Pennisetum squamulatum: Is the Predominant Cytotype Hexaploid or Octaploid?

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Apomixis is a mode of asexual reproduction where maternal clones are produced through seeds. Consequently, genetic segregation is prevented in hybrid progenies. Pennisetum squamulatum has been used to transfer apomixis into the related sexual species Pennisetum glaucum by the introgression of an apospory-specific genomic region (ASGR)–carrier chromosome. Crosses between Pennisetum purpureum and Pennisetum squamulatum have been used to transfer apomixis into the related sexual species Pennisetum glaucum by the introgression of an apospory-specific genomic region (ASGR)–carrier chromosome. Crosses between Pennisetum purpureum and Pennisetum squamulatum have been used to transfer apomixis into the related sexual species Pennisetum glaucum by the introgression of an apospory-specific genomic region (ASGR)–carrier chromosome. Crosses between Pennisetum purpureum and Pennisetum squamulatum have been used to transfer apomixis into the related sexual species Pennisetum glaucum by the introgression of an apospory-specific genomic region (ASGR)–carrier chromosome. Crosses between Pennisetum purpureum and Pennisetum squamulatum have been used to transfer apomixis into the related sexual species Pennisetum glaucum by the introgression of an apospory-specific genomic region (ASGR)–carrier chromosome. Crosses between Pennisetum purpureum and Pennisetum squamulatum have been used to transfer apomixis into the related sexual species Pennisetum glaucum by the introgression of an apospory-specific genomic region (ASGR)–carrier chromosome.

Pennisetum squamulatum is one of the major plant species in which apomixis has been studied (Ozias-Akins and others 2003). Apomixis is a form of asexual reproduction where maternal clones can be produced through seeds. The utility of apomixis is the ability to prevent genetic segregation in the progeny of a hybrid plant. Therefore, apomixis has attracted attention from both commercial and academic groups. Although the apomitic trait has not been introduced into major crops, it has been transferred from Pennisetum squamulatum into pearl millet (Pennisetum glaucum) (Dujardin and Hanna 1989a). Molecular cytological study revealed that apomictic pearl millet has a chromosome containing the apospory-specific genomic region (ASGR) from Pennisetum squamulatum, and the ASGR-carrier chromosome is necessary and sufficient for the expression of apomixis (Goel and others 2003). In apomictic pearl millet and Pennisetum squamulatum, the size of the ASGR has been estimated to be at least 50 Mbp by high-resolution fluorescence in situ hybridization (FISH) (Akiyama and others 2004). Study of the transmission of the ASGR-carrier chromosome in Pennisetum squamulatum has raised the question whether it has the characteristics of a supernumerary or B chromosome (Roche and others 2001) or whether it is part of the normal chromosome complement of this species. Further research has established that the ASGR-carrier chromosome is heteromorphic but does have a homologue in the Pennisetum squamulatum genome (Akiyama and others 2004).

The basic number of chromosomes in the genus Pennisetum varies from 5 to 9 (Jauhar 1981). Raman and others (1959) reported that Pennisetum squamulatum had 54 chromosomes. Patil and others (1961) proposed, based on the observation of chromosome paring in meiosis, that Pennisetum squamulatum was an autoallohexaploid species. Sindhe (1976) observed 2 extra chromosomes defined as B chromosomes (n = 54 + 2 Bs). Dujardin and Hanna (1984) reported that Pennisetum squamulatum accession PS24 has 54 chromosomes and is hexaploid (x = 9). In spite of these multiple reports of 54 chromosomes in Pennisetum squamulatum, Goel and others (2003) observed that Pennisetum squamulatum accession PS26 has 56 chromosomes, and 8 chromosomes had 18S-5.8S-26S rDNA loci. Moreover, 28 chromosomes from Pennisetum squamulatum have been observed by genomic in situ hybridization in the 42-chromosome F1 hybrid offspring between tetraploid Pennisetum glaucum and Pennisetum squamulatum.
(Goel and others 2003). Dujardin and Hanna (1983) reported 41-chromosome F₁ hybrids in contrast to 42 reported by Goel and others (2003). The inconsistency could be due to differences between accessions in chromosome number. Cells with large chromosome numbers often show overlapping chromosomes in a spread that makes counting difficult and prone to errors. Application of FISH with a centromeric probe is considered to be more effective for counting an accurate number of chromosomes.

A highly repetitive KpnI fragment was reported in the genus Pennisetum (Ingham and others 1993), and it was mapped on the centromere of P. squamulatum by FISH (Goel and others 2003; Akiyama and others 2004). Using this centromeric repeat and 18S-5.8S-26S rDNA as probes for FISH, we confirmed the number of chromosomes in 5 accessions of P. squamulatum. The ASGR-carrier chromosome also was characterized among the 5 accessions by its hybridization to a bacterial artificial chromosome (BAC) clone containing repetitive sequences abundant in ASGR-carrier chromosomes.

Materials and Methods

Plant Materials

Pennisetum squamulatum accession numbers PS24 (PI248534, Africa), PS26 (PI319196, Africa), PS158 (received from the International Crops Research Institute for the Semi-Arid Tropics [ICRISAT]), PS262 (same as PS24 but received at another time), and PS523 (Australia No. 37914 from B. Pengelly) were grown in the greenhouse in the summer at another time), and PS523 (Australia No. 37914 from B. Pengelly) were grown in the greenhouse in the summer of 2002. For chromosome preparations, root tips of all accessions were collected and pretreated for 3 h by soaking in a saturated solution of 2-bromonaphthalene at 4 °C prior to fixation in 3:1 ethanol:acetic acid.

Probes

Isolation of ASGR-linked BAC clone P208 (containing sequence-characterized amplified region [SCAR] ugr197) was reported previously (Roche and others 2002). BAC clone P602 containing SCAR marker X18 and a large amount of repetitive DNA was isolated by polymerase chain reaction screening of pooled BAC DNAs (Akiyama and others 2004). For 18S-5.8S-26S rDNA, the 18S rDNA–coding region isolated from rice was provided by Dr Nobuko Ohmido, Kobe University, and was used according to Fukui and others (1994).

Chromosome Preparation and FISH

Cytological experiments were carried out according to Akiyama and others (2004). Chromosome spreads were prepared by the enzymatic maceration method (Fukui 1996). After pretreatment with pepsin, slides were denatured at 85 °C with 70% formamide in 2× standard saline citrate (SSC) for 90 s. The slides were incubated with denatured hybridization mixture (ca. 1 ng/ml biotin- or digoxigenin-labeled probe, 5% dextran sulfate [molecular weight = 500,000], 50% formamide, 2× SSC) in a humidified chamber at 37 °C overnight. The digoxigenin-labeled probes were detected with fluorescein using a fluorescent antibody enhancer set for digoxigenin detection (Roche, Indianapolis, IN). Biotin-labeled probes were detected with Texas-red streptavidin (Vector Laboratories, Burlingame, CA) and biotinylated anti-streptavidin (Vector Laboratories) for a second layer of Texas-red streptavidin. After detection, the slides were mounted in Vectashield (Vector Laboratories) containing 1.5 μg/ml 4’,6 diamidino-2-phenylindole (DAPI) and observed under a fluorescence microscope, Olympus BX50. Images of chromosomes were captured by a Sensys charge-coupled device camera (Photometrics, Tucson, AZ) and IMAGE PRO ver. 4.1 software (Media Cybernetics, Silver Spring, MD). The number of chromosomes was counted on digital images with ADOBE PHOTOSHOP 6.0.1.

Image Analysis

ASGR-carrier chromosomes in each accession were analyzed by using OBJECT-IMAGE 2.10 (http://simon.bio.uva.nl/object-image.html) with CHIAS3 (Kato and Fukui 1998) on a Macintosh Power Book. Analysis of variance (ANOVA) was carried out by the Analysis ToolPak add-in option of Microsoft Excel.

Results

Characteristics of the ASGR-Carrier Chromosome across Accessions

In all accessions, BAC P602 hybridized as 2 repetitive blocks on the telomeric region of the hemizygous ASGR-carrier chromosome (Figure 1a–e). The length of the ASGR-carrier chromosome and arm ratios ranged from 5.62 to 6.91 μm and from 1.23 to 1.34, respectively (Table 1). One-way ANOVA showed no significant difference among accessions for either the chromosome length (P = 0.16) or the arm ratio (P = 0.61).

The Number of Chromosomes in P. squamulatum

Chromosomes from at least 14 DAPI-stained spreads from each accession were counted by drawing a line on each chromosome in the digital images. This process facilitated accurate counting. The majority of spreads (90%) across the 5 accessions showed 56 chromosomes (Table 1). Hybridization of a centromeric probe was carried out to confirm that all accessions had 56 centromeric signals. The intensity of the centromeric signal varied among chromosomes. A strong centromeric signal was observed on 25–28 of the chromosomes, whereas the others had a weak signal (Figure 1f). In all accessions, 8 chromosomes were detected by FISH with the 18S-5.8S-26S rDNA probe, which was consistent with our previous report on PS24 (Goel and others 2003) (Figure 1f). Four of the 8 chromosomes consistently had strong signals from rDNA.

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Discussion

The partially hemizygous and heteromorphic ASGR-carrier chromosome was found in all accessions of *P. squamulatum* and had similar morphological characteristics regardless of the genetic background. This result is consistent with a previous report for uniformity of the ASGR-carrier chromosome among BC3, PS24, and PS26 (Goel and others 2003; Akiyama and others 2004).

For *P. squamulatum* chromosome number, we observed fewer or greater than 56 chromosomes in a spread less than 10% of the time. The anomalous chromosome numbers were most likely due to our use of the enzymatic maceration method in which fragile protoplasts can rupture prematurely and disperse their chromosomes. The number of chromosomes and the ploidy level in *P. squamulatum* had been previously reported to be 2n = 56 (Raman and others 1969; Patil and others 1961). Sindhe (1976) observed 2 extra chromosomes in *P. squamulatum* and defined them as B chromosomes, although we did not find any chromosomes that clearly fit the definition of a B chromosome or were acrocentric as described by Sindhe. The number of chromosomes that had a strong centromeric signal fluctuated because of the variation in FISH signal intensity among spreads but could be up to 28. Groups of chromosomes with strong and weak centromeric signals imply that there are at least 2 genomes in *P. squamulatum*. Half the number of 18S-5.8S-26S rDNA chromosomes showed strong centromeric signals, which also supports this supposition. Furthermore, an ASGR-linked BAC, containing a marker that was shown to recombine with apospory, physically mapped to the ASGR-carrier chromosome and its putative homologue as well as to 2 additional, and potentially homeologous, chromosomes (Goel and others 2006).

The rDNA loci have been localized in numerous species by FISH. The number of rDNA loci does not directly reflect the ploidy level in many species (Raina and Mukai 1999; Krishnan and others 2001; Mishima and others 2002) because duplication, deletion, and/or translocation have occurred during chromosomal evolution. Recently, cytological study revealed that the number of rDNA loci could be changed with alternative rearrangements in chromosome structure by cross-hybridization between *Arabidopsis* species (Pontes and others 2004). The number of rDNA loci does increase, however, with genome duplication, and the information can be helpful to decide the ploidy level. Colchicine-induced tetraploids in *Rubus* showed the number of rDNA loci corresponding to the ploidy level (Lim and others 1998). In the genus *Sanguisorba*, duplicated 18S-5.8S-26S rDNA sites were all conserved after each polyploidization (Mishima and others 2002). Eight chromosomes bearing 18S-5.8S-26S rDNA loci in all accessions of *P. squamulatum* suggest that this species is octaploid. Evolutionary changes in rDNA loci may occur more slowly in apomictic than in sexual species as has been shown for concerted evolution of such loci in the *Amelanchier* agamic complex of Rosaceae (Campbell and others 1997).

Martel and others (1997) reported the DNA content of several *Pennisetum* species. According to their data, the DNA amount per haploid chromosome set for the species with a basic chromosome number of x = 9 ranged from 0.85 to 0.95 pg with the exception of *P. squamulatum* whose DNA content was 0.93 pg. This result is consistent with the ploidy level of 56 reported for this species. Additional investigation is needed to confirm the exact ploidy level of *P. squamulatum*.

![Figure 1. FISH of *Pennisetum squamulatum* chromosomes with a BAC clone that localizes to the ASGR-carrier chromosome (P602), 18S rDNA, and a centromeric probe. (a–e) ASGR-carrier chromosome detected by P602 (red). (f) A chromosome spread of PS262 hybridized with 18S-5.8S-26S rDNA (green with green arrows), P208 (red with white arrow), and centromeric probe (red with red arrows to indicate strong centromeric signals). Bars equal 10 µm.](https://academic.oup.com/jhered/article-abstract/97/5/521/2187626)

**Table 1.** Morphology of the ASGR-carrier chromosome and the number of chromosomes in *Pennisetum squamulatum*

<table>
<thead>
<tr>
<th>Accession</th>
<th>ASGR-carrier chromosome</th>
<th>Chromosome number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>54</td>
</tr>
<tr>
<td>PS24</td>
<td>55</td>
<td>1 (7.1%)</td>
</tr>
<tr>
<td>PS26</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>PS158</td>
<td>6</td>
<td>1 (5.3%)</td>
</tr>
<tr>
<td>PS262</td>
<td>6</td>
<td>2 (13.3%)</td>
</tr>
<tr>
<td>PS523</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>4 (4.2%)</td>
<td>2 (2.1%)</td>
</tr>
</tbody>
</table>

*According to Akiyama and others (2004).*
DNA amount per haploid genome was 1.59 pg. The $x = 7$ species are distributed in the primary (P. glaucum), secondary (Pennisetum purpureum), and tertiary (Pennisetum schweinfurthii) gene pools based on their interfertility (Harlan and de Wet 1971). The secondary gene pool species P. purpureum has a DNA amount of 1.15 pg per haploid genome and hybridizes readily with P. glaucum (Hanna 1990). Pennisetum squamulatum also hybridizes well with P. glaucum, although only at the tetraploid level (Dujardin and Hanna 1983, 1989b). If P. squamulatum is considered to be octaploid with a basic chromosome number of $x = 7$, the DNA amount per haploid genome would be 1.19, which is similar to P. purpureum. Pennisetum squamulatum has also been crossed with P. purpureum cv. Merkeron (hybrid Napier grass, Burton 1989) giving 4 hybrids from 2 pollinations (WW Hanna, unpublished data). These hybrids were partially male fertile (9–51%) and showed up to 69% seed set. Because P. squamulatum is an outlier in the $x = 9$ group with respect to the average DNA amount per haploid genome, produces fertile hybrids with P. purpureum, and shares a substantial number of random amplified polymorphic DNA fragments with P. purpureum (Ozias-Akins and others 1993), we propose an octaploid status for P. purpureum. This work was supported by the US Department of Agriculture National Research Initiative (award no. 02–35301-12283).

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References


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