RESEARCH NOTE

Karyotype of asparagus by physical mapping of 45S and 5S rDNA by FISH

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Introduction

Garden asparagus (*Asparagus officinalis* L.) is an economically important plant with 2n = 2x = 20 chromosomes and a haploid genome size of 1323 Mb (Bennett and Leitch 2003). The karyotype of asparagus consists of five long (L), one medium (M) and four small (S) chromosomes (Löptien 1976). Plants of this dioecious species differ in producing either male or female flowers. The sexual dimorphism in asparagus is controlled by a region located on a pair of homomorphic sex chromosomes (chromosome L5) (Löptien 1979) termed the M locus (Flory 1932; Uno et al. 2002). Maleness is dominant with males normally heterogametic (Mm) and females homogametic (mm). Supernumaries (MM) can be produced from anther culture or from rarely occurring andromonoecious plants. The identification of chromosome L5 is important for studying sex chromosome evolution in asparagus. However, it was observed that the sex chromosome pair is homomorphic: X and Y chromosomes do not differ in morphology.

Telgmann-Rauber et al. (2007) tried to clone the region determining sex in asparagus from its position in the genome. Molecular cytogenetic and sequence analyses of bacterial artificial chromosomes (BACs) flanking M locus indicate that the BAC contain highly repetitive sequences that localize to centromeric and pericentromeric locations on all asparagus chromosomes. However, the L5 chromosome could not be distinguished by BAC-FISH (Telgmann-Rauber et al. 2007).

Recently, FISH has become a powerful and useful tool for the direct detection of specific DNA in genome. In this technique, ribosomal DNA genes (45S and 5S rDNA) are commonly used as markers for the physical mapping of plant chromosomes to analyse genomic organization (Nakayama et al. 2001; Lan et al. 2006; Grabowska-Joachimiak et al. 2011; Novotná et al. 2011). To date, there is no report on rDNA loci in *A. officinalis* L. detected by FISH. The objective of this study was to detect 45S and 5S rDNA in asparagus chromosomes using FISH.

Materials and methods

Plant materials and chromosome preparation

Seeds of *A. officinalis* were germinated and seedings were cultivated in greenhouse. The radicles were collected and pretreated according to Kato et al. (2004). Excised root tips were treated with nitrous-oxide gas for 2 h. They were fixed in ice-cold 90% acetic acid for 10 min and stored in 70% ethanol at −20°C until use. After washing in water on ice, the section containing dividing cells was dissected and digested in 1% pectolyase Y23 (Yakult Pharmaceutical, Tokyo, Japan) and 2% cellulose Onozuka R-10 (Yakult Pharmaceutical) solution for 50 min at 37°C (one section per tube with 20 μL of enzyme solution). After digestion, the root sections were washed once in ice-cold-distilled water and then twice in 100% ethanol for 5 min. The meristematic region of the root was carefully broken using a needle, and vortexed at maximum speed in 100% ethanol for 30 s at room temperature to separate cells from one another. The cells were collected at the bottom of the tube by centrifugation and resuspended in acetic acid: ethanol (9:1 dilution) solution. The cell suspension was dropped onto glass slides in a box lined with wet paper towels and dried slowly.

Probe labelling

For somatic cell analysis, 45S rDNA that consist of a 18s-5.8s-25s rDNA gene cluster (Sano and Sano 1990) and 5S rDNA were labelled with Texas-red-dCTP using nick translation reactions according to Birchler et al. (2008).

Keywords. Asparagus; fluorescence in situ hybridization; 45S rDNA; 5S rDNA; *Asparagus officinalis* L.
Fluorescence in-situ hybridization (FISH)

The FISH procedure was according to Gao et al. (2011). The slides were UV-crosslinked for 2 min, washed in 2× SSC (3 × 5 min) and rinsed in 70%, 95%, 100% ethanol for 5 min each and air dried for 30 min. After application of 6 μL probe solution (4 ng/μL of each probe in 2× SSC and 1× TE buffer, previously denatured for 5 min in boiling water and then placed on ice), the slides were heated for 5 min at 100°C, incubated at 55°C overnight in a humid chamber. After hybridization, slides were washed in 2× SSC and mounted in Vectashield mounting medium (containing 1.5 μg/mL DAPI, Vector Laboratories, Burlingame, USA). The FISH images were recorded using a Zeiss universal microscope (Jena, Germany); images were captured with a Magnafire charge-coupled device camera (Tokyo, Japan) and processed with Photoshop 7.0 (http://www.adobe.com/products/photoshop.html).

Results and discussion

Karyotype of male and female plants of asparagus

All plants examined had a basic chromosome number of 2n = 20 (figure 1, A&B). The chromosome complement was composed of six long submetacentrics, four long metacentrics (contain two satellite chromosomes), two medium submetacentrics, two small submetacentrics and six small metacentrics (figure 1, C&D and figure 2). The results were the same as previous studies (Löptien 1976). Chromosome L5 was a long metacentric chromosome with satellite.

Physical mapping of 45S and 5S rDNA loci

Texas red hybridization signals on the chromosomes stained by DAPI (4',6-diamidino-2-phenylindole) are shown in figure 1, A&B. Six obvious 45S rDNA sites labelled with texas red were clearly observed as red signals on the three pairs of chromosomes (figure 1, A&C and figure 2A). Four hybridization signals of the 45S rDNA were located on the long arm of chromosomes 1 and 2. Two hybridization signals of the 45S rDNA were located at the distal end of chromosome L5 with satellite. With respect to the chromosomal localization of 5S rRNA genes, six obvious and two weak 5S rDNA sites labelled with texas red were observed on the four pairs of chromosomes (figure 1, B&D and figure 2B). Six obvious hybridization signals of the 5S rDNA were overlapped with the 45S hybridization signals on chromosomes 1, 2 and 5. Two weak hybridization signals of the 5S rDNA were located on the short arm of chromosome 4. The results showed that the hybridization signals of both 45S

Figure 1. Cytological analysis of asparagus by FISH using 45S rDNA and 5S rDNA as probes. (A) 45S rDNA is in red; (B) 5S rDNA is in red; (C) the karyotype of asparagus using 45S rDNA; (D) the karyotype of asparagus using 5S rDNA.