A New 45S rDNA Locus and Its Genomic Origin in the Amphidiploid of Scilla scilloides Druce

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Summary Two amphidiploids of Scilla scilloides collected in Matsuyama, Japan, had new secondary constrictions at the interstitial region of the long arm of one subtelocentric chromosome in addition to the secondary constriction at the proximal region of the short arms of a pair of subtelocentric b1 chromosomes commonly found in amphidiploids. In situ hybridization using a 45S rDNA probe revealed 3 strong signals at the secondary constrictions and 2 weak signals. Genomic in situ hybridization using 45S rDNA and genomic DNA from either genome A or B indicated that the subtelocentric chromosome with the new 45S rDNA locus was chromosome a3 of genome A. Genomic Southern analysis showed that the rDNA at the new locus of chromosome a3 originated from the 45S rDNA of genome B.

Key words Genomic in situ hybridization, Scilla scilloides, Secondary constriction, 45S rDNA locus.

In plants, the location, size, shape, and number of secondary constrictions and related structures sometimes differ among species or among populations or individuals of the same species. The number of secondary constrictions or nucleolar organizer regions (NORs) varies in number in many species. The size of the secondary constriction also fluctuates in organisms such as Ornithogalum montanum, as revealed by conventional staining (Maggini et al. 1980), and Pseudotsuga menziesii, shown by chromomycin A3 banding (Hizume and Akiyama 1992). The size differences are explained by variation in the number of 45S rDNA genes as a result of unequal recombination. Variation in the number of rDNA repeats among individuals has been demonstrated using molecular techniques in flax (Timmis and Ingle 1973), Zea mays (Givens and Phillips 1976), Pisum sativum (Cullis and Davies 1975), Allium fistulosum (Yampol and Pospelov 1978), O. montanum (Maggini et al. 1980), and P. menziesii (Strauss and Tsai 1988). The addition of a secondary constriction with the ability to behave as a mobile genetic element was reported in Allium hybrid plants (Schubert 1984) and Oryza species (Shishido et al. 2000). Despite widespread reports of variation in the number of secondary constrictions, the origin of these differences has not been investigated.

The Scilla scilloides complex is comprised of diploids and polyploids of genomes A and B. In the amphidiploid plants (AABB), except for plants growing in the Okinawa islands, and polyploids (ABB, ABBB) derived from the amphidiploid, secondary constrictions of genome B remain, but the secondary constriction of genome A is lost (Araki 1972, Araki et al. 1979, Haga and Noda 1976). In situ hybridization (ISH) using a 45S rDNA probe revealed that loss of the secondary constriction was a consequence of a large deletion of 45S rDNA (Hizume and Araki 1994), not of differential ampliplasty, as in interspecific hybrids of Crepis (Nawashin 1934), Hordeum (Subrahmanyan and Azad 1978), triticale (Lacadena et al. 1984), wheat (Mukai et al. 1991), and Aegilopus (Mukai 1996). The secondary constriction of the longest chromosome of genome B (b1 chromosome) moved as a result of a translocation of the chromosome arm carrying the secondary constriction in

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AABB (Noda 1961, Uchino and Miyazaki 1986) and ABBB plants (Hizume and Araki 1996). A new 45S rDNA locus was discovered in certain populations of AABB plants. This study aimed to identify the chromosome carrying the new 45S rDNA locus and to reveal the genomic origin of the rDNA contained within that locus.

Materials and methods

Two amphidiploid plants (2n=34, AABB) of Scilla scilloides Druce carrying a new secondary constriction were discovered in natural populations collected on the bank of the Shigenobu River, Izumi, Matsuyama, Japan. The population is composed of numerous AABB amphidiploids. Plants homozygous for the new secondary constriction were found in progeny of the variants. For genomic DNA preparation, plants were collected from Yongdang-dong, Migpo, Jeonra-namdo, Republic of Korea (AA diploids) and from Nakabaru, Miyoki, Saga, Japan (BB diploids). The diploid plants were supplied from the S. scilloides stock collection at the Fukuoka University of Education. Materials used in this study are preserved in the experimental garden at Faculty of Education, Ehime University.

Root-tips were collected, treated in 2 mM 8-hydroxquinoline for 3 h, and fixed in acetic acid: ethanol (1:3). For ISH experiment, chromosomes were prepared by acid maceration with 45% acetic acid at 60°C for at least 2 min. For genomic in situ hybridization (GISH), chromosomes were prepared by enzymatic maceration with 2% Cellulase Onozuka RS (Yakult, Tokyo) and 0.5% pectolyase Y-23 (Seishin Pharma. Co., Tokyo) at 37°C for 1 h. The macerated materials were squashed in 45% acetic acid and allowed to air-dry after dry-ice coverslip removal. Young flower buds were fixed in Carnoy’s fixative (ethanol: chloroform: acetic acid, 2:1:1), and pollen mother cells (PMCs) were squashed in 45% acetic acid and air-dried.

Cloned wheat 45S rDNA (pTa71; Gerlack and Bedbrook 1979) and total genomic DNA, extracted by the CTAB method (Murray and Thompson 1980), from leaves of Korean AA diploids and Japanese BB diploids were used as probes for ISH and GISH. One μg of probe DNA was biotin-labeled by nick translation using the BioNick Labeling system (BRL), according to the manufacturer’s instructions. Probes were dissolved in 400 μl of 2×SSC/50% formamide/5% dextran sulfate. ISH and GISH procedures were described previously (Hizume and Araki 1994, 1996).

Chromosome designations were done according to Araki (1971) and Ono et al. (1994). Chromosomes of genomes A and B are designated in descending order of length, a1 to a8 and b1 to b9, respectively. The a2 and b1 chromosomes in each diploid genome possess the secondary constriction on the proximal region of the short arm.

Genomic DNA from leaves of Korean AA diploids, Japanese BB diploids, standard AABB plants, and variants carrying the new 45S rDNA locus was digested with 13 different endonucleases: EcoRI, HindIII, BamHI, EcoRV, PstI, KpnI, Sall, PvuII, Alul, Hpal, Mbol, HaeIII, and RsaI (Takara). Fragments of the digested DNA were separated on 1.5% agarose gels and blotted to nylon membranes (Magnagraph). The wheat 45S rDNA probe was labeled with digoxigenin (DIG, Roche Diagnostics). Labeling, hybridization, and detection were done according to the instructions for the DIG DNA Labeling and Detection Kit (Roche Diagnostics). Lambda DNA digested with HindIII and pTa71 digested with EcoRI and BamHI were used as molecular weight markers.

Results and discussion

Standard amphidiploids (2n=4x=34, AABB) of S. scilloides have 2 secondary constrictions at the proximal region of the subtelocentric b1 chromosomes and form 1 or 2 nucleoli in each nucleus (Sato 1940, Haga and Noda 1974). The secondary constriction of chromosome b1 contains large amounts of 45S rDNA; chromosome a1 has a small amount of rDNA at the proximal region of the
short arm (Hizume and Araki 1994). Two plants having nuclei with 3 nucleoli and 3 secondary constrictions on one chromosome compliment were found in a population collected in Matsuyama, Japan. The plants had 2n=34 chromosomes and karyotypes similar to the standard amphidiploid plant. In addition to the 2 secondary constrictions at the proximal region of the short arm of b2 chromosome observed in standard amphidiploids, the variants had an additional secondary constriction at the interstitial region of the long arm of one subtelocentric chromosome. ISH was performed, using the 45S rDNA probe, and revealed 3 strong and 2 weak signals in one nucleus and chromosome complement (Fig. 1). The strong signals appeared on nucleoli, and the weak signals appeared in the nucleoplasm (Fig. 1A). At somatic metaphase (Fig. 1B), 2 strong signals were located at the secondary constriction of the b1 chromosome, and 2 small signals were found at the proximal region of the a3 chromosomes, as observed in standard AABB plants (Hizume and Araki 1994). Another strong signal was localized at the secondary constriction of one subtelocentric chromosome (arrow in Fig. 1B). At metaphase in the first meiotic division, 17 bivalents appeared in the pollen mother cells. One bivalent of the b2 chromosome had 2 strong signals and the other had one...
The chromosomes heterozygous for the additional rDNA locus formed normal bivalents in all PMCs scored, indicating no large chromosome segment was translocated. A new secondary constriction at the interstitial region of the long arm of a subtelo-centric chromosome was also reported in an ABBB plant collected in Kochi (Hizume and Araki 1996).

In order to determine to which genome the subtelo-centric chromosome carrying the secondary constriction or new 45S rDNA locus belonged, GISH was performed on the somatic chromosomes of the variants. A mixture of genomic DNA from each genome and 45S rDNA was biotin-labeled and used as a probe. When probed with the AA diploid genomic DNA, the sixteen chromosomes of genome A were hybridized with the probe. Additionally, two strong 45S rDNA signals were observed at the secondary constriction of the b1 chromosomes (Fig. 1D). One of the subtelo-centric chromosomes the AA diploid genomic DNA possessed a new strong 45S rDNA signal in the interstitial region of the long arm (arrow in Fig. 1D). Probing with the BB diploid genomic DNA resulted in strong signals on 18 genome B chromosomes. One of the subtelo-centric chromosomes with a strong 45S rDNA signal in the interstitial region of the long arm did not result in a GISH signal (arrow in Fig. 1E). One a1 chromosome had a chromosome segment from genome B at the terminal region of one arm. The GISH results indicate that the chromosome carrying the new secondary constriction or strong rDNA signal at the interstitial region of the long arm was identified the a3 chromosome of genome A.

Genomic DNA extracted from the AA and BB diploids and from the amphidiploid (AABB), either with or without the new 45S rDNA locus, were digested with 13 endonucleases and analyzed by Southern hybridization with a 45S rDNA probe. BamHI digests resulted in restriction fragment length polymorphisms (RFLP) in 2 DNA fragments (Fig. 2). Fragments of 1.05 and 2.75 kb are common in the A and B genomes. The 2 largest fragments of genome A were shorter than those of genome B by approximately 900 bp (genome A, 6.2 and 7.5 kb; genome B, 7.1 and 8.4 kb). The standard AABB plants had digest patterns similar to genome B, supporting the conclusion that most of the 45S rDNA of genome A was lost from the a2 chromosomes in the AABB plants and that the secondary constriction was absent (Hizume and Araki 1994). The AABB plants carrying one or 2 new 45S rDNA loci also had RFLP patterns similar to genome B (Fig. 2, lanes 4, 5). This result indicates that the 45S rDNA at the new 45S rDNA locus on the a3 chromosome is from genome B.

We can speculate as to when the new 45S rDNA locus arose in certain amphidiploid plants. The new locus may have originated by addition of the genome B 45S rDNA locus after the establishment of amphidiploid plants. Karyotypes of amphidiploids have been reported in many populations in China, Korea, and Japan (Araki 1972, Haga and Noda 1976, Araki et al. 1979, Choi and Bang 1990, Yu and Araki 1991, Ding et al. 1998). However, this variation of the secondary constriction...
has not been reported in Chinese or Korean populations, and may have occurred in a Japanese population in the Shikoku and/or Kyushu districts. The GISH results (Fig. 1D, E) and meiotic configuration (Fig. 1C) results indicate that the intergenomic translocation between the rDNA-containing b1 chromosome and the a3 chromosome did not involve movement of 45S rDNA. Small accessory chromosomes observed in BB diploids and ABB triploids collected in Kyushu district had rDNA signals over the entire chromosome (Hizume and Araki 1994). The accessory chromosomes might be involved in the addition of 45S rDNA to chromosome a3. In Allium hybrids (Schubert 1984) and Oryza (Shishido et al. 2000) the NORs behave as mobile genetic elements, but the mechanism of the movement of the 45S rDNA in S. scilloides is unknown.

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References


