrata* Ladiz. (A_pA_p) and *A. damascena* (A_dA_d), *A. sativa* (AACCDD) and *A. macrocarpa* Moench (AACCDD), *A. strigosa* (A_sA_s) and *A. murphyi* Ladiz. (AACC; Fig. 5).

Physical mapping of the 18S-26S rRNA genes

A total of 12 18S-26S rRNA sites on the *A. sativa* chromosomes were visualised using pTa71 as a probe. Six of them were major, detected on the satellited chromosomes, while the other six were found as minor sites (Fig. 6).

Discussion

Polyploidy can induce various types of genomic alterations in wheat (Zohary and Feldman 1962; Dvorak and Zhang 1990; Feldman et al. 1997; Liu et al. 1998; Ozkan et al. 2001), Brassica (Song et al. 1995), Arabidopsis (Comai 2000; Lee and Chen 2000; Madlung et al. 2002), cotton (Hanson et al. 1998; Zhao et al. 1998) and in synthesised *Cucumis* × *hytivus* allotetraploids (Chen et al. 2007). Changes include sequence elimination, intergenomic exchanges, cytosine methylation, gene repression, novel gene activation, genetic dominance, subfunctionalization

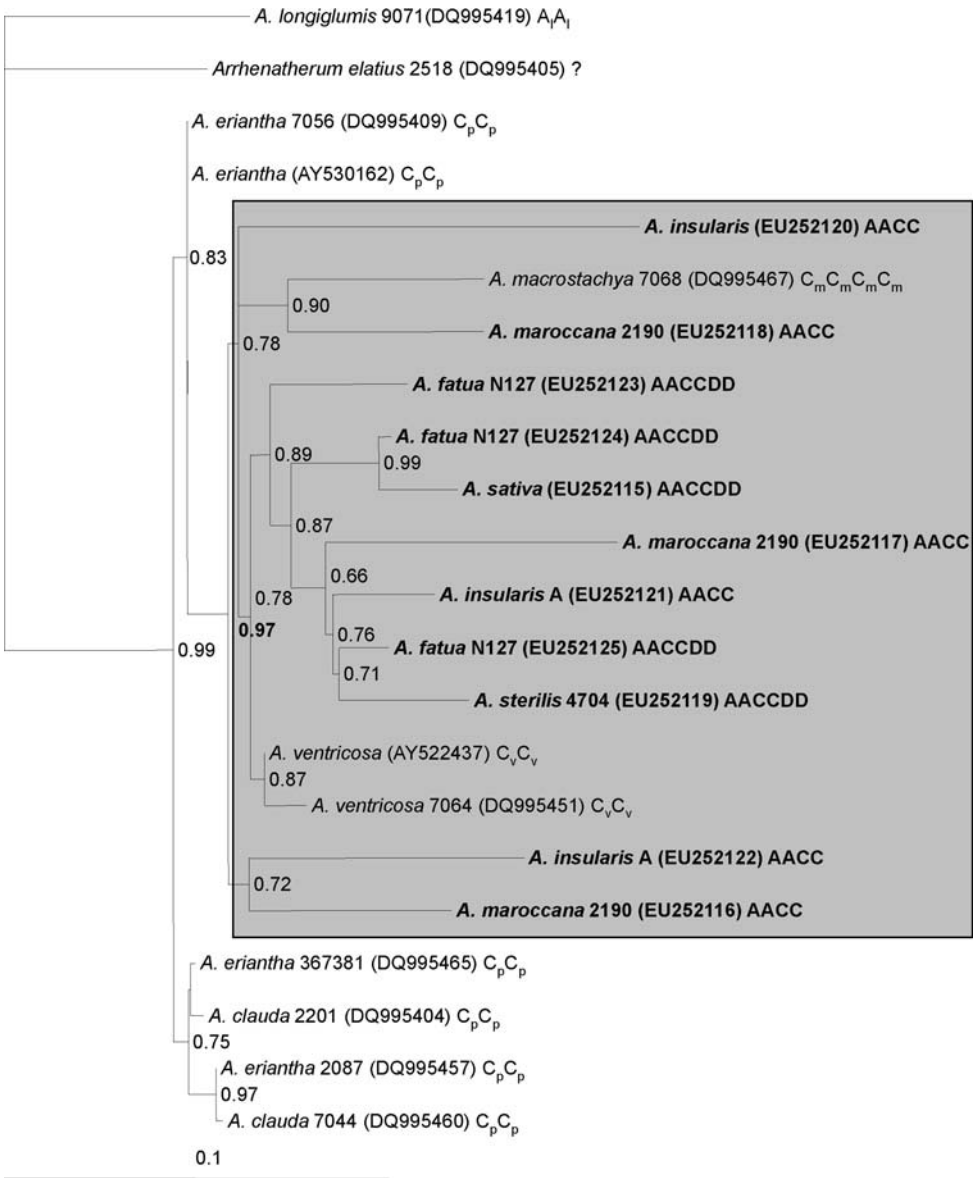

Fig. 3 Phylogenetic relationships of C-genome-derived rRNA sequences among diploid and polyploid species. Accessions in *bold* represent clones of polyploids sequenced for the present study. High affinity with C_v sequences and differentiation from C_p sequences is expressed by high branch support values

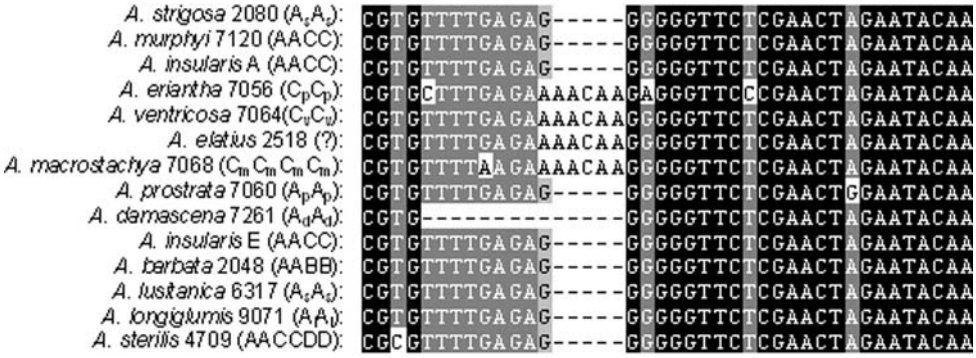

Fig. 4 Details of the trnL intron nucleotide polymorphism observed among 13 *Avena* spp. accessions and *Arrhenatherum elatius*. All *Avena* spp. except C-genome species and *Arrhenatherum elatius* include a 5′-AA-CAA-3′ nucleotide deletion

and transposon activation (Chen and Ni 2006). Especially, in newly developed allopolyploids, rapid genomic changes often occur to stabilize and bring into equilibrium the genomes (Song et al. 1995; Feldman et al. 1997; Liu et al. 1998; Comai 2000; Ozkan et al. 2001; Madlung et al. 2002; Ma and Gustafson 2005).

Genomic changes that have been studied extensively at the cytogenetic and the molecular level include the nucleolar

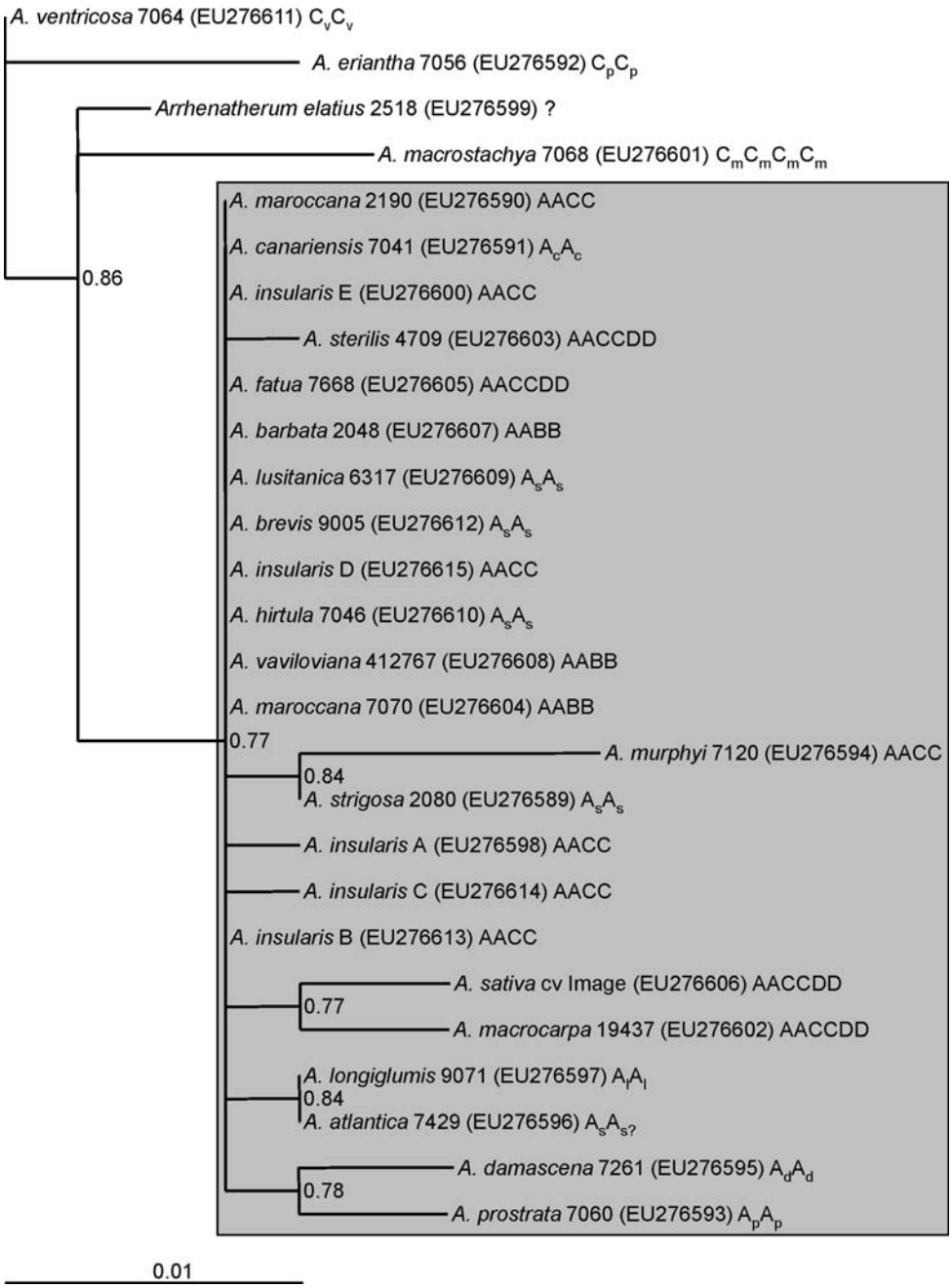

Fig. 5 Phylogram of *Avena* spp. based on the trnL intron. Sequences included in the *shaded box* are from diploids and polyploids containing the A-genome. Separation from C-genome diploids is evident

organising regions. Nucleolar dominance results from selective silencing of one of the parental rRNA gene loci in interspecific hybrids or allopolyploids (Pikaard 2000). Reeder (1985) proposed the 'enhancer-imbalance' model, suggesting that active rRNA genes have more and stronger enhancers, hence longer spacers than inactive genes. This was confirmed in hexaploid wheat (Flavell 1986). However, Nikoloudakis et al. (2008) reported equal lengths of the Inter-Genic Spacers in *Avena* spp., and thus nuclear dominance in *Avena* is probably not related with enhancer length. Silenced rRNA genes in Arabidopsis, allotetraploid Brassicas (Chen and Pikaard 1997) and Triticale (Neves et al. 1995) were reactivated by chemical inhibition of DNA methylation or histone deacetylation or both, suggesting that rRNA gene silencing presumably is associated with inactive chromatin structure. However, sequence elimination of C-genome rRNA in AACC and AACCDD polyploids has been reported (Jellen et al. 1994; Fominaya et al. 1995; Linares et al. 1996), narrowing the possibility of epigenetic modifications in allopolyploids. On the other hand, in situ hybridization revealed that the barbata tetraploid group (AABB) contains twice as many major sites of rRNA sites as the diploid A_s-genome species, although these tetraploid and diploid species have the same number of satellited chromosomes (Leggett and Markhand 1995; Katsiotis et al. 1997).

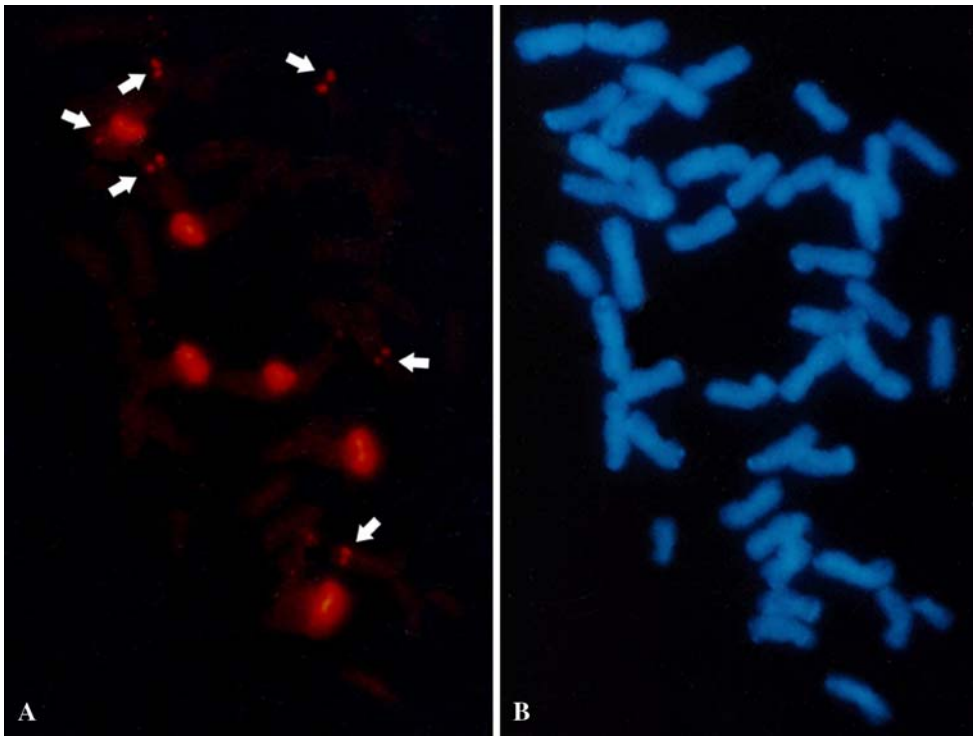

Fig. 6 Localization of 18S-26S rRNA sequences in *A. sativa* cv. 'Sun II', using the pTa71 biotin-labelled probe. Hybridization sites were detected using the Streptavidin-Cy3 conjugate. Chromosomes were counter-stained with DAPI. The six minor sites are indicated by *arrows*

Using in situ hybridization and pTa71 as a probe, Yang et al. (1999) identified three major pairs of rRNA sites located on A-genome/D-genome chromosomes and a minor pair on C-genome chromosomes in *A. fatua*. These results were in agreement with findings in other hexaploid species of *Avena*, which showed that none of the C-genome chromosomes carried a major site for 18S-26S rRNA (Chen and Armstrong 1994; Fominaya et al. 1995; Linares et al. 1996; Irigoyen et al. 2002). Minor rRNA sites were localised on a pair of submedian C-genome chromosomes. Fominaya et al. (1995), using pTa71 and a C-genome specific probe (pAm1), identified a minute rRNA site in *Avena* hexaploids on the short arm of a C-genome chromosome. Therefore, it is likely that polyploid formation causes elimination of these sequences as suggested by Jellen et al. (1994). We were able to identify six major and six minor 18S-26S rRNA sites on the chromosomes of *A. sativa*, which is in general agreement with the previous studies.

Recently, Shelukhina et al. (2007), using C-banding and in situ hybridization techniques, were able to conduct a comparative cytogenetic analysis among the AACC tetraploids. Thus, *A. insularis* and *A. magna* were found to contain four major and six minor sites when pTa71 was used as a probe, while four major sites and only two minor were detected in *A. murphyi*. All minor sites were assigned to C-genome chromosomes.

By means of genome-specific SCAR markers, we were able to isolate and identify C-genome ITS1-5.8S-ITS2 clones in AACC/AACCDD *Avena* polyploids. Based on colony counts and positive PCR amplifications, C-genome rRNA sequences have been extensively reduced to an approximate 1.5:100 ratio of C-genome rRNA loci to A-genome rRNA sequences.

Data analysis of 11 C-genome clones isolated from AACC and AACCDD genome species revealed extensive sequence similarities to *A. ventricosa* (C_vC_v) ITS1-5.8S-ITS2 locus, supporting its active role in the evolution of *Avena* polyploids. Furthermore, major dissimilarities to *A. eriantha* and *A. clauda* Dur. species (C_pC_p) were observed, diminishing the possibility of their participation in the *Avena* allopolyploid formation. Molecular data concur with chromosome pairing results, since pairing studies in interspecific hybrids minimise the likelihood of *A. eriantha* contribution to the formation of *A. maroccana* (Leggett 1998), while *A. ventricosa* could be considered the donor of the C-genome in allopolyploids (Rajhathy and Thomas 1967). Thus, according to sequence results of the present study along with previous reports (Rajhathy 1966; Loskutov 1999), it is evident that *A. ventricosa* is the C-genome donor in tetraploid and hexaploid species.

The perennial autotetraploid species *A. macrostachya* ($C_mC_mC_mC_m$) was also clustered within the C_v-genome group. In previous studies based on nucleotide and RFLP data (Nikoloudakis et al. 2008), clustering of *A. macrostachya* reflects a closer affinity to C_v-genome species rather than to C_p-genome species. Major similarities among *A. eriantha* and *A. clauda* and dissimilarities to

C_mC_m-genome species and C_v-genome species were also observed. These results are consistent with Malzew studies (Malzew 1930), revealing major differences in the ecology and morphology of these taxa and categorising two series of C-genome diploid species: *A. eriantha* and *A. clauda,* and *A. ventricosa* and *A. bruhnsiana* Grun. Crosses among species within each series yield fertile offsprings, while complete sterility was demonstrated in crosses among species between the two series (Loskutov 2001).

Murai and Tsunewaki (1987) analysed cpDNA of the most representative *Avena* species, by means of an RFLP technique, and classified chloroplast genomes into five types (I–V). Type I cpDNA included the majority of the A-genome species (A_s, A_c, A_d, A_p) and all polyploids. Type II cpDNA included *A. longiglumis* (A_lA_l) and was isolated by only one mutation from type I group. C-genome taxa were classified as type III (*A. clauda*; C_pC_p), type IV (*A. eriantha*; C_pC_p) and type V (*A. ventricosa*; C_vC_v) chloroplast genomes. TrnL intron nucleotide data from the present study are in general agreement with the above classification. High affinity among the A-genome species and polyploids was observed. In addition, notable similarities between *A. longiglumis* and *A. atlantica*, as well as *A. prostrata* and *A. damascena*, were shown. Nikoloudakis et al. (2008) using nuclear sequences (ITS1-5.8S-ITS2 sequences and IGS-RFLP) also revealed close proximity among the above species. C-genome diploid species have major cytoplasmic differences from species carrying the A-genome. It is noteworthy that none of the allopolyploids studied was clustered with C-genome diploids. This clearly demonstrates that during interspecific hybridization events generating AACC allopolyploids, the C-genome taxon was the male donor, since plastids are cytoplasm-inherited. Finally, the possibility of parallel evolutionary events cannot be ruled out uncritically, considering the high affinity observed between *A. murphyi* and *A. strigosa*.

In conclusion, the present work expands information on the evolutionary pathways that gave rise to *Avena* polyploid species and provides molecular evidence that *A. ventricosa* is the C-genome diploid donor. Major C-genome rRNA sequence elimination is revealed in allopolyploids and nucleotide analysis from cpDNA supports the ♀ A-genome × ♂ C-genome crossing direction for the AACC interspecific hybrids, which were a genetic bridge to the cultivated hexaploid oats.

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