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RESEARCH ARTICLE

Karyotype analysis and physical mapping of 45S rDNA in eight species of *Sophora*, *Robinia*, and *Amorpha*

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Abstract The karyotype analysis and physical locations of 45S rDNA were carried out by means of fluorescence *in situ* hybridization in three species, and two forms of *Sophora*, two species of *Robina,* and one species of *Amorpha. S. japonica* L., *S. japonica* L. f. *oligophylla* Franch., *S. japonica* L. f. *pendula* Loud., and *S. xanthantha* C. Y. Ma. are all tetraploids with 2n = 28. There were four 45S rDNA sites in pericentromeric regions of two pairs of chromosomes in each of them. *S. rubriflora* Tsoong. is a triploid with 2n = 21, and three sites were located in each satellite of group 5 chromosomes. In *R. pseudoacacia* L. (2n = 2*x* = 22), we examined four intensive signals in telomeric regions of two pairs of satellite chromosomes. In *R. hispida* L. (2n = 2*x* = 30), there were four other signals in centromeric regions besides those like in *R. pseudoacacia. Amorpha fruticosa* L. has most chromosomes (2n = 40) among the eight materials, however, there were only six 45S rDNA loci and they laid in centromeric regions, and satellites of three pairs of chromosomes. 45S rDNA is a valuable chromosomal landmark in karyotype analysis. The distribution and genomic organization of rDNA in the three genera were also discussed.

Keywords *Sophora*, *Robinia*, *Amorpha*, karyotype analysis, 45S rDNA, fluorescence *in situ* hybridization (FISH)

1 Introduction

One of the most extensively studied genetic units in the plant genome is the ribosomal RNA genes (18S-5.8S-25S and 5S rDNA) and their chromosomal localization via fluorescence *in situ* hybridization (FISH). Ribosomal DNAs are highly conserved and repeated gene families with hundreds to thousands of copies, and concentrated in one or more clusters of one and/or multiple chromosome pairs (Pedersen and Linde-Laursen, 1994). Owing to easy visualization of rDNA sites by FISH, these genes are excellent cytological markers for karyotype analysis especially in species with many small and equal chromosomes. Zoldos et al. (1999) characterized the chromosome distribution of rDNA loci in 11 *Quercus* species, and six chromosomes can be unambigu- ously distinguished among 24 chromosomes with similar morphology. In spite of their high conservation and stable physical location of chromosomes, rDNA is changeable during species evolution. Both the position and number of rDNA loci and their copy number can be discrepant among species even with close relationship and even within a species. Naganowska and Zielińska (2002) analyzed some interspecific variation among five *Lupinus* species through the number and size of the rDNA loci. Torrell et al. (2003) developed cytotaxonomy in seven *Artemisia* species by rDNA-FISH combined with fluorochrome banding. Thomas et al. (2001) characterized the position and number of rDNA loci of eight genotypes of *Lolium rigidum*, which revealed extensive chromosome structural variations among these genotypes. Galasso et al. (1998) observed the increasing number of rDNA loci in *Vigna unguiculata* during its domestication. Taketa et al. (1999) examined the physical locations of rDNA in nine wild *Hordeum* species and cytotypes, and they found that distribution of rDNA is very valuable for the study on karyotype evolution and phylog- eny of the genus *Hordeum*. Hanson et al. (1996), Singh et al. (2001), and Gu and Xiao (2003) considered the distribution of rDNA loci as molecular cytogenetic clue for polyploid origin study on *Gossypium*, *Glycine*, and *Camellia,*, respectively. *Sophora*, *Robinia*, and *Amorpha* are all belongs to the family Leguminoseae. *Sophora* is deciduous or evergreen arbor and shrub including about 70 species with large distribution ranged from tropic to temperate zones. There are 21 species, 14 varieties, and two forms of *Sophora* are

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existing in China. *S. japonica* L. is a good shade trees and nectar source. The two forms of *S. japonica* (*S. japonica* L. f. *oligophylla* Franch. and *S. japonica* L. f. *pendula* Loud.) and two other *Sophora* species (*S. xanthantha* C. Y. Ma. and *S. rubriflora* Tsoong.) are important garden tree species. *Robina pseudoacacia* L., *R. hispida* L., and *Amorpha fruticosa* L. are garden trees and species in plantation establishments (Chen et al., 1994). Although Chen et al. (2003) have performed karyotype analysis in these species, studies on molecular cytogenetics on them are rare. In this study, we localized rDNA loci on metaphase chromosomes of the eight tree species by FISH, aimed to provide chromosome markers for detailed karyotype analysis, and studied genomic organization of rDNA and interspecific variations in *Sophora*, *Robinia*, and *Amorpha.*

2 Material and method

2.1 Material

S. japonica L. (voucher number: Chen Ry 2003-123), *S. japonica* L. f. *oligophylla* Franch. (Chen Ry 2003-124), *S. japonica* L. f. *pendula* Loud. (Chen Ry 2003-125), *S. xanthantha* C. Y. Ma. (Chen Ry 2003-126), *S. rubriflora* Tsoong. (Chen Ry 2003-61), *R. pseudoacacia* L. (Chen Ry 2003-121), and *R. hispida* L. (Chen Ry 2003-122) were collected in the campus of Nankai University, China. Seeds of *A. fruticosa* L. (Chen Ry 2003-136) were collected from Laoting in Hebei province, China. The specimens were kept in College of Life Sciences, Nankai University, China.

2.2 Preparation of metaphase chromosomes

The tender buds were collected in early spring for chromosome analysis except that *A. fruticosa* root tips were used. In brief, materials were immersed in saturated *p*-dichlorobenzene for 3 hours and fixed in fixative (methanol: acetic acid = 3: 1). The chromosome spreads were prepared based on Chen et al. (1979). Specimens with good morphology and few overlapping were selected for FISH.

2.3 Probe preparation and FISH

Plasmids containing 45S rDNA were provided by Prof. Song Yunchun, Wuhan University, China. The 9.1 kbp long 45S rDNA sequence from tomato (Lycopersicon pennellii LA716, Arumuganathan et al., 1994) included 18S, 5.8S, 28S and non-transcribed spacer sequences. The 45S rDNA was labeled with digoxigenin-11-dUTP (Roche) by randomly primed DNA synthesis. FISH and signal detection were carried out as described by Qi et al. (2002).

3 Results

3.1 Conventional karyotype analysis

We have performed karyotype analysis in the eight species previously (Chen et al. 2003), and the results were shown in Table 1.

3.2 Physical mapping of 45S rDNA in the eight tree species

Chromosome spreads and FISH results with 45S rDNA probe were shown in Fig. 1. Chromosome pairing and alignment were shown in Fig. 2. The chromosome number bearing 45S rDNA were shown in Table 1. Among the eight species, we did not find multiple 45S rDNA loci located in a single chromosome.

In *S. japonica* L., a pair of signals with slight difference in size was located in pericentromeric regions of submetacentric chromosome 2. Another pair of signals was located in pericentromeric regions of metacentric chromosome 7 and they were larger than the signals on chromosome 2. In addition, one signal of this pair has much more intensity than the other that it almost overlapped the short arm.

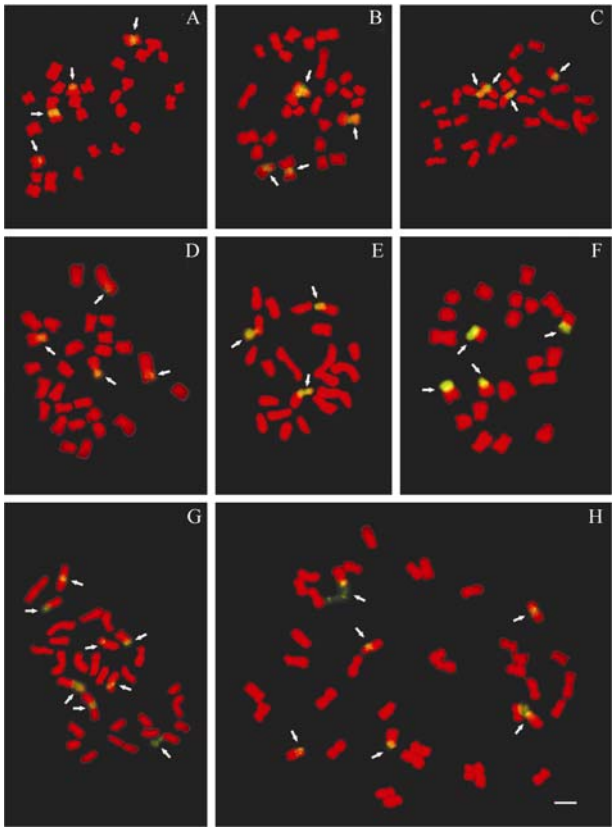
In *S. japonica* L. f. *oligophylla* Franch., a pair of signals with similar intensity was localized in pericentromeric regions of metacentric chromosome 2, one of which almost overlapped the short arm. Another pair of signals was localized in pericentromeric regions of submetacentric chromosome 4 and they were different both in size and intensity.

Table1 The karyotypic data and 45S rDNA loci of the 8 species in this study

Taxa	Karyotype formula	A.A.R	Lt/St	P.C.A/%	As. K/%	Type	rDNA loci[a]
S. japonica	2n=4*x*=28=18m+10sm	1.678	2.04	29	61.70	2B	2, 7
S. japonica f. *oligophylla*	2n=4*x*=28=14m+10sm+4st	1.913	2.38	29	63.48	2B	2, 4
S. japonica f. *pendula*	2n=4*x*=28=18m+8sm+2st	1.717	2.34	36	61.38	2B	2, 7
S. xanthoantha	2n=4*x*=28=14m+12sm+2st	1.962	1.97	21	62.38	2A	1, 3
S. rubriflora	2n=3*x*=21=6m+6sm+6st+3t	3.631	1.72	57	69.97	3A	5*
R. pseudoacacia	2n=2*x*=22=4m+8sm+10st	3.562	2.38	82	71.67	3B	6*, 9*
R. hispida	2n=2*x*=30=10m+12sm+6st+2t	2.770	2.06	60	66.41	3B	2, 6, 11*, 13*
A. fruticosa	2n=2*x*=40=32m+8sm	1.427	1.70	0	58.27	1A	8, 10*, 13

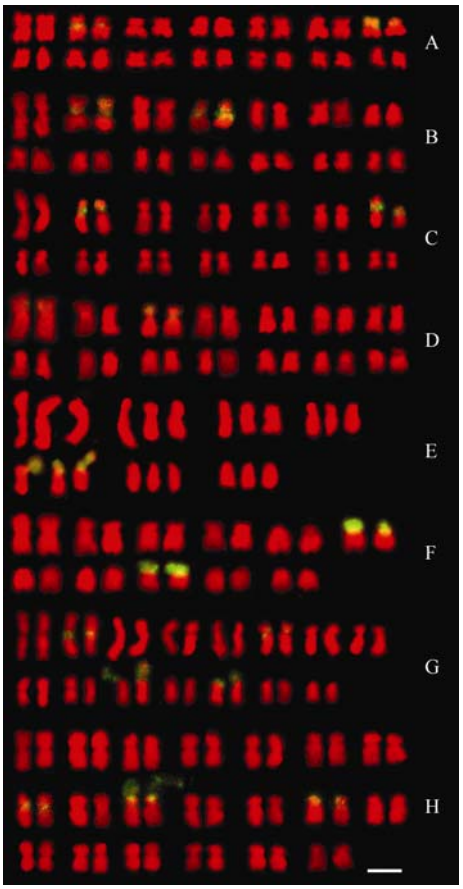
[a]: Shows the chromosome number bearing 45S rDNA loci; ∗:Denote satellite chromosomes;

Abbreviations: A.A.R = Average arm ratio, Lt = Longest arm, St = Shortest arm, P.C.A. = Percentage of chromosome with arm ratio > 2



3A: S. joponica; B: S. japonica f. oligophylla; C: S. japonica f. pendula; D: S. xanthantha; E: S. rubriflora; F: R. pseudoacacia; G: R. hispida; H: A. fruticosa; Bar = 5μm

Fig. 1 Fluorescence *in situ* hybridization with 45S rDNA on metaphase chromosomes of the eight species



A: S. joponica; B: S. japonica f. oligophylla; C: S. japonica f. pendula; D: S. xanthantha; E: S. rubriflora; F: R. pseudoacacia; G: R. hispida; H: *A. fruticosa*; Bar = 5μm

Fig. 2 The karyotypes of the eight species

In *S. japonica* L. f. *pendula* Loud., two pairs of signals with slightly different intensities were localized in pericentromeric regions of submetacentric chromosomes 2 and 7, respectively. However, there was one signal of each pair almost overlapping the short arms.

In *S. xanthantha* C. Y. Ma., the signal intensity was the faintest among the five *Sophora* species. The pair of signals on the short arms of metacentric chromosome 1 was almost invisible. Another pair of signals in pericentromeric regions of submetacentric chromosome 3 had a little stronger intensity, one of which overlapped the short arm.

In *S. rubriflora* Tsoong. group 5 were satellite chromosomes, two of which had large and distinct satellites and one had a small satellite attached to its end. There were three signals with strong intensity localized on the secondary constriction and the whole satellites of the three chromosomes.

In *R. pseudoacacia* L., the signal intensity was the strongest among all the materials in this study. The two pairs of signals were also showed intensity differences among each pair, and were localized on the satellites of subtelomeric chromosomes 6 and 9, respectively.

In *R. hispida* L., two pairs of smaller signals were localized in pericentromeric regions of metacentric chromosome 2 and submetacentric chromosome 6, respectively. The other two pairs of larger signals were localized on the secondary constrictions and the whole satellites of subtelocentric chromosome 11 and telocentric chromosome 13, respectively.

In *A. fruticosa* L., a pair of large signals was localized on the satellites of submetacentric chromosome 10 and their intensity increased at the end of the chromosomes. The other two pairs of smaller signals were localized in pericentromeric regions of metacentric chromosome 10 and submetacentric chromosome 13, respectively.

4 Discussion

4.1 Detailed karyotyping and comparison based on rDNA loci

According to the Stebbins (1970) classification, *A. fruticosa* L. that karyotype fall into "1A" type is relatively ancient, and the genus *Robinia* showing "3B" type is relatively young, whereas the genus *Sophora* is between *Amorpha* and *Robinia*.

In the genus *Sophora*, the karyotype of *S. japonica* and its two forms, *S. japonica* f. *oligophylla* and *S. japonica* f. *pendula*, fall into "2B" type with similar karyotype data (Table 1). In addition, all of them have a pair of 45S rDNA loci on their chromosome 2, especially for *S. japonica* f. *pendula*, in which the genomic distribution pattern of 45S rDNA is completely the same with that in *S. japonica*. These similarities suggest that chromosome structural variation may not occurred in the two forms compared with their original species. The karyotype of *S. xanthantha* falls into "2A" type shows more symmetry, whereas *S. rubriflora* that belongs to "3A" type is more evolutional. The average arm ratio of *S. xanthantha* and *S. rubriflora* is greater than that of *S. japonica* (Table 1), which indicates that the asymmetry of chromosome arms increases markedly in these two species. In our study, none of the satellites was found in the four tetraploids of the genus *Sophora,* and the rDNA loci were only located in pericentromeric regions near the short arms of chromosome (Fig. 1 A−D). Chen et al. (2003) presented chromosome 4 of *S. japonica* as satellite chromosomes because the short arm of one chromosome of this pair was a little distant from its long arm so that this short arm was presented as a satellite (data not shown, and it looks like chromosome 7 of *S. japonica* f. *pendula* in Fig. 2 C). However, according to our rDNA-FISH results and observation in a number of chromosome spreads of *S. japonica*, it is more sensible to represent the "satellite" as the short arm of chromosome 4, and the location of 45S rDNA on this pair is centromeric but not secondary constriction. Because the length of the portion that has been presented as satellites previously has not taken into account into chromosome length, the alignment of chromosomes of *S. japonica* is modified, in which chromosome 4 in Chen et al. (2003) has been presented as chromosome 2 in this paper. It is clear that the location of rDNA can assist in more precise karyotype analysis and partially increases the reliability of homology pairing, especially for species with small chromosomes in similar morphology.

S. japonica, *S. japonica* f. *oligophylla*, *S. japonica* f. *pendula*, and *S. xanthantha* are all tetraploids, and four 45S rDNA loci are found in each of them (Fig. 1A−D). In triploid *S. rubriflora*, three 45S rDNA loci are found (Fig. 1E). These results suggest that the number of rDNA loci is in positive correlation with the ploidy levels in the genus *Sophora*. The two pairs of signal in each of the four *Sophor* tetraploids share some common traits. One of the signals in a single pair is larger and strong, whereas the other one is smaller and weak. In addition, most of the large signals overlap the whole short arms, whereas the smaller ones concentrate in the pericentromeric regions of the chromosomes. So we speculate that these traits of rDNA loci maybe exist widely in the genus *Sophor* and even their diploid ancestors. However, this should be approved by localization of rDNA loci in many other *Sophor* species.

4.2 Genomic organization and evolution of 45S rDNA in the eight tree species

Except *S. rubriflora*, the rDNA hybridization signals of different loci showed prominent differences in size and intensity, which also happen in many other plant species (Hanson et al., 1996; Vanzela et al. 2002; Muravenko et al. 2003). In our study, the size and intensity of hybridization signals were also found different between homologous sites of 45S rDNA. Zoldos et al. (1999) found difference between homologous sites of rDNA in the genus *Quercus,* and one site of the locus was not always detectable. However, it does not occur so commonly. Although *in situ* hybridization cannot show the number of gene copies directly, the size and intensity of hybridization signals can reflect the number of gene copies indirectly, so that FISH is considered to be a semi-quantitative technique (Maluszynska and Heslop-Harrison, 1993). The variation in copy number of genes is related to the amplification, deletion, or unequal crossing over of genes, which may be the main cause for signal differences.

The 45S rDNA multigene families were located in a chromosomal nucleolus organizer region (NOR), which was cytologically visible as a secondary constriction (SC) associated with distal satellite. In this study, all the satellite chromosomes showed prominent hybridization signals that were not only located in SCs but also overlaps the whole satellites (Fig. 1 E-H). This result suggests that the satellites in the three genera are mostly composed with multicopies of ribosomal RNA gene families. In *R. hispida* and *A. fruticosa*, there are also rDNA signals located in pericentromeric regions and short arms. In *Cucumis sativus* (Koo et al., 2002) and the four *Sophor* tetraploids in this study. All the rDNA loci were located in pericentromeric regions. Many *in situ* hybridization experiments by the use of 45S rDNA as a probe have revealed the presence of minor 45S rDNA sites in addition to the major 45S rDNA sites in SCs and satellites in various species. The hybridization signals in the minor sites were always smaller than those in SCs and many of them were without transcription activity. The origin of minor 45S rDNA sites was not well understood (Taketa et al., 1999). 45S rDNAs were mostly located in centromeric regions and with short arms, whereas the loci on long arms were seldom. Lima-De-Faria (1976) analyzed the nucleolus-organizing citrons in over 700 species and reported that, in 87% cases, the nucleolus was located on the short arm of the chromosomes. Such striking conservatism in karyotype morphology suggests some molecular or physical constraints for chromosome arms to associate with the nucleolus (Lim et al., 2001).

The 45SrDNA sites were useful chromosome landmarks and provide valuable evidence about genome evolution at both molecular and chromosomal levels (Taketa et al., 1999). They can reflect the degree of discrepancy among species or genera (Thomas et al., 2001). In the four *Sophor* tetraploids, the number of 45S rDNA loci was completely the same and their genomic distribution was similar. These results suggested the little discrepancy among the four species at chromosomal level.

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